Synthesis of 3-\(O\)- and 4-\(O\)-(2-aminoethylphosphonono) derivatives of methyl \(L\)-glycero-\(\alpha\)-\(D\)-manno-heptopyranoside

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Abstract Phosphoethanolamine derivatives of the bacterial saccharide \(L\)-glycero-\(\alpha\)-\(D\)-manno-heptose have been prepared using a phosphoramidite-based coupling reaction at position 4 of a side-chain-protected 2,3-\(O\)-orthoester methyl heptoside and at position 3 of a 3,4-diol heptoside, respectively. Global deprotection afforded the corresponding 2-aminoethylphosphodiester derivatives as substrates for crystallographic and binding studies with lectins and antibodies targeting the inner core structure of bacterial lipopolysaccharides.

Graphical abstract

Keywords Lipopolysaccharide · Phosphorylations · Heptose · Carbohydrates · Glycosides

Introduction

The outer membrane of the cell wall of Gram-negative bacteria harbors higher carbon sugars as characteristic components, which occur in the inner core region of bacterial lipopolysaccharides (LPS), but have also been detected in capsular polysaccharides (CPS) [1]. Heptoses of the \(L\)-glycero-\(\alpha\)-\(D\)-manno configuration (\(L\),\(D\)-Hep), in particular, constitute a structurally conserved domain in Enterobacteriaceae, such as in \(Escherichia coli\), \(Salmonella\) or \(Yersinia\), and are common LPS core determinants in the genera \(Haemophilus\), \(Pseudomonas\), \(Helicobacter\), or \(Neisseria\) [2, 3]. Heptoses contribute toward many biomedically important interactions with the complement system, antibodies and lectins, and these features have been substantiated by recent data from crystallographic and glycan array studies [4–9]. These binding interactions and specificities are further modulated by additional phosphate substituents in the pyranose ring as well as at the exocyclic side chain.

Among the phosphate containing appendices, 2-aminoethyl phosphodiester (PEtn) groups have been found at positions 3, 4, 6, and 7 of \(L\),\(D\)-Hep, and the group of O scarson has successfully prepared 4-\(O\)-, 6-\(O\)-, and 7-\(O\)-substituted (2-aminoethyl)phosphate monoheptosides as well as various 3-\(O\)- and 6-\(O\)-PEtn substituted LPS oligosaccharides to unravel the structural basis for cross-reactive antibodies against \(Neisseria meningitidis\) and \(Haemophilus influenzae\), respectively [10–13]. Recently, the structure of an antigen-binding fragment (Fab) from the bactericidal monoclonal antibody LPT3-1 complexed to an inner core octasaccharide fragment of \(N. meningitidis\) has been solved, which had been isolated via KOH treatment from the bacterial lipooligosaccharide [14]. The isolation protocol, however, leads to hydrolysis of the...
phosphoethanolamine units. As the 3-O-PEtn substituent is present in ~70% of *N. meningitidis* strains and constitutes a relevant epitope for the neutralizing antibodies, chemical synthesis is needed to provide material for binding and crystallographic studies [15]. For this purpose, we have set out to access both 3-O- and 4-O-substituted heptosides starting from a common intermediate with a minimum number of protecting group manipulations.

