# Synthesis, molecular dynamics simulations, and biology of a carba-analogue of the trisaccharide repeating unit of *Streptococcus pneumoniae* 19F capsular polysaccharide†

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The synthesis of a carba-analogue corresponding to the trisaccharide repeating unit of *Streptococcus pneumoniae* type 19F capsular polysaccharide, where a residue of carba-L-rhamnose has been inserted into the natural trisaccharide in place of L-rhamnose, is described. The conformational properties of the analogue were investigated with the aid of molecular dynamics simulations and were strictly analogous to those of the natural compound. The biological activity of the carba-analogue was comparable to that of the corresponding natural repeating unit, thus suggesting that this compound, more stable to hydrolysis, is a good mimic of the natural structure.

## Introduction

Infections elicited by *Streptococcus pneumoniae* (pneumonia, otitis media, sinusitis, meningitis) are frequently occurring diseases that are associated with considerable morbidity and mortality even in developed countries. Pneumococcal diseases primarily affect elderly people and toddlers.[1] The World Health Organization (WHO) estimates that every year up to one million children under 5 years of age die from pneumococcal infection.[2] Vaccination of infants and young children against pneumococcal diseases has become a global health priority in order to reduce and/or control antibiotic resistance to *Streptococcus pneumoniae*.[3] A 23-valent pneumococcal polysaccharide vaccine (PPV23), containing purified capsular polysaccharides (CPS) from 23 serotypes, and a pneumococcal 7-valent conjugate vaccine are now available and have demonstrated effectiveness in older children and adults, or in children younger than 2 years, respectively.[4,5] Nevertheless, with the need to prevent these diseases in the developing world an urgent priority, there is increasing attention on the improvement of vaccine formulations for maximum public-health impact. Strategic decisions should consider the possibility of broadening the serotype coverage and to provide optimum immunological protection in vaccinated individuals.

Recently, the intrinsic problems of manufacturing and safety connected to conventional vaccines, obtained from bacterial cultures and traditionally conjugated to carriers, have led to the development of synthetic vaccines that offer advantages in terms of purity, lot-to-lot consistency, and a high specificity in eliciting immune responses.[6] Indeed, different studies in the field of bacterial infections have shown the potential of synthetic carbohydrate antigens as highly sensitive and specific detection systems or for the development of glycoconjugate vaccines providing effective prophylaxis against the diseases. Successful examples have been reported in the literature for *Shigella dysenteriae* type 1,[7] *Haemophilus influenzae* type b,[8] and *Bacillus anthracis*.[9]

In this context, we are currently involved in a project aimed at a rational development of glycoconjugate vaccines,[10] exploiting chemical synthesis as a tool for obtaining new molecular entities related to natural structures. The aim of the design is to identify molecules that are endowed with the natural biological activity, but superior in terms of stability, potency or efficacy. In particular we have focused on the capsular polysaccharide of *Streptococcus pneumoniae* (SP) type 19F,[11] which is one of the most commonly isolated serotypes causing pneumococcal disease, and is covered up from all licensed vaccines.[12]

The capsular polysaccharide of SP type 19F comprises the trisaccharide repeating unit **1**, (→4)-β-D-Man*p*NAc-(1→4)-α-D-Glc*p*-(1→2)-α-L-Rha*p*-(1-$OPO_3^-$→), with a phosphodiester bridge connecting a residue of rhamnose at the reducing end of one unit to the non-reducing end of the following unit (Fig. 1). The lability of polysaccharide structures is one of the problems associated with conventional vaccines that necessitate transporting and storing in cold conditions due to their limited shelf-life at room temperature. In this case, the presence of a phosphate group at the anomeric position of rhamnose is the cause of the limited stability in water of structure **1**, due to the high propensity to hydrolysis of this group at an acetalic position.[13] The replacement of the pyranose oxygen of L-rhamnose with a methylene group

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† Electronic supplementary information (ESI) available: $\phi_2/\psi_2$ scatter plots and history of $\phi_3$ from the MD simulations of the mono- and disaccharide substructures in vacuum and using explicit water as solvent (Figs. S1 and S2); $^{13}C$ and $^{1}H$ spectra of compounds **9**, **12**, **6**, **17** and **4**. See DOI: 10.1039/b911323a

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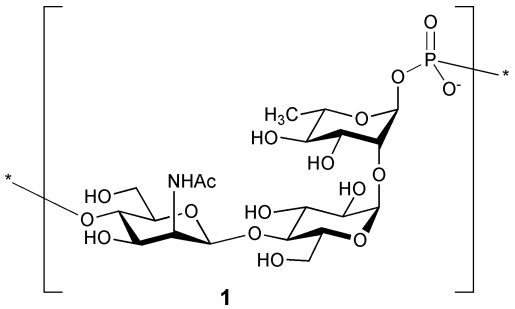


