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Synthesis of lipid A type carboxymethyl derivatives with ether chains instead of ester chains and their LPS-antagonistic activities

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Abstract

Synthesis of lipid A type carboxymethyl derivatives having ether chains at both the C-3 and C-3′ positions and their LPS-antagonistic activities toward human U937 cells are described.

Keywords: Lipid A; Endotoxin; LPS-agonist; LPS-antagonist; TNFα production inhibitor

1. Introduction

Endotoxin (lipopolysaccharide; LPS)[1] is a toxic substance from Gram-negative bacteria and one of the components of their outer surface membrane. A variety of responses, both beneficial and harmful, can be elicited by LPS. It is also a highly potent stimulator of the immune system.[2] Therefore, LPS and its related compounds have mainly been investigated as anti-cancer drugs[3] that function as LPS-agonists by activating macrophages. Most of the biological activities of LPS reside in a relatively small portion of the molecule, that is, the terminal disaccharide phospholipid subunit known as lipid A,[4] which is a hydrophobic anchor substance holding an essentially linear polysaccharide chain to the cell wall. Many lipid A type disaccharide analogues were synthesized to investigate their biological activities.[5] Monosaccharide analogues of both the non-reducing distal subunit and the reducing sugar part of Lipid A are usually still biologically active.[6] However, many of these compounds usually show fatal endotoxic shock (bacterial sepsis) caused as a consequence of acute inflammatory response.

In recent years, lipid A-related compounds have been studied as LPS-antagonists,[7] which may have potential as immunosuppressants,[8] and in autoimmune diseases[8] and septicemia[9] by deactivating LPS-induced aggressive macrophages. For example, Qureshi's group[10] isolated a non-toxic lipid A-related compound from *Rhodobacter sphaeroides* as an LPS antagonist, and an Eisai group recently developed a related compound, E5564,[9,11] as a highly potent anti-septicemia drug.

On the other hand, during our investigation of the biological activities of compounds related to GLA-60,[6a] which is a non-reducing distal subunit analogue in the Lipid A molecule, we also found that most of them had LPS-agonistic activity, but a few of them behaved as LPS antagonists. The α anomeric carboxymethyl GLA-60 analogue **A**[12] exhibited fairly strong LPS-antagonistic activity ($IC_{50} \approx 5$ nM), and also lipid A-type disaccharide **B**[13] constructed from anomeric pyrancarboxylic acid and *O*-ether side chains showed a strong LPS-antagonistic activity ($IC_{50} = 0.6$ nM) toward human U937 cells. Therefore, we are interested in the activity of lipid A type α-carboxymethyl derivatives, which have partially each component of compounds **A** and **B**. And also we anticipated that the *O*-ether chains instead of ester chains would stabilize the compound

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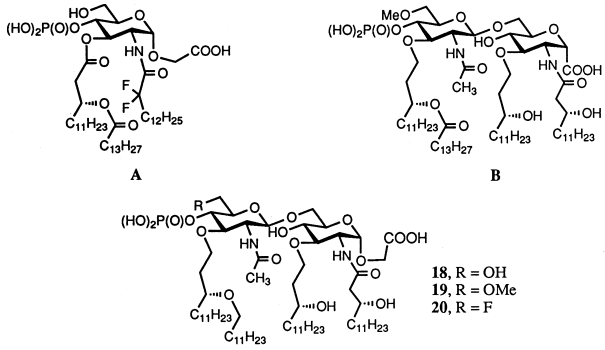


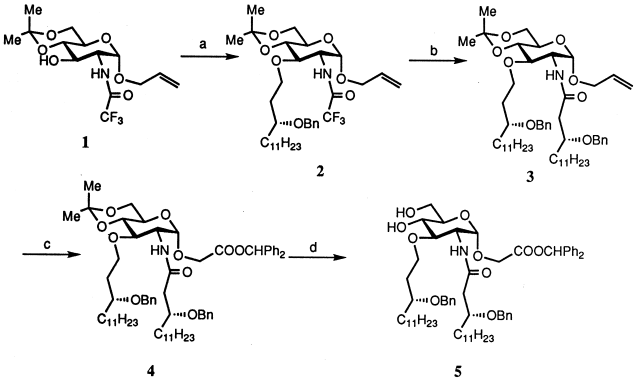
Fig. 1. Structures of compounds **A**, **B**, **18**, **19** and **20**.

and increase the activity. Herein we report the synthesis of compounds **18**, **19** and **20** and their activities (Fig. 1).

