

Compounds for complexation of rare earth elements and/or s-, p-, d- block metals, their coordination compounds, peptide conjugates, method of their preparation and use thereof

State of Art

5

The present invention relates to new macrocyclic compounds suitable as cross-bridged chelators for complexation of rare earth elements and/or s-, p-, d- block metals, forming extremely stable coordination compounds. These coordination compounds are suitable for use as labels for quantitative detection of peptide conjugates, and/or as contrast agents for magnetic resonance imaging. The invention further relates to a method
10 of preparation of the chelators and to a method of tracing peptide/protein containing medicaments.

Background Art

Metal elements find biomedical applications such as imaging contrast agents, radiotherapeutic agents or
15 bioanalytical labels. For most of these applications, it is necessary to bind the metal in a stable chelate that can be covalently linked to other molecules, such as targeting vectors based on peptides or antibodies. The most universal example of a chelator applicable to majority of metal elements is DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) and its derivatives. A broad range of other chelators have been developed specifically for particular metal elements. [Price E. W., Orvig C. (2014), *Chem. Soc. Rev.* **43**(1), 260
20 – 290].

Rare earth elements (scandium - Sc, yttrium - Y, lanthanum - La, cerium - Ce, praseodymium - Pr, neodymium - Nd, promethium - Pm, samarium - Sm, europium - Eu, gadolinium - Gd, terbium - Tb, dysprosium - Dy, holmium - Ho, erbium - Er, thulium - Tm, ytterbium - Yb and lutetium - Lu) are a group of metals that offer a
25 broad range of medical applications. Radiopharmaceuticals based on ^{90}Y , ^{153}Sm and ^{177}Lu are approved by FDA, clinical trials are ongoing with ^{166}Ho , and others show advantageous properties for Positron Emission Tomography (PET), Single-Photon Emission Computed Tomography (SPECT) or therapy (^{44}Sc , ^{47}Sc , ^{86}Y , ^{149}Pm , ^{159}Gd , ^{149}Tb , ^{161}Tb , ^{165}Dy , ^{161}Ho , ^{169}Er and ^{175}Yb). Stable, non-radioactive Gd chelates are in clinical use as contrast agents for Magnetic Resonance Imaging (MRI). Stable isotopes of rare earth elements also serve as
30 labels for analytical purposes. Compounds of interest labeled in this way can be visualized, traced and quantified based on unique luminescence or isotope mass of the elements that have zero background in biological systems. The unique isotope mass is especially useful, as it allows many compounds to be quantified simultaneously in a single analysis (multiplexing), typically with the use of inductively-coupled plasma mass spectrometry (ICP-MS) [Bodenmiller B. *et al.* (2012), *Nat. Biotechnol.* **30**(9), 858-867]. Other metal elements

from s-, p- and d-block of the periodic system, such as ^{89}Sr , ^{223}Ra , $^{117\text{m}}\text{Sn}$, ^{212}Pb , ^{213}Bi , ^{64}Cu , ^{225}Ac , find use in radiopharmaceutical compounds for imaging or therapy.

For practical use, metal chelates must be very stable, so that the metal ion cannot easily escape from the chelator and detach from the carrier molecule. In this respect, thermodynamic stability constant provides insufficient information, because most applications proceed under conditions far from thermodynamic equilibrium (e.g. *in-vivo*). Instead, kinetic inertness that characterizes the rate at which the metal ion escapes from the chelate under given conditions must be considered. Kinetic inertness strongly correlates with rigidity of the chelator. Acyclic chelators of the type DTPA (diethylenetriaminepentaacetic acid) have more flexible structure and provide lower kinetic inertness than macrocyclic chelators of the type DOTA, which are more rigid. Chelates with high kinetic inertness are desirable particularly for *in-vivo* applications, where the metal chelate is challenged with excess of competing biogenic chelators and metal ions. For this reason, the rigid and kinetically inert macrocyclic chelators are preferred to the flexible acyclic ones. For example, practically all radiopharmaceuticals based on the radionuclide ^{177}Lu (half-life 6.7 days) that are approved or in development make use of the macrocyclic chelator DOTA, as the metal must remain bound to the targeting molecule *in-vivo* for weeks in order to have the desired curative effect. Similarly, in MRI contrast agents, macrocyclic chelates of gadolinium(III) are preferred to the acyclic DTPA type. It has been recently found that significant amount of free gadolinium is released *in-vivo* from the acyclic agents and deposited in human brain for long time. [Fur M. L., Caravan P. (2019), *Metallomics* 11(2), 240-254] In response to this finding and to prevent potential harm to patients, the use of the acyclic MRI contrast agents has been severely restricted around the globe by drug-regulating agencies. It is therefore likely that the importance of kinetic inertness of metal chelates will grow in medical and other applications, and chelators providing higher inertness will be needed.

Methods to further increase kinetic inertness rely on reinforcement and rigidification of the macrocyclic chelators with additional rings. For example, a cross-bridged macrocyclic chelator cb-TE2A (4,11-bis(carboxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane) provides much more inert chelates with copper(II) ion than the non-cross-bridged analogues [Boswell C. A. *et al.* (2004), *J. Med. Chem.* 47(6), 1465-1474]. Another similar chelator cb-TEDPA (6,6'-((1,4,8,11-Tetraazabicyclo[6.6.2]hexadecane-4,11-diyl)bis(methylene))dipicolinic acid) was shown to provide exceptionally inert lanthanide(III) chelates [Rodríguez-Rodríguez A. *et al.* (2016), *Inorg. Chem.* 55(5), 2227-2239]. Another cyclen-based cross-bridged chelator provided extremely inert copper(II) chelates [Esteves, C. V. *et al.* (2013), *Inorg. Chem.* 52(9), 5138-5153]. A common feature of these chelators is that the bridge is independent from the coordinating pendant arms and is oriented on the opposite side from the pendant arms in the chelate. Chelators where the bridge connected the pendant arms themselves have also been made, but the effect on stability of the chelates was strongly negative [Vipond, J. *et al.* (2007), *Inorg. Chem.* 46(7), 2584-2595].

There are also practical limitations when too high kinetic inertness becomes counterproductive. This is because the energy barrier that the metal ion must overcome on the way out of the chelate is similar to the barrier that it has to overcome on the way in during chelate formation. Generally, the more inert a chelate is, the more difficult it is to make. The formation kinetics can be accelerated with high temperature and long reaction times. However, such reaction conditions are often incompatible with sensitive targeting vectors (e.g. antibodies) and/or half-life of metal radionuclides in preparation of radiopharmaceuticals.

There remains a strong need for new types of chelators, which would form highly kinetically inert metal complexes quickly and under mild synthetic conditions.

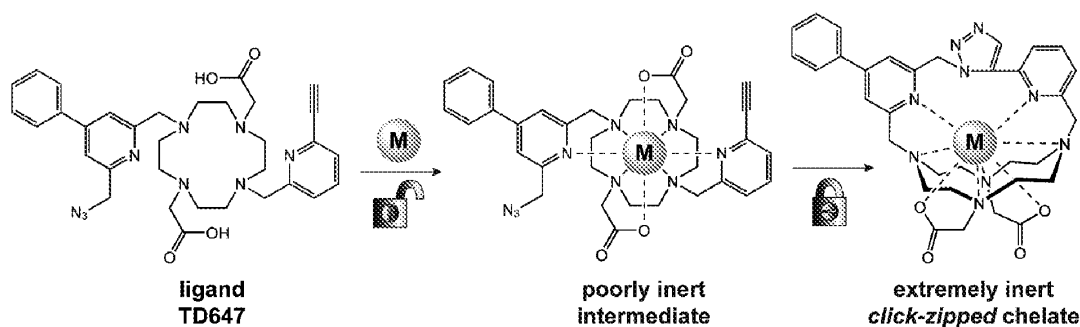
Disclosure of Invention

We have circumvented problems of the background art with a new type of chelators, which form a bridge after complexation of the metal ion that rigidifies the structure and increases kinetic inertness. The bridge is formed between two coordinating pendant arms based on cycloaddition reaction between alkyne and azide substituents on the opposite pendant arms. This reaction does not require catalyzation by Cu(I) ions as is usually the case, and occurs spontaneously after formation of a non-bridged chelate. The chelator therefore acts as a one-way trap for the metal ion. Initial formation of the non-bridged chelate is relatively fast. Then, a bridge is formed that traps the metal inside the chelator. For rare earth elements, the resulting bridged chelates show extremely high kinetic inertness that is up to 6 orders of magnitude higher than for the analogous chelates with DOTA. Such unusually high inertness allows new uses of the chelates as analytical labels that can withstand harsh hydrolytic conditions in concentrated acids and can be quantified in the lysate as intact chelates with liquid chromatography – mass spectrometry (LC-MS). The use of LC-MS is advantageous, as it is much more common instrument than ICP-MS. The extremely high inertness is also advantageous for potential *in-vivo* use of the chelates, such as MRI contrast agents or radiopharmaceuticals, as no free metal is released *in-vivo* from the coordination compounds, therefore no metal deposit occurs in human or animal body.

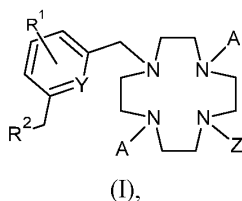
The compounds according to the present invention are capable of acting as extremely efficient molecular traps, which can coordinate metal ions in an extremely stable, rigid and well defined manner. The ligands can be prepared in relatively a few number of steps of organic synthesis. After coordination of the metal ion, the click reaction is performed between the azide group and a triple bond present in opposite pendant arms of the macrocycle, forming a triazole bridge, and enclosing the metal ion within the cage (forming the so-called click-zipped complex). The formation of triazole is irreversible and is depicted in Scheme 1 below.

Scheme 1. Schematic representation of the *click-zip* principle illustrated on the example of ligand **TD647**.

4



In first aspect, the subject of the present invention relates to compounds of general formula (I)



5

wherein

Y is selected from a group consisting of nitrogen (N); N-oxide (N⁺-O⁻);

R¹ is selected from the group consisting of H; halogen; -OH; -N₃; -(CH₂)_nN₃, wherein *n* is an integer in the range of from 1 to 3; -NR₂, wherein R is independently selected from H or C₁ to C₆ alkyl, which may be branched or linear; -(CH₂)_nNR₂, wherein *n* and R are as defined above; C₆ to C₁₀ aryl, which can optionally be substituted with -NH₂, -NO₂, -N₃, ξ -≡CH, -COOH, -CH₂Cl and/or -CH₂COOH; C₇ to C₁₀ arylalkyl, which can optionally be substituted with -NH₂, -NO₂, -N₃, ξ -≡CH, -COOH, -CH₂Cl and/or -CH₂COOH;

-CF₃; -COOR, wherein R is as defined above; -(CH₂)_nCOOR, wherein *n* and R are as defined above;

15 ξ -≡CH-Si(iPr)₃; -CH₂CH(OMe)₂; ξ -≡CH; -SH; -SO₃H; -SO₂Ar, wherein Ar is phenyl; NO₂;

R² is ξ -N₃; or ξ -[CH₂]_n-N₃, wherein *n* is an integer in the range of from 1 to 3; preferably, R² is ξ -N₃ or ξ -CH₂-N₃; more preferably R² is ξ -N₃;

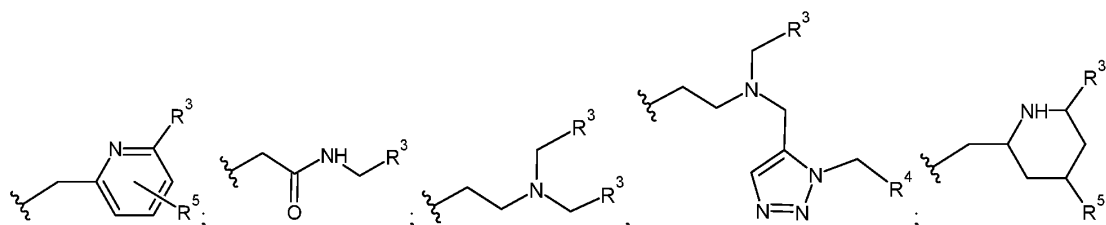
A are independently selected from the group consisting of H; -(CH₂)_nCOOH, wherein *n* is an integer from 1 to 3; -CH(CH₃)COOH; -CH((CH₂)_nCH₃)COOH, wherein *n* is as defined above; -CH₂P(=O)(OR)₂, wherein

20 R is as defined above; -CH((CH₂)_nCOOH)COOH, wherein *n* is an integer from 1 to 3;

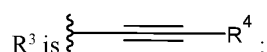
-CH((CH₂)_nNH₂)COOH, wherein *n* is an integer from 1 to 3;

$-\text{CH}_2\text{C}(=\text{O})(\text{NH}_2)$; $-\text{CH}_2\text{C}(=\text{O})(\text{NH})-\text{CH}_2\text{COOH}$; $-\text{CH}_2\text{P}(=\text{O})(\text{OH})(\text{Ar})$, wherein Ar is phenyl, which can optionally be substituted with C_1 to C_6 alkyl;

Z is selected from a group consisting of



5 wherein

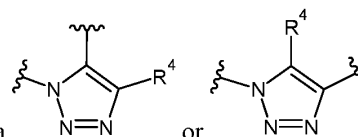


R^4 is selected from the group consisting of H; halogen; piperidinyl; trimethylsilyl; triisopropylsilyl; phenyl; C_1 to C_6 alkyl, which may be branched or linear; C_3 to C_6 cycloalkyl; $-\text{CF}_3$; $-(\text{CH}_2)_n\text{NHR}^6$, wherein n is an integer in the range of from 1 to 3 and R^6 is selected from H, fluorenylmethoxycarbonyl, tert-butyloxycarbonyl and

10 benzyloxycarbonyl; $-\text{C}(\text{CH}_3)_2(\text{NHR}^6)$, wherein R^6 is as defined above; adamantyl; $\text{---}\equiv\text{CH}$; $-(\text{CH}_2)_n\text{COOH}$, wherein n is as defined above; C_6 to C_{10} aryl, which can optionally be substituted with $-\text{NH}_2$, $-\text{NO}_2$, $-\text{N}_3$, $\text{---}\equiv\text{CH}$, $-\text{COOH}$, $-\text{CH}_2\text{Cl}$ and/or $-\text{CH}_2\text{COOH}$;

R^5 is R^1 or $\text{---}\equiv\text{---}\text{R}^4$, wherein R^4 is as defined above; preferably R^5 is H; $-\text{CF}_3$; halogen; $-\text{OH}$; C_7 to C_{10} arylalkyl, which can optionally be substituted with $-\text{NH}_2$, $-\text{NO}_2$, $-\text{COOH}$, $-\text{CH}_2\text{Cl}$ and/or

15 $-\text{CH}_2\text{COOH}$; or $\text{---}\equiv\text{---}\text{R}^4$;



and/or R^2 and R^3 together form a 1,2,3-triazole group of formula or ; wherein R^4 is defined above;

with the proviso that at most one A is H.

When the compounds of this invention contain a chiral centre, then all enantiomers, mixtures of enantiomers and racemates fall within the framework of the present invention. The present invention further includes the compounds of general formula (I) in the form of salts with alkali metals, ammonium or amines, as well as in the form of addition salts with acids.

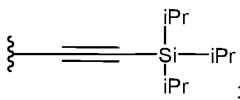
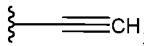
The term „halogen“ means any isotope of F, Cl, Br, and I; in particular, this term includes non-radioactive isotopes, such as ^{19}F , ^{35}Cl , ^{37}Cl , ^{79}Br , ^{81}Br , ^{127}I , and radioisotopes, such as ^{18}F , ^{36}Cl , ^{77}Br , ^{83}Br , ^{123}I , ^{124}I , ^{125}I , ^{131}I .

In one embodiment, A are independently selected from the group consisting of H; $-(CH_2)_nCOOH$, wherein n is an integer from 1 to 3; $-CH(CH_3)COOH$; $-CH((CH_2)_nCH_3)COOH$, wherein n is an integer from 1 to 3; $-CH_2P(=O)(OR)_2$, wherein R is as defined above; $-CH_2C(=O)(NH_2)$; $-CH_2C(=O)(NH)-CH_2COOH$; $-CH_2P(=O)(OH)(Ar)$, wherein Ar is phenyl, which can optionally be substituted with C_1 to C_6 alkyl.

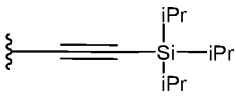
5

In one embodiment, R^1 is selected from the group consisting of H; halogen; $-OH$; $-N_3$; $-CH_2N_3$; $-NR_2$, wherein R is independently selected from H or C_1 to C_6 alkyl, which may be branched or linear; $-CH_2NR_2$, wherein R are as defined above; C_6 to C_{10} aryl, which can optionally be substituted with $-NH_2$, $-NO_2$, $-COOH$ and/or $-CH_2COOH$; C_7 to C_{10} arylalkyl, which can optionally be substituted with $-NH_2$, $-NO_2$, $-COOH$, $-CH_2Cl$ and/or $-CH_2COOH$; $-CF_3$; $-COOR$, wherein R is as defined above; $-(CH_2)_nCOOR$, wherein n and R are as

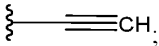
10

defined above; ; $-CH_2CH(OMe)_2$; ; $-SH$; $-SO_3H$; $-SO_2Ar$, wherein Ar is phenyl; NO_2 ;

preferably R^1 is selected from H; halogen; $-OH$; $-N_3$; $-CH_2N_3$; $-N(CH_3)_2$; phenyl; carboxyphenyl; $-CF_3$;

$-COOR$, wherein R is as defined above; and .

15

In one embodiment, R^4 is selected from the group consisting of H; halogen; piperidinyl; trimethylsilyl; triisopropylsilyl; phenyl; C_1 to C_6 alkyl, which may be branched or linear; C_3 to C_6 cycloalkyl; $-CF_3$; $-CH_2NHR^6$, wherein R^6 is selected from H, fluorenylmethyloxycarbonyl, tert-butyloxycarbonyl and benzyloxycarbonyl; $-C(CH_3)_2(NHR^6)$, wherein R^6 is as defined above; adamantyl; .

20

preferably R^4 is selected from the group consisting of H; trifluoromethyl; 4-piperidinyl; $-CH_2-NH_2$; phenyl; cyclopropyl; adamantyl; tert-butyl; trimethylsilyl (TMS); triisopropylsilyl (TIPS); halogen (F, Cl, Br, I); $-C(CH_3)_2NH_2$; and $-C(CH_3)_2NHBoc$. More preferably, R^4 is hydrogen.

In one embodiment of the present invention, the compounds of general formula (I) comprises the following substituents:

25

Y is nitrogen (N);

R^1 is selected from the group consisting of H; halogen; $-OH$; $-N_3$; $-CH_2N_3$; $-NR_2$, wherein R is independently selected from H or C_1 to C_6 alkyl, which may be branched or linear; C_6 to C_{10} aryl, which can optionally be substituted with $-NH_2$, $-NO_2$, $-COOH$ and/or $-CH_2COOH$; C_7 to C_{10} arylalkyl, which can optionally be

30

substituted with $-NH_2$, $-NO_2$, $-COOH$, $-CH_2Cl$ and/or $-CH_2COOH$;

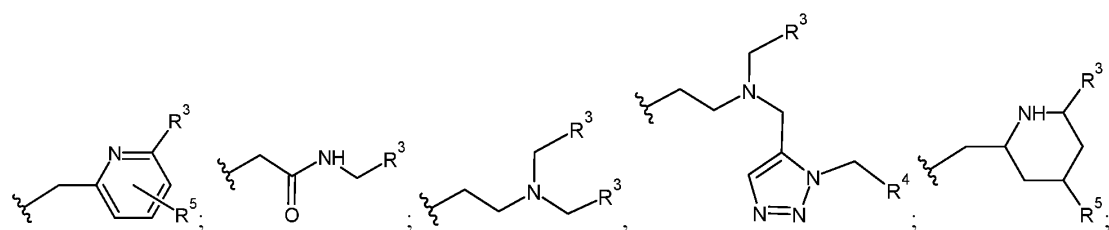
7

$-\text{CF}_3$; $-\text{COOR}$, wherein R is as defined above; $\text{---}\equiv\text{Si}(\text{iPr})_3$; $-\text{CH}_2\text{CH}(\text{OMe})_2$; $\text{---}\equiv\text{CH}$;
 R^2 is $\text{---}\text{N}_3$;

A are independently selected from the group consisting of H; $-(\text{CH}_2)_n\text{COOH}$, wherein n is an integer from 1 to 3; $-\text{CH}(\text{CH}_3)\text{COOH}$; $-\text{CH}((\text{CH}_2)_n\text{CH}_3)\text{COOH}$, wherein n is as defined above; $-\text{CH}_2\text{P}(=\text{O})(\text{OR})_2$, wherein

5 R is as defined above; $-\text{CH}_2\text{C}(=\text{O})(\text{NH}_2)$; $-\text{CH}_2\text{C}(=\text{O})(\text{NH})-\text{CH}_2\text{COOH}$; $-\text{CH}_2\text{P}(=\text{O})(\text{OH})(\text{Ar})$, wherein Ar is phenyl, which can optionally be substituted with C_1 to C_6 alkyl;

Z is selected from a group consisting of

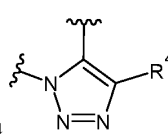
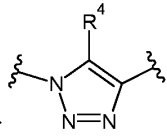


wherein

10 R^3 is $\text{---}\equiv\text{R}^4$;

R^4 is selected from the group consisting of H; halogen; piperidinyl; trimethylsilyl; triisopropylsilyl; phenyl; C_1 to C_6 alkyl, which may be branched or linear; C_3 to C_6 cycloalkyl; $-\text{CF}_3$; $-\text{CH}_2\text{NHR}^6$, wherein R^6 is selected from H, fluorenylmethyloxy carbonyl and benzyloxycarbonyl; $-\text{C}(\text{CH}_3)_2(\text{NHR}^6)$, wherein R^6 is as defined above; adamantyl; $\text{---}\equiv\text{CH}$;

15 R^5 is H; $-\text{CF}_3$; halogen; $-\text{OH}$; C_7 to C_{10} arylalkyl, which can optionally be substituted with $-\text{NH}_2$, $-\text{NO}_2$, $-\text{COOH}$, $-\text{CH}_2\text{Cl}$ and/or $-\text{CH}_2\text{COOH}$; or $\text{---}\equiv\text{R}^4$, wherein R^4 is as defined above;

and/or R^2 and R^3 together form a 1,2,3-triazole group of formula  or ; wherein R^4 is defined above;

with the proviso that at most one A is H.

20

In one preferred embodiment, Y is nitrogen, thus forming a pendant arm comprising a pyridyl moiety, substituted with R^1 and R^2 .

In one embodiment, R^1 is in meta-position from Y, and para-position from R^2-CH_2- .

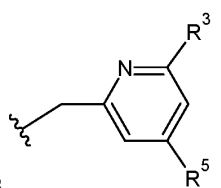
In one embodiment, R^1 is in meta-position from Y, and ortho-position from R^2 -CH₂-.

In another embodiment, R^1 is in para-position from Y.

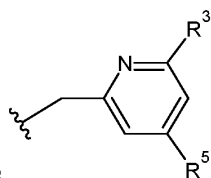
In one preferred embodiment, R^2 is ξ -N₃.

Preferably, R^2 is ξ -N₃ and R^1 is selected from the group comprising H, phenyl, carboxyphenyl, halogen
 5 (preferably Cl), trifluoromethyl, carboxyl, carboxylic ester or amine, more preferably R^1 is H or carboxyphenyl.

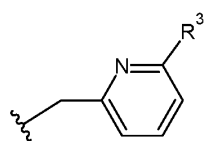
In one embodiment, Z is



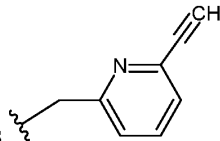
In one preferred embodiment, Z is



and R^3 and R^5 are as defined above. Preferably, Z is



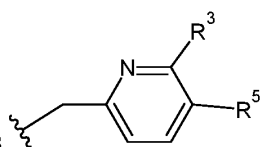
, even more preferably, Z is



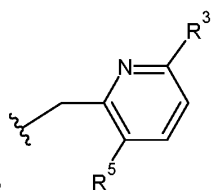
10

In the compound of general formula (I), A can be the same or different. Preferably, A are the same.

In one embodiment, Z is



In one embodiment, Z is



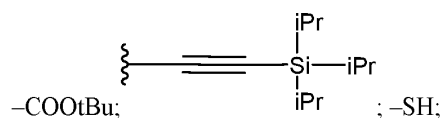
15

In one embodiment, A is selected from the group comprising $-(CH_2)COOH$; $-(CH_2)_2COOH$;
 $-CH_2P(=O)(OEt)_2$; $-CH_2P(=O)(OH)_2$; $-CH_2P(=O)(OH)(OEt)$; $-CH_2P(=O)(Ph)(OH)$; $-(CH_2)COONH_2$;

$-(CH_2)C(=O)NH-CH_2COOH$; $-(CH_2)COO^tBu$; $-CH(CH_2COOH)COOH$.

Preferably, A is selected from the group comprising $-(CH_2)COOH$; $-CH_2P(=O)(OEt)_2$; $-CH_2P(=O)(OH)_2$; $-CH_2P(=O)(OH)(OEt)$; and $-CH_2P(=O)(Ph)(OH)$. Most preferably, A is $-(CH_2)COOH$.

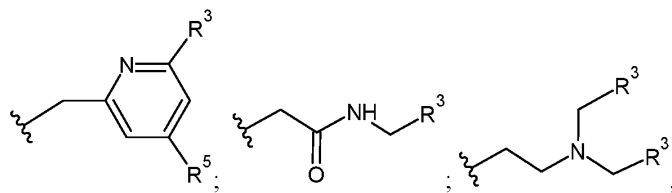
- 5 In one embodiment, Y is nitrogen; R^1 is selected from the group consisting of H; Cl; $-N(CH_3)_2$; phenyl, which can optionally be substituted with $-COOH$ or $-CH_2COOH$; benzyl, which can optionally be substituted with $-COOH$ or $-CH_2COOH$; $-CF_3$; $-COOCH_3$; $-COOCH(CH_3)_2$;



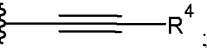
A are independently selected from the group consisting of H; $-(CH_2)_nCOOH$, wherein n is 1 or 2;

- 10 $-CH_2P(=O)(OH)_2$; $-CH_2P(=O)(OH)(OEt)$; $-CH_2P(=O)(OEt)_2$; $-CH_2C(=O)(NH_2)$; $-CH_2C(=O)(NH)-CH_2COOH$; $-CH_2P(=O)(OH)(Ph)$;

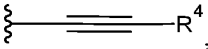
Z is selected from a group consisting of



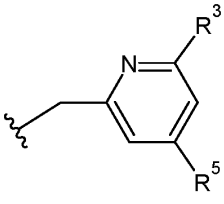
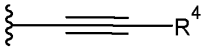
wherein

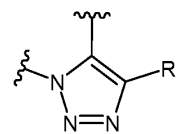
- 15 R^3 is ;

R^4 is selected from the group consisting of H; halogen; piperidinyl; trimethylsilyl; triisopropylsilyl; phenyl; cyclopropyl; *tert*-butyl; $-CF_3$; $-CH_2NH_2$; $-CH_2N(H)(Fmoc)$; $-C(CH_3)_2(NH_2)$; $-C(CH_3)_2(NHBoc)$; adamantyl;

R^5 is H or , wherein R^4 is as defined above;

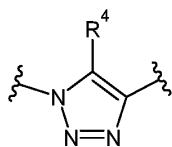
with the proviso that at most one A is H.

- 20 In one preferred embodiment, Z is ; R^3 is ; R^4 and R^5 are as defined above, preferably R^4 and R^5 are H.



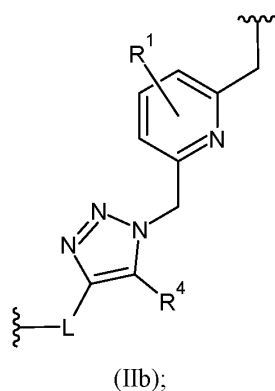
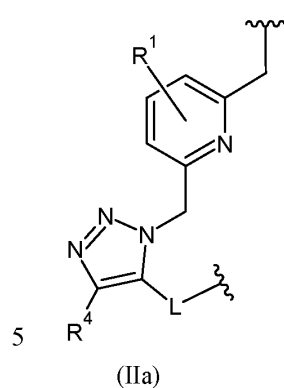
In another embodiment, R^2 and R^3 together form a 1,2,3-triazole group of formula

or



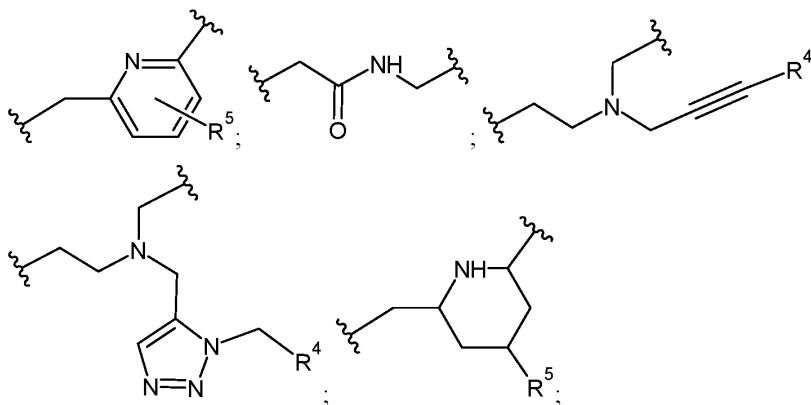
; wherein R^4 is as defined above, preferably R^4 is hydrogen. In such embodiment,

a bridge is formed between two opposite nitrogen atoms of the cyclen moiety, and the compound of general formula (I) thus forms a cage-like structure. The bridge is an entity of general formula (IIa) or (IIb)

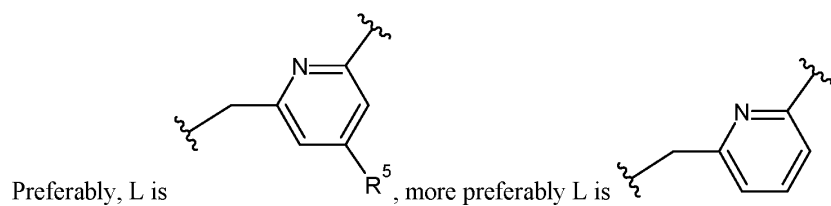


wherein

L is a linker selected from the group consisting of

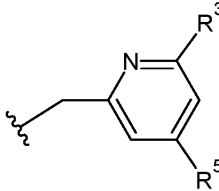
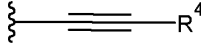


and R^1 , R^4 and R^5 are as defined above. The linker L is derived from the group Z defined above.

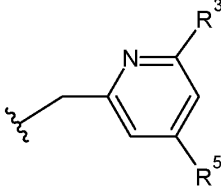
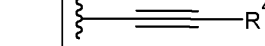
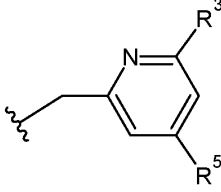
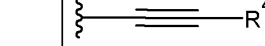
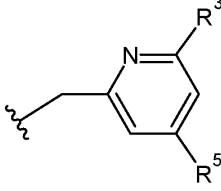
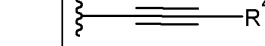


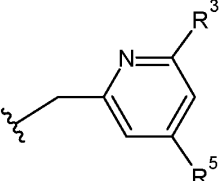
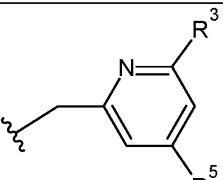
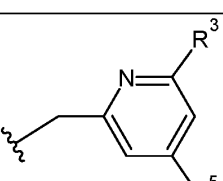
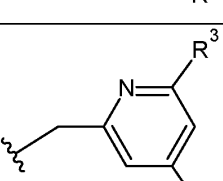
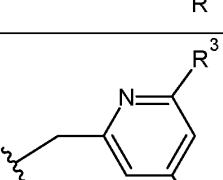
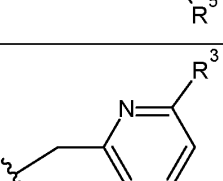
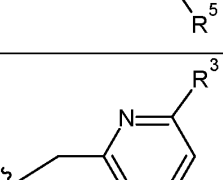
In one preferred embodiment, the compound of general formula (I) is selected from the group comprising compounds with the following combinations of substituents:

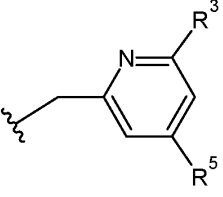
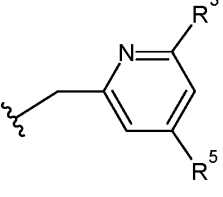
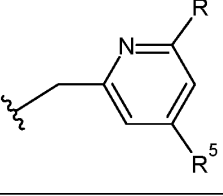
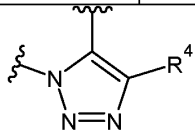
- 5 Y is N^+-O^- ; R^1 , R^4 and R^5 is H:

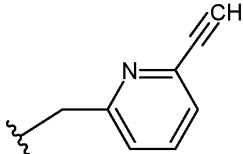
Compound	A	Z	R^2	R^3
TD604	$-(\text{CH}_2)\text{COOH}$			

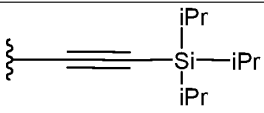
Y is N; R^1 , R^4 and R^5 is H:

Compound	A	Z	R^2	R^3
TD556	$-(\text{CH}_2)\text{COOH}$			
TD1204	$-(\text{CH}_2)\text{COOH}$			
TD703	$-(\text{CH}_2)\text{COOH}$ and H			

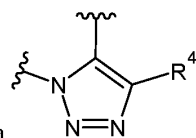
TD705	$-\text{CH}_2\text{P}(=\text{O})(\text{OEt})_2$ and $-(\text{CH}_2)\text{COO}^t\text{Bu}$		$\xi-\text{N}_3$	$\xi-\text{C}\equiv\text{C}-\text{R}^4$
TD714	$-\text{CH}_2\text{P}(=\text{O})(\text{OH})_2$ and $-(\text{CH}_2)\text{COOH}$		$\xi-\text{N}_3$	$\xi-\text{C}\equiv\text{C}-\text{R}^4$
TD921	$-\text{CH}_2\text{P}(=\text{O})(\text{Ph})(\text{OH})$		$\xi-\text{N}_3$	$\xi-\text{C}\equiv\text{C}-\text{R}^4$
TD580	$-\text{CH}_2\text{P}(=\text{O})(\text{OEt})_2$		$\xi-\text{N}_3$	$\xi-\text{C}\equiv\text{C}-\text{R}^4$
TD582	$-\text{CH}_2\text{P}(=\text{O})(\text{OH})(\text{OEt})$		$\xi-\text{N}_3$	$\xi-\text{C}\equiv\text{C}-\text{R}^4$
TD581	$-\text{CH}_2\text{P}(=\text{O})(\text{OH})_2$		$\xi-\text{N}_3$	$\xi-\text{C}\equiv\text{C}-\text{R}^4$
TD801	$-(\text{CH}_2)_2\text{COOH}$		$\xi-\text{N}_3$	$\xi-\text{C}\equiv\text{C}-\text{R}^4$

TD1346	$-\text{CH}(\text{CH}_3)\text{COOH}$		---N_3	$\text{---}\equiv\text{---R}^4$
TD1408	$-(\text{CH}_2)\text{COOH}$		$\text{---CH}_2\text{---N}_3$	$\text{---}\equiv\text{---R}^4$
cz-TD556	$-(\text{CH}_2)\text{COOH}$			

Y is N; Z is  : and R² is ---N_3 :

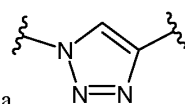
Compound	R ¹	A
TD827		$-(\text{CH}_2)\text{COOH}$
TD575	Ph	$-\text{CH}_2\text{P}(=\text{O})(\text{OEt})_2$
TD576	Ph	$-\text{CH}_2\text{P}(=\text{O})(\text{OH})_2$
TD764	Cl	$-(\text{CH}_2)\text{COOH}$
TD1063	$-\text{CF}_3$	$-(\text{CH}_2)\text{COOH}$
TD1092	$-\text{COOCH}_3$	$-(\text{CH}_2)\text{COOH}$
TD1148	$-\text{COO}^i\text{Pr}$	$-(\text{CH}_2)\text{COOH}$
TD1160	$-\text{COO}^t\text{Bu}$	$-(\text{CH}_2)\text{COOH}$
TD1176	$-\text{N}(\text{CH}_3)_2$	$-(\text{CH}_2)\text{COOH}$
TD647	Ph	$-(\text{CH}_2)\text{COOH}$
TD742	4-carboxyphenyl	$-(\text{CH}_2)\text{COOH}$
TD779	Ph	$-(\text{CH}_2)\text{COONH}_2$

TD778	Ph	$-(\text{CH}_2)\text{CONH-CH}_2\text{COOH}$
TD1504	H	$-\text{CH}(\text{CH}_2\text{COOH})\text{COOH};$ $-\text{CH}(\text{CH}_3)\text{COOH}$



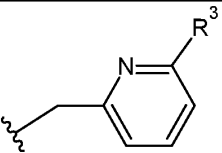
Y is N; and R^2 and R^3 together form a 1,2,3-triazole group of formula

Compound	R^1	A	Z	R^4
cz-TD425	Ph	$-(\text{CH}_2)\text{COOH}$		H
cz-TD764	Cl	$-(\text{CH}_2)\text{COOH}$		H
cz-TD1063	$-\text{CF}_3$	$-(\text{CH}_2)\text{COOH}$		H
TD1188	H	$-(\text{CH}_2)\text{COOH}$		$-\text{CF}_3$
TD650	Ph	$-(\text{CH}_2)\text{COOH}$		H

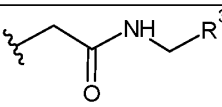
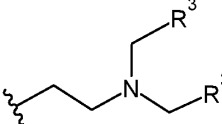


Y is N; and R^2 and R^3 together form a 1,2,3-triazole group of formula

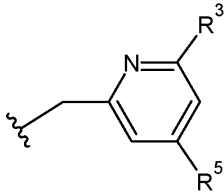
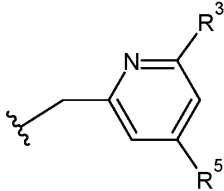
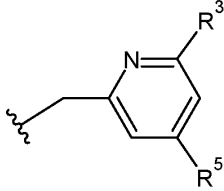
Compound	R^1	A	Z
----------	--------------	---	---

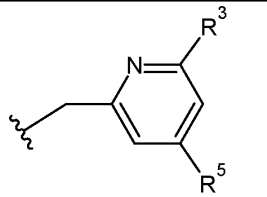
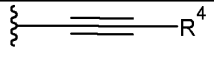
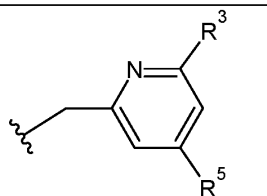
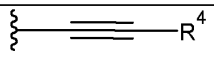
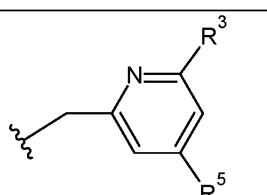
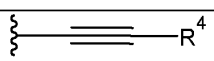
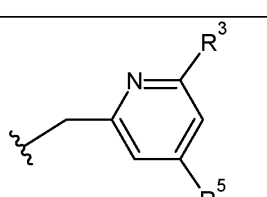
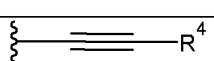
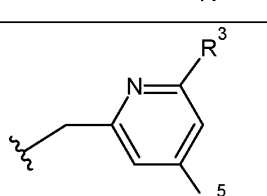
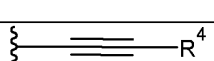
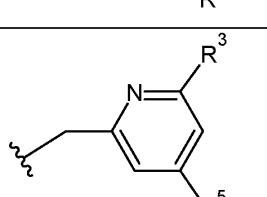
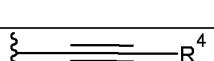
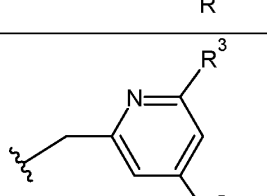
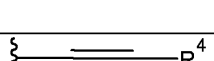
TD871	Ph	$-(CH_2)COOH$	
--------------	----	---------------	---

Y is N; R¹ is phenyl; and R² is ---N_3 ; and R³ is $\text{---}\equiv\text{CH}$.

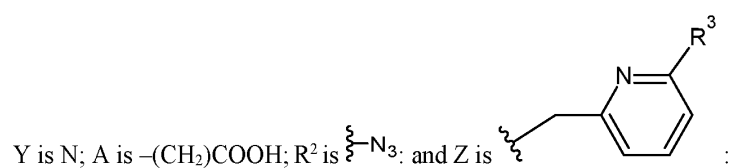
Compound	A	Z
TD425	$-(CH_2)COOH$	
TD669	$-(CH_2)COOH$	

Y is N; A is $-(CH_2)COOH$; R¹ is H; and R² is ---N_3 .

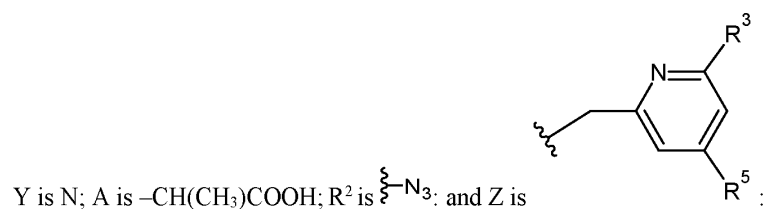
Compound	Z	R ³	R ⁴	R ⁵
TD810		$\text{---}\equiv\text{---R}^4$	H	$\text{---}\equiv\text{---R}^4$
TD718		$\text{---}\equiv\text{---R}^4$	TMS	H
TD728		$\text{---}\equiv\text{---R}^4$	TIPS	H

TD1204			I	H
TD944			Ph	H
TD943			cyclopropyl	H
TD959			tBu	H
TD992			adamantyl	H
TD1105			- C(CH ₃) ₂ N HBoc	H
TD1127			4- piperidiny1	H

17



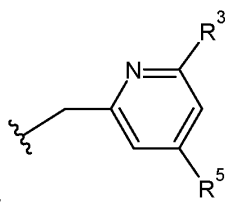
Compound	R^1	R^3	R^4
TD722	Ph		$-CH_2NH_2$
TD1054	Ph		$-C(CH_3)_2NH_2$
TD744	4-carboxyphenyl		$-CH_2-NH_2$



5

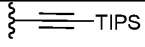
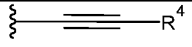
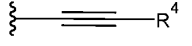
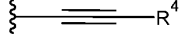
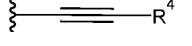
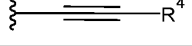
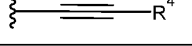
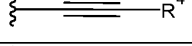
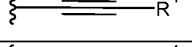
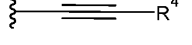
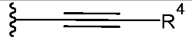
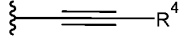
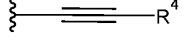
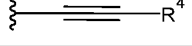
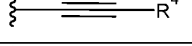
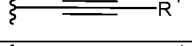
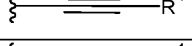
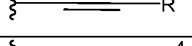
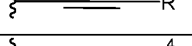
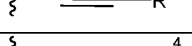
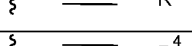
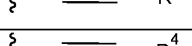
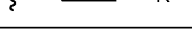
Compound	R^1	R^3	R^4	R^5	R^6
TD1451	$-CF_3$		$-C(CH_3)_2(NHR^6)$	$-COOH$	tert-butylloxycarbonyl

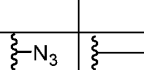
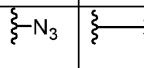
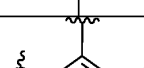
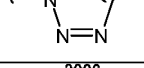
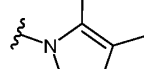
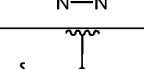
In the most preferred embodiment, the compound of general formula (I) is selected from the group



comprising compounds, wherein Y is nitrogen, Z is present in the following combinations:

Compound	R^1	A	R^2	R^3	R^4	R^5
TD556	H	$-(CH_2)COOH$	$\text{---}N_3$		H	H
TD810	H	$-(CH_2)COOH$	$\text{---}N_3$		H	

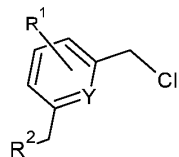
TD827		-(CH ₂)COOH			H	H
TD718	H	-(CH ₂)COOH			TMS	H
TD728	H	-(CH ₂)COOH			TIPS	H
TD1204	H	-(CH ₂)COOH			I	H
TD944	H	-(CH ₂)COOH			Ph	H
TD943	H	-(CH ₂)COOH			cyclopropyl	H
TD959	H	-(CH ₂)COOH			tBu	H
TD992	H	-(CH ₂)COOH			adamantyl	H
TD705	H	-CH ₂ P(=O)(OEt) ₂ and -(CH ₂)COO ^t Bu			H	H
TD714	H	-CH ₂ P(=O)(OH) ₂ and -(CH ₂)COOH			H	H
TD921	H	-CH ₂ P(=O)(Ph)(OH)			H	H
TD580	H	-CH ₂ P(=O)(OEt) ₂			H	H
TD582	H	-CH ₂ P(=O)(OH)(OEt)			H	H
TD581	H	-CH ₂ P(=O)(OH) ₂			H	H
TD575	Ph	-CH ₂ P(=O)(OEt) ₂			H	H
TD576	Ph	-CH ₂ P(=O)(OH) ₂			H	H
TD764	Cl	-(CH ₂)COOH			H	H
TD1063	-CF ₃	-(CH ₂)COOH			H	H
TD1092	-COOCH ₃	-(CH ₂)COOH			H	H
TD1148	-COO ^t Pr	-(CH ₂)COOH			H	H
TD1160	-COO ^t Bu	-(CH ₂)COOH			H	H
TD1176	-N(CH ₃) ₂	-(CH ₂)COOH			H	H
TD647	Ph	-(CH ₂)COOH			H	H
TD722	Ph	-(CH ₂)COOH			-CH ₂ NH ₂	H

TD1054	Ph	-(CH ₂)COOH			-C(CH ₃) ₂ NH ₂	H
TD1105	H	-(CH ₂)COOH			-C(CH ₃) ₂ NHBoc	H
TD1127	H	-(CH ₂)COOH			4-piperidinyl	H
TD742	4-carboxyphenyl	-(CH ₂)COOH			H	H
TD744	4-carboxyphenyl	-(CH ₂)COOH			-CH ₂ -NH ₂	H
TD779	Ph	-(CH ₂)COONH ₂			H	H
TD778	Ph	-(CH ₂)CONH-CH ₂ COOH			H	H
c _z -TD556	H	-(CH ₂)COOH			H	H
c _z -TD764	Cl	-(CH ₂)COOH			H	H
c _z -TD1063	-CF ₃	-(CH ₂)COOH			H	H
TD1188	H	-(CH ₂)COOH			-CF ₃	H
TD650	Ph	-(CH ₂)COOH			H	H
TD871	Ph	-(CH ₂)COOH			H	H

In another aspect, the subject of the present invention is a method of synthesis of the compounds of general formula (I), comprising the following steps:

i) providing an alkyne intermediate of general formula Z-Cl, wherein Z is as defined above;

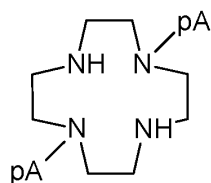
ii) providing an azide intermediate of general formula (III)



(III);

wherein Y, R¹ and R² are as defined above;

5 iii) providing a cyclen derivative of general formula (IV)

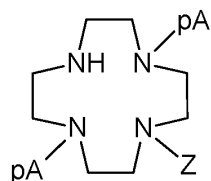


(IV),

wherein pA is selected from the group comprising $-(CH_2)_nCOO^tBu$, wherein n is an integer from 1 to 3; benzyloxycarbonyl; $-CH(CH_3)COO^tBu$; $-CH(CH_3)COOR$, wherein R is tert-butyl, methyl or ethyl;

10 $-CH((CH_2)_nCH_3)COOR$, wherein n and R are as defined above; $-CH((CH_2)_nCOOR)COOR$, wherein n is as defined above and R are independently selected from tert-butyl, methyl or ethyl; $-CH_2P(=O)(OR)_2$, wherein R is C_1 to C_6 alkyl, which may be branched or linear; $-CH_2C(=O)(NH_2)$; $-CH_2C(=O)(NH)CH_2COOH$; $-CH_2P(=O)(OH)(Ar)$, wherein Ar is phenyl, which can optionally be substituted with C_1 to C_6 alkyl; preferably pA is selected from the group comprising $-CH_2COOtBu$; $-CH_2P(O)(OEt)_2$; $-(CH_2)_2COOtBu$;

15 iv-A) reacting the cyclen derivative of general formula (IV) with alkyne intermediate Z-Cl to obtain an intermediate of general formula (V)

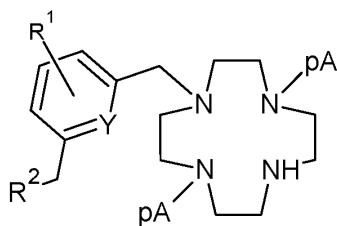


(V),

wherein Z and pA are as defined above;

20 or

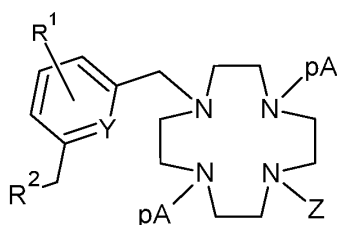
iv-B) reacting the cyclen derivative of general formula (IV) with the azide intermediate of general formula (III) to obtain an intermediate of general formula (VI)



(VI),

wherein Y, R¹, R² and pA are as defined above;

- 5 v-A) reacting the intermediate of general formula (V) with the azide intermediate of general formula (III) to obtain an intermediate of general formula (VII)



(VII)

wherein Y, R¹, R², pA and Z are as defined above;

10 or

v-B) reacting the intermediate of general formula (VI) with the alkyne intermediate Z-Cl to obtain the intermediate of general formula (VII);

vi) optionally, hydrolysis of protecting groups, resulting in the compound of general formula (I).

- 15 In general, compounds of general formula Z-Cl can be obtained commercially or can be synthesised using Pd(0) catalyzed Sonogashira cross-coupling reaction from corresponding 2-halopyridines (preferably Br, I) and terminal alkynes or silyl-protected acetylenes.

Compounds of general formula (III) can be obtained from 2,6-bis(halomethyl)pyridines (preferably Cl) by desymetrization using sodium azide as a source of azide moiety.

- 20 Cyclene derivatives of general formula (IV) are commercially available or may be prepared by reaction of cyclen or *trans-N*-bis-protected cyclen (preferably carbamate protected) with alkyl haloacetates (alkylation), alkyl acrylates (Michael addition) or trialkylphosphites in the presence of (para)formaldehyde (Kabachnik-Fields reaction).

- Steps iv-A), iv-B), v-A) and v-B) take place in a solvent selected from the group comprising MeCN (preferably), DMSO or DMF. The reaction takes place at room temperature for usually several hours up to
25 several days.

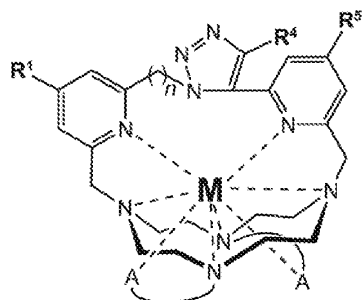
Step vi) of deprotecting pA groups is optional, for example if pA is $-\text{CH}_2\text{P}(\text{O})(\text{OEt})_2$, then if no deprotection takes place, the resulting A in general formula (I) equals pA. Specific conditions for deprotection of the protecting group depend on the chemical nature of the protecting group, and are known to the person skilled in the art. Typically, *tert*-butyl ester protecting groups and Boc protecting group are hydrolyzed under acidic conditions (TFA) or thermally, methyl or isopropyl protecting groups are hydrolyzed under alkaline conditions, ethyl phosphonate ester are converted to corresponding phosphonic acids by transesterification using trimethylsilylbromide in presence of pyridine base.

Another subject of the present invention is a coordination compound of the compound of the general formula (I) as defined above with a metal cation, selected from the group consisting of lanthanide(III) cations, Na^+ , Ba^{2+} , Pb^{2+} , Sr^{2+} , Ca^{2+} , Cd^{2+} , Zn^{2+} , Mn^{2+} , Pt^{2+} , Cu^{2+} , Ni^{2+} , Sc^{3+} , Y^{3+} , Bi^{3+} , In^{3+} , Ru^{3+} , Ir^{3+} , Ga^{3+} , Tl^{3+} , Pd^{2+} , preferably the metal cation is selected from the group consisting of La^{3+} , Ce^{3+} , Pr^{3+} , Nd^{3+} , Sm^{3+} , Eu^{3+} , Gd^{3+} , Tb^{3+} , Dy^{3+} , Ho^{3+} , Er^{3+} , Tm^{3+} , Yb^{3+} , Lu^{3+} , Y^{3+} , Sc^{3+} , Bi^{3+} , In^{3+} , Tl^{3+} , Pb^{2+} , Ca^{2+} , Cd^{2+} , Zn^{2+} , Cu^{2+} , Ni^{2+} , Mn^{2+} , Pd^{2+} , Na^+ .

The metal ions shall be understood as comprising any isotope of the particular metal, including stable isotopes such as ^{40}Ca , ^{42}Ca , ^{43}Ca , ^{44}Ca , ^{46}Ca , ^{48}Ca , ^{45}Sc , ^{89}Y , ^{138}La , ^{139}La , ^{136}Ce , ^{138}Ce , ^{140}Ce , ^{142}Ce , ^{141}Pr , ^{142}Nd , ^{143}Nd , ^{144}Nd , ^{145}Nd , ^{146}Nd , ^{148}Nd , ^{150}Nd , ^{144}Sm , ^{147}Sm , ^{148}Sm , ^{149}Sm , ^{150}Sm , ^{152}Sm , ^{154}Sm , ^{151}Eu , ^{153}Eu , ^{152}Gd , ^{154}Gd , ^{155}Gd , ^{156}Gd , ^{157}Gd , ^{158}Gd , ^{160}Gd , ^{159}Tb , ^{156}Dy , ^{158}Dy , ^{160}Dy , ^{161}Dy , ^{162}Dy , ^{163}Dy , ^{164}Dy , ^{165}Ho , ^{162}Er , ^{164}Er , ^{166}Er , ^{167}Er , ^{168}Er , ^{170}Er , ^{169}Tm , ^{168}Yb , ^{170}Yb , ^{171}Yb , ^{172}Yb , ^{173}Yb , ^{174}Yb , ^{176}Yb , ^{175}Lu , ^{176}Lu , and radioisotopes such as ^{44}Sc , ^{47}Sc , ^{64}Cu , ^{67}Cu , ^{86}Y , ^{90}Y , ^{140}Nd , ^{149}Pm , ^{151}Pm , ^{153}Sm , ^{159}Gd , ^{149}Tb , ^{161}Tb , ^{165}Dy , ^{161}Ho , ^{166}Ho , ^{169}Er , ^{167}Tm , ^{175}Yb , ^{177}Lu .

Lanthanides (Ln) are defined as chemical elements comprising 15 metallic chemical elements with atomic numbers in the range of from 57 to 71 (from lanthanum through lutetium).

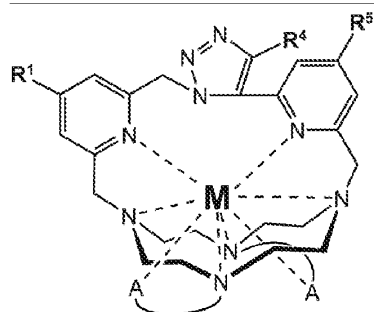
Preferably, the coordination compound has a general formula (VIII)



(VIII);

wherein M is the metal cation, R^1 , R^4 , R^5 and A are as defined above, and n is selected from 1, 2, 3 and 4;

more preferably, the coordination compound has a general formula (VIIIa)



(VIIIa).

- 5 The coordination compounds according to the present invention comprise also solvates and salts of pharmaceutically acceptable acids. The positive charge of the metal cation is counterbalanced by two negative charges arising from A pendant arms (e.g. acetates). Therefore, the overall charge of the coordination compound is -1 (for monovalent metal ions), 0 (for divalent metal ions) or +1 (trivalent metal ions). The counterion, compensating for the overall charge of the coordination compound, is selected from the group,
- 10 comprising anions derived from the following pharmaceutically acceptable acids:
- 1-hydroxy-2-naphthoic acid; 2,2-dichloroacetic acid; 2-hydroxyethanesulfonic acid; 2-oxoglutaric acid; 4-acetamidobenzoic acid; 4-aminosalicylic acid; acetic acid; adipic acid; ascorbic acid (L); aspartic acid (L); benzenesulfonic acid; benzoic acid; camphoric acid (+); camphor-10-sulfonic acid (+); capric acid (decanoic acid); caproic acid (hexanoic acid); caprylic acid (octanoic acid); carbonic acid; cinnamic acid; citric acid;
- 15 cyclamic acid; dodecylsulfuric acid; ethane-1,2-disulfonic acid; ethanesulfonic acid; formic acid; fumaric acid; galactaric acid; gentisic acid; glucoheptonic acid (D); gluconic acid (D); glucuronic acid (D); glutamic acid; glutaric acid; glycerophosphoric acid; glycolic acid; hippuric acid; hydrobromic acid; hydrochloric acid; isobutyric acid; lactic acid (DL); lactobionic acid; lauric acid; maleic acid; malic acid (- L); malonic acid; mandelic acid (DL); methanesulfonic acid; naphthalene-1,5-disulfonic acid; naphthalene-2-sulfonic acid;
- 20 nicotinic acid; nitric acid; oleic acid; oxalic acid; palmitic acid; pamoic acid; phosphoric acid; propionic acid; pyroglutamic acid (- L); salicylic acid; sebacic acid; stearic acid; succinic acid; sulfuric acid; tartaric acid (+ L); thiocyanic acid; toluenesulfonic acid (*p*); undecylenic acid; trifluoroacetic acid.

- The coordination compounds can be prepared according to Scheme 1 above from the free ligand of general formula (I) (preferably the non-caged, therefore preferably without the triazole bridge) and a metal salt (typically water soluble metal salt, preferably selected from a group comprising chloride, nitrate, acetate, formate, trifluoroacetate, trifluoromethylsulfonate, more preferably chloride or nitrate salt). By stirring of these reactants in an aqueous environment (pH 5-7) at temperature in the range of from 25 °C to 100 °C, the metal cation
- 25

coordinates into the compound of general formula (I) and the R^2 and R^3 substituents then “click-zip” the metal cation inside the cage by forming the triazole bridge (reaction temperatures and times are strongly dependent on the choice of ligand and metal cation).

However, the suitable ligands are not limited to the non-caged compounds of general formula (I), even though they are preferred because wider range of metal cations (such as lanthanides) may form the caged coordination compounds. The bridged (caged) compounds of general formula (I) with 1,5-triazole bridge are capable of forming coordination compounds with metal cations, typically selected from the group comprising Tl^{3+} , Pb^{2+} , Bi^{3+} , In^{3+} , Cd^{2+} , Ca^{2+} , Cu^{2+} , Ni^{2+} , Mn^{2+} , Pd^{2+} , Na^+ . The bridged (caged) compounds of general formula (I) with 1,4-triazole bridge are capable of forming coordination compounds with metal cations, typically selected from the group comprising La^{3+} , Ce^{3+} , Pr^{3+} , Nd^{3+} , Sm^{3+} , Eu^{3+} , Gd^{3+} , Tb^{3+} , Dy^{3+} , Ho^{3+} , Er^{3+} , Tm^{3+} , Yb^{3+} , Lu^{3+} , Y^{3+} , Tl^{3+} , Pb^{2+} , Bi^{3+} , In^{3+} , Cd^{2+} , Ca^{2+} , Cu^{2+} , Ni^{2+} , Mn^{2+} , Pd^{2+} , Na^+ .

The resulting coordination compounds are very inert and stable, therefore suitable for various applications, such as MRI contrast agents, radiodiagnostics or radiotherapy, peptide or protein containing drug tracing.

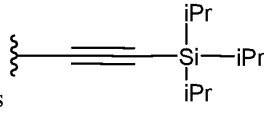
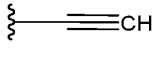
The coordination of the compound of general formula (I) to a metal cation takes place via the macrocyclic nitrogen atoms of the cyclene moiety, oxygen atoms present in groups A (pendant arms) and Y group (when Y is N-oxide), and via nitrogen atoms of Y group (when Y is nitrogen) and nitrogen atom present in Z group.

In one embodiment, the coordination compounds according to the present invention may undergo a post-clickzip transformation. It means that after the metal cation is coordinated within the cage of the compound of general formula (I) by either following the Scheme 1 or coordinating with the caged compound of general formula (I), some functional groups, if present, may undergo a further reaction. Typically, such functional group is a halogen, preferably Cl, NO_2 or SH, present within substituent R^1 or R^5 of the coordination compound, however, post-clickzip transformations may include reactions of R^1 , R^4 , R^5 and/or A groups (such as amidic coupling reactions of non-coordinated carboxylic or amino groups).

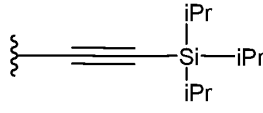
For example, when R^1 is halogen, preferably Cl; or R^1 is C_6 to C_{10} aryl, substituted with $-CH_2Cl$, then the coordination compound of general formula (VIII) may undergo post-click transformation using Pd(0) catalyzed Suzuki cross-coupling reaction with phenylboronic acids (3-borono-5-nitrobenzoic acid or 2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetic acid or 2-(4-boronophenyl)acetic acid), during which the halogen is replaced, thus the resulting coordination compound of general formula (VIII) would have $R^1 = COOH$ or C_6 to C_{10} aryl, substituted with $-COOH$ or $-CH_2COOH$. The resulting carboxylate moiety can be then utilized in amide coupling reaction for synthesis of peptide conjugates. Conjugates with oligoarginines are capable of crossing cell membrane, allowing metal cages to be incorporated to the cells.

Another possible post-click transformation is the replacement of halogen present within R^1 , R^4 and/or R^5 substituent by $-N_3$ group by reaction with NaN_3 . The resulting azide can be used for coupling of the metal cage to another alkyne-bearing moiety using copper-catalysed azide-alkyne cycloaddition (CuAAC) reaction.

Another possible post-click transformation is the hydrolysis of TIPS protecting group present in R^1 , R^4 and/or

- 5 R^5 substituent and protecting the triple bond (R^1 , R^3 and/or R^5 is ). The hydrolysis is performed using aqueous K_2CO_3 , and results in R^1 , R^3 and/or R^5 being . The resulting deprotected triple bond is suitable for further conjugations via reaction with the alkyne, for example click reactions with azides and forming triazole bridges (e.g. CuAAC reaction).

- 10 In another embodiment, the post-click transformation may be addition reaction of methanol to R^1 , R^4 and/or

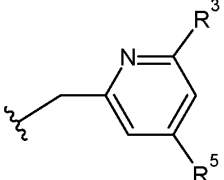
R^5 , wherein R^1 , R^3 and/or R^5 is , thus transforming the triple bond to dimethyl acetal (protected version of an aldehyde). This demonstrates conversion of one functional group (alkyne) to another (aldehyde). Aldehydes are usable for conjugation reactions based on formation of Schiff bases with amines, hydrazines and acid hydrazides.

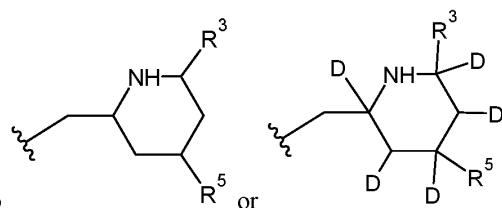
15

In yet another embodiment, the post-click transformation may be deuteration reaction of CH_2 groups of the pendant arms in D_2O in presence of DBU, transforming them into CD_2 groups. This conversion does not alter the physico-chemical properties of the molecule (in terms of stability or reactivity), but changes the overall mass of the molecule, to be entirely distinguishable from the parent non-deuterated molecule using mass spectrometry. This means, that the metal ion cage with one particular metal can be used as two different mass tags.

20

In yet another embodiment, the post-click transformation may be a selective reduction of pyridyl cycle of

 Z substituent, wherein Z is R^3 and R^5 are as defined above, using $NaBH_4$ or $NaBD_4$



as the reducing agent, thus transforming the Z group into respectively. Similarly to the deuteration of CH₂ groups, this conversion changes the mass of the molecule for metal tags purposes.

- 5 In yet another embodiment, the post-click transformation may be reaction of NH₂ group, if present, of R¹ and/or R⁴ and/or R⁵ and/or A, wherein R¹ and/or R⁴ and/or R⁵ contains –NH₂ or –(CH₂)_nNH₂, with FmocCl, resulting in transformation of –NH₂ group into –NHFmoc group. Fmoc group is the most commonly encountered amine protecting group used in solid-phase peptide synthesis (SPPS). Introducing of Fmoc group therefore facilitates use of the metal cages for SPPS.

10

In yet another embodiment, the post-click transformation may be reaction of NH₂ group, if present, of R¹ and/or R⁴ and/or R⁵ and/or A, with a carboxyl group of another molecule of general formula (I) or of its metal complex as defined above, resulting in conjugation of two or more compounds of general formula (I) or of their metal complexes into conjugates bound via a peptide (amide) bond.

15

In yet another embodiment, the post-click transformation may be reaction of non-coordinating –COOH group, if present, in R¹ and/or R⁴ and/or R⁵ and/or A, with an amino group of an aminoacid or peptide, thus forming a peptide bond for conjugation purposes.

20

In yet another embodiment, the post-click transformation may be S-alkylation reaction of a halogen, preferably Cl, of R¹, R⁴ and/or R⁵ substituent with a thiol, resulting in transforming the halogen into sulfide. The thiol may be e.g. Na₂S, NaSH, N-Boc-cysteine or a cysteine-containing peptide and the reaction may take place in presence of DIPEA. Incorporating cysteine (aminoacid) into the structure of the coordination compound according to the present invention opens further possibilities for amide bonds (e.g. peptide attachment).

25

In yet another embodiment, the post-click transformation may be reaction of $\text{---}\equiv\text{CH}$ group of R¹, R³ and/or R⁵, wherein R¹, R³ and/or R⁵ contains $\text{---}\equiv\text{CH}$, with an azide, preferably with alkyl- or arylazide, thereby forming a triazole bridge connecting the alkyl- or aryl- to R¹, R³ and/or R⁵ group. The arylazide is preferably C₆ to C₁₀ arylazide, e.g. benzyl azide, optionally substituted with –COOH or –CH₂COOH. The alkyl azide is preferably C₁ to C₇ alkyl azide, wherein the alkyl may be linear or branched.

30

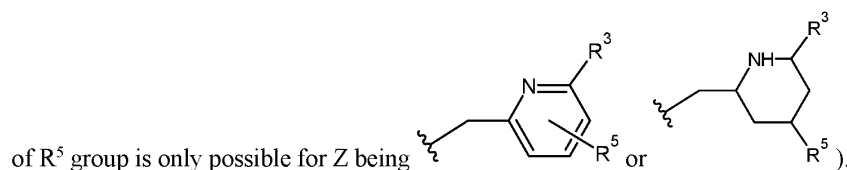
In yet another embodiment, the post-click transformation may be reaction of $-N_3$ group of R^1 or R^5 , wherein R^1 or R^5 contains $-N_3$ group, with a substituent comprising a carbon-carbon triple bond, thereby forming a triazole bridge connecting the substituent with the coordination compound. The substituent comprising a triple bond
 5 may be for example C7 to C10 arylalkynyl, optionally substituted with $-COOH$ or $-CH_2COOH$, e.g. phenylacetylene, wherein the phenyl moiety may optionally be substituted with $-COOH$ or $-CH_2COOH$.

In yet another embodiment, the post-clickzip transformation may be reaction of $-SH$ group of R^1 and/or R^5 , with a substituent comprising maleimide, thereby forming thiosuccinimide linkage connecting the substituent
 10 with the coordination compound; or reaction of $-SH$ group of R^1 and/or R^5 , with a substituent comprising another $-SH$ group, thereby forming a disulfide bridge connecting the substituent with the coordination compound; or reaction of $-SH$ group of R^1 and/or R^5 , with alkyl- or arylhalogenide, thereby forming a thioether linkage connecting the alkyl- or aryl- to the coordination compound.

15 In yet another embodiment, the post-clickzip transformation may be reaction of $-NO_2$ group of R^1 and/or R^5 , with a substituent comprising $-SH$ group, thereby forming a thioether linkage connecting the substituent with the coordination compound.

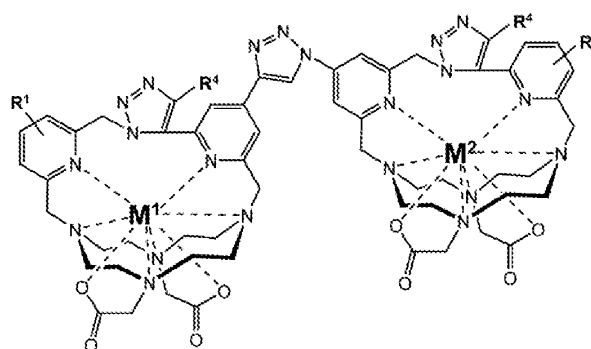
In one embodiment, the post-click transformation may include reaction with another coordination compound
 20 of the present invention, thereby forming a chain or a dimer. The coordination compound chain or dimer may contain the same metal cation in both click-zipped cages or it may contain different metal cations in each of the chelates. The advantage of the chains and dimers lies in the possibility to combine properties of the two caged chelates, such as their unique weights to produce mass tags with higher variability of weights.

Typically, the coordination compounds are bound into the chain by forming a triazole bridge between R^1 or R^5
 25 group of the first coordination compound and R^1 or R^5 group of the following coordination compound (the use

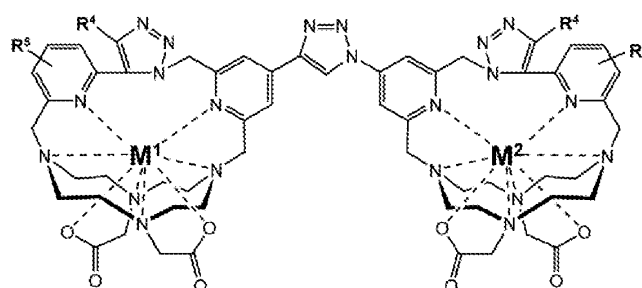


The general basic structure of the coordination compound chain is thus (IXa) or (IXb)

28



(IXa)



(IXb).

5

In one embodiment, the coordination compounds are bound into the chain by amide bonds formed between a substituent of one coordination compound containing an amine group (R^1 and/or R^4 and/or R^5 and/or A) and a substituent of another coordination compound containing a carboxyl group (R^1 and/or R^4 and/or R^5 and/or A).

10

The chain usually contains two coordination compounds according to the present invention, however it may contain three, four or more coordination compounds.

In yet another embodiment the two types of linkage, described above, are possible in one chain, for example two neighbouring coordination compounds may be linked by one triazole bridge, leaving R^5 or R^1 substituent available for linking another coordination compound according to the present invention, either via a triazole bridge or, if amino or carboxyl groups are present, via an amide bond.

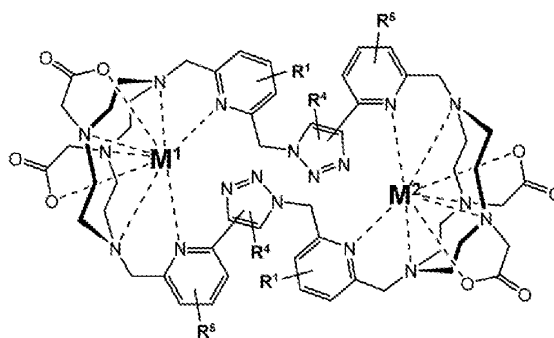
15

In one embodiment, the coordination compounds are bound into the chain by disulfide bonds (-S-S-) formed between a substituent R^1 or R^5 of one coordination compound containing -SH group, and a substituent R^1 or R^5 of another coordination compound containing -SH group.

20

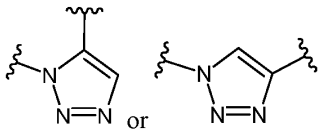
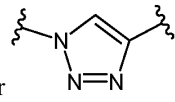
The coordination compound dimer contains two coordination compounds according to the present invention, bound together by forming a triazole bridge between R^1 group of the first coordination compound and R^5 group of the second coordination compound, and, at the same time, by forming a triazole bridge between R^5 group of the first coordination compound and R^1 group of the second coordination compound.

- 5 The general structure of the coordination compound dimer is thus (X)



(X).

- The synthesis of the coordination compound chain or dimer is based on a click reaction between an azido group of the first coordination compound and a triple bond of the second coordination compound, thereby forming a triazole bridge, linking the two coordination compound into a dimer. The triazole bridge is selected from the group comprising:

1,2,3-triazole group of formula  or  formed between $-N_3$ group of R^1 or R^4 or R^5 of first coordination compound and the triple bond of R^1 or R^4 or R^5 of the second coordination compound.

Preferably, the metal cations are different, for example the metal cations are selected from Tb^{3+} and $^{176}Yb^{3+}$.

- 15 Yet another object of the present invention is a conjugate suitable for use as markers for drug tracing. Many drugs are based on peptides or proteins, and their biodistribution can be traced by attachment of a fluorescent or radioactive marker to the peptide or protein structure. The coordination compounds and dimers of the present invention are suitable for their attachment to the peptide or protein of a drug to be traced. The isolated cell culture or a tissue can then be analysed for the presence of the metal complex using conventional methods, such as LC-MS, which is commonly used and relatively inexpensive instrument. The cells or tissues can be entirely hydrolyzed in a strong acid without any decomposition of the coordination compound according to the present invention. The coordination compound can then be detected in a hydrolyzed sample. Such method is unexpensive and reliable thanks to the extremely high stability of the coordination compounds. LC-MS method

also allows for using a plurality of different coordination compounds with different metal isotopes (multiplexing), which may then be all analyzed during one LC-MS analysis.

The conjugate according to the present invention contains the coordination compound according to the present invention as defined above or the coordination compound dimer or chain as defined above, conjugated to a peptide or to a protein. The conjugation takes place via an amide bond, formed between the amino group of the peptide or the protein and a carboxyl group of the coordination compound or of the chain or dimer or vice versa. Thus, the coordination compound or the chain or dimer need to contain either $-NH_2$ or $-COOH$ group prior to peptide or protein conjugation. Preferably, the $-NH_2$ or $-COOH$ group is part of the R^1 and/or R^4 and/or R^5 group, therefore the conjugates are formed from coordination compounds or coordination compound dimers, which have R^1 and/or R^4 and/or R^5 group selected from the group comprising $-NH_2$; $-(CH_2)_nNH_2$; $-COOH$; $-(CH_2)_nCOOH$; C_6 to C_{10} aryl, substituted with $-NH_2$, $-COOH$, $-(CH_2)_nNH_2$, or $-(CH_2)_nCOOH$; wherein n is an integer in the range of from 1 to 3.

The conjugation reaction conditions are known to the person skilled in the art, typically the reaction takes place at $25^\circ C$ in DMSO, using commonly used peptide coupling agents (preferably HATU, PyAOP) in the presence of organic base (triethylamine, ethyldiisopropylamine). Reaction are usually completed within several minutes. The peptide is preferably selected from the group comprising oligopeptides of 3 to 20 aminoacids; The protein is preferably selected from the group comprising antibodies, preferably monoclonal antibodies.

Another object of the present invention is a method of drug tracing, preferably of tracing of peptide-based or protein-based drugs, which comprises the following steps:

- i) providing a cell culture or a tissue to be analyzed, containing at least one conjugate defined above, wherein the peptide or the protein is of the peptide-based or protein-based drug to be traced;
- ii) hydrolyzing the cell culture or tissue from step i) using a strong acid, preferably non-oxidizing aq. HCl, obtaining a hydrolyzate;
- iii) qualitative and/or quantitative analysis of the hydrolyzate of step ii) for the presence of the coordination compound or of the coordination compound dimer according to the present invention, preferably using LC-MS.

Further object of the present invention is the *in vitro* use of the coordination compound or of the coordination compound dimer or of the conjugate according to the present invention in pharmacy, preferably for development and testing of new drugs, more preferably for drug marking and tracing.

Another object of the present invention is the use of the coordination compounds or of the coordination compound dimers according to the present invention in medical diagnostics, preferably as MRI contrast agents. Preferably, Gd^{3+} coordination compounds or dimers shall be used as MRI contrast agents, as Gd has a very

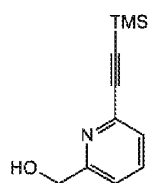
high relaxivity. The extremely high kinetic inertness of the coordination compounds (approximately 100x higher than for GdDOTA, a commonly used MRI contrast agent) allows for using even higher dosages and no free gadolinium, which itself is toxic for humans or animals, is released from the coordination compounds according to the present invention. The claimed coordination compounds are thus expected to be safe for use in human or animal beings.

Another object of the present invention is the use of the coordination compound or the coordination compound chain or the coordination compound dimer according to the present invention, wherein M is a radionuclide, preferably selected from the group comprising ^{44}Sc , ^{47}Sc , ^{64}Cu , ^{67}Cu , ^{86}Y , ^{90}Y , ^{140}Nd , ^{149}Pm , ^{151}Pm , ^{153}Sm , ^{159}Gd , ^{149}Tb , ^{161}Tb , ^{165}Dy , ^{161}Ho , ^{166}Ho , ^{169}Er , ^{167}Tm , ^{175}Yb , ^{177}Lu , in medicine as radiodiagnostic and/or radiopharmaceutic agents. Alternatively, a radionuclide may also be present in the side chain of the coordination compound such as a radioisotope of a halogen, e.g. R^1 and/or R^5 may be ^{18}F .

The present invention is further demonstrated by the following examples.

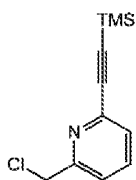
Examples

Example 1: *Synthesis of intermediates of alkyne pendant arms*



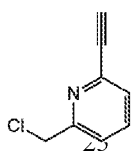
Synthesis of TD701: Pear-shape glass flask (50 mL) was charged with **(6-Bromopyridin-2-yl)methanol** (2.47 g; 13.1 mmol; 1.0 equiv.) and magnetic stirrer and three times secured with argon. Solid **CuI** (149 mg; 782 μmol ; 6.0 mol%) and **[Pd(PPh₃)₂Cl₂]** (225 mg; 321 μmol ; 2.4 mol%) were subsequently added followed by another securing with argon (three times). Under constant flow of argon was then added dry THF (25 mL) through septum followed by addition of **Ethynyltrimethylsilane** (2.0 mL; 14.5 mmol; 1.1 equiv.) and **TEA** (5.5 mL; 39.5 mmol; 3.0 equiv.) after which the mixture changed color to dark brown. The flask was left stirring under septum (but without external argon) for 3 h at RT. The mixture was then transferred to a separatory funnel with EtOAc (150 mL) and H₂O (100 mL). After shaking, the dark colored (and not entirely homogenous) organic layer was separated and the aqueous layer was further extracted with EtOAc (5 $\times$ 50 mL). Combined organic layers were filtered through cotton plug and the filtrate was further dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. Resulting dark brown oily residue was purified on flash chromatography (330 g SiO₂, 100% DCM to 10% EtOAc in DCM). Combined fractions with product were evaporated to dryness and further co-evaporated once with DCM. The residue was further dried on high vacuum overnight to give product in the form of free base as dark yellow oil. **Yield:** 2.16 g (80%; 1 step; based on **(6-Bromopyridin-2-yl)methanol**). **NMR (DMSO-d₆):** ^1H δ_{H} 0.24 (CH_3 , s, 9H); 4.52 (CH_2 , d, 2H, $^3J_{\text{HH}} = 6$); 5.48 (OH, t, 1H, $^3J_{\text{HH}} = 6$); 7.39 (arom., dd, 1H, $^3J_{\text{HH}} = 8$; $^4J_{\text{HH}} = 1$); 7.46 (arom., dd, 1H, $^3J_{\text{HH}} = 8$; $^4J_{\text{HH}} = 1$); 7.80 (arom., t, 1H, $^3J_{\text{HH}} = 8$).

$^{13}\text{C}\{^1\text{H}\}$ δ_{C} -0.3 (CH_3 , s); 63.9 (CH_2 , s); 93.4 and 104.5 ($\text{C}\equiv\text{C}$; $2 \times \text{s}$); 120.2 (*arom.*, s); 125.4 (*arom.*, s); 137.2 (*arom.*, s); 140.8 (*arom.*, s); 162.7 (*arom.*, s). **ESI-HRMS**: 206.0995 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{11}\text{H}_{16}\text{N}_1\text{O}_1\text{Si}_1]^+ = 206.0996$).



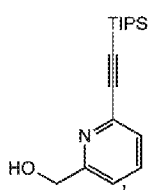
Synthesis of TD557: In a round-bottom glass flask (100 mL), **TD701** (2.16 g; 10.5 mmol; 1.0 equiv.) was dissolved in DCM (40 mL). Freshly prepared solution of **SOCl₂** (1.50 mL; 20.7 mmol; 2.0 equiv.) in DCM (10 mL) was then added and the open flask was stirred 1 h at RT. Dil. aq. solution of **NaHCO₃** (50 mL) was then added and resulting biphasic mixture was vigorously stirred for additional 1 h at RT, during which bubbles of gas slowly evolved.

Mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (4×50 mL). Combined organic layers were dried with anhydrous **Na₂SO₄**, filtered through glass frit (S3) and evaporated to dryness. The brown residue (already pure according to ^1H NMR) was purified on flash chromatography (330 g **SiO₂**, 60% P.E. in DCM to 20% P.E. in DCM) to remove the intensively colored impurities. Combined fractions with product were evaporated to dryness and further co-evaporated once with DCM. The residual nearly colorless oil was left in a freezer to solidify. Resulting solid was crushed and further dried on high vacuum overnight to give product in the form of free base as fine white powder. **Yield**: 2.14 g (87%; 1 step; based on **TD701**). **NMR (DMSO-*d*₆)**: ^1H δ_{H} 0.25 (CH_3 , s, 9H); 4.76 (CH_2 , s, 2H); 7.50 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$; $^4J_{\text{HH}} = 1$); 7.55 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$; $^4J_{\text{HH}} = 1$); 7.86 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$). $^{13}\text{C}\{^1\text{H}\}$ δ_{C} -0.4 (CH_3 , s); 46.3 (CH_2 , s); 94.3 and 103.8 ($\text{C}\equiv\text{C}$; $2 \times \text{s}$); 123.3 (*arom.*, s); 126.7 (*arom.*, s); 138.1 (*arom.*, s); 141.5 (*arom.*, s); 157.0 (*arom.*, s). **ESI-HRMS**: 224.0657 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{11}\text{H}_{15}\text{N}_1\text{Cl}_1\text{Si}_1]^+ = 224.0657$).

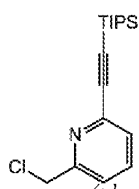


Synthesis of TD558: In a pear-shaped glass flask (100 mL), **TD557** (1.05 g; 4.69 mmol; 1.0 equiv.) was dissolved in MeCN (40 mL). Freshly prepared solution of **KF** (406 mg; 7.0 mmol; 1.5 equiv.) in **H₂O** (7 mL) was then added and the flask was vigorously stirred 2 h at RT. The mixture was concentrated to ~10 mL and then diluted with DCM (50 mL) and dil. aq. **NaHCO₃**

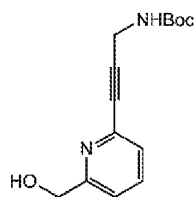
(50 mL). The mixture was transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (4×25 mL). Combined organic layers were dried with anhydrous **Na₂SO₄**, filtered through glass frit (S3) and evaporated to dryness. The residue was further briefly dried on high vacuum to give product in the form of free base as pale yellow oil. **Yield**: 702 mg (99%; 1 step; based on **TD557**). **NMR (DMSO-*d*₆)**: ^1H δ_{H} 4.37 ($\text{C}\equiv\text{CH}$, s, 1H); 4.76 (CH_2 , s, 2H); 7.53 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$; $^4J_{\text{HH}} = 1$); 7.57 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$; $^4J_{\text{HH}} = 1$); 7.87 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$). $^{13}\text{C}\{^1\text{H}\}$ 46.3 (CH_2 , s); 80.6 and 82.6 ($\text{C}\equiv\text{CH}$; $2 \times \text{s}$); 123.4 (*arom.*, s); 126.8 (*arom.*, s); 138.1 (*arom.*, s); 141.3 (*arom.*, s); 157.0 (*arom.*, s). **ESI-HRMS**: 152.0262 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_8\text{H}_7\text{N}_1\text{Cl}_1]^+ = 152.0262$).



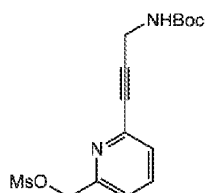
Synthesis of TD712: Pear-shape glass flask (10 mL) was charged with **(6-Bromopyridin-2-yl)methanol** (284 mg; 1.51 mmol; 1.0 equiv.) and magnetic stirrer and three times secured with argon. Solid **CuI** (14.5 mg; 76 μ mol; 5.0 mol%) and **[Pd(PPh₃)₂Cl₂]** (21 mg; 30 μ mol; 2.0 mol%) were subsequently added followed by another securing with argon (three times). Under constant flow of argon was then added dry THF (4 mL) through septum followed by addition of **Ethynyltriisopropylsilane** (370 μ L; 1.65 mmol; 1.1 equiv.) and **TEA** (630 μ L; 4.52 mmol; 3.0 equiv.) after which the mixture changed color to dark brown. The flask was left stirring under septum (but without external argon) for 16 h at RT. The mixture was then transferred to a separatory funnel with EtOAc (30 mL) and H₂O (30 mL). After shaking, the dark colored (and not entirely homogenous) organic layer was separated and the aqueous layer was further extracted with EtOAc (5 $\times$ 25 mL). Combined organic layers were filtered through cotton plug and the filtrate was further dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. Resulting dark brown oily residue was purified on flash chromatography (120 g SiO₂, 100% DCM to 10% EtOAc in DCM). Combined fractions with product were evaporated to dryness and further co-evaporated once with DCM. The residue was further dried on high vacuum overnight to give product in the form of free base as yellow oil. **Yield:** 387 mg (89%; 1 step; based on **(6-Bromopyridin-2-yl)methanol**). **NMR (DMSO-d₆):** ¹H δ_H 0.92–1.26 (*i-Pr*, m, 21H); 4.53 (CH₂, d, 2H, ³J_{HH} = 6); 5.48 (OH, t, 1H, ³J_{HH} = 6); 7.40 (*arom.*, dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.47 (*arom.*, dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.80 (*arom.*, t, 1H, ³J_{HH} = 8). ¹³C{¹H} δ_C 10.7 (*i-Pr*, s); 18.5 (*i-Pr*, s); 64.0 (CH₂, s); 89.4 and 106.6 (C $\equiv$ C; 2 $\times$ s); 120.2 (*arom.*, s); 125.8 (*arom.*, s); 137.2 (*arom.*, s); 140.9 (*arom.*, s); 162.7 (*arom.*, s). **ESI-HRMS:** 290.1932 [M+H]⁺ (theor. [C₁₇H₂₈N₁O₁Si₁]⁺ = 290.1935).



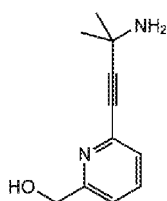
Synthesis of TD723: In a round-bottom glass flask (25 mL), **TD712** (384 g; 1.33 mmol; 1.0 equiv.) was dissolved in DCM (5 mL). Freshly prepared solution of **SOCl₂** (200 μ L; 2.75 mmol; 2.1 equiv.) in DCM (1 mL) was then added and the open flask was stirred 1 h at RT. Dil. aq. solution of NaHCO₃ (10 mL) was then added and resulting biphasic mixture was vigorously stirred for additional 1 h at RT, during which bubbles of gas slowly evolved. Mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (4 $\times$ 10 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The brown residue (already pure according to ¹H NMR) was purified on flash chromatography (120 g SiO₂, 20% DCM in P.E. to 30% DCM in P.E.) to remove the intensively colored impurities. Combined fractions with product were evaporated to dryness and further co-evaporated once with DCM. The residual colorless oil was further dried on high vacuum overnight to give product in the form of free base as colourless solid. **Yield:** 356 mg (87%; 1 step; based on **TD712**). **NMR (DMSO-d₆):** ¹H δ_H 0.91–1.28 (*i-Pr*, m, 21H); 4.78 (CH₂, s); 7.52 (*arom.*, dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.57 (*arom.*, dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.86 (*arom.*, t, 1H, ³J_{HH} = 8). **ESI-HRMS:** 308.1593 [M+H]⁺ (theor. [C₁₇H₂₇N₁Cl₁Si₁]⁺ = 308.1596).



