Bioorganic Chemistry 39 (2011) 88–93

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Synthesis of the essential core of the human glycosylphosphatidylinositol (GPI) anchor

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ARTICLE INFO

Article history:
Received 8 November 2010
Available online 17 December 2010

Keywords:
GPI anchor
Glycosylations
PNH disease

ABSTRACT

The biological role of GPI anchors is of paramount importance; however, we are still far from fully understanding the structure–function relationship of these molecules. One major limiting factor has been the tiny quantities available from natural sources; obtaining homogeneous and well-defined GPI structures by synthesis, is both a challenge and an attractive goal. We report here the convergent synthesis of the essential core of the human GPI anchor **1**, exploiting a common precursor to obtain the trisaccharidic donor **2** and a novel protecting groups sequence. The final product, prepared for the first time, is biologically active.

1. Introduction

Glycosyl phosphatidylinositols (GPIs) are a family of glycolipids defined by the structural motif Manα1-4 GlcNα 1-6 myo-inositol-P-lipid. GPIs anchor a variety of functionally diverse hydrophilic molecules (proteins, proteoglycans and phosphoglycans) to cell membranes in eukaryotic cells [1,2]. GPIs are found in all eukaryotes, but their structures vary; in fact, although the carbohydrate backbone is universally conserved, additional phosphoethanolamine carbohydrate side chains and different phospholipid tails differentiate the more than 20 GPI anchors that are known. Approximately 150 proteins are modified by GPI, which is vital for embryonic development, immune response and neurogenesis in mammals [3].

Although GPI anchors play a pivotal role in numerous biological functions, a full understanding of their structure–function relationship is still elusive, largely because of the extremely limited quantities of GPI anchors available from natural sources [4–6]. Thus, for example, while the role of the GPI lipid moiety is clearly related to embedding the anchor in the lipid bilayer of the membrane, the function of the glycan moiety is not yet completely understood [7,8].

Chemical synthesis provides a valuable tool for tackling these issues, as it can supply native and non-native GPI anchors for biological studies. Several efforts have been made in the organic synthesis of GPIs [9–20]: however, due to molecular complexity, these are not easily amenable to structural modifications. As a matter of fact, the lack/insertion of a monosaccharidic unit or of a phospholipidic chain often mean a re-designing of the all synthesis. For this reason the availability of new strategies to get homogeneous and well-defined GPIs and related structures remains an attractive target.

Recently, it has been proposed that an autoimmune attack against GPI anchors may be a key factor to explain the expansion of hematopoietic cells deficient in all GPI-linked proteins (GPI^-) in the serious rare blood disorder paroxysmal nocturnal hemoglobinuria (PNH) [21]. PNH is characterized by complex clinical manifestations, of which the most spectacular, intravascular haemolysis producing hemoglobinuria, is a direct consequence of the deficiency from the red cell membrane of the two complement-regulatory proteins CD59 and CD55 [21,22]. Studies of the pathogenetic mechanisms of PNH have been hampered by the fact that the mammalian GPI molecule is not available. This has been the main goal driving the work reported in this paper.

The choice of the specific target was not easy and, to some extent arbitrary. We have selected structure **1** (Fig. 1) based on the following considerations.

(a) Most GPI-anchored proteins on the cell surface do not have phosphoethanolamine (EtNP) on Man2 of GPI [7]; we therefore thought that the GPI glycan core of structure **1** might be the major precursor for protein attachment in the endoplasmatic reticulum.

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0045-2068/$ - see front matter © 2011 Published by Elsevier Inc.
doi:10.1016/j.bioorg.2010.12.002

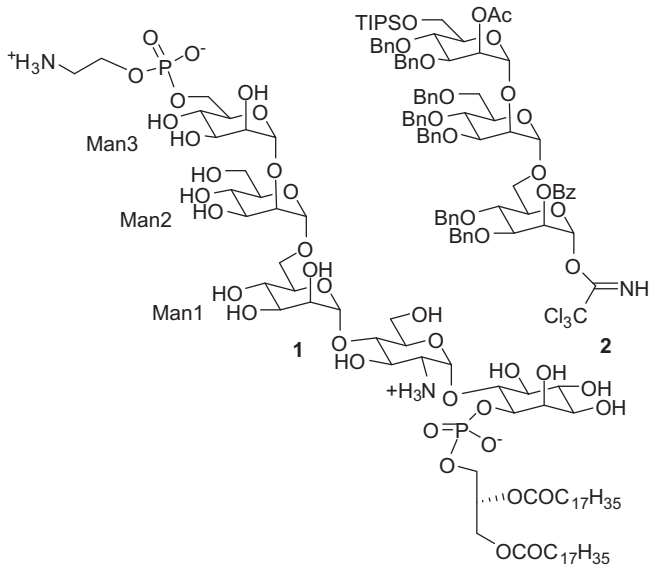


Fig. 1. Structure of GPI anchor **1** and trisaccharidic donor **2**.

