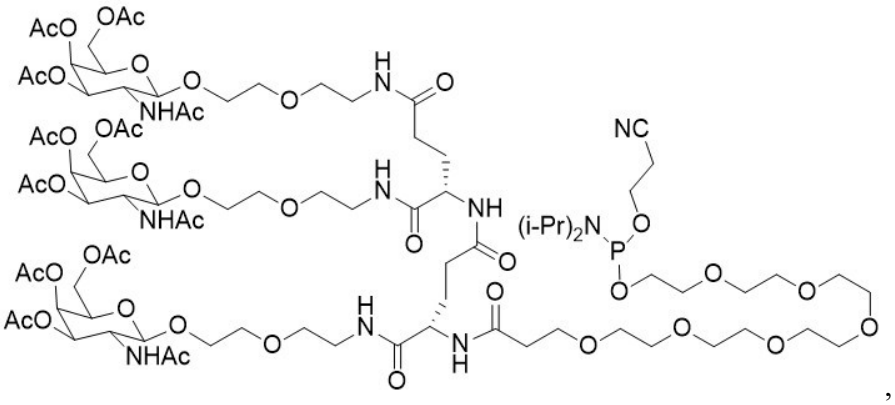
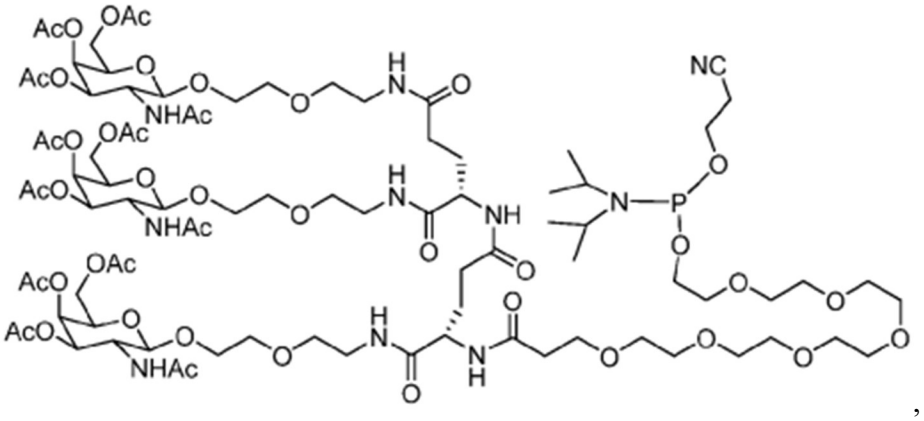


wherein Ac is acetyl (or -(C=O)CH_3) and i-Pr is isopropyl (or 1-methylethyl).

SUMMARY

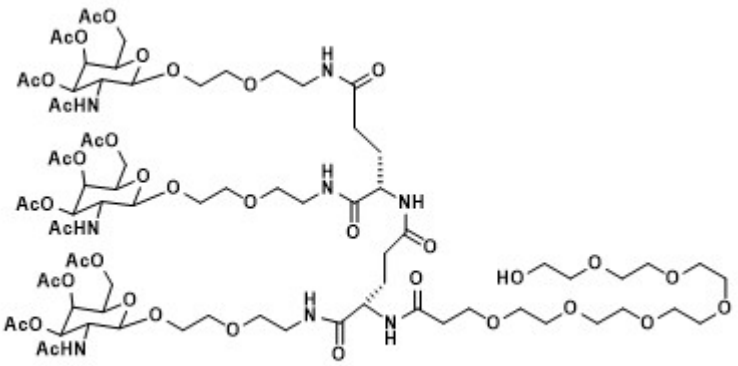
[0005] Described herein are processes for preparing NAG-25, which arose from recognition of the need for NAG-25 manufacturing processes that are more efficient, streamlined, environmentally friendly, and/or economical, including processes that result in NAG-25 and its intermediates having increased purities and/or yields. Such processes contain no chromatography steps, minimize or prevent formation of impurities, reduce or reject reactive impurities, and/or improve isolation points and physical properties of intermediates. Therefore, described herein is a process whereby NAG-25 is prepared without the use of column chromatography. Further described herein is a process whereby NAG-25 is prepared using a crystalline form of the Triacid intermediate. Additional new improved processes are further described herein. As a result of the process steps described, isolation of NAG-25 intermediates and yields have been improved, impurities have been reduced, and waste has been reduced. Further described herein are compositions comprising NAG-25, or one of its intermediates (e.g., t-Butyl Core, Triacid or its salts, Alcohol-Z, NAG-Z, NAG-H or its salt, Triol, unprotected Triol, TGZ, TG Amine or its salt, TG PEG, and/or salts of any of the foregoing), with reduced levels of impurities such as the impurities and the percentages of impurities described herein.

[0005a] In one aspect, the present invention refers to a process for preparing NAG-25:



wherein said process comprises

Step 6: a process comprising reacting TG PEG



TG PEG

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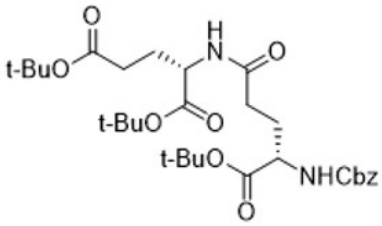
with an activator and a phosphitylating reagent (P-reagent), wherein the activator is tetrazole, 4,5-dicyanoimidazole (DCI), 5-Ethylthio-lH-Tetrazole (ETT), or Benzothiotetrazole (BTT); and/or wherein the phosphitylating reagent is 2-cyanoethyl-N, N, N', N'-tetraisopropylphosphorodiamidite or 2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite;

and

wherein the process of Step 6 is performed in the presence of a base; and

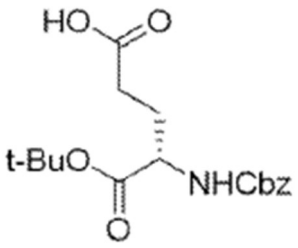
optionally wherein the step 6 process comprises reacting TG PEG with 2-cyanoethyl-N, N, N', N'-tetraisopropylphosphorodiamidite in the presence of tetrazole and NMI in dichloromethane.

[0005b] In another aspect, the present invention refers to a process or preparing t-Butyl Core:



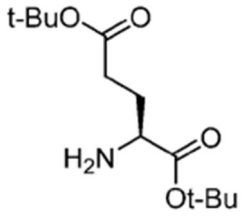
t-Butyl Core

said process comprising reacting GluZ:



GluZ

with GluOtBu or salt thereof:



GluOtBu

to produce t-Butyl Core, wherein the reacting is performed in the presence of a coupling reagent, a base, and a solvent; optionally wherein:

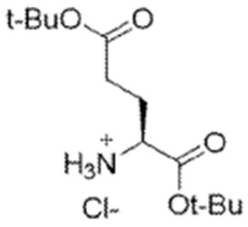
a) the:

(i) coupling reagent is EDC/Oxyma, TFFH, PyOxim, CDI, PivCl, T3P, or COMU;

(ii) base is N-methylmorpholine (NMM); and/or

(iii) solvent is IPAc, MeTHF, MIBK, or MTBE; and/or

b) GluOtBu or salt thereof is a GluOtBu hydrochloride salt:



,

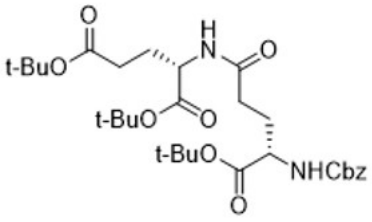
c) the coupling reagent is PivCl, solvent is MTBE; optionally wherein the GluOtBu is about 1-1.5 eq.; or PivCl is about 1-1.5 eq; orNMM is about 3-4 eq.; or MTBE is about 8-12 vol, each relative to GluZ; and/or

d) a first solution comprising GluZ and NMM in a solvent is added to a second solution comprising the solvent and coupling reagent; optionally wherein the coupling reagent is in excess and/or GluOtBu is in excess, optionally wherein the coupling reagent is PivCl; and/or

e) the process further comprises addition of an antisolvent, optionally over a time period of about 2-5 hours; and/or

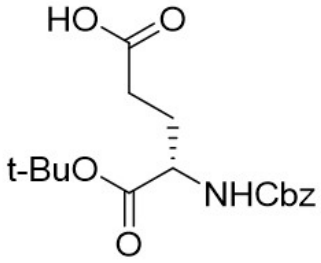
f) the process further comprises transforming the t-Butyl Core into NAG-25.

[0006] Disclosed herein are processes for preparing t-Butyl Core



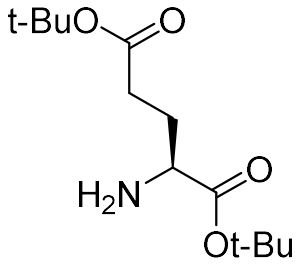
t-Butyl Core

said process comprising reacting GluZ:



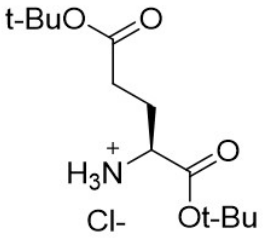
GluZ

with GluOtBu or salt thereof:



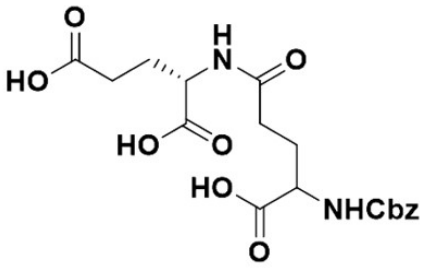
GluOtBu or salt thereof

to produce t-Butyl Core. In some examples, the GluOtBu or salt thereof is a GluOtBu hydrochloride salt:



. In some examples, the reaction is performed in the presence of comprising a coupling reagent, a base, and a solvent. In some examples, the coupling reagent is EDC/Oxyma, TFFH, PyOxim, CDI, PivCl, T3P, or COMU. In some examples, the coupling reagent is T3P or PivCl. In some examples, the couple reagent is PivCl. In some examples, the base is N-methylmorpholine (NMM). In some examples, the solvent is IPAc, MeTHF (also referred to as 2-MeTHF), MIBK, or MTBE. In some examples, the solvent is MTBE. In some examples, the antisolvent is heptane. In some examples, the solvent is MTBE and the antisolvent is heptane. In some examples, a first solution comprising GluZ and NMM in a solvent is added to a second solution comprising the solvent and PivCl. In some examples, PivCl is in excess. In some examples, GluOtBu is in excess. In some examples, conversion to t-Butyl Core is greater than about 90%, based on the amount of GluZ.

[0007] Disclosed herein are processes for preparing Triacid:

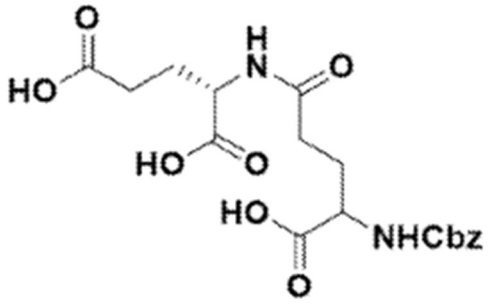


Triacid

said process comprising reacting t-Butyl core with an acid. In some examples, the acid is phosphoric acid (H_3PO_4), TFA, HCl, benzenesulfonic acid, or p-toluenesulfonic acid. In some examples, the acid is phosphoric acid (H_3PO_4). In some examples, the reaction is conducted in a solvent selected from 2-MeTHF, acetonitrile, THF, DMA, sulfolane, and DME. In some examples, the solvent is 2-MeTHF, THF, or acetonitrile. In some examples, the solvent is 2-MeTHF or THF. In some examples, the solvent is 2-MeTHF. In some examples, the Triacid is isolated as a crystalline solid. In some examples, the Triacid has greater than or equal to 95% purity as measured by LC. In some examples, the solvent further comprises water. In some examples, the solvent is a mixture of 2-MeTHF and water. In some examples, about 3.0 - 4.0 vol of 2-MeTHF and about 0.5 – 2 vol water is used. In some examples, the reaction is conducted at a temperature of about 40-60ºC. In some examples, the reaction is conducted at a temperature of about 45-55ºC. In some examples, the reaction is conducted at a temperature of about 50ºC. In some examples, the process further comprising removing phosphoric acid with at least one organic/aqueous wash. In some examples, the organic/aqueous wash comprises isopropylacetate (iPAC) as organic layer and ammonium sulfate as aqueous layer. In some examples, about 8-18 vol of iPAC and about 3-7 vol 20 wt% of ammonium sulfate are used. In some examples, about 10 vol of iPAC and about 5 vol 20 wt% of ammonium sulfate are used. In some examples, the process further comprising crystallization of Triacid using acetone/toluene, 2-MeTHF/IPAc/, 2-MeTHF/CPME, acetone/heptane, or MeTHF/acetonitrile. In some examples, the process further comprising crystallization of Triacid using acetone/toluene. In some examples, the acetone/toluene is in ratio of 2:3, 1:1, 3:2, or 1:2. In some examples, the acetone/toluene is in ratio of 2:3, 1:1, or 1:2. In some examples, processes produces the Triacid in a crystalline form characterized by an X-ray powder diffractogram

having at least a signal at three two-theta values chosen from 7.4 ± 0.2, 9.2 ± 0.2, 21.0 ± 0.2, 14.6 ± 0.2, 18.3 ± 0.2, and 19.3 ± 0.2.