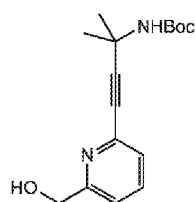
- Synthesis of TD530:** Pear-shape glass flask (50 mL) was charged with **(6-Bromopyridin-2-yl)methanol** (1.00 g; 5.35 mmol; 1.0 equiv.), **N-Boc-propargylamine** (1.00 g; 6.44 mmol; 1.2 equiv.) and magnetic stirrer and three times secured with argon. Solid **CuI** (102 mg; 536 μ mol; 10.0 mol%) and **[Pd(PPh₃)₂Cl₂]** (187 mg; 266 μ mol; 5.0 mol%) were subsequently added followed by another securing with argon (three times). Under constant flow of argon was then added dry THF (27 mL) through septum followed by addition of **TEA** (2.24 mL; 16.1 mmol; 3.0 equiv.) after which the mixture changed color to dark brown. The flask was left stirring under septum (but without external argon) for 18 h at RT. The mixture was then transferred to a separatory funnel with EtOAc (30 mL) and H₂O (50 mL). After shaking, the dark colored (and not entirely homogenous) organic layer was separated and the aqueous layer was further extracted with EtOAc (3 $\times$ 30 mL). Combined organic layers were filtered through cotton plug and the filtrate was further dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. Resulting dark brown oily residue was purified on flash chromatography (220 g SiO₂, 100% DCM to 100% EtOAc). Combined fractions with product were evaporated to dryness and further co-evaporated once with DCM. The residue was further dried on high vacuum overnight to give product in the form of free base as yellow oil. **Yield:** 610 mg (43%; 1 step; based on **(6-Bromopyridin-2-yl)methanol**). **NMR (DMSO-d₆):** ¹H δ _H 1.40 (CH₃, s, 9H); 3.99 (CH₂, d, 2H, ³J_{HH} = 6); 4.51 (CH₂, d, 2H, ³J_{HH} = 6); 5.47 (OH, t, 1H, ³J_{HH} = 6); 7.32 (arom., dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.40 (NH, t, 1H, ³J_{HH} = 6); 7.44 (arom., dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.79 (arom., t, 1H, ³J_{HH} = 8). ¹³C{¹H} δ _C 28.2 (CH₃, s); 29.9 (CH₂, s); 64.0 (CH₂, s); 78.4 (C-CH₃, s); 81.3 and 86.9 (C $\equiv$ C; 2 $\times$ s); 119.8 (arom., s); 125.0 (arom., s); 137.2 (arom., s); 141.1 (arom., s); 155.3 (CO, s); 162.5 (arom., s). **ESI-HRMS:** 263.1390 [M+H]⁺ (theor. [C₁₄H₁₉N₂O₃]⁺ = 263.1390).



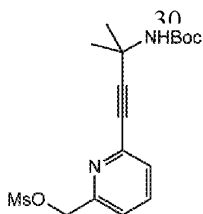
- Synthesis of TD538:** In a round-bottom glass flask (250 mL), **TD530** (568 mg; 2.17 mmol; 1.0 equiv.) was dissolved in DCM (20 mL) followed by addition of **TEA** (900 μ L; 6.46 mmol; 3.0 equiv.). Freshly prepared solution of **MsCl** (355 μ L; 4.33 mmol; 2.0 equiv.) in DCM (5 mL) was then added. Resulting solution was stirred 20 min at RT. Mixture was then diluted with DCM (50 mL) followed by addition of dil. aq. solution of NaHCO₃ (50 mL). The resulting biphasic mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 $\times$ 50 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. **ESI-MS (LC-MS):** 341.1 [M+H]⁺ (theor. [C₁₅H₂₁N₂O₅Si]⁺ = 341.1). All of **TD538** obtained in this way was directly used for **TD539** without further purification or characterization.



- Synthesis of TD1045:** Pear-shaped glass flask (25 mL) was charged with **(6-Bromopyridin-2-yl)methanol** (500 mg; 2.67 mmol; 1.0 equiv.), **1,1-Dimethylpropargylamine** (420 μ L; 3.99 mmol; 1.5 equiv.) and magnetic stirrer and three times secured with argon. Solid **CuI** (21 mg; 110 μ mol; 4.0 mol%) and **[Pd(PPh₃)₂Cl₂]** (19 mg; 27 μ mol; 1.0 mol%) were subsequently added followed by another securing with argon (three times). Under constant flow of argon was then added dry THF (10 mL) through septum followed by addition of **TEA** (1.10 mL; 7.89 mmol; 3.0 equiv.) after which the mixture changed color to dark brown. The flask was left stirring under septum (but without external argon) for 18 h at RT. The mixture was filtered with syringe microfilter (PTFE) and the solid phase was further washed with MeCN. Filtrate was evaporated to dryness and the resulting dark brown solid residue was purified on flash chromatography (120 g SiO₂, 100% EtOAc to 40% EtOAc in MeOH). Combined fractions with product were evaporated to dryness and further co-evaporated once with DCM. The residue was further dried on high vacuum overnight to give product in the form of free base as yellow solidified oil. **Yield:** 283 mg (56%; 1 step; based on **(6-Bromopyridin-2-yl)methanol**). **NMR (DMSO-d₆):** ¹H δ _H 1.38 (CH₃, s, 6H); 3.17 (NH₂, s, 2H); 4.51 (CH₂, s, 2H); 5.44 (OH, bs, 1H); 7.26 (*arom.*, dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.40 (*arom.*, dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.76 (*arom.*, t, 1H, ³J_{HH} = 8). **ESI-HRMS:** 191.1180 [M+H]⁺ (theor. [C₁₁H₁₅N₂O]⁺ = 191.1180).



- Synthesis of TD1048:** Pear-shaped glass flask (25 mL) was charged with **TD1045** (280 mg; 1.47 mmol; 1.0 equiv.) followed by addition of MeCN (10 mL) and solution of **Boc₂O** (2.0 M in dry THF; 1.0 mL; 2.00 mmol; 1.4 equiv.). The resulting mixture was stirred 16 h at RT. Mixture was then evaporated to dryness and the resulting yellow residue was purified on flash chromatography (120 g SiO₂, 100% DCM to 100% EtOAc). Combined fractions with product were evaporated to dryness and further co-evaporated once with DCM. The residue was further dried on high vacuum overnight to give product in the form of free base as colourless solid. **Yield:** 242 mg (57%; 1 step; based on **TD1045**). **NMR (DMSO-d₆):** ¹H δ _H 1.40 (CH₃, s, 9H), 1.53 (CH₃, s, 6H); 4.51 (CH₂, d, 2H, ³J_{HH} = 4); 5.44 (OH, t, 1H, ³J_{HH} = 4); 7.12 (NH, bs, 1H); 7.25 (*arom.*, dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.41 (*arom.*, dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.77 (*arom.*, t, 1H, ³J_{HH} = 8). **ESI-HRMS:** 291.1704 [M+H]⁺ (theor. [C₁₆H₂₃N₂O₃]⁺ = 291.1703).

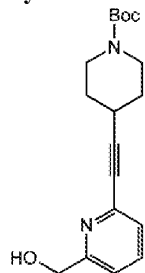


- Synthesis of TD1050:** In a glass vial (20 mL), **TD1048** (19 mg; 65 μ mol; 1.0 equiv.) was dissolved in DCM (3 mL) followed by addition of **TEA** (28 μ L; 201 μ mol; 3.1 equiv.). Freshly prepared solution of **MsCl** (10 μ L; 129 μ mol; 2.0 equiv.) in DCM (1 mL) was then added. Resulting solution was stirred 20 min at RT. Mixture was then diluted with DCM (15 mL) followed by addition of dil. aq. solution of NaHCO₃ (15 mL). The resulting biphasic mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated.

Aqueous phase was further extracted with DCM (3 × 20 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. **ESI-MS** (LC-MS): 369.1 [M+H]⁺ (theor. [C₁₇H₂₅N₂O₅Si]⁺ = 369.1). All of **TD1050** obtained in this way was directly used for **TD1054** (and analogically for **TD1105**) without further purification or characterization.

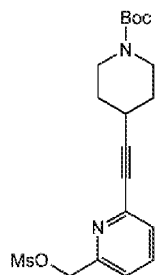
5

Synthesis of TD966: Pear-shape glass flask (100 mL) was charged with **(6-Bromopyridin-2-yl)methanol** (1.00 g; 5.35 mmol; 1.0 equiv.), **N-Boc-4-ethynylpiperidine** (1.23 g; 5.88 mmol; 1.1 equiv.)



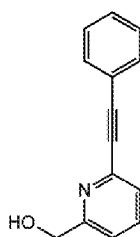
and magnetic stirrer and three times secured with argon. Solid **CuI** (41 mg; 215 μmol; 4.0 mol%) and **[Pd(PPh₃)₂Cl₂]** (75 mg; 107 μmol; 2.0 mol%) were subsequently added followed by another securing with argon (three times). Under constant flow of argon was then added dry THF (40 mL) through septum followed by addition of **TEA** (2.20 mL; 15.7 mmol; 3.0 equiv.) after which the mixture changed color to dark brown. The flask was left stirring under septum (but without external argon) for 2 d at RT. The mixture was then transferred to a separatory funnel with EtOAc (30 mL) and H₂O (50 mL). After shaking, the dark colored (and not entirely homogenous) organic layer was separated and the aqueous layer was further extracted with EtOAc (3 × 30 mL). Combined organic layers were filtered through cotton plug and the filtrate was further dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. Resulting dark brown oily residue was purified on flash chromatography (220 g SiO₂, 100% DCM to 100% EtOAc). Combined fractions with product were evaporated to dryness and further co-evaporated once with DCM. The residue was further dried on high vacuum overnight to give product in the form of free base as yellow oil. **Yield:** 1.47 g (87%; 1 step; based on **(6-Bromopyridin-2-yl)methanol**). **NMR (DMSO-d₆):** ¹H δ_H 1.40 (CH₃, s, 9H); 1.44–1.57 (CH₂, m, 2H); 1.75–1.89 (CH₂, m, 2H); 2.87 (CH–C≡C tt, 1H, ³J_{HH} = 8, ³J_{HH} = 4); 3.01–3.17 (CH₂, m, 2H); 3.59–3.73 (CH₂, m, 2H); 4.51 (CH₂–O, d, 2H, ³J_{HH} = 6); 5.44 (OH, t, 1H, ³J_{HH} = 6); 7.32 (arom., dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.41 (arom., dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.76 (arom., t, 1H, ³J_{HH} = 8). **ESI-HRMS:** 317.1860 [M+H]⁺ (theor. [C₁₈H₂₅N₂O₃]⁺ = 317.1860).

25

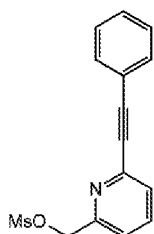


Synthesis of TD1117: In a glass vial (20 mL), **TD952** (50.0 mg; 158 μmol; 1.0 equiv.) was dissolved in DCM (6 mL) followed by addition of **TEA** (66 μL; 473 μmol; 3.0 equiv.) and neat **MsCl** (25 μL; 323 μmol; 2.0 equiv.). Resulting solution was stirred 20 min at RT followed by addition of DCM (10 mL) dil. aq. solution of NaHCO₃ (10 mL). The resulting biphasic mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (4 × 10 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. **ESI-MS** (LC-MS): 395.2 [M+H]⁺ (theor. [C₁₉H₂₇N₂O₅Si]⁺ = 395.2). All of **TD1117** obtained in this way was directly used for **TD1118** without further purification or characterization.

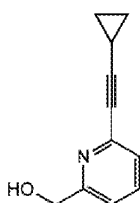
35



Synthesis of TD936: Glass vial (4 mL) was charged with **(6-Bromopyridin-2-yl)methanol** (135 mg; 722 μmol ; 1.0 equiv.), **CuI** (5.5 mg; 29 μmol ; 4.0 mol%), **[Pd(PPh₃)₂Cl₂]** (10.0 mg; 14 μmol ; 2.0 mol%) and magnetic stirrer and three times secured with argon. Under constant flow of argon was then added dry THF (3 mL) through septum followed by addition of **phenylacetylene** (95 μL ; 865 μmol ; 1.2 equiv.) and **TEA** (300 μL ; 2.15 mmol; 3.0 equiv.) after which the mixture changed color to dark brown. The vial was left stirring under septum (but without external argon) for 16 h at RT. The mixture was then filtered through syringe microfilter (PTFE) followed by washing with MeCN. Filtrate was evaporated to dryness, re-dissolved in MeCN (3 mL) and directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions containing product were neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in a mixture of DCM (50 mL) and H₂O (50 mL) and transferred to a separatory funnel. After brief shaking, the bottom phase was separated and the aq. layer was further extracted with DCM (4 $\times$ 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residual solid was briefly dried on high vacuum to give product in the form of free base as a yellow oil. **Yield:** 141 mg (93%; 1 step; based on **(6-Bromopyridin-2-yl)methanol**). **NMR (DMSO-*d*₆):** ¹H δ_{H} 4.57 (CH₂, d, 2H, ³J_{HH} = 6); 5.50 (OH, t, 1H, ³J_{HH} = 6); 7.42–7.54 (*arom.*, *Ph*, m, 2+3H); 7.57–7.64 (*Ph*, m, 2H); 7.85 (*arom.*, t, 1H, ³J_{HH} = 8). **ESI-MS:** 210.0913 [M+H]⁺ (theor. [C₁₄H₁₂N₁O₁]⁺ = 210.0913).

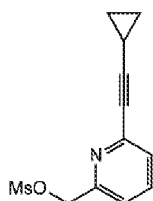


Synthesis of TD939: In a glass vial (20 mL), **TD936** (22 mg; 105 μmol ; 1.0 equiv.) was dissolved in DCM (6 mL) followed by addition of **TEA** (44 μL ; 316 μmol ; 3.0 equiv.) and neat **MsCl** (16 μL ; 207 μmol ; 2.0 equiv.). Resulting solution was stirred 20 min at RT followed by addition of dil. aq. solution of NaHCO₃ (5 mL). The resulting biphasic mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (4 $\times$ 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. **ESI-MS (LC-MS):** 288.1 [M+H]⁺ (theor. [C₁₅H₁₄N₁O₃S₁]⁺ = 288.1). All of **TD939** obtained in this way was directly used for **TD944** without further purification or characterization.

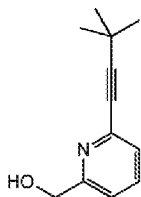


Synthesis of TD937: Glass vial (4 mL) was charged with **(6-Bromopyridin-2-yl)methanol** (135 mg; 722 μmol ; 1.0 equiv.), **CuI** (5.5 mg; 29 μmol ; 4.0 mol%), **[Pd(PPh₃)₂Cl₂]** (10.0 mg; 14 μmol ; 2.0 mol%) and magnetic stirrer and three times secured with argon. Under constant flow of argon was then added dry THF (3 mL) through septum followed by addition of **Cyclopropylacetylene** (75 μL ; 886 μmol ; 1.2 equiv.) and **TEA** (300 μL ; 2.15 mmol; 3.0 equiv.) after which the mixture changed color to dark brown. The vial was left stirring under septum (but without external argon) for 16 h at RT. The mixture was then filtered through syringe microfilter (PTFE)

followed by washing with MeCN. Filtrate was evaporated to dryness, re-dissolved in MeCN (3 mL) and directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions containing product were neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in a mixture of DCM (50 mL) and H₂O (50 mL) and transferred to a separatory funnel. After brief shaking, the bottom phase was separated and the aq. layer was further extracted with DCM (4 × 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residual solid was briefly dried on high vacuum to give product in the form of free base as a yellow oil. **Yield:** 98 mg (78%; 1 step; based on (6-Bromopyridin-2-yl)methanol). **NMR (DMSO-d₆):** ¹H δ_H 0.69–0.83 (CH₂–CH, m, 2H); 0.83–0.99 (CH₂–CH, m, 2H); 1.56 (CH, tt, ³J_{HH} = 8, ³J_{HH} = 5); 4.49 (CH₂, d, 2H, ³J_{HH} = 6); 5.42 (OH, t, 1H, ³J_{HH} = 6); 7.27 (arom., dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.38 (arom., dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.74 (arom., t, 1H, ³J_{HH} = 8). **ESI-HRMS:** 174.0913 [M+H]⁺ (theor. [C₁₁H₁₂N₁O₁]⁺ = 174.0913).



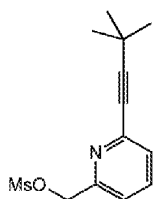
Synthesis of TD940: In a glass vial (20 mL), **TD937** (26 mg; 150 μmol; 1.0 equiv.) was dissolved in DCM (9 mL) followed by addition of **TEA** (63 μL; 452 μmol; 3.0 equiv.) and neat **MsCl** (23 μL; 297 μmol; 2.0 equiv.). Resulting solution was stirred 20 min at RT followed by addition of dil. aq. solution of NaHCO₃ (5 mL). The resulting biphasic mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (4 × 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. **ESI-MS (LC-MS):** 252.0 [M+H]⁺ (theor. [C₁₂H₁₄N₁O₃S₁]⁺ = 252.1). All of **TD940** obtained in this way was directly used for **TD943** without further purification or characterization.



Synthesis of TD952: Glass vial (4 mL) was charged with (6-Bromopyridin-2-yl)methanol (150 mg; 802 μmol; 1.0 equiv.), **CuI** (6.1 mg; 32 μmol; 4.0 mol%), [Pd(PPh₃)₂Cl₂] (11.5 mg; 16 μmol; 2.0 mol%) and magnetic stirrer and three times secured with argon. Under constant flow of argon was then added dry THF (3 mL) through septum followed by addition of *tert*-butylacetylene (200 μL; 1.62 mmol; 2.0 equiv.) and **TEA** (340 μL; 2.44 mmol; 3.0 equiv.) after which the mixture changed color to dark brown. The vial was left stirring under septum (but without external argon) for 24 h at RT. The mixture was then filtered through syringe microfilter (PTFE) followed by washing with MeCN. Filtrate was evaporated to dryness, re-dissolved in MeCN (3 mL) and directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions containing product were neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in a mixture of DCM (50 mL) and H₂O (50 mL) and transferred to a separatory funnel. After brief shaking, the bottom phase was separated and the aq. layer was further extracted with DCM (4 × 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residual solid was briefly

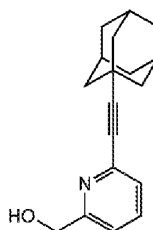
dried on high vacuum to give product in the form of free base as a yellow oil. **Yield:** 146 mg (96%; 1 step; based on (6-Bromopyridin-2-yl)methanol). **NMR (DMSO- d_6):** ^1H δ_{H} 1.30 (CH_3 , s, 9H); 4.51 (CH_2 , d, 2H, $^3J_{\text{HH}} = 6$); 5.43 (OH , t, 1H, $^3J_{\text{HH}} = 6$); 7.26 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.39 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.74 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$). **ESI-HRMS:** 190.1227 [$\text{M}+\text{H}$] $^+$ (theor. [$\text{C}_{12}\text{H}_{16}\text{N}_1\text{O}_1$] $^+$ = 190.1226).

5



Synthesis of TD956: In a glass vial (20 mL), **TD952** (8 mg; 42 μmol ; 1.0 equiv.) was dissolved in DCM (3 mL) followed by addition of **TEA** (18 μL ; 129 μmol ; 3.0 equiv.) and neat **MsCl** (6.5 μL ; 84 μmol ; 2.0 equiv.). Resulting solution was stirred 20 min at RT followed by addition of DCM (5 mL) dil. aq. solution of NaHCO_3 (5 mL). The resulting biphasic mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (4×10 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. **ESI-MS (LC-MS):** 268.1 [$\text{M}+\text{H}$] $^+$ (theor. [$\text{C}_{13}\text{H}_{18}\text{N}_1\text{O}_3\text{S}_1$] $^+$ = 268.1). All of **TD956** obtained in this way was directly used for **TD959** without further purification or characterization.

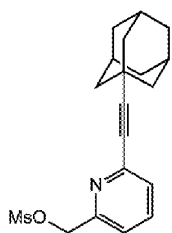
15



Synthesis of TD965: Glass vial (4 mL) was charged with (6-Bromopyridin-2-yl)methanol (53 mg; 283 μmol ; 1.0 equiv.), *1-Ethynyladamantane* (50 mg; 312 μmol ; 1.1 equiv.), **CuI** (2.7 mg; 14 μmol ; 5.0 mol%), $[\text{Pd}(\text{PPh}_3)_2\text{Cl}_2]$ (5.0 mg; 7 μmol ; 2.5 mol%) and magnetic stirrer and three times secured with argon. Under constant flow of argon was then added dry THF (1.5 mL) through septum followed by addition of **TEA** (120 μL ; 861 μmol ; 3.0 equiv.) after which the mixture changed color to dark brown. The vial was left stirring under septum (but without external argon) for 2 d at RT. The mixture was then filtered through syringe microfilter (PTFE) followed by washing with MeCN. Filtrate was evaporated to dryness, re-dissolved in MeCN (3 mL) and directly purified by preparative HPLC (C18; H_2O –MeCN gradient with TFA additive). Fractions containing product were neutralized with dil. aq. NaHCO_3 and evaporated to dryness. Residue was dissolved in a mixture of DCM (25 mL) and H_2O (25 mL) and transferred to a separatory funnel. After brief shaking, the bottom phase was separated and the aq. layer was further extracted with DCM (4×10 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. The residual solid was briefly dried on high vacuum to give product in the form of free base as a colourless oil. **Yield:** 67 mg (89%; 1 step; based on (6-Bromopyridin-2-yl)methanol). **NMR (DMSO- d_6):** ^1H δ_{H} 1.61–1.76 (*Adm.*, m, 6H); 1.82–1.93 (*Adm.*, m, 6H); 1.94–2.00 (*Adm.*, m, 3H); 4.50 (CH_2 , d, 2H, $^3J_{\text{HH}} = 6$); 5.43 (OH , t, 1H, $^3J_{\text{HH}} = 6$); 7.25 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.38 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.74 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$). **ESI-HRMS:** 268.1694 [$\text{M}+\text{H}$] $^+$ (theor. [$\text{C}_{18}\text{H}_{22}\text{N}_1\text{O}_1$] $^+$ = 268.1696).

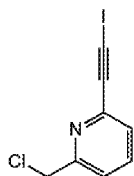
25

30



Synthesis of TD990: In a glass vial (20 mL), **TD965** (21 mg; 79 μmol ; 1.0 equiv.) was dissolved in DCM (3 mL) followed by addition of **TEA** (33 μL ; 237 μmol ; 3.0 equiv.) and neat **MsCl** (12 μL ; 155 μmol ; 2.0 equiv.). Resulting solution was stirred 20 min at RT followed by addition of DCM (10 mL) dil. aq. solution of NaHCO_3 (10 mL). The resulting biphasic mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3×15 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. **ESI-MS** (LC-MS): 346.1 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{19}\text{H}_{24}\text{N}_1\text{O}_3\text{S}_1]^+ = 346.1$). All of **TD990** obtained in this way was directly used for **TD992** without further purification or characterization.

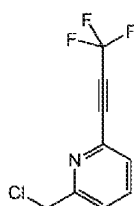
10



15

Synthesis of TD1194: In a glass vial (4 mL), **TD558** (110 mg; 726 μmol ; 1.0 equiv.) was dissolved in MeCN (3.3 mL) followed by addition of **NIS** (180 mg; 800 μmol ; 1.1 equiv.) and **AcOH** (50 μL ; 875 μmol ; 1.2 equiv.) and the resulting mixture was stirred 3 h at 80 $^\circ\text{C}$. Mixture was then directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions containing product were joined and diluted with DCM (50 mL). The resulting biphasic mixture was transferred to a separatory funnel followed by addition of dil. aq. NaHCO_3 and aq. $\text{Na}_2\text{S}_2\text{O}_3$. After brief shaking, the bottom phase was separated and the aq. layer was further extracted with DCM (3×25 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. The residue was briefly dried on high vacuum to give product in the form of free base as a pale red solid. **Yield:** 61.5 mg (31%; 1 step; based on **TD558**). **NMR** ($\text{DMSO}-d_6$): ^1H δ_{H} 4.74 (CH_2 , s, 2H); 7.48 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.54 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.85 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$). **ESI-HRMS:** 277.9230 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_8\text{H}_6\text{N}_1\text{I}_1\text{Cl}_1]^+ = 277.9228$).

20



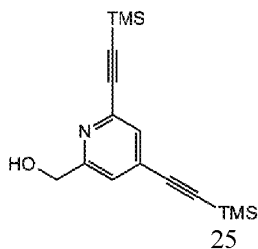
30

Synthesis of TD1178: Glass vial (20 mL) was charged with **CuI** (60.0 mg; 315 μmol ; 40 mol%), **Phen** (114 mg; 633 μmol ; 80.0 mol%), **KHCO₃** (159 mg; 1.59 mmol; 2.0 equiv.) and magnetic stirrer and three times secured with argon. Under constant flow of argon was then added solution of **TD558** (120 mg; 792 μmol ; 1.0 equiv.) and **Togni I** (288 mg; 872 μmol ; 1.1 equiv.) in dry DCM (8 mL) through septum and the resulting mixture was stirred 2 d at RT. Mixture was then filtered through syringe microfilter (PTFE) and the solid phase was further washed with DCM. Filtrate was evaporated to dryness and the residue was resuspended in MeCN (3 mL). Mixture was again filtered through syringe microfilter (PTFE) and the filtrate was purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions containing product were joined and diluted with DCM (50 mL). The resulting biphasic mixture was transferred to a separatory funnel followed by addition of dil. aq. NaHCO_3 . After brief shaking, the bottom phase was separated and the aq. layer was further extracted with DCM (3×25 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness.

35

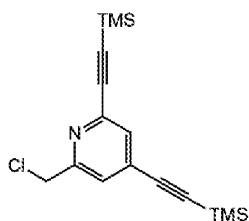
The resulting yellow oil was briefly dried on high vacuum (until the oil crystallized) to give product in the form of free base as pale yellow solid (portion of the product sublimed as colourless crystals on the upper part of the flask). **Yield:** 48.5 mg (28%; 1 step; based on **TD558**). **NMR (DMSO- d_6):** ^1H δ_{H} 4.83 (CH_2 , s, 2H); 7.78 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.88 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 8.04 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$). $^{19}\text{F}\{^1\text{H}\}$ δ_{F} -49.2 (s). **ESI-HRMS:** 220.0140 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_9\text{H}_6\text{N}_1\text{F}_3\text{Cl}_1]^+ = 220.0135$).

Synthesis of TD797: In a round-bottom glass flask (50 mL), **Methyl 4,6-dibromopicolinate** (200 mg; 678 μmol ; 1.0 equiv.) was dissolved in a mixture of THF (4 mL) and MeOH (2 mL). To the resulting slightly yellow solution was added portion wise (over course of 10 min, with no stopper on the flask) solid **NaBH_4** (205 mg; 5.42 mmol; 8.0 equiv.) during which hydrogen gas evolved intensively and the color of the mixture changed to nearly colorless. Mixture was then transferred to a separatory funnel and diluted with DCM (100 mL) and H_2O (100 mL). After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3×50 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as white solid. **Yield:** 179 mg (99%; 1 step; based on **Methyl 4,6-dibromopicolinate**). **NMR (DMSO- d_6):** ^1H δ_{H} 4.53 (CH_2 , s, 2H); 5.68 (OH , bs, 1H); 7.67 (*arom.*, m, 1H); 7.89 (*arom.*, m, 1H). **ESI-HRMS:** 265.8809 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_6\text{H}_6\text{N}_1\text{O}_1\text{Br}_2]^+ = 265.8811$).

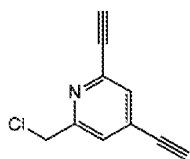


Synthesis of TD804: Glass vial (4 mL) was charged with **TD797** (100 mg; 375 μmol ; 1.0 equiv.) and magnetic stirrer and three times secured with argon. Solid **CuI** (4.3 mg; 23 μmol ; 6.0 mol%) and **$[\text{Pd}(\text{PPh}_3)_2\text{Cl}_2]$** (8.0 mg; 11 μmol ; 3.0 mol%) were subsequently added followed by another securing with argon (three times). Under constant flow of argon was then added dry THF (1.5 mL) through septum followed by addition of **Ethynyltrimethylsilane** (160 μL ; 1.16 mmol; 3.1 equiv.) and **TEA** (160 μL ; 1.15 mmol; 3.1 equiv.) after which the mixture changed color to dark brown. The flask was left stirring under septum (but without external argon) for 16 h at RT. The mixture was then transferred to a separatory funnel with EtOAc (50 mL) and H_2O (50 mL). After shaking, the dark colored organic layer was separated and the aqueous layer was further extracted with EtOAc (3×25 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. Resulting dark brown oily residue was purified on flash chromatography (120 g SiO_2 , 100% DCM to 20% EtOAc in DCM). Combined fractions with product were evaporated to dryness and further co-evaporated once with DCM. The residue was further dried on high vacuum overnight to give product in the form of free base as dark brown oil. **Yield:** 99 mg (88%; 1 step; based on **TD797**). **NMR (DMSO- d_6):** ^1H δ_{H} 0.24 (CH_3 , s, 9H); 0.25 (CH_3 , s, 9H);

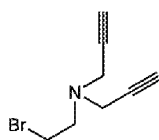
4.51 (CH_2 , d, 2H, $^3J_{HH} = 6$); 5.53 (OH, t, 1H, $^3J_{HH} = 6$); 7.40 (arom., d, 1H, $^4J_{HH} = 2$); 7.42 (arom., d, 1H, $^4J_{HH} = 2$). **ESI-HRMS**: 302.1389 $[M+H]^+$ (theor. $[C_{16}H_{24}N_1O_1Si_2]^+ = 302.1391$).



Synthesis of TD806: In a pear-shaped glass flask (50 mL), **TD804** (98 mg; 325 μ mol; 1.0 equiv.) was dissolved in DCM (3 mL). Freshly prepared solution of **SOCl₂** (47 μ L; 647 μ mol; 2.0 equiv.) in DCM (1 mL) was then added and the open flask was stirred 1 h at RT. Reaction mixture was diluted with DCM (16 mL) and dil. aq. solution of NaHCO₃ (20 mL) was then added and resulting biphasic mixture was vigorously stirred for additional 1 h at RT, during which bubbles of gas slowly evolved. Mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 $\times$ 20 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as brown oil. **Yield**: 102 mg (98%; 1 step; based on **TD804**). **NMR (DMSO-d₆)**: 1H δ_H 0.25 (CH_3 , s, 18H); 4.74 (CH_2 , s, 2H); 7.53 (arom., d, 1H, $^4J_{HH} = 2$); 7.60 (arom., d, 1H, $^4J_{HH} = 2$). **ESI-HRMS**: 320.1051 $[M+H]^+$ (theor. $[C_{16}H_{23}N_1O_1Cl_1Si_2]^+ = 320.1052$).



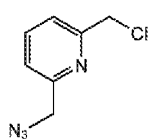
Synthesis of TD807: In a round-bottom glass flask (50 mL), **TD806** (101 mg; 316 μ mol; 1.0 equiv.) was dissolved in MeCN (5 mL). Freshly prepared solution of **KF** (46.4 mg; 800 μ mol; 2.5 equiv.) in H₂O (800 μ L) was then added and the flask was stirred 5 h at RT. The mixture was concentrated to ~1 mL and diluted with DCM (50 mL) and dil. aq. NaHCO₃ (50 mL). The mixture was transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 $\times$ 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as brown solid. **Yield**: 48 mg (87%; 1 step; based on **TD806**). **NMR (DMSO-d₆)**: 1H δ_H 4.48 ($C\equiv CH$, s, 1H); 4.75 ($C\equiv CH$, s, 1H); 4.76 (CH_2 , s, 2H); 7.61 (arom., d, 1H, $^4J_{HH} = 1$); 7.65 (arom., d, 1H, $^4J_{HH} = 1$). **ESI-HRMS**: 176.0267 $[M+H]^+$ (theor. $[C_{10}H_7N_1Cl_1]^+ = 176.0267$).



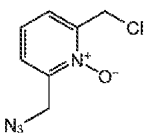
Synthesis of TD662: In a glass vial (4 mL), **Dipropargylamine** (116 μ L; 1.12 mmol; 1.0 equiv.) was dissolved in MeCN (2.5 mL) followed by addition of neat **1,2-Dibromoethane** (965 μ L; 11.2 mmol; 10 equiv.) and **NaHCO₃** (113 mg; 1.35 mmol; 1.2 equiv.) and the resulting mixture was stirred 2 days at 80 °C. Mixture was then diluted with DCM (50 mL) followed by addition of dil. aq. solution of NaHCO₃ (50 mL). The resulting biphasic mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 $\times$ 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through

glass frit (S3) and evaporated to dryness. Residue was further purified by column chromatography (SiO₂; 25 g; DCM). Combined fractions with product were evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as nearly colorless oil. **Yield:** 69 mg (31%; 1 step; based on **Dipropargylamine**). **NMR (DMSO-d₆):** ¹H δ_H 2.86 (CH₂, t, 2H, ³J_{HH} = 7); 3.21 (C≡CH, t, 2H, ⁴J_{HH} = 2); 3.21 (CH₂, t, 4H, ⁴J_{HH} = 2); 3.55 (CH₂, t, 2H, ³J_{HH} = 7). ¹³C{¹H} δ_C 30.3 (CH₂, s); 41.6 (CH₂, s); 53.9 (CH₂, s); 76.4 and 78.5 (C≡CH, 2 × s). **ESI-HRMS:** 200.0070 [M+H]⁺ (theor. [C₈H₁₁N₁Br₁]⁺ = 200.0070).

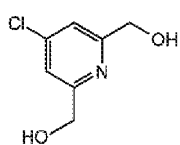
Example 2: Synthesis of intermediates of azide pendant arms



Synthesis of TD406: In a round-bottom glass flask (250 mL), **2,6-Bis(chloromethyl)pyridine** (6.17 g; 35.0 mmol; 1.2 equiv.) was dissolved in MeCN (80 mL) followed by addition of solid NaN₃ (1.90 g; 29.2 mmol; 1.0 equiv.) and anhydrous K₂CO₃ (4.04 g; 29.2 mmol; 1.0 equiv.) and the resulting suspension was stirred 5 days at 50 °C. The mixture was filtered on glass frit S3 and the solid residue was washed with MeCN. Filtrate was evaporated to dryness and the orange oily residue was purified by column chromatography (SiO₂, 30% P.E. in DCM to 100% DCM). Fractions with product were combined and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as faint yellow oil. **Yield:** 2.62 g (49%; 1 step; based on NaN₃). **Recovery:** 2.21 g of **2,6-Bis(chloromethyl)pyridine** (36% of the initial amount used). **NMR (DMSO-d₆):** ¹H δ_H 4.53 (CH₂, s, 2H); 4.78 (CH₂, s, 2H); 7.40 (arom., d, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.52 (arom., d, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.89 (arom., t, 1H, ³J_{HH} = 8). ¹³C{¹H} δ_C 46.6 (CH₂, s); 54.2 (CH₂, s); 121.9 (arom., s); 122.6 (arom., s); 138.5 (arom., s); 155.7 (arom., s); 156.3 (arom., s). **ESI-HRMS:** 183.0433 [M+H]⁺ (theor. [C₇H₈N₄Cl]⁺ = 183.0432).

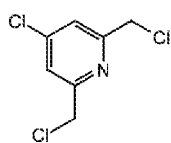


Synthesis of TD595: In a glass vial (20 mL), **TD406** (30 mg; 162 μmol; 1.0 equiv.) was dissolved in DCM (5 mL) followed by addition of freshly prepared solution of **MCPBA** (77%; 72 mg; 320 μmol; 2.0 equiv.) in DCM (1 mL). The resulting solution was stirred 3 h at RT. Mixture was then diluted with DCM (20 mL) and dil. aq. NaHCO₃ (25 mL) and transferred to a separatory funnel. After shaking, the bottom layer was separated and aqueous layer was further extracted with DCM (3 × 20 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. **ESI-MS (LC-MS):** 199.0 [M+H]⁺ (theor. [C₇H₈N₄O₁Cl]⁺ = 199.0). All of **TD595** obtained in this way was directly used for **TD596** without further purification or characterization.

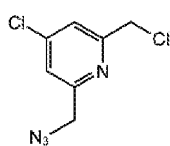


Synthesis of TD726: In a round-bottom glass flask (1000 mL), **Dimethyl 4-chloropyridine-2,6-dicarboxylate** (15.0 g; 65.3 mmol; 1.0 equiv.) was gradually dissolved in a mixture of THF (540 mL) and MeOH (120 mL). The resulting slightly yellow solution was cooled with an ice bath (~5 °C) followed by portion wise additions (over course of 1 h,

with no stopper on the flask) of solid **NaBH₄** (12.4 g; 328 mmol; 5.0 equiv.) during which hydrogen gas evolved intensively and the color of the mixture changed to red, orange and eventually yellow. After the addition, the flask was allowed to warm up to RT and was further stirred 16 h at RT. Resulting faint yellow opalescent solution was filtered on glass frit (S3), the filtrate was evaporated to dryness and further co-evaporated with DCM (as a suspension). Residue was dissolved in boiling H₂O (~600 mL) and the resulting highly alkaline solution was continuously extracted by DCM overnight. The organic layer (containing partly crystallized product) was evaporated to dryness. The residue was mechanically crushed and further dried on high vacuum (till constant mass) to give product in the form of free base as nearly colorless microcrystalline powder. **Yield:** 10.84 g (96%; 1 step; based on **Dimethyl 4-chloropyridine-2,6-dicarboxylate**). **NMR (DMSO-d₆):** ¹H δ_H 4.53 (CH₂, d, 4H, ³J_{HH} = 6); 5.54 (OH, t, 2H, ³J_{HH} = 6); 7.36 (arom., s, 2H). ¹³C{¹H} δ_C 63.7 (CH₂, s); 118.0 (arom., s); 144.0 (arom., s); 163.5 (arom., s). **ESI-HRMS:** 174.0317 [M+H]⁺ (theor. [C₇H₉N₁O₂Cl₁]⁺ = 174.0316). **EA** (C₇H₈N₁O₂Cl₁, M_R = 173.6): C 48.4 (48.5); H 4.7 (4.5); N 7.7 (8.1); Cl 20.4 (21.2).

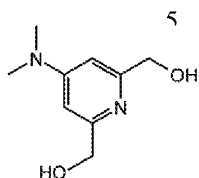


Synthesis of TD759: In a round-bottom glass flask (100 mL), **TD726** (626 mg; 3.61 mmol; 1.0 equiv.) was suspended in DCM (20 mL). Freshly prepared solution of **SOCl₂** (780 μL; 10.7 mmol; 3.0 equiv.) in DCM (10 mL) was then added and the open flask was stirred 90 min at RT, producing clear solution. Dil. aq. solution of NaHCO₃ (40 mL) was then added and resulting biphasic mixture was vigorously stirred for additional 1 h at RT, during which bubbles of gas slowly evolved. Mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 × 50 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The resulting crystallized residue was crushed and further dried on high vacuum overnight to give product in the form of free base as nearly colorless microcrystalline powder. **Yield:** 711 mg (94%; 1 step; based on **TD726**). **NMR (DMSO-d₆):** ¹H δ_H 4.79 (CH₂, s, 4H); 7.70 (arom., s, 2H). ¹³C{¹H} δ_C 45.7 (CH₂, s); 122.8 (arom., s); 144.4 (arom., s); 158.2 (arom., s). **ESI-HRMS:** 209.9638 [M+H]⁺ (theor. [C₇H₇N₁Cl₃]⁺ = 209.9639).

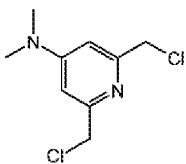


Synthesis of TD760: In a glass vial (20 mL), **TD759** (708 mg; 3.36 mmol; 1.3 equiv.) was dissolved in MeCN (20 mL) followed by addition of solid **NaN₃** (168 mg; 2.58 mmol; 1.0 equiv.) and dried **K₂CO₃** (360 mg; 2.61 mmol; 1.0 equiv.) and the resulting suspension was stirred 24 h at 80 °C. The mixture was filtered on through syringe microfilter (PTFE) and the solid residue was washed with MeCN. Filtrate was evaporated to dryness and yellow oily residue was purified by column chromatography (SiO₂, 40% P.E. in DCM to 10% P.E. in DCM). Fractions with product were combined and evaporated to dryness. The residue was further dried on high vacuum 1 h to give product in the form of free base as faint yellow oil. **Yield:** 323 mg (58%; 1 step; based on **NaN₃**). **Recovery:** 218 mg of **TD759** (31% of the initial amount used). **NMR (DMSO-d₆):** ¹H δ_H 4.56 (CH₂, s, 2H); 4.78 (CH₂, s, 2H); 7.58

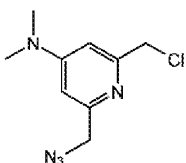
(*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 7.69 (*arom.*, d, 1H, $^4J_{\text{HH}} = 8$). $^{13}\text{C}\{^1\text{H}\}$ δ_{C} 45.8 (CH_2 , s); 53.6 (CH_2 , s); 121.9 (*arom.*, s); 122.5 (*arom.*, s); 144.4 (*arom.*, s); 157.9 (*arom.*, s); 158.2 (*arom.*, s). **ESI-HRMS**: 217.0040 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_7\text{H}_7\text{N}_4\text{Cl}_2]^+ = 217.0042$).



- Synthesis of TD1146:** In a glass vial (20 mL), **TD726** (495 mg; 2.85 mmol; 1.0 equiv.) was dissolved in **DMF** (11.0 mL; 143 mmol; 50 equiv.) followed by addition of freshly ground **KOH** (1.6 g; 28.5 mmol; 10 equiv.). The resulting suspension was stirred 3 days at 100 °C in the presence of air (vial septum was punctured by two thick needles). The mixture was then filtered through syringe microfilter (PTFE; the solids were washed with
- 10 **DMF**). Filtrate was evaporated to dryness and purified by preparative HPLC (C18; H_2O – MeCN gradient with TFA additive). Fractions with product were combined and directly lyophilized to give product in the form of trifluoroacetate salt as white foam. **Yield**: 187 mg (22%; 1 step; based on **TD726**). **NMR (DMSO- d_6)**: ^1H δ_{H} 3.18 (CH_3 , s, 6H); 4.59 (CH_2 , d, 4H, $^3J_{\text{HH}} = 6$); 5.91 (*OH*, t, 2H, $^3J_{\text{HH}} = 6$); 6.84 (*arom.*, s, 2H). **ESI-HRMS**: 183.1128 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_9\text{H}_{15}\text{N}_2\text{O}_2]^+ = 183.1128$). **EA** ($\text{C}_9\text{H}_{14}\text{N}_2\text{O}_2 \cdot 1.0\text{TFA}$, $M_{\text{R}} = 296.2$): C 44.6 (44.1); H
- 15 5.1 (4.9); N 9.5 (9.1); F 19.2 (18.2).



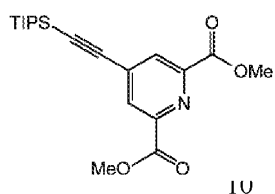
- Synthesis of TD1154:** In a round-bottom glass flask (100 mL), **TD1146**·1.0TFA (173.3 mg; 585 μmol ; 1.0 equiv.) was suspended in **DCM** (17 mL) followed by addition of neat **SOCl_2** (780 μL ; 2.34 mmol; 4.0 equiv.). The resulting mixture was stirred 90 min at RT, producing clear solution. Dil. aq. solution of NaHCO_3 (20 mL) was then added and resulting biphasic mixture was vigorously stirred for additional 1 h RT, during which
- 25 bubbles of gas slowly evolved. Mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with **DCM** (3×25 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as white solid. **Yield**: 105.6 mg (82%; 1 step; based on **TD726**). **NMR (DMSO- d_6)**: ^1H δ_{H} 2.98 (CH_3 , s, 6H); 4.60 (CH_2 , s, 4H); 6.73 (*arom.*, s, 2H). **ESI-HRMS**: 219.0450 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_9\text{H}_{13}\text{N}_2\text{Cl}_2]^+ = 219.0450$).



- Synthesis of TD1163:** In a glass vial (20 mL), **TD1154** (103.9 mg; 474 μmol ; 1.3 equiv.) was dissolved in **MeCN** (7 mL) followed by addition of solid **NaN_3** (23.7 mg; 365 μmol ; 1.0 equiv.) and dried **K_2CO_3** (50.3 mg; 364 μmol ; 1.0 equiv.) and the resulting suspension was stirred 24 h at 70 °C. The mixture was filtered on through syringe microfilter (PTFE) and the solid residue was washed with **MeCN**. Filtrate was evaporated to dryness and the residue was purified by column chromatography (SiO_2 , 5% **EtOAc** in **DCM** to 10% **EtOAc** in **DCM**). Fractions
- 35 with product were combined and evaporated to dryness. The residue was further dried on high vacuum 1 h to

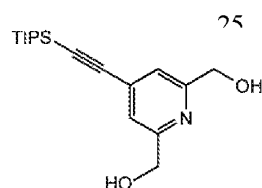
give product in the form of free base as colourless oil. **Yield:** 33.8 mg (41%; 1 step; based on NaN_3). **Recovery:** 11.0 mg of **TD1154** (11% of the initial amount used). **NMR (DMSO- d_6):** ^1H δ_{H} 2.99 (CH_3 , s, 6H); 4.32 (CH_2 , s, 2H); 4.60 (CH_2 , s, 2H); 6.60 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 6.73 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$). **ESI-HRMS:** 226.0853 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_9\text{H}_{13}\text{N}_3\text{Cl}_1]^+ = 226.0854$).

5



Synthesis of TD1024: Glass vial (100 mL) was charged with **CuI** (70 mg; 0.37 mmol; 4.0 mol%) and **[Pd(PPh₃)₂Cl₂]** (125 mg; 0.18 mmol; 2.0 mol%) and magnetic stirrer and three times secured with argon. Solution of **Dimethyl 4-iodopyridine-2,6-dicarboxylate** (2.93 g; 9.13 mmol; 1.0 equiv.) in mixture of dry THF (40 mL), dry Toluene (40 mL) and dry DMF (2 mL) was subsequently added through septum followed by addition of **Ethynyltriisopropylsilane** (2.25 mL; 10.0 mmol; 1.1 equiv.) and **TEA** (3.80 mL; 27.3 mmol; 3.0 equiv.) after which the mixture changed color to pale red. The flask was left stirring under septum (but without external argon) for 20 h at RT. The resulting dark mixture was filtered through syringe microfilter (PTFE) and the filtrate was evaporated to dryness. Residue was dissolved in DCM (250 mL) and the resulting red solution was washed once with aq. solution of $\text{Na}_2\text{S}_2\text{O}_3$. The organic layer was separated, dried with Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. Residue was purified on flash chromatography (220 g SiO_2 , 100% DCM to 20% EtOAc in DCM). Combined fractions with product were evaporated to dryness and further co-evaporated once with DCM. The residue was further dried on high vacuum overnight to give pre-purified product in the form of free base as orange oil. **Yield:** 3.16 g (92%; 1 step; based on **Dimethyl 4-iodopyridine-2,6-dicarboxylate**). **NMR (DMSO- d_6):** ^1H δ_{H} 0.92–1.27 (*i-Pr*, m, 21H); 3.92 (CH_3 , s, 6H); 8.16 (*arom.*, s, 2H). $^{13}\text{C}\{^1\text{H}\}$ δ_{C} 10.6 (*i-Pr*, s); 18.4 (*i-Pr*, s); 52.9 (CH_3 , s); 99.0 ($\text{C}\equiv\text{C}-\text{Si}$, s); 102.5 ($\text{C}\equiv\text{C}-\text{arom.}$, s); 129.3 (*arom.*, s); 132.7 (*arom.*, s); 148.4 (*arom.*, s); 164.0 (CO , s). **ESI-HRMS:** 376.1939 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{20}\text{H}_{30}\text{N}_1\text{O}_4\text{Si}_1]^+ = 376.1939$).

20

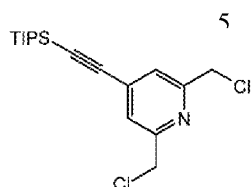


Synthesis of TD808: In a round-bottom glass flask (250 mL), **TD1024** (3.15 g; 8.39 mmol; 1.0 equiv.) was dissolved in a mixture of THF (60 mL) and MeOH (30 mL). Then, solid **NaBH_4** (2.55 g; 67.4 mmol; 8 equiv.) was added portion wise over the course of 1 h (during which hydrogen gas evolved intensively) and the color of the mixture changed to dark red. After the addition, the flask was further stirred 1 h at RT. The mixture was evaporated to dryness. Residue was resuspended in DCM (200 mL) and H_2O (200 mL) and transferred to a separatory funnel. After shaking, the bottom phase (as a suspension) was separated. Aqueous phase was further extracted with DCM (3×100 mL). Combined organic layers were diluted with EtOAc (500 mL). The resulting brown solution was then dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as pale brown solid. **Yield:** 2.61 g (97%; 1 step; based on **TD1024**). **NMR (DMSO- d_6):**

30

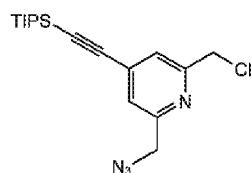
35

^1H δ_{H} 1.04–1.17 (*i*-Pr, m, 21H); 4.51 (CH_2 , d, 4H, $^3J_{\text{HH}} = 6$); 5.48 (OH, t, 2H, $^3J_{\text{HH}} = 6$); 7.31 (*arom.*, s, 2H). $^{13}\text{C}\{^1\text{H}\}$ δ_{C} 10.6 (*i*-Pr, s); 18.5 (*i*-Pr, s); 63.9 (CH_2 , s); 94.6 ($\text{C}\equiv\text{C}-\text{Si}$, s); 105.1 ($\text{C}\equiv\text{C}-\text{arom.}$, s); 119.8 (*arom.*, s); 130.8 (*arom.*, s); 161.9 (*arom.*, s). **ESI-HRMS**: 320.2036 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{18}\text{H}_{30}\text{N}_1\text{O}_2]^+ = 320.2040$).



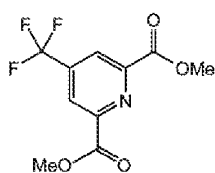
Synthesis of TD815: In a round-bottom glass flask (250 mL), pre-purified **TD808** (2.61 g; 8.17 mmol; 1.0 equiv.) was suspended in DCM (100 mL). Freshly prepared solution of SOCl_2 (1.80 mL; 24.8 mmol; 3.0 equiv.) in DCM (10 mL) was then added and the open flask was stirred 1 h at RT. Dil. aq. solution of NaHCO_3 (100 mL) was then added and resulting biphasic mixture was vigorously stirred for additional 1 h

at RT, during which bubbles of gas slowly evolved. Mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3×75 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as yellow oil. **Yield**: 2.87 g (99%; 1 step; based on **TD808**). **NMR (DMSO- d_6)**: ^1H δ_{H} 0.97–1.20 (*i*-Pr, m, 21H); 4.78 (CH_2 , s, 4H); 7.59 (*arom.*, s, 2H). $^{13}\text{C}\{^1\text{H}\}$ δ_{C} 10.6 (*i*-Pr, s); 18.4 (*i*-Pr, s); 45.9 (CH_2 , s); 96.6 ($\text{C}\equiv\text{C}-\text{Si}$, s); 103.5 ($\text{C}\equiv\text{C}-\text{arom.}$, s); 124.6 (*arom.*, s); 132.0 (*arom.*, s); 157.1 (*arom.*, s). **ESI-HRMS**: 356.1368 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{18}\text{H}_{28}\text{N}_1\text{Cl}_2\text{Si}_1]^+ = 356.1368$).



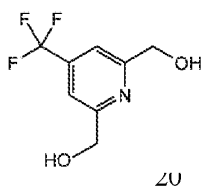
Synthesis of TD817: In a round-bottom glass flask (250 mL), **TD815** (2.86 g; 8.02 mmol; 1.2 equiv.) was dissolved in MeCN (130 mL) followed by addition of solid NaN_3 (435 mg; 6.69 mmol; 1.0 equiv.) and dried K_2CO_3 (920 mg; 6.67 mmol; 1.0 equiv.) and the resulting suspension was stirred 16 h at 80 °C. The mixture was filtered through syringe microfilter (PTFE) and the solid residue was washed with

MeCN. Filtrate was evaporated to dryness and orange oily residue was purified by column chromatography (220 g SiO_2 , 30% P.E. in DCM to 10% P.E. in DCM). Fractions with product were combined and evaporated to dryness. The residue was further dried on high vacuum 1 h to give product in the form of free base as faint yellow oil. **Yield**: 1.23 g (51%; 1 step; based on NaN_3). **Recovery**: 709 mg of **TD815** (25% of the initial amount used). **NMR (DMSO- d_6)**: ^1H δ_{H} 1.01–1.21 (*i*-Pr, m, 21H); 4.55 (CH_2 , s, 2H); 4.78 (CH_2 , s, 2H); 7.47 (*arom.*, d, 1H, $^4J_{\text{HH}} = 1$); 7.58 (*arom.*, d, 1H, $^4J_{\text{HH}} = 1$). $^{13}\text{C}\{^1\text{H}\}$ δ_{C} 10.6 (*i*-Pr, s); 18.4 (*i*-Pr, s); 46.0 (CH_2 , s); 53.7 (CH_2 , s); 96.5 ($\text{C}\equiv\text{C}-\text{Si}$, s); 103.7 ($\text{C}\equiv\text{C}-\text{arom.}$, s); 123.7 (*arom.*, s); 124.3 (*arom.*, s); 131.8 (*arom.*, s); 156.6 (*arom.*, s); 157.1 (*arom.*, s). **ESI-HRMS**: 363.1763 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{18}\text{H}_{28}\text{N}_4\text{Cl}_1\text{Si}_1]^+ = 363.1766$).



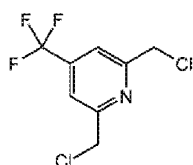
Synthesis of TD1052: In a glass vial (20 mL), **Dimethyl 4-iodopyridine-2,6-dicarboxylate** (500 mg; 1.56 mmol; 1.0 equiv.), **CuI** (600 mg; 3.15 mmol, 2.0 equiv.) and **[(dppf)PdCl₂]** (7.0 mg, 10 μmol; 0.6 mol%) were dissolved in dry DMF (8 mL) followed by addition of **Methyl 2,2-difluoro-2-(fluorosulfonyl)acetate** (600 mg; 3.12 mmol; 2.0 equiv.) in dry DMF (2 mL). The resulting dark mixture was stirred 16 h at 100 °C. After cooling down, the mixture was diluted with DCM (10 mL) and filtered through syringe microfilter (PTFE). The solids were further washed with DCM. Filtrate was further diluted with DCM (50 mL) and washed with dil. aq. NaHCO₃ (5 × 25 mL). Organic layer was dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. Residue was purified by column chromatography (120 g SiO₂, 100 % DCM to 15% EtOAc in DCM). Fractions with product were combined and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as colorless solid. **Yield:** 366 mg (89%; 1 step; based on **Dimethyl 4-iodopyridine-2,6-dicarboxylate**). **NMR (DMSO-d₆):** ¹H δ_H 3.97 (CH₃, s, 6H); 8.49 (arom., q, 2H, ⁴J_{HF} = 1). ¹⁹F{¹H} δ_F -63.4 (s). **ESI-HRMS:** 264.0480 [M+H]⁺ (theor. [C₁₀H₉N₁O₄F₃]⁺ = 264.0478).

15



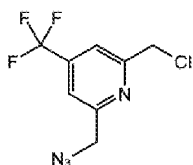
Synthesis of TD1053: In a round-bottom glass flask (100 mL), **TD1052** (365 mg; 1.39 mmol; 1.0 equiv.) was dissolved in a mixture of MeOH (5 mL) and THF (10 mL). The resulting colourless solution was cooled with an ice bath (~5 °C) followed by portion wise additions (over course of 30 min, with no stopper on the flask) of solid **NaBH₄** (420 mg; 11.1 mmol; 8.0 equiv.) during which hydrogen gas evolved intensively and the color of the mixture changed to red and orange. After the addition, the flask was allowed to warm up to RT and was further stirred 30 min at RT. Resulting yellow solution was evaporated to dryness. Residue was purified by column chromatography (120 g SiO₂, solid load technique, 100 % EtOAc to 15% MeOH in EtOAc). Fractions with product were combined and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as yellow oil. **Yield:** 171 mg (60%; 1 step; based on **TD1052**). **NMR (DMSO-d₆):** ¹H δ_H 4.62 (CH₂, s, 4H); 5.65 (OH, s, 2H); 7.60 (arom., q, 2H, ⁴J_{HF} = 1). ¹⁹F{¹H} δ_F -63.6 (s). **ESI-HRMS:** 208.0580 [M+H]⁺ (theor. [C₈H₉N₁O₂F₃]⁺ = 208.0580).

25



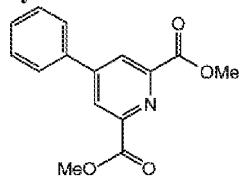
Synthesis of TD1055: In a round-bottom glass flask (100 mL), **TD1053** (169 mg; 816 μmol; 1.0 equiv.) was dissolved in DCM (20 mL). Freshly prepared solution of **SOCl₂** (237 μL; 3.26 mmol; 4.0 equiv.) in DCM (5 mL) was then added and the resulting mixture was stirred 16 h at RT. Dil. aq. solution of NaHCO₃ (25 mL) was then added and resulting biphasic mixture was vigorously stirred for additional 10 min at RT, during which bubbles of gas slowly evolved. Mixture was then transferred to a separatory funnel. After shaking, the bottom phase

was separated. Aqueous phase was further extracted with DCM (3×25 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as yellow oil. **Yield:** 168 mg (84%; 1 step; based on **TD1053**). **NMR (DMSO- d_6):** ^1H δ_{H} 4.90 (CH_2 , s, 4H); 7.94 (*arom.*, s, 2H). $^{19}\text{F}\{^1\text{H}\}$ δ_{F} -63.4 (s). **APCI-HRMS:** 243.9902 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_8\text{H}_7\text{N}_1\text{F}_3\text{Cl}_2]^+ = 243.9902$).

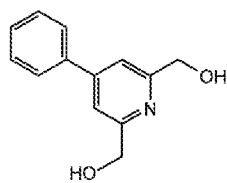


Synthesis of TD1057: In a glass vial (4 mL), **TD1055** (143 mg; 586 μmol ; 1.8 equiv.) was dissolved in MeCN (3.5 mL) followed by addition of solid NaN_3 (21.5 mg; 331 μmol ; 1.0 equiv.) and dried K_2CO_3 (46 mg; 333 μmol ; 1.0 equiv.) and the resulting suspension was stirred 3 d at 70 $^\circ\text{C}$. The mixture was filtered through syringe microfilter (PTFE) and the solid residue was washed with MeCN. Filtrate was evaporated to dryness and orange oily residue was purified by column chromatography (80 g SiO_2 , 30% P.E. in DCM to 40% DCM in P.E.). Fractions with product were combined and evaporated to dryness. The residue was further dried on high vacuum 1 h to give product in the form of free base as colourless oil. **Yield:** 43.9 g (53%; 1 step; based on NaN_3). **Recovery:** 32.1 mg of **TD1055** (22% of the initial amount used). **NMR (DMSO- d_6):** ^1H δ_{H} 4.69 (CH_2 , s, 2H); 4.90 (CH_2 , s, 2H); 7.81 (*arom.*, d, 1H, $^4J_{\text{HH}} = 1$); 7.93 (*arom.*, d, 1H, $^4J_{\text{HH}} = 1$). $^{19}\text{F}\{^1\text{H}\}$ δ_{F} -63.4 (s). **APCI-HRMS:** 251.0306 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_8\text{H}_7\text{N}_4\text{F}_3\text{Cl}_1]^+ = 251.0306$).

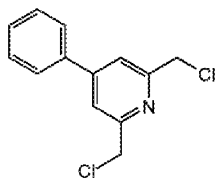
Synthesis of TD549: Pear-shape glass flask (250 mL) was charged with **Dimethyl 4-chloropyridine-2,6-dicarboxylate** (5.00 g; 21.8 mmol; 1.0 equiv.), **Phenylboronic acid** (3.20 g; 26.2 mmol; 1.2 equiv.) and **XPhos Pd G2** (510 mg; 648 μmol ; 3.0 mol%) and was then three times secured with argon. Under constant flow of argon was then added dry DMF (110 mL) through septum followed by addition of freshly dried (using heat gun



under high vacuum) Cs_2CO_3 (15.6 g; 47.9 mmol; 2.2 equiv.; the flask was briefly opened for addition). The mixture was then stirred 20 h under septum (but without external argon) at 80 $^\circ\text{C}$. Resulting dark mixture was filtered through glass frit (S3) and the filtrate was poured to a stirred beaker with H_2O (500 mL). Precipitate was collected on glass frit (S2), washed with H_2O and further dried on high vacuum overnight to give product in the form of free base as off white solid. **Yield:** 3.01 g (51%; 1 step; based on **Dimethyl 4-chloropyridine-2,6-dicarboxylate**). **NMR (DMSO- d_6):** ^1H δ_{H} 3.95 (CH_3 , s, 6H); 7.45–7.57 (*Ph*, m, 3H); 7.82–8.00 (*Ph*, m, 2H); 8.48 (*arom.*, s, 2H); $^{13}\text{C}\{^1\text{H}\}$ δ_{C} 52.7 (CH_3 , s); 125.0 (*arom.*, s); 127.2 (*Ph.*, s); 129.5 (*Ph.*, s); 130.2 (*Ph.*, s); 135.5 (*Ph.*, s); 148.6 (*arom.*, s); 150.0 (*arom.*, s); 164.7 (CO , s). **ESI-HRMS:** 272.0916 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{15}\text{H}_{14}\text{N}_1\text{O}_4]^+ = 272.0917$).

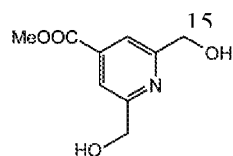


Synthesis of TD563: In a round-bottom glass flask (500 mL), **TD549** (2.98 g; 11.0 mmol; 1.0 equiv.) was dissolved in a mixture of MeOH (100 mL) and THF (100 mL). The resulting slightly yellow solution was cooled with an ice bath ($\sim 5^{\circ}\text{C}$) followed by portion wise additions (over course of 30 min, with no stopper on the flask) of solid **NaBH₄** (2.90 g; 76.7 mmol; 9.0 equiv.) during which hydrogen gas evolved intensively and the color of the mixture changed to red and orange. After the addition, the flask was allowed to warm up to RT and was further stirred 30 min at RT. Resulting faint yellow opalescent solution was filtered through syringe microfilter (PTFE). The filtrate was evaporated to dryness and further co-evaporated with DCM (as a suspension). Residue was dissolved in a mixture of H₂O (300 mL) and DCM (300 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (8×75 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The resulting crystallized residue was crushed and further dried on high vacuum overnight to give product in the form of free base as nearly colorless solid. **Yield:** 2.29 g (97%; 1 step; based on **TD549**). **NMR (DMSO-*d*₆):** ¹H δ_{H} 4.59 (CH₂, s, 4H); 5.45 (OH, s, 2H); 7.45–7.57 (Ph, m, 3H); 7.60 (arom., s, 2H); 7.68–7.82 (Ph, m, 2H). ¹³C{¹H} δ_{C} 64.2 (CH₂, s); 115.7 (arom., s); 126.7 (Ph, s); 129.1 (Ph, s); 129.3 (Ph, s); 138.1 (Ph, s); 148.2 (arom., s); 161.7 (arom., s). **ESI-HRMS:** 216.1022 [M+H]⁺ (theor. [C₁₃H₁₄N₁O₂]⁺ = 216.1019).

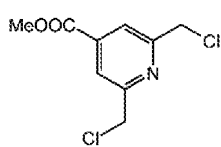


Synthesis of TD564: In a round-bottom glass flask (500 mL), **TD563** (2.25 g; 10.5 mmol; 1.0 equiv.) was dissolved (with gentle heating) in DCM (250 mL). Freshly prepared solution of **SOCl₂** (2.27 mL; 31.3 mmol; 3.0 equiv.) in DCM (10 mL) was then added, after which precipitate started to form. After 1 h of stirring at RT, all of the previously formed precipitate was dissolved again. Dil. aq. solution of NaHCO₃ (150 mL) was then added to the clear yellow solution and the resulting biphasic mixture was vigorously stirred for additional 1 h at RT, during which bubbles of gas slowly evolved. Mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3×100 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as faint yellow solid. **Yield:** 2.59 g (98%; 1 step; based on **TD563**). **NMR (DMSO-*d*₆):** ¹H δ_{H} 4.88 (CH₂, s, 4H); 7.47–7.58 (Ph, m, 3H); 7.77–7.85 (Ph, m, 2H); 7.86 (arom., s, 2H). ¹³C{¹H} δ_{C} 46.6 (CH₂, s); 120.3 (arom., s); 126.9 (Ph, s); 129.3 (Ph, s); 129.7 (Ph, s); 136.5 (Ph, s); 149.5 (arom., s); 157.1 (arom., s); 157.1 (arom., s). **ESI-HRMS:** 252.0342 [M+H]⁺ (theor. [C₁₃H₁₂N₁Cl₂]⁺ = 252.0341).

Synthesis of TD566: In a pear-shaped glass flask (100 mL), **TD564** (1.60 g; 6.35 mmol; 1.3 equiv.) was dissolved (with gentle heating) in MeCN (60 mL) followed by addition of solid NaN_3 (320 mg; 4.92 mmol; 1.0 equiv.) and dried K_2CO_3 (680 mg; 4.93 mmol; 1.0 equiv.) and the resulting suspension was stirred 24 h at 80 °C. The mixture was filtered on glass frit S3 and the solid residue was washed with MeCN. Filtrate was evaporated to dryness and the orange oily residue was purified by column chromatography (SiO_2 , 15% P.E. in DCM to 100% DCM). Fractions with product were combined and evaporated to dryness. The residue was further dried 1 h on high vacuum to give product in the form of free base as white solid. **Yield:** 691 mg (54%; 1 step; based on NaN_3). **Recovery:** 613 mg of **TD564** (38% of the initial amount used). **NMR (DMSO- d_6):** ^1H δ_{H} 4.59 (CH_2 , s, 2H); 4.84 (CH_2 , s, 2H); 7.46–7.60 (*Ph*, m, 3H); 7.74 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 7.77–7.85 (*Ph*, m, 2H); 7.86 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$). $^{13}\text{C}\{^1\text{H}\}$ δ_{C} 46.7 (CH_2 , s); 54.2 (CH_2 , s); 119.4 (*arom.*, s); 120.0 (*arom.*, s); 126.9 (*Ph*, s); 129.3 (*Ph*, s); 129.7 (*Ph*, s); 136.6 (*Ph*, s); 149.3 (*arom.*, s); 156.6 (*arom.*, s); 157.1 (*arom.*, s). **ESI-HRMS:** 259.0747 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{13}\text{H}_{12}\text{N}_4\text{Cl}]^+ = 259.0745$).



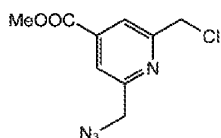
Synthesis of TD1083: In a round-bottom glass flask (100 mL), **methyl isonicotinate** (250 mg; 1.83 mmol; 1.0 equiv.) was dissolved in MeOH (10 mL) followed by addition of conc. H_2SO_4 (25 μL). The resulting mixture was stirred 30 min at 55 °C. After cooling down, solution of $(\text{NH}_4)_2\text{S}_2\text{O}_8$ (4.16 g; 18.2 mmol; 10 equiv.) in H_2O (10 mL) was added dropwise and the resulting mixture was then further stirred 16 h at 55 °C. Reaction was then carefully quenched by aq. NaHCO_3 . Mixture was then transferred to a separatory funnel and extracted with EtOAc (5×50 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. Residue was purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of free base as white fluffy solid. **Yield:** 99.5 mg (28%; 1 step; based on **methyl isonicotinate**). **NMR (DMSO- d_6):** ^1H δ_{H} 3.92 (CH_3 , s, 3H); 4.60 (CH_2 , d, 4H, $^3J_{\text{HH}} = 6$); 5.58 (*OH*, t, 2H, $^3J_{\text{HH}} = 6$); 7.80 (*arom.*, s, 2H). **ESI-HRMS:** 198.0759 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_9\text{H}_{12}\text{N}_1\text{O}_4]^+ = 198.0761$). **EA** ($\text{C}_9\text{H}_{11}\text{N}_1\text{O}_4$, $M_{\text{R}} = 197.2$): C 54.8 (54.8); H 5.6 (5.5); N 7.1 (7.0).



Synthesis of TD1087: In a round-bottom glass flask (50 mL), **TD1083** (96.2 mg; 488 μmol ; 1.0 equiv.) was dissolved in DCM (20 mL) followed by addition of SOCl_2 (106 μL ; 1.46 mmol; 3.0 equiv.). The mixture was stirred 1 h at RT. Mixture was then quenched by addition of dil. aq. solution of NaHCO_3 (20 mL). The resulting biphasic mixture was vigorously stirred for additional 1 h at RT, during which bubbles of gas slowly evolved. Mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3×30 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered

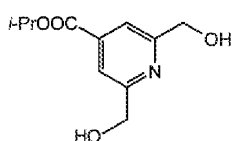
through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight (where product slowly crystallized) to give product in the form of free base as bright yellow solid. **Yield:** 112.8 mg (99%; 1 step; based on **TD1083**). **NMR (DMSO-*d*₆):** ¹H δ_H 3.92 (CH₃, s, 3H); 4.90 (CH₂, s, 4H); 7.98 (*arom.*, s, 2H). **ESI-HRMS:** 234.0085 [M+H]⁺ (theor. [C₉H₁₀N₁O₂Cl₂]⁺ = 234.0083).

5



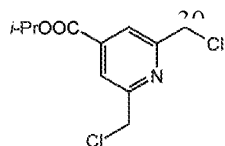
Synthesis of TD1089: In a glass vial (4 mL), **TD1087** (112.0 mg; 478 μmol; 1.2 equiv.) was dissolved in MeCN (2 mL) followed by addition of solid NaN₃ (26.0 mg; 400 μmol; 1.0 equiv.) and dried K₂CO₃ (55.0 mg; 400 μmol; 1.0 equiv.) and the resulting suspension was stirred 2 h at 70 °C. The mixture was filtered on through syringe

10 microfilter (PTFE) and the solid residue was washed with MeCN. Filtrate was evaporated to dryness and oily residue was purified by column chromatography (SiO₂, DCM to 10% EtOAc in DCM). Fractions with product were combined and evaporated to dryness. The residue was further dried on high vacuum overnight to give pre-purified product (containing ~20% of bis azide byproduct) in the form of free base as yellow oil. **Yield:** 49.6 mg. **ESI-HRMS:** 241.0490 [M+H]⁺ (theor. [C₉H₁₀N₄O₂Cl]⁺ = 241.0487). Part of **TD1089** obtained in
15 this way was directly used for **TD1092** without further purification or characterization.



Synthesis of TD1101: In a round-bottom glass flask (100 mL), **isopropyl isonicotinate** (600 mg; 3.63 mmol; 1.0 equiv.) was dissolved in MeOH (20 mL) followed by addition of conc. H₂SO₄ (50 μL). Then, solution of (NH₄)₂S₂O₈ (8.32 g; 36.5 mmol; 10 equiv.) in H₂O (20 mL) was added dropwise and the resulting mixture

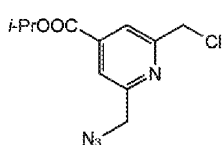
was further stirred 16 h at 70 °C. Reaction was then carefully neutralized by aq. NaHCO₃. Mixture was then transferred to a separatory funnel and extracted with EtOAc (5 × 50 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and
25 directly lyophilized to give product in the form of free base as white fluffy solid. **Yield:** 284 mg (35%; 1 step; based on **isopropyl isonicotinate**). **NMR (DMSO-*d*₆):** ¹H δ_H 1.34 (CH₃, d, 6H, ³J_{HH} = 6); 4.59 (CH₂, d, 4H, ³J_{HH} = 6); 5.19 (CH, hept, 1H, ³J_{HH} = 6); 5.57 (OH, t, 2H, ³J_{HH} = 6); 7.78 (*arom.*, s, 2H). **ESI-HRMS:** 226.1076 [M+H]⁺ (theor. [C₁₁H₁₆N₁O₄]⁺ = 226.1074).



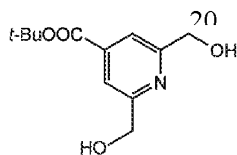
Synthesis of TD1109: In a round-bottom glass flask (100 mL), **TD1101** (283 mg; 1.26 mmol; 1.0 equiv.) was dissolved in DCM (40 mL) followed by addition of SOCl₂ (275 μL; 3.79 mmol; 3.0 equiv.). The mixture was stirred 1 h at RT. Mixture was then quenched by addition of dil. aq. solution of NaHCO₃ (40 mL). The resulting biphasic

mixture was vigorously stirred for additional 1 h at RT, during which bubbles of gas slowly evolved. Mixture
35 was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was

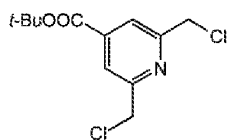
further extracted with DCM (3×50 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight (where product slowly crystallized) to give product in the form of free base as pale yellow solid. **Yield:** 322 mg (98%; 1 step; based on **TD1101**). **NMR (DMSO- d_6):** ^1H δ_{H} 1.35 (CH_3 , d, 6H, $^3J_{\text{HH}} = 6$); 4.90 (CH_2 , s, 4H); 5.20 (CH , hept, 1H, $^3J_{\text{HH}} = 6$); 7.96 (*arom.*, s, 2H). **ESI-HRMS:** 262.0399 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{11}\text{H}_{14}\text{N}_1\text{O}_2\text{Cl}_2]^+ = 262.0396$).



Synthesis of TD1112: In a glass vial (20 mL), **TD1109** (321 mg; 1.23 mmol; 1.2 equiv.) was dissolved in MeCN (6 mL) followed by addition of solid NaN_3 (66.5 mg; 1.02 mmol; 1.0 equiv.) and dried K_2CO_3 (141 mg; 1.02 mmol; 1.0 equiv.) and the resulting suspension was stirred 16 h at 70 °C. The mixture was filtered on through syringe microfilter (PTFE) and the solid residue was washed with MeCN. Filtrate was evaporated to dryness and oily residue was purified by column chromatography (SiO_2 , DCM to 3% EtOAc in DCM). Fractions with product were combined and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as colourless oil. **Yield:** 116 mg (42%; 1 step; based on NaN_3). **Recovery:** 124 mg of **TD1109** (39% of the initial amount used). **NMR (DMSO- d_6):** ^1H δ_{H} 1.35 (CH_3 , d, 6H, $^3J_{\text{HH}} = 6$); 4.67 (CH_2 , s, 2H); 4.89 (CH_2 , s, 4H); 5.19 (CH , hept, 1H, $^3J_{\text{HH}} = 6$); 7.83 (*arom.*, d, 1H, $^4J_{\text{HH}} = 1$); 7.95 (*arom.*, d, 1H, $^4J_{\text{HH}} = 1$). **ESI-HRMS:** 269.0800 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{11}\text{H}_{14}\text{N}_4\text{O}_2\text{Cl}_1]^+ = 269.0800$).

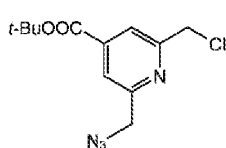


Synthesis of TD1119: In a glass vial (40 mL), *tert*-butyl isonicotinate (486 mg; 2.71 mmol; 1.0 equiv.) was dissolved in MeOH (15 mL) followed by addition of conc. H_2SO_4 (40 μL). Then, solution of $(\text{NH}_4)_2\text{S}_2\text{O}_8$ (6.20 g; 27.2 mmol; 10 equiv.) in H_2O (15 mL) was added dropwise and the resulting mixture was further stirred 30 min at 80 °C. Reaction was then carefully neutralized by aq. NaHCO_3 . Mixture was then transferred to a separatory funnel and extracted with EtOAc (5×30 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. Residue was purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of free base as white fluffy solid. **Yield:** 161 mg (25%; 1 step; based on *tert*-butyl isonicotinate). **NMR (DMSO- d_6):** ^1H δ_{H} 1.57 (CH_3 , s, 9H); 4.58 (CH_2 , d, 4H, $^3J_{\text{HH}} = 4$); 5.54 (*OH*, t, 2H, $^3J_{\text{HH}} = 4$); 7.73 (*arom.*, s, 2H). **ESI-HRMS:** 240.1230 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{12}\text{H}_{18}\text{N}_1\text{O}_4]^+ = 240.1230$).

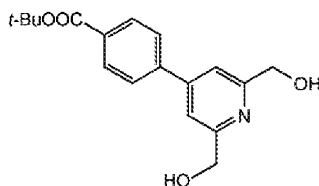


Synthesis of TD1129: In a round-bottom glass flask (100 mL), **TD1119** (159 mg; 664 μmol ; 1.0 equiv.) was dissolved in DCM (20 mL) followed by addition of SOCl_2 (145 μL ; 2.00 mmol; 3.0 equiv.). The mixture was stirred 1 h at RT. Mixture was then quenched by addition of dil. aq. solution of NaHCO_3 (20 mL). The resulting biphasic

mixture was vigorously stirred for additional 1 h at RT, during which bubbles of gas slowly evolved. Mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 × 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight (where product slowly crystallized) to give product in the form of free base as faint yellow solid. **Yield:** 175 mg (95%; 1 step; based on **TD1119**). **NMR (DMSO-d₆):** ¹H δ_H 1.57 (CH₃, s, 9H); 4.89 (CH₂, s, 4H); 7.91 (arom., s, 2H). **ESI-HRMS:** 276.0552 [M+H]⁺ (theor. [C₁₂H₁₆N₁O₂Cl₂]⁺ = 276.0553).

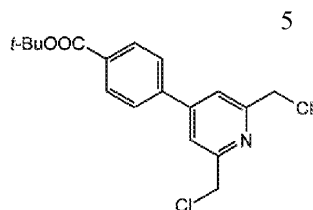


Synthesis of TD1130: In a glass vial (4 mL), **TD1129** (174.0 mg; 630 μmol; 1.2 equiv.) was dissolved in MeCN (3 mL) followed by addition of solid NaN₃ (34.0 mg; 523 μmol; 1.0 equiv.) and dried K₂CO₃ (72.0 mg; 522 μmol; 1.0 equiv.) and the resulting suspension was stirred 6 h at 70 °C. The mixture was filtered on through syringe microfilter (PTFE) and the solid residue was washed with MeCN. Filtrate was evaporated to dryness and oily residue was purified by column chromatography (SiO₂, DCM to 3% EtOAc in DCM). Fractions with product were combined and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as colourless oil. **Yield:** 58.2 mg (39%; 1 step; based on NaN₃). **Recovery:** 90.5 mg of **TD1129** (52% of the initial amount used). **NMR (DMSO-d₆):** ¹H δ_H 1.57 (CH₃, s, 9H); 4.66 (CH₂, s, 2H); 4.89 (CH₂, s, 2H); 7.79 (arom., d, 1H, ⁴J_{HH} = 2); 7.91 (arom., d, 1H, ⁴J_{HH} = 2). **ESI-HRMS:** 305.0772 [M+Na]⁺ (theor. [C₁₂H₁₅N₄O₂Cl₁Na₁]⁺ = 305.0776).



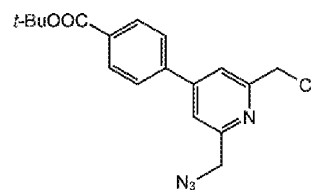
Synthesis of TD725: Glass vial (40 mL) was charged with **TD726** (628 mg; 3.62 mmol; 1.0 equiv.), **(4-(tert-Butoxycarbonyl)phenyl)boronic acid** (880 mg; 3.96 mmol; 1.1 equiv.) and **XPhos Pd G2** (57 mg; 7.2 μmol; 2.0 mol%) and was then three times secured with argon. Under constant flow of argon was then added dry 1,4-Dioxane (16 mL) through septum followed by addition of freshly prepared (and briefly washed with argon prior addition) solution of K₃PO₄·H₂O (920 mg; 4.00 mmol; 1.1 equiv.) in H₂O (8 mL). The mixture was then stirred 16 h under septum (but without external argon) at 80 °C. Resulting dark mixture was transferred to a separatory funnel and diluted with EtOAc (40 mL) and H₂O (50 mL). After shaking, upper layer was separated and aqueous layer was further extracted with EtOAc (5 × 30 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. Residue was purified on flash chromatography (120 g SiO₂, 100% EtOAc to 30% MeOH in EtOAc). Combined fractions with product were evaporated to dryness and further co-evaporated once with DCM. The residue was further dried on high vacuum overnight to give pre-purified product in the form of free base as off-white powder. **Yield:** 1.04 g. **NMR (DMSO-d₆):** ¹H δ_H 1.57 (CH₃, s, 9H); 4.61 (CH₂, d, 4H, ³J_{HH} = 6); 5.48 (OH, t, 2H, ³J_{HH} = 6); 7.64 (arom., s, 2H); 7.88 (arom., dm, 2H, ³J_{HH} = 9); 8.04 (arom., dm, 2H, ³J_{HH}

= 9). $^{13}\text{C}\{^1\text{H}\}$ δ_{C} 27.8 (CH_3 , s); 64.2 (CH_2 , s); 81.0 ($\text{C}-\text{CH}_3$, s); 115.8 (*arom.*, s); 127.0 (*arom.*, s); 129.9 (*arom.*, s); 131.6 (*arom.*, s); 142.2 (*arom.*, s); 147.0 (*arom.*, s); 162.0 (*arom.*, s); 164.6 (CO , s). **ESI-HRMS**: 316.1539 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{18}\text{H}_{22}\text{N}_4\text{O}_4]^+ = 316.1543$).



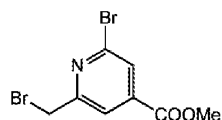
5 Synthesis of TD730: In a round-bottom glass flask (250 mL), pre-purified **TD725** (1.04 g; ≤ 3.30 mmol; 1.0 equiv.) was suspended in DCM (35 mL). Freshly prepared solution of SOCl_2 (721 μL ; 9.93 mmol; ≥ 3.0 equiv.) in DCM (5 mL) was then added. Clear solution was quickly formed and the open flask was stirred 1 h at RT. Mixture was then quenched by addition of dil. aq.

10 solution of NaHCO_3 (60 mL). The resulting biphasic mixture was vigorously stirred for additional 1 h at RT, during which bubbles of gas slowly evolved. Mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (5×30 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. The residue was purified on flash chromatography (120 g SiO_2 , 100% DCM to 20% EtOAc in DCM).
 15 Combined fractions with product were evaporated to dryness and further co-evaporated once with DCM. The residual faint yellow oil slowly crystallized. The solid was crushed and further dried on high vacuum overnight to give product in the form of free base as off-white powder. **Yield**: 839 mg (66%; 2 steps; based on **TD726**). **NMR (DMSO- d_6)**: ^1H δ_{H} 1.58 (CH_3 , s, 9H); 4.86 (CH_2 , s, 4H); 7.92 (*arom.*, s, 2H); 7.94 (*arom.*, dm, 2H, $^3J_{\text{HH}} = 9$); 8.05 (*arom.*, dm, 2H, $^3J_{\text{HH}} = 9$). $^{13}\text{C}\{^1\text{H}\}$ δ_{C} 27.8 (CH_3 , s); 46.5 (CH_2 , s); 81.2 ($\text{C}-\text{CH}_3$, s); 120.6 (*arom.*, s); 127.2 (*arom.*, s); 129.9 (*arom.*, s); 132.1 (*arom.*, s); 140.6 (*arom.*, s); 148.3 (*arom.*, s); 157.3 (*arom.*, s);
 20 164.5 (CO , s). **ESI-HRMS**: 352.0866 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{18}\text{H}_{20}\text{N}_4\text{O}_2\text{Cl}_2]^+ = 352.0866$).

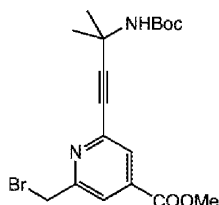


Synthesis of TD733: In a glass vial (20 mL), **TD730** (837 mg; 2.38 mmol; 1.3 equiv.) was dissolved in MeCN (18 mL) followed by addition of solid NaN_3 (119 mg; 1.83 mmol; 1.0 equiv.) and dried K_2CO_3 (253 mg; 1.83 mmol; 1.0 equiv.) and the resulting suspension was stirred 24 h at 80 $^\circ\text{C}$. The mixture was filtered on through syringe microfilter (PTFE) and the solid residue was washed with MeCN. Filtrate was evaporated to dryness and oily residue was purified by column chromatography (SiO_2 , 20% Pentane in DCM to 1% EtOAc in DCM). Fractions with product were combined and evaporated to dryness. The resulting nearly colorless oil was further dried on high vacuum overnight (where product slowly crystallized) to give product in the form of free base as colorless solid. **Yield**: 230 mg (35%; 1 step; based on NaN_3). **Recovery**: 442 mg of **TD730** (53% of the initial amount used). **NMR (DMSO- d_6)**: ^1H δ_{H} 1.57 (CH_3 , s, 9H); 4.62 (CH_2 , s, 2H); 4.86 (CH_2 , s, 2H); 7.80 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 7.91 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 7.94 (*arom.*, dm, 2H, $^3J_{\text{HH}} = 9$); 8.05 (*arom.*, dm, 2H, $^3J_{\text{HH}} = 9$). $^{13}\text{C}\{^1\text{H}\}$ δ_{C} 27.8 (CH_3 , s); 46.6 (CH_2 , s); 54.2 (CH_2 , s); 81.2 ($\text{C}-\text{CH}_3$, s); 119.6 (*arom.*, s); 120.3 (*arom.*, s); 127.2 (*arom.*, s); 129.9 (*arom.*, s); 132.1
 30
 35

(*arom.*, s); 140.7 (*arom.*, s); 148.1 (*arom.*, s); 156.8 (*arom.*, s); 157.3 (*arom.*, s); 164.5 (CO, s). **ESI-HRMS:** 359.1270 [M+H]⁺ (theor. [C₁₈H₂₀N₄O₂Cl]⁺ = 359.1269).

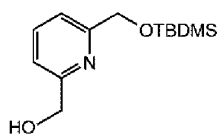


Synthesis of TD1425: Glass vial (40 mL) was charged **methyl 2-bromo-6-methylisonicotinate** (1.00 g; 4.35 mmol; 1.0 equiv.), **NBS** (recrystallized from boiling H₂O; 770 mg; 4.33 mmol; 1.0 equiv.), **(BnO)₂** (53 mg; 219 μmol; 5 mol%) and magnetic stirrer and three times secured with argon. Under constant flow of argon was then added CCl₄ (21 mL) through septum. The vial was left stirring under septum (but without external argon) for 24 h at 75 °C. After cooling down, the mixture was filtered through syringe microfilter (PTFE) and the solids were washed with DCM. Filtrate was evaporated to dryness, re-suspended in *c*-Hex (25 ml) and filtered through syringe microfilter (PTFE). Filtrate was evaporated to dryness and the residue was purified by flash chromatography (120 g SiO₂, 100% *c*-Hex to 80% DCM in *c*-Hex). Combined fractions with product were evaporated to dryness. The residual oil was further dried on high vacuum overnight (where the product crystallized) to give product in the form of free base as faint yellow solid. **Yield:** 571 mg (43%; 1 step; based on **methyl 2-bromo-6-methylisonicotinate**). **Recovery:** 314 mg of **methyl 2-bromo-6-methylisonicotinate** (31% of the initial amount used). **NMR (DMSO-*d*₆):** ¹H δ_H 3.91 (CH₃, s, 3H); 4.79 (CH₂, s, 2H); 7.94 (*arom.*, d, 1H, ⁴J_{HH} = 1); 8.05 (*arom.*, d, 1H, ⁴J_{HH} = 1). **ESI-HRMS:** 307.8916 [M+H]⁺ (theor. [C₈H₈N₁O₂Br₂]⁺ = 307.8916).