(b) The glycolipid residues are required for mooring the entire anchor to the cell membrane. Since the precise structural requirements (e.g. length) are still unclear [7], we have opted for two $C_{17}H_{35}$ acyl residues.
(c) We have omitted any acyl group on inositol, because this does not appear in the mammalian GPI biosynthetic pathway [7,23].

Although we have capitalized on previous syntheses of GPI anchors, the molecule described herein is novel.

2. Results and discussion

The synthetic strategy we have adopted has some features in common with previous approaches to the total synthesis of GPI [11,24–28]; however the protecting groups strategy, the selection of glycosylation methods, as well as the choice of some key intermediates have been specifically devised for compound **1**.

The trisaccharidic donor **2** [24] (Fig. 1) represents the first target of the proposed total synthesis. Trichloroacetimidates donors **3** and **4** and mannopyranoside acceptor **5** have been chosen (Scheme 1). The three mannopyranosides were obtained from a common precursor, the commercially available *ortho*ester **6**, in high yielding steps (Scheme 1).

After deacetylation under basic conditions, **6** was monosilylated at O-6 and dibenzylated to afford **7**. Reaction of the *ortho*ester **7** [29] with allyl alcohol in the presence of a Lewis acid formed the corresponding allyl glycoside **8**, as α anomer, in 95% yield. The axial acetyl group of **8** was removed and replaced, using standard conditions, by a benzoyl group. Subsequent deprotection of the tri*iso*propylsilyl ether gave the orthogonally protected acceptor **5** in 98% yield (Scheme 1). Treatment of the *ortho*ester **7** with *para*-toluensulfonic acid afforded the mannopyranoside **9**, which was activated as trichloroacetimidate under Schmidt's conditions, affording donor **3** (Scheme 1). Donor **4**, in turn, was prepared from the tribenzyl *ortho*ester **10** which, after treatment with *para*-toluensulfonic acid (98%) and activation as trichloroacetimidate under Schmidt's conditions, gave the suitably protected donor **4** (91%, α anomer) (see Scheme 1).

Glycosylation of **5** with donor **4** by treatment with trimethylsilyl triflate in dichloromethane at room temperature, afforded the α 1–6 dimannoside **11** (95%) (Scheme 2). Dimannoside **11** was deacetylated [30] and glycosidated (α 1–2), under the same reaction conditions reported above, with donor **3** giving the trimannoside **12** (83%). Sequential deprotection of the allyl group under standard conditions (94%) and activation as trichloroacetimidate afforded the desired trisaccharidic donor **2** (70%) (Scheme 2).

The pseudo-disaccharide **13** (Scheme 3) was obtained reacting the activated derivative **14** [31] and allylinositol **15** [26,32] at room temperature with trimethylsilyl triflate in dichloromethane [14,25,33].

After acetyl removal, the obtained azide **16** (α anomer, 53%) was firstly treated with benzaldehyde dimethylacetal, then the acetal was reductively opened using an acid and sodium cyanoboronhydride to afford the pseudo-disaccharidic acceptor **13** (see Scheme 3). The assembly of the pseudo-pentasaccharide **17** [24] was obtained in excellent yield by coupling the trisaccharide **2** with the glucosamine-inositol pseudo-disaccharide **13** (Scheme 4).

