Modeling of synthetic phosphono and carba analogues of *N*-acetyl-α-D-mannosamine 1-phosphate, the repeating unit of the capsular polysaccharide from *Neisseria meningitidis* serovar A†

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The conformational behavior of methyl (2-acetamido-2-deoxy-α-D-mannopyranosyl)phosphate **1**, and its analogues, methyl *C*-(2-acetamido-2-deoxy-α-D-mannopyranosyl)methanephosphonate **2** and methyl *O*-(2-acetamido-2-deoxy-5a-carba-α-D-mannopyranosyl)phosphate **3**, where a methylene group replaces, respectively, the anomeric and the pyranose oxygen atom, was investigated at the B3LYP/6-311+G(d,p) level [6-311+G(2df,p) for the phosphorus atom]. The energy of the optimized structures was recalculated using the continuum solvent model C-PCM choosing water as the solvent. The compounds exhibited several populated conformations, but they all showed a marked preference for the $^4C_1$ geometry of the pyranose ring; this preference was almost complete for **1**, very large for the phosphono analogue **2**, and large for the carba analogue **3**. To give experimental support to these results, compounds **2** and **3** were synthesized and characterized by NMR spectroscopy. The comparison of the theoretical and experimental vicinal coupling constants confirmed the marked preference for the $^4C_1$ geometry in the case of **2** and suggested that the same holds true for compound **3**.

Introduction

Neisseria meningitidis[1] belongs to the class of encapsulated bacteria, whose extremely powerful virulence is due to the presence of a saccharide capsule, embodying the bacterium cell, which delays the host's immune response. A possible defense against infections from encapsulated bacteria can be mediated by the presence of antibodies directed towards their capsular polysaccharides (CPSs).[2] Accordingly, a vaccine including a mixture of the CPSs associated to the most virulent serogroups induce a long term protection.[3] Among thirteen clinically significant capsular serogroups of *N. meningitidis*, serotype A (MenA), the main responsible for African outbreaks and epidemics of meningitis, is one of the most virulent.[4] The MenA capsule is constituted of *N*-acetyl-α-D-mannosamine residues (1→6)-linked through phosphodiester bridges (Chart 1).[5]

Recent studies have shown the potential of effective synthetic carbohydrate vaccines against bacteria-induced diseases. Successful examples have been reported in the literature for *Shigella dysenteriae* type 1,[6] *Haemophilus influenzae*,[7] and *Bacillus anthracis*.[8] The design of an effective vaccine against MenA is hampered by the major problem of the chemical lability of anomeric glycosyl phosphates, which makes it difficult to store and manipulate the saccharide fragments in the formulation of the conjugated vaccines.[9] To overcome this problem, we became interested in designing stable synthetic analogues of the repeating unit of MenA CPS, which could be incorporated into a saccharide chain in order to obtain more stable oligomers. These, after conjugation with a proper immunogenic protein carrier, should elicit the proliferation of protective antibodies cross-reacting with the natural CPS.[3a,10]

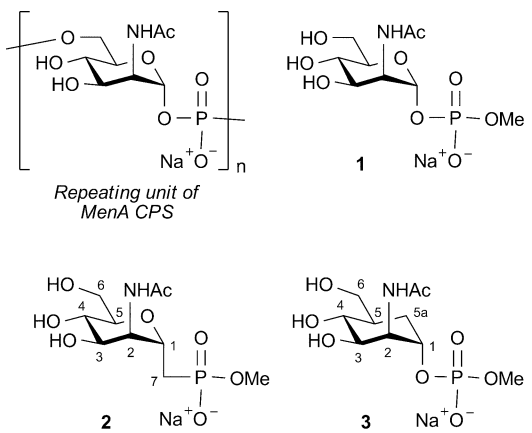

Chart 1

We envisaged two main approaches in order to increase the stability of the phosphodiester-linked oligomers of MenA CPS. The first kind of analogue can be obtained by replacing the anomeric oxygen of the phosphodiester with a methylene group.[11] Following this approach, we recently reported the synthesis of phosphonoester-linked oligomers of MenA CPS.[12] Their biological evaluation showed that they are recognized by a human polyclonal anti-MenA serum, confirming that the replacement of the anomeric oxygen atom with a methylene group still permits antibody binding.[12a] However, this result does not imply that

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the overall geometrical features of the natural compound are preserved in the synthetic oligomers. Due to the high complexity of their ^{1}H NMR spectra, the experimental NMR data of a suitable monomeric *N*-acetylmannosamine 1-phosphonate should allow easier determination of the conformational preferences of this kind of compound.

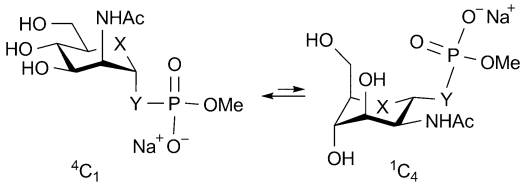
An alternative approach is the replacement of the pyranose oxygen atom with a methylene group to give a carbocyclic analogue.[13] In this way, the acetal character of the phosphodiester is lost, and it is expected to be of comparable stability to that of oligonucleotides.