[0007a] In another aspect, the present disclosure refers to a process of preparing Triacid:



Triacid,

said process comprising reacting t-Butyl core with an acid, wherein the acid is phosphoric acid (H_3PO_4), TFA, HCl, benzenesulfonic acid, or p-toluenesulfonic acid,

optionally wherein:

a) the reaction is conducted in a solvent selected from 2-MeTHF, acetonitrile, THF, DMA, sulfolane, and DME, optionally wherein the solvent is mixed with water, optionally wherein the solvent is 2-MeTHF, optionally wherein the solvent is about 3.0 - 4.0 vol of 2-MeTHF and is mixed with about 0.5 - 2vol water; and/or

b) the reaction is conducted at a temperature of about 40-60°C; and/or

c) the Triacid is isolated as a crystalline solid.

d) the process further comprises crystallization of Triacid using acetone/toluene, 2-MeTHF/IPAc, 2-MeTHF/CPME, acetone/heptane, or MeTHF/acetonitrile; and/or

e) the Triacid is obtained in a crystalline form characterized by an X-ray powder diffractogram having at least a signal at three two-theta values chosen from 7.4 ± 0.2, 9.2 ± 0.2, 21.0 ± 0.2, 14.6 ± 0.2, 18.3 ± 0.2, and 19.3 ± 0.2 or characterized by an X-ray powder diffractogram having at least a signal at three two-theta values selected from 7.8 ± 0.2, 9.7 ± 0.2, 13.3 ± 0.2, 15.7± 0.2, 17.1 ± 0.2, 18.9 ± 0.2, 20.2± 0.2, and 21.0 ± 0.2; and/or

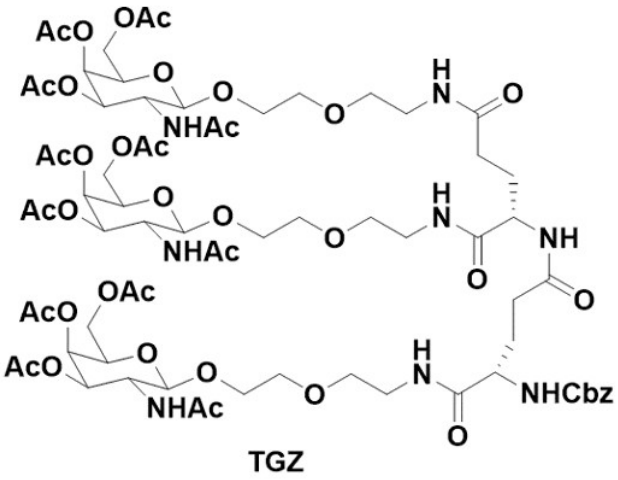
f) the process further comprises transforming the Triacid into NAG-25.

[0008] Disclosed herein is a crystalline Form I of Triacid, characterized by an X-ray powder diffractogram having at least a signal at three two-theta values selected from 7.4 ± 0.2, 9.2 ± 0.2, 21.0 ±

0.2, 14.6 ± 0.2, 18.3 ± 0.2, and 19.3 ± 0.2. In some examples, the crystalline Form I of Triacid, characterized by an X-ray powder diffractogram having a signal at two-theta values of 7.4 ± 0.2, 9.2 ± 0.2, and 21.0 ± 0.2. In some examples, the crystalline Form I of Triacid, characterized by an X-ray powder diffractogram substantially similar to that in Figure 2. In some examples, the crystalline Form II of Triacid, characterized by an X-ray powder diffractogram substantially similar to that in Figure 3.

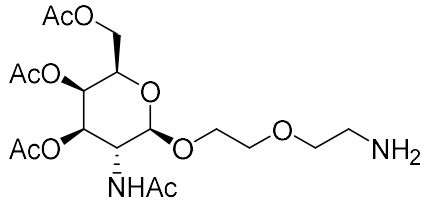
[0008a] In another aspect, the present disclosure refers to a crystalline form of Triacid, wherein: a) the crystalline form is crystalline Form I of Triacid, characterized by an X-ray powder diffractogram having at least a signal at three two-theta values selected from 7.4 ± 0.2, 9.2 ± 0.2, 21.0 ± 0.2, 14.6 ± 0.2, 18.3 ± 0.2, and 19.3 ± 0.2 or characterized by an X-ray powder diffractogram substantially similar to that in Figure 2; or b) the crystalline form is crystalline Form II of Triacid, characterized by an X-ray powder diffractogram having at least a signal at three two-theta values selected from 7.8 ± 0.2, 9.7 ± 0.2, 13.3 ± 0.2, 15.7± 0.2, 17.1 ± 0.2, 18.9 ± 0.2, 20.2± 0.2, and 21.0 ± 0.2 or characterized by an X-ray powder diffractogram substantially similar to that in Figure 3.

[0009] Disclosed herein is a process of preparing TGZ

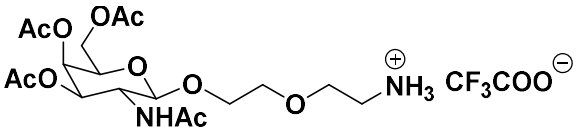


TGZ

said process comprising reacting Triacid with NAG-H:

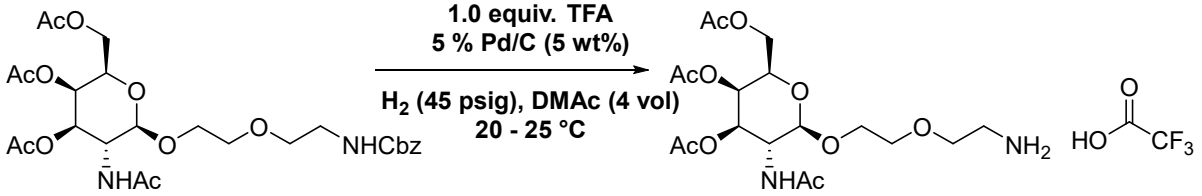


, or salt thereof, in the presence of a coupling reagent and a base. In some examples, the NAG-H or salt thereof is a TFA salt of NAG-H:



. In some examples, the coupling reagent is TBTU, HATU or TCFH. In some examples, the coupling reagent is TBTU. In some examples, the base is DIPEA, NMI, NMM, or TMP. In some examples, the base is NMI. In some examples, the process further comprising at least one buffer wash. In some examples, the buffer wash is a phosphate buffer. In some examples, the buffer wash is about pH 5-7. In some examples, the buffer wash is about pH 6. In some examples, the process is conducted in a solvent selected from DCM, DMF, MeCN, and DMAc, or combination thereof. In some examples, the solvent is DMAc. In some examples, the solvent is DCM. In some examples, the process further comprising adding an anti-solvent to a

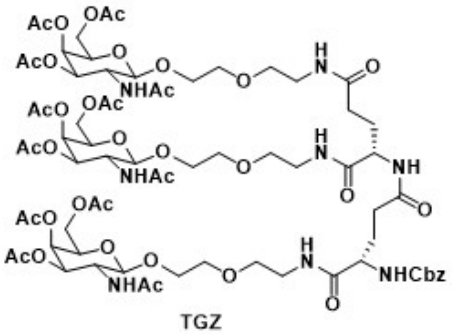
solution comprising TGZ and the solvent. In some examples, the anti-solvent is an ethereal solvent. In some examples, the ethereal solvent is DME, 2-MeTHF, or MTBE. In some examples, the solvent is DCM and the anti-solvent is MTBE. In some examples, the TGZ is prepared with reduced impurities. In some examples, the impurity side product is des-acyl. In some examples, the process further comprises precipitation of TGZ from solvent/antisolvent system. In some examples, the solvent/antisolvent system is DCM/MTBE. In some examples, the TGZ is obtained in at least 95 % purity without use of column chromatography. In some examples, the NAG-H, or salt thereof, was carried forward as a solution from a prior reaction to react with Triacid without isolation of the NAG-H, or salt thereof, from the prior reaction. In some examples, NAG-H, or salt thereof, is prepared in a prior reaction comprising hydrogenation of NAG-Z the presence of a Pd/C catalyst and in DCM or dimethylacetamide (DMAc). In some examples, the DCM is about 3 volumes to about 6 volumes. In some examples, the DMAc is about 3 volumes to about 5 volumes. In some examples, the DMAc is about 4 volumes. In some examples, the NAG-H or salt thereof is NAG-H TFA. In some examples, the Pd/C catalyst is 5% Pd/C. In some examples, NAG-H TFA is prepared in a prior reaction comprising the reagents in the below scheme:



NAG-Z **NAG-H TFA**

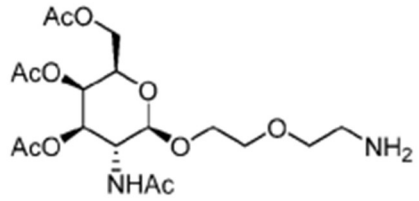
.

[0009a] In another aspect, the present disclosure refers to a process of preparing TGZ



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said process comprising reacting Triacid with NAG-H:

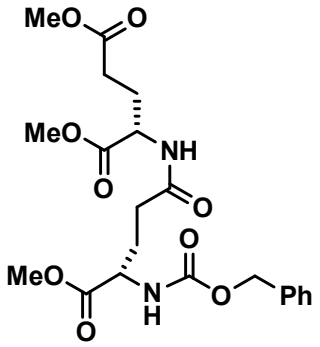


NAG-H , or salt thereof, in the presence of a coupling reagent and a base; precipitating TGZ from a solvent/antisolvent system,

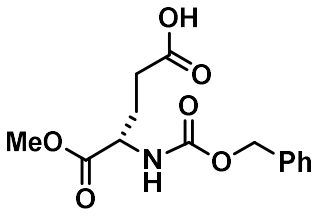
optionally wherein:

a) the coupling reagent is TBTU, HATU or Chloro-N,N,N',N'-tetramethylformamidinium hexafluorophosphate (TCFH), optionally wherein the coupling reagent is TBTU; and/or b) the base is diisopropylethylamine (DIPEA), N-methylimidazole (NMI), *N*-methylmorpholine (NMM), or 2,2,6,6-tetramthylpiperidine (TMP), optionally wherein the base is NMI; and/or c) the process further comprises at least one buffer wash, optionally wherein the buffer wash is about pH-5-7; and/or d) said reacting is conducted in a solvent selected from DCM, DMF, MeCN, and DMAc, or combination thereof; and/or e) an anti-solvent is added to a solution comprising TGZ and the solvent, optionally wherein the anti-solvent is an ethereal solvent, optionally wherein the ethereal solvent is DME, 2-MeTHF, or MTBE; and/or f) TGZ is prepared with reduced levels of one or more impurities, wherein the impurities is mono-NAG, DI-NAG, DI-NAG-AZLACTONE, des-acyl, di-NAG-OH, di-NAG-OAc, NAG-H guanidine, or NAG-H trifluoroacetamide; and/or g) TGZ is obtained in at least 95 % purity without use of column chromatography; and/or h) the NAG-H, or salt thereof, is a solution carried forward from another process without isolation of the NAG-H, or salt thereof; and/or i) the process further comprises transforming the TGZ into NAG-25.

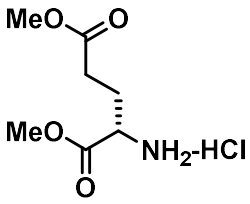
[0010] Disclosed herein is a process for preparing Methyl Core:



Methyl Core , said process comprising reacting z-L-Glu-OMe:

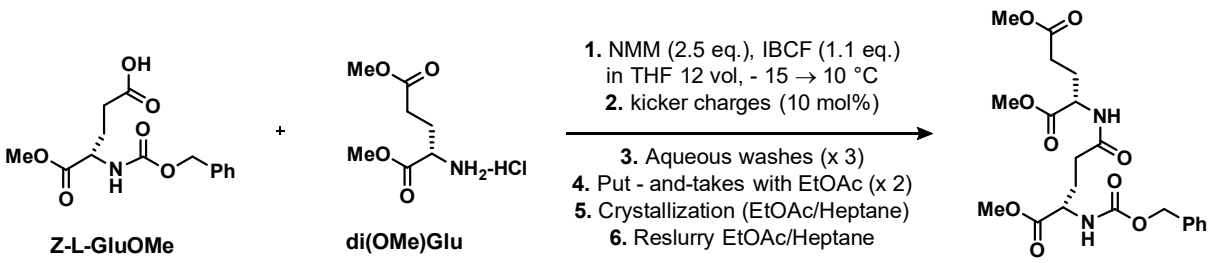


Z-L-GluOMe with L-Glutamic acid dimethyl ester (di(OMe)Glu):

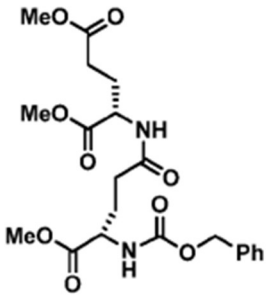


di(OMe)Glu to produce Methyl Core.