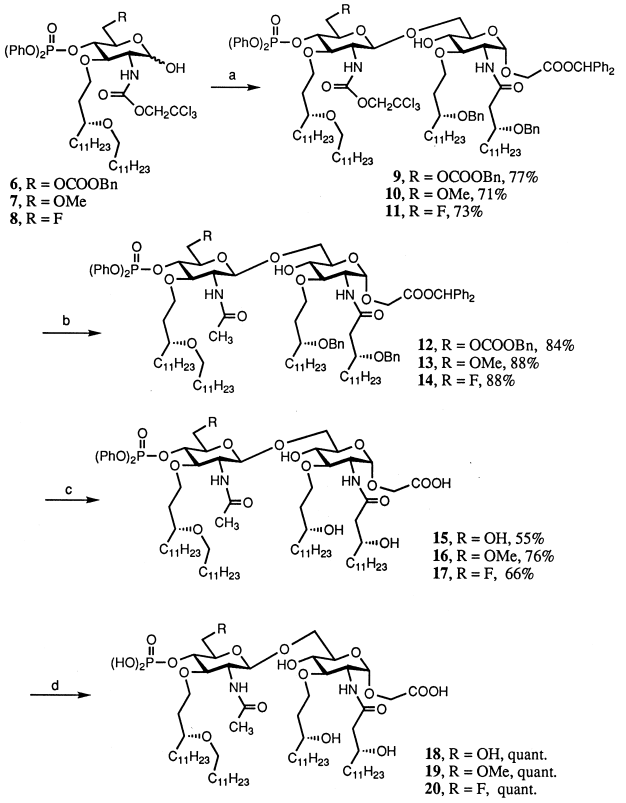
2. Results and discussion

Firstly, the carboxymethyl glucosamine derivative **5** on the right half moiety was synthesized from trifluoroacetamide **1** as shown in Scheme 1. Compound **1**[14] was alkylated with (*R*)-3-benzyloxy-1-(methylsulfonyloxy)tetradecane[15] and NaH in *N*,*N*-dimethylformamide (DMF) to yield ether **2**, and the protecting trifluoroacetyl group was cleaved by aqueous NaOH at 60 °C, and then the liberated amine was treated with (*R*)-3-benzyloxytetradecanoic acid using dicyclohexylcarbodiimide (DCC) as a dehydrating agent and 4-dimethylaminopyridine (DMAP) as a catalyst to give amide **3**. The double bond of allyl group was oxidized with OsO_4 and 4-methylmorpholine *N*-oxide (NMO), and the liberated vicinal diol was cleaved with $Pb(OAc)_4$ to afford the aldehyde, which was further oxidized to carboxylic acid by $NaClO_2$ according to the reported procedure.[5] Finally the carboxylic acid was esterified with Ph_2CN_2 to give benzhydryl ester **4** in four steps in 76% yield. The isopropylidene group was deprotected with aqueous 80% AcOH at 60 °C to give diol **5**.

Secondly, the left half compounds **6**, **7** and **8** synthesized from a common starting material **1** according to the reported method[13] were treated with trichloroacetonitrile using Cs_2CO_3 as a catalyst to yield corresponding imidates, which were reacted with the diol **5** obtained above to give corresponding disaccharides **9**, **10** and **11** as shown in Scheme 2 according to the reported method.[4a,4c] Treatment of each compound (**9**, **10** or **11**) with Zn–acetic acid, and successive acetylation with acetic anhydride–pyridine gave the corresponding acetamide (**12**, **13** or **14**, respectively), which was hydrogenolyzed with 20% $Pd(OH)_2$–C to give the acid (**15**, **16** or **17**, respectively). Finally, each acid (**15**, **16** or **17**) was treated with hydrogen using Pt as a catalyst to give phosphoric acid (**18**, **19** or **20**, respectively).



Scheme 1. Reagents and conditions: (a) (*R*)-3-benzyloxy-1-(methylsulfonyloxy)tetradecane, NaH, DMF, rt, 6 h, 74%; (b) (1) 1 M aq NaOH, EtOH, 60 °C, 5 h; (2) (*R*)-3-(benzyloxy)tetradecanoic acid, DCC, DMAP, CH_2Cl_2, rt, 18 h, two steps 89%; (c) (1) OsO_4, NMO, THF–*t*-BuOH–H_2O, rt, 3 h; (2) $Pb(OAc)_4$, benzene, rt, 1 h; (3) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH–H_2O, rt, 18 h; (4) Ph_2CN_2, EtOAc, rt, 18 h, four steps 76%; (d) 80% aq AcOH, 60 °C, 4 h, 86%.



Scheme 2. Reagents and conditions: (a) (1) Cl_3CCN, cat. Cs_2CO_3, CH_2Cl_2, rt, 1 h; (2) **5**, TMSOTf, MS 4 Å, CH_2Cl_2, −40 °C, 1 h; (b) (1) Zn, AcOH, rt, 2 h; (2) Ac_2O, pyridine, THF–H_2O, rt, 1 h; (c) H_2, 20% $Pd(OH)_2$–C, EtOH, rt, 18 h; (d) H_2, PtO_2, THF, rt, 20 h.

2.1. Biological activity

The inhibitory activity of compounds **18**, **19** and **20** on LPS-induced TNFα production was investigated in

vitro using human monoblastic U937 cells. Compounds **18**, **19** and **20**, which have four chains in their molecules, inhibited TNFα production as LPS-antagonists toward human monoblastic U937 cells, and the IC_{50} values of these three compounds were 6.5, 6.1 and 12.4 nM, respectively. Judging from the results reported in Ref. 13 (that is, the IC_{50} values of the corresponding pyran–carboxylic acid analogues of compounds **18**, **19** and **20** were 11, 6.4 and 10 nM, respectively), the values were almost the same. Therefore, the difference of the anomeric substituents between the pyrancarboxylic acid type acid in Ref. 13 and the carboxymethyl type acid in this paper did not greatly affect the inhibitory activity toward human monoblastic U937 cells. The C-6 methoxy group[11] of compound **19** did not enhance the activity compared with C-6 hydroxyl group of compound **18**. The C-6 fluoride[16] of compound **20** weakened the activity in comparison with compounds **18** and **19**. However, the LPS-antagonistic activity of these compounds was much less than that of compound **B** ($IC_{50} = 0.6$ nM). The structural feature of compound **B** possessed an ester side chain in 3-(tetradecanoyloxy)tetradecyl group instead of the corresponding ether side chain. The existence of an ester bond in the side chain in the 3-position may suggest a significant role for this activity.

3. Experimental

Melting points are uncorrected. Optical rotations were obtained by the use of a JASCO P-1030 polarimeter. IR absorption spectra were recorded on a JASCO IR A-2 spectrophotometer. ^{1}H NMR spectra were recorded with a JEOL-GSX 400 spectrometer using Me_4Si as an internal standard, and mass spectra were obtained with a JMS-700 mass spectrometer. Separation of the compounds by column chromatography was carried out with silica gel 60 (E. Merck, 0.040–0.063 mm) at slightly elevated pressure (1.1–1.8 atm) for easy elution, and the quantity of the used silica gel was 50–100 times the weight of the purified compounds. Thin-layer chromatography was performed on E. Merck silica gel 60-F_{254} (cat. no. 5715) plates. Tetrahydrofuran was distilled in the presence of radical anions generated by Na–benzophenone ketyl. Dichloromethane was dried by being passed through an ICN Alumina B-Super I, and DMF and pyridine were dried by storage over 4 Å molecular sieves.