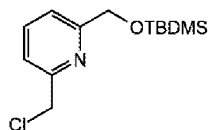


Synthesis of TD1428: Pear-shaped glass flask (25 mL) was charged with **TD1425** (523 mg; 1.69 mmol; 1.0 equiv.), ***N*-Boc-1,1-dimethylpropargylamine** (310 mg; 1.69 mmol; 1.0 equiv.) and magnetic stirrer and three times secured with argon. Solid **CuI** (13.0 mg; 68 μmol; 4.0 mol%) and **[Pd(PPh₃)₂Cl₂]** (24 mg; 34 μmol; 2.0 mol%) were subsequently added followed by another securing with argon (three times). Under constant flow of argon was then added dry THF (7.5 mL) through septum followed by addition of **DIPEA** (885 μL; 5.08 mmol; 3.0 equiv.). The flask was left stirring under septum (but without external argon) for 2 d at RT. The mixture was evaporated to dryness, re-suspended in DCM (15 mL) and filtered through syringe microfilter (PTFE) and the solid phase was further washed with DCM. Filtrate was purified on flash chromatography (120 g SiO₂, 10% EtOAc in *c*-Hex to 50% EtOAc in *c*-Hex). Combined fractions with product were evaporated to dryness and once re-purified on flash chromatography (80 g SiO₂, 100% DCM to 5% EtOAc in DCM). Combined fractions with product were evaporated to dryness and further co-evaporated once with DCM. The residue was further dried on high vacuum overnight to give product in the form of free base as faint yellow solidified oil. **Yield:** 175 mg (25%; 1 step; based on **TD1425**). **NMR (DMSO-*d*₆):** ¹H δ_H 1.42 (CH₃-C-O, s, 9H); 1.56 (CH₃-C-N, s, 6H); 3.91 (CH₃-O, s, 3H); 4.79 (CH₂, s, 2H); 7.23 (NH,

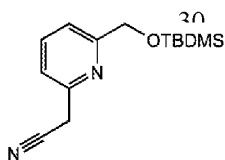
bs, 1H); 7.71 (*arom.*, d, 1H, $^4J_{\text{HH}} = 1$); 7.97 (*arom.*, d, 1H, $^4J_{\text{HH}} = 1$). **ESI-HRMS**: 411.0913 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{18}\text{H}_{24}\text{N}_2\text{O}_4\text{Br}_1]^+ = 411.0914$).



Synthesis of TD1386: In a round-bottom glass flask (100 mL), **pyridine-2,6-diyl dimethanol** (2.00 g; 14.4 mmol; 1.0 equiv.) and **Imidazole** (0.98 g; 14.4 mmol; 1.0 equiv.) were dissolved in DMF (20 mL) followed by addition of **TBDMSCl** (2.17 g; 14.4 mmol; 1.0 equiv.). The resulting solution was stirred 2 h at RT. Mixture was evaporated to dryness and the residue dissolved in a mixture of DCM (50 mL) and dil. aq. NaHCO_3 (50 mL) and transferred to a separatory funnel. After brief shaking, the bottom phase was separated and the aq. layer was further extracted with DCM (5×30 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. Residue was purified by column chromatography (100 g SiO_2 , DCM to 50% EtOAc in DCM). Fractions with product were combined and evaporated to dryness and once co-evaporated with DCM. The residue was further dried on high vacuum overnight to give product in the form of free base as nearly colorless oil. **Yield**: 1.54 g (42%; 1 step; based on **pyridine-2,6-diyl dimethanol**). **NMR (DMSO- d_6)**: ^1H δ_{H} 0.09 (CH_3 , s, 6H); 0.92 (CH_3 , s, 9H); 4.51 (CH_2 , d, 2H, $^3J_{\text{HH}} = 5$); 4.71 (CH_2 , s, 2H); 5.37 (OH, t, 1H, $^3J_{\text{HH}} = 5$); 7.28 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$; $^4J_{\text{HH}} = 1$); 7.34 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$; $^4J_{\text{HH}} = 1$); 7.81 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$). **ESI-HRMS**: 254.1569 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{13}\text{H}_{24}\text{N}_1\text{O}_2\text{Si}_1]^+ = 254.1571$).

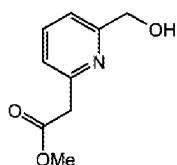


Synthesis of TD1389: In a round-bottom glass flask (250 mL), **TD1386** (1.54 g; 6.08 mmol; 1.0 equiv.) was dissolved in DCM (60 mL) followed by addition of SOCl_2 (885 μL ; 12.0 mmol; 2.0 equiv.). The mixture was stirred 1 h at RT. Mixture was then quenched by addition of dil. aq. solution of NaHCO_3 (60 mL). The resulting biphasic mixture was vigorously stirred for additional 30 min at RT, during which bubbles of gas slowly evolved. Mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3×50 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as nearly colorless oil. **Yield**: 1.63 g (99%; 1 step; based on **TD1386**). **NMR (DMSO- d_6)**: ^1H δ_{H} 0.10 (CH_3 , s, 6H); 0.92 (CH_3 , s, 9H); 4.74 (CH_2 , s, 2H); 4.75 (CH_2 , s, 2H); 7.40 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$; $^4J_{\text{HH}} = 1$); 7.42 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$; $^4J_{\text{HH}} = 1$); 7.87 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$). **ESI-HRMS**: 272.1231 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{13}\text{H}_{23}\text{N}_1\text{O}_1\text{Cl}_1\text{Si}_1]^+ = 272.1232$).

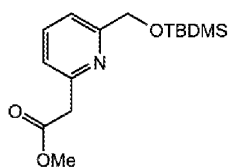


Synthesis of TD1390: In a glass flask (20 mL), **KCN** (390 mg; 5.99 mmol; 1.05 equiv.) was dissolved in H_2O (2 mL) followed by addition of **TD1389** (1.55 g; 5.70 mmol; 1.0 equiv.) in DMF (6 mL). The resulting mixture was vigorously stirred for 70 min at 100 $^\circ\text{C}$. After cooling to RT, mixture was diluted with H_2O (4 mL) and MeCN (8 mL) and

filtered through syringe microfilter (PTFE). Filtrate was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (100 mL) and H₂O (100 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (4 × 50 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residual pale yellow oil was left in a freezer to solidify. Resulting solid was crushed and further dried on high vacuum overnight to give product in the form of free base as faint yellow powder. **Yield:** 1.23 g (82%; 1 step; based on **TD1389**). **NMR (DMSO-*d*₆):** ¹H δ_H 0.10 (CH₃, s, 6H); 0.92 (CH₃, s, 9H); 4.17 (CH₂, s, 2H); 4.75 (CH₂, s, 2H); 7.30 (*arom.*, dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.40 (*arom.*, dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.86 (*arom.*, t, 1H, ³J_{HH} = 8). **ESI-HRMS:** 263.1573 [M+H]⁺ (theor. [C₁₄H₂₃N₂O₁Si]⁺ = 263.1574).

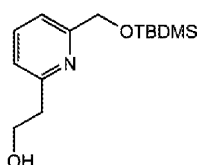


Synthesis of TD1393: In a pear-shaped glass flask (100 mL), **TD1390** (700 mg; 2.67 mmol; 1.00 equiv.) was dissolved in MeOH (2.70 mL; 66.7 mmol; 25 equiv.) followed by addition of **TMSCI** (2.37 mL; 18.7 mmol; 7.0 equiv.). The resulting mixture was vigorously stirred for 2 h at 60 °C. After cooling to RT, mixture was quenched with H₂O (4 mL) and partly neutralized (to pH 3–4) by slow addition of sat. aq. NaHCO₃ (5 mL). Resulting biphasic mixture was carefully evaporated to dryness followed by addition of H₂O (10 mL) to the residue. Mixture was then filtered through syringe microfilter (RC) and the filtrate was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (100 mL) and H₂O (100 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (4 × 50 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further briefly dried on high vacuum to give product in the form of free base as faint yellow oil. **Yield:** 404 mg (84%; 1 step; based on **TD1390**). **NMR (DMSO-*d*₆):** ¹H δ_H 3.61 (CH₃, s, 3H); 3.81 (CH₂, s, 2H); 4.51 (CH₂, d, 2H, ³J_{HH} = 5); 5.39 (OH, t, 1H, ³J_{HH} = 5); 7.20 (*arom.*, dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.36 (*arom.*, dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.76 (*arom.*, t, 1H, ³J_{HH} = 8). **ESI-HRMS:** 182.0811 [M+H]⁺ (theor. [C₉H₁₂N₁O₃]⁺ = 182.0812).

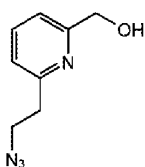


Synthesis of TD1395: In a round-bottom glass flask (50 mL), **TD1393** (340 mg; 1.88 mmol; 1.0 equiv.) and **Imidazole** (190 mg; 2.79 mmol; 1.5 equiv.) were dissolved in DMF (6 mL) followed by addition of **TBDMSCl** (425 mg; 2.82 mmol; 1.5 equiv.). The resulting solution was stirred 16 h at RT. Mixture was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (50 mL) and H₂O (50 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase

was further extracted with DCM (4 × 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as faint yellow oil. **Yield:** 537 mg (97%; 1 step; based on **TD1393**). **NMR (DMSO-d₆):** ¹H δ_H 0.09 (CH₃, s, 6H); 0.91 (CH₃, s, 9H); 3.61 (CH₃, s, 3H); 3.81 (CH₂, s, 2H); 4.70 (CH₂, s, 2H); 7.23 (arom., dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.32 (arom., dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.79 (arom., t, 1H, ³J_{HH} = 8). **ESI-HRMS:** 296.1676 [M+H]⁺ (theor. [C₁₅H₂₆N₁O₃Si₁]⁺ = 296.1677).

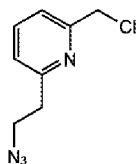


Synthesis of TD1396: In a round-bottom glass flask (100 mL), **TD1395** (537 mg; 1.82 mmol; 1.0 equiv.) was dissolved in a mixture of THF (9 mL) and MeOH (9 mL). To the resulting colourless solution was added portion wise (over course of 10 min, with no stopper on the flask) solid NaBH₄ (2.06 g; 54.5 mmol; 30 equiv.) during which hydrogen gas evolved intensively. Mixture was further stirred 1 h at RT, during which was twice diluted with MeOH (9 mL each) to ensure stirring. Mixture was then diluted with DCM (100 mL) and H₂O (100 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (4 × 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as nearly colourless oil. **Yield:** 483 mg (99%; 1 step; based on **TD1395**). **NMR (DMSO-d₆):** ¹H δ_H 0.09 (CH₃, s, 6H); 0.92 (CH₃, s, 9H); 2.84 (CH₂, t, 2H, ³J_{HH} = 7); 3.71 (CH₂, td, 2H, ³J_{HH} = 7, ³J_{HH} = 5); 4.62 (OH, t, 1H, ³J_{HH} = 5); 4.71 (CH₂, s, 2H); 7.14 (arom., dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.24 (arom., dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.70 (arom., t, 1H, ³J_{HH} = 8). **ESI-HRMS:** 268.1726 [M+H]⁺ (theor. [C₁₄H₂₆N₁O₂Si₁]⁺ = 268.1727).

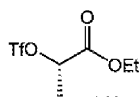


Synthesis of TD1401: In a round-bottom glass flask (50 mL), **TD1396** (94 mg; 352 μmol; 1.0 equiv.) was dissolved in DCM (3.5 mL) followed by addition of SOCl₂ (51 μL; 702 μmol; 2.0 equiv.). The mixture was stirred 3 h at RT. Mixture was then diluted with DCM (20 mL) and quenched by addition of dil. aq. solution of NaHCO₃ (10 mL). The resulting biphasic mixture was vigorously stirred for additional 30 min at RT, during which bubbles of gas slowly evolved. Mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 × 15 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was dissolved in DMF (1.7 mL) followed by addition of solid NaN₃ (46 mg; 708 μmol; 2.0 equiv.). The resulting mixture was stirred at 80 °C overnight. After cooling, the mixture was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (25 mL) and H₂O (25 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (4 × 10 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass

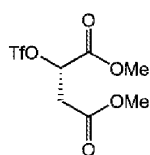
frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as colorless oil. **Yield:** 17.6 mg (28%; 2 steps; based on **TD1396**). **NMR (DMSO- d_6):** ^1H δ_{H} 2.98 (CH_2 , t, 2H, $^3J_{\text{HH}} = 7$); 3.69 (CH_2 , td, 2H, $^3J_{\text{HH}} = 7$, $^3J_{\text{HH}} = 5$); 4.53 (CH_2 , d, 2H, $^3J_{\text{HH}} = 6$); 5.37 (OH, t, 1H, $^3J_{\text{HH}} = 6$); 7.18 (arom., dd, 1H, $^3J_{\text{HH}} = 8$; $^4J_{\text{HH}} = 1$); 7.32 (arom., dd, 1H, $^3J_{\text{HH}} = 8$; $^4J_{\text{HH}} = 1$); 7.73 (arom., t, 1H, $^3J_{\text{HH}} = 8$). **ESI-HRMS:** 201.0746 $[\text{M}+\text{Na}]^+$ (theor. $[\text{C}_8\text{H}_{10}\text{N}_4\text{O}_1\text{Na}_1]^+ = 201.0747$).



Synthesis of TD1406: In a round-bottom glass flask (25 mL), **TD1401** (16.5 mg; 92.6 μmol ; 1.0 equiv.) was dissolved in DCM (1.85 mL) followed by addition of **SOCl_2** (13.5 μL ; 186 μmol ; 2.0 equiv.). The mixture was stirred 1 h at RT. Mixture was then diluted with DCM (10 mL) and quenched by addition of dil. aq. solution of NaHCO_3 (10 mL). The resulting biphasic mixture was vigorously stirred for additional 30 min at RT, during which bubbles of gas slowly evolved. Mixture was then transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3×10 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as colourless solid. **Yield:** 17.7 mg (97%; 1 step; based on **TD1401**). **NMR (DMSO- d_6):** ^1H δ_{H} 3.02 (CH_2 , t, 2H, $^3J_{\text{HH}} = 7$); 3.71 (CH_2 , td, 2H, $^3J_{\text{HH}} = 7$, $^3J_{\text{HH}} = 5$); 4.75 (CH_2 , s, 2H); 7.32 (arom., dd, 1H, $^3J_{\text{HH}} = 8$; $^4J_{\text{HH}} = 1$); 7.41 (arom., dd, 1H, $^3J_{\text{HH}} = 8$; $^4J_{\text{HH}} = 1$); 7.79 (arom., t, 1H, $^3J_{\text{HH}} = 8$). **ESI-HRMS:** 197.0589 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_8\text{H}_{10}\text{N}_4\text{Cl}_1]^+ = 197.0589$).



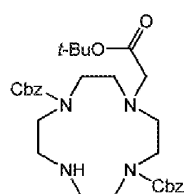
Synthesis of TD1339: Pear-shaped glass flask (250 mL) was charged with **ethyl 1-lactate** (3.54 g; 30.0 mmol; 1.00 equiv.) and magnetic stirrer and three times briefly secured with argon. Under constant flow of argon was then added dry DCM (100 mL) through septum and the mixture was cooled with an ice bath (5°C) followed by dropwise addition of **triflic anhydride** (5.3 mL; 31.5 mmol; 1.05 equiv.) and immediately followed by dropwise addition of dry **pyridine** (2.54 mL; 31.5 mmol; 1.05 equiv.). The resulting mixture was stirred at 5°C for 30 min. Resulting suspension was then directly purified by column chromatography (140 g SiO_2 , DCM). Combined fractions with product were evaporated to dryness and briefly dried on high vacuum to give product as faintly pinkish oil. **Yield:** 5.90 g (79%; 1 step; based on **ethyl 1-lactate**). **NMR (DMSO- d_6):** ^1H δ_{H} 1.26 ($\text{CH}_3\text{-CH}_2$, t, 3H, $^3J_{\text{HH}} = 7$); 1.51 ($\text{CH}_3\text{-CH}$, d, 3H, $^3J_{\text{HH}} = 7$); 4.19–4.31 (CH_2 , m, 2H); 5.27 (CH, q, 1H, $^3J_{\text{HH}} = 7$). $^{19}\text{F}\{^1\text{H}\}$ δ_{F} -77.7 (s). **CI-HRMS:** 251.0193 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_6\text{H}_{10}\text{O}_5\text{Si}_1\text{F}_3]^+ = 251.0196$).



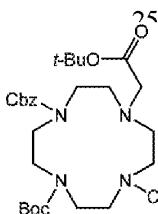
Synthesis of TD1488: Pear-shaped glass flask (25 mL) was charged with **dimethyl L-malate** (200 mg; 1.23 mmol; 1.00 equiv.) and magnetic stirrer and three times briefly secured with argon. Under constant flow of argon was then added dry DCM (4 mL) through septum and the mixture was cooled with an ice bath (5°C) followed by dropwise addition of **triflic anhydride** (220 μL ; 1.31 mmol; 1.06 equiv.) and immediately followed

by dropwise addition of dry **pyridine** (105 μ L; 1.30 mmol; 1.06 equiv.). The resulting mixture was stirred at RT for 30 min. Resulting suspension was then directly purified by column chromatography (30 g SiO₂, DCM). Combined fractions with product were evaporated to dryness and briefly dried on high vacuum to give product as colourless oil. **Yield:** 258 mg (77%; 1 step; based on **dimethyl L-malate**). **NMR (DMSO-d₆):** ¹H δ _H 3.08 (CH₂, dd, 1H, ²J_{HH} = 17, ³J_{HH} = 5); 3.13 (CH₂, dd, 1H, ²J_{HH} = 17, ³J_{HH} = 6); 4.65 (CH₃, s, 3H); 3.78 (CH₃, s, 3H); 5.48 (CH, dd, 1H, ³J_{HH} = 6, ³J_{HH} = 5). ¹⁹F{¹H} δ _F -77.7 (s). **ESI-HRMS:** 316.9910 [M+Na]⁺ (theor. [C₇H₉O₇F₃SiNa]⁺ = 316.9913).

Example 3: Synthesis of protected macrocyclic intermediates

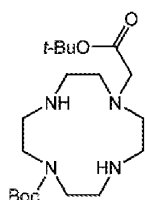


Synthesis of TD680: In a pear-shape glass flask (250 mL), **Cbz2cyclen** (free base; 1.54 g; 3.50 mmol; 1.6 equiv.) was dissolved in MeCN (100 mL) followed by addition of dried **K₂CO₃** (300 mg; 2.17 mmol; 1.0 equiv.). To the vigorously stirred reaction mixture, solution of **tert-Butyl bromoacetate** (427 mg; 2.19 mmol; 1.0 equiv.) in dry MeCN (25 mL) was added dropwise (over the course of 2 h). Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (150 mL) and H₂O (150 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 × 75 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as nearly colorless oil. **Yield:** 624 mg (51%; 1 step; based on **tert-Butyl bromoacetate**). **NMR (CD₃CN):** ¹H δ _H 1.32–1.49 (CH₃, m, 9H); 2.57–2.76 (mc, m, 4H); 2.81–2.98 (mc, m, 4H); 3.23–3.64 (mc, CH₂–CO, m, 8+2H); 5.00–5.15 (CH₂–Ph, m, 4H); 7.19–7.48 (Ph, m, 10H). **ESI-HRMS:** 555.3171 [M+H]⁺ (theor. [C₃₀H₄₃N₄O₆]⁺ = 555.3177).

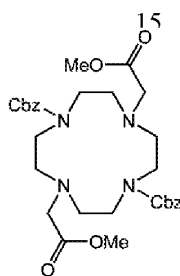


Synthesis of TD686: In a glass vial (4 mL), **TD680** (150 mg; 270 μ mol; 1.0 equiv.) was dissolved in MeCN (3 mL) followed by addition of **Boc₂O** (2.0 M solution in THF; 200 μ L; 400 μ mol; 1.5 equiv.). Resulting mixture was stirred 16 h at RT. Reaction mixture was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, immediately neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (50 mL) and H₂O (50 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 × 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as colorless oil. **Yield:** 170 mg (96%; 1 step; based on **TD680**). **NMR (CD₃CN):** ¹H δ _H 1.17–1.56

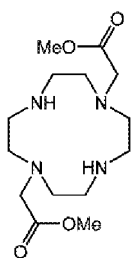
(CH₃, m, 18H); 2.62–2.99 (*mc*, m, 4H); 3.10–3.53 (*mc*, CH₂–CO, m, 12+2H); 4.96–5.16 (CH₂–Ph, m, 4H); 7.20–7.47 (*Ph*, m, 10H). **ESI-HRMS**: 655.3691 [M+H]⁺ (theor. [C₃₅H₅₁N₄O₈]⁺ = 655.3701).



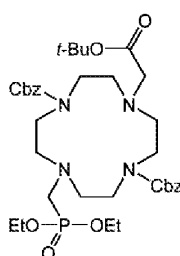
Synthesis of TD691: Pear-shape glass flask (25 mL) was charged with **TD686** (170 mg; 260 μmol) and magnetic stirrer and three times secured with argon. Solid **Pd@C** (17 mg) was then added followed by another securing with argon (three times). Under constant flow of argon was then added MeOH (10 mL) through septum. Argon input was then removed and **H₂** gas (from balloon) was allowed to bubble through the mixture for 30 min at RT. Catalyst was then filtered off using syringe microfilter (PTFE; filter was further washed with MeOH). Filtrate was evaporated to dryness and once co-evaporated with DCM. The residue was further dried on high vacuum overnight to give product in the form of free base as colorless oil. **Yield:** 100 mg (≥99%; 1 step; based on **TD686**). **ESI-MS** (LC-MS): 387.3 [M+H]⁺ (theor. [C₁₉H₃₉N₄O₄]⁺ = 387.3). All of **TD691** was directly used for **TD692** without further characterization.



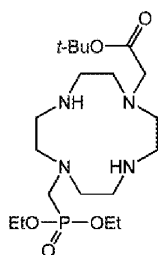
Synthesis of TD1106: In a pear-shape glass flask (50 mL), **Cbz2cyclen** (free base; 500 mg; 1.14 mmol; 1.0 equiv.) was dissolved in MeCN (10 mL) followed by addition of dried **K₂CO₃** (870 mg; 5.69 mmol; 5.0 equiv.) and **Methyl bromoacetate** (235 μL; 2.51 mmol; 2.2 equiv.) in MeCN (5 mL). The resulting suspension was stirred 2 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (50 mL) and H₂O (50 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 × 50 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as colorless oil. **Yield:** 422 mg (64%; 1 step; based on **Cbz2cyclen**). **NMR (CD₃CN):** ¹H δ_H 2.63–2.88 (*mc*, m, 8H); 3.19–3.49 (*mc*, CH₂–CO, m, 8+4H); 3.59 (CH₃, s, 6H); 5.07 (CH₂–Ph, s, 4H); 7.21–7.41 (*Ph*, m, 10H). **ESI-LCMS:** 585.3 [M+H]⁺ (theor. [C₃₀H₄₁N₄O₈]⁺ = 585.3).



Synthesis of TD1116: Pear-shape glass flask (50 mL) was charged with **Pd@C** (42 mg) and magnetic stirrer and three times secured with argon. Under constant flow of argon was then added solution of **TD1106** (418 mg; 715 μmol) in MeOH (25 mL) through septum. Argon input was then removed and **H₂ gas** (from balloon) was allowed to bubble through the mixture for 1 h at RT. Catalyst was then filtered off using syringe microfilter (PTFE; filter was further washed with MeOH). Filtrate was evaporated to dryness and once co-evaporated with DCM. The residue was further dried on high vacuum overnight to give product in the form of free base as colorless oil that crystallized on standing. **Yield:** 223 mg (99%; 1 step; based on **TD1106**). **NMR (CD₃CN):** ¹H δ_{H} 2.48–2.57 (*mc*, m, 8H); 2.63–2.74 (*mc*, m, 8H); 3.36 (*CH₂-CO*, s, 4H); 3.64 (*CH₃*, s, 6H). **ESI-MS (LC-MS):** 317.2 [M+H]⁺ (theor. [C₁₄H₂₉N₄O₄]⁺ = 317.2).

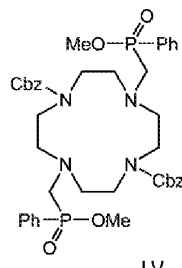


Synthesis of TD687: In a glass vial (2 mL), **TD680** (150 mg; 270 μmol ; 1.0 equiv.) and **P(OEt)₃** (232 μL ; 1.35 mmol; 5.0 equiv.) were mixed together followed by addition of solid **(CH₂O)_n** (12 mg; 400 μmol ; 1.5 equiv.). The resulting suspension was stirred 3 d at RT. Reaction mixture was then diluted with MeCN (700 μL) and filtered through syringe microfilter (PTFE). Filter was further washed with MeCN. Filtrate was then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, immediately neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (25 mL) and H₂O (25 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 $\times$ 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as slightly yellow oil. **Yield:** 167 mg (87%; 1 step; based on **TD680**). **ESI-MS (LC-MS):** 705.4 [M+H]⁺ (theor. [C₃₅H₅₄N₄O₉P]⁺ = 705.4). All of **TD687** was directly used for **TD695** without further characterization.

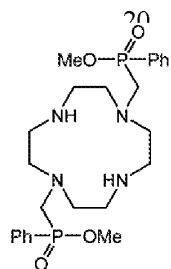


Synthesis of TD695: Pear-shape glass flask (25 mL) was charged with **TD687** (167 mg; 237 μmol) and magnetic stirrer and three times secured with argon. Solid **Pd@C** (17 mg) was then added followed by another securing with argon (three times). Under constant flow of argon was then added MeOH (10 mL) through septum. Argon input was then removed and **H₂ gas** (from balloon) was allowed to bubble through the mixture for 30 min at RT. The reaction mixture was further stirred under hydrogen atmosphere (from balloon) 16 h at RT. Catalyst was then filtered off using syringe microfilter (PTFE; filter was further washed with MeOH). Filtrate was evaporated to dryness and once co-evaporated with DCM. The residue was further dried on high vacuum overnight to give product in the form of free base as slightly yellow oil. **Yield:** 100 mg (97%; 1 step;

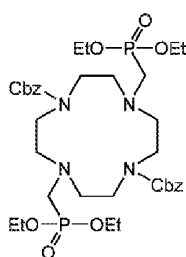
based on **TD686**). **ESI-MS** (LC-MS): 437.2 $[M+H]^+$ (theor. $[C_{19}H_{41}N_4O_5P_1]^+ = 437.3$). All of **TD695** was directly used for **TD700** without further characterization.



- Synthesis of TD913:** In a glass vial (20 mL), **Cbz2cyclen** (free base; 242 mg; 549 μ mol; 1.0 equiv.) was dissolved in MeCN (12 mL) followed by addition of solid $(CH_2O)_n$ (50 mg; 1.67 mmol; 3.0 equiv.) and **PhP(OMe)₂** (350 μ L; 2.2 mmol; 4.0 equiv.). The resulting suspension was stirred 24 h at 80°C. Reaction mixture was then filtered through syringe microfilter (PTFE) and the filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (75 mL) and H₂O (75 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 $\times$ 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as nearly colourless oil. **Yield:** 259 mg (61%; 1 step; based on **Cbz2cyclen**). **NMR (CD₃CN):** ¹H δ_H 2.30–3.38 (*mc*, CH₂–P, m, 16+4H); 3.47 (CH₃, d, 6H, ³J_{HP} = 11); 5.00–5.12 (CH₂–Ph, m, 4H); 7.27–7.40 (*Ph*, m, 10H); 7.40–7.51 (*Ph*, m, 4H); 7.51–7.60 (*Ph*, m, 2H); 7.60–7.82 (*Ph*, m, 4H). ³¹P δ_P 42.8 (m). **ESI-HRMS:** 777.3178 $[M+H]^+$ (theor. $[C_{40}H_{51}N_4O_8P_2]^+ = 777.3177$).

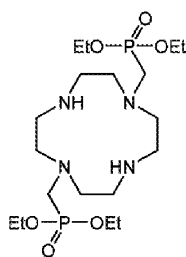


- Synthesis of TD910:** Pear-shape glass flask (50 mL) was charged with **TD913** (250 mg; 322 μ mol) and magnetic stirrer and three times secured with argon. Solid **Pd@C** (50 mg) was then added followed by another securing with argon (three times). Under constant flow of argon was then added MeOH (25 mL) through septum. Argon input was then removed and **H₂ gas** (from balloon) was allowed to bubble through the mixture for 2 h at RT. Catalyst was then filtered off using syringe microfilter (PTFE; filter was further washed with MeOH). Filtrate was evaporated to dryness and once co-evaporated with DCM. The residue was further dried on high vacuum overnight to give product in the form of free base as nearly colourless oil. **Yield:** 161 mg (98%; 1 step; based on **TD913**). **NMR (CD₃CN):** ¹H δ_H 2.28–3.14 (*mc*, CH₂–P, m, 16+4H); 3.53 (CH₃, d, 3H, ³J_{HP} = 11); 3.54 (CH₃, d, 3H, ³J_{HP} = 11); 7.46–7.63 (*Ph*, m, 6H); 7.71–7.83 (*Ph*, m, 4H). ³¹P δ_P 43.3 (m). **ESI-HRMS:** 509.2438 $[M+H]^+$ (theor. $[C_{24}H_{39}N_4O_4P_2]^+ = 509.2441$).



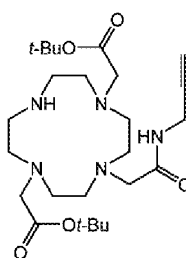
Synthesis of TD571: In a glass vial (4 mL), **Cbz2cyclen** (free base; 1.00 g; 2.27 mmol; 1.0 equiv.) and **P(OEt)₃** (2.00 mL; 11.7 mmol; 5.1 equiv.) were mixed together followed by addition of solid **(CH₂O)_n** (164 mg; 5.47 mmol; 2.4 equiv.). The resulting suspension was stirred 24 h at RT. Reaction mixture was then evaporated to dryness and the residue was purified by column chromatography (SiO₂; 80 g; DCM–MeOH–aq. NH₃ 150:10:1). Fractions with product were combined and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as slightly yellow oil. **Yield:** 1.29 g (77%; 1 step; based on **Cbz2cyclen**). **ESI-MS** (LC-MS): 741.3 [M+H]⁺ (theor. [C₃₄H₅₅N₄O₁₀P₂]⁺ = 741.3). All of **TD571** was directly used for **TD573** without further characterization.

10



Synthesis of TD573: Pear-shape glass flask (100 mL) was charged with **TD571** (1.29 g; 1.74 mmol) and magnetic stirrer and three times secured with argon. Solid **Pd@C** (129 mg) was then added followed by another securing with argon (three times). Under constant flow of argon was then added EtOH (96%, 70 mL) through septum. Argon input was then removed and **H₂ gas** (from balloon) was allowed to bubble through the mixture for 30 min at RT. The reaction mixture was further stirred under hydrogen atmosphere (from balloon) 16 h at RT. Catalyst was then filtered off using syringe microfilter (PTFE; filter was further washed with EtOH). Filtrate was evaporated to dryness and once co-evaporated with DCM. The residue was further dried on high vacuum overnight to give product in the form of free base as slightly yellow oil. **Yield:** 776 mg (95%; 1 step; based on **TD571**). **ESI-MS** (LC-MS): 473.2 [M+H]⁺ (theor. [C₁₈H₄₃N₄O₄P₂]⁺ = 473.3). Major portion of **TD573** was directly used for **TD579** without further characterization.

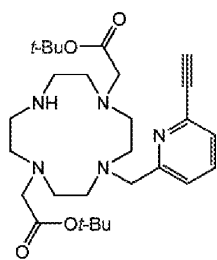
20



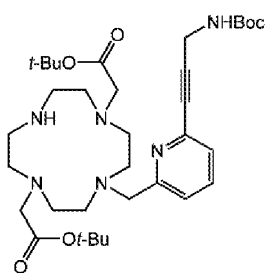
Synthesis of TD423: In a pear-shape glass flask (25 mL), **tBuDO2A** (free base; 410 mg; 1.03 mmol; 1.4 equiv.) was dissolved in dry MeCN (8 mL) followed by addition of **CS₂CO₃** (840 mg; 2.58 mmol; 3.4 equiv.) and of **KI** (172 mg; 1.04 mmol; 1.4 equiv.). Solution of **2-Chloro-N-(prop-2-yn-1-yl)acetamide** (100 mg; 760 μmol; 1.0 equiv.) in dry MeCN (2 mL) was added and the resulting mixture was stirred 3 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (100 mL) and H₂O (100 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 × 50 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness to give pre-purified product in the form of free base as yellow oil. **Yield:** 180 mg (~75% of **TD423** in a mixture with ~25%

30

of bis(substituted) by-product). **ESI-MS** (LC-MS): 496.4 $[M+H]^+$ (theor. $[C_{25}H_{46}N_5O_5]^+ = 496.3$). All of **TD423** was directly used for **TD425** without further purification and characterization.

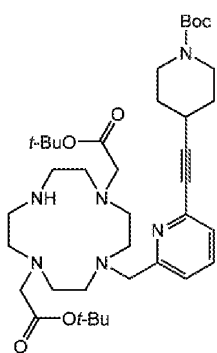


- Synthesis of TD635:** In a pear-shape glass flask (250 mL), **tBuDO2A** (free base; 1.21 g; 3.02 mmol; 2.3 equiv.) was dissolved in MeCN (150 mL). To the vigorously stirred reaction mixture, solution of **TD558** (1202 mg; 1.33 mmol; 1.0 equiv.) in MeCN (100 mL) was added dropwise (over the course of 2 h). Resulting mixture was further stirred 2 d at RT after which was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (100 mL) and H₂O (100 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (4 × 50 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as faint yellow oil that crystallized on standing. **Yield:** 453 mg (66%; 1 step; based on **TD558**). **NMR** (CD₃CN): ¹H δ_H 1.39 (CH₃, s, 18H); 2.53 (*mc*, m, 4H); 2.61 (*mc*, m, 4H); 2.73 (*mc*, m, 4H); 2.77 (*mc*, m, 4H); 3.01 (CH₂–CO, s, 4H); 3.44 (C≡CH, s, 1H); 3.66 (CH₂–arom., s, 2H); 7.38 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.70 (*arom.*, t, 1H, ³J_{HH} = 8); 7.76 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1). ¹³C{¹H} δ_C 28.4 (CH₃, s); 48.2 (*mc*, s); 51.8 (*mc*, s); 52.6 (*mc*, s); 54.9 (*mc*, s); 57.6 (CH₂–CO, s); 59.3 (CH₂–arom., s); 78.0 (C≡CH, s); 81.3 (C–CH₃, s); 84.0 (C≡CH, s); 124.9 (*arom.*, s); 126.5 (*arom.*, s); 137.8 (*arom.*, s); 141.6 (*arom.*, s); 162.8 (*arom.*, s); 172.0 (CO, s). **ESI-HRMS:** 516.3542 $[M+H]^+$ (theor. $[C_{28}H_{46}N_5O_4]^+ = 516.3544$).

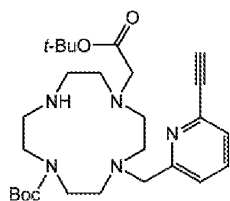


- Synthesis of TD539:** In a pear-shape glass flask (100 mL), **tBuDO2A** (free base; 1.73 g; 4.32 mmol; ≥2.0 equiv.) was dissolved in dry MeCN (15 mL) followed by addition of dried K₂CO₃ (900 mg; 6.52 mmol; ≥3.0 equiv.). To the vigorously stirred reaction mixture, solution of freshly prepared and isolated **TD538** (≤2.17 mmol; 1.0 equiv.) in dry MeCN (10 mL) was added dropwise (over the course of 15 min). Resulting mixture was further stirred 20 h at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (150 mL) and H₂O (150 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 × 100 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as yellow solidified oil. **Yield:** 653 mg (47%;

2 steps; based on **TD530**). **NMR (CD₃CN):** ¹H δ_H 1.39 (CH₃, s, 18H); 1.43 (CH₃, s, 9H); 2.51–2.84 (*mc*, m, 16H); 3.01 (CH₂–CO, s, 4H); 3.64 (CH₂–arom., s, 2H); 4.05 (CH₂–C≡C, bd, 2H, ³J_{HH} = 5); 5.97 (NH–CO, bt, 1H, ³J_{HH} = 5); 7.27 (*arom.*, dd, 1H, ³J_{HH} = 7, ⁴J_{HH} = 2); 7.66–7.75 (*arom.*, m, 2H). ¹³C{¹H} δ_C 28.4 (CH₃, s); 28.6 (CH₃, s); 31.2 (CH₂–C≡C, bs); 48.1 (*mc*, s); 51.8 (*mc*, s); 52.8 (*mc*, s); 55.1 (*mc*, s); 57.6 (CH₂–CO, s); 59.6 (CH₂–arom., s); 81.3 (C–CH₃, 2 × s); 82.6 and 87.0 (C≡C, 2 × s); 124.3 (*arom.*, s); 126.0 (*arom.*, s); 137.7 (*arom.*, s); 142.3 (*arom.*, s); 162.7 (*arom.*, s); 172.0 (CO, s). **ESI-HRMS:** 645.4331 [M+H]⁺ (theor. [C₃₄H₅₇N₆O₆]⁺ = 645.4334).

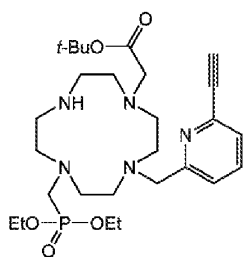


Synthesis of TD1118: In a pear-shape glass flask (50 mL), **tBuDO2A** (free base; 160 mg; 399 μmol; ≥2.5 equiv.) was dissolved in MeCN (10 mL) followed by addition of dried **K₂CO₃** (65 mg; 470 μmol; ≥3 equiv.). To the vigorously stirred reaction mixture, solution of freshly prepared and isolated **TD1117** (≤470 mmol; 1.0 equiv.) in MeCN (10 mL) was added dropwise (over the course of 5 min). Resulting mixture was further stirred 16 h at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (50 mL) and H₂O (50 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 × 30 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as colourless solidified oil. **Yield:** 62.2 mg (57%; 2 steps; based on **TD966**). **NMR (CD₃CN):** ¹H δ_H 1.40 (CH₃, s, 18H); 1.43 (CH₃, s, 9H); 1.53–1.64 (CH₂, m, 2H); 1.82–1.91 (CH₂, m, 2H); 2.47–2.79 (*mc*, m, 16H); 2.79–2.88 (CH–C≡C, m, 1H); 3.01 (CH₂–CO, s, 4H); 3.06–3.18 (CH₂, m, 2H); 3.63 (CH₂–arom., s, 2H); 3.71–3.82 (CH₂, m, 2H); 7.27 (*arom.*, dd, 1H, ³J_{HH} = 7, ⁴J_{HH} = 2); 7.62–7.72 (*arom.*, m, 2H). **ESI-HRMS:** 699.4801 [M+H]⁺ (theor. [C₃₈H₆₃N₆O₆]⁺ = 699.4804).

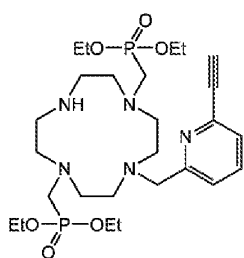


Synthesis of TD692: In a pear-shape glass flask (250 mL), **TD681** (100 mg; 259 μmol; 1.5 equiv.) was dissolved in MeCN (25 mL). To the vigorously stirred reaction mixture, solution of **TD558** (26 mg; 172 μmol; 1.0 equiv.) in MeCN (25 mL) was added dropwise (over the course of 1 h). Resulting mixture was further stirred 2 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, immediately neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (25 mL) and H₂O (25 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted

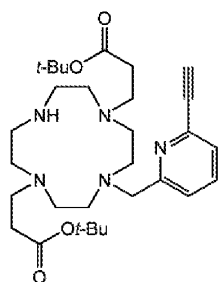
with DCM (3 × 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as yellow oil. **Yield:** 38 mg (44%; 1 step; based on **TD558**). **ESI-MS** (LC-MS): 502.4 [M+H]⁺ (theor. [C₂₇H₄₄N₅O₄]⁺ = 502.3). All of **TD692** was directly used for **TD703** without further characterization.



Synthesis of TD700: In a pear-shape glass flask (100 mL), **TD695** (100 mg; 229 μmol; 1.5 equiv.) was dissolved in MeCN (25 mL) followed by addition of dried K₂CO₃ (21 mg; 152 μmol; 1.0 equiv.). To the vigorously stirred reaction mixture, solution of **TD558** (23 mg; 152 μmol; 1.0 equiv.) in MeCN (25 mL) was added dropwise (over the course of 1 h). Resulting mixture was further stirred 16 h at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (75 mL) and H₂O (75 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 × 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as yellow oil. **Yield:** 20 mg (24%; 1 step; based on **TD558**). **ESI-MS** (LC-MS): 552.3 [M+H]⁺ (theor. [C₂₇H₄₇N₅O₅P₁]⁺ = 552.3). All of **TD700** was directly used for **TD705** without further characterization.

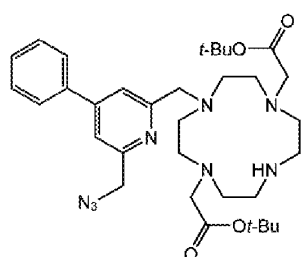


Synthesis of TD579: In a pear-shape glass flask (25 mL), **TD573** (600 mg; 1.27 mmol; 2.0 equiv.) was dissolved in MeCN (10 mL). To the vigorously stirred reaction mixture, solution of **TD558** (30 mg; 488 μmol; 1.0 equiv.) in MeCN (10 mL) was added dropwise (over the course of 30 min). Resulting mixture was further stirred 16 h at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (125 mL), H₂O (125 mL) and aq. NaOH (1%; 20 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 × 50 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as yellow oil. **Yield:** 137 mg (48%; 1 step; based on **TD558**). **ESI-MS** (LC-MS): 588.3 [M+H]⁺ (theor. [C₂₆H₄₈N₅O₆P₂]⁺ = 588.3). **TD579** was directly used for **TD575** and **TD580** and without further characterization.



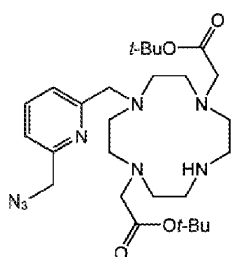
Synthesis of TD799: In a pear-shape glass flask (100 mL), **tBuDO2Prop** (free base; 165 mg; 385 μ mol; 2.0 equiv.) was dissolved in MeCN (30 mL). To the vigorously stirred reaction mixture, solution of **TD558** (29 mg; 191 μ mol; 1.0 equiv.) in MeCN (30 mL) was added dropwise (over the course of 6 h). Resulting mixture was further stirred 10 h at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (50 mL) and

H₂O (50 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 $\times$ 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as slightly yellow oil. **Yield:** 47 mg (45%; 1 step; based on **TD558**). **NMR (CD₃CN):** ¹H δ _H 1.38 (CH₃, s, 18H); 2.19–2.25 (CH₂–CO, m, 4H); 2.44–2.64 (*mc*, CH₂–CH₂–CO, m, 16+4H); 3.41 (C $\equiv$ CH, s, 1H); 3.66 (CH₂–arom., s, 2H); 7.36 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.70 (*arom.*, t, 1H, ³J_{HH} = 8); 7.77 (*arom.*, bd, 1H, ³J_{HH} = 8). **ESI-HRMS:** 544.3854 [M+H]⁺ (theor. [C₃₀H₅₀N₅O₄]⁺ = 544.3857).

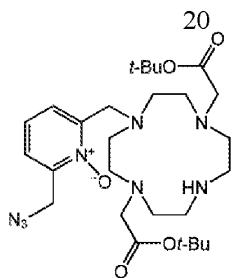


Synthesis of TD663: In a pear-shape glass flask (50 mL), **tBuDO2A** (free base; 210 mg; 524 μ mol; 2.7 equiv.) was dissolved in MeCN (15 mL) followed by addition of dried K₂CO₃ (27 mg; 195 μ mol; 1.0 equiv.). To the vigorously stirred reaction mixture, solution of **TD566** (50 mg; 193 μ mol; 1.0 equiv.) in MeCN (15 mL) was added dropwise (over the course of 15 min). Resulting mixture was further stirred 2 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified

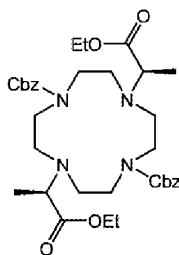
by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (50 mL) and H₂O (50 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 $\times$ 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as nearly colorless oil. **Yield:** 83 mg (69%; 1 step; based on **TD566**). **NMR (CD₃CN):** ¹H δ _H 1.33 (CH₃, s, 18H); 2.62 (*mc*, m, 4H); 2.66 (*mc*, m, 4H); 2.74 (*mc*, m, 4H); 2.80 (*mc*, m, 4H); 2.82 (CH₂–CO, s, 4H); 3.74 (CH₂–arom., s, 2H); 4.46 (CH₂–N₃, s, 2H); 2.46–7.58 (*Ph*, *arom.*, m, 3+1H); 7.78–7.87 (*Ph*, m, 2H); 8.18 (*arom.*, bs, 1H). **ESI-HRMS:** 623.4022 [M+H]⁺ (theor. [C₃₃H₅₁N₈O₄]⁺ = 623.4028).



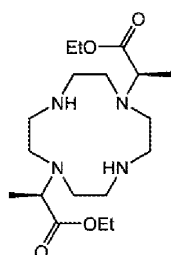
Synthesis of TD711: In a pear-shape glass flask (100 mL), **tBuDO2A** (free base; 564 mg; 1.41 mmol; 2.5 equiv.) was dissolved in MeCN (40 mL). To the vigorously stirred reaction mixture, solution of **TD406** (103 mg; 564 μ mol; 1.0 equiv.) in MeCN (40 mL) was added dropwise (over the course of 2 h). Resulting mixture was further stirred 3 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (100 mL) and H₂O (100 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 $\times$ 50 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as nearly colourless oil. **Yield:** 217 mg (70%; 1 step; based on **TD406**). **NMR (CD₃CN):** ¹H δ _H 1.39 (CH₃, s, 18H); 2.55 (*mc*, m, 4H); 2.60 (*mc*, m, 4H); 2.76 (*mc*, m, 8H); 3.03 (CH₂–CO, s, 4H); 3.70 (CH₂–arom., s, 2H); 4.39 (CH₂–N₃, s, 2H); 7.21 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.67 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.73 (*arom.*, t, 1H, ³J_{HH} = 8). ¹³C{¹H} δ _C 28.4 (CH₃, s); 48.3 (*mc*, s); 51.8 (*mc*, s); 52.7 (*mc*, s); 54.7 (*mc*, s); 56.1 (CH₂–N₃, s); 57.7 (CH₂–CO, s); 59.0 (CH₂–arom., s); 81.2 (C–CH₃, s); 121.2 (*arom.*, s); 124.1 (*arom.*, s); 138.2 (*arom.*, s); 155.6 (*arom.*, s); 161.9 (*arom.*, s); 172.0 (CO, s). **ESI-HRMS:** 547.3716 [M+H]⁺ (theor. [C₂₇H₄₇N₈O₄]⁺ = 547.3715).



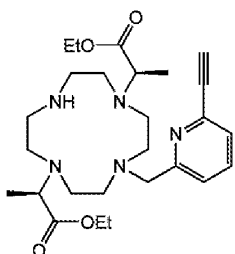
Synthesis of TD596: In a pear-shape glass flask (25 mL), **tBuDO2A** (free base; 162 mg; 405 μ mol; $\geq$ 2.5 equiv.) was dissolved in MeCN (10 mL). To the vigorously stirred reaction mixture, solution of crude **TD633** ($\leq$ 162 μ mol; 1.0 equiv.) in MeCN (10 mL) was added dropwise (over the course of 15 min). Resulting mixture was further stirred 2 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (50 mL) and H₂O (50 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated and the aqueous phase was further extracted with DCM (3 $\times$ 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as nearly colorless oil. **Yield:** 44 mg (48%; 2 steps; based on **TD406**). **ESI-MS (LC-MS):** 563.4 [M+H]⁺ (theor. [C₂₇H₄₇N₈O₅]⁺ = 563.4). All of **TD596** was directly used for **TD604** without further characterization.



- Synthesis of TD1340:** In a pear-shaped glass flask (50 mL), **Cbz2cyclen** (free base; 1.32 g; 3.0 mmol; 1.0 equiv.) was dissolved in dry MeCN (15 mL) followed by addition of dried K_2CO_3 (1.65 g; 12.0 mmol; 4.0 equiv.). Then, solution of **TD1339** (1.50 g; 6.0 mmol; 2.0 equiv.) in dry MeCN (5 mL) was added and the resulting suspension was stirred 8 h at RT. Solids were filtered off using syringe microfilter (PTFE) and the filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO_3 and evaporated to dryness. Residue was dissolved in DCM (150 mL) and H_2O (150 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (4×50 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. Residue was further dried on high vacuum overnight to give product in the form of free base as colourless oil. **Yield:** 1.57 g (82%; 1 step; based on **Cbz2cyclen**). **NMR (CD_3CN):** ^1H δ_{H} 1.09 (CH_3 –CH, d, 6H, $^3J_{\text{HH}} = 7$); 1.19 (CH_3 –CH₂, t, 6H, $^3J_{\text{HH}} = 7$); 2.58–2.70 (mc, m, 4H); 2.76–2.98 (mc, m, 4H); 3.23–3.52 (mc, m, 8H); 3.52–3.79 (CH, m, 2H); 4.06 (CH_2 –CH₃, q, 4H, $^3J_{\text{HH}} = 7$); 5.00–5.13 (CH_2 –Ph, m, 4H); 7.24–7.41 (Ph, m, 10H). **ESI-HRMS:** 641.3539 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{34}\text{H}_{49}\text{N}_4\text{O}_8]^+ = 641.3545$).

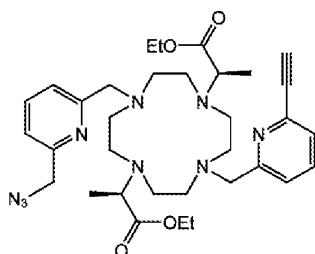


- Synthesis of TD1341:** Pear-shaped glass flask (100 mL) was charged with **Pd@C** (127 mg) and magnetic stirrer and three times secured with argon. Solution of **TD1340** (1.27 g; 1.98 mmol) in MeOH (50 mL) was then added through septum. Argon input was then removed and H_2 gas (from balloon) was allowed to bubble through the mixture for 1 h at RT. Catalyst was then filtered off using syringe microfilter (PTFE; filter was further washed with MeOH). Filtrate was evaporated to dryness and once co-evaporated with DCM. The residue was further dried on high vacuum overnight to give product in the form of free base as nearly colourless oil. **Yield:** 723 mg (98%; 1 step; based on **TD1340**). **NMR (CD_3CN):** ^1H δ_{H} 1.16–1.33 (CH_3 , m, 12H); 2.35–2.45 (mc, m, 4H); 2.56–2.65 (mc, m, 4H); 2.65–2.76 (mc, m, 4H); 2.76–2.85 (mc, m, 4H); 3.54 (CH, q, 2H, $^3J_{\text{HH}} = 7$); 4.00–4.24 (CH_2 –CH₃, q, 4H, $^3J_{\text{HH}} = 7$). **ESI-HRMS:** 372.2808 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{18}\text{H}_{37}\text{N}_4\text{O}_4]^+ = 373.2809$).

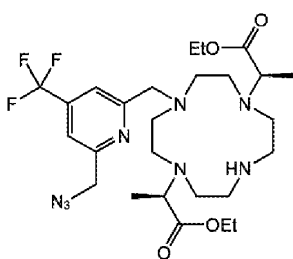


- Synthesis of TD1343:** In a pear-shaped glass flask (50 mL), **TD1341** (143 mg; 384 μmol ; 2.0 equiv.) was dissolved in MeCN (15 mL) followed by addition of dried K_2CO_3 (26.3 mg; 191 μmol ; 1.0 equiv.). To the vigorously stirred reaction mixture, solution of **TD558** (29.0 mg; 191 μmol ; 1.0 equiv.) in MeCN (15 mL) was added dropwise (over the course of 15 min). Resulting mixture was stirred 2 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO_3 and evaporated to

dryness. Residue was dissolved in DCM (25 mL) and H₂O (25 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 × 10 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further briefly dried on high vacuum to give product in the form of free base as nearly colourless oil. **Yield:** 46.0 mg (49%; 1 step; based on **TD558**). **NMR (CD₃CN):** ¹H δ_H 1.14 (CH₃-CH, d, 6H, ³J_{HH} = 7); 1.19 (CH₃-CH₂, t, 6H, ³J_{HH} = 7); 2.28–2.38 (mc, m, 4H); 2.45–2.59 (mc, m, 4H); 2.59–2.94 (mc, m, 8H); 3.23 (CH, q, 2H, ³J_{HH} = 7); 3.41 (CH₂-arom., d, 1H, ²J_{HH} = 15); 3.45 (C≡CH, s, 1H); 3.88 (CH₂-arom., d, 1H, ²J_{HH} = 15); 4.06 (CH₂-CH₃, q, 4H, ³J_{HH} = 7); 7.35–7.42 (arom., m, 1H); 7.66–7.79 (arom., m, 2H). **ESI-HRMS:** 488.3231 [M+H]⁺ (theor. [C₂₆H₄₂N₅O₄]⁺ = 488.3231).

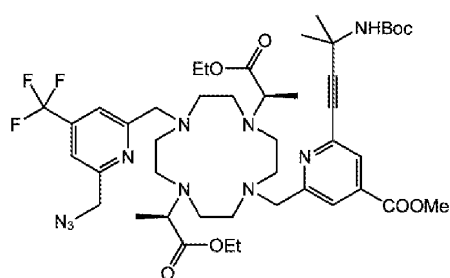


Synthesis of TD1345: In a pear-shaped glass flask (25 mL), **TD1343** (45.0 mg; 92 μmol; 1.0 equiv.) was dissolved in MeCN (2 mL) followed by addition of dried K₂CO₃ (51 mg; 196 μmol; 4.0 equiv.). Solution of **TD406** (20.0 mg; 110 μmol; 1.2 equiv.) in MeCN (2 mL) was then added and the resulting suspension was stirred 7 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (25 mL) and H₂O (25 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (4 × 10 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further briefly dried on high vacuum to give product in the form of free base as nearly colourless oil. **Yield:** 52.9 mg (90%; 1 step; based on **TD1343**). **ESI-HRMS:** 634.3825 [M+H]⁺ (theor. [C₃₃H₄₈N₉O₄]⁺ = 634.3824). All of **TD1345** was directly used for **TD1346** without further characterization.



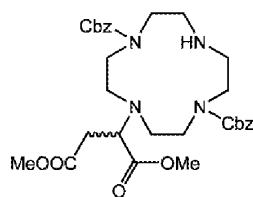
Synthesis of TD1447: In a pear-shaped glass flask (100 mL), **TD1341** (722 mg; 1.94 mmol; 2.0 equiv.) was dissolved in MeCN (40 mL) followed by addition of dried K₂CO₃ (133 mg; 964 μmol; 1.0 equiv.). To the vigorously stirred reaction mixture, solution of **TD1057** (241 mg; 962 μmol; 1.0 equiv.) in MeCN (40 mL) was added dropwise (over the course of 2 h). Resulting mixture was further stirred 16 h at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (100 mL) and H₂O (100 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (4 × 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3)

and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of formate salt as faint yellow oil. **Yield:** 367 mg (60% assuming **M**·1.0FA; $M_R = 632.7$; 1 step; based on **TD1057**). **Recovery:** 352 mg of **TD1341** (49% of the initial amount used). **NMR (CD₃CN):** ¹H δ_H 1.18 (CH₃–CH, d, 6H, ³J_{HH} = 7); 1.21 (CH₃–CH₂, t, 6H, ³J_{HH} = 7); 2.52–2.62 (mc, m, 4H); 2.64–2.76 (mc, m, 4H); 2.81–2.94 (mc, m, 4H); 2.95–3.06 (mc, m, 4H); 3.39 (CH–CH₃, q, 2H, ³J_{HH} = 7); 3.81 (CH₂–arom., d, 1H, ²J_{HH} = 15); 3.90 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.09 (CH₂–CH₃, q, 4H, ³J_{HH} = 7); 4.62 (CH₂–N₃, s, 2H); 7.58 (arom., d, 1H, ⁴J_{HH} = 2); 7.66 (arom., d, 1H, ⁴J_{HH} = 2). ¹⁹F δ_F –65.10 (s). **ESI-HRMS:** 587.3266 [M+H]⁺ (theor. [C₂₆H₄₂N₈O₄F₃]⁺ = 587.3276).



Synthesis of TD1449: In a pear-shaped glass flask (25 mL), **TD1447** (assuming **M**·FA; 263.0 mg; 418 μmol; 1.0 equiv.) was dissolved in MeCN (5 mL) followed by addition of dried **K₂CO₃** (231 mg; 1.67 μmol; 4.0 equiv.). Solution of **TD1428** (173.0 mg; 421 μmol; 1.0 equiv.) in MeCN (10 mL) was then added and the resulting suspension was stirred 1 d at 40 °C. Solids were filtered off using syringe microfilter (PTFE) and filtrate

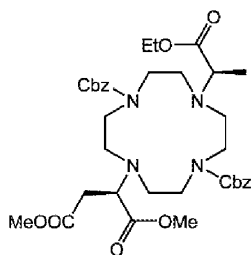
was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (50 mL) and H₂O (50 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (4 × 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further briefly dried on high vacuum to give product in the form of free base as yellow waxy oil. **Yield:** 309 mg (81%; 1 step; based on **TD1447**). **NMR (CD₃CN):** ¹H δ_H 1.10 (CH₃–CH, d, 6H, ³J_{HH} = 7); 1.13 (CH₃–CH₂, t, 6H, ³J_{HH} = 7); 1.43 (CH₃–C–O, s, 9H); 1.61 (CH₃–C–N, s, 6H); 2.53–2.80 (mc, m, 12H); 2.89–3.03 (mc, m, 4H); 3.32 (CH–CH₃, q, 2H, ³J_{HH} = 7); 3.68 (CH₂–arom., s, 2H); 3.71 (CH₂–arom., d, 1H, ²J_{HH} = 15); 3.77 (CH₂–arom., d, 1H, ²J_{HH} = 15); 3.89 (CH₃–O, s, 3H); 3.99 (CH₂–CH₃, q, 4H, ³J_{HH} = 7); 4.52 (CH₂–N₃, s, 2H); 5.56 (NH, bs, 1H); 7.50 (arom., d, 1H, ⁴J_{HH} = 2); 7.71 (arom., d, 1H, ⁴J_{HH} = 2); 8.00 (arom., d, 1H, ⁴J_{HH} = 2); 8.16 (arom., d, 1H, ⁴J_{HH} = 2). ¹⁹F δ_F –64.98 (s). **ESI-HRMS:** 917.4851 [M+H]⁺ (theor. [C₄₄H₆₄N₁₀O₈F₃]⁺ = 917.4855).



Synthesis of *rac*-TD1489: In a glass vial (20 mL), **Cbz2cyclen** (free base; 178 mg; 404 μ mol; 1.0 equiv.) was dissolved in MeCN (2 mL) followed by addition of dried **K₂CO₃** (225 mg; 1.63 mmol; 4.0 equiv.). Then, solution of **TD1488** (250 mg; 850 μ mol; 2.1 equiv.) in MeCN (2 mL) was added and the resulting suspension was stirred 4 d at 40 °C (racemization occurred during

the course of the reaction). Solids were filtered off using syringe microfilter (PTFE) and further washed with MeCN. The filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and concentrated on rotary evaporator to remove most of MeCN.

- 10 Mixture was diluted with DCM (25 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 $\times$ 10 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. Residue was further dried on high vacuum overnight to give product in the form of free base as colourless oil. **Yield:** 172 mg (73%; 1 step; based on **Cbz2cyclen**). **ESI-MS (LC-MS):** 585.3
- 15 $[M+H]^+$ (theor. $[C_{30}H_{41}N_4O_8]^+ = 585.3$).

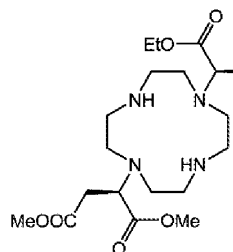


Synthesis of TD1493: In a glass vial (20 mL), *rac*-**TD1489** (free base; 172 mg; 294 μ mol; 1.0 equiv.) was dissolved in MeCN (2 mL) followed by addition of dried **K₂CO₃** (162 mg; 1.17 mmol; 4.0 equiv.). Then, solution of **TD1399** (148 mg; 592 μ mol; 2.0 equiv.) in MeCN (2 mL) was added and the resulting suspension was stirred 70 min at 40 °C. Solids were filtered off using syringe microfilter (PTFE) and further washed with MeCN. The filtrate was

evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with pure product (the more hydrophobic diastereomer differing in chirality of the malate arm was successfully separated) were combined, neutralized with dil. aq. NaHCO₃ and concentrated on rotary evaporator to remove most of MeCN. Mixture was diluted with DCM (25 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 $\times$ 10 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. Residue was further dried on high vacuum overnight to give product in the form of free base as colourless oil. **Yield:** 88.1 mg (44%; 1

- 25 step; based on *rac*-**TD1489**). **NMR (CD₃CN):** ¹H δ_H 1.09 (CH₃–CH, d, 3H, ³J_{HH} = 7); 1.19 (CH₃–
- 30

CH₂, t, 3H, ³J_{HH} = 7); 2.44 (CH₂–CH, dd, 1H, ²J_{HH} = 17, ³J_{HH} = 6); 2.55–2.70 (*mc*, CH₂–CH, m, 3+2H); 2.73–7.43 (*Ph*, m, 10H). **ESI-HRMS**: 685.3439 [M+H]⁺ (theor. [C₃₅H₄₉O₁₀N₄]⁺ = 685.3443).

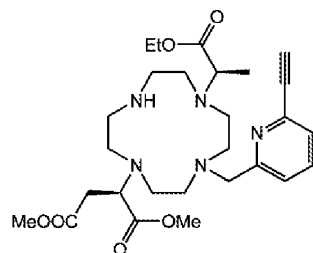


Synthesis of TD1495: Pear-shaped glass flask (25 mL) was charged with **Pd@C** (9 mg) and magnetic stirrer and three times secured with argon. Solution of **TD1493** (88.0 mg; 129 μmol) in MeOH (6 mL) was then added through septum. Argon input was then removed and **H₂ gas** (from balloon) was allowed to bubble through the mixture for 1 h at RT. Catalyst was then filtered off using syringe microfilter (PTFE; filter was further washed with

MeOH). Filtrate was evaporated to dryness and once co-evaporated with DCM. The residue was

10 further dried on high vacuum overnight to give product in the form of free base as colourless oil.

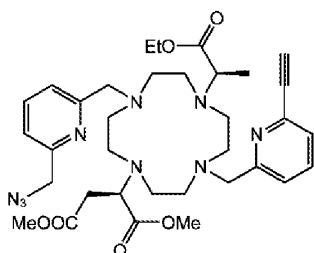
Yield: 52.6 mg (98%; 1 step; based on **TD1493**). **ESI-HRMS**: 417.2706 [M+H]⁺ (theor. [C₁₉H₃₇O₆N₄]⁺ = 417.2708).



Synthesis of TD1497: In a pear-shaped glass flask (25 mL), **TD1495** (55 mg; 132 μmol; 1.7 equiv.) was dissolved in MeCN (5 mL) followed by addition of dried **K₂CO₃** (11.0 mg; 80 μmol; 1.0 equiv.). To the vigorously stirred reaction mixture, solution of **TD558** (12.0 mg; 79 μmol; 1.0 equiv.) in MeCN (5 mL) was added dropwise (over the course of 15 min). Resulting mixture was stirred 4 d at RT. Solids were filtered

off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by
20 preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (25 mL) and H₂O (25 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 × 10 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness.

25 The residue was further briefly dried on high vacuum to give product in the form of free base as nearly colourless oil. **Yield:** 12.1 mg (29%; 1 step; based on **TD558**). **ESI-MS (LC-MS)**: 532.3 [M+H]⁺ (theor. [C₂₇H₄₂N₅O₆]⁺ = 532.3).

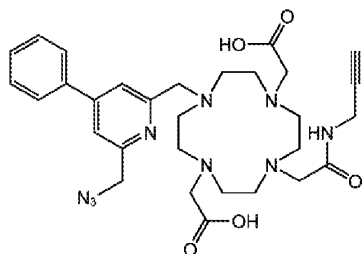


Synthesis of TD1500: In a glass vial (4 mL), **TD1497** (12.1 mg; 23 μmol ; 1.0 equiv.) was dissolved in solution of **TD406** (5.8 mg; 32 μmol ; 1.4 equiv.) in MeCN (2 mL) followed by addition of dried **K₂CO₃** (17.5 mg; 127 μmol ; 4.0 equiv.). The resulting suspension was stirred 1 d at 40 °C. Solids were filtered off using syringe microfilter (PTFE) and filtrate was diluted with H₂O (1 mL). Resulting solution was purified by

preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (25 mL) and H₂O (25 mL) and transferred to a separatory funnel. After shaking, the bottom
 10 phase was separated. Aqueous phase was further extracted with DCM (4 $\times$ 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further briefly dried on high vacuum to give product in the form of free base as faint yellow oil. **Yield:** 11.9 mg (77%; 1 step; based on **TD1497**). **ESI-MS (LC-MS):** 678.3 [M+H]⁺ (theor. [C₃₄H₄₈N₉O₆]⁺ = 678.4).

15

Example 4: Synthesis of non-caged macrocyclic ligands

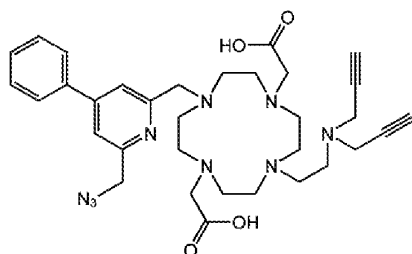


Synthesis of TD425: In a pear-shape glass flask (50 mL), crude **TD423** (~75% in a mixture with ~25% of bis(substituted) by-product; 180 mg; ≥ 1.0 equiv.) was dissolved in dry MeCN (10 mL) followed by addition of **Cs₂CO₃** (360 mg; 1.11 mmol; 3.8 equiv.). Solution of **TD566** (75 mg; 290 μmol ; 1.0 equiv.) in MeCN (5 mL) was then added and the resulting suspension was stirred 2 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was

purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with *tert*-butyl protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in nearly zwitterionic form as white fluffy solid. **Yield:** 129 mg (27%; 3
 25 steps; based on **2-Chloro-N-(prop-2-yn-1-yl)acetamide**). **NMR (D₂O, pD ~5):** ¹H δ_{H} 2.62 (C $\equiv$ CH, t, 1H, ⁴J_{HH} = 3); 2.89–3.74 (mc, CH₂–CO, m, 16+6H); 3.79 (CH₂–C $\equiv$ CH, t, 2H, ⁴J_{HH} = 3); 4.03 (CH₂–arom., bs, 2H); 4.68 (CH₂–N₃, s, 2H); 7.55–7.67 (Ph, arom., m, 3+1H); 7.78 (arom., d, 1H, ⁴J_{HH} = 2); 7.85–7.92 (Ph, m, 2H); 8.17

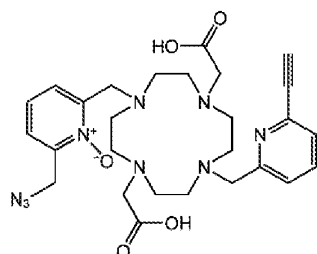
30

(*arom.*, d, 1H, $^4J_{\text{HH}} = 2$). **ESI-HRMS**: 606.3151 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{30}\text{H}_{40}\text{N}_9\text{O}_5]^+ = 606.3147$). **EA** ($\text{C}_{30}\text{H}_{39}\text{N}_9\text{O}_5 \cdot 0.1\text{FA} \cdot 1.3\text{H}_2\text{O}$, $M_{\text{R}} = 633.7$): C 57.0 (56.8); H 6.6 (6.3); N 19.9 (19.5).



Synthesis of TD669: In a glass vial (4 mL), **TD663** (30 mg; 48 μmol , 1.0 equiv.) was dissolved in dry MeCN (500 μL) followed by addition of K_2CO_3 (20 mg; 145 μmol ; 3 equiv.). Solution of **TD662** (10 mg; 50 μmol ; 1.0 equiv.) in MeCN (500 μL) was then added and the resulting suspension was stirred 2 d at RT. Another portion of **TD662** (6 mg; 30 μmol ; 0.6 equiv.) in MeCN (200 μL) was then added and the mixture was further stirred 2 d at RT. Solids were

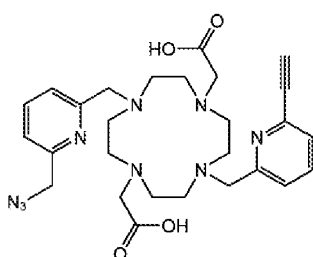
- 10 filtered off using syringe microfilter (PTFE) and filtrate was directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with *tert*-butyl protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was purified by preparative HPLC (C18; H_2O –MeCN
- 15 gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in nearly zwitterionic form as white fluffy solid. **Yield**: 27 mg ($\leq 88\%$ assuming $\text{M} \cdot 0.1\text{FA} \cdot x\text{H}_2\text{O}$; $M_{\text{R}} = 634.4$; 2 steps; based on **TD663**). **NMR (D_2O , pD ~ 5)**: ^1H δ_{H} 2.59–3.82 (*mc*, $\text{CH}_2\text{--CO}$, $\text{CH}_2\text{--CH}_2\text{--N}(\text{CH}_2\text{--C}\equiv\text{CH})_2$, m, 16+4+10H); 4.00 ($\text{CH}_2\text{--arom.}$, bs, 2H); 4.64 ($\text{CH}_2\text{--N}_3$, s, 2H); 7.55–7.65 (*Ph*, m, 3H); 7.74 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 7.83–7.91 (*Ph*, m, 2H); 8.17 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$). **ESI-HRMS**: 630.3510 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{33}\text{H}_{44}\text{N}_9\text{O}_4]^+ = 630.3511$).
- 20



Synthesis of TD604: In a glass vial (4 mL), **TD596** (44 mg; 78 μmol ; 1.0 equiv.) was dissolved in MeCN (3 mL) followed by addition of dried K_2CO_3 (33 mg; 239 μmol ; 3.0 equiv.). Solution of **TD558** (13 mg; 86 μmol ; 1.1 equiv.) in MeCN (600 μL) was then added and the resulting suspension was stirred 16 d at RT. Then additional portion of **TD558** (2 mg; 13 μmol ; 0.2 equiv.) in MeCN (400 μL). Reaction mixture was further stirred 2 d at RT.

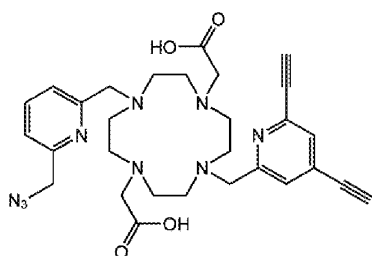
- Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with *tert*-butyl protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in nearly zwitterionic form as white fluffy solid. **Yield**: 38 mg (81%; 2 steps; based on **TD596**). **NMR (D_2O , pD ~ 4)**: ^1H δ_{H} 2.80–3.68 (*mc*, $\text{CH}_2\text{--CO}$, m, 16+4H); 3.72 ($\text{C}\equiv\text{CH}$, s, 1H); 3.93 ($\text{CH}_2\text{--arom.}$, bs, 2H); 4.17 ($\text{CH}_2\text{--arom.}$, s, 2H); 4.74
- 30
- 35

- (CH₂-N₃, s, 2H); 7.48–7.67 (*arom.*, m, 3H); 7.74–7.95 (*arom.*, m, 3H). ¹³C{¹H} δ_C 48.3 (*mc*, s); 48.9 (*mc*, s); 50.6 (CH₂-N₃, s); 51.4 (CH₂-*arom.*, s); 51.5 (*mc*, s); 51.7 (*mc*, s); 56.7 (CH₂-CO, s); 58.8 (CH₂-*arom.*, s); 80.7 and 82.2 (C≡CH; 2 × s); 126.0 (*arom.*, s); 126.5 (*arom.*, s); 127.9 (*arom.*, s); 128.5 (*arom.*, s); 131.5 (*arom.*, s); 140.3 (*arom.*, s); 141.2 (*arom.*, s); 148.4 (*arom.*, s); 148.7 (*arom.*, s); 157.3 (*arom.*, s); 169.5 (CO, s). **ESI-**
 5 **HRMS**: 566.2836 [M+H]⁺ (theor. [C₂₇H₃₆N₉O₅]⁺ = 566.2834). **EA** (C₂₇H₃₅N₉O₅·0.1FA·1.6H₂O, M_R = 599.1): C 54.3 (54.4); H 6.5 (6.1); N 21.0 (20.7).



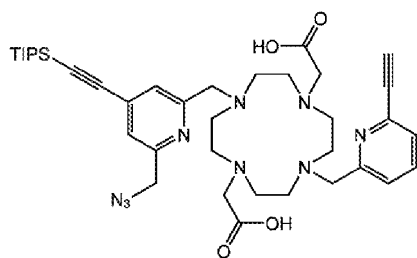
Synthesis of TD556: In a pear-shape glass flask (25 mL), **TD635** (216 mg; 419 μmol; 1.0 equiv.) was dissolved in MeCN (12 mL) followed by addition of dried K₂CO₃ (174 mg; 1.26 mmol; 3.0 equiv.). Solution of **TD406** (92 mg; 504 μmol; 1.2 equiv.) in MeCN (3 mL) was then added and the resulting suspension was stirred 3 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive).

- 15 Fractions with *tert*-butyl protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in nearly zwitterionic form as white
 20 fluffy solid. **Yield**: 161 mg (66%; 2 steps; based on **TD635**). **NMR** (D₂O, pD ~4): ¹H δ_H 2.80–3.60 (*mc*, CH₂-CO, m, 16+4H); 3.74 (C≡CH, s, 1H); 3.86 (CH₂-*arom.*, bs, 2H); 3.94 (CH₂-*arom.*, bs, 2H); 4.72 (CH₂-N₃, s, 2H); 7.65 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.67 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.95–8.03 (*arom.*, m, 3H); 8.12 (*arom.*, t, 1H, ³J_{HH} = 8); ¹³C{¹H} δ_C 48.3 (*mc*, s); 48.4 (*mc*, s); 51.4 (*mc*, s); 51.5 (*mc*, s); 54.3 (CH₂-N₃, s); 56.6 (CH₂-CO, s); 58.0 (CH₂-*arom.*, s); 58.9 (CH₂-*arom.*, s); 80.9 and 82.0 (C≡CH; 2 × s); 124.5 (*arom.*,
 25 s); 126.2 (*arom.*, s); 126.3 (*arom.*, s); 128.5 (*arom.*, s); 140.5 (*arom.*, s); 141.2 (*arom.*, s); 142.7 (*arom.*, s); 155.0 (*arom.*, s); 155.5 (*arom.*, s); 157.2 (*arom.*, s); 169.2 (CO, s). **ESI-HRMS**: 550.2880 [M+H]⁺ (theor. [C₂₇H₃₆N₉O₄]⁺ = 550.2885). **EA** (C₂₇H₃₅N₉O₄·0.1FA·1.6H₂O, M_R = 583.1): C 55.8 (55.5); H 6.6 (6.1); N 21.6 (21.2).



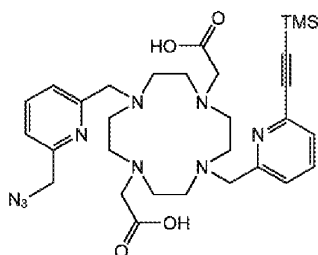
Synthesis of TD810: In a glass vial (20 mL), **TD711** (103 mg; 188 μmol; 1.0 equiv.) was dissolved in MeCN (5 mL) followed by addition of dried K₂CO₃ (130 mg; 942 μmol; 5.0 equiv.). Solution of **TD807** (33 mg; 188 μmol; 1.0 equiv.) in MeCN (2 mL) was then added and the resulting suspension was stirred 3 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient

with FA additive). Fractions with *tert*-butyl protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of mixed trifluoroacetate/formate salt as off white fluffy solid. **Yield:** 55 mg (43%; 2 steps; based on **TD711**). **NMR (D₂O, pD ~4):** ¹H δ_H 2.79–3.63 (*mc*, CH₂–CO, m, 16+4H); 3.74 (C≡CH, s, 1H); 3.82 (CH₂–arom., bs, 2H); 3.87 (CH₂–arom., bs, 2H); 3.92 (C≡CH, s, 1H); 4.63 (CH₂–N₃, s, 2H); 7.55 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.72 (*arom.*, d, 1H, ⁴J_{HH} = 1); 8.10 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 8.16 (*arom.*, t, 1H, ³J_{HH} = 8); 8.26 (*arom.*, d, 1H, ⁴J_{HH} = 1). **ESI-HRMS:** 574.2882 [M+H]⁺ (theor. [C₂₉H₃₆N₉O₄]⁺ = 574.2885). **EA** (C₂₉H₃₅N₉O₄·0.5TFA·0.1FA·2.1H₂O, *M_R* = 673.1): C 53.7 (53.8); H 6.0 (6.2); N 18.7 (18.7).



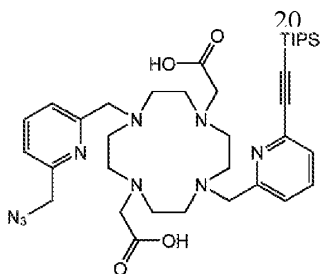
Synthesis of TD827: In a glass vial (20 mL), **TD635** (157 mg; 304 μmol; 1.0 equiv.) was dissolved in dry MeCN (7 mL) followed by addition of dried K₂CO₃ (210 mg; 1.52 mmol; 5.0 equiv.). Solution of **TD817** (110 mg; 303 μmol; 1.0 equiv.) in dry MeCN (3 mL) was then added and the resulting suspension was stirred 2 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC

(C18; H₂O–MeCN gradient with FA additive). Fractions with *tert*-butyl protected product were combined, concentrated to approx. half volume (to remove most of MeCN) and then diluted with DCM (125 mL). The resulting biphasic mixture was transferred to a separatory funnel. Aqueous phase was only then neutralized with dil. aq. NaHCO₃. After shaking, the bottom phase was separated and the aqueous phase was further extracted with DCM (3 × 50 mL). Combined organic layers were with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and once co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of formate salt as off white fluffy solid. **Yield:** 149 mg (62%; 2 steps; based on **TD711**). **NMR (D₂O+FA, pD ~3):** ¹H δ_H 0.86–1.33 (*i*-Pr, m, 21H); 2.58–3.78 (*mc*, CH₂–CO, C≡CH, bm, 16+4+1H); 3.93 (CH₂–arom., bs, 2H); 4.06 (CH₂–arom., bs, 2H); 4.56 (CH₂–N₃, s, 2H); 7.37 (*arom.*, s, 1H); 7.53 (*arom.*, d, 1H, ³J_{HH} = 8); 7.74 (*arom.*, s, 1H); 7.80 (*arom.*, d, 1H, ³J_{HH} = 8); 7.96 (*arom.*, t, 1H, ³J_{HH} = 8). **ESI-HRMS:** 730.4216 [M+H]⁺ (theor. [C₃₈H₅₆N₉O₄Si₁]⁺ = 730.4219). **EA** (C₃₈H₅₅N₉O₄Si₁·0.8FA·1.6H₂O, *M_R* = 795.6): C 58.6 (58.7); H 7.6 (7.7); N 15.8 (15.7); Si 3.5 (3.2).



Synthesis of TD718: In a pear-shape glass flask (10 mL), **TD711** (31 mg; 57 μmol ; 1.0 equiv.) was dissolved in dry MeCN (2 mL) followed by addition of dried K_2CO_3 (31 mg; 225 μmol ; 4.0 equiv.). Solution of **TD706** (13 mg; 58 μmol ; 1.0 equiv.) in dry MeCN (2 mL) was then added and the resulting suspension was stirred 24 h at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive).

Fractions with *tert*-butyl protected product were combined, diluted with DCM (100 mL) and transferred to a separatory funnel. Aqueous phase was only then neutralized with dil. aq. NaHCO_3 . After shaking, bottom phase was separated, dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and once co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in zwitterionic form as white fluffy solid. **Yield:** 23 mg (61%; 2 steps; based on **TD706**). **NMR (D_2O , $\text{pD} \sim 5$):** ^1H δ_{H} 0.28 (CH_3 , s, 9H); 2.28–3.67 (*mc*, CH_2 –CO, m, 16+4H); 3.89 (CH_2 –arom., bs, 4H); 4.57 (CH_2 – N_3 , s, 2H); 7.49 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 7$, $^4J_{\text{HH}} = 2$); 7.58–7.64 (*arom.*, m, 1H); 7.78–7.98 (*arom.*, m, 4H). **ESI-HRMS:** 622.6277 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{30}\text{H}_{44}\text{N}_9\text{O}_4\text{Si}_1]^+ = 622.3280$). **EA** ($\text{C}_{30}\text{H}_{43}\text{N}_9\text{O}_4\text{Si}_1 \cdot 2.6\text{H}_2\text{O}$, $M_{\text{R}} = 668.7$): C 53.9 (53.9); H 7.3 (7.0); N 18.9 (18.7).

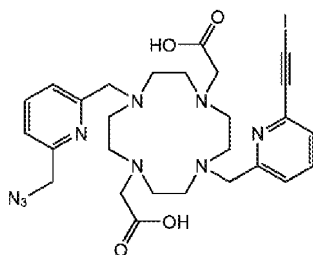


Synthesis of TD728: In a pear-shape glass flask (10 mL), **TD711** (66 mg; 121 μmol ; 1.0 equiv.) was dissolved in MeCN (2 mL) followed by addition of dried K_2CO_3 (67 mg; 486 μmol ; 4.0 equiv.). Solution of **TD723** (37 mg; 120 μmol ; 1.0 equiv.) in MeCN (2 mL) was then added and the resulting suspension was stirred 24 h at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive).

Fractions with *tert*-butyl protected product were combined, diluted with DCM (150 mL) and the resulting biphasic mixture was transferred to a separatory funnel. Aqueous phase was only then neutralized with dil. aq. NaHCO_3 . After shaking, the bottom phase was separated and the aqueous phase was further extracted with DCM (2×50 mL). Combined organic layers were with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and once co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in zwitterionic form as white fluffy solid. **Yield:** 64 mg (71%; 2 steps; based on **TD723**). **NMR (D_2O , $\text{pD} \sim 5$):** ^1H δ_{H} 0.84–1.34 (*i-Pr*, m, 21H); 2.41–3.78 (*mc*,

$\text{CH}_2\text{-CO}$, m, 16+4H); 3.89 ($\text{CH}_2\text{-arom.}$, bs, 2H); 3.95 ($\text{CH}_2\text{-arom.}$, bs, 2H); 4.52 ($\text{CH}_2\text{-N}_3$, s, 2H); 7.42 (*arom.*, d, 1H, $^3J_{\text{HH}} = 8$); 7.46 (*arom.*, d, 1H, $^3J_{\text{HH}} = 8$); 7.74 (*arom.*, d, 1H, $^3J_{\text{HH}} = 8$); 7.78 (*arom.*, d, 1H, $^3J_{\text{HH}} = 8$); 7.85 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$); 7.87 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$). **ESI-HRMS**: 706.4221 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{36}\text{H}_{56}\text{N}_9\text{O}_4\text{Si}_1]^+ = 706.4219$). **EA** ($\text{C}_{36}\text{H}_{55}\text{N}_9\text{O}_4\text{Si}_1 \cdot 2.6\text{H}_2\text{O}$, $M_R = 752.8$): C 57.4 (57.7); H 8.1 (8.0); N 16.7 (16.5); Si 3.7 (3.8).

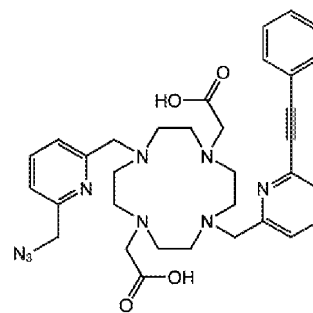
5



Synthesis of TD1204: In a glass vial (20 mL), **TD711** (35.0 mg; 64.0 μmol ; 1.0 equiv.) was dissolved in MeCN (1 mL) followed by addition of dried K_2CO_3 (44.2 mg; 320 μmol ; 5.0 equiv.). Solution of **TD1194** (21.4 mg; 77.1 μmol ; 1.2 equiv.) in MeCN (2 mL) was then added and the resulting suspension was stirred 2 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H_2O -MeCN gradient with FA additive).

Fractions with *tert*-butyl protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H_2O -MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield**: 23.3 mg (49%; 2 steps; based on **TD711**). **NMR** (D_2O , $\text{pD} \sim 7$): ^1H δ_{H} 2.83–3.67 (*mc*, $\text{CH}_2\text{-CO}$, m, 16+4H); 3.88 ($\text{CH}_2\text{-arom.}$, bs, 2H); 3.92 ($\text{CH}_2\text{-arom.}$, bs, 2H); 4.56 ($\text{CH}_2\text{-N}_3$, s, 2H); 7.49 (*arom.*, d, 1H, $^3J_{\text{HH}} = 8$); 7.54 (*arom.*, d, 1H, $^3J_{\text{HH}} = 8$); 7.87–8.00 (*arom.*, m, 4H). **ESI-HRMS**: 676.1855 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{27}\text{H}_{35}\text{N}_9\text{O}_4\text{I}_1]^+ = 676.1851$). **EA** ($\text{C}_{27}\text{H}_{34}\text{N}_9\text{O}_4\text{I}_1 \cdot 0.3\text{FA} \cdot 2.7\text{H}_2\text{O}$, $M_R = 738.0$): C 44.4 (44.6); H 5.5 (5.1); N 17.1 (16.7).

20

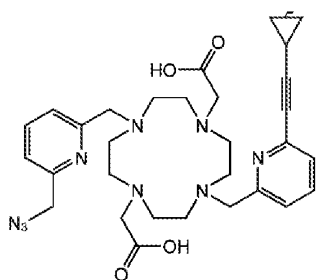


Synthesis of TD944: In a pear-shape glass flask (10 mL), **TD711** (58 mg; 106 μmol ; 1.0 equiv.) was dissolved in MeCN (2 mL) followed by addition of dried K_2CO_3 (87 mg; 629 μmol ; 6.0 equiv.). Solution of freshly prepared and isolated **TD939** (≤ 105 μmol ; ≤ 1.0 equiv.) in MeCN (2 mL) was then added and the resulting suspension was stirred 2 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H_2O -MeCN gradient with FA additive). Fractions with *tert*-butyl protected product were combined,

evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H_2O -MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in zwitterionic form as faint yellow solid. **Yield**: 38 mg (55%; 3 steps; based on **TD936**). **NMR**

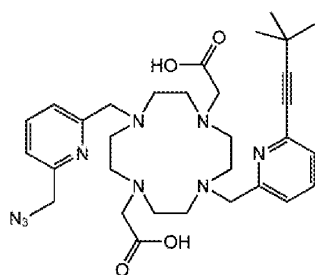
35

(D₂O, pD ~7): ¹H δ_H 2.82–3.67 (*mc*, CH₂–CO, m, 16+4H); 3.91 (CH₂–arom., bs, 2H); 3.95 (CH₂–arom., bs, 2H); 4.58 (CH₂–N₃, s, 2H); 7.40–8.07 (*Ph*, *arom.*, m, 5+6H). **ESI-HRMS**: 626.3197 [M+H]⁺ (theor. [C₃₃H₄₀N₉O₄]⁺ = 626.3198). **EA** (C₃₀H₃₉N₉O₄·1.5H₂O, *M_R* = 652.8): C 60.7 (61.0); H 6.5 (6.3); N 19.3 (18.8).



Synthesis of TD943: In a pear-shape glass flask (10 mL), **TD711** (82 mg; 150 μmol; 1.0 equiv.) was dissolved in MeCN (2 mL) followed by addition of dried K₂CO₃ (125 mg; 904 μmol; 6.0 equiv.). Solution of freshly prepared and isolated **TD940** (≤150 μmol; ≤1.0 equiv.) in MeCN (2 mL) was then added and the resulting suspension was stirred 2 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with

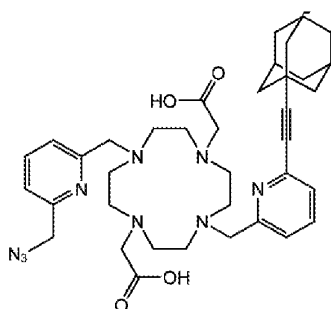
FA additive). Fractions with *tert*-butyl protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in zwitterionic form as faint yellow solid. **Yield**: 66 mg (71%; 3 steps; based on **TD937**). **NMR** (D₂O, pD ~7): ¹H δ_H 0.74–1.06 (CH₂–CH, m, 4H); 1.35–1.66 (CH₂–CH, m, 1H); 2.69–3.65 (*mc*, CH₂–CO, m, 16+4H); 3.86 (CH₂–arom., bs, 2H); 3.92 (CH₂–arom., bs, 2H); 4.57 (CH₂–N₃, s, 2H); 7.42–7.53 (*arom.*, m, 2H); 7.83–8.01 (*arom.*, m, 4H). **ESI-HRMS**: 590.3196 [M+H]⁺ (theor. [C₃₀H₄₀N₉O₄]⁺ = 590.3198). **EA** (C₃₀H₃₉N₉O₄·1.5H₂O, *M_R* = 616.7): C 58.4 (58.6); H 6.9 (6.9); N 20.4 (20.3).