[0011] In some examples, the reaction is performed in the presence of a base and a coupling reagent. In some examples, the base is added to a z-L-GluOMe solution prior to addition of the coupling reagent and di(OMe)Glu to the reaction. In some examples, the coupling reagent is added to the solution of z-L-Glu-OMe and base prior to addition of di(OMe)Glu to the reaction. In some examples, the reaction further comprising a solvent. In some examples, the base is N-Methylmorpholine (NMM) or di-isopropylethylamine. In some examples, the coupling reagent is isobutyl chloroformate (IBCF), TBTU, HATU, EDC, or DCC. In some examples, the solvent is THF or MeTHF.In some examples, the reaction temperature prior to and during the reaction with coupling reagent is about -20°C to about -10°C. In some examples, the reaction temperature is about -18°C to about -13°C. In some examples, the reaction temperature after addition of di(OMe)Glu is increased to about 5°C to about 15°C. In some examples, the reaction temperature is increased to about 7°C to about 12°C. In some examples, the reaction further comprises an additional charge of coupling reagent, base, and/or z-L-Glu-OMe.In some examples, the reaction further comprising washing the reaction with aqueous acid followed by washing the reaction with aqueous base. In some examples, the reaction further comprises crystallization by adding anti-solvent. In some examples, the process comprises the reagents and conditions in the below scheme:

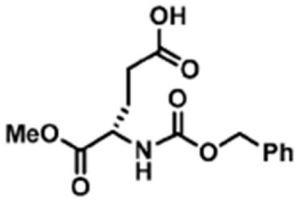


[0011a] In yet another aspect, the present disclosure refers to a process for preparing Methyl Core:



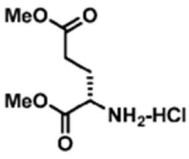
Methyl Core,

said process comprising reacting z-L-Glu-OMe:



Z-L-GluOMe

with L-Glutamic acid dimethyl ester (di(OMe)Glu):



di(OMe)Glu to produce Methyl Core, optionally:

a) in the presence of a base and a coupling reagent, optionally wherein:

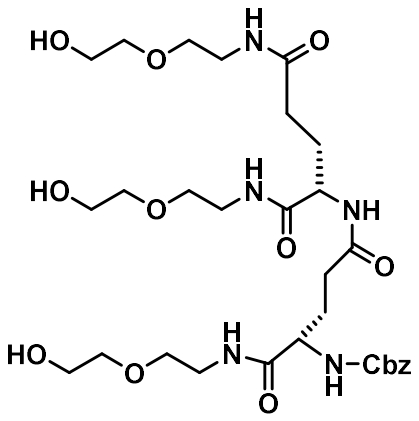
(i) the base is added to a z-L-GluOMe solution prior to addition of the coupling reagent and di(OMe)Glu to the reaction; or wherein the coupling reagent is added to the solution of z-L-Glu-OMe and base prior to addition of di(OMe)Glu to the reaction; and/or

(ii) the base is N-Methylmorpholine (NMM) or di-isopropylethylamine and/or the coupling reagent is isobutyl chloroformate (IBCF), TBTU, HATU, EDC, or DCC

b) further comprising a solvent, optionally wherein the solvent is THF or MeTHF; and/or

c) further comprising transforming the Methyl Core into NAG-25.

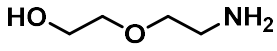
[0012] Disclosed herein is a process for preparing Triol:

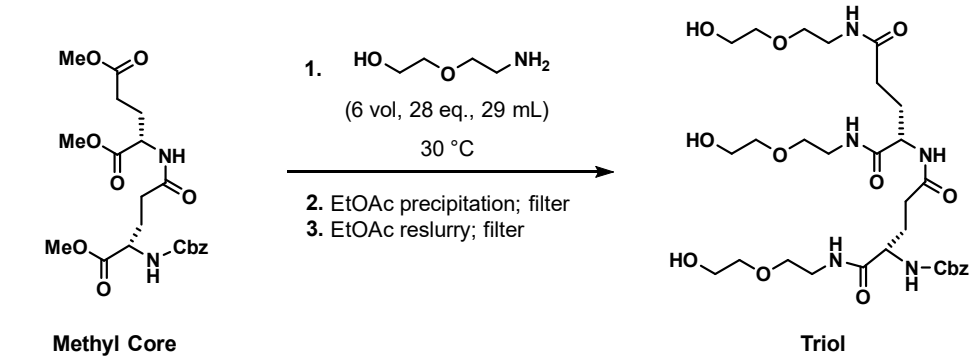


Triol

,

the process comprising reacting Methyl Core with 2-(2-aminoethoxy)ethanol:

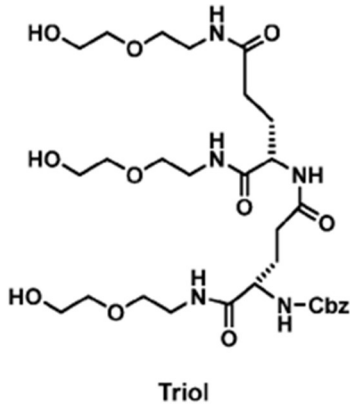
 to produce Triol. In some examples, the process is performed with neat 2-(2-aminoethoxy)ethanol. In some examples, the reaction temperature is at about 25°C to 35°C. In some examples, the reaction temperature is about 30°C. In some examples, the reaction further comprising adding an anti-solvent to result in precipitation of the Triol. In some examples, the anti-solvent is ethyl acetate, methyl-tert-butyl ether, or iso-propylacetate. In some examples, the process further comprises adding a Triol seed crystal. In some examples, the process comprises the reagents and conditions in the below scheme:



(97 w%, 5.0 g, 1.0 eq.)

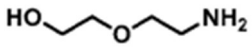
.

[0012a] In yet another, the present disclosure refers to a process for preparing Triol:



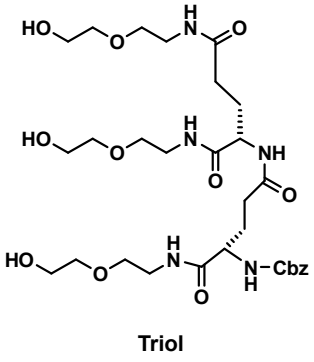
,

the process comprising reacting Methyl Core with 2-(2-aminoethoxy)ethanol:

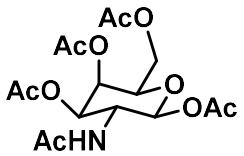


to produce Triol, optionally wherein the Triol is transformed into NAG-25.

[0013] Disclosed is an alternative process for preparing TGZ, the process comprising reacting Triol:



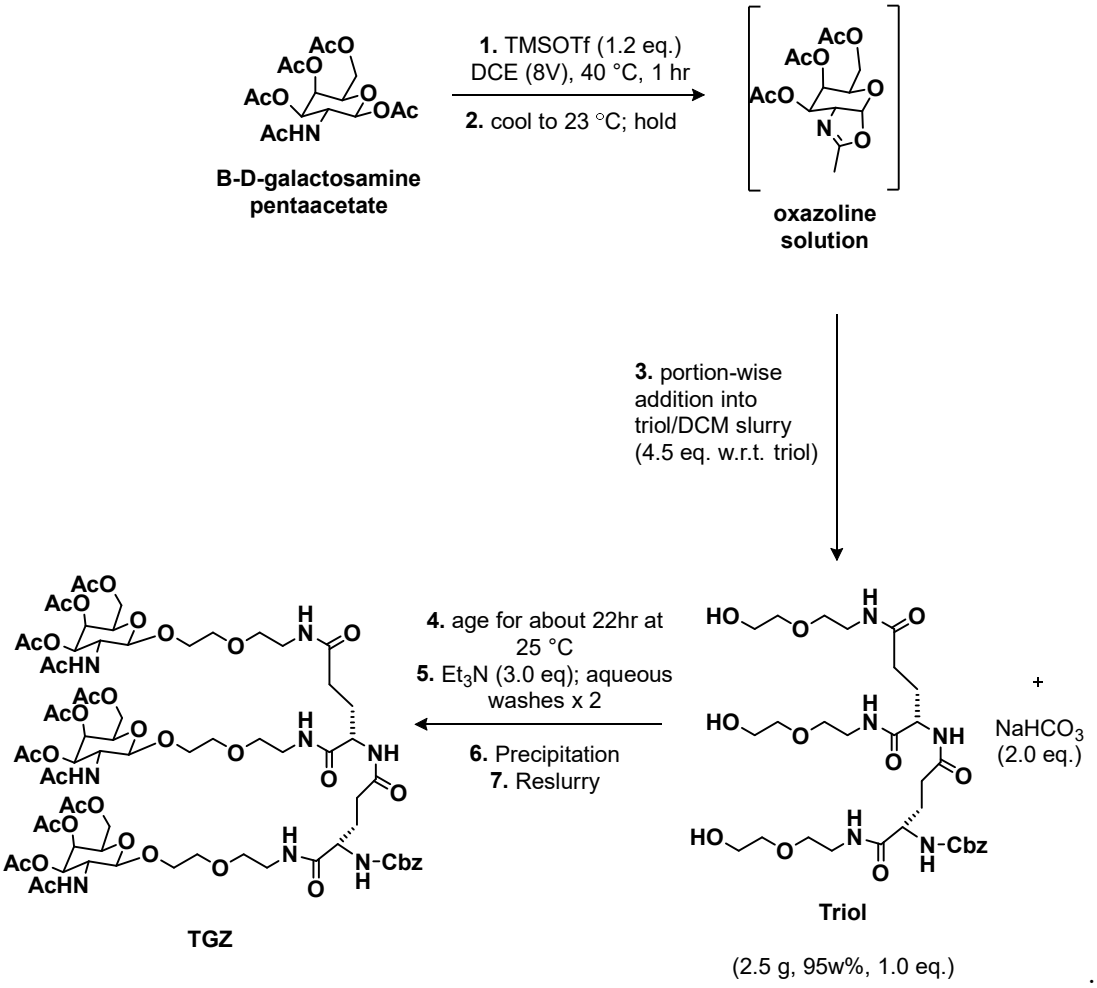
with Beta-D-Galactosamine pentaacetate:



B-D-galactosamine pentaacetate to produce TGZ.

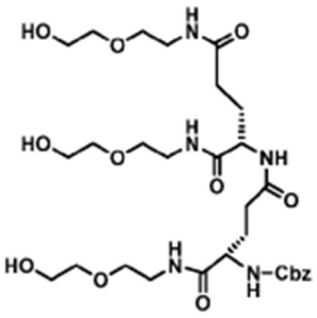
[0014] In some examples, the Beta-D-Galactosamine pentaacetate is first reacted with a silyl-triflate to produce an oxazoline solution. In some examples, the silyl triflate is Trimethylsilyl trifluoromethanesulfonate (TMSOTf) or Triisopropylsilyl Trifluoromethanesulfonate (TIPSOTf). In some examples, the reaction further comprises solvent. In some examples, the solvent is dichloroethane (DCE) or dichloromethane (DCM). In some examples, the reaction temperature is about 35ºC to about 45ºC. In some examples, the reaction temperature is about 40ºC.In some examples, the reaction temperature is decreased to about 20ºC to about 28ºC. In some examples, the reaction temperature is about 23ºC. In some examples, the Triol is mixed with sodium bicarbonate ($NaHCO_3$)

to produce a slurry mixture of Triol and $NaHCO_3$ prior to addition of Beta-D-Galactosamine pentaacetate or its oxazoline solution. In some examples, the slurry mixture of Triol and $NaHCO_3$ further comprises a solvent. In some examples, the solvent is dichloromethane (DCM), dichloroethane, or acetonitrile. In some examples, the oxazoline solution is added to the slurry mixture of triol and $NaHCO_3$. In some examples, the reaction temperature is about 20°C to about 30°C. In some examples, the reaction temperature is about 25°C. In some examples, TGZ is further precipitated from a solvent/antisolvent system. In some examples, an anti-solvent is added to a solution comprising TGZ and the solvent. In some examples, the anti-solvent is dimethoxyethane. In some examples, the process comprises the reagents and conditions in the below scheme:



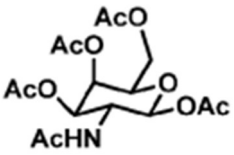
[0015] In some examples, the reaction further comprising any one of claims for steps A-2 or A-1.