**Results and discussion**

The previously reported 6,7-O-TBDPS protected heptoside 1 served as a versatile precursor for the introduction of the PEtn moiety via intermediate 2,3-orthoester formation as shown for the synthesis of 4-O-monophosphate derivatives [16]. In our hands, a three step procedure in a one-pot reaction could be elaborated to give a fair yield of the phosphotriester derivative 5 (Scheme 1). First, the reaction of 1 with *x*,*x*,*x*-triethoxytoluene (2) in the presence of camphorsulfonic acid (CSA) led to the intermediate orthoester 3, which was followed by the application of the phosphoramidite procedure with [2-(benzyloxy-diisopropylamino-phosphanyloxy)ethyl]-carbamic acid benzyl ester (4) promoted by 1H-tetrazole, and the ensuing oxidation of the resulting phosphate with *meta*-chloroperbenzoic acid (*m*CPBA) [17–19]. Since the phosphorylated orthoester 5 was present as a mixture of four diastereoisomers, the product mixture was then separated into individual components to exclude the presence of potential impurities in the subsequent deprotection steps. MPLC separation allowed the isolation of a 1:3 mixture of the phosphorylated *endo* orthobenzoates 5a, 5b and *exo*-isomers 6a, 6b in 56% overall yield for three steps, followed by further HPLC separation of the phosphate diastereomers; no attempts, however, for assignment of the stereogenic center at phosphorus were undertaken. Assignment of the *endo* configuration was based on the high-field shift of the *exo*-oriented OCH2 group at 3.30 ppm compared to the corresponding low-field shifted signal of the *endo*-isomer at 3.80 ppm [20].

Next, the *endoexo*-orthoester derivatives 5a and 6a (representing one of the diastereomeric forms on phosphorus) were subjected to acid-promoted orthoester opening, which produced the homogeneous 2-O-benzoyl derivative 7 in 91% yield. Compound 7 is equipped with an orthogonal protecting group pattern which allows access to chain elongation at position 3, as well as at the exocyclic side-chain positions. Removal of the 1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl group was achieved by treatment of 7 with triethylamine trifluoride (TREAT). The reaction had to be monitored until full removal of the monofluorinated silyl intermediate (Ref. [16]) by TLC, to afford triol 8 in 83% yield. Hydrogenation of 8 was uneventful and gave the phosphodiester 9 in 93% yield. Cleavage of the benzoyl ester under Zemplén transesterification conditions was sluggish but eventually provided the 4-O-PEtn derivative 10 in good yield. Optical rotation values and 13C NMR data matched the previously reported data of 10, which, however, had been synthesized via a different route based on *H*-phosphonate coupling chemistry [10].

The orthoester approach was then applied for the synthesis of the 3-O-substituted derivative 16 (Scheme 2). 1 was subjected to CSA-promoted orthoester formation with 1,1,1-trimethoxyethane to give 2,3-O-orthoacetate 11, which was not isolated but directly converted into the 2-O-acetate 12 in 71% yield. The structure of ester 12 was readily assigned on the basis of the low-field shifted H-2 signal at 5.02 ppm. Based on previous evidence that a
hydroxyl group adjacent to an axial one in a cis-vicinal diol is more reactive, and that the 4-OH group in a manno-pyranoside is much less reactive, a direct regioselective phosphorylation was expected to directly lead to the 3-O-substituted phosphoester, thereby avoiding additional protecting group manipulations [21, 22]. Thus, phosphorylation of diol 12 using 4 and 1H-tetrazole was followed by oxidation with mCPBA. The 3-O-substituted derivative 13 could then be separated from additional phosphorylated species by chromatography, and was isolated as a diastereomeric mixture in 32% yield.

The structural assignment of 13 was based on the \(^{1}H\)\(^{31}P\) correlated HMBC spectrum, which showed the connectivity of H-3 to the phosphate unit, as well as on the low-field shifted H-3 signal at 4.21 ppm.

Similar to 6, phosphotriester 13 was then treated with TREAT to give triol 14 in 70% yield, which was hydrogenated in the presence of 10% Pd–C in MeOH containing 1% acetic acid. The addition of the acid was needed to prevent formation of N-methylated products [23]. Removal of the 2-O-acetyl group was carried out by reaction of 15 with triethylamine in aqueous MeOH and furnished the deprotected zwitterionic glycoside 16 in 63% yield [24].

The structures of 10 and 16 were fully confirmed by their \(^{13}C\) NMR data, which showed a characteristic downfield shift of the respective carbons C-4 and C-3, respectively, involved in the phosphodiester linkage as well as heteronuclear \(^{1}J_{CP}\) coupling interactions of C-4 and C-5 for 9 and C-3 and C-4 for 16 (Table 1). Similar \(^{1}J_{CP}\) coupling interactions were also seen for the ethanolamine fragments. These assignments were further supported by \(^{1}H\)\(^{31}P\) HMBC connectivity data.