3.1. Allyl 3-*O*-[(*R*)-3-(benzyloxy)tetradecyl]-2-deoxy-4,6-*O*-isopropylidene-2-trifluoroacetamido-α-D-glucopyranoside (2)

To a solution of allyl 2-deoxy-4,6-*O*-isopropylidene-2-trifluoroacetamido-α-D-glucopyranoside (**1**) (3.53 g, 9.93 mmol) in DMF (40 mL) was gradually added NaH (60% oil dispersion, 482 mg, 12.0 mmol) at 0 °C with stirring. After 15 min, (*R*)-3-benzyloxy-1-methylsulfonyloxytetradecane (3.31 g, 8.30 mmol) was added to this solution, which was stirred at room temperature (rt) for 6 h. The reaction mixture was quenched with water, extracted with EtOAc, washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture that was chromatographed on a silica gel column. Elution with 4:1 C_6H_{14}–EtOAc gave **2** (4.05 g, 74%). IR (cm^{-1}): $\nu_{max}(CHCl_3)$ 3430, 2928, 2856, 1734. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (3 H, t, *J* 6.6 Hz), 1.26 (18 H, brs), 1.40 (3 H, s), 1.48 (3 H, s), 1.51–1.75 (4 H, m), 3.43–3.49 (2 H, m), 3.59 (1 H, m), 3.66–3.69 (2 H, m), 3.75 (1 H, t, *J* 10.3 Hz), 3.84–3.91 (2 H, m), 3.98 (1 H, dd, *J* 5.9, 12.5 Hz), 4.14–4.20 (2 H, m), 4.42, 4.51 (2 H, AB-q, *J* 11.7 Hz), 4.86 (1 H, d, *J* 3.7 Hz), 5.25–5.31 (2 H, m), 5.87 (1 H, m), 6.42 (1 H, d, *J* 9.5 Hz, NH), 7.26–7.34 (5 H, m). FABMS (positive-ion): *m*/*z* 658 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{35}H_{55}F_3NO_7$: 658.3931. Found: 658.3904. Anal. Calcd for $C_{35}H_{54}F_3NO_7$ (657.8): C, 63.91; H, 8.28; F, 8.66; N, 2.13. Found: C, 64.09; H, 8.30; F, 8.46; N, 2.11.

3.2. Allyl 2-[(*R*)-3-(benzyloxy)tetradecanamido]-3-*O*-[(*R*)-3-(benzyloxy)tetradecyl]-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside (3)

A solution of **2** (2.73 g, 4.15 mmol) in EtOH (10 mL) and 1 M aq NaOH (10 mL) was stirred at 60 °C for 5 h. The solution was concentrated in vacuo, diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, filtered, and concentrated to give an amine, which was dissolved in CH_2Cl_2 (30 mL). (*R*)-3-(Benzyloxy)tetradecanoic acid (1.50 g, 4.48 mmol), DCC (963 mg, 4.67 mmol), and DMAP (574 mg, 4.70 mmol) were added to this solution, which was stirred for 18 h at rt, filtered, and concentrated in vacuo to give a mixture. The mixture was chromatographed on a silica gel column. Elution with 4:1 C_6H_{14}–EtOAc gave **3** (3.25 g, 89%) as a gum. IR (cm^{-1}): $\nu_{max}(CHCl_3)$ 3441, 3363, 2928, 2856, 1666. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (6 H, t, *J* 6.6–7.3 Hz), 1.24–1.81 (48 H, m, containing two 3 H, s, at 1.46 and 1.38 ppm), 2.32 (1 H, dd, *J* 7.3, 14.6 Hz), 2.43 (1 H, dd, *J* 3.7, 14.6 Hz), 3.40 (1 H, t, *J* 10.3 Hz), 3.45–3.56 (2 H, m), 3.61–3.85 (7 H, m), 4.04 (1 H, dd, *J* 5.9, 12.5 Hz), 4.20 (1 H, td, *J* 9.5, 3.7 Hz), 4.45, 4.47 (2 H, AB-q, *J* 11.0 Hz), 4.51, 4.54 (2 H, AB-q, *J* 11.0 Hz), 4.78 (1 H, d, *J* 3.7 Hz), 5.12–5.23 (2 H, m), 5.77 (1 H, m), 6.40 (1 H, d, *J* 9.5 Hz, NH), 7.26–7.35 (10 H, m). FABMS (positive-ion): *m*/*z* 900 $(M + Na)^+$, 878 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{54}H_{88}NO_8$: 878.6510. Found: 878.6506. Anal. Calcd for $C_{54}H_{87}NO_8$ (878.3): C, 73.86; H, 9.98; N, 1.59. Found: C, 73.92; H, 9.84; N, 1.67.

3.3. (Diphenylmethoxycarbonyl)methyl 2-[(*R*)-3-(benzyloxy)tetradecanamido]-3-*O*-[(*R*)-3-(benzyloxy)tetradecyl]-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside (4)

To a solution of **3** (2.80 g, 3.19 mmol) in THF (10 mL)–*t*-BuOH (10 mL)–water (1 mL) were added 4-methylmorpholine *N*-oxide (1.12 g, 9.56 mmol) and OsO_4 in *t*-BuOH (2.5%, 6.5 mL, 0.518 mmol). After vigorous stirring for 3 h at rt, the mixture was quenched with satd aq $Na_2S_2O_3$, and extracted with EtOAc. The organic layer was washed with satd aq $Na_2S_2O_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo to give the crude diol, which was used without further purification for the following oxidation.

