

Figure 1

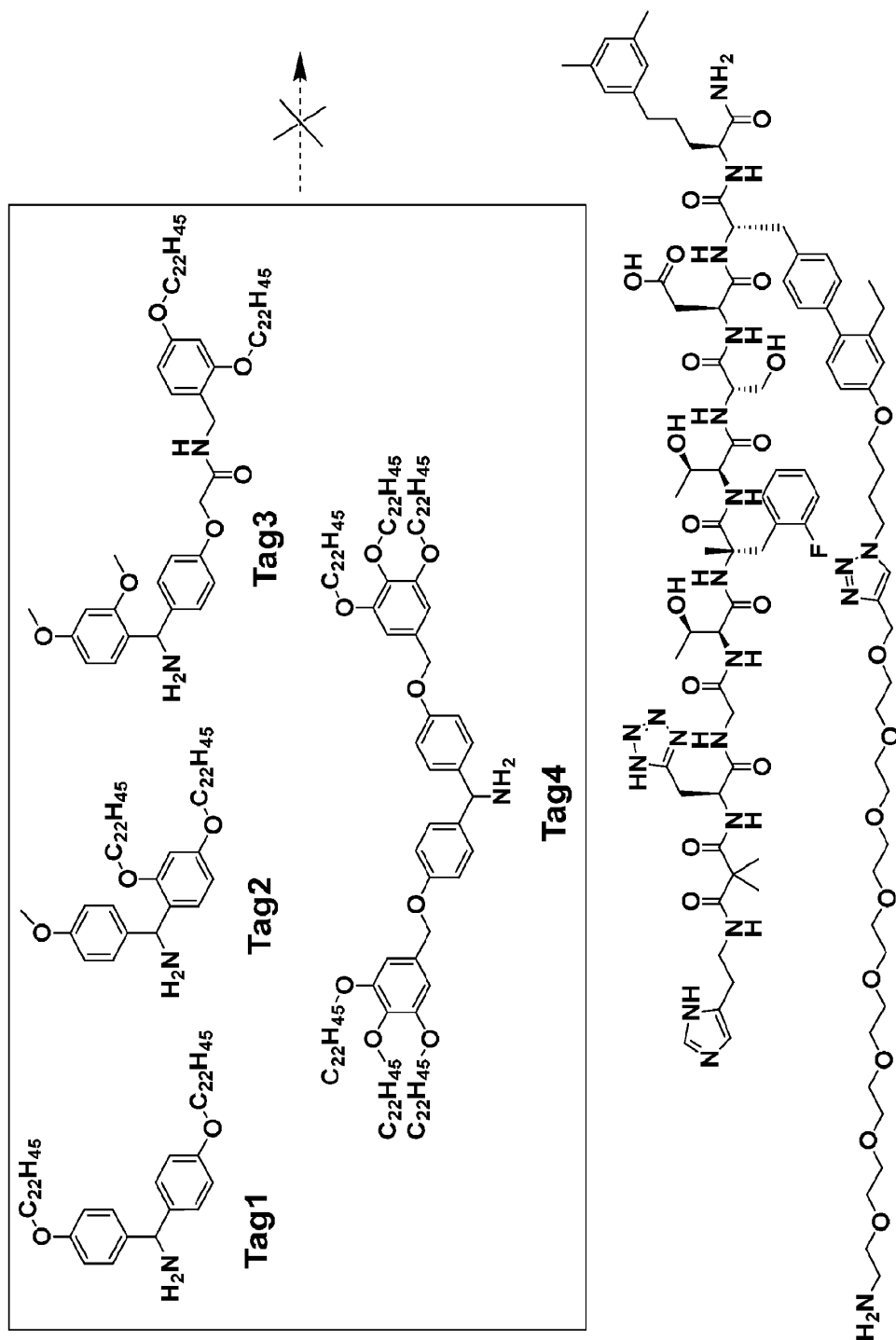
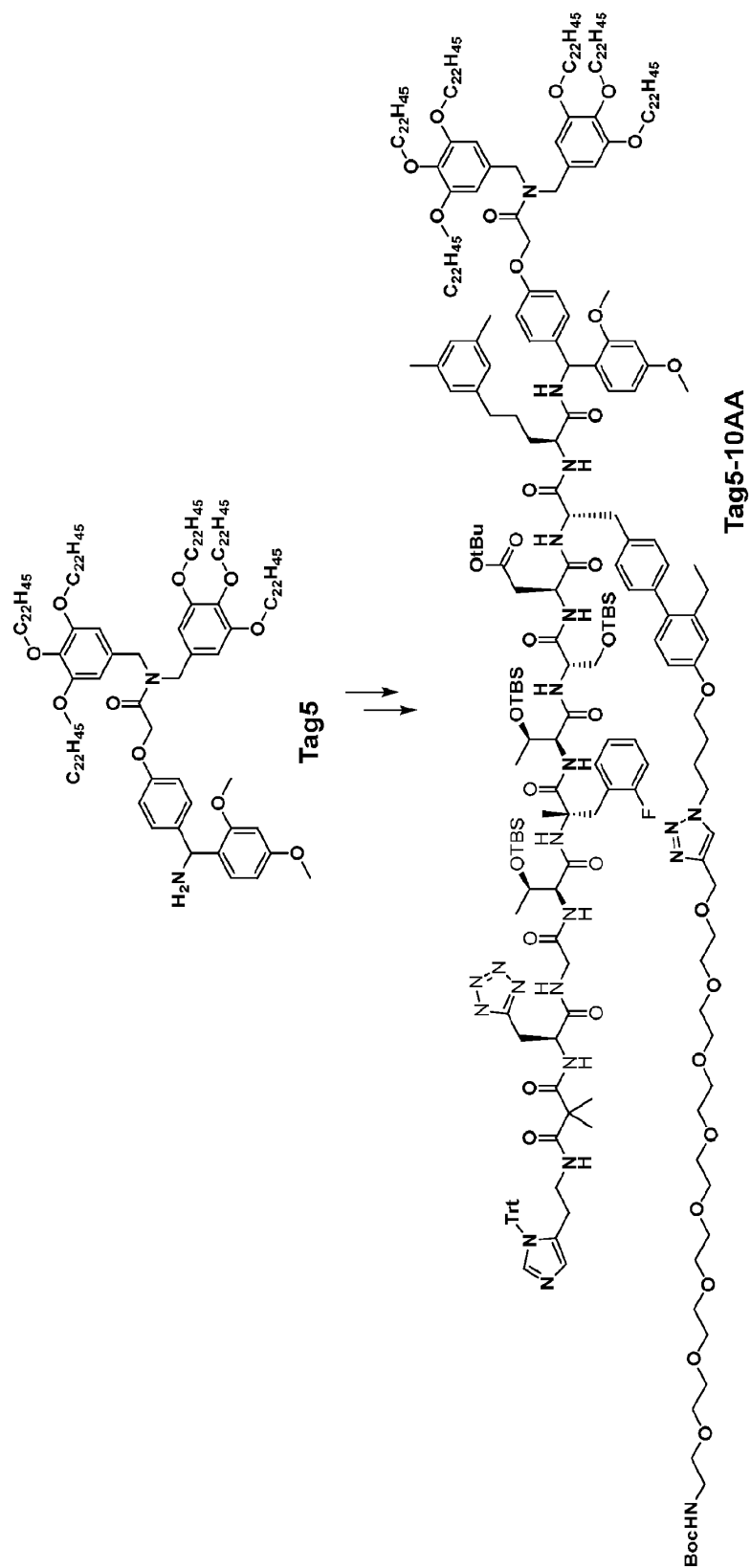


Figure 1 (cont.)



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Figure 1 (cont.)

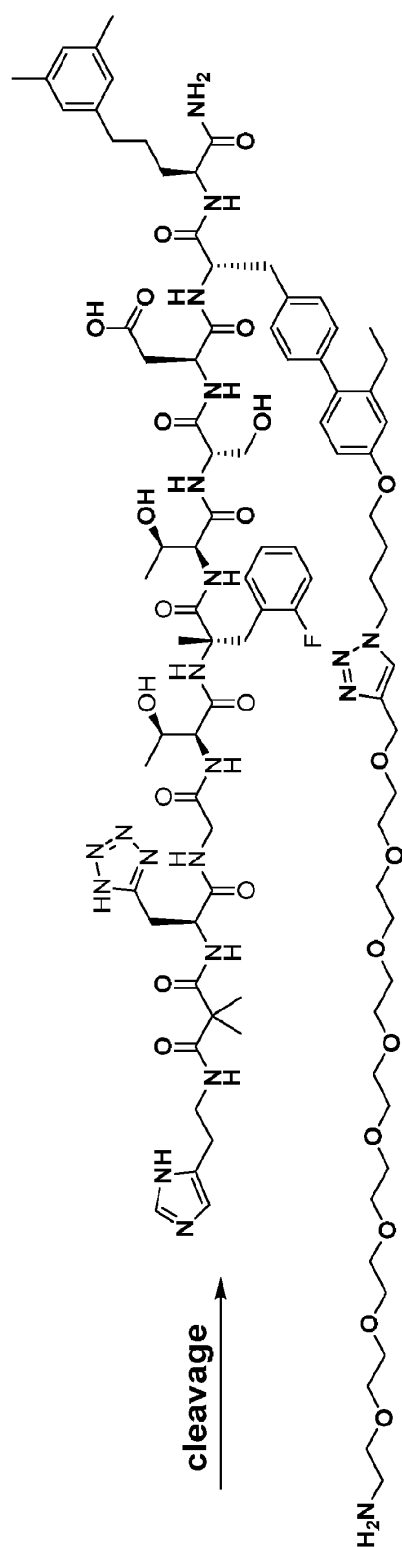


Figure 2

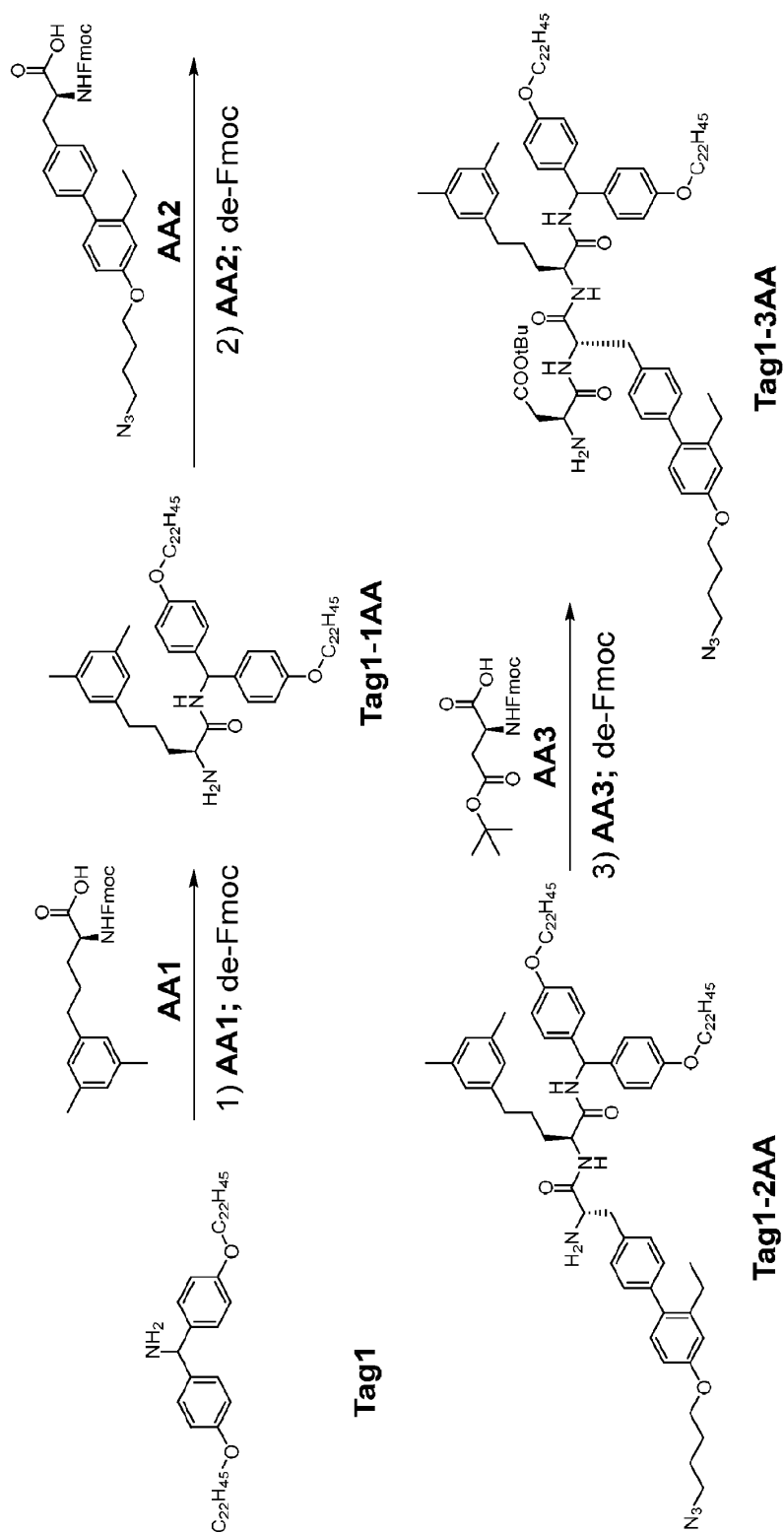
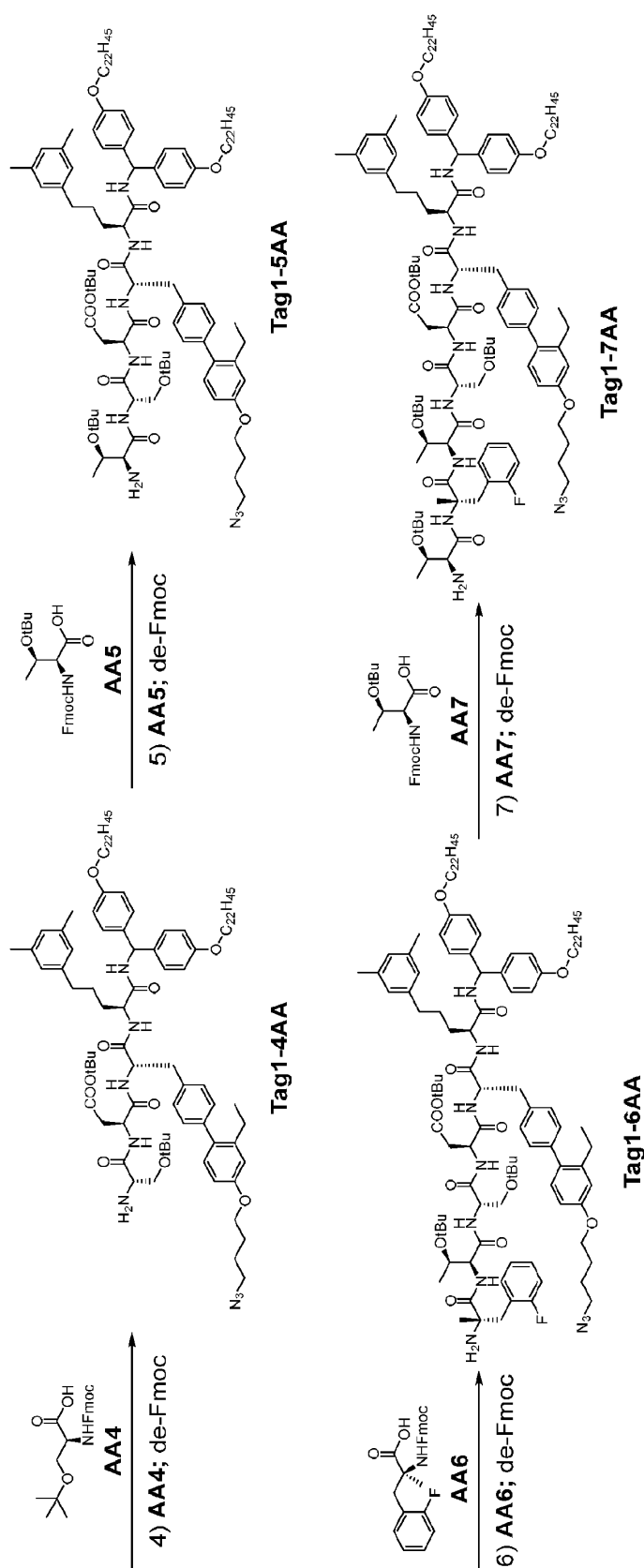


Figure 2 (cont.)



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The reaction scheme illustrates the synthesis of Tag1-10AA. In step 8, the starting material AA10-AA9-AA8-OH, which features a triazole-linked aromatic core with a carboxylic acid group and a trityl-protected amine, is converted to Tag1-10AA. This step involves the introduction of a long alkyl chain with a terminal azide group and the formation of a complex peptide-like structure with multiple amide bonds and side chains, including a long alkyl chain with a terminal azide group. In step 9, the Tag1-10AA intermediate is subjected to cleavage, resulting in the final product, Tag1-10AA, which is a complex molecule with a triazole-linked aromatic core and a long alkyl chain with a terminal azide group.