### Experimental Section

All purchased chemicals were used without further purification unless stated otherwise. Solvents were dried over activated 4 Å (CH\(_2\)Cl\(_2\), pyridine) molecular sieves. Cation-exchange resin DOWEX 50 H\(^+\) was regenerated by consecutive washing with HCl (3 M), water, and dry MeOH. Aqueous solutions of salts were saturated unless stated otherwise. Concentration of organic solutions was performed under reduced pressure \(<\)40 °C. Optical rotations were measured with a Perkin-Elmer 243 B Polarimeter. Thin-layer chromatography was performed on Merck precoated plates: generally, on 5 × 10 cm, layer thickness 0.25 mm, silica gel 60F 254; alternatively on HPTLC plates with 2.5 cm concentration zone (Merck). Spots were detected by dipping reagent (anisaldehyde-H\(_2\)SO\(_4\)). For column chromatography, silica gel (0.040–0.063 mm) was used. HPL-column chromatography was performed on prepacked columns (YMC-Pack SIL-06, 0.005 mm, 25 × 1 cm and 25 × 2 cm). NMR spectra were recorded with a Bruker Avance III 600 instrument (600.22 MHz for \(^{1}H\), 150.93 MHz for \(^{13}C\), and 242.97 MHz for \(^{31}P\)) using the standard Bruker NMR software. \(^{1}H\) spectra were referenced to 7.26 (CDCl\(_3\)) and 0.00 (D\(_2\)O, external calibration to 2,2-dimethyl-2-silapentane-5-sulfonic acid) ppm unless stated otherwise. \(^{13}C\) spectra were referenced to 77.00 (CDCl\(_3\)), 49.00 (MeOD), and 67.40 (D\(_2\)O, external calibration to 1,4-dioxane) ppm. \(^{31}P\) spectra were referenced to 0.00 ppm (orthophosphoric acid) for solutions in D\(_2\)O and according to [25] for solutions in CDCl\(_3\), ESI–MS data were obtained on a Waters Micromass Q-TOF Ultima Global instrument.
Methyl 2,3-O-(1-endo-ethoxybenzylidene)-4-O-[benzyl-2-(benzoxycarbonylamino)ethylphosphono]-6,7-O-(1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl)-l-glycero-ω-dmanno-heptopyranoside (5a, 5b, C_{46}H_{68}NO_{14}PS_{12}) and methyl 2,3-O-(1-exo-ethoxybenzylidene)-4-O-[benzyl-2-(benzoxycarbonylamino)ethylphosphono]-6,7-O-(1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl)-l-glycero-ω-dmanno-heptopyranoside (6a, 6b, C_{46}H_{68}NO_{14}PS_{12}) Compound 1 (57.5 mg, 0.123 mmol) was coevaporated with dry toluene, and the residue was dissolved in 1.4 cm³ dry CH₂Cl₂ under Ar. a, a, a-Triethylamine (33.5 mm³, 0.269 mmol) was then added, and the solution was concentrated, and the residue was coevaporated with toluene. The residue was dissolved in 1.4 cm³ dry CH₂Cl₂, and 79.3 mm³ phosphoramidite reagent 4 (0.18 mmol) was added followed by dropwise addition of 0.36 cm³ (of a 0.45 M solution of 1M methyl 2,3-O-(1-exo-ethoxybenzylidene)-4-O-[benzyl-2-(benzoxycarbonylamino)ethylphosphono]-6,7-O-(1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl)-l-glycero-ω-dmanno-heptopyranoside) was added, the solution was concentrated, and the residue was coevaporated with toluene. The residue was dissolved in 1.4 cm³ dry CH₂Cl₂, and 79.3 mm³ phosphoramidite reagent 4 (0.18 mmol) was added followed by dropwise addition of 0.36 cm³ (of a 0.45 M solution of 1H-tetrazole in acetonitrile. Additional amounts of 4 (4 x 30 mm³, 0.277 mmol) and the 0.45 M solution of 1H-tetrazole in acetonitrile (1 x 0.17 cm³ and 3 x 0.13 cm³, 0.269 mmol) were added in four portions over 200 min.