[0015a] In yet another, the present disclosure refers to a process for preparing TGZ, the process comprising reacting protected Triol:



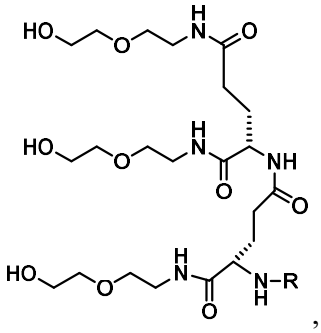
Triol

with Beta-D-Galactosamine pentaacetate:



B-D-galactosamine pentaacetate

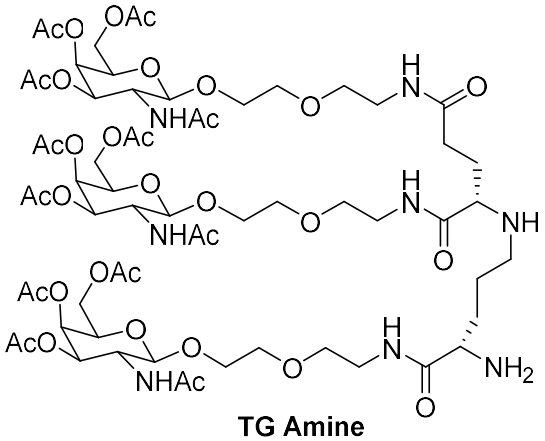
to produce TGZ. optionally wherein the TGZ is transformed into NAG-25.



[0016] In yet another aspect, the present disclosure refers to a compound , wherein R is H or a Cbz protecting group, which is useful for preparing TGZ as described herein.

[0017] Disclosed herein is a process for preparing a NAG-25, said process comprising any one of the examples for preparing the Methyl Core or Triol using the alternative processes described. Disclosed herein is are alternative processes for preparing NAG-25, said process comprising a Triol intermediate. In some examples, the alternative processes further comprises any one of examples for step 5a, step 5b, or step 6. In some examples, the alternative processes comprises reacting z-L-Glu-OMe and L-Glutamic acid dimethyl ester to form the Methyl Core, and transforming the Methyl Core into NAG-25. In some examples, the alternative processes comprises reacting Methyl Core with 2-(2-aminoethoxy)ethanol to form the Triol, and transforming the Triol into NAG-25. In some examples, the alternative processes comprises reacting triol with Beta-D-Galactosamine pentaacetate to form TGZ, and transforming the TGZ into NAG-25. In some examples, the TGZ is further precipitated from a solvent/antisolvent system as described herein.

[0018] Disclosed herein is a process of preparing TG Amine

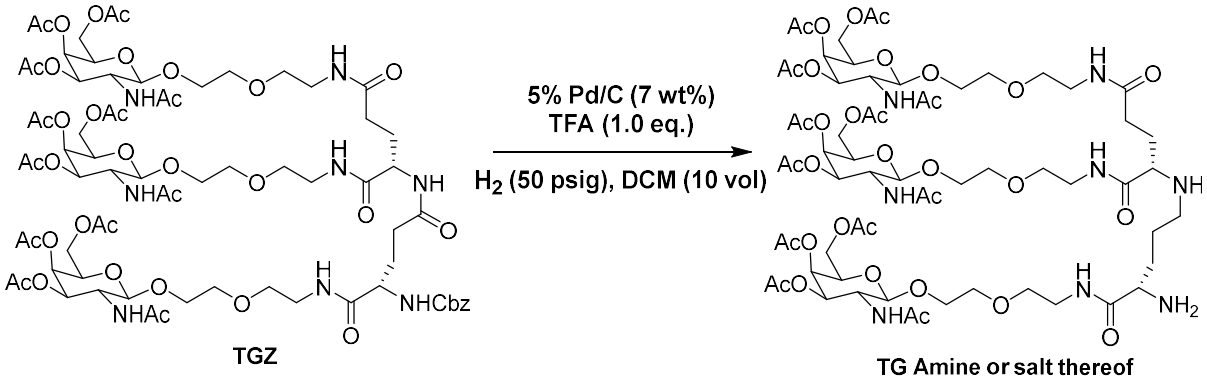


TG Amine

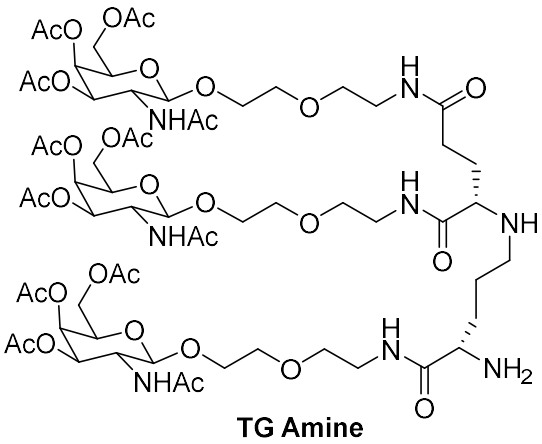
, or a salt thereof,

said process comprising a high pressure hydrogenolysis for a carboxybenzyl deprotection of TGZ to form the TG Amine or a salt thereof. In some examples, the process comprises a palladium source for hydrogenolysis. In some examples, the palladium source is palladium on carbon (Pd/C) catalyst. In some examples, the palladium source is 5% Pd/C.4d. In some examples, the catalyst loading is

less than about 10%, 9%, 8%, 7%, 6% Pd/C. In some examples, the catalyst used is 5% Pd/C. In some examples, the hydrogenolysis is performed in the presence of an acid. In some examples, the acid is TFA, oxalic acid, HCl, AcOH, H_3PO_4, citric.In some examples, the acid is TFA or oxalic acid. In some examples, the acid is TFA. In some examples, said hydrogenolysis is performed in a solvent, and the solvent is DCM, IPAc, or MeOH. In some examples, the solvent is DCM. In some examples, the process reduces formation of des-acyl impurities. In some examples, the TG Amine or salt thereof is a TG Amine acid salt. In some examples, the TG Amine acid salt is a phosphate, formate, acetate, trifluoroacetate, or oxalate salt. In some examples, the TG Amine acid salt is trifluoroacetate or oxalate salt. In some examples, the TG Amine acid salt is trifluoroacetate salt. In some examples, the process results in high purity of the TG PEG product of Step 5b while reducing the amount of a TG Amine acetamide side product. In some examples, the TG Amine or salt thereof comprises less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, or 2% acetamide side product. In some examples, the TG Amine or salt thereof produced from the improved step 5a process has greater than or equal to about 97%, 98%, or 99% purity as measured by LC. In some examples, the TG Amine or salt thereof is telescoped into Step 5b without isolation of the TG Amine or salt thereof. In some examples, the process comprises the reagents in the below scheme:



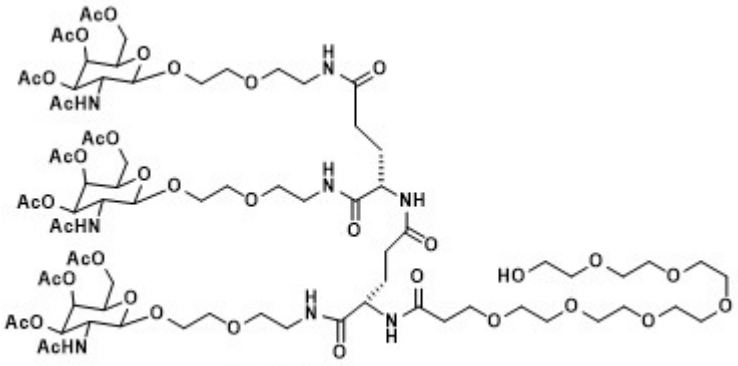
[0018a] In yet another, the present disclosure refers to a process of preparing TG Amine:



, or a salt thereof,

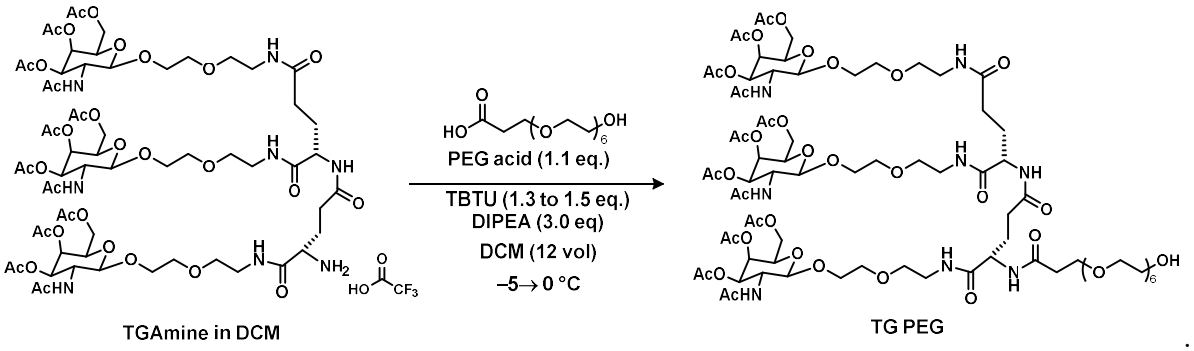
said process comprising a high pressure hydrogenolysis of TGZ to form the TG Amine or a salt thereof, wherein said hydrogenolysis is performed in the presence of an acid, optionally: a) comprising a palladium source for hydrogenolysis, optionally wherein the palladium source is palladium on carbon (Pd/C) catalyst, optionally wherein the palladium source is 5% Pd/C; and/or b) wherein the acid is trifluoroacetic acid (TFA), oxalic acid, hydrochloride acid (HC1), acetic acid (AcOH), phosphoric acid (H3PO4), or citric acid; optionally wherein the acid is no more than about 1.1 eq; and/or c) wherein said hydrogenolysis is performed in a solvent, optionally wherein the solvent is DCM, isopropyl acetate (IPAc), or methanol (MeOH); and/or d) wherein the process reduces formation of one or more impurities that arc TG Amine des-acyl, TG Amine di-NAG-OH, TG Amine guanidyl, TG Amine acyl, TG Acetamide, and/or NAG-H guanidine; and/or e) wherein the TG Amine or salt thereof is carried forward as a solution of TG Amine or salt thereof and without isolation of the TG Amine or salt thereof into another process of preparing TG PEG; and/or f) wherein the TG Amine or salt thereof is a TG Amine acid salt, optionally, a phosphate, formate, acetate, trifluoroacetate, or oxalate salt; and/or g) further comprising transforming the TG Amine or salt thereof into NAG-25.