Figure 3

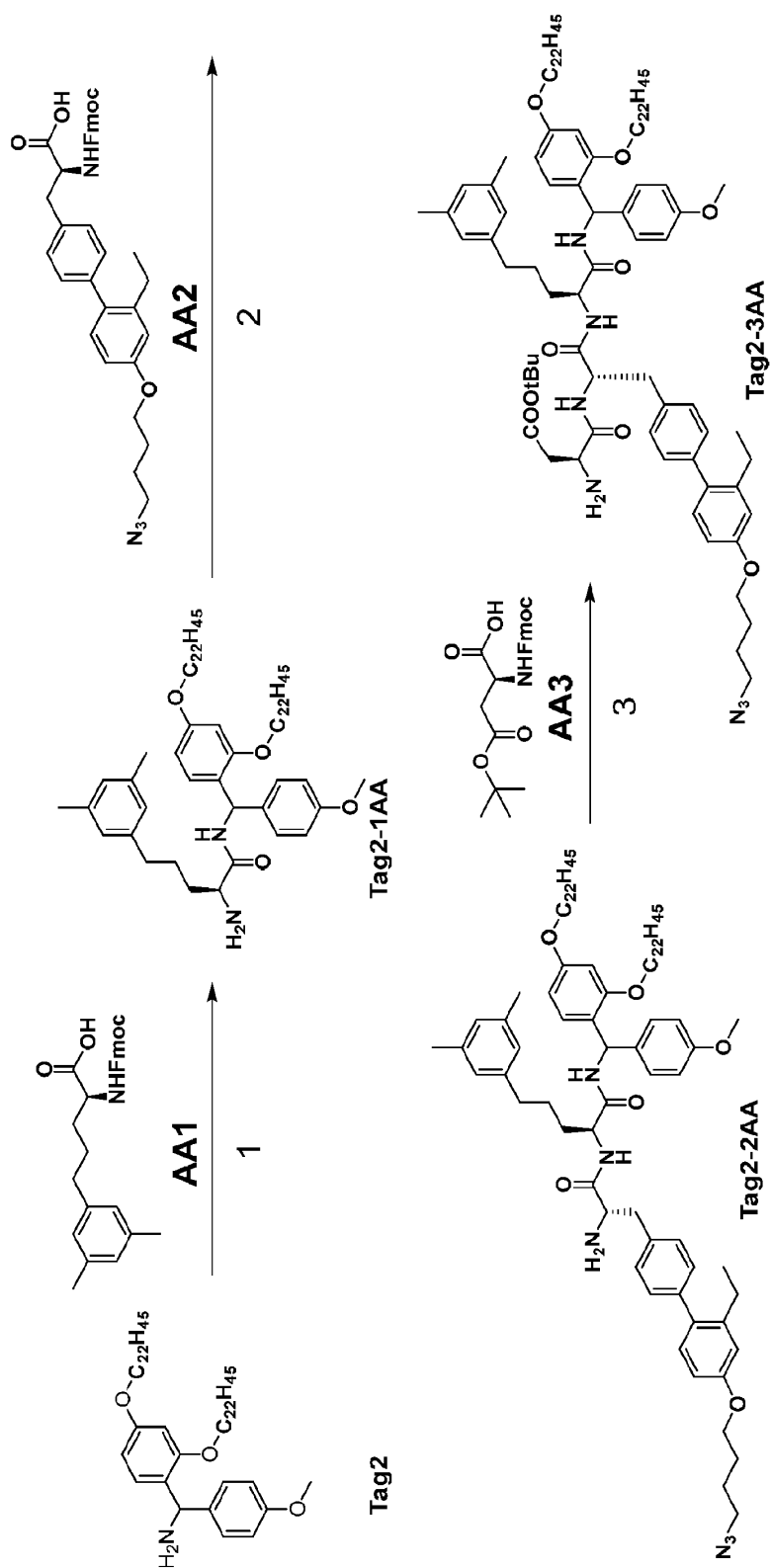
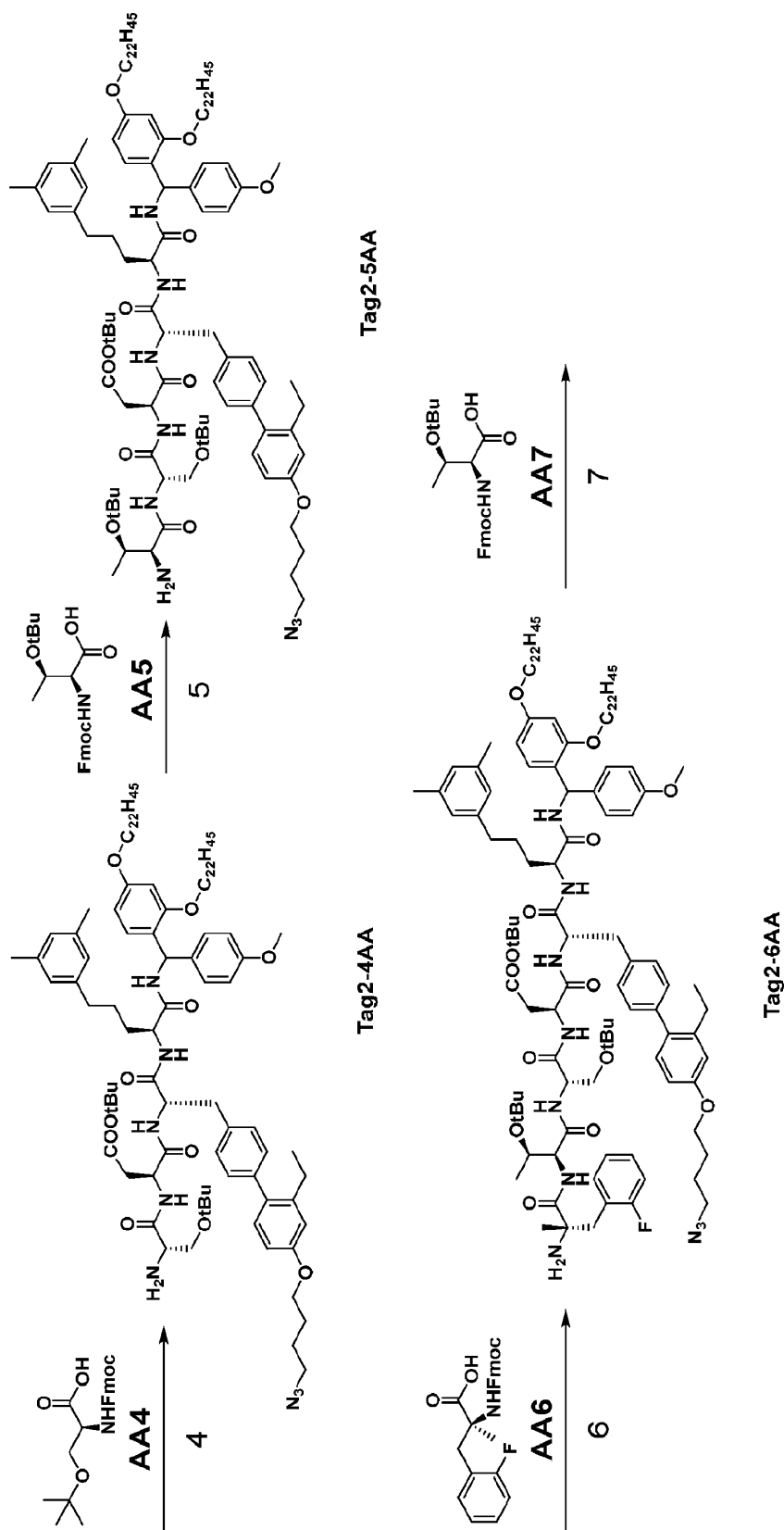


Figure 3 (cont.)



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Figure 3 (cont.)

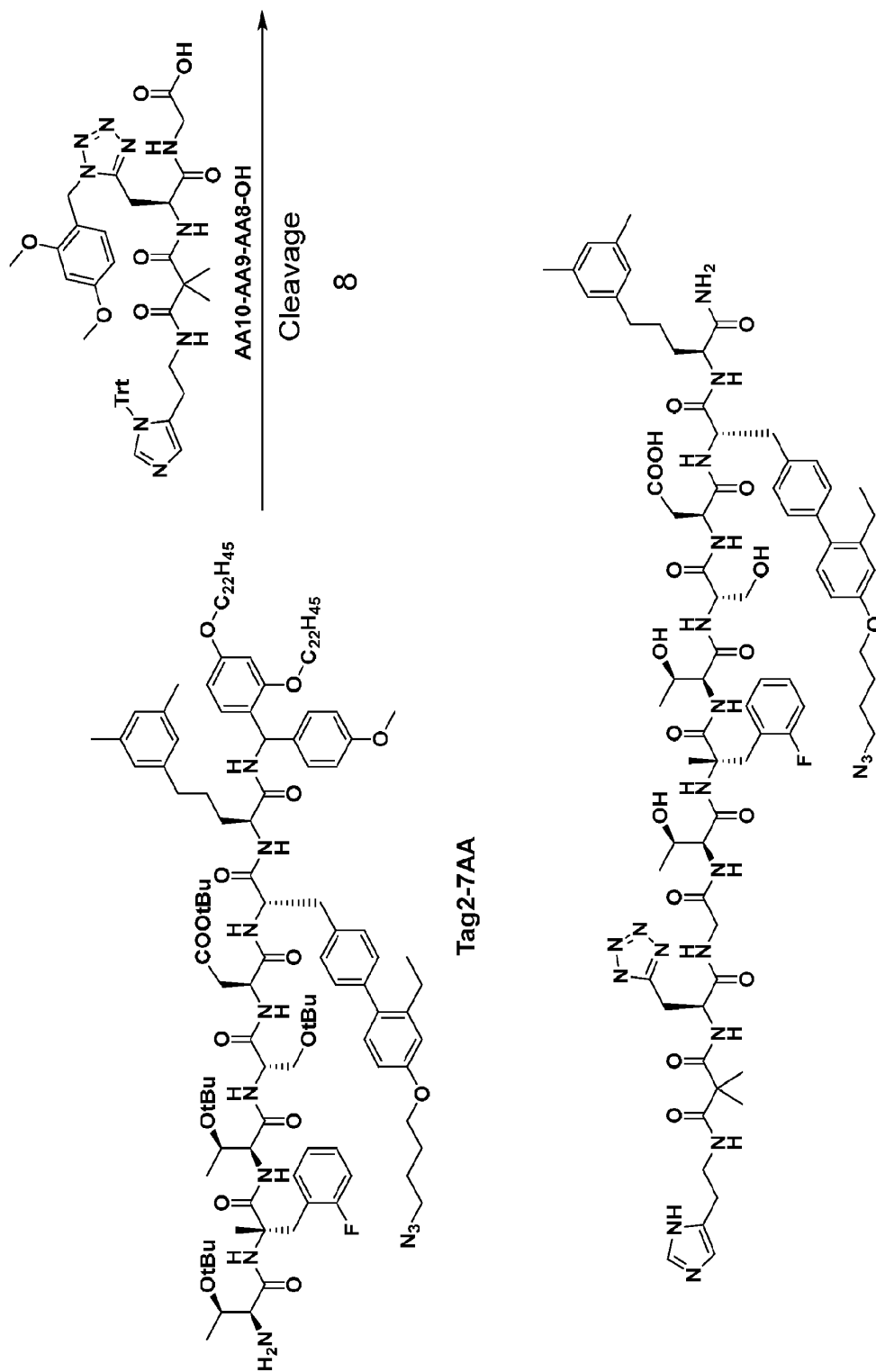


Figure 4

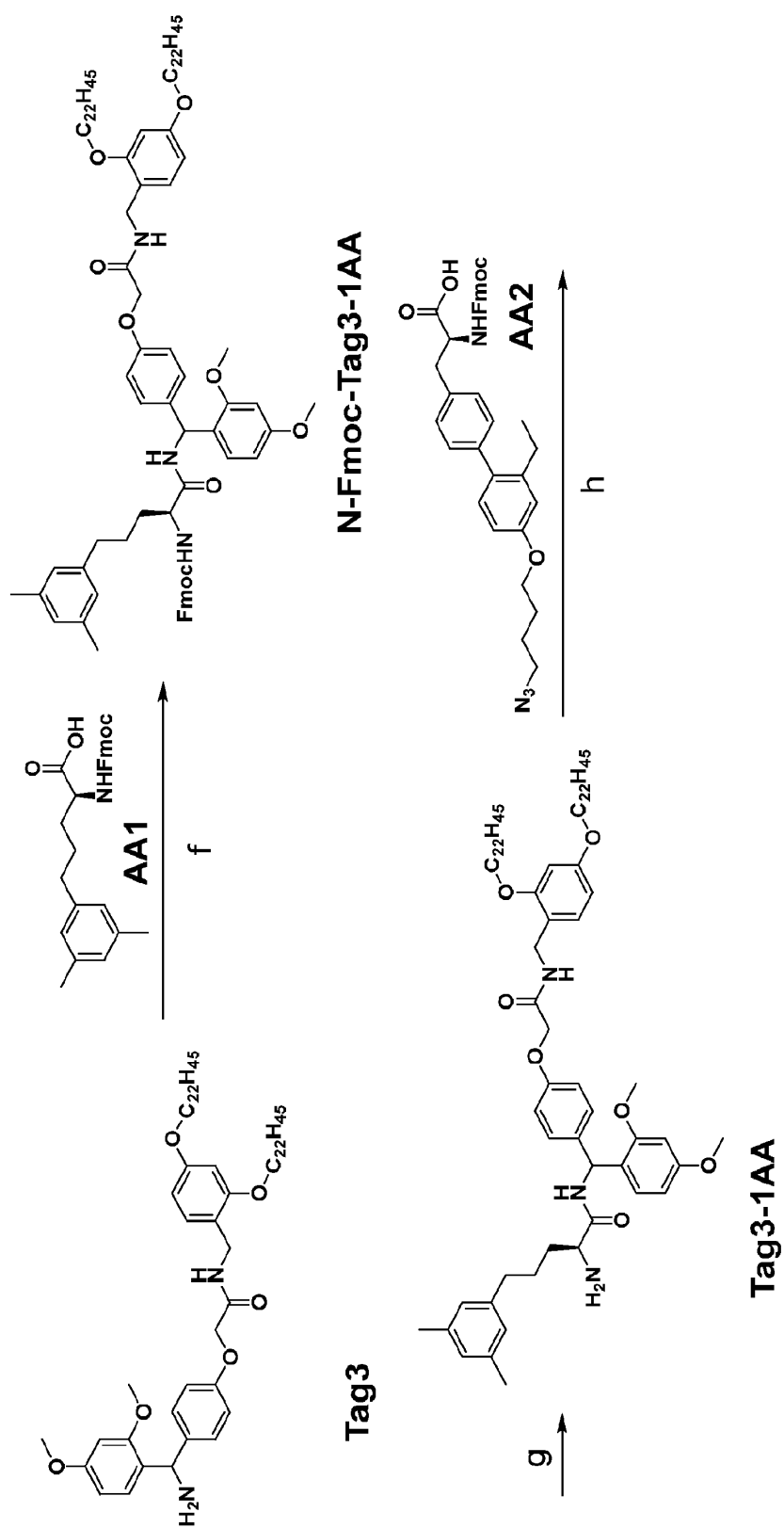
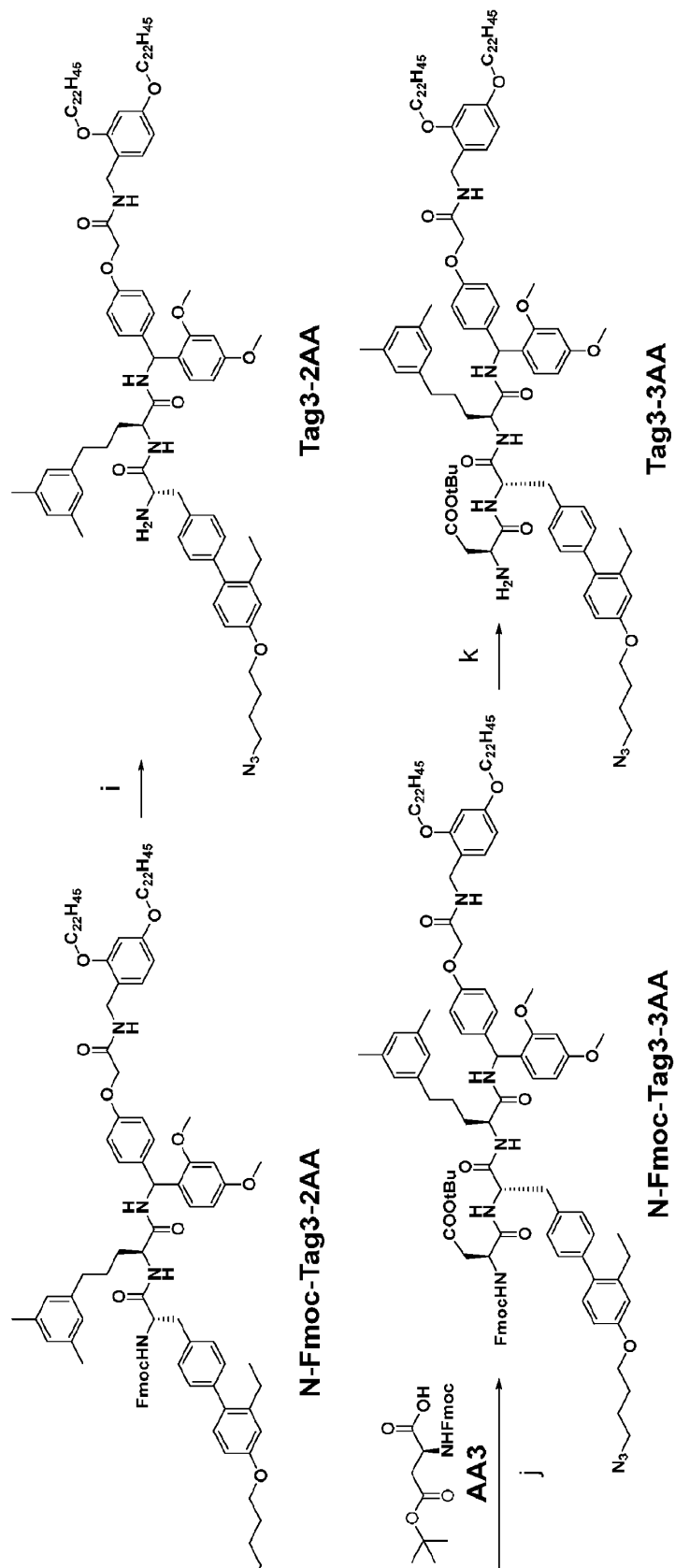
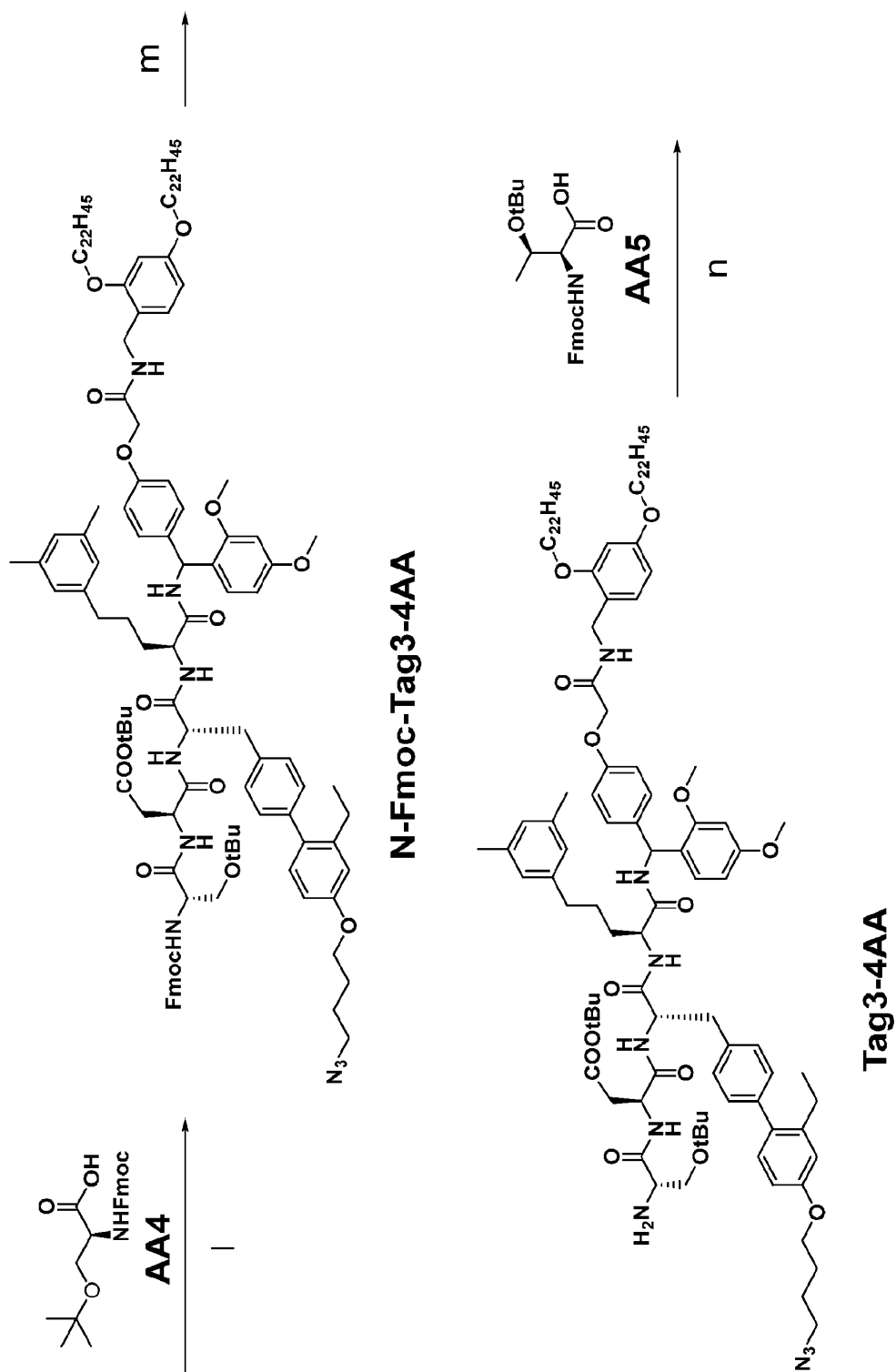


