Chemical synthesis and functionalization of clickable glycosylphosphatidylinositol anchors

Electronic Supplementary Information

Benjamin M. Swarts and Zhongwu Guo*

Department of Chemistry, Wayne State University, 5101 Cass Avenue, Detroit, MI 48202, USA

zwguo@chem.wayne.edu

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I. Abbreviations

Ac = acetyl
All = allyl
BARAC = biarylazacyclooctynone
Bn = benzyl
BRSM = based on recovered starting material
COD = 1,5-cyclooctadiene
CSA = camphor sulfonic acid
DAST = diethylamino sulfur trifluoride
DBU = diazo(1,3)bicyclo[5.4.0]undecene
DCC = dicyclohexyl carbodiimide
DIBAL-H = diisobutylaluminum hydride
DMAP = 4-dimethylamino pyridine
DMF = N,N-dimethylformamide
DMTST = dimethyl(methylthio)sulfonyl trifluoromethanesulfonate
EDCI = 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
ESI MS = electrospray ionization mass spectrometry
Fmoc = 9-fluorenlymethoxycarbonyl
GPI = glycosylphosphatidylinositol
HOBt = 1-hydroxybenzotriazole
HPLC = high performance liquid chromatography
MALDI MS = matrix-assisted laser desorption ionization mass spectrometry
MS 4 Å = molecular sieves 4 Å
NIS = N-iodosuccinimide
PI = phosphatidylinositol
PMB = para-methoxybenzyl
PMP = para-methoxyphenyl
PMTrt = para-methoxytrityl
TBAF = tetrabutylammonium fluoride
TBS = tert-butyldimethylsilyl
TES = triethylsilyl
THF = tetrahydrofuran
Tf = trifluoromethane sulfonyle
TFA = trifluoroacetic acid
TLC = thin layer chromatography
TMS = trimethylsilyl

\( p\text{-TolSCl} = \text{para-toluenesulfenyl chloride} \)

Ts = 4-toluenesulfonyl

TTBP = 2,4,6-tri-\textit{tert}-butylpyrimidine
II. Fig. S-1

Failed coupling using Man-I 2-O-PMB-protected trimannosyl donor. During early exploration of the key glycosylation reaction, we identified a structural feature in the trimannose donor that was essential for successful coupling. The glycosylation was first attempted using trimannose donor I, which carried orthogonal protection at the Man-I 3-O-position – the position we initially selected as the late-stage functionalization site. After I was converted into donor II, its reaction with pseudodisaccharide acceptor 35 was performed under standard Schmidt glycosylation conditions, which failed to provide the desired GPI intermediate III. Rather, upon activation the glycosyl donor collapsed to form 1,2-anhydro sugar V, presumably via loss of the Man-I 2-O-PMB group from an intermediate oxocarbenium cation IV as shown above. It is noteworthy that in the preparation of II, thioglycoside I was readily hydrolyzed to the hemiacetal without suffering from anhydro sugar formation. Furthermore, no anhydro sugar was detected when monosaccharide donor 12, also bearing a 2-O-PMB group, was employed in the successful glycosylation of dimannose 44 (see Scheme 6 in article text). These observations suggested that the poorly nucleophilic hydroxyl group of pseudodisaccharide acceptor 35 was the major contributing factor to the failure of the coupling reaction between 35 and II, and that usage of 2-O-PMB groups in glycosyl donors should not be universally excluded.
II. Experimental

**General Methods.** Materials were obtained from commercial sources without further purification unless otherwise noted. Anhydrous solvents were obtained either commercially or from an MBRAUN alumina column solvent purification system. Molecular sieves were flame-dried under hi-vacuum and used immediately after cooling. All reactions were carried out in oven-dried glassware under argon unless otherwise noted. Analytical thin-layer chromatography (TLC) was performed on Whatman silica gel 60 Å plates (thickness 0.25 mm) and detected by UV lamp, charring with phosphomolybdic acid in EtOH, or charring with 5% H$_2$SO$_4$ in EtOH. Column chromatography was performed using Dynamic Adsorbents flash silica gel (32-63 μm).

$^1$H NMR spectra were recorded at 400 or 500 MHz with the chemical shifts reported in ppm (δ) relative to CHCl$_3$ (7.26 ppm) or tetramethylsilane (0.00 ppm). $^{13}$C NMR spectra were recorded at 100 or 125 MHz relative to the $^{13}$C signal of CDCl$_3$ (77.23 ppm). Coupling constants ($J$) are reported in hertz (Hz). Mass spectrometry was performed using either a Bruker Daltonics Ultraflex MALDI TOF MS or Waters LCT Premier XE high resolution ESI MS. Optical rotation values were recorded using a Rudolph Research Analytical Autopol III automatic polarimeter. Microscale sample measurements were made using a Mettler Toledo AX205 DeltaRange balance (for max 81 g, d = 0.01 mg).

Compounds below are in order of appearance in the manuscript. Intermediates that were not numbered in the manuscript receive numbers beginning with 67.
(±)-1,6-di-O- Allyl-myo-inositol (68)

To a solution of (±)-67 (9.00 g, 21.4 mmol) in anhydrous CH₂Cl₂-MeOH (2:1, 150 mL) stirring under an Ar atmosphere at rt was added acetyl chloride (AcCl, 1.0 mL) dropwise. After 3 h, the reaction was quenched with triethylamine and concentrated in vacuum. The crude material was purified by silica gel column chromatography to give (±)-68 (5.55 g, 98%) as a white solid. ¹H NMR (CD₃OD, 500 MHz): δ 5.94-5.84 (m, 2 H), 5.23 (dd, J = 2.0, 17.5 Hz, 1 H), 5.17 (dd, J = 1.5, 17 Hz, 1 H), 5.06 (dd, J = 1.0, 10.5 Hz, 1 H), 5.01 (dd, J = 1.5, 10.5 Hz, 1 H), 4.22 (ddd, J = 6.0, 12.5, 19.5 Hz, 2 H), 4.11 (dd, J = 5.5, 13 Hz, 1 H), 4.02-3.99 (m, 2 H), 3.53 (t, J = 9.5 Hz, 1 H), 3.47 (t, J = 9.5 Hz, 1 H), 3.22-3.19 (m, 1 H), 3.17 (dd, J = 3.0, 10 Hz, 1 H), 3.14 (t, J = 9.0 Hz, 1 H). ¹³C NMR (CD₃OD, 125 MHz): δ 137.11, 136.53, 117.06, 116.54, 82.08, 81.17, 76.09, 75.27, 74.17, 73.13, 72.22, 71.11. HR ESI MS: calcd. for C₁₂H₂₀O₆Na[M+Na]⁺ m/z 283.1158; found, 283.1152. To identify the desired enantiomer of compound 8, an analogous route was developed starting with optically active inositol (+)-13, which was prepared using a known enzymatic resolution procedure.¹ Optical rotation for the correct enantiomer of 68 generated by authentication route: [α]D²⁵ = -21° (c 1.0, CH₃OH)
(±)-1,6-di-O-Allyl-2,3,4,5-tetra-O-(para-methoxybenzyl)-myo-inositol (17)

To a solution of (±)-68 (5.30 g, 20.3 mmol) in anhydrous DMF (100 mL) stirring under Ar at 0 ºC was slowly added NaH (60% dispersion in mineral oil, 3.65 g, 91.4 mmol). After stirring for 30 min, para-methoxybenzyl chloride (10.3 mL, 98.3 mmol) was added dropwise. The reaction was stirred overnight at rt, then quenched with MeOH, diluted with EtOAc, and poured into water. The aqueous layer was extracted 3 times with EtOAc, after which the combined organic layer was washed with brine, dried over Na₂SO₄, and concentrated in vacuum. The residue was purified by silica gel column chromatography to give (±)-17 (11.0 g, 73%) as a syrup. ¹H NMR (CDCl₃, 500 MHz): δ 7.35-7.25 (m, 8 H), 6.89-6.84 (m, 8 H), 6.04-5.97 (m, 1 H), 5.96-5.88 (m, 1 H), 5.31 (dd, J = 1.0, 17 Hz, 1 H), 5.29 (dd, J = 1.0, 17.5 Hz, 1 H), 5.19-5.15 (m, 2 H), 4.84-4.75 (m, 6 H), 4.60 (d, J = 11.5 Hz, 1 H), 4.55 (d, J = 11 Hz, 1 H), 4.39 (dd, J = 5.5, 12 Hz, 1 H), 4.31 (dd, J = 6.0, 12.5 Hz, 1 H), 4.13-4.05 (m, 2 H), 4.00-3.95 (m, 2 H), 3.86 (t, J = 9.0 Hz, 1 H), 3.83 (s, 3 H), 3.81 (s, 3 H), 3.80 (s, 6 H), 3.38 (t, J = 9.5 Hz, 1 H), 3.32 (dd, J = 2.0, 10 Hz, 1 H), 3.18 (dd, J = 1.5, 9.5 Hz, 1 H). ¹³C NMR (CDCl₃, 125 MHz): δ 159.38, 159.34, 159.19, 135.83, 135.32, 131.50, 131.45, 131.41, 130.90, 129.91, 129.75, 129.63, 129.38, 116.72, 116.64, 113.99, 113.73, 83.71, 81.75, 81.63, 80.94, 80.89, 75.81, 75.69, 74.78, 74.19, 73.81, 72.64, 71.89, 55.51. HR ESI MS: calcd. for C₄₄H₅₂O₁₀Na [M+Na]⁺ m/z, 763.3458; found, 763.3448. Optical rotation for the correct enantiomer of 17 generated by authentication route: [α]D²⁵ = -9.0° (c 1.0, CHCl₃).
(±)-2,3,4,5-tetra-O-(para-Methoxybenzyl)-myo-inositol (18)

To a solution of (±)-17 (10.5 g, 14.2 mmol) and titanium(IV) isopropoxide (9.20 mL, 31.2 mmol) in anhydrous THF (100 mL) was slowly added cyclohexylmagnesium chloride (2.0 M solution in anhydrous THF, 85 mL) via a dropping funnel under an Ar atmosphere at rt. After 2 h, the reaction was poured into 0.5 N HCl and the aqueous layer was extracted 3 times with Et<sub>2</sub>O. The combined organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated in vacuum. The resulting residue was purified by silica gel column chromatography to obtain (±)-18 (7.9 g, 85%) as a syrup. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 7.30-7.26 (m, 8 H), 6.89-6.85 (m, 8 H), 4.98 (d, <i>J</i> = 11.0 Hz, 1 H), 4.88 (d, <i>J</i> = 11.0 Hz, 1 H), 4.87 (d, <i>J</i> = 10.5 Hz, 1 H), 4.77 (d, <i>J</i> = 10 Hz, 1 H), 4.69 (d, <i>J</i> = 11 Hz, 1 H), 4.68 (d, <i>J</i> = 11.5 Hz, 1 H), 4.63 (d, <i>J</i> = 11.5 Hz, 1 H), 4.59 (d, <i>J</i> = 11.5 Hz, 1 H), 3.99-3.95 (m, 2 H), 3.83 (s, 3 H), 3.82 (s, 6 H), 3.81 (s, 3 H), 3.77 (t, <i>J</i> = 9.5 Hz, 1 H), 3.44 (dd, <i>J</i> = 2.0, 9.5 Hz, 1 H), 3.37-3.33 (m, 1 H), 3.28 (t, <i>J</i> = 9.0 Hz, 1 H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz): δ 159.48, 131.12, 129.94, 129.75, 129.67, 129.52, 114.18, 114.10, 114.05, 114.01, 82.94, 81.44, 81.37, 77.14, 75.65, 75.22, 74.71, 74.12, 73.08, 72.33, 55.52. HR ESI MS: calcd. for C<sub>38</sub>H<sub>44</sub>O<sub>10</sub>Na [M+Na]<sup>+</sup> <i>m/z</i>, 683.2832; found, 683.2834. Optical rotation for the correct enantiomer of 18 generated by authentication route: [α]<sub>25</sub><sup>D</sup> = +7.0° (c 1.0, CHCl<sub>3</sub>).

Electronic Supplementary Material (ESI) for Chemical Science
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(±)-1-O- Allyl-2,3,4,5-tetra-O-(para-methoxybenzyl)-myo-inositol (8)

A mixture of (±)-18 (7.00 g, 10.6 mmol) and dibutyltin oxide (2.90, 11.7 mmol) in anhydrous toluene (150 mL) was refluxed with azeotropic removal of water using a Dean-Stark apparatus for 2 h. After concentration in vacuum, the residue was dissolved in anhydrous DMF (100 mL) and cooled to 0 °C, after which cesium fluoride (8.2 g, 54 mmol) and allyl bromide (4.6 mL, 54 mmol) were added to the solution. After stirring overnight under an Ar atmosphere at rt, the reaction mixture was diluted with EtOAc, washed with brine, dried over Na₂SO₄, and concentrated in vacuum. After passing the crude products through a silica gel plug, the two regioisomers were separated by semi-preparative HPLC (Waters Nova-Pak Silica 6 μm, 300 x 19 mm, eluent 33% EtOAc in hexanes, 10 mL/min, tᵣ = 13.4 min) to give the desired product (±)-8 (5.39 g, 72%) as a white solid. ¹H NMR (CDCl₃, 500 MHz): δ 7.33-7.25 (m, 8 H), 6.90-6.85 (m, 8 H), 5.94-5.86 (m, 1 H), 5.28 (dd, J = 1.5, 17 Hz, 1 H), 5.20 (dd, J = 1.0, 10 Hz, 1 H), 4.85 (d, J = 10.5 Hz, 1 H), 4.84 (d, J = 11 Hz, 1 H), 4.80-4.73 (m, 4 H), 4.63 (d, J = 11 Hz, 1 H), 4.57 (d, J = 11 Hz, 1 H), 4.10-3.97 (m, 5 H), 3.83 (s, 3 H), 3.82 (s, 6 H), 3.81 (s, 1 H), 3.35 (dd, J = 2.5, 9.5 Hz, 1 H), 3.34 (t, J = 9.0 Hz, 1 H), 3.09 (dd, J = 2.0, 10.0 Hz, 1 H). ¹³C NMR (CDCl₃, 125 MHz): δ 159.43, 159.37, 159.26, 134.84, 131.38, 131.24, 130.81, 129.94, 129.70, 129.64, 129.41, 117.50, 114.08, 114.02, 113.98, 113.79, 83.44, 81.43, 81.19, 80.08, 75.68, 75.19, 73.82, 73.17, 72.94, 72.77, 71.32, 55.52. HR ESI MS: calcd. for C₄₁H₄₈O₁₀Na [M+Na]+ m/z, 723.3145; found, 723.3110. Optical rotation for the correct enantiomer of 8 generated by authentication route: [α]D²⁵ = -8.5° (c 1.0, CHCl₃).
To a solution of (±)-8 (5.10 g, 7.27 mmol), triethylamine (2.0 mL, 15 mmol), and 4-dimethylamino pyridine (300 mg) in anhydrous CH₂Cl₂ (100 mL) under an Ar atmosphere at 0 °C was added (1S)-(−)-camphanic chloride (freshly prepared 1.0 M solution in anhydrous CH₂Cl₂, 12.9 mL) dropwise. After stirring for 1 h, the reaction was poured into water and extracted 3 times with CH₂Cl₂, after which the combined organic layer was washed with saturated aqueous NaHCO₃, brine, dried over Na₂SO₄, and concentrated in vacuum. The residue was then subjected to silica gel column chromatography, which gave a 1:1 mixture of diastereomers. Preparative HPLC (Waters Nova-Pak Silica 6 μm, 300 x 19 mm, eluent 25% EtOAc in hexanes, 10 mL/min, tᵣ = 21.4 min) was used to separate the diastereomers, affording optically pure (−)-19 (2.97 g, 46%) as a white solid. **¹H NMR** (CDCl₃, 500 MHz): δ 7.35-7.32 (m, 2 H), 7.27-7.25 (m, 2 H), 7.21-7.18 (m, 4 H), 6.89-6.81 (m, 8 H), 5.83-5.76 (m, 1 H), 5.71 (t, J = 10 Hz, 1 H, 6-O-position shifted downfield from δ 4.08 after acylation, confirming regiochemistry of previous step), 5.24 (dd, J = 1.5, 17 Hz, 1 H), 5.15 (dd, J = 1.5, 10.5 Hz, 1 H), 4.76 (m, 4 H), 4.70 (d, J = 10 Hz, 1 H), 4.62 (d, J = 10.5 Hz, 1 H), 4.61 (d, J = 11 Hz, 1 H), 4.54 (d, J = 11.5 Hz, 1 H), 4.09 (t, J = 10 Hz, 1 H), 4.03 (t, J = 1.5 Hz, 1 H), 3.97 (dd, J = 5.0, 12.5 Hz, 1 H), 3.86-3.83 (m, 4 H), 3.81 (s, 3 H), 3.80 (s, 3 H), 3.79 (s, 3 H), 3.52 (t, J = 9.5 Hz, 1 H), 3.34 (dd, J = 2.5, 9.5 Hz, 1 H), 3.29 (dd, J = 2.0, 10.0 Hz, 1 H), 2.36 (dd, J = 1.0, 4.0, 10 Hz, 1 H), 1.91-1.78 (m, 2 H), 1.64-1.59 (m, 1 H), 1.10 (s, 3 H), 1.06 (s, 3 H), 0.96 (s, 3 H). **¹³C NMR** (CDCl₃, 125 MHz): δ 178.94, 166.94, 159.46,
(-)-1-O-Allyl-2,3,4,5-tetra-(para-methoxybenzyl)-myo-inositol (8)

To a solution of (-)-19 (400 mg, 0.45 mmol) in anhydrous THF (4 mL) was added a 2.0 M solution of NaOH in anhydrous MeOH (4 mL) at rt. After stirring at rt for 3 h, the reaction was neutralized with AcOH. After concentration, the residue was dissolved in EtOAc, and the resulting organic layer was washed with brine 3 times, dried over Na2SO4, and concentrated in vacuum. The residue was purified by silica gel column chromatography to give optically pure (-)-8 (302 mg, 95%) as a white solid. The product was confirmed as the correct enantiomer by comparison of optical rotation with a standard compound, which was prepared from optically active (+)-13\textsuperscript{1} using an identical route. (-)-8: [α]\textsubscript{D}\textsuperscript{25} = -7.5° (c 1.0, CHCl\textsubscript{3}), authentic standard: [α]\textsubscript{D}\textsuperscript{25} = -8.5° (c 1.0, CHCl\textsubscript{3}). All spectroscopic data for (-)-8 are identical to those obtained for (+)-8.
Allyl 3,4,6-tri-O-acetyl-2-azido-2-deoxy-D-glucopyranoside (21)

To a mixture of 20\(^2\) (3.80 g, 10.2 mmol), allyl alcohol (2.0 mL, 29 mmol), and MS 4 Å (1.0 g) in anhydrous CH\(_2\)Cl\(_2\) stirring under Ar at 0 ºC was slowly added SnCl\(_4\) (1.0 M solution in dry CH\(_2\)Cl\(_2\), 30.0 mL). After the reaction stirred overnight at rt, additional SnCl\(_4\) (1.0 M solution in dry CH\(_2\)Cl\(_2\), 30.0 mL) was added and stirring continued for another 48 h (total ~72 h). After filtration through celite to remove MS, the reaction was quenched by pouring into cold, saturated aqueous NaHCO\(_3\), which was extracted 3 times with CH\(_2\)Cl\(_2\). The combined organic layer was then washed with saturated aqueous NaHCO\(_3\) and brine, dried over Na\(_2\)SO\(_4\), and concentrated in vacuum. The residue was purified by silica gel column chromatography to give α,β-mixture 21 (3.0 g, 81%) as a syrup. NMR data is consistent with those reported in the literature\(^3\) (\(^1\)H NMR and HR ESI MS spectra are included in the supplemental information). HR ESI MS: calcd. for C\(_{15}\)H\(_{21}\)N\(_3\)O\(_8\)Na [M+Na]\(^+\) m/z, 394.1226; found, 394.1231.

Allyl 2-azido-2-deoxy-4,6-O-(para-methoxybenzylidene)-D-glucopyranoside (22)

After a solution of 21 (2.6 g, 7.0 mmol) in 33 mL of 0.05 M NaOMe in anhydrous methanol was stirred at rt for 30 min, it was neutralized to pH 6-7 using Amberlyst H\(^+\) resin. The solution was filtered off and concentrated to afford a white powder (1.75 g), which was directly used for the next step. After the crude triol was dissolved in anhydrous DMF (40 mL), para-anisaldehyde dimethyl acetal (1.6 mL, 9.1 mmol) and camphor sulfonic acid (160 mg, 0.70 mmol) were added.
The reaction was stirred for 12 h under an Ar atmosphere at rt while periodically removing MeOH under reduced pressure. After dilution with EtOAc, the organic layer was washed with saturated aqueous NaHCO₃ and brine, dried over Na₂SO₄, and concentrated in vacuum. The residue was purified by silica gel column chromatography to obtain α,β-mixture 22 (1.94 g, 77%, two steps) as a syrup. 

\[ ^1H \text{ NMR (CDCl}_3, 500 \text{ MHz)} \] of β anomer: δ 7.42-7.40 (m, 2 H), 6.92-6.89 (m, 2 H), 6.00-5.93 (m, 1 H), 5.50 (s, 1 H), 5.37 (dd, \( J = 1.5, 17 \text{ Hz), 5.27 (dd, } J = 1.0, 10 \text{ Hz, 1 H), 4.46 (d, } J = 8.0 \text{ Hz, 1 H), 4.41 (dd, } J = 5.0, 12.5 \text{ Hz, 1 H), 4.32 (dd, } J = 5.5, 11 \text{ Hz, 1 H), 4.18 (dd, } J = 6.0, 12.5 \text{ Hz, 1 H), 3.81 (s, 3 H), 3.77 (t, } J = 10 \text{ Hz, 1 H), 3.64 (dt, } J = 2.0, 9.5 \text{ Hz, 1 H), 3.53 (t, } J = 9.5 \text{ Hz, 1 H), 3.43 (dd, } J = 8.0, 9.5 \text{ Hz, 1 H), 3.41-3.36 (m, 1 H), 2.82 (d, } J = 2.0 \text{ Hz, 1 H).} \]

\[ ^{13}C \text{ NMR (CDCl}_3, 125 \text{ MHz)} \] of β anomer: δ 160.56, 133.34, 129.48, 127.83, 118.49, 113.98, 102.15, 101.66, 80.80, 72.24, 70.98, 68.70, 66.62, 66.39, 55.55. \[ [\alpha]^{D}_{25} \] of β anomer = -53º (c 1.0, CHCl₃). HR ESI MS: calcd. for C₁₇H₂₂N₅O₆ [M+H]+ \( m/z \), 364.1509; found, 364.1511.