The crude diol thus obtained was dissolved in C_6H_6 (20 mL). To this solution was added $Pb(OAc)_4$ (1.88 g, 3.82 mmol). After stirring for 1 h at rt, the mixture was filtered through a silica gel column using EtOAc as an eluent. After removal of the solvent in vacuo, the crude aldehyde was dissolved in *t*-BuOH (16 mL) and water (4 mL). To this solution was added NaH_2PO_4 (561 mg, 3.60 mmol), 2-methyl-2-butene (1.06 g, 15.1 mmol), and $NaClO_2$ (1.05 g, 9.17 mmol) at rt. After 18 h, the reaction mixture was acidified with 1 M aq HCl and extracted with EtOAc. The extract was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give a residue, which was dissolved in EtOAc (20 mL). To this solution was added Ph_2CN_2 (1.15 g, 5.92 mmol) at rt. After stirring for 18 h, the reaction mixture was quenched with AcOH and concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with 9:1 → 7:3 C_6H_{14}–EtOAc gave **4** (2.57 g, 76%) as a solid. IR (cm^{-1}): ν_{max}($CHCl_3$) 3692, 3360, 2927, 2855, 1755, 1666. ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (6 H, t, *J* 6.6 Hz), 1.25–1.79 (48 H, m, containing two 3 H, s, at 1.44 and 1.36 ppm), 2.36 (1 H, dd, *J* 6.6, 14.6 Hz), 2.43 (1 H, dd, *J* 4.4, 14.6 Hz), 3.38–3.57 (3 H, m), 3.63–3.84 (6 H, m), 4.01 (2 H, s), 4.22 (1 H, td, *J* 10.3, 3.7 Hz), 4.44 (2 H, s), 4.46 (2 H, s), 4.73 (1 H, d, *J* 3.7 Hz), 6.74 (1 H, d, *J* 9.5 Hz, NH), 6.91 (1 H, s), 7.19–7.36 (20 H, m). FABMS (positive-ion): *m*/*z* 1084 $(M + Na)^+$, 1062 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{66}H_{96}NO_{10}$: 1062.7034. Found: 1062.7009. Anal. Calcd for $C_{66}H_{95}NO_{10}$ (1062.5): C, 74.61; H, 9.01; N, 1.32. Found: C, 74.26; H, 8.72; N, 1.35.

3.4. (Diphenylmethoxycarbonyl)methyl 2-[(*R*)-3-(benzyloxy)tetradecanamido]-3-*O*-[(*R*)-3-(benzyloxy)tetradecyl]-2-deoxy-α-D-glucopyranoside (5)

A solution of **4** (2.35 g, 2.21 mmol) in 80% aq AcOH (20 mL) was stirred at 60 °C for 4 h. The solution was diluted with EtOAc, washed with aq $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, concentrated in vacuo, and chromatographed on a silica gel column. Elution with 2:3 C_6H_{14}–EtOAc gave **5** (1.95 mg, 86%) as a white powder. IR (cm^{-1}): ν_{max}(KBr) 3319, 3065, 3033, 2925, 2854, 1756, 1642. ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (6 H, t, *J* 6.6 Hz), 1.26–1.78 (42 H, m), 2.40–2.43 (2 H, m), 3.40 (1 H, t, *J* 9.5 Hz), 3.46–3.56 (3 H, m), 3.62–3.75 (4 H, m), 3.84 (1 H, m), 4.06 (2 H, s), 4.19 (1 H, td, *J* 10.3, 3.7 Hz), 4.40–4.48 (4 H, m), 4.74 (1 H, d, *J* 3.7 Hz), 6.83 (1 H, d, *J* 9.5 Hz, NH), 6.92 (1 H, s), 7.20–7.37 (20 H, m). FABMS (positive-ion): *m*/*z* 1060 $(M + K)^+$ (on addition of KI), 1044 $(M + Na)^+$, 1022 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{63}H_{91}KNO_{10}$: 1060.6280. Found: 1060.6273. Anal. Calcd for $C_{63}H_{91}NO_{10}$ (1022.4): C, 74.01; H, 8.97; N, 1.37. Found: C, 73.82; H, 8.90; N, 1.48.

3.5. (Diphenylmethoxycarbonyl)methyl 6-*O*-[6-*O*-benzyloxycarbonyl-2-deoxy-4-*O*-diphenylphosphono-3-*O*-[(*R*)-3-(dodecyloxy)tetradecyl]-2-[(2,2,2-trichloroethoxycarbonyl)amino]-β-D-glucopyranosyl]-2-[(*R*)-3-(benzyloxy)tetradecanamido]-3-*O*-[(*R*)-3-(benzyloxy)tetradecyl]-2-deoxy-α-D-glucopyranoside (9)

To a solution of **6** (352 mg, 0.320 mmol) in CH_2Cl_2 (5 mL) were added Cl_3CCN (0.32 mL, 3.20 mmol) and Cs_2CO_3 (53 mg, 0.163 mmol). After stirring for 1 h at rt, the reaction mixture was quenched with satd aq $NaHCO_3$ (40 mL), and extracted with EtOAc. The extract was washed with brine and dried over Na_2SO_4. Removal of the solvent in vacuo gave the crude imidate (399 mg), which was immediately used for subsequent glycosylation without further purification. In a nitrogen atmosphere, a solution of the imidate (339 mg) thus obtained, diol **5** (299 mg, 0.292 mmol), and molecular sieves 4 Å (420 mg) in CH_2Cl_2 (5 mL) was stirred at rt. After stirring for 1 h, a catalytic amount of TMSOTf (6 μL, 0.033 mmol) was added to the mixture at −40 °C. After stirring for 1 h at −40 °C, the mixture was quenched with satd aq $NaHCO_3$, diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with 3:2 C_6H_{14}–EtOAc gave **9** (471 mg, 77%) as a gum. IR (cm^{-1}): ν_{max}($CHCl_3$) 3442, 2928, 2855, 1748, 1667. ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (12 H, t, *J* 6.6 Hz), 1.25–1.77 (84 H, m), 2.36–2.38 (2 H, m), 3.08 (1 H, brs, OH), 3.20–3.27 (4 H, m), 3.33 (1 H, t, *J* 9.5 Hz), 3.41–3.45 (2 H, m), 3.57–3.76 (7 H, m), 3.82 (1 H, m), 3.89–3.96 (2 H, m), 4.04 (2 H, s), 4.17 (1 H, td, *J* 10.3, 3.7 Hz), 4.23 (1 H, dd, *J* 5.1, 12.5 Hz), 4.36–4.56 (6 H, m, containing 1 H, d, *J* 3.7 Hz, δ 4.43), 4.67–4.74 (3 H, m), 4.86 (1 H, m), 5.04, 5.09 (2 H, AB-q, *J* 12.5 Hz), 5.52 (1 H, m), 6.75 (1 H, d, *J* 9.5 Hz, NH), 6.89 (1 H, s), 7.11–7.34 (35 H, m). FABMS (positive-ion): *m*/*z* 2141 $(M + K)^+$ (on addition of KI),

2125 $(M + Na)^+$. HRFABMS (positive-ion); Calcd for $C_{118}H_{170}Cl_3KN_2O_{22}P$: 2142.0686. Found: 2142.0625. Anal. Calcd for $C_{118}H_{170}Cl_3N_2O_{22}P$ (2105.9): C, 67.30; H, 8.14; Cl, 5.05; N, 1.33; P, 1.47. Found: C, 67.10; H, 7.95; Cl, 5.02; N, 1.31; P, 1.53.