[0019] Disclosed herein is a process of preparing TG PEG

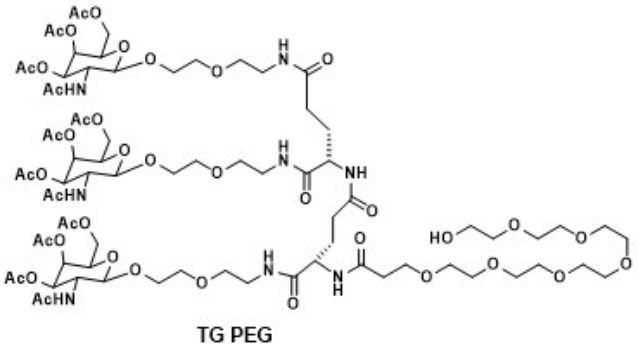


TG PEG

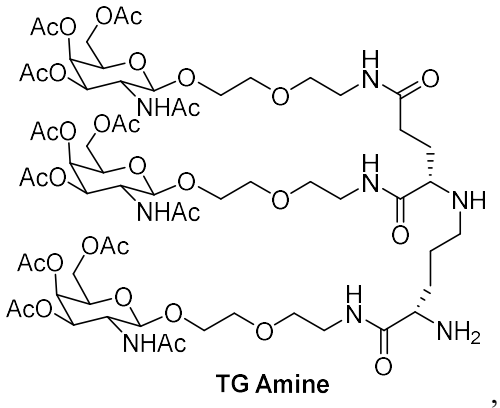
said process comprising treating a solution of TG Amine and PEG acid with TBTU. In some examples, the solution further comprises a base. In some examples, the base is N,N-Diisopropylethylamine (DIPEA). In some examples, the TBTU is added over a period of time of about 30min to about 1.5 hours. In some examples, the TBTU is added over a period of time of about 1-1.5 hour. In some examples, the TG PEG is produced in greater than or equal to about 90% purity as measured by LC. In some examples, TG PEG dimer impurity is present at the end of reaction at an amount of less than 10% as measured by LC. In some examples, the process comprises the reagents in the below scheme:



[0019a] In another aspect, the present disclosure refers to a process for preparing TG PEG



said process comprising reacting a solution of TG Amine:

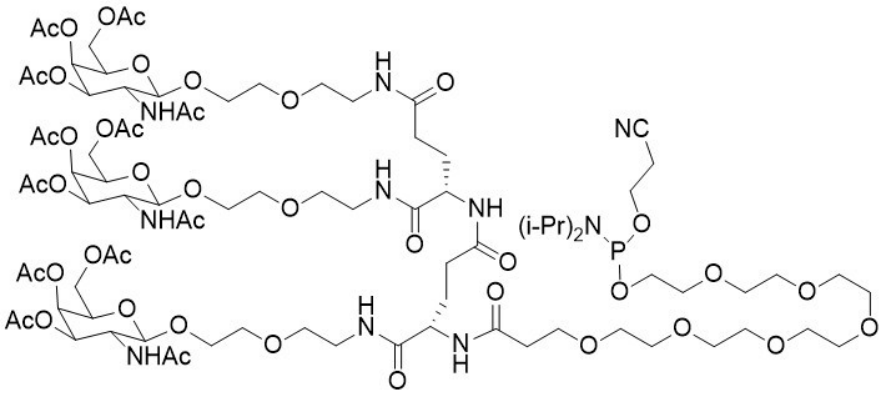


, or a salt thereof, and PEG acid with a coupling reagent, wherein the coupling reagent is added to a solution of PEG acid and TG Amine or salt thereof over a period of time of about 30min to about 1.5 hours,

optionally wherein: a) solution further comprises a base, optionally wherein the base is N,N-Diisopropylethylamine (DIPEA); and/or b) the coupling reagent is 2-(1H-Benzotriazole-l-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU) or N-[(Dimethylamino)-1H-l,2,3-triazolo-[4,5-b]pyridin-l-ylmethylene]-N-methylmethanaminium hexafluorophosphate N-oxide (HATU); and/or c) the TG PEG is produced in greater than or equal to about 90% purity as measured by LC or wherein TG PEG dimer impurity is present at the end of reaction at an amount of less than 10% as measured by LC; and/or d) the solution of TG Amine or a salt thereof is carried forward from a separate process without isolation of the TG Amine or salt thereof, optionally wherein the TG Amine or salt thereof is TG Amine trifluoroacetate (TFA) salt; and/or e) the process further comprises transforming the TG PEG into NAG-25.

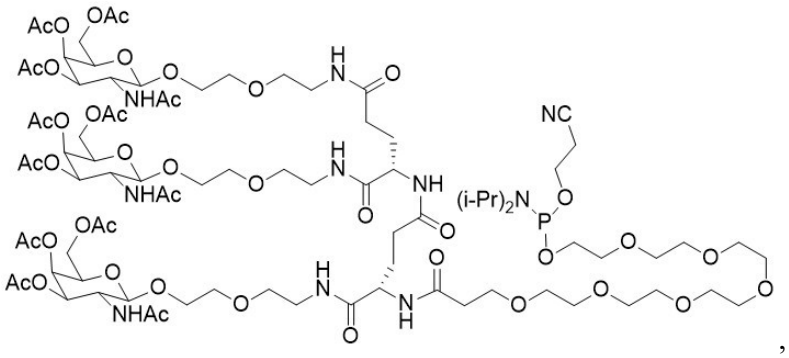
[0020] Disclosed herein is a process of preparing NAG-25 comprising treating TG PEG with an activator and a phosphitylating reagent. In some examples, the activator is tetrazole, 4,5-dicyanoimidazole (DCI), ETT, or Benzothiotetrazole (BTT). In some examples, the activator is tetrazole, DCI, or ETT. In some examples, the activator is tetrazole. In some examples, the tetrazole is added in about 0.2eq to 1.2 eq. In some examples, the activator is DCI. In some examples, the DCI is added in about 0.02eq to 1.0 eq. In some examples, said process is performed in the presence of a base. In some examples, the base is NMI. In some examples, the phosphitylating reagent is 2-cyanoethyl-N, N, N', N'-tetraisopropylphosphorodiamidite or 2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite. In some examples, the process further comprises extraction, filtration, precipitation, and drying. In some examples, the precipitation step comprises precipitation of NAG-25 from DCM/heptane.

[0021] Disclosed herein is a process of preparing a NAG-25:

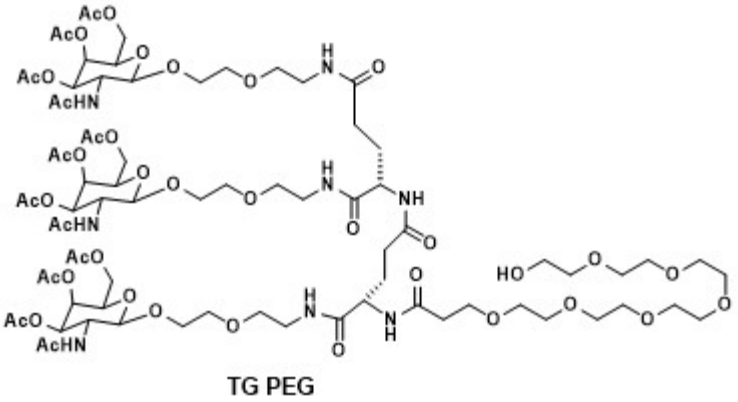


said process comprising any one of the examples disclosed herein for steps 1, 2, 3, 4a, 4, 5, or 6. In some examples the process comprises preparing t-Butyl Core by any one of the examples disclosed herein for preparing t-Butyl Core and transforming the t-Butyl Core into NAG-25. In some examples the process comprises preparing Triacid by any one of the examples disclosed herein for preparing Triacid and transforming the Triacid into NAG-25. In some examples, the process comprises preparing TGZ by any one of the examples disclosed herein for preparing TGZ and transforming the TGZ into NAG-25. In some examples, the process comprises preparing TG Amine by any one of the examples disclosed herein for preparing TG Amine and transforming the TG Amine into NAG-25. In some examples, the process comprises preparing TG PEG by any one of the examples disclosed herein for TG PEG and transforming the TG PEG into NAG-25.

[0022] Disclosed herein is a process of preparing NAG-25:



comprising treating a mixture of TG Amine and PEG acid with TBTU to afford TG PEG

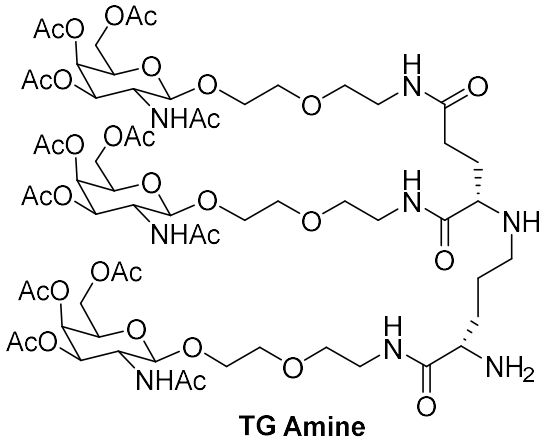


,

and

transforming TG PEG into NAG-25. Any of the foregoing examples and further examples described herein related to treating a mixture of TG Amine and PEG acid with TBTU to afford TG PEG would be useful for preparing NAG-25.

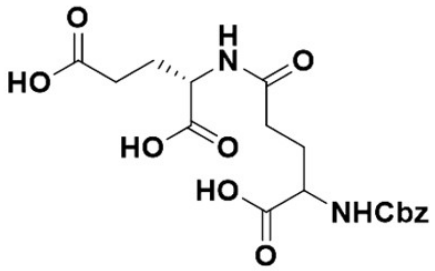
[0023] Disclosed herein is a process of preparing NAG-25, comprising a high pressure hydrogenolysis for a carboxybenzyl deprotection of TGZ to form the TG Amine:



, or a salt thereof,

and transforming TG Amine, or a salt thereof, into NAG-25. Any of the foregoing examples and further examples described herein related to a high pressure hydrogenolysis for a carboxybenzyl deprotection of TGZ to form the TG Amine would be useful for preparing NAG-25.

[0024] Disclosed herein is a process of preparing NAG-25, comprising reacting Triacid



with NAG-H or salt thereof in the presence of a coupling reagent and a base to form TGZ, and transforming TGZ into NAG-25. In some examples, TGZ is further precipitated from a solvent/antisolvent system. Any of the foregoing examples and further examples described herein related to reacting Triacid with NAG-H or salt thereof would be useful for preparing NAG-25.

[0025] Disclosed herein is a process of preparing NAG-25, comprising reacting t-Butyl core with an acid to form the Triacid, and transforming the Triacid into NAG-25. In some examples, the Triacid is crystalline. In some examples, the Triacid is crystalline Form I. Any of the foregoing examples and further examples described herein related to reacting t-Butyl core with an acid to form the Triacid would be useful for preparing NAG-25.

[0026] Disclosed herein is a process of preparing NAG-25, comprising reacting GluZ with GluOtBu or salt thereof to form the t-Butyl core, and transforming the t-Butyl Core into NAG-25. Any of the foregoing examples and further examples described herein related to processes comprising reacting GluZ with GluOtBu or salt thereof to form the t-Butyl core would be useful for preparing NAG-25.

[0027] Disclosed herein is a process of preparing a NAG-25, said process comprising a precipitation of TGZ from solvent/antisolvent system. Any of the foregoing examples and further examples described herein related to a precipitation of TGZ from solvent/antisolvent system would be useful for preparing NAG-25.

[0028] Disclosed herein is a process of preparing a NAG-25, said process comprising a Triacid intermediate that is crystalline. In some examples, the crystalline Triacid is crystalline Form I of Triacid, characterized by an X-ray powder diffractogram having at least a signal at three two-theta values chosen from 7.4 ± 0.2, 9.2 ± 0.2, 21.0 ± 0.2, 14.6 ± 0.2, 18.3 ± 0.2, and 19.3 ± 0.2. In some examples, the crystalline Triacid is crystalline Form I of Triacid, characterized by an X-ray powder diffractogram having a signal at two-theta values of 7.4 ± 0.2, 9.2 ± 0.2, and 21.0 ± 0.2. In some examples, the crystalline Triacid is crystalline Form I of Triacid, characterized by an X-ray powder diffractogram substantially similar to that in Figure 2. In some examples, the crystalline Triacid is crystalline Form

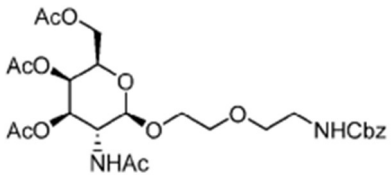
II of Triacid, characterized by an X-ray powder diffractogram substantially similar to that in Figure 3. Any of the foregoing examples and further examples described herein related to crystalline Triacid would be useful for preparing NAG-25.

[0029] Disclosed herein is a process for preparing t-Butyl Core comprising reacting GluZ with GluOtBu or salt thereof to produce t-Butyl Core, wherein the reacting is performed in the presence of a coupling reagent, a base, and a solvent; optionally wherein the a) coupling reagent is EDC/Oxyma, TFFH, PyOxim, CDI, PivCl, T3P, or COMU; or b) base is N-methylmorpholine (NMM); and/or c) solvent is IPAc, MeTHF, MIBK, or MTBE.

[0030] Disclosed herein is a process of preparing Triacid comprising reacting t-Butyl core with an acid, wherein the acid is phosphoric acid (H3PO4), TFA, HCl, benzenesulfonic acid, or p-toluenesulfonic acid.

[0031] Disclosed herein is a process for preparing NAG-Z comprising reacting Acyl GalNAc with Alcohol-Z to produce NAG-Z, wherein the reaction is performed in the presence of acid.