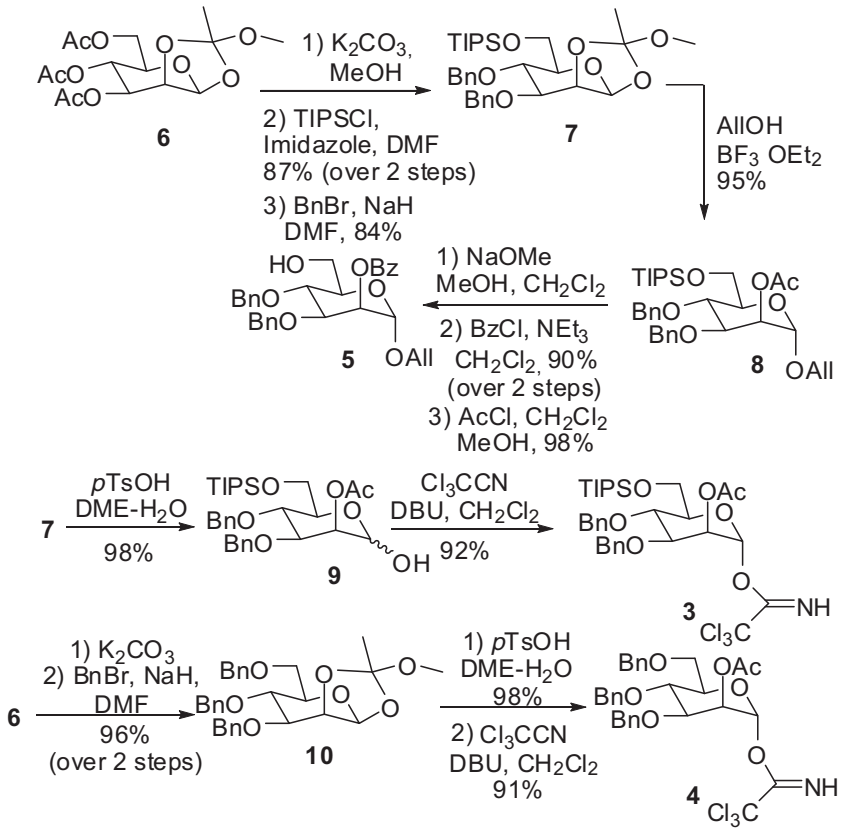
Acetyl and benzoyl groups of the pseudo-pentasaccharide **17** [24] were converted into a benzyl protecting group; the allyl residue on inositol moiety was then removed by treatment with palladium (II) chloride in the presence of acetic acid leading to the pseudo-pentasaccharide **20** [34] (67%). Coupling with the phospholipid **21** [25] afforded the phosphate derivative **22**. Mild cleavage of the tri*iso*propyl group with scandium (III) triflate [25] gave **23**, to which the phosphoethanol amine complex **24** [25,28] was attached, giving **25** (Scheme 5).

As previously reported by Fraser-Reid for a similar molecule [35], the final global debenzylation and azide reduction, performed in a single step, proved to be rather unpredictable. In our hands, the following approach was successful (any variation of this procedure was rather unsatisfactory, producing partially deprotected material or causing loss of the phospholipid tail): starting with dichloromethane/*iso*propanol (1:1) mixture as solvent, in the presence of palladium (II) hydroxide (20% on charcoal) and an excess of formic acid [25], with balloon pressure for eight hours at room temperature (see Section 4).

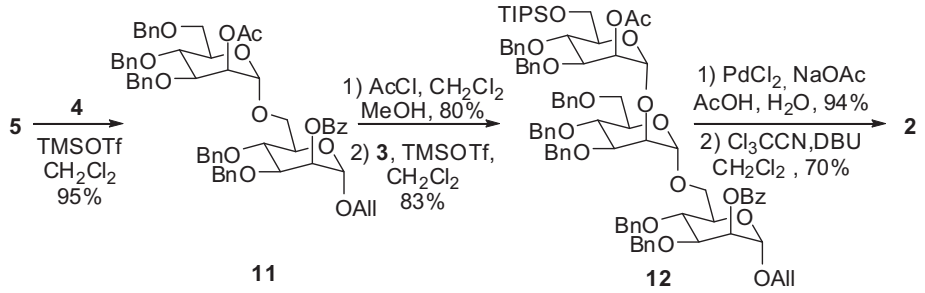
After filtration and evaporation of the solvent, the recovered material was re-dissolved in a mixture of dichloromethane/*iso*propanol/water (1:1:0.2) and further treated with palladium (II) hydroxide (20% on charcoal) and an excess of formic acid for additional 8 h at room temperature. The crude product purified by HPLC (see Fig. S1) afforded the desired GPI anchor **1** in 75% yield. A flow injection analysis of **1** in methanol/formic acid in a triple quadrupole MS instrument equipped with an electrospray source, gave *m*/*z* 1638 $[M+H]^+$ as quasi-molecular ion. ESI-HRMS confirmed the structure of **1** depicted in Fig. 1 (see Section 4).