**Allyl 2-azido-2-deoxy-3-O-(para-methoxybenzyl)-4,6-O-(para-methoxybenzylidene)-D-glucopyranoside (23)**

To a solution of 22 (1.70 g, 4.67 mmol) in anhydrous DMF (40 mL) stirring under Ar at 0 ºC was slowly added NaH (60% dispersion in mineral oil, 0.92 g, 5.9 mmol). After stirring for 30 min, para-methoxybenzyl chloride (0.62 mL, 5.9 mmol) was added dropwise. The reaction was stirred overnight at rt, then quenched with MeOH, diluted with EtOAc, and poured into water. The aqueous layer was extracted 3 times with EtOAc, after which the combined organic layer
was washed with brine, dried over Na₂SO₄, and concentrated in vacuum. The residue was purified by silica gel column chromatography to give α,β-mixture 23 (2.20 g, 97%) as a syrup.

¹H NMR (CDCl₃, 500 MHz) of β anomer: δ 7.43-7.41 (m, 2 H), 7.32-7.30 (m, 2 H), 6.94-6.87 (m, 4 H), 6.00-5.92 (m, 1 H), 5.55 (s, 1 H), 5.37 (dd, J = 1.5, 17.5 Hz, 1 H), 5.26 (dd, J = 1.5, 10.5 Hz, 1 H), 5.34 (d, J = 11 Hz, 1 H), 4.74 (d, J = 11 Hz, 1 H), 4.40 (d, J = 8.0 Hz, 1 H), 4.39-4.37 (m, 1 H), 4.33 (d, J = 4.5, 10.5 Hz, 1 H), 4.17 (dd, J = 6.0, 12.5 Hz, 1 H), 3.84 (s, 3 H), 3.81 (s, 3 H), 3.79 (t, J = 10 Hz, 1 H), 3.69 (t, J = 9.5 Hz, 1 H), 3.46 (dd, J = 8.0, 9.5 Hz, 1 H), 3.40-3.35 (m, 1 H). ¹³C NMR (CDCl₃, 125 MHz) of β anomer: δ 160.34, 159.64, 133.48, 130.18, 129.88, 127.56, 118.39, 114.03, 113.88, 101.63, 101.54, 81.77, 78.86, 74.80, 70.92, 68.77, 66.47, 66.34, 55.55, 55.51. [α]D²⁵ of α anomer = +12° (c 1.0, CHCl₃). HR ESI MS: calcd. for C₂₅H₃₀N₃O₇[M+H]⁺ m/z, 484.2084; found, 484.2097.

Allyl 2-azido-2-deoxy-3,6-di-O-(para-methoxybenzyl)-D-glucopyranoside (69)

To a mixture of 23 (630 mg, 1.3 mmol), NaBH₃CN (818 mg, 13 mmol), and MS 4 Å (100 mg) in anhydrous THF (10 mL) stirring under Ar at 0 ºC was added dry hydrogen chloride (1.0 M solution in Et₂O) dropwise until a pH of ~1 was reached. After 1 h, the reaction was filtered through celite to remove MS. The filtrate was then diluted with EtOAc and washed with saturated aqueous NaHCO₃ and brine, dried over Na₂SO₄, and concentrated in vacuum. The residue was purified by silica gel column chromatography to give α,β-mixture 69 (448 mg, 71%) as a syrup. ¹H NMR (CDCl₃, 500 MHz) of β anomer: δ 7.27-7.25 (m, 2 H), 7.34-7.32 (m, 2 H), 6.92-6.88 (m, 4 H), 5.99-5.91 (m, 1 H), 5.35 (dd, J = 1.5, 17 Hz, 1 H), 5.23 (dd, J = 1.5, 10.5 Hz,
$^1$H NMR (CDCl$_3$, 500 MHz) of β anomer: δ 7.31-7.26 (m, 4 H), 6.90-6.87 (m, 4 H), 6.01-5.94 (m, 1 H), 5.36 (dd, $J = 2.0, 17.5$ Hz, 1 H), 5.24 (dd, $J = 1.5, 10$ Hz, 1 H), 4.83 (d, $J = 10.5$ Hz, 1 H), 4.65 (d, $J = 10.5$ Hz, 1 H), 4.60 (d, $J = 12$ Hz, 1 H), 4.46-4.41 (m, 2 H), 4.37 (d, $J = 8.5$ Hz, 1 H), 4.17 (dd, $J = 6.0, 13$ Hz, 1 H), 3.82 (s, 3 H), 3.81 (s, 3 H), 3.73 (dd, $J = 2.0, 11.0$ Hz, 1 H), 3.56-3.51 (m, 2 H), 3.43-3.36 (m, 2 H), 3.20 (dd, $J = 8.5, 9.5$ Hz, 1 H), 0.86 (s, 9 H), 0.04 (s, 3 H), 0.00 (s, 3 H). $^{13}$C NMR (CDCl$_3$, 125 MHz) of β anomer: δ 159.41, 159.32, 133.86, 130.61, 130.57, 129.45, 129.40, 117.84,
114.01, 113.93, 101.28, 83.25, 76.73, 74.97, 73.28, 71.28, 70.48, 69.18, 67.00, 55.53, 55.50,
26.17, 18.22, -3.42, -4.49. [α]_D^{25} of β anomer = +18º (c 1.0, CHCl₃). HR ESI MS: calcd. for C₃₁H₄₅N₇O₇NaSi [M+Na]^+ m/z, 622.2924; found, 622.2927.

2-Azido-4-O-(tert-butyldimethylsilyl)-2-deoxy-3,6-di-O-(para-methoxybenzyl)-D-glucopyranose (25)

A solution of [Ir(COD)(PMePh₂)₂]PF₆ (72 mg, 80 μmol) in anhydrous THF (5 mL) was stirred under H₂ at rt until the color turned from red to colorless to pale yellow (15 min). After the H₂ atmosphere was exchanged with Ar, 24 (410 mg, 0.68 mmol, solution in 5 mL THF) was added slowly. TLC showed that isomerization of the allyl ether to the corresponding vinyl ether was complete after stirring at rt for 1 h. The reaction was concentrated in vacuum, then dissolved in acetone-H₂O (9:1, 10 mL) and treated with HgCl₂ (950 mg, 3.5 mmol) and HgO (17 mg, 80 μmol). The mixture was stirred at rt under an Ar atmosphere for 15 min, then concentrated and purified by silica gel column chromatography to give hemiacetal 25 (323 mg, 85%) as a syrup.

¹H NMR (CDCl₃, 500 MHz, all signals): δ 7.32-7.26 (m, 8 H), 6.90-6.87 (m, 8 H), 5.36 (d, J = 3.5 Hz, 1 H), 4.81 (d, J = 10.5 Hz, 1 H), 4.79 (d, J = 10.5 Hz, 1 H), 4.66-4.58 (m, 4 H), 4.42 (d, J = 12 Hz, 2 H), 4.07-4.04 (m, 1 H), 3.81 (s, 12 H), 3.77 (dd, J = 9.0, 10.5 Hz, 1 H), 3.68-3.64 (m, 2 H), 3.53 (t, J = 8.5 Hz, 1 H), 3.50-3.42 (m, 5 H), 3.35-3.30 (m, 2 H), 3.18 (dd, J = 8.0, 10 Hz, 1 H), 0.85 (s, 9 H), 0.82 (s, 9 H), -0.02 (s, 6 H), -0.03 (s, 3 H), -0.05 (s, 3 H). ¹³C NMR (CDCl₃, 125 MHz, all signals): δ 159.62, 159.58, 159.31, 159.29, 130.51, 130.02, 129.91, 129.85, 129.78, 129.46, 129.34, 114.11, 114.07, 113.94, 113.90, 96.52, 92.28, 83.02, 80.13, 76.38, 75.10, 75.04,
73.34, 73.23, 72.08, 71.93, 71.42, 69.18, 69.12, 68.06, 64.68, 55.53, 55.50, 26.12, 26.09, 18.18, 18.15, -3.39, -3.44, -4.43, -4.50. HR ESI MS: calcd. for C_{28}H_{41}N_{3}O_{7}NaSi [M+Na]^+ m/z, 582.2611; found, 582.2604.

6-O-[2-Azido-4-O-(tert-butyldimethylsilyl)-2-deoxy-3,6-di-O-(para-methoxybenzyl)-α-D-glucopyranosyl]-1-O-allyl-2,3,4,5-tetra-O-(para-methoxybenzyl)-myo-inositol (31)

DBU (1 drop) was added to a solution of hemiacetal 25 (320 mg, 0.57 mmol) and trichloroacetonitrile (0.5 mL) in anhydrous CH_{2}Cl_{2} (5 mL) stirring under an Ar atmosphere at 0 °C. After 1 h, the reaction mixture was concentrated in vacuum and purified with a triethylamine-neutralized silica gel column to give 9 (335 mg, 83%). A mixture of purified trichloroacetimidate 9 (904 mg, 1.28 mmol), acceptor 8 (450 mg, 0.64 mmol), and MS 4 Å (100 mg) in anhydrous toluene-1,4-dioxane (pre-dried over activated MS 4 Å, 5 mL) was stirred under an Ar atmosphere at rt for 1 h. After cooling to 0 °C, TMSOTf (12 μl, 64 μmol) was added and the reaction was stirred for 10 min. Neutralization with triethylamine was followed by filtration through celite, concentration in vacuum, and purification by silica gel column chromatography to give an α,β-mixture of the pseudodisaccharide 30 (632 mg, 80%) as a syrup. Semi-preparative HPLC (Waters Nova-Pak Silica 6 μm, 300 x 19 mm, eluent 20% EtOAc in hexanes, 10 mL/min, α-t_{R} = 16.1 min, β-t_{R} = 17.8 min) showed an α/β ratio of 2.3:1, and purification using this method could be performed to obtain 31-α as a white solid for characterization (see below). Alternatively, the α,β-mixture can be taken directly to the next step and purified thereafter on a large scale.
using silica gel column chromatography. $^1$H NMR of 31-α (CDCl$_3$, 500 MHz): δ 7.38-7.22 (m, 10 H), 7.12-7.10 (m, 2 H), 6.92-6.83 (m, 10 H), 6.83-6.77 (m, 2 H), 6.00-5.92 (m, 1 H), 5.78 (d, $J = 3.5$ Hz, 1 H), 5.30 (dd, $J = 2.0$, 17.5 Hz, 1 H), 5.20 (d, $J = 1.5$, 10.5 Hz, 1 H), 4.94 (d, $J = 11.5$ Hz, 1 H), 4.86 (d, $J = 10$ Hz, 1 H), 4.85 (d, $J = 10.5$ Hz, 1 H), 4.80-4.78 (m, 3 H), 4.74 (d, $J = 10.5$ Hz, 1 H), 4.67 (d, $J = 10$ Hz, 1 H), 4.62 (d, $J = 11.5$ Hz, 1 H), 4.57 (d, $J = 11.5$ Hz, 1 H), 4.43 (d, $J = 11.5$ Hz, 1 H), 4.35 (d, $J = 11.5$ Hz, 1 H), 4.28 (t, $J = 9.5$ Hz, 1 H), 4.09 (t, $J = 9.5$ Hz, 1 H), 4.03-3.99 (m, 4 H), 3.84 (s, 3 H), 3.83 (s, 3 H), 3.82 (s, 3 H), 3.81 (s, 3 H), 3.80 (s, 3 H), 3.79 (s, 3 H), 3.78 (t, $J = 9.0$ Hz, 1 H), 3.73 (t, $J = 10.0$ Hz, 1 H), 3.40 (t, $J = 9.5$ Hz, 1 H), 3.39-3.35 (m, 3 H), 3.33 (dd, $J = 2.0$, 11 Hz, 1 H), 3.22 (dd, $J = 4.0$, 10.5 Hz, 1 H), 0.80 (s, 9 H), 0.01 (s, 3 H), -0.03 (s, 3 H). $^{13}$C NMR of 31-α (CDCl$_3$, 125 MHz): δ 159.46, 159.33, 159.31, 159.19, 158.97, 134.64, 131.24, 131.20, 130.86, 130.81, 130.75, 129.90, 129.72, 129.46, 129.39, 129.15, 128.57, 117.11, 114.04, 113.92, 113.87, 113.81, 97.99, 82.22, 82.03, 81.31, 80.93, 80.39, 75.67, 75.63, 74.84, 74.29, 73.90, 73.07, 72.68, 72.59, 71.67, 70.97, 68.27, 64.22, 55.55, 55.54, 55.52, 26.33, 18.29, -3.49, -4.61. [α]$_D^{25}$ of 31-α = +51º (c 1.0, CHCl$_3$). HR ESI MS of 31-α: calcd. for C$_{69}$H$_{87}$N$_3$O$_{16}$NaSi [M+Na]$^+$ m/z, 1264.5753; found, 1264.5693.

6-O-[2-Azido-4-O-(tert-butyldimethylsilyl)-2-deoxy-3,6-di-O-(para-methoxybenzyl)-α-D-glucopyranosyl]-2,3,4,5-tetra-O-(para-methoxybenzyl)-myo-inositol (6)

A solution of [Ir(COD)(PMePh$_2$)$_2$]PF$_6$ (6 mg, 7 μmol) in anhydrous THF (2.5 mL) was stirred under H$_2$ at rt until the color turned from red to colorless to pale yellow (10 min). After the H$_2$ atmosphere was exchanged with Ar, 31-α (88 mg, 70 μmol, solution in 2.5 mL THF) was added
slowly. Stirring at rt for 1 h resulted in complete isomerization of the allyl ether to the vinyl ether as indicated by TLC. The reaction was concentrated in vacuum, then dissolved in acetone-H$_2$O (9:1, 5 mL) and treated with HgCl$_2$ (95 mg, 0.35 mmol) and HgO (2 mg, 7 μmol). The mixture was stirred at rt under an Ar atmosphere for 5 min, then concentrated and purified by silica gel column chromatography to give pseudodisaccharide 6 (81 mg, 96%) as a syrup. $^1$H NMR (CDCl$_3$, 500 MHz): δ 7.33-7.28 (m, 8 H), 7.19-7.17 (m, 4 H), 6.90-6.80 (m, 12 H), 5.53 (d, $J$ = 4.0 Hz, 1 H), 4.96 (d, $J$ = 11 Hz, 1 H), 4.91 (d, $J$ = 10.5 Hz, 1 H), 4.87 (d, $J$ = 10 Hz, 1 H), 4.80 (d, $J$ = 11 Hz, 1 H), 4.76-4.62 (m, 6 H), 4.30 (s, 2 H), 4.04 (t, $J$ = 9.5 Hz, 1 H), 3.97 (t, $J$ = 9.0 Hz, 1 H), 3.97-3.95 (m, 1 H), 3.92-3.89 (m, 1 H), 3.83 (s, 3 H), 3.82 (s, 3 H), 3.81 (s, 6 H), 3.80 (s, 6 H), 3.77 (t, $J$ = 8.5 Hz, 1 H), 3.72 (t, $J$ = 9 Hz, 1 H), 3.63-3.60 (m, 1 H), 3.46-3.41 (m, 3 H), 3.38 (t, $J$ = 9.5 Hz, 1 H), 3.27 (dd, $J$ = 2.0, 10.5 Hz, 1 H), 3.17 (d, $J$ = 7.0 Hz, 1 H), 0.85 (s, 9 H), 0.02 (s, 3 H), 0.00 (s, 3 H). $^{13}$C NMR (CDCl$_3$, 125 MHz): δ 159.52, 159.36, 159.34, 159.20, 159.14, 131.21, 131.15, 130.93, 130.58, 130.52, 130.37, 129.95, 129.84, 129.61, 129.10, 129.04, 114.10, 113.97, 113.94, 113.91, 113.87, 98.15, 81.84, 81.36, 80.90, 80.83, 80.29, 77.12, 75.62, 74.99, 74.79, 74.60, 73.52, 73.02, 72.91, 72.51, 70.91, 68.14, 64.98, 55.53, 55.49, 26.28, 18.24, -3.44, -4.60. [α]$_D$$_{25}^0$ = +10º (c 1.0, CHCl$_3$). HR ESI MS: calcd. for C$_{66}$H$_{83}$N$_3$O$_{16}$NaSi [M+Na]$^+$ m/z, 1224.5440; found, 1224.5420.

(2-Cyanoethoxy)-(diisopropylamino)-(2,3-di-O-stearoyl-sn-glycerol)-phosphine (34)

To a solution of known glycerolipid 70 (1.04 g, 1.66 mmol) and commercially available bis(diisopropylamino)(2-cyanoethoxy)phosphine (970 mg, 0.32 mmol) in anhydrous CH$_2$Cl$_2$-
acetonitrile (3:1, 12 mL) was added diisopropylammonium tetrazolide (266 mg, 1.66 mmol). After the reaction stirred at rt under Ar for 1 h, it was diluted with CH$_2$Cl$_2$ and poured into saturated aqueous NaHCO$_3$. The aqueous layer was extracted 3 times with CH$_2$Cl$_2$, after which the combined organic layers were dried over Na$_2$SO$_4$ and concentrated in vacuum. Purification using a triethylamine-neutralized silica gel column gave a 1:1 diastereomeric mixture (originating at phosphorus) of phosphoramidite 34 (1.25 g, 90%) as a white solid. $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 5.21-5.16 (m, 1 H), 4.37-4.30 (m, 1 H), 4.20-4.13 (m, 1 H), 3.89-3.48 (m, 6 H), 2.63 (t, $J$ = 6.4 Hz, 2 H), 2.33-2.28 (m, 4 H), 1.65-1.58 (m, 4 H), 1.34-1.24 (m, 56 H), 1.17 (t, $J$ = 7.2 Hz, 12 H), 0.87 (t, $J$= 7.2 Hz, 6 H). $^{31}$P NMR (CDCl$_3$, 160 MHz): $\delta$ 150.64, 150.48.