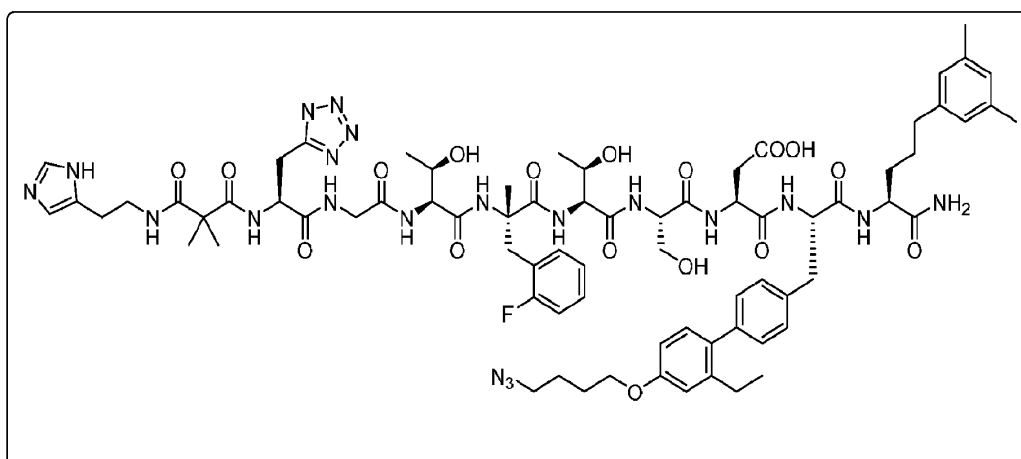
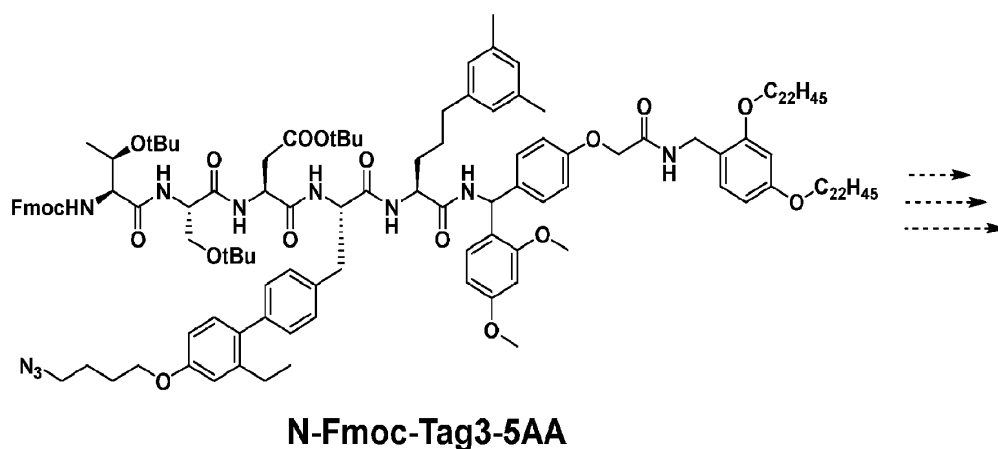
Figure 4 (cont.)



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Figure 4 (cont.)





Reagents and conditions: f) **AA1** (1.5 eq.), DIC (1.5 eq.), HOAt (1.5 eq.), DCM (2 mL), 20 °C, 12 h, 78%; g) 20% piperidine in THF, 20 °C, 2 h, 95%; h) **AA2** (1.5 eq.), DIC (1.5 eq.), HOAt (1.5 eq.), DCM (2 mL), 20 °C, 12 h, 63%; i) 20% piperidine in THF, 20 °C, 2 h, 97%; j) **AA3** (1.5 eq.), DIC (1.5 eq.), HOAt (1.5 eq.), DCM (4 mL), 20 °C, 12 h, 74%; k) 20% piperidine in THF, 20 °C, 2 h, 89%; l) **AA4** (1.5 eq.), DIC (1.5 eq.), HOAt (1.5 eq.), DCM (4 mL), 20 °C, 12 h, 89%; m) 20% piperidine in THF, 20 °C, 2 h, 91%; n) **AA5** (1.5 eq.), DIC (1.5 eq.), HOAt (1.5 eq.), DCM (4 mL), 20 °C, 12 h, 90%.

Figure 4 (cont.)

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Figure 5

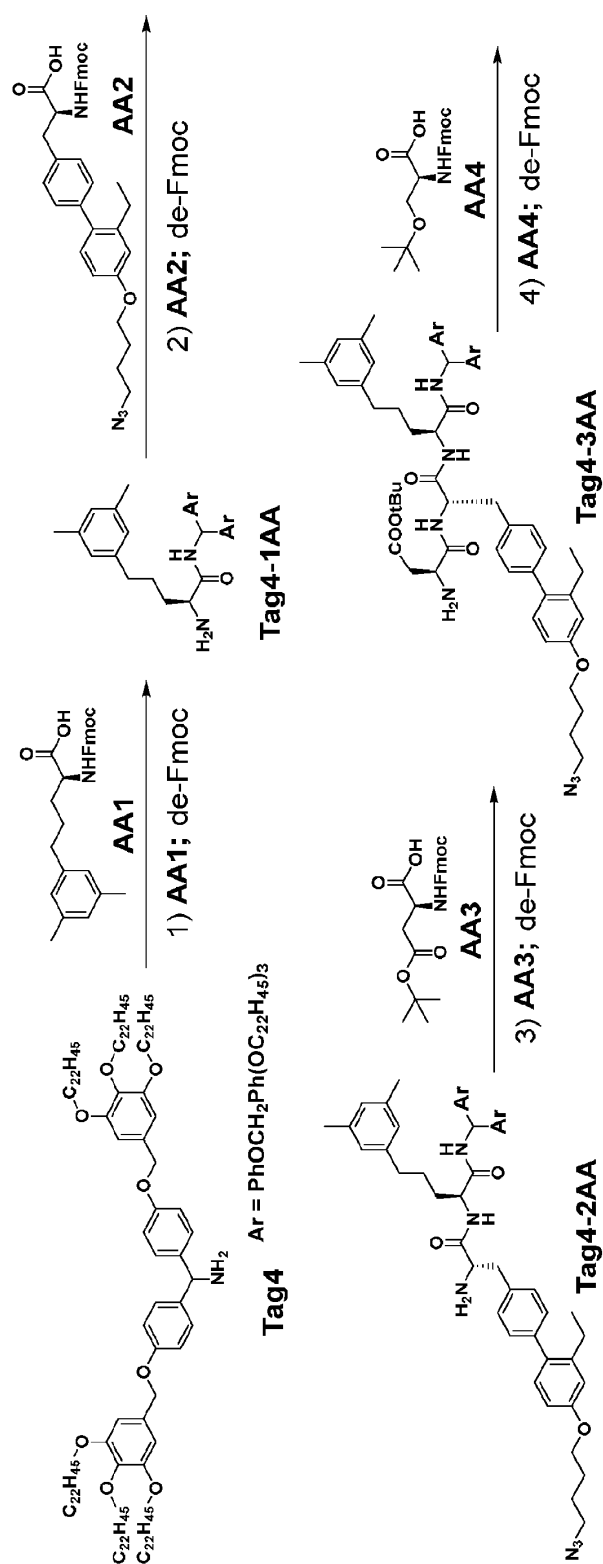


Figure 5 (cont.)