3.6. (Diphenylmethoxycarbonyl)methyl 2-[(*R*)-3-(benzyloxy)tetradecanamido]-3-*O*-[(*R*)-3-(benzyloxy)tetradecyl]-2-deoxy-6-*O*-[2-deoxy-4-*O*-diphenylphosphono-3-*O*-[(*R*)-3-(dodecyloxy)tetradecyl]-6-*O*-methyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-α-D-glucopyranoside (10)

Compound **7** (410 mg, 0.418 mmol) was treated as described in the formation of **9** from **6** to give **10** (537 mg, 71%) as a gum. IR (cm^{-1}): $\nu_{max}(CHCl_3)$ 3441, 2928, 2855, 1747, 1667. ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (12 H, t, *J* 6.6 Hz), 1.25–1.78 (84 H, m), 2.36–2.37 (2 H, m), 3.12–3.29 (8 H, m, containing 3 H, s, δ 3.20), 3.34 (1 H, t, *J* 9.5 Hz), 3.46–3.97 (14 H, m), 4.04 (2 H, s), 4.17 (1 H, td, *J* 10.3, 3.7 Hz), 4.38–4.48 (4 H, m), 4.55 (1 H, q, *J* 9.5 Hz), 4.71–4.74 (3 H, m), 4.84 (1 H, m), 5.45 (1 H, m), 6.74 (1 H, d, *J* 9.5 Hz, NH), 6.90 (1 H, s), 7.15–7.36 (30 H, m). FABMS (positive-ion): *m*/*z* 2005 $(M + Na)^+$. HRFABMS (positive-ion); Calcd for $C_{111}H_{166}Cl_3N_2NaO_{20}P$: 2006.0735. Found: 2006.0745. Anal. Calcd for $C_{111}H_{166}Cl_3N_2O_{20}P$ (1985.84): C, 67.14; H, 8.43; Cl, 5.36; N, 1.41; P, 1.56. Found: C, 67.19; H, 8.11; Cl, 5.59; N, 1.68; P, 1.68.

3.7. (Diphenylmethoxycarbonyl)methyl 2-[(*R*)-3-(benzyloxy)tetradecanamido]-3-*O*-[(*R*)-3-(benzyloxy)tetradecyl]-2-deoxy-6-*O*-[2,6-dideoxy-4-*O*-diphenylphosphono-3-*O*-[(*R*)-3-(dodecyloxy)tetradecyl]-6-fluoro-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-α-D-glucopyranoside (11)

Compound **8** (420 mg, 0.433 mmol) was treated as described in the formation of **9** from **6** to give **11** (570 mg, 73%) as a gum. IR (cm^{-1}): $\nu_{max}(CHCl_3)$ 3443, 3358, 2928, 2855, 1745, 1667. ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (12 H, t, *J* 6.6 Hz), 1.25–1.77 (84 H, m), 2.33–2.38 (2 H, m), 3.21–3.27 (5 H, m), 3.34 (1 H, t, *J* 9.5 Hz), 3.43 (1 H, t, *J* 9.5 Hz), 3.48–3.75 (8 H, m), 3.82 (1 H, m), 3.90–4.05 (2 H, m), 4.05 (2 H, s), 4.17 (1 H, td, *J* 10.3, 3.7 Hz), 4.37–4.59 (7 H, m), 4.71–4.73 (3 H, m), 4.90 (1 H, m), 5.54 (1 H, m), 6.77 (1 H, d, *J* 9.5 Hz, NH), 6.90 (1 H, s), 7.17–7.35 (30 H, m). FABMS (positive-ion): *m*/*z* 1993 $(M + Na)^+$. HRFABMS (positive-ion); Calcd for $C_{110}H_{163}Cl_3FN_2NaO_{19}P$: 1994.0535. Found: 1994.0587. Anal. Calcd for $C_{110}H_{163}Cl_3FN_2O_{19}P$ (1973.8): C, 66.94; H, 8.32; Cl, 5.39; N, 1.42; P, 1.57. Found: C, 67.03; H, 8.24; Cl, 5.36; N, 1.43; P, 1.65.

3.8. (Diphenylmethoxycarbonyl)methyl 6-*O*-[2-acetamido-6-*O*-benzyloxycarbonyl-2-deoxy-4-*O*-diphenylphosphono-3-*O*-[(*R*)-3-(dodecyloxy)tetradecyl]-β-D-glucopyranosyl]-2-[(*R*)-3-(benzyloxy)tetradecanamido]-3-*O*-[(*R*)-3-(benzyloxy)tetradecyl]-2-deoxy-α-D-glucopyranoside (12)