3. Conclusion

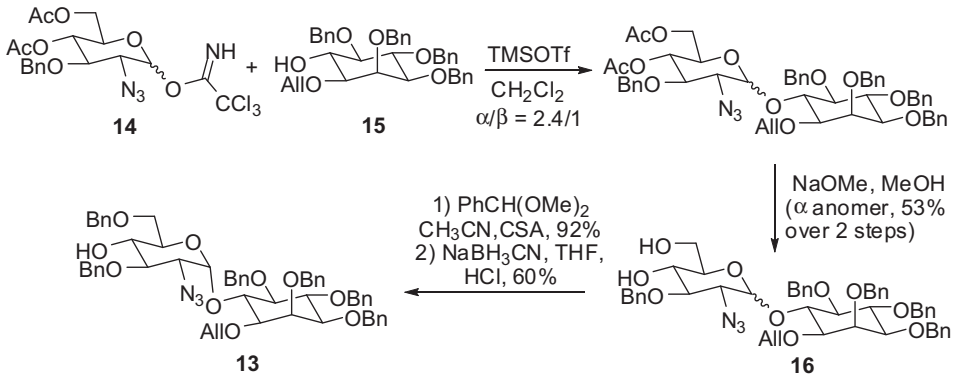
Over the past decade, the arduous task of *de novo* synthesis has been achieved for GPI anchors resembling those of several organisms (*Trypanosoma brucei* [11,13], *Plasmodium falciparum* [35], *Toxoplasma gondii* [14], *Saccharomyces cerevisiae* [36], *Trypanosoma cruzi* [28,37], *Plasmodium falciparum* [38]); but in only two cases those of mammals (rat brain Thy [10], and human sperm CD52 [17]). However, in these two cases (a pseudo heptasaccharide with two EtNP and a GPI-anchored peptide lacking the glycerolipid residue), the complexity of the molecules makes these not easily amenable to structural modifications. The work described here is the first report of a tailored synthesis of the essential core of the human GPI anchor structure **1**. To satisfactorily obtain GPI **1** we used a common precursor to produce the trisaccharidic donor **2** and developed a convenient protection/deprotection strategy. The final crucial step (removal of 15 benzyl groups, one benzyloxy residue



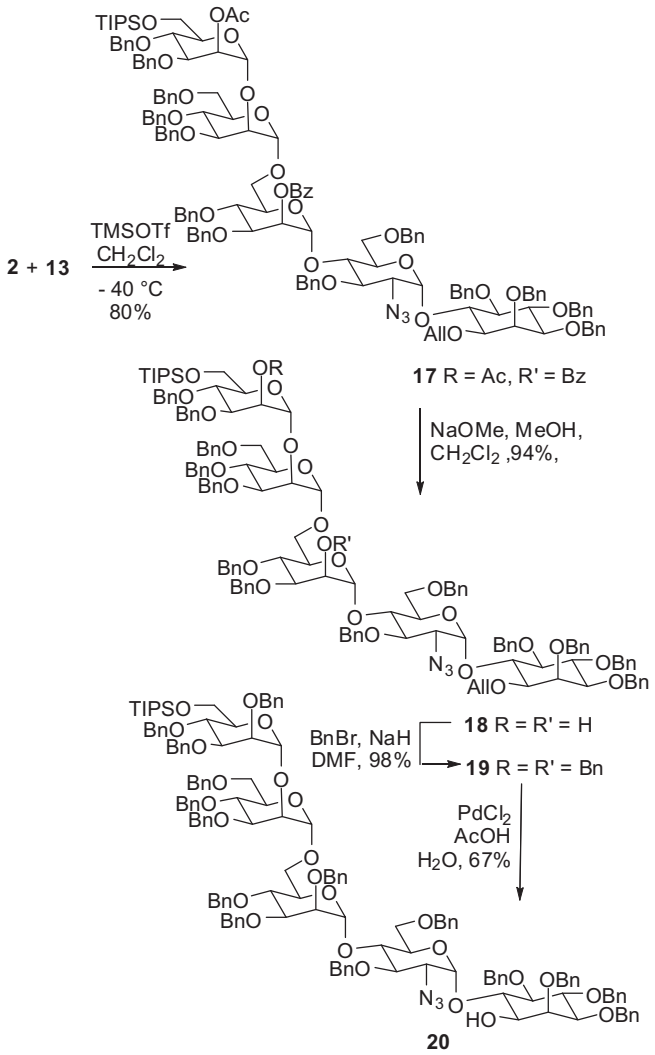
Scheme 1. Synthesis of donors **3** and **4** and acceptor **5**.



Scheme 2. Synthesis of trisaccharidic donor **2**.



Scheme 3. Synthesis of pseudo-disaccharide **13**.



Scheme 4. Assembly of pseudo-pentasaccharide **20**.

and reduction of the azido group), was optimized and enabled us to obtain a sample of **1** suitable for biological tests (see Section 4).

With respect to syntheses reported for different GPIs structures, the strategy we followed gave the desired essential core of human GPI, employing orthogonal protecting groups and decreasing the protection–deprotection steps.

This compound is currently investigated for *in vitro* studies, aiming to identifying the target molecules of a T cell-mediated autoimmune process underlying the pathogenesis of PNH. At present, we have found that after loading CD1d glycoprotein with **1** we can detect in the peripheral blood of normal subjects a discrete (and very small) population of T lymphocytes that presumably recognize the GPI molecule. This indicates that our synthetic GPI is biologically active. Biological tests and activity will be published in detail in the due corse.