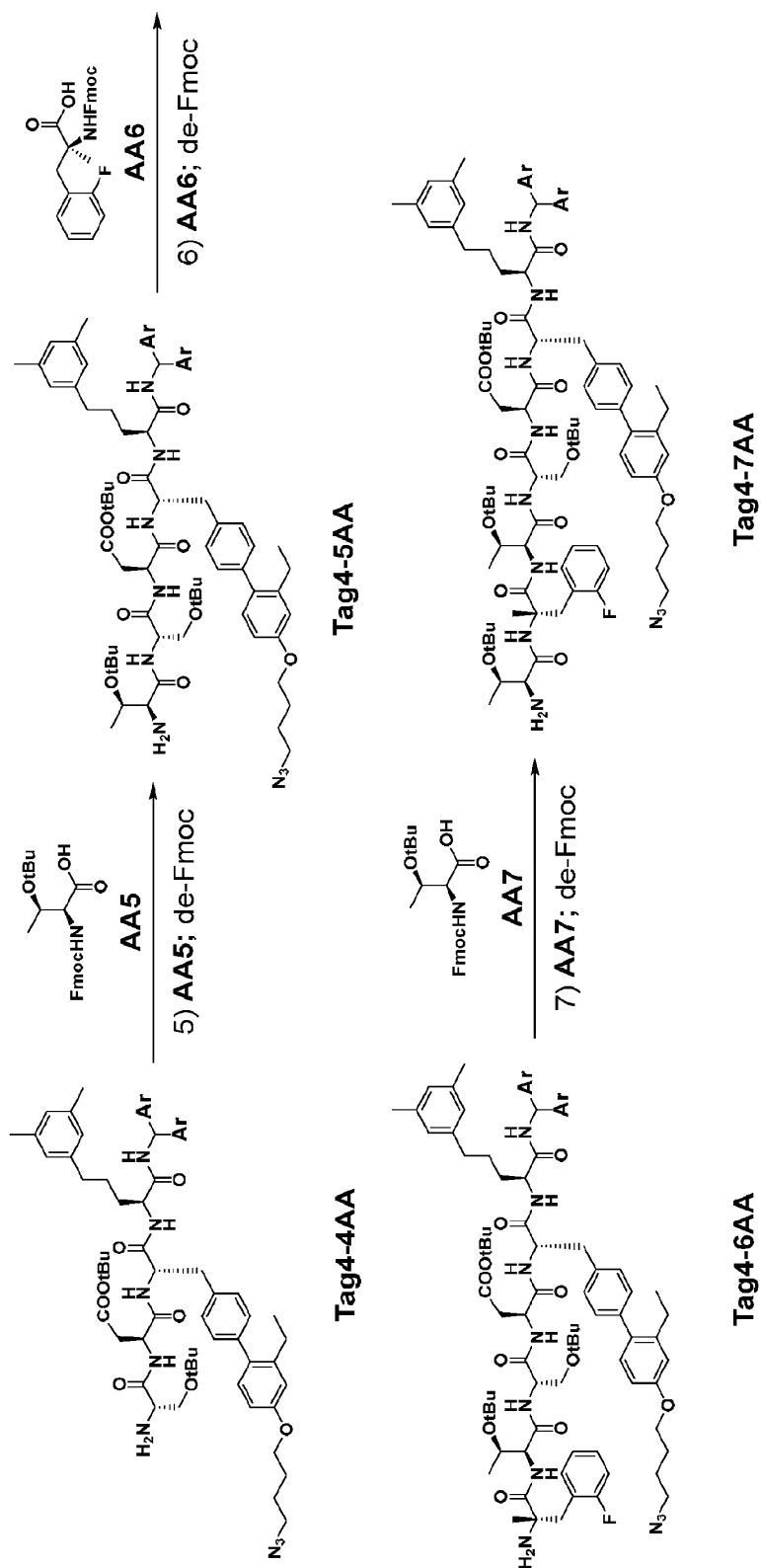
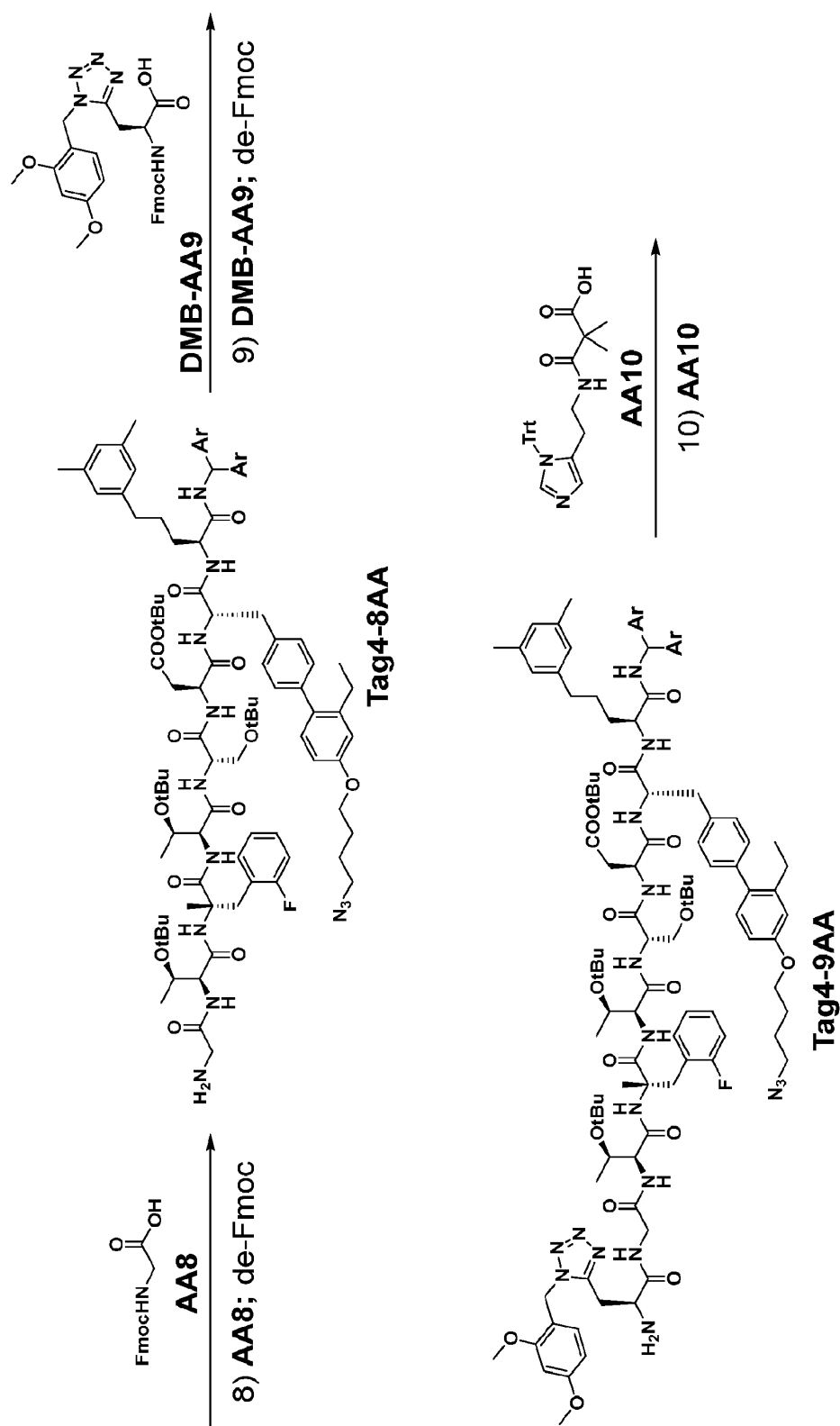
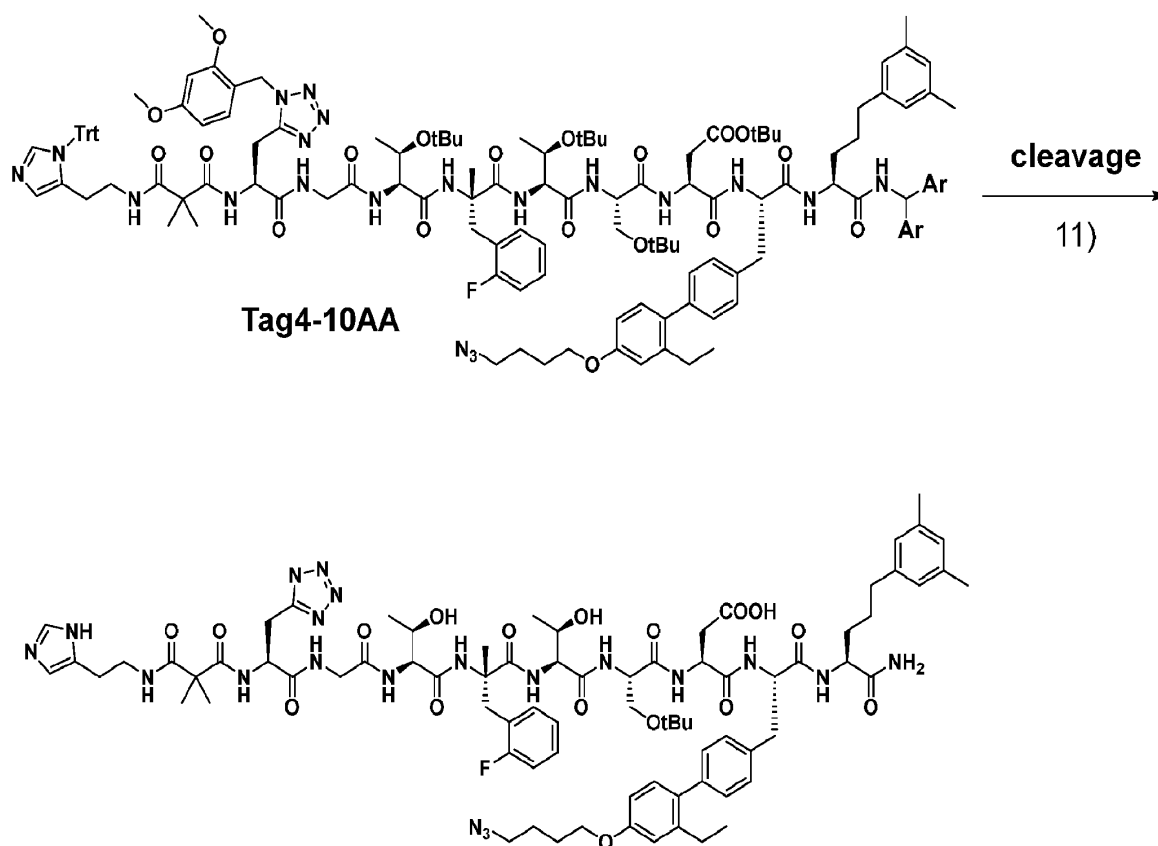


Figure 5 (cont.)





Reagents and conditions:

1) AA1 (1.5 eq.), DIC (1.5 eq.), HOAt (1.5 eq.), DCM (2 mL), 20 °C, 12 h; 20% piperidine in THF, 20 °C, 2 h; 2) AA2 (1.5 eq.), DIC (1.5 eq.), HOAt (1.5 eq.), DCM (2 mL), 20 °C, 12 h; 20% piperidine in THF, 20 °C, 2 h; 3) AA3 (1.5 eq.), DIC (1.5 eq.), HOAt (1.5 eq.), DCM (4 mL), 20 °C, 12 h; 20% piperidine in THF, 20 °C, 2 h; 4) AA4 (1.5 eq.), DIC (1.5 eq.), HOAt (1.5 eq.), DCM (4 mL), 20 °C, 12 h; 20% piperidine in THF, 20 °C, 2 h; 5) AA5 (1.5 eq.), DIC (1.5 eq.), HOAt (1.5 eq.), DCM (4 mL), 20 °C, 12 h; 20% piperidine in THF, 20 °C, 2 h; 6) AA6 (1.5 eq.), DIC (1.5 eq.), HOAt (1.5 eq.), DCM (4 mL), 20 °C, 12 h; 20% piperidine in THF, 20 °C, 2 h; 7) AA7 (1.5 eq.), DIC (1.5 eq.), HOAt (1.5 eq.), DCM (4 mL), 20 °C, 12 h; 20% piperidine in THF, 20 °C, 2 h; 8) AA8 (1.5 eq.), DIC (1.5 eq.), HOAt (1.5 eq.), DCM (4 mL), 20 °C, 12 h; 20% piperidine in THF, 20 °C, 2 h; 9) AA9 (1.5 eq.), DIC (1.5 eq.), HOAt (1.5 eq.), DCM (4 mL), 20 °C, 12 h; 20% piperidine in THF, 20 °C, 2 h; 10) AA10 (1.5 eq.), DIC (1.5 eq.), HOAt (1.5 eq.), DCM (4 mL), 20 °C, 12 h; 11) TFA cocktail (TFA:Tips:H₂O = 95:2.5:2.5), 20 °C, 1.5 h.

Figure 5 (cont.)

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Figure 6

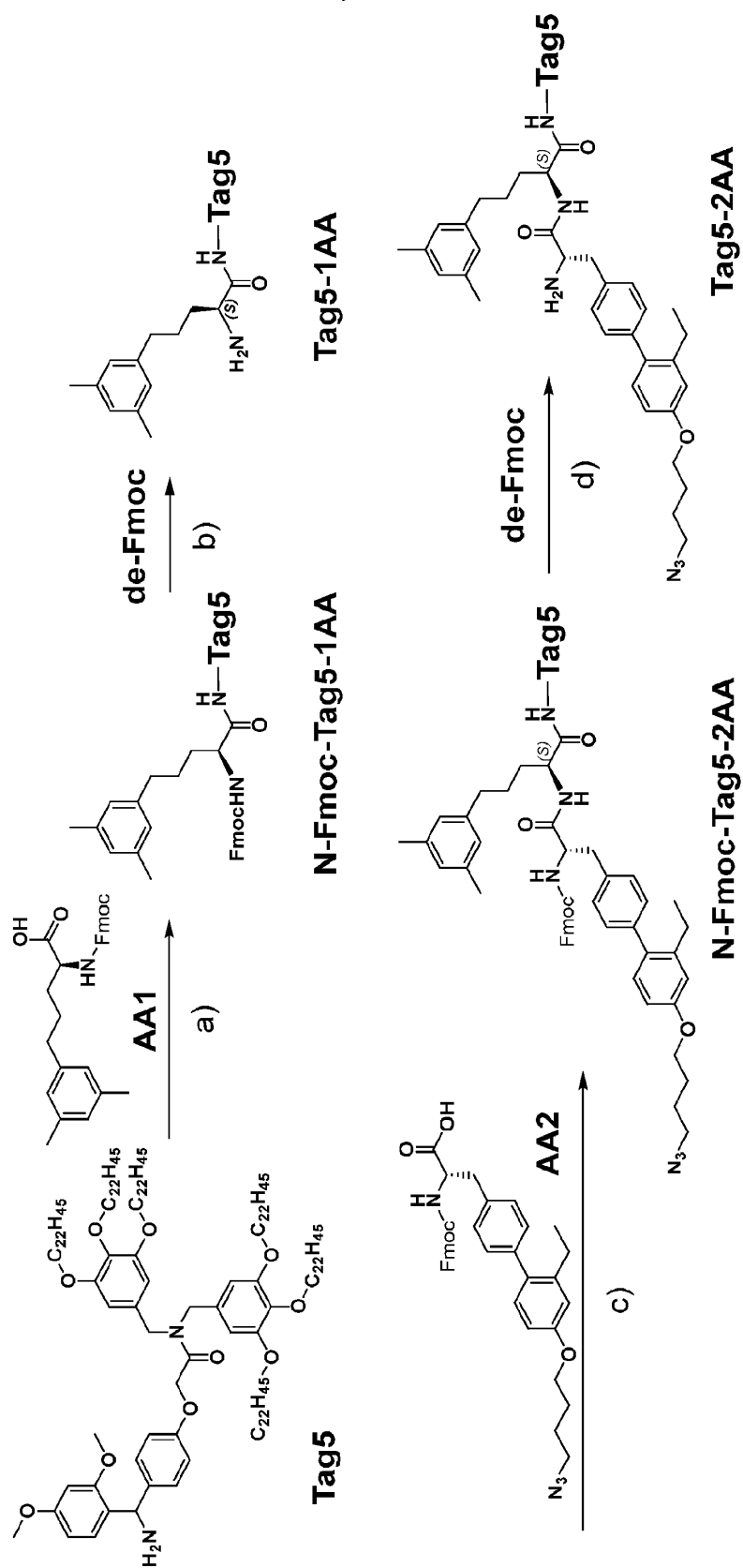


Figure 6 (cont.)

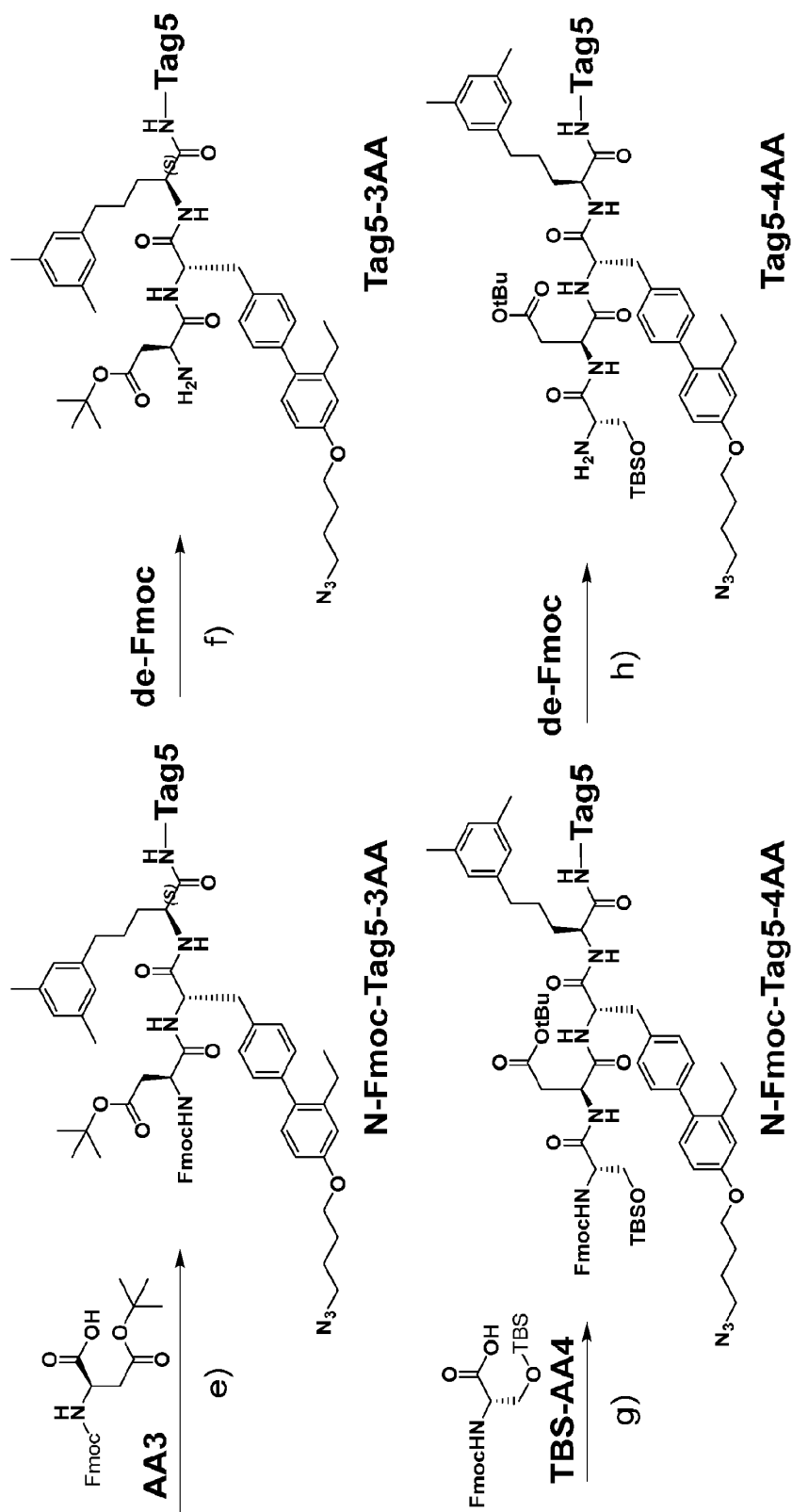


Figure 6 (cont.)