Since we planned the synthesis of short MenA CPS oligomers incorporating *carba*-*N*-acetylmannosamine units, we decided to perform preliminary modeling studies on a simpler monomeric structure to verify on theoretical grounds the preservation of the overall geometrical properties in this class of analogues. Thus, looking at the structure of methyl(2-acetamido-2-deoxy-α-D-mannopyranosyl)phosphate anion **1** (Chart 1) as the minimum substructure representing the *N*-acetylmannosamine moiety in MenA CPS, we report herein a comparative modeling study of **1** with its phosphono and carba analogues **2** and **3**, respectively. Moreover, we also describe the synthesis of compounds **2** and **3**, whose spectral data were employed to give experimental support to the modeling study.

Results and discussion

The pyranose ring of D-mannopyranose and its derivatives usually assumes the ^{4}C$_1$ conformation, even when the configuration at C1 is α, the anomeric effect being one of the factors that stabilizes this geometry with respect to the inverted ^{1}C$_4$ chair conformation. The 2-acetamido-2-deoxy-α-D-mannopyranosyl moiety conforms to this general behavior also when a phosphate group is linked at the anomeric position. In fact, complete NMR assignment of MenA fragments synthesized by Poszgay *et al.*[14] allowed most of the vicinal ^{1}H–^{1}H coupling constants to be measured, confirming that the pyranose rings exclusively assume the ^{4}C$_1$ geometry ($J_{1,2} \approx 1.6$ Hz, $J_{2,3} \approx 4.7$ Hz, $J_{3,4} \approx 10.0$ Hz, $J_{4,5} \approx 9.7$ Hz).

In compounds **2** and **3**, conversely, the equilibrium depicted in Chart 2 cannot be excluded. Although methylene and oxygen can be considered isosteric groups, their stereoelectronic properties are quite different, and the replacement of one of the acetalic oxygen atoms with a methylene could, in principle, cause major changes in the conformational behavior. The loss of the anomeric effect, which in the case of **1** favorably cooperates with the axial orientation of the substituent at C1, together with the steric hindrance of both the substituents at C1 and C2, might cause the stability of the ^{1}C$_4$ conformations in **2** and **3** to become comparable with that of ^{4}C$_1$.



2 X = O, Y = CH_2

3 X = CH_2, Y = O

Chart 2

Previous theoretical studies,[15] as well as experimental evidence of the effects of the oxygen/methylene substitution in α-L-rhamnopyranosyl derivatives,[16] showed that the preferred ring conformation in the analogues is the same as in rhamnopyranosyl phosphate, though the analogues present smaller energy differences between the ^{1}C$_4$ and ^{4}C$_1$ conformations. 2-Acetamido-2-deoxy-D-mannose and L-rhamnose belong to opposite steric series but share the same relative arrangement of the ring substituents, the main difference being the bulkier substituent at C2 in mannosamine. The higher steric requirements of the acetamido group might contribute to a further reduction of the energy difference between the conformers, making the ^{1}C$_4$–^{4}C$_1$ equilibrium significant.

The modeling study of compounds **1–3** was performed on the anions, without considering the sodium cation, through optimizations within the density functional approach at the B3LYP level.[17] Large basis sets were used: 6-311+G(d,p) for all the atoms and 6-311+G(2df,p) for the third-row atom phosphorus. Diffuse functions were required by the anionic nature of the structures. All the degrees of conformational freedom were considered, including rotation around single bonds of the substituents and the different rotamers of the hydroxymethyl group. The formation of intramolecular hydrogen bonds among the three hydroxyl groups and with the several H-bond acceptors in the molecules as well as the orientation of the phosphate or phosphonate groups were taken into consideration. The conformational mobility of the pyranose or carbocyclic ring was investigated considering the ^{4}C$_1$ and ^{1}C$_4$ conformations as well as the twisted-boat geometries. The energy of the conformations optimized in vacuum was recalculated using a continuum solvent model, C-PCM,[18] choosing water as the solvent, to obtain values comparable with water solutions. The results are gathered in Table 1, which reports the energy values and some geometrical features of the most significant conformations, together with their percentage contributions to the overall population determined through the Boltzmann equation. Each conformation represents an ensemble of conformers differing in the orientation of the hydroxymethyl group and of all the hydroxyl groups; only the conformer energetically favored in each ensemble is reported. The ring geometry and the three torsional angles ϕ, ψ_1, and ψ_2, describe the differences among the conformers in Table 1 and represent the main conformational features of the compounds under investigation.

As expected, the reference compound **1** showed an almost complete preference for the ^{4}C$_1$ geometry, populated to a level >99% (*vs.* 0.2% of the inverted ^{1}C$_4$ chair), whereas the twisted-boat conformations gave no contribution to the overall population. Moreover, the value of the torsional angles ϕ shown by all the conformers of compound **1** corresponds to that of the *exo*-anomeric effect. The lowest energy ^{1}C$_4$ conformation (not reported in Table 1) showed an energy >3 kcal/mol higher than the global minimum **1A**.