6-O-[2-Azido-2-deoxy-3,6-di-O-(para-methoxybenzyl)-$\alpha$-D-glucopyranosyl]-1-O-[2-cyanoethoxyl)-(2,3-di-O-stearoyl-sn-glycerol)-phosphono]-2,3,4,5-tetra-O-(para-methoxybenzyl)-myo-inositol (35)

To a solution of pseudodisaccharide 6 (150 mg, 125 μmol) and 1H-tetrazole (0.45 M solution in acetonitrile, 1.66 mL, 0.75 mmol) stirring in anhydrous CH$_2$Cl$_2$-CH$_3$CN (3:1, 8.0 mL) under Ar at rt was slowly added a solution of freshly prepared phosphoramidite 34 (326 mg in 1.00 mL anhydrous CH$_2$Cl$_2$, 375 μmol). After the reaction stirred at rt under Ar for 20 min, it was cooled to 0 ºC and treated with tert-butyl hydroperoxide (5.5 M solution in decane, 0.15 mL, 0.83 mmol). The solution stirred for 1 h while warming to rt, and was then diluted with CH$_2$Cl$_2$. The
mixture was poured into saturated aqueous NaHCO₃ and extracted 3 times with CH₂Cl₂. The combined organic layers were dried over Na₂SO₄ and concentrated in vacuum. Purification by silica gel column chromatography gave the desired partially purified intermediate (characterized by MALDI MS), which was then dissolved in anhydrous THF-CH₃CN (1:1, 4.0 mL) and treated with Et₃N·3HF (2.0 mL) under Ar at rt. After stirring for 7 days at rt, the reaction was quenched by dropwise addition of saturated aqueous NaHCO₃. The mixture was extracted 3 times with CH₂Cl₂, and the combined organic layer was dried over Na₂SO₄, concentrated in vacuum, and purified by silica gel column chromatography to give 35 (124 mg, 54%, two steps) as a ~1:1 mixture of diastereomers (originating at phosphorus). Semi-preparative HPLC (Waters Nova-Pak Silica 6 µm, 300 x 19 mm, eluent 30% acetone in hexanes, 10 mL/min, isomer 1-<i>t</i><sub>R</sub> = 18.4 min, isomer 2-<i>t</i><sub>R</sub> = 19.6 min) was used to separate the mixture, and isomer 1 was characterized and carried forward to complete the synthesis. ¹H NMR (CDCl₃, 500 MHz), isomer 1: δ 7.36-7.34 (m, 2 H), 7.33-7.31 (m, 2 H), 7.29-7.27 (m, 2 H), 7.22-7.20 (m, 2 H), 7.15-7.13 (m, 4 H), 6.90-6.83 (m, 8 H), 6.80-6.76 (m, 4 H), 5.40 (d, <i>J</i> = 4.0 Hz, 1 H), 5.23 (pent, <i>J</i> = 5.5 Hz, 1 H), 4.94 (d, <i>J</i> = 10.5 Hz, 1 H), 4.92 (d, <i>J</i> = 11 Hz, 1 H), 4.86 (d, <i>J</i> = 10 Hz, 1 H), 4.83-4.78 (m, 2 H), 4.70-4.67 (m, 3 H), 4.62 (d, <i>J</i> = 10.5 Hz, 1 H), 4.61 (d, <i>J</i> = 11 Hz, 1 H), 4.43-4.32 (m, 5 H), 4.29-4.21 (m, 4 H), 4.17-4.12 (m, 1 H), 4.10 (dd, <i>J</i> = 6.0, 12 Hz, 1 H), 4.04-4.00 (m, 2 H), 3.82 (s, 3 H), 3.81 (s, 3 H), 3.80 (s, 3 H), 3.79 (s, 3 H), 3.78 (s, 3 H), 3.78-3.77 (m, 1 H), 3.76 (s, 3 H), 3.67 (dt, <i>J</i> = 3.0, 10 Hz, 1 H), 3.51 (dd, <i>J</i> = 1.5, 9.5 Hz, 1 H), 3.40 (t, <i>J</i> = 9.0 Hz, 1 H), 3.33-3.26 (m, 2 H), 3.18 (dd, <i>J</i> = 3.5, 10.5 Hz, 1 H), 2.84-2.73 (m, 2 H), 2.29 (t, <i>J</i> = 7.5 Hz, 4 H), 2.09 (d, <i>J</i> = 4.0 Hz, 1 H), 1.61-1.56 (m, 4 H), 1.32-1.26 (m, 56 H), 0.89 (t, <i>J</i> = 6.5 Hz, 1 H). ¹³C NMR (CDCl₃, 125 MHz), isomer 1: δ 173.41, 173.06, 159.69, 159.43, 159.41, 159.35, 159.29, 131.11, 130.98, 130.57, 130.52, 130.49, 130.24, 130.06, 129.70, 129.53, 129.50, 129.29, 116.60, 114.21, 114.04, 113.96,
Phenyl 2-O-allyl-3-O-(para-methoxybenzyl)-4,6-O-(para-methoxybenzylidene)-1-thio-α-D-mannopyranoside (39)

A mixture of 37\(^5\) (1.36 g, 3.48 mmol) and dibutyltin oxide (0.95 g, 3.8 mmol) in anhydrous toluene (50 mL) was refluxed with azeotropic removal of water using a Dean-Stark apparatus for 4 h. After cooling to rt and concentration in vacuum, the residue was dissolved in anhydrous DMF (20 mL) and cooled to 0 °C, after which cesium fluoride (1.22 g, 8.00 mmol) and para-methoxybenzyl chloride (1.0 mL, 7.3 mmol) were added to the solution. After stirring overnight under an Ar atmosphere at rt, the reaction mixture was filtered through celite into cold saturated aqueous NaHCO\(_3\) and EtOAc. The organic layer was washed with brine, dried over Na\(_2\)SO\(_4\), and concentrated in vacuum. The residue was purified by silica gel column chromatography to give 38, which was taken directly to the next step. To a solution of 38 in anhydrous DMF (30 mL) under Ar at 0 °C was added NaH (60% dispersion in mineral oil, 350 mg, 8.7 mmol). After stirring for 30 min, allyl bromide (0.73 mL, 8.7 mmol) was added dropwise and the reaction was stirred for 1 h. The reaction was quenched with MeOH and diluted with EtOAc. The organic layer was washed with brine, dried over Na\(_2\)SO\(_4\), and concentrated in vacuum. The residue was
purified by silica gel column chromatography to give 39 (1.3 g, 68%, two steps) as a syrup. $^1$H NMR (CDCl$_3$, 500 MHz): δ 7.46-7.44 (m, 4 H), 7.34-7.29 (m, 5 H), 6.93-6.89 (m, 4 H), 5.97-5.89 (m, 1 H), 5.60 (s, 1 H), 5.53 (d, $J = 1.0$ Hz, 1 H), 5.30 (dd, $J = 2.0$, 17.5 Hz, 1 H), 5.21 (dd, $J = 1.5$, 10.5 Hz, 1 H), 4.81 (d, $J = 11.5$ Hz, 1 H), 4.66 (d, $J = 11.5$ Hz, 1 H), 4.31-4.16 (m, 5 H), 3.96-3.93 (m, 2 H), 3.87 (t, $J = 10$ Hz, 1 H), 3.83 (s, 3 H), 3.83 (s, 3 H). $^{13}$C NMR (CDCl$_3$, 125 MHz): δ 160.21, 159.48, 134.81, 134.15, 131.73, 130.69, 130.39, 129.62, 129.37, 127.82, 127.65, 118.19, 114.02, 113.79, 101.74, 87.53, 79.22, 78.38, 75.97, 73.07, 72.77, 68.71, 65.67, 55.54. [$\alpha$]$^D_{25} = +154^\circ$ (c 1.0, CHCl$_3$). HR ESI MS: calcd. for C$_{31}$H$_{34}$O$_7$Na [M+Na]$^+$ m/z, 573.1923; found, 573.1925.

**Phenyl 2-O-allyl-3,4-di-O-(para-methoxybenzyl)-1-thio-α-D-mannopyranoside (10)**

![Structural diagram]

To a solution of 39 (550 mg, 1.0 mmol) in anhydrous CH$_2$Cl$_2$ (15 mL) stirring under Ar at 0 ºC was added DIBAL-H (1.0 M solution in toluene, 2.0 mL, 2.0 mmol) dropwise over 1 h. After stirring at 0 ºC for an additional hour, saturated aqueous Na-K-tartrate was added and the mixture was stirred vigorously for 2 h. After separation of the two phases, the organic layer was washed with brine, dried over Na$_2$SO$_4$, and concentrated in vacuum. The residue was purified by silica gel column chromatography to give 10 (471 mg, 85%) as a syrup. $^1$H NMR (CDCl$_3$, 500 MHz): δ 7.46-7.44 (m, 2 H), 7.36-7.26 (m, 7 H), 6.93-6.88 (m, 4 H), 5.97-5.89 (m, 1 H), 5.52 (d, $J = 1.5$ Hz, 1 H), 5.30 (dd, $J = 1.5$, 17.5 Hz, 1 H), 5.22 (dd, $J = 2.0$, 10.5 Hz, 1 H), 4.88 (d, $J = 10.5$ Hz, 1 H), 4.68 (s, 2 H), 4.59 (d, $J = 11$ Hz, 1 H), 4.16 (d, $J = 6.0$ Hz, 2 H), 4.13-4.09 (m, 1 H), 3.95 (t, $J = 9.5$ Hz, 1 H), 3.92 (dd, $J = 2.0$, 3.0 Hz, 1 H), 3.88 (dd, $J = 3.0$, 9.5 Hz, 1 H), 3.84
(s, 3 H), 3.83-3.76 (m, 2 H), 3.82 (s, 3 H). $^{13}$C NMR (CDCl$_3$, 125 MHz): δ 159.60, 159.57, 134.87, 134.27, 132.02, 130.79, 130.47, 129.98, 129.85, 129.35, 127.86, 118.20, 114.14, 114.12, 86.34, 79.91, 76.65, 75.21, 74.80, 73.45, 72.29, 72.00, 62.51, 55.54. $[\alpha]^D_{25} = +18^\circ$ (c 1.0, CHCl$_3$).

HR ESI MS: calcd. for C$_{31}$H$_{36}$O$_7$NaS [M+Na]$^+$ m/z, 575.2079; found, 575.2063.

**3,4,6-tri-O-(para-Methoxybenzyl)-1,2-O-(1-methoxyethylidene)-β-D-mannopyranoside (71)**

![Structural diagram of 71]

After a solution of 40$^6$ (2.00 g, 5.52 mmol) in 15 mL of 0.05 M NaOMe in methanol was stirred at rt for 20 min, it was concentrated to afford a white powder (note: Neutralization with Amberlyst H$^+$ resin caused significant decomposition of the orthoester, so the reaction was taken directly to the next step). To the crude triol in anhydrous DMF (30 mL) stirring under Ar at 0 ℃ was slowly added NaH (60% dispersion in mineral oil, 1.10 g, 27.5 mmol). After stirring for 30 min, para-methoxybenzyl chloride (2.30 mL, 22.0 mmol) was added dropwise. The reaction was stirred for 3 h while warming to rt and then quenched with MeOH and diluted with EtOAc. The solution was washed with brine, dried over Na$_2$SO$_4$, and concentrated in vacuum. The residue was purified by silica gel column chromatography to give 71 (2.50 g, 76 %, two steps) as a syrup.

$^1$H NMR (CDCl$_3$, 500 MHz): δ 7.32-7.30 (m, 2 H), 7.25-7.24 (m, 2 H), 7.14-7.12 (m, 2 H), 6.87-6.82 (m, 6 H), 5.32 (d, J = 2.5 Hz, 1 H), 4.78 (d, J = 10.5 Hz, 1 H), 4.73 (d, J = 12 Hz, 1 H), 4.70 (d, J = 11.5 Hz, 1 H), 4.54-4.47 (m, 3 H), 4.34 (dd, J = 2.5, 4.0 Hz, 1 H), 3.83 (t, J = 9.5 Hz, 1 H), 3.80 (s, 6 H), 3.79 (s, 3 H), 3.68-3.65 (m, 3 H), 3.38 (dt, J = 3.5, 9.0 Hz, 1 H), 3.28 (s, 3 H), 1.72 (s, 3 H). $^{13}$C NMR (CDCl$_3$, 125 MHz): δ 129.95, 129.86, 129.46, 114.13, 114.01, 113.94, 97.76,
78.83, 75.10, 74.44, 74.19, 73.23, 72.25, 68.90, 55.51, 49.98, 24.62. [α]$_D^{25}$ = +12º (c 1.0, CHCl$_3$).

HR ESI MS: calcd. for C$_{33}$H$_{40}$O$_{10}$Na [M+Na]$^+$ m/z, 619.2519; found, 619.2524.

2-O-Acetyl-3,4,6-tri-O-(para-methoxybenzyl)-D-mannopyranose (72)

To a round bottom flask containing 71 (2.30 g, 3.85 mmol) was added AcOH/H$_2$O (1:1, 20 mL). The poorly soluble substrate went into solution as the reaction was stirred vigorously at rt over 3 h, after which the solution was diluted with CH$_2$Cl$_2$ and poured into a separatory funnel containing cold, saturated aqueous NaHCO$_3$. The aqueous phase was extracted with CH$_2$Cl$_2$ two additional times, and the combined organic layers were washed with saturated aqueous NaHCO$_3$ and brine, dried over Na$_2$SO$_4$, and concentrated in vacuum. The resulting residue was purified by silica gel column chromatography to give hemiacetal 72 (1.57 g, 70%) as a glassy syrup, while the minor product was identified as the regioisomer containing 1-O-acetyl and 2-OH functional groups. $^1$H NMR (CDCl$_3$, 500 MHz): δ 7.27-7.25 (m, 4 H), 7.05-7.03 (m, 2 H), 6.86-6.81 (m, 6 H), 5.35 (dd, $J$ = 2.0, 3.0 Hz, 1 H), 5.20 (d, $J$ = 2.0 Hz, 1 H), 4.75 (d, $J$ = 10.5 Hz, 1 H), 4.63 (d, $J$ = 11 Hz, 1 H), 4.54 (d, $J$ = 12 Hz, 1 H), 4.46 (d, $J$ = 10 Hz, 1 H), 4.44 (d, $J$ = 12 Hz, 1 H), 4.35 (d, $J$ = 10.5 Hz, 1 H), 4.04-4.00 (m, 1 H), 3.99 (dd, $J$ = 3.0, 9.0 Hz, 1 H), 3.80 (s, 3 H), 3.79 (s, 3 H), 3.78 (s, 3 H), 3.68 (t, $J$ = 10 Hz, 1 H), 3.66-3.60 (m, 2 H), 3.37 (d, $J$ = 3.5 Hz, 1 H), 2.14 (s, 3 H). $^{13}$C NMR (CDCl$_3$, 125 MHz): δ 170.74, 159.50, 159.44, 130.73, 130.37, 130.17, 129.96, 129.95, 129.76, 114.04, 113.99, 113.94, 92.75, 77.55, 74.96, 74.49, 73.24, 71.67, 71.41, 69.31, 69.03, 55.50, 55.48, 55.45, 21.40. HR ESI MS: calcd. for C$_{32}$H$_{38}$O$_{10}$Na [M+Na]$^+$ m/z, 605.2363; found, 605.2392.
**Allyl 6-O-(para-methoxytriphenylmethyl)-α-D-mannopyranoside (41)**

After a solution of allyl mannoside $73^7$ (2.45 g, 7.24 mmol) in 20 mL of 0.05 M NaOMe in methanol was stirred at rt for 30 min, it was neutralized to pH 6-7 using Amberlyst H$^+$ resin. The solution was filtered off and concentrated to afford a white powder, which was directly used for the next step. After the crude tetraol was dissolved in pyridine (30 mL), *para*-methoxymethyl chloride (5.60 g, 18.1 mmol) was added and the reaction was stirred at rt for 2 h, then concentrated in vacuum and purified by silica gel column chromatography to afford $41$ (2.30 g, 66%, two steps) as a white foam. $^1$H NMR (CDCl$_3$, 500 MHz): δ 7.45-7.43 (m, 4 H), 7.33-7.28 (m, 6 H), 7.24-7.21 (m, 2 H), 6.84-6.83 (m, 2 H), 5.95-5.87 (m, 1 H), 5.30 (dd, $J$ = 1.5, 17 Hz, 1 H), 5.21 (dd, $J$ = 1.5, 10.8 Hz, 1 H), 4.86 (d, $J$ = 1.5 Hz, 1 H), 4.19 (dd, $J$ = 5.5, 13.5 Hz, 1 H), 3.99 (dd, $J$ = 6.5, 13 Hz, 1 H), 3.93 (dd, $J$ = 1.5, 3.5 Hz, 1 H), 3.84-3.83 (m, 1 H), 3.79 (s, 3 H), 3.74-3.71 (m, 2 H), 3.43 (dd, $J$ = 4.5, 9.5 Hz, 1 H), 3.39 (dd, $J$ = 4.5, 9.5 Hz, 1 H). $^{13}$C NMR (CDCl$_3$, 125 MHz): δ 158.92, 144.37, 144.25, 135.39, 133.89, 130.57, 128.54, 128.20, 127.31, 117.84, 113.49, 98.86, 87.33, 71.86, 70.72, 70.58, 70.15, 65.05, 55.46. [$\alpha$]$_{D}^{25}$ = +17º (c 1.0, CHCl$_3$). HR ESI MS: calcd. for C$_{29}$H$_{32}$O$_7$Na[M+Na]$^+$ $m/z$, 515.2046; found, 515.2054.
Allyl 2,3,4-tri-O-(para-methoxybenzyl)-6-O-(para-methoxytriphenylmethyl)-α-D-mannopyranoside (74)

To a solution of 41 (2.10 g, 4.26 mmol) in anhydrous DMF (40 mL) stirring under Ar at 0 °C was slowly added NaH (60% dispersion in mineral oil, 756 mg, 18.9 mmol). After stirring for 30 min, para-methoxybenzyl chloride (1.97 mL, 18.9 mmol) was added dropwise. The reaction was stirred overnight at rt and then quenched with MeOH and diluted with EtOAc. The solution was washed with saturated aqueous NaHCO₃ and brine, dried over Na₂SO₄, and concentrated in vacuum. The residue was purified by silica gel column chromatography to give 74 (2.61 g, 73 %) as a white foam. ¹H NMR (CDCl₃, 500 MHz): δ 7.52-7.51 (m, 4 H), 7.37-7.33 (m, 4 H), 7.30-7.20 (m, 8 H), 6.87-6.79 (m, 8 H), 6.71-6.70 (m, 2 H), 5.93-5.85 (m, 1 H), 5.25 (dd, J = 1.5, 17 Hz, 1 H), 5.17 (dd, J = 1.0, 10.5 Hz, 1 H), 4.92 (d, J = 1.5 Hz, 1 H), 4.76 (d, J = 12 Hz, 1 H), 4.65-4.63 (m, 2 H), 4.59 (d, J = 12 Hz, 1 H), 4.56 (d, J = 11 Hz, 1 H), 4.24 (dd, J = 4.5, 12.5 Hz, 1 H), 4.18 (d, J = 10 Hz, 1 H), 3.99 (dd, J = 6.0, 13 Hz, 1 H), 3.94 (t, J = 9.5 Hz, 1 H), 3.87 (dd, J = 3.0, 9.5 Hz, 1 H), 3.81 (s, 3 H), 3.80-3.78 (m, 2 H), 3.79 (s, 3 H), 3.78 (s, 3 H), 3.77 (s, 3 H), 3.47 (dd, J = 1.5, 9.5 Hz, 1 H), 3.24 (dd, J = 5.5, 9.5 Hz, 1 H). ¹³C NMR (CDCl₃, 125 MHz): δ 159.30, 158.67, 145.02, 144.84, 136.08, 134.20, 131.09, 130.95, 130.79, 130.71, 130.07, 129.50, 129.44, 128.91, 128.83, 127.95, 126.89, 117.45, 113.97, 113.78, 113.24, 97.02, 86.13, 80.23, 75.32, 75.03, 74.93, 72.53, 72.23, 67.77, 63.12, 55.50, 55.48, 55.39. [α]D²⁵ = +13° (c 1.0, CHCl₃).

HR ESI MS: calcd. for C₅₃H₅₆O₁₀Na [M+Na]+ m/z, 875.3771; found, 875.3762.
Allyl 2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannopyranoside (75)

After a solution of 74 (2.40 g, 2.81 mmol) in AcOH/CH₂Cl₂/H₂O (15:4:1, 20 mL) was stirred at rt for 2.5 h, it was diluted with CH₂Cl₂ and poured into cold, saturated aqueous NaHCO₃. The organic layer was washed with saturated aqueous NaHCO₃ and brine, then dried over Na₂SO₄. After concentration in vacuum, the residue was purified by silica gel column chromatography to give 75 (1.53 g, 94%) as a syrup. ¹H NMR (CDCl₃, 500 MHz): δ 7.29-7.28 (m, 4 H), 7.24-7.22 (m, 2 H), 6.88-6.85 (m, 6 H), 5.86-5.78 (m, 1 H), 5.20 (dd, J = 2.0, 17.5 Hz, 1 H), 5.15 (dd, J = 1.5, 10.5, 1 H), 4.85 (d, J = 10.5 Hz, 1 H), 4.79 (d, J = 1.5 Hz, 1 H), 4.71 (d, J = 12 Hz, 1 H), 4.61 (d, J = 12, 1 H), 4.57-4.55 (m, 3 H), 4.10 (dd, J = 5.0, 13 Hz, 1 H), 3.91-3.87 (m, 3 H), 3.81 (s, 3 H), 3.80 (s, 3 H), 3.79 (s, 3 H), 3.79-3.74 (m, 3 H), 3.64-3.61 (m, 1 H). ¹³C NMR (CDCl₃, 125 MHz): δ 159.50, 159.39, 133.88, 130.91, 130.83, 130.53, 129.97, 129.75, 129.45, 117.49, 114.07, 114.01, 97.69, 80.16, 75.12, 74.90, 74.62, 72.81, 72.41, 72.15, 68.05, 62.70, 55.51. [α]D²⁵ = +35° (c 1.0, CHCl₃). HR ESI MS: calcd. for C₃₃H₄₀O₉Na [M+Na]^⁺ m/z, 603.2570; found, 603.2549.

Allyl 6-O-(tert-butyldimethylsilyl)-2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannopyranoside (42)

A solution of 75 (1.50 g, 2.58 mmol) and TBSCI (777 mg, 5.16 mmol) in pyridine (25 mL) was stirred under Ar overnight at rt. After concentration to remove most of the pyridine, the residue
was dissolved in CH$_2$Cl$_2$ and poured into water. The aqueous layer was extracted twice with CH$_2$Cl$_2$ and the combined organic layers were washed with saturated aqueous NaHCO$_3$ and brine, then dried over Na$_2$SO$_4$ and concentrated in vacuum. The resulting residue was purified by silica gel column chromatography to obtain 42 (1.62 g, 88%) as a glassy syrup.

$^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 7.29-7.27 (m, 4 H), 7.23-7.21 (m, 2 H), 6.87-6.84 (m, 6 H), 5.86-5.80 (m, 1 H), 5.20 (dd, $J = 1.5, 17$ Hz, 1 H), 5.13 (dd, $J = 1.5, 10.5$ Hz, 1 H), 4.83 (d, $J = 10.5$ Hz, 1 H), 4.66 (d, $J = 12.5$ Hz, 1 H), 4.59 (d, $J = 12$ Hz, 1 H), 4.56-4.52 (m, 3 H), 4.12 (dd, $J = 4.5, 13$ Hz, 1 H), 3.91-3.80 (m, 4 H), 3.81 (s, 3 H), 3.80 (s, 3 H), 3.80 (s, 3 H), 3.79-3.77 (m, 1 H), 3.73 (dd, $J = 2.0, 3.5$ Hz, 1 H), 3.57 (ddd, $J = 2.0, 5.0, 9.0$ Hz, 1 H), 0.89 (s, 9 H), 0.064 (s, 3 H), 0.058 (s, 3 H). $^{13}$C NMR (CDCl$_3$, 125 MHz): $\delta$ 159.41, 159.35, 134.20, 131.20, 131.09, 130.78, 129.86, 129.58, 129.48, 117.24, 114.00, 113.95, 113.90, 97.01, 80.21, 74.96, 74.90, 74.79, 73.55, 72.34, 72.05, 67.66, 63.09, 55.50, 26.16, -4.90, -5.04. [α]$_D^{25} = +23^\circ$ (c 1.0, CHCl$_3$). HR ESI MS: calcd. for C$_{39}$H$_{54}$O$_9$NaSi [M+Na]$^+$ m/z, 717.3435; found, 717.3434.