Synthesis of TD959: In a pear-shape glass flask (10 mL), **TD711** (23 mg; 42 μmol; 1.0 equiv.) was dissolved in MeCN (1 mL) followed by addition of dried K₂CO₃ (35 mg; 253 μmol; 6.0 equiv.). Solution of freshly prepared and isolated **TD956** (≤42 μmol; ≤1.0 equiv.) in MeCN (1 mL) was then added and the resulting suspension was stirred 2 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA

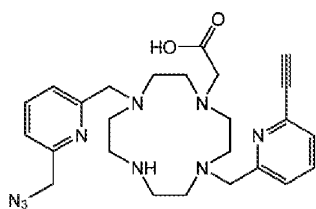
additive). Fractions with *tert*-butyl protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in zwitterionic form as nearly colourless solid. **Yield**: 18 mg (67%; 3 steps; based on **TD952**). **NMR** (D₂O, pD ~6): ¹H δ_H 1.34

(CH₃, s, 9H); 2.72–3.69 (*mc*, CH₂–CO, m, 16+4H); 3.88 (CH₂–arom., bs, 2H); 3.92 (CH₂–arom., bs, 2H); 4.57 (CH₂–N₃, s, 2H); 7.46–7.55 (*arom.*, m, 2H); 7.84–8.03 (*arom.*, m, 4H). **ESI-HRMS**: 606.3513 [M+H]⁺ (theor. [C₃₁H₄₄N₉O₄]⁺ = 606.3511). **EA** (C₃₁H₄₃N₉O₄·1.8H₂O, *M_R* = 638.2): C 58.3 (58.7); H 7.4 (7.0); N 19.8 (19.4



Synthesis of TD992: In a glass vial (4 mL), **TD711** (43 mg; 79 μmol; 1.0 equiv.) was dissolved in MeCN (1 mL) followed by addition of dried **K₂CO₃** (44 mg; 318 μmol; 4.1 equiv.). Solution of freshly prepared and isolated **TD990** (≤79 μmol; ≤1.0 equiv.) in MeCN (1 mL) was then added and the resulting suspension was stirred 16 h at 40 °C. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with *tert*-butyl protected product were combined,

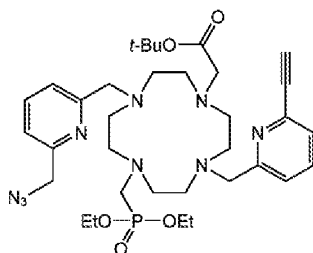
evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of formate salt as off white fluffy solid. **Yield**: 42.0 mg (73%; 3 steps; based on **TD965**). **NMR (D₂O, pD ~5)**: ¹H δ_H 1.62–1.83 (*Adm.*, m, 6H); 1.86–2.04 (*Adm.*, m, 9H); 2.77–3.70 (*mc*, CH₂–CO, m, 16+4H); 3.90 (CH₂–arom., bs, 2H); 3.94 (CH₂–arom., bs, 2H); 4.57 (CH₂–N₃, s, 2H); 7.44–7.56 (*arom.*, m, 2H); 7.82–8.00 (*arom.*, m, 4H). **ESI-HRMS**: 684.3982 [M+H]⁺ (theor. [C₃₇H₅₀N₉O₄]⁺ = 684.3980). **EA** (C₃₇H₄₉N₉O₄·0.3FA·1.9H₂O, *M_R* = 731.9): C 61.2 (61.2); H 7.1 (7.1); N 17.2 (17.0).



Synthesis of TD703: In a glass vial (20 mL), **TD692** (38 mg; 76 μmol; 1.0 equiv.) was dissolved in MeCN (3 mL) followed by addition of dried **K₂CO₃** (31 mg; 217 μmol; 3.0 equiv.). Solution of **TD406** (14 mg; 77 μmol; 1.0 equiv.) in MeCN (2 mL) was then added and the resulting suspension was stirred 16 h at RT. Then, another portion of dried **K₂CO₃** (31 mg; 217 μmol; 3.0 equiv.) and of **TD406** (10 mg; 55 μmol; 0.7 equiv.) in MeCN (1 mL) was

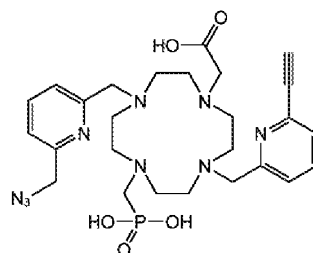
added and the resulting suspension was further stirred 3 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with protected product were combined, evaporated to dryness and further two-times co-evaporated with MeOH. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and three-times co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the

form of mixed trifluoroacetate/formate salt as slightly yellow viscous oil. **Yield:** 16 mg (34%; 2 steps; based on **TD692**). **NMR (D₂O, pD ~5):** ¹H δ_H 2.59–3.73 (*mc*, CH₂–CO, m, 16+2H); 3.75 (C≡CH, s, 1H); 3.84 (CH₂–arom., bs, 2H); 4.29–4.62 (CH₂–arom., CH₂–N₃, m, 2+2H); 7.37 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.45 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.52 (*arom.*, t, 1H, ³J_{HH} = 8); 7.56 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.59 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.89 (*arom.*, t, 1H, ³J_{HH} = 8). **ESI-HRMS:** 492.2829 [M+H]⁺ (theor. [C₂₅H₃₄N₉O₂]⁺ = 492.2830). **EA** (C₂₅H₃₃N₉O₂·0.3TFA·0.7FA·3.5H₂O, *M_R* = 621.1): C 50.9 (51.1); H 6.8 (6.8); N 20.3 (20.0).



Synthesis of TD705: In a glass vial (20 mL), **TD700** (20 mg; 36 μmol; 1.0 equiv.) was dissolved in MeCN (3 mL) followed by addition of dried **K₂CO₃** (20 mg; 145 μmol; 4.0 equiv.). Solution of **TD406** (9 mg; 49 μmol; 1.4 equiv.) in MeCN (1 mL) was then added and the resulting suspension was stirred 2 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were

combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (50 mL) and H₂O (50 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 × 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as nearly colorless oil. **Yield:** 22 mg (87%; 1 step; based on **TD406**). **ESI-MS (LC-MS):** 698.4 [M+H]⁺ (theor. [C₃₄H₅₃N₉O₅P₁]⁺ = 698.4). All of **TD705** was directly used for **TD714** without further characterization.

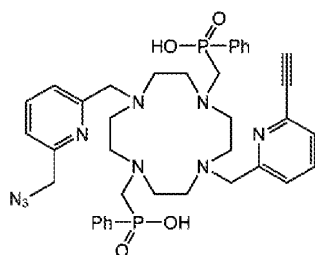


Synthesis of TD714: In a glass vial (4 mL), **TD705** (22 mg; 32 μmol; 1.0 equiv.) was dissolved in dry Pyridine (1 mL) followed by addition of neat **TMSBr** (50 μL; 379 μmol; 12.0 equiv.) and the resulting mixture was stirred 16 h at RT. Reaction was then quenched with 50% aq. MeOH (1 mL) and the mixture was further stirred 1 h at RT. The mixture was then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with *tert*-butyl protected product were combined, evaporated to dryness and

further two-times co-evaporated with MeOH. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in nearly zwitterionic form as white fluffy solid. **Yield:** 12 mg (≤64% assuming **M**·0.1FA·*x*H₂O; *M_R* = 590.2; 2 steps; based on **TD705**). **NMR (D₂O, pD ~5):** ¹H δ_H 2.73–3.71 (*mc*, CH₂–CO, CH₂–P, m, 16+2+1H); 3.74 (C≡CH, s, 1H);

3.84 (CH_2 -arom, bs, 1H); 3.99 (CH_2 -arom., bs, 1H); 4.17 (CH_2 -arom., bs, 2H); 4.47 (CH_2 -P; bd, $^2J_{\text{H}} = 13$); 4.67 (CH_2 -N₃, s, 1H); 4.68 (CH_2 -N₃, s, 1H); 7.54 (arom., d, 1H, $^3J_{\text{HH}} = 8$); 7.61 (arom., d, 1H, $^3J_{\text{HH}} = 8$); 7.62 (arom., d, 1H, $^3J_{\text{HH}} = 8$); 7.64 (arom., d, 1H, $^3J_{\text{HH}} = 8$); 7.90 (arom., t, 1H, $^3J_{\text{HH}} = 8$); 7.93 (arom., t, 1H, $^3J_{\text{HH}} = 8$). **ESI-HRMS**: 586.2649 [M+H]⁺ (theor. [C₂₆H₃₇N₉O₅P₁]⁺ = 586.2650).

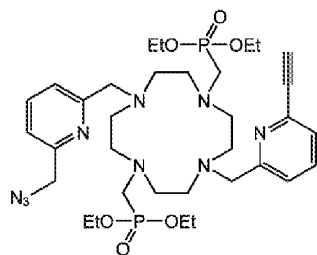
5



Synthesis of TD921: In a pear-shape glass flask (25 mL), **TD910** (160 mg; 315 μmol ; 2.0 equiv.) was dissolved in MeCN (10 mL) followed by addition of solution of dried **K₂CO₃** (22 mg; 160 μmol ; 1.0 equiv.). Solution of **TD558** (24 mg; 158 μmol ; 1.0 equiv.) in MeCN (5 mL) was then added dropwise over the course of 15 min and the resulting suspension was stirred for 24 h at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O-

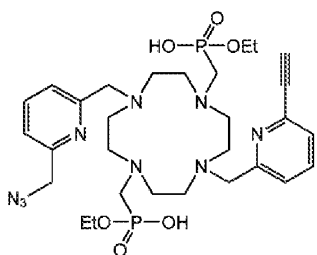
-MeCN gradient with TFA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (50 mL) and H₂O (50 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 $\times$ 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The resulting alkyne bearing intermediate (together with partially *N*-methylated/demethylated by-products as a consequence of ongoing self-methylation; 49 mg; ≤ 78 μmol ; ≤ 1.0 equiv.) was dissolved in MeCN (7 mL) followed by addition of **TD406** (18 mg; 119 μmol ; ≥ 1.5 equiv.) and K₂CO₃ (54 mg; 391 μmol ; ≥ 5.0 equiv.) and the resulting suspension was stirred 16 h at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O-MeCN gradient with TFA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO₃ and evaporated to dryness. Residue was dissolved in DCM (50 mL) and H₂O (50 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3 $\times$ 25 mL). Combined organic layers were dried with anhydrous Na₂SO₄, filtered through glass frit (S3) and evaporated to dryness. The resulting methyl ester of product (together with partially *N*-methylated/demethylated by-products as a consequence of ongoing self-methylation; 37 mg; ≤ 48 μmol ; ≤ 1.0 equiv.) was dissolved in dry Pyridine (2 mL) followed by addition of neat **TMSBr** (65 μL ; 492 μmol ; ≥ 12.0 equiv.) and the resulting mixture was stirred 16 h at RT. Reaction was then quenched with 50% aq. MeOH (1 mL) and the mixture was further stirred 1 h at RT. The mixture was then directly purified by preparative HPLC (C18; H₂O-MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in zwitterionic form as white fluffy solid. **Yield**: 25.6 mg (20%; 3 steps; based on **TD558**). **NMR (D₂O, pD ~4)**: ¹H δ_{H} 2.56–3.75 (*mc*, CH₂-P, C $\equiv$ CH, m, 16+4+1H); 4.20 (CH₂-arom., bs, 2H); 4.29 (CH₂-arom., bs, 2H); 4.45 (CH₂-N₃, s, 2H); 7.23–7.77 (*Ph*, arom., m, 10+4H); 7.96 (arom., t, 1H, $^3J_{\text{HH}} = 8$); 7.97

(*arom.*, t, 1H, $^3J_{\text{HH}} = 8$). $^{31}\text{P}\{^1\text{H}\}$ δ_{P} 25.1 (bs). **ESI-HRMS**: 796.2334 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{44}\text{H}_{34}\text{N}_{10}\text{O}_2\text{P}_2]^+ = 796.2336$). **EA** ($\text{C}_{37}\text{H}_{45}\text{N}_9\text{O}_4\text{P}_2 \cdot 4.6\text{H}_2\text{O}$, $M_{\text{R}} = 824.6$): C 53.9 (54.5); H 6.6 (6.3); N 15.3 (14.7).



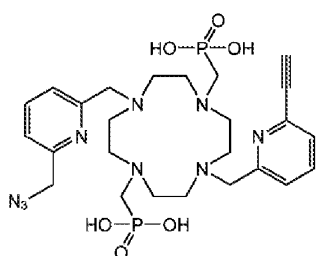
Synthesis of TD580: In a glass vial (20 mL), **TD579** (137 mg; 233 μmol ; 1.0 equiv.) was dissolved in MeCN (7 mL) followed by addition of dried K_2CO_3 (141 mg; 1.02 mmol; 4.4 equiv.). Solution of **TD406** (50 mg; 274 μmol ; 1.2 equiv.) in MeCN (3 mL) was then added and the resulting suspension was stirred 3 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were

combined, neutralized with dil. aq. NaHCO_3 and evaporated to dryness. Residue was dissolved in DCM (50 mL) and H_2O (50 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3×50 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as colorless oil. **Yield**: 125 mg (73%; 1 step; based on **TD579**). **NMR** (CD_3CN): ^1H δ_{H} 1.21 (CH_3 , t, 12H, $^3J_{\text{HH}} = 7$); 2.90 (*mc*, m, 8H); 2.97 ($\text{CH}_2\text{--P}$, d, 4H, $^2J_{\text{HP}} = 10$); 2.99 (*mc*, m, 4H); 3.24 (*mc*, m, 8H); 3.55 ($\text{C}\equiv\text{CH}$, s, 1H); 4.01 ($\text{CH}_2\text{--O--P}$, m, 8H); 4.21 ($\text{CH}_2\text{--arom.}$, s, 2H); 4.48 ($\text{CH}_2\text{--N}_3$, s, 2H); 4.55 ($\text{CH}_2\text{--arom.}$, s, 2H); 7.37 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.49 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.51 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.54 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.80 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$); 7.84 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$). $^{31}\text{P}\{^1\text{H}\}$ δ_{P} 24.8 (s). **ESI-MS** (LC-MS): 734.5 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{33}\text{H}_{54}\text{N}_9\text{O}_6\text{P}_2]^+ = 734.4$).



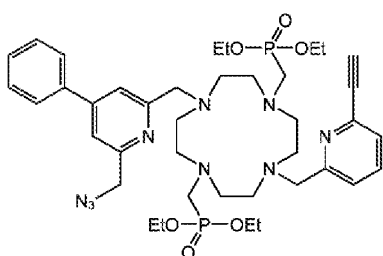
Synthesis of TD582: In a glass vial (4 mL), **TD580** (25 mg; 34 μmol ; 1.0 equiv.) was dissolved in a mixture of aq. NaOH (10%; 1 mL) and EtOH (300 μL) and the resulting solution was stirred 2 d at RT. Reaction was then quenched with AcOH (200 μL) and the mixture was directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in nearly zwitterionic form as white fluffy solid. **Yield**: 9 mg ($\leq 39\%$ assuming

$\text{M} \cdot 0.1\text{FA} \cdot x\text{H}_2\text{O}$; $M_{\text{R}} = 682.3$; 1 step; based on **TD580**). **ESI-MS** (LC-MS): 678.4 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{29}\text{H}_{46}\text{N}_9\text{O}_6\text{P}_2]^+ = 678.3$).



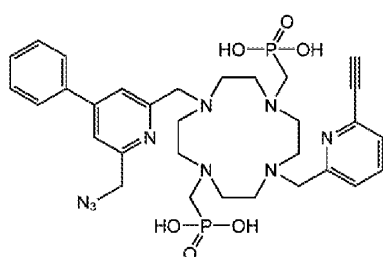
Synthesis of TD581: In a glass vial (4 mL), **TD580** (25 mg; 34 μmol ; 1.0 equiv.) was dissolved in dry Pyridine (1 mL) followed by addition of neat **TMSBr** (110 μL ; 833 μmol ; ~ 25 equiv.) and the resulting mixture was stirred 16 h at RT. Reaction was then quenched with 50% aq. MeOH (1 mL) and the mixture was further stirred 1 h at RT. The mixture was then directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in nearly zwitterionic form as slightly yellow fluffy solid. **Yield:** 6 mg ($\leq 28\%$ assuming $\text{M} \cdot 0.1\text{FA} \cdot x\text{H}_2\text{O}$; $M_R = 626.2$; 1 step; based on **TD580**). **ESI-MS** (LC-MS): 622.3 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{25}\text{H}_{38}\text{N}_9\text{O}_6\text{P}_2]^+ = 622.2$).

10



Synthesis of TD575: In a glass vial (20 mL), **TD579** (51 mg; 87 μmol ; 1.2 equiv.) was dissolved in MeCN (3 mL) followed by addition of dried K_2CO_3 (30 mg; 217 μmol ; 3.0 equiv.). Solution of **TD566** (20 mg; 73 μmol ; 1.0 equiv.) in MeCN (2 mL) was then added and the resulting suspension was stirred 16 h at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were combined, neutralized with dil. aq. NaHCO_3 and evaporated to dryness. Residue was dissolved in DCM (50 mL) and H_2O (50 mL) and transferred to a separatory funnel. After shaking, the bottom phase was separated. Aqueous phase was further extracted with DCM (3×25 mL). Combined organic layers were dried with anhydrous Na_2SO_4 , filtered through glass frit (S3) and evaporated to dryness. The residue was further dried on high vacuum overnight to give product in the form of free base as nearly colorless oil. **Yield:** 46 mg (90%; 1 step; based on **TD566**). **ESI-MS** (LC-MS): 810.4 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{39}\text{H}_{58}\text{N}_9\text{O}_6\text{P}_2]^+ = 810.4$). **TD575** was directly used for **TD576** and without further characterization.

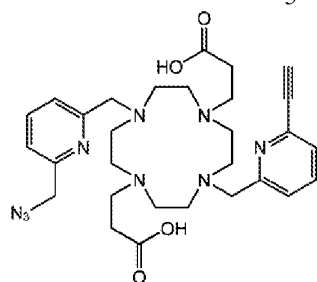
20



Synthesis of TD576: In a glass vial (4 mL), **TD575** (23 mg; 28 μmol ; 1.0 equiv.) was dissolved in dry Pyridine (1 mL) followed by addition of neat **TMSBr** (220 μL ; 1.67 mmol; ~ 60 equiv.) and the resulting mixture was stirred 16 h at RT. Reaction was then quenched with 50% aq. MeOH (1 mL) and the mixture was further stirred 1 h at RT. The mixture was then directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in nearly zwitterionic form as white fluffy solid. **Yield:** 15 mg ($\leq 75\%$ assuming $\text{M} \cdot 0.1\text{FA} \cdot x\text{H}_2\text{O}$; $M_R = 702.3$; 1 step; based on **TD575**). **NMR** ($\text{D}_2\text{O}+\text{NaOD}$, pD ~ 8): ^1H δ_{H} 2.54–3.76 (mc, $\text{CH}_2\text{--P}$, m, 16+4H); 3.93 ($\text{CH}_2\text{--arom.}$, bs, 2H); 4.10 ($\text{CH}_2\text{--arom.}$, bs, 2H); 4.60 ($\text{CH}_2\text{--N}_3$, s, 2H); 7.44

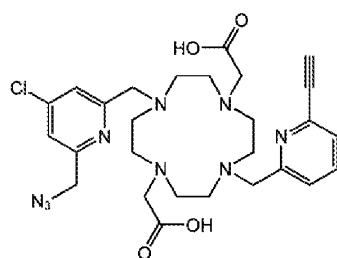
25

(*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.46–7.51 (*Ph*, m, 3H); 7.53 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.66–7.70 (*Ph*, m, 2H); 7.71 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 7.77 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 7.81 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$). $^{31}\text{P}\{^1\text{H}\}$ δ_{P} 8.2 (bs). **ESI-HRMS**: 696.2575 $[\text{M}-\text{H}]^-$ (theor. $[\text{C}_{31}\text{H}_{40}\text{N}_9\text{O}_6\text{P}_2]^- = 696.2582$).



5 **Synthesis of TD801**: In a glass vial (20 mL), **TD799** (46 mg; 85 μmol ; 1.0 equiv.) was dissolved in MeCN (5 mL) followed by addition of dried K_2CO_3 (48 mg; 348 μmol ; 4.1 equiv.). Solution of **TD406** (20 mg; 110 μmol ; 1.3 equiv.) in MeCN (1 mL) was then added and the resulting suspension was stirred 2 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with *tert*-butyl

protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in nearly zwitterionic form as white fluffy solid. **Yield**: 33 mg (48%; 2 steps; based on **TD799**). **NMR** (D_2O , $\text{pD} \sim 3$): ^1H δ_{H} 2.41–3.78 (*mc*, $\text{CH}_2\text{--CO}$, $\text{CH}_2\text{--P}$, m, 16+2+2H); 3.89 ($\text{CH}_2\text{--arom.}$, bs, 2H); 3.95 ($\text{CH}_2\text{--arom.}$, bs, 2H); 4.52 ($\text{CH}_2\text{--N}_3$, s, 2H); 7.42 (*arom.*, d, 1H, $^3J_{\text{HH}} = 8$); 7.46 (*arom.*, d, 1H, $^3J_{\text{HH}} = 8$); 7.74 (*arom.*, d, 1H, $^3J_{\text{HH}} = 8$); 7.78 (*arom.*, d, 1H, $^3J_{\text{HH}} = 8$); 7.85 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$); 7.87 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$). **ESI-HRMS**: 578.3208 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{29}\text{H}_{39}\text{N}_9\text{O}_4]^+ = 578.3203$). **EA** ($\text{C}_{29}\text{H}_{39}\text{N}_9\text{O}_4 \cdot 2.0\text{TFA}$, $M_{\text{R}} = 805.7$): C 49.2 (49.3); H 5.1 (5.4); N 15.6 (15.7).

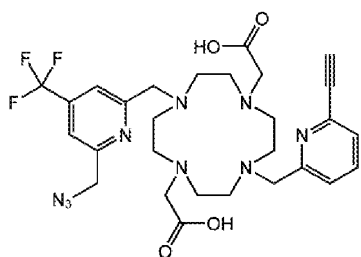


Synthesis of TD764: In a glass vial (20 mL), **TD635** (304 mg; 589 μmol ; 1.1 equiv.) was dissolved in MeCN (7 mL) followed by addition of dried K_2CO_3 (305 mg; 2.21 mmol; 4.0 equiv.). Solution of **TD760** (120 mg; 553 μmol ; 1.0 equiv.) in MeCN (3 mL) was then added and the resulting suspension was stirred 2 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H_2O –MeCN gradient with FA

additive). Fractions with *tert*-butyl protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield**: 196 mg (51%; 2 steps; based on **TD635**). **NMR** (D_2O , $\text{pD} \sim 3$):

^1H δ_{H} 2.86–3.60 (*mc*, $\text{CH}_2\text{-CO}$, m, 16+4H); 3.77 ($\text{C}\equiv\text{CH}$, s, 1H); 3.79–4.06 ($\text{CH}_2\text{-arom.}$, bm, 4H); 4.62 ($\text{CH}_2\text{-N}_3$, s, 2H); 7.62 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 7.67–7.73 (*arom.*, m, 1H); 8.07–8.14 (*arom.*, m, 2H); 8.17 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$). **ESI-HRMS**: 582.2344 $[\text{M-H}]^-$ (theor. $[\text{C}_{27}\text{H}_{33}\text{N}_9\text{O}_4\text{Cl}_1]^- = 582.2350$). **EA** ($\text{C}_{27}\text{H}_{34}\text{N}_9\text{O}_4\text{Cl}_1 \cdot 0.7\text{TFA} \cdot 1.7\text{H}_2\text{O}$, $M_{\text{R}} = 694.5$): C 49.1 (49.0); H 5.5 (5.4); N 18.2 (18.4); Cl 5.1 (5.3).

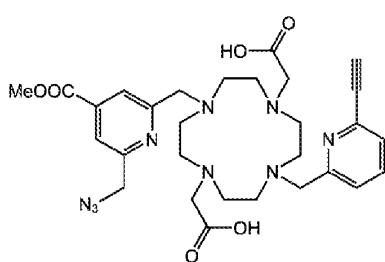
5



Synthesis of TD1063: In a glass vial (4 mL), **TD635** (50 mg; 97 μmol ; 1.0 equiv.) was dissolved in MeCN (1 mL) followed by addition of dried K_2CO_3 (67 mg; 485 μmol ; 5.0 equiv.). Solution of **TD1057** (24 mg; 96 μmol ; 1.0 equiv.) in MeCN (1 mL) was then added and the resulting suspension was stirred 2 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H_2O –MeCN gradient with FA

additive). Fractions with *tert*-butyl protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in nearly zwitterionic form as white fluffy solid. **Yield**: 33.4 mg (55%; 2 steps; based on **TD635**). **NMR (D_2O , pD ~7)**: ^1H δ_{H} 2.81–3.68 (*mc*, $\text{CH}_2\text{-CO}$, m, 16+4H); 3.72 ($\text{C}\equiv\text{CH}$, s, 1H); 3.82 ($\text{CH}_2\text{-arom.}$, bs, 2H); 4.05 ($\text{CH}_2\text{-arom.}$, bs, 2H); 4.70 ($\text{CH}_2\text{-N}_3$, s, 2H); 7.62–7.69 (*arom.*, m, 1H); 7.82 (*arom.*, s, 1H); 7.92–7.99 (*arom.*, m, 2H); 8.38 (*arom.*, s, 1H). $^{19}\text{F}\{^1\text{H}\}$ δ_{F} –64.2 (s). **ESI-HRMS**: 618.2759 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{28}\text{H}_{35}\text{N}_9\text{O}_4\text{F}_3]^+ = 618.2759$). **EA** ($\text{C}_{28}\text{H}_{34}\text{N}_9\text{O}_4\text{F}_3 \cdot 0.1\text{FA} \cdot 1.2\text{H}_2\text{O}$, $M_{\text{R}} = 643.8$): C 52.4 (52.8); H 5.7 (5.4); N 19.6 (19.4); F 8.8 (7.8).

20

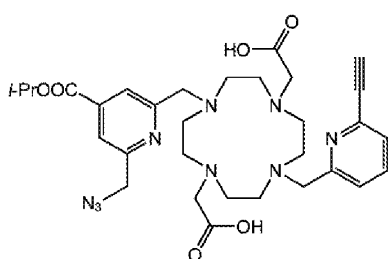


30

Synthesis of TD1092: In a glass vial (4 mL), **TD635** (35 mg; 68 μmol ; 1.0 equiv.) was dissolved in MeCN (2 mL) followed by addition of dried K_2CO_3 (44 mg; 318 μmol ; 4.7 equiv.). Solution of pre-purified **TD1089** (containing ~20% of the bis azide byproduct; 20 mg; <83 μmol ; <1.2 equiv.) in MeCN (1 mL) was then added and the resulting suspension was stirred 24 h at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was

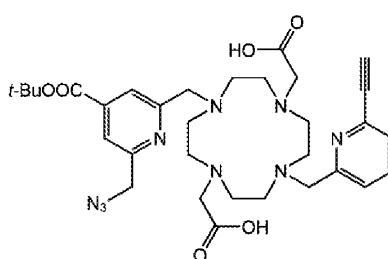
purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with *tert*-butyl protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly

purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in nearly zwitterionic form as white fluffy solid. **Yield:** 31.9 mg (74%; 2 steps; based on **TD635**). **NMR (D₂O, pD ~6):** ¹H δ_H 2.76–3.62 (*mc*, CH₂–CO, m, 16+4H); 3.73 (C≡CH, s, 1H); 3.83 (CH₂–arom., bs, 2H); 3.99 (CH₃, s, 3H); 4.05 (CH₂–arom., bs, 2H); 4.69 (CH₂–N₃, s, 2H); 7.70 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 8.02 (*arom.*, d, 1H, ⁴J_{HH} = 1); 8.05 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 8.15 (*arom.*, t, 1H, ³J_{HH} = 8); 8.58 (*arom.*, d, 1H, ⁴J_{HH} = 1). **ESI-HRMS:** 608.2939 [M+H]⁺ (theor. [C₂₉H₃₈N₉O₆]⁺ = 608.2940). **EA** (C₂₉H₃₇N₉O₆·0.1FA·1.1H₂O, *M_R* = 632.1): C 55.3 (55.1); H 6.3 (6.0); N 19.9 (19.6).



Synthesis of TD1148: In a glass vial (4 mL), **TD635** (44 mg; 85 μmol; 1.0 equiv.) was dissolved in MeCN (2.5 mL) followed by addition of dried **K₂CO₃** (47 mg; 340 μmol; 4.0 equiv.). Solution of **TD1112** (23 mg; 86 μmol; 1.0 equiv.) in MeCN (1 mL) was then added and the resulting suspension was stirred 3 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–

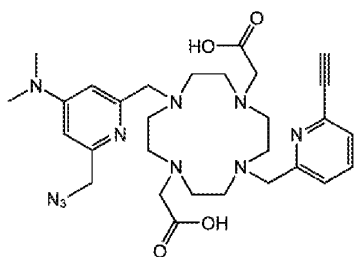
MeCN gradient with FA additive). Fractions with *tert*-butyl protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in nearly zwitterionic form as white fluffy solid. **Yield:** 30.7 mg (55%; 2 steps; based on **TD635**). **NMR (D₂O, pD ~6):** ¹H δ_H 1.40 (CH₃, d, 6H, ³J_{HH} = 6); 2.77–3.63 (*mc*, CH₂–CO, m, 16+4H); 3.70 (C≡CH, s, 1H); 3.80 (CH₂–arom., bs, 2H); 4.06 (CH₂–arom., bs, 2H); 4.65 (CH₂–N₃, s, 2H); 5.26 (CH, hept, 1H, ³J_{HH} = 6); 7.65 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.93–8.00 (*arom.*, m, 2H); 8.12 (*arom.*, t, 1H, ³J_{HH} = 8); 8.45 (*arom.*, d, 1H, ⁴J_{HH} = 2). **ESI-HRMS:** 636.3247 [M+H]⁺ (theor. [C₃₁H₄₂N₉O₆]⁺ = 636.3253). **EA** (C₃₁H₄₁N₉O₆·0.1FA·1.0H₂O, *M_R* = 658.3): C 56.7 (56.4); H 6.6 (6.4); N 19.1 (18.8).



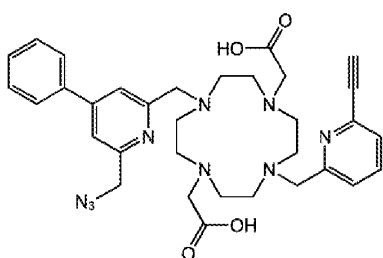
Synthesis of TD1160: In a pear-shape glass flask (100 mL), **TD1116** (50 mg; 158 μmol; 2.0 equiv.) was dissolved in MeCN (20 mL) followed by addition of dried **K₂CO₃** (11 mg; 80 μmol; 1.0 equiv.). Solution of **TD558** (12 mg; 79 μmol; 1.0 equiv.) in MeCN (20 mL) was then added dropwise over the course of 2 h and the resulting suspension was stirred 7 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue

was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with mono

alkylated intermediate were combined and directly lyophilized. Resulting solid (15.4 mg; $\leq 36 \mu\text{mol}$; 1.0 equiv.) was dissolved in MeCN (2 mL) followed by addition of dried K_2CO_3 (25 mg; $181 \mu\text{mol}$; ≥ 5.0 equiv.). Solution of **TD1130** (15 mg; $53 \mu\text{mol}$; ≥ 1.5 equiv.) in MeCN (1 mL) was then added and the resulting suspension was stirred 7 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with methyl protected product were joined and directly lyophilized. Residue was re-dissolved in a mixture of MeOH (2 mL) and H_2O (1 mL) followed by addition of solid $\text{LiOH} \cdot \text{H}_2\text{O}$ (15 mg; $360 \mu\text{mol}$; >10 equiv.). The resulting mixture was stirred 15 min at RT, quenched with FA (15 μL) and then directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product as white solid. **Yield:** 1.7 mg ($<5\%$; 3 steps; based on **TD558**). **ESI-HRMS:** 650.3407 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{32}\text{H}_{44}\text{N}_9\text{O}_6]^+ = 650.3409$).

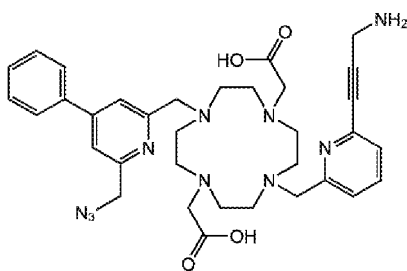


Synthesis of TD1176: In a glass vial (4 mL), **TD635** (43.5 mg; $84.4 \mu\text{mol}$; 1.1 equiv.) was dissolved in MeCN (2 mL) followed by addition of dried K_2CO_3 (42.5 mg; $308 \mu\text{mol}$; 4.0 equiv.). Solution of **TD1163** (17.3 mg; $76.7 \mu\text{mol}$; 1.0 equiv.) in MeCN (1 mL) was then added and the resulting suspension was stirred 2 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with *tert*-butyl protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 43.6 mg (80%; 2 steps; based on **TD1163**). **NMR (D_2O , $\text{pD} \sim 7$):** ^1H δ_{H} 2.74–3.62 (*mc*, $\text{CH}_2\text{--CO}$, CH_3 , m, 16+4+6H); 3.70 ($\text{C}\equiv\text{CH}$, s, 1H); 3.76 ($\text{CH}_2\text{--arom.}$, bs, 2H); 3.84 ($\text{CH}_2\text{--arom.}$, bs, 2H); $\sim 4.7\text{--}4.8$ ($\text{CH}_2\text{--N}_3$, obscured by HOD signal); 6.85 (*arom.*, bs, 1H); 7.02 (*arom.*, bs, 1H); 7.64 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.88 (*arom.*, bt, 1H, $^3J_{\text{HH}} = 8$); 7.94 (*arom.*, bd, 1H, $^3J_{\text{HH}} = 8$). **ESI-HRMS:** 593.3310 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{29}\text{H}_{41}\text{N}_{10}\text{O}_4]^+ = 593.3307$). **EA** ($\text{C}_{29}\text{H}_{40}\text{N}_{10}\text{O}_4 \cdot 0.8\text{FA} \cdot 4.4\text{H}_2\text{O}$, $M_{\text{R}} = 708.8$): C 50.5 (50.8); H 7.2 (7.1); N 19.8 (19.5).



Synthesis of TD647: In a pear-shape glass flask (50 mL), **TD635** (375 mg; 727 μmol ; 1.0 equiv.) was dissolved in dry MeCN (20 mL) followed by addition of dried K_2CO_3 (300 mg; 2.17 mmol; 3.0 equiv.). Solution of **TD566** (189 mg; 731 μmol ; 1.0 equiv.) in dry MeCN (5 mL) was then added and the resulting suspension was stirred 24 h at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative

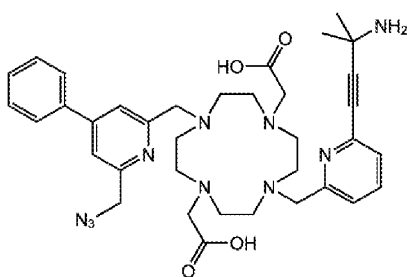
HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with *tert*-butyl protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in nearly zwitterionic form as white fluffy solid. **Yield:** 342 mg (72%; 2 steps; based on **TD635**). **NMR (D_2O , $\text{pD} \sim 4$):** ^1H δ_{H} 2.70–3.59 (*mc*, $\text{CH}_2\text{--CO}$, m, 16+4H); 3.66 ($\text{CH}_2\text{--arom.}$, bs, 2H); 3.69 ($\text{C}\equiv\text{CH}$, s, 1H); 3.90 ($\text{CH}_2\text{--arom.}$, bs, 2H); 4.64 ($\text{CH}_2\text{--N}_3$, s, 2H); 7.46–7.60 (*Ph*, *arom.*, m, 3+1H); 7.64 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 2$); 7.68 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$); 7.69 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 7.79–7.88 (*Ph*, m, 2H); 8.28 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$). $^{13}\text{C}\{^1\text{H}\}$ δ_{C} 48.2 (*mc*, s); 48.3 (*mc*, s); 51.2 (*mc*, s); 51.3 (*mc*, s); 54.7 ($\text{CH}_2\text{--N}_3$, s); 56.4 ($\text{CH}_2\text{--CO}$, s); 57.8 ($\text{CH}_2\text{--arom.}$, s); 59.1 ($\text{CH}_2\text{--arom.}$, s); 80.3 and 82.4 ($\text{C}\equiv\text{CH}$; $2 \times$ s); 121.9 (*arom.*, s); 124.4 (*arom.*, s); 125.6 (*arom.*, s); 128.2 (*Ph*, s); 128.5 (*Ph*, s); 130.0 (*Ph*, s); 130.8 (*arom.*, s); 137.7 (*Ph*, s); 140.3 (*arom.*, s); 141.2 (*arom.*, s); 153.6 (*arom.*, s); 155.4 (*arom.*, bs); 156.2 (*arom.*, s); 157.6 (*arom.*, s); 168.7 (CO, s). **ESI-HRMS:** 626.3195 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{33}\text{H}_{40}\text{N}_9\text{O}_4]^+ = 626.3198$). **EA** ($\text{C}_{33}\text{H}_{39}\text{N}_9\text{O}_4 \cdot 0.2\text{FA} \cdot 0.9\text{H}_2\text{O}$, $M_{\text{R}} = 651.2$): C 61.2 (61.6); H 6.4 (6.1); N 19.4 (19.1).



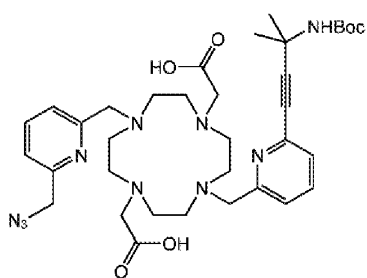
Synthesis of TD722: In a glass vial (20 mL), **TD539** (350 mg; 543 μmol ; 1.0 equiv.) was dissolved in MeCN (15 mL) followed by addition of dried K_2CO_3 (375 mg; 2.72 mmol; 5.0 equiv.). Solution of **TD566** (140 mg; 541 μmol ; 1.0 equiv.) in MeCN (3 mL) was then added and the resulting suspension was stirred 24 h at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC

(C18; H_2O –MeCN gradient with FA additive). Fractions with *tert*-butyl/Boc protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (5 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to

give product in the form of trifluoroacetate salt as slightly yellow solid. **Yield:** 295 mg (69%; 2 steps; based on **TD566**). **NMR (D₂O, pD ~7):** ¹H δ_H 2.74–3.64 (*mc*, CH₂–CO, m, 16+4H); 3.72 (CH₂–arom., bs, 2H); 3.98 (CH₂–arom., bs, 2H); 4.10 (CH₂–C≡C, s, 2H); 4.65 (CH₂–N₃, s, 2H); 7.47–7.69 (*Ph*, *arom.*, m, 3+3H); 7.72 (*arom.*, d, 1H, ⁴J_{HH} = 2); 7.85–7.96 (*Ph*, m, 2H); 8.41 (*arom.*, d, 1H, ⁴J_{HH} = 2). **ESI-HRMS:** 655.3460 [M+H]⁺ (theor. [C₃₄H₄₃N₁₀O₄]⁺ = 655.3463). **EA** (C₃₄H₄₂N₁₀O₄·0.7TFA·2.0H₂O, *M_R* = 770.6): C 55.2 (54.9); H 6.1 (5.8); N 18.2 (18.3); F 5.2 (5.3).

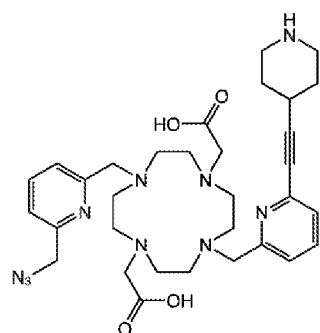


Synthesis of TD1054: In a glass vial (4 mL), **TD663** (30.0 mg; 45.3 μmol; 1.0 equiv.) was dissolved in MeCN (1 mL) followed by addition of dried K₂CO₃ (37.5 mg; 271 μmol; 5.0 equiv.). Solution of freshly prepared and isolated **TD1050** (≤65.3 μmol; ≤1.5 equiv.) in MeCN (1 mL) was then added and the resulting suspension was stirred 3 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with *tert*-butyl/Boc protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of mixed trifluoroacetate/formate salt as colourless solid. **Yield:** 23.0 mg (65%; 2 steps; based on **TD663**). **NMR (D₂O, pD ~8):** ¹H δ_H 1.72 (CH₃, s, 6H); 2.67–3.61 (*mc*, CH₂–CO, m, 16+4H); 3.70 (CH₂–arom., bs, 2H); 3.97 (CH₂–arom., bs, 2H); 4.64 (CH₂–N₃, s, 2H); 7.45–7.67 (*Ph*, *arom.*, m, 3+3H); 7.72 (*arom.*, d, 1H, ⁴J_{HH} = 2); 7.86–7.93 (*Ph*, m, 2H); 8.40 (*arom.*, d, 1H, ⁴J_{HH} = 2). **ESI-HRMS:** 683.3775 [M+H]⁺ (theor. [C₃₆H₄₇N₁₀O₄]⁺ = 683.3776). **EA** (C₃₆H₄₆N₁₀O₄·0.3TFA·0.8FA·2.3H₂O, *M_R* = 777.3): C 57.8 (58.0); H 6.5 (6.5); N 18.0 (17.7).

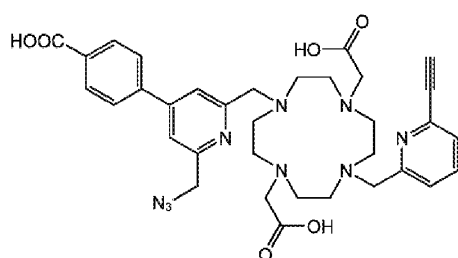


Synthesis of TD1105: In a glass vial (4 mL), **TD711** (101 mg; 185 μmol; 1.0 equiv.) was dissolved in MeCN (2 mL) followed by addition of dried K₂CO₃ (102 mg; 738 μmol; 4.0 equiv.). Solution of freshly prepared and isolated **TD1050** (≤222 μmol; ≤1.2 equiv.) in MeCN (1 mL) was then added and the resulting suspension was stirred 24 h at 40 °C. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with *tert*-butyl/Boc protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3

mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with fully deprotected intermediate were joined and directly lyophilized. The resulting solid was dissolved in a mixture of H₂O (6 mL), MeCN (6 mL) and THF (2 mL) followed by addition of **Boc₂O** (2.0 M in dry THF; 315 μ L; 630 μ mol; ≥ 3.4 equiv.) and **NaHCO₃** (210 mg; 2.50 mmol; ≥ 13.5 equiv.) The resulting mixture was stirred 2 d at RT after which another portion of **Boc₂O** (2.0 M in dry THF; 315 μ L; 630 μ mol; ≥ 3.4 equiv.) was added. The mixture was further stirred 16 h at RT. Mixture was evaporated to dryness and the residue was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined to give product in nearly zwitterionic form as white fluffy solid. **Yield:** 36.8 mg (27%; 3 steps; based on **TD711**). **NMR (D₂O, pD ~7):** ¹H δ_{H} 1.47 (CH₃, s, 9H); 1.65 (CH₃, s, 6H); 2.85–3.65 (*mc*, CH₂–CO, m, 16+4H); 3.91 (CH₂–arom., bs, 2H); 3.95 (CH₂–arom., bs, 2H); 4.59 (CH₂–N₃, s, 2H); 7.51 (*arom.*, dd, 1H, ³J_{HH} = 7, ⁴J_{HH} = 2); 7.59 (*arom.*, dd, 1H, ³J_{HH} = 7, ⁴J_{HH} = 2); 7.89–8.05 (*arom.*, m, 4H). **ESI-HRMS:** 707.3988 [M+H]⁺ (theor. [C₃₅H₅₁N₁₀O₆]⁺ = 707.3988). **EA** (C₃₅H₅₀N₁₀O₆·0.1FA·2.0H₂O, *M_R* = 747.5): C 56.4 (56.7); H 7.3 (7.0); N 18.7 (18.5).

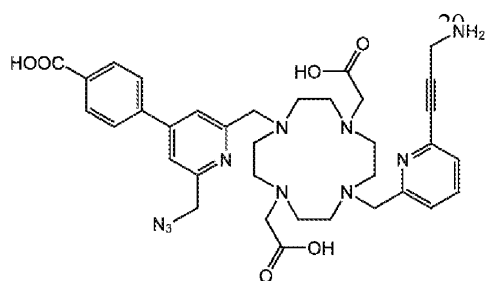


Synthesis of TD1127: In a glass vial (20 mL), **TD1118** (56.9 mg; 81.4 μ mol; 1.0 equiv.) was dissolved in MeCN (2 mL) followed by addition of dried **K₂CO₃** (45.0 mg; 326 μ mol; 4.0 equiv.) and **TD406** (16.0 mg; 87.6 μ mol; 1.1 equiv.) in MeCN (1 mL). The resulting suspension was stirred 16 h at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with *tert*-butyl/Boc protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of mixed trifluoroacetate/formate salt as colourless solid. **Yield:** 49.0 mg (71%; 2 steps; based on **TD1118**). **NMR (D₂O, pD ~7):** ¹H δ_{H} 1.96–2.11 (CH₂, m, 2H); 2.14–2.29 (CH₂, m, 2H); 2.86–3.63 (*mc*, CH₂–CO, CH₂, CH, m, 16+4+4+1H); 3.90 (CH₂–arom., bs, 2H); 3.94 (CH₂–arom., bs, 2H); 4.59 (CH₂–N₃, s, 2H); 7.49–7.55 (*arom.*, m, 1H); 7.60 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 2); 7.92–8.04 (*arom.*, m, 4H). **ESI-HRMS:** 633.3612 [M+H]⁺ (theor. [C₃₂H₄₅N₁₀O₄]⁺ = 633.3620). **EA** (C₃₂H₄₄N₁₀O₄·1.8TFA·0.1FA, *M_R* = 878.6): C 50.9 (50.7); H 5.5 (5.9); N 16.6 (16.9).



Synthesis of TD742: In a glass vial (20 mL), **TD635** (90 mg; 163 μmol ; 1.1 equiv.) was dissolved in MeCN (6 mL) followed by addition of dried K_2CO_3 (85 mg; 616 μmol ; 4.0 equiv.). Solution of **TD733** (55 mg; 153 μmol ; 1.0 equiv.) in MeCN (2 mL) was then added and the resulting suspension was stirred 18 h at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was

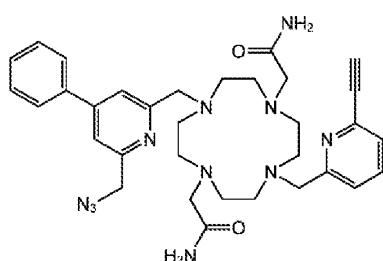
purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with *tert*-butyl protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in nearly zwitterionic form as white fluffy solid. **Yield:** 87 mg (80%; 2 steps; based on **TD733**). **NMR (D_2O , pD ~4):** ^1H δ_{H} 2.76–3.64 (*mc*, $\text{CH}_2\text{--CO}$, m, 16+4H); 3.69 ($\text{C}\equiv\text{CH}$, s, 1H); 3.72 ($\text{CH}_2\text{--arom.}$, bs, 2H); 3.99 ($\text{CH}_2\text{--arom.}$, bs, 2H); 4.77 ($\text{CH}_2\text{--N}_3$, s, 2H); 7.54–7.70 (*arom.*, m, 3H); 7.85 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 7.96 (*arom.*, d, 2H, $^3J_{\text{HH}} = 8$); 8.11 (*arom.*, d, 2H, $^3J_{\text{HH}} = 8$); 8.38 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$). **ESI-HRMS:** 670.3094 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{34}\text{H}_{40}\text{N}_9\text{O}_6]^+ = 670.3096$). **EA** ($\text{C}_{34}\text{H}_{40}\text{N}_9\text{O}_6 \cdot 0.2\text{FA} \cdot 1.6\text{H}_2\text{O}$, $M_{\text{R}} = 707.8$): C 58.0 (58.4); H 6.1 (5.9); N 17.8 (17.5).



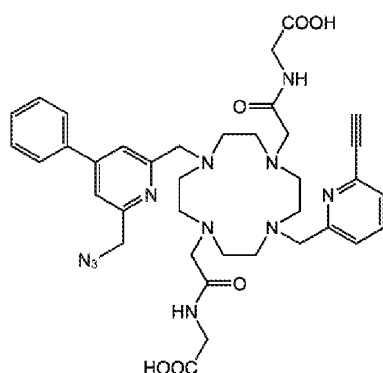
Synthesis of TD744: In a glass vial (20 mL), **TD539** (80 mg; 124 μmol ; 1.1 equiv.) was dissolved in MeCN (3 mL) followed by addition of dried K_2CO_3 (65 mg; 471 μmol ; 4.0 equiv.). Solution of **TD733** (42 mg; 117 μmol ; 1.0 equiv.) in MeCN (3 mL) was then added and the resulting suspension was stirred 24 h at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness.

Residue was purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of mixed trifluoroacetate/formate salt as off white fluffy solid. **Yield:** 44 mg (45%; 2 steps; based on **TD733**). **NMR (D_2O , pD ~4):** ^1H δ_{H} 2.71–3.64 (*mc*, $\text{CH}_2\text{--CO}$, m, 16+4H); 3.70 ($\text{CH}_2\text{--arom.}$, bs, 2H); 3.98 ($\text{CH}_2\text{--arom.}$, bs, 2H); 4.12 ($\text{CH}_2\text{--C}\equiv\text{C}$, s, 2H); 4.70 ($\text{CH}_2\text{--N}_3$, s, 2H); 7.50–7.59 (*arom.*, m, 2H); 7.63 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 7$, $^4J_{\text{HH}} = 2$); 7.78 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 7.95

(*arom.*, d, 2H, $^3J_{\text{HH}} = 9$); 8.07 (*arom.*, d, 2H, $^3J_{\text{HH}} = 9$); 8.42 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$). **ESI-HRMS**: 699.3358 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{35}\text{H}_{43}\text{N}_{10}\text{O}_6]^+ = 699.3362$). **EA** ($\text{C}_{35}\text{H}_{42}\text{N}_{10}\text{O}_6 \cdot 1.0\text{TFA} \cdot 1.3\text{H}_2\text{O}$, $M_{\text{R}} = 836.2$): C 53.1 (52.8); H 5.5 (5.8); N 16.8 (16.9). **Synthesis of TD750**: In a glass vial (2 mL), **TD744** ($1.0\text{TFA} \cdot 1.3\text{H}_2\text{O}$ (1.5 mg; ~ 1.8 μmol ; 1.0 equiv.)) was dissolved in aq. **Borate/NaOH buffer** (200 mM; pH 9.0; 900 μL ; 180 mmol; ~ 100 equiv.). followed by addition of freshly prepared solution of **FmocCl** (0.6 mg; ~ 2.3 μmol ; 1.3 equiv.) in MeCN (900 μL). The resulting clear solution was stirred 2 h at RT. Mixture was then directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in nearly zwitterionic form as white fluffy solid. **Yield**: 0.8 mg ($\leq 48\%$ assuming **M** $\cdot 0.2\text{FA} \cdot x\text{H}_2\text{O}$; $M_{\text{R}} = 930.2$; 1 step; based on **TD744** $\cdot 1.0\text{TFA} \cdot 1.3\text{H}_2\text{O}$). **ESI-HRMS**: 921.4044 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{50}\text{H}_{53}\text{N}_{10}\text{O}_8]^+ = 921.4042$).

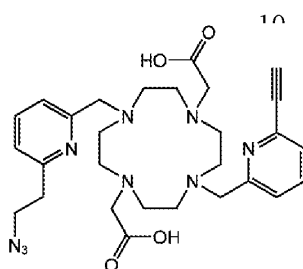


Synthesis of TD779: In a glass vial (4 mL), **TD647** ($0.2\text{FA} \cdot 0.9\text{H}_2\text{O}$ (10 mg; 15 μmol ; 1.0 equiv.) and **Ammonium chloride** (2 mg; 38 μmol ; 2.5 equiv.) were dissolved in DMSO (2 mL) followed by addition of **DIPEA** (22 μL ; 126 μmol ; 8.2 equiv.) and solid **HATU** (18 mg; 47 μmol ; 3.1 equiv.). The resulting yellow solution was stirred 15 min at RT. Mixture was then quenched with **FA** (6 μL ; 169 μmol ; 10 equiv.) and directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in nearly zwitterionic form as white fluffy solid. **Yield**: 7 mg ($\leq 73\%$ assuming **M** $\cdot 0.1\text{FA} \cdot x\text{H}_2\text{O}$; $M_{\text{R}} = 628.4$; 1 step; based on **TD647** $\cdot 0.2\text{FA} \cdot 0.9\text{H}_2\text{O}$). **NMR** (**D₂O**+**FA**, **pD** ~ 3): ^1H δ_{H} 2.83–3.74 (*mc*, $\text{CH}_2\text{--CO}$, *bm*, 16+4H); 3.65 ($\text{C}\equiv\text{CH}$, s, 1H); 4.13–4.68 ($\text{CH}_2\text{--arom.}$, *bm*, 4H); 4.69 ($\text{CH}_2\text{--N}_3$, s, 2H); 7.52–7.66 (*Ph*, *arom.*, m, 3+1H); 7.71 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.80–7.88 (*Ph*, m, 2H); 7.90 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 7.91 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$); 8.00 (*arom.*, bs, 1H). **ESI-HRMS**: 624.3503 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{33}\text{H}_{42}\text{N}_{11}\text{O}_2]^+ = 624.3504$).



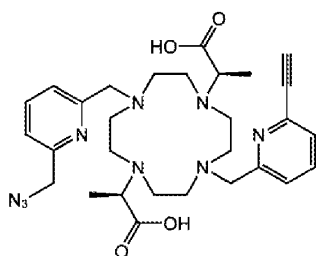
Synthesis of TD778: In a glass vial (4 mL), **TD647** ($0.2\text{FA} \cdot 0.9\text{H}_2\text{O}$ (12 mg; 18 μmol ; 1.0 equiv.) and **Glycine tert-butyl ester hydrochloride** (8 mg; 48 μmol ; 2.6 equiv.) were dissolved in DMSO (2 mL) followed by addition of **DIPEA** (26 μL ; 149 μmol ; 8.1 equiv.) and solid **HATU** (21 mg; 55 μmol ; 3.0 equiv.). The resulting yellow solution was stirred 20 min at RT. Mixture was then quenched with **FA** (7 μL ; 186 μmol ; 10 equiv.) and directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with *tert*-butyl protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture

was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in nearly zwitterionic form as white fluffy solid. **Yield:** 9 mg (62%; 2 steps; based on **TD647**·0.2FA·0.9H₂O). **NMR (D₂O, pD ~5):** ¹H δ_H 2.83–3.93 (*mc*, CH₂–CO, C≡CH, bm, 16+8+1H); 4.35–4.69 (CH₂–arom., CH₂–N₃., bm, 4+2H); 7.49 (*arom.*, d, 1H, ³J_{HH} = 8); 7.56–7.73 (*Ph*, *arom.*, m, 3+1H); 7.75–7.92 (*Ph*, *arom.*, m, 2+2H); 8.06 (*arom.*, s, 1H). **ESI-HRMS:** 738.3471 [M–H][–] (theor. [C₃₇H₄₄N₁₁O₆][–] = 738.3482). **EA** (C₃₇H₄₅N₁₁O₆·0.1FA·2.4H₂O, M_R = 787.7): C 56.6 (55.9); H 6.4 (5.8); N 19.6 (20.3).



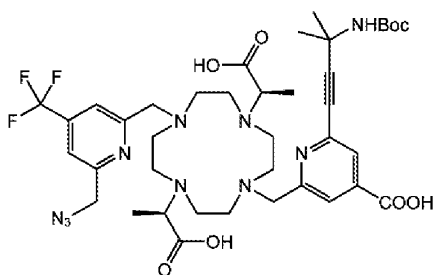
Synthesis of TD1408: In a glass vial (25 mL), **TD635** (51 mg; 99 μmol; 1.1 equiv.) was dissolved in MeCN (1 mL) followed by addition of dried K₂CO₃ (54 mg; 391 μmol; 4.4 equiv.). Solution of **TD1406** (17.5 mg; 89 μmol; 1.0 equiv.) in MeCN (1 mL) was then added and the resulting suspension was stirred 16 h at 40 °C. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with *tert*-butyl

protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in nearly zwitterionic form as white fluffy solid. **Yield:** 41.7 mg (77%; 2 steps; based on **TD1406**). **NMR (D₂O, pD ~7):** ¹H δ_H 2.74–4.16 (*mc*, CH₂–CO, CH₂–arom., CH₂–CH₂–N₃, C≡CH, m, 16+4+4+4+1H); 7.37–7.45 (*arom.*, m, 1H); 7.65 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.85–7.93 (*arom.*, m, 2H); 7.96 (*arom.*, t, 1H, ³J_{HH} = 8); 8.06 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1). **ESI-HRMS:** 564.3044 [M+H]⁺ (theor. [C₂₈H₃₈N₉O₄]⁺ = 564.3041). **EA** (C₂₈H₃₇N₉O₄·0.1FA·2.4H₂O, M_R = 611.5): C 55.2 (56.0); H 6.9 (6.7); N 20.6 (19.8).



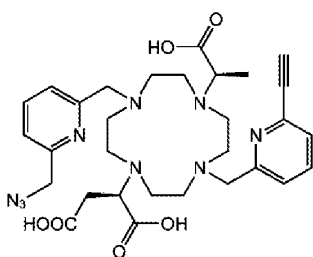
Synthesis of TD1346: In a pear-shaped glass flask (25 mL), **TD1345** (52.7 mg; 83 μmol; 1.0 equiv.) was dissolved in 60 % aq. MeCN (2.7 mL) followed by addition of freshly prepared aq. LiOH (1.0 M; 830 μL; 830 μmol; 10 equiv.). Resulting solution was stirred at RT for 5 h. Mixture was quenched by addition of FA (31 μL; 822 μmol; 10 equiv.). The resulting solution was briefly concentrated to remove most of MeCN and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of free base as white fluffy solid.

Yield: 35.4 mg (71%; 1 step; based on **TD1345**). **NMR (D₂O, pD ~7):** ¹H δ_H 1.24–1.42 (CH₃, m, 6H); 2.44–3.81 (*mc*, CH₂–arom., C≡CH, m, 16+4+1H); 4.41–4.53 (CH–CH₃, m, 2H); 4.56 (CH₂–N₃, s, 2H); 7.49 (*arom.*, d, 1H, ³J_{HH} = 8); 7.63 (*arom.*, d, 1H, ³J_{HH} = 8); 7.93 (*arom.*, t, 1H, ³J_{HH} = 8); 7.96 (*arom.*, t, 1H, ³J_{HH} = 8); 8.05 (*arom.*, d, 1H, ³J_{HH} = 8); 8.10 (*arom.*, d, 1H, ³J_{HH} = 8). **ESI-HRMS:** 578.3197 [M+H]⁺ (theor. [C₂₉H₄₀N₉O₄]⁺ = 578.3198). **EA** (C₂₉H₃₉N₉O₄·1.2H₂O, M_R = 599.3): C 58.1 (58.0); H 7.0 (6.5); N 21.0 (20.6).



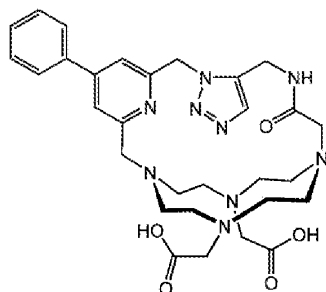
Synthesis of TD1451: In a pear-shaped glass flask (25 mL), **TD1449** (302 mg; 330 μmol; 1.0 equiv.) was dissolved in 60 % aq. MeCN (17 mL) followed by addition of freshly prepared aq. **LiOH** (1.0 M; 3.3 mL; 3.3 mmol; 10 equiv.). Resulting solution was stirred at RT for 2 h. Mixture was briefly concentrated to remove most of MeCN and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions

with product were joined and directly lyophilized to give product in nearly zwitterionic form as white fluffy solid. **Yield:** 257 mg (87%; 1 step; based on **TD1449**). **NMR (D₂O, pD ~3):** ¹H δ_H 1.37–1.60 (CH₃–C–O, CH₃–CH, m, 9+6H); 1.67 (CH₃–C–N, s, 6H); 2.66–4.30 (*mc*, CH₂–arom., CH–CH₃, m, 16+4+2H); 4.68 (CH₂–N₃, s, 2H); 7.80 (*arom.*, s, 1H); 7.92 (*arom.*, s, 1H); 7.98 (*arom.*, s, 1H); 8.02 (*arom.*, s, 1H). ¹⁹F δ_F –64.44 (s). **ESI-HRMS:** 847.4070 [M+H]⁺ (theor. [C₃₉H₅₄N₁₀O₈F₃]⁺ = 847.4070). **EA** (C₃₉H₅₃N₁₀O₈F₃·0.1FA·2.7H₂O, M_R = 900.1): C 52.2 (53.1); H 6.6 (6.2); N 15.6 (15.5); 6.3 (5.4).



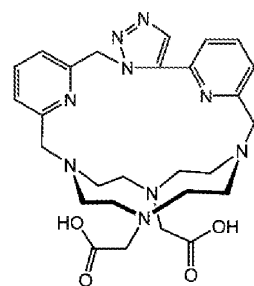
Synthesis of TD1504: In a glass vial (25 mL), **TD1500** (11.9 mg; 18 μmol; 1.0 equiv.) was dissolved in MeCN (800 μL) and H₂O (550 μL) followed by addition of freshly prepared aq. **LiOH** (1.0 M; 263 μL; 263 μmol; 15 equiv.). Resulting solution was stirred at RT for 3 h. Mixture was quenched by addition of **FA** (8 μL; 212 μmol; 12 equiv.). The resulting solution was diluted with H₂O (2 mL) and then directly

purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of free base as white fluffy solid. **Yield:** 8.9 mg (~77 % assuming **M**·2H₂O; M_R = 657.7; 2 steps; based on **TD1500**). **NMR (D₂O, pD ~3):** ¹H δ_H 1.28–1.45 (CH₃, m, 3H); 2.49–3.70 (*mc*, CH–CH₃, CH–CH₂, CH₂–arom., m, 16+1+3+4H); 3.76 (C≡CH, s, 1H); 4.35–4.51 (CH₂–N₃, m, 2H); 7.69 (*arom.*, dd, 2H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.98 (*arom.*, t, 1H, ³J_{HH} = 8); 8.05–8.21 (*arom.*, m, 3H). **ESI-MS (LC-MS):** 622.3 [M+H]⁺ (theor. [C₃₀H₄₀N₉O₆]⁺ = 622.3).

Example 5: Synthesis of caged macrocyclic ligands (bridged with a triazole group)

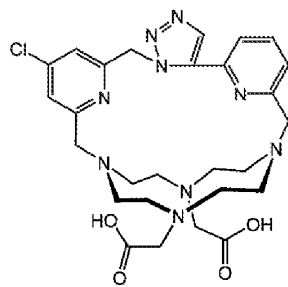
Synthesis of cz-TD425: Obtained as side product during synthesis of **TD425** in nearly zwitterionic form as white fluffy solid. **Yield:** 3 mg ($\leq 1\%$ assuming $\mathbf{M} \cdot 0.1\text{FA} \cdot x\text{H}_2\text{O}$; $M_R = 609.9$; 3 steps; based on **2-Chloro-N-(prop-2-yn-1-yl)acetamide**). **NMR (D_2O , $\text{pD} \sim 3$):** ^1H δ_{H} 2.52–5.20 (*mc*, $\text{CH}_2\text{-CO}$, $\text{CH}_2\text{-NH-CO-CH}_2$, $\text{CH}_2\text{-arom.}$, *bm*, 16+4+4+2H); 6.24 ($\text{CH}_2\text{-N}_3$, *bs*, 2H); 7.56–7.72 (*Ph*, *m*, 3H); 7.88 (CH-N_3 , *s*, 1H); 7.88–7.94 (*Ph*, *m*, 2H); 8.33 (*arom.*, *d*, 1H, $^4J_{\text{HH}} = 2$); 8.73 (*arom.*, *d*, 1H, $^4J_{\text{HH}} = 2$). **ESI-HRMS:** 606.3143

10 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{30}\text{H}_{40}\text{N}_9\text{O}_5]^+ = 606.3147$).



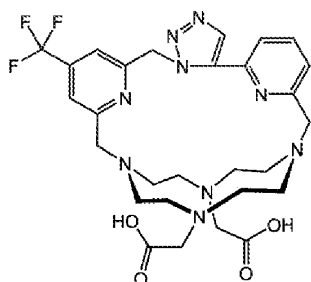
Synthesis of cz-TD556: Obtained as side product during synthesis of **TD556** in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 9 mg ($\sim 2\%$; 2 steps; based on **TD635**). **NMR (D_2O , $\text{pD} \sim 3$):** ^1H δ_{H} 2.54–4.06 (*mc*, $\text{CH}_2\text{-CO}$, *bm*, 16+4H); 4.59 ($\text{CH}_2\text{-arom.}$, *bs*, 2H); 5.02 ($\text{CH}_2\text{-arom.}$, *bs*, 2H); 5.86 ($\text{CH}_2\text{-N}_3$, *bs*, 2H); 7.67 (*arom.*, *bd*, 1H, $^3J_{\text{HH}} = 8$); 7.70 (*arom.*, *bd*, 1H, $^3J_{\text{HH}} = 8$); 7.83 (*arom.*, *bd*, 1H, $^3J_{\text{HH}} = 8$); 7.87 (*arom.*, *bd*, 1H, $^3J_{\text{HH}} = 8$); 8.14 (*arom.*, *bt*, 1H, $^3J_{\text{HH}} = 8$); 8.16 (CH-N_3 , *bs*, 1H); 8.17 (*arom.*, *bt*, 1H, $^3J_{\text{HH}} = 8$). **ESI-HRMS:** 550.2889 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{27}\text{H}_{36}\text{N}_9\text{O}_4]^+ = 550.2885$). **EA** ($\text{C}_{27}\text{H}_{35}\text{N}_9\text{O}_4 \cdot 2.7\text{TFA} \cdot 0.5\text{H}_2\text{O}$, $M_R = 866.4$): C 44.9 (44.8); H 4.5 (4.8); N 14.5 (14.8).

20

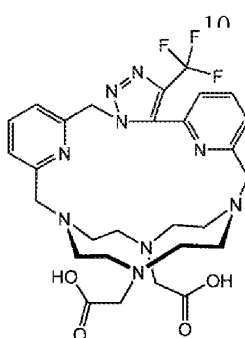


Synthesis of cz-TD764: Obtained as side product during synthesis of **TD764** in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 9 mg ($\sim 2\%$; 2 steps; based on **TD635**). **NMR (D_2O , $\text{pD} \sim 3$):** ^1H δ_{H} 2.48–3.94 (*mc*, $\text{CH}_2\text{-CO}$, *bm*, 16+4H); 4.57 ($\text{CH}_2\text{-arom.}$, *bs*, 2H); 5.00 ($\text{CH}_2\text{-arom.}$, *bs*, 2H); 5.94 ($\text{CH}_2\text{-N}_3$, *bs*, 2H); 7.64 (*arom.*, *d*, 1H, $^3J_{\text{HH}} = 8$); 7.79 (*arom.*, *s*, 1H); 7.84 (*arom.*, *d*, 1H, $^3J_{\text{HH}} = 8$); 7.92 (*arom.*, *s*, 1H); 8.14 (*arom.*, *t*, 1H, $^3J_{\text{HH}} = 8$); 8.15 (CH-N_3 , *s*, 1H). **ESI-HRMS:** 582.2344 $[\text{M}-\text{H}]^-$ (theor. $[\text{C}_{27}\text{H}_{33}\text{N}_9\text{O}_4\text{Cl}_1]^- = 582.2350$). **EA** ($\text{C}_{27}\text{H}_{34}\text{N}_9\text{O}_4\text{Cl}_1 \cdot 1.8\text{TFA} \cdot 3.3\text{H}_2\text{O}$, $M_R = 848.7$): C 43.3 (43.3); H 5.0 (4.5); N 14.9 (14.5); Cl 4.2 (4.2); F 12.1 (12.0).

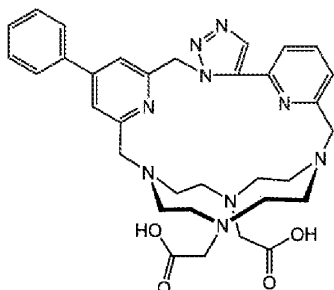
30



Synthesis of cz-TD1063: Obtained as side product during synthesis of **TD1113** in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 1.7 mg. **NMR (D₂O, pD ~3):** ¹H δ_H 2.57–3.84 (*mc*, CH₂–CO, m, 16+4H); 4.70 (CH₂–arom., s, 2H); 4.99 (CH₂–arom., bs, 2H); 6.03 (CH₂–N₃, bs, 2H); 7.65 (*arom.*, d, 1H, ³J_{HH} = 8); 7.86 (*arom.*, d, 1H, ³J_{HH} = 8); 8.05 (*arom.*, s, 1H); 8.12–8.18 (*arom.*, CH–N₃, m, 1+1H); 8.19 (*arom.*, s, 1H). ¹⁹F δ_F –65.82 (s). **NMR (~0.5 mM cz-TD1063 in ~50 mM MOPS/NaOH buffer in 10% H₂O, pH 7.0):** ¹⁹F δ_F –65.66 (s; ΔH_{1/2} = 4.6 Hz). **ESI-HRMS:** 618.2754 [M+H]⁺ (theor. [C₂₉H₃₅N₉O₄F₃]⁺ = 618.2759).

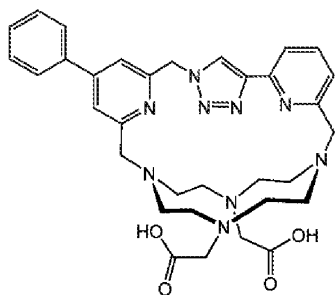


Synthesis of TD1188: In a glass vial (4 mL), **TD711** (70.0 mg; 128 μmol; 1.0 equiv.) was dissolved in MeCN (1.5 mL) followed by addition of dried **K₂CO₃** (71 mg; 514 μmol; 4.0 equiv.). Solution of **TD1178** (31 mg; 141 μmol; 1.1 equiv.) in MeCN (1.5 mL) was then added and the resulting suspension was stirred 7 d at RT. Solids were filtered off using syringe microfilter (PTFE) and filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with *tert*-butyl protected product were combined, evaporated to dryness and twice co-evaporated with MeOH to remove most of MeCN. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and twice co-evaporated with MeOH to remove most of TFA. Residue was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in zwitterionic form as white fluffy solid. **Yield:** 9.5 mg (10%; 2 steps; based on **TD711**). **NMR (D₂O, pD ~5):** ¹H δ_H 2.11–4.00 (*mc*, CH₂–CO, bm, 16+4H); 4.52 (CH₂–arom., bs, 2H); 4.88–6.58 (CH₂–arom., CH₂–N₃, bm, 4H); 7.66 (*arom.*, bd, 1H, ³J_{HH} = 8); 7.68 (*arom.*, d, 1H, ³J_{HH} = 8); 7.75 (*arom.*, bd, 1H, ³J_{HH} = 8); 7.85 (*arom.*, d, 1H, ³J_{HH} = 8); 8.08 (*arom.*, bt, 1H, ³J_{HH} = 8); 8.16 (*arom.*, t, 1H, ³J_{HH} = 8). ¹⁹F δ_F –59.35 (s). **ESI-HRMS:** 618.2756 [M+H]⁺ (theor. [C₂₈H₃₅N₉O₄F₃]⁺ = 618.2759). **EA** (C₂₈H₃₄N₉O₄F₃·0.2TFA·0.4FA·4.3H₂O, *M_R* = 736.3): C 47.0 (46.6); H 6.0 (5.6); N 17.1 (16.8); F 9.3 (10.1).

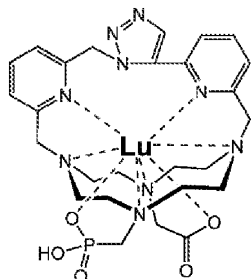


Synthesis of TD650: In a glass vial (40 mL), **TD647**·0.2FA·0.9H₂O (130 mg; 200 μmol; 1.0 equiv.) was dissolved in H₂O (20 mL) followed by addition aq. **Citric acid** (100 mM; 20 mL; 2.0 mmol; 10 equiv.) to reach pH 2.4. Resulting mixture was then stirred 2 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined

and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 109 mg (77%; 1 step; based on **TD647**·0.2FA·0.9H₂O). **NMR (D₂O, pD ~5, 95 °C):** ¹H δ_H 2.69–3.63 (*mc*, CH₂–CO, m, 16+4H); 4.22 (CH₂–arom., s, 2H); 4.58 (CH₂–arom., s, 2H); 6.22 (CH₂–N₃, s, 2H); 7.61 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.60–7.67 (*Ph*, m, 3H); 7.83 (*arom.*, s, 1H); 7.85 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.86–7.92 (*Ph*, m, 2H); 8.00 (*arom.*, s, 1H); 8.11 (*arom.*, t, 1H, ³J_{HH} = 8); 8.21 (CH–N₃, s, 1H). ¹³C{¹H} δ_C 48.5 (*mc*, s); 49.9 (*mc*, s); 50.2 (*mc*, s); 50.4 (*mc*, s); 53.4 (CH₂–N₃, s); 55.6 (CH₂–CO, s); 57.8 (CH₂–arom., s); 59.2 (CH₂–arom., s); 122.6 (*arom.*, s); 124.2 (*arom.*, s); 125.4 (*arom.*, s); 125.9 (*arom.*, s); 127.8 (*Ph*, s); 129.9 (*Ph*, s); 130.7 (*Ph*, s); 134.5 (CH–N₃, s); 137.0 (*Ph*, s); 138.0 (*arom.*, s); 139.9 (*arom.*, s); 147.1 (*arom.*, s); 152.3 (*arom.*, bs); 153.6 (*arom.*, bs); 153.8 (*arom.*, bs); 156.8 (*arom.*, s); 172.6 (CO, s). **ESI-HRMS:** 626.3200 [M+H]⁺ (theor. [C₃₃H₄₀N₉O₄]⁺ = 626.3198). **EA** (C₃₃H₃₉N₉O₄·0.9FA·2.0H₂O, *M_R* = 703.2): C 57.9 (58.3); H 6.4 (5.9); N 17.9 (17.5).



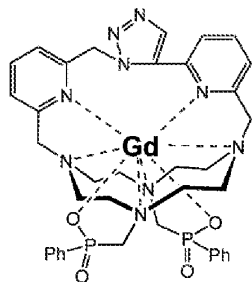
Synthesis of TD871: In a glass vial (40 mL), **TD647**·1.3TFA (78.0 mg; 103 μmol; 1.0 equiv.) was dissolved in H₂O (34 mL) followed by addition aq. **Borate/CsOH buffer** (200 mM; pH 9.0; 6.0 mL; 1.2 mmol; ~12 equiv.). Resulting mixture was then stirred 6 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 49.3 mg (70%; 1 step; based on **TD647**·1.3TFA). **NMR (D₂O, pD ~5, 95 °C):** ¹H δ_H 2.93–3.56 (*mc*, CH₂–CO, m, 16+4H); 4.41 (CH₂–arom., s, 2H); 4.49 (CH₂–arom., s, 2H); 6.00 (CH₂–N₃, s, 2H); 7.54 (*arom.*, dd, 1H, ³J_{HH} = 7, ⁴J_{HH} = 2); 7.56–7.66 (*Ph*, m, 3H); 7.77 (*arom.*, d, 1H, ⁴J_{HH} = 1); 7.79–7.87 (*Ph*, m, 2H); 7.94 (*arom.*, d, 1H, ⁴J_{HH} = 1); 8.05 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 2); 8.07 (*arom.*, dd, 1H, ³J_{HH} = 8, ³J_{HH} = 7); 8.93 (CH–N₃, s, 1H). ¹³C{¹H} δ_C 50.0 (*mc*, s); 50.7 (*mc*, bs); 52.0 (*mc*, s); 52.3 (*mc*, s); 54.8 (CH₂–N₃, s); 56.6 (CH₂–CO, s); 59.9 (CH₂–arom., s); 60.2 (CH₂–arom., bs); 121.2 (*arom.*, s); 121.7 (*arom.*, s); 123.3 (*arom.*, s); 124.5 (*arom.*, s); 127.0 (CH–N₃, s); 127.7 (*Ph*, s); 129.9 (*Ph*, s); 130.5 (*Ph*, s); 137.1 (*Ph*, s); 140.2 (*arom.*, s); 148.0 (*arom.*, s); 150.3 (*arom.*, s); 151.7 (*arom.*, s); 152.5 (*arom.*, bs); 154.9 (*arom.*, s); 155.0 (*arom.*, s); 174.4 (CO, s). **ESI-HRMS:** 626.3199 [M+H]⁺ (theor. [C₃₃H₄₀N₉O₄]⁺ = 626.3198). **EA** (C₃₃H₃₉N₉O₄·0.6FA·2.3H₂O, *M_R* = 696.8): C 58.1 (57.9); H 6.5 (6.2); N 18.1 (18.2).

Example 6: Synthesis of coordination compounds

Synthesis of TD734: In a glass vial (20 mL), **TD714**·0.1FA·xH₂O (6 mg; ~10 μmol; 1.0 equiv.) was dissolved in H₂O (4 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 1.00 mL; 500 μmol; ~50 equiv.) and aq. **LuCl₃** (100 mM; 110 μL; 11 μmol; ~1.1 equiv.) and the mixture was stirred 16 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly

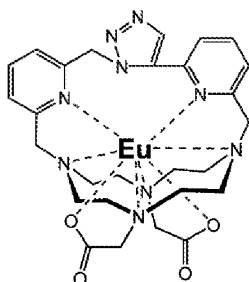
lyophilized to give product as a mixture of two diastereoisomer (~11:1) in the form of formate salt as white

10 fluffy solid. **Yield:** 5 mg (≤61% assuming $[M]^+[FA]^- \cdot xH_2O$; $M_R = 803.6$; 1 step; based on **TD714**·0.1FA·xH₂O). **NMR (D₂O, pD ~4, signals of major isomer):** ¹H δ_H 2.50–3.82 (*mc*, CH₂–CO, CH₂–P, m, 16+2+1H); 3.85–4.00 (CH₂–arom., CH₂–P, m, 1+1H); 4.10 (CH₂–arom., d, 1H, ²J_{HH} = 16); 4.23 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.99 (CH₂–arom., d, 1H, ²J_{HH} = 15); 5.53 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.56 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.67 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.73 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.81 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 2); 7.83 (CH–N₃, s, 1H); 7.98 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 2); 7.18 (*arom.*, t, 1H, ³J_{HH} = 8); 8.20 (*arom.*, t, 1H, ³J_{HH} = 8); 8.35 (FA, s, 1H). ¹³C{¹H} δ_C 48.9 (*mc*, s); 51.3 (*mc*, s); 51.6 (CH₂–P, d, ¹J_{CP} = 73); 52.0 (*mc*, s); 52.3 (*mc*, s); 53.4 (*mc*, s); 53.5 (CH₂–N₃, s); 53.6 (*mc*, s); 55.0 (*mc*, s); 56.3 (*mc*, s); 59.7 (CH₂–arom., s); 60.0 (CH₂–arom., s); 61.0 (CH₂–CO, s); 125.9 (*arom.*, s); 127.6 (*arom.*, s); 128.9 (*arom.*, s); 130.4 (*arom.*, s); 136.4 (CH–N₃, s); 138.7 (*arom.*, s); 142.0 (*arom.*, s); 142.9 (*arom.*, s); 146.2 (*arom.*, s); 156.8 (*arom.*, s); 157.6 (*arom.*, s); 158.6 (*arom.*, s); 169.5 (FA, s); 178.2 (CO, s). ³¹P{¹H} δ_P 17.8 (s). **ESI-HRMS:** 758.1822 $[M]^+$ (theor. $[C_{26}H_{34}N_9O_5P_1Lu_1]^+ = 758.1823$).

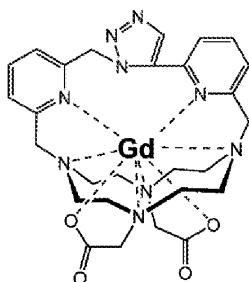


Synthesis of TD925: In a glass vial (20 mL), **TD921**·4.6H₂O (6.1 mg; 7.4 μmol; 1.0 equiv.) was dissolved in H₂O (18 mL) and *i*-PrOH (1 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 400 μL; 200 μmol; 27 equiv.) and aq. **GdCl₃** (100 mM; 95 μL; 9.5 μmol; 1.3 equiv.) and the mixture was stirred 7 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined

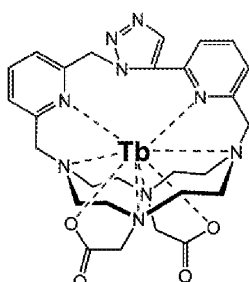
30 and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 2.5 mg (≤33% assuming $[M]^+[TFA]^- \cdot xH_2O$; $M_R = 1010.0$; 1 step; based on **TD921**·4.6H₂O). **ESI-HRMS:** 897.2158 $[M]^+$ (theor. $[C_{37}H_{43}N_9O_4P_2Gd_1]^+ = 897.2149$).



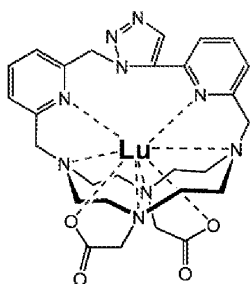
- Synthesis of TD560:** In a glass vial (20 mL), **TD556**·0.1FA·1.6H₂O (39 mg; 69 μmol; 1.0 equiv.) was dissolved in H₂O (15 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 3.40 mL; 1.70 mmol; 25 equiv.) and aq. **EuCl₃** (100 mM; 745 μL; 75 μmol; 1.1 equiv.) and the mixture was stirred 4 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 24 mg (43%; 1 step; based on **TD556**·0.1FA·1.6H₂O). **ESI-HRMS:** 700.1867 [M]⁺ (theor. [C₂₇H₃₃N₉O₄Eu]⁺ = 700.1862). **EA** ([C₂₇H₃₃N₉O₄Eu]⁺[FA]⁻·4.5H₂O, *M_R* = 825.7): C 40.7 (40.7); H 5.2 (4.8); N 15.3 (15.0); Eu 18.4 (17.7).



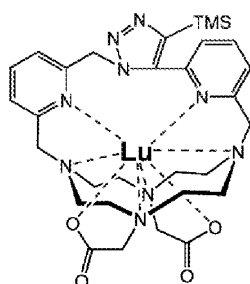
- Synthesis of TD561:** In a glass vial (20 mL), **TD556**·0.1FA·1.6H₂O (40 mg; 69 μmol; 1.0 equiv.) was dissolved in H₂O (15 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 3.40 mL; 1.70 mmol; 25 equiv.) and aq. **GdCl₃** (100 mM; 745 μL; 75 μmol; 1.1 equiv.) and the mixture was stirred 4 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 24 mg (42%; 1 step; based on **TD556**·0.1FA·1.6H₂O). **ESI-HRMS:** 705.1893 [M]⁺ (theor. [C₂₇H₃₃N₉O₄Gd]⁺ = 705.1891). **EA** ([C₂₇H₃₃N₉O₄Gd]⁺[FA]⁻·4.6H₂O, *M_R* = 832.8): C 40.4 (40.8); H 5.2 (4.9); N 15.1 (15.5); Gd 18.9 (18.1).



- Synthesis of TD562:** In a glass vial (20 mL), **TD556**·0.1FA·1.6H₂O (40 mg; 69 μmol; 1.0 equiv.) was dissolved in H₂O (15 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 3.40 mL; 1.70 mmol; 25 equiv.) and aq. **TbCl₃** (100 mM; 745 μL; 75 μmol; 1.1 equiv.) and the mixture was stirred 4 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 27 mg (47%; 1 step; based on **TD556**·0.1FA·1.6H₂O). **ESI-HRMS:** 706.1906 [M]⁺ (theor. [C₂₇H₃₃N₉O₄Tb]⁺ = 706.1903). **EA** ([C₂₇H₃₃N₉O₄Tb]⁺[FA]⁻·4.7H₂O, *M_R* = 836.2): C 40.2 (40.5); H 5.2 (4.9); N 15.1 (14.7); Tb 19.0 (18.7).

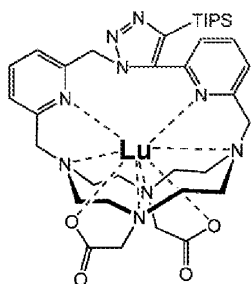


Synthesis of TD1069: In a glass vial (20 mL), **TD556**·0.1FA·1.6H₂O (20.8 mg; 35.7 μmol; 1.0 equiv.) was dissolved in H₂O (17 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 1.80 mL; 900 μmol; 25 equiv.) and aq. **LuCl₃** (100 mM; 400 μL; 40.0 μmol; 1.1 equiv.) and the mixture was stirred 16 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 26.6 mg (82%; 1 step; based on **TD556**·0.1FA·1.6H₂O). **NMR (D₂O, pD ~3):** ¹H δ_H 2.64–3.82 (*mc*, CH₂–CO, m, 16+4H); 3.95 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.15 (CH₂–arom., d, 1H, ²J_{HH} = 16); 4.29 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.85 (CH₂–arom., d, 1H, ²J_{HH} = 16); 5.60 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.10 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.73 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.80 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.85 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.88 (CH–N₃, s, 1H); 8.02 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 8.25 (*arom.*, t, 1+1H, ³J_{HH} = 8). **ESI-HRMS:** 722.2060 [M]⁺ (theor. [C₂₇H₃₃N₉O₄Lu₁]⁺ = 722.2058). **EA** ([C₂₇H₃₃N₉O₄Lu₁]⁺[TFA][−]·0.3TFA·2.4H₂O, M_R = 913.0): C 38.9 (38.7); H 4.2 (3.8); N 13.8 (13.7); Lu 19.2 (16.5).



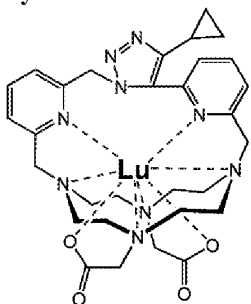
Synthesis of TD751: In a glass vial (20 mL), **TD718**·2.6H₂O (14 mg; 21 μmol; 1.0 equiv.) was dissolved in H₂O (18 mL) followed by addition aq. **MES/NaOH buffer** (500 mM; pH 5.2; 1.30 mL; 650 μmol; 31 equiv.) and aq. **LuCl₃** (100 mM; 236 μL; 24 μmol; 1.1 equiv.) and the mixture was stirred 3 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 13 mg (65%; 1 step; based on **TD718**·2.6H₂O). **NMR (D₂O, pD ~6):** ¹H δ_H 0.10 (CH₃, s, 9H); 2.61–3.83 (*mc*, CH₂–CO, m, 16+4H); 3.94 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.24 (CH₂–arom., d, 1H, ²J_{HH} = 16); 4.24 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.84 (CH₂–arom., d, 1H, ²J_{HH} = 16); 5.54 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 6.92 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.70 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.75 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.85 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.98 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 8.22 (*arom.*, t, 1H, ³J_{HH} = 8); 8.26 (*arom.*, t, 1H, ³J_{HH} = 8); 8.45 (*FA*, s, 1H). ¹³C{¹H} δ_C −1.85 (CH₃, s); 48.4 (*mc*, s); 48.7 (*mc*, s); 50.7 (*mc*, s); 51.9 (*mc*, s); 52.0 (CH₂–N₃, s); 53.2 (*mc*, s); 53.8 (*mc*, s); 54.4 (*mc*, s); 55.5 (*mc*, s); 58.4 (CH₂–CO, s); 59.0 (CH₂–arom., s); 59.1 (CH₂–arom., s); 60.9 (CH₂–CO, s); 125.9 (*arom.*, s); 126.7 (*arom.*, s); 128.3 (*arom.*, s); 130.4 (*arom.*, s); 141.4 (*arom.*, s); 142.3 (*arom.*, s); 142.6 (*arom.*, s); 146.3 (*arom.*, s); 147.5 (*arom.*, s); 155.1 (*arom.*, s); 157.7 (*arom.*, s); 158.7 (*arom.*, s); 171.0 (*FA*, s); 176.8 (CO, s); 178.6 (CO, s). **ESI-HRMS:** 794.2445 [M]⁺ (theor. [C₃₀H₄₁N₉O₄Si₁Lu₁]⁺ =

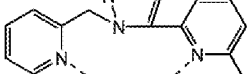
794.2453). **EA** ($[\text{C}_{30}\text{H}_{41}\text{N}_9\text{O}_4\text{Si}_1\text{Lu}_1]^{+}[\text{FA}]^{-} \cdot 6.6\text{H}_2\text{O}$, $M_{\text{R}} = 958.7$): C 38.8 (39.0); H 5.8 (5.3); N 13.1 (13.0); Si 2.9 (3.0); Lu 18.2 (17.4).