As far as the data of the analogues **2** and **3** are concerned, Table 1 shows that their minimum energy conformers **2A** and **3A**, respectively, still assume the ^{4}C$_1$ conformation and present the *exo*-anomeric effect. However, the relative energy of the most stable ^{1}C$_4$ conformations **2D** and **3F**, 1.94 and 1.72 kcal/mol, respectively, is lower than the corresponding conformation of compound **1**

Table 1 Relative energy, recalculated using the continuum solvent model C-PCM, equilibrium percentages at 298 K, and selected geometrical data of the B3LYP/6-311+G(d,p)[a] calculated minimum energy conformations of compounds **1–3**

	E_{rel} (kcal/mol)	Equilibrium %	Ring conf.	ϕ^b (°)	$\psi_1{}^c$ (°)	$\psi_2{}^d$ (°)
1A	0.00	38.3	$^4C_1$	62	167	–75
1B	0.09	32.9	$^4C_1$	60	53	80
1C	0.70	11.7	$^4C_1$	62	163	75
1D	1.11	5.9	$^4C_1$	84	–94	–84
1E	1.31	4.2	$^4C_1$	88	–85	101
1F	1.49	3.1	$^4C_1$	58	43	–122
1G	1.82	1.8	$^4C_1$	82	–90	–168
Others		2.1				
2A	0.00	56.8	$^4C_1$	49	174	–88
2B	0.42	28.1	$^4C_1$	46	50	89
2C	1.01	10.3	$^4C_1$	44	49	–119
2D	1.94	2.1	$^1C_4$	145	–65	–85
Others		2.7				
3A	0.00	46.5	$^4C_1$	68	–97	–81
3B	0.33	26.7	$^4C_1$	72	62	74
3C	0.95	9.3	$^4C_1$	57	155	–72
3D	1.11	7.1	$^4C_1$	79	73	–169
3E	1.51	3.6	$^4C_1$	69	–94	–168
3F	1.72	2.6	$^1C_4$	133	–67	–79
3G	1.87	2.0	$^1C_4$	113	73	–172
3H	2.16	1.2	$^1C_4$	140	–90	77
Others		1.0				

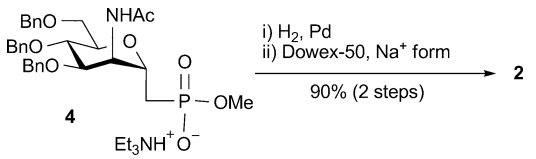
[a] B3LYP/6-311+G(2df,p) level for the phosphorus atom. [b] ϕ: O5-C1-O-P for **1**, O5-C1-C7-P for **2**, C5a-C1-O-P for **3**. [c] ψ_1: C1-O-P-O for **1** and **3**, C1-C7-P-O for **2**. [d] ψ_2: O-P-O-CH_3 for **1** and **3**, C7-P-O-CH_3 for **2**.

(E_{rel} >3 kcal/mol). Actually, the percentage contribution to the overall population of the $^1C_4$ conformations was 3% for **2** and 7% for **3**, showing the possibility of an equilibrium between the $^4C_1$ and $^1C_4$ conformations.

It is worth pointing out that the results are strongly influenced by the computational approach and by the basis set chosen for the calculations. In fact, if the zero-point energy corrections are not taken into consideration (data not shown), the percentage contributions of the $^1C_4$ conformations to the populations of **2** and **3** are predicted to be much greater than those reported in Table 1.[19] Moreover, a similar overestimation of the $^1C_4$ percentage is found if the medium basis set 6-31G(d) is used (data not shown). This should suggest the use of a basis set even larger than 6-311+G(d,p); however, the molecular flexibility would make the computational cost too high, since the optimization of a very large number of conformations is required. Moreover, it should be also emphasized that modeling of carbohydrates bearing charged groups contains a degree of uncertainty higher than modeling of non polar molecules. For all these reasons, we performed the synthesis of compounds **2** and **3** to obtain the validation of the theoretical results by comparing the experimental scalar coupling constants with those calculated for the predicted conformations.

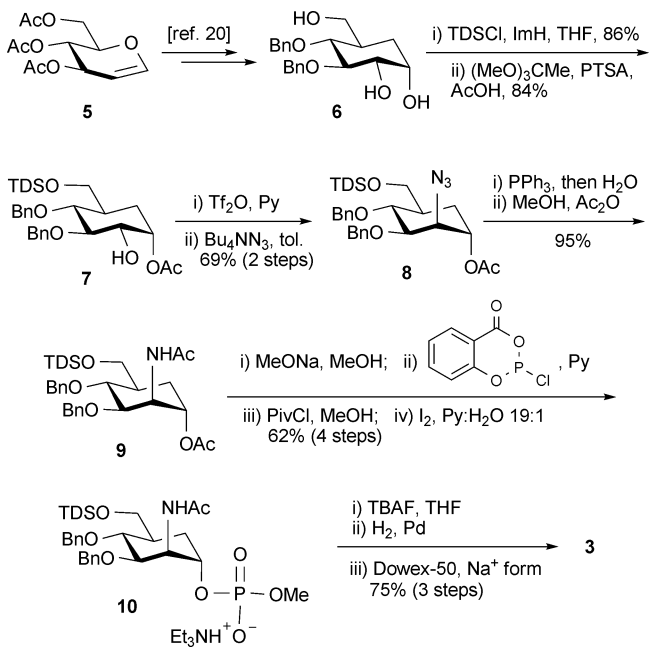
The synthesis of compound **2** was carried out starting from known monomethyl phosphonate derivative **4**.[12a] Catalytic hydrogenolysis of the benzyl groups of **4** and subsequent elution through ion exchange resin Dowex-50W X8 (Na^+ form), provided the final phosphonate analogue **2** in 90% overall yield (Scheme 1).