To a solution of **9** (271 mg, 0.128 mmol) in AcOH (3 mL) was added Zn dust (168 mg, 2.57 mmol). After vigorous stirring for 2 h at rt, the solution was filtered to remove Zn and concentrated in vacuo to give a crude product. The product was diluted with EtOAc, washed with satd aq $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give a crude product, which was dissolved in THF (2 mL). Water (1 mL), pyridine (52 μL) and Ac_2O (60 μL) were added to this solution. After stirring for 1 h at rt, the mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with 1:1 C_6H_{14}–EtOAc gave **12** (212 mg, two steps, 84%) as a gum. IR (cm^{-1}): $\nu_{max}(CHCl_3)$ 3450, 2928, 2855, 1751, 1668. ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (12 H, t, *J* 6.6 Hz), 1.20–1.75 (84 H, m), 1.95 (3 H, s), 2.36 (2 H, d, *J* 6.6 Hz), 3.08 (1 H, m), 3.24–3.36 (4 H, m), 3.46–3.48 (3 H, m, containing OH), 3.62–3.73 (7 H, m), 3.83 (1 H, m), 3.94 (1 H, m), 4.02–4.06 (3 H, m, containing 2 H, s, at 4.02 ppm), 4.15–4.23 (2 H, m), 4.36–4.51 (6 H, m), 4.73 (1 H, d, *J* 3.7 Hz), 5.04, 5.09 (2 H, AB-q, *J* 12.1 Hz), 5.22 (1 H, d, *J* 8.1 Hz), 6.16 (1 H, d, *J* 6.6 Hz, NH), 6.72 (1 H, d, *J* 9.5 Hz, NH), 6.89 (1 H, s), 7.11–7.34 (35 H, m). FABMS (positive-ion): *m*/*z* 1993 $(M + Na)^+$. HRFABMS (positive-ion); Calcd for $C_{117}H_{171}N_2NaO_{21}P$: 1994.2010. Found: 1994.2020. Anal. Calcd for $C_{117}H_{171}N_2O_{21}P$ (1972.6): C, 71.24; H, 8.74; N, 1.42; P, 1.57. Found: C, 71.70; H, 8.69; N, 1.39; P, 1.56.

3.9. (Diphenylmethoxycarbonyl)methyl 6-*O*-[2-acetamido-2-deoxy-4-*O*-diphenylphosphono-3-*O*-[(*R*)-3-(dodecyloxy)tetradecyl]-6-*O*-methyl-β-D-glucopyranosyl]-2-[(*R*)-3-(benzyloxy)tetradecanamido]-3-*O*-[(*R*)-3-(benzyloxy)tetradecyl]-2-deoxy-α-D-glucopyranoside (13)

Compound **10** (340 mg, 0.171 mmol) was treated as described in the formation of **12** from **9** to give **13** (280 mg, 88%) as a gum. IR (cm^{-1}): $\nu_{max}(CHCl_3)$ 3692, 3451, 3363, 2928, 2855, 1755, 1668. ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (12 H, t, *J* 6.6 Hz), 1.20–1.76 (84 H, m), 1.96 (3 H, s), 2.36 (2 H, d, *J* 5.9 Hz), 3.14–3.20 (4 H, m, containing 3 H, s, at 3.20 ppm), 3.22–3.37 (5 H, m), 3.46–3.77 (11 H, m, containing OH), 3.83 (1 H, m), 3.96–4.05 (4 H, m, containing 2 H, s, at 4.02 ppm), 4.18 (1 H, dt, *J* 10.3, 3.7 Hz), 4.38–4.45 (4 H, m), 4.51 (1 H, q, *J* 9.5 Hz), 4.73 (1 H, d, *J* 3.7 Hz), 5.16 (1 H,

d, *J* 8.1 Hz), 6.09 (1 H, d, *J* 6.6 Hz, NH), 6.73 (1 H, d, *J* 9.5 Hz, NH), 6.89 (1 H, s), 7.16–7.36 (30 H, m). FABMS (positive-ion): *m*/*z* 1873 $(M + Na)^+$. HR-FABMS (positive-ion); Calcd for $C_{110}H_{167}N_2NaO_{19}P$: 1874.1798. Found: 1874.1810. Anal. Calcd for $C_{110}H_{167}N_2O_{19}P$ (1852.5): C, 71.32; H, 9.09; N, 1.51; P, 1.67. Found: C, 71.68; H, 9.18; N, 1.54; P, 1.72.

3.10. (Diphenylmethoxycarbonyl)methyl 6-*O*-[2-acetamido-2,6-dideoxy-4-*O*-diphenylphosphono-3-*O*-[(*R*)-3-(dodecyloxy)tetradecyl]-6-fluoro-β-D-glucopyranosyl]-2-[(*R*)-3-(benzyloxy)tetradecanamido]-3-*O*-[(*R*)-3-(benzyloxy)tetradecyl]-2-deoxy-α-D-glucopyranoside (14)

Compound **11** (339 mg, 0.172 mmol) was treated as described in the formation of **12** from **9** to give **14** (279 mg, 88%) as a gum. IR (cm^{-1}): ν_{max}($CHCl_3$) 3692, 3451, 3360, 2928, 2855, 1754, 1669. ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (12 H, t, *J* 6.6 Hz), 1.20–1.77 (84 H, m), 1.95 (3 H, s), 2.36–2.37 (2 H, m), 3.08 (1 H, m), 3.26–3.37 (5 H, m), 3.48–3.50 (3 H, m, containing OH), 3.61–3.72 (6 H, m), 3.83 (1 H, m), 3.97 (1 H, m), 4.02 (2 H, d, *J* 4.4 Hz), 4.10 (1 H, dt, *J* 9.5, 10.3 Hz), 4.18 (1 H, dt, *J* 10.3, 3.7 Hz), 4.38–4.58 (7 H, m), 4.73 (1 H, d, *J* 3.7 Hz), 6.18 (1 H, d, *J* 5.9 Hz, NH), 6.75 (1 H, d, *J* 9.5 Hz, NH), 6.89 (1 H, s), 7.17–7.34 (30 H, m). FABMS (positive-ion): *m*/*z* 1862 $(M + Na)^+$. HR-FABMS (positive-ion); Calcd for $C_{109}H_{164}FN_2NaO_{18}P$: 1862.1599. Found: 1862.1622. Anal. Calcd for $C_{109}H_{164}FN_2O_{18}P$ (1840.4): C, 71.13; H, 8.98; F, 1.03; N, 1.52; P, 1.68. Found: C, 71.41; H, 9.01; F, 1.08; N, 1.50; P, 1.77.