The reaction mixture was then cooled to −78 °C, and a solution of 166 mg meta-chloroperoxybenzoic acid (77% content, 0.739 mmol) in 1.5 cm³ dry CH₂Cl₂ was added and stirred at −78 °C for 45 min. TEA (0.137 cm³) was then added, and the mixture allowed to warm up to rt. The reaction mixture was then transferred into a two-phase solution of aqueous NaHCO₃ and EtOAc (each 20 cm³) followed by phase separation. The aqueous phase was extracted once more with 10 cm³ EtOAc. The combined organic phases were washed with brine, dried (Na₂SO₄), and concentrated. The residue was subjected to chromatography on silica gel (2 g, hexane/EtOAc = 3/1 → 2.5/1, containing 0.5% TEA) which gave a product fraction (67 mg) and a fraction containing byproducts (49 mg). The latter fraction was rechromatographed to afford an additional amount of product (25.9 mg). The combined product fractions were then submitted to MPLC separation (hexane/EtOAc = 3.5/1, containing 0.3% TEA) which afforded a mixture of endo-orthoester 5a and 5b (16.1 mg, 14%) and exo-orthoester 6a and 6b (49.7 mg, 42%) as oils. Both fractions were further separated by HPLC (hexane/EtOAc = 3/1, containing 0.3% TEA) which gave endo-isomer 5a (5.0 mg) and endo-isomer 5b; R₁ = 0.57 (hexane/EtOAc = 1:1); oil.