The synthesis of the carba-analogue **3** started from triol **6**, obtained from triacetyl glucal **5** as described in the literature.[20]



Scheme 1

Compound **6** was protected at the primary position as thexyldimethylsilyl ether, and the axial hydroxyl was regioselectively acetylated, providing intermediate **7** in 72% overall yield (Scheme 2). The C2 hydroxyl of **7** was then activated to S_N2 reaction by transformation into a triflate ester; this was reacted with freshly prepared tetrabutylammonium azide, providing the carba-mannosamine derivative **8** in 69% overall yield, in which the presence of the azido group was confirmed by IR spectroscopy. Conversion of the azide into the acetamido group was achieved by Staudinger reaction, followed by *N*-acetylation to give compound **9** in 95% yield. The synthesis of methylphosphate **3** was then completed by deacetylation of compound **9** under Zémplen conditions, followed by the introduction of the monomethyl phosphate group at the free hydroxyl employing the *H*-phosphonate protocol,[21] to afford phosphodiester **10** in 65% overall yield. Removal of thexyldimethylsilyl (tetrabutylammonium fluoride in THF) and benzyl groups (hydrogenolysis over palladium-on-charcoal), followed by final elution through a column of Dowex-50W X8 resin (Na^+ form) provided the carba-analogue **3** in 75% overall yield. To the best of our knowledge, this is the first synthesis of the carba-analogue of *N*-acetylmannosamine 1-phosphate reported in the literature.



Scheme 2

Compounds **2** and **3** were characterized by ^1H NMR, and the experimental values of the vicinal coupling constants of the ring hydrogen atoms, diagnostic for the conformational preferences of the compounds, were determined (Table 2). For every populated conformation of **2** and **3**, the theoretical values of the coupling

Table 2 Experimental ^{1}H NMR coupling constants (Hz) of compounds **2** and **3** in comparison with the values calculated with the Haasnoot *et al.* equation[a] for their $^4C_1$ and $^1C_4$ conformations and for the entire set of populated conformations

		Calculated values		
	Exp. values	$^4C_1$	$^1C_4$	All
Compound **2**				
$J_{1,2}$	2.4	1.3	9.2	1.5
$J_{2,3}$	4.3	3.3	3.1	3.3
$J_{3,4}$	8.8	8.5	2.2	8.4
$J_{4,5}$	—	9.1	1.0	8.9
Compound **3**				
$J_{1,2}$	3.2	3.0	9.3	3.4
$J_{2,3}$	4.8	3.7	3.0	3.6
$J_{3,4}$	9.8	9.2	2.6	8.7
$J_{4,5}$	10.0	11.0	1.7	10.3
$J_{5,5aax}$	—	12.4	4.6	11.8
$J_{5,5aeq}$	—	3.4	1.9	3.3
$J_{1,5aax}$	2.8	2.5	11.3	3.1
$J_{1,5aeq}$	3.5	3.7	4.1	3.7

[a] Ref. 22.

constants were calculated by using the equation of Haasnoot *et al.*,[22] an electronegativity-modified Karplus relationship, applicable to a wide variety of compounds, that reproduces the effects of the presence and orientation of electronegative atoms on the coupling constants. The theoretical values were weighted averaged on the basis of the population percentages separately for the $^4C_1$ and the $^1C_4$ groups of conformations, as well as for the entire set of populated conformations. In Table 2 these three series of computed vicinal coupling constants are reported in comparison with the experimental values.

Measurement of the experimental coupling constant values at 400 MHz are reasonably accurate and precise, especially with regard to the high values of axial–axial pairs of protons. Thus, in very good agreement with the theoretical predictions, ^{1}H NMR data of compound **2** confirmed its strong preference for the $^4C_1$ conformations. Actually, the coupling constant values calculated both for the entire conformational set and for the sole $^4C_1$ conformations (Table 2) agree with the experimental data, as the small computed contribution of the $^1C_4$ conformations (3%) is below the threshold that the approximations connected with the Haasnoot equation allow one to detect.

The ring coupling constants of **3** calculated for the entire conformational set have some differences from those determined for the only $^4C_1$ conformations (Table 2), but in both the cases they are in fair agreement with the experimental data. The values of $J_{3,4}$ and $J_{4,5}$ close to 10 Hz ensure that the $^4C_1$ conformations largely prevail, as they would be significantly reduced in the case of an appreciable contribution of the $^1C_4$ conformations.[23] These pieces of evidence suggest that, for compound **3** in water, the $^4C_1$ conformations are also preferred to a considerable extent, and that the contribution of the $^1C_4$ conformations corresponds to that computed, or is even lower.

Conclusions

The conformational behavior of methyl(2-acetamido-2-deoxy-α-D-mannopyranosyl)phosphate **1**, together with its analogues **2** and **3** in which a methylene group replaces either the anomeric or the pyranose oxygen atom, was investigated through a modeling study with a DFT approach. The energy of the optimized structures was recalculated using a continuum solvent model, C-PCM, choosing water as the solvent. The compounds exhibited several populated conformations so that the overall properties of flexibility and mobility were evaluated with particular attention to the possibility of the pyranose ring inversion and to the orientation of the phosphate or phosphonate aglycone, as well as of the hydroxymethyl group. The $^4C_1$ geometry was preferred by all the compounds; this preference was almost complete in the natural reference compound **1**, very large in the phosphono analogue **2**, and large for the carba analogue **3**.

To give experimental support to these results, compounds **2** and **3** were synthesized. Their NMR spectroscopic characterization and the comparison of the theoretical and experimental vicinal coupling constants confirmed the marked preference for the $^4C_1$ geometry in the case of **2** and suggested that the same holds true in compound **3**.