**Fig. 1** *Streptococcus pneumoniae* 19F polysaccharide.

would transform the natural sugar in a more stable carba-sugar analogue, *i.e.* carba-L-rhamnose, where the instability associated with the acetalic anomeric phosphate has been removed. Recently, within a theoretical quantum mechanical study on the conformational behavior of α-L-rhamnose-1-phosphate analogues, a series of stable mimics of the minimum substructure representing the rhamnose moiety in the trisaccharide unit, *i.e.* methyl(2-*O*-methyl-α-L-rhamnopyranosyl)-phosphate (**2**, Fig. 2), have been compared.[14] Carba-L-rhamnose analogue **3** emerged as the best candidate to mimic the rhamnosyl phosphate **2**, as it preserves the geometrical properties of the reference compound. In fact, in spite of the lack of the anomeric and *exo*-anomeric effects, calculations showed that compound **3** maintains (as does **2**) a strong preference for the $^1C_4$ conformation, every $^4C_1$ conformation being almost 5 kcal/mol higher in energy than the global minimum. This suggests that the carbocycle **3**, when inserted into a trisaccharidic unit in the place of a rhamnose residue, could produce an oligosaccharide analogue having immunogenic activity. For these reasons, we have focused our attention on trisaccharide **4**, where a residue of carba-L-rhamnose replaces the L-rhamnose present in the natural repeating unit. Compound **4**, together with the corresponding natural trisaccharide repeating unit **5**, was first submitted to a modeling study to confirm that the carba-L-rhamnose, also when embedded in the trisaccharide, maintains the geometrical preference reported for compound **3**. Then, compound **4** was prepared and subjected to ELISA tests to assess its activity in comparison with **5**[11b] and the native SP 19F polysaccharide.

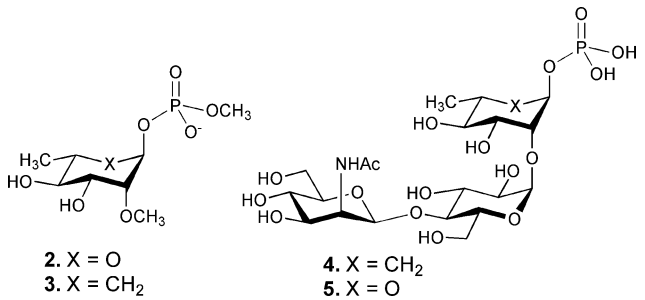


**Fig. 2**

## Results and discussion

### Modeling

In a stepwise approach to the modeling of trisaccharide **5** and its carba-analogue **4**, we first carried out MD simulations, both in vacuum and using explicit water as solvent, on the monosaccharide and disaccharide substructures, α-L-Rha*p*-1-$OPO_3H_2$ and α-D-Glc*p*-(1→2)-α-L-Rha*p*-1-$OPO_3H_2$, and the corresponding carba-analogues (see ESI†). The results in vacuum and in water were very similar, so only vacuum simulations were performed for the larger molecules **4** and **5**. The corresponding torsion angle analyses are given in Fig. 3, where scatter plots of the populated conformations at the two inter-residue glycosidic linkages are presented as well as the trajectory of the $\phi_3$ torsional angle.

As expected,[15] the conformational behavior of the two trisaccharide structures at both the glycosidic linkages was quite superimposable in consequence of the fact that the rhamnose ring oxygen (or methylene) is far enough away not to affect the $\phi_1/\psi_1$ and $\phi_2/\psi_2$ torsional angles. In the β-D-Man*p*NAc-(1→4)-α-D-Glc*p* glycosidic linkage two major conformations are present. They correspond for the $\phi_1$ torsion to that of an *exo*-anomeric effect ($\phi_1 > 0$) and for the $\psi_1$ torsion to those alternating between positive and negative values. Population at the non-*exo*-anomeric conformations where $\phi_1 < 0$ is very limited for the region with $\psi_1$ positive, whereas it is higher for the region with $\psi_1$ negative. In the α-D-Glc*p*-(1→2)-α-L-Rha*p* linkage two conformational regions exist, also in this case corresponding to the *exo*-anomeric effect ($\phi_2 < 0$); in this case one is much more populated than the other, and corresponds to values of $\phi_2$ and $\psi_2$ both negative. The second region is centered at values of the $\psi_2$ torsional angle shifted towards positive values.

The orientation of the phosphate group is described by the $\phi_3$ torsional angle. Toma *et al.*[14] had shown that at the B3LYP/6-31G(d) DFT level of theory the mG conformation ($\phi_3 \approx -60°$) is the most populated (80%) in **2** because of the *exo*-anomeric effect, the remaining percentage being attributable to the T conformation ($\phi_3 \approx 180$ °), whereas the percentage of the G conformation ($\phi_3 \approx 60$ °) is negligible. In **3**, in spite of the absence of such an effect, the mG conformation remains the most populated (61%), followed by the T conformation, indicating that the oxygen/methylene replacement exerts only a slight influence on the orientation of the phosphate group.

Though the computational approach used herein is very different, similar results are obtained by the MD simulation on **4** and **5**. In fact, an analysis of the $\phi_3$ trajectories reported in Fig. 3 shows that only the mG and T conformations are populated, whereas the G conformation was never visited. The analysis allows one to determine percentages of the mG conformations of 61 and 43% for **5** and **4**, respectively, confirming that the effect of the oxygen/methylene replacement consists in a small lowering of the conformation corresponding to the *exo*-anomeric effect that, however, remains highly populated also in **4**. Moreover, a closer inspection of the trajectories shows that the mG/T transition is easier for the carba-analogue than for the natural compound; in fact, it occurs much more frequently in **4** than in **5**. This seems to indicate a lowering of the energy barrier between mG and T in **4** as a consequence of the oxygen/methylene replacement.

### Synthesis of the carba-analogue repeating unit 4

Protected trisaccharide **6** (Fig. 4) is the intermediate planned for the synthesis of the target compound **4**, namely the carba-L-rhamnose analogue of the repeating unit of *Streptococcus pneumoniae* capsular polysaccharide type 19F. The hydroxyl