1H NMR (600 MHz, CDCl₃) for 5a: δ = 7.52–7.48 (m, 2H, HAr), 7.38–7.35 (m, 2H, HAr), 7.35–7.26 (m, 11H, H₃), 5.40 (bs, 1H, NH), 5.14 (dd, Jₗ,ₗ = 7.8 Hz, J = 11.9 Hz, 1H, POCH₂Ph), 5.07 (dd, Jₗ,ₗ = 9.2 Hz, J = 11.9 Hz, 1H, POCH₂Ph), 5.03–5.00 (m, 2H, CH₂Ph), 5.00 (dd, Jₗ,ₗ = 8.9 Hz, J = 6.9, 10.0 Hz, 1H, H₃), 4.94 (s, 1H, H-1), 4.54 (app t, J = 6.9 Hz, 1H, H-3), 4.35 (app d, J = 8.7 Hz, 1H, H-6), 4.13 (dd, J = 8.8, J = 12.2 Hz, 1H, H-7a), 4.17–4.10 (m, 2H, NCH₂CO₂), 3.98 (dd, J = 0.8, J = 6.8 Hz, 1H, H-2), 3.86 (dd, J = 1.4, J = 12.2 Hz, 1H, H-7b), 3.82–3.73 (m, 2H, OCH₂CH₂), 3.65 (dd, J = 1.4, J = 10.1 Hz, 1H, H-5), 3.46–3.40 (m, 2H, CH₂N), 3.37 (s, 3H, OCH₃), 1.17 (t, J = 7.1 Hz, 3H, OCH₂CH₂), 1.12–0.92 (m, 28H, TIPDS) ppm; 13C NMR (151 MHz, CDCl₃): δ = 138.8 (C-1⁴), 129.0, 128.6, 128.4, 128.2, 128.1, 128.0, 127.9, 125.9 (C-²⁴), 121.8 (Cq, orthoester), 98.3 (C-1), 76.6 (C-3), 75.4 (C-2), 74.9 (d, Jₗ,ₗ = 5.5 Hz, C-4), 73.6 (C-6), 69.6 (d, Jₗ,ₗ = 5.5 Hz, POCH₂), 68.9 (d, Jₗ,ₗ = 9.9 Hz, C-5), 68.3 (C-7), 67.2 (d, Jₗ,ₗ = 5.5 Hz, NCH₂CH₂OP), 66.7 (CH₃Ph), 59.2 (OCH₂CH₂), 55.4 (OCH₃), 41.5 (d, Jₗ,ₗ = 7.7 Hz, NCH₂), 17.7, 17.6, 17.4, 17.34, 17.28, 17.25, 17.2 (8 × TIPDS-CH₃), 14.9 (OCH₂CH₂), 13.33, 13.30, 12.7, 12.6 (4 × TIPDS-CH) ppm; 31P NMR (243 MHz, CDCl₃): δ = −2.36 ppm.
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δ = 165.8 (C=O), 156.3 (NC=O), 136.4 (C-1Ar), 135.4 (d, J_{CP} = 6.7 Hz, C-1Ar), 133.3 (C-4Ar), 129.9 (C-2/C-6Ar), 129.6 (C-3), 128.8, 128.68, 128.67, 128.5, 128.3, 128.03, 128.01, 127.97 (CAr), 98.4 (C-1), 76.9 (d, J_{CP} = 6.6 Hz, C-4), 73.4 (C-6), 72.4 (C-2), 71.0 (d, J_{CP} = 9.5 Hz, C-5), 70.1 (d, J_{CP} = 5.5 Hz, POCH_2), 68.4 (C-3), 68.1 (C-7), 67.6 (d, J_{CP} = 5.4 Hz, NCH_2CH_2OP), 66.7 (CH_2Ph), 55.2 (OCH_3), 41.3 (d, J_{CP} = 5.5 Hz, NCH_2), 17.6, 17.5, 17.34, 17.33, 17.30, 17.25, 17.2, 17.1 (8 \times TIPDS-CH_3), 13.4, 13.3, 12.6 (4 \times TIPDS-CH ppm); 31P NMR (243 MHz, CDCl_3): δ = −0.38 ppm; HRMS ([ESI-TOF]; m/z calcld for C_{32}H_{38}NO_{13}P (M+H^+) 918.3676, found 918.3673.

**Methyl 2-O-benzoyl-4-O-(benzyl-2-(benzoyloxy)carbonyl-amino)ethylphosphonoj,l-glycero-α-d-manno-heptopyranoside** (8, C_{32}H_{38}NO_{13}P)