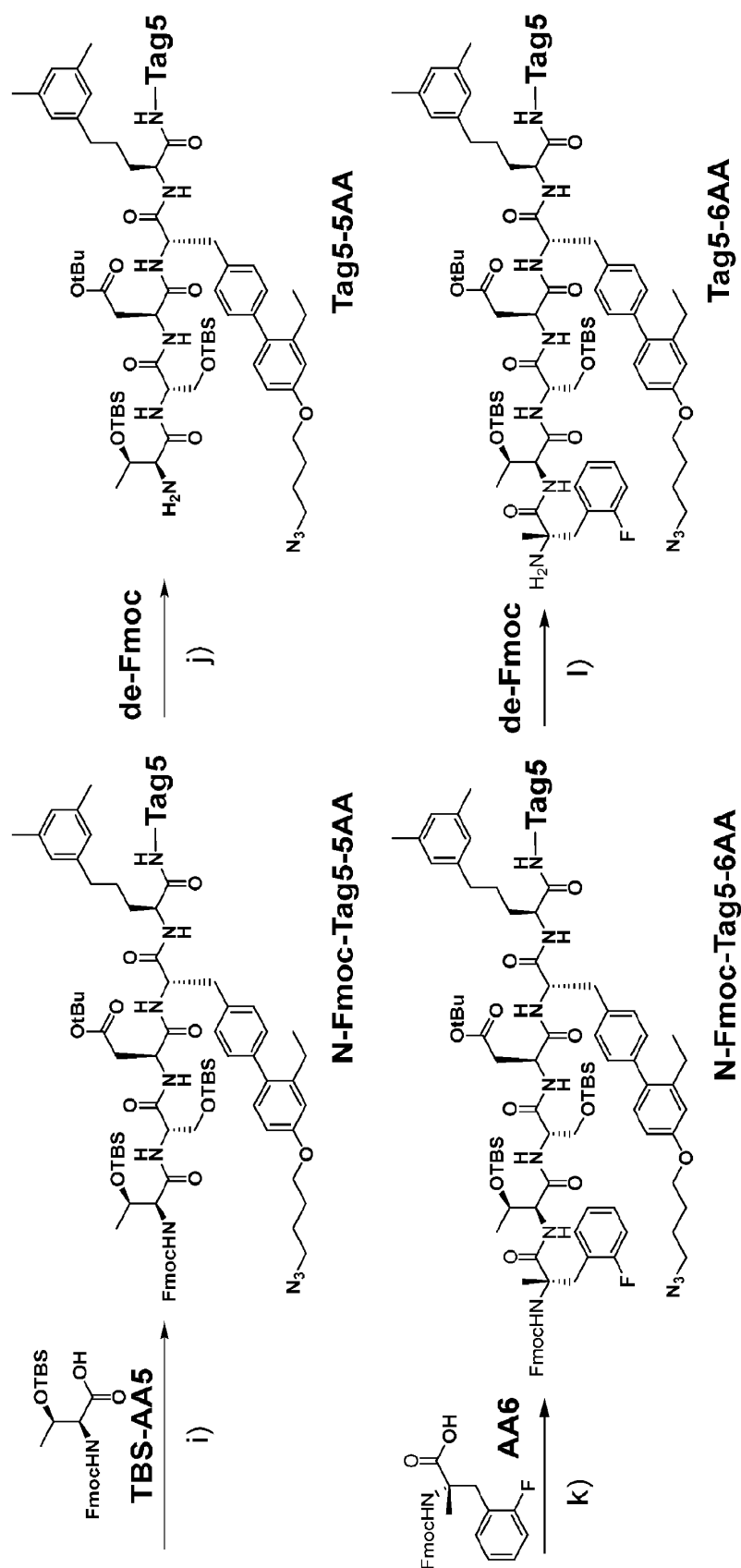


Figure 6 (cont.)

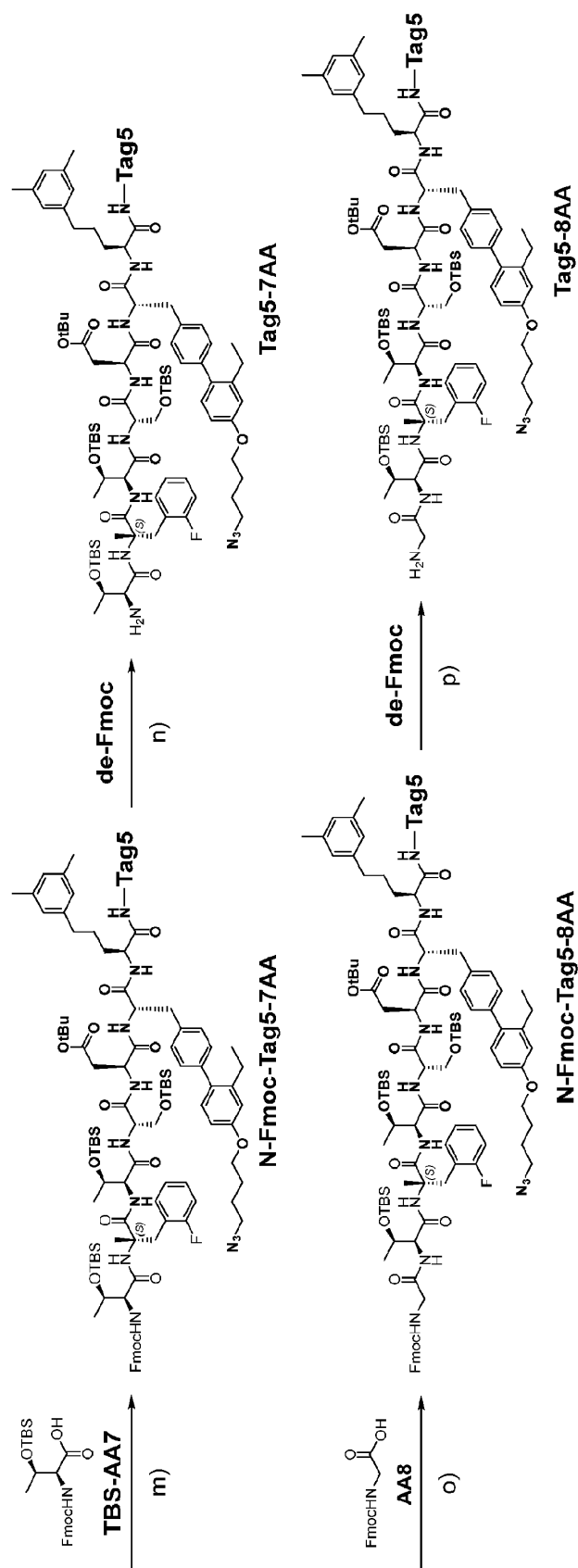
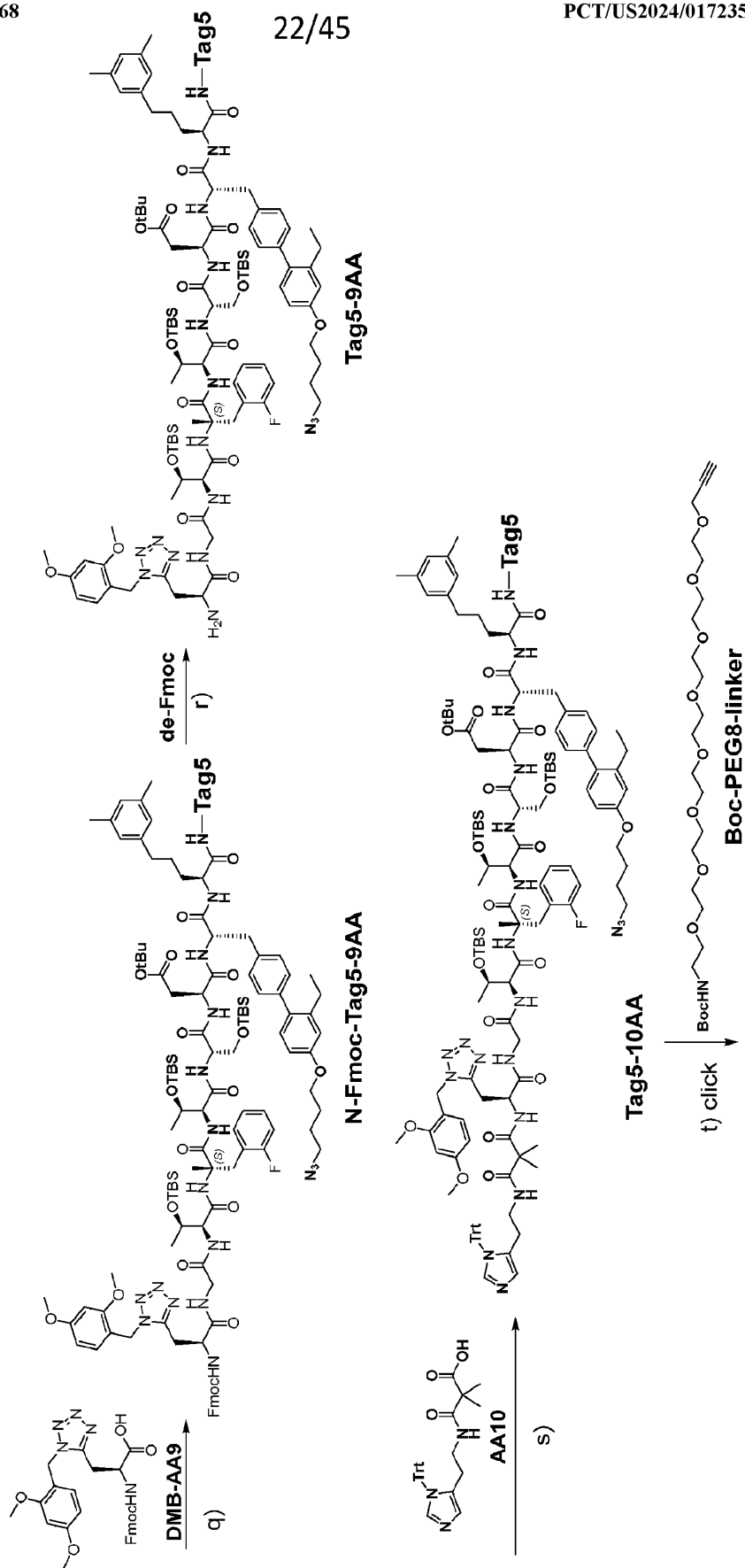


Figure 6 (cont.)



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Figure 6 (cont.)