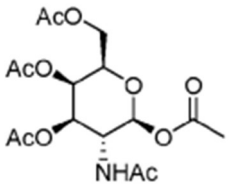
[0031a] In yet another aspect, the present disclosure refers to a process for preparing NAG-Z:



NAG-Z

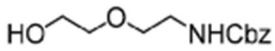
,

said process comprising reacting Acyl GaINAc:



Acyl GalNAc

with Alcohol-Z:



Alcohol-Z

to produce NAG-Z, wherein the reaction is performed in the presence of acid,

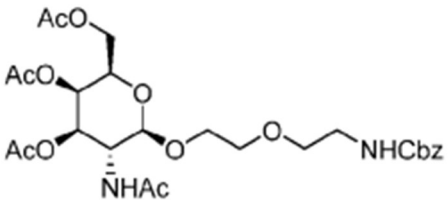
optionally wherein:

a) the acid is bismuth trifluoromethane sulfonate ($Bi(OTf)_3$), boron trifluoride etherate (BF_3OEt_2), tert-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf), triisopropylsilyl trifluoromethanesulfonate

(TIPSOTf), indium triflate ($In(OTf)_3$), or copper triflate ($Cu(OTf)_2$); and/or b) the reaction is performed in the presence of a solvent selected from acetonitrile, dichloromethane, and dichloroethane, optionally wherein the solvent is acetonitrile and antisolvent is added is added to crystalline the NAG-Z, optionally wherein the antisolvent is water or MTBE; and/or c) the: Acyl GaINAc is present at about 1.0-2.0 equivalents, relative to Alcohol-Z; or acid is present at about 0.01-0. 1 equivalents, relative to Alcohol-Z; and/or d) the reaction temperature is heated to about 55-65°C or 58-62°C; and/or the process further comprises transforming the NAG-Z into NAG-25.

[0032] Disclosed herein is a process of preparing NAG-H, or salt thereof, comprising hydrogenation of NAG-Z in the presence of a Pd/C catalyst and solvent, optionally wherein the solvent is dimethylacetamide (DMAc) or DCM.

[0032a] In yet another aspect, the present disclosure refers to a process of preparing NAG-H, or salt thereof, comprising hydrogenation of NAG-Z:



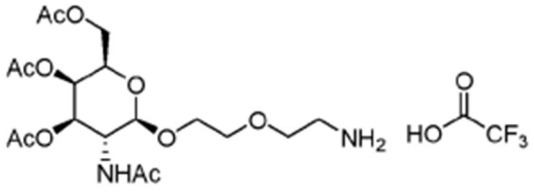
NAG-Z

in the presence of a Pd/C catalyst and solvent, optionally wherein the solvent is dimethylacetamide (DMAc) or DCM,

optionally wherein:

a) the DMAc or DCM is about 3 volumes to about 6 volumes; and/or b) the process further comprises acid, optionally wherein the acid is trifluoroacetic acid (TFA), acetic acid (AcOH), or Pivalic acid (PivOH), optionally wherein the acid is present at 0.01-0.04 equivalents, relative to Alcohol-Z; and/or

c) the NAG-H or salt thereof is NAG-H TFA:



NAG-H TFA

; and/or

d) the Pd/C catalyst is 5-10% Pd/C; optionally wherein the Pd/C is 5% Pd/C; and/or e) the process further comprises transforming the NAG-H or salt thereof into NAG-25.

[0033] Disclosed herein is a process of preparing TGZ comprising reacting Triacid with NAG-H, or a salt thereof, in the presence of a coupling reagent and a base; and precipitating TGZ from a solvent/antisolvent system.

[0034] Disclosed herein is a process of preparing TG Amine, or a salt thereof, comprising a high pressure hydrogenolysis of TGZ to form the TG Amine or a salt thereof, wherein said hydrogenolysis is performed in the presence of an acid.

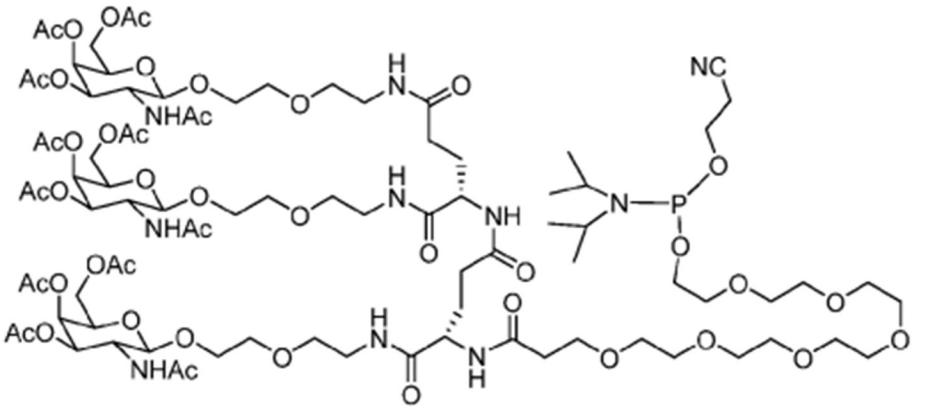
[0035] Disclosed herein is a process for preparing TG PEG comprising reacting a solution of TG Amine, or a salt thereof, and PEG acid with a coupling reagent, wherein the coupling reagent is added to a solution of PEG acid and TG Amine or salt thereof over a period of time of about 30min to about 1.5 hours.

[0036] Disclosed herein is a process of preparing NAG-25 comprising reacting TG PEG with an activator and a phosphitylating reagent, wherein the activator is tetrazole, 4,5-dicyanoimidazole (DCI), 5-Ethylthio-1H-Tetrazole (ETT), or Benzothiotetrazole (BTT); and/or wherein the phosphitylating reagent is 2-cyanoethyl-N, N, N', N'-tetraisopropylphosphorodiamidite or 2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite.

[0036a] In yet another aspect, the present disclosure refers to a process of preparing Alcohol-Z, said process comprising reacting benzyl chloroformate with aminoalcohol in presence of a solvent and a base, optionally wherein the solvent is dichloromethane (DCM) and the base is triethylamine (TEA), and/or wherein the aminoalcohol is present at 1.1 equivalents, relative to benzyl chloroformate, and/or the reaction temperature is about 30-40°C.

CLAIMS

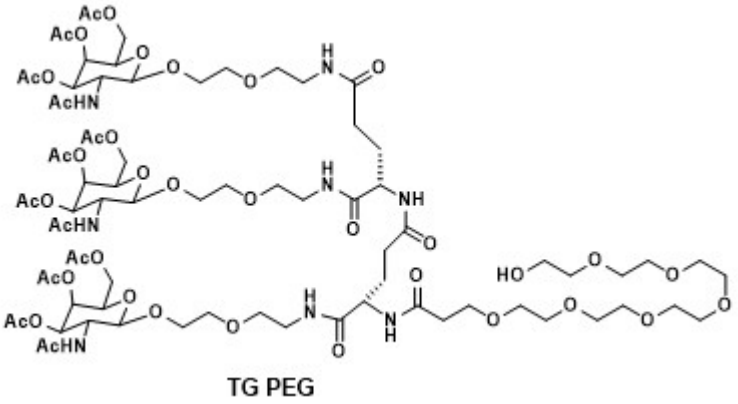
1. A process for preparing NAG-25:



,

wherein said process comprises

Step 6: a process comprising reacting TG PEG



TG PEG

,

with an activator and a phosphitylating reagent (P-reagent), wherein the activator is tetrazole, 4,5-dicyanoimidazole (DCI), 5-Ethylthio-lH-Tetrazole (ETT), or Benzothiotetrazole (BTT); and/or wherein the phosphitylating reagent is 2-cyanoethyl-N, N, N', N'-tetraisopropylphosphorodiamidite or 2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite;

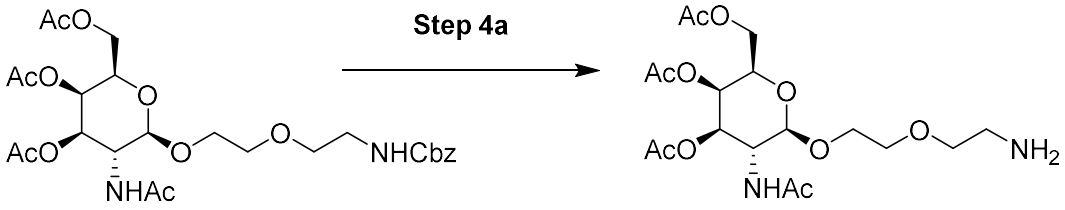
and

wherein the process of Step 6 is performed in the presence of a base; and

optionally wherein the step 6 process comprises reacting TG PEG with 2-cyanoethyl-N, N, N', N'-tetraisopropylphosphorodiamidite in the presence of tetrazole and NMI in dichloromethane.

2. The process of claim 1, wherein said process further comprises one or more of the following sequence of steps:

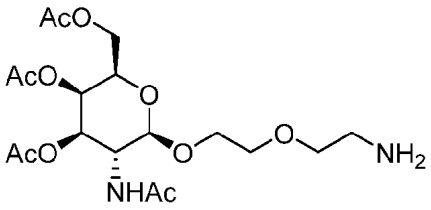
Step 4a: a process comprising hydrogenation of NAG-Z in the presence of a Pd/C catalyst and a solvent to obtain NAG-H, or a salt thereof,



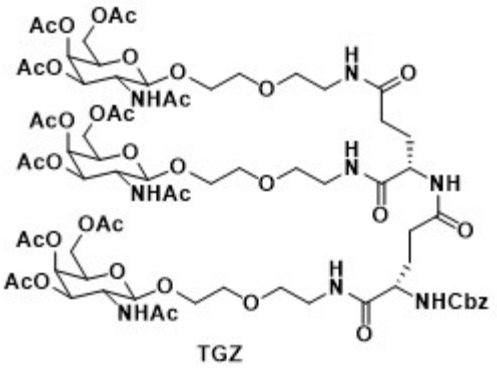
,

wherein the solvent is dimethylacetamide (DMAc) or dichloromethane (DCM);

Step 4b: a process comprising reacting Triacid

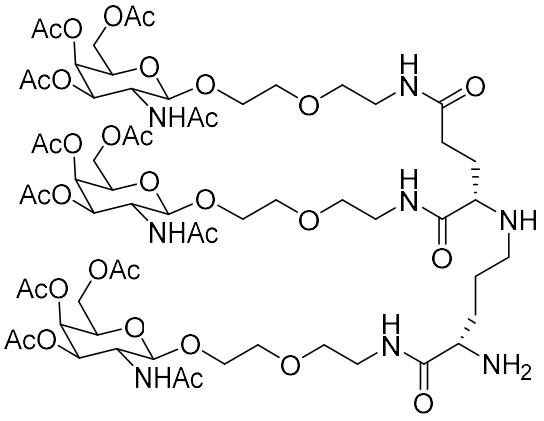


(Triacid), with NAG-H , or a salt thereof, in the presence of a coupling reagent and a base; and precipitating the product from a solvent/antisolvent system to obtain TGZ,



;

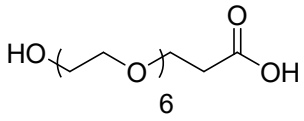
Step 5a: a process comprising high pressure hydrogenolysis of TGZ to form TG Amine,



(TG Amine), or a salt thereof, wherein the reaction solvent is DCM, said hydrogenolysis is performed in the presence of an acid and a palladium source for hydrogenolysis, wherein the palladium source is palladium on carbon (Pd/C) catalyst;

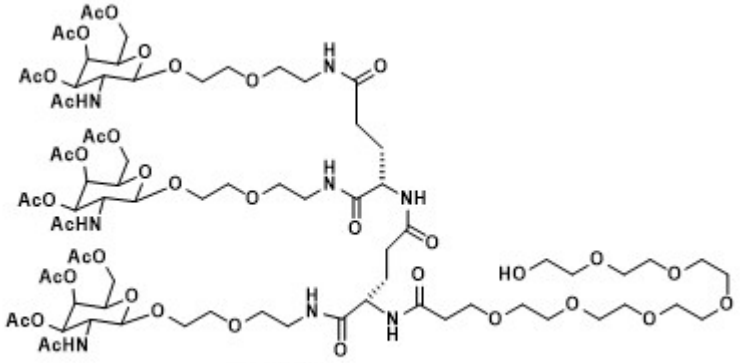
and/or

Step 5b: a process comprising reacting a solution of TG Amine, or a salt thereof, and PEG acid,



PEG Acid

, with a coupling reagent, wherein the coupling reagent is added to a solution of PEG acid and TG Amine or salt thereof over a period of time of 30min to 1.5 hours, to obtain TG PEG,

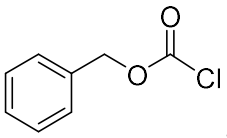
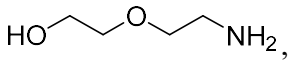
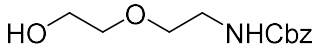


TG PEG

;.

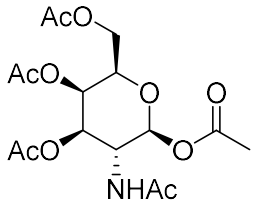
3. The process of claim 1, wherein said process comprises each of Step 4a, Step 4b, Step 5a, Step 5b, and wherein the step 6 process comprises reacting TG PEG with 2-cyanoethyl-N, N, N', N'-tetraisopropylphosphorodiamidite in the presence of tetrazole and N-methylimidazole (NMI).