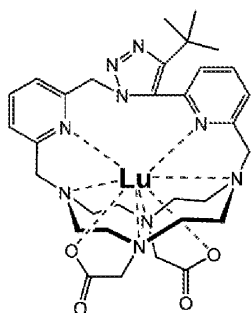
Synthesis of TD737: In a glass vial (20 mL), **TD728**·2.6H₂O (15 mg; 20 μmol; 1.0 equiv.) was dissolved in H₂O (17 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 2.00 mL; 1.00 mmol; 50 equiv.) and aq. **LuCl₃** (100 mM; 220 μL; 22 μmol; 1.1 equiv.) and the mixture was stirred 5 days at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 12 mg (58%; 1 step; based on **TD728**·2.6H₂O). **NMR (D₂O, pD ~5):** δ_H 0.96 (*i-Pr*, d, 9H, ³J_{HH} = 8); 0.99 (*i-Pr*, d, 9H, ³J_{HH} = 8); 1.26 (*i-Pr*, hept, 3H, ³J_{HH} = 8); 2.62–3.84 (*mc*, CH₂–CO, m, 16+4H); 4.05 (CH₂–arom., s, 2H); 4.29 (CH₂–arom., d, 1H, ²J_{HH} = 17); 4.88 (CH₂–arom., d, 1H, ²J_{HH} = 17); 5.43 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 6.74 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.67 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.82 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.83 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.94 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 8.20 (*arom.*, t, 1H, ³J_{HH} = 8); 8.22 (*arom.*, t, 1H, ³J_{HH} = 8); 8.43 (*FA*, s, 1H). **¹³C{¹H}** δ_C 12.1 (*i-Pr*, s); 18.8 (*i-Pr*, 2 × s); 47.9 (*mc*, bs); 50.0 (*mc*, bs); 50.4 (*mc*, bs); 52.2 (*mc*, bs); 53.0 (CH₂–N₃, s); 54.0 (*mc*, s); 55.2 (*mc*, s); 57.2 (*mc*, s); 57.6 (*mc*, s); 59.3 (CH₂–CO, s); 60.0 (CH₂–arom., s); 62.3 (CH₂–arom., bs); 64.0 (CH₂–CO, s); 126.1 (*arom.*, s); 127.4 (*arom.*, s); 128.9 (*arom.*, s); 130.8 (*arom.*, s); 141.6 (*arom.*, s); 143.3 (*arom.*, s); 143.8 (*arom.*, s); 144.6 (*arom.*, s); 147.1 (*arom.*, s); 156.1 (*arom.*, s); 158.4 (*arom.*, s); 159.6 (*arom.*, s); 171.5 (*FA*, s); 178.0 (CO, s); 179.1 (CO, s). **ESI-HRMS:** 878.3396 [M]⁺ (theor. [C₃₆H₅₃N₉O₄Si₁Lu₁]⁺ = 878.3392). **EA** ([C₃₆H₅₃N₉O₄Si₁Lu₁]⁺[FA][−]·5.9H₂O, M_R = 1030.2): C 43.1 (43.4); H 12.2 (11.8); N 12.2 (11.8); Si 2.7 (2.9); Lu 17.0 (16.9).

25 **Synthesis of TD946:** In a glass vial (20 mL), **TD943**·1.5H₂O (28.1 mg; 45.6 μmol; 1.0 equiv.) was dissolved



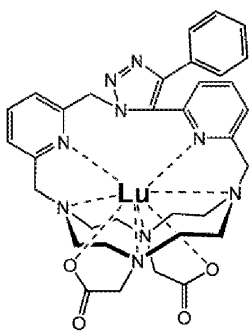

 in H₂O (16 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 3.00 mL; 1.50 mmol; 33 equiv.) and aq. **LuCl₃** (100 mM; 540 μL; 54 μmol; 1.2 equiv.) and the mixture was stirred 3 days at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 32.6 mg (77%; 1 step; based on **TD943**·1.5H₂O). **NMR (D₂O, pD ~5):** δ_H 0.69–0.94 (CH₂–CH, m, 2H); 0.94–1.07 (CH₂–CH, m, 2H); 1.65 (CH₂–CH, tt, 1H, ³J_{HH} = 8, ³J_{HH} = 5); 2.58–3.86 (*mc*, CH₂–CO, m, 16+4H); 3.97 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.19 (CH₂–arom., d, 1H, ²J_{HH} = 16); 4.29 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.87 (CH₂–arom., d, 1H, ²J_{HH}

= 16); 5.53 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 6.99 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 7.73 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.86 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.93 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.99 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 8.24 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$); 8.29 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$). **ESI-HRMS**: 762.2375 $[\text{M}]^+$ (theor. $[\text{C}_{30}\text{H}_{37}\text{N}_9\text{O}_4\text{Lu}_1]^+ = 762.2371$). **EA** ($[\text{C}_{30}\text{H}_{37}\text{N}_9\text{O}_4\text{Lu}_1]^+[\text{TFA}]^- \cdot 0.2\text{TFA} \cdot 1.8\text{H}_2\text{O}$, $M_R = 930.8$): C 41.8 (42.2); H 4.4 (4.2); N 13.5 (13.1); F 7.3 (7.0).



Synthesis of TD961: In a glass vial (20 mL), **TD959**·1.5H₂O (14.7 mg; 23.0 μmol ; 1.0 equiv.) was dissolved in H₂O (16 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 1.50 mL; 0.75 mmol; 33 equiv.) and aq. **LuCl₃** (100 mM; 280 μL ; 28 μmol ; 1.2 equiv.) and the mixture was stirred 3 days at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid.

Yield: 20.1 mg (90%; 1 step; based on **TD959**·1.8H₂O). **NMR (D₂O, pD ~4)**: δ_{H} 1.18 (CH_3 , s, 9H); 2.64–3.86 (*mc*, $\text{CH}_2\text{-CO}$, m, 16+4H); 4.01 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 4.16 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 4.35 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 17$); 4.87 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 17$); 5.48 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 6.66 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 7.70 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.84 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.87 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.93 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 8.22 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$); 8.26 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$). **ESI-HRMS**: 778.2685 $[\text{M}]^+$ (theor. $[\text{C}_{31}\text{H}_{41}\text{N}_9\text{O}_4\text{Lu}_1]^+ = 778.2684$). **EA** ($[\text{C}_{31}\text{H}_{41}\text{N}_9\text{O}_4\text{Lu}_1]^+[\text{TFA}]^- \cdot 0.2\text{TFA} \cdot 3.0\text{H}_2\text{O}$, $M_R = 968.5$): C 41.4 (41.5); H 4.9 (4.6); N 13.0 (12.7); F 7.1 (7.4).

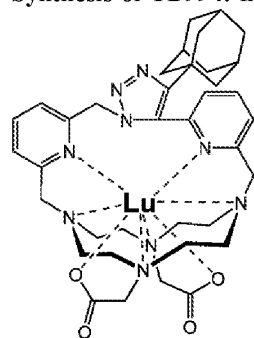


Synthesis of TD951: In a glass vial (20 mL), **TD944**·1.5H₂O (14.4 mg; 22.1 μmol ; 1.0 equiv.) was dissolved in H₂O (18 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 1.20 mL; 600 μmol ; 27 equiv.) and aq. **LuCl₃** (100 mM; 265 μL ; 26.5 μmol ; 1.2 equiv.) and the mixture was stirred 4 days at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid.

Yield: 17.4 mg (80%; 1 step; based on **TD944**·1.5H₂O). **NMR (D₂O, pD ~2)**: δ_{H} 2.69–3.88 (*mc*, $\text{CH}_2\text{-CO}$, m, 16+4H); 3.97 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 4.16–4.36 ($\text{CH}_2\text{-arom.}$, m, 2H); 4.88 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 17$); 5.51 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 6.97 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 7.16–7.25 (*Ph*, m, 2H); 7.28–7.41 (*Ph*, m, 3H); 7.49 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.73 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.83 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 8.03 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 8.06 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$); 8.25 (*arom.*, t, 1H,

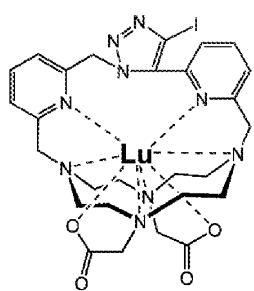
$^3J_{\text{HH}} = 8$). **ESI-HRMS:** 798.2379 $[\text{M}]^+$ (theor. $[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Lu}_1]^+ = 798.2371$). **EA** ($[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Lu}_1]^+[\text{TFA}]^- \cdot 0.2\text{TFA} \cdot 3.0\text{H}_2\text{O}$, $M_{\text{R}} = 988.5$): C 43.0 (43.0); H 4.4 (4.3); N 12.8 (12.6); F 6.9 (6.9).

Synthesis of TD994: In a glass vial (20 mL), **TD992**·0.3FA·1.9H₂O (41.6 mg; 56.8 μmol ; 1.0 equiv.) was dissolved in H₂O (16 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 2.85 mL; 1.43 mmol; 25 equiv.) and aq. **LuCl₃** (100 mM; 625 μL ; 62.5 μmol ; 1.1 equiv.) and the mixture was stirred 8 days at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 52.3 mg (86%; 1 step; based on **TD992**·0.3FA·1.9H₂O). **NMR (D₂O, pD ~4):** δ_{H} 1.52–1.78



(*Adm.*, m, 9H); 1.80–1.95 (*Adm.*, m, 6H); 2.60–3.83 (*mc*, $\text{CH}_2\text{--CO}$, m, 16+4H); 3.99 ($\text{CH}_2\text{--arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 4.11 ($\text{CH}_2\text{--arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 4.33 ($\text{CH}_2\text{--arom.}$, d, 1H, $^2J_{\text{HH}} = 17$); 4.84 ($\text{CH}_2\text{--arom.}$, d, 1H, $^2J_{\text{HH}} = 17$); 5.43 ($\text{CH}_2\text{--N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 6.58 ($\text{CH}_2\text{--N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 7.66 (*arom.*, d, 1H, $^3J_{\text{HH}} = 8$); 7.80 (*arom.*, d, 1H, $^3J_{\text{HH}} = 8$); 7.88–7.92 (*arom.*, m, 2H); 8.18 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$); 8.22 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$). **ESI-HRMS:** 856.3154 $[\text{M}]^+$ (theor. $[\text{C}_{37}\text{H}_{47}\text{N}_9\text{O}_4\text{Lu}_1]^+ = 856.3153$). **EA** ($[\text{C}_{37}\text{H}_{47}\text{N}_9\text{O}_4\text{Lu}_1]^+[\text{TFA}]^- \cdot 0.4\text{TFA} \cdot 3.0\text{H}_2\text{O}$, $M_{\text{R}} = 1069.4$): C 44.7 (44.3); H 5.0 (4.6); N 11.8 (11.5); F 7.5 (7.4); Lu 16.4 (14.0).

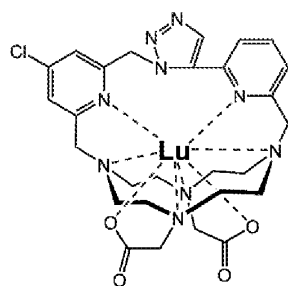
20



Synthesis of TD1221: In a glass vial (20 mL), **TD1204**·0.3FA·2.7H₂O (22.5 mg; 30.5 μmol ; 1.0 equiv.) was dissolved in H₂O (18 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 1.57 mL; 785 μmol ; 26 equiv.) and aq. **LuCl₃** (100 mM; 375 μL ; 37.5 μmol ; 1.2 equiv.) and the mixture was stirred 24 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 24.5 mg (74%; 1 step; based on **TD1204**·0.3FA·2.7H₂O). **NMR (D₂O, pD ~2):** ^1H δ_{H} 2.59–3.83 (*mc*, $\text{CH}_2\text{--CO}$, m, 16+4H); 3.95 ($\text{CH}_2\text{--arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 4.23 ($\text{CH}_2\text{--arom.}$, d, 1H, $^2J_{\text{HH}} = 17$); 4.27 ($\text{CH}_2\text{--arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 4.85 ($\text{CH}_2\text{--arom.}$, d, 1H, $^2J_{\text{HH}} = 17$); 5.62 ($\text{CH}_2\text{--N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 7.01 ($\text{CH}_2\text{--N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 7.72 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.87 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 7.90 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.97 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 8.23 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$); 8.29 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$). **ESI-HRMS:** 848.1020 $[\text{M}]^+$ (theor. $[\text{C}_{27}\text{H}_{32}\text{N}_9\text{O}_4\text{I}_1\text{Lu}_1]^+ = 848.1024$). **EA**

30

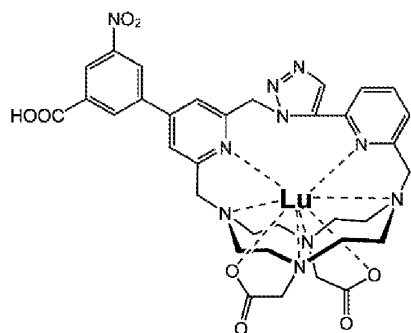
([C₂₇H₃₂N₉O₄Lu₁]⁺[TFA]⁻·0.5TFA·4.1H₂O, *M_R* = 1092.3): C 33.0 (33.8); H 3.8 (3.6); N 11.5 (11.8); I 11.6 (10.9); F 7.8 (7.0).



Synthesis of TD782: In a glass vial (40 mL), **TD764**·0.7TFA·1.7H₂O (190 mg; 274 μmol; 1.0 equiv.) was dissolved in H₂O (21 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 15.4 mL; 7.70 mmol; 28 equiv.) and aq. **LuCl₃** (100 mM; 3.30 mL; 330 μmol; 1.2 equiv.) and the mixture was stirred 16 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions

with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 224 mg (85%; 1 step; based on **TD764**·0.7TFA·1.7H₂O). **NMR (D₂O, pD ~3):** ¹H δ_H 2.61–3.84 (*mc*, CH₂–CO, m, 16+4H); 3.94 (CH₂–arom., d, 1H, ²*J*_{HH} = 15); 4.15 (CH₂–arom., d, 1H, ²*J*_{HH} = 16); 4.29 (CH₂–arom., d, 1H, ²*J*_{HH} = 15); 4.84 (CH₂–arom., d, 1H, ²*J*_{HH} = 16); 5.58 (CH₂–N₃, d, 1H, ²*J*_{HH} = 15); 7.10 (CH₂–N₃, d, 1H, ²*J*_{HH} = 15); 7.80 (*arom.*, dd, 1H, ³*J*_{HH} = 8, ⁴*J*_{HH} = 1); 7.83 (*arom.*, d, 1H, ⁴*J*_{HH} = 2); 7.85 (*arom.*, dd, 1H, ³*J*_{HH} = 8, ⁴*J*_{HH} = 1); 7.88 (CH–N₃, s, 1H); 8.13 (*arom.*, d, 1H, ⁴*J*_{HH} = 2); 8.24 (*arom.*, t, 1H, ³*J*_{HH} = 8).

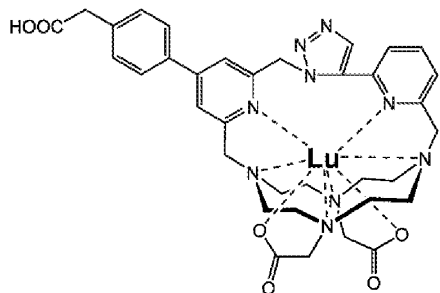
ESI-HRMS: 756.1664 [M]⁺ (theor. [C₂₇H₃₂N₉O₄Cl₁Lu₁]⁺ = 756.1668). **EA** ([C₂₇H₃₂N₉O₄Cl₁Lu₁]⁺[TFA]⁻·5.1H₂O, *M_R* = 961.9): C 36.2 (36.4); H 4.4 (4.0); N 13.1 (12.9); Cl 3.7 (3.7); F 5.9 (6.4); Lu 18.2 (16.8).



Synthesis of TD819: Glass vial (4 mL) was charged with [TD782]⁺[TFA]⁻·5.1H₂O (10.0 mg; 10.4 μmol; 1.0 equiv.), **3-borono-5-nitrobenzoic acid** (4.4 mg; 21 μmol; 2.0 equiv.) and **XPhos Pd G2** (0.5 mg; ~0.6 μmol; 6 mol%) and was then three times secured with argon. Under constant flow of argon was then added dry DMF (420 μL) through septum followed by addition of freshly prepared (and briefly washed with argon prior addition) aq. solution of **K₃PO₄·H₂O** (326 mM; 160 μL; 52 μmol; 5.0 equiv.). The mixture was then stirred 16 h under septum (but without external argon) at

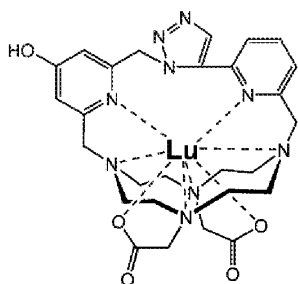
80 °C. Resulting heterogenous mixture was diluted with H₂O (2 mL) and the resulting slightly opalescent pale-yellow solution was filtered through syringe microfilter (RC). Filtrate was then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product as white fluffy solid. **Yield:** 10 mg (≤96% assuming [M]⁺[TFA]⁻·xH₂O; *M_R* = 1000.7; 1 step; based on [TD782]⁺[TFA]⁻·5.1H₂O). **NMR (D₂O, pD ~3):** ¹H δ_H 2.66–3.84 (*mc*, CH₂–CO, m, 16+4H); 4.07 (CH₂–arom., d, 1H, ²*J*_{HH} = 15); 4.17 (CH₂–arom., d, 1H, ²*J*_{HH} = 16); 4.39 (CH₂–arom., d, 1H,

$^2J_{\text{HH}} = 15$); 4.87 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 5.72 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 7.19 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 7.83 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.87 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.90 (CH-N_3 , s, 1H); 8.14 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 8.26 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$); 8.45 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 8.82 (*arom.*, dm, 1H, $^4J_{\text{HH}} = 2$); 8.92 (*arom.*, dd, 1H, $^4J_{\text{HH}} = 2$, $^4J_{\text{HH}} = 1$); 8.97 (*arom.*, dm, 1H, $^4J_{\text{HH}} = 2$). **ESI-HRMS**: 887.2121 $[\text{M}]^+$ (theor. $[\text{C}_{34}\text{H}_{36}\text{N}_{10}\text{O}_8\text{Lu}_1]^+ = 887.2120$).

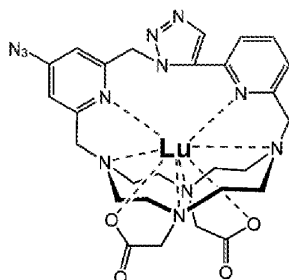


Synthesis of TD891: Glass vial (4 mL) was charged with $[\text{TD782}]^+[\text{TFA}]^- \cdot 5.1\text{H}_2\text{O}$ (15.0 mg; 15.6 μmol ; 1.0 equiv.), **2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetic acid** (8.0 mg; 30.5 μmol ; 2.0 equiv.) and **XPhos Pd G2** (0.7 mg; $\sim 0.9 \mu\text{mol}$; 6 mol%) and was then three times secured with argon. Under constant flow of argon was then added dry DMF (600 μL) through septum followed by addition of freshly prepared (and briefly washed with argon prior addition) aq. solution of

K₃PO₄·H₂O (391 mM; 200 μL ; 78 μmol ; 5.0 equiv.). The mixture was then stirred 16 h under septum (but without external argon) at 80 °C. Resulting heterogenous mixture was diluted with H₂O (2 mL) and the resulting slightly opalescent pale-yellow solution was filtered through syringe microfilter (RC). Filtrate was then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product as white fluffy solid. **Yield**: 7.2 mg (41%; 1 step; based on $[\text{TD782}]^+[\text{TFA}]^- \cdot 5.1\text{H}_2\text{O}$). **NMR (D₂O, pD ~3)**: ^1H δ_{H} 2.60–3.82 (*mc*, $\text{CH}_2\text{-CO}$, m, 16+4H); 3.86 ($\text{OC-CH}_2\text{-arom.}$, s, 2H); 3.99 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 4.15 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 16$); 4.32 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 4.84 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 16$); 5.65 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 7.13 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 7.54 (*arom.*, dm, 2H, $^3J_{\text{HH}} = 8$); 7.81 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.85 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.90 (CH-N_3 , s, 1H); 7.92 (*arom.*, dm, 2H, $^3J_{\text{HH}} = 8$); 7.99 (*arom.*, d, 1H, $^4J_{\text{HH}} = 1$); 8.25 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$); 8.30 (*arom.*, d, 1H, $^4J_{\text{HH}} = 1$). **ESI-HRMS**: 856.2418 $[\text{M}]^+$ (theor. $[\text{C}_{35}\text{H}_{39}\text{N}_9\text{O}_6\text{Lu}_1]^+ = 856.2426$). **EA** ($[\text{C}_{35}\text{H}_{39}\text{N}_9\text{O}_6\text{Cl}_1\text{Lu}_1]^+[\text{TFA}]^- \cdot 0.7\text{TFA} \cdot 4.6\text{H}_2\text{O}$, $M_{\text{R}} = 1132.4$): C 40.7 (40.6); H 4.4 (4.3); N 11.1 (11.3).

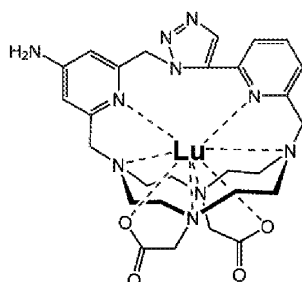


Synthesis of TD891-OH: **TD891-OH** was obtained (in the form of trifluoroacetate salt as white fluffy solid) as by-product during synthesis **TD891**. **Yield**: 5.7 mg. **NMR (D₂O, pD ~3)**: ^1H δ_{H} 2.60–3.83 (*mc*, $\text{CH}_2\text{-CO}$, $\text{CH}_2\text{-arom.}$, m, 16+4+1H); 4.13 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 16$); 4.20 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 4.82 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 16$); 5.43 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 6.97 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 7.09 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 7.40 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 7.79 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.84 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.89 (CH-N_3 , s, 1H); 8.23 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$). **ESI-HRMS**: 738.2003 $[\text{M}]^+$ (theor. $[\text{C}_{27}\text{H}_{33}\text{N}_9\text{O}_5\text{Lu}_1]^+ = 738.2007$).



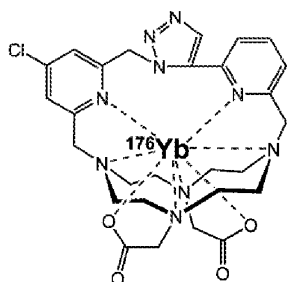
Synthesis of TD786: In a glass vial (4 mL), [TD782]⁺[TFA][−]·5.1H₂O (37 mg; 38 μmol; 1.0 equiv.) was dissolved in DMSO (3 mL) followed by addition solid NaN₃ (54 mg; 831 μmol; 20 equiv.) and the mixture was stirred 2 h at 80 °C. Mixture was then concentrated to remove most of DMSO. Residue was dissolved in H₂O and lyophilized. Crude product was purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 21 mg (55%; 1 step; based on

[TD782]⁺[TFA][−]·5.1H₂O). **NMR (D₂O, pD ~4):** ¹H δ_H 2.62–3.81 (*mc*, CH₂–CO, m, 16+4H); 3.87 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.14 (CH₂–arom., d, 1H, ²J_{HH} = 16); 4.25 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.83 (CH₂–arom., d, 1H, ²J_{HH} = 16); 5.52 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.05 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.38 (*arom.*, d, 1H, ⁴J_{HH} = 2); 7.71 (*arom.*, d, 1H, ⁴J_{HH} = 2); 7.80 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.84 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.88 (CH–N₃, s, 1H); 8.24 (*arom.*, t, 1H, ³J_{HH} = 8). **ESI-HRMS:** 763.2075 [M]⁺ (theor. [C₂₇H₃₂N₁₂O₄Lu]⁺ = 763.2072). **EA** ([C₂₇H₃₂N₁₂O₄Lu]⁺[TFA][−]·0.2TFA·5.2H₂O, M_R = 993.0): C 35.6 (35.6); H 4.3 (4.0); N 16.9 (16.6); F 6.9 (6.8).



Synthesis of TD787: Obtained as side product during synthesis of TD786 in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 5 mg (~10%; 1 step; based on TD782·5.1H₂O). **NMR (D₂O, pD ~2):** ¹H δ_H 2.59–3.79 (*mc*, CH₂–CO, CH₂–arom., m, 16+4+1H); 4.02–4.19 (CH₂–arom., m, 2H); 4.25 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.79 (CH₂–arom., d, 1H, ²J_{HH} = 15); 5.27 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 6.74 (*arom.*, d, 1H, ⁴J_{HH} = 2); 6.83 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.03 (*arom.*, d, 1H, ⁴J_{HH} = 2); 7.78 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.82 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.89 (CH–N₃, s, 1H); 8.22 (*arom.*, t, 1H, ³J_{HH} = 8). **ESI-HRMS:** 737.2165 [M]⁺ (theor. [C₂₇H₃₄N₁₀O₄Lu]⁺ = 737.2167).

25

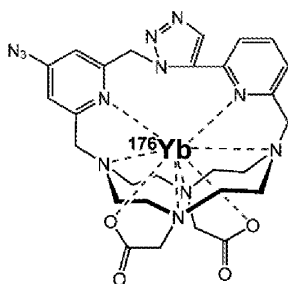


Synthesis of TD830: In a glass vial (20 mL), TD764·0.7TFA·1.7H₂O (60 mg; 86 μmol; 1.0 equiv.) was dissolved in H₂O (5 mL) followed by addition aq. MOPS/NaOH buffer (500 mM; pH 7.0; 5.0 mL; 2.50 mmol; 29 equiv.) and freshly prepared aq. ¹⁷⁶YbCl₃ (~90 μmol; ~1.0 eq.; obtained by dissolving 18.1 mg of ¹⁷⁶Yb₂O in 0.5 mL of 6 M HCl at 80 °C for several hours, followed by evaporation of the acid and adding 10 mL of H₂O to the residue). Mixture was then stirred 6 h at 80 °C after which was filtered through syringe microfilter (RC).

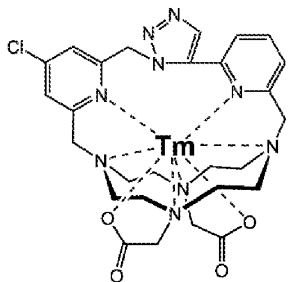
The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 71 mg (≤94%)

35

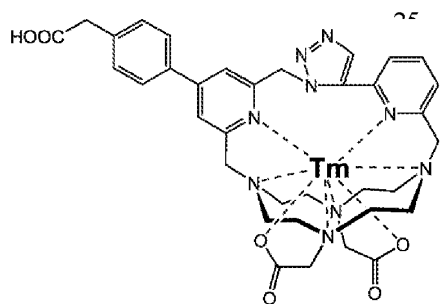
assuming $[M]^+[TFA]^- \cdot xH_2O$; $M_R = 871.0$; 1 step; based on **TD764**·0.7TFA·1.7H₂O). **ESI-HRMS**: 757.1680 $[M]^+$ (theor. $[C_{27}H_{32}N_9O_4Cl]^{176}Yb_1]^+ = 757.1686$).



Synthesis of TD836: In a glass vial (4 mL), **[TD830]⁺[TFA]⁻·xH₂O** (12.1 mg; ~14 μ mol; 1.0 equiv.) was dissolved in DMSO (500 μ L) followed by addition solid **NaN₃** (9.0 mg; 138 μ mol; ~10 equiv.) and the mixture was stirred 30 min at 80 °C. Mixture was then diluted with H₂O (3 mL) and directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product as white fluffy solid. **Yield**: 9.9 mg ($\leq$ 81% assuming $[M]^+[TFA]^- \cdot xH_2O$; $M_R = 877.6$; 1 step; based on **[TD830]⁺[TFA]⁻·xH₂O**). **ESI-HRMS**: 764.2085 $[M]^+$ (theor. $[C_{27}H_{32}N_{12}O_4]^{176}Yb_1]^+ = 764.2090$).

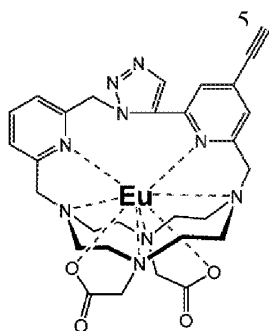


Synthesis of TD888: In a glass vial (20 mL), **TD764**·0.6TFA·1.0H₂O (56.5 mg; 84.3 μ mol; 1.0 equiv.) was dissolved in H₂O (15 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 4.0 mL; 2.00 mmol; 24 equiv.) and aq. **TmCl₃** (100 mM; 1.00 mL; 100 μ mol; 1.2 equiv.) and the mixture was stirred 6 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield**: 68.0 mg (86%, 1 step; based on **TD764**·0.6TFA·1.0H₂O). **ESI-HRMS**: 750.1608 $[M]^+$ (theor. $[C_{27}H_{32}N_9O_4Cl_1Tm_1]^+ = 750.1602$). **EA** ($[C_{27}H_{32}N_9O_4Cl_1Tm_1]^+[TFA]^- \cdot 0.2TFA \cdot 2.6H_2O$, $M_R = 933.6$): C 37.8 (37.7); H 4.0 (3.8); N 13.5 (13.4); Cl 3.8 (3.9); F 7.3 (7.3); Tm 18.1 (17.8).



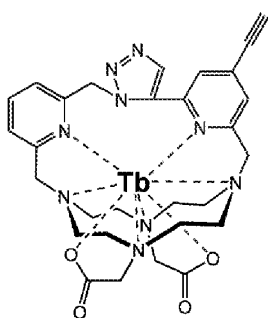
Synthesis of TD899: Glass vial (4 mL) was charged with **[TD888]⁺[TFA]⁻·0.2TFA·2.6H₂O** (15.0 mg; 16.1 μ mol; 1.0 equiv.), **2-(4-boronophenyl)acetic acid** (4.2 mg; 23.3 μ mol; 1.5 equiv.) and **XPhos Pd G2** (0.7 mg; ~0.9 μ mol; 6 mol%) and was then three times secured with argon. Under constant flow of argon was then added dry *1,4*-Dioxane (600 μ L) through septum followed by addition of freshly prepared (and briefly washed with argon prior addition) aq. solution of **K₃PO₄·H₂O** (391 mM; 200 μ L; 78 μ mol; 4.9 equiv.). The mixture was then stirred 16 h under septum (but without external argon) at 80 °C. Resulting heterogenous mixture was evaporated to dryness. Residue was re-dissolved in H₂O (3 mL), filtered through syringe microfilter (RC) and purified by preparative HPLC (C18; H₂O–MeCN gradient with

TFA additive). Fractions with product were joined and directly lyophilized to give product as white fluffy solid. **Yield:** 10.1 mg (57%; 1 step; based on $[\text{TD888}]^+[\text{TFA}]^- \cdot 0.2\text{TFA} \cdot 2.6\text{H}_2\text{O}$). **ESI-HRMS:** 850.2362 $[\text{M}]^+$ (theor. $[\text{C}_{35}\text{H}_{39}\text{N}_9\text{O}_6\text{TM}_1]^+ = 850.2360$). **EA** ($[\text{C}_{35}\text{H}_{39}\text{N}_9\text{O}_6\text{TM}_1]^+[\text{TFA}]^- \cdot 0.2\text{TFA} \cdot 7.0\text{H}_2\text{O}$, $M_R = 1112.6$): C 40.4 (40.6); H 4.8 (4.7); N 11.3 (11.1); F 6.1 (6.4).



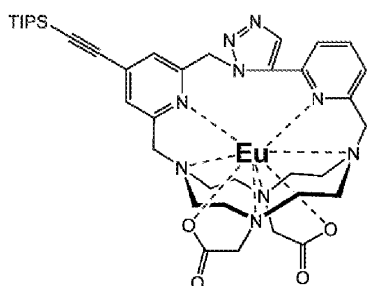
Synthesis of TD822: In a glass vial (20 mL), $\text{TD810} \cdot 0.5\text{TFA} \cdot 0.1\text{FA} \cdot 2.1\text{H}_2\text{O}$ (26 mg; 39 μmol ; 1.0 equiv.) was dissolved in H_2O (17 mL) followed by addition aq. **AcOH/NaOH buffer** (500 mM; pH 5.8; 2.1 mL; 1.05 mmol; 27 equiv.) and aq. **EuCl₃** (100 mM; 510 μL ; 51 μmol ; 1.3 equiv.) and the mixture was stirred 3 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H_2O –MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 19 mg ($\leq 59\%$ assuming $[\text{M}]^+[\text{TFA}]^- \cdot x\text{H}_2\text{O}$; $M_R = 836.6$; 1 step; based on $\text{TD810} \cdot 0.5\text{TFA} \cdot 0.1\text{FA} \cdot 2.1\text{H}_2\text{O}$). **ESI-HRMS:** 724.1861 $[\text{M}]^+$ (theor. $[\text{C}_{29}\text{H}_{33}\text{N}_9\text{O}_4\text{Eu}_1]^+ = 724.1862$).

15



Synthesis of TD823: In a glass vial (20 mL), $\text{TD810} \cdot 0.5\text{TFA} \cdot 0.1\text{FA} \cdot 2.1\text{H}_2\text{O}$ (28 mg; 42 μmol ; 1.0 equiv.) was dissolved in H_2O (17 mL) followed by addition aq. **AcOH/NaOH buffer** (500 mM; pH 5.8; 2.3 mL; 1.15 mmol; 28 equiv.) and aq. **TbCl₃** (100 mM; 530 μL ; 53 μmol ; 1.3 equiv.) and the mixture was stirred 2 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H_2O –MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 22 mg ($\leq 63\%$ assuming $[\text{M}]^+[\text{TFA}]^- \cdot x\text{H}_2\text{O}$; $M_R = 843.6$; 1 step; based on $\text{TD810} \cdot 0.5\text{TFA} \cdot 0.1\text{FA} \cdot 2.1\text{H}_2\text{O}$). **ESI-HRMS:** 730.1899 $[\text{M}]^+$ (theor. $[\text{C}_{29}\text{H}_{33}\text{N}_9\text{O}_4\text{Tb}_1]^+ = 730.1903$).

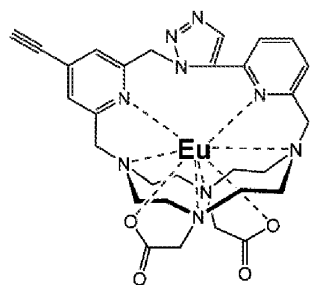
25



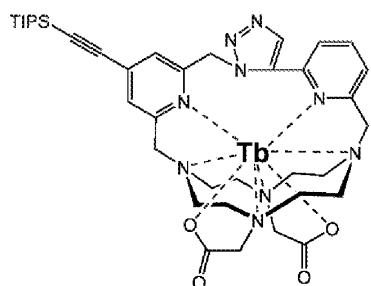
Synthesis of TD831: In a glass vial (20 mL), $\text{TD827} \cdot 0.8\text{FA} \cdot 1.6\text{H}_2\text{O}$ (50 mg; 63 μmol ; 1.0 equiv.) was dissolved in a mixture of abs. EtOH (8 mL) and H_2O (7.5 mL) followed by addition aq. **AcOH/NaOH buffer** (500 mM; pH 5.8; 3.3 mL; 1.65 mmol; 26 equiv.) and aq. **EuCl₃** (100 mM; 740 μL ; 74 μmol ; 1.2 equiv.) and the mixture was stirred 4 d at 80 °C. Mixture was then diluted with EtOH (100 mL) and filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator. The residue was purified by preparative HPLC (C18; H_2O –MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy

35

solid. **Yield:** 36 mg ($\leq 58\%$ assuming $[M]^+[TFA]^- \cdot xH_2O$; $M_R = 993.0$; 1 step; based on **TD827**·0.8FA·1.6H₂O). **ESI-HRMS:** 880.3189 $[M]^+$ (theor. $[C_{38}H_{53}N_9O_4Si_1Eu_1]^+ = 880.3197$).

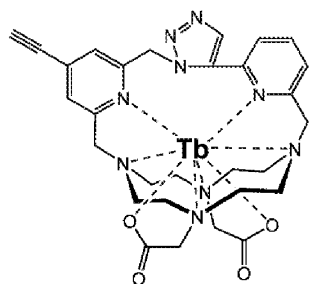


Synthesis of TD885: In a glass vial (4 mL), **[TD831]⁺[TFA]⁻·xH₂O** (30.5 mg; 30.7 μ mol; 1.0 equiv.) was dissolved in a H₂O (3.4 mL) followed by addition aq. **K₂CO₃** (1.0 M; 155 μ L; 155 μ mol; ~ 5 equiv.) and the mixture was stirred 40 min at 80 °C. The resulting orange-brown (and not entirely homogenous) mixture was then filtered through syringe microfilter (RC) and the filtrate was purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 15.4 mg ($\leq 60\%$ assuming $[M]^+[TFA]^- \cdot xH_2O$; $M_R = 836.6$; 1 step; based on **[TD831]⁺[TFA]⁻·xH₂O**). **ESI-HRMS:** 724.1858 $[M]^+$ (theor. $[C_{29}H_{33}N_9O_4Eu_1]^+ = 724.1862$).



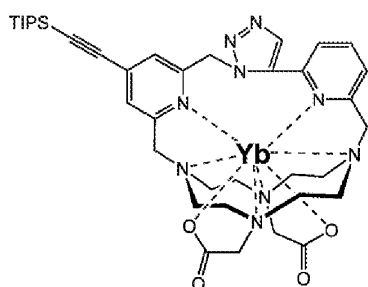
Synthesis of TD832: In a glass vial (20 mL), **TD827**·0.8FA·1.6H₂O (47 mg; 59 μ mol; 1.0 equiv.) was dissolved in a mixture of abs. EtOH (8 mL) and H₂O (8 mL) followed by addition aq. **AcOH/NaOH buffer** (500 mM; pH 5.8; 3.1 mL; 1.55 mmol; 26 equiv.) and aq. **EuCl₃** (100 mM; 720 μ L; 72 μ mol; 1.2 equiv.) and the mixture was stirred 4 d at 80 °C. Mixture was then diluted with EtOH (100 mL) and filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator. The residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 46 mg ($\leq 78\%$ assuming $[M]^+[TFA]^- \cdot xH_2O$; $M_R = 999.9$; 1 step; based on **TD827**·0.8FA·1.6H₂O). **ESI-HRMS:** 886.3229 $[M]^+$ (theor. $[C_{38}H_{53}N_9O_4Si_1Tb_1]^+ = 886.3238$).

25

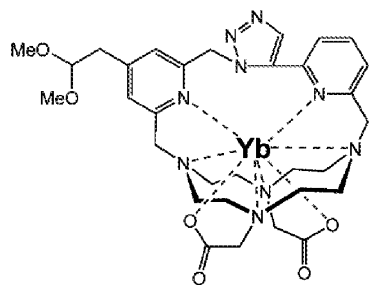


Synthesis of TD886: In a glass vial (4 mL), **[TD832]⁺[TFA]⁻·xH₂O** (46.2 mg; 46.2 μ mol; 1.0 equiv.) was dissolved in a H₂O (3.3 mL) followed by addition aq. **K₂CO₃** (1.0 M; 230 μ L; 230 μ mol; ~ 5 equiv.) and the mixture was stirred 40 min at 80 °C. The resulting orange-brown (and not entirely homogenous) mixture was then filtered through syringe microfilter (RC) and the filtrate was purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 29.3 mg ($\leq 75\%$ assuming $[M]^+[TFA]^- \cdot xH_2O$; $M_R = 843.6$; 1 step; based on **[TD832]⁺[TFA]⁻·xH₂O**). **ESI-HRMS:** 730.7901 $[M]^+$ (theor. $[C_{29}H_{33}N_9O_4Tb_1]^+ = 730.1903$).

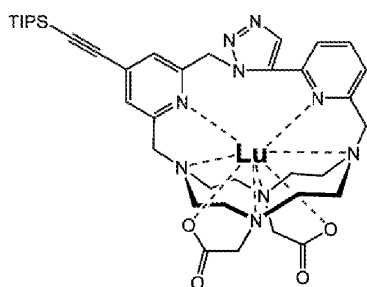
35



Synthesis of TD829: In a glass vial (20 mL), **TD827**·0.8FA·1.6H₂O (31 mg; 39 μmol; 1.0 equiv.) was dissolved in a mixture of abs. EtOH (11 mL) and H₂O (6 mL) followed by addition aq. **AcOH/NaOH buffer** (500 mM; pH 5.8; 2.0 mL; 1.00 mmol; 26 equiv.) and aq. **YbCl₃** (100 mM; 400 μL; 40 μmol; 1.0 equiv.) and the mixture was stirred 8 h at 80 °C. Mixture was then diluted with MeOH (20 mL) and filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator. The residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 38 mg (≤96% assuming [M]⁺[TFA][−]·xH₂O; *M_R* = 1014.0; 1 step; based on **TD827**·0.8FA·1.6H₂O). **ESI-HRMS:** 901.3372 [M]⁺ (theor. [C₃₈H₅₃N₉O₄Si₁Yb₁]⁺ = 901.3373).

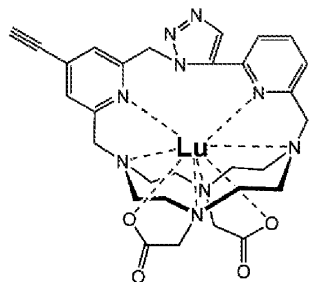


Synthesis of TD834: In a glass vial (4 mL), [**TD829**]⁺[TFA][−]·xH₂O (37.9 mg; 37.4 μmol; 1.0 equiv.) was dissolved in a 50% aq. **MeOH** (18 mL) followed by addition aq. **K₂CO₃** (100 mM; 2.0 mL μL; 200 μmol; ~5 equiv.) and the mixture was stirred 1 h at 80 °C. The resulting mixture was then concentrated to ~3 mL and then purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 22.5 mg (≤65% assuming [M]⁺[TFA][−]·xH₂O; *M_R* = 921.8; 1 step; based on [**TD829**]⁺[TFA][−]·xH₂O). **ESI-HRMS:** 809.2570 [M]⁺ (theor. [C₃₁H₄₁N₉O₆Yb₁]⁺ = 809.2563).



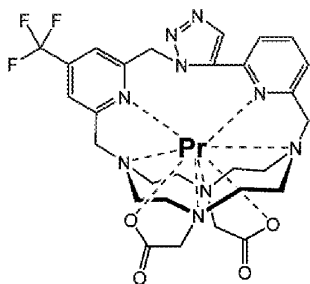
Synthesis of TD833: In a glass vial (20 mL), **TD827**·0.8FA·1.6H₂O (64.8 mg; 81 μmol; 1.0 equiv.) was dissolved in a mixture of *i*-PrOH (10 mL) and H₂O (5 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 4.1 mL; 2.05 mmol; 25 equiv.) and aq. **LuCl₃** (100 mM; 900 μL; 90 μmol; 1.1 equiv.) and the mixture was stirred 20 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator. The residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 76.3 mg (78%; 1 step; based on **TD827**·0.8FA·1.6H₂O). **NMR (D₂O, pD ~3):** ¹H δ_H 1.06–1.32 (*i*-Pr, m, 21H); 2.63–3.81 (*mc*, CH₂–CO, m, 16+4H); 3.90 (CH₂–arom., d, 1H, ²*J*_{HH} = 15); 4.15 (CH₂–arom., d, 1H, ²*J*_{HH} = 16); 4.28 (CH₂–arom., d, 1H, ²*J*_{HH} = 15); 4.84 (CH₂–arom., d, 1H, ²*J*_{HH} = 16); 5.55

(CH₂-N₃, d, 1H, ²J_{HH} = 16); 7.09 (CH₂-N₃, d, 1H, ²J_{HH} = 16); 7.76 (*arom.*, d, 1H, ⁴J_{HH} = 1); 7.80 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.85 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.88 (CH-N₃, s, 1H); 8.06 (*arom.*, d, 1H, ⁴J_{HH} = 1); 8.24 (*arom.*, t, 1H, ³J_{HH} = 8). **ESI-HRMS**: 902.3386 [M]⁺ (theor. [C₃₈H₅₃N₉O₄Si₁Lu₁]⁺ = 902.3392). **EA** ([C₃₈H₅₃N₉O₄Si₁Lu₁]⁺[TFA]⁻·1.0TFA·4.0H₂O, *M_R* = 1202.0): C 42.0 (41.7); H 5.2 (5.0); N 10.5 (10.4); Si 2.3 (1.9); F 9.5 (9.4).



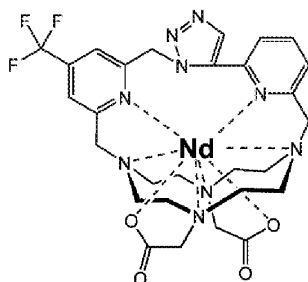
Synthesis of MIS007: In a glass vial (40 mL), [TD833]⁺[TFA]⁻·1.0TFA·4.0H₂O (19 mg; 16 μmol; 1.0 equiv.) was dissolved in a 50% aq. MeCN (20 mL) followed by addition aq. K₂CO₃ (100 mM; 2.0 mL; 200 μmol; ~10 equiv.) and the mixture was stirred 1 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated to ~3 mL and then purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and

directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 13 mg (≤81% assuming [M]⁺[TFA]⁻·xH₂O; *M_R* = 1016.0; 1 step; based on [TD833]⁺[TFA]⁻·1.0TFA·4.0H₂O). **NMR (D₂O, pD ~5):** ¹H δ_H 2.68–3.84 (*mc*, CH₂-CO, m, 16+4H); 3.96 (CH₂-*arom.*, d, 1H, ²J_{HH} = 15); 4.17 (CH₂-*arom.*, d, 1H, ²J_{HH} = 16); 4.18 (C≡CH, s, 1H); 4.30 (CH₂-*arom.*, d, 1H, ²J_{HH} = 15); 4.86 (CH₂-*arom.*, d, 1H, ²J_{HH} = 16); 5.61 (CH₂-N₃, d, 1H, ²J_{HH} = 16); 7.11 (CH₂-N₃, d, 1H, ²J_{HH} = 16); 7.82 (*arom.*, d, 1H, ⁴J_{HH} = 2); 7.82 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.87 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.90 (CH-N₃, s, 1H); 8.11 (*arom.*, d, 1H, ⁴J_{HH} = 2); 8.27 (*arom.*, t, 1H, ³J_{HH} = 8). **ESI-HRMS**: 746.2059 [M]⁺ (theor. [C₂₉H₃₃N₉O₄Lu₁]⁺ = 746.2058).

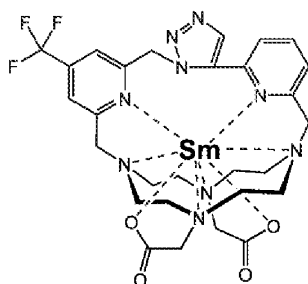


Synthesis of TD1113: In a glass vial (20 mL), TD1063·0.1FA·1.2H₂O (4.0 mg; 6.2 μmol; 1.0 equiv.) was dissolved in H₂O (10 mL) followed by addition aq. MOPS/NaOH buffer (500 mM; pH 7.0; 310 μL; 155 μmol; 25 equiv.) and aq. PrCl₃ (100 mM; 67 μL; 6.7 μmol; 1.1 equiv.) and the mixture was stirred 10 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form

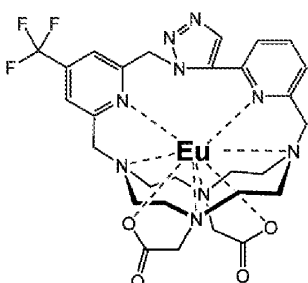
of trifluoroacetate salt as white fluffy solid. **Yield:** 0.6 mg (≤10% assuming [M]⁺[TFA]⁻·1.0TFA·xH₂O; *M_R* = 985.9; 1 step; based on TD1063·0.1FA·1.2H₂O). **NMR (D₂O, pD ~3):** ¹⁹F δ_F -66.45 (bs). **NMR (~0.5 mM TD1113 in ~50 mM MOPS/NaOH buffer in 10% D₂O, pH 7.0):** ¹⁹F δ_F -66.49 (bs; ΔH_{1/2} = 135.2 Hz). **ESI-HRMS**: 756.1593 [M]⁺ (theor. [C₂₈H₃₂N₉O₄F₃Pr₁]⁺ = 756.1600).



Synthesis of TD1114: In a glass vial (20 mL), **TD1063**·0.1FA·1.2H₂O (4.0 mg; 6.2 μmol; 1.0 equiv.) was dissolved in H₂O (10 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 310 μL; 155 μmol; 25 equiv.) and aq. **NdCl₃** (100 mM; 67 μL; 6.7 μmol; 1.1 equiv.) and the mixture was stirred 10 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 2.0 mg (≤33% assuming [M]⁺[TFA][−]·0.9TFA·xH₂O; *M_R* = 975.5; 1 step; based on **TD1063**·0.1FA·1.2H₂O). **NMR (D₂O, pD ~3):** ¹⁹F δ_F −66.75 (s). **NMR (~0.5 mM TD1114 in ~50 mM MOPS/NaOH buffer in 10% D₂O, pH 7.0):** ¹⁹F δ_F −66.76 (s; Δ*H*_{1/2} = 47.4 Hz). **ESI-HRMS:** 757.1595 [M]⁺ (theor. [C₂₈H₃₂N₉O₄F₃Nd]⁺ = 757.1601).



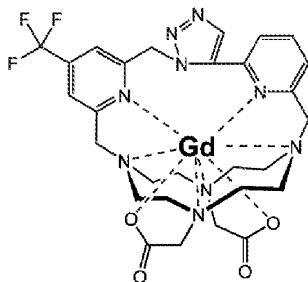
Synthesis of TD1115: In a glass vial (20 mL), **TD1063**·0.1FA·1.2H₂O (4.0 mg; 6.2 μmol; 1.0 equiv.) was dissolved in H₂O (10 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 310 μL; 155 μmol; 25 equiv.) and aq. **SmCl₃** (100 mM; 67 μL; 6.7 μmol; 1.1 equiv.) and the mixture was stirred 10 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 3.6 mg (≤63% assuming [M]⁺[TFA][−]·0.3TFA·xH₂O; *M_R* = 913.2; 1 step; based on **TD1063**·0.1FA·1.2H₂O). **NMR (D₂O, pD ~2):** ¹⁹F δ_F −66.03 (s). **NMR (~0.5 mM TD1115 in ~50 mM MOPS/NaOH buffer in 10% D₂O, pH 7.0):** ¹⁹F δ_F −66.06 (s; Δ*H*_{1/2} = 1.9 Hz). **ESI-HRMS:** 767.1713 [M]⁺ (theor. [C₂₈H₃₂N₉O₄F₃Sm]⁺ = 767.1721).



Synthesis of TD1072: In a glass vial (20 mL), **TD1063**·0.1FA·1.2H₂O (2.9 mg; 4.6 μmol; 1.0 equiv.) was dissolved in H₂O (10 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 225 μL; 113 μmol; 25 equiv.) and aq. **EuCl₃** (100 mM; 55 μL; 5.5 μmol; 1.2 equiv.) and the mixture was stirred 8 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form

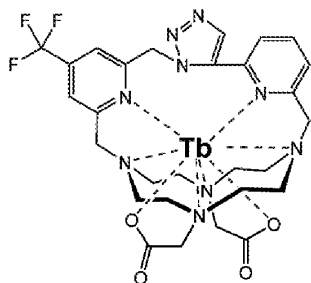
of trifluoroacetate salt as white fluffy solid. **Yield:** 2.7 mg ($\leq 66\%$ assuming $[M]^+[TFA]^- \cdot 0.3TFA \cdot xH_2O$; $M_R = 914.8$; 1 step; based on **TD1063**·0.1FA·1.2H₂O). **NMR (D₂O, pD ~2):** ¹⁹F δ_F -62.77 (s). **NMR (~0.5 mM TD1072 in ~50 mM MOPS/NaOH buffer in 10% D₂O, pH 7.0):** ¹⁹F δ_F -62.79 (s; $\Delta H_{1/2} = 3.3$ Hz). **ESI-HRMS:** 768.1738 $[M]^+$ (theor. $[C_{28}H_{32}N_9O_4F_3Eu_1]^+ = 768.1736$).

5



Synthesis of TD1179: In a glass vial (20 mL), **TD1063**·0.1FA·1.2H₂O (4.0 mg; 6.5 μ mol; 1.0 equiv.) was dissolved in H₂O (13 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 326 μ L; 163 μ mol; 25 equiv.) and aq. **GdCl₃** (100 mM; 78 μ L; 7.8 μ mol; 1.2 equiv.) and the mixture was stirred 4 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give

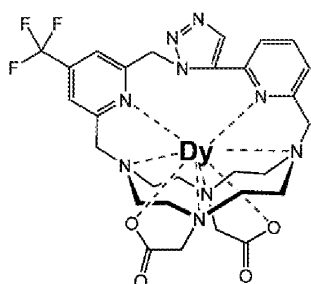
product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 2.2 mg ($\leq 36\%$ assuming $[M]^+[TFA]^- \cdot 0.9TFA \cdot xH_2O$; $M_R = 988.5$; 1 step; based on **TD1063**·0.1FA·1.2H₂O). **NMR (D₂O, pD ~2):** ¹⁹F δ_F -62.98 (bs). **NMR (~0.5 mM TD1179 in ~50 mM MOPS/NaOH buffer in 10% D₂O, pH 7.0):** ¹⁹F δ_F -62.97 (bs; $\Delta H_{1/2} = 342.9$ Hz). **ESI-HRMS:** 773.1779 $[M]^+$ (theor. $[C_{28}H_{32}N_9O_4F_3Gd_1]^+ = 773.1765$).



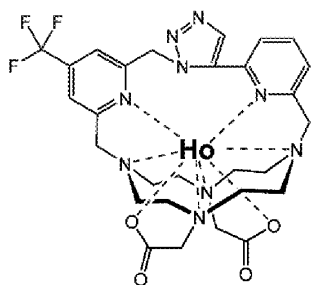
Synthesis of TD1073: In a glass vial (20 mL), **TD1063**·0.1FA·1.2H₂O (2.9 mg; 4.6 μ mol; 1.0 equiv.) was dissolved in H₂O (10 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 225 μ L; 113 μ mol; 25 equiv.) and aq. **TbCl₃** (100 mM; 55 μ L; 5.5 μ mol; 1.2 equiv.) and the mixture was stirred 6 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form

of trifluoroacetate salt as white fluffy solid. **Yield:** 2.8 mg ($\leq 64\%$ assuming $[M]^+[TFA]^- \cdot 0.7TFA \cdot xH_2O$; $M_R = 967.3$; 1 step; based on **TD1063**·0.1FA·1.2H₂O). **NMR (D₂O, pD ~2):** ¹⁹F δ_F -107.22 (bs). **NMR (~0.5 mM TD1073 in ~50 mM MOPS/NaOH buffer in 10% D₂O, pH 7.0):** ¹⁹F δ_F -107.50 (bs; $\Delta H_{1/2} = 708.6$ Hz). **ESI-HRMS:** 774.1779 $[M]^+$ (theor. $[C_{28}H_{32}N_9O_4F_3Tb_1]^+ = 774.1777$).

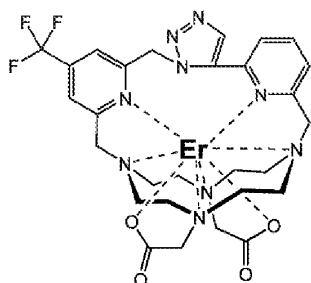
30



Synthesis of TD1074: In a glass vial (20 mL), **TD1063**·0.1FA·1.2H₂O (2.9 mg; 4.6 μmol; 1.0 equiv.) was dissolved in H₂O (10 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 225 μL; 113 μmol; 25 equiv.) and aq. **DyCl₃** (100 mM; 55 μL; 5.5 μmol; 1.2 equiv.) and the mixture was stirred 5 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 3.3 mg (≤75% assuming [M]⁺[TFA][−]·0.7TFA·xH₂O; $M_R = 970.9$; 1 step; based on **TD1063**·0.1FA·1.2H₂O). **NMR (D₂O, pD ~2):** ¹⁹F δ_F −142.86 (s). **NMR (~0.5 mM TD1074 in ~50 mM MOPS/NaOH buffer in 10% D₂O, pH 7.0):** ¹⁹F δ_F −143.22 (s; ΔH_{1/2} = 47.2 Hz). **ESI-HRMS:** 779.1816 [M]⁺ (theor. [C₂₈H₃₂N₉O₄F₃Dy]⁺ = 779.1816).



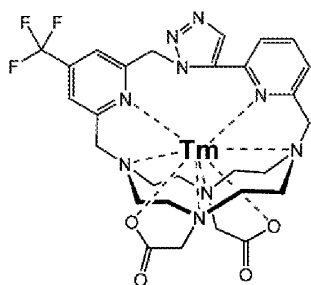
Synthesis of TD1075: In a glass vial (20 mL), **TD1063**·0.1FA·1.2H₂O (2.9 mg; 4.6 μmol; 1.0 equiv.) was dissolved in H₂O (10 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 225 μL; 113 μmol; 25 equiv.) and aq. **HoCl₃** (100 mM; 55 μL; 5.5 μmol; 1.2 equiv.) and the mixture was stirred 5 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 4.0 mg (≤90% assuming [M]⁺[TFA][−]·0.8TFA·xH₂O; $M_R = 984.7$; 1 step; based on **TD1063**·0.1FA·1.2H₂O). **NMR (D₂O, pD ~2):** ¹⁹F δ_F −85.69 (bs). **NMR (~0.5 mM TD1075 in ~50 mM MOPS/NaOH buffer in 10% D₂O, pH 7.0):** ¹⁹F δ_F −85.79 (bs; ΔH_{1/2} = 185.8 Hz). **ESI-HRMS:** 780.1829 [M]⁺ (theor. [C₂₈H₃₂N₉O₄F₃Ho]⁺ = 780.1827).



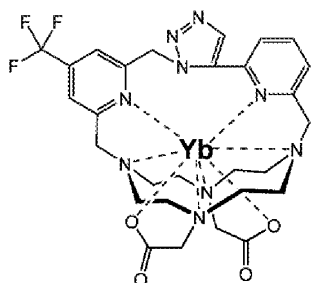
Synthesis of TD1076: In a glass vial (20 mL), **TD1063**·0.1FA·1.2H₂O (2.9 mg; 4.6 μmol; 1.0 equiv.) was dissolved in H₂O (10 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 225 μL; 113 μmol; 25 equiv.) and aq. **ErCl₃** (100 mM; 55 μL; 5.5 μmol; 1.2 equiv.) and the mixture was stirred 4 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white

fluffy solid. **Yield:** 4.1 mg ($\leq 92\%$ assuming $[M]^+[TFA]^- \cdot 1.0TFA \cdot xH_2O$; $M_R = 987.1$; 1 step; based on **TD1063**·0.1FA·1.2H₂O). **NMR (D₂O, pD ~2):** ^{19}F δ_F -47.48 (s). **NMR (~0.5 mM TD1076 in ~50 mM MOPS/NaOH buffer in 10% D₂O, pH 7.0):** ^{19}F δ_F -47.42 (s; $\Delta H_{1/2} = 19.3$ Hz). **ESI-HRMS:** 781.1824 $[M]^+$ (theor. $[C_{28}H_{32}N_9O_4F_3Er_1]^+ = 781.1827$).

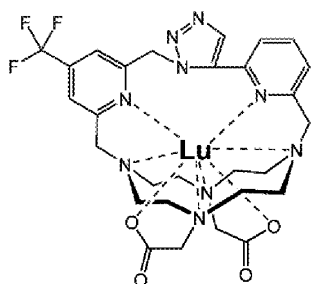
5



Synthesis of 1077: In a glass vial (20 mL), **TD1063**·0.1FA·1.2H₂O (2.9 mg; 4.6 μ mol; 1.0 equiv.) was dissolved in H₂O (10 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 225 μ L; 113 μ mol; 25 equiv.) and aq. **TmCl₃** (100 mM; 55 μ L; 5.5 μ mol; 1.2 equiv.) and the mixture was stirred 4 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 4.2 mg ($\leq 92\%$ assuming $[M]^+[TFA]^- \cdot 1.0TFA \cdot xH_2O$; $M_R =$
 15 1011.6; 1 step; based on **TD1063**·0.1FA·1.2H₂O). **NMR (D₂O, pD ~2):** ^{19}F δ_F -57.57 (bs). **NMR (~0.5 mM TD1077 in ~50 mM MOPS/NaOH buffer in 10% D₂O, pH 7.0):** ^{19}F δ_F -57.51 (bs; $\Delta H_{1/2} = 138.72$ Hz). **ESI-HRMS:** 784.1863 $[M]^+$ (theor. $[C_{28}H_{32}N_9O_4F_3Tm_1]^+ = 784.1866$).



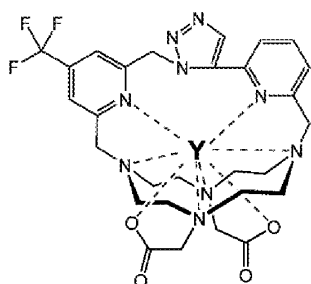
Synthesis of 1078: In a glass vial (20 mL), **TD1063**·0.1FA·1.2H₂O (2.9 mg; 4.6 μ mol; 1.0 equiv.) was dissolved in H₂O (10 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 225 μ L; 113 μ mol; 25 equiv.) and aq. **YbCl₃** (100 mM; 55 μ L; 5.5 μ mol; 1.2 equiv.) and the mixture was stirred 16 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 3.7 mg ($\leq 82\%$ assuming $[M]^+[TFA]^- \cdot 0.9TFA \cdot xH_2O$; $M_R = 1004.3$; 1 step; based on **TD1063**·0.1FA·1.2H₂O). **NMR (D₂O, pD ~2):** ^{19}F δ_F -59.01 (s). **NMR (~0.5 mM TD1078 in ~50 mM MOPS/NaOH buffer in 10% D₂O, pH 7.0):** ^{19}F δ_F -59.00 (s; $\Delta H_{1/2} = 18.4$ Hz). **ESI-HRMS:** 789.1913 $[M]^+$
 30 (theor. $[C_{28}H_{32}N_9O_4F_3Yb_1]^+ = 789.1912$).



Synthesis of TD1079: In a glass vial (20 mL), **TD1063**·0.1FA·1.2H₂O (14.7 mg; 22.8 μmol; 1.0 equiv.) was dissolved in H₂O (18 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 1150 μL; 575 μmol; 25 equiv.) and aq. **LuCl₃** (100 mM; 250 μL; 25.0 μmol; 1.1 equiv.) and the mixture was stirred 16 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive).

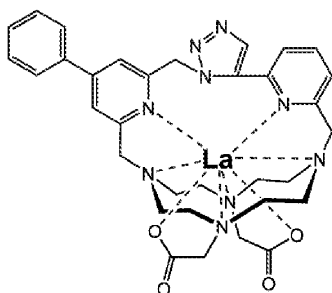
Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 16.1 mg (70%; 1 step; based on **TD1063**·0.1FA·1.2H₂O). **NMR (D₂O, pD ~2):**

- 10 ¹H δ_H 2.69–3.85 (*mc*, CH₂–CO, m, 16+4H); 4.12 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.19 (CH₂–arom., d, 1H, ²J_{HH} = 16); 4.38 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.88 (CH₂–arom., d, 1H, ²J_{HH} = 16); 5.75 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.23 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.83 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.88 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.90 (CH–N₃, s, 1H); 8.12 (*arom.*, d, 1H, ⁴J_{HH} = 2); 8.28 (*arom.*, t, 1H, ³J_{HH} = 8), 8.43 (*arom.*, d, 1H, ⁴J_{HH} = 2). ¹⁹F δ_F –66.10 (s). **NMR (~0.5 mM TD1079 in ~50 mM MOPS/NaOH buffer in 10% D₂O, pH 7.0):**
- 15 ¹⁹F δ_F –66.13 (s; ΔH_{1/2} = 2.4 Hz). **ESI-HRMS:** 790.1931 [M]⁺ (theor. [C₂₈H₃₂N₉O₄F₃Lu]⁺ = 790.1932). **EA** ([C₂₈H₃₂N₉O₄LuF₃]⁺[TFA][–]·0.2TFA·4.1H₂O, *M_R* = 1000.2): C 36.5 (36.2); H 4.1 (3.6); N 12.6 (12.3); F 12.5 (12.0).



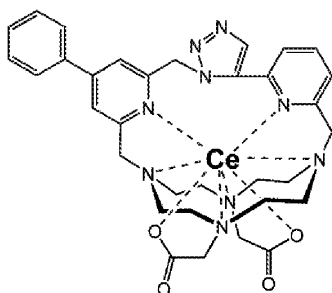
Synthesis of TD1080: In a glass vial (20 mL), **TD1063**·0.1FA·1.2H₂O (2.9 mg; 4.6 μmol; 1.0 equiv.) was dissolved in H₂O (10 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 225 μL; 113 μmol; 25 equiv.) and aq. **YCl₃** (100 mM; 55 μL; 5.5 μmol; 1.2 equiv.) and the mixture was stirred 16 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions

- 25 with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 3.6 mg (≤94% assuming [M]⁺[TFA][–]·0.3TFA·xH₂O; *M_R* = 851.7; 1 step; based on **TD1063**·0.1FA·1.2H₂O). **NMR (D₂O, pD ~2):** ¹H δ_H 2.66–3.87 (*mc*, CH₂–CO, m, 16+4H); 4.11 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.18 (CH₂–arom., d, 1H, ²J_{HH} = 16); 4.42 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.89 (CH₂–arom., d, 1H, ²J_{HH} = 16); 5.80 (CH₂–N₃, d, 1H, ²J_{HH} = 16); 7.16 (CH₂–N₃, d, 1H, ²J_{HH} = 16); 7.84 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.87 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.90 (CH–N₃, s, 1H); 8.13 (*arom.*, d, 1H, ⁴J_{HH} = 2); 8.28 (*arom.*, t, 1H, ³J_{HH} = 8), 8.43 (*arom.*, d, 1H, ⁴J_{HH} = 2). ¹⁹F δ_F –66.10 (s).
- 30 **NMR (~0.5 mM TD1080 in ~50 mM MOPS/NaOH buffer in 10% D₂O, pH 7.0):** ¹⁹F δ_F –66.12 (s; ΔH_{1/2} = 2.1 Hz). **ESI-HRMS:** 704.1583 [M]⁺ (theor. [C₂₈H₃₂N₉O₄F₃Y]⁺ = 704.1582).



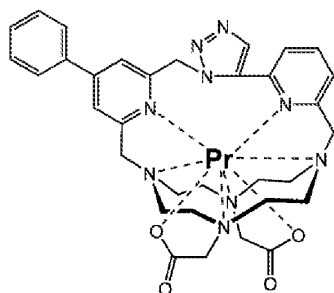
Synthesis of TD651: In a glass vial (20 mL), **TD647**·0.2FA·0.9H₂O (30 mg; 46 μmol; 1.0 equiv.) was dissolved in H₂O (17 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 2.30 mL; 1.15 mmol; 25 equiv.) and aq. **LaCl₃** (100 mM; 500 μL; 50 μmol; 1.1 equiv.) and the mixture was stirred 14 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give

product in the form of formate salt as white fluffy solid. **Yield:** 15 mg (36%; 1 step; based on **TD647**·0.2FA·0.9H₂O). **NMR (D₂O, pD ~5):** ¹H δ_H 2.54–3.63 (*mc*, CH₂–CO, m, 16+3H); 3.96 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.00 (CH₂–arom., d, 1H, ²J_{HH} = 14); 4.09 (CH₂–CO, 1H, d, ²J_{HH} = 17); 4.23 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.25 (CH₂–arom., d, 1H, ²J_{HH} = 14); 5.72 (CH₂–N₃, d, 1H, ²J_{HH} = 16); 6.62 (CH₂–N₃, d, 1H, ²J_{HH} = 16); 7.58–7.66 (*Ph*, m, 3H); 7.70 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.81 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.83–7.95 (*Ph*, *arom.*, m, 2+1H); 8.06 (*arom.*, d, 1H, ⁴J_{HH} = 2); 8.16 (CH–N₃, s, 1H); 8.22 (*arom.*, t, 1H, ³J_{HH} = 8); 8.42 (FA, s, 1H). ¹³C{¹H} δ_C 53.5 (CH₂–N₃, s); 56.3 (*mc*, s); 58.7 (*mc*, s); 58.9 (*mc*, s); 59.2 (*mc*, s); 59.3 (*mc*, s); 60.0 (*mc*, s); 61.9 (*mc*, s); 62.5 (CH₂–CO, s); 63.5 (*mc*, s); 65.1 (CH₂–CO, s); 65.2 (CH₂–arom., s); 66.6 (CH₂–arom., s); 124.1 (*arom.*, s); 124.6 (*arom.*, s); 127.1 (*arom.*, s); 127.9 (*arom.*, s); 128.1 (*Ph*, s); 130.2 (*Ph*, s); 131.5 (*Ph*, s); 136.1 (*arom.*, s); 136.2 (*Ph*, s); 136.8 (CH–N₃, s); 142.0 (*arom.*, s); 144.9 (*arom.*, s); 153.6 (*arom.*, s); 157.0 (*arom.*, s); 157.5 (*arom.*, s); 159.0 (*arom.*, s); 171.0 (FA, s); 179.0 (CO, s); 180.4 (CO, s). **ESI-HRMS:** 762.2024 [M]⁺ (theor. [C₃₃H₃₇N₉O₄La]⁺ = 762.2027). **EA** ([C₃₃H₃₇N₉O₄La]⁺[FA][−]·4.8H₂O, M_R = 894.1): C 45.7 (46.1); H 5.4 (4.7); N 14.1 (13.6); La 15.5 (15.0).

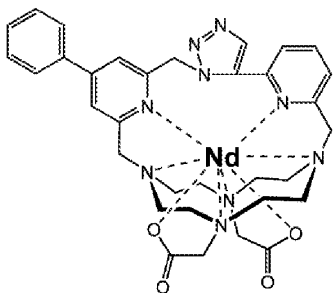


Synthesis of TD870: In a glass vial (20 mL), **TD647**·1.3TFA (30.5 mg; 39.4 μmol; 1.0 equiv.) was dissolved in H₂O (17 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 2.30 mL; 1.15 mmol; 29 equiv.) and aq. **CeCl₃** (100 mM; 545 μL; 54.5 μmol; 1.4 equiv.) and the mixture was stirred 14 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give

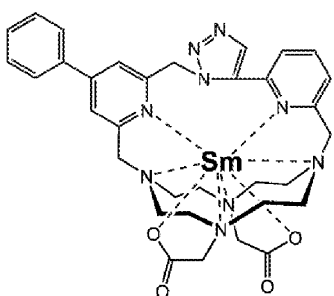
product in the form of formate salt as white fluffy solid. **Yield:** 21.2 mg (58%; 1 step; based on **TD647**·1.3TFA). **ESI-HRMS:** 763.2012 [M]⁺ (theor. [C₃₃H₃₇N₉O₄Ce]⁺ = 763.2017). **EA** ([C₃₃H₃₇N₉O₄Ce]⁺[FA][−]·6.5H₂O, M_R = 925.9): C 44.1 (44.7); H 5.6 (5.1); N 13.6 (13.1); Ce 15.1 (14.5).



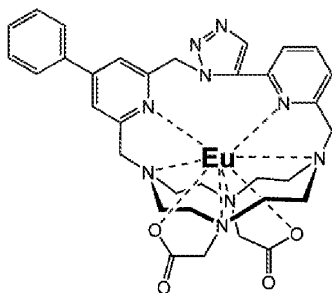
Synthesis of TD869: In a glass vial (20 mL), **TD647**·1.3TFA (26.4 mg; 34.1 μmol ; 1.0 equiv.) was dissolved in H_2O (17 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 2.40 mL; 1.20 mmol; 35 equiv.) and aq. **PrCl₃** (100 mM; 500 μL ; 50.0 μmol ; 1.5 equiv.) and the mixture was stirred 14 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 17.7 mg (56%; 1 step; based on **TD647**·1.3TFA). **ESI-HRMS:** 764.2035 $[\text{M}]^+$ (theor. $[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Pr}_1]^+ = 764.2040$). **EA** ($[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Pr}_1]^+[\text{FA}]^- \cdot 6.4\text{H}_2\text{O}$, $M_R = 924.9$): C 44.2 (44.7); H 5.5 (5.0); N 13.6 (13.1); Pr 15.2 (14.8).



Synthesis of TD868: In a glass vial (20 mL), **TD647**·1.3TFA (25.7 mg; 33.2 μmol ; 1.0 equiv.) was dissolved in H_2O (17 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 2.30 mL; 1.15 mmol; 35 equiv.) and aq. **NdCl₃** (100 mM; 460 μL ; 46.0 μmol ; 1.4 equiv.) and the mixture was stirred 12 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 19.8 mg (65%; 1 step; based on **TD647**·1.3TFA). **ESI-HRMS:** 765.2039 $[\text{M}]^+$ (theor. $[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Nd}_1]^+ = 765.2040$). **EA** ($[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Nd}_1]^+[\text{FA}]^- \cdot 5.6\text{H}_2\text{O}$, $M_R = 913.9$): C 44.7 (45.0); H 5.4 (4.6); N 13.8 (13.4); Nd 15.8 (15.2).



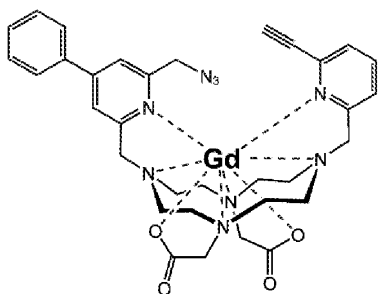
Synthesis of TD867: In a glass vial (20 mL), **TD647**·1.3TFA (25.6 mg; 33.1 μmol ; 1.0 equiv.) was dissolved in H_2O (17 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 2.30 mL; 1.15 mmol; 35 equiv.) and aq. **SmCl₃** (100 mM; 460 μL ; 46.0 μmol ; 1.4 equiv.) and the mixture was stirred 12 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 22.3 mg (73%; 1 step; based on **TD647**·1.3TFA). **ESI-HRMS:** 775.2158 $[\text{M}]^+$ (theor. $[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Sm}_1]^+ = 775.2160$). **EA** ($[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Sm}_1]^+[\text{FA}]^- \cdot 5.8\text{H}_2\text{O}$, $M_R = 923.6$): C 44.2 (44.7); H 5.4 (4.9); N 13.6 (13.3); Nd 16.3 (15.8).



Synthesis of TD599: In a glass vial (20 mL), **TD647**·1.3H₂O (50 mg; 77 μmol; 1.0 equiv.) was dissolved in H₂O (15 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 3.75 mL; 1.88 mmol; 24 equiv.) and aq. **EuCl₃** (100 mM; 825 μL; 83 μmol; 1.1 equiv.) and the mixture was stirred 3 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give

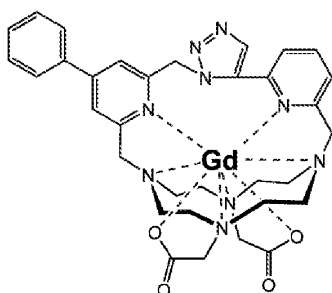
product in the form of formate salt as white fluffy solid. **Yield:** 44 mg (63%; 1 step; based on **TD647**·1.3H₂O).

10 **ESI-HRMS:** 776.2171 [M]⁺ (theor. [C₃₃H₃₇N₉O₄Eu]⁺ = 776.2175). **EA** ([C₃₃H₃₇N₉O₄Eu]⁺[FA][−]·4.6H₂O, *M_R* = 903.6): C 45.2 (45.3); H 5.3 (5.1); N 14.0 (13.7); Eu 16.8 (16.5).



Synthesis of TD451: In a glass vial (2 mL), **TD647**·2.2H₂O (2.0 mg; 3.1 μmol; 1.0 equiv.) was dissolved in aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 155 μL; 77.5 μmol; 25 equiv.) and aq. **GdCl₃** (100 mM; 34 μL; 120 μmol; 1.1 equiv.) and the mixture was stirred 2 d at RT. Mixture was then diluted with H₂O and filtered through syringe microfilter (RC). The filtrate was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined

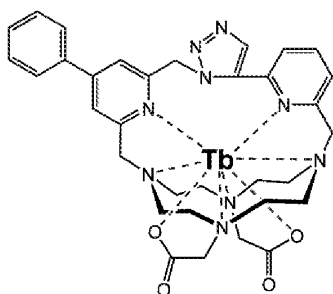
20 and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 0.3 mg.



Synthesis of TD527: In a glass vial (40 mL), **TD647**·2.2H₂O (75 mg; 113 μmol; 1.0 equiv.) was dissolved in H₂O (32 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 4.60 mL; 2.30 mmol; 20 equiv.) and aq. **GdCl₃** (100 mM; 1.20 mL; 120 μmol; 1.1 equiv.) and the mixture was stirred 2 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give

product in the form of formate salt as white fluffy solid. **Yield:** 58 mg (56%; 1 step; based on **TD647**·2.2H₂O).

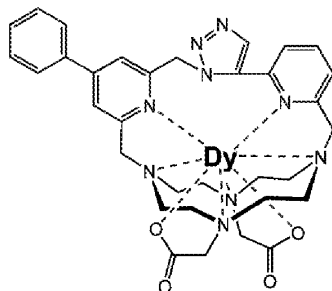
50 **ESI-HRMS:** 781.2218 [M]⁺ (theor. [C₃₃H₃₇N₉O₄Gd]⁺ = 781.2204). **EA** ([C₃₃H₃₇N₉O₄Gd]⁺[FA][−]·5.3H₂O, *M_R* = 921.5): C 44.3 (44.7); H 5.3 (4.7); N 13.7 (13.4); Gd 17.1 (16.2).



Synthesis of TD600: In a glass vial (20 mL), **TD647**·1.3H₂O (50 mg; 77 μmol; 1.0 equiv.) was dissolved in H₂O (15 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 3.75 mL; 1.88 mmol; 24 equiv.) and aq. **TbCl₃** (100 mM; 825 μL; 83 μmol; 1.1 equiv.) and the mixture was stirred 3 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give

product in the form of formate salt as white fluffy solid. **Yield:** 49 mg (69%; 1 step; based on **TD647**·1.3H₂O).

10 **ESI-HRMS:** 782.2211 [M]⁺ (theor. [C₃₃H₃₇N₉O₄Tb]⁺ = 781.2217). **EA** ([C₃₃H₃₇N₉O₄Tb]⁺[FA][−]·4.9H₂O, *M_R* = 915.9): C 44.6 (44.7); H 5.3 (5.1); N 13.8 (13.6); Tb 17.4 (17.1).

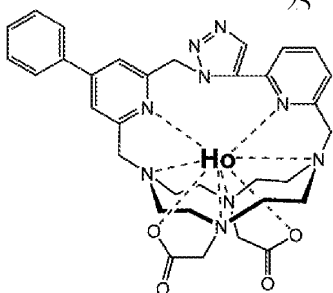


20

Synthesis of TD866: In a glass vial (20 mL), **TD647**·1.3TFA (20.0 mg; 25.8 μmol; 1.0 equiv.) was dissolved in H₂O (17 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 1.84 mL; 920 μmol; 36 equiv.) and aq. **DyCl₃** (100 mM; 370 μL; 37.0 μmol; 1.4 equiv.) and the mixture was stirred 46 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to

give product in the form of formate salt as white fluffy solid. **Yield:** 22.2 mg (89%; 1 step; based on

TD647·1.3TFA). **ESI-HRMS:** 787.2248 [M]⁺ (theor. [C₃₃H₃₇N₉O₄Dy]⁺ = 787.2255). **EA** ([C₃₃H₃₇N₉O₄Dy]⁺[FA][−]·7.7H₂O, *M_R* = 970.0): C 42.1 (42.5); H 5.5 (5.0); N 13.0 (12.6); Dy 16.8 (16.1).



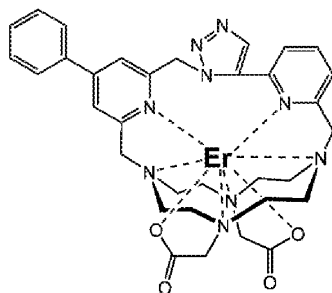
25

Synthesis of TD648: In a glass vial (20 mL), **TD647**·0.2FA·0.9H₂O (22 mg; 34 μmol; 1.0 equiv.) was dissolved in H₂O (17 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 1.72 mL; 860 μmol; 25 equiv.) and aq. **HoCl₃** (100 mM; 375 μL; 38 μmol; 1.1 equiv.) and the mixture was stirred 20 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give

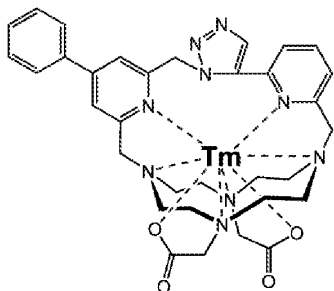
product in the form of formate salt as white fluffy solid. **Yield:** 24 mg (76%; 1 step; based on

TD647·0.2FA·0.9H₂O). **ESI-HRMS:** 788.2265 [M]⁺ (theor. [C₃₃H₃₇N₉O₄Ho]⁺ = 788.2266). **EA** ([C₃₃H₃₇N₉O₄Ho]⁺[FA][−]·5.0H₂O, *M_R* = 923.7): C 44.2 (44.2); H 5.2 (4.8); N 13.6 (13.3); Ho 17.9 (17.3).

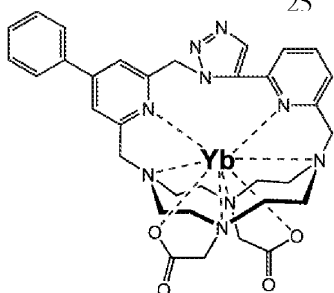
35



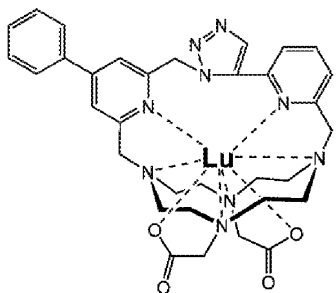
Synthesis of TD865: In a glass vial (20 mL), **TD647**·1.3TFA (20.5 mg; 26.5 μmol ; 1.0 equiv.) was dissolved in H_2O (17 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 1.84 mL; 920 μmol ; 35 equiv.) and aq. **ErCl₃** (100 mM; 370 μL ; 37.0 μmol ; 1.4 equiv.) and the mixture was stirred 22 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 22.8 mg (90%; 1 step; based on **TD647**·1.3TFA). **ESI-HRMS:** 791.2280 $[\text{M}]^+$ (theor. $[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Er}_1]^+ = 791.2302$). **EA** ($[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Er}_1]^+[\text{FA}]^- \cdot 6.9\text{H}_2\text{O}$, $M_R = 960.3$): C 42.5 (42.4); H 5.4 (4.9); N 13.1 (12.7); Er 17.4 (16.8).



Synthesis of TD601: In a glass vial (20 mL), **TD647**·1.3H₂O (49 mg; 75 μmol ; 1.0 equiv.) was dissolved in H_2O (15 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 3.75 mL; 1.88 mmol; 25 equiv.) and aq. **TmCl₃** (100 mM; 825 μL ; 83 μmol ; 1.1 equiv.) and the mixture was stirred 20 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 51 mg (73%; 1 step; based on **TD647**·1.3H₂O). **ESI-HRMS:** 792.2299 $[\text{M}]^+$ (theor. $[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Tm}_1]^+ = 792.2305$). **EA** ($[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Tm}_1]^+[\text{FA}]^- \cdot 4.7\text{H}_2\text{O}$, $M_R = 922.3$): C 44.3 (44.4); H 5.2 (5.1); N 13.7 (13.5); Tm 18.3 (18.3).

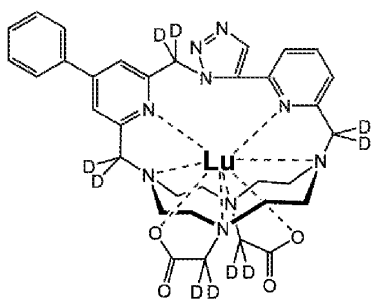


Synthesis of TD602: In a glass vial (20 mL), **TD647**·1.3H₂O (49 mg; 75 μmol ; 1.0 equiv.) was dissolved in H_2O (15 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 3.75 mL; 1.88 mmol; 25 equiv.) and aq. **YbCl₃** (100 mM; 825 μL ; 83 μmol ; 1.1 equiv.) and the mixture was stirred 20 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 53 mg (77%; 1 step; based on **TD647**·1.3H₂O). **ESI-HRMS:** 797.2347 $[\text{M}]^+$ (theor. $[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Yb}_1]^+ = 797.2352$). **EA** ($[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Yb}_1]^+[\text{FA}]^- \cdot 4.2\text{H}_2\text{O}$, $M_R = 917.4$): C 44.5 (44.1); H 5.1 (5.1); N 13.7 (13.3); Yb 18.4 (18.7).



Synthesis of TD522: In a glass vial (20 mL), **TD647**·1.3TFA (30.0 mg; 38.8 μmol ; 1.0 equiv.) was dissolved in H_2O (17 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 2.30 mL; 1.15 mmol; 30 equiv.) and aq. **LuCl₃** (100 mM; 500 μL ; 50.0 μmol ; 1.3 equiv.) and the mixture was stirred 4 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly

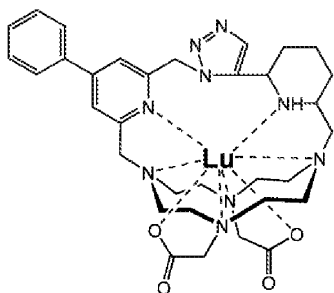
lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 32.6 mg (89%; 1 step; based on **TD647**·1.3TFA). **NMR (D₂O, pD ~5):** ¹H δ_{H} 2.62–3.83 (*mc*, $\text{CH}_2\text{--CO}$, m, 16+4H); 3.99 ($\text{CH}_2\text{--arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 4.16 ($\text{CH}_2\text{--arom.}$, d, 1H, $^2J_{\text{HH}} = 16$); 4.32 ($\text{CH}_2\text{--arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 4.84 ($\text{CH}_2\text{--arom.}$, d, 1H, $^2J_{\text{HH}} = 16$); 5.66 ($\text{CH}_2\text{--N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 7.13 ($\text{CH}_2\text{--N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 7.60–7.67 (*Ph*, m, 3H); 7.81 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.86 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.90 (CH--N_3 , s, 1H); 7.91–7.97 (*Ph*, m, 2H); 7.99 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 8.25 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$); 8.31 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 8.38 (*FA*, s, 1H). ¹³C{¹H} δ_{C} 48.9 (*mc*, bs); 49.1 (*mc*, bs); 51.8 (*mc*, bs); 51.9 (*mc*, bs); 52.9 ($\text{CH}_2\text{--N}_3$, s); 53.8 (*mc*, s); 53.9 (*mc*, bs); 54.9 (*mc*, s); 56.0 (*mc*, s); 59.2 ($\text{CH}_2\text{--CO}$, s); 59.6 ($\text{CH}_2\text{--arom.}$, s); 59.7 ($\text{CH}_2\text{--arom.}$, s); 61.0 ($\text{CH}_2\text{--CO}$, s); 123.7 (*arom.*, s); 126.5 (*arom.*, s); 127.7 (*arom.*, s); 128.3 (*Ph*, s); 130.3 (*Ph*, s); 130.5 (*arom.*, s); 131.8 (*Ph*, s); 136.0 (*Ph*, s); 136.4 (CH--N_3 , s); 138.4 (*arom.*, s); 142.6 (*arom.*, s); 146.4 (*arom.*, s); 154.7 (*arom.*, s); 156.0 (*arom.*, s); 158.7 (*arom.*, s); 158.8 (*arom.*, s); 170.0 (*FA*, s); 177.9 (*CO*, s); 179.4 (*CO*, s). **ESI-HRMS:** 798.2377 [M]⁺ (theor. $[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Lu}_1]^+ = 798.2371$). **EA** ($[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Lu}_1]^+[\text{FA}]^- \cdot 5.5\text{H}_2\text{O}$, $M_{\text{R}} = 942.8$): C 43.3 (43.7); H 5.2 (4.6); N 13.4 (13.1); Lu 18.6 (18.0).