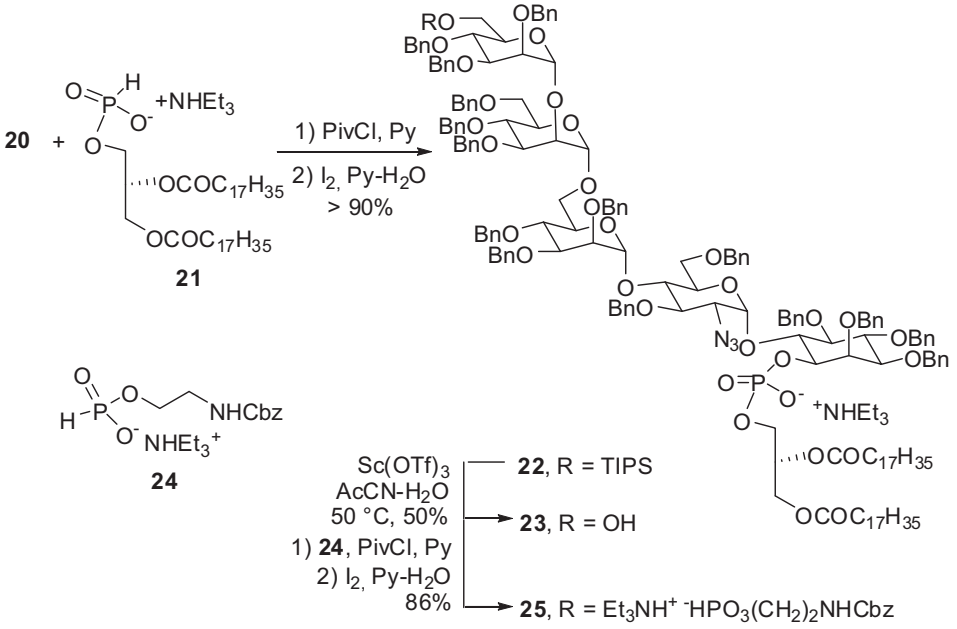
4. Experimental

4.1. Materials and methods

All solvents were of reagent grade quality and purchased commercially. All starting materials were purchased commercially and used without further purification. NMR spectra used for characterization of products and binding experiments were recorded on a Varian Inova and Mercury 400 instruments and Bruker 700 instrument. The NMR spectra were referenced to solvent. Mass spectra were recorded on an Agilent Technologies 6110 Quadrupole LC/MS. ESI-MS analysis was performed both in positive or negative ion mode. HRMS were performed on a LTQ-IT-Orbitrap with a spray voltage of 2.10 kV and a resolution of 100,000. C, H and N elemental analysis was performed on a Perkin–Elmer 2400 elemental analyser.

4.2. Synthesis of (3,4-di-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl)-(1 → 2)-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1 → 6)-(3,4-di-O-benzyl-α-D-mannopyranosyl)-(1 → 4)-(2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1 → 6)-1-O-allyl-2,3,4,5-tetra-O-benzyl-D-myo-inositol (**18**)

To a stirred solution of 17 (1.8 g, 0.760 mmol) in a mixture of $CHCl_3$/MeOH 1:2 (13 mL), 4.2 mL of 0.5 M solution of MeONa in



Scheme 5. Synthesis of derivative **25**.

MeOH at rt were added. The mixture was warmed at 45 °C for 24 h then the pH adjusted to neutrality with HCl (10% in MeOH). Evaporation of the solvent under vacuum gave a crude which was dissolved in CH_2Cl_2 (100 mL) and washed with brine (1 × 20 mL). The organic phase was dried over Na_2SO_4 and concentrated to dryness. The crude product was purified by flash chromatography on silica gel (petroleum ether:EtOAc 2:1) to give 18 (1.58 g, 94%) as glass solid. $[\alpha]_D^2$5 + 30.81 (*c* 0.65 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42–7.04 (m. 65H), 5.98–5.88 (m, 1H), 5.75 (d, *J* = 4.0 Hz, 1H), 5.30–5.25 (m, 1H), 5.23 (d, *J* = 1.6 Hz, 1H), 5.21 (d, *J* = 1.6 Hz, 1H), 5.21–5.17 (m, 1H), 4.99–4.78 (m, 8H), 4.73–4.25 (m, 19H), 4.13–3.96 (m, 9H), 3.88–3.72 (m, 10H), 3.69–3.65 (m, 2H), 3.58–3.28 (m, 9H), 3.23–3.17 (m, 2H), 2.19 (d, *J* = 4.0 Hz, 1H), 2.05 (d, *J* = 2.4 Hz, 1H), 1.05–1.04 (m, 21H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.9, 138.8, 138.74, 138.72, 138.58, 138.54, 138.3, 138.25, 138.21, 138.1, 134.1, 128.49, 128.45, 128.40, 128.36, 128.34, 128.26, 128.21, 128.18, 128.13, 127.97, 127.92, 127.86, 127.80, 127.7, 127.66, 127.63, 127.5, 127.49, 127.47, 127.44, 127.41, 127.3, 127.26, 127.22, 127.05, 127.02, 117.1, 101.5, 100.1, 99.1, 97.4, 81.8, 81.4, 80.8, 80.3, 79.9, 79.8, 79.5, 75.7, 75.3, 75.1, 75.0, 74.9, 74.56, 74.52, 74.3, 74.0,73.7, 73.5, 73.1, 72.9, 72.79, 72.71, 72.4, 72.0, 71.9, 71.7, 71.6, 71.1, 70.7, 69.5, 69.0, 68.6, 68.5, 65.8, 63.3, 62.4, 18.06, 18.02, 11.9; HRMS-ESI (*m*/*z*): Calcd for $[M+H+NH_4]^{2+}$ $C_{133}H_{158}O_{25}N_4Si$, 1119.54867. Found: 1119.54870.