A solution of 20.5 mg 7 (22.3 μmol) in 1 cm^3 CH_2Cl_2 was transferred to a Teflon-flask and cooled to an ice-bath temperature. TREAT (73 mm^3, 0.45 μmol) was added, and the solution was vigorously stirred at rt. Additional portions of TREAT (2×27 μmol, 0.17 μmol) were added after 4 and 7.5 h reaction time. After 10 h, the solution was transferred into an ice-cold solution of aqueous NaHCO_3 (5 cm^3) followed by extraction with 10 cm^3 portions of EtOAc. The combined organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The crude residue (17.4 mg) was purified by chromatography on a Biotage Isolute Flash SI column using hexane/EtOAc = 1/2 → EtOAc for elution, which afforded 11.5 mg (83%) 8 as colorless syrup. R_f = 0.19 (EtOAc); [α]_{D}^{20} = +0.3° cm^2 g^−1 (c = 1.25, CHCl_3); 1H NMR (600 MHz, CDCl_3): δ = 8.05–8.02 (m, 2H, H-2/H-6 Ar), 7.58–7.54 (m, 1H, H-4 Ar), 7.44–7.40 (m, 2H, H-3/H-5 Ar), 7.40–7.27 (m, 10H, HAr), 5.42 (bs, 1H, NH), 5.37 (dd, J = 1.6, J = 3.6 Hz, 1H, H-2), 5.13 (d, J_{HP} = 8.8 Hz, 2H, POCH_2Ph), 5.01 (d, J = 12.1 Hz, 1H, CH_2Ph), 4.98 (br s, 1H, H-1), 4.84 (br s, 1H, H-1), 4.79 (br s, 1H, H-1), 4.61 (app q, J = 9.5 Hz, 1H, H-4), 4.21–4.15 (m, 2H, H-3, CH_2OP), 4.15–4.08 (m, 2H, H-6, CH_2OP), 3.77 (app d, J = 9.2 Hz, 1H, H-5), 3.75 (dd, J = 6.9, J = 10.5 Hz, 1H, H-7a), 3.69 (dd, J = 6.6, J = 10.7 Hz, 1H, H-7b), 3.41 (s, 3H, OCH_3), 3.19–3.11 (m, 2H, CH_2N) ppm; 13C NMR (151 MHz, MeOD): δ = 167.4 (C-O), 134.5 (C-4 Ar), 131.2 (C-1 Ar), 131.0 (C-2/C-6 Ar), 129.5 (C-3/C-5 Ar), 100.1 (C-1), 74.1 (C-2), 73.2 (d, J_{CP} = 5.8 Hz, C-4), 71.8 (d, J_{CP} = 5.5 Hz, C-5), 70.7 (C-3), 70.03 (C-6), 63.5 (C-7), 63.2 (d, J_{CP} = 5.3 Hz, CH_2OP), 55.6 (OCH_3), 41.5 (d, J_{CP} = 7.7 Hz, CH_2N) ppm; 31P NMR (243 MHz, D_2O): δ = 0.99 ppm; HRMS ([ESI-TOF]; m/z calcld for C_{17}H_{27}NO_{14}P (M+H^+) 452.1316, found 452.1323.

**Methyl 4-O-(2-aminoethylphosphono)-l-glycero-α-d-manno-heptopyranoside** (10, C_{17}H_{27}NO_{14}P)

A solution of 11.8 mg 9 (26 μmol) was coevaporated with toluene. The residue was dissolved in 1.2 cm^3 dry MeOH and stirred with 0.1 cm^3 1 M methanolic NaOMe for 4.5 h at rt. The solution was made neutral by the addition of Dowex 50 cation-exchange resin (H^+-form). The suspension was filtered, and the filtrate was concentrated. The residue was dissolved in 1 cm^3 D_2O and extracted three times with 1.5 cm^3 portions of diethylether. The combined organic phase was re-extracted with 0.7 cm^3 D_2O, filtered over glass-wool, and purged with argon to remove residual ether. The aqueous phase was lyophilized to give 7.1 mg (78%) 10 as amorphous solid. R_f = 0.17 (CHCl_3/MeOH/H_2O = 5/4/1); [α]_{D}^{20} = +43° cm^2 g^−1 (c = 0.7, H_2O) (Ref. [10]; [α]_{D}^{20} = +37° cm^2 g^−1 (c = 1.0, H_2O)); 1H NMR (600 MHz, D_2O, pD ~7.5 to 8.0): δ = 4.74 (d, J = 1.5 Hz, 1H, H-1), 4.31 (app q, J = 9.5 Hz, 1H, H-4), 4.19–4.10 (m, 2H, CH_2OP), 4.04 (ddd, J = 1.3,
Synthesis of 3-O- and 4-O-(2-aminoethylphosphono) derivatives of methyl L-glycero-\(\alpha\)-d-manno-heptopyranoside