6-O-(tert-Butyldimethylsilyl)-2,3,4-tri-O-(para-methoxybenzyl)-D-mannopyranose (76)

To a solution of 42 (491 mg, 0.707 mmol) in CH$_2$Cl$_2$/AcOH (1:1, 8 mL) was added H$_2$O (5 drops), sodium acetate (373 mg, 4.50 mmol), and palladium chloride (248 mg, 1.41 mmol). After stirring under Ar at rt for 5 h, the reaction was concentrated down to a volume of ~1 mL and diluted with EtOAc. After catalyst filtration through celite, the organic layer was washed with saturated aqueous NaHCO$_3$ and brine, then dried over Na$_2$SO$_4$ and concentrated in vacuum. The residue was purified by silica gel column chromatography to give an α,β-mixture of hemiacetal...
Phenyl O-[3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-2-O-allyl-3,4-di-
O-(para-methoxybenzyl)-1-thio-α-D-mannopyranoside (44)

DBU (5 drops) was added to a solution of hemiacetal 72 (735 mg, 1.26 mmol) and trichloroacetonitrile (0.5 mL) in anhydrous \( \text{CH}_2\text{Cl}_2 \) (12 mL) stirring under an Ar atmosphere at 0 °C. After 20 min, the reaction mixture was concentrated in vacuum and purified with a triethylamine-neutralized silica gel column to give 11 (706 mg, 77%). A mixture of the resulting trichloroacetimidate 11 (706 mg, 0.97 mmol), acceptor 10 (413 mg, 0.75 mmol), and MS 4 Å (150 mg) in anhydrous \( \text{CH}_2\text{Cl}_2 \) (10 mL) was stirred under an Ar atmosphere at rt for 1 h. After cooling to 0 °C, TMSOTf (10 μL, 55 μmol) was added and the reaction was stirred for 5 min. Neutralization with triethylamine was followed by filtration through celite to remove MS, concentration in vacuum, and purification by silica gel column chromatography to give crude 43,
which was dissolved in anhydrous MeOH (10 mL) and treated with K₂CO₃ (to pH ~9). After stirring for 1 h, the reaction was neutralized by Amberlyst H⁺ resin, filtered, and concentrated in vacuum. The residue was purified by silica gel column chromatography to afford α anomer 44 (530 mg, 66%, two steps) as a syrup. ¹H NMR (CDCl₃, 500 MHz): δ 7.45-7.43 (m, 2 H), 7.35-7.33 (m, 2 H), 7.28-7.25 (m, 5 H), 7.20-7.19 (m, 2 H), 7.08-7.06 (m, 2 H), 6.92-6.90 (m, 2 H), 6.85-6.81 (m, 10 H), 5.97-5.89 (m, 1 H), 5.52 (d, J = 1.5 Hz, 1 H), 5.31 (dd, J = 1.5, 17 Hz, 1 H), 5.20 (dd, J = 1.5, 10.5 Hz, 1 H), 4.99 (d, J = 1.5 Hz, 1 H), 4.85 (d, J = 11 Hz, 1 H), 4.74 (d, J = 10 Hz, 1 H), 4.69-4.63 (m, 2 H), 4.61-4.54 (m, 3 H), 4.45 (d, J = 10.5 Hz, 1 H), 4.41 (d, J = 12 Hz, 1 H), 4.38 (d, J = 10 Hz, 1 H), 4.20-4.09 (m, 3 H), 4.05 (s, broad, 1 H), 3.93-3.86 (m, 3 H), 3.83 (s, 3 H), 3.81 (s, 3 H), 3.78 (s, 3 H), 3.77 (s, 3 H), 3.75 (s, 3 H), 3.84-3.75 (m, 3 H), 3.72-3.66 (m, 3 H), 3.59 (dd, J = 1.5, 10.5 Hz, 1 H), 2.41 (s, broad, 1 H). ¹³C NMR (CDCl₃, 125 MHz): δ 159.59, 159.57, 159.45, 159.38, 159.34, 153.02, 134.94, 131.15, 131.05, 130.80, 130.60, 130.44, 130.29, 129.90, 129.85, 129.81, 129.78, 129.75, 129.35, 127.43, 118.03, 114.16, 114.12, 114.03, 113.94, 113.84, 99.82, 85.96, 80.09, 79.64, 76.65, 75.11, 74.91, 74.65, 74.12, 73.15, 72.72, 72.13, 71.63, 71.51, 71.23, 68.61, 68.30, 66.71, 55.53, 55.51, 55.45. [α]D²⁵ = +78º (c 1.0, CHCl₃). HR ESI MS: calcd. for C₆₁H₇₀O₁₅NaS [M+Na]⁺ m/z, 1097.4333; found, 1097.4349.
Phenyl [6-O-(tert-butyldimethylsilyl)-2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)-[3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-2-O-allyl-3,4-di-O-(para-methoxybenzyl)-1-thio-α-D-mannopyranoside (7)

DBU (2 drops) was added to a solution of hemiacetal 76 (144 mg, 0.22 mmol) and trichloroacetonitrile (0.3 mL) in anhydrous CH₂Cl₂ (3 mL) stirring under an Ar atmosphere at 0 °C. After 1 h, the reaction mixture was concentrated in vacuum and purified with a triethylamine-neutralized silica gel column to give 12 (129 mg, 73%). A mixture of the resulting trichloroacetimidate 12 (129 mg, 0.16 mmol), acceptor 44 (121 mg, 0.11 mmol), and MS 4 Å (50 mg) in anhydrous Et₂O (5 mL) was stirred under an Ar atmosphere at rt for 1 h. After cooling to 0 °C, TMSOTf (3 μl, 16 μmol) was added and the reaction was stirred for 5 min. Neutralization with triethylamine was followed by filtration through celite to remove MS, concentration in vacuum, and purification by silica gel column chromatography to give 7 (145 mg, 76%) as a syrup. ¹H NMR (CDCl₃, 500 MHz) : δ 7.46-7.45 (m, 2 H), 7.34-7.32 (m, 2 H), 7.26-7.17 (m, 14 H), 7.15-7.10 (m, 3 H), 6.91-6.75 (m, 16 H), 5.95-5.87 (m, 1 H), 5.51 (d, J = 1.5 Hz, 1 H), 5.30 (dd, J = 1.5, 17 Hz, 1 H), 5.23 (d, J = 1.0 Hz, 1 H), 5.18 (dd, J = 1.0, 10 Hz, 1 H), 4.85 (d, J = 10.5 Hz, 1 H), 4.82 (d, J = 2.0 Hz, 1 H), 4.82 (d, J = 10.5 Hz, 1 H), 4.77 (d, J = 10.5 Hz, 1 H), 4.66 (d, J = 11.5 Hz, 1 H), 4.63 (d, J = 11.5 Hz, 1 H), 4.60 (d, J = 12 Hz, 1 H), 4.55 (d, J = 10.5 Hz, 1 H), 4.51-4.47 (m, 2 H), 4.46 (d, J = 10.5 Hz, 1 H), 4.45 (d, J = 10.5 Hz, 1 H), 4.42-4.36 (m, 5 H), 4.22-4.15 (m, 2 H), 4.10-4.07 (m, 2 H), 3.96-3.91 (m, 3 H), 3.89-3.84 (m, 4 H), 3.83 (s, 3
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H), 3.83-3.82 (m, 2 H), 3.81 (s, 3 H), 3.80 (s, 3 H), 3.79-3.77 (m, 1 H), 3.76 (s, 3 H), 3.75 (s, 3 H), 3.74 (s, 3 H), 3.74-3.72 (m, 2 H), 3.69 (s, 3 H), 3.68 (s, 3 H), 3.66-3.63 (m, 2 H), 3.62-3.57 (m, 2 H), 0.88 (s, 9 H), 0.05 (s, 3 H), 0.04 (s, 3 H). ¹³C NMR (CDCl₃, 125 MHz): δ 159.59, 159.45, 159.36, 159.32, 159.29, 159.09, 159.12, 159.15, 134.94, 131.56, 131.27, 131.23, 131.07, 130.98, 130.89, 130.84, 130.64, 130.43, 129.89, 129.87, 129.81, 129.70, 129.61, 129.54, 129.38, 127.36, 118.05, 114.11, 114.05, 113.98, 113.93, 113.87, 113.84, 113.70, 99.50, 98.74, 86.16, 80.28, 80.08, 79.65, 76.65, 74.94, 74.85, 74.74, 74.69, 74.67, 74.59, 73.77, 73.08, 73.02, 72.46, 72.14, 72.08, 71.94, 71.66, 71.48, 69.14, 66.86, 62.93, 55.52, 55.48, 55.43, 55.41, 55.37, 55.32, 26.22, 18.59, -4.74, -4.98. The configurations of all three anomeric positions were established as α by coupled ¹³C NMR JCH values (125 MHz): 99.50 (JCH = 171 Hz, Man-II), 98.74 (JCH = 171 Hz, Man-III), 86.16 (JCH = 167 Hz, Man-I). [α]D²⁵ = +37º (c 1.0, CHCl₃). HR ESI MS: calcd. for C₉₇H₁₁₈O₂₃NaSiS [M+Na]⁺ m/z, 1733.7452; found, 1733.7410.

[6-O-(tert-Butyldimethylsilyl)-2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)-[3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-2-O-allyl-3,4-di-O-(para-methoxybenzyl)-D-mannopyranose (77)

To a solution of 7 (85 mg, 49.6 μmol) and 2,4,6-tri-tert-butylpyrimidine (62 mg, 248 μmol) in CH₂Cl₂ (2.0 mL) at -60 °C was added silver triflate (51 mg in 1.0 mL Et₂O, 199 μmol). Then, p-TolSCl (12 μl, 59.5 μmol) was added directly to the mixture to pre-activate the substrate. After
stirring for 1 min, acetone-H₂O (10:1, 105 μl) was added and the reaction was warmed to rt gradually and stirred for 2 h. After quenching with triethylamine and concentration in vacuum, the resulting residue was redissolved in CH₂Cl₂ and filtered to remove solids. The filtrate was then washed with water, dried over Na₂SO₄, concentrated, and purified by silica gel column chromatography to afford unreacted starting material 7 (15 mg) and hemiacetal 77 (40 mg, 50% yield, 68% BRSM). The desired product, an anomeric mixture, was quickly characterized by ^1H NMR and HR ESI MS (see S121 for spectra). HR ESI MS: calcd. for C₉₁H₁₁₄O₂₄NaSi [M+Na]^+ m/z 1641.7367; found, 1641.7310.

6-O-[[2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-1→2]-[3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-1→6]-[2-O-allyl-3,4-di-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-1→4]-[2-azido-2-deoxy-3,6-di-O-(para-methoxybenzyl)-α-D-glucopyranosyl]-1-O-[(2-cyanoethoxy)-(2,3-di-O-stearoyl-sn-glycerol)-phosphono]-2,3,4,5-tetra-O-(para-methoxybenzyl)-myo-inositol (46)

DBU (1 drop) was added to a solution of hemiacetal 77 (36 mg, 22.2 μmol) and trichloroacetonitrile (0.25 mL) in anhydrous CH₂Cl₂ (2 mL) stirring under an Ar atmosphere at 0
°C. After 1 h, the reaction mixture was concentrated in vacuum and purified with a triethylamine-neutralized silica gel column to give 45 (36 mg, 92%). A mixture of the resulting trichloroacetimide 45 (36 mg, 20.4 μmol), pseudodisaccharide acceptor 35 (23 mg, 12.6 μmol), and MS 4 Å (10 mg) in anhydrous Et₂O (0.5 mL) was stirred under an Ar atmosphere at rt for 1 h. After cooling to 0 °C, TMSOTf (0.5 μl, 0.1 equiv) was added and the reaction was stirred for 5 min. Saturated aqueous NaHCO₃ and Et₂O were then added, and the resulting mixture was passed through celite to remove MS. After extraction of the aqueous layer with Et₂O 3 times, the combined organic phase was dried over Na₂SO₄, concentrated in vacuum, and taken directly to the next step. The crude intermediate was dissolved in anhydrous THF-CH₃CN (1:1, 2 mL) and treated with Et₃N·3HF (0.5 mL) under Ar at rt. After stirring overnight, the reaction was quenched by dropwise addition of saturated aqueous NaHCO₃. The aqueous layer was extracted 3 times with Et₂O, and the combined organic layer was dried over Na₂SO₄, concentrated in vacuum, and purified by silica gel column chromatography to give 46 (35 mg, 83%, two steps) as a syrup. ¹H NMR (CDCl₃, 500 MHz, resolved signals): δ 5.67-5.75 (m, 1 H), 5.42 (d, J = 4.0 Hz, 1 H), 5.20 (s, 1 H), 5.13 (dd, J = 2.0, 17.5 Hz, 1 H), 5.08 (d, J = 1.5 Hz, 1 H), 4.99 (dd, J = 2.0, 10.5 Hz, 1 H), 4.94-4.89 (m, 4 H), 4.85-4.79 (m, 5 H), 4.74 (d, J = 10.5 Hz, 1 H), 4.69-4.59 (m, 8 H), 4.55-4.47 (m, 9 H), 4.45-4.40 (m, 8 H), 4.37-4.21 (m, 14 H), 4.15-4.09 (m, 4 H), 4.07-4.04 (m, 1 H), 4.01 (t, J = 9.0 Hz, 1 H), 3.92 (t, J = 9.5 Hz, 1 H), 3.87-3.84 (m, 5 H), 3.53-3.48 (m, 4 H), 3.42-3.37 (m, 6 H), 3.33-3.31 (m, 1 H), 3.25 (dd, J = 3.5, 11 Hz, 1 H), 3.20 (dd, J = 3.5, 10 Hz, 1 H), 2.80-2.70 (m, 2 H), 2.32 (t, J = 8.0 Hz, 4 H), 1.60-1.54 (m, 4 H), 1.31-1.25 (m, 56 H), 0.89 (t, J = 7.0 Hz, 6 H). ¹³C NMR (CDCl₃, 125 MHz): δ 173.40, 173.05, 159.43, 159.37, 159.32, 159.29, 159.23, 159.13, 135.49, 131.15, 131.06, 130.99, 130.93, 130.73, 130.59, 130.49, 130.32, 130.18, 129.77, 129.72, 129.622, 129.52, 129.41, 129.28, 129.25,
(2-Cyanoethoxyl)-[2-(9-fluorenylmethoxycarbonylamino)ethoxy]-(diisopropylamino)-phosphine (47)

To a solution of N-Fmoc-ethanolamine (38 mg, 132 μmol) and commercially available bis(diisopropylamino)(2-cyanoethoxy)phosphine (84 μl, 264 μmol) in anhydrous CH₂Cl₂-CH₃CN (2:1, 3 mL) was added diisopropylammonium tetrazolide (24 mg, 135 μmol). After the reaction stirred at rt under Ar for 1 h, it was diluted with CH₂Cl₂ and poured into saturated aqueous NaHCO₃. The aqueous layer was extracted 3 times with CH₂Cl₂, after which the combined organic layers were dried over Na₂SO₄ and concentrated in vacuum. Purification using a triethylamine-neutralized silica gel column gave phosphoramidite 47 (59 mg, 92%) as a white
solid. $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 7.78 (m, 2 H), 7.62-7.61 (m, 2 H), 7.43-7.40 (m, 2 H), 7.34-7.31 (m, 2 H), 5.27 (s, broad, 1 H), 4.42 (d, $J = 7.0$ Hz, 2 H), 4.24 (t, $J = 7.0$ Hz, 1 H), 3.90-3.84 (m, 1 H), 3.82-3.58 (m, 5 H), 3.44 (q, $J = 5.0$ Hz, 2 H), 2.61 (t, $J = 6.5$ Hz, 2 H), 1.20 (t, $J = 8.0$ Hz, 12 H). $^{13}$C NMR (CDCl$_3$, 125 MHz): $\delta$ 156.65, 144.21, 141.55, 127.92, 127.28, 125.30, 120.21, 117.90, 66.91, 63.06, 62.93, 58.69, 58.53, 47.79, 43.41, 43.31, 42.41, 24.92, 24.87, 24.81, 20.64, 20.58. $^{31}$P NMR (CDCl$_3$, 160 MHz): $\delta$ 149.65.

**6-O-[(6-O-[(2-cyanoethoxyl)-(2-9-fluorenylmethoxycarbonylamino)ethoxy]-phosphono]-2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)-[3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-[2-O-allyl]-3,4-di-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→4)-[2-azido]-2-deoxy]-3,6-di-O-(para-methoxybenzyl)-α-D-glucopyranosyl]-1-O-[(2-cyanoethoxyl)-(2,3-di-O-stearoyl-sn-glycerol)-phosphono]-2,3,4,5-tetra-O-(para-methoxybenzyl)-myo-inositol (48)**

To a solution of pseudopentasaccharide alcohol 46 (32 mg, 9.7 μmol) and MS 4 Å (100 mg) stirring in anhydrous CH$_2$Cl$_2$ (1 mL) under Ar at rt was added a solution of freshly prepared phosphoramidite 47 (23 mg in dry CH$_2$Cl$_2$, 48 μmol) and $1H$-tetrazole (0.45 M solution in
acetonitrile, 0.22 mL, 97 μmol). After stirring at rt under Ar for 1 h, the reaction was cooled to -40 °C and treated with tert-butyl hydroperoxide (5.5 M solution in decane, 53 μL, 0.29 mmol). The solution stirred for 1 h at -40 °C, then Me₂S (43 μl, 0.58 mmol) was added and stirring continued for 1 h at -40 °C. The mixture was poured into saturated aqueous NaHCO₃ and extracted 3 times with CH₂Cl₂. The combined organic layers were dried over Na₂SO₄ and concentrated in vacuum. Purification by silica gel column chromatography afforded 48 as a ~1:1 diastereomeric mixture (29 mg, 81%). Preparative HPLC (Waters Nova-Pak Silica 6 μm, 300 x 19 mm, eluent 35% acetone in hexanes, 10 mL/min, isomer 1-τᵣ = 14.6 min, isomer 2-τᵣ = 15.7 min) was used to separate the mixture to facilitate characterization. ¹H NMR (CDCl₃, 500 MHz, isomer 1): δ 7.74 (d, J = 7.5 Hz, 2 H), 7.58 (d, J = 7.0 Hz, 1 H), 7.39-7.36 (m, 2 H), 7.32-7.28 (m), 7.11 (m), 7.06-7.04 (m, 2 H), 6.88-6.72 (m), 5.75-5.67 (m, 1 H), 5.53 (t, J = 7.0 Hz, 1 H), 5.42 (d, J = 3.5 Hz, 1 H), 5.23 (pent, J = 4.5 Hz, 1 H), 5.20 (s, 1 H), 5.15-5.12 (m, 5 H), 5.00 (d, J = 10.5 Hz, 1 H), 4.93-4.89 (m, 2 H), 4.86-4.77 (m, 5 H), 4.73 (d, J = 10.5 Hz, 1 H), 4.69-4.56 (m, 8 H), 4.52-4.47 (m, 6 H), 4.45-4.36 (m, 9 H), 4.33-4.21 (m, 17 H), 4.19-4.05 (m, 8 H), 4.02-3.93 (m, 3 H), 3.91-3.86 (m, 7 H), 3.82 (s, 3 H), 3.80-3.79 (m, 3 H), 3.78 (s, 3 H), 3.77 (s, 3 H), 3.76 (s, 6 H), 3.74 (s, 12 H), 3.73 (s, 3 H), 3.72-3.69 (m, 4 H), 3.65 (s, 3 H), 3.64 (s, 3 H), 3.62 (s, 3 H), 3.59-3.49 (m, 5 H), 3.45 (s, 1 H), 3.41 (s, 3 H), 3.39-3.37 (m, 3 H), 3.34-3.32 (m, 2 H), 3.27 (dd, J = 4.0, 10.5 Hz, 1 H), 3.22 (dd, J = 3.5, 10 Hz, 1 H), 2.77-2.69 (m, 2 H), 2.27 (m), 2.05 (m), 1.32-1.22 (m), 0.89 (t, J = 6.5 Hz, 6 H). ¹³C NMR (CDCl₃, 125 MHz): δ 173.40, 173.05, 159.45, 159.38, 159.32, 159.26, 159.20, 156.62, 144.21, 141.49, 135.47, 135.44, 131.19, 131.09, 131.04, 130.94, 130.89, 130.81, 130.67, 130.63, 130.52, 130.49, 130.36, 130.17, 129.84, 129.77, 129.72, 129.64, 129.61, 129.52, 129.48, 129.27, 128.77, 127.88, 127.32, 125.43, 125.27, 120.13, 117.19, 116.65, 116.44, 114.10, 114.07, 114.04, 113.99, 113.92, 113.87, 113.80, 113.78, 100.95,
99.67, 99.56, 97.92, 81.43, 80.61, 79.85, 76.65, 75.08, 75.02, 74.92, 74.81, 74.79, 74.59, 74.49, 73.44, 73.11, 72.79, 72.56, 72.43, 71.99, 71.94, 71.86, 71.37, 71.34, 70.09, 69.59, 69.55, 68.96, 68.56, 68.21, 67.36, 67.28, 67.10, 66.68, 66.39, 66.35, 63.00, 62.71, 62.68, 62.32, 62.28, 61.74, 55.45, 55.34, 55.27, 55.24, 47.39, 41.54, 34.33, 34.18, 32.44, 32.17, 29.95, 29.91, 29.77, 29.60, 29.55, 29.38, 29.34, 26.63, 25.85, 25.05, 22.93, 19.88, 19.83, 19.36, 19.31, 14.35.