*4.3. Synthesis of (2,3,4-tri-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl)-(1 → 2)-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1 → 6)-(2,3,4-tri-O-benzyl-α-D-mannopyranosyl)-(1 → 4)-(2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1 → 6)-2,3,4,5-tetra-O-benzyl-D-myo-inositol (**20**)*

To an ice cooled solution of 18 (1.28 g, 0.575 mmol) in dry DMF (16.0 mL), BnBr (0.590 g, 0.410 mL, 3.45 mmol) and NaH (0.115 g, 4.60 mmol) were added. The mixture was warmed to rt and stirred for 2.0 h. After this time, 0.5 mL of MeOH and a saturated solution of NH_4Cl (15 mL) were added. The mixture was then diluted with Et_2O (150 mL) and the organic phase was washed with brine (2 × 20 mL) and dried over Na_2SO_4. Evaporation of the solvent under vacuum gave a crude which was purified by flash chromatography on silica gel (petroleum ether:EtOAc 5:1) to give 19 (1.35 g, 98%) as an oily. To a stirred solution of 19 (1.33 g, 0.553 mmol) in AcOH (15 mL), H_2O (0.761 mL), NaOAc (0.636 g, 7.75 mmol) and $PdCl_2$ (0.686 g, 3.87 mmol) were added. The mixture was stirred at rt for 6.0 h then diluted with AcOEt and washed with H_2O (2 × 10 mL), with a saturated solution of $NaHCO_3$ (3 × 10 mL) and brine (1 × 10 mL). The organic phase was dried over Na_2SO_4 and concentrated to dryness. The crude product was purified by flash chromatography on silica gel (petroleum ether:EtOAc 4:1) to give 20 (0.875 g, 67%) as an oily. The spectroscopic and analytical data were in agreement with those reported previously.

*4.4. Synthesis of triethylammonium-(2,3,4-tri-O-benzyl-6-O-triisopropylsilyl-α-D-manno pyranosyl)-(1 → 2)-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1 → 6)-(2,3,4-tri-O-benzyl-α-D-mannopyranosyl)-(1,4)-(2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1 → 6)-2,3,4,5-tetra-O-benzyl-1-O-(1,2-di-O-octadecanoyl-sn-glyceryl-phosphonato)-D-myo-inositol (**22**)*