4. The process of any one of claims 1-3, further comprising the following steps:

Step 3a: a process comprising reacting benzyl chloroformate, , with aminoalcohol, , in presence of a solvent and a base to obtain Alcohol Z,  (Alcohol Z);

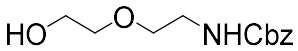
and

Step 3b: a process comprising reacting Acyl GalNAc:



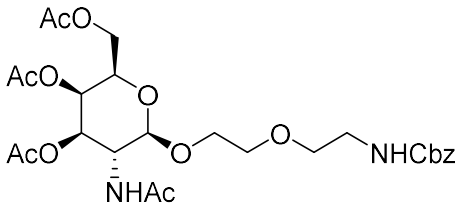
Acyl GalNAc

with Alcohol-Z:



Alcohol-Z

to produce NAG-Z,

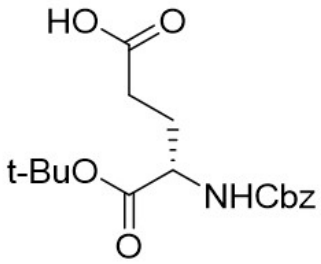


NAG-Z

wherein the reaction is performed in the presence of acid.

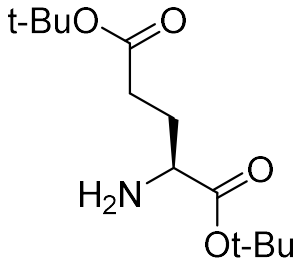
5. The process of any one of claims 1-4, further comprising

Step 1: a process comprising reacting GluZ:

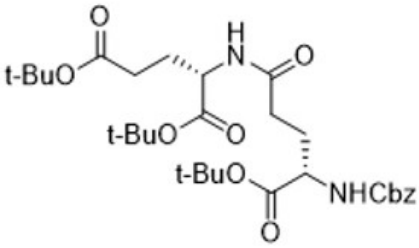


(GluZ)

with GluOtBu or salt thereof:



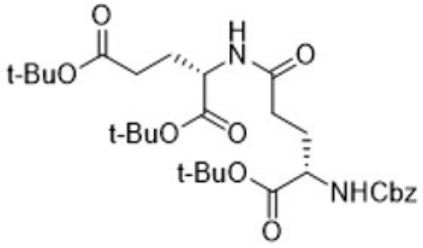
GluOtBu



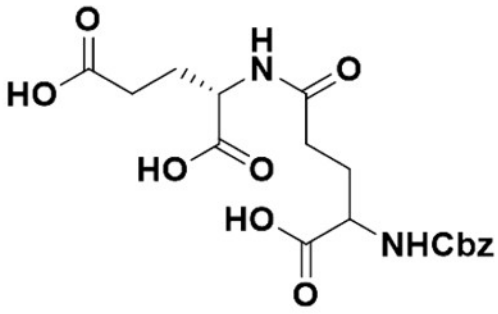
to produce t-Butyl Core, (t-Butyl Core), wherein the reacting is performed in the presence of a coupling reagent, a base, and a solvent; optionally wherein the a) coupling reagent is EDC/Oxyma, TFFH, PyOxim, CDI, PivCl, T3P, or COMU; or b) base is N-methylmorpholine (NMM); and/or c) solvent is IPAc, MeTHF, MIBK, or MTBE

and

Step 2:



a process comprising reacting t-Butyl Core, (t-Butyl Core), with an acid, wherein the acid is phosphoric acid (H_3PO_4), TFA, HCl, benzenesulfonic acid, or p-toluenesulfonic acid, to obtain Triacid,



(Triacid).

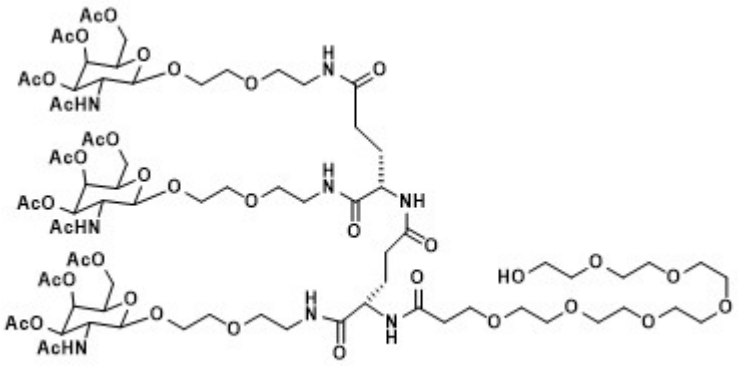
6. The process of any one of claims 1-5 wherein the step 6 process:

a) comprises reacting TG PEG with 2-cyanoethyl-N, N, N', N'-tetraisopropylphosphorodiamidite in the presence of tetrazole and N-methylimidazole (NMI) in dichloromethane; and/or

b) (i) the TG PEG is in a solution and wherein an activator and a phosphitylating reagent are combined in another solution; and (ii) the solution of TG PEG is added to the solution of activator and P-reagent over a period of time, optionally wherein the period of time is about 2.5-10 hour or optionally wherein the solution of TG PEG is allowed to react with the solution of P-reagent and activator at about 35-45 °C; and/or

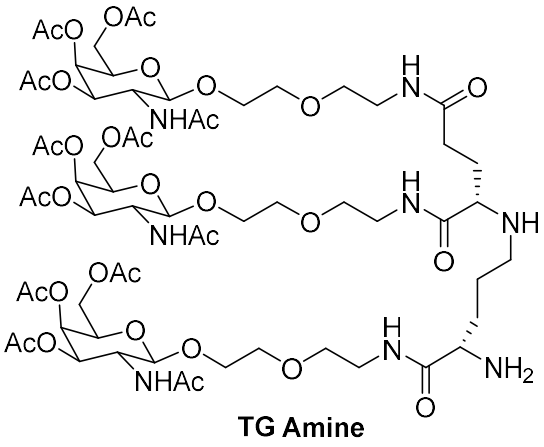
c) NAG-25 is precipitated using inverse precipitation into antisolvent, optionally where precipitation comprises precipitation of NAG-25 from dichloromethane (DCM)/heptane as solvent/antisolvent system.

7. A process for preparing TG PEG



TG PEG

said process comprising reacting a solution of TG Amine:



TG Amine

, or a salt thereof, and PEG acid with a coupling reagent, wherein the coupling reagent is added to a solution of PEG acid and TG Amine or salt thereof over a period of time of about 30min to about 1.5 hours,

optionally wherein:

a) solution further comprises a base, optionally wherein the base is N,N-Diisopropylethylamine (DIPEA); and/or

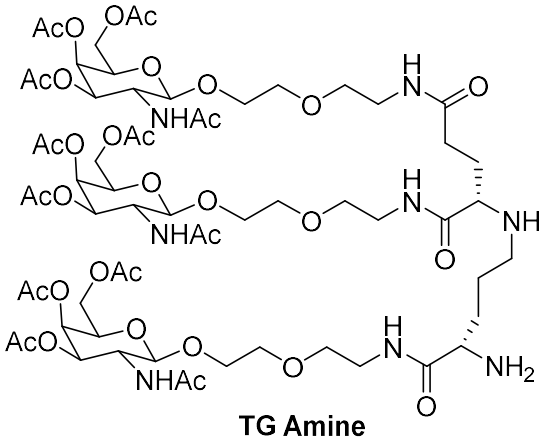
b) the coupling reagent is 2-(1H-Benzotriazole-l-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU) or N-[(Dimethylamino)-1H-l,2,3-triazolo-[4,5-b]pyridin-l-ylmethylene]-N-methylmethanaminium hexafluorophosphate N-oxide (HATU); and/or

c) the TG PEG is produced in greater than or equal to about 90% purity as measured by LC or wherein TG PEG dimer impurity is present at the end of reaction at an amount of less than 10% as measured by LC; and/or

d) the solution of TG Amine or a salt thereof is carried forward from a separate process without isolation of the TG Amine or salt thereof, optionally wherein the TG Amine or salt thereof is TG Amine trifluoroacetate (TFA) salt; and/or

e) the process further comprises transforming the TG PEG into NAG-25.

8. A process of preparing TG Amine:



TG Amine

, or a salt thereof,

said process comprising a high pressure hydrogenolysis of TGZ to form the TG Amine or a salt thereof, wherein said hydrogenolysis is performed in the presence of an acid, optionally:

a) comprising a palladium source for hydrogenolysis, optionally wherein the palladium source is palladium on carbon (Pd/C) catalyst, optionally wherein the palladium source is 5% Pd/C; and/or

b) wherein the acid is trifluoroacetic acid (TFA), oxalic acid, hydrochloride acid (HC1), acetic acid (AcOH), phosphoric acid (H3PO4), or citric acid; optionally wherein the acid is no more than about 1.1 eq; and/or

c) wherein said hydrogenolysis is performed in a solvent, optionally wherein the solvent is DCM, isopropyl acetate (IPAc), or methanol (MeOH); and/or

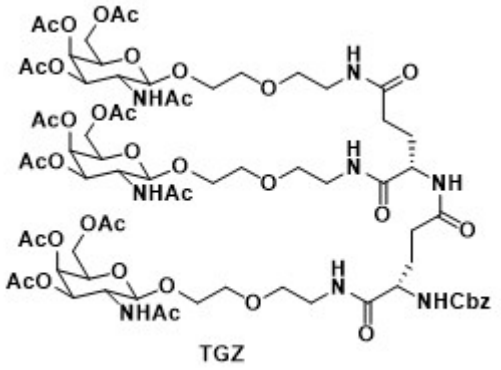
d) wherein the process reduces formation of one or more impurities that arc TG Amine des-acyl, TG Amine di-NAG-OH, TG Amine guanidyl, TG Amine acyl, TG Acetamide, and/or NAG-H guanidine; and/or

e) wherein the TG Amine or salt thereof is carried forward as a solution of TG Amine or salt thereof and without isolation of the TG Amine or salt thereof into another process of preparing TG PEG; and/or

f) wherein the TG Amine or salt thereof is a TG Amine acid salt, optionally, a phosphate, formate, acetate, trifluoroacetate, or oxalate salt; and/or

g) further comprising transforming the TG Amine or salt thereof into NAG-25.

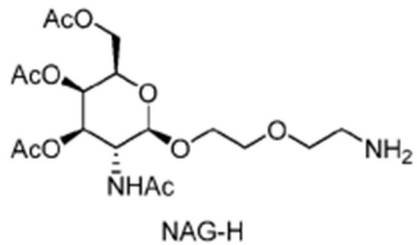
9. A process of preparing TGZ



TGZ

,

said process comprising reacting Triacid with NAG-H:



NAG-H

, or salt thereof, in the presence of a coupling reagent and a base; precipitating TGZ from a solvent/antisolvent system,

optionally wherein:

a) the coupling reagent is TBTU, HATU or Chloro-N,N,N',N'-tetramethylformamidinium hexafluorophosphate (TCFH), optionally wherein the coupling reagent is TBTU; and/or

b) the base is diisopropylethylamine (DIPEA), N-methylimidazole (NMI), *N*-methylmorpholine (NMM), or 2,2,6,6-tetramthylpiperidine (TMP), optionally wherein the base is NMI; and/or

c) the process further comprises at least one buffer wash, optionally wherein the buffer wash is about pH-5-7; and/or

d) said reacting is conducted in a solvent selected from DCM, DMF, MeCN, and DMAc, or combination thereof; and/or

e) an anti-solvent is added to a solution comprising TGZ and the solvent, optionally wherein the anti-solvent is an ethereal solvent, optionally wherein the ethereal solvent is DME, 2-MeTHF, or MTBE; and/or

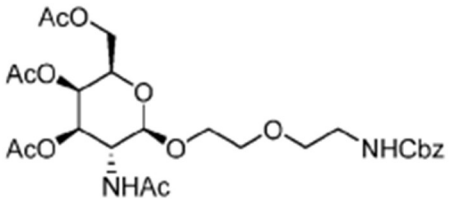
f) TGZ is prepared with reduced levels of one or more impurities, wherein the impurities is mono-NAG, DI-NAG, DI-NAG-AZLACTONE, des-acyl, di-NAG-OH, di-NAG-OAc, NAG-H guanidine, or NAG-H trifluoroacetamide; and/or

g) TGZ is obtained in at least 95 % purity without use of column chromatography; and/or

h) the NAG-H, or salt thereof, is a solution carried forward from another process without isolation of the NAG-H, or salt thereof; and/or

i) the process further comprises transforming the TGZ into NAG-25.