$^{31}$P NMR (CDCl$_3$, 160 MHz): $\delta$ -0.32, -1.25. HR ESI MS: calcd. for C$_{207}$H$_{268}$N$_{51}$O$_{51}$NaP$_2$ [M+NH$_4$]$^+$ m/z, 3729.8068; found, 3729.7986.

6-O-[[6-O-[2-(cyanoethoxyl)-2-(9-fluorenylmethoxycarbonylamino)ethoxy]phosphono]-2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)-[3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-[3,4-di-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→4)-[2-azido-2-deoxy-3,6-di-O-(para-methoxybenzyl)-α-D-glucopyranosyl]]-1-O-[((2-cyanoethoxyl)-(2,3-di-O-stearoyl-sn-glycerol)-phosphono]-2,3,4,5-tetra-O-(para-methoxybenzyl)-myo-inositol (49)

A solution of [Ir(COD)(PMePh$_2$)$_2$]PF$_6$ (9.0 mg, 11 μmol) in anhydrous THF (0.5 mL) was stirred under H$_2$ at rt until the color turned from red to colorless to pale yellow (10 min). After the H$_2$
atmosphere was exchanged with Ar, 48 (27 mg, 7.3 μmol, solution in 0.5 mL THF, as a ~1:1 diastereomeric mixture) was added slowly. After 2 h, the reaction was concentrated in vacuum, then dissolved in acetone/H₂O (9:1, 1 mL) and treated with HgCl₂ (6 mg, 22 μmol) and HgO (0.2 mg, 0.7 μmol). Note: The isomerization reaction could not be monitored by TLC; we took small aliquots of the reaction forward to treatment with Hg(II), then checked MALDI MS to determine reaction progress. The mixture was stirred at rt under an Ar atmosphere for 2 h, then concentrated and purified by silica gel column chromatography to give 49 (24 mg, 90%), which was taken directly to the next step after characterization by MALDI mass spectrometry. MALDI TOF MS (positive mode): calcd. for C₂₀₄H₂₆₀N₆O₅₁NaP₂ [M+Na]⁺ m/z, 3694.7; found, 3695.0.
To a mixture of 49 (12 mg, 3.2 μmol, as a ~1:1 diastereomeric mixture from previous phosphorylation) and MS 4 Å (~10 mg) in CH₂Cl₂ were added succinic anhydride (3.2 mg, 32 μmol) and DMAP (4.0 mg, 32 μmol) at rt under Ar. After stirring for 6 h, additional succinic anhydride (3.2 mg, 32 μmol) and DMAP (4.0 mg, 32 μmol) were added. The reaction was stirred overnight, treated with AcOH, concentrated, and purified by silica gel column chromatography to afford 50 (8.1 mg, 67%). ¹H NMR (CDCl₃, 500 MHz, mixture of 2 diastereomers from previous phosphorylation reaction): See page S125 for spectrum. ³¹P NMR (CDCl₃, 160 MHz): δ -2.05, -2.36. MALDI TOF MS (positive mode): calcd. for C₂₀₈H₂₆₄N₆O₅₄Na₁P₂ [M+Na]⁺ m/z, 3794.7; found, 3794.1.
To a solution of 50 (2.0 mg, 0.53 μmol, as a ~1:1 diastereomeric mixture) in CH₂Cl₂ stirring under an Ar atmosphere at rt was added HOBt (solution in CH₂Cl₂/DMF, total 0.17 mg, 1.2 μmol) and EDCI (solution in CH₂Cl₂, total 0.23 mg, 1.2 μmol). After stirring for 15 min, propargylamine (solution in CH₂Cl₂, total 0.05 mg, 0.83 μmol) was added. The reaction was stirred overnight, treated with 1 drop AcOH, concentrated, and purified by silica gel column chromatography to afford 51 (1.5 mg, 75%). ¹H NMR (CDCl₃, 500 MHz, mixture of 2 diastereomers): See page S126 for spectrum. HR ESI MS: calcd. for C₂₁₁H₂₆₇N₇O₅₃Na₂P₂ [M+2Na]²⁺ m/z, 1927.3944; found, 1927.3900.
6-O-[(2-Aminoethyl)-phosphono]-α-D-mannopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→6)-[2-O-(N-propargyl-succinamide)-α-D-mannopyranosyl)-(1→4)-(2-amino-2-deoxy-α-D-glucopyranosyl)]-1-O-[(2,3-di-O-stearoyl-sn-glycerol)-phosphono]-myo-inositol (1)

To a solution of 51 (1.02 mg, 0.26 μmol, as a ~1:1 mixture of diastereomers) in CH₂Cl₂ (250 μl) was added acetic acid (1 drop) and activated zinc powder (1 mg). After stirring for 1 h, MALDI TOF MS showed complete reduction of the azide [MALDI TOF MS (positive mode): calcd. for C₂₁₁H₂₆₉N₅O₅₃NaP₂ [M+Na]⁺ m/z, 3805.8; found, 3805.2]. The mixture was filtered and condensed in vacuum to remove acetic acid, and the resulting residue was re-dissolved in CH₂Cl₂ (250 μl) and treated with DBU (1 μl). The solution stirred for 1 h, after which MALDI TOF MS confirmed removal of the Fmoc and cyanoethoxyl protecting groups [MALDI TOF MS (positive mode): calcd. for C₁₉₀H₂₅₁N₂O₅₁Na₃P₂ [M-2H+3Na]⁺ m/z, 3521.6; found, 3522.2]. Then, 20% TFA in CH₂Cl₂ (250 μl) was added directly to the reaction, giving a final concentration of ~10% TFA. After stirring for 30 min, the reaction was co-evaporated with toluene to remove most solvent and TFA. Two drops of Et₃N were added to neutralize any residual TFA, and the mixture was co-evaporated with toluene 5 times. Purification of the crude product by Sephadex LH-20
size exclusion chromatography (CHCl₃-MeOH-H₂O 3:3:1) gave 1 (0.40 mg, 85%). ¹H NMR (CDCl₃-CD₃OD-D₂O 3:3:1, 500 MHz, anomeric region): δ 5.39 (1 H), 5.35 (2 H), 5.27 (1 H), 5.18 (1 H), 5.11 (1 H), 4.97 (1 H). ³¹P NMR (CDCl₃, 160 MHz): δ -0.38, -0.35. MALDI TOF MS (positive mode): calcd. for C₇₈H₁₃₉N₃O₇Na₃P₂ [M-2H⁺3Na]⁺ m/z, 1840.8; found, 1841.4.

Phenyl [6-O-(tert-butyldimethylsilyl)-2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)[3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-3,4-di-O-(para-methoxybenzyl)-1-thio-α-D-mannopyranoside (52)

To a solution of 7 (56 mg, 32 μmol) in anhydrous THF (5 mL) was added titanium(IV) isopropoxide (25 mg in 0.5 mL hexanes, 80 μmol). Cyclopentylmagnesium chloride (2.0 M solution in THF, 160 μL) was added dropwise under an Ar atmosphere at rt. After 2 h, saturated aqueous Na-K-tartrate was poured into the reaction, which was stirred vigorously for 2 h. The mixture was extracted 3 times with CH₂Cl₂, and then the combined organic layer was washed with brine, dried over Na₂SO₄, and concentrated in vacuum. The resulting residue was purified by silica gel column chromatography to obtain 52 (45 mg, 82%) as a syrup. ¹H NMR (CDCl₃, 500 MHz): δ 7.44-7.43 (m, 2 H), 7.32-7.30 (m, 2 H), 7.26-7.18 (m, 14 H), 7.15-7.11 (m, 3 H), 6.91-6.90 (m, 2 H), 6.87-6.75 (m, 14 H), 5.53 (d, J = 1.0 Hz, 1 H), 5.24 (d, J = 1.5 Hz, 1 H), 4.83 (d, J = 2.0 Hz, 1 H), 4.82-4.80 (m, 2 H), 4.77 (d, J = 10.5 Hz, 1 H), 4.65 (s, 2 H), 4.59 (d, J = 12 Hz, 1 H), 4.55 (d, J = 10.5 Hz, 1 H), 4.50-4.44 (m, 4 H), 4.41-4.37 (m, 5 H), 4.25-4.22 (m, 2 H),
4.07 (t, J = 2.0 Hz, 1 H), 3.95-3.84 (m, 6 H), 3.83 (s, 3 H), 3.81 (s, 3 H), 3.80 (s, 3 H), 3.82-3.79 (m, 2 H), 3.77 (s, 3 H), 3.76 (s, 3 H), 3.74 (s, 3 H), 3.74-3.72 (m, 3 H), 3.70 (s, 3 H), 3.68 (s, 3 H), 3.65-3.57 (m, 4 H), 2.72 (s, broad, 1 H), 0.88 (s, 9 H), 0.06 (s, 3 H), 0.05 (s, 3 H). $^{13}$C NMR (CDCl$_3$, 125 MHz): δ 159.78, 159.44, 159.33, 159.30, 159.20, 159.15, 131.09, 130.91, 130.86, 130.65, 130.58, 129.96, 129.84, 129.81, 129.74, 129.64, 129.58, 129.56, 129.41, 127.42, 114.27, 114.05, 114.04, 113.94, 113.86, 113.84, 113.72, 99.54, 98.56, 87.57, 80.43, 79.96, 79.64, 74.98, 74.93, 74.85, 74.78, 74.59, 74.19, 73.74, 73.08, 72.89, 72.24, 72.10, 71.94, 71.52, 70.07, 69.15, 66.56, 62.94, 55.52, 55.49, 55.44, 55.41, 55.32, 29.95, 26.23, 18.61, -4.74, -4.97. $[\alpha]_{D}^{25} = +58^\circ$ (c 1.0, CHCl$_3$). HR ESI MS: calcd. for C$_{94}$H$_{114}$O$_{23}$NaSiS [M+Na]$^+$ m/z, 1693.7139; found, 1693.7136.

Phenyl [6-O-(tert-butyldimethylsilyl)-2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)-[3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-3,4-di-O-(para-methoxybenzyl)-2-O-propargyl-1-thio-α-D-mannopyranoside (53)

To a solution of 52 (95 mg, 57 μmol) in anhydrous DMF (3 mL) stirring under Ar at 0 ºC was slowly added NaH (60% dispersion in mineral oil, 5 mg, 114 μmol). After stirring for 15 min, propargyl bromide (80% solution in toluene, 13 μL, 114 mmol) was added dropwise. The reaction stirred for 1 hr at 0 ºC, then quenched with MeOH, diluted with EtOAc, and poured into water. The aqueous layer was extracted 3 times with EtOAc, after which the combined organic
layer was washed with brine, dried over Na$_2$SO$_4$, and concentrated in vacuum. The residue was purified by silica gel column chromatography to give 53 (76 mg, 78%) as a syrup. $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 7.47-7.46 (m, 2 H), 7.35-7.34 (m, 2 H), 7.26-7.19 (m, 14 H), 7.15-7.10 (m, 3 H), 6.91-6.75 (m, 14 H), 5.58 (d, $J = 1.0$ Hz, 1 H), 5.23 (d, $J = 1.0$ Hz, 1 H), 4.85 (d, $J = 10.5$ Hz, 1 H), 4.83 (d, $J = 1.5$ Hz, 1 H), 4.82 (d, $J = 10.5$ Hz, 1 H), 4.77 (d, $J = 10.5$ Hz, 1 H), 4.74 (d, $J = 11$ Hz, 1 H), 4.61 (t, $J = 11.5$ Hz, 2 H), 4.55 (d, $J = 10.5$ Hz, 1 H), 4.50 (s, 2 H), 4.47-4.44 (m, 2 H), 4.42-4.37 (m, 6 H), 4.31-4.28 (m, 2 H), 4.22 (dd, $J = 5.0$, 9.5 Hz, 1 H), 4.09 (t, $J = 2.0$ Hz, 1 H), 3.95-3.90 (m, 2 H), 3.88-3.85 (m, 4 H), 3.83 (s, 3 H), 3.81 (s, 3 H), 3.80 (s, 3 H), 3.79-3.78 (m, 1 H), 3.77 (s, 3 H), 3.76 (s, 3 H), 3.75 (s, 3 H), 3.73-3.72 (m, 1 H), 3.70 (s, 3 H), 3.68 (s, 3 H), 3.66-3.57 (m, 3 H), 2.44 (t, $J = 2.0$ Hz, 1 H), 0.88 (s, 9 H), 0.05 (s, 3 H), 0.04 (s, 3 H). $^{13}$C NMR (CDCl$_3$, 125 MHz): $\delta$ 159.63, 159.45, 159.38, 159.32, 159.29, 159.20, 159.19, 159.17, 135.05, 131.53, 131.26, 131.20, 131.10, 130.96, 130.88, 130.75, 130.62, 130.20, 130.06, 129.86, 129.81, 129.72, 129.65, 129.55, 129.42, 129.39, 127.44, 114.14, 114.05, 113.99, 113.88, 113.84, 113.71, 99.45, 98.74, 85.79, 80.10, 80.05, 79.73, 79.64, 75.63, 75.57, 75.02, 74.95, 74.86, 74.75, 74.68, 74.68, 74.58, 74.55, 73.78, 73.10, 73.02, 72.48, 72.12, 72.01, 71.94, 71.51, 69.15, 66.71, 62.94, 57.59, 55.53, 55.49, 55.43, 55.41, 55.38, 55.32, 26.23, 18.60, -4.74, -4.98. [α]$_D^{25}$ = +38° (c 1.0, CHCl$_3$). HR ESI MS: calcd. for C$_{97}$H$_{116}$O$_{23}$NaSiS [M+Na]$^+$ $m/z$, 1731.7295; found, 1731.7350.
[6-O-(tert-Butyldimethylsilyl)-2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-
(1→2)-[3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-3,4-di-O-(para-
methoxybenzyl)-2-O-propargyl-D-mannopyranose (54)

To a solution of 53 (60 mg, 35 μmol) and 2,4,6-tri-tert-butyldipyrimidine (35 mg, 140 μmol) in
wet CH₂Cl₂ (1.0 mL) at -78 ºC was added silver triflate (27 mg, 105 μmol). Then, a solution of
para-nitrobenzenesulfonyl chloride in dry CH₂Cl₂ (0.14 M solution, 0.5 mL) was slowly added
and the reaction was warmed to 0 ºC. After stirring for 30 min, saturated aqueous NaHCO₃ was
added to quench the reaction. The mixture was extracted 3 times with CH₂Cl₂, after which the
combined organic layer was dried over Na₂SO₄, concentrated in vacuum, and purified by silica
gel column chromatography to give anomeric mixture 54 (44 mg, 77%). ¹H NMR (CDCl₃, 500
MHz, anomeric signals of major isomer): δ 5.13 (S, 1 H), 5.01 (s, broad, 1 H), 4.85 (s, 1 H). See
page S134 for spectrum. ¹³C NMR (CDCl₃, 125 MHz, anomeric signals): 99.39, 97.72,
92.22. HR ESI MS: calcd. for C₉₁H₁₁₄O₂₄NaSi [M+Na]⁺ m/z, 1641.7367; found, 1641.7310.
6-O-[[2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)-[3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-[3,4-di-O-(para-methoxybenzyl)-2-O-propargyl-α-D-mannopyranosyl]-(1→4)-[2-azido-2-deoxy-3,6-di-O-(para-methoxybenzyl)-α-D-glucopyranosyl]]-1-O-[(2-cyanoethoxyl)-(2,3-di-O-stearoyl-sn-glycerol)-phosphono]-2,3,4,5-tetra-O-(para-methoxybenzyl)-myo-inositol (56)

DBU (1 drop) was added to a solution of hemiacetal 54 (37 mg, 23 μmol) and trichloroacetonitrile (0.2 mL) in anhydrous CH₂Cl₂ (1.0 mL) stirring under an Ar atmosphere at 0 ºC. After 1 h, the reaction mixture was concentrated in vacuum and purified with a triethylamine-neutralized silica gel column to give 55 (36 mg, 90%). A mixture of the resulting trichloroacetimidate 55 (30.7 mg, 17.4 μmol), acceptor 35 (16.0 mg, 8.7 μmol), and MS 4 Å (50 mg) in anhydrous Et₂O (0.5 mL) was stirred under an Ar atmosphere at rt for 1 h. After cooling to 0 ºC, TMSOTf (0.5 μl, 2 μmol) was added and the reaction was stirred for 10 min. Saturated aqueous NaHCO₃ and Et₂O were then added, and the resulting mixture was passed through celite to remove MS 4 Å. After extraction of the aqueous layer with Et₂O 3 times, the combined organic phase was dried over Na₂SO₄, concentrated in vacuum, and taken directly to the next step. The crude intermediate was dissolved in anhydrous THF-CH₃CN (1:1, 1.0 mL) and treated with triethylamine trihydrofluoride (0.5 mL) under Ar at rt. After stirring overnight, the reaction
was quenched by dropwise addition of saturated aqueous NaHCO₃. The aqueous layer was extracted 3 times with CH₂Cl₂, and the combined organic layer was dried over Na₂SO₄, concentrated in vacuum, and purified by silica gel column chromatography to give 56 (23.5 mg, 82%, two steps) as a syrup.

¹H NMR (CDCl₃, 500 MHz, resolved signals): δ 5.41 (d, J = 4.0 Hz, 1 H), 5.31 (s, 1 H), 5.24 (pent, J = 5.0 Hz, 1 H), 5.12 (s, 1 H), 4.94 (s, 1 H), 4.94-4.90 (m, 2 H), 3.31-3.29 (m, 1 H), 3.23 (dd, J = 2.5, 11 Hz, 1 H), 3.20 (dd, J = 4.0, 10 Hz, 1 H), 2.80-2.70 (m, 2 H), 2.32-2.27 (m, 5 H), 1.60-1.54 (m, 4 H), 1.31-1.25 (m, 56 H), 0.90 (t, J = 7.0 Hz, 6 H).

¹³C NMR (CDCl₃, 125 MHz): δ 173.44, 173.09, 159.45, 159.37, 159.30, 159.23, 159.20, 159.12, 131.13, 131.06, 130.98, 130.89, 130.70, 130.57, 130.52, 130.49, 130.33, 130.14, 129.79, 129.69, 129.62, 129.54, 129.44, 129.31, 129.27, 129.12, 128.96, 116.65, 114.14, 114.08, 114.05, 113.99, 113.93, 113.82, 100.35, 99.76, 99.68, 98.02, 81.46, 80.76, 80.61, 80.47, 79.85, 79.63, 79.14, 76.86, 76.59, 75.62, 75.40, 75.24, 75.12, 75.00, 74.86, 74.76, 74.62, 74.49, 74.04, 73.67, 73.19, 73.12, 73.05, 72.79, 72.35, 72.04, 71.96, 71.64, 70.20, 69.60, 69.52, 69.11, 68.34, 66.41, 66.37, 66.07, 62.99, 62.69, 62.62, 62.50, 61.74, 58.22, 55.48, 55.42, 55.35, 55.32, 55.28, 34.34, 34.19, 32.18, 29.98, 29.93, 29.80, 29.63, 29.60, 29.40, 29.35, 25.06, 22.96, 19.91, 19.85, 14.40.

Configurations of anomeric positions were established as α by coupled ¹³C NMR JCH values (125 MHz): 100.35 (JCH = 172 Hz, Man-1), 99.76 (JCH = 171 Hz, Man-1), 99.68 (JCH = 177 Hz, Man-1), 98.02 (JCH = 178 Hz, GlcN3-1).

³¹P NMR (CDCl₃, 160 MHz): δ -1.32. [α]D²⁵ = +33° (c 1.0, CHCl₃).

HR ESI MS: calcd. for C₁₈₇H₂₄₃N₄O₄₆NaP [M+Na]+ m/z, 3334.6434; found, 3334.6477.
6-O-[6-O-[(2-cyanoethoxyl)-2-(9-fluorenylmethoxycarbonylamino)ethoxy]-phosphono]-2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl-(1→2)-[3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-[3,4-di-O-(para-methoxybenzyl)-2-O-propargyl-α-D-mannopyranosyl]-(1→4)-[2-azido-2-deoxy-3,6-di-O-(para-methoxybenzyl)-α-D-glucopyranosyl]-1-O-[(2-cyanoethoxy)-(2,3-di-O-stearoyl-sn-glycerol)-phosphono]-2,3,4,5-tetra-O-(para-methoxybenzyl)-myo-inositol (57)

To a solution of pseudopentasaccharide alcohol 56 (22.0 mg, 6.2 μmol) and MS 4 Å (20 mg) stirring in anhydrous CH₂Cl₂ (1 mL) under Ar at rt was added a solution of freshly prepared phosphoramidite 47 (24 mg in dry CH₂Cl₂, 50 μmol) and 1H-tetrazole (0.45 M solution in acetonitrile, 0.22 mL, 100 μmol). After stirring at rt under Ar for 1 h, the reaction was cooled to -40 ºC and treated with tert-butyl hydroperoxide (5.5 M solution in decane, 36 μL, 0.20 mmol). The solution stirred for 1 h at -40 ºC, then Me₂S (30 μL, 0.40 μmol) was added and stirring continued for 1 h at -40 ºC. The mixture was filtered through celite, poured into saturated aqueous NaHCO₃, and extracted 3 times with CH₂Cl₂. The combined organic layers were dried over Na₂SO₄ and concentrated in vacuum. Purification by silica gel column chromatography afforded 57 as an inconsequential ~1:1 diastereomeric mixture (17.6 mg, 72%) that was briefly
characterized and taken to the final step. $^1$H NMR (CDCl$_3$, 500 MHz, resolved signals): $\delta$ 7.75 (d, $J = 7.5$ Hz, 2 H), 7.59 (d, $J = 7.0$ Hz, 2 H), 5.54 (s, broad, 1 H), 5.41 (d, $J = 3.0$ Hz, 1 H), 5.31 (s, 1 H), 5.24 (pent, $J = 5.0$ Hz, 1 H), 5.16 (d, $J = 6.5$ Hz, 1 H), 2.80-2.70 (m, 4 H). $^{31}$P NMR (CDCl$_3$, 160 MHz): $\delta$ -0.28 (1 P), -0.37 (1 P), -1.22 (2 P). MALDI TOF MS (positive mode): calcd. for C$_{207}$H$_{263}$N$_6$O$_{51}$NaP$_2$ [M+Na]$^+$ $m/z$, 3709.8; found, 3709.3.