3.11. Carboxymethyl 6-*O*-[2-acetamido-2-deoxy-4-*O*-diphenylphosphono-3-*O*-[(*R*)-3-(dodecyloxy)tetradecyl]-β-D-glucopyranosyl]-2-deoxy-2-[(*R*)-3-hydroxytetradecanamido]-3-*O*-[(*R*)-3-hydroxytetradecyl]-α-D-glucopyranoside (15)

A solution of **12** (159 mg, 0.081 mmol) in EtOH (5 mL) containing 20% $Pd(OH)_2$-on-carbon (88.5 mg) was stirred vigorously under hydrogen for 18 h at rt. The reaction mixture was filtered and concentrated in vacuo to give a crude product, which was purified by preparative silica gel thin-layer chromatography developed by 8:1 $CHCl_3$–MeOH to give **15** (65.4 mg, 55%) as an amorphous. IR (cm^{-1}): ν_{max}(CH_3OH) 3430 (broad), 3326 (broad), 2927, 2855, 1729, 1653. ^1H NMR (400 MHz, $CDCl_3$): δ 0.90 (12 H, t, *J* 6.6 Hz), 1.29–1.73 (84 H, m), 2.00 (3 H, s), 2.36–2.41 (2 H, m), 3.26–3.42 (5 H, m), 3.52–3.80 (11 H, m), 3.91 (1 H, m), 4.00–4.03 (2 H, m), 4.12 (1 H, m), 4.10, 4.23 (2 H, AB-q, *J* 16.8 Hz), 4.54 (1 H, m), 4.59 (1 H, d, *J* 7.3 Hz), 4.78 (1 H, d, *J* 3.7 Hz), 7.19–7.40 (10 H, m). FABMS (positive-ion): *m*/*z* 1513 $(M + Na)^+$. HRFABMS (positive-ion); Calcd for $C_{82}H_{143}N_2NaO_{19}P$: 1513.9920. Found: 1513.9908.

3.12. Carboxymethyl 6-*O*-[2-acetamido-2-deoxy-4-*O*-diphenylphosphono-3-*O*-[(*R*)-3-(dodecyloxy)tetradecyl]-6-*O*-methyl-β-D-glucopyranosyl]-2-deoxy-2-[(*R*)-3-hydroxytetradecanamido]-3-*O*-[(*R*)-3-hydroxytetradecyl]-α-D-glucopyranoside (16)

Compound **13** (231 mg, 0.125 mmol) was treated as described in the formation of **15** from **12** to give **16** (143 mg, 76%) as an amorphous. IR (cm^{-1}): ν_{max}(KBr) 3308, 3073, 2924, 2854, 1730, 1657. ^1H NMR (400 MHz, CD_3OD): δ 0.90 (12 H, t, *J* 6.6 Hz), 1.21–1.70 (84 H, m), 2.01 (3 H, s), 2.33–2.43 (2 H, m), 3.21 (3 H, s), 3.22–3.34 (5 H, m), 3.38 (1 H, t, *J* 9.5 Hz), 3.48 (2 H, m), 3.56 (1 H, m), 3.63–3.93 (8 H, m), 3.96–4.05 (2 H, m), 4.05, 4.17 (2 H, AB-q, *J* 16.8 Hz), 4.21 (1 H, m), 4.55 (1 H, q, *J* 8.8 Hz), 4.63 (1 H, d, *J* 8.1 Hz), 4.73 (1 H, d, *J* 2.9 Hz), 7.20–7.41 (10 H, m). FABMS (positive-ion): *m*/*z* 1527 $(M + Na)^+$. HRFABMS (positive-ion); Calcd for $C_{83}H_{145}N_2NaO_{19}P$: 1528.0077. Found: 1528.0076. Anal. Calcd for $C_{83}H_{145}N_2O_{19}P$ (1506.0): C, 66.19; H, 9.71; N, 1.86; P, 2.06. Found: C, 65.86; H, 9.86; N, 1.58; P, 2.00.

3.13. Carboxymethyl 6-*O*-[2-acetamido-2,6-dideoxy-4-*O*-diphenylphosphono-3-*O*-[(*R*)-3-(dodecyloxy)tetradecyl]-6-fluoro-β-D-glucopyranosyl]-2-deoxy-2-[(*R*)-3-hydroxytetradecanamido]-3-*O*-[(*R*)-3-hydroxytetradecyl]-α-D-glucopyranoside (17)

Compound **14** (236 mg, 0.128 mmol) was treated as described in the formation of **15** from **12** to give **17** (125 mg, 66%) as an amorphous. IR (cm^{-1}): ν_{max}(KBr) 3305 (broad), 3074, 2924, 2854, 1731, 1646. ^1H NMR (400 MHz, CD_3OD): δ 0.89 (12 H, t, *J* 6.6 Hz), 1.29–1.70 (84 H, m), 2.00 (3 H, s), 2.33–2.42 (2 H, m), 3.27–3.39 (4 H, m), 3.54 (1 H, t, *J* 11.0–8.8 Hz), 3.65–3.82 (8 H, m), 3.90 (1 H, m), 4.00–4.04 (2 H, m), 4.09–4.27 (4 H, m, containing 2 H, AB-q, *J* 16.8 Hz, δ 4.10, 4.25), 4.36–4.56 (3 H, m), 4.64 (1 H, d, *J* 8.1 Hz), 4.78 (1 H, d, *J* 2.9 Hz), 7.18–7.39 (10 H, m). FABMS (positive-ion): *m*/*z* 1515 $(M + Na)^+$. HRFABMS (positive-ion); Calcd for $C_{82}H_{142}FN_2NaO_{18}P$: 1515.9877. Found: 1515.9871. Anal. Calcd for $C_{82}H_{142}FN_2O_{18}P$ (1494.0): C, 65.92; H, 9.58; F; 1.27; N, 1.88; P, 2.07. Found: C, 66.09; H, 9.60; F, 1.27; N, 1.59; P, 1.96.