10. A process of preparing NAG-H, or salt thereof, comprising hydrogenation of NAG-Z:



NAG-Z

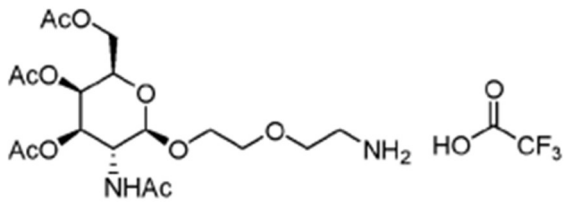
in the presence of a Pd/C catalyst and solvent, optionally wherein the solvent is dimethylacetamide (DMAc) or DCM,

optionally wherein:

a) the DMAc or DCM is about 3 volumes to about 6 volumes; and/or

b) the process further comprises acid, optionally wherein the acid is trifluoroacetic acid (TFA), acetic acid (AcOH), or Pivalic acid (PivOH), optionally wherein the acid is present at 0.01-0.04 equivalents, relative to Alcohol-Z; and/or

c) the NAG-H or salt thereof is NAG-H TFA:



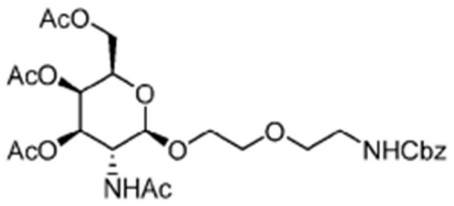
NAG-H TFA

; and/or

d) the Pd/C catalyst is 5-10% Pd/C; optionally wherein the Pd/C is 5% Pd/C; and/or

e) the process further comprises transforming the NAG-H or salt thereof into NAG-25.

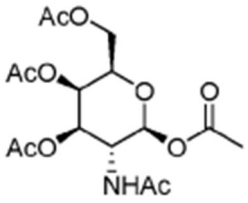
11. A process for preparing NAG-Z:



NAG-Z

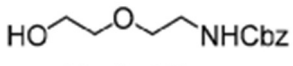
,

said process comprising reacting Acyl GaINAc:



Acyl GalNAc

with Alcohol-Z:



Alcohol-Z

to produce NAG-Z, wherein the reaction is performed in the presence of acid,

optionally wherein:

a) the acid is bismuth trifluoromethane sulfonate ($Bi(OTf)_3$), boron trifluoride etherate (BF_3OEt_2), tert-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf), triisopropylsilyl trifluoromethanesulfonate (TIPSOTf), indium triflate ($In(OTf)_3$), or copper triflate ($Cu(OTf)_2$); and/or

b) the reaction is performed in the presence of a solvent selected from acetonitrile, dichloromethane, and dichloroethane, optionally wherein the solvent is acetonitrile and antisolvent is added is added to crystalline the NAG-Z, optionally wherein the antisolvent is water or MTBE; and/or

c) the: Acyl GaINAc is present at about 1.0-2.0 equivalents, relative to Alcohol-Z; or acid is present at about 0.01-0. 1 equivalents, relative to Alcohol-Z; and/or

d) the reaction temperature is heated to about 55-65°C or 58-62°C; and/or

the process further comprises transforming the NAG-Z into NAG-25.

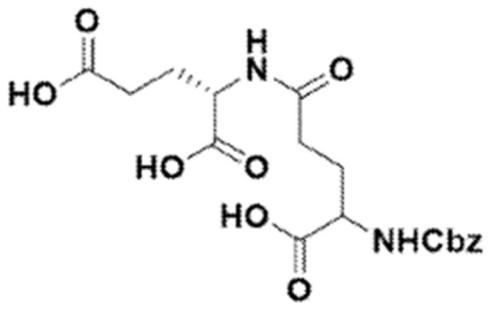
12. A process of preparing Alcohol-Z, said process comprising reacting benzyl chloroformate with aminoalcohol in presence of a solvent and a base, optionally wherein the solvent is dichloromethane (DCM) and the base is triethylamine (TEA), and/or wherein the aminoalcohol is present at 1.1 equivalents, relative to benzyl chloroformate, and/or the reaction temperature is about 30-40°C.

13. A crystalline form of Triacid, wherein:

a) the crystalline form is crystalline Form I of Triacid, characterized by an X-ray powder diffractogram having at least a signal at three two-theta values selected from 7.4 ± 0.2, 9.2 ± 0.2, 21.0 ± 0.2, 14.6 ± 0.2, 18.3 ± 0.2, and 19.3 ± 0.2 or characterized by an X-ray powder diffractogram substantially similar to that in Figure 2; or

b) the crystalline form is crystalline Form II of Triacid, characterized by an X-ray powder diffractogram having at least a signal at three two-theta values selected from 7.8 ± 0.2, 9.7 ± 0.2, 13.3 ± 0.2, 15.7± 0.2, 17.1 ± 0.2, 18.9 ± 0.2, 20.2± 0.2, and 21.0 ± 0.2 or characterized by an X-ray powder diffractogram substantially similar to that in Figure 3.

14. A process of preparing Triacid:



Triacid,

said process comprising reacting t-Butyl core with an acid, wherein the acid is phosphoric acid (H_3PO_4), TFA, HCl, benzenesulfonic acid, or p-toluenesulfonic acid,

optionally wherein:

a) the reaction is conducted in a solvent selected from 2-MeTHF, acetonitrile, THF, DMA, sulfolane, and DME, optionally wherein the solvent is mixed with water, optionally wherein the solvent is 2-MeTHF, optionally wherein the solvent is about 3.0 - 4.0 vol of 2-MeTHF and is mixed with about 0.5 - 2vol water; and/or

b) the reaction is conducted at a temperature of about 40-60°C; and/or

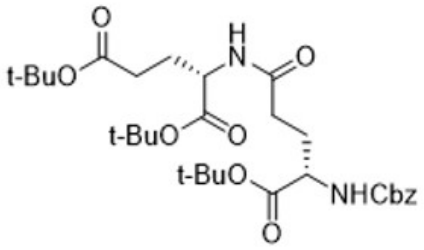
c) the Triacid is isolated as a crystalline solid.

d) the process further comprises crystallization of Triacid using acetone/toluene, 2-MeTHF/IPAc, 2-MeTHF/CPME, acetone/heptane, or MeTHF/acetonitrile; and/or

e) the Triacid is obtained in a crystalline form characterized by an X-ray powder diffractogram having at least a signal at three two-theta values chosen from 7.4 ± 0.2, 9.2 ± 0.2, 21.0 ± 0.2, 14.6 ± 0.2, 18.3 ± 0.2, and 19.3 ± 0.2 or characterized by an X-ray powder diffractogram having at least a signal at three two-theta values selected from 7.8 ± 0.2, 9.7 ± 0.2, 13.3 ± 0.2, 15.7± 0.2, 17.1 ± 0.2, 18.9 ± 0.2, 20.2± 0.2, and 21.0 ± 0.2; and/or

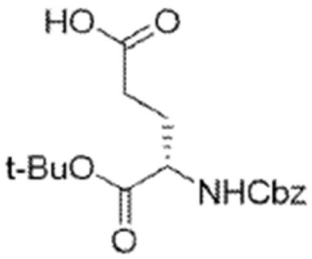
f) the process further comprises transforming the Triacid into NAG-25.

15. A process for preparing t-Butyl Core:



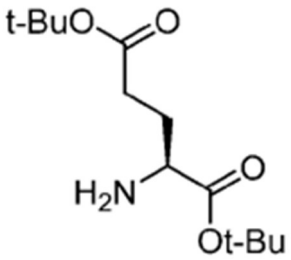
t-Butyl Core

said process comprising reacting GluZ:



GluZ

with GluOtBu or salt thereof:



GluOtBu

to produce t-Butyl Core, wherein the reacting is performed in the presence of a coupling reagent, a base, and a solvent; optionally wherein:

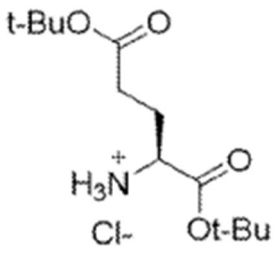
a) the:

(i) coupling reagent is EDC/Oxyma, TFFH, PyOxim, CDI, PivCl, T3P, or COMU;

(ii) base is N-methylmorpholine (NMM); and/or

(iii) solvent is IPAc, MeTHF, MIBK, or MTBE; and/or

b) GluOtBu or salt thereof is a GluOtBu hydrochloride salt:



,

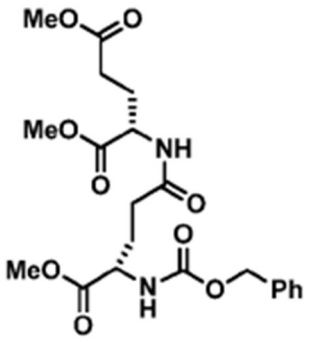
c) the coupling reagent is PivCl, solvent is MTBE; optionally wherein the GluOtBu is about 1-1.5 eq.; or PivCl is about 1-1.5 eq; orNMM is about 3-4 eq.; or MTBE is about 8-12 vol, each relative to GluZ; and/or

d) a first solution comprising GluZ and NMM in a solvent is added to a second solution comprising the solvent and coupling reagent; optionally wherein the coupling reagent is in excess and/or GluOtBu is in excess, optionally wherein the coupling reagent is PivCl; and/or

e) the process further comprises addition of an antisolvent, optionally over a time period of about 2-5 hours; and/or

f) the process further comprises transforming the t-Butyl Core into NAG-25.

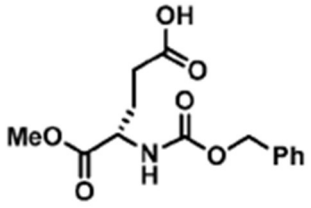
16. A process for preparing Methyl Core:



Methyl Core

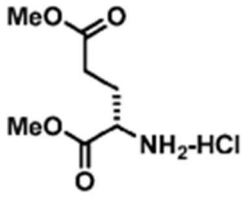
,

said process comprising reacting z-L-Glu-OMe:



Z-L-GluOMe

with L-Glutamic acid dimethyl ester (di(OMe)Glu):



di(OMe)Glu

to produce Methyl Core, optionally:

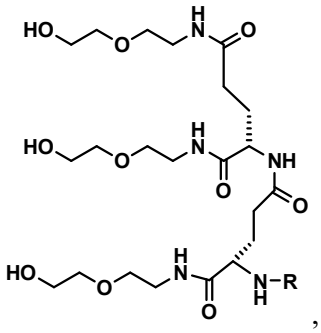
a) in the presence of a base and a coupling reagent, optionally wherein:

(i) the base is added to a z-L-GluOMe solution prior to addition of the coupling reagent and di(OMe)Glu to the reaction; or wherein the coupling reagent is added to the solution of z-L-Glu-OMe and base prior to addition of di(OMe)Glu to the reaction; and/or

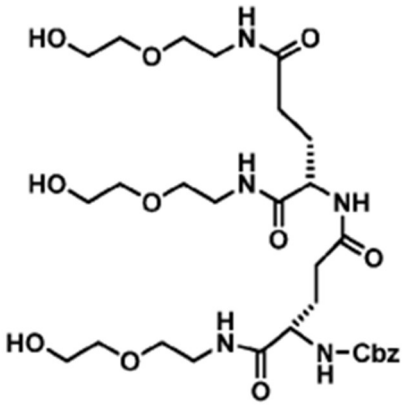
(ii) the base is N-Methylmorpholine (NMM) or di-isopropylethylamine and/or the coupling reagent is isobutyl chloroformate (IBCF), TBTU, HATU, EDC, or DCC

b) further comprising a solvent, optionally wherein the solvent is THF or MeTHF; and/or

c) further comprising transforming the Methyl Core into NAG-25.

17. A compound , wherein R is H (unprotected Triol) or a Cbz protecting group (Triol).

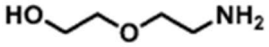
18. A process for preparing Triol:



Triol

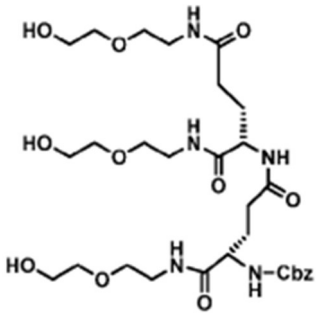
,

the process comprising reacting Methyl Core with 2-(2-aminoethoxy)ethanol:



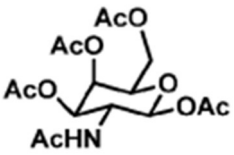
to produce Triol, optionally wherein the Triol is transformed into NAG-25.

19. A process for preparing TGZ, the process comprising reacting protected Triol:



Triol

with Beta-D-Galactosamine pentaacetate:



B-D-galactosamine pentaacetate

to produce TGZ. optionally wherein the TGZ is transformed into NAG-25.