6-O-[(6-O-[2-Aminoethyl]-phosphono]-α-D-mannopyranosyl]-(1→2)-(α-D-mannopyranosyl)-(1→6)-(2-O-propargyl-α-D-mannopyranosyl)-(1→4)-(2-amino-2-deoxy-α-D-glucopyranosyl)-1-O-[(2,3-di-O-stearyl-sn-glycerol)-phosphono]-myo-inositol (2)

To a solution of 57 (2.43 mg, 0.655 μmol) in CH$_2$Cl$_2$ (300 μl) was added acetic acid (1 drop) and activated zinc powder (10 mg). After stirring for 1 h, MALDI TOF MS showed complete reduction of the azide [MALDI TOF MS (positive mode): calcd. for C$_{207}$H$_{264}$N$_4$O$_{51}$NaP$_2$ [M+Na]$^+$ $m/z$, 3683.8; found, 3684.0]. The mixture was filtered and condensed in vacuum to remove acetic acid, and the resulting residue was re-dissolved in CH$_2$Cl$_2$ (300 μl) and treated with DBU (1 μl). The solution stirred for 1 h, after which MALDI TOF MS confirmed removal of the Fmoc and cyanoethoxyl protecting groups [MALDI TOF MS (negative mode): calcd. for
C$_{186}$H$_{247}$N$_2$O$_{49}$P$_2$ [M-H]$^-$ m/z, 3355.7; found, 3354.7]. Then, 20% TFA in CH$_2$Cl$_2$ (300 μl) was added directly to the reaction, giving a final concentration of ~10% TFA. After stirring for 30 min, the reaction was co-evaporated with toluene to remove most solvent and TFA. Two drops of Et$_3$N were added to neutralize any residual TFA, and the mixture was co-evaporated with toluene 5 times. Purification of the crude product by Sephadex LH-20 size exclusion chromatography (CHCl$_3$-MeOH-H$_2$O 3:3:1) gave 2 (1.03 mg, 93%). $^1$H NMR (CDCl$_3$-CD$_3$OD-D$_2$O 3:3:1, 500 MHz, anomic region): δ 5.41 (s, 1 H), 5.34 (s, 2 H), 5.27 (m, 2 H), 5.10 (s, 1 H), 4.97 (s, 1 H). $^{31}$P NMR (CDCl$_3$, 160 MHz): δ 0.31, -0.20. MALDI TOF MS (negative mode): calcd. for C$_{74}$H$_{135}$N$_2$O$_{35}$P$_2$ [M-H]$^-$ m/z, 1674.8; found, 1674.9.

6-O-[4-O-(tert-Butyldimethylsilyl)-2-deoxy-2-(9-fluorenylmethoxycarbonylamino)-3,6-di-O-(para-methoxybenzyl)-α-D-glucopyranosyl]-2,3,4,5-tetra-O-(para-methoxybenzyl)-myo-inositol (58)

To a solution of 6 (110 mg, 91.4 μmol) in CH$_2$Cl$_2$-AcOH (10:1, 2.2 mL) was added activated zinc dust (110 mg). After stirring under Ar at rt for 1 h, the reaction was filtered through cotton and co-evaporated with toluene 3 times. After drying under high vacuum, the intermediate amine was dissolved in 1,4-dioxane-H$_2$O (10:1, 3.3 mL) and treated with NaHCO$_3$ (31 mg, 0.37 mmol) and FmocCl (35.5 mg, 137 μmol). The reaction was stirred at rt for 1 h, then concentrated and suspended in water. The suspension was extracted with CH$_2$Cl$_2$ 3 times, and the combined organic layer was dried over Na$_2$SO$_4$, concentrated in vacuum, and purified by silica gel column chromatography to give pseudodisaccharide 58 (99 mg, 78%) as a syrup. $^1$H NMR (CDCl$_3$, 500
MHz): δ 7.75 (d, J = 7.5 Hz, 2 H), 7.54-7.48 (m, 2 H), 7.38-7.12 (m, 16 H), 6.91-6.70 (m, 12 H), 5.96 (d, J = 10 Hz, 1 H), 5.30 (d, J = 3.0 Hz, 1 H), 4.96 (d, J = 11.5 Hz, 1 H), 4.88-4.83 (m, 4 H), 4.76-4.65 (m, 8 H), 4.53 (d, J = 11 Hz, 1 H), 4.34-4.26 (m, 4 H), 4.13 (t, J = 7.5 Hz, 1 H), 4.04-3.99 (m, 5 H), 3.91-3.87 (m, 3 H), 3.84 (s, 3 H), 3.81 (s, 3 H), 3.79 (s, 3 H), 3.78 (s, 3 H), 3.74 (t, J = 9.0 Hz, 1 H), 3.65 (s, 3 H), 3.62 (s, 3 H), 3.59-3.51 (m, 3 H), 3.48-3.43 (m, 4 H), 3.38 (t, J = 9.0 Hz, 1 H), 2.72 (d, J = 9.0 Hz, 1 H), 0.85 (s, 9 H), 0.02 (s, 3 H), 0.00 (s, 3 H). 13C NMR (CDCl3, 125 MHz): δ 159.56, 159.38, 159.19, 159.00, 156.42, 144.46, 144.27, 141.45, 141.41, 131.28, 131.13, 130.84, 130.66, 130.49, 129.85, 129.80, 129.59, 129.44, 129.36, 129.20, 127.79, 127.27, 125.51, 125.47, 120.05, 114.14, 114.08, 113.99, 113.93, 113.84, 113.68, 99.13, 81.55, 81.34, 81.03, 80.88, 75.59, 74.64, 74.48, 74.41, 73.54, 73.21, 73.09, 72.33, 70.93, 69.03, 67.04, 55.74, 55.53, 55.51, 55.48, 55.33, 47.42, 26.27, 18.30, -3.49, -4.63. HR ESI MS: calcd. for C81H95NO18NaSi [M+Na]+ m/z, 1420.6216; found, 1420.6266.

6-O-[2-Deoxy-2-(9-fluorenylmethoxycarbonylamino)-3,6-di-O-(para-methoxybenzyl)-α-D-glucopyranosyl]-1-O-[(2-cyanoethoxyl)-(2,3-di-O-stearoyl-sn-glycerol)-phosphono]-2,3,4,5-tetra-O-(para-methoxybenzyl)-myo-inositol (604)

To a solution of 58 (63 mg, 45 μmol) and MS 4 Å (100 mg) stirring in anhydrous CH2Cl2-CH3CN (3:1, 4 mL) under Ar at rt was added a solution of freshly prepared phosphoramidite 34 (202 mg in dry CH2Cl2, 232 μmol) and 1H-tetrazole (0.45 M solution in acetonitrile, 1.0 mL, 464
μmol). After stirring at rt under Ar for 30 min, the reaction was cooled to 0 °C and treated with tert-butyl hydroperoxide (5.5 M solution in decane, 55 μL, 300 μmol). After stirring for 1 h, the mixture was filtered through celite, poured into saturated aqueous NaHCO₃, and extracted 3 times with CH₂Cl₂. The combined organic layers were dried over Na₂SO₄ and concentrated in vacuum. Purification by silica gel column chromatography gave the desired intermediate 59 (characterized by MALDI TOF MS), which was then dissolved in anhydrous THF-CH₃CN (1:1, 4 mL) and treated with triethylamine trihydrofluoride (2 mL) under Ar at rt. After stirring for 7 days at rt, the reaction was quenched by dropwise addition of saturated aqueous NaHCO₃. The mixture was extracted 3 times with CH₂Cl₂, and the combined organic layer was dried over Na₂SO₄, concentrated in vacuum, and purified by silica gel column chromatography to give 60 (62.5 mg, 67%, two steps) as a mixture of diastereomers (originating at phosphorus). Preparative HPLC (Waters Nova-Pak Silica 6 μm, 300 x 19 mm, eluent 30% acetone in hexanes, 10 mL/min, tᵣ ≈ 20 min) was used to partially separate the diastereomeric mixture, and the fraction primarily containing isomer 1 was characterized and carried forward to complete the synthesis. ¹H NMR (CDCl₃, 500 MHz, resolved signals of isomer 1): δ 5.57 (d, J = 10 Hz, 1 H), 5.28 (d, J = 4.0 Hz, 1 H), 5.24 (pent, J = 5.0 Hz, 1 H), 3.82 (s, 3 H), 3.80 (s, 3 H), 3.79 (s, 3 H), 3.77 (s, 3 H), 3.74 (s, 3 H), 3.69 (s, 3 H), 3.60 (t, J = 9.5 Hz, 1 H), 2.54 (s, broad, 1 H), 2.30-2.22 (m, 4 H), 1.60-1.50 (m, 4 H), 0.90 (t, J = 7.0 Hz, 6 H). ¹³C NMR (CDCl₃, 125 MHz): δ 173.45, 173.11, 159.45, 159.36, 159.30, 156.57, 144.24, 141.55, 141.43, 130.98, 130.92, 130.82, 130.67, 130.45, 130.34, 130.08, 129.79, 129.72, 129.67, 129.53, 129.47, 129.39, 127.94, 127.38, 125.50, 125.42, 120.19, 116.90, 114.04, 113.99, 113.92, 113.89, 99.70, 81.76, 81.33, 80.41, 80.08, 79.62, 76.23, 76.02, 75.59, 75.47, 74.92, 73.42, 73.31, 72.79, 72.71, 71.45, 70.96, 69.56, 69.51, 69.39, 67.06, 66.36, 62.65, 61.72, 55.50, 55.46, 55.40, 54.76, 47.55, 34.34, 34.17, 32.19, 29.98, 29.93, 29.80, 29.63,
29.58, 29.40, 29.36, 25.06, 22.96, 14.40. $^3$P NMR (CDCl$_3$, 160 MHz): $\delta$ -1.06. $[\alpha]_D^{25} = +15^\circ$ (c 1.0, CHCl$_3$). HR ESI MS: calcd. for C$_{117}$H$_{159}$N$_2$O$_{25}$NaP $[M+Na]^+$ $m/z$, 2046.0867; found, 2046.0914.

**Phenyl [6-O-(tert-butyldimethylsilyl)-2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)[3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-2-O-(2-tert-butoxy-2-oxoethyl)-3,4-di-O-(para-methoxybenzyl)-1-thio-α-D-mannopyranoside (78)**

To a solution of 52 (110 mg, 66 μmol) in anhydrous DMF (2 mL) stirring under Ar at 0 ºC was added NaH (60% dispersion in mineral oil, 4.0 mg, 99 μmol). After stirring for 15 min, tert-butyl bromoacetate (10 μL, 70 μmol) was added dropwise. The reaction was stirred for 1 h at 0 ºC, then diluted with EtOAc. The organic layer was washed 3 times with brine, and the resulting aqueous layer was back-extracted with EtOAc. The combined organic layers were dried over Na$_2$SO$_4$ and concentrated in vacuum, giving a residue that was purified by silica gel column chromatography to provide 78 (90 mg, 77%) as a syrup. $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 7.48-7.46 (m, 2 H), 7.36-7.34 (m, 2 H), 7.27-7.14 (m, 14 H), 7.16-7.14 (m, 2 H), 7.10-7.07 (m, 1 H), 6.91-6.75 (m, 16 H), 5.70 (d, $J = 1.0$ Hz, 1 H), 5.25 (s, 1 H), 4.86 (d, $J = 11$ Hz, 1 H), 4.82 (d, $J = 11$ Hz, 1 H), 4.81 (s, 1 H), 4.79-4.74 (m, 2 H), 4.67 (d, $J = 11$ Hz, 1 H), 4.62 (d, $J = 11.5$ Hz, 1 H), 4.55 (d, $J = 10.5$ Hz, 1 H), 4.83-4.45 (m, 4 H), 4.42-4.35 (m, 6 H), 4.26-4.22 (m, 1 H), 4.22-4.14 (m, 2 H), 4.08 (s, 1 H), 4.04 (s, 1 H), 3.95-3.85 (m, 9 H), 3.83 (s, 3 H), 3.81 (s, 3 H), 3.80 (s,
3 H), 3.79-3.78 (m, 1 H), 3.77 (s, 3 H), 3.76 (s, 3 H), 3.75 (s, 3 H), 3.71 (s, 3 H), 3.67 (s, 3 H), 3.65-3.57 (m, 4 H), 1.43 (s, 9 H), 0.89 (s, 9 H), 0.07 (s, 3 H), 0.06 (s, 3 H).

$^{13}$C NMR (CDCl$_3$, 125 MHz): $\delta$ 169.79, 159.63, 159.44, 159.37, 159.29, 159.20, 159.16, 135.15, 131.53, 131.28, 131.00, 130.87, 130.76, 130.69, 130.29, 130.17, 129.90, 129.80, 129.74, 129.63, 129.57, 129.40, 127.29, 114.15, 114.03, 113.99, 113.94, 113.86, 113.83, 113.70, 99.36, 98.61, 86.59, 81.94, 80.82, 80.21, 79.66, 78.03, 74.99, 74.93, 74.88, 74.69, 74.59, 73.77, 73.06, 72.99, 72.56, 72.48, 72.15, 72.09, 71.92, 71.42, 68.69, 66.83, 62.97, 55.52, 55.50, 55.44, 55.42, 55.39, 55.31, 53.72, 28.33, 26.25, 18.63, -4.69, -4.94. [$\alpha$]$_D^{25}$ = +32º (c 1.0, CHCl$_3$). HR ESI MS: calcd. for C$_{100}$H$_{124}$O$_{25}$NaSiS [M+Na]$^+$ m/z, 1807.7819; found, 1807.7859.

Phenyl [6-O-(tert-butyldimethylsilyl)-2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)[3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-2-O-(2-hydroxyethyl)-3,4-di-O-(para-methoxybenzyl)-1-thio-α-D-mannopyranoside (61)

To a solution of 78 (80 mg, 45 μmol) in anhydrous THF (5 mL) stirring under Ar at 0 ºC was added LiAlH$_4$ (18 mg, 0.45 mmol). The reaction was warmed to 50 ºC and stirred for 1 h, after which it was diluted with EtOAc and treated with saturated aqueous Na-K-tartrate and stirred for an additional hour at rt. The aqueous layer was extracted 3 times with EtOAc, and the combined organic layers were dried over Na$_2$SO$_4$ and concentrated in vacuum. The resulting residue was purified by silica gel column chromatography to provide 61 (59 mg, 77%) as a syrup. $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 7.45-7.44 (m, 2 H), 7.35-7.33 (m, 2 H), 7.28-7.13 (m, 17 H), 6.92-6.76 (m,
16 H), 5.52 (s, 1 H), 5.24 (s, 1 H), 4.84 (d, J = 11 Hz, 1 H), 4.76 (d, J = 10.5 Hz, 1 H), 4.71 (d, J = 11 Hz, 1 H), 4.67 (d, J = 11.5 Hz, 1 H), 4.58 (d, J = 12 Hz, 1 H), 4.55-4.47 (m, 4 H), 4.44 (d, J = 10 Hz, 1 H), 4.42-4.35 (m, 6 H), 4.21 (d, J = 5.5, 8.5 Hz, 1 H), 4.13 (s, 1 H), 3.96 (dd, J = 5.5, 11 Hz, 1 H), 3.92-3.85 (m, 8 H), 3.83 (s, 3 H), 3.80 (s, 3 H), 3.79-3.78 (m, 1 H), 3.77 (s, 3 H), 3.75 (s, 3 H), 3.74 (s, 3 H), 3.74-3.72 (m, 2 H), 3.70 (s, 3 H), 3.69 (s, 3 H), 3.66-3.58 (m, 8 H), 0.88 (s, 9 H), 0.05 (s, 3 H), 0.04 (s, 3 H).

$^{13}$C NMR (CDCl$_3$, 125 MHz): δ 159.75, 159.44, 159.34, 159.21, 159.16, 134.90, 131.45, 131.12, 130.87, 130.81, 130.78, 130.64, 130.59, 130.20, 129.88, 129.74, 129.66, 129.59, 129.46, 127.54, 114.20, 114.04, 113.95, 113.89, 113.85, 113.71, 99.59, 98.76, 86.19, 80.17, 80.07, 79.57, 78.75, 75.10, 74.99, 74.88, 74.79, 74.56, 73.79, 73.08, 72.89, 72.44, 72.34, 71.92, 71.48, 69.16, 66.72, 62.96, 61.71, 55.53, 55.50, 55.44, 55.39, 55.33, 29.97, 26.23, 18.62, -4.78, -4.98. [$\alpha$]$_{D}^{25} = +43^\circ$ (c 1.0, CHCl$_3$). HR ESI MS: calcd. for C$_{96}$H$_{118}$O$_{24}$NaSiS [M+Na]$^+$ m/z, 1737.7401; found, 1737.7438.

**Phenyl [6-O-(tert-butyldimethylsilyl)-2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)-[3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-2-O-(2-azidoethyl)-3,4-di-O-(para-methoxybenzyl)-1-thio-α-D-mannopyranoside (62)**

![Chemical Structure](image)

To a solution of 61 (57 mg, 33 μmol) and diisopropylethylamine (15 μL, 83 μmol) in anhydrous CH$_2$Cl$_2$ (2 mL) stirring under Ar at 0 °C was added mesyl chloride (7.5 mg, 66 μmol). The reaction was warmed to rt and stirred for 30 min, after which it was quenched with saturated aqueous NaHCO$_3$. The aqueous layer was then extracted 3 times with CH$_2$Cl$_2$, dried over
Na$_2$SO$_4$, and concentrated in vacuum. The resulting residue was dissolved in anhydrous DMF (1 mL) and treated with NaN$_3$ (6.5 mg, 99 μmol). The reaction was heated to 90 ºC and stirred for 1 h, after which it was diluted with EtOAc, washed with brine, dried over Na$_2$SO$_4$, and concentrated in vacuum. The crude product was purified by silica gel column chromatography to provide 62 (47 mg, 82%) as a syrup. $^1$H NMR (CDCl$_3$, 500 MHz): δ 7.47-7.46 (m, 2 H), 7.35 (m, 2 H), 7.26-7.09 (m, 17 H), 6.92-6.75 (m, 16 H), 5.52 (d, $J$ = 1.0 Hz, 1 H), 5.26 (s, 1 H), 4.86 (d, $J$ = 10.5 Hz, 1 H), 4.83 (d, $J$ = 11 Hz, 1 H), 4.82 (s, 1 H), 4.78 (d, $J$ = 10 Hz, 1 H), 4.70 (d, $J$ = 12 Hz, 1 H), 4.67 (d, $J$ = 11.5 Hz, 1 H), 4.62 (d, $J$ = 11.5 Hz, 1 H), 4.55 (d, $J$ = 10.5 Hz, 1 H), 4.50-4.45 (m, 5 H), 4.41-4.35 (m, 6 H), 4.24 (t, $J$ = 6.5 Hz, 1 H), 4.10 (s, 1 H), 3.95-3.85 (m, 8 H), 3.83 (s, 3 H), 3.82 (s, 3 H), 3.80 (s, 3 H), 3.79-3.78 (m, 2 H), 3.77 (s, 3 H), 3.76 (s, 3 H), 3.75 (s, 3 H), 3.74-3.72 (m, 1 H), 3.71 (s, 3 H), 3.70-3.68 (m, 1 H), 3.67 (s, 3 H), 3.66-3.58 (m, 4 H), 3.41-3.28 (m, 2 H), 0.89 (s, 9 H), 0.06 (s, 3 H), 0.05 (s, 3 H). $^{13}$C NMR (CDCl$_3$, 125 MHz): δ 159.58, 159.42, 159.38, 159.31, 159.17, 159.14, 153.05, 131.52, 131.29, 131.22, 131.13, 130.98, 130.85, 130.73, 130.44, 129.85, 129.79, 129.73, 129.71, 129.57, 129.44, 129.40, 127.47, 114.15, 114.02, 113.99, 113.94, 113.86, 113.83, 113.69, 99.34, 98.67, 86.23, 80.43, 80.33, 79.65, 78.91, 74.98, 74.87, 74.78, 74.59, 73.77, 73.03, 72.44, 72.35, 72.10, 71.92, 71.43, 70.27, 69.06, 66.73, 62.95, 55.53, 55.49, 55.44, 55.42, 55.40, 55.32, 51.07, 26.23, 18.61, -4.73, -4.97. [$\alpha$]$_D^{25}$ = +45º (c 1.0, CHCl$_3$). HR ESI MS: calcd. for C$_96$H$_{117}$N$_5$O$_{23}$NaSiS $[M+Na]^+$ $m/z$, 1762.7466; found, 1762.7423.
To a mixture of 62 (45 mg, 26 μmol), 2,4,6-tri-tert-butyldimethylsilyl)-2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-
(1→2)-[3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-2-O-(2-azidoethyl)-
3,4-di-O-(para-methoxybenzyl)-D-mannopyranose (79)
6-O-[(2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)-[3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-[2-O-(2-azidoethyl)-3,4-di-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→4)-[2-deoxy-2-(9-fluorenylmethoxycarbonylamino)-3,6-di-O-(para-methoxybenzyl)-α-D-glucopyranosyl]-1-O-[(2-cyanoethoxyl)-(2,3-di-O-stearoyl-sn-glycerol)-phosphono]-2,3,4,5-tetra-O-(para-methoxybenzyl)-myo-inositol (64)