These experimental results indicate that replacement of either the anomeric or the pyranose oxygen atom with a methylene group allows the preservation of the overall geometrical properties in both classes of analogues. Moreover, the comparison between the experimental and the theoretical data highlights the importance of a proper choice of the calculation level to obtain reliable computational results in the modeling of these compounds.

Experimental

General

NMR spectra were recorded on a Bruker Avance 400 spectrometer at 298 K, unless otherwise reported. In ^{13}C NMR spectra, signals corresponding to aromatic carbons are omitted. Chemical shifts are reported on the δ (ppm) scale and in ^{31}P spectra they are relative to H_3PO_4. J values are given in Hz. Peak assignments were based on analysis of 2D spectra (H,H-COSY and HSQC or HMQC spectra). IR spectra were recorded on a Jasco 4100 FT-IR instrument and wave number values are reported in cm^{-1}. Optical rotations were measured at rt with a Perkin–Elmer 241 polarimeter. Elemental analyses were performed using the Carlo Erba elemental analyser 1108. TLC and HPTLC were carried out on Merck silica gel 60 F-254 plates (0.25 mm and 0.2 mm thickness, respectively), and spots were viewed by spraying with a solution containing H_2SO_4 (31 mL), ammonium molybdate (21 g) and $Ce(SO_4)_2$ (1 g) in 500 mL water, followed by heating at 110 °C for 5 min. Column chromatography was performed by the flash procedure on Merck silica gel 60 (230–400 mesh). Solvents were dried by standard procedures.

Methyl *C*-(2-acetamido-2-deoxy-α-D-mannopyranosyl)methanephosphonate sodium salt (2). Compound **4** (130 mg, 0.19 mmol) was dissolved in a 3:2 MeOH:H_2O mixture (5 mL) at rt, together with 130 mg of Pd/C catalyst. The reaction was stirred overnight under hydrogen atmosphere, then it was filtered through a Celite pad and concentrated. The residue was dissolved in water and passed through a column of Dowex-50W X8 (Na^+ form) ion exchange resin. The solvent was evaporated, giving **2** (57 mg, 90%); $[\alpha]_D^{25}$ +3.2 (*c* 1 in H_2O); (Found: C, 35.95; H, 5.11; N, 4.18. Calc. for $C_{10}H_{19}NNaO_8P$: C, 35.83; H, 5.71; N, 4.18); δ_H(400 MHz; D_2O) 1.98 (3H, s, NHAc), 2.00 (1H, ddd, $J_{7,1}$ 7.0,

$J_{7,7'}$ 15.4, $J_{7,P}$ 18.8, 7-H), 2.06 (1H, ddd, $J_{7',1}$ 7.7, $J_{7',P}$ 18.2, 7′-H), 3.49 (3H, d, J 10.8, CH_3OP), 3.50–3.58 (2H, m, 4-H, 5-H), 3.72 (1H, dd, $J_{6,5}$ 1.9, $J_{6,6'}$ 12.2, 6-H), 3.80 (1H, dd, $J_{6',5}$ 4.6, 6′-H), 3.93 (1H, dd, $J_{3,4}$ 8.8, $J_{2,3}$ 4.3, 3-H), 4.1 (1H, dddd, $J_{1,2}$ 2.4, $J_{1,P}$ 9.6, 1-H), 4.28 (1H, dd, 2-H); δ_C(100.6 MHz; H_2O) 22.0 (NHAc); 26.7 (d, $J_{7,P}$ 133, 7-C), 51.2 (d, $J_{Me,P}$ 5, OMe), 52.8 (d, $J_{2,P}$ 9, 2-C), 60.4 (6-C), 67.3, 72.9 (4-C, 5-C), 69.3 (3-C), 73.9 (1-C), 174.4 (C=O); δ_P(162 MHz; D_2O) 24.5.

3,4-Di-*O*-benzyl-5a-carba-α-D-glucopyranose (6). Compound **6** was prepared according to the procedure reported in ref. 20, and its identity was checked by NMR and elemental analysis. $[\alpha]_D^{25}$ +85.5 (*c* 1 in $CHCl_3$); (Found: C, 70.45; H, 7.38. Calc. for $C_{21}H_{26}O_5$: C, 70.37; H, 7.31); δ_H(400 MHz; $CDCl_3$) 1.40 (1H, ddd, $J_{5a,1}$ 2.6, $J_{5a,5a'}$ 15.1, $J_{5a,5}$ 13.1, $5a_{ax}$-H), 1.86 (1H, ddd, $J_{5a',1}$ 3.7, $J_{5a',5}$ 3.7, $5a_{eq}$-H), 2.15 (1H, m, 5-H), 3.42 (1H, dd, $J_{4,3}$ 9.2, $J_{4,5}$ 10.4, 4-H), 3.49 (1H, dd, $J_{2,1}$ 3.1, $J_{2,3}$ 9.3, 2-H), 3.58 (1H, dd, $J_{6,5}$ 4.5, $J_{6,6'}$ 10.8, 6-H), 3.70 (1H, dd, $J_{6,5}$ 4.0, 6′-H), 3.78 (1H, t, 3-H), 4.06 (1H, m, 1-H), 4.71, 4.95 (2H, AB system, J 11.1, CH_2Ph), 4.75, 5.00 (2H, AB system, J 11.4, CH_2Ph), 7.32–7.39 (10H, m, H_{Ar}); δ_C(100.6 MHz; $CDCl_3$) 29.9 (5a-C), 38.5 (5-C), 63.9 (6-C), 68.1 (1-C), 74.4 (2-C), 74.7, 76.7 (2 *C*H_2Ph), 81.9 (4-C), 83.6 (3-C).