A suspension of 15 mg 12 (29 \(\mu\)mol), 26 mg 4 (59 \(\mu\)mol), and molecular sieves 4 Å in 0.2 cm\(^3\) dry CH\(_2\)Cl\(_2\) was stirred for 1 h at rt under Ar. Then, a 0.45 M solution of 1H-tetrazine in acetonitrile (131 mm\(^3\), 59 \(\mu\)mol) was added, and stirring was continued for 2 h. The reaction mixture was warmed to \(-78 \, ^\circ\text{C}\) and treated with 10 mg mCPBA (59 \(\mu\)mol) was added. After 1 h, the reaction was quenched by addition of 10 mm\(^3\) triethylamine and warmed up to rt. The suspension was diluted with CH\(_2\)Cl\(_2\) and washed with saturated aqueous NaHCO\(_3\) and brine. The organic layer was dried (Na\(_2\)SO\(_4\)) and concentrated. The residue was purified by HPLC (YMC-Pack-Sil-06, hexane/EtOAc = 2/1 \(\rightarrow 1/2\)) which gave 8.2 mg (32\%) 13 as diastereomeric mixture. \(R_t = 0.45\) (hexane/EtOAc = 1/1); \(1^H\) NMR (600 MHz, CDCl\(_3\)) for major isomer a: \(\delta = 7.38\)–7.28 (m, 10H, H-\(\text{Ar}\)), 5.38 (bs, 1H, NH), 5.19 (dd, 1H, \(J = 1.9, J = 3.5\) Hz, H-2), 5.10–5.08 (m, 4H, 2 \(\times\) CH\(_2\)Ph), 4.65 (br s, 1H, H-1), 4.63 (ddd, 1H, \(J = 9.5, J_{\text{p3}} = 7.6\) Hz, H-3), 4.35 (dt, 1H, \(J = 8.9, J = 1.5\) Hz, H-6), 4.13 (t, 1H, \(J = 9.5\) Hz, H-4), 4.13–4.09 (m, 1H, POCH\(_2\)CH\(_2\)), 4.07–4.02 (m, 1H, POCH\(_2\)CH\(_2\)), 4.03 (ddd, 1H, \(J = 12.0, J = 8.7\) Hz, H-7a), 3.85 (br d, 1H, \(J = 11.9\) Hz, H-7b), 3.51 (br d, 1H, \(J = 9.5\) Hz, H-4), 4.11–4.01 (m, 3H, POCH\(_2\)CH\(_2\)), 3.87 (dd, 1H, \(J = 1.05\) Hz, H-7b), 3.58 (br s, 1H, H-1), 3.50 (dd, 1H, \(J = 9.5, J = 1.6\) Hz, H-5), 3.42–3.36 (br s, 2H, CH\(_2\)COOH), 2.95 (s, 3H, OAc), 1.34–0.93 (m, 28H, TIPS) ppm; \(3^P\) NMR (243 MHz, CDCl\(_3\)); \(\delta = 0.52\) ppm; \(1^H\) NMR (600 MHz, CDCl\(_3\)) for minor isomer b: \(\delta = 7.41\)–7.28 (m, 10H, H-\(\text{Ar}\)), 5.28 (bs, 1H, NH), 5.16 (dd, 1H, \(J = 1.9, J = 3.5\) Hz, H-2), 5.12–5.05 (m, 4H, 2 \(\times\) CH\(_2\)Ph), 4.65 (d, 1H, \(J = 1.9\) Hz, H-1), 4.59 (ddd, 1H, \(J = 3.3, J_{\text{p1}} = 7.5, J = 9.6\) Hz, H-3), 4.36 (br d, 1H, \(J = 8.9\) Hz, H-6), 4.15 (t, 1H, \(J_{\text{p4,5}} = J_{\text{p1,2}} = 9.5\) Hz, H-4), 4.11–4.01 (m, 3H, POCH\(_2\)CH\(_2\)), 3.87 (dd, 1H, \(J = 1.05\) Hz, H-7b), 3.58 (br s, 1H, H-1), 3.50 (dd, 1H, \(J = 9.5, J = 1.6\) Hz, H-5), 3.42–3.36 (br s, 2H, CH\(_2\)COOH), 2.95 (s, 3H, OAc), 1.34–0.93 (m, 28H, TIPS) ppm; \(3^P\) NMR (243 MHz, CDCl\(_3\)); \(\delta = 0.45\) ppm; \(1^C\) NMR (150 MHz, CDCl\(_3\)); \(\delta = 170.1\) (C=O), 156.4 (NC=O), 134.7, 134.55, 128.81, 128.75, 128.17, 128.6, 128.5, 128.2, 128.11, 128.08, 128.03, 128.00, 127.5 (C\(^\\beta\)), 54.91 (OCH\(_3\)); additional signals for isomer a: 98.3 (C-1), 77.6 (\(J_{\text{CP}} = 6.0\) Hz, C-3), 73.5 (C-6), 72.51 (C-5), 70.1 (CH\(_2\)Ph), 70.0 (C-2), 68.0 (C-7), 67.4 (\(J_{\text{CP}} = 6.2\) Hz, CH\(_2\)Ph), 66.1 (C-4) and 41.3 (NCH\(_3\)) ppm; additional signals for isomer b: 98.2 (C-1), 77.5 (\(J_{\text{CP}} = 6.3\) Hz, C-3), 73.6 (C-6), 72.5 (C-5), 70.1 (CH\(_2\)Ph), 70.0 (C-2), 68.0 (C-7), 67.4 (\(J_{\text{CP}} = 6.2\) Hz, CH\(_2\)Ph), 66.1 (C-4), 41.2 (NCH\(_3\)) ppm; HRMS (ESI): \(m/z\) calecd for C\(_{39}\)H\(_{56}\)O\(_{14}\)P\(_{3}\)Si\(_2\) + H\(^+\) ([M+H\(^+\)]) 856.3519, found 856.3528.
EtOAc = 1/7 → EtOAc → EtOAc/EtOH = 9/1) which gave 14 (6 mg, 70%) as syrup. 