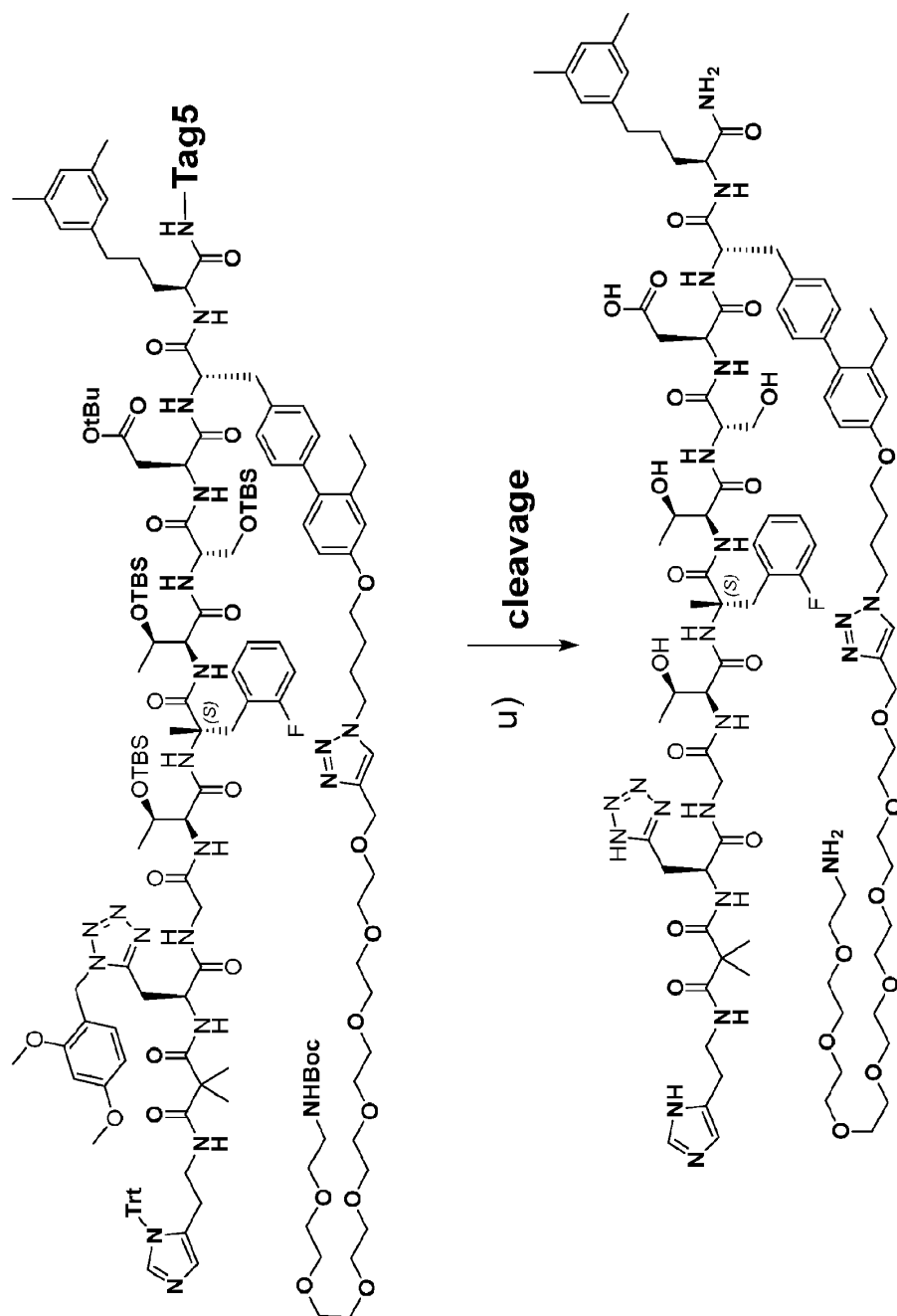


Figure 7

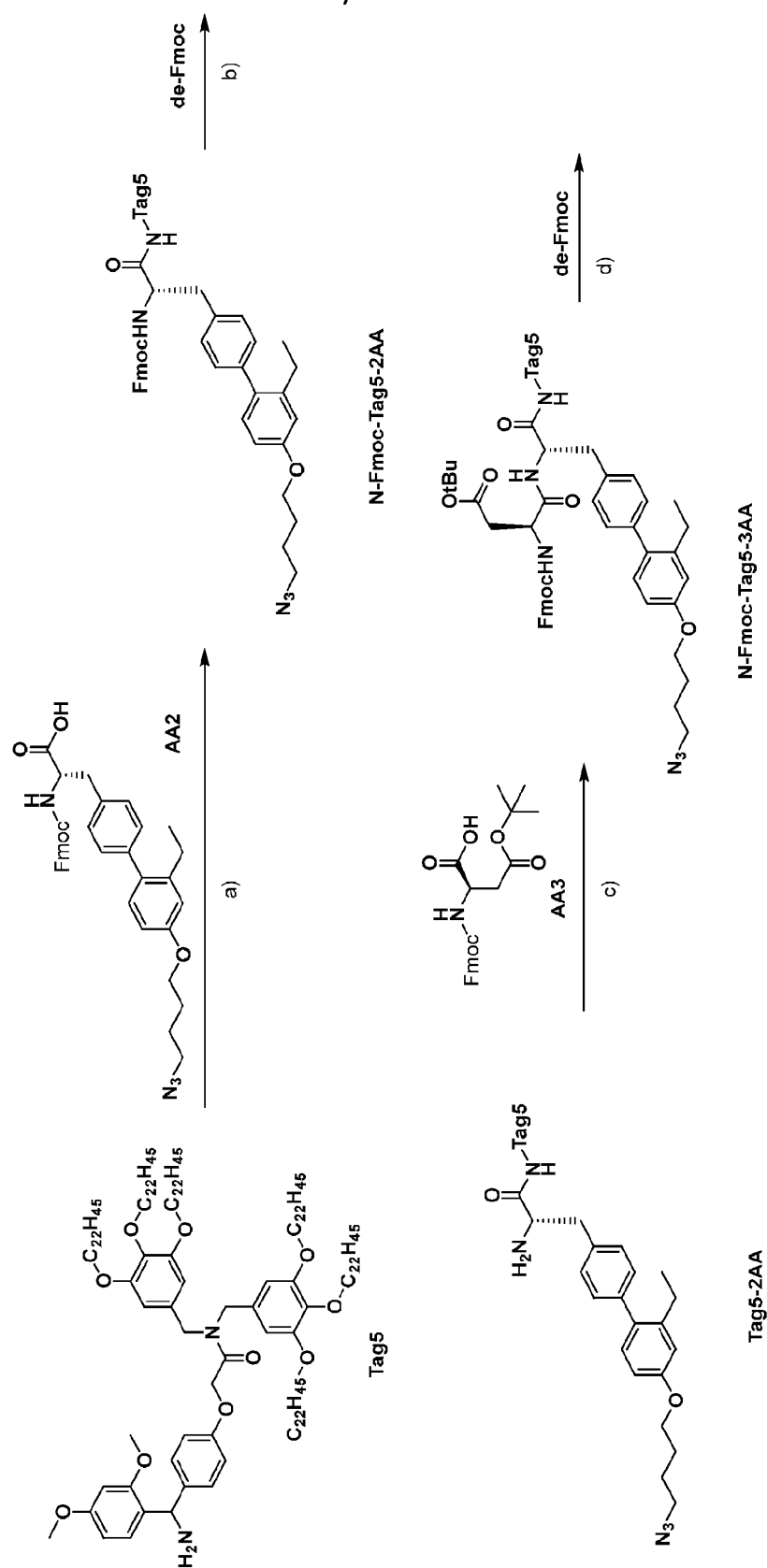
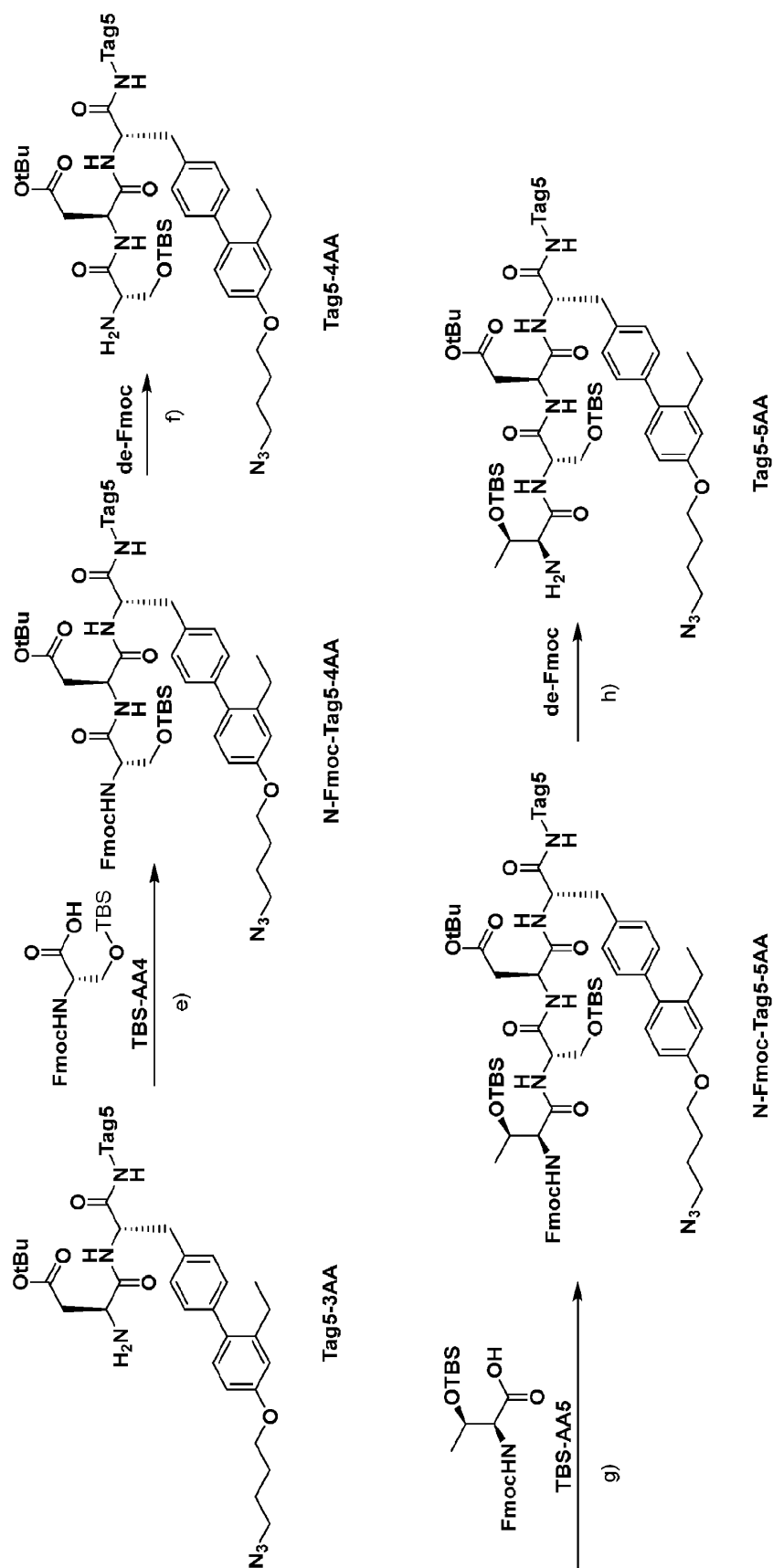
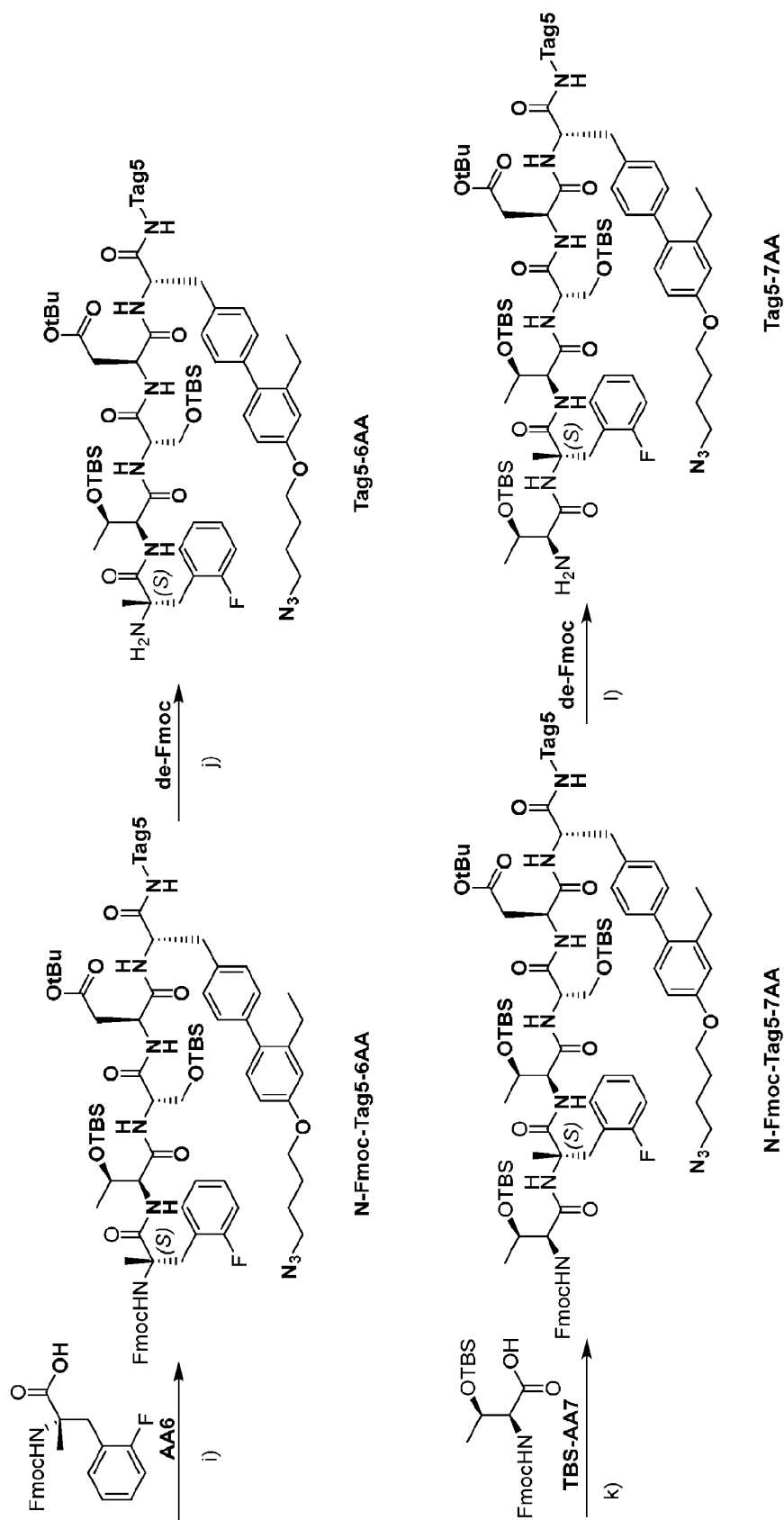


Figure 7 (cont.)



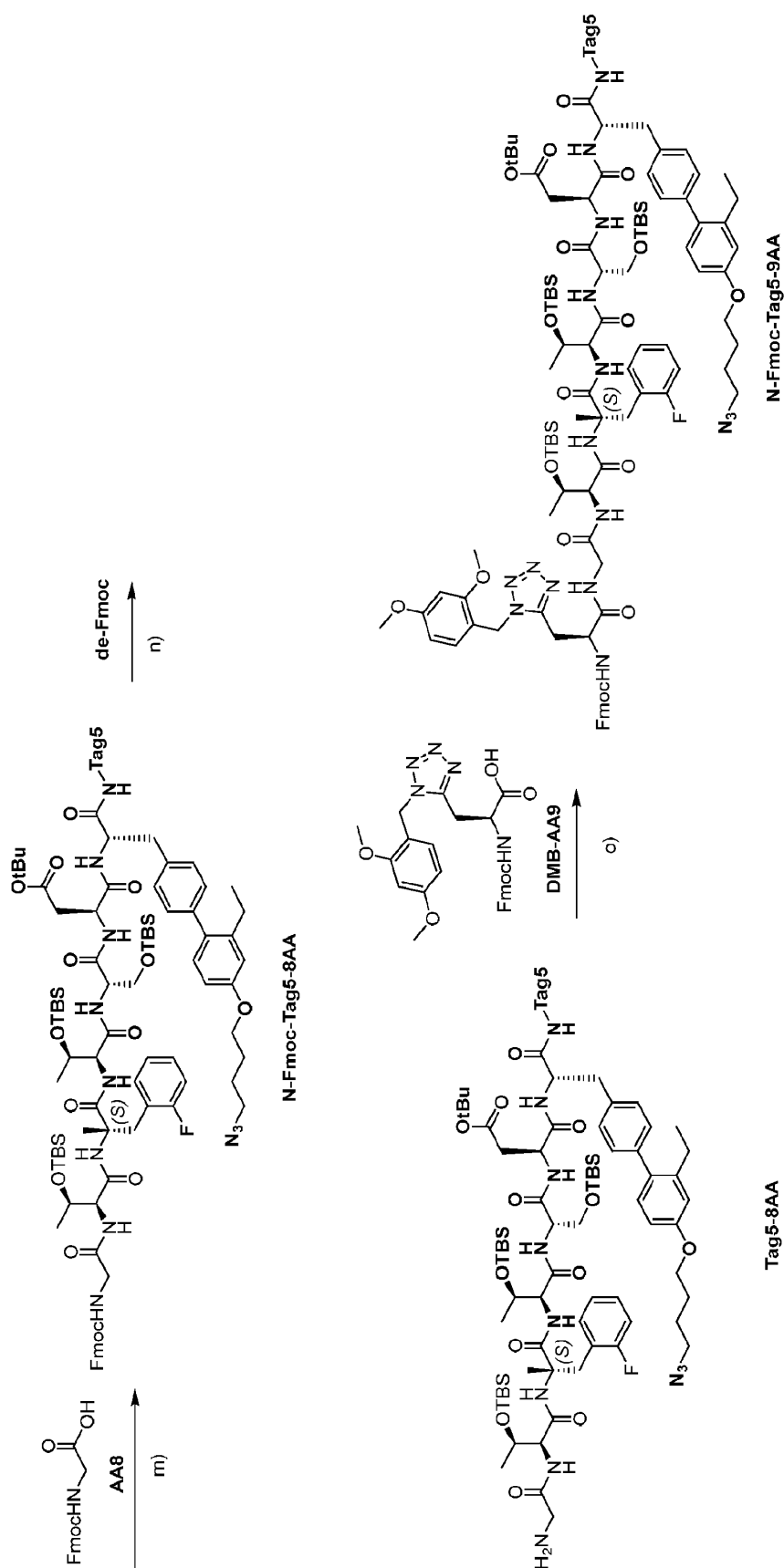
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Figure 7 (cont.)



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Figure 7 (cont.)



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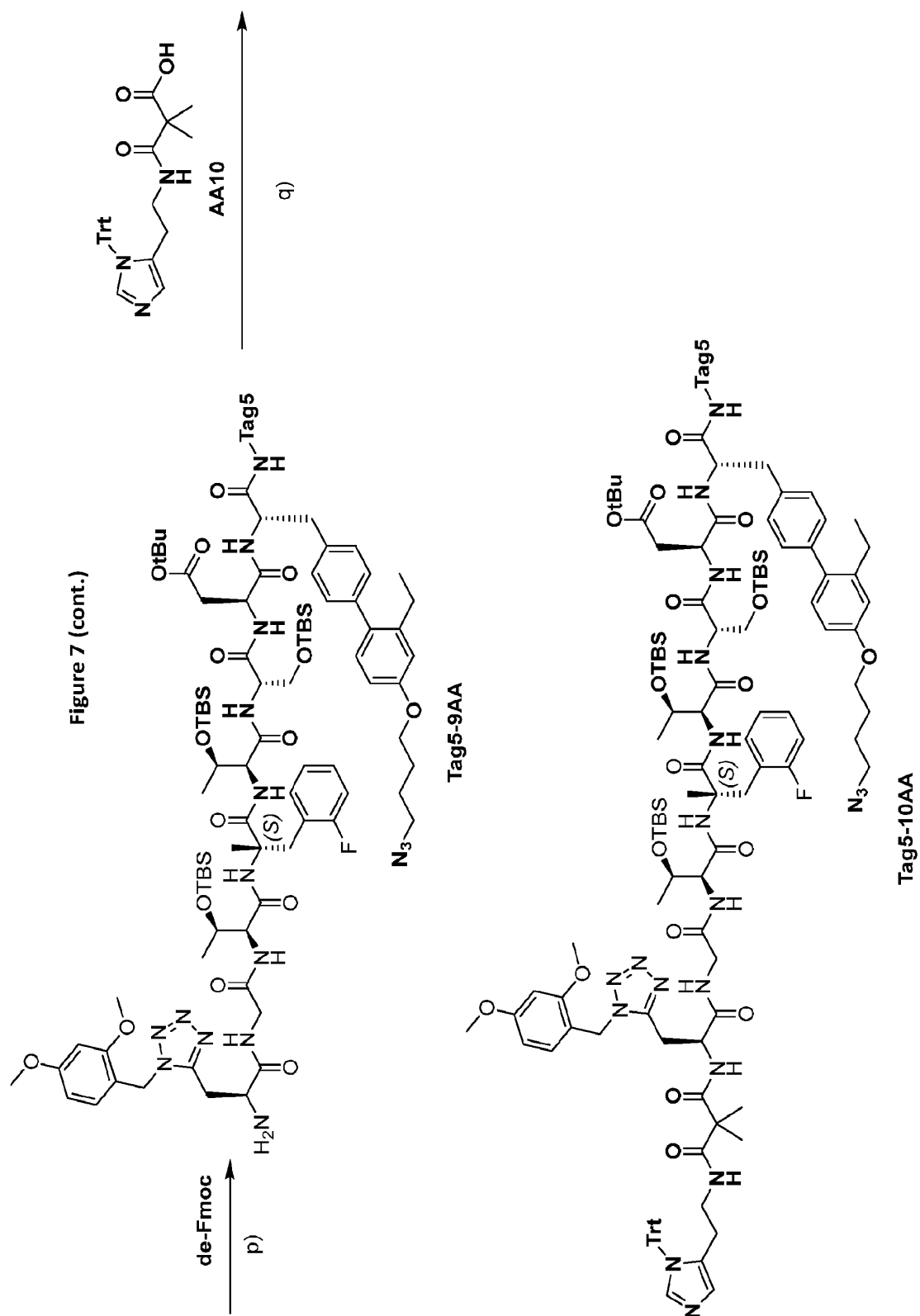
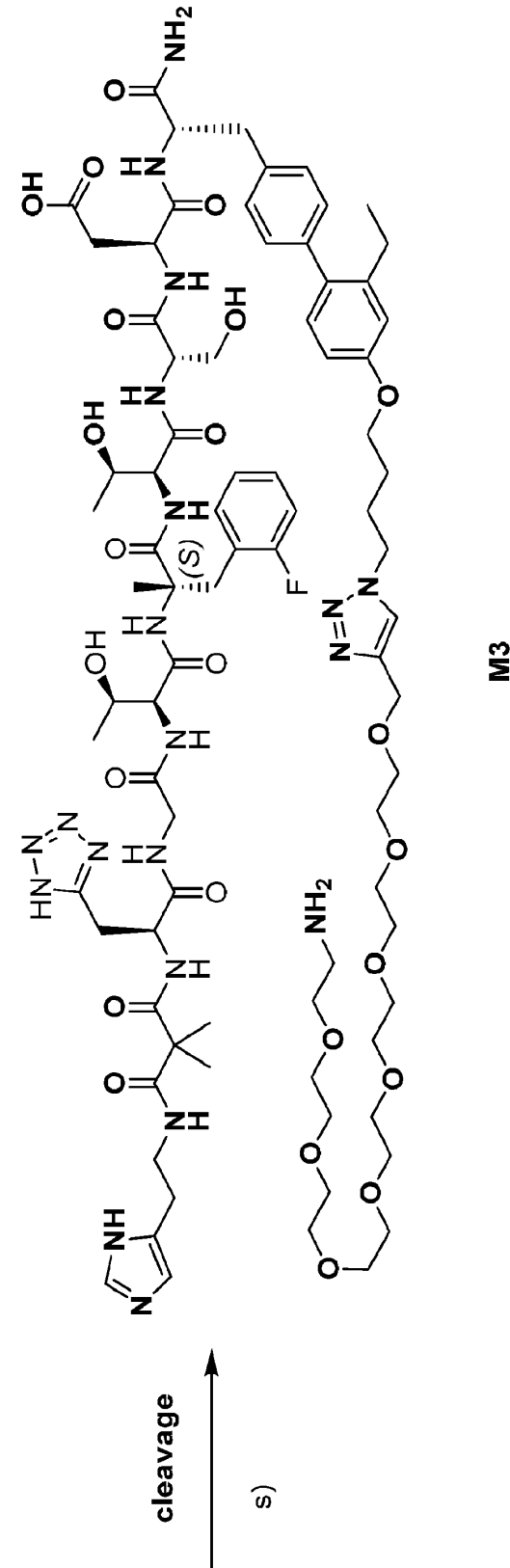
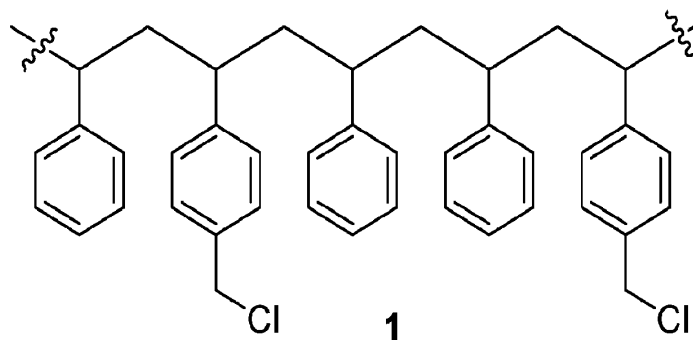