1-*O*-Acetyl-3,4-di-*O*-benzyl-6-*O*-thexyldimethylsilyl-5a-carba-α-D-glucopyranose (7). To a solution of **6** (204 mg, 0.57 mmol) in THF (3 mL), imidazole (58 mg, 0.85 mmol) and thexyldimethylsilyl chloride (123 μL, 0.63 mmol) were added at rt. After 24 h, satd $NaHCO_3$ (10 mL) was added, followed by extraction with EtOAc (3 × 10 mL). The organic phases were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by a short filtration on flash silica gel (eluent hexane:EtOAc 7:3). It was successively dissolved in dry acetonitrile under nitrogen atmosphere, then trimethyl orthoacetate (154 μL, 1.23 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added at rt. After 10 min, 80% acetic acid (7.5 mL) was added and stirring was continued for 15 min. Dichloromethane was added and the organic layer was washed with satd $NaHCO_3$, dried with Na_2SO_4, filtered, and concentrated. The crude compound was purified by flash chromatography (eluent hexane:EtOAc 6:4), providing **7** (217 mg, 72% over 2 steps); $[\alpha]_D^{25}$ +22.4 (*c* 1 in $CHCl_3$); (Found: C, 68.70; H, 8.49. Calc. for $C_{31}H_{46}O_6Si$: C, 68.60; H, 8.54); δ_H(400 MHz; $CDCl_3$) 0.09, 0.10 (6H, 2 s, 2 CH_3Si), 0.87 (6H, s, 2 C$H_{3\text{-thexyl}}$), 0.90 (6H, d, J 6.9, 2 CH_3CH_{thexyl}), 1.61–1.68 (2H, m, $5a_{ax}$-H, CH_{thexyl}), 1.88 (1H, ddd, $J_{5a,5a'}$ 14.8, $J_{5a',1}$ 3.7, $J_{5a',5}$ 3.7, $5a_{eq}$-H), 1.95 (1H, m, 5-H), 2.10 (3H, s, OAc), 2.36 (1H, br s, OH), 3.54 (1H, dd, $J_{6,5}$ 2.0, $J_{6,6'}$ 9.7, 6-H), 3.57 (1H, t, $J_{4,3}$ 9.4, $J_{4,5}$ 9.4, 4-H), 3.64 (1H, dd, $J_{2,1}$ 2.9, $J_{2,3}$ 9.5, 2-H), 3.79 (1H, t, 3-H), 3.97 (1H, dd, $J_{6',5}$ 3.4, 6′-H), 4.70, 4.92 (2H, AB system, J 10.6, CH_2Ph), 4.81, 4.99 (2H, AB system, J 11.1, CH_2Ph), 5.29 (1H, m, 1-H), 7.31–7.39 (10H, m, H_{Ar}); δ_C(100.6 MHz, $CDCl_3$, signals from HSQC NMR spectrum) -3.0 (2 *C*H_3Si), 18.6 (2 *C*$H_{3\text{-thexyl}}$), 20.3 (2 *C*H_3CH_{thexyl}), 21.2 (OAc); 28.8 (5a-C), 34.3 (*C*H_{thexyl}), 39.7 (5-C), 61.7 (6-C), 71.6 (1-C), 73.3 (2-C), 75.1, 75.6 (2 *C*H_2Ph), 80.3 (4-C), 84.1 (3-C).

1-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranose (8). To a solution of **7** (108 mg, 0.20 mmol) in dry dichloromethane (2 mL), pyridine (64 μL, 0.80 mmol) and triflic anhydride (98 μL, 0.60 mmol) were added. After 45 min, the reaction mixture was extracted with dichloromethane (2 × 5 mL), dried (Na_2SO_4), and concentrated. The residue was co-evaporated with toluene (3 × 1 mL) and dried in high-vacuum pump. Then it was dissolved in dry toluene (2 mL) and dropped into a solution of freshly prepared tetrabutylammonium azide in dry toluene (2 mL) under argon atmosphere. The reaction mixture was stirred overnight at 80 °C, then the solvent was evaporated and the residue was purified by flash chromatography (eluent toluene:EtOAc 98:2) giving **8** (78 mg, 69%); $[\alpha]_D^{25}$ +22.9 (*c* 2 in $CHCl_3$); (Found: C, 65.61; H, 8.05; N, 7.30. Calc. for $C_{31}H_{45}N_3O_5Si$: C, 65.58; H, 7.99; N, 7.40); ν_{max}/cm^{-1} 1737.07, 2108.30 and 2399.98; δ_H(400 MHz, $CDCl_3$) 0.09, 0.10 (6H, 2 s, 2 CH_3Si), 0.88 (6H, s, 2 C$H_{3\text{-thexyl}}$), 0.92 (6H, d, J 6.8, 2 CH_3CH_{thexyl}), 1.62–1.68 (2H, m, $5a_{ax}$-H, CH_{thexyl}), 1.87–1.95 (2H, m, 5-H, $5a_{eq}$-H), 2.02 (3H, s, OAc), 3.56 (1H, dd, $J_{6,5}$ 1.9, $J_{6,6'}$ 9.8, 6-H), 3.83–3.92 (4H, m, 2-H, 3-H, 4-H, 6′-H), 4.64, 4.91 (2H, AB system, J 10.7, CH_2Ph), 4.71–4.79 (2H, AB system, J 11.0, CH_2Ph), 5.00 (1H, m, 1-H), 7.28–7.41 (10H, m, H_{Ar}); δ_C(100.6 MHz, $CDCl_3$) -3.0 (2 *C*H_3Si), 18.6 (2 *C*$H_{3\text{-thexyl}}$), 20.0 (2 *C*H_3CH_{thexyl}), 21.1 (OAc), 27.1 (5a-C), 34.3 (*C*H_{thexyl}), 39.8 (5-C), 61.4 (2-C), 62.1 (6-C), 70.5 (1-C), 73.2, 75.6 (2 *C*H_2Ph); 76.8 (4-C), 81.3 (3-C), 169.7 (C=O).