DBU (1 drop) was added to a solution of hemiacetal 79 (29.0 mg, 17.5 μmol) and trichloroacetonitrile (0.1 mL) in anhydrous CH₂Cl₂ (1.0 mL) stirring under an Ar atmosphere at 0 °C. After 30 min, the reaction mixture was concentrated in vacuum and purified with a triethylamine-neutralized silica gel column to give 63 (30.2 mg, 96%). A mixture of the resulting trichloroacetimidate 63 (29.0 mg, 16.3 μmol), acceptor 60 (19 mg, 9.3 μmol), and MS 4 Å (10 mg) in anhydrous Et₂O (1.0 mL) was stirred under an Ar atmosphere at rt for 1 h. After cooling to 0 °C, TMSOTf (1.0 μl, 5 μmol) was added and the reaction was stirred for 30 min, at which point the trichloroacetimidate donor was consumed. Saturated aqueous NaHCO₃ and Et₂O were then added, and the resulting mixture was passed through celite to remove MS 4 Å. After extraction of the aqueous layer with Et₂O 3 times, the combined organic phase was dried over Na₂SO₄, concentrated in vacuum, and taken directly to the next step. The crude intermediate was
dissolved in anhydrous THF-CH$_3$CN (1:1, 1.0 mL) and treated with triethylamine trihydrofluoride (0.5 mL) under Ar at rt. After stirring overnight, the reaction was quenched by dropwise addition of saturated aqueous NaHCO$_3$. The aqueous layer was extracted 3 times with CH$_2$Cl$_2$, and the combined organic layer was dried over Na$_2$SO$_4$, concentrated in vacuum, and purified by silica gel column chromatography to give recovered acceptor 60 (11 mg, 58%) and pseudopentasaccharide 64 (10 mg, 30%, two steps, 88% based on recovered starting material) as a syrup. $^1$H NMR (CDCl$_3$, 500 MHz, resolved signals in anomeric region): δ 5.41 (d, $J = 10.5$ Hz, 1 H), 5.16 (s, 1 H), 5.14 (d, $J = 3.0$ Hz, 1 H), 5.15-5.13 (m, 1 H), 5.05 (s, 1 H), 5.01 (s, 1 H), 4.80 (s, 1 H). $^{13}$C NMR (CDCl$_3$, 125 MHz): δ 173.42, 173.05, 159.45, 159.38, 159.32, 159.26, 159.12, 156.45, 144.16, 144.12, 141.47, 141.27, 131.14, 131.96, 130.84, 130.70, 130.53, 130.43, 129.79, 129.72, 129.65, 129.58, 129.50, 129.45, 129.35, 129.26, 128.40, 127.90, 127.83, 127.30, 125.62, 125.27, 120.08, 116.92, 114.10, 114.02, 113.97, 113.94, 113.86, 113.82, 101.06, 99.76, 99.61, 98.48, 81.67, 80.97, 80.69, 80.43, 79.74, 79.54, 79.20, 75.77, 75.66, 75.45, 75.14, 75.00, 74.85, 74.67, 74.07, 73.53, 73.13, 73.00, 72.76, 72.23, 72.03, 71.93, 71.84, 70.76, 70.17, 69.58, 69.48, 60.03, 68.84, 67.07, 66.33, 66.09, 62.63, 62.47, 61.67, 55.49, 55.44, 55.39, 55.32, 55.27, 55.09, 50.94, 47.39, 34.32, 34.16, 32.18, 29.98, 29.93, 29.79, 29.62, 29.58, 29.39, 29.35, 25.05, 22.95, 14.39. Configurations of anomeric positions were established as α by coupled $^{13}$C NMR $J_{CH}$ values (125 MHz): 101.06 ($J_{CH} = 175$ Hz, Man-1), 99.76 ($J_{CH} = 166$ Hz, Man-1), 99.61 ($J_{CH} = 174$ Hz, Man-1), 98.48 ($J_{CH} = 177$ Hz, GlcN-1). $^{31}$P NMR (CDCl$_3$, 160 MHz): δ -1.01. HR ESI MS: calcd. for C$_{201}$H$_{256}$N$_5$O$_{48}$NaP [M+Na]$^+$ m/z, 3561.7380; found, 3561.7419.
6-O-[(6-O-[(2-Cyanoethoxyl)-(2-(9-fluorenylmethoxycarbonylamino)ethoxy]-phosphono]-2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-{(1→2)-[3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-[2-O-(2-azidoethyl)-3,4-di-O-(para-methoxybenzyl)-α-D-mannopyranosyl]-(1→4)-[2-deoxy-2-(9-fluorenylmethoxycarbonylamino)-3,6-di-O-(para-methoxybenzyl)-α-D-glucopyranosyl]}-1-O-[(2-cyanoethoxyl)-(2,3-di-O-stearoyl-sn-glycerol)-phosphono]-2,3,4,5-tetra-O-(para-methoxybenzyl)-myo-inositol (80)

To a solution of pseudopentasaccharide alcohol 64 (6.0 mg, 1.7 μmol) and MS 4 Å (10 mg) stirring in anhydrous CH₂Cl₂ (1 mL) under Ar at rt was added a solution of freshly prepared phosphoramidite 55 (8.2 mg in dry CH₂Cl₂, 17 μmol) and 1H-tetrazole (0.45 M solution in acetonitrile, 75 μL, 34 μmol). After stirring at rt under Ar for 1 h, the reaction was cooled to 0 °C and treated with tert-butyl hydroperoxide (5.5 M solution in decane, 6 μL, 33 μmol). After stirring for 2 h, the mixture was filtered through celite, poured into saturated aqueous NaHCO₃, and extracted 3 times with CH₂Cl₂. The combined organic layers were dried over Na₂SO₄ and concentrated in vacuum. Purification by Sephadex LH-20 size exclusion chromatography followed by silica gel column chromatography afforded 80 as an inconsequential ~1:1
diastereomeric mixture (4.1 mg, 61%) that was briefly characterized and taken to the final step.

$^1$H NMR (CDCl$_3$, 500 MHz, resolved signals): $\delta$ 7.67-7.61 (m, 4 H), 7.52-7.46 (m, 4 H), 5.53 (s, 1 H), 5.43-5.41 (m, 1 H), 5.28 (s, 1 H), 5.23-5.20 (pent, $J = 5.0$ Hz, 4 H). $^{31}$P NMR (CDCl$_3$, 160 MHz): $\delta$ -0.30 (1 P), -0.41 (1 P), -0.94 (2 P). MALDI TOF MS (positive mode): calcd. for C$_{221}$H$_{273}$N$_7$O$_{53}$NaP$_2$ [M+Na]$^+$ m/z, 3959.9; found, 3960.2.

6-O-[(6-O-[(2-Aminoethyl)-phosphono]-α-D-mannopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→6)-[2-O-(2-azidoethyl)-α-D-mannopyranosyl)-(1→4)-(2-amino-2-deoxy-α-D-glucopyranosyl)]-1-O-[(2,3-di-O-stearoyl-sn-glycerol)-phosphono]-myo-inositol (3)

To a solution of 80 (1.80 mg, 0.46 μmol) in CH$_2$Cl$_2$ (200 μL) was added DBU (1 μL) at rt. The solution stirred for 1 h, after which MALDI TOF MS confirmed removal of the Fmoc and cyanoethoxyyl protecting groups [MALDI TOF MS (positive mode): calcd. for C$_{185}$H$_{250}$N$_5$O$_{49}$P$_2$ [M+H]$^+$ m/z, 3387.7; found, 3387.8]. Then, 20% TFA in CH$_2$Cl$_2$ (200 μL) was added directly to the reaction, giving a final concentration of ~10% TFA. After stirring for 30 min, the reaction was co-evaporated with toluene to remove most solvent and TFA. Two drops of Et$_3$N were
added to neutralize any residual TFA, and the mixture was co-evaporated with toluene 5 times. Purification of the crude product by Sephadex LH-20 size exclusion chromatography (CHCl₃-MeOH-H₂O 3:3:1) gave 3 (0.76 mg, 97%) as a white powder. ¹H NMR (CDCl₃-CD₂OD-D₂O 3:3:1, 500 MHz, anomeric region): δ 5.35-5.27 (m, 3 H), 5.18 (s, 1 H), 5.09 (s, 1 H), 4.96 (s, 1 H). ³¹P NMR (CDCl₃, 160 MHz): δ 0.59, -0.60. MALDI TOF MS (negative mode): calcd. for C₇₃H₁₃₆N₅O₃₅P₂ [M-H]⁻ m/z, 1704.9; found, 1704.9.

**GPI-Fluor conjugate (4)**

To a solution of 2 (~50 μg, 0.03 μmol) and Azide-Fluor 488 65 (86 μg, 0.15 μmol, purchased from Click Chemistry Tools) in THF-MeOH-H₂O (300 μl) was added CuSO₄·5H₂O (0.3 equivalents with respect to 2) and sodium ascorbate (2 equivalents with respect to 2). After stirring overnight at 37 °C, MALDI TOF MS showed moderate conversion to product, and additional CuSO₄·5H₂O (6 equivalents with respect to 2) and sodium ascorbate (4 equivalents with respect to 2) were added. Stirring continued for 2 days at 37 °C, after which MALDI TOF MS showed complete conversion of starting material to product 4. MALDI TOF MS (negative mode): calcd. for C₁₀₃H₁₆₅N₆O₄₂P₂ [M-H]⁻ m/z, 2248.1; found, 2248.7.
GPI-Biotin conjugate (5)

Compound 3 (~0.15 mg) was dissolved in CHCl₃-MeOH-H₂O (3:3:1, 0.5 mL). 10 equivalents of BARAC-biotin 66 [solution in CHCl₃-MeOH-H₂O (3:3:1), 0.5 mL] were added, and the reaction was allowed to stand at rt. After 24 h, MALDI TOF MS showed ~50% conversion from starting material to the desired triazole. After 48 h, MALDI TOF MS showed complete conversion of the starting material to GPI-biotin conjugate 5. MALDI TOF MS (negative mode): calcd. for C₁₂₀H₁₉₂N₁₁O₄₄P₂ [M-H]- m/z, 2585.2; found, 2585.2.

References for Electronic Supplementary Information

III. NMR and MS spectra, HPLC chromatograms

(±)-68

$^1$H NMR (500 MHz, CD$_3$OD)

(±)-68

$^{13}$C NMR (125 MHz, CD$_3$OD)
**Single Mass Analysis**

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
357 formula(e) evaluated with 2 results within limits (up to 50 best isotopic matches for each mass)

Elements Used:
C: 0-500  H: 0-1000  N: 0-5  O: 0-10  23Na: 0-1

Guo- Ben Swarts BMS-V1-97 mw260 LCT0100 in meoh 1uL
Shay 2008-07b.pro
2008_0822_0100_1314 (0.301) Cm (12:17:1.8x2.000)

**HR ESI MS**

LCT Premier 22-Aug-2008 14:34:37
1: TOF MS ES+
1.43e+004

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(±)-17

$^1$H NMR (500 MHz, CDCl$_3$)

(±)-17

$^{13}$C NMR (125 MHz, CDCl$_3$)
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
393 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-500 H: 0-1000 N: 0-1 O: 0-10 23Na: 0-1
Guo-Ben Swarts BMS-L-98 LCT0299 mwf/40 0.5uL meth
Shay 2008-07b pro
2009_0109_0269 11 (0.230) Cm (2:11-2:7x2.000)

Minimum:
Maximum:

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$^{1}$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (125 MHz, CDCl$_3$)
Single Mass Analysis
Tolerance = 0.0 PPM / DEB: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
178 formula(e) evaluated with 3 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-500 H: 0-1000 O: 0-10 23Na: 0-1
Guo: Ben Swartz BMS-V1-0S mw650 LCT0339 2uL mech 3cm stk
DOPH: LCT Premier
2007_0720_0039_14_12 (0.247) Cm (11.12-1:5x1.200)

Minimum: 100.0 6.0 -1.5
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$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (125 MHz, CDCl$_3$)
Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 60.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
258 formula(e) evaluated with 5 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-60 H: 0-200 O: 0-15 23Na: 0-1

gua Ben Swarts BMS-VI-28-A mw700 LCT0111 100pg/uI meoh 0.1ul 1ul stk Shay 2008-07b.pro
2008_0822_0111_30 13 (0.300) Cm (10:15:1:7x2.000)

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HR ESI MS
LCT Premier 22-Aug-2008 16:58:39
1: TOF MS ES+
4.43e+003
HPLC chromatogram for purification of (±)-9

**Conditions:** Waters Nova-Pak Silica 6 µm, 300 x 19 mm eluent 33% EtOAc in hexanes, 10 mL/min

\[ \text{(±)-8} \]

\[ \text{PMBO \ OPMB \ OPMB} \]
\[ \text{HO \ AllO \ OPMB} \]

\[ \text{PMBO \ OPMB \ OPMB} \]
\[ \text{HO \ AllO \ OPMB} \]

\[ \text{(-)-19} \]

\[ \text{^{1}H NMR (500 MHz, CDCl\textsubscript{3})} \]
$(-)-19$

$^{13}$C NMR (125 MHz, CDCl$_3$)

$(-)-19$

COSY (500 MHz, CDCl$_3$)
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 60.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
276 formula(e) evaluated with 3 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-113  H: 0-116  O: 0-13  23Na: 0-1
guo Ben Swarts BMS-VI-41 mw880 LCT0106 10pg/ul mech 1 ul 1ul sk
Shay 2006-07b pro 2008_0322_0105_22 11 (0.230) Cm (9:11-2:6x2.000)

Minimum:
Maximum: 8.0  5.0  -1.5

Mass  Calc. Mass  mDa  PPM  DBE  i-FIT  i-FIT (Norm)  Formula
903.3889  903.3932  -4.3  -4.8  21.5  87.6  0.1  C51 H60 O13 23Na ±
903.3897  -0.8  -0.9  33.5  90.9  3.3  C60 H55 O6
903.3873  1.6  1.8  30.5  91.2  3.6  C58 H56 O8 23Na

HPLC chromatogram for purification of (-)-22

Conditions: Waters Nova-Pak Silica 6 μm, 300 x 19 mm
eluent 25% ETOAc in hexanes, 10 mL/min
**Single Mass Analysis**

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
412 formula(e) evaluated with 3 results within limits (up to 50 best isotopic matches for each mass)

Elements Used:
C: 0-500 H: 0-1000 N: 0-4 O: 0-8 23Na: 0-1

Guo- Ben Swarts BMS-VF-75 LCT0221 mw371 5ul. meth
Shay 2008-07b pro
2008_1125_0221_01 15 (0.318) Cm (1220-4(8+30.37)x3.000)

LCT Premier 25-Nov-2008 09:46:03
1: TOF MS ESI+
3.29e+004

**S77**

1H NMR (500 MHz, CDCl₃)
$\text{PMP} - \text{O} - \text{O} - \text{O} - \text{OAll}$

$^1\text{H NMR (500 MHz, CDCl}_3)$

$\text{PMP} - \text{O} - \text{O} - \text{O} - \text{OAll}$

$^{13}\text{C NMR (125 MHz, CDCl}_3)$
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
310 formula(e) evaluated with 2 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-500  H: 0-1000  N: 0-4  O: 0-6  23Na: 0-1
Guo- Ben Swarts BMS-VI-02 LCT0230 mx363.05, 50.0, mesh
Shay 2008-07b pro
2008_1202_0230_03 18 (0.368) Cm (18:19-(2:4+28-32)x2.000)

Minimum: 36.0 36.0 -1.5
Maximum: 5.0 5.0 50.0

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364.1525  -1.4 -3.8  9.5  47.0  1.4  C20 H23 N O4
23Na
$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (125 MHz, CDCl$_3$)
**Single Mass Analysis**

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

602 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 0-40  H: 0-83  N: 0-4  O: 0-10  23Na: 0-1

Guo BEN SWENY/DSMS-VI-84 LCT0229 mw483 0.5ul. meoh
Shay 2008-07-b.m
2008_1202_0229_02 11 (0.226) Cm (9.11-16x6.000)

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$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (125 MHz, CDCl$_3$)
**Single Mass Analysis**

- **Tolerance**: 5.0 PPM / DBE: min = -1.5, max = 50.0
- **Element prediction**: Off
- **Number of isotope peaks used for i-FIT**: 3

**Monoisotopic Mass, Even Electron ions**

483 formula(e) evaluated with 4 results within limits (up to 50 best isotopic matches for each mass)

**Elements Used:**
- C: 0-500
- H: 0-1000
- O: 0-7
- N: 0-4
- Na: 0-1

**Inputs:**
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- 2006_1002_0160_01.20 (0.423) Cm (20:23:46)x2.000

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$^{1}H$ NMR (500 MHz, CDCl$_3$)

$^{13}C$ NMR (125 MHz, CDCl$_3$)
Single Mass Analysis
Tolerance = 1.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
256 formula(e) evaluated with 1 result within limits (up to 50 best isotopic matches for each mass)

Elements Used:
C: 0-40 H: 0-50 N: 0-3 O: 0-7 Na: 0-1 Sc: 0-1

Guo-Hebei/analytical/V1-73 mw598 LCT01641ul mech RF150
Shay 2008-07b.pro
2008_1007_0164_05 14 (0.300) Cm (11:17-33:43x2.000)

Minimum: 5.0 1.0 -1.5
Maximum: 1.0 50.0

Mass Calc. Mass mDa PPM DBE i-FIT i-FIT (Norm) Formula
622.2927 622.2924 0.3 11.5 39.3 0.0 C44H45N3O7 Na

LCT Premier 07-Oct-2008 2:6:3
1: TOF MS ES+ 3.50e+004
$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (125 MHz, CDCl$_3$)
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
1331 formula(e) evaluated with 11 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-50  H: 0-100  N: 0-3  O: 0-12  Na: 0-1  Si: 0-1

Ben Swarts  BMS-VI-90
Shay 2008-07b.pro
2008_1125_0235 14 (0.300) Cm (11:17:4:16+25:32)x3.000

Minimum:
Mass  Calc. Mass  mDa  PPM  DBE  i-FIT  i-FIT (Norm)  Formula
582.2604  582.2611  -0.7  -1.2  10.5  22.9  0.0  C28 H41 N3 O7

Na  Si
$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (125 MHz, CDCl$_3$)
Single Mass Analysis
Tolerance = 12.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
335 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-70 H: 0-1000 N: 0-3 O: 0-18 23Na: 0-1 Si: 0-1
Guo- Ben Swarts BMS-VI-51 mw1241 LCT0140 0.5uL meth added
Shay 2006-07b.pro
2008_0909_0140_12 17 (0.334) Cm (17:18-33:35x1.200)

Minimum:                  5.0       12.0    -1.5
Maximum:                  50.0      126.0
Mass          Calc. Mass     mDa      PPM   DBE  i-FIT  i-FIT (Norm)  Formula
1264.5693  1264.5753       -6.0      -4.7  28.5  56.6     0.0  C69 H87 N3 016 23Na Si  

1H NMR (500 MHz, CDCl3)
$^{13}$C NMR (125 MHz, CDCl$_3$)

COSY (500 MHz, CDCl$_3$)
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
5564 formula(e) evaluated with 20 results within limits (up to 50 best isotopic matches for each mass)

Elements Used:
C: 0-500  H: 0-1000  N: 0-5  O: 0-16  Na: 0-1  Si: 0-1
Guc- Ben Swarts BMS-VI-93 LCT0245 mw1201.5 0.5uL mech
Shay 2008-07b.pro
2008_1125_0245 15 (0.300) Cmr (13:18-(5.9+30.35)x6.000)

Minimum: 5.0  5.0  -1.5
Maximum: 50.0

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Electronic Supplementary Material (ESI) for Chemical Science
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$OCH_2CH_2CN$

$N(i-Pr)_2P-O$

$OCOC_{17}H_{35}$

$OCOC_{17}H_{35}$

$^1H$ NMR (500 MHz, CDCl$_3$)

$NCCH_2CH_2O-P=O$

$OCOC_{17}H_{35}$

$OCOC_{17}H_{35}$

$^1H$ NMR (500 MHz, CDCl$_3$)
$^{13}$C NMR (125 MHz, CDCl$_3$)
**Single Mass Analysis**

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0  
Element prediction: Off  
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions  
12591 formula(e) evaluated with 68 results within limits (up to 50 best isotopic matches for each mass)  
Elements Used:  
C: 0-500  H: 0-1000  N: 0-5  O: 0-25  23Na: 0-1  P: 0-1

BEN SWARTS  
BMS-VII-122-A  
2008-07b prc  
2009-0506_0423 9 (0.136) Cn (7:13:19:26)x2.000

1: TOF MS ESI+  
2:16e+003

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$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (125 MHz, CDCl$_3$)
**Single Mass Analysis**

**Tolerance = 5.0 PPM / DBE:** min = -1.5, max = 100.0

**Element prediction:** Off

**Number of isotope peaks used for i-FIT = 3**

**Monoisotopic Mass, Even Electron Ions**

256 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)

**Elements Used:**

C: 25-40  H: 20-60  N: 0-4  O: 0-10  23Na: 0-1  S: 1-1

Ben Swarts  BM-D-VII-68

Lew 2008-07b.pro

2009_0318_0336.13 (0.284) Cm (11:16-(3.8+24:36)x10.000)

**Minimum: 5.0  **  **Maximum: 100.0**

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$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (125 MHz, CDCl$_3$)
Single Mass Analysis

Tolerance = 5.0 PPM  /  DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ion
N127 formula(e) evaluated with 6 results within limits (up to 50 best isotopic matches for each mass)