H-phosphonate 21 (0.900 g, 1.14 mmol) and 20 (0.408 g, 0.203 mmol) were co-evaporated with anhydrous pyridine (2 × 5 mL) and dried under vacuum for 2.0 h. After this time, the mixture was dissolved in pyridine (20 mL) and pivaloyl chloride (0.293 g, 0.298 mL, 2.43 mmol) was added. The resulting mixture was stirred for 3.0 h then iodine (0.309 g, 1.22 mmol) in a mixture of pyridine/water (19:1, 1.7 mL) was added. The reaction mixture was further stirred for 1.5 h, then diluted with $CHCl_3$ (100 mL) and washed with a saturated solution of $Na_2S_2O_3$ (1 × 10 mL). The organic phase was washed with TEAB buffer (3 × 20 mL), dried over Na_2SO_4 and concentrated to dryness. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2:MeOH 18:1 + 1%NEt_3) to give 22 (0.635 g, 99%) as glass solid. $[\alpha]_D^2$5 + 43.2 (c 0.5 CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41–6.99 (m, 75H), 5.91 (d, *J* = 3.6 Hz, 1H), 5.28–5.20 (m, 2H), 5.03–5.00 (A part of an AB system, *J* = 12.0 Hz, 1H), 4.94–4.83 (m, 7H), 4.78–4.74 (m, 4H), 4.68–4.30 (m, 22H), 4.25–3.99 (m, 11H), 3.94–3.30 (m, 21H), 3.12 (dd, *J* = 10.4 Hz, 3.6 Hz, 1H), 2.91 (q, *J* = 7.2 Hz, 6H), 2.26–2.21 (m, 4H), 1.54 (bs, 4H), 1.26–1.17 (m, 65H), 1.05–1.03 (m, 21H), 0.88 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.3, 173.0, 139.7, 139.1, 138.9, 138.8, 138.75, 138.72, 138.6, 138.58, 138.55, 138.50, 138.39, 138.36, 137.9, 128.5, 128.36, 128.32, 128.28, 128.23, 128.20, 128.14, 128.12, 128.0, 127.99, 127.96, 127.90, 127.8, 127.71, 127.6, 127.4, 127.39, 127.32, 127.2, 127.1, 127.0, 126.99, 126.9, 126.8, 100.9, 99.2, 98.4, 96.4, 81.7, 80.9, 80.0, 79.6, 78.2, 75.9. 75.6, 74.9, 74.8, 74.6, 74.5, 74.3, 73.7, 73.6, 73.1, 72.9, 72.7, 72.2, 72.1, 72.0, 71.9, 71.8, 71.4, 70.5, 69.2, 68.9, 66.4, 63.6, 63.1, 62.7, 45.3, 34.2, 34.0, 31.9, 29.7, 29.6, 29.5, 29.3, 29.1, 27.1, 24.88, 24.80, 22.6, 18.07, 18.02, 14.1, 12.0, 8.4; ^{31}P NMR (81 MHz, $CDCl_3$): δ −1.74; HRMS-ESI (*m*/*z*): $[M+Na+K]^{2+}$ Calcd for $C_{183}H_{236}N_3O_{32}PSiNaK$, 1554.29813. Found: 1554.29705.

*4.5. Synthesis of triethylammonium-(2,3,4-tri-O-benzyl-α-D-mannopyranosyl)-(1 → 2)-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1 → 6)-(2,3,4-tri-O-benzyl-α-D-mannopyranosyl)-(1 → 4)-(2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1 → 6)-2,3,4,5-tetra-O-benzyl-1-O-(1,2-di-O-octadecanoyl-sn-glyceryl-phosphonato)-D-myo-inositol (**23**)*

A solution of 22 (0.442 g, 0.143 mmol) in a mixture of $CHCl_3$/MeOH (1:1) was passed through a column with a cation-exchanger resin (Amberlite IR-120, Na^+ form). The eluate was collected and concentrated to dryness. The residue was then dissolved in CH_3CN (28 mL) and water (14 μL) and $Sc(OTf)_3$ (0.283 g, 0.575 mmol) were added. The reaction mixture was warmed at 50 °C for 10 h then a few drops of pyridine were added. The resulting mixture was diluted with $CHCl_3$ (50 mL) and washed with brine (2 × 10 mL). The organic phase was dried over Na_2SO_4 and concentrated to dryness. The crude product was purified by flash chromatography on silica gel ($CHCl_3$:MeOH 30:1 + 0.5%NEt_3) to give 23 (0.214 g, 50%) as a glass solid. $[\alpha]_D^2$5 + 39.2 (c 0.75 CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41–7.40(m, 2H), 7.32–7.00 (m, 73H), 5.85 (bs, 1H), 5.25–5.22 (m, 2H), 5.043 (d, *J* = 2.0 Hz, 1H), 5.02–4.99 (A part of an AB system, *J* = 11.6 Hz, 1H), 4.95–4.55 (m, 16H), 4.49–4.30 (m, 16H), 4.25–4.22 (m, 1H), 4.17–4.02 (m, 7H), 3.97–3.37 (m, 24H), 3.17 (dd, *J* = 10.4 Hz, *J* = 4.0 Hz, 1H), 2.94–2.88 (m, 6H), 2.26–2.21 (m, 4H), 2.00 (bs, 5H), 1.54 (m, 4H), 1.32–1.18 (m, 60H), 0.88 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.3, 172.9, 139.6, 138.9, 138.65, 138.62, 138.5, 138.4, 138.38, 138.34, 138.0, 137.8, 128.5, 128.4, 128.3, 128.2,8, 128.24, 128.22, 128.18, 128.16, 128.0, 127.9, 127.8, 127.76, 127.7, 127.65, 127.61, 127.5, 127.44, 127.42, 127.36, 127.32, 127.25, 127.22, 127.15, 127.10, 127.0, 126.9, 126.8, 100.7, 99.5, 99.3, 96.5, 81.7, 81.6, 80.9, 79.7, 79.2, 75.7, 75.6, 75.1, 74.95, 74.90, 74.7, 74.5, 74.3, 74.1, 73.7, 73.2, 73.0, 72.7, 72.3, 72.2, 72.19, 72.13, 72.0, 71.9, 71.7, 70.3, 69.5, 69.0, 68.7, 66.0, 63.0, 62.6, 62.1, 45.3, 34.2, 34.0, 31.9, 29.69, 29.64, 29.52, 29.34, 29.30, 29.13, 24.87, 24.84, 22.66, 17.6, 14.0, 12.2, 8.3; ^{31}P NMR (81 MHz, $CDCl_3$): δ −1.66; HRMS–ESI (*m*/*z*): $[M+NH_4]^+$ Calcd for $C_{174}H_{220}N_4O_{32}P$, 2908.54428. Found: 2908.53941.