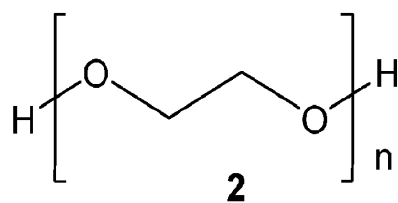
Figure 7 (cont.)



cleavage
s)
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Chloromethylated polystyrene polymer



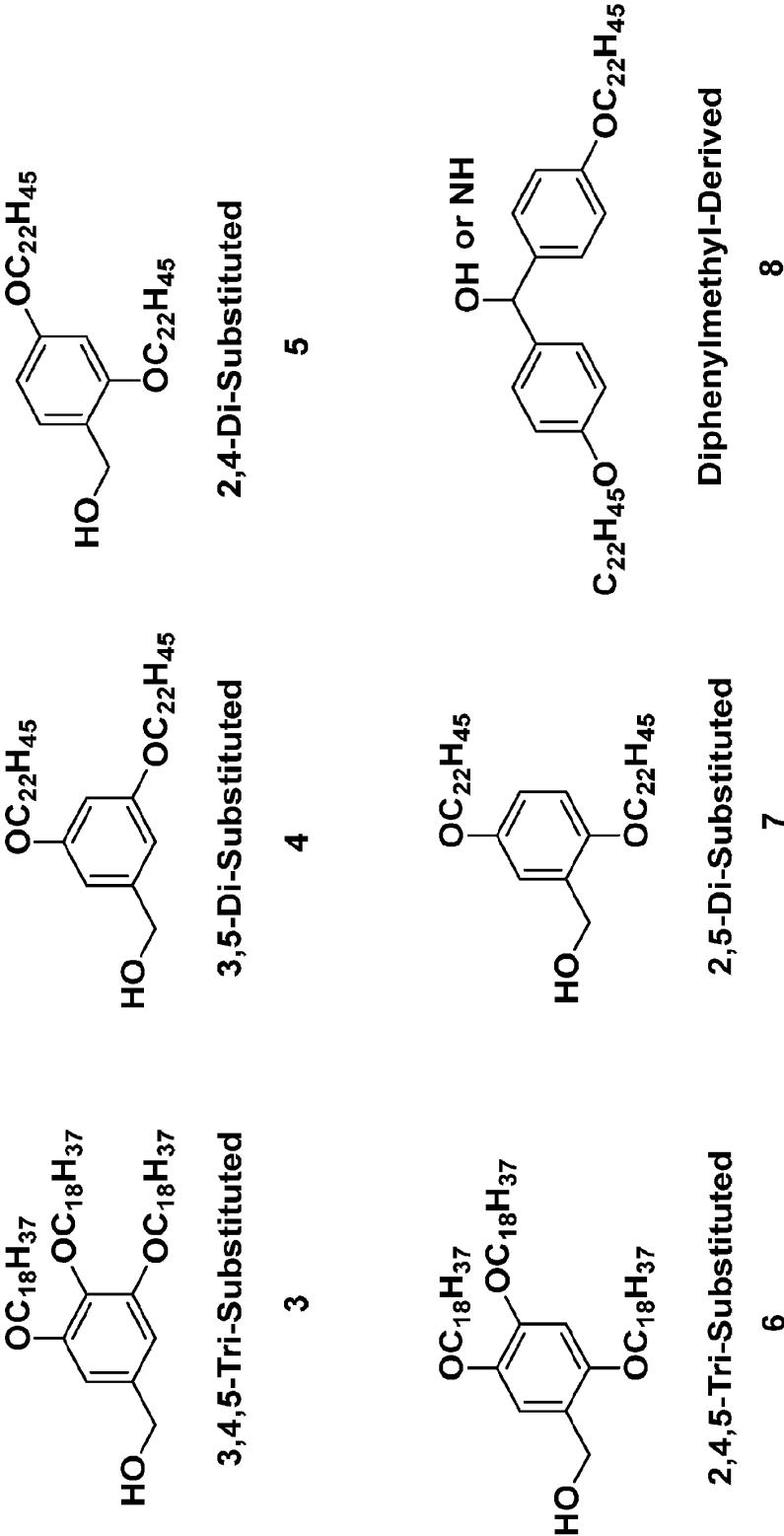
**PEG (molecular
weights 2000 to 20000)**



abbreviated as

Figure 8

Figure 8 (cont.)



A series of hydrophobic benzyl alcohols tags

Figure 8 (cont.)

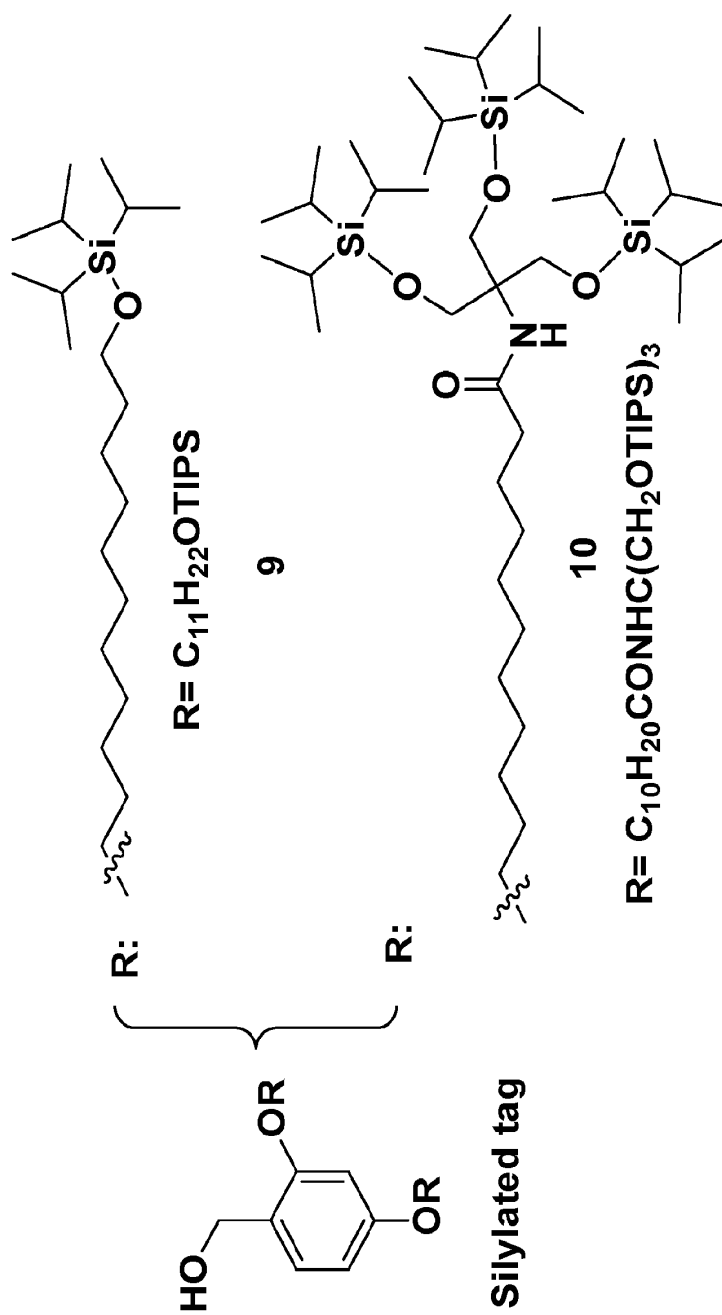


Figure 9

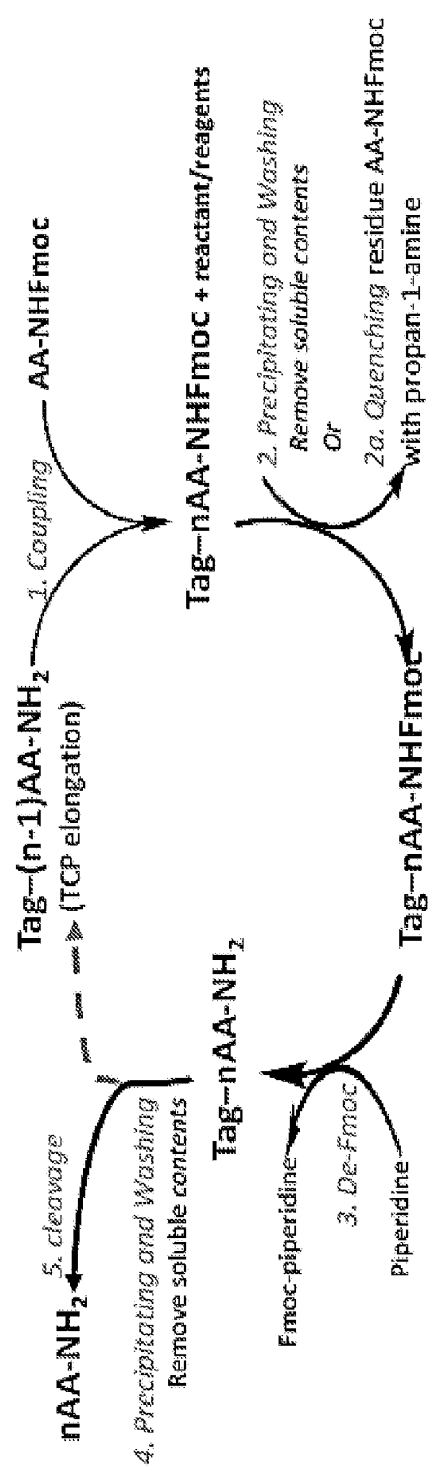


Figure 10A

Amide coupling reaction					de-Fmoc		Product			
Step #	amino acid	DIC	HOBt	Crude yield ^d	Step #	20% piperidine in THF	Crude yield	#	Found MS (M+H) ⁺	LC-MS method
a	AA1	1.5 eq.	1.5 eq.	84.2%	b	30 V	99.0%	Tag5-1AA	221.1 [M-Tag5+2H] ⁺	A
c	AA2	1.5 eq.	1.5 eq.	86.7%	d	30 V	99.4%	Tag5-2AA	585.4 [M-TAG+2H] ⁺	A
e	AA3	1.5 eq.	1.5 eq.	99.9%	f	20 V	98.7%	Tag5-3AA	778.5 [M-TAG+2H+Na] ⁺	B
g	TBS-AA4	1.5 eq.	1.5 eq.	85.0%	h	20 V	99.0%	Tag5-4AA	787.4 [M-TAG-tBu-TBS+4H] ⁺	A
i	TBS-AA5	1.5 eq.	1.5 eq.	94.3%	j	20 V	84.2%	Tag5-5AA	1002.7 [M-TAG-tBu-TBS+4H] ⁺	B
k	AA6	1.5 eq.	1.5 eq.	96.8%	l	20 V	94.7%	Tag5-6AA	1067.7 [M-TAG-tBu-2TBS+5H] ⁺	B
m	TBS-AA7	10 eq.	10 eq.	100.0%	n	20 V	99.1%	Tag5-7AA	1282.7 [M-TAG-tBu-2TBS+5H] ⁺	B
o	AA8	1.5 eq.	1.5 eq.	78.2%	p	20 V	98.8%	Tag5-8AA	1225.6 [M-TAG-tBu-3TBS+6H] ⁺	A
q	DMB-AA9	1.5 eq.	1.5 eq.	90.5%	r	20 V	99.1%	Tag5-9AA	1364.7 [M-TAG-tBu-3TBS-DMB+7H] ⁺	B
s	AA10	1.5 eq.	1.5 eq.	96.6%				Tag5-10AA	786.7 [M-TAG-tBu-3TBS-DMB-Ttt+9H] ²⁺	A
t	Click with PEG8-Linker (3 eq.) in THF, CuSO ₄ ·H ₂ O (1.5 eq.) and Sodium ascorbate (1.5 eq.) in H ₂ O, at RT for 12 hrs							Tag5-M1	990.4 [M-Tag5-tBu-3TBS-DMB-Ttt-Boc+10H] ²⁺	
u	Cleavage with TFA cocktail (TFA/TIPS/H ₂ O = 95/2.5/2.5) in DCM (3/1, 100 V), 20°C, 12hr							M1	990.5 [M+2H] ²⁺	

Figure 10B

<i>coupling</i>	amino acid	DIC	HOAt	<i>Quench</i> w/ $C_6H_5NH_2$	<i>de-Fmoc</i>	20% piperidine in THF	yield
<i>a</i>	AA1	1.5 eq.	1.5 eq.		<i>b</i>	20 V	99.0%
<i>c</i>	AA2	1.5 eq.	1.5 eq.		<i>d</i>	20 V	99.4%
<i>e</i>	AA3	1.5 eq.	1.5 eq.		<i>f</i>	20 V	98.7%
<i>g</i>	TBS-AA4	1.5 eq.	1.5 eq.		<i>h</i>	20 V	99.0%
<i>i</i>	TBS-AA5	1.5 eq.	1.5 eq.		<i>j</i>	20 V	84.2%
<i>k</i>	AA6	1.5 eq.	1.5 eq.		<i>l</i>	20 V	94.7%
<i>m</i>	TBS-AA7	10 eq.	10 eq.		<i>n</i>	20 V	99.1%
<i>o</i>	AA8	1.5 eq.	1.5 eq.		<i>p</i>	20 V	98.8%
<i>q</i>	DMB-AA9	1.5 eq.	1.5 eq.		<i>r</i>	20 V	99.1%
<i>s</i>	AA10	1.5 eq.	1.5 eq.		<i>t</i>	Click reaction	99.4%

Figure 11A

Amide coupling reaction Molar eq per Tag5 (0.04 mmol)					de-Fmoc (V stands 1 mL of solvent per gram of AA#)			Product	
Step #	Amino acid	DIC	HOBt	Crude yield	Step#	20% piperidine in THF	Crude yield	#	Found MS (M+H) ⁺ After Cleavage to remove Tag5 and protective groups
a	AA1	1.5	1.5	84.2%	b	20 V	99.0%	Tag5- 1AA	221.1 [M-TAG+2H] ⁺
c	AA2	1.5	1.5	86.3%	d	20 V	99.4%	Tag5- 2AA	585.4 [M-TAG+2H] ⁺
e	AA3	1.5	1.5	99.9%	f	20 V	98.7%	Tag5- 3AA	778.5 [M-TAG+2H+Na] ⁺
g	TBS-AA4	1.5	1.5	85.0%	h	20 V	99.0%	Tag5- 4AA	787.4 [M-TAG-tBu- TBS+4H] ⁺
i	TBS-AA5	1.5	1.5	94.3%	j	20 V	84.2%	Tag5- 5AA	1002.7 [M-TAG-tBu- TBS+4H] ⁺
k	AA6	1.5	1.5	96.8%	l	20 V	94.7%	Tag5- 6AA	1067.7 [M-TAG-tBu- 2TBS+5H] ⁺
m	TBS-AA7	10	10	100.0%	n	20 V	99.1%	Tag5- 7AA	1282.7 [M-TAG-tBu- 2TBS+5H] ⁺
o	AA8	1.5	1.5	78.2%	p	20 V	98.8%	Tag5- 8AA	1225.6 [M-TAG-tBu- 3TBS+6H] ⁺
q	DMB-AA9	1.5	1.5	90.5%	r	20 V	99.1%	Tag5- 9AA	1364.7 [M-TAG-tBu-3TBS- DMB+7H] ⁺
s	AA10	1.5	1.5	96.6%				Tag5- 10AA	786.7 [M-TAG-tBu-3TBS- DMB-Ttt+9H] ²⁺
t					Click reaction			Tag5-M1	990.4 [M-Tag5-tBu-3TBS- DMB-Ttt-Boc+10H] ²⁺