3.14. Carboxymethyl 6-*O*-[2-acetamido-2-deoxy-3-*O*-[(*R*)-3-(dodecyloxy)tetradecyl]-4-*O*-phosphono-β-D-glucopyranosyl]-2-deoxy-2-[(*R*)-3-hydroxytetradecanamido]-3-*O*-[(*R*)-3-hydroxytetradecyl]-α-D-glucopyranoside (18)

A solution of **15** (55 mg, 0.037 mmol) in THF (5 mL) containing PtO_2 (28 mg) as a catalyst was stirred vigorously under hydrogen for 20 h at rt. The reaction mixture was filtered and concentrated in vacuo to give

a residue. The residue was dissolved in $CHCl_3$ (5 mL), MeOH (10 mL) and 0.1 M aq HCl (4 mL). To this solution was added another volume of $CHCl_3$ (5 mL) and 0.1 M aq HCl (5 mL) to separate the solution into two phases. The lower $CHCl_3$ phase was collected and concentrated to give **18** (49 mg, 98%) as a white powder: mp 194.0–196.5 °C; $[\alpha]_D^{23}$ −13.0° (*c* 0.3, $CHCl_3$). IR (cm^{-1}): ν_{max}(KBr) 3289 (broad), 3087, 2923, 2853, 1733, 1654. ^1H NMR (400 MHz, 1:1 CD_3OD–$CDCl_3$): δ 0.89 (12 H, t, *J* 6.8 Hz), 1.16–1.55 (82 H, m), 1.74–1.88 (2 H, m), 2.01 (3 H, s), 2.30–2.43 (2 H, m), 3.39–3.47 (5 H, m), 3.54 (1 H, t, *J* 10.7–8.8 Hz), 3.61–3.71 (6 H, m), 3.86–3.87 (5 H, m), 3.98 (1 H, m), 4.05–4.15 (3 H, m), 4.08, 4.25 (2 H, AB-q, *J* 16.6 Hz), 4.61 (1 H, d, *J* 4.9 Hz), 4.77 (1 H, d, *J* 2.9 Hz). FABMS (positive-ion): *m/z* 1361 $(M+Na)^+$, 1339 $(M+H)^+$. HRFABMS (positive-ion); Calcd for $C_{70}H_{135}N_2NaO_{19}P$: 1361.9294. Found: 1361.9294. Anal. Calcd for $C_{70}H_{135}N_2O_{19}P$ (1339.8): C, 62.75; H, 10.16; N, 2.09; P, 2.31. Found: C, 62.64; H, 10.01 N, 2.02; P, 2.21.

3.15. Carboxymethyl 6-*O*-[2-acetamido-2-deoxy-3-*O*-[(*R*)-3-(dodecyloxy)tetradecyl]-6-*O*-methyl-4-*O*-phosphono-β-D-glucopyranosyl]-2-deoxy-2-[(*R*)-3-hydroxytetradecanamido]-3-*O*-[(*R*)-3-hydroxytetradecyl]-α-D-glucopyranoside (19)

Compound **16** (113 mg, 0.075 mmol) was treated as described in the formation of **18** from **15** to give **19** (102 mg, quant) as a white powder: mp. 199–201 °C; $[\alpha]_D^{23}$ +8.3° (*c* 0.3, $CHCl_3$). IR (cm^{-1}): ν_{max}(KBr) 3283 (broad), 3091, 2924, 2854, 1734, 1655. ^1H NMR (400 MHz, 5:1 CD_3OD–$CDCl_3$): δ 0.89 (12 H, t, *J* 6.8 Hz), 1.28–1.59 (82 H, m), 1.75–1.83 (2 H, m), 2.01 (3 H, s), 2.34 (1 H, dd, *J* 8.6, 14.6 Hz), 2.41 (1 H, dd, *J* 3.7, 14.6 Hz), 3.35–3.46 (7 H, m, containing 3 H, s, at 3.41 ppm), 3.52–3.75 (9 H, m), 3.81–3.91 (4 H, m), 3.93–4.11 (4 H, m), 4.07, 4.24 (2 H, AB-q, *J* 16.7 Hz), 4.58 (1 H, d, *J* 8.2 Hz), 4.78 (1 H, d, *J* 3.4 Hz). FABMS (positive-ion): *m/z* 1375 $(M+Na)^+$, 1353 $(M+H)^+$. HRFABMS (positive-ion); Calcd for $C_{71}H_{137}N_2NaO_{19}P$: 1375.9451. Found: 1375.9497. Anal. Calcd for $C_{71}H_{137}N_2O_{19}P$ (1353.8): C, 62.99; H, 10.20; N, 2.07; P, 2.29. Found: C, 62.70; H, 10.37; N, 1.92; P, 2.11.

3.16. Carboxymethyl 6-*O*-[2-acetamido-2,6-dideoxy-3-*O*-[(*R*)-3-(dodecyloxy)tetradecyl]-6-fluoro-4-*O*-phosphono-β-D-glucopyranosyl]-2-deoxy-2-[(*R*)-3-hydroxytetradecanamido]-3-*O*-[(*R*)-3-hydroxytetradecyl]-α-D-glucopyranoside (20)

Compound **17** (85 mg, 0.057 mmol) was treated as described in the formation of **18** from **15** to give **20** (77 mg, quant) as a white powder: mp 215.0–217.0 °C; $[\alpha]_D^{24}$ +19.1° (*c* 0.3, $CHCl_3$). IR (cm^{-1}): ν_{max}(KBr) 3280 (broad), 3093, 2924, 2854, 1733, 1654. ^1H NMR (400 MHz, 5:1 CD_3OD–$CDCl_3$): δ 0.89 (12 H, t, *J* 6.8 Hz), 1.20–1.59 (82 H, m), 1.71–1.83 (2 H, m), 2.01 (3 H, s), 2.34 (1 H, dd, *J* 8.7, 14.6 Hz), 2.41 (1 H, dd, *J* 3.8, 14.6 Hz), 3.38–3.47 (4 H, m), 3.55 (1 H, t, *J* 9.3–10.2 Hz), 3.61–3.69 (5 H, m), 3.75–3.92 (4 H, m), 3.95–4.14 (5 H, m), 4.08, 4.25, (2 H, AB-q, *J* 16.8 Hz), 4.56–4.71 (2 H, m), 4.63 (1 H, d, *J* 7.6 Hz), 4.78 (1 H, d, *J* 3.3 Hz). FABMS (positive-ion): *m/z* 1363 $(M+Na)^+$, 1341 $(M+H)^+$. HRFABMS (positive-ion); Calcd for $C_{70}H_{134}FN_2NaO_{18}P$: 1363.9251. Found: 1363.9291. Anal. Calcd for $C_{70}H_{134}FN_2O_{18}P$ (1341.8): C, 62.66; H, 10.07; F, 1.42; N, 2.09; P, 2.31. Found: C, 62.58; H, 10.05; F, 1.34; N, 1.95; P, 2.14.

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