Synthesis of TD776: In a glass vial (4 mL), **[TD522]⁺[FA]⁻·5.5H₂O** (9.0 mg; 9.5 μmol ; 1.0 equiv.) was dissolved in D_2O (500 μL) and evaporated to dryness. Residue was re-dissolved in D_2O (500 μL) followed by addition of neat **DBU** (29 μL ; 194 μmol ; 20 equiv.) and the resulting solution was stirred 2 d at 80 °C. Mixture was then evaporated to dryness. Residue was re-dissolved in D_2O (500 μL) followed by addition of another portion of neat **DBU** (15 μL ; 100 μmol ; ~10 equiv.) and the resulting solution was further stirred 16 h at 80 °C. Mixture was then

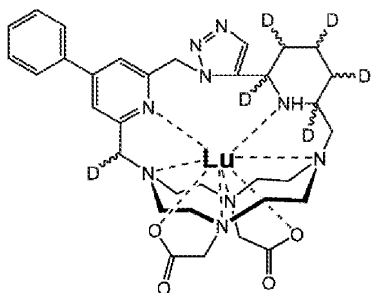
quenched by addition of **FA** (75 μL ; 2.92 mmol; ~300 equiv.) and directly purified by preparative HPLC (C18; H_2O –MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give crude product in the form of formate salt as white fluffy solid (¹H NMR analysis found ~1 equiv. of DBU or its byproducts apart from otherwise pure deka-deuterated chelate). Bulk of the material was therefore re-purified by preparative HPLC (C18; H_2O –MeCN gradient with TFA additive). Fractions with product were

joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 7.6 mg ($\leq 87\%$ assuming $[M]^+[TFA]^- \cdot xH_2O$; $M_R = 921.7$; 1 step; based on $[TD522]^+[FA]^- \cdot 5.5H_2O$). **NMR (D_2O , pD ~4):** 1H δ_H 2.64–3.83 (*mc*, m, 16H); 7.59–7.67 (*Ph*, m, 3H); 7.80 (*arom.*, dd, 1H, $^3J_{HH} = 8$, $^4J_{HH} = 1$); 7.84 (*arom.*, dd, 1H, $^3J_{HH} = 8$, $^4J_{HH} = 1$); 7.89 (*CH-N₃*, s, 1H); 7.90–7.96 (*Ph*, m, 2H); 7.98 (*arom.*, d, 1H, $^4J_{HH} = 2$); 8.24 (*arom.*, t, 1H, $^3J_{HH} = 8$); 8.30 (*arom.*, d, 1H, $^4J_{HH} = 2$); 8.39 (*FA*, s, 1H). $^{13}C\{^1H\}$ δ_C 48.9 (*mc*, bs); 49.2 (*mc*, bs); 51.8 (*mc*, bs); 51.9 (*mc*, bs); 52.0–52.9 (*CD₂-N₃*, bm); 53.8 (*mc*, s); 53.9 (*mc*, bs); 54.8 (*mc*, s); 55.9 (*mc*, s); 58.2–59.7 (*CD₂-CO*, $2 \times CD_2$ -*arom.*, bm); 59.7–60.9 (*CD₂-CO*, s); 123.8 (*arom.*, s); 126.5 (*arom.*, s); 127.8 (*arom.*, s); 128.3 (*Ph*, s); 130.3 (*Ph*, s); 130.5 (*arom.*, s); 131.8 (*Ph*, s); 136.1 (*Ph*, s); 136.4 (*CH-N₃*, s); 138.5 (*arom.*, s); 142.6 (*arom.*, s); 146.4 (*arom.*, s); 154.8 (*arom.*, s); 156.0 (*arom.*, s); 158.7 (*arom.*, s); 158.8 (*arom.*, s); 170.5 (*FA*, s); 178.0 (*CO*, s); 179.4 (*CO*, s). **ESI-HRMS:** 808.3004 $[M]^+$ (theor. $[C_{33}H_{27}D_{10}N_9O_4Lu]^+ = 808.2998$).



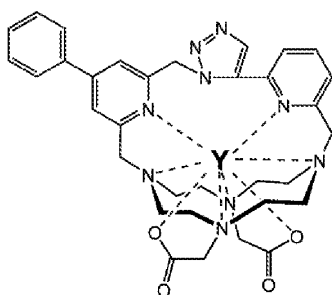
Synthesis of TD688: In a glass vial (2 mL), $[TD522]^+[FA]^- \cdot 5.5H_2O$ (1 mg; $\sim 1 \mu\text{mol}$; 1.0 equiv.) was dissolved in MeOH (1 mL) followed by addition of solid $NaBH_4$ (4 mg; $\sim 100 \text{ mmol}$; $\sim 100 \text{ equiv.}$). The resulting open vial was stirred 1 h at RT after which further portions of $NaBH_4$ were added until all starting material was consumed (analyzed by LC-MS). The mixture was then diluted with H_2O (2 mL), neutralized with neat FA (200 μL) and filtered through syringe microfilter (RC). Filtrate was directly purified by preparative HPLC (C18; H_2O -MeCN gradient with FA additive). Fractions

with product were joined and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** $< 1 \text{ mg}$. **NMR (D_2O , pD ~6):** 1H δ_H 1.42–2.23 (*Pip.*, m, 6H); 2.52–3.70 (*mc*, CH_2 -CO, CH_2 -*Pip.*, m, 16+3+2H); 3.76 (*Pip.*, m, 1H); 4.16 (CH_2 -CO, d, $^2J_{HH} = 17$); 4.22 (CH_2 -*arom.*, d, 1H, $^2J_{HH} = 16$); 4.32 (CH_2 -*arom.*, d, 1H, $^2J_{HH} = 16$); 4.78 (*Pip.*, m, 1H); 5.66 (CH_2 - N_3 , d, 1H, $^2J_{HH} = 16$); 6.88 (CH_2 - N_3 , d, 1H, $^2J_{HH} = 16$); 7.68–7.74 (*Ph*, m, 3H); 7.91 (*CH-N₃*, s, 1H); 7.98 (*arom.*, d, 1H, $^4J_{HH} = 2$); 7.98–8.02 (*Ph*, m, 2H); 8.25 (*arom.*, d, 1H, $^4J_{HH} = 2$); 8.52 (*FA*, s). $^{13}C\{^1H\}$ δ_C 22.8 (*Pip.*, s); 27.0 (*Pip.*, s); 29.5 (*Pip.*, s); 50.1 (*Pip.*, s); 51.4 (CH_2 - N_3 , s); 52.1 (*mc*, s); 53.4 (*mc*, s); 54.4 (*mc*, s); 54.5 (*Pip.*, s); 55.4 (*mc*, s); 55.6 (*mc*, s); 56.0 (*mc*, s); 56.1 (*mc*, s); 56.8 (*mc*, s); 56.0 (CH_2 -*Pip.*, s); 64.7 (CH_2 -CO, s); 64.8 (CH_2 -*arom.*, s); 65.4 (CH_2 -*Pip.*, s); 119.8 (*arom.*, s); 123.7 (*arom.*, s); 126.9 (*Ph*, s); 129.0 (*Ph*, s); 130.5 (*Ph*, s); 131.6 (CH - N_3 , s); 135.7 (*Ph*, s); 138.9 (*arom.*, s); 153.1 (*arom.*, s); 155.9 (*arom.*, s); 159.0 (*arom.*, s); 170.5 (*FA*, s); 178.8 (*CO*, s); 179.5 (*CO*, s). **ESI-HRMS:** 804.2841 $[M]^+$ (theor. $[C_{33}H_{43}N_9O_4Lu]^+ = 804.2840$).



Synthesis of TD739: In a glass vial (2 mL), [TD522]⁺[FA]⁻·5.5H₂O (10 mg; 11 μmol; 1.0 equiv.) was dissolved in CD₃OD (1 mL) followed by addition of solid NaBD₄ (44 mg; 1.05 mmol; ~100 equiv.). The resulting open vial was stirred 1 h at RT after which another portion of NaBD₄ (28 mg; 0.67 mmol; ~60 equiv.) was added. Mixture was further stirred with 1 h at RT. The mixture was then diluted with H₂O (2 mL), neutralized with neat FA (160 μL; ~400 equiv.) and filtered through syringe microfilter (RC). Filtrate was directly purified by preparative

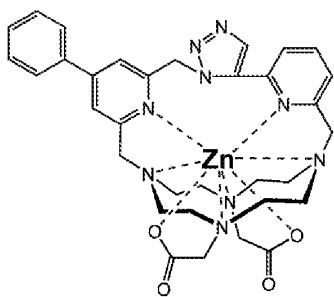
HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product (major diastereoisomer) were joined and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 6 mg (~60%; 1 step; based on [TD522]⁺[FA]⁻·5.5H₂O). **NMR (D₂O, pD ~5):** 1.33–1.77 (*Pip.*, m, 1H); 1.93–2.09 (*Pip.*, m, 2H); 2.42–3.62 (*mc*, CH₂–CO, CH₂–*Pip.*, m, 16+3H+1H); 4.07 (CH₂–CO, d, 1H, ²J_{HH} = 17); 4.19 (CHD–*arom.*, s, 1H); 5.56 (CH₂–N₃, d, 1H, ²J_{HH} = 16); 6.78 (CH₂–N₃, d, 1H, ²J_{HH} = 16); 7.59–7.65 (*Ph*, m, 3H); 7.82 (CH–N₃, s, 1H); 7.87 (*arom.*, d, 1H, ⁴J_{HH} = 2); 7.88–7.93 (*Ph*, m, 2H); 8.14 (*arom.*, d, 1H, ⁴J_{HH} = 2); 8.43 (*FA*, s, 1H). **¹³C{¹H} δ_C** 23.4 (*Pip.*, bs); 27.7 (*Pip.*, bs); 30.3 (*Pip.*, bs); 50.9 (*Pip.*, bs); 52.7 (CH₂–N₃, s); 53.4 (*mc*, s); 54.7 (*mc*, s); 55.3 (*Pip.*, bs); 55.7 (*mc*, s); 56.7 (*mc*, s); 56.8 (*mc*, s); 57.3 (*mc*, s); 57.4 (*mc*, s); 58.1 (*mc*, s); 61.2 (CH₂–CO, s); 65.8 (CHD–*arom.*, bs); 66.0 (CH₂–*Pip.*, s); 66.8 (CH₂–CO, s); 121.1 (*arom.*, s); 125.0 (*arom.*, s); 128.2 (*Ph*, s); 130.3 (*Ph*, s); 131.8 (*Ph*, s); 132.9 (*arom.*, s); 135.9 (*Ph*, s); 140.2 (*arom.*, s); 154.3 (*arom.*, s); 157.2 (*arom.*, s); 160.3 (*arom.*, s); 171.4 (*FA*, s); 180.1 (CO, s); 180.8 (CO, s). **ESI-HRMS:** 810.3215 [M]⁺ (theor. [C₃₃H₃₇D₆N₉O₄Lu]⁺ = 810.3217).



Synthesis of TD649: In a glass vial (20 mL), TD647·0.2FA·0.9H₂O (38 mg; 59 μmol; 1.0 equiv.) was dissolved in H₂O (16 mL) followed by addition aq. MOPS/NaOH buffer (500 mM; pH 7.0; 2.96 mL; 1.48 mmol; 25 equiv.) and aq. YCl₃ (100 mM; 650 μL; 65 μmol; 1.1 equiv.) and the mixture was stirred 20 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give

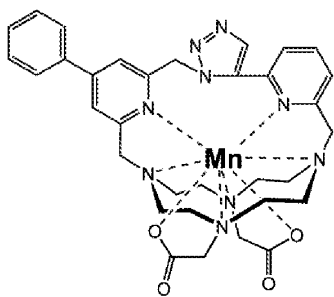
product in the form of formate salt as white fluffy solid. **Yield:** 32 mg (65%; 1 step; based on TD647·0.2FA·0.9H₂O). **NMR (D₂O, pD ~5):** ¹H δ_H 2.58–3.86 (*mc*, CH₂–CO, m, 16+4H); 3.99 (CH₂–*arom.*, d, 1H, ²J_{HH} = 15); 4.15 (CH₂–*arom.*, d, 1H, ²J_{HH} = 16); 4.37 (CH₂–*arom.*, d, 1H, ²J_{HH} = 15); 4.85 (CH₂–*arom.*, d, 1H, ²J_{HH} = 16); 5.71 (CH₂–N₃, d, 1H, ²J_{HH} = 16); 7.07 (CH₂–N₃, d, 1H, ²J_{HH} = 16); 7.58–7.68 (*Ph*, m, 3H); 7.78–7.86 (*arom.*, m, 2H); 7.89 (CH–N₃, s, 1H); 7.91–7.97 (*Ph*, m, 2H); 7.99 (*arom.*, d, 1H, ⁴J_{HH} = 2); 8.26 (*arom.*, t, 1H, ³J_{HH} = 8); 8.31 (*arom.*, d, 1H, ⁴J_{HH} = 2); 8.42 (*FA*, s, 1H). **¹³C{¹H} δ_C** 49.0 (*mc*, s); 49.2 (*mc*, s);

51.4 (*mc*, s); 51.7 (*mc*, s); 53.0 ($\text{CH}_2\text{-N}_3$, s); 53.9 (*mc*, s); 54.1 (*mc*, s); 54.7 (*mc*, s); 55.7 (*mc*, s); 59.5 ($\text{CH}_2\text{-CO}$, s); 59.6 ($\text{CH}_2\text{-arom.}$, s); 59.8 ($\text{CH}_2\text{-arom.}$, s); 61.4 ($\text{CH}_2\text{-CO}$, s); 123.9 (*arom.*, s); 126.5 (*arom.*, s); 127.6 (*arom.*, s); 128.4 (*Ph*, s); 130.1 (*arom.*, s); 130.3 (*Ph*, s); 131.8 (*Ph*, s); 136.2 (*Ph*, s); 136.8 (CH-N_3 , s); 138.2 (*arom.*, s); 142.8 (*arom.*, s); 146.0 (*arom.*, s); 155.0 (*arom.*, s); 155.6 (*arom.*, s); 158.6 (*arom.*, s); 158.7 (*arom.*, s); 171.2 (*FA*, s); 177.7 (CO , s); 179.0 (CO , s). **ESI-HRMS:** 712.2017 $[\text{M}]^+$ (theor. $[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Y}_1]^+ = 712.2022$). **EA** ($[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Y}_1]^+[\text{FA}]^- \cdot 4.5\text{H}_2\text{O}$, $M_R = 838.7$): C 48.7 (48.6); H 5.6 (5.2); N 15.0 (14.7); Y 10.6 (10.3).



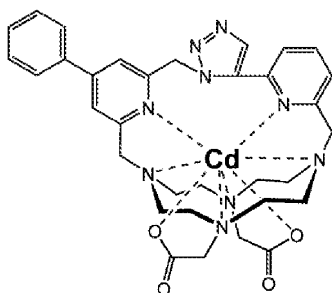
Synthesis of TD890: In a glass vial (4 mL), **TD650**·1.1FA·1.6H₂O (15.2 mg; 21.6 μmol ; 1.0 equiv.) was dissolved in H₂O (2 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 1.10 mL; 550 μmol ; 25 equiv.) and aq. **Zn(NO₃)₂** (51.7 mM; 460 μL ; 23.8 μmol ; 1.1 equiv.) and the mixture was stirred 2 h at 80 °C. Mixture was then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with no additive). Fractions with product were joined and directly lyophilized to give product as white fluffy solid. **Yield:** 15.5 mg (91%; 1 step; based on **TD650**·1.1FA·1.6H₂O).

NMR (D₂O, pD ~8): ¹H δ_{H} 2.43–3.38 (*mc*, $\text{CH}_2\text{-CO}$, bm, 16+4H); 4.14 ($\text{CH}_2\text{-arom.}$, s, 2H); 4.25 ($\text{CH}_2\text{-arom.}$, bs, 2H); 6.48 ($\text{CH}_2\text{-N}_3$, bs, 2H); 7.60 (*arom.*, dd, 1H, ³ $J_{\text{HH}} = 8$, ⁴ $J_{\text{HH}} = 1$); 7.62–7.68 (*Ph*, m, 3H); 7.85 (*arom.*, dd, 1H, ³ $J_{\text{HH}} = 8$, ⁴ $J_{\text{HH}} = 1$); 7.86 (*arom.*, d, 1H, ⁴ $J_{\text{HH}} = 2$); 7.91–7.98 (*Ph*, m, 2H); 8.09 (*arom.*, t, 1H, ³ $J_{\text{HH}} = 8$); 8.21 (CH-N_3 , s, 1H); 8.31 (*arom.*, d, 1H, ⁴ $J_{\text{HH}} = 2$). **ESI-HRMS:** 688.2339 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{33}\text{H}_{38}\text{N}_9\text{O}_4\text{Zn}_1]^+ = 688.2333$). **EA** ($[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Zn}_1] \cdot 5.5\text{H}_2\text{O}$, $M_R = 788.2$): C 50.3 (50.2); H 6.1 (5.8); N 16.0 (15.7); Zn 8.3 (8.5).



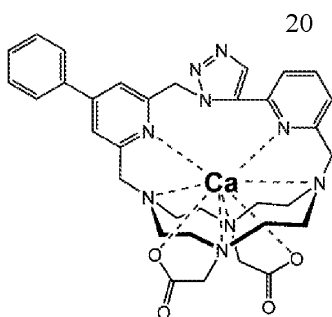
Synthesis of TD1195: In a glass vial (4 mL), **TD650**·1.1FA·1.6H₂O (9.0 mg; 12.8 μmol ; 1.0 equiv.) was dissolved in H₂O (780 μL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 650 μL ; 325 μmol ; 25 equiv.) and aq. **MnCl₂** (25.0 mM; 570 μL ; 14.3 μmol ; 1.1 equiv.) and the mixture was stirred 1 h at 80 °C. Mixture was then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product as white fluffy solid. **Yield:** 7.5 mg (74%; 1 step; based on **TD650**·1.1FA·1.6H₂O).

ESI-HRMS: 679.2423 $[\text{M}+\text{H}]^+$ (theor. $[\text{C}_{33}\text{H}_{38}\text{N}_9\text{O}_4\text{Mn}_1]^+ = 679.2422$). **EA** ($[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Mn}_1] \cdot 6.4\text{H}_2\text{O}$, $M_R = 794.0$): C 49.9 (49.5); H 6.3 (5.9); N 15.9 (15.7); Mn 6.9 (6.2).



Synthesis of TD880: In a glass vial (4 mL), **TD650**·1.1FA·1.6H₂O (35.0 mg; 49.8 μmol; 1.0 equiv.) was dissolved in aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 2.50 mL; 1.25 mmol; 25 equiv.) and aq. **CdCl₂** (62.6 mM; 875 μL; 54.8 μmol; 1.1 equiv.) and the mixture was stirred 1 h at 80 °C. Mixture was then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with no additive). Fractions with product were joined and directly lyophilized to give product as white fluffy solid. **Yield:** 37.1 mg (90%; 1 step; based on **TD650**·1.1FA·1.6H₂O). **NMR (D₂O, pD ~7):** ¹H δ_H 2.24–

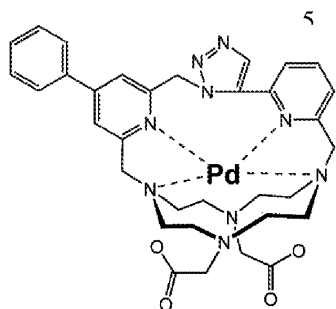
3.54 (*mc*, CH₂–CO, m, 16+4H); 3.60–3.79 (CH₂–arom., m, 2H); 4.03 (CH₂–arom., d, 1H, ²J_{HH} = 13); 4.75 (CH₂–arom., d, 1H, ²J_{HH} = 14); 5.51 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 6.95 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.58–7.67 (*Ph*, m, 3H); 7.71 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.80 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.89 (*arom.*, d, 1H, ⁴J_{HH} = 2); 7.93–7.98 (*Ph*, m, 2H); 8.04 (CH–N₃, s, 1H); 8.15 (*arom.*, t, 1H, ³J_{HH} = 8); 8.21 (*arom.*, d, 1H, ⁴J_{HH} = 2); ¹³C{¹H} δ_C 47.8 (*mc*, s); 48.3 (*mc*, s); 49.3 (*mc*, s); 50.3 (*mc*, s); 50.9 (*mc*, s); 51.1 (*mc*, s); 51.2 (*mc*, s); 51.5 (*mc*, s); 53.4 (CH₂–N₃, s); 56.6 (CH₂–CO, s); 57.4 (CH₂–arom., s); 57.6 (CH₂–CO, s); 58.6 (CH₂–arom., s); 124.3 (*arom.*, s); 125.2 (*arom.*, s); 128.2 (*Ph*, s); 128.3 (*arom.*, s); 128.9 (*arom.*, s); 130.2 (*Ph*, s); 131.3 (*Ph*, s); 135.3 (CH–N₃, s); 136.6 (*Ph*, s); 138.8 (*arom.*, s); 141.3 (*arom.*, s); 147.4 (*arom.*, s); 153.6 (*arom.*, s); 156.9 (*arom.*, s); 157.9 (*arom.*, s); 158.3 (*arom.*, s); 175.7 (CO, s); 177.4 (CO, s). **ESI-HRMS:** 738.2079 [M+H]⁺ (theor. [C₃₃H₃₈N₉O₄Cd]⁺ = 738.2075). **EA** ([C₃₃H₃₇N₉O₄Cd]⁺·5.1H₂O, M_R = 828.0): C 47.9 (47.6); H 5.7 (5.0); N 15.2 (14.6); Cd 13.6 (13.5).



Synthesis of TD863: In a glass vial (20 mL), **TD650**·1.1FA·1.6H₂O (8.2 mg; 11.7 μmol; 1.0 equiv.) was dissolved in H₂O (2 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 1.35 mL; 675 μmol; 58 equiv.) and aq. **Ca(NO₃)₂** (100 mM; 200 μL; 20 μmol; 1.7 equiv.) and the mixture was stirred 24 h at 80 °C. Mixture was then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with no additive). Fractions with product were joined and directly lyophilized to give product as white fluffy solid. **Yield:** 7.4 mg (81%; 1 step; based on **TD650**·1.1FA·1.6H₂O).

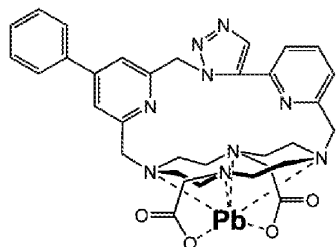
NMR (D₂O, pD ~6): ¹H δ_H 2.20–3.58 (*mc*, CH₂–CO, m, 16+4H); 3.62 (CH₂–arom., d, 1H, ²J_{HH} = 14); 3.76 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.09 (CH₂–arom., d, 1H, ²J_{HH} = 14); 4.54 (CH₂–arom., d, 1H, ²J_{HH} = 15); 5.56 (CH₂–N₃, d, 1H, ²J_{HH} = 16); 7.02 (CH₂–N₃, d, 1H, ²J_{HH} = 16); 7.57–7.65 (*Ph*, m, 3H); 7.66 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.72 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.83 (*arom.*, d, 1H, ⁴J_{HH} = 2); 7.89 (CH–N₃, s, 1H); 7.90–7.95 (*Ph*, m, 2H); 8.09 (*arom.*, t, 1H, ³J_{HH} = 8); 8.12 (*arom.*, d, 1H, ⁴J_{HH} = 2); ¹³C{¹H} δ_C 48.4 (*mc*, s); 48.5 (*mc*, s); 50.6 (*mc*, s); 50.7 (*mc*, s); 52.6 (*mc*, s); 52.7 (*mc*, s); 53.0 (*mc*, s); 53.3 (CH₂–N₃, s); 53.6 (*mc*, s); 57.9 (CH₂–CO, s); 59.1 (CH₂–arom., s); 59.2 (CH₂–arom., s); 59.3 (CH₂–CO, s); 123.3 (*arom.*, s); 124.7 (*arom.*, s); 127.0 (*arom.*, s); 128.1 (*Ph*, s); 128.2 (*arom.*, s); 130.1 (*Ph*, s); 130.9 (*Ph*, s); 136.7 (CH–N₃, s); 137.3 (*Ph*,

s); 139.4 (*arom.*, s); 140.9 (*arom.*, s); 146.7 (*arom.*, s); 153.1 (*arom.*, s); 155.5 (*arom.*, s); 159.3 (*arom.*, s); 159.5 (*arom.*, s); 178.4 (CO, s); 179.7 (CO, s). **ESI-HRMS**: 664.2663 [M+H]⁺ (theor. [C₃₃H₃₈N₉O₄Ca]⁺ = 664.2667). **EA** ([C₃₃H₃₇N₉O₄Ca]⁺·6.7H₂O, *M_R* = 784.5): C 50.5 (50.5); H 6.5 (6.0); N 16.1 (15.6); Ca 5.1 (4.8).



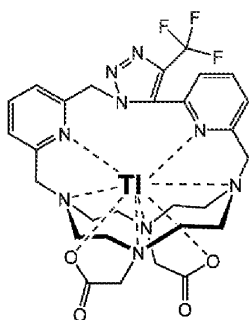
Synthesis of TD1214: In a glass vial (20 mL), **TD650**·1.1FA·1.6H₂O (9.0 mg; 12.8 μmol; 1.0 equiv.) was dissolved in H₂O (780 μL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 650 μL; 325 μmol; 25 equiv.) and aq. **Na₂[PdCl₄]** (25 mM; 570 μL; 14.3 μmol; 1.1 equiv.) and the mixture was stirred 4 d at 80 °C. Mixture was then filtered through syringe microfilter (RC) and directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product as white fluffy solid. **Yield**: 6.4 mg

(58%; 1 step; based on **TD650**·1.1FA·1.6H₂O). **NMR (D₂O, pD ~5)**: 1.54–3.82 (*mc*, CH₂–CO, bm, 16+4H); 4.21–5.10 (CH₂–*arom.*, bm, 4H overlapped with water signal); 5.69 (CH₂–N₃, bs, 1H); 7.42–7.54 (*Ph*, *arom.*, m, 3+1H); 7.60 (*arom.*, d, 1H, ³*J*_{HH} = 8); 7.65 (CH₂–N₃, bs, 1H); 7.69–7.76 (*Ph*, m, 2H); 8.01 (*arom.*, d, 1H, ³*J*_{HH} = 8); 8.08–8.15 (*arom.*, m, 2H); 8.47 (CH–N₃, s, 1H). **ESI-HRMS**: 730.2088 [M+H]⁺ (theor. [C₃₃H₃₈N₉O₄Pd]⁺ = 730.2076). **EA** ([C₃₃H₃₇N₉O₄Pd]⁺·0.2FA·7.0H₂O, *M_R* = 865.2): C 46.1 (46.4); H 6.0 (5.7); N 14.6 (14.4); Pd 12.3 (9.6).

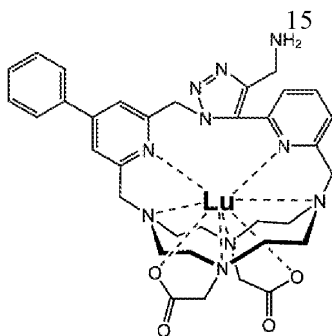


Synthesis of TD876: In a glass vial (20 mL), **TD650**·1.1FA·1.6H₂O (8.2 mg; 11.7 μmol; 1.0 equiv.) was dissolved in H₂O (2.5 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 580 μL; 290 μmol; 25 equiv.) and aq. **Pb(NO₃)₂** (52.4 mM; 300 μL; 15.7 μmol; 1.3 equiv.) and the mixture was stirred 5 min at RT. Mixture was then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with no additive). Fractions with product were joined and directly lyophilized to give product as white

fluffy solid. **Yield**: 10.4 mg (94%; 1 step; based on **TD650**·1.1FA·1.6H₂O). **NMR (D₂O, pD ~6)**: δ_H 2.40–3.62 (*mc*, CH₂–CO, bm, 16+4H); 4.20 (CH₂–*arom.*, bm, 4H); 5.72 (CH₂–N₃, bd, 1H, ²*J*_{HH} = 15); 6.18 (CH₂–N₃, bd, 1H, ²*J*_{HH} = 15); 7.56–7.67 (*Ph*, m, 3H); 7.74 (*arom.*, dd, 1H, ³*J*_{HH} = 8, ⁴*J*_{HH} = 1); 7.79 (*arom.*, dd, 1H, ³*J*_{HH} = 8, ⁴*J*_{HH} = 1); 7.86–7.90 (*Ph*, m, 2H); 7.91 (*arom.*, d, 1H, ⁴*J*_{HH} = 2); 7.99 (CH–N₃, s, 1H); 8.08 (*arom.*, d, 1H, ⁴*J*_{HH} = 2); 8.16 (*arom.*, t, 1H, ³*J*_{HH} = 8). **ESI-HRMS**: 832.2806 [M+H]⁺ (theor. [C₃₃H₃₈N₉O₄Pb]⁺ = 832.2808). **EA** ([C₃₃H₃₇N₉O₄Pb]⁺·6.4H₂O, *M_R* = 946.2): C 41.9 (42.6); H 5.3 (4.7); N 13.3 (12.6); Pb 21.9 (21.4).

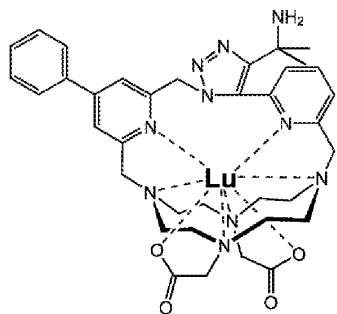


Synthesis of TD1238: **Synthesis:** In a glass vial (20 mL), **TD1188**·0.2TFA·0.4FA·4.3H₂O (6.5 mg; 8.6 μmol; 1.0 equiv.) was dissolved in H₂O (19 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 950 μL; 475 μmol; 56 equiv.) and solid **Ti(NO₃)₃·3H₂O** (4.2 mg; 9.5 μmol; 1.1 equiv.) and the mixture was stirred 2 h at 80 °C. Then, another portion of **Ti(NO₃)₃·3H₂O** (19.0 mg; 43 μmol; 5.0 equiv.) was added and the mixture was further stirred 20 h at 80 °C. Mixture was then filtered through syringe microfilter (RC) and the filtrate was evaporated to dryness. Residue was re-dissolved in H₂O and directly purified by preparative (HPLC C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product as white solid. **Yield:** 1.4 mg (≤18% assuming [M]⁺[TFA][−]·xH₂O; *M_R* = 933.0; 1 step; based on **TD1188**·0.2TFA·0.4FA·4.3H₂O). **NMR (D₂O, pD ~4, two set of signals on ¹H, but one on ¹⁹F):** δ_H 2.17–5.34 (*mc*, CH₂–CO, CH₂–arom., m, 16+4H+4H); 5.54–5.70 (CH₂–N₃, m, 1H); 6.93–7.07 (CH₂–N₃, m, 1H); 7.69–8.44 (*arom.*, m, 6H). **¹⁹F** δ_F −58.70 (s). **ESI-HRMS:** 820.2265 [M]⁺ (theor. [C₂₈H₃₂N₉O₄F₃Ti]⁺ = 820.2264).



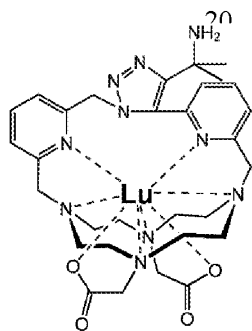
Synthesis of TD735: In a glass vial (20 mL), **TD722**·0.7TFA·2.0H₂O (100 mg; 130 μmol; 1.0 equiv.) was dissolved in H₂O (30 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 7.00 mL; 3.50 mmol; 27 equiv.) and aq. **LuCl₃** (100 mM; 1.50 mL; 150 μmol; 1.2 equiv.) and the mixture was stirred 16 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 134.2 mg (91%; 1 step; based on **TD722**·0.7TFA·2.0H₂O). **NMR (D₂O, pD ~4): ¹H** δ_H 2.65–3.84 (*mc*, CH₂–CO, m, 16+4H); 4.01 (CH₂–arom., d, 1H, ²*J*_{HH} = 15); 4.04 (CH₂–NH₂, d, 1H, ²*J*_{HH} = 15); 4.23 (CH₂–arom., d, 1H, ²*J*_{HH} = 16); 4.31 (CH₂–NH₂, d, 1H, ²*J*_{HH} = 15); 4.33 (CH₂–arom., d, 1H, ²*J*_{HH} = 15); 4.86 (CH₂–arom., d, 1H, ²*J*_{HH} = 16); 5.68 (CH₂–N₃, d, 1H, ²*J*_{HH} = 15); 7.08 (CH₂–N₃, d, 1H, ²*J*_{HH} = 15); 7.60–7.69 (*Ph*, m, 3H); 7.72 (*arom.*, dd, 1H, ³*J*_{HH} = 8, ⁴*J*_{HH} = 1); 7.91 (*arom.*, dd, 1H, ³*J*_{HH} = 8, ⁴*J*_{HH} = 1); 7.92–7.99 (*Ph*, m, 2H); 8.03 (*arom.*, d, 1H, ⁴*J*_{HH} = 2); 8.31 (*arom.*, t, 1H, ³*J*_{HH} = 8); 8.33 (*arom.*, d, 1H, ⁴*J*_{HH} = 2). **¹³C{¹H}** δ_C 34.8 (CH₂–C≡C, s); 48.9 (*mc*, s); 49.2 (*mc*, s); 51.8 (*mc*, s); 52.0 (*mc*, s); 53.4 (CH₂–N₃, s); 53.9 (*mc*, s); 54.1 (*mc*, s); 55.2 (*mc*, s); 56.1 (*mc*, s); 59.2 (CH₂–CO, s); 59.7 (CH₂–arom., s); 60.0 (CH₂–arom., s); 61.1 (CH₂–CO, s); 124.0 (*arom.*, s); 126.8 (*arom.*, s); 128.2 (*arom.*, s); 128.4 (*Ph*, s); 130.3 (*Ph*, s); 130.8 (*arom.*, s); 131.9 (*Ph*, s); 136.0 (*Ph*, s); 136.2 (*arom.*, s); 140.5 (*arom.*, s); 142.8 (*arom.*, s); 144.4 (*arom.*, s); 155.0 (*arom.*, s); 156.0 (*arom.*, s); 158.9 (*arom.*, s); 160.0 (*arom.*, s); 170.7 (*FA*, s); 178.8 (CO, s); 179.3 (CO, s). **ESI-HRMS:** 827.2637 [M]⁺ (theor. [C₃₄H₄₀N₁₀O₄Lu]⁺ = 827.2636). **EA**

([C₃₄H₄₀N₁₀O₄Lu₁]⁺[TFA]⁻·1.2TFA·3.1H₂O, *M_R* = 1133.3): C 40.7 (40.9); H 4.2 (3.9); N 12.4 (12.1); Lu 11.1 (11.0).



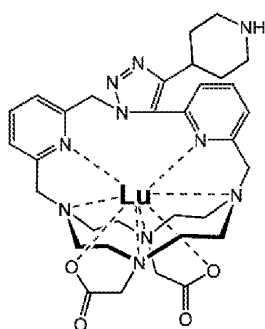
Synthesis of TD1059: In a glass vial (20 mL), **TD1054**·0.3TFA·0.8FA·2.3H₂O (10.0 mg; 12.9 μmol; 1.0 equiv.) was dissolved in H₂O (19 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 800 μL; 400 μmol; 31 equiv.) and aq. **LuCl₃** (100 mM; 150 μL; 15.0 μmol; 1.2 equiv.) and the mixture was stirred 16 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with

product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 10.8 mg (71%; 1 step; based on **TD1054**·0.3TFA·0.8FA·2.3H₂O). **NMR (D₂O, pD ~6):** ¹H δ_H 1.18 (CH₃, s, 3H); 1.79 (CH₃, s, 3H); 2.64–3.85 (*mc*, CH₂–CO, m, 16+4H); 4.03 (CH₂–arom., d, 1H, ²*J*_{HH} = 15); 4.30 (CH₂–arom., d, 1H, ²*J*_{HH} = 16); 4.36 (CH₂–arom., d, 1H, ²*J*_{HH} = 18); 4.86 (CH₂–arom., d, 1H, ²*J*_{HH} = 18); 5.62 (CH₂–N₃, d, 1H, ²*J*_{HH} = 15); 6.83 (CH₂–N₃, d, 1H, ²*J*_{HH} = 15); 7.60–7.68 (*Ph*, m, 3H); 7.83 (*arom.*, d, 1H, ³*J*_{HH} = 8); 7.89 (*arom.*, d, 1H, ³*J*_{HH} = 8); 7.91–7.98 (*Ph*, m, 2H); 8.03 (*arom.*, d, 1H, ⁴*J*_{HH} = 2); 8.28 (*arom.*, t, 1H, ³*J*_{HH} = 8); 8.30 (*arom.*, d, 1H, ⁴*J*_{HH} = 2). **ESI-HRMS:** 855.2951 [M]⁺ (theor. [C₃₆H₄₄N₁₀O₄Lu₁]⁺ = 855.2949). **EA** ([C₃₇H₄₅N₁₀O₄Lu₁]⁺[TFA]⁻·1.0TFA·4.6H₂O, *M_R* = 1178.6): C 41.8 (41.4); H 4.7 (4.2); N 11.9 (11.7); Lu 14.8 (13.2).



Synthesis of TD1124: In a glass vial (20 mL), **TD1105**·0.1FA·2.0H₂O (35.2 mg; 47.1 μmol; 1.0 equiv.) was dissolved in H₂O (17 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 2.35 mL; 1.18 mmol; 25 equiv.) and aq. **LuCl₃** (100 mM; 520 μL; 52.0 μmol; 1.1 equiv.) and the mixture was stirred 4 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white

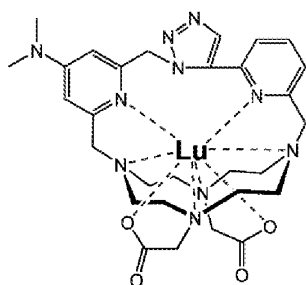
fluffy solid. **Yield:** 41.2 mg (77%; 1 step; based on **TD1105**·0.1FA·2.0H₂O). **NMR (D₂O, pD ~3):** ¹H δ_H 1.17 (CH₃, s, 3H); 1.78 (CH₃, s, 3H); 2.64–3.84 (*mc*, CH₂–CO, m, 16+4H); 3.97 (CH₂–arom., d, 1H, ²*J*_{HH} = 15); 4.25 (CH₂–arom., d, 1H, ²*J*_{HH} = 15); 4.35 (CH₂–arom., d, 1H, ²*J*_{HH} = 17); 4.86 (CH₂–arom., d, 1H, ²*J*_{HH} = 17); 5.56 (CH₂–N₃, d, 1H, ²*J*_{HH} = 15); 6.80 (CH₂–N₃, d, 1H, ²*J*_{HH} = 15); 7.73 (*arom.*, d, 1H, ³*J*_{HH} = 8); 7.82 (*arom.*, d, 1H, ³*J*_{HH} = 8); 7.88 (*arom.*, d, 1H, ³*J*_{HH} = 8); 7.99 (*arom.*, d, 1H, ³*J*_{HH} = 8); 8.24 (*arom.*, t, 1H, ³*J*_{HH} = 8); 8.27 (*arom.*, t, 1H, ³*J*_{HH} = 8). **ESI-HRMS:** 779.2636 [M]⁺ (theor. [C₃₀H₄₀N₁₀O₄Lu₁]⁺ = 779.2636). **EA** ([C₃₀H₄₀N₁₀O₄Lu₁]⁺[TFA]⁻·1.5TFA·3.9H₂O, *M_R* = 1133.9): C 37.1 (37.3); H 4.4 (3.9); N 12.4 (12.0); F 12.6 (12.1).



Synthesis of TD1137: In a glass vial (20 mL), **TD1127**·1.8TFA·0.1FA (45.9 mg; 52.2 μ mol; 1.0 equiv.) was dissolved in H₂O (16 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 3.15 mL; 1.58 mmol; 30 equiv.) and aq. **LuCl₃** (100 mM; 690 μ L; 69.0 μ mol; 1.3 equiv.) and the mixture was stirred 2 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt

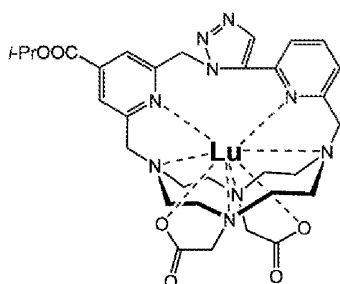
as white fluffy solid. **Yield:** 58.2 mg (98%; 1 step; based on **TD1127**·1.8TFA·0.1FA). **NMR (D₂O, pD ~4):**

- 10 ¹H δ_{H} 1.92–2.13 (*CH*₂, m, 3H); 2.17–2.33 (*CH*₂, m, 1H); 2.63–3.83 (*mc*, *CH*₂–CO, *CH*₂, *CH*, m, 16+4+4+1H); 3.98 (*CH*₂–arom., d, 1H, ²*J*_{HH} = 15); 4.22 (*CH*₂–arom., d, 1H, ²*J*_{HH} = 15); 4.34 (*CH*₂–arom., d, 1H, ²*J*_{HH} = 17); 4.87 (*CH*₂–arom., d, 1H, ²*J*_{HH} = 17); 5.50 (*CH*₂–N₃, d, 1H, ²*J*_{HH} = 15); 6.93 (*CH*₂–N₃, d, 1H, ²*J*_{HH} = 15); 7.67–7.76 (*arom.*, m, 2H); 7.85 (*arom.*, dd, 1H, ³*J*_{HH} = 8, ⁴*J*_{HH} = 1); 7.98 (*arom.*, dd, 1H, ³*J*_{HH} = 8, ⁴*J*_{HH} = 1); 8.23 (*arom.*, t, 1H, ³*J*_{HH} = 8); 8.27 (*arom.*, t, 1H, ³*J*_{HH} = 8). **ESI-HRMS:** 805.2786 [M]⁺ (theor. [C₃₂H₄₂N₁₀O₄Lu]⁺ = 805.2793). **EA** ([C₃₂H₄₂N₁₀O₄Lu]⁺[TFA][−]·1.0TFA·5.5H₂O, *M_R* = 1131.8): C 38.2 (38.7); H 4.8 (4.5); N 12.4 (11.9); F 10.1 (9.9).
- 15



Synthesis of TD1182: In a glass vial (20 mL), **TD1176**·0.8FA·4.4H₂O (39.3 mg; 55.4 μ mol; 1.0 equiv.) was dissolved in H₂O (16 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 2.77 mL; 1.39 mmol; 25 equiv.) and aq. **LuCl₃** (100 mM; 610 μ L; 61.0 μ mol; 1.1 equiv.) and the mixture was stirred 16 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive).

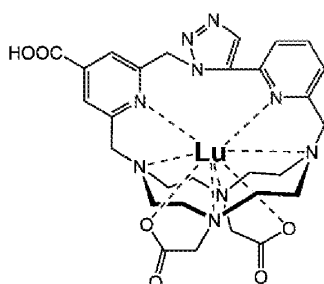
- 25 Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 44.8 mg (77%; 1 step; based on **TD1176**·0.8FA·4.4H₂O). **NMR (D₂O, pD ~2):** ¹H δ_{H} 2.55–3.78 (*mc*, *CH*₂–CO, *CH*₃, *CH*₂–arom., m, 16+4+6+2H); 4.11 (*CH*₂–arom., d, 1H, ²*J*_{HH} = 16); 4.13 (*CH*₂–arom., d, 1H, ²*J*_{HH} = 14); 5.31 (*CH*₂–N₃, d, 1H, ²*J*_{HH} = 15); 6.73 (*arom.*, d, 1H, ⁴*J*_{HH} = 2); 6.83 (*CH*₂–N₃, d, 1H, ²*J*_{HH} = 15); 7.07 (*arom.*, d, 1H, ⁴*J*_{HH} = 2); 7.78 (*arom.*, dd, 1H, ³*J*_{HH} = 8, ⁴*J*_{HH} = 1); 7.82 (*arom.*, dd, 1H, ³*J*_{HH} = 8, ⁴*J*_{HH} = 1); 7.89 (*CH*–N₃, s, 1H); 8.22 (*arom.*, t, 1H, ³*J*_{HH} = 8). **ESI-HRMS:** 765.2487 [M]⁺ (theor. [C₂₉H₃₉N₁₀O₄Lu]⁺ = 765.2486). **EA** ([C₂₉H₃₈N₁₀O₄Lu]⁺[TFA][−]·1.0TFA·3.4H₂O, *M_R* = 1053.9): C 37.6 (37.5); H 4.4 (4.1); N 13.3 (13.1); F 10.8 (10.5).
- 30



Synthesis of TD1152: In a glass vial (20 mL), **TD1148**·0.1FA·1.0H₂O (27.0 mg; 41.0 μmol; 1.0 equiv.) was dissolved in H₂O (17 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 2.05 mL; 1.03 mmol; 25 equiv.) and aq. **LuCl₃** (100 mM; 450 μL; 45.0 μmol; 1.1 equiv.) and the mixture was stirred 3 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient

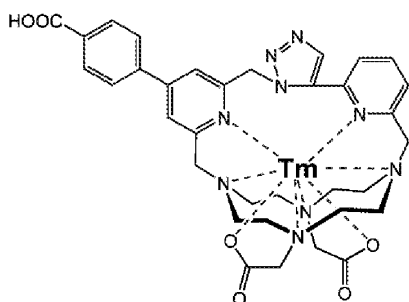
with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 24.5 mg (60%; 1 step; based on **TD1148**·0.1FA·1.0H₂O).

- 10 **NMR (D₂O, pD ~3):** ¹H δ_H 1.43 (CH₃, d, 6H, ³J_{HH} = 6); 2.65–3.85 (*mc*, CH₂–CO, m, 16+4H); 4.06 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.16 (CH₂–arom., d, 1H, ²J_{HH} = 16); 4.34 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.86 (CH₂–arom., d, 1H, ²J_{HH} = 16); 5.34 (CH, hept, 1H, ³J_{HH} = 6); 5.69 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.17 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.81 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.86 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.88 (CH–N₃, s, 1H); 8.22 (*arom.*, d, 1H, ⁴J_{HH} = 2); 8.25 (*arom.*, t, 1H, ³J_{HH} = 8); 8.53 (*arom.*, d, 1H, ⁴J_{HH} = 2). **ESI-HRMS:**
- 15 808.2425 [M]⁺ (theor. [C₃₁H₃₉N₉O₆Lu₁]⁺ = 808.2426). **EA** ([C₃₁H₃₉N₉O₆Lu₁]⁺[TFA][−]·0.3TFA·2.5H₂O, *M_R* = 1000.9): C 40.3 (40.7); H 4.5 (4.2); N 12.6 (12.2); F 7.4 (7.6).



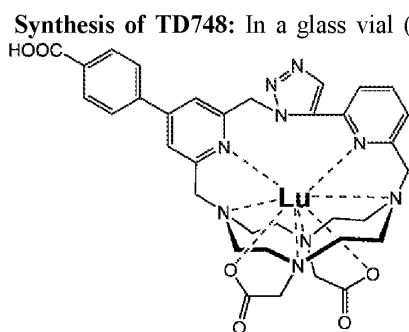
Synthesis of TD1123: In a glass vial (20 mL), **TD1092**·0.1FA·1.1H₂O (25.9 mg; 41.0 μmol; 1.0 equiv.) was dissolved in H₂O (17 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 2.05 mL; 1.03 mmol; 25 equiv.) and aq. **LuCl₃** (100 mM; 450 μL; 45.0 μmol; 1.1 equiv.) and the mixture was stirred 16 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA

- 25 additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 24.7 mg (60%; 1 step; based on **TD1092**·0.1FA·1.1H₂O).
- NMR (D₂O, pD ~2):** ¹H δ_H 2.65–3.86 (*mc*, CH₂–CO, m, 16+4H); 4.09 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.18 (CH₂–arom., d, 1H, ²J_{HH} = 16); 4.36 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.87 (CH₂–arom., d, 1H, ²J_{HH} = 16); 5.73 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.19 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.83 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.88 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.90 (CH–N₃, s, 1H); 8.21 (*arom.*, d, 1H, ⁴J_{HH} = 2); 8.27 (*arom.*, t, 1H, ³J_{HH} = 8); 8.50 (*arom.*, d, 1H, ⁴J_{HH} = 2). **ESI-HRMS:** 766.1948 [M]⁺ (theor. [C₂₈H₃₃N₉O₆Lu₁]⁺ = 766.1956). **EA** ([C₂₈H₃₃N₉O₆Lu₁]⁺[TFA][−]·0.6TFA·3.6H₂O, *M_R* = 1012.8): C 37.0 (36.7); H 4.1 (3.6); N 12.4 (12.0); F 9.0 (8.6).
- 30



Synthesis of TD897: In a glass vial (20 mL), **TD742**·0.1FA·3.2H₂O (48.6 mg; 66.4 μmol; 1.0 equiv.) was dissolved in H₂O (15 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 3.4 mL; 1.70 mmol; 26 equiv.) and aq. **TmCl₃** (100 mM; 800 μL; 80.0 μmol; 1.2 equiv.) and the mixture was stirred 8 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive).

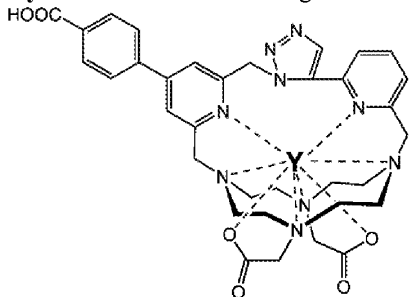
Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 65.2 mg (88%; 1step; based on **TD742**·0.1FA·3.2H₂O). **ESI-HRMS:** 836.2206 [M]⁺ (theor. [C₃₄H₃₇N₉O₆Tm]⁺ = 836.2203). **EA** ([C₃₄H₄₀N₁₀O₄Tm]⁺[TFA][−]·1.1TFA·2.6H₂O, *M_R* = 1121.9): C 40.9 (40.9); H 3.9 (3.8); N 11.2 (11.2); Tm 15.1 (15.2); F 10.7 (10.7).



Synthesis of TD748: In a glass vial (20 mL), **TD742**·0.2FA·1.6H₂O (85 mg; 120 μmol; 1.0 equiv.) was dissolved in H₂O (12 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 6.00 mL; 3.00 mmol; 25 equiv.) and aq. **LuCl₃** (100 mM; 1.3 mL; 130 μmol; 1.1 equiv.) and the mixture was stirred 16 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and

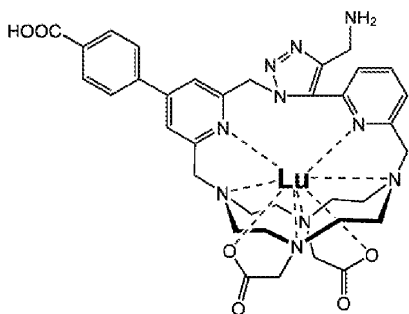
directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 105 mg (75%; 1step; based on **TD742**·0.1FA·2.9H₂O). **NMR (D₂O, pD ~2):** ¹H δ_H 2.64–3.86 (*mc*, CH₂–CO, *m*, 16+4H); 3.98 (CH₂–arom., *d*, 1H, ²*J*_{HH} = 15); 4.18 (CH₂–arom., *d*, 1H, ²*J*_{HH} = 16); 4.34 (CH₂–arom., *d*, 1H, ²*J*_{HH} = 15); 4.87 (CH₂–arom., *d*, 1H, ²*J*_{HH} = 16); 5.71 (CH₂–N₃, *d*, 1H, ²*J*_{HH} = 15); 7.17 (CH₂–N₃, *d*, 1H, ²*J*_{HH} = 15); 7.83 (*arom.*, *dd*, 1H, ³*J*_{HH} = 8, ⁴*J*_{HH} = 1); 7.88 (*arom.*, *dd*, 1H, ³*J*_{HH} = 8, ⁴*J*_{HH} = 1); 7.92 (*arom.*, *d*, 1H, ⁴*J*_{HH} = 2); 7.94 (CH–N₃, *s*, 1H); 7.97 (*arom.*, *dm*, 2H, ³*J*_{HH} = 9); 8.15 (*arom.*, *dm*, 2H, ³*J*_{HH} = 9); 8.27 (*arom.*, *t*, 1H, ³*J*_{HH} = 8); 8.43 (*arom.*, *d*, 1H, ⁴*J*_{HH} = 2). **ESI-HRMS:** 842.2259 [M]⁺ (theor. [C₃₄H₃₇N₉O₆Lu]⁺ = 842.2269). **EA** ([C₃₄H₄₀N₁₀O₄Lu]⁺[TFA][−]·1.4TFA·3.2H₂O, *M_R* = 1172.9): C 39.7 (40.0); H 3.9 (3.5); N 10.7 (10.3); Lu 14.9 (14.9); F 11.7 (11.8).

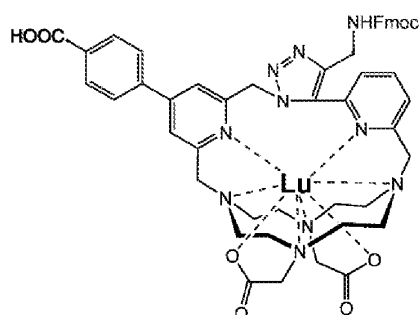
Synthesis of TD785: In a glass vial (20 mL), **TD742**·0.1FA·2.9H₂O (13 mg; 18 μmol; 1.0 equiv.) was dissolved in H₂O (18 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 1.20 mL; 600 μmol; 31 equiv.) and aq. **YCl₃** (100 mM; 197 μL; 20 μmol; 1.1 equiv.) and the mixture was stirred 22 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and



directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 13 mg (71%; 1 step; based on **TD742**·0.1FA·2.9H₂O). **NMR (D₂O, pD ~2):** ¹H δ_H 2.60–3.88 (*mc*, CH₂–CO, m, 16+4H); 3.98 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.16 (CH₂–arom., d, 1H, ²J_{HH} = 16); 4.38 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.86 (CH₂–arom., d, 1H, ²J_{HH} = 16); 5.94 (CH₂–N₃, d, 1H, ²J_{HH} = 16); 7.09 (CH₂–N₃, d, 1H, ²J_{HH} = 16); 7.82 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.85 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.91 (CH–N₃, s, 1H); 7.98 (*arom.*, d, 1H, ⁴J_{HH} = 2); 8.00 (*arom.*, dm, 2H, ³J_{HH} = 9); 8.02 (*arom.*, dm, 2H, ³J_{HH} = 9); 8.26 (*arom.*, t, 1H, ³J_{HH} = 8); 8.40 (*arom.*, d, 1H, ⁴J_{HH} = 2). **ESI-HRMS:** 756.1925 [M]⁺ (theor. [C₃₄H₃₇N₉O₆Y]⁺ = 756.1920). **EA** ([C₃₄H₃₇N₉O₆Y]⁺[TFA][–]·0.6TFA·4.8H₂O, M_R = 1024.5): C 43.6 (43.9); H 4.6 (4.2); N 12.3 (12.0); Y 8.7 (7.9); F 8.9 (8.5).

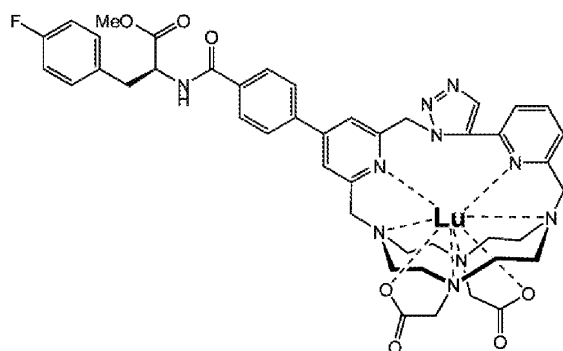
Synthesis of TD904: In a glass vial (20 mL), **TD744**·1.0TFA·1.3H₂O (38.6 mg; 46.2 μmol; 1.0 equiv.) was dissolved in H₂O (16 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 2.80 mL; 1.40 mmol; 30 equiv.) and aq. **LuCl₃** (100 mM; 550 μL; 55.0 μmol; 1.2 equiv.) and the mixture was stirred 16 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 42.2 mg (75%; 1 step; based on **TD744**·1.0TFA·1.3H₂O). **NMR (D₂O, pD ~3):** ¹H δ_H 2.62–3.87 (*mc*, CH₂–CO, m, 16+4H); 4.03 (CH₂–NH₂, d, 1H, ²J_{HH} = 15); 4.05 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.24 (CH₂–arom., d, 1H, ²J_{HH} = 16); 4.32 (CH₂–NH₂, d, 1H, ²J_{HH} = 15); 4.35 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.87 (CH₂–arom., d, 1H, ²J_{HH} = 16); 5.71 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.10 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.73 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.92 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 8.03 (*arom.*, dm, 2H, ³J_{HH} = 9); 8.07 (*arom.*, d, 1H, ⁴J_{HH} = 2); 8.21 (*arom.*, dm, 2H, ³J_{HH} = 9); 8.31 (*arom.*, t, 1H, ³J_{HH} = 8); 8.40 (*arom.*, d, 1H, ⁴J_{HH} = 2). **ESI-HRMS:** 871.2536 [M]⁺ (theor. [C₃₅H₄₀N₁₀O₆Lu]⁺ = 871.2535). **EA** ([C₃₅H₄₀N₁₀O₆Lu]⁺[TFA][–]·1.1TFA·6.0H₂O, M_R = 1218.2): C 38.6 (38.6); H 4.4 (4.1); N 11.5 (11.2); F 9.8 (9.5).





Synthesis of 905: In a glass vial (2 mL), [TD904]⁺[TFA]⁻·1.1TFA·6.0H₂O (10.0 mg; 8.2 μmol; 1.0 equiv.) was dissolved in aq. **Borate/NaOH buffer** (200 mM; pH 9.0; 2.00 mL; 400 mmol; 50 equiv.). followed by addition of freshly prepared solution of **FmocCl** (3.3 mg; 12.8 μmol; 1.6 equiv.) in MeCN (1.5 mL). The resulting clear solution was stirred 30 min at RT. Mixture was then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined

10 and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 9.8 mg (≤99% assuming [M]⁺[TFA]⁻·xH₂O; *M_R* = 1207.0; 1step; based on [TD904]⁺[TFA]⁻·1.1TFA·6.0H₂O). **NMR (CD₃CN; compound is soluble in D₂O but shows broad ¹H signals):** ¹H δ_H 2.52–3.64 (*mc*, CH₂–CO, m, 16+4H); 3.89 (CH₂–arom., d, 1H, ²*J*_{HH} = 15); 4.03 (CH₂–NH–Fmoc, dd, 1H, ²*J*_{HH} = 15, ³*J*_{HH} = 3); 4.05 (CH₂–arom., d, 1H, ²*J*_{HH} = 16); 4.18 (*Fmoc*, t, 1H, ³*J*_{HH} = 7); 4.26–4.35 (*Fmoc*, m, 2H); 4.36 (CH₂–arom., d, 1H, ²*J*_{HH} = 15); 4.52 (CH₂–NH–Fmoc, dd, 1H, ²*J*_{HH} = 15, ³*J*_{HH} = 7); 4.78 (CH₂–arom., d, 1H, ²*J*_{HH} = 16); 5.50 (CH₂–N₃, d, 1H, ²*J*_{HH} = 15); 6.54 (NH–Fmoc, dd, 1H, ³*J*_{HH} = 7, ³*J*_{HH} = 3); 7.27–7.33 (*Fmoc*, m, 2H); 7.35–7.43 (*Fmoc*, CH₂–N₃, m, 2+1H); 7.57–7.65 (*Fmoc*, CH₂–arom., m, 2+1H); 7.70 (*arom.*, dd, 1H, ³*J*_{HH} = 8, ⁴*J*_{HH} = 1); 7.78–7.84 (*Fmoc*, m, 2H); 7.91 (*arom.*, d, 1H, ⁴*J*_{HH} = 2); 7.97 (*arom.*, dm, 2H, ³*J*_{HH} = 9); 8.14 (*arom.*, t, 1H, ³*J*_{HH} = 8); 8.18 (*arom.*, dm, 2H, ³*J*_{HH} = 9); 8.26 (*arom.*, d, 1H, ⁴*J*_{HH} = 2). **ESI-HRMS:** 1093.3219 [M]⁺ (theor. [C₅₀H₅₀N₁₀O₈Lu₁]⁺ = 1093.3215).

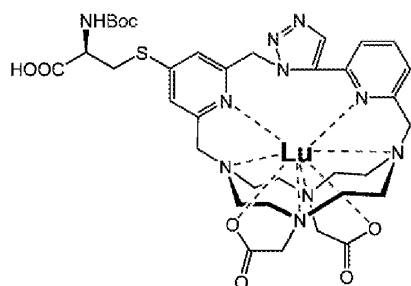


Synthesis of TD757: In a glass vial (2 mL), [TD748]⁺[FA]⁻·6.4H₂O (0.7 mg; 0.7 μmol; 1.0 equiv.) was dissolved in DMSO (75 μL) followed by addition of **4-Fluoro-L-phenylalanine hydrochloride** (50 mM in DMSO; 22 μL; 1.1 μmol; 1.5 equiv.), **DIPEA** (100 mM in DMSO; 22 μL; 2.2 μmol; 3.1 equiv.) and **HATU** (freshly prepared 50 mM in DMSO; 24 μL; 1.2 μmol; 1.7 equiv.) and the resulting yellow solution was stirred 5 min at RT.

30 Mixture was then diluted with H₂O (750 μL) and directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product as white fluffy solid. **Yield:** 0.7 mg (≤90% assuming [M]⁺[FA]⁻·xH₂O; *M_R* = 1066.9; 1 step; based on [TD748]⁺[FA]⁻·6.4H₂O). **NMR (D₂O, pD ~4):** ¹H δ_H 2.56–3.79 (*mc*, CH₂–CO, CH₂–CH, m, 16+4+2H); 3.82 (CH₃, s, 3H); 4.02 (CH₂–arom., d, 1H, ²*J*_{HH} = 15); 4.16 (CH₂–arom., d, 1H, ²*J*_{HH} = 16); 4.35 (CH₂–arom., d, 1H, ²*J*_{HH} = 15); 4.86 (CH₂–arom., d, 1H, ²*J*_{HH} = 16); 4.96 (CH₂–CH, dd, 1H, ³*J*_{HH} = 10, ³*J*_{HH} = 5); 5.68 (CH₂–

N₃, d, 1H, $^2J_{\text{HH}} = 15$); 7.04–7.20 ($\text{CH}_2\text{-N}_3$, arom., m, 1+2H); 7.27–7.34 (arom., dd, 2H, $^3J_{\text{HF}} = 9$, $^3J_{\text{HH}} = 6$); 7.78–7.88 (arom., m, 4H); 7.90 (CH-N_3 , s, 1H); 7.95–8.06 (arom., m, 3H); 8.26 (arom., t, 1H, $^3J_{\text{HH}} = 8$); 8.35 (arom., d, 1H, $^4J_{\text{HH}} = 2$); 8.46 (FA, s, 1H). ^{19}F $\delta_{\text{F}} -116.5$ (tt, $^3J_{\text{FH}} = 9$, $^4J_{\text{FH}} = 5$). **ESI-HRMS**: 1021.3014 $[\text{M}]^+$ (theor. $[\text{C}_{44}\text{H}_{47}\text{N}_{10}\text{O}_7\text{F}_1\text{Lu}_1]^+ = 1021.3015$).

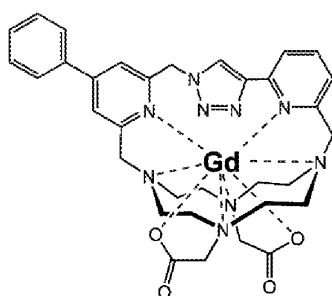
5



Synthesis of TD793: In a glass vial (2 mL), $[\text{TD782}]^+[\text{TFA}]^- \cdot 5.1\text{H}_2\text{O}$ (4.4 mg; 4.6 μmol ; 1.0 equiv.) was dissolved in DMSO (460 μL) followed by addition of *N*-Boc-cysteine (50 mM in DMSO; 185 μL ; 9.3 μmol ; 2.0 equiv.) and DIPEA (8 μL ; 46 μmol ; 10 equiv.) and the resulting solution was stirred 16 h RT. Mixture was then diluted with H₂O (2 mL) and directly purified by preparative HPLC (C18; H₂O–MeCN gradient

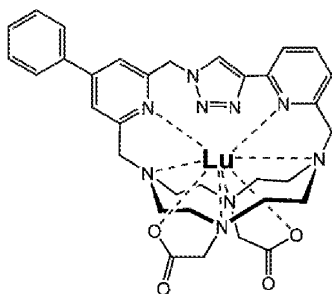
with TFA additive). Fractions with product were joined and directly lyophilized to give product as white fluffy solid. **Yield:** 2.0 mg ($\leq 41\%$ assuming $[\text{M}]^+[\text{TFA}]^- \cdot x\text{H}_2\text{O}$; $M_{\text{R}} = 1054.8$; 1 step; based on

15 $[\text{TD782}]^+[\text{TFA}]^- \cdot 5.1\text{H}_2\text{O}$). **NMR (D₂O, pD ~3):** ^1H δ_{H} 1.28–1.54 (CH_3 , m, 9H); 2.63–3.95 (*mc*, $\text{CH}_2\text{-CO}$, $\text{CH}_2\text{-S}$, $\text{CH}_2\text{-arom.}$, m, 16+4+2+1H); 4.16 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 16$); 4.26 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 4.48–4.63 (NH-CH-COOH , m, 1H); 4.84 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 16$); 5.45–5.59 ($\text{CH}_2\text{-N}_3$, m, 1H); 7.03 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 7.59–7.65 (arom., m, 1H); 7.81 (arom., dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.86 (arom., dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.90 (CH-N_3 , s, 1H); 7.90–7.95 (arom., m, 1H); 8.26 (arom., t, 1H, $^3J_{\text{HH}} = 8$). **ESI-**
20 **HRMS**: 941.2614 $[\text{M}]^+$ (theor. $[\text{C}_{35}\text{H}_{46}\text{N}_{10}\text{O}_8\text{S}_1\text{Lu}_1]^+ = 941.2623$).

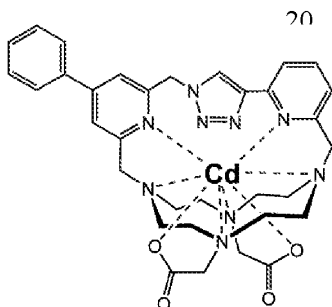


Synthesis of TD875: In a glass vial (20 mL), $\text{TD871} \cdot 0.6\text{FA} \cdot 2.3\text{H}_2\text{O}$ (10.0 mg; 14.4 μmol ; 1.0 equiv.) was dissolved in H₂O (2 mL) followed by addition of aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 750 μL ; 375 μmol ; 26 equiv.) and aq. **GdCl₃** (100 mM; 180 μL ; 18.0 μmol ; 1.3 equiv.). The resulting suspension was stirred 20 d at 80 °C. Then, the mixture was diluted with additional H₂O (16 mL) followed by another portion of aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 750 μL ; 375 μmol ; 26 equiv.) and aq. **GdCl₃** (100 mM; 180 μL ; 18.0 μmol ; 1.3 equiv.). The resulting

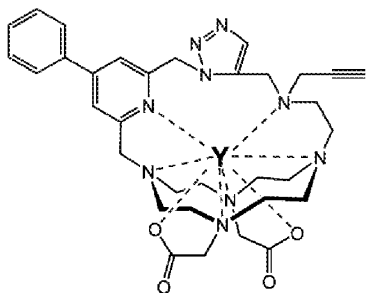
30 suspension was further stirred 10 d at 80 °C. Mixture was filtered through syringe microfilter (RC). The filtrate was evaporated to dryness and then resuspended in H₂O (3.6 mL). Mixture was once more filtered through syringe microfilter (RC) and the filtrate was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product as white fluffy solid. **Yield:** 2.1 mg ($\leq 18\%$ assuming $[\text{M}]^+[\text{FA}]^- \cdot x\text{H}_2\text{O}$; $M_{\text{R}} = 826.0$; 1 step; based on $\text{TD871} \cdot 0.6\text{FA} \cdot 2.3\text{H}_2\text{O}$).
35 **ESI-HRMS**: 781.2205 $[\text{M}]^+$ (theor. $[\text{C}_{33}\text{H}_{37}\text{N}_9\text{O}_4\text{Gd}_1]^+ = 781.2204$).



Synthesis of TD874: In a glass vial (20 mL), **TD871**·0.6FA·2.3H₂O (10.0 mg; 14.4 μmol; 1.0 equiv.) was dissolved in H₂O (2 mL) followed by addition of aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 750 μL; 375 μmol; 26 equiv.) and aq. **LuCl₃** (100 mM; 180 μL; 18.0 μmol; 1.3 equiv.). The resulting suspension was stirred 20 d at 80 °C. Then, the mixture was diluted with additional H₂O (16 mL) followed by another portion of aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 750 μL; 375 μmol; 26 equiv.) and aq. **LuCl₃** (100 mM; 180 μL; 18.0 μmol; 1.3 equiv.). The resulting suspension was further stirred 10 d at 80 °C. Mixture was filtered through syringe microfilter (RC). The filtrate was evaporated to dryness and then resuspended in H₂O (3.6 mL). Mixture was once more filtered through syringe microfilter (RC) and the filtrate was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product as white fluffy solid. **Yield:** 8.6 mg (≤71% assuming [M]⁺[FA][−]·xH₂O; *M_R* = 843.2; 1 step; based on **TD871**·0.6FA·2.3H₂O). **NMR (D₂O, pD ~6):** ¹H δ_H 2.13–3.76 (*mc*, CH₂–CO, CH₂–arom., m, 16+3+1H); 4.13 (CH₂–CO, d, 1H, ²*J*_{HH} = 18); 4.32 (CH₂–arom., d, 1H, ²*J*_{HH} = 16); 4.45 (CH₂–arom., d, 1H, ²*J*_{HH} = 16); 4.67 (CH₂–arom., d, 1H, ²*J*_{HH} = 13); 5.81 (CH₂–N₃, d, 1H, ²*J*_{HH} = 14); 6.08 (CH₂–N₃, d, 1H, ²*J*_{HH} = 14); 7.56–7.64 (*Ph*, arom., m, 3+1H); 7.67 (arom., d, 1H, ⁴*J*_{HH} = 2); 7.79–7.85 (*Ph*, m, 2H); 7.90 (arom., dd, 1H, ³*J*_{HH} = 8, ⁴*J*_{HH} = 1); 8.09 (arom., d, 1H, ⁴*J*_{HH} = 2); 8.16 (arom., t, 1H, ³*J*_{HH} = 8); 8.45 (*FA*, s, 1H); 8.72 (CH–N₃, s, 1H). **ESI-HRMS:** 798.2376 [M]⁺ (theor. [C₃₃H₃₇N₉O₄Lu]⁺ = 798.2371).

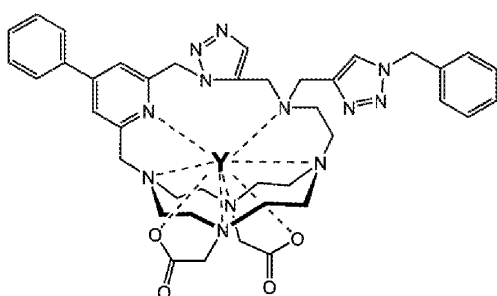


Synthesis of TD900: In a glass vial (4 mL), **TD871**·0.6FA·2.3H₂O (4.3 mg; 6.2 μmol; 1.0 equiv.) was dissolved in aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 640 μL; 320 μmol; 52 equiv.) and aq. **CdCl₂** (62.6 mM; 120 μL; 7.5 μmol; 1.2 equiv.) and the mixture was stirred 1 h at 80 °C. Mixture was then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with no additive). Fractions with product were joined and directly lyophilized to give product as white fluffy solid. **Yield:** 4.2 mg (≤92% assuming [M]·xH₂O; *M_R* = 736.1; 1 step; based on **TD871**·0.6FA·2.3H₂O). **NMR (D₂O, pD ~7):** ¹H δ_H 2.00–4.41 (*mc*, CH₂–CO, CH₂–arom., bm, 16+4+4H); 5.93 (CH₂–N₃, bs, 2H); 7.44 (arom., dd, 1H, ³*J*_{HH} = 8, ⁴*J*_{HH} = 1); 7.53–7.63 (*Ph*, m, 3H); 7.73–7.84 (*Ph*, arom., m, 2+1H); 7.88–7.96 (arom., m, 2H); 8.01 (arom., t, 1H, ³*J*_{HH} = 8); 9.74 (CH–N₃, s, 1H). **ESI-HRMS:** 738.2081 [M+H]⁺ (theor. [C₃₃H₃₈N₉O₄Cd]⁺ = 738.2075).



Synthesis of TD685: In a glass vial (20 mL), **TD669**·0.1FA·xH₂O (17 mg; ~27 μmol; 1.0 equiv.) was dissolved in H₂O (17 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 2.10 mL; 1.05 mmol; ~40 equiv.) and aq. **YCl₃** (100 mM; 290 μL; 29 μmol; ~1.1 equiv.) and the mixture was stirred 6 weeks at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined

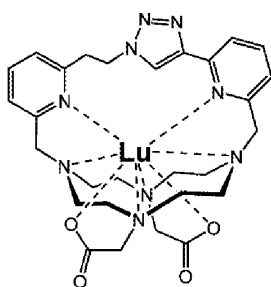
and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 4 mg (≤21% assuming [M]⁺[FA][−]·xH₂O; *M_R* = 761.7; 1 step; based on **TD669**·0.1FA·xH₂O). **NMR (D₂O, pD ~5):** ¹H δ_H 2.63–3.09 (*mc*, CH₂–CH₂–N–CH₂–C≡CH, m, 4+2H); 3.11 (C≡CH, t, 1H, ⁴*J*_{HH} = 2); 3.13–3.75 (*mc*, CH₂–CH₂–N–CH₂–C≡CH, CH₂–CO, 12+2+2H); 3.78 (CH₂–CO, d, 1H, ²*J*_{HH} = 19); 3.79 (CH₂–C≡CH, dm, 1H, ²*J*_{HH} = 18); 3.91 (CH₂–C≡CH, dd, 1H, ²*J*_{HH} = 18, ⁴*J*_{HH} = 2); 4.01 (CH₂–CO, d, 1H, ²*J*_{HH} = 19); 4.09 (CH₂–arom., d, 1H, ²*J*_{HH} = 15); 4.25 (CH₂–arom., d, 1H, ²*J*_{HH} = 15); 4.39 (CH₂–C–N₃, d, 1H, ²*J*_{HH} = 15); 4.75 (CH₂–C–N₃, d, 1H, ²*J*_{HH} = 15); 5.67 (CH₂–N₃, d, 1H, ²*J*_{HH} = 15); 7.00 (CH₂–N₃, d, 1H, ²*J*_{HH} = 15); 7.67–7.73 (*Ph*, m, 3H); 7.94 (*arom.*, d, 1H, ⁴*J*_{HH} = 2); 7.96–8.00 (*Ph*, m, 2H); 8.13 (CH–N₃, s, 1H); 8.30 (*arom.*, d, 1H, ⁴*J*_{HH} = 2); 8.52 (*FA*, s, 1H). ¹³C{¹H} δ_C 41.2 (CH₂–C≡CH, s); 47.8 (CH₂–C–N₃, s); 52.6 (CH₂–CH₂–N–CH₂–C≡CH, s); 54.1 (CH₂–CH₂–N–CH₂–C≡CH, s); 54.4 (CH₂–N₃, s); 55.1 (*mc*, s); 57.2 (*mc*, s); 58.0 (*mc*, s); 59.0 (*mc*, s); 59.4 (*mc*, s); 59.5 (*mc*, s); 59.9 (*mc*, s); 60.1 (*mc*, bs); 66.0 (CH₂–CO, s); 67.2 (CH₂–CO, s); 67.6 (CH₂–arom., s); 75.3 (C≡CH, s); 81.8 (C≡CH, s); 124.8 (*arom.*, s); 127.7 (*arom.*, s); 130.0 (*Ph*, s); 132.1 (*Ph*, s); 133.6 (*Ph*, s); 143.4 (*arom.*, s); 137.5 (*Ph*, s); 138.1 (CH–N₃, s); 156.1 (*arom.*, s); 158.6 (*arom.*, s); 161.4 (*arom.*, s); 173.3 (*FA*, s); 181.2 (CO, s); 181.5 (CO, s). **ESI-HRMS:** 716.2327 [M]⁺ (theor. [C₃₃H₄₁N₉O₄Y₁]⁺ = 716.2335).



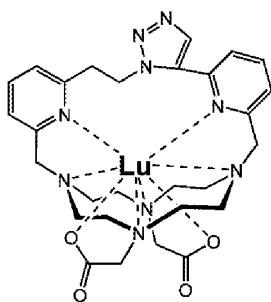
Synthesis of TD780: In a glass vial (4 mL), [TD685]⁺[FA][−]·xH₂O (3 mg; ~4 μmol; 1.0 equiv.) was dissolved in aq. **MES/NaOH buffer** (500 mM; pH 5.2; 375 μL; 188 μmol; ~50 equiv.) followed by addition of *t*-BuOH (240 μL), **Benzyl azide** (500 mM in *t*-BuOH; 12 μL; 6 μmol; ~1.6 equiv.) and aq. solution of **CuSO₄** (500 mM; 3 μL; 1.5 μmol; ~0.4 equiv.). To the resulting faint light blue solution was added freshly prepared aq. solution of **Sodium**

ascorbate (100 mM; 15 μL; 1.5 μmol; ~0.4 equiv.) and the color of the solution immediately changed to yellow-green. Mixture was stirred 1 h at RT after which was diluted with H₂O (2.4 mL) and filtered through syringe microfilter (RC). The filtrate was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of

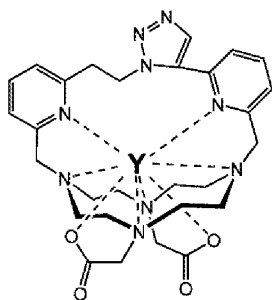
trifluoroacetate salt as white fluffy solid. **Yield:** 3 mg ($\leq 74\%$ assuming $[M]^+[TFA]^- \cdot xH_2O$; $M_R = 962.8$; 1 step; based on **TD685**· xH_2O). **NMR (D_2O , pD ~3):** 1H δ_H 2.46–4.59 (*mc*, CH_2 -CO, CH_2 -arom., CH_2 - CH_2 -N- $(CH_2$ -C=C) $_2$, m, 16+4+2+8H); 5.60 (CH_2 -N $_3$, d, 1H, $^2J_{HH} = 16$); 5.71 (CH_2 -Ph, s, 2H); 6.94 (CH_2 -N $_3$, d, 1H, $^2J_{HH} = 15$); 7.37–7.50 (*Ph*, m, 5H); 7.59–7.65 (*Ph*, m, 3H); 7.88 (*arom.*, d, 1H, $^4J_{HH} = 2$); 7.89–7.93 (*Ph*, m, 2H); 8.21 (*arom.*, d, 1H, $^4J_{HH} = 2$); 8.34 (CH -N $_3$, s, 1H); 8.35 (CH -N $_3$, s, 1H). **ESI-HRMS:** 849.2970 $[M]^+$ (theor. $[C_{40}H_{48}N_{12}O_4]^+ = 849.2975$).



Synthesis of 1,4-TD1413: In a glass vial (20 mL), **TD1408**·0.1FA·2.4H $_2$ O (10.0 mg; 16.4 μ mol; 1.0 equiv.) was dissolved in H $_2$ O (19 mL) followed by addition of aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 820 μ L; 410 μ mol; 25 equiv.) and aq. **LuCl $_3$** (100 mM; 196 μ L; 19.6 μ mol; 1.2 equiv.). The resulting suspension was stirred 1 w at 80 °C. Mixture was filtered through syringe microfilter (RC). The filtrate was evaporated to dryness and the residue was purified by preparative HPLC (C18; H $_2$ O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 9.3 mg ($\leq 73\%$ assuming $[M]^+[FA]^- \cdot xH_2O$; $M_R = 781.6$; 1 step; based on **TD1408**·0.1FA·2.4H $_2$ O). **NMR (D_2O , pD ~5):** 1H δ_H 1.73–3.16 (*mc*, CH_2 -CO, m, 12+1H); 3.27 (CH_2 -CO, d, $^2J_{HH} = 13$); 3.42–3.77 (*mc*, CH_2 - CH_2 -N $_3$, CH_2 -CO, m, 4+2+1H); 4.03 (CH_2 - CH_2 -N $_3$, ddd, 1H, $^2J_{HH} = 14$, $^3J_{HH} = 12$, $^3J_{HH} = 16$); 4.10 (CH_2 -arom., d, 1H, $^2J_{HH} = 17$); 4.19 (CH_2 -arom., d, 1H, $^2J_{HH} = 16$); 4.34 (CH_2 -arom., d, 1H, $^2J_{HH} = 16$); 4.56 (CH_2 -CO, d, 1H, $^2J_{HH} = 13$); 4.92–5.03 (CH_2 - CH_2 -N $_3$, CH_2 -arom., m, 1+1H); 7.18 (*arom.*, dd, 1H, $^3J_{HH} = 8$, $^4J_{HH} = 1$); 7.54–7.60 (*arom.*, m, 2H); 7.71–7.77 (*arom.*, m, 2H); 8.11 (*arom.*, t, 1H, $^3J_{HH} = 8$); 8.13 (CH -N $_3$, s, 1H); 8.44 (*FA*, s, 1H). **ESI-HRMS:** 736.2217 $[M]^+$ (theor. $[C_{28}H_{35}N_9O_4Lu_1]^+ = 736.2214$).

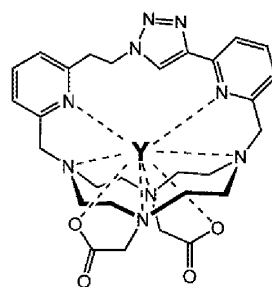


Synthesis of 1,5-TD1413: Obtained as a side product during synthesis of **1,4-TD1413** in the form of formate salt as white fluffy solid. **Yield:** 4.0 mg ($\leq 31\%$ assuming $[M]^+[FA]^- \cdot xH_2O$; $M_R = 781.6$; 1 step; based on **TD1408**·0.1FA·2.4H $_2$ O). **NMR (D_2O , pD ~6):** 1H δ_H 2.62–4.60 (*mc*, CH_2 -CO, CH_2 -arom., CH_2 - CH_2 -N $_3$, CH_2 - CH_2 -N $_3$, m, 16+4+3+2+1H); 4.85 (CH_2 -arom., m, 1H); 5.45 (CH_2 - CH_2 -N $_3$, bs, 1H); 7.44 (*arom.*, dd, 1H, $^3J_{HH} = 8$, $^4J_{HH} = 1$); 7.67–7.78 (*arom.*, m, 2H); 7.81–7.90 (*arom.*, CH -N $_3$, m, 1+1H); 8.02 (*arom.*, t, 1H, $^3J_{HH} = 8$); 8.25 (*arom.*, t, 1H, $^3J_{HH} = 8$); 8.45 (*FA*, s, 1H). **ESI-HRMS:** 736.2218 $[M]^+$ (theor. $[C_{28}H_{35}N_9O_4Lu_1]^+ = 736.2214$).

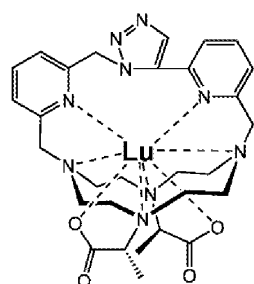


Synthesis of 1,5-TD1414: In a glass vial (20 mL), **TD1408**·0.1FA·2.4H₂O (10.0 mg; 16.4 μmol; 1.0 equiv.) was dissolved in H₂O (19 mL) followed by addition of aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 820 μL; 410 μmol; 25 equiv.) and aq. **YCl₃** (100 mM; 196 μL; 19.6 μmol; 1.2 equiv.). The resulting suspension was stirred 1 w at 80 °C. Mixture was filtered through syringe microfilter (RC). The filtrate was evaporated to dryness and the residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of formate salt as white

fluffy solid. **Yield:** 6.6 mg (≤58% assuming [M]⁺[FA][−]·xH₂O; $M_R = 695.6$; 1 step; based on **TD1408**·0.1FA·2.4H₂O). **NMR (D₂O, pD ~6):** ¹H δ_H 2.56–4.00 (*mc*, CH₂–CO, CH₂–CH₂–N₃, CH₂–arom., m, 16+4+2+1H); 4.06 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.38 (CH₂–arom., d, 1H, ²J_{HH} = 16); 4.42–4.54 (CH₂–CH₂–N₃, m, 1H); 4.93 (CH₂–arom., d, 1H, ²J_{HH} = 15); 5.72–5.91 (CH₂–CH₂–N₃, m, 1H); 7.45 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.69–7.80 (*arom.*, m, 2H); 7.84 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.87 (CH–N₃, s, 1H); 8.01 (*arom.*, t, 1H, ³J_{HH} = 8); 8.26 (*arom.*, t, 1H, ³J_{HH} = 8); 8.46 (FA, s, 1H). **ESI-HRMS:** 650.1867 [M]⁺ (theor. [C₂₈H₃₅N₉O₄Y]⁺ = 650.1865).



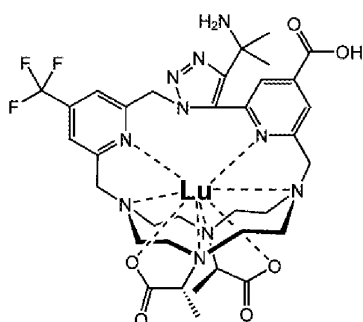
Synthesis of 1,4-TD1414: Obtained as a side product during synthesis of **1,5-TD1414** in the form of formate salt as white fluffy solid. **Yield:** 4.0 mg (≤35% assuming [M]⁺[FA][−]·xH₂O; $M_R = 695.6$; 1 step; based on **TD1408**·0.1FA·2.4H₂O). **NMR (D₂O, pD ~5):** ¹H δ_H 1.76–3.19 (*mc*, CH₂–CO, m, 12+1H); 3.23 (CH₂–CO, d, ²J_{HH} = 13); 3.43–3.79 (*mc*, CH₂–CH₂–N₃, CH₂–CO, m, 4+2+1H); 4.06–4.24 (CH₂–CH₂–N₃, CH₂–arom., m, 1+2H); 4.34 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.60 (CH₂–CO, d, 1H, ²J_{HH} = 12); 4.89–5.05 (CH₂–CH₂–N₃, CH₂–arom., m, 1+1H); 7.17 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.50–7.58 (*arom.*, m, 2H); 7.72 (*arom.*, t, 1H, ³J_{HH} = 8); 7.74 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 8.09 (*arom.*, t, 1H, ³J_{HH} = 8); 8.14 (CH–N₃, s, 1H); 8.43 (FA, s, 1H). **ESI-HRMS:** 650.1868 [M]⁺ (theor. [C₂₈H₃₅N₉O₄Y]⁺ = 650.1865).



Synthesis of TD1350: In a glass vial (20 mL), **TD1346**·0.1FA·3.0H₂O (6.9 mg; 10.8 μmol; 1.0 equiv.) was dissolved in H₂O (19 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 550 μL; 275 μmol; 25 equiv.) and aq. **LuCl₃** (100 mM; 120 μL; 12.0 μmol; 1.1 equiv.) and the mixture was stirred 16 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined

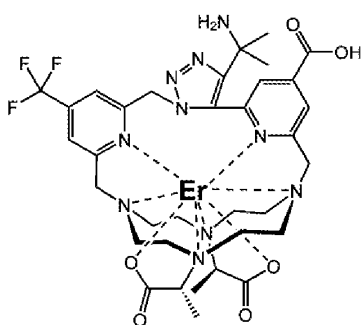
and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 41.4 mg (84%; 1 step; based on **TD1346**·0.1FA·3.0H₂O). **NMR (D₂O, pD ~3):** ¹H δ_H 1.03 (CH₃, d, 3H, ³J_{HH} = 7); 1.12

(CH₃, d, 3H, ³J_{HH} = 7); 2.69–3.81 (*mc*, CH–CH₃, m, 16+2H); 3.90 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.10 (CH₂–arom., d, 1H, ²J_{HH} = 16); 4.23 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.78 (CH₂–arom., d, 1H, ²J_{HH} = 16); 5.58 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.11 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.70 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.78 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.83 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.84 (CH–N₃, s, 1H); 8.01 (*arom.*, dd, 1H, ³J_{HH} = 8, ⁴J_{HH} = 1); 8.22 (*arom.*, t, 1+1H, ³J_{HH} = 8). **ESI-HRMS**: 750.2370 [M]⁺ (theor. [C₂₉H₃₇N₉O₄Lu₁]⁺ = 750.2371). **EA** ([C₂₉H₃₇N₉O₄Lu₁]⁺[TFA][−]·0.3TFA·2.1H₂O, M_R = 935.6): C 40.6 (40.6); H 4.5 (4.2); N 13.5 (13.2); F 7.9 (8.1).