$R_f = 0.35$ (EtOAc/ EtOH = 9/1); 

$^1H$ NMR (600 MHz, CDCl$_3$):

- δ = 7.39–7.29 (m, 10H, H$_{Ar}$), 5.42 (br s, $\sim$0.5H, NH-a), 5.34 (br s, $\sim$0.5H, N'OH-B), 5.24 (dd, $\sim$0.5H, J = 1.5, J = 3.5 Hz, H-2b), 5.20–5.18 (m, 0.5H, H-2a), 5.11–5.06 (m, 4H, 2 $\times$ CH$_2$Ph), 4.65 (m, 1H, H-1a,b), 4.63 (ddd, 1H, J = 3.7, J$_{3P}$ = 7.6, J = 9.4 Hz, H-3a,b), 4.12 (t, 1H, J$_{4,5}$ = J$_{4,3}$ = 9.8 Hz, H-4a,b), 4.14–4.02 (m, 3H, 6-H$_2$,a,b, OPCH$_2$CH$_2$), 3.85–3.78 (m, 1H, H-7a,b), 3.63–3.58 (m, 1H, H-5), 3.45–3.67 (m, 2H, NCH$_2$), 3.34 (s, 1.7H, OCH$_3$-a), 3.32 (s, 1.3H, OCH$_3$-b), 2.06 and 2.05 (s, 3H, OAc-a,b) ppm; 

$^{13}$C NMR (150 MHz, CDCl$_3$, selected HSQC data): δ = 128.9, 128.7, 128.5, 128.0 (C$_{Ar}$), 98.6 (C-1), 77.3 (C-3), 72.5 (C-5), 70.2 (CH$_2$Ph), 70.0, 69.8 (C-2), 69.9 (C-6), 67.4 (POCH$_2$CH$_2$), 66.9 (CH$_2$Ph), 66.3 (C-4), 64.4 (C-7), 55.2 (OCH$_3$), 41.5 (NCH$_2$), 20.6 (OAc) ppm; 

$^{31}$P NMR (243 MHz, CDCl$_3$): δ = 0.37, 0.22 ppm; HRMS (ESI): m/z calcd for C$_{27}$H$_{36}$NO$_{13}$P + H$^+$ ([M+H$^+$]) 614.1997, found 614.1995.

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