2-Acetamido-1-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranose (9). A mixture of **8** (115 mg, 0.20 mmol) and PPh_3 (160 mg, 0.60 mmol) in dry THF was stirred overnight at 60 °C under nitrogen atmosphere. After addition of water (0.5 mL), the reaction was stirred for 24 h at the same temperature, then the solvent was evaporated. The residue was dissolved in methanol and acetic anhydride (380 μL, 4.04 mmol) was added. After 24 h the solvent was evaporated and the crude compound was purified by flash chromatography (eluent hexane:EtOAc 7:3), providing **9** (111 mg, 95%); $[\alpha]_D^{25}$ +25.2 (*c* 1.15 in $CHCl_3$); (Found: C, 68.00; H, 8.42; N, 2.35. Calc. for $C_{33}H_{49}NO_6Si$: C, 67.89; H, 8.46; N, 2.40); δ_H(400 MHz, $CDCl_3$) 0.09, 0.12 (6H, 2 s, 2 CH_3Si), 0.88 (6H, s, 2 C$H_{3\text{-thexyl}}$), 0.93 (6H, d, J 6.8, 2 CH_3CH_{thexyl}), 1.66 (1H, m, CH_{thexyl}), 1.81–2.16 (3H, m, 5-H, 5a-H, 5a′-H), 1.92 (3H, s, NHAc), 2.04 (3H, s, OAc), 3.67 (1H, dd, $J_{6,5}$ 5.7, $J_{6,6'}$ 9.5, 6-H), 5.71 (1H, dd, $J_{4,3}$ 5.8, $J_{4,5}$ 5.8, 4-H), 3.83–3.90 (2H, m, 3-H, 6′-H), 4.41–4.49 (2H, m, 2-H, C*H*HPh); 4.58–4.65 (2H, AB system, CH_2Ph), 4.74 (1H, d, J 11.3, CH*H*Ph), 5.17 (1H, m, 1-H), 5.63 (1H, d, $J_{NH,2}$ 7.8, NH), 7.30–7.43 (10H, m, H_{Ar}); δ_C(100.6 MHz, $CDCl_3$) -3.6, -3.5 (2 *C*H_3Si), 18.7 (2 *C*$H_{3\text{-thexyl}}$), 20.4 (2 *C*H_3CH_{thexyl}), 21.1 (OAc), 23.4 (NHAc), 27.1 (5a-C), 34.3 (*C*H_{thexyl}), 39.8 (5-C), 50.6 (2-C), 62.5 (6-C), 69.5 (1-C), 72.2, 73.4 (2 *C*H_2Ph), 74.3 (4-C), 79.0 (3-C), 170.0, 170.6 (2 C=O).

Methyl *O*-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranosyl)phosphate triethylammonium salt (10). Compound **9** (107 mg, 0.18 mmol) was dissolved into a 1M mixture of MeONa in methanol (3 mL) and stirred at rt for 2 h, then the reaction mixture was neutralized to pH 7 with IR-120 resin (H^+ form), filtered, and concentrated. The residue was dissolved into dry CH_3CN (1.8 mL), then pyridine (0.80 mL) and a 1M solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphinin-4-one in CH_3CN (550 μL, 0.22 mmol) were added at rt. The mixture was stirred under nitrogen for 45 min, then a pyridine:H_2O 1:1 mixture (1.8 mL) was added. After addition of dichloromethane, the reaction was washed with water (2 × 10 mL) and a 1M solution of triethylammonium hydrogen carbonate (TEAB, 2 × 10 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was filtered through a short silica