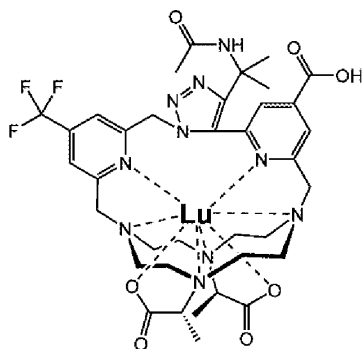
Synthesis of TD1452: In a glass vial (40 mL), **TD1451**·0.1FA·2.7H₂O (101 mg; 112 μmol; 1.0 equiv.) was dissolved in H₂O (30 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 7.0 mL; 3.50 mmol; 31 equiv.) and aq. **LuCl₃** (100 mM; 1.40 mL; 140 μmol; 1.3 equiv.) and the mixture was stirred 18 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product

were joined and directly lyophilized to give product in the form of trifluoroacetate salt as faint yellow fluffy solid. **Yield**: 115 mg (76%; 1 step; based on **TD1451**·0.1FA·2.7H₂O). **NMR (D₂O, pH ~3)**: ¹H δ_H 1.12 (CH₃–CH, d, 3H, ³J_{HH} = 7); 1.18 (CH₃–C–NH₂, s, 3H); 1.19 (CH₃–CH, d, 3H, ³J_{HH} = 7); 1.80 (CH₃–C–NH₂, s, 3H); 2.77–3.90 (*mc*, CH–CO, m, 16+2H); 4.08 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.31 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.44 (CH₂–arom., d, 1H, ²J_{HH} = 18); 4.87 (CH₂–arom., d, 1H, ²J_{HH} = 18); 5.71 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 6.97 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 8.05 (*arom.*, d, 1H, ⁴J_{HH} = 2); 8.11 (*arom.*, d, 1H, ⁴J_{HH} = 1); 8.16 (*arom.*, d, 1H, ⁴J_{HH} = 2); 8.41 (*arom.*, d, 1H, ⁴J_{HH} = 1); ¹⁹F δ_F −66.1 (s). **NMR (~0.5 mM TD1452 in ~50 mM MOPS/NaOH buffer in 10% D₂O, pH 7.0)**: ¹⁹F δ_F −66.12 (s). **ESI-HRMS**: 919.2725 [M]⁺ (theor. [C₃₄H₄₃N₁₀O₆F₃Lu₁]⁺ = 919.2721). **EA** ([C₃₃H₄₃N₁₀O₆Lu₁F₃]⁺[TFA][−]·1.5TFA·8.3H₂O, M_R = 1353.2): C 34.6 (35.4); H 4.6 (3.7); N 10.4 (9.7); F 14.7 (14.3).



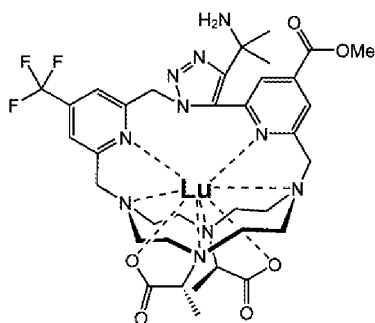
Synthesis of TD1453: In a glass vial (20 mL), **TD1451**·0.1FA·2.7H₂O (21.7 mg; 24.1 μmol; 1.0 equiv.) was dissolved in H₂O (18 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 1.50 mL; 750 μmol; 31 equiv.) and aq. **ErCl₃** (100 mM; 300 μL; 30.0 μmol; 1.2 equiv.) and the mixture was stirred 10 d at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield**: 22.6 mg

(~70% assuming $[M]^+[TFA]^- \cdot 1.5TFA \cdot 8.3H_2O$; $M_R = 1345$; 1 step; based on **TD1451**·0.1FA·2.7H₂O). **NMR** (~0.5 mM **TD1453** in ~50 mM MOPS/NaOH buffer in 10% D₂O, pH 7.0): ¹⁹F δ_F −47.85 (s). **ESI-HRMS**: 910.2620 $[M]^+$ (theor. $[C_{34}H_{43}N_{10}O_6F_3Er_1]^+ = 910.2617$).



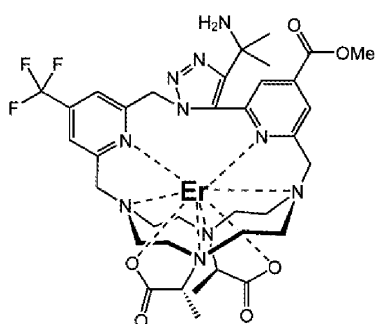
Synthesis of TD1472: In a glass vial (4 mL), $[TD1452]^+[TFA]^- \cdot 1.5TFA \cdot 8.3H_2O$ (20.0 mg; 14.8 μmol; 1.0 equiv.) was dissolved in DMSO (1.5 mL) followed by addition of **AcOH** (42 μL; 735 μmol; 50 equiv.) and **DIPEA** (181 μL; 1.04 mmol; 70 equiv.). Freshly prepared solution of **PyAOP** (200 mM in DMSO; 368 μL; 73.7 μmol; 5.0 equiv.) was then added and the resulting mixture was stirred 5 min at RT. The resulting biphasic mixture was diluted with DMF (800 μL), and the resulting solution was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined

and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 11.5 mg (64%; 1 step; based on $[TD1452]^+[TFA]^- \cdot 1.5TFA \cdot 8.3H_2O$). **NMR** (~0.5 mM **TD1472** in ~50 mM MOPS/NaOH buffer in 10% D₂O, pH 7.0): ¹⁹F δ_F −66.10 (s). **EA** ($[C_{36}H_{45}N_{10}O_7Lu_1F_3]^+[TFA]^- \cdot 0.8TFA \cdot 3.1H_2O$, $M_R = 1221.8$): C 38.9 (39.1); H 4.3 (4.5); N 11.5 (11.3).

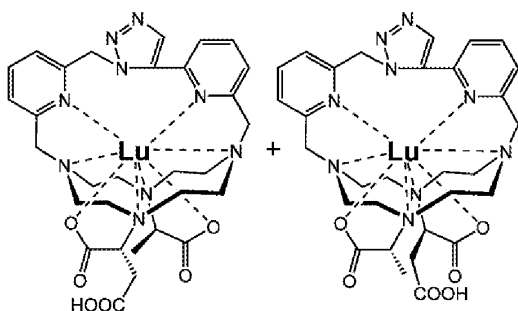


Synthesis of TD1473: In a glass vial (4 mL), $[TD1452]^+[TFA]^- \cdot 1.5TFA \cdot 8.3H_2O$ (16.2 mg; 12.0 μmol; 1.0 equiv.) was dissolved in a **MeOH** (1.20 mL) followed by addition of **SOCl₂** (8.8 μL; 121 μmol; 10 equiv.). The resulting clear solution was stirred 2 d at 50 °C. Mixture was then diluted with H₂O (2 mL) and directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:**

13.2 mg (91%; 1 step; based on $[TD1452]^+[TFA]^- \cdot 1.5TFA \cdot 8.3H_2O$). **NMR** (~0.5 mM **TD1473** in ~50 mM MOPS/NaOH buffer in 10% D₂O, pH 7.0): ¹⁹F δ_F −66.13 (s). **EA** ($[C_{35}H_{45}N_{10}O_6Lu_1F_3]^+[TFA]^- \cdot 0.2TFA \cdot 7.2H_2O$, $M_R = 1199.2$): C 37.5 (37.1); H 5.0 (4.0); N 11.7 (10.7).

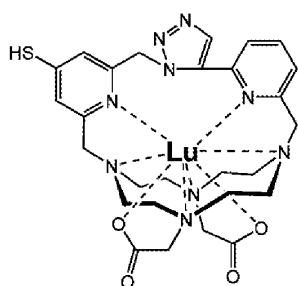


Synthesis of TD1475: In a glass vial (4 mL), **TD1453** (assuming $[\text{TD1453}]^+[\text{TFA}]^- \cdot 1.5\text{TFA} \cdot 8.3\text{H}_2\text{O}$; $M_R = 1345$; 20.6 mg; 15.3 μmol ; 1.0 equiv.) was dissolved in a **MeOH** (1.50 mL) followed by addition of **SOCl₂** (11.2 μL ; 154 μmol ; 10 equiv.). The resulting clear solution was stirred 2 d at 50 °C. Mixture was then diluted with **H₂O** (2 mL) and directly purified by preparative HPLC (C18; **H₂O**–**MeCN** gradient with **TFA** additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 15.5 mg (~85% assuming $[\text{M}]^+[\text{TFA}]^- \cdot 0.2\text{TFA} \cdot 7.2\text{H}_2\text{O}$; $M_R = 1192$; 1 step; based on **TD1453**). **NMR** (~0.5 mM **TD1475** in ~50 mM **MOPS/NaOH** buffer in 10% **D₂O**, pH 7.0): ^{19}F δ_F –48.45 (s).



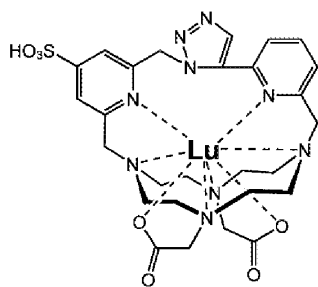
Synthesis of TD1506: In a glass vial (20 mL), **TD1504** (assuming $\text{M} \cdot 2.0\text{H}_2\text{O}$; $M_R = 657.7$, 8.7 mg; 13.2 μmol ; 1.0 equiv.) was dissolved in **H₂O** (19 mL) followed by addition aq. **MOPS/NaOH** buffer (500 mM; pH 7.0; 595 μL ; 398 μmol ; 30 equiv.) and aq. **LuCl₃** (100 mM; 159 μL ; 15.9 μmol ; 1.2 equiv.) and the mixture was stirred 16 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; **H₂O**–**MeCN** gradient with **FA** additive). Fractions with products (both diastereoisomers were fully separated) were joined and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 6.9+3.4 mg ($\leq 62+31\%$ assuming $[\text{M}]^+[\text{FA}]^- \cdot x\text{H}_2\text{O}$; $M_R = 836.6$; 1 step; based on **TD1504**·2.0H₂O). **NMR (first isomer, D₂O, pD ~4):** ^1H δ_H 1.06 (**CH₃**, d, 3H, $^3J_{\text{HH}} = 7$); 2.53–2.65 (**CH₂–CO**, m, 2H); 2.76–3.84 (*mc*, **CH–CH₂**, **CH–CH₃**, m, 16+1+1H); 3.96 (**CH₂–arom.**, d, 1H, $^2J_{\text{HH}} = 15$); 4.13 (**CH₂–arom.**, d, 1H, $^2J_{\text{HH}} = 16$); 4.27 (**CH₂–arom.**, d, 1H, $^2J_{\text{HH}} = 15$); 4.77 (**CH₂–arom.**, d, 1H, $^2J_{\text{HH}} = 16$); 5.61 (**CH₂–N₃**, d, 1H, $^2J_{\text{HH}} = 15$); 7.11 (**CH₂–N₃**, d, 1H, $^2J_{\text{HH}} = 15$); 7.78 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.81 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.85 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.87 (**CH–N₃**, s, 1H); 8.03 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 8.25 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$); 8.28 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$); 8.40 (**FA**, s, 1H). **NMR (second isomer, D₂O, pD ~5):** ^1H δ_H 1.14 (**CH₃**, d, 3H, $^3J_{\text{HH}} = 7$); 2.38–2.61 (**CH₂–CO**, m, 2H); 2.76–3.82 (*mc*, **CH–CH₂**, **CH–CH₃**, m, 16+1+1H); 3.92 (**CH₂–arom.**, d, 1H, $^2J_{\text{HH}} = 15$); 4.16 (**CH₂–arom.**,

d, 1H, $^2J_{\text{HH}} = 16$); 4.25 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 4.80 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 16$); 5.61 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 7.14 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 7.73 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.82 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.88 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.90 (CH-N_3 , s, 1H); 8.03 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 8.24 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$); 8.27 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$); 8.45 (*FA*, s, 1H). **ESI-MS** (LC-MS): 794.2 $[\text{M}]^+$ (theor. $[\text{C}_{30}\text{H}_{37}\text{N}_9\text{O}_6\text{Lu}_1]^+ = 794.2$).



Synthesis of TD1512: In a glass vial (2 mL), $[\text{TD782}]^+[\text{TFA}]^- \cdot 5.1\text{H}_2\text{O}$ (0.5 mg; 0.52 μmol ; 1.0 equiv.) was dissolved in solution of freshly prepared **DIPEA** (100 mM in DMSO; 104 μL ; 10.4 μmol ; 20 equiv.) followed by addition of freshly prepared solution of **NaSH** (500 mM in DMSO; 4.2 μL ; 2.1 μmol ; 4 equiv.) and the resulting mixture was stirred 15 min at RT. Mixture was diluted with H_2O (800 μL) and purified by preparative HPLC (C18; H_2O -MeCN gradient with TFA additive).

Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 0.2 mg ($\leq 44\%$ assuming $[\text{M}]^+[\text{TFA}]^- \cdot x\text{H}_2\text{O}$; $M_{\text{R}} = 867.6$; 1 step; based on $[\text{TD782}]^+[\text{TFA}]^- \cdot 5.1\text{H}_2\text{O}$). **NMR (D_2O , pD ~4): ^1H δ_{H} 2.63–3.79 (*mc*, $\text{CH}_2\text{-CO}$, $\text{CH}_2\text{-arom.}$, m, 16+4+1H); 4.05–4.17 ($\text{CH}_2\text{-arom.}$, m, 2H); 4.79 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 5.28 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 6.83 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 7.47 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 7.72 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 7.79 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.83 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.88 (CH-N_3 , s, 1H); 8.23 (*arom.*, t, 1H, $^3J_{\text{HH}} = 8$). **ESI-MS** (LC-MS): 754.2 $[\text{M}]^+$ (theor. $[\text{C}_{27}\text{H}_{33}\text{N}_9\text{O}_4\text{S}_1\text{Lu}_1]^+ = 754.2$).**



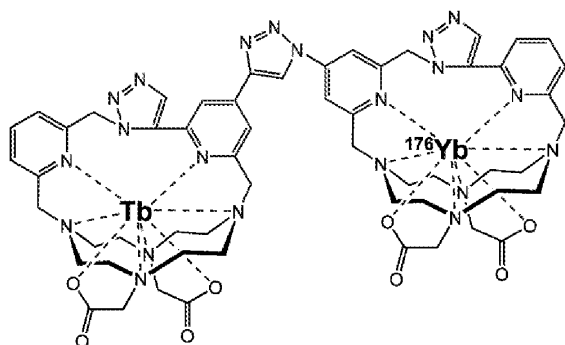
Synthesis of TD1512-SO₃H: In a glass vial (2 mL), $[\text{TD782}]^+[\text{TFA}]^- \cdot 5.1\text{H}_2\text{O}$ (1.0 mg; 1.0 μmol ; 1.0 equiv.) was dissolved in H_2O (156 μL) followed by addition of aq. solution of **Na₂S** (100 mM; 52 μL ; 5.2 μmol ; 5 equiv.) and the resulting mixture was stirred at 80 °C overnight. Mixture was diluted with H_2O (800 μL) and purified by preparative HPLC (C18; H_2O -MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized

to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 0.3 mg ($\leq 32\%$ assuming $[\text{M}]^+[\text{TFA}]^- \cdot x\text{H}_2\text{O}$; $M_{\text{R}} = 915.6$; 1 step; based on $[\text{TD782}]^+[\text{TFA}]^- \cdot 5.1\text{H}_2\text{O}$). **NMR (D_2O , pD ~7): ^1H δ_{H} 2.60–3.83 (*mc*, $\text{CH}_2\text{-CO}$, $\text{CH}_2\text{-arom.}$, m, 16+4H); 4.09 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 4.16 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 16$); 4.35 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 4.82 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 16$);**

5.72 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 7.16 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 7.82 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.86 (*arom.*, dd, 1H, $^3J_{\text{HH}} = 8$, $^4J_{\text{HH}} = 1$); 7.88 (CH-N_3 , s, 1H); 8.06 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$); 8.25 *arom.*, t, 1H, $^3J_{\text{HH}} = 8$); 8.36 (*arom.*, d, 1H, $^4J_{\text{HH}} = 2$). **ESI-MS (LC-MS):** 802.1 $[\text{M}]^+$ (theor. $[\text{C}_{27}\text{H}_{33}\text{N}_9\text{O}_7\text{S}_1\text{Lu}_1]^+ = 802.2$).

5

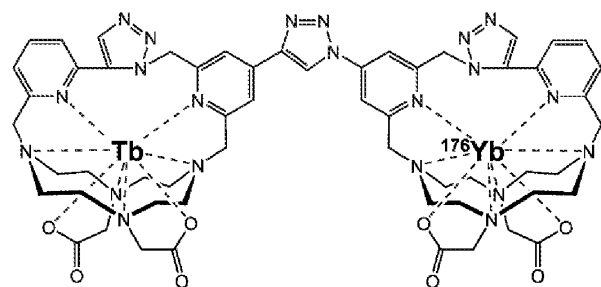
Example 7: Synthesis of chains of coordination compounds



Synthesis of TD837: In a glass vial (4 mL), aq. solution of $[\text{TD836}]^+[\text{TFA}]^- \cdot x\text{H}_2\text{O}$ (~20 mM; 274 μL ; ~5.5 μmol ; 1.0 equiv.) was mixed with aq. solution of $[\text{TD823}]^+[\text{TFA}]^- \cdot x\text{H}_2\text{O}$ (~20 mM; 274 μL ; ~5.5 μmol ; 1.0 equiv.) followed by addition of aq. **MES/NaOH buffer** (500 mM; pH 5.2; 548 μL ; 274 μmol ; ~50 equiv.), *t*-BuOH (1.1 mL) and aq. solution of **CuSO₄** (100 mM; 11 μL ; 1.1 μmol ; ~0.2 equiv.). To the resulting solution was added freshly

prepared aq. solution of **sodium ascorbate** (100 mM; 11 μL ; 1.1 μmol ; ~0.2 equiv.) and the mixture was stirred 2 h at RT. The mixture was then concentrated to ~1 mL and then purified by preparative HPLC (C18; H_2O –MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 4.7 mg ($\leq 50\%$ assuming $[\text{M}]^{2+}[\text{TFA}]_2^{2-} \cdot x\text{H}_2\text{O}$; $M_R = 1721$; 1 step; based on $[\text{TD836}]^+[\text{TFA}]^- \cdot x\text{H}_2\text{O}$). **ESI-HRMS:** 747.1998 $[\text{M}]^{2+}$ (theor. $[\text{C}_{56}\text{H}_{65}\text{N}_{21}\text{O}_8\text{Tb}_1^{176}\text{Yb}_1]^{2+} = 747.1997$).

20

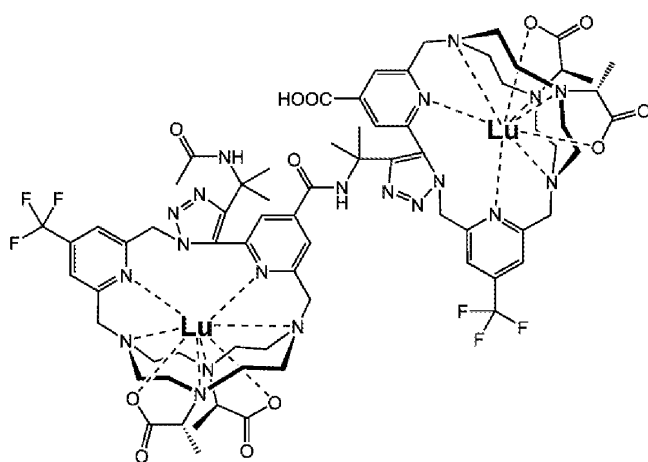


Synthesis of TD889: In a glass vial (4 mL), aq. solution of $[\text{TD836}]^+[\text{TFA}]^- \cdot x\text{H}_2\text{O}$ (~20 mM; 274 μL ; ~5.5 μmol ; 1.0 equiv.) was mixed with aq. solution of $[\text{TD886}]^+[\text{TFA}]^- \cdot x\text{H}_2\text{O}$ (~20 mM; 274 μL ; ~5.5 μmol ; 1.0 equiv.) followed by addition of aq. **MES/NaOH buffer** (500 mM; pH 5.2; 548 μL ; 274 μmol ; ~50 equiv.), *t*-BuOH (1.1 mL) and aq. solution of **CuSO₄** (100 mM; 28 μL ; 2.8

30

μmol ; ~0.5 equiv.). To the resulting solution was added freshly prepared aq. solution of **sodium ascorbate** (100 mM; 28 μL ; 2.8 μmol ; ~0.5 equiv.) and the mixture was stirred 30 min at RT. The mixture was then concentrated to ~1 mL and then purified by preparative HPLC (C18; H_2O –MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt

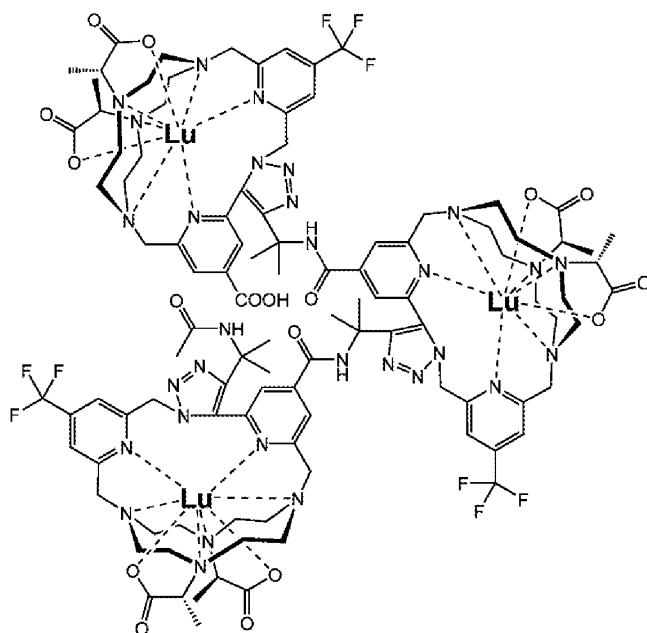
as white fluffy solid. **Yield:** 7.8 mg ($\leq 82\%$ assuming $[M]^{2+}[TFA]_2^{-} \cdot xH_2O$; $M_R = 1721$; 1 step; based on $[TD836]^+[TFA]^{-} \cdot xH_2O$). **ESI-HRMS:** 747.2002 $[M]^{2+}$ (theor. $[C_{56}H_{65}N_{21}O_8Tb_1^{176}Yb_1]^{2+} = 747.1997$).



Synthesis in TD1483: In a glass vial (4 mL), $[TD1472]^+[TFA]^{-} \cdot 0.8TFA \cdot 3.1H_2O$ (7.8 mg; 6.4 μmol ; 1.0 equiv.) and $[TD1473]^+[TFA]^{-} \cdot 0.2TFA \cdot 7.2H_2O$ (7.5 mg; 6.4 μmol ; 1.0 equiv.) were dissolved in DMSO (335 μL) followed by addition of **DIPEA** (400 mM in DMF; 240 μL ; 96 μmol ; 15 equiv.). Freshly prepared solution of **PyAOP** (200 mM in DMSO; 64 μL ; 12.8 μmol ; 2.0 equiv.) was then added and the resulting mixture was vortexed for 1 min and

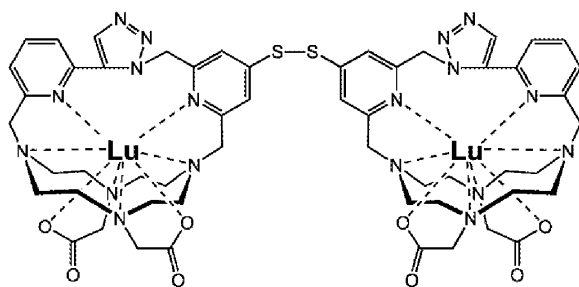
15 further left standing for 5 min at RT. Mixture

was then diluted with H_2O (1 mL), vortexed for 1 min and further left standing for 5 min at RT. Reaction mixture was then directly purified by preparative HPLC (C18; H_2O –MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid. **Yield:** 10.8 mg ($\leq 80\%$ assuming $[M]^{2+}[TFA]_2^{-} \cdot xH_2O$; $M_R = 2090$; 1 step; based on $[TD1472]^+[TFA]^{-} \cdot 0.8TFA \cdot 3.1H_2O$). **NMR (~0.5 mM TD1483 in ~50 mM MOPS/NaOH buffer in 10% D_2O , pH 7.0):** ^{19}F δ_F –66.10 (CF_3 , s, 3F); –66.11 (CF_3 , s, 3F).



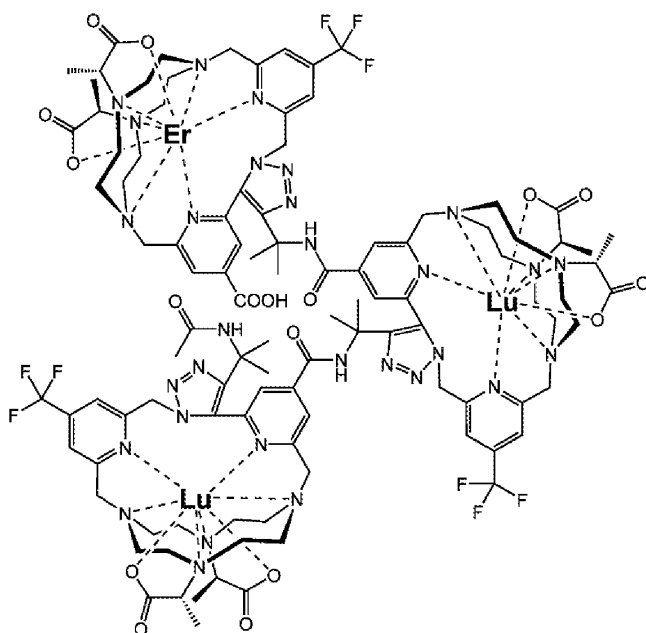
Synthesis of TD1484: In a glass vial (4 mL), **TD1483** (~20 mM in DMSO; 20 μ L; 0.4 μ mol; 1.0 equiv.), **TD1473** (~20 mM in DMSO; 20 μ L; 0.4 μ mol; 1.0 equiv.), **DIPEA** (400 mM in DMF; 15 μ L; 6.0 μ mol; 15 equiv.) and DMSO (21 μ L) were mixed together followed by addition of **PyAOP** (200 mM in DMSO; 4 μ L; 0.8 μ mol; 2.0 equiv.) and the resulting mixture was stirred 30 min at RT. Mixture was then diluted with H₂O (2 mL) and further stirred 30 min at RT. Mixture was then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly

lyophilized. All of the obtained product was used for ¹⁹F NMR characterization. **NMR (~0.5 mM TD1484 in ~50 mM MOPS/NaOH buffer in 10% D₂O, pH 7.0):** ¹⁹F δ_F –65.86 (CF₃, s, 3F); –66.09 (CF₃, s, 3F); –66.10 (CF₃, s, 3F).



Synthesis of TD1512-SS: Obtained as a side product (in the form of trifluoroacetate salt) during synthesis and purification of TD1512.

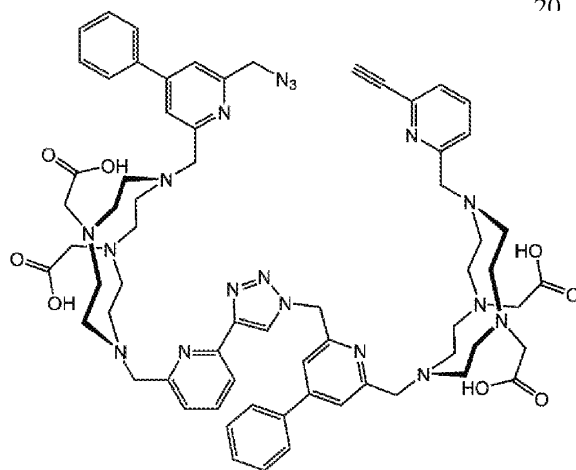
Yield: 0.1 mg. **ESI-MS (LC-MS):** 753.3 [M]²⁺ (theor. [C₅₄H₆₄Lu₂N₁₈O₈S₂]²⁺ = 753.2).



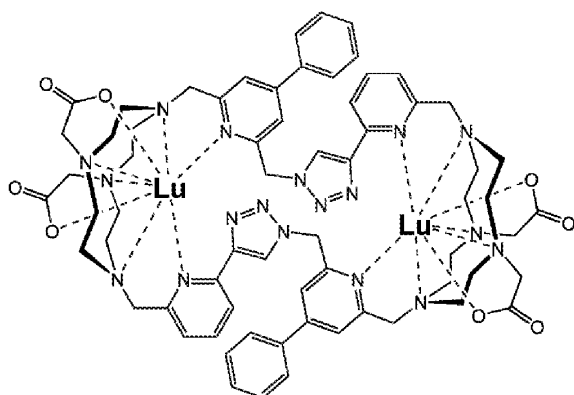
Synthesis: In a glass vial (4 mL), **TD1483** (~20 mM in DMSO; 20 μ L; 0.4 μ mol; 1.0 equiv.), **TD1475** (~20 mM in DMSO; 20 μ L; 0.4 μ mol; 1.0 equiv.), **DIPEA** (400 mM in DMF; 15 μ L; 6.0 μ mol; 15 equiv.) and DMSO (21 μ L) were mixed together followed by addition of **PyAOP** (200 mM in DMSO; 4 μ L; 0.8 μ mol; 2.0 equiv.) and the resulting mixture was stirred 30 min at RT. Mixture was then diluted with H₂O (2 mL) and further stirred 30 min at RT. Mixture was then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized. All of the

obtained product was used for ¹⁹F NMR characterization. **NMR (~0.5 mM TD1485 in ~50 mM MOPS/NaOH buffer in 10% D₂O, pH 7.0):** ¹⁹F δ_F –48.05 (CF₃, s, 3F); –65.13 (CF₃, s, 3F); –67.24 (CF₃, s, 3F).

Example 8: Synthesis of dimers of coordination compounds



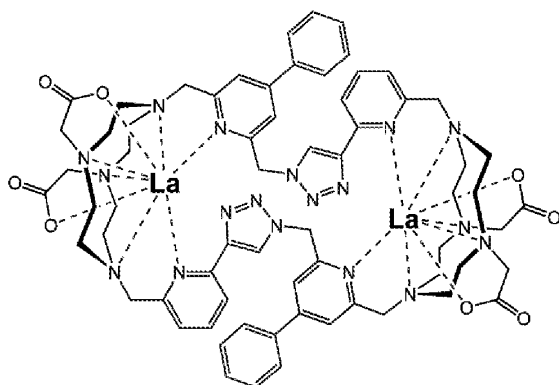
Synthesis of sc-TD647: Obtained as side product during synthesis of **TD647**. **Yield:** ~8 mg. **NMR (D₂O):** ¹H δ_H 2.69–3.65 (*mc* and CH₂–CO, 32+8H); 3.68 (C $\equiv$ CH, s, 1H); 3.70 (CH₂–arom., bs, 2H); 3.75–4.12 (CH₂–arom., 6H); 4.59 (CH₂–N₃, s, 2H); 5.89 (CH₂–N₃, s, 2H); 7.36–7.97 (*Ph* and *arom.*, 10+8H); 8.15 (*arom.*, bs, 1H); 8.32 (*arom.*, bs, 1H); 8.64 (CH–N₃, s, 1H). **ESI-HRMS:** 1251.6313 [M+H]⁺ (theor. [C₆₆H₇₉N₁₈O₈]⁺ = 1251.6323).



Synthesis of TD613: In a glass vial (20 mL), **sc-TD647**·xH₂O (5.0 mg; ~4 μmol; 1.0 equiv.) was dissolved in H₂O (14 mL) followed by addition aq. **MOPS/NaOH buffer** (500 mM; pH 7.0; 800 μL; 400 mmol; ~100 equiv.) and aq. **LuCl₃** (100 mM; 88 μL; 8.8 μmol; ~2.2 equiv.) and the mixture was stirred 1 h at 80 °C. Mixture was then filtered through syringe microfilter (RC). The filtrate was concentrated on rotary evaporator and then directly purified by preparative HPLC (C18; H₂O–MeCN

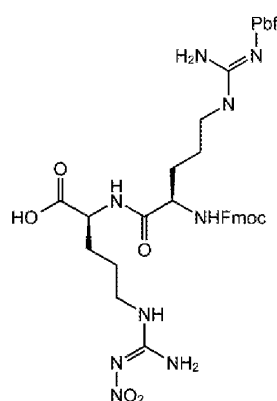
gradient with FA additive). Fractions with product were joined and directly lyophilized to give product in the form of formate salt as white fluffy solid. **Yield:** 4 mg (~60%; 1 step; based on **sc-TD647**·xH₂O). **ESI-HRMS:** 798.2368 [M]²⁺ (theor. [C₆₆H₇₄N₁₈O₈Lu₂]²⁺ = 798.2371).

- 15 **Synthesis of TD598:** Obtained as side product during synthesis of **TD651** in the form of formate salt as white fluffy solid. **Yield:** 6 mg. **NMR (D₂O, pD ~5):** ¹H δ_H 2.47–3.99 (*mc*, CH₂–CO, CH₂–arom, m, 32+8+4H); 4.42



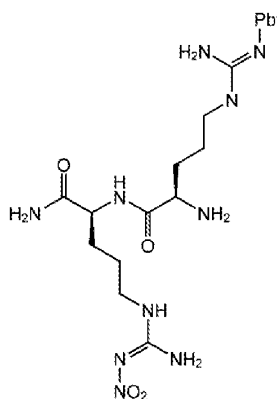
(CH₂–arom., d, 2H, ²J_{HH} = 17); 4.75 (CH₂–arom., d, 2H, ²J_{HH} = 17); 5.90 (CH₂–N₃, d, 2H, ²J_{HH} = 16); 6.20 (CH₂–N₃, d, 2H, ²J_{HH} = 16); 7.07 (*arom.*, d, 2H, ⁴J_{HH} = 2); 7.44 (*arom.*, d, 2H, ⁴J_{HH} = 2); 7.47–7.63 (*Ph*, m, 10H); 7.66 (*arom.*, dd, 2H, ³J_{HH} = 8, ⁴J_{HH} = 1); 7.89 (*arom.*, dd, 2H, ³J_{HH} = 8, ⁴J_{HH} = 1); 8.15 (*arom.*, t, 2H, ³J_{HH} = 8); 8.42 (*FA*, s); 8.74 (CH–N₃, s, 2H). ¹³C{¹H} δ_C 49.3 (*mc*, s); 49.4 (*mc*, s); 51.0 (*mc*, s); 51.6 (*mc*, s); 52.5 (*mc*, s); 53.9 (*mc*, s); 54.0 (*mc*, s); 54.3 (*mc*, s); 55.6 (CH₂–N₃, s); 59.2 (CH₂–arom., s); 59.8 (CH₂–CO, s); 61.2 (CH₂–arom., s); 61.9 (CH₂–CO, s); 120.7 (*arom.*, s); 121.2 (*arom.*, s); 122.2 (*arom.*, s); 125.4 (*arom.*, s); 126.7 (CH–N₃, s); 128.2 (*Ph*, s); 129.6 (*Ph*, s); 131.6 (*Ph*, s); 135.2 (*Ph*, s); 143.0 (*arom.*, s); 145.5 (*arom.*, s); 148.9 (*arom.*, s); 150.2 (*arom.*, s); 157.2 (*arom.*, s); 157.3 (*arom.*, s); 158.9 (*arom.*, s); 170.8 (*FA*, s); 177.9 (CO, s); 180.5 (CO, s). **ESI-HRMS:** 762.2025 [M]²⁺

- 30 (theor. [C₆₆H₇₄N₁₈O₈La₂]²⁺ = 762.2027).

Example 9: Synthesis of conjugates of coordination compounds with peptides

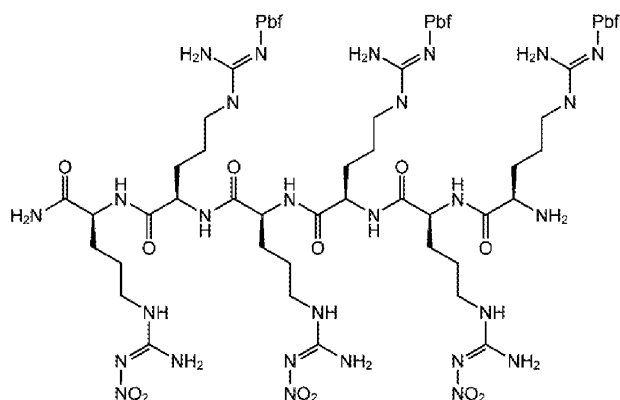
Synthesis of TD981: In a glass vial #1 (20 mL), **HO-R(*N*_o-NO₂)-Fmoc** (886 mg; 2.01 mmol; 1.1 equiv.) was dissolved (with the help of vortexing/gentle heating) in DMSO (6 mL) followed by addition of **DBU** (550 μ L; 3.68 mmol; 2.0 equiv.) and the resulting solution was briefly vortexed and further stirred 2 mins at RT. In a glass vial #2 (20 mL), **HO-^oR(Pbf)-Fmoc** (1.20 g; 1.85 mmol; 1.0 equiv.) was dissolved (with the help of vortexing/gentle heating) in a mixture of DMSO (9 mL) and **NMM** (1.0 mL; 9.09 mmol; 4.9 equiv.) followed by addition of solid **PyAOP** (965 mg; 1.85 mmol; 1.0 equiv.). The mixture was briefly vortexed and the resulting slightly yellow solution was further stirred 2 mins at RT. Solution from vial #1 was then added in one portion (via syringe) to the intensively stirred

solution in vial #2. Resulting bright yellow solution was further gently stirred 5 mins at RT after which neat **TFA** (430 μ L; 5.62 mmol; 3.0 equiv.) was added. Mixture was then directly purified by flash chromatography (C18; H₂O–MeCN gradient with TFA additive). Fractions with pure product were joined and directly lyophilized. Fractions containing impure product were joined, lyophilized and subsequently re-purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive) and re-lyophilized. Both solids were then joined and homogenized to give product as off-white solid. **Yield:** 1.21 g (63%; 2 steps; based on **HO-^oR(Pbf)-H**). **ESI-MS** (LC-MS): 850.4 [M+H]⁺ (theor. [C₄₀H₅₂N₉O₁₀S₁]⁺ = 850.3). **EA** (C₄₀H₅₁N₉O₁₀S₁·1.4TFA·1.4H₂O, *M_R* = 1034.8): C 49.7 (50.0); H 5.4 (5.1); N 12.2 (11.8); F 7.7 (7.8).



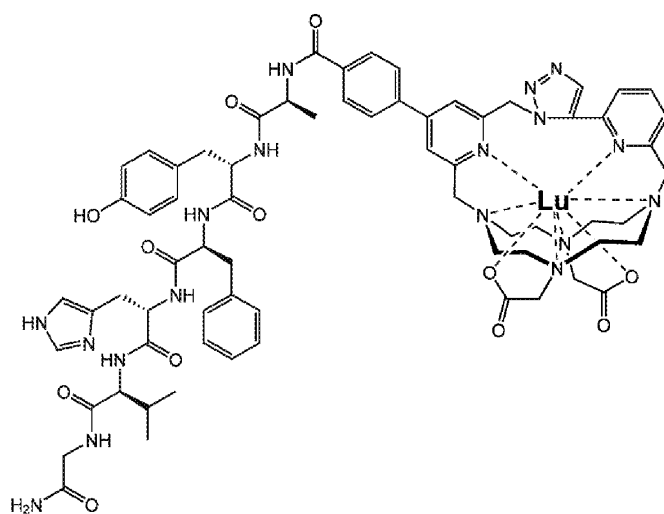
Synthesis of TD982: In a glass vial (20 mL), **TD981**·1.4TFA·1.4H₂O (360 mg; 348 μ mol; 1.1 equiv.) and **NH₄Cl** (27 mg; 505 μ mol; 1.5 equiv.) were dissolved (with the help of vortexing/gentle heating) in DMSO (2 mL) followed by addition of **NMM** (140 μ L; 1.27 mmol; 3.0 equiv.) and of solid **PyAOP** (264 mg; 506 μ mol; 1.5 equiv.). Mixture was briefly vortexed and the resulting bright yellow solution was further stirred 5 mins at RT followed by addition of **DBU** (380 μ L; 2.54 mmol; 7.3 equiv.). Mixture was briefly vortexed and the resulting bright yellow solution was further stirred 2 mins at RT after which neat **TFA** (160 μ L; 2.09 mmol; 6.0 equiv.) was added. Mixture was then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions

with pure product were joined and directly lyophilized to give product as white fluffy solid. **Yield:** 237 mg (74%; 2 steps; based on **TD981**·1.4TFA·1.4H₂O). **ESI-MS** (LC-MS): 627.4 [M+H]⁺ (theor. [C₂₅H₄₂N₁₀O₇S₁]⁺ = 627.3). **EA** (C₂₅H₄₂N₁₀O₇S₁·2.3TFA·1.9H₂O, *M_R* = 923.2): C 38.5 (38.7); H 5.3 (4.9); N 15.2 (14.8); F 14.2 (14.0).



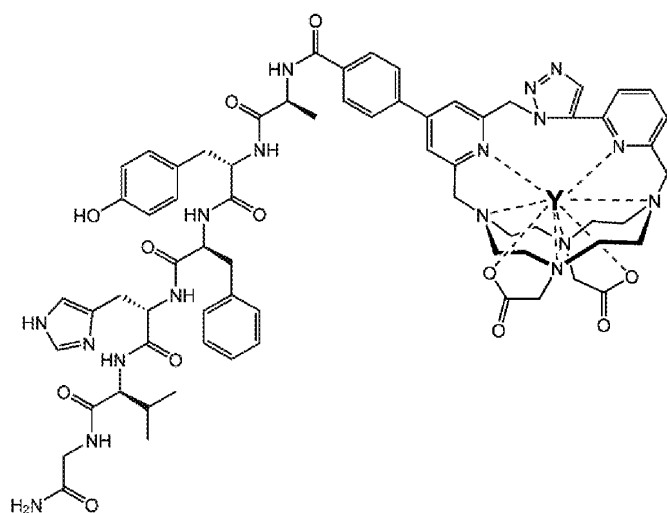
Synthesis of TD986: In a glass vial (20 mL), **TD981**·1.4TFA·1.4H₂O (258 mg; 249 μmol; 1.0 equiv.) and **TD982**·2.3TFA·1.9H₂O (222 mg; 240 μmol; 1.0 equiv.) were dissolved (with the help of vortexing/gentle heating) in DMSO (2 mL) followed by addition of **NMM** (220 μL; 2.00 mmol; 8.3 equiv.) and of solid **PyAOP** (136 mg; 261 μmol; 1.1 equiv.). Mixture was briefly vortexed and the resulting bright yellow solution was further stirred 15

- mins at RT followed by addition of **DBU** (221 μL; 1.48 mmol; 6.2 equiv.). Mixture was briefly vortexed and the resulting bright yellow solution was further stirred 15 mins at RT after which another portion of **DBU** (36 μL; 241 μmol; 1.0 equiv.) was added. Mixture was briefly vortexed and the resulting bright yellow solution was further stirred 5 mins at RT after which neat **TFA** (58 μL; 758 μmol; 3.2 equiv.) was added. Mixture was then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with pure product were joined and directly lyophilized. The resulting **tetraarginine intermediate NH₂-R(N_o-NO₂)^pR(Pbf)R(N_o-NO₂)^pR(Pbf)-H** (≤238 μmol; 1.0 equiv.) was transferred to a glass vial (20 mL) followed by addition of **TD981**·1.4TFA·1.4H₂O (259 mg; 250 μmol; ≥1.1 equiv.), DMSO (2 mL; dissolved with the help of vortexing/gentle heating), **NMM** (276 μL; 2.51 mmol; ≥10.5 equiv.) and of solid **PyAOP** (144 mg; 277 μmol; ≥1.2 equiv.). Mixture was briefly vortexed and the resulting bright yellow solution was further stirred 20 mins at RT followed by addition of **DBU** (263 μL; 1.76 mmol; ≥7.0 equiv.). Mixture was briefly vortexed and the resulting bright yellow solution was further stirred 5 mins at RT after which neat **TFA** (77 μL; 1.00 mmol; ≥4.0 equiv.) was added. Mixture was then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with pure product were joined and directly lyophilized to give product as white fluffy solid. **Yield:** 368 mg (66%; 4 steps; based on **TD982**·2.3TFA·1.9H₂O). **ESI-MS** (LC-MS): 923.6 [M+2H]²⁺ (theor. [C₇₅H₁₂₀N₂₈O₂₁S₃]²⁺ = 923.4). **EA** (C₇₅H₁₂₀N₂₈O₂₁S₃·3.2TFA·5.3H₂O, M_R = 2306.4): C 42.4 (42.8); H 5.8 (5.4); N 17.0 (16.6); F 7.9 (7.6).



Synthesis of TD802: In a glass vial (4 mL), $[\text{TD748}]^+[\text{TFA}]^- \cdot 1.4\text{TFA} \cdot 3.2\text{H}_2\text{O}$ (3.3 mg; 2.8 μmol ; 1.0 equiv.) and $\text{H}_2\text{N-GVHFYA-H} \cdot 1.0\text{TFA} \cdot x\text{H}_2\text{O}$ (3.4 mg; ~ 4.2 μmol ; ~ 1.5 equiv.) were dissolved in DMSO (1 mL) followed by addition of DIPEA (5.0 μL ; 29 μmol ; ~ 10 equiv.) and PyOAP (freshly prepared 100 mM in DMSO; 52 μL ; 5.2 μmol ; 1.9 equiv.) and the resulting yellow solution was stirred 20 min at RT. Mixture was then directly purified by preparative HPLC (C18; H_2O –MeCN gradient with TFA additive).

Fractions with product were joined and directly lyophilized to give product as white fluffy solid. **Yield:** 4.4 mg ($\leq 96\%$ assuming $[\text{M}]^+[\text{TFA}]^- \cdot x\text{H}_2\text{O}$; $M_R = 1629.5$; 1 step; based on $[\text{TD748}]^+[\text{TFA}]^- \cdot 1.4\text{TFA} \cdot 3.2\text{H}_2\text{O}$). **NMR (D_2O , $\text{pD} \sim 5$):** ^1H δ_{H} 0.93 ($^{\text{V}}\text{CH}_3$, d, 3H, $^3J_{\text{HH}} = 7$); 0.95 ($^{\text{V}}\text{CH}_3$, d, 3H, $^3J_{\text{HH}} = 7$); 1.42 ($^{\text{A}}\text{CH}_3$, d, 3H, $^3J_{\text{HH}} = 7$); 2.03 ($^{\text{V}}\text{CH}-(\text{CH}_3)_2$, d, 1H, $^3J_{\text{HH}} = 7$, $^3J_{\text{HH}} = 7$); 2.62–3.81 ($^{\text{mc}}$, $\text{CH}_2\text{-CO}$, $^{\text{H}}\text{CH}_2$, $^{\text{F}}\text{CH}_2$, $^{\text{V}}\text{CH}_2$, m, 16+4+2+2+2H); 3.84 ($^{\text{G}}\text{CH}_2$, d, 1H, $^2J_{\text{HH}} = 17$); 3.95 ($^{\text{G}}\text{CH}_2$, d, 1H, $^2J_{\text{HH}} = 17$); 4.01 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 4.02 ($^{\text{V}}\text{CH}$, d, 1H, $^3J_{\text{HH}} = 7$); 4.15 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 16$); 4.35 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 4.45 ($^{\text{A}}\text{CH}$, q, 1H, $^3J_{\text{HH}} = 7$); 4.52–4.66 ($^{\text{H}}\text{CH}$, $^{\text{F}}\text{CH}$, $^{\text{V}}\text{CH}$, m, 1+1+1H); 4.85 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 16$); 5.67 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 15$); 6.59 ($^{\text{V}}\text{arom.}$, dm, 2H, $^3J_{\text{HH}} = 8$); 6.96 ($^{\text{V}}\text{arom.}$, dm, 2H, $^3J_{\text{HH}} = 8$); 7.08–7.18 ($\text{CH}_2\text{-N}_3$, $^{\text{F}}\text{arom.}$, m, 1+2H); 7.19 ($^{\text{H}}\text{arom.}$, s, 1H); 7.23–7.30 ($^{\text{F}}\text{arom.}$, m, 3H); 7.81 (arom. , dd, 1H, $^3J_{\text{HH}} = 8$; $^4J_{\text{HH}} = 1$); 7.82–7.87 (arom. , m, 3H); 7.89 (CH-N_3 , s, 1H); 8.00 (arom. , dm, 2H, $^3J_{\text{HH}} = 8$); 8.03 (arom. , d, 1H, $^4J_{\text{HH}} = 2$); 8.24 (arom. , t, 1H, $^3J_{\text{HH}} = 8$); 8.33 (arom. , d, 1H, $^4J_{\text{HH}} = 2$); 8.48 ($^{\text{H}}\text{arom.}$, bs, 1H). **ESI-HRMS:** 1515.5593 $[\text{M}]^+$ (theor. $[\text{C}_{68}\text{H}_{80}\text{N}_{18}\text{O}_{12}\text{Lu}]^+ = 1515.5605$).

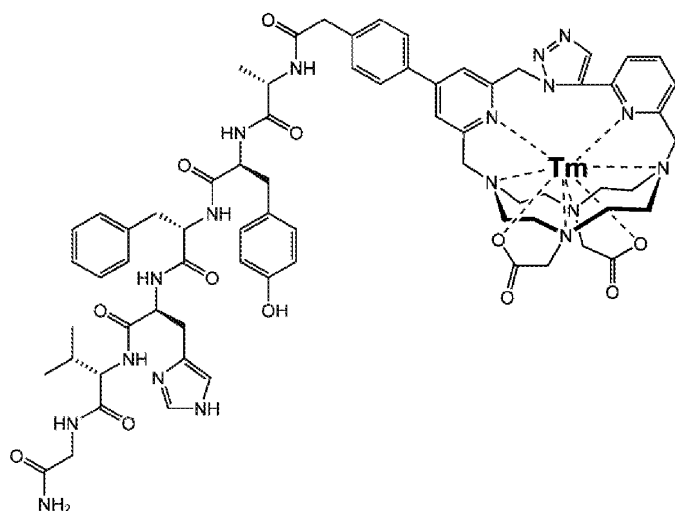


Synthesis of TD792: In a glass vial (4 mL), $[\text{TD785}]^+[\text{TFA}]^- \cdot 0.6\text{TFA} \cdot 4.8\text{H}_2\text{O}$ (3.0 mg; 2.9 μmol ; 1.0 equiv.) and $\text{H}_2\text{N-GVHFYA-H} \cdot 1.0\text{TFA} \cdot x\text{H}_2\text{O}$ peptide (3.4 mg; $\sim 4.2 \mu\text{mol}$; ~ 1.4 equiv.) were dissolved in DMSO (1 mL) followed by addition of **DIPEA** (5.0 μL ; 29 μmol ; ~ 10 equiv.) and **PyOAP** (freshly prepared 100 mM in DMSO; 52 μL ; 5.2 μmol ; 1.8 equiv.) and the resulting yellow solution was stirred 20 min at RT. Mixture was then directly purified by preparative HPLC (C18; H_2O –MeCN gradient with TFA

additive). Fractions with product were joined and directly lyophilized to give product as white fluffy solid.

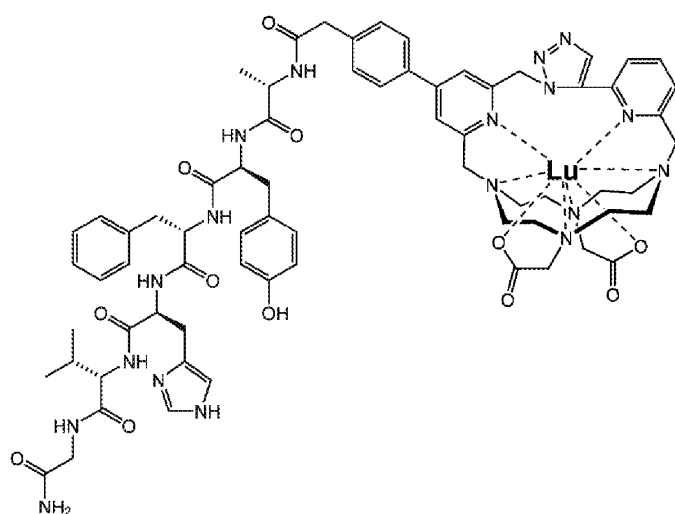
- 15 **Yield:** 4.0 mg ($\leq 89\%$ assuming $[\text{M}]^+[\text{TFA}]^- \cdot x\text{H}_2\text{O}$; $M_R = 1543.3$; 1 step; based on $[\text{TD785}]^+[\text{TFA}]^- \cdot 0.6\text{TFA} \cdot 4.8\text{H}_2\text{O}$). **NMR (D_2O , $\text{pD} \sim 5$):** ^1H δ_{H} 0.93 ($^{\text{V}}\text{CH}_3$, d, 3H, $^3J_{\text{HH}} = 7$); 0.95 ($^{\text{V}}\text{CH}_3$, d, 3H, $^3J_{\text{HH}} = 7$); 1.42 ($^{\text{A}}\text{CH}_3$, d, 3H, $^3J_{\text{HH}} = 7$); 2.03 ($^{\text{V}}\text{CH}-(\text{CH}_3)_2$, d, 1H, $^3J_{\text{HH}} = 7$, $^3J_{\text{HH}} = 7$); 2.62–3.81 ($^{\text{mc}}$, $\text{CH}_2\text{-CO}$, $^{\text{H}}\text{CH}_2$, $^{\text{F}}\text{CH}_2$, $^{\text{Y}}\text{CH}_2$, m, 16+4+2+2+2H); 3.84 ($^{\text{G}}\text{CH}_2$, d, 1H, $^2J_{\text{HH}} = 17$); 3.95 ($^{\text{G}}\text{CH}_2$, d, 1H, $^2J_{\text{HH}} = 17$); 4.01 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 4.02 ($^{\text{V}}\text{CH}$, d, 1H, $^3J_{\text{HH}} = 7$); 4.14 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 16$); 4.39 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 15$); 4.45 ($^{\text{A}}\text{CH}$, q, 1H, $^3J_{\text{HH}} = 7$); 4.52–4.67 ($^{\text{H}}\text{CH}$, $^{\text{F}}\text{CH}$, $^{\text{Y}}\text{CH}$, m, 1+1+1H); 4.85 ($\text{CH}_2\text{-arom.}$, d, 1H, $^2J_{\text{HH}} = 16$); 5.72 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 16$); 6.59 ($^{\text{Y}}\text{arom.}$, dm, 2H, $^3J_{\text{HH}} = 8$); 6.96 ($^{\text{Y}}\text{arom.}$, dm, 2H, $^3J_{\text{HH}} = 8$); 7.09 ($\text{CH}_2\text{-N}_3$, d, 1H, $^2J_{\text{HH}} = 16$); 7.10–7.16 ($^{\text{F}}\text{arom.}$, m, 2H); 7.21 ($^{\text{H}}\text{arom.}$, s, 1H); 7.24–7.30 ($^{\text{F}}\text{arom.}$, m, 3H); 7.78–7.86 (arom. , m, 4H); 7.89 (CH-N_3 , s, 1H); 8.00 (arom. , dm, 2H, $^3J_{\text{HH}} = 8$); 8.03 (arom. , d, 1H, $^4J_{\text{HH}} = 2$); 8.25 (arom. , t, 1H, $^3J_{\text{HH}} = 8$); 8.33 (arom. , d, 1H, $^4J_{\text{HH}} = 2$); 8.53 ($^{\text{H}}\text{arom.}$, t, 1H, $^4J_{\text{HH}} = 2$). **ESI-HRMS:**
- 25 1429.5264 $[\text{M}]^+$ (theor. $[\text{C}_{68}\text{H}_{80}\text{N}_{18}\text{O}_{12}\text{Y}_1]^+ = 1429.5256$).

157



–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product as white fluffy solid. **Yield:** 2.0 mg ($\leq 85\%$ assuming $[M]^+[TFA]^- \cdot xH_2O$; $M_R = 1637.5$; 1 step; based on $[TD899]^+[TFA]^- \cdot 0.2TFA \cdot 7.0H_2O$). **ESI-HRMS:** 762.2885 $[M+H]^{2+}$ (theor. $[C_{69}H_{83}N_{18}O_{12}Tm_1]^{2+} = 762.2887$).

15



Synthesis of TD902: In a glass vial (4 mL), $[TD891]^+[TFA]^- \cdot 0.7TFA \cdot 4.6H_2O$ (1.6 mg; 1.4 μ mol; 1.0 equiv.) and H_2N -GVHFYA-H $\cdot 1.0TFA \cdot xH_2O$ (1.6 mg; ~ 2.0 μ mol; ~ 1.4 equiv.) were dissolved in DMSO (500 μ L) followed by addition of DIPEA (2.3 μ L; 13 μ mol; 9.4 equiv.) and PyOAP (freshly prepared 100 mM in DMSO; 25 μ L; 2.5 μ mol; 1.8 equiv.) and the resulting yellow solution was stirred 40 min at RT. Mixture was then directly purified by preparative HPLC (C18; H_2O -MeCN gradient with TFA additive).

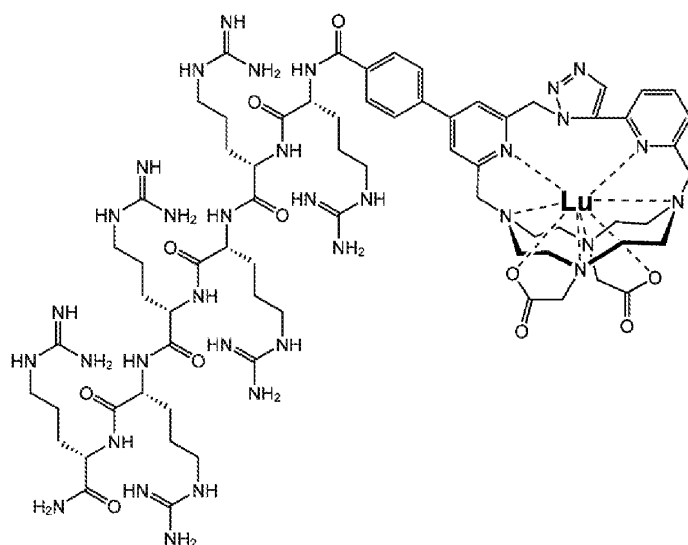
30

Fractions with product were joined and directly lyophilized to give product as white fluffy solid. **Yield:** 1.8 mg ($\leq 78\%$ assuming $[M]^+[TFA]^- \cdot xH_2O$; $M_R = 1643.5$; 1 step; based on $[TD891]^+[TFA]^- \cdot 0.7TFA \cdot 4.6H_2O$). **NMR (D_2O , pD ~ 4 , assignment was complicated by two distinguishable diastereomeric set of most of the signals):** 1H δ_H 0.91 (VCH_3 , d, 3H, $^3J_{HH} = 7$); 0.93 (VCH_3 , d, 3H, $^3J_{HH} = 7$); 1.36 (ACH_3 , d, 3H, $^3J_{HH} = 7$); 1.90–4.21 ($^VCH-(CH_3)_2$, mc, CH_2-CO , HCH_2 , FCH_2 , YCH_2 , GCH_2 , CH_2 -arom., VCH , ACH , m,

35

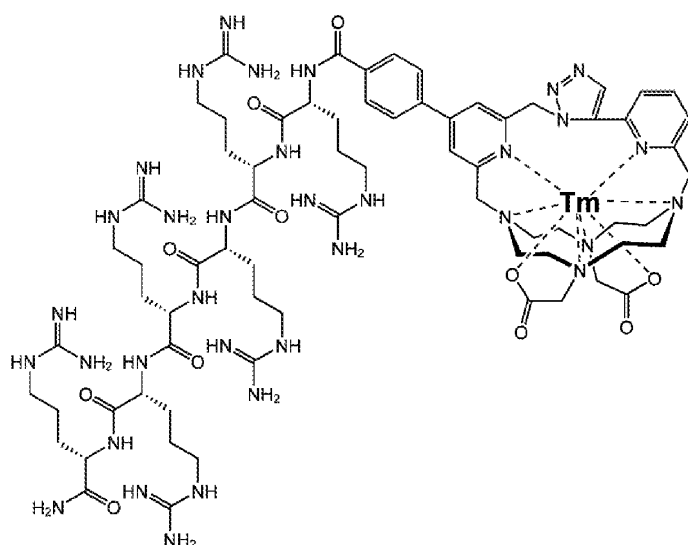
1+16+4+2+2+2+2+5+1+1H); 4.21–4.56 (^1HCH , $^{\text{F}}\text{CH}$, $^{\text{Y}}\text{CH}$, m, 1+1+1H); 4.81–4.90 (CH_2 -arom., m, 1H); 5.52–5.62 (CH_2 -N₃, m, 1H); 6.77–7.18 ($^{\text{X}}\text{arom.}$, CH_2 -N₃, $^{\text{F}}\text{arom.}$, $^{\text{H}}\text{arom.}$, $^{\text{F}}\text{arom.}$, m, 4+1+2+1+3H); 7.34–8.10 (arom., CH -N₃, 8+1H); 8.21–8.30 (arom., m, 1H); 8.52–8.58 ($^{\text{H}}\text{arom.}$, m, 1H). **ESI-HRMS**: 765.2919 $[\text{M}+\text{H}]^{2+}$ (theor. $[\text{C}_{69}\text{H}_{83}\text{N}_{18}\text{O}_{12}\text{Lu}_1]^{2+} = 765.2917$).

5



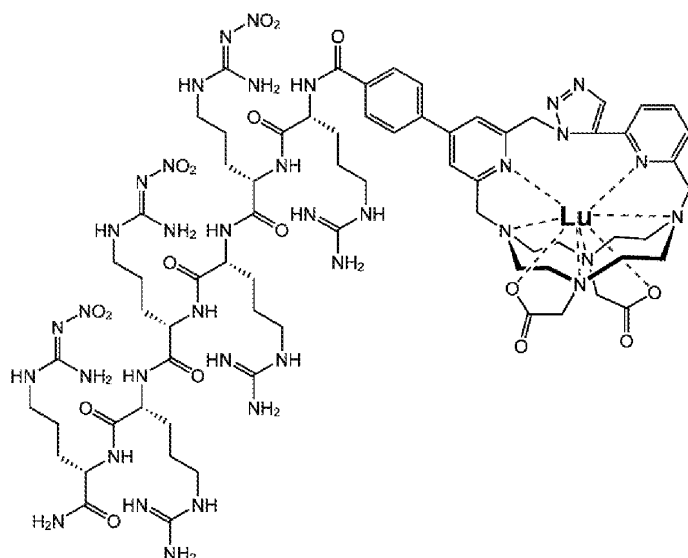
Synthesis of TD975: In a glass vial (4 mL), $[\text{TD748}]^+[\text{TFA}]^- \cdot 1.4\text{TFA} \cdot 3.2\text{H}_2\text{O}$ (7.1 mg; 6.1 μmol ; 1.0 equiv.) and $\text{H}_2\text{N}-[\text{R}(\text{Pbf})^{\text{P}}\text{R}(\text{Pbf})]_3\text{-H} \cdot 1.0\text{TFA} \cdot x\text{H}_2\text{O}$ (21 mg; $\sim 8.1 \mu\text{mol}$; ~ 1.3 equiv.) were dissolved in DMSO (1 mL) followed by addition of NMM (20 μL ; 182 μmol ; 30 equiv.) and **PyOAP** (15.5 mg; 29.7 μmol ; 5.0 equiv.) and the resulting yellow solution was stirred 20 min at RT. Mixture was then directly purified by preparative HPLC (C18; H_2O -MeCN gradient with TFA additive).

Fractions with Pbf protected product were joined, evaporated to dryness and twice co-evaporated with MeOH. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and the residue was directly purified by preparative HPLC (C18; H_2O -MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid (the amount of TFA was determined by qNMR using external capillary solution of 1,3,5-tris(trifluoromethyl)benzene in CCl_4). **Yield:** 13.0 mg ($\sim 85\%$ assuming $[\text{M}]^+[\text{TFA}]^- \cdot 5.4\text{TFA} \cdot x\text{H}_2\text{O}$; $M_{\text{R}} = 2510$; 2 steps; based on $[\text{TD748}]^+[\text{TFA}]^- \cdot 1.4\text{TFA} \cdot 3.2\text{H}_2\text{O}$). **NMR (D_2O , $\text{pD} \sim 3$):** ^1H δ_{H} 1.41–2.07 ($^{\text{R}}\text{CH}_2$, m, 24H); 2.63–3.88 (mc, CH_2 -CO, $^{\text{R}}\text{CH}_2$, m, 16+4+12H); 4.05 (CH_2 -arom., d, 1H, $^2J_{\text{HH}} = 15$); 4.18 (CH_2 -arom., d, 1H, $^2J_{\text{HH}} = 16$); 4.21–4.57 ($^{\text{R}}\text{CH}$, CH_2 -arom., m, 5+1H); 4.53 ($^{\text{R}}\text{CH}$, t, 1H, $^3J_{\text{HH}} = 7$); 4.87 (CH_2 -arom., d, 1H, $^2J_{\text{HH}} = 16$); 5.69 (CH_2 -N₃, d, 1H, $^2J_{\text{HH}} = 15$); 7.19 (CH_2 -N₃, d, 1H, $^2J_{\text{HH}} = 15$); 7.82 (arom., dd, 1H, $^3J_{\text{HH}} = 8$; $^4J_{\text{HH}} = 1$); 7.88 (arom., dd, 1H, $^3J_{\text{HH}} = 8$; $^4J_{\text{HH}} = 1$); 7.91 (CH -N₃, s, 1H); 8.03 (arom., dm, 2H, $^3J_{\text{HH}} = 8$); 8.06–8.12 (arom., m, 3H); 8.27 (arom., t, 1H, $^3J_{\text{HH}} = 8$); 8.39 (arom., d, 1H, $^4J_{\text{HH}} = 2$). **MALDI-HRMS**: 1777.8487 $[\text{M}]^+$ (theor. $[\text{C}_{70}\text{H}_{110}\text{N}_{34}\text{O}_{11}\text{Lu}_1]^+ = 1777.8496$).



Synthesis of TD989: In a glass vial (4 mL), [TD897]⁺[TFA]⁻·1.1TFA·2.6H₂O (15.0 mg; 13.4 μmol; 1.0 equiv.) and TD986·3.2TFA·5.3H₂O (30.9 mg; 13.4 μmol; 1.0 equiv.) were dissolved in DMSO (1.5 mL) followed by addition of solution of NMM (400 mM, 335 μL; 134 μmol; ~10 equiv.) in DMSO and freshly prepared solution of PyOAP (110 mM; 210 μL; 23.1 μmol; 1.7 equiv.) in DMSO and the resulting yellow solution was stirred 30 min at RT. Mixture was then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with

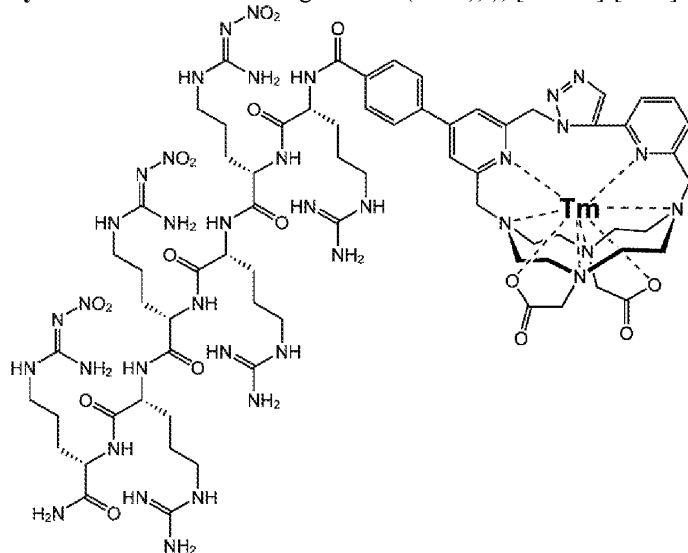
- 15 TFA additive). Fractions with fully protected product were joined, evaporated to dryness and twice co-evaporated with MeOH. Residue was dissolved in TFA (3 mL) and stirred overnight at RT to remove Pbf groups. Resulting blue solution was evaporated to dryness and the residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with partly protected product were joined, and evaporated to dryness. Residue was re dissolved in H₂O (10 mL) and transferred via syringe through septum to
- 20 a glass flask (25 mL) already filled with Pd/C (30 mg) and argon secured. Hydrogen gas was then left bubbling through the suspension for 2 days at RT. Mixture was then filtered through syringe microfilter (RC), and the filtrate was evaporated to dryness. Residue was purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product in the form of
- 25 14.6 mg (~45% assuming [M]⁺[TFA]⁻·5.4TFA·xH₂O; $M_R = 2505$; 3 steps; based on [TD897]⁺[TFA]⁻·1.1TFA·2.6H₂O). **MALDI-HRMS:** 1771.8412 [M]⁺ (theor. [C₇₀H₈₃N₃₄O₁₁Tm]⁺ = 1771.8435).



Synthesis of TD1011: In a glass vial (4 mL), [TD748]⁺[TFA]⁻·1.4TFA·3.2H₂O (15.0 mg; 12.8 μmol; 1.0 equiv.) and TD986·3.2TFA·5.3H₂O (31 mg; 13.4 μmol; 1.0 equiv.) were dissolved in DMSO (1.5 mL) followed by addition of solution of NMM (400 mM, 340 μL; 136 μmol; 11 equiv.) in DMSO and freshly prepared solution of PyOAP (110 mM; 210 μL; 23.1 μmol; 1.8 equiv.) in DMSO and the resulting yellow solution was stirred 30 min at RT. Mixture was then directly purified by preparative HPLC (C18; H₂O–MeCN gradient with

- 15 TFA additive). Fractions with Pbf protected product were joined, evaporated to dryness and twice co-evaporated with MeOH. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and the residue was suspended in 20 % MeCN (3 mL). Mixture was filtered through syringe microfilter (RC) and the filtrate was directly purified by preparative HPLC (C18; H₂O–MeCN gradient with TFA additive). Fractions with product were joined and directly lyophilized to give product
- 20 in the form of trifluoroacetate salt as white fluffy solid (the amount of TFA was determined by qNMR using external capillary solution of 1,3,5-tris(trifluoromethyl)benzene in CCl₄). **Yield:** 18.4 mg (~55% assuming [M]⁺[TFA]⁻·4.5TFA·xH₂O; *M_R* = 2540; 2 steps; based on [TD748]⁺[TFA]⁻·1.4TFA·3.2H₂O). **NMR (D₂O, pD ~5):** ¹H δ_H 1.39–2.07 (^RCH₂, m, 24H); 2.63–3.85 (*mc*, CH₂–CO, ^RCH₂, m, 16+4+12H); 4.04 (CH₂–arom., d, 1H, ²J_{HH} = 15); 4.16 (CH₂–arom., d, 1H, ²J_{HH} = 16); 4.21–4.66 (^RCH, CH₂–arom., m, 6+1H); 4.86 (CH₂–arom., d, 1H, ²J_{HH} = 16); 5.69 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.18 (CH₂–N₃, d, 1H, ²J_{HH} = 15); 7.81 (*arom.*, dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.86j (*arom.*, dd, 1H, ³J_{HH} = 8; ⁴J_{HH} = 1); 7.90 (CH–N₃, s, 1H); 7.95–8.09 (*arom.*, m, 5H); 8.25 (*arom.*, t, 1H, ³J_{HH} = 8); 8.37 (*arom.*, d, 1H, ⁴J_{HH} = 2). **MALDI-HRMS:** 1912.8054 [M]⁺ (theor. [C₇₀H₁₀₇N₃₇O₁₇Lu₁]⁺ = 1912.8048).
- 25

Synthesis of TD1010: In a glass vial (4 mL), $[\text{TD897}]^+[\text{TFA}]^- \cdot 1.1\text{TFA} \cdot 2.6\text{H}_2\text{O}$ (10.0 mg; 8.9 μmol ; 1.0



equiv.) and $\text{TD986} \cdot 3.2\text{TFA} \cdot 5.3\text{H}_2\text{O}$ (21 mg; 9.1 μmol ; 1.0 equiv.) were dissolved in DMSO (1.0 mL) followed by addition of solution of **NMM** (400 mM, 230 μL ; 92 μmol ; 10 equiv.) in DMSO and freshly prepared solution of **PyOAP** (110 mM; 140 μL ; 15.4 μmol ; 1.7 equiv.) in DMSO and the resulting yellow solution was stirred 30 min at RT. Mixture was then directly purified by preparative HPLC (C18; H_2O –MeCN gradient with TFA additive). Fractions with Pbf protected product were joined,

- 15 evaporated to dryness and twice co-evaporated with MeOH. Residue was dissolved in TFA (3 mL) and the resulting clear mixture was stirred 16 h at RT. Mixture was evaporated to dryness and the residue was suspended in 20 % MeCN (3 mL). Mixture was filtered through syringe microfilter (RC) and the filtrate was directly purified by preparative HPLC (C18; H_2O –MeCN gradient with TFA additive). Fractions with product
- 20 were joined and directly lyophilized to give product in the form of trifluoroacetate salt as white fluffy solid (the amount of TFA was set arbitrary from analogous **TD1011**). **Yield:** 11.6 mg (~50% assuming $[\text{M}]^+[\text{TFA}]^- \cdot 4.5\text{TFA} \cdot x\text{H}_2\text{O}$; $M_R = 2530$; 2 steps; based on $[\text{TD897}]^+[\text{TFA}]^- \cdot 1.1\text{TFA} \cdot 2.6\text{H}_2\text{O}$). **MALDI-HRMS:** 1906.7973 $[\text{M}]^+$ (theor. $[\text{C}_{70}\text{H}_{107}\text{N}_{37}\text{O}_{17}\text{Tm}_1]^+ = 1906.7982$).

Example 10: Charts

Chart 1. Formation of 1,5-cz-[Ln(Lig)] from Lig and Ln^{III} ions (*metal dependent click-zip*): conversion determined after 1 hour at 80 °C by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at λ ; rounded to integers; aliquots from reaction mixtures were diluted with H₂O, vortexed, centrifuged and then injected to HPLC). Initial component concentrations: 0.5 mM aq. Lig (1.0 equiv.); 1.0 mM aq. Ln^{III} (2.0 equiv.); 50 mM aq. MOPS/NaOH buffer pH 7.0 (100 equiv.). Performed in glass vials with magnetic stirrers. Metal ions are sorted by decreasing ionic radius.

5

Lig	λ , nm	La ^{III}	Ce ^{III}	Pr ^{III}	Nd ^{III}	Sm ^{III}	Eu ^{III}	Gd ^{III}	Tb ^{III}	Dy ^{III}	Ho ^{III}	Er ^{III}	Tm ^{III}	Yb ^{III}	Lu ^{III}
TD425	275	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
TD556	275	1%	1%	1%	2%	3%	4%	5%	11%	16%	24%	37%	52%	64%	71%
TD576	280	?	?	?	?	?	?	?	?	?	?	?	?	?	?
TD582 [↓]	275	0%	0%	5%	6%	12%	14%	15%	18%	18%	16%	15%	14%	12%	5%
TD604	280	0%	0%	0%	0%	0%	0%	0%	1%	1%	1%	1%	2%	2%	1%
TD647	280	1%	1%	1%	1%	3%	4%	5%	9%	13%	21%	32%	48%	61%	64%
TD669	275	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
TD714 [*]	270	0%	1%	2%	3%	5%	7%	9%	17%	23%	26%	34%	40%	40%	37%
TD722	280	0%	0%	0%	0%	1%	1%	2%	3%	5%	9%	17%	27%	38%	49%
TD728 [/]	275	0%	0%	0%	0%	0%	0%	0%	0%	1%	1%	1%	1%	2%	2%
TD742	285	1%	1%	1%	1%	3%	4%	5%	9%	15%	22%	34%	48%	55%	66%
TD778 [‡]	285	24%	20%	13%	9%	4%	4%	4%	9%	13%	17%	25%	31%	30%	26%
TD779 [‡]	280	0%	0%	0%	0%	1%	2%	3%	6%	8%	10%	12%	9%	9%	5%
TD801 [†]	275	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
TD827 [/]	275	1%	2%	2%	3%	7%	9%	11%	21%	30%	43%	56%	68%	75%	77%
TD921 [↓]	275	0%	0%	5%	4%	1%	1%	0%	1%	1%	1%	1%	1%	1%	1%
TD943	280	0%	0%	0%	0%	0%	0%	0%	1%	1%	2%	2%	3%	3%	5%

164

* Pendant arms of *l*,5-cz-[Ln(Lig)] are coordinated in deprotonated iminol form (each bearing -1 charge). As a consequence, these species undergo hydrolysis to acetate arms (i.e. *in situ* yielding *l*,5-cz-[Ln(TD647)]).

⁴⁷ Values in italics: *l*,5-*c*-[Ln(Lig)] with hydrolysed group in the side chain (Me ester in the case of **TD1092**, *i*-Pr ester in the case of **TD1148**, *t*-Bu ester in the case of **TD1160** and Boc in the case of **TD1105**).

— Not performed.

— Not performed.

Chart 2. Formation of *1,5-cz*-[Ln(Lig)] from **Lig** and Ln^{III} ions (*metal dependent click-zip*): conversion determined after 1 week at 80 °C by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at λ ; rounded to integers; aliquots from reaction mixtures were diluted with H₂O, vortexed, centrifuged and then injected to HPLC). Initial component concentrations: 0.5 mM aq. **Lig** (1.0 equiv.); 1.0 mM aq. Ln^{III} (2.0 equiv.); 50 mM aq. MOPS/NaOH buffer pH 7.0 (100 equiv.). Performed in glass vials with magnetic stirrers. Metal ions are sorted by decreasing ionic radius.

[illegible]

TD801 [†]	275	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
TD827 [†]	275	46%	22%	79%	84%	91%	92%	93%	95%	96%	96%	96%	96%	97%	97%	97%	97%	97%
TD921 [†]	275	0%	0%	55%	43%	42%	31%	28%	41%	37%	31%	26%	18%	15%	10%			
TD943	280	5%	4%	10%	16%	29%	36%	40%	56%	65%	77%	88%	94%	97%	97%			
TD944	270	4%	3%	6%	8%	16%	19%	23%	37%	51%	68%	84%	93%	95%	94%			
TD959	275	18%	11%	27%	34%	48%	51%	54%	68%	76%	84%	93%	97%	98%	99%			
TD992	275	22%	20%	33%	40%	55%	59%	61%	73%	81%	89%	95%	98%	99%	99%			
TD1054	280	4%	4%	10%	11%	22%	28%	33%	55%	70%	79%	88%	94%	97%	98%			
TD1063	278	5%	1%	35%	52%	57%	73%	74%	83%	86%	90%	93%	93%	95%	95%			
TD1092 [†]	280	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%			
TD1105 [†]	275	0%	0%	0%	0%	1%	1%	2%	1%	0%	1%	1%	1%	1%	1%			
TD1127	278	18%	7%	15%	25%	46%	53%	56%	72%	80%	86%	91%	93%	95%	97%			
TD1148 [†]	280	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%			
TD1160 [†]	280	–	–	–	–	0%	–	–	0%	–	–	–	–	–	0%			
TD1176	285	73%	60%	82%	85%	92%	93%	91%	94%	96%	96%	96%	96%	97%	96%			
TD1204	278	0%	0%	0%	1%	4%	5%	5%	10%	17%	23%	39%	62%	76%	83%			

Legend: [†] Obtained data might not accurately reflect abundance of 1,5-cz-[Ln(Lig)] in the whole mixture as non-negligible variation of overall signal intensity was observed throughout the series.

[†] Propionate pendants arms detach during course of the reaction, yielding a mixture of 1,4-cz-TD801 and 1,5-TD801 and their corresponding derivatives with either one or both propionate arms removed.

n. d. Could not be precisely determined due to signal overlapping and/or decomplexation during HPLC analysis.

- *

Sum of two diastereoisomers.
- /

Performed in a mixture of 50% *i*-PrOH in aq. MOPS/NaOH Buffer pH 7.0.
- ‡

Pendant arms of *l*,5-*cz*-[Ln(**Lig**)] are coordinated in deprotonated iminol form (each bearing –1 charge). As a consequence, these species undergo hydrolysis to acetate arms (i.e. *in situ* yielding *l*,5-*cz*-[Ln(**TD647**)]).
- err.

Accidental evaporation of the whole reaction solvent due to untighten vial cap.
- ‡

Values in italics: *l*,5-*cz*-[Ln(**Lig**)] with hydrolysed group in the side chain (Me ester in the case of **TD1092**, *i*-Pr ester in the case of **TD1148**, *t*-Bu ester in the case of **TD1160** and Boc in the case of **TD1105**).
- Not performed.

Chart 3. Formation of *l*,5-*cz*-[Ln(**Lig**)] from **Lig** and Ln^{III} ions (*metal dependent click-zip*): conversion determined after 1 hour at 80 °C (**A**) and 18 hours at 80 °C (**B**) by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at λ; rounded to integers; aliquots from reaction mixtures were diluted with H₂O, vortexed, centrifuged and then injected to HPLC). Initial component concentrations: 0.5 mM aq. **Lig** (1.0 equiv.); 1.0 mM aq. Ln^{III} (2.0 equiv.); 50 mM aq. MES/NaOH buffer pH 5.2 (100 equiv.). Performed in glass vials with magnetic stirrers. Metal ions are sorted by decreasing ionic radius.

A	λ, nm	La ^{III}	Ce ^{III}	Pr ^{III}	Nd ^{III}	Sm ^{III}	Eu ^{III}	Gd ^{III}	Tb ^{III}	Dy ^{III}	Ho ^{III}	Er ^{III}	Tm ^{III}	Yb ^{III}	Lu ^{III}
TD750	270	–	–	–	–	–	–	3%	–	–	–	–	–	–	19%
B	λ, nm	La ^{III}	Ce ^{III}	Pr ^{III}	Nd ^{III}	Sm ^{III}	Eu ^{III}	Gd ^{III}	Tb ^{III}	Dy ^{III}	Ho ^{III}	Er ^{III}	Tm ^{III}	Yb ^{III}	Lu ^{III}
TD750	270	–	–	–	–	–	–	9%	–	–	–	–	–	–	71%

Legend: – Not performed.

Chart 4. Formation of *l*,5-*cz*-[M(**Lig**)] from **Lig** and M^{III} ions (*metal dependent click-zip*): conversion determined after 1 hour at 80 °C by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at λ; rounded to integers; aliquots from reaction mixtures were diluted with H₂O, vortexed, centrifuged and then injected to HPLC). Initial component concentrations: 0.5 mM aq. **Lig** (1.0 equiv.); 1.0 mM aq. M^{III} (2.0 equiv.); 50 mM aq. MOPS/NaOH buffer pH 7.0 (100 equiv.). Performed in glass vials with magnetic stirrers. Metal ions are sorted by decreasing ionic radius.

Lig	λ, nm	[†] Bi ^{III}	Y ^{III}	In ^{III}	Sc ^{III}	Ru ^{III}	Ir ^{III}	Ga ^{III}
TD425	275	–	0%	–	0%	–	–	–

TD556	275	-	22%	-	2%	-	-	-	-
TD582	275	-	11%	-	0%	-	-	-	-
TD604	280	-	1%	-	1%	-	-	-	-
TD639	275	-	0%	-	0%	-	-	-	-
TD647	280	29%	20%	0%	7%	-	-	0%	0%
TD669	275	-	0%	-	0%	-	-	-	-
TD714 *	270	-	22%	-	prec.	-	-	-	-
TD722	280	-	9%	-	3%	-	-	-	-
TD728 /	275	5%	1%	-	0%	0%	0%	-	-
TD742	285	-	22%	-	7%	-	-	-	-
TD764	278	31%	-	-	-	-	-	-	-
TD778 ‡	285	-	17%	-	0%	-	-	-	-
TD779 ‡	280	-	4%	-	0%	-	-	-	-
TD801	275	-	0%	-	0%	-	-	-	-
TD827 /	275	-	42%	-	2%	-	-	-	-
TD921	275	-	0%	-	0%	-	-	-	-
TD943	280	-	1%	-	0%	-	-	-	-
TD944	270	-	1%	-	0%	-	-	-	-
TD959	275	-	1%	-	0%	-	-	-	-
TD992	275	-	2%	-	0%	-	-	-	-
TD1054	280	-	16%	-	4%	-	-	-	-
TD1063	278	-	20%	-	1%	-	-	-	-
TD1063	278	-	20%	-	1%	-	-	-	-
TD1092 †	280	-	6%	-	1%	-	-	-	-

				12%		0%			
TD1105 [‡]	275	–		2%	–	0%	–		–
				1%		0%			
TD1127	278	–		2%	–	0%	–		–
TD1148 [‡]	280	–		18%	–	0%	–		–
				3%		0%			
TD1160 [‡]	280	–		1%	–	–	–		–
				17%					
TD1176	285	–		30%	–	6%	–		–
TD1204	278	–		3%	–	0%	–		–

Legend: prec. Could not be reasonably determined due to formation of precipitate.

- *

Sum of two diastereoisomers.
- /

Performed in a mixture of 50% *i*-PrOH in aq. MOPS/NaOH Buffer pH 7.0.
- †

Performed in a mixture of 4% DMSO in aq. MOPS/NaOH Buffer pH 7.0.
- ‡

Pendant arms of 1,5-cz-[Ln(Lig)] are coordinated in deprotonated iminol form (each bearing –1 charge). As a consequence, these species undergo hydrolysis to acetate arms (i.e. *in situ* yielding 1,5-cz-[Ln(TD647)]).
- ‡

Values in italics: 1,5-cz-[Ln(Lig)] with hydrolysed group in the side chain (Me ester in the case of TD1092, *i*-Pr ester in the case of TD1148, *t*-Bu ester in the case of TD1160 and Boc in the case of TD1105).
- Not performed.

Chart 5. Formation of 1,5-cz-[M(Lig)] from Lig and M^{III} (*metal dependent click-zip*): conversion determined after 1 week at 80 °C by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at λ; rounded to integers; aliquots from reaction mixtures were diluted with H₂O, vortexed, centrifuged and then injected to HPLC). Initial component concentrations: 0.5 mM aq. Lig (1.0 equiv.); 1.0 mM aq. M^{III} (2.0 equiv.); 50 mM aq. MOPS/NaOH buffer pH 7.0 (100 equiv.). Performed in glass vials with magnetic stirrers. Metal ions are sorted by decreasing ionic radius.

Lig	λ, nm	[†] B ^{III}	Y ^{III}	In ^{III}	Sc ^{III}	Ru ^{III}	Ir ^{III}	Ga ^{III}
-----	-------	-------------------------------	------------------	-------------------	-------------------	-------------------	-------------------	-------------------

TD425	275	—	0%	—	0%	—	—	—	—	—
TD556	275	—	95%	—	23%	—	—	—	—	—
TD582	275	—	68%	—	0%	—	—	—	—	—
TD604	280	—	58%	—	10%	—	—	—	—	—
TD639	275	—	0%	—	0%	—	—	—	—	—
TD647	280	73%	93%	8%	35%	—	—	—	0%	—
TD669	275	—	12%	—	0%	—	—	—	—	—
TD714 *	270	—	89%	—	prec.	—	—	—	—	—
TD722	280	—	71%	—	14%	—	—	—	—	—
TD728 ¹	275	29%	50%	—	2%	0%	0%	—	—	—
TD742	285	—	94%	—	52%	—	—	—	—	—
TD764	278	85%	95%	—	5%	—	—	—	—	—
TD778 [‡]	285	—	27%	—	0%	—	—	—	—	—
TD779 [‡]	280	—	0%	—	0%	—	—	—	—	—
TD801	275	—	0%	—	0%	—	—	—	—	—
TD827 ¹	275	—	96%	—	55%	—	—	—	—	—
TD921	275	—	14%	—	0%	—	—	—	—	—
TD943	280	—	74%	—	5%	—	—	—	—	—
TD944	270	—	63%	—	4%	—	—	—	—	—
TD959	275	—	82%	—	1%	—	—	—	—	—
TD992	275	—	87%	—	2%	—	—	—	—	—
TD1054	280	—	80%	—	61%	—	—	—	—	—
TD1063	278	—	91%	—	8%	—	—	—	—	—
TD1092 [‡]	280	—	0%	—	0%	—	—	—	—	—

				95%		17%			
TD1105 [‡]	275	–	0% 87%	–	0% 60	–	–	–	–
TD1127	278	–	83%	–	29%	–	–	–	–
TD1148 [‡]	280	–	0% 94%	–	0% 11%	–	–	–	–
TD1160 [‡]	280	–	0% 90%	–	–	–	–	–	–
TD1176	285	–	95%	–	31%	–	–	–	–
TD1204	278	–	22%	–	16%	–	–	–	–

Legend: prec. Could not be reasonably determined due to formation of precipitate.

- *

Sum of two diastereoisomers.
- /

Performed in a mixture of 50% *i*-PrOH in aq. MOPS/NaOH Buffer pH 7.0.
- †

Performed in a mixture of 4% DMSO in aq. MOPS/NaOH Buffer pH 7.0.
- ‡

Pendant arms of *l*,5-*cz*-[Ln(**Lig**)] are coordinated in deprotonated iminol form (each bearing –1 charge). As a consequence, these species undergo hydrolysis to acetate arms (i.e. *in situ* yielding *l*,5-*cz*-[Ln(**TD647**)]).
- ‡

Values in italics: *l*,5-*cz*-[Ln(**Lig**)] with hydrolysed group in the side chain (Me ester in the case of **TD1092**, *i*-Pr ester in the case of **TD1148**, *t*-Bu ester in the case of **TD1160** and Boc in the case of **TD1105**).
- Not performed.

Chart 6. Formation of *l*,5-*cz*-[M(**Lig**)] from **Lig** and M^{II} ions (*metal dependent click-zip*): conversion determined after 1 hour at 80 °C by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at λ; rounded to integers; aliquots from reaction mixtures were diluted with H₂O, vortexed, centrifuged and then injected to HPLC). Initial component concentrations: 0.5 mM aq. **Lig** (1.0 equiv.); 1.0 mM aq. M^{II} (2.0 equiv.); 50 mM aq. MOPS/NaOH buffer pH 7.0 (100 equiv.). Performed in glass vials with magnetic stirrers. Metal ions are sorted by decreasing ionic radius.

Lig	λ, nm	Ba ^{II}	Pb ^{II}	Sr ^{II}	Ca ^{II}	Cd ^{II}	Pd ^{II}	Mn ^{II}	Pt ^{II}	Zn ^{II}	Cu ^{II}	Ni ^{II}
------------	-------	------------------	------------------	------------------	------------------	------------------	------------------	------------------	------------------	------------------	------------------	------------------

171

TD639	275	0%	–	0%	0%	–	–	–	–	–	–	–	–
TD647	280	0%	2%	0%	16%	64%	–	–	–	0%	prec.	0%	–
TD714*	270	0%	–	0%	0%	89%	–	–	–	–	–	–	–
TD728/	275	–	0%	–	0%	1%	1%	0%	0%	–	–	–	–
TD764	278	–	1%	–	16%	53%	10%	1%	–	–	–	–	–
TD921	275	0%	–	0%	0%	92%	–	–	–	–	–	–	–
TD959	275	–	–	–	1%	2%	–	–	–	–	–	–	–

Legend: prec. Could not be reasonably determined due to formation of precipitate.

- * Sum of two diastereoisomers.
- / Performed in a mixture of 50% *i*-PrOH in aq. MOPS/NaOH Buffer pH 7.0.
- Not performed.

5

Chart 7. Formation of *1,5-cz*-[M(Lig)] from Lig and M^{II} ions (*metal dependent click-zip*): conversion determined after 1 week at 80 °C by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at λ ; rounded to integers; aliquots from reaction mixtures were diluted with H₂O, vortexed, centrifuged and then injected to HPLC). Initial component concentrations: 0.5 mM aq. Lig (1.0 equiv.); 1.0 mM aq. M^{II} (2.0 equiv.); 50 mM aq. MOPS/NaOH buffer pH 7.0 (100 equiv.). Performed in glass vials with magnetic stirrers. Metal ions are sorted by decreasing ionic radius.

Lig	λ , nm	Ba ^{II}	Pb ^{II}	Sr ^{II}	Ca ^{II}	Cd ^{II}	Pd ^{II}	Mn ^{II}	Pt ^{II}	Zn ^{II}	Cu ^{II}	Ni ^{II}
TD639	275	0%	–	0%	0%	–	–	–	–	–	–	–
TD647	280	0%	54%	0%	87%	86%	–	–	–	0%	prec.	0%
TD714*	270	0%	–	0%	6%	92%	–	–	–	–	–	–
TD728/	275	–	14%	–	37%	36%	1%	1%	0%	–	–	–
TD764	278	–	37%	–	95%	96%	9%	35%	–	–	–	–
TD921	275	0%	–	0%	0%	96%	–	–	–	–	–	–
TD959	275	–	–	–	83%	96%	–	–	–	–	–	–

Legend: prec. Could not be reasonably determined due to formation of precipitate.

10

- * Sum of two diastereoisomers.
- / Performed in a mixture of 50% *t*-PrOH in aq. MOPS/NaOH Buffer pH 7.0.
- Not performed.

5 **Chart 8.** Formation of *1,5-cz-[Ln(Lig)]* from *1,5-cz-Lig* (metal dependent direct complexation): conversion determined after 1 hour at 80 °C (A), 1 week at 80 °C (B) and ~ 6 months at 80 °C (C) by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at λ; rounded to integers; aliquots from reaction mixtures were diluted with H₂O, vortexed, centrifuged and then injected to HPLC). Initial component concentrations: 0.5 mM aq. *1,5-cz-Lig* (1.0 equiv.); 1.0 mM aq. Ln^{III} (2.0 equiv.); 50 mM aq. MOPS/NaOH buffer pH 7.0 (100 equiv.). Performed in glass vials with magnetic stirrers. Metal ions are sorted by decreasing ionic radius.

A	λ, nm	La ^{III}	Ce ^{III}	Pr ^{III}	Nd ^{III}	Sm ^{III}	Eu ^{III}	Gd ^{III}	Tb ^{III}	Dy ^{III}	Ho ^{III}	Er ^{III}	Tm ^{III}	Yb ^{III}	Lu ^{III}
TD650	280	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
B	λ, nm	La ^{III}	Ce ^{III}	Pr ^{III}	Nd ^{III}	Sm ^{III}	Eu ^{III}	Gd ^{III}	Tb ^{III}	Dy ^{III}	Ho ^{III}	Er ^{III}	Tm ^{III}	Yb ^{III}	Lu ^{III}
TD650	280	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
C	λ, nm	La ^{III}	Ce ^{III}	Pr ^{III}	Nd ^{III}	Sm ^{III}	Eu ^{III}	Gd ^{III}	Tb ^{III}	Dy ^{III}	Ho ^{III}	Er ^{III}	Tm ^{III}	Yb ^{III}	Lu ^{III}
TD650	280	–	–	–	–	–	–	0%	–	–	–	–	–	–	0%

10 Legend: – Not performed.

Chart 9. Formation of *1,5-cz-[Ln(Lig)]* from *1,5-cz-Lig* (metal dependent direct complexation): conversion determined after 1 min at RT (A), 1 hour at RT (B), 1 hour at 80 °C (C) and 1 week at 80 °C (D) by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at λ; rounded to integers; aliquots from reaction mixtures were diluted with H₂O, vortexed, centrifuged and then injected to HPLC). Initial component concentrations: 0.5 mM aq. *1,5-cz-Lig* (1.0 equiv.); 1.0 mM aq. M^{II} or M^{III} (2.0 equiv.); 50 mM aq. MOPS/NaOH buffer pH 7.0 (100 equiv.). Performed in glass vials with magnetic stirrers. Metal ions are sorted by decreasing ionic radius.

A	λ, nm	Ba ^{II}	Pb ^{II}	Sr ^{II}	[†] Bi ^{III}	Ca ^{II}	Cd ^{II}	Y ^{III}	In ^{III}	Sc ^{III}	Zn ^{II}	Cu ^{II}	Ni ^{II}	Ga ^{II}
TD650	280	–	98%	–	–	–	–	–	–	–	–	43%	–	–

15

B	λ , nm	Ba ^{II}	Pb ^{II}	Sr ^{II}	[†] Bi ^{III}	Ca ^{II}	Cd ^{II}	Y ^{III}	In ^{III}	Sc ^{III}	Zn ^{II}	Cu ^{II}	Ni ^{II}	Ga ^{II}
TD650	280	–	99%	–	2%	0%	58%	–	–	–	59%	47%	0%	–
C	λ , nm	Ba ^{II}	Pb ^{II}	Sr ^{II}	[†] Bi ^{III}	Ca ^{II}	Cd ^{II}	Y ^{III}	In ^{III}	Sc ^{III}	Zn ^{II}	Cu ^{II}	Ni ^{II}	Ga ^{II}
TD650	280	–	98%	–	96%	23%	98%	–	–	–	91%	49%	9%	–
D	λ , nm	Ba ^{II}	Pb ^{II}	Sr ^{II}	[†] Bi ^{III}	Ca ^{II}	Cd ^{II}	Y ^{III}	In ^{III}	Sc ^{III}	Zn ^{II}	Cu ^{II}	Ni ^{II}	Ga ^{II}
TD650	280	0%	98%	0%	90%	96%	99%	0%	0%	0%	99%	78%	11%	0%

Legend: [†] Performed in a mixture of ~4% DMSO in aq. MOPS/NaOH Buffer pH 7.0.

– Not performed.

Chart 10. Formation of *1,4-cz-[Ln(Lig)]* from *1,4-cz-Lig* (metal dependent direct complexation): conversion determined after 1 week at 80 °C (A) by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at λ ; rounded to integers; aliquots from reaction mixtures were diluted with H₂O, vortexed, centrifuged and then injected to HPLC). Initial component concentrations: 0.5 mM aq. *1,4-cz-Lig* (1.0 equiv.); 1.0 mM aq. Ln^{III} (2.0 equiv.); 50 mM aq. MOPS/NaOH buffer pH 7.0 (100 equiv.). Performed in glass vials with magnetic stirrers. Metal ions are sorted by decreasing ionic radius.

A	λ , nm	La ^{III}	Ce ^{III}	Pr ^{III}	Nd ^{III}	Sm ^{III}	Eu ^{III}	Gd ^{III}	Tb ^{III}	Dy ^{III}	Ho ^{III}	Er ^{III}	Tm ^{III}	Yb ^{III}	Lu ^{III}
TD622	275	0%	0%	3%	6%	34%	45%	46%	61%	66%	68%	70%	71%	51%	53%

Chart 11. Formation of *1,4-cz-[Ln(Lig)]* from *1,4-cz-Lig* (metal dependent direct complexation): conversion determined after 1 hour at 80 °C (A) and 1 week at 80 °C (B) by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at λ ; rounded to integers; aliquots from reaction mixtures were diluted with H₂O, vortexed, centrifuged and then injected to HPLC). Initial component concentrations: 0.5 mM aq. *1,4-cz-Lig* (1.0 equiv.); 1.0 mM aq. M^{II} or M^{III} (2.0 equiv.); 50 mM aq. MOPS/NaOH buffer pH 7.0 (100 equiv.). Performed in glass vials with magnetic stirrers. Metal ions are sorted by decreasing ionic radius.

A	λ , nm	Ba ^{II}	Pb ^{II}	Sr ^{II}	[†] Bi ^{III}	Ca ^{II}	Cd ^{II}	Y ^{III}	In ^{III}	Sc ^{III}	Zn ^{II}	Cu ^{II}	Ni ^{II}	Ga ^{II}
TD622	275	–	98%	–	98%	20%	98%	–	–	–	88%	42%	41%	–
B	λ , nm	Ba ^{II}	Pb ^{II}	Sr ^{II}	[†] Bi ^{III}	Ca ^{II}	Cd ^{II}	Y ^{III}	In ^{III}	Sc ^{III}	Zn ^{II}	Cu ^{II}	Ni ^{II}	Ga ^{II}

TD622	275	0%	99%	0%	91%	21%	99%	51%	13%	0%	99%	48%	44%	8%
--------------	-----	----	-----	----	-----	-----	-----	-----	-----	----	-----	-----	-----	----

Legend: † Performed in a mixture of ~4% DMSO in aq. MOPS/NaOH Buffer pH 7.0.

– Not performed.

Chart 12. Formation of *1,5-cz*-[Lu(**Lig**)] from **Lig** (*metal dependent click-zip*) with various absolute concentrations: conversion determined after 1 hour at 80 °C (A), 1 day at 80 °C (B) and 1 week at 80 °C (C) by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at 280 nm; rounded to integers; aliquots from reaction mixtures were diluted with H₂O, vortexed, centrifuged and then injected to HPLC). Performed with constant ratio of reagents (**Lig** : Lu^{III} : aq. MOPS/NaOH buffer pH 7.0 = 1.0 : 1.5 : 50). Performed in glass vials with magnetic stirrers.

Lig , mM	5.0 × 10 ¹	5.0 × 10 ⁰	5.0 × 10 ⁻¹	5.0 × 10 ⁻²	5.0 × 10 ⁻³
Lu ^{III} , mM	7.5 × 10 ¹	7.5 × 10 ⁰	7.5 × 10 ⁻¹	7.5 × 10 ⁻²	7.5 × 10 ⁻³
aq. MOPS/NaOH buffer pH 7.0, mM	2.5 × 10 ³	2.5 × 10 ²	2.5 × 10 ¹	2.5 × 10 ⁰	2.5 × 10 ⁻¹
TD647 (A)	71%	74%	70%	60%	15%
TD647 (B)	84%	96%	97%	96%	59%
TD647 (C)	86%	96%	97%	96%	57%

Chart 13. pH dependent formation of *1,5*- and *1,4-cz*-**Lig** isomers from **Lig** (*pH dependent click-zip*): conversion determined after 1 hour at 80 °C (A) and 1 day at 80 °C (B) by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at λ ; rounded to integers; aliquots from reaction mixtures were diluted with H₂O, vortexed and then injected to HPLC). Initial component concentrations: 0.5 mM aq. **Lig** (1.0 equiv.); 1.0 M HClO₄ (pH ~0)/0.1 M HClO₄ (pH ~1)/50 mM aq. buffer (100 equiv.). Performed in glass vials with magnetic stirrers.

A	λ , nm	Syste	aq. HClO ₄		aq. Citrate/NaOH buffer						aq. Borate/NaOH			
		m									buffer			
		pH	~0	~1	2.2	3.0	3.8	4.6	5.4	6.2	7.0	8.6	9.4	10.2
	275	1,5	13%	13%	13%	10%	3%	1%	1%	0%	0%	0%	1%	1%

TD42 5		<i>1,4</i>	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	280	<i>1,5</i>	9%	44%	17%	6%	1%	1%	1%	1%	1%	2%	8%	17%
		<i>1,4</i>	2%	3%	2%	1%	1%	1%	1%	1%	2%	3%	3%	2%
B	λ , nm	Syste m	aq. HClO ₄			aq. Citrate/NaOH buffer						aq. Borate/NaOH buffer		
		pH	~0	~1	2.2	3.0	3.8	4.6	5.4	6.2	7.0	8.6	9.4	10.2
		<i>1,5</i>	88%	90%	93%	89%	57%	15%	6%	6%	8%	10%	11%	12%
TD42 5	275	<i>1,4</i>	0%	0%	0%	0%	0%	0%	0%	1%	4%	5%	4%	2%
		<i>1,5</i>	72%	91%	88%	69%	22%	13%	12%	11%	19%	26%	60%	80%
TD64 7	280	<i>1,4</i>	8%	5%	6%	6%	6%	5%	6%	12%	23%	32%	17%	5%

Chart 14. Alkali metal dependent formation of *1,5-ez-Lig* and *1,4-ez-Lig* isomers from **Lig** (*alkali metal dependent click-zip*) at pH 10.2: conversion determined after 1 hour at 80 °C (A) and 1 day at 80 °C (B) by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at λ ; rounded to integers; aliquots from reaction mixtures were diluted with H₂O, vortexed and then injected to HPLC). Initial component concentrations: 0.5 mM aq. **Lig** (1.0 equiv.); 50 mM aq. Borate/(M^I)₂CO₃ buffer (M^I = Li^I – Cs^I, 100 equiv.). Performed in plastic Eppendorf test tubes with magnetic stirrers.

A	λ , nm	aq. Borate/(M ^I) ₂ CO ₃ buffer (pH 10.2)											
		System	M ^I	Li ^I	Na ^I	K ^I	Rb ^I	Cs ^I					
TD425	275	<i>1,5</i>		0%	1%	0%	1%	1%					
		<i>1,4</i>		0%	0%	1%	1%	1%					
TD647	280	<i>1,5</i>		0%	15%	1%	0%	0%					
		<i>1,4</i>		0%	1%	2%	2%	2%					
TD921	275	<i>1,5</i>		12%	30%	2%	2%	2%					

		<i>1,4</i>	1%	2%	2%	2%	2%
B	λ , nm	System	aq. Borate/(M ¹) ₂ CO ₃ buffer (pH 10.2)				
		M ¹	^Ψ Li ¹	Na ¹	K ¹	Rb ¹	Cs ¹
TD425	275	<i>1,5</i>	2%	9%	10%	10%	11%
		<i>1,4</i>	0%	1%	10%	10%	10%
TD647	280	<i>1,5</i>	9%	85%	10%	6%	7%
		<i>1,4</i>	2%	1%	40%	41%	39%
TD582	275	<i>1,5</i>	53%	69%	11%	10%	10%
		<i>1,4</i>	3%	2%	25%	25%	26%
TD581	280	<i>1,5</i>	10%	25%	7%	7%	6%
		<i>1,4</i>	19%	26%	32%	32%	31%
TD703	270	<i>1,5</i>	24%	75%	14%	14%	13%
		<i>1,4</i>	14%	10%	20%	20%	20%
TD714	270	<i>1,5</i>	15%	81%	10%	9%	9%
		<i>1,4</i>	12%	7%	46%	45%	44%
TD921	275	<i>1,5</i>	86%	89%	23%	21%	21%
		<i>1,4</i>	6%	4%	31%	31%	31%

Legend: ^Ψ Reaction mixture contained a visible precipitate, which was shown to be analyte free (all forms).

Chart 15. Relaxivities of Gd^{III} chelates **TD451** and **TD527** determined by standard inversion recovery pulse sequence on relaxometer (the minispec mq20 NMR analyzer; 0.5T; Bruker) at 40.0 °C (concentration of chelates: 1.0 mM in aq. MOPS/NaOH buffer, pH 7.0).

species	formally	relaxivity, mM ⁻¹ s ⁻¹
TD451	[Gd(TD647)]	2.39

TD527	<i>l</i> ,5- <i>cz</i> - [Gd(TD647)]	1.48
-------	---	------

Chart 17. Dissociation of chelates in aq. 6.0 M HCl at 80 °C (*acid decomplexation kinetic inertness*): fraction of intact chelate determined after 1 hour, 1 day, 1 week and 1 month by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at 280 nm; rounded to integers; aliquots from reaction mixtures were appropriately diluted to prevent re-complexation, vortexed, centrifuged and then injected to HPLC). Performed in glass vials with magnetic stirrers.

5

chelate	formally	λ , nm	1 hour	1 day	1 week	1 month	$t_{1/2}$, hours [/]
TD875 ^A	<i>l</i> ,4- <i>cz</i> -[Gd ^{III} (TD647)]	278	0%	0%	0%	0%	too unstable
TD874 ^A	<i>l</i> ,4- <i>cz</i> -[Lu ^{III} (TD647)]	278	0%	0%	0%	0%	too unstable
TD900 ^A	<i>l</i> ,4- <i>cz</i> -[Cd ^{II} (TD647)]	278	0%	0%	0%	0%	too unstable
TD651 ^B	<i>l</i> ,5- <i>cz</i> -[La ^{III} (TD647)]	278	0%	0%	0%	0%	too unstable
TD870 ^B	<i>l</i> ,5- <i>cz</i> -[Ce ^{III} (TD647)]	278	0%	0%	0%	0%	too unstable
TD869 ^B	<i>l</i> ,5- <i>cz</i> -[Pr ^{III} (TD647)]	278	0%	0%	0%	0%	too unstable
TD868 ^B	<i>l</i> ,5- <i>cz</i> -[Nd ^{III} (TD647)]	278	0%	0%	0%	0%	too unstable
TD867 ^B	<i>l</i> ,5- <i>cz</i> -[Sm ^{III} (TD647)]	278	0%	0%	0%	0%	too unstable
TD599 ^B	<i>l</i> ,5- <i>cz</i> -[Eu ^{III} (TD647)]	278	0%	0%	0%	0%	too unstable
TD527 ^B	<i>l</i> ,5- <i>cz</i> -[Gd ^{III} (TD647)]	278	2%	0%	0%	0%	1.7E ⁻¹
TD600 ^B	<i>l</i> ,5- <i>cz</i> -[Tb ^{III} (TD647)]	278	72%	0%	0%	0%	2.7E ⁰
TD866 ^B	<i>l</i> ,5- <i>cz</i> -[Dy ^{III} (TD647)]	278	95%	25%	0%	0%	1.2E ¹ ± 1.3E ⁻¹
TD648 ^B	<i>l</i> ,5- <i>cz</i> -[Ho ^{III} (TD647)]	278	99%	81%	17%	0%	6.8E ¹ ± 2.7E ⁰
TD865 ^B	<i>l</i> ,5- <i>cz</i> -[Er ^{III} (TD647)]	278	99%	95%	71%	24%	3.4E ² ± 2.5E ⁰
TD601 ^B	<i>l</i> ,5- <i>cz</i> -[Tm ^{III} (TD647)]	278	100%	99%	92%	69%	1.4E ³ ± 1.2E ¹
TD602 ^B	<i>l</i> ,5- <i>cz</i> -[Yb ^{III} (TD647)]	278	100%	100%	98%	90%	4.7E ³ ± 2.1E ¹

TD522^B	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (TD647)] [∞]	278	100%	100%	99%	98%	3.3E⁴ ± 1.4E³
TD649^B	<i>l</i> ,5- <i>cz</i> -[Y ^{III} (TD647)]	278	98%	69%	7%	0%	4.5E¹ ± 3.1E⁻¹
TD876^A	<i>l</i> ,5- <i>cz</i> -[Pb ^{II} (TD647)]	278	0%	0%	0%	0%	too unstable
TD863^A	<i>l</i> ,5- <i>cz</i> -[Ca ^{II} (TD647)]	278	0%	0%	0%	0%	too unstable
TD880^A	<i>l</i> ,5- <i>cz</i> -[Cd ^{II} (TD647)]	278	43%	0%	0%	0%	1.2E⁰
TD890^A	<i>l</i> ,5- <i>cz</i> -[Zn ^{II} (TD647)]	278	0%	0%	0%	0%	too unstable
TD624-La^{A,*}	↓ [La ^{III} (<i>p</i> -NO ₂ BnDOTA)]	285	0%	0%	0%	0%	too unstable
TD624-Gd^{A,*}	↓ [Gd ^{III} (<i>p</i> -NO ₂ BnDOTA)]	285	0%	0%	0%	0%	too unstable
TD624-Lu^{A,*}	↓ [Lu ^{III} (<i>p</i> -NO ₂ BnDOTA)]	285	0%	0%	0%	0%	too unstable
TD925^B	<i>l</i> ,5- <i>cz</i> -[Gd ^{III} (TD921)]	275	77%	0%	0%	0%	2.6E⁰
TD748^B	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (TD742)] [∞]	285	100%	100%	100%	97%	1.5E⁴ ± 2.9E²
TD735^B	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (TD722)] [∞]	280	100%	100%	99%	98%	2.3E⁴ ± 8.3E²
TD739^{B,*}	--	275	100%	90%	43%	15%	1.5E² ± 2.6E¹
TD734^{B,*}	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (TD714)] [∞]	280	100%	100%	98%	95%	1.6E⁴ ± 1.5E³
TD737^B	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (TD728)]	280	100%	100%	99%	98%	—

Legend: ^A Aliquots (2 μL) were neutralized with 500 mM FA/NaOH buffer (pH 3.6; 200 μL) prior measurement.

^B Aliquots (2 μL) were neutralized with 500 mM MOPS/NaOH buffer (pH 7.0; 200 μL) prior measurement.

^f Dissociation data were fitted by exponential decay (equation $y = \exp(-x/t)$; y = fraction of intact chelate; x = time;

t = decay constant). Chelate stability is expressed as half-life $t_{1/2}$ (in hours; highlighted bold), derived from decay

constant ($t_{1/2} = \ln(2) \times t$). The error of the fitting is not displayed for single data point fitting.

^{*} Sum of two diastereoisomers.

↓ Stock solutions preparation: In Eppendorf tube (0.5 mL), **2,2',2'',2'''-(2-(4-nitrobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetraacetic acid tetrahydrochloride** (10.0 mM in H₂O; 100 μL; 1 μmol; 1.0 equiv.) was mixed with corresponding aq. **LnCl₃** (100

mm; 23.5 μ L; 1.1 μ mol; 1.1 equiv.), H₂O (59 μ L) and aq. **NaOH** (400 mM; 17.5 μ L; 7.0 μ mol; 7.0 equiv.). The resulting mixtures were then stirred 16 h at RT (full conversion was confirmed by LC-MS) followed by centrifugation. Supernatants were then directly used as stock solutions for the dissociation assay.

- Isolated only as a click-zipped complex (no parent ligand exists).
- [∞] Additional data points were collected over the period of 6 months (included in the fitting).
- [‡] Values in brackets: chelate with intact TIPS group/chelate with hydrolysed TIPS group.
- The half-life cannot be rigorously determined due to a parallel hydrolysis of TIPS group.

Chart 18. Dissociation of chelates in aq. 1.0 M HCl at 80 °C (*acid decomplexation kinetic inertness*): fraction of intact chelate determined after 1 hour, 1 day, 1 week and 1 month by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at 280 nm; rounded to integers; aliquots from reaction mixtures were neutralized with aq. MOPS/NaOH Buffer pH 7.0, vortexed, centrifuged and then injected to HPLC). Performed in glass vials with magnetic stirrers.

chelate	formally	λ , nm	1 hour	1 day	1 week	1 month	$t_{1/2}$, hours /
TD875 ^A	<i>1,4-cz</i> -[Gd ^{III} (TD647)]	278	0%	0%	0%	0%	too unstable
TD874 ^A	<i>1,4-cz</i> -[Lu ^{III} (TD647)]	278	0%	0%	0%	0%	too unstable
TD900 ^A	<i>1,4-cz</i> -[Cd ^{II} (TD647)]	278	0%	0%	0%	0%	too unstable
TD651 ^B	<i>1,5-cz</i> -[La ^{III} (TD647)]	278	0%	0%	0%	0%	too unstable
TD870 ^B	<i>1,5-cz</i> -[Ce ^{III} (TD647)]	278	0%	0%	0%	0%	too unstable
TD869 ^B	<i>1,5-cz</i> -[Pr ^{III} (TD647)]	278	0%	0%	0%	0%	too unstable
TD868 ^B	<i>1,5-cz</i> -[Nd ^{III} (TD647)]	278	0%	0%	0%	0%	too unstable
TD867 ^B	<i>1,5-cz</i> -[Sm ^{III} (TD647)]	278	62%	0%	0%	0%	1.5E⁰
TD599 ^B	<i>1,5-cz</i> -[Eu ^{III} (TD647)]	278	92%	10%	0%	0%	7.3E⁰ ± 2.6E⁻¹
TD527 ^B	<i>1,5-cz</i> -[Gd ^{III} (TD647)]	278	98%	58%	1%	0%	3.0E¹ ± 4.9E⁻¹
TD600 ^B	<i>1,5-cz</i> -[Tb ^{III} (TD647)]	278	99%	96%	78%	23%	3.7E² ± 3.2E¹

TD866 ^B	<i>l</i> ,5- <i>cz</i> -[Dy ^{III} (TD647)]	278	100%	99%	96%	87%	3.6E ³ ± 5.0E ¹
TD648 ^B	<i>l</i> ,5- <i>cz</i> -[Ho ^{III} (TD647)]	278	100%	100%	99%	96%	1.3E ⁴ ± 2.6E ²
TD865 ^B	<i>l</i> ,5- <i>cz</i> -[Er ^{III} (TD647)]	278	100%	100%	100%	99%	5.9E ⁴ ± 1.1E ³
TD601 ^B	<i>l</i> ,5- <i>cz</i> -[Tm ^{III} (TD647)]	278	100%	100%	100%	100%	too stable
TD602 ^B	<i>l</i> ,5- <i>cz</i> -[Yb ^{III} (TD647)]	278	100%	100%	100%	100%	too stable
TD522 ^B	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (TD647)]	278	100%	100%	100%	100%	too stable
TD649 ^B	<i>l</i> ,5- <i>cz</i> -[Y ^{III} (TD647)]	278	100%	100%	98%	92%	5.9E ³ ± 1.4E ²
TD876 ^A	<i>l</i> ,5- <i>cz</i> -[Pb ^{II} (TD647)]	278	0%	0%	0%	0%	too unstable
TD863 ^A	<i>l</i> ,5- <i>cz</i> -[Ca ^{II} (TD647)]	278	0%	0%	0%	0%	too unstable
TD880 ^A	<i>l</i> ,5- <i>cz</i> -[Cd ^{II} (TD647)]	278	100%	99%	90%	63%	1.1E ³ ± 7.7E ⁰
TD890 ^A	<i>l</i> ,5- <i>cz</i> -[Zn ^{II} (TD647)]	278	0%	0%	0%	0%	too unstable
TD624-La ^{A,*}	[La ^{III} (<i>p</i> -NO ₂ BnDOTA)]	285	0%	0%	0%	0%	too unstable
TD624-Gd ^{A,*}	[Gd ^{III} (<i>p</i> -NO ₂ BnDOTA)]	285	15%	0%	0%	0%	3.7E ⁻¹
TD624-Lu ^{A,*}	[Lu ^{III} (<i>p</i> -NO ₂ BnDOTA)]	285	12%	0%	0%	0%	3.3E ⁻¹
TD925 ^B	<i>l</i> ,5- <i>cz</i> -[Gd ^{III} (TD921)]	275	100%	82%	21%	0%	7.7E ¹ ± 2.3E ⁰

Legend: ^A Aliquots (2 µL) were neutralized with 125 mM FA/NaOH buffer (pH 3.6; 200 µL) prior measurement.

^B Aliquots (2 µL) were neutralized with 500 mM MOPS/NaOH buffer (pH 7.0; 200 µL) prior measurement.

^f Dissociation data were fitted by exponential decay (equation $y = \exp(-x/t)$; y = fraction of intact chelate; x = time;

t = decay constant). Chelate stability is expressed as half-life $t_{1/2}$ (in hours, highlighted bold), derived from decay constant ($t_{1/2} = \ln(2) \times t$). The error of the fitting is not displayed for single data point fitting.

* Sum of two diastereoisomers.

↓ Stock solutions preparation: In Eppendorf tube (0.5 mL), **2,2',2'',2'''-(2-(4-nitrobenzyl))-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl** tetraacetic acid tetrahydrochloride (10.0 mM in H₂O; 100 µL; 1 µmol; 1.0 equiv.) was mixed with corresponding aq. **LnCl₃** (100 mM; 23.5 µL; 1.1 µmol; 1.1 equiv.), H₂O (59 µL) and aq. **NaOH** (400 mM; 17.5 µL; 7.0 equiv.). The resulting mixtures were

then stirred 16 h at RT (full conversion was confirmed by LC-MS) followed by centrifugation. Supernatants were then directly used as stock solutions for the dissociation assay.

Chart 19. Dissociation of chelates in aq. 0.1 M HCl at 80 °C (*acid decomplexation kinetic inertness*): fraction of intact chelate determined after 1 hour, 1 day, 1 week and 1 month by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at 280 nm; rounded to integers; aliquots from reaction mixtures were diluted with H₂O, vortexed and then injected to HPLC). Performed in glass vials with magnetic stirrers.

chelate	formally	λ , nm	1 hour	1 day	1 week	1 month	$t_{1/2}$, hours <i>f</i>
TD875^A	<i>l</i> ,4-cz-[Gd ^{III} (TD647)]	278	0%	0%	0%	0%	too unstable
TD874^A	<i>l</i> ,4-cz-[Lu ^{III} (TD647)]	278	0%	0%	0%	0%	too unstable
TD900^A	<i>l</i> ,4-cz-[Cd ^{II} (TD647)]	278	56%	0%	0%	0%	1.2E⁰
TD651^B	<i>l</i> ,5-cz-[La ^{III} (TD647)]	278	0%	0%	0%	0%	too unstable
TD870^B	<i>l</i> ,5-cz-[Ce ^{III} (TD647)]	278	0%	0%	0%	0%	too unstable
TD869^B	<i>l</i> ,5-cz-[Pr ^{III} (TD647)]	278	23%	0%	0%	0%	4.7E⁻¹
TD868^B	<i>l</i> ,5-cz-[Nd ^{III} (TD647)]	278	76%	0%	0%	0%	1.2E⁰
TD867^B	<i>l</i> ,5-cz-[Sm ^{III} (TD647)]	278	99%	86%	35%	1%	1.1E² ± 7.5E⁻¹
TD599^B	<i>l</i> ,5-cz-[Eu ^{III} (TD647)]	278	100%	97%	83%	21%	3.8E² ± 5.8E¹
TD527^B	<i>l</i> ,5-cz-[Gd ^{III} (TD647)]	278	100%	99%	95%	82%	2.5E³ ± 3.7E¹
TD600^B	<i>l</i> ,5-cz-[Tb ^{III} (TD647)]	278	100%	100%	99%	99%	3.2E⁴ ± 3.3E³
TD866^B	<i>l</i> ,5-cz-[Dy ^{III} (TD647)]	278	100%	100%	100%	100%	9.5E⁴ ± 3.0E³
TD648^B	<i>l</i> ,5-cz-[Ho ^{III} (TD647)]	278	100%	100%	100%	100%	too stable
TD865^B	<i>l</i> ,5-cz-[Er ^{III} (TD647)]	278	100%	100%	100%	100%	too stable
TD601^B	<i>l</i> ,5-cz-[Tm ^{III} (TD647)]	278	100%	100%	100%	100%	too stable
TD602^B	<i>l</i> ,5-cz-[Yb ^{III} (TD647)]	278	100%	100%	100%	100%	too stable
TD522^B	<i>l</i> ,5-cz-[Lu ^{III} (TD647)]	278	100%	100%	100%	100%	too stable

TD649 ^B	<i>l</i> ,5-cz-[Y ^{III} (TD647)]	278	100%	100%	100%	100%	too stable
TD876 ^A	<i>l</i> ,5-cz-[Pb ^{II} (TD647)]	278	0%	0%	0%	0%	too unstable
TD863 ^A	<i>l</i> ,5-cz-[Ca ^{II} (TD647)]	278	0%	0%	0%	0%	too unstable
TD880 ^A	<i>l</i> ,5-cz-[Cd ^{II} (TD647)]	278	100%	100%	100%	100%	too stable
TD890 ^A	<i>l</i> ,5-cz-[Zn ^{II} (TD647)]	278	46%	0%	0%	0%	4.7E ⁻¹
TD624-La ^{A,*}	[La ^{III} (<i>p</i> -NO ₂ BnDOTA)]	285	9%	0%	0%	0%	2.9E ⁻¹
TD624-Gd ^{A,*}	[Gd ^{III} (<i>p</i> -NO ₂ BnDOTA)]	285	88%	2%	0%	0%	4.9E ⁰ ± 2.9E ⁻¹
TD624-Lu ^{A,*}	[Lu ^{III} (<i>p</i> -NO ₂ BnDOTA)]	285	76%	2%	0%	0%	2.5E ⁰ ± 9.6E ⁻²
TD925 ^B	<i>l</i> ,5-cz-[Gd ^{III} (TD921)]	275	100%	98%	90%	60%	9.9E ² ± 2.2E ¹

Legend: ^A Aliquots (2 µL) were diluted with H₂O (200 µL) prior measurement.

^B Aliquots (2 µL) were neutralized with 500 mM MOPS/NaOH buffer (pH 7.0; 200 µL) prior measurement.

^f Dissociation data were fitted by exponential decay (equation $y = \exp(-x/t)$; y = fraction of intact chelate; x = time; t = decay constant). Chelate stability is expressed as half-life $t_{1/2}$ (in hours; highlighted bold), derived from decay constant ($t_{1/2} = \ln(2) \times t$). The error of the fitting is not displayed for single data point fitting.

^{*} Sum of two diastereoisomers.

[†] Stock solutions preparation: In Eppendorf tube (0.5 mL), **2,2',2'',2'''-(2-(4-nitrobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetraacetic acid tetrahydrochloride** (10.0 mM in H₂O; 100 µL; 1 µmol; 1.0 equiv.) was mixed with corresponding aq. **LnCl₃** (100 mM; 23.5 µL; 1.1 µmol; 1.1 equiv.), H₂O (59 µL) and aq. **NaOH** (400 mM; 17.5 µL; 7.0 µmol; 7.0 equiv.). The resulting mixtures were then stirred 16 h at RT (full conversion was confirmed by LC-MS) followed by centrifugation. Supernatants were then directly used as stock solutions for the dissociation assay.

Chart 20. Dissociation of chelates in aq. 0.1 M HCl at RT (*acid decomplexation kinetic inertness*): fraction of intact chelate determined after 1 hour, 1 day, 1 week and 1 month by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at 280 nm; rounded to integers; aliquots from reaction mixtures were diluted with H₂O, vortexed and then injected to HPLC). Performed in glass vials with magnetic stirrers.

chelate	formally	λ , nm	1 hour	1 day	1 week	1 month	$t_{1/2}$, hours ^f
TD875^A	<i>l</i> ,4- <i>cz</i> -[Gd ^{III} (TD647)]	278	98%	58%	6%	0%	3.2E¹ ± 2.1E⁰
TD874^A	<i>l</i> ,4- <i>cz</i> -[Lu ^{III} (TD647)]	278	99%	68%	14%	0%	5.1E¹ ± 4.8E⁰
TD900^A	<i>l</i> ,4- <i>cz</i> -[Cd ^{II} (TD647)]	278	100%	99%	91%	63%	1.3E³ ± 5.8E⁰
TD651^B	<i>l</i> ,5- <i>cz</i> -[La ^{III} (TD647)]	278	76%	4%	0%	0%	2.5E⁰ ± 2.5E⁻¹
TD870^B	<i>l</i> ,5- <i>cz</i> -[Ce ^{III} (TD647)]	278	95%	37%	0%	0%	1.7E¹ ± 2.9E⁻¹
TD869^B	<i>l</i> ,5- <i>cz</i> -[Pr ^{III} (TD647)]	278	98%	71%	12%	0%	5.2E¹ ± 2.2E⁰
TD868^B	<i>l</i> ,5- <i>cz</i> -[Nd ^{III} (TD647)]	278	99%	90%	54%	2%	1.8E² ± 1.2E¹
TD867^B	<i>l</i> ,5- <i>cz</i> -[Sm ^{III} (TD647)]	278	100%	100%	99%	96%	1.3E⁴ ± 4.4E²
TD599^B	<i>l</i> ,5- <i>cz</i> -[Eu ^{III} (TD647)]	278	100%	100%	100%	100%	too stable
TD527^B	<i>l</i> ,5- <i>cz</i> -[Gd ^{III} (TD647)]	278	100%	100%	100%	100%	too stable
TD600^B	<i>l</i> ,5- <i>cz</i> -[Tb ^{III} (TD647)]	278	100%	100%	100%	100%	too stable
TD866^B	<i>l</i> ,5- <i>cz</i> -[Dy ^{III} (TD647)]	278	100%	100%	100%	100%	too stable
TD648^B	<i>l</i> ,5- <i>cz</i> -[Ho ^{III} (TD647)]	278	100%	100%	100%	100%	too stable
TD865^B	<i>l</i> ,5- <i>cz</i> -[Er ^{III} (TD647)]	278	100%	100%	100%	100%	too stable
TD601^B	<i>l</i> ,5- <i>cz</i> -[Tm ^{III} (TD647)]	278	100%	100%	100%	100%	too stable
TD602^B	<i>l</i> ,5- <i>cz</i> -[Yb ^{III} (TD647)]	278	100%	100%	100%	100%	too stable
TD522^B	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (TD647)]	278	100%	100%	100%	100%	too stable
TD649^B	<i>l</i> ,5- <i>cz</i> -[Y ^{III} (TD647)]	278	100%	100%	100%	100%	too stable
TD876^A	<i>l</i> ,5- <i>cz</i> -[Pb ^{II} (TD647)]	278	6%	0%	0%	0%	2.4E⁻¹
TD863^A	<i>l</i> ,5- <i>cz</i> -[Ca ^{II} (TD647)]	278	39%	0%	0%	0%	7.3E⁻¹
TD880^A	<i>l</i> ,5- <i>cz</i> -[Cd ^{II} (TD647)]	278	100%	100%	100%	100%	too stable
TD890^A	<i>l</i> ,5- <i>cz</i> -[Zn ^{II} (TD647)]	278	100%	93%	54%	5%	1.9E² ± 8.5E⁰
TD624-La^{A*}	[La ^{III} (<i>p</i> -NO ₂ BnDOTA)]	285	95%	36%	0%	0%	1.6E¹ ± 1.8E⁻¹

TD624-Gd^{A,*}	[Gd ^{III} (<i>p</i> -NO ₂ BnDOTA)]	285	100%	99%	92%	79%	2.1E ³ ± 1.2E ²
TD624-Lu^{A,*}	[Lu ^{III} (<i>p</i> -NO ₂ BnDOTA)]	285	99%	98%	87%	67%	1.2E ³ ± 8.7E ¹
TD925^B	<i>l</i> ,5- <i>cz</i> -[Gd ^{III} (TD921)]	275	100%	100%	100%	100%	too stable

Legend: ^A Aliquots (2 μL) were diluted with H₂O (200 μL) prior measurement.

^B Aliquots (2 μL) were neutralized with 500 mM MOPS/NaOH buffer (pH 7.0; 200 μL) prior measurement.

^f Dissociation data were fitted by exponential decay (equation $y = \exp(-x/t)$; y = fraction of intact chelate; x = time;

t = decay constant). Chelate stability is expressed as half-life $t_{1/2}$ (in hours, highlighted bold), derived from decay

constant ($t_{1/2} = \ln(2) \times t$). The error of the fitting is not displayed for single data point fitting.

^{*} Sum of two diastereoisomers.

[↓] Stock solutions preparation: In Eppendorf tube (0.5 mL), **2,2',2'',2'''-(2-(4-nitrobenzyl))-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra-yl)tetraacetic acid tetrahydrochloride** (10.0 mM in H₂O; 100 μL; 1.0 equiv.) was mixed with corresponding aq. **LnCl₃** (100 mM; 23.5 μL; 1.1 μmol; 1.1 equiv.), H₂O (59 μL) and aq. **NaOH** (400 mM; 17.5 μL; 7.0 μmol; 7.0 equiv.). The resulting mixtures were then stirred 16 h at RT (full conversion was confirmed by LC-MS) followed by centrifugation. Supernatants were then directly used as stock solutions for the dissociation assay.

Chart 21. Cleavage of model hexapeptide-chelate conjugates into parent chelate and individual amino acids in aq. 3.0 M HCl at 80 °C over the course of 1 week (*acid peptide hydrolysis kinetics*): fractions of starting chelate-peptide conjugate (*S*), chelate-containing intermediates (*I*), parent chelate (*P*) and free ligand (*L*) determined by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at 285 nm; rounded to integers; aliquots from reaction mixtures were neutralized with MOPS buffer, pH 7.0, vortexed and then injected to HPLC). Performed in glass vials with magnetic stirrers.

chelate	species	1 h	1 d	2 d	3 d	4 d	5 d	6 d	7 d
TD802^f	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (Lig -AYFHV G -NH ₂)]	<i>S</i>	0%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (Lig -AYFHV G -OH)]	<i>I</i>	48%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (Lig -AYFHV V -OH)]	<i>I</i>	2%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (Lig -AYF H -OH)]	<i>I</i>	5%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (Lig -AYF F -OH)]	<i>I</i>	9%	0%	0%	0%	0%	0%	0%

chelate	species	1 h	1 d	2 d	3 d	4 d	5 d	6 d	7 d
TD901 "	<i>l</i> ,5- <i>cz</i> -[Tm ^{III} (Lig-AYFHV _G -NH ₂)]	<i>S</i> 0%	0%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Tm ^{III} (Lig-AYFHV _G -OH)]	<i>I</i> 34%	0%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Tm ^{III} (Lig-AYFHV-OH)]	<i>I</i> 1%	0%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Tm ^{III} (Lig-AYFH-OH)]	<i>I</i> 4%	0%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Tm ^{III} (Lig-AYF-OH)]	<i>I</i> 6%	0%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Tm ^{III} (Lig-AY-OH)]	<i>I</i> 4%	0%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Tm ^{III} (Lig-A-OH)]	<i>I</i> 12%	0%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Tm ^{III} (Lig)]	<i>P</i> 37%	100%	100%	100%	100%	100%	100%	99%
	<i>l</i> ,5- <i>cz</i> -Lig	<i>L</i> 0%	0%	0%	0%	0%	0%	0%	1%

Legend: ' Hexapeptide is connected to the chelate through aromatic amide moiety.

" Hexapeptide is connected to the chelate through aliphatic amide moiety.

Sum of *l*,5-*cz*-Lig and *l*,5-*cz*-Lig-A-OH.

5 **Chart 22.** Cleavage of model hexapeptide-chelate conjugates into parent chelate and individual amino acids in aq. 2.0 M HCl at 80 °C over the course of 1 week (*acid peptide hydrolysis kinetics*): fractions of starting chelate-peptide conjugate (*S*), chelate-containing intermediates (*I*), parent chelate (*P*) and free ligand (*L*) determined by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at 285 nm; rounded to integers; aliquots from reaction mixtures were neutralized with MOPS buffer, pH 7.0, vortexed and then injected to HPLC). Performed in glass vials with magnetic stirrers.

chelate	species	1 h	1 d	2 d	3 d	4 d	5 d	6 d	7 d
TD802 '	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (Lig-AYFHV _G -NH ₂)]	<i>S</i> 0%	0%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (Lig-AYFHV _G -OH)]	<i>I</i> 65%	0%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (Lig-AYFHV-OH)]	<i>I</i> 1%	0%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (Lig-AYFH-OH)]	<i>I</i> 4%	0%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (Lig-AYF-OH)]	<i>I</i> 7%	0%	0%	0%	0%	0%	0%	0%

[illegible]

chelate	species	1 h	1 d	2 d	3 d	4 d	5 d	6 d	7 d
TD901 "	<i>l</i> ,5- <i>cz</i> -[Tm ^{III} (Lig-AYFHV _G -NH ₂)]	<i>S</i>	0%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Tm ^{III} (Lig-AYFHV _G -OH)]	<i>I</i>	50%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Tm ^{III} (Lig-AYFHV-OH)]	<i>I</i>	1%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Tm ^{III} (Lig-AYFH-OH)]	<i>I</i>	3%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Tm ^{III} (Lig-AYF-OH)]	<i>I</i>	5%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Tm ^{III} (Lig-AY-OH)]	<i>I</i>	3%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Tm ^{III} (Lig-A-OH)]	<i>I</i>	8%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Tm ^{III} (Lig)]	<i>P</i>	30%	100%	100%	100%	100%	100%	100%
	<i>l</i> ,5- <i>cz</i> -Lig	<i>L</i>	0%	0%	0%	0%	0%	0%	0%

Legend: " Hexapeptide is connected to the chelate through aromatic amide moiety.

" Hexapeptide is connected to the chelate through aliphatic amide moiety.

Sum of *l*,5-*cz*-Lig and *l*,5-*cz*-Lig-A-OH.

5

Chart 23. Cleavage of model hexapeptide-chelate conjugates into parent chelate and individual amino acids in aq. 1.0 M HCl at 80 °C over the course of 1 week (*acid peptide hydrolysis kinetics*): fractions of starting chelate-peptide conjugate (*S*), chelate-containing intermediates (*I*), parent chelate (*P*) and free ligand (*L*) determined by analytical HPLC (H₂O–MeCN system with 0.1% FA additive; detection at 285 nm; rounded to integers; aliquots from reaction mixtures were neutralized with MOPS buffer, pH 7.0, vortexed and then injected to HPLC). Performed in glass vials with magnetic stirrers.

chelate	species	1 h	1 d	2 d	3 d	4 d	5 d	6 d	7 d
TD802 '	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (Lig-AYFHV _G -NH ₂)]	<i>S</i>	4%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (Lig-AYFHV _G -OH)]	<i>I</i>	80%	1%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (Lig-AYFHV-OH)]	<i>I</i>	1%	0%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (Lig-AYFH-OH)]	<i>I</i>	2%	1%	0%	0%	0%	0%	0%
	<i>l</i> ,5- <i>cz</i> -[Lu ^{III} (Lig-AYF-OH)]	<i>I</i>	3%	2%	0%	0%	0%	0%	0%

	<i>l,5-cz-[Lu^{III}(Lig-AY-OH)]</i>	<i>I</i>	3%	5%	1%	0%	0%	0%	0%	0%	0%
	<i>l,5-cz-[Lu^{III}(Lig-A-OH)]</i>	<i>I</i>	6%	55%	40%	28%	17%	10%	6%	3%	
	<i>l,5-cz-[Lu^{III}(Lig)]</i>	<i>P</i>	2%	34%	59%	72%	83%	90%	94%	97%	
	<i>l,5-cz-Lig</i>	<i>L</i>	0%	0%	0%	0%	0%	0%	0%	0%	
	species		1 h	1 d	2 d	3 d	4 d	5 d	6 d	7 d	
TD792'	<i>l,5-cz-[Y^{III}(Lig-AYFHVG-NH₂)]</i>	<i>S</i>	5%	0%	0%	0%	0%	0%	0%	0%	
	<i>l,5-cz-[Y^{III}(Lig-AYFHVG-OH)]</i>	<i>I</i>	77%	1%	0%	0%	0%	0%	0%	0%	
	<i>l,5-cz-[Y^{III}(Lig-AYFHV-OH)]</i>	<i>I</i>	1%	0%	0%	0%	0%	0%	0%	0%	
	<i>l,5-cz-[Y^{III}(Lig-AYFH-OH)]</i>	<i>I</i>	2%	1%	0%	0%	0%	0%	0%	0%	
	<i>l,5-cz-[Y^{III}(Lig-AYF-OH)]</i>	<i>I</i>	4%	2%	0%	0%	0%	0%	0%	0%	
	<i>l,5-cz-[Y^{III}(Lig-AY-OH)]</i>	<i>I</i>	3%	5%	1%	0%	0%	0%	0%	0%	
	<i>l,5-cz-[Y^{III}(Lig-A-OH)]</i>	<i>I</i>	6%	56%	35%	26%	16%	8%	5%	4%	
	<i>l,5-cz-[Y^{III}(Lig-COOH)]</i>	<i>P</i>	2%	35%	64%	73%	83%	91%	93%	95%	
	<i>l,5-cz-Lig[#]</i>	<i>L</i>	0%	0%	0%	1%	1%	1%	2%	2%	
	species		1 h	1 d	2 d	3 d	4 d	5 d	6 d	7 d	
TD902''	<i>l,5-cz-[Lu^{III}(Lig-AYFHVG-NH₂)]</i>	<i>S</i>	3%	0%	0%	0%	0%	0%	0%	0%	
	<i>l,5-cz-[Lu^{III}(Lig-AYFHVG-OH)]</i>	<i>I</i>	67%	0%	0%	0%	0%	0%	0%	0%	
	<i>l,5-cz-[Lu^{III}(Lig-AYFHV-OH)]</i>	<i>I</i>	1%	0%	0%	0%	0%	0%	0%	0%	
	<i>l,5-cz-[Lu^{III}(Lig-AYFH-OH)]</i>	<i>I</i>	2%	0%	0%	0%	0%	0%	0%	0%	
	<i>l,5-cz-[Lu^{III}(Lig-AYF-OH)]</i>	<i>I</i>	3%	0%	0%	0%	0%	0%	0%	0%	
	<i>l,5-cz-[Lu^{III}(Lig-AY-OH)]</i>	<i>I</i>	2%	0%	0%	0%	0%	0%	0%	0%	
	<i>l,5-cz-[Lu^{III}(Lig-A-OH)]</i>	<i>I</i>	4%	3%	0%	0%	0%	0%	0%	0%	
	<i>l,5-cz-[Lu^{III}(Lig)]</i>	<i>P</i>	19%	97%	100%	100%	100%	100%	100%	100%	
	<i>l,5-cz-Lig</i>	<i>L</i>	0%	0%	0%	0%	0%	0%	0%	0%	

Chelate	species	1 h	1 d	2 d	3 d	4 d	5 d	6 d	7 d
		<i>S</i>	0%	0%	0%	0%	0%	0%	0%
TD901 "	<i>I,5-cz-[Tm^{III}(Lig-AYFHV-G-NH₂)]</i>	<i>S</i>	2%	0%	0%	0%	0%	0%	0%
	<i>I,5-cz-[Tm^{III}(Lig-AYFHV-G-OH)]</i>	<i>I</i>	68%	1%	0%	0%	0%	0%	0%
	<i>I,5-cz-[Tm^{III}(Lig-AYFHV-OH)]</i>	<i>I</i>	1%	0%	0%	0%	0%	0%	0%
	<i>I,5-cz-[Tm^{III}(Lig-AYFH-OH)]</i>	<i>I</i>	2%	0%	0%	0%	0%	0%	0%
	<i>I,5-cz-[Tm^{III}(Lig-AYF-OH)]</i>	<i>I</i>	3%	0%	0%	0%	0%	0%	0%
	<i>I,5-cz-[Tm^{III}(Lig-AY-OH)]</i>	<i>I</i>	2%	0%	0%	0%	0%	0%	0%
	<i>I,5-cz-[Tm^{III}(Lig-A-OH)]</i>	<i>I</i>	4%	4%	0%	0%	0%	0%	0%
	<i>I,5-cz-Lig</i>	<i>P</i>	19%	95%	100%	100%	100%	100%	100%
		<i>L</i>	0%	0%	0%	0%	0%	0%	0%

Legend: " Hexapeptide is connected to the chelate through aromatic amide moiety.

" Hexapeptide is connected to the chelate through aliphatic amide moiety.

Sum of *I,5-cz-Lig* and *I,5-cz-Lig-A-OH*.

5 **Chart 24.** Cell penetrating properties of **TD975**, **TD989**, **TD1010** and **TD1011** (CPP-conjugates). Protocol: Grown cells (CCRF-CEM; T-lymphoblastic leukemia) were centrifuged (250 × g, RT), washed with PBS and resuspended in RPMI medium Dutch modification (with HEPES buffer, without antibiotics) to a concentration 2.5 × 10⁷ mL⁻¹. Suspension was transferred into 12 (4 CPP-conjugate combinations, each in triplicate) Eppendorf tubes (400 µL of suspension to each). Stock solution of CPP conjugates (5.0 mM, 50 µL) were added to reach 0.5 mM final concentration of given CPP-conjugate. Cells were then incubated 2 h at 37 °C. After incubation, samples were centrifuged (900 × g, RT). Medium was carefully pipetted out and the pellets twice washed with 1 mL PBS. Resulting pellets were treated with HCl (3.0 M, 500 µL) and stirred 7 days at 80 °C. Resulting solutions were then analyzed for cell uptake.

CPP conjugates incubated with cells		cell uptake, %		
0.5 mM TD975 + 0.5 mM TD989	TD975 ^a	0.54±0.012% ^b	0.57±0.039 ^c	TD989 ^a 0.47±0.049 ^b 0.47±0.052 ^c
0.5 mM TD1011 + 0.5 mM TD1010	TD1011 ^a	0.13±0.013% ^b	0.15±0.017 ^c	TD1010 ^a 0.13±0.003 ^b 0.14±0.019 ^c

0.5 mM TD975 + 0.5 mM TD1010	TD975 ^a	0.72±0.006% ^b	0.73±0.020 ^c	TD1010 ^a	0.06±0.036 ^b	0.06±0.027 ^c
0.5 mM TD1011 + 0.5 mM TD989	TD1011 ^a	0.07±0.014% ^b	0.07±0.049 ^c	TD989 ^a	0.69±0.003 ^b	0.66±0.018 ^c

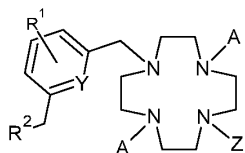
Legend: ^a analysed as **TD748** and **TD897** after acid hydrolysis of parent CPP conjugates.
^b quantified by LC-MS: UV integration at 285 nm; ESI-MS integration of EIC⁺ 841–843 *m/z* (**TD748**⁺) and 835–837 *m/z* (**TD897**⁺). The quantification was based on calibration (of both UV and ESI-MS modes) using stock solutions of **TD748** and **TD897** with known concentrations.
^c quantified by ICP.

Abbreviations and definitions

	cyclene	1,4,7,10-tetraazacyclododecane
	Cbz	benzylchloroformate
5	^t Bu	terc-butyl
	Boc	benzyloxycarbonyl
	DCM	dichloromethane
	TFA	trifluoroacetic acid
	TIPS	triisopropylsilyl
10	TMS	trimethylsilyl
	Ph	phenyl
	Fmoc	fluorenylmethyloxykarbonyl
	iPr	isopropyl
	MOPS	3-(N-morpholino)propanesulfonic acid
15	DIPEA	N,N-Diisopropylethylamine
	LC-MS	liquid chromatography – mass spectrometry
	MRI	magnetic resonance imaging
	aryl	substituent derived from at least one aromatic hydrocarbon ring by removal of one hydrogen atom from the aromatic ring, e.g. phenyl or naphthyl
20	alkyl	substituent derived from an alkane (linear or branched) which is derived from an alkane by removal of one hydrogen atom, e.g. methyl or ethyl
	arylalkyl	substituent derived from an aryl-substituted alkane (linear or branched) which is derived from an alkane by removal of one hydrogen atom from the alkane chain., e.g. benzyl
25	arylalkynyl	substituent derived from an aryl-substituted alkyne by removing one hydrogen (thus containing a triple bond), which may be linear or branched
	click-zip	closed cage ligand or complex, wherein the click reaction between the azide and the triple bond took place and the triazole bridge was formed, thereby “zipping” the metal coordination place.
30	1,5-cz-[M(Lig)] from Lig and M ions is a general notation of the coordination compounds according to the present invention. “1,5-cz-“ stands for a 1,5-triazole bridged cage, [M(Lig)] stands for a coordination compound of a metal cation and a ligand (the compound according to general formula (I)).	

CLAIMS

1. Compound of general formula (I)



5 (I),

wherein

Y is selected from a group consisting of nitrogen; N-oxide;

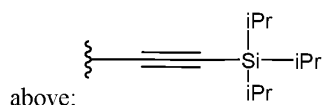
R¹ is selected from the group consisting of H; halogen; -OH; -N₃; -(CH₂)_nN₃, wherein *n* is an integer in the range of from 1 to 3; -NR₂, wherein R is independently selected from H or C₁ to C₆ alkyl, which may be

10 branched or linear; -(CH₂)_nNR₂, wherein *n* and R are as defined above; C₆ to C₁₀ aryl, which can optionally be

substituted with -NH₂, -NO₂, -N₃, ξ -C≡CH, -COOH, -CH₂Cl and/or -CH₂COOH; C₇ to C₁₀ arylalkyl,

which can optionally be substituted with -NH₂, -NO₂, -N₃, ξ -C≡CH, -COOH, -CH₂Cl and/or

-CH₂COOH; -CF₃; -COOR, wherein R is as defined above; -(CH₂)_nCOOR, wherein *n* and R are as defined



above; ξ -CH₂CH(OMe)₂; ξ -C≡CH; -SH; -SO₃H; -SO₂Ar, wherein Ar is phenyl;

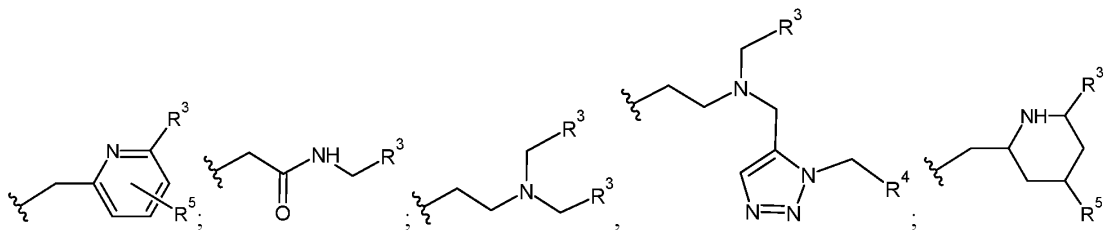
15 NO₂;

R² is ξ -N₃; or ξ -[CH₂]_n-N₃, wherein *n* is an integer in the range of from 1 to 3;

A are independently selected from the group consisting of H; -(CH₂)_nCOOH, wherein *n* is an integer from 1 to 3; -CH(CH₃)COOH; -CH((CH₂)_nCH₃)COOH, wherein *n* is as defined above; -CH₂P(=O)(OR)₂, wherein R is as defined above; -CH((CH₂)_nCOOH)COOH, wherein *n* is an integer from 1 to 3; -CH((CH₂)_nNH₂)COOH,

20 wherein *n* is an integer from 1 to 3; -CH₂C(=O)(NH₂); -CH₂C(=O)(NH)-CH₂COOH; -CH₂P(=O)(OH)(Ar), wherein Ar is phenyl, which can optionally be substituted with C₁ to C₆ alkyl;

Z is selected from a group consisting of



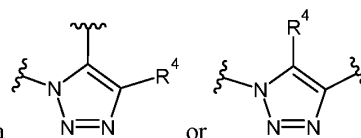
wherein

R^3 is $\text{---}\equiv\text{---}R^4$;

R^4 is selected from the group consisting of H; halogen; piperidinyl; trimethylsilyl; triisopropylsilyl; phenyl; C_1 to C_6 alkyl, which may be branched or linear; C_3 to C_6 cycloalkyl; $-\text{CF}_3$; $-(\text{CH}_2)_n\text{NHR}^6$, wherein n is an integer in the range of from 1 to 3 and R^6 is selected from H, fluorenylmethyloxycarbonyl, tert-butylloxycarbonyl and benzyloxycarbonyl; $-\text{C}(\text{CH}_3)_2(\text{NHR}^6)$, wherein R^6 is as defined above; adamantyl; $\text{---}\equiv\text{CH}$; $-(\text{CH}_2)_n\text{COOH}$, wherein n is as defined above; C_6 to C_{10} aryl, which can optionally be substituted with $-\text{NH}_2$, $-\text{NO}_2$, $-\text{N}_3$, $\text{---}\equiv\text{CH}$, $-\text{COOH}$, $-\text{CH}_2\text{Cl}$ and/or $-\text{CH}_2\text{COOH}$;

R^5 is R^1 or $\text{---}\equiv\text{---}R^4$, wherein R^4 is as defined above;

and/or R^2 and R^3 together form a 1,2,3-triazole group of formula



or ; wherein

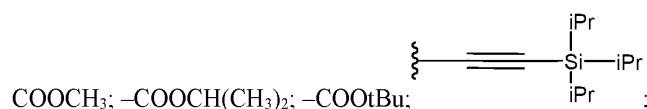
R^4 is defined above;

with the proviso that at most one A is H.

2. Compound of general formula (I) according to claim 1, wherein:

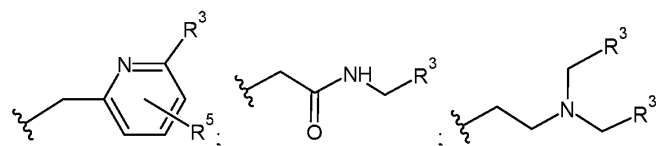
Y is nitrogen;

15 R^1 is selected from the group consisting of H; Cl; $-\text{N}(\text{CH}_3)_2$; phenyl, which can optionally be substituted with $-\text{COOH}$ or $-\text{CH}_2\text{COOH}$; benzyl, which can optionally be substituted with $-\text{COOH}$ or $-\text{CH}_2\text{COOH}$; $-\text{CF}_3$; $-\text{COOCH}_3$; $-\text{COOCH}(\text{CH}_3)_2$; $-\text{COOtBu}$;



A are independently selected from the group consisting of H; $-(\text{CH}_2)_n\text{COOH}$, wherein n is 1 or 2; $-\text{CH}_2\text{P}(=\text{O})(\text{OH})_2$; $-\text{CH}_2\text{P}(=\text{O})(\text{OH})(\text{OEt})$; $-\text{CH}_2\text{P}(=\text{O})(\text{OEt})_2$; $-\text{CH}_2\text{C}(=\text{O})(\text{NH}_2)$; $-\text{CH}_2\text{C}(=\text{O})(\text{NH})-\text{CH}_2\text{COOH}$; $-\text{CH}_2\text{P}(=\text{O})(\text{OH})(\text{Ph})$;

20 Z is selected from a group consisting of



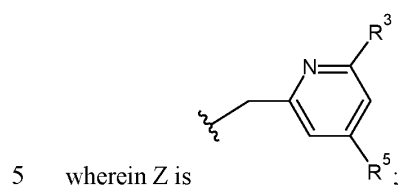
wherein

R^3 is $\text{---}\equiv\text{---}R^4$;

25 R^4 is selected from the group consisting of H; halogen; piperidinyl; trimethylsilyl; triisopropylsilyl; phenyl; cyclopropyl; *tert*-butyl; $-\text{CF}_3$; $-\text{CH}_2\text{NH}_2$; $-\text{CH}_2\text{N}(\text{H})(\text{Fmoc})$; $-\text{C}(\text{CH}_3)_2(\text{NH}_2)$; $-\text{C}(\text{CH}_3)_2(\text{NHBoc})$; adamantyl;

R^5 is H or $\text{---}\equiv\text{---}R^4$, wherein R^4 is as defined above;
with the proviso that at most one A is H.

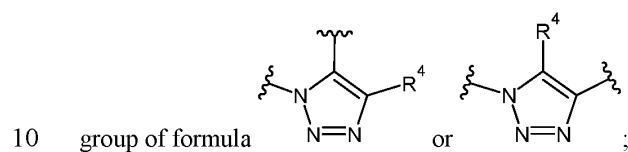
3. Compound according to claim 1 or 2,



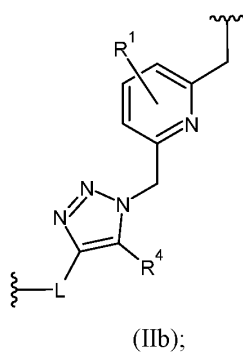
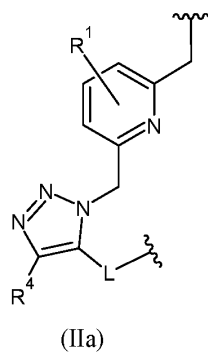
R^3 is $\text{---}\equiv\text{---}R^4$;

R^4 and R^5 are as defined above, preferably R^4 and R^5 are H.

4. Compound of general formula (I) according to claim 1, wherein R^2 and R^3 together form a 1,2,3-triazole

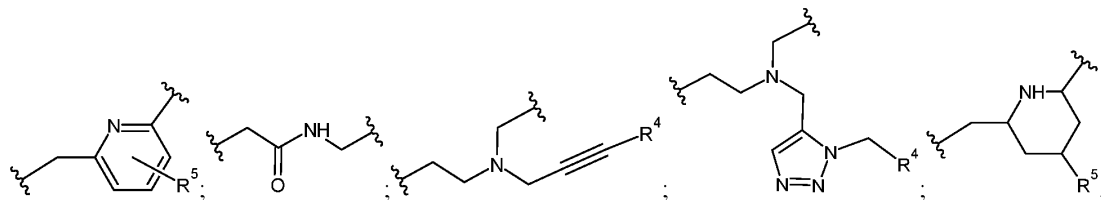


such that the bridge between two opposite nitrogen atoms of the cyclen moiety is an entity of general formula (IIa) or (IIb)

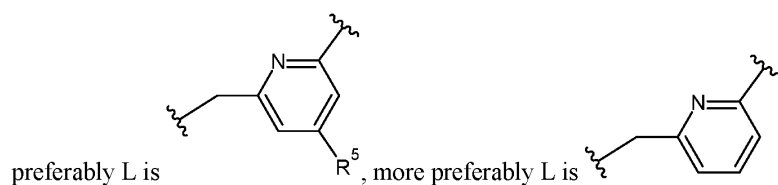


15 wherein

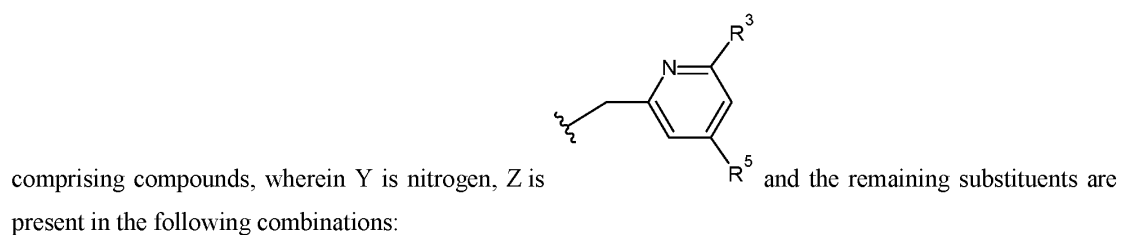
L is selected from the group consisting of



and R¹, R⁴ and R⁵ are as defined in claim 1;

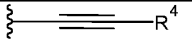
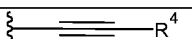
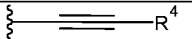
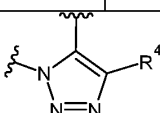
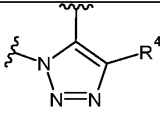
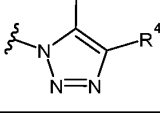
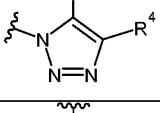
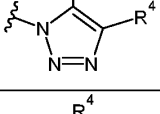
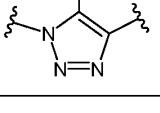


5. Compound of general formula (I) according to any one of the preceding claims, selected from the group



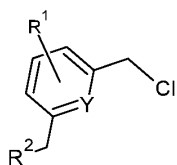
Compound	R ¹	A	R ²	R ³	R ⁴	R ⁵
TD556	H	-(CH ₂)COOH	 -N ₃	 -≡-R ⁴	H	H
TD810	H	-(CH ₂)COOH	 -N ₃	 -≡-R ⁴	H	 -≡CH
TD827	 -≡-TIPS	-(CH ₂)COOH	 -N ₃	 -≡-R ⁴	H	H
TD718	H	-(CH ₂)COOH	 -N ₃	 -≡-R ⁴	TMS	H
TD728	H	-(CH ₂)COOH	 -N ₃	 -≡-R ⁴	TIPS	H
TD1204	H	-(CH ₂)COOH	 -N ₃	 -≡-R ⁴	I	H
TD944	H	-(CH ₂)COOH	 -N ₃	 -≡-R ⁴	Ph	H
TD943	H	-(CH ₂)COOH	 -N ₃	 -≡-R ⁴	cyclopropyl	H
TD959	H	-(CH ₂)COOH	 -N ₃	 -≡-R ⁴	tBu	H
TD992	H	-(CH ₂)COOH	 -N ₃	 -≡-R ⁴	adamantyl	H
TD705	H	-CH ₂ P(=O)(OEt) ₂ and -(CH ₂)COO ^t Bu	 -N ₃	 -≡-R ⁴	H	H
TD714	H	-CH ₂ P(=O)(OH) ₂ and	 -N ₃	 -≡-R ⁴	H	H

		$-(CH_2)COOH$				
TD921	H	$-CH_2P(=O)(Ph)(OH)$	$\zeta-N_3$	$\zeta \equiv R^4$	H	H
TD580	H	$-CH_2P(=O)(OEt)_2$	$\zeta-N_3$	$\zeta \equiv R^4$	H	H
TD582	H	$-CH_2P(=O)(OH)(OEt)$	$\zeta-N_3$	$\zeta \equiv R^4$	H	H
TD581	H	$-CH_2P(=O)(OH)_2$	$\zeta-N_3$	$\zeta \equiv R^4$	H	H
TD575	Ph	$-CH_2P(=O)(OEt)_2$	$\zeta-N_3$	$\zeta \equiv R^4$	H	H
TD576	Ph	$-CH_2P(=O)(OH)_2$	$\zeta-N_3$	$\zeta \equiv R^4$	H	H
TD764	Cl	$-(CH_2)COOH$	$\zeta-N_3$	$\zeta \equiv R^4$	H	H
TD1063	$-CF_3$	$-(CH_2)COOH$	$\zeta-N_3$	$\zeta \equiv R^4$	H	H
TD1092	$-COOCH_3$	$-(CH_2)COOH$	$\zeta-N_3$	$\zeta \equiv R^4$	H	H
TD1148	$-COO^iPr$	$-(CH_2)COOH$	$\zeta-N_3$	$\zeta \equiv R^4$	H	H
TD1160	$-COO^iBu$	$-(CH_2)COOH$	$\zeta-N_3$	$\zeta \equiv R^4$	H	H
TD1176	$-N(CH_3)_2$	$-(CH_2)COOH$	$\zeta-N_3$	$\zeta \equiv R^4$	H	H
TD647	Ph	$-(CH_2)COOH$	$\zeta-N_3$	$\zeta \equiv R^4$	H	H
TD722	Ph	$-(CH_2)COOH$	$\zeta-N_3$	$\zeta \equiv R^4$	$-CH_2NH_2$	H
TD1054	Ph	$-(CH_2)COOH$	$\zeta-N_3$	$\zeta \equiv R^4$	$-C(CH_3)_2NH_2$	H
TD1105	H	$-(CH_2)COOH$	$\zeta-N_3$	$\zeta \equiv R^4$	$-C(CH_3)_2NHBoc$	H
TD1127	H	$-(CH_2)COOH$	$\zeta-N_3$	$\zeta \equiv R^4$	4-piperidinyl	H
TD742	4-carboxyphenyl	$-(CH_2)COOH$	$\zeta-N_3$	$\zeta \equiv R^4$	H	H
TD744	4-carboxyphenyl	$-(CH_2)COOH$	$\zeta-N_3$	$\zeta \equiv R^4$	$-CH_2-NH_2$	H
TD779	Ph	$-(CH_2)COONH_2$	$\zeta-N_3$	$\zeta \equiv R^4$	H	H
TD778	Ph	$-(CH_2)CONH-CH_2COOH$	$\zeta-N_3$	$\zeta \equiv R^4$	H	H
TD1346	H	$-CH(CH_3)COOH$	$\zeta-N_3$	$\zeta \equiv R^4$	H	H

TD1408	H	-(CH ₂)COOH			H	H
TD1451	-CF ₃	-CH(CH ₃)COOH			-C(CH ₃) ₂ NHBoc	-COOH
TD1504	H	-CH(CH ₂ COOH)COOH; -CH(CH ₃)COOH			H	H
cZ- TD556	H	-(CH ₂)COOH			H	H
cZ- TD764	Cl	-(CH ₂)COOH			H	H
cZ- TD1063	-CF ₃	-(CH ₂)COOH			H	H
TD1188	H	-(CH ₂)COOH			-CF ₃	H
TD650	Ph	-(CH ₂)COOH			H	H
TD871	Ph	-(CH ₂)COOH			H	H

6. A method of preparation of the compound of the general formula (I) according to any one of the claims 1 to 5, **characterized in that** it comprises the following steps:

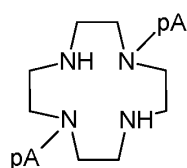
- i) providing an alkyne intermediate of general formula Z-Cl, wherein Z is as defined in claim 1;
- 5 ii) providing an azide intermediate of general formula (III)



(III);

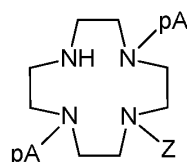
wherein Y, R¹ and R² are as defined in claim 1;

- iii) providing a cyclen derivative of general formula (IV)



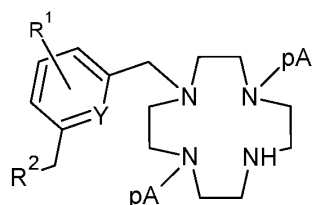
(IV),

- wherein pA is selected from the group comprising $-(CH_2)_nCOO^tBu$, wherein n is an integer from 1 to 3; benzyloxycarbonyl; $-CH(CH_3)COOR$, wherein R is tert-butyl, methyl or ethyl; $-CH((CH_2)_nCH_3)COOR$, wherein n is an integer from 1 to 3 and R is tert-butyl, methyl or ethyl; $-CH((CH_2)_nCOOR)COOR$, wherein n is an integer from 1 to 3 and R are independently selected from tert-butyl, methyl or ethyl; $-CH_2P(=O)(OR)_2$, wherein R is C_1 to C_6 alkyl, which may be branched or linear; $-CH_2C(=O)(NH_2)$; $-CH_2C(=O)(NH)-CH_2COOH$; $-CH_2P(=O)(OH)(Ar)$, wherein Ar is phenyl, which can optionally be substituted with C_1 to C_6 alkyl;
- 10 iv-A) reacting the cyclen derivative of general formula (IV) with alkyne intermediate Z-Cl to obtain an intermediate of general formula (V)



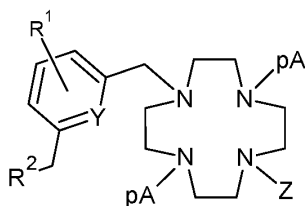
(V),

- wherein Z is as defined in claim 1, and pA is defined above;
- 15 or
- iv-B) reacting the cyclen derivative of general formula (IV) with the azide intermediate of general formula (III) to obtain an intermediate of general formula (VI)



(VI),

- 20 wherein Y, R^1 , R^2 and pA are as defined above;
- v-A) reacting the intermediate of general formula (V) with the azide intermediate of general formula (III) to obtain an intermediate of general formula (VII)



(VII)

wherein Y, R¹, R², pA and Z are as defined above;

or

- 5 v-B) reacting the intermediate of general formula (VI) with the alkyne intermediate Z-Cl to obtain the intermediate of general formula (VII);

vi) optionally, hydrolysis of protecting groups, resulting in the compound of general formula (I).

7. A coordination compound of the compound of the general formula (I) according to any one of the
 10 claims 1 to 5 with a metal cation, selected from the group consisting of lanthanide(III) cations, Na⁺, Ba²⁺, Pb²⁺, Sr²⁺, Ca²⁺, Cd²⁺, Zn²⁺, Mn²⁺, Pt²⁺, Cu²⁺, Ni²⁺, Sc³⁺, Y³⁺, Bi³⁺, In³⁺, Ru³⁺, Ir³⁺, Ga³⁺, Tl³⁺, Pd²⁺; preferably the metal cation is selected from the group consisting of La³⁺, Ce³⁺, Pr³⁺, Nd³⁺, Sm³⁺, Eu³⁺, Gd³⁺, Tb³⁺, Dy³⁺, Ho³⁺, Er³⁺, Tm³⁺, Yb³⁺, Lu³⁺, Y³⁺, Sc³⁺, Bi³⁺, In³⁺, Tl³⁺, Pb²⁺, Ca²⁺, Cd²⁺, Zn²⁺, Cu²⁺, Ni²⁺, Mn²⁺, Pd²⁺, Na⁺.

- 15 8. A method of preparation of the coordination compound according to claim 7, **characterized in that** it comprises the following steps:

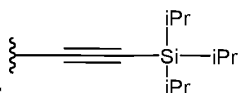
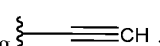
- i) synthesis of the compound of the general formula (I) according to claim 6;
 ii) providing a salt of an inorganic acid and a metal cation, selected from the group consisting of lanthanide(III) cations, Na⁺, Ba²⁺, Pb²⁺, Sr²⁺, Ca²⁺, Cd²⁺, Zn²⁺, Mn²⁺, Pt²⁺, Cu²⁺, Ni²⁺, Sc³⁺, Y³⁺, Bi³⁺, In³⁺, Ru³⁺, Ir³⁺, Ga³⁺, Tl³⁺,
 20 Pd²⁺; preferably, the salt is selected from a group comprising chloride, nitrate, acetate, formate, trifluoroacetate, trifluoromethylsulfonate;

- iii) mixing the compound of the general formula (I) from step i) with the metal salt from step ii) in aqueous solution, preferably for at least 1 hour at temperature in the range of from 25 to 100 °C, resulting in chelation of the metal cation by the compound of general formula (I) and formation of the coordination compound
 25 according to claim 7;

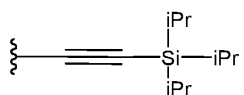
- iv) optionally, transformation of the coordination compound from step iii), wherein the transformation is selected from at least one of the following reactions:

- substitution reaction of halogen group present in R¹ and/or R⁴ and/or R⁵, with a phenylboronic acid, thus transforming the halogen substituent into -COOH;
 30 - substitution reaction of halogen group present in R¹ and/or R⁴ and/or R⁵, with -N₃ group by reaction with NaN₃;

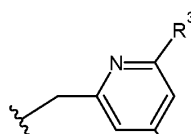
- hydrolysis of TIPS protecting group present in R^1 and/or R^4 and/or R^5 substituent, wherein R^1 and/or R^3 and/or

R^5 is , resulting in R^1 and/or R^3 and/or R^5 being ;

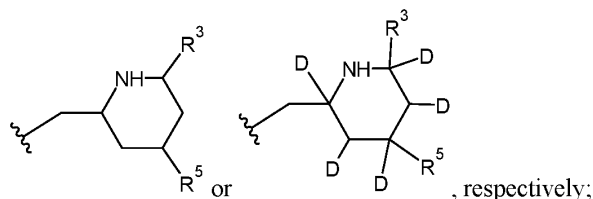
- addition reaction of methanol to R^1 and/or R^4 and/or R^5 , wherein R^1 and/or R^3 and/or R^5 is

, thus transforming the triple bond to dimethyl acetal;

- 5 - deuteration of CH_2 groups of the pendant arms in D_2O in presence of DBU, transforming them into CD_2 groups;



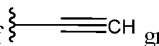
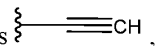
- selective reduction of pyridyl cycle of Z substituent, wherein Z is defined in claim 1, using $NaBH_4$ or $NaBD_4$ as the reducing agent, thus transforming the Z group into



- 10 - reaction of NH_2 group of R^1 and/or R^4 and/or R^5 and/or A, wherein R^1 and/or R^4 and/or R^5 and/or A contains $-NH_2$ or $-(CH_2)_nNH_2$, with FmocCl, resulting in transformation of $-NH_2$ group into $-NHFmoc$ group;

- reaction of $-COOH$ group present in R^1 and/or R^4 and/or R^5 and/or A, with an amino group of an amino acid or peptide, thus forming a peptide bond;

- S-alkylation reaction of a halogen, preferably Cl, of R^1 , R^4 and/or R^5 substituent with a thiol, resulting in transforming the halogen into sulfide;

- 15 - reaction of  group of R^1 , R^3 and/or R^5 , wherein R^1 , R^3 and/or R^5 contains , with an azide, preferably with alkyl- or arylazide, thereby forming a triazole bridge connecting the alkyl- or aryl- to R^1 , R^3 and/or R^5 group;

- reaction of $-N_3$ group of R^1 and/or R^5 , wherein R^1 and/or R^5 contains $-N_3$ group, with a substituent comprising a carbon-carbon triple bond, thereby forming a triazole bridge connecting the substituent with the coordination compound;

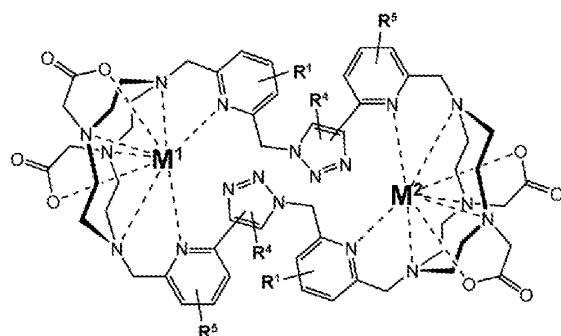
- 20 - reaction of $-SH$ group of R^1 and/or R^5 , with a substituent comprising maleimide, thereby forming thiosuccinimide linkage connecting the substituent with the coordination compound;

- reaction of –SH group of R^1 and/or R^5 , with a substituent comprising another –SH group, thereby forming a disulfide bridge connecting the substituent with the coordination compound;
- reaction of –SH group of R^1 and/or R^5 , with alkyl- or arylhalogenide, thereby forming a thioether linkage connecting the alkyl- or aryl- to the coordination compound;
- 5 - reaction of –NO₂ group of R^1 and/or R^5 , with a substituent comprising –SH group, thereby forming a thioether linkage connecting the substituent with the coordination compound.

9. A coordination compound chain containing at least two coordination compounds according to claim 7, bound via a triazole bridge formed between R^1 or R^5 group of the first coordination compound and R^1 or R^5 group of the following coordination compound and/or via amide bond formed between a substituent R^1 or R^4 or R^5 or A of one coordination compound containing an amine group and a substituent R^1 or R^4 or R^5 or A of another coordination compound containing a carboxyl group and/or via disulfide bonds formed between a substituent R^1 or R^5 of one coordination compound containing –SH group, and a substituent R^1 or R^5 of another coordination compound containing –SH group.

15

10. A coordination compound dimer of general formula (X)



(X),

wherein M^1 and M^2 are independently metal cations as defined in claim 7, and R^1 , R^4 and R^5 are as defined in claim 1.

20

11. A conjugate for drug tracing, which contains the coordination compound according to claim 7 or the coordination compound chain according to claim 9, conjugated to a peptide or to a protein; wherein the coordination compound according to claim 7 or the coordination compound chain according to claim 9 contain R^1 and/or R^4 and/or R^5 group comprising –NH₂ or –COOH group; preferably R^1 and/or R^4 and/or R^5 is selected from –NH₂; –(CH₂)_nNH₂; –COOH; –(CH₂)_nCOOH; C₆ to C₁₀ aryl, substituted with –NH₂, –COOH, –(CH₂)_nNH₂, or –(CH₂)_nCOOH; wherein n is an integer in the range of from 1 to 3;

25

which is conjugated to the peptide or the protein via an amide bond formed between –COOH group present in R¹ and/or R⁴ and/or R⁵ and amino group of the peptide or the protein or via an amide bond formed between –NH₂ group present in R¹ and/or R⁴ and/or R⁵ and carboxyl group of the peptide or the protein;
and wherein the peptide is selected from the group comprising oligopeptides of 3 to 20 aminoacids;
5 and wherein the protein is selected from the group comprising antibodies, preferably monoclonal antibodies.

12. A method of drug tracing, **characterized in that** it comprises the following steps:

- i) providing a cell culture or a tissue to be analyzed, containing at least one conjugate according to claim 11, wherein the peptide or the protein is of the peptide-based or protein-based drug to be traced;
10 ii) hydrolyzing the cell culture or tissue from step i) using a strong acid, preferably HCl, obtaining a hydrolyzate;
iii) qualitative and/or quantitative analysis of the hydrolyzate of step ii) for the presence of the coordination compound of claim 7 or of the coordination compound chain of claim 9, preferably using LC-MS.

- 15 13. Use *in vitro* of the coordination compound according to claim 7 or of the coordination compound chain according to claim 9 or of the coordination compound dimer according to claim 10 or of the conjugate according to claim 11 in pharmacy, preferably for development and testing of new drugs, more preferably for drug marking and tracing.

- 20 14. The coordination compound according to claim 7 or the coordination compound chain according to claim 9 or the coordination compound dimer according to claim 10 for use in medical diagnostics, preferably as MRI contrast agents.

- 15 15. The coordination compound according to claim 7 or the coordination compound chain according to claim 9 or the coordination compound dimer according to claim 10, wherein M is selected from the group comprising ⁴⁴Sc, ⁴⁷Sc, ⁶⁴Cu, ⁶⁷Cu, ⁸⁶Y, ⁹⁰Y, ¹⁴⁰Nd, ¹⁴⁹Pm, ¹⁵¹Pm, ¹⁵³Sm, ¹⁵⁹Gd, ¹⁴⁹Tb, ¹⁶¹Tb, ¹⁶⁵Dy, ¹⁶¹Ho, ¹⁶⁶Ho, ¹⁶⁹Er, ¹⁶⁷Tm, ¹⁷⁵Yb, ¹⁷⁷Lu, and/or R¹, R⁴ and/or R⁵ contains a radioactive halogen, for use in medicine as radiodiagnostic and/or radiopharmaceutic agents.