Elements Used:
C: 0-500  H: 0-1000  N: 0-3  O: 0-10  23Na: 0-1  S: 0-1

Guo: Ben Swarts  BMS-VIII-76
Lev 2008-07b pro
2009_0326_0255  18 (0.389) Cm (18.21-2.7x2.000)

1: TOF MS ES+
2.00e+003

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S100

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (125 MHz, CDCl$_3$)
**Single Mass Analysis**

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
334 formula(e) evaluated with 2 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-500 H: 0-1000 O: 0-30 Na: 0-1
Guo; Ben Swarts BMS-V-22 mw595 LCT0017 10pg/ml meoh 1cm stk inj3ul pluno
OA-FIA-1min DOP
LCT Premier

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**HR ESI MS**

12:28:28 03-Jul-2008
16/29 pushes/sec
1 TOF MS E5+
5.21s+004

`^1^H NMR (500 MHz, CDCl3)`
Single Mass Analysis

Tolerance = 5.0 PPM / DEB: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
314 formula(e) evaluated with 3 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-500  H: 0-1000  O: 0-30  Na: 0-1
Gnu: Ben Swarts SMB-V-24 mw562 LCTQ019 10pg/ul meth 1cm stk inj/ul plnue
OA-FIA-1min DOP
LCT Premier

2008_0703_0019_13 16 (0.311) Cm (13:18-28:30)

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HR ESI MS

14:13:13 03-Jul-2008
16/29 pushes/sec
1: 10F MS ES+ 2.19e+604
$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (125 MHz, CDCl$_3$)
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
259 formula(e) evaluated with 2 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-500 H: 0-1000 O: 0-30 Na: 0-1
Guo: Ban Swarts BMS-V-66 mw492 LCT0015 10pg/inj meoh 1cm sil3 inj3 plun
OA-FIA-1min DOP
LCT Premier

2008_0703_0015_0916 (0.311) Cm (11:21-31:49)

Minimum: 6.0 5.0 -1.5
Maximum: 515.2054 515.2046 0.8 1.6 13.5 5.3

Calc. Mass mDa PPM DBE i-FIT Formula
515.2054 515.2046 0.8 1.6 13.5 5.3 C29 H32 O7 Na
515.2070 -1.6 -3.1 16.5 27.1 C31 H31 O7
$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (125 MHz, CDCl$_3$)
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
525 formula(e) evaluated with 5 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-500 H: 0-1000 O: 0-30 Na: 0-1
Guar: Ben Swarts BMS-V-63 ma652 LCT0020 10pg/u1 meth 1cm sti j3ul pluno
OA-FIA 1min DOP
LDT Premier

2008_0703_0020_17 16 (0.311) Cm (1:21:32:45)

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$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (125 MHz, CDCl$_3$)
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
323 formula(e) evaluated with 2 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-500 H: 0-1000 O: 0-30 Na: 0-1
Guo, Ben Swarta BMS-V-75A mwr580 LCT70016 10ppgul mech 1cm 3ik jnJul plano
OA-FBA-1min DOP
LCT Premier

2008_0703_0018_10:16 (0.311) Cm (12:20-34:48)

Minimum: 6.0 5.0 -1.5
Maximum: 340.9431 311.9313 340.957

Mass Calc. Mass mDa PPM DBE i-FIT Formula
603.2549 603.2570 -2.1 -3.5 13.5 72.8 C33 H40 O12 Na
603.2535 1.4 2.3 25.5 1273.3 C42 H35 D9

HR ESI MS

11:55:08 03-Jul-2008
16129 pushes/sec
I: T0F MS ES+ 7.28e104
$\text{S109}$

$\text{HCDCl}_3$ NMR-V-76-9 24mg
Pulse sequence: x2pul

42

$^{1}H$ NMR (500 MHz, CDCl$_3$)

$\text{Varian 500 MHz spectrometer}$

$\text{TBSO} \quad \text{OPMB}$
$\text{OMBO} \quad \text{PMBO}$

42

$\text{OAll}$

$\text{13C NMR (125 MHz, CDCl}_3\text{)}$
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
785 formula(e) evaluated with 6 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-600  H: 0-1000  O: 0-30  Na: 0-1  Si: 0-1

Guo: Ben Swarts BMS-V-70-B mw604 LCT0018 10pg/ul meoh 1cm stk inj3ul plu0
OA-FIA-1min DOP  LCT Premier
2008_0703_0018_12 16 (0.311) Cm (13.18-25.35)

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$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (125 MHz, CDCl$_3$)
**Single Mass Analysis**

**Tolerance = 5.0 PPM / DBE:** min = -1.5, max = 50.0

**Element prediction:** Off

**Number of isotope peaks used for i-FIT = 3**

**Monoisotopic Mass, Even Electron Ions**

314 formula(e) evaluated with 4 results within limits (up to 50 best isotopic matches for each mass)

**Elements Used:**

- C: 0-500
- H: 0-1000
- O: 0-9
- 23Na: 0-1
- Si: 0-1

**Guo- Ben Swarts BMS-V1-9smw654 LCT0093 in meoh 1uL 3x stk Shay 2008-07b.pro**

**2008_0822_0093_01 14 (0.300) Cm (13.16)-(3.7+35.42)x10.000**

**Minimum:**

- Mass: 677.3103
- Calc. Mass: 677.3090
- PPM: 1.3
- DBE: 17.5
- i-FIT: 31.9
- i-FIT (Norm): 0.4
- Formula: C40 H46 O8 23Na

**Maximum:**

- Mass: 677.3103
- Calc. Mass: 677.3090
- PPM: 1.3
- DBE: 17.5
- i-FIT: 31.9
- i-FIT (Norm): 0.4
- Formula: C40 H46 O8 23Na

---

**HR ESI MS**

**LCT Premier 22-Aug-2008 11:36:35**

**I: TOF MS ES+**

**6.04e+003**

---

**COSY (400 MHz, CDCl₃)**

---

**TBSO**

**OPMB**

**PMBO**

**76**

**OH**

---

**S112**
$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (125 MHz, CDCl$_3$)
COSY & HMQC
(500 MHz, CDCl₃)
**Single Mass Analysis**

**Mass** | **Calc. Mass** | **mDa** | **PPM** | **DBE** | **i-FIT** | **i-FIT (Norm)** | **Formula**
---|---|---|---|---|---|---|
1097.4349 | 1097.4333 | 1.6 | 1.5 | 26.5 | 49.5 | 1.0 | C61 H70 O15 23Na
1097.4367 | -1.8 | -1.6 | 21.5 | 49.8 | 1.2 | C59 H74 O15 23Na
1097.4357 | -0.8 | -0.7 | 29.5 | 50.0 | 1.5 | C63 H69 O15 S
1097.4391 | -4.2 | -3.0 | 24.5 | 52.0 | 3.5 | C60 H73 O15 S2
1097.4299 | 5.0 | 4.6 | 31.5 | 52.7 | 4.1 | C64 H66 O15 23Na

**1H NMR (500 MHz, CDCl3)**
$\text{S116}$

$^{13}\text{C NMR} \ (125 \text{ MHz, CDCl}_3)$

$\text{COSY} \ (500 \text{ MHz, CDCl}_3)$
**Single Mass Analysis**

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
96 formula(s) evaluated with 11 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:

BEN SWARTS BMS-VII-901
2008-07b.pro
2009_0330_0357 14 (0.284) Cn (10:20-(1.8+29:39)x2.000)

LCT Premier 30-Mar-2009 11:49:41
1. TOF MS ESI+
8.22e+30/

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HMOC (500 MHz, CDCl₃)
& HR ESI MS
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

11082 formula(e) evaluated with 65 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-500  H: 0-1000  N: 0-5  O: 0-25  23Na: 0-1  Si: 0-1

BMS-VII-83
2008-07b pro
2009_0508_0424 15 (0.300) Cm (11:20-(18+23:30)x2.000)

LCT Premier: 13-May-2009 14:07:37
1: TOF MS ES+
6,23e+002

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\[ ^1H \text{ NMR (500 MHz, CDCl}_3 \text{)} \]
& HR ESI MS (below)
Compound 46
$^1$H NMR (500 MHz, CDCl$_3$)

Compound 46
$^{13}$C NMR (125 MHz, CDCl$_3$)
Compound 46
COSY (500 MHz, CDCl₃)

Compound 46
HMOC (500 MHz, CDCl₃)
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 150.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
356 formula(e) evaluated with 17 results within limits (up to 50 best isotopic matches for each mass)

Elements Used:

BEN SWARTS
BSM-VII-119
2008-07b Core
2009_0516_0436a 19 (0.389) Cm ((17+19:20+22:24;-(4.6+36.43)x2 000)

Minimum:
5.0 5.0 -1.5
Maximum:
3336.6597 3336.6590 67.5 15.2 3.0 C187 H245 N4 O46

Compound 46
HR ESI MS

\[
\text{OCH}_2\text{CH}_2\text{CN} \quad \text{N}(i-\text{Pr})_2^+ \quad \text{OCH}_2\text{CH}_2\text{NHFmoc} \\
47
\]

\[
^1\text{H} \text{NMR} (500 \text{ MHz}, \text{CDCl}_3)
\]
OCH₂CH₂CN
N(i-Pr)₂\(\text{P}^\downarrow\)OCH₂CH₂NHFmoc

47

\(^{13}\text{C} \text{ NMR (125 MHz, CDCl}_3\))

---

Compound 48

\(^{1}\text{H} \text{ NMR (500 MHz, CDCl}_3\))
Compound 48
$^{13}$C NMR (125 MHz, CDCl$_3$)

Compound 48
COSY (500 MHz, CDCl$_3$)
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 150.0
Element prediction: Off
Number of isotope peaks used for i-PIT = 3

Monoisotopic Mass, Odd and Even Electron Loss
1022 formula(s) evaluated with 78 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
DEN-CHAIN
BISM-VII-132
2008-07b pro
2009_6528_0445b 15 (0.300) Cm (12:22-3.7+35.44)x2.000

1: TOF MS ES+
3.54e+002

Minimum:
Maximum:
Mass Calc. Mass mDa PPM DBE i-PIT i-PIT (Norm) Formula
3729.7986 3729.8001 -1.5 -0.4 70.0 59.8 5.2 C199 H270 N8 O56
3729.8154 -15.8 -4.5 74.0 57.8 3.1 P2
3729.8041 -5.5 -1.5 74.0 59.2 4.5 C204 H270 N6 O54
3729.8044 -5.8 -1.6 75.5 59.1 4.4 P2
3729.7929 6.7 1.6 74.0 60.6 5.9 C205 H269 N7 O51
3729.7932 5.4 1.4 75.5 60.6 5.9 P2
3729.7942 4.4 1.2 79.0 60.4 5.7 C206 H269 N5 O52
3729.8068 -8.2 -2.2 78.5 58.7 4.0 P2

Compound 48
HR ESI MS
Compound 50

$^1$H NMR (500 MHz, CDCl$_3$)
mixture of 2 diastereomers

Comment 1  BMS_VII_Succinylation Product
Comment 2  DHB Positive

m/z 3794.089  SN 69.5  Intens. 77.02  Area 199  Res. 6513

Compound 50
MALDI TOF MS
Compound 51

$^1$H NMR (500 MHz, CDCl$_3$)
mixture of 2 diastereomers

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = 0.0, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Odd and Even Electron ions
196 formulae evaluated with 50 results (up to 1000) for each mass
Elements Used:

BEN SWARTS
BSM-VII-186
1.2e+03

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<td>n/a</td>
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HR ESI MS
Alkynyl-GPI 1
$^1$H NMR (500 MHz, CDCl$_3$-CD$_3$OD-D$_2$O 3:3:1)

$^{31}$P NMR (160 MHz, CDCl$_3$-CD$_3$OD-D$_2$O 3:3:1)
Alkynyl-GPI 1
MALDI TOF MS
Electronic Supplementary Material (ESI) for Chemical Science
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$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (125 MHz, CDCl$_3$)
COSY & HMBC
(500 MHz, CDCl$_3$)
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

717 formula(e) evaluated with 24 results within limits (up to 50 closest results for each mass)

Elements Used:


BEN SWARTS
BSM-VII-157
2008-07b pro
2009.0629_0472a 14 (0.284) Cm (12:20-(1.9+30.36)x100.000)

Minimum:

Maximum:

Mass   Calc. Mass   mDa   PPM   DBE   i-FIT   i-FIT (Norm)   Formula
1693.7136 1693.7136  0.0   0.0   38.5   68.1   2.7   C93 H114 O24 23Na
1693.7135 1693.7135  0.1   0.1   45.5   68.6   3.2   S12  C99 H113 O19 S12
1693.7138 1693.7138 -0.2  -0.1   45.5   68.4   3.0   S2    C100 H113 O18 S2
1693.7139 1693.7139 -0.3  -0.2   38.5   67.9   2.5   S1 S  C94 H114 O23 23Na

53
SPh

1H NMR (500 MHz, CDCl3)
$^{13}$C NMR (125 MHz, CDCl$_3$)

COSY & HMQC (below) (500MHz, CDCl$_3$)
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron ions
2579 formula(e) evaluated with 7 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 80-120  H: 0-1000  O: 0-25  Na: 0-1  Si: 0-1  S: 0-1

BEN SWARTS
LCT2008.07b.prm 2010-01-22
2010_0613_90013 (0.247) Cm (11.13-2.6x2.000)

Minimum:
Maximum:
Mass  Calc. Mass  mDa  PPM  DBE  i-FIT  i-FIT (Norm)  Formula
1731.7350  1731.7319  3.1  1.8  43.5  35.2  1.3  C99 H115 O23 Si
1731.7288  1731.7416  6.2  3.6  48.5  35.8  1.8  C101 H111 O22 S
1731.7295  5.5  3.2  40.5  36.0  2.1  C97 H116 O23 23Na Si
**1H NMR (500 MHz, CDCl₃)**

**13C NMR (125 MHz, CDCl₃)**
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron ions
3134 formula(s) evaluated with 14 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 80-120  H: 0-1000  O: 0-25  23Na: 0-1  S: 0-1
BEN SWARTS
LCT2008-07b pro 2010-cvf flux 2010_0519_951 21 (0.422) Cm (21.26-1.8x2.000)

Minimum: 5.0  Maximum: 5.0  T  99.9

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<th>PPM</th>
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<th>i-FIT</th>
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\( ^1 \text{H NMR (500 MHz, CDCl}_3 \)
Compound 56

$^{13}$C NMR (125 MHz, CDCl$_3$)

Compound 56
COSY (500MHz)
**Elemental Composition Report**

**Single Mass Analysis**

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

256 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 150-200  H: 200-300  N: 3-5  O: 44-48  Na: 0-1  P: 1-1

**BEN SWARTS**

**RMAS IX 28**

**LCT2009-07b-2010-24d-4b**

2010_0723_1140 13 (0.246) Cm (12.17-2.9x2.000)

---

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<td>3334.6477</td>
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Minimum: 5.0  5.0  -1.5  100.0

Maximum: 5.0  5.0  -1.5  100.0

---

**Compound 56**

HR ESI MS


**Comment 1**
BMS-IX Alkynyl Pseudopentasaccharide Phosphorylation-Oxidation

**Comment 2**
DHB Positive

---

**Compound 57**
MALDI TOF MS

**D:\Data\Guo_lab\Ben\GPI Synthesis\BMS-IX\BMS-IX Alkynyl Pseudopentasaccharide**

**Phosphorylation-Oxidation**

---

**m/z** | **SN** | **Intens.** | **Area** | **Res.**
---|---|---|---|---
3732.281 | 33.1 | 32.90 | 133 | 51.89

---

**Expansion**
Alkynyl-GPI 2

$^1$H NMR (500 MHz, CDCl$_3$-CD$_3$OD-D$_2$O 3:3:1)

Alkynyl-GPI 2

$^{31}$P NMR (160 MHz, CDCl$_3$-CD$_3$OD-D$_2$O 3:3:1)
**Comment 1**

BMS-IX-48 Final Deprotection Neg

**Comment 2**

TFA Treatment

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Alkynyl-GPI 2
MALDI TOF MS

Expansion

\[ [M-H]^- \]

\[ [M-2H+Na]^+ \]

**Formula 58**

$^1$H NMR (500 MHz, CDCl$_3$)
$^{13}$C NMR (125 MHz, CDCl$_3$)

COSY (500 MHz, CDCl$_3$)
Elemental Composition Report

**Single Mass Analysis**

- Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
- Element prediction: Off
- Number of isotope peaks used for i-FIT = 3

**Monoisotopic Mass, Even Electron Ions**

3001 formula(e) evaluated with 25 results within limits (all results up to 1000) for each mass

**Elements Used:**
- C: 0-100
- H: 0-150
- N: 0-3
- O: 0-20
- 23Na: 0-1
- Si: 0-1

**BEN SWARTS**

BMS-IX-11

LCT2008-07D pro 2010-09-01

**LCT Premier 26 Apr 2010 14:33:58**

1: TOF MS ES+

**1.50e+004**

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**58**

HR ESI MS
$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (125 MHz, CDCl$_3$)
## Elemental Composition Report

### Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = 1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

**Monoisotopic Mass, Even Electron Ions**
5256 formula(e) evaluated with 28 results within limits (up to 700 best isotopic matches for each mass)

Elements Used:
- C: 110-120
- H: O-1000
- N: O-18
- O: 0-30
- Na: 0-1
- P: 1-1

**BEN SWARTS**
LCT2006-07b pro 2010-crpl
2010_0514_95Ac 50 (C,0.050) Cm (30.35-1:62:0.00)

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$^{1}H$ NMR (500 MHz, CDCl$_3$)

$^{13}C$ NMR (125 MHz, CDCl$_3$)
### Elemental Composition Report

**Single Mass Analysis**

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of sotope peaks used for i-FIT = 3

**Monoisotopic Mass, Even Electron Ions**

4766 formula(e) evaluated with 16 results within limits (all results (up to 1000) for each mass)

Elements Used:
- C: 0-500
- H: 0-1000
- O: 0-30
- Na: 0-1
- S: 0-1
- Si: 0-1

BEN SWARTS
BMS-IX-36
LCT2009-07b.pro 2016-cif.spl
2016_0723_1138 17 (0.334) Cm (17.25-1.8x2.000)

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Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
5742 formula(s) evaluated with 59 results within limits (all results up to 1000) for each mass
Elements Used:
C: 0-500 H: 0-1000 O: 0-40 23Na: 0-1 Si: 0-1 S: 0-1

BEN SWARTS
LCT2008-07b.proc.2010-off.spl
2010_0722_113720 (0.40E) Cm (20.25-1.7x2.000)

Minimum: 5.0 5.0 100.0
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S152

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (125 MHz, CDCl$_3$)
Electronic Supplementary Material (ESI) for Chemical Science

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Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element precision: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron ions
23075 formula(0) evaluated with 68 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-500  H: 0-1000  N: 0-4  O: 0-30  23Na: 0-1  S: 0-1

BEN SWARTS  BMS-IX-41
LCT2008-07b.proc 2010-05.spl
2010_0723_114120 (0.425) Cn (20:29:18x2 000)

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Maximum: 5.0  5.0  50.0

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$^{1}H$ NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (125 MHz, CDCl$_3$)
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = 1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
13729 formula(e) evaluated with 68 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-500  H: 0-1000  N: 0-4  O: 0-40  23Na: 0-1  Si: 0-1

BEN SWARTS
BMX-42
LCT2006-07b pro 2010-off.spl
2010_0727_1149 15 (0.300) Cm (15.25-1.8x2.000)

Minimum: 5.0 5.0  -1.5
Maximum: 100.0

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1670.7381  0.1  0.1  36.5  65.6  6.9  C90 H113 N3 O24

1H NMR (500 MHz, CDCl3)

64

P=O-OCOC17H38
OCOC17H38

Compound 64
HMOC (500MHz)
### Elemental Composition Report

**Single Mass Analysis**

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

**Monoisotopic Mass, Even Electron Ions**

2803 formula(e) evaluated with 34 results within limits (all results up to 10000 for each mass)

Elements Used:

- C: 195-210
- H: 0-1000
- N: 4-7
- O: 45-55
- 23Na: 0-1
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**B.M.S. IX-44**

**LCT Premier 24-Aug-2010 14:12:36**

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**Notes:**

- Minimum: 100.0
- Maximum: 100.0

**Formula:** Compound 64
Comment 1  BMS-IX-46
Comment 2  Man-III 6-O-Phosphorylation

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Compound 80
MALDI TOF MS

Expansion
Azido-GPI 3

$^1$H NMR (500 MHz, CDCl$_3$-CD$_3$OD-D$_2$O 3:3:1)

Azido-GPI 3

$^{31}$P NMR (160 MHz, CDCl$_3$-CD$_3$OD-D$_2$O 3:3:1)
Azido-GPI 3
MALDI TOF MS

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Expansion

[M-H]^-

[M-2H+Na]^−
MALDI TOF MS monitoring of Cu-catalyzed click reaction to form GPI-Fluor 4

Cu-catalyzed click reaction after 3 days at 37 °C

GPI-Fluor 4

Alkynyl-GPI 2

Expansion of above spectrum

GPI-Fluor 4

Electronic Supplementary Material (ESI) for Chemical Science
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MALDI TOF MS monitoring of Cu-free click reaction to form GPI-Biotin 5

**Comment 1:** BMS-IX-49 1 day CHCl3-MeOH-H2O Neg
**Comment 2:** Cu-Free Click

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starting material
Azido-GPI 3
Cu-free click reaction after standing ~24 h
GPI-Biotin 5

**Comment 1:** BMS-IX-49 2 days CHCl3-MeOH-H2O
**Comment 2:** DHB Negative

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triazole coupling product
GPI-Biotin 5
starting material consumed
Azido-GPI 3

Cu-free click reaction after standing ~48 h