*4.6. Synthesis of bistriethylammonium-(2,3,4-tri-O-benzyl-6-O-(2-(N-benzyloxycarbonyl) aminoethyl-phosphonato)-α-D-mannopyranosyl)-(1 → 2)-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1 → 6)-(2,3,4-tri-O-benzyl-α-D-mannopyranosyl)-(1 → 4)-(2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1 → 6)-2,3,4,5-tetra-O-benzyl-1-O-(1,2-di-O-octadecanoyl-sn-glyceryl-phosphonato)-D-myo-inositol (**25**)*

H-phosphonate **24** (0.149 g, 0.414 mmol) and **23** (0.080 g, 0.026 mmol) were co-evaporated with anhydrous pyridine (2 × 3 mL) and dried under vacuum for 2.0 h. After this time, the mixture was dissolved in pyridine (4.3 mL) and pivaloyl chloride (0.100 g, 0.102 mL, 0.829 mmol) was added. The resulting mixture was stirred for 3.0 h then iodine (0.105 g, 0.414 mmol) in a mixture of pyridine/water (19:1, 0.413 mL) was added. The reaction mixture was further stirred for 1.5 h, then diluted with $CHCl_3$ (50 mL) and washed with a saturated solution of $Na_2S_2O_3$ (1 × 5 mL). The organic phase was washed with TEAB buffer (3 × 20 mL), dried over Na_2SO_4 and concentrated to dryness. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2:MeOH 18:1 + 0.5%NEt_3) to give **25** (0.061 g, 86%) as glass solid. $[\alpha]_D^2$5 + 37.84 (*c* 0.65 $CHCl_3$); ^{31}P NMR (81 MHz, $CDCl_3$): δ −1.79,+1.07; HRMS–ESI (*m*/*z*): $[M+Na+K]^{2+}$ Calcd for $C_{184}H_{228}N_4O_{37}P_2KNa$, 1604.75407. Found: 1604.75175.

*4.7. Synthesis of (6-O-aminoethylphosphonato-α-D-mannopyranosyl)-(1 → 2)-α-D-manno pyranosyl-(1 → 6)-α-D-mannopyranosyl-(1 → 4)-(2-amino-2-deoxy-α-D-glucopyranosyl)-(1 → 6)-1-O-(1,2-di-O-octadecanoyl-sn-glyceryl-phosphonato)-D-myo-inositol (**1**)*

To a stirred solution of **25** (93 mg, 0.029 mmol) in a mixture of CH_2Cl_2/iPrOH 1:1 (28 mL), $Pd(OH)_2$/C (20% Pd content) (93 mg) and formic acid (6 μL, 0.145 mmol) were added. The mixture was stirred at rt for 8.0 h then filtered through a pad of Celite. Evaporation of the solvent under vacuum gave a crude which was dissolved in a mixture of CH_2Cl_2/iPrOH/H_2O 1:1:0.2 (22 mL) and $Pd(OH)_2$/C (20% Pd content) (100 mg) and formic acid (6 μL, 0.145 mmol) were added. The mixture was stirred at rt for 8.0 h then filtered through a pad of Celite. Evaporation of the solvent under vacuum gave 1 (40 mg, 75%) as glassy solid. HPLC purification of 1 (10 mg) [Phenomenex Synergi 4 μm Polar-RP 80A, from 90% water (+0.1% formic acid) and 10 methanol (+0.1% formic acid), to 10% water (+0.1% formic acid) and 90% (+0.1% formic acid)] allowed to obtain **1** (8 mg) as pure compound.

Acknowledgments

Authors thank Istituto Toscano Tumori (ITT), Florence, Italy for financial support (cap. 26/6, 2007) and Prof. S. Oscarson (University College, Dublin, Ireland) for intermediates' samples provided.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bioorg.2010.12.002.

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