gel column (eluent CH_2Cl_2:MeOH 9:1 + 1% triethylamine) to afford a yellow oil. The crude compound was dissolved in dry pyridine (1 mL) under argon atmosphere, then methanol (20 μL, 0.50 mmol) and pivaloyl chloride (52 μL, 0.43 mmol) were added at rt. After 40 min. a freshly prepared solution of iodine in pyridine:H_2O 19:1 was added, then after 15 min. the reaction was diluted with chloroform and washed with a 1 M solution of $Na_2S_2O_4$ (2 × 10 mL) and a 1 M solution of TEAB (2 × 10 mL), dried (Na_2SO_4), filtered, and concentrated. The crude compound was purified by flash chromatography (eluent CH_2Cl_2:MeOH 9:1 + 1% triethylamine) to afford **10** as a glass (84 mg, 62%); $[\alpha]_D^{25}$ +8.0 (*c* 1 in $CHCl_3$); (Found: C, 62.01; H, 8.85; N, 3.74. Calc. for $C_{38}H_{65}N_2O_8PSi$: C, 61.93; H, 8.89; N, 3.80); δ_H(400 MHz, $CDCl_3$) 0.05, 0.06 (6H, 2 s, CH_3Si), 0.83, 0.84 (6H, 2 s, 2 C$H_{3\text{-thexyl}}$), 0.89 (6H, d, *J* 6.8, 2 CH_3CH_{thexyl}), 1.30 (9H, t, *J* 7.2, CH_3CH_2NH), 1.62 (1H, m, CH_{thexyl}), 1.71–182 (2H, m, 5a-H, 5a′-H), 1.92 (3H, s, NHAc), 2.07 (1H, m, 5-H), 3.02 (6H, q, CH_3CH_2NH), 3.48–3.69 (2H, m, 4-H, 6-H), 3.54 (d, $J_{H,P}$ 10.4, CH_3OP), 3.86 (1H, m, 6′-H), 4.07 (1H, dd, $J_{3,4}$ 7.6, $J_{3,2}$ 4.4, 3-H), 4.48–4.61 (5H, m, 1-H, 2-H, CH_2Ph, C*H*HPh), 4.81 (1H, d, *J* 11.2, CH*H*Ph), 7.22–7.36 (10H, m, H_{Ar}); δ_C(100.6 MHz; $CDCl_3$) -3.7, -3.5 (2 *C*H_3Si), 8.7 (3 *C*H_3CH$_2$NH), 18.6, 18.7 (2 *C*$H_{3\text{-thexyl}}$), 20.4, 20.5 (2 *C*H_3CH_{thexyl}), 23.3 (NHAc), 28.7 (5a-C), 34.3 (*C*H$_{\text{thexyl}}$), 39.0 (5-C), 45.3 (3 CH$_3$*C*H$_2$NH), 52.1 (2-C), 52.8 (*C*H$_3$OP), 62.4 (6-C), 70.4 (1-C), 71.8 (2 *C*H$_2$Ph), 76.0 (4-C, from HSQC NMR spectrum), 78.5 (3-C), 171.5 (C=O); δ_P(162 MHz; $CDCl_3$) 2.91.

Methyl *O*-(2-acetamido-2-deoxy-5a-carba-α-D-mannopyranosyl)phosphate sodium salt (3). Compound **10** (110 mg, 0.15 mmol) was dissolved in THF (5 mL), then TBAF (1M in THF + 5% water, 630 μL) was added and the reaction was stirred overnight at rt. The reaction was extracted with chloroform (5 × 10 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was dissolved in a 1:1 MeOH:H_2O mixture (5 mL) at rt, together with 100 mg of Pd/C catalyst. The reaction was stirred overnight under hydrogen atmosphere, then it was filtered through a Celite pad and concentrated. The residue was dissolved in water and passed through a column of Dowex-50W X8 ion exchange resin (Na^+ form). The solvent was evaporated, giving **3** (38 mg, 75%); $[\alpha]_D^{25}$ +1.4 (*c* 0.75 in H_2O); (Found: C, 35.91; H, 5.68; N, 4.10. Calc. for $C_{10}H_{19}NNaO_8P$: C, 35.83; H, 5.71; N, 4.18); δ_H(400 MHz, D_2O) 1.60 (1H, m, 5a-H), 1.87–1.93 (2H, m, 5a′-H, 5-H), 2.00 (3H, s, NHAc), 3.52 (1H, dd, $J_{3,4}$ 9.8, $J_{4,5}$ 10.0, 4-H), 3.56 (3H, d, $J_{H,P}$ 10.8, CH_3OP), 3.68 (1H, dd, $J_{6,6'}$ 16.7, $J_{6,5}$ 5.5, 6-H), 3.71 (1H, dd, $J_{6',5}$ 3.6, 6′-H), 3.91 (1H, dd, $J_{2,3}$ 4.8, 3-H) 4.27 (1H, ddd, $J_{1,2}$ 3.2, $J_{1,5a}$ 2.8, $J_{1,5a'}$ 3.5, 1-H), 4.36 (1H, dd, 2-H); δ_C(100.6 MHz, H_2O) 22.1 (NHAc), 28.0 (5a-C), 38.7 (5-C), 53.0 (2-C), 53.6 (*C*H$_3$OP), 62.1 (6-C), 70.1 (4-C), 70.4 (3-C), 72.1 (1-C), 174.8 (C=O); δ_P(162 MHz, D_2O) 1.22.

Computational methods

Calculations were carried out using the Gaussian03 program package[24] through optimizations in the gas phase at the B3LYP/6-311+G(2df,p) level for phosphorus and 6-311+G(d,p) level for the other atoms. The high level of calculation was necessary to correctly describe compounds **1–3** that contain a phosphorus atom, and the inclusion of diffuse functions was required by their anionic nature. Vibrational frequencies were computed to verify that the optimized structures were minima. The energy of the conformations was recalculated in water at the same level through single-point calculations using a continuum solvent model (C-PCM).[18] A zero-point correction was carried out. For the most populated conformers the proton vicinal coupling constants were calculated with the electronegativity-modified Karplus relationship.[22]

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