Figure 11B

Amide coupling reaction Molar eq per Tag5 (0.5mmol)					de-Fmoc (V stands 1 mL of solvent per gram of AA#)			Product	
Step#	amino acid	DIC	HOAt	Quench w/ C ₆ H ₅ NH ₂	Step#	20% piperidine in THF	Crude yield	#	Found MS (M+H) ⁺ (Detect after cleavage to remove Tag5 and protective groups)
a	AA1	1.5	1.5	2	b	20 V	99.0%	Tag5-1AA	221.1 [M-TAG+2H] ⁺
c	AA2	1.5	1.5	2	d	20 V	99.4%	Tag5-2AA	585.4 [M-TAG+2H] ⁺
e	AA3	1.5	1.5	2	f	20 V	98.7%	Tag5-3AA	778.5 [M-TAG+2H+Na] ⁺
g	TBS-AA4	1.5	1.5	2	h	20 V	99.0%	Tag5-4AA	787.4 [M-TAG-tBu-TBS+4H] ⁺
i	TBS-AA5	1.5	1.5	2	j	20 V	84.2%	Tag5-5AA	1002.7 [M-TAG-tBu-TBS+4H] ⁺
k	AA6	1.5	1.5	2	l	20 V	94.7%	Tag5-6AA	1067.7 [M-TAG-tBu-2TBS+5H] ⁺
m	TBS-AA7	10	10	2	n	20 V	99.1%	Tag5-7AA	1282.7 [M-TAG-tBu-2TBS+5H] ⁺
o	AA8	1.5	1.5	2	p	20 V	98.8%	Tag5-8AA	1225.6 [M-TAG-tBu-3TBS+6H] ⁺
q	DMB-AA9	1.5	1.5	2	r	20 V	99.1%	Tag5-9AA	1364.7 [M-TAG-tBu-3TBS-DMB+7H] ⁺
s	AA10	1.5	1.5	2				Tag5-10AA	786.7 [M-TAG-tBu-3TBS-DMB-Ttt+9H] ²⁺
t	Click reaction				Click reaction				990.4 [M-Tag5-tBu-3TBS-DMB-Ttt-Boc+10H] ²⁺
u	Cleavage				Cleavage				990.5 [M+2H] ²⁺

Figure 12

Amide coupling reaction Molar eq per Tag5 (0.04 mmol)					De-Fmoc (V stands 1 mL of solvent per gram of AA#)			Product	
Step#	amino acid (eq.)	DIC (eq.)	HQAt (eq.)	Yield	Step#	20% piperidine in THF	Yield	#	Found MS (M+H) ⁺ After Cleavage to remove Tag5 and protective groups
a	AA2	1.5	1.5	86.7%	d	40V	94.1%	Tag5-2AA	626.28 [M-TAG+H+Na] ⁺
c	AA3	3.0	3.0	99.9%	f	40V	100%	Tag5-3AA	719.30 [M-TAG-tBu+2H] ⁺
e	TBS-AA4	3.0	3.0	85.0%	h	40V	93.4%	Tag5-4AA	851.70 [M-TAG-tBu-OTBS+4H] ⁺
g	TBS-AA5	3.0	3.0	94.3%	j	40V	98.6%	Tag5-5AA	907.50 [M-TAG-tBu-2OTBS+5H] ⁺
i	AA6	2.0	3.0	96.8%	l	40V	96.8%	Tag5-6AA	1086.20 [M-TAG-tBu-2OTBS+5H] ⁺
k	TBS-AA7	10	10	100.0%	n	40V	96.2%	Tag5-7AA	1187.40 [M-TAG-tBu-3OTBS+6H] ⁺
m	AA8	10	10	78.2%	p	40V	92.8%	Tag5-8AA	1244.57 [M-TAG-tBu-3OTBS+6H] ⁺
o	DMB-AA9	3.0	3.0	90.5%	r	40V	92.2%	Tag5-9AA	1439.30 [M-TAG-PMB-3OTBS-tBu+7H] ⁺
q	AA10	3.0	3.0	96.6%	/	/	/	Tag5-10AA	1368.20 [M-TAG-PMB-3OTBS-tBu-Trt+9H] ⁺
r	Click with PEG8-Linker (3 eq.) in THF, CuSO ₄ ·H ₂ O (1.5 eq.) and Sodium ascorbate (1.5 eq.) in H ₂ O, at 40°C for 12 hr.				/			Tag5-21	888.60, [M-TAG-Trt-PMB-3TBS-Boc-tBu+10H] ²⁺
s	Cleavage with TFA cocktail (TFA/TIPS/H ₂ O = 95/2.5/2.5) in DCM (3/1, 100 V), 20°C, 2hr				/			M3	888.60, [M+2H] ²⁺
Total yield					9.30%				

Route	Steps	Total yield	Commercial SMs in a large quantity	Purification steps		Reported In the patent	Challenge for scaled-up
				Column	slurry		
Rt 1 AA2	10	6~9%	YES	6	/	YES	Route 1 is the longest route.
Rt 2 AA2	9	3.9%	YES	4	2	No	Route 2 includes two difficulty steps: the purification is very difficult in 3 rd step, and the yield in Suzuki reaction in 5 th step is low and the purification is difficult, too.
Rt 3 AA2+ Linker	9 for AA2	23%	YES	4	1	No	Route 3 includes two steps with low yields. In step 3, compound 3 was easily converted to dehalogenation and difficult to be purified. In step 5, the Suzuki reaction for compound 6 to compound 7 is also difficult to be purified via silica gel column.
	10 for AA2+Linker	28.7% for last step		1	/		
Rt 4 AA2	8	15.2%	NO	N/A	N/A	No	Route 4 is only 8 steps. It also avoids the dangerous operation of hydrogenation. But No suppliers provide large amounts of SM (4-bromo-3-ethylphenol).

Figure 13

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Route	Steps	Total yield	Commercial SMs in a large quantity	Purification steps		Reported In the patent	Challenge for scaled-up
				Column	slurry		
Rt 5 AA2+Linker	8 for AA2	31.09 %	YES	6		No	Route 5 is the optimized route 3 with modifications to reduce the double bond before the Suzuki reaction, because the double in Cmpd 3 is also the reaction site in Pd catalyst C-C bond formation, which is not suitable for scale-up. Route 5 is used to generate 3.5kg of AA2+Linker.
	9 for AA2+Linker	75.8% for last step	YES	1	/	No	

Figure 13 (cont.)

Figure 14

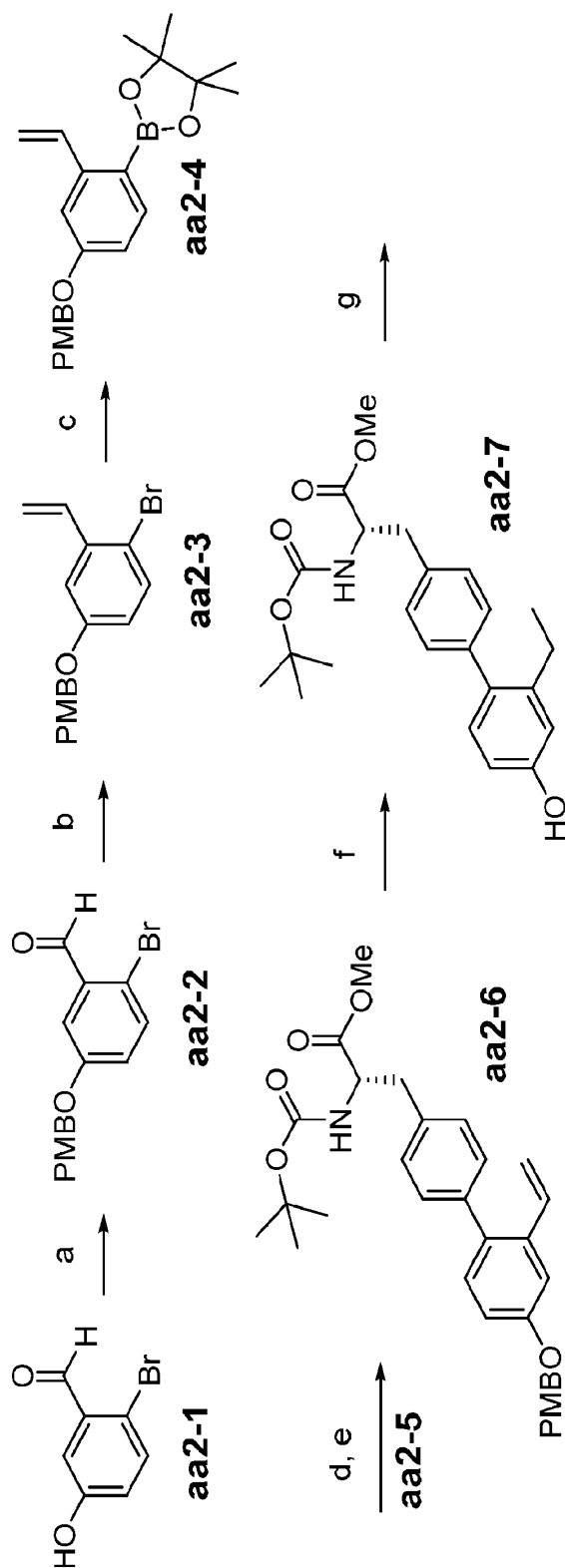
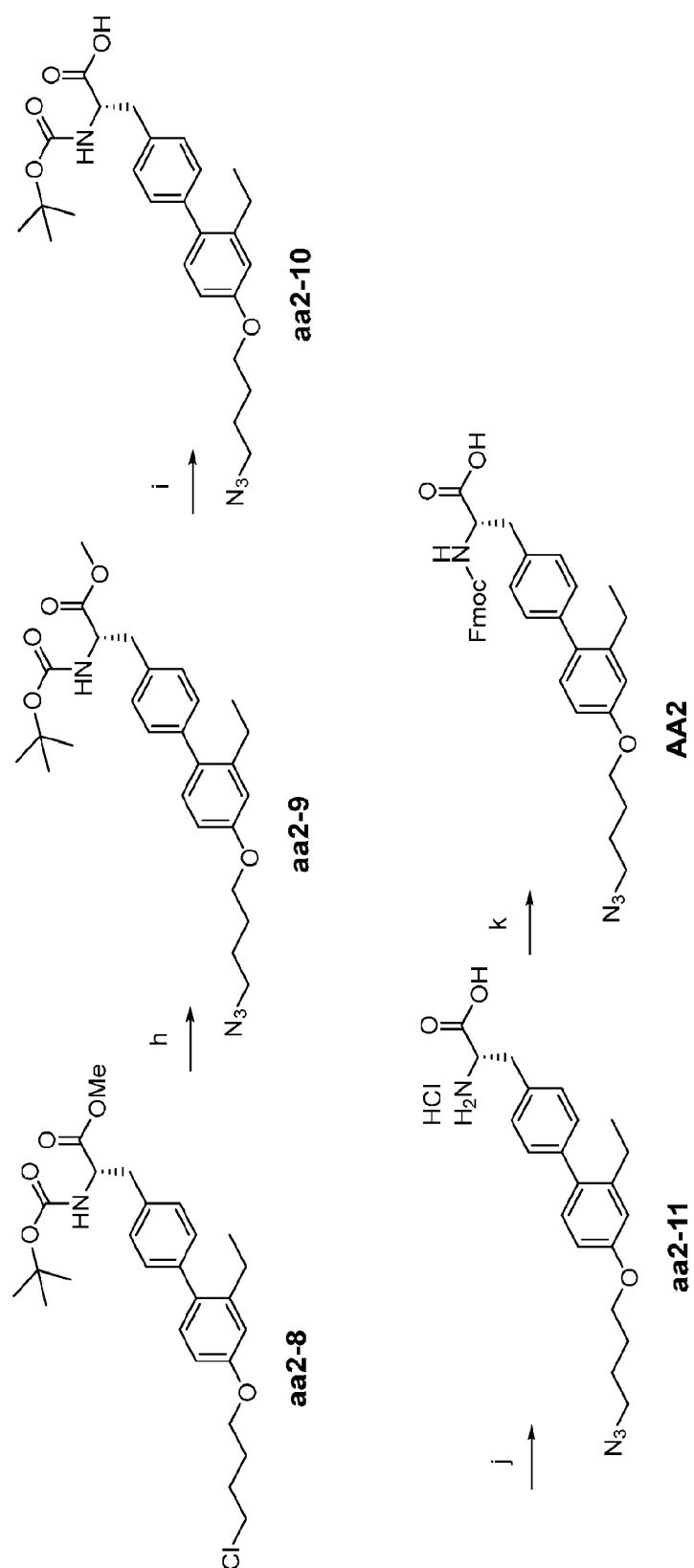
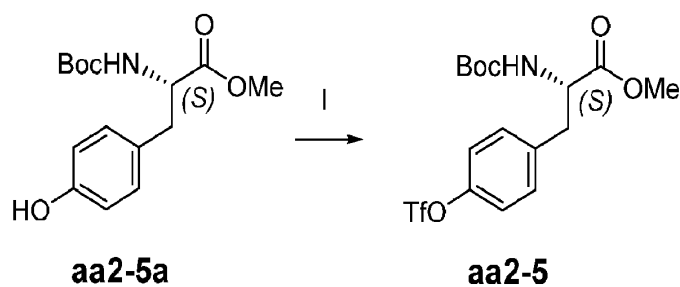


Figure 14 (cont.)





Reagents and Conditions: (a) K_2CO_3 , PMB-Cl, DMF, 25 °C, 12h; (b) $\text{Ph}_3\text{PCH}_2\text{Br}$, t-BuOK, THF, 0-25°C, 4h, 71.91%; (c) $\text{Pd}(\text{dppf})\text{Cl}_2$, KOAc, 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane, 1,4-dioxane, 80 °C, 20 h, 42.75%; (d) aa 2-6, K_2CO_3 , $\text{Pd}(\text{PPh}_3)_4$, 1,4-dioxane and H_2O , 80 °C, 2.5 h, 76.20%; (e) Pd/C, H_2 (15 psi), MeOH, 25 °C, 48 h, 85.01%; (f) K_2CO_3 , 1-chloro-4-iodo-butane, DMF, 50 °C, 12 h, 79.2%; (g) K_2CO_3 , KI, NaN_3 , DMF, 50 °C, 7 h. (h) LiOH (2.0 eq.), THF/ H_2O (2:1), 0 ~25°C, 1 h, (i) HCl/EtOAc; (j) HCl/EtOAc; (k) Fmoc-Osu (1.0 eq.), NaHCO_3 (2.0 eq.), THF/ H_2O (2:1), 25°C, 12 h. 65.44% in totally 4 steps; (l) Py, Trf_2O , DCM, 0 °C, 1 hr.

Figure 14 (cont.)

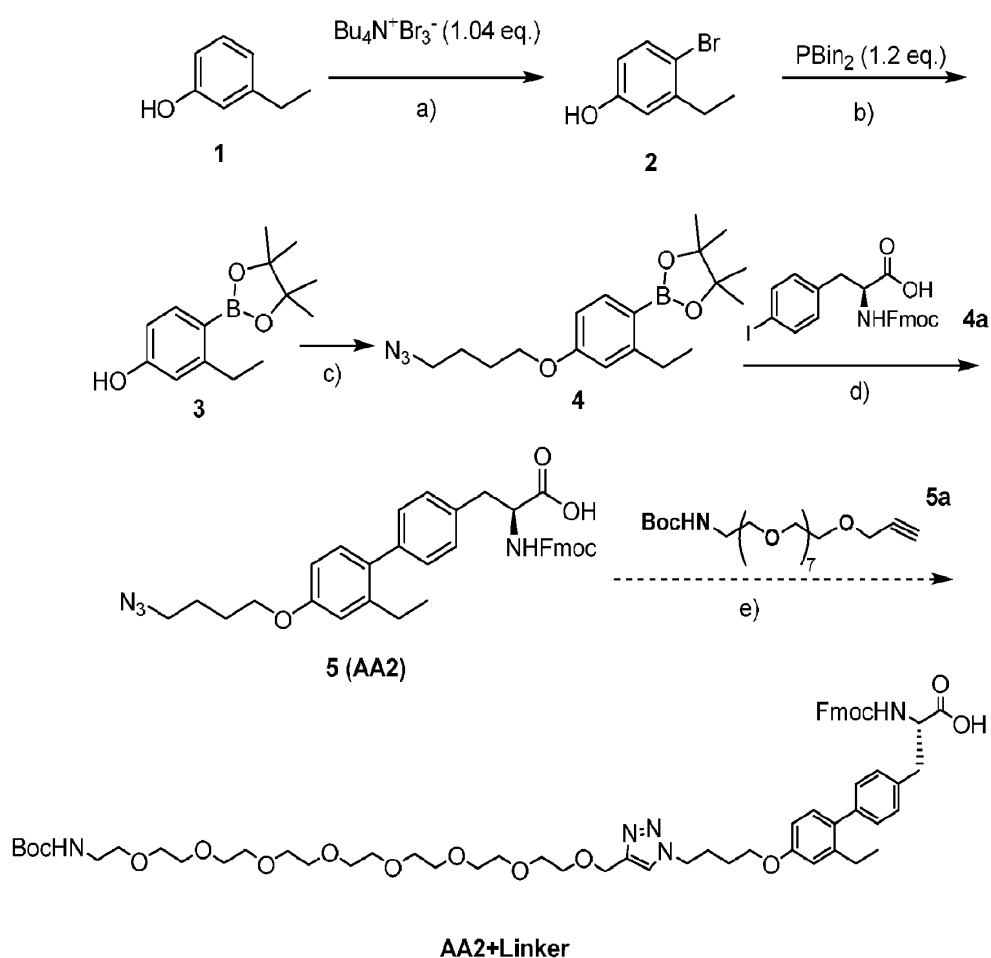


Figure 15

SUBSTITUTE SHEET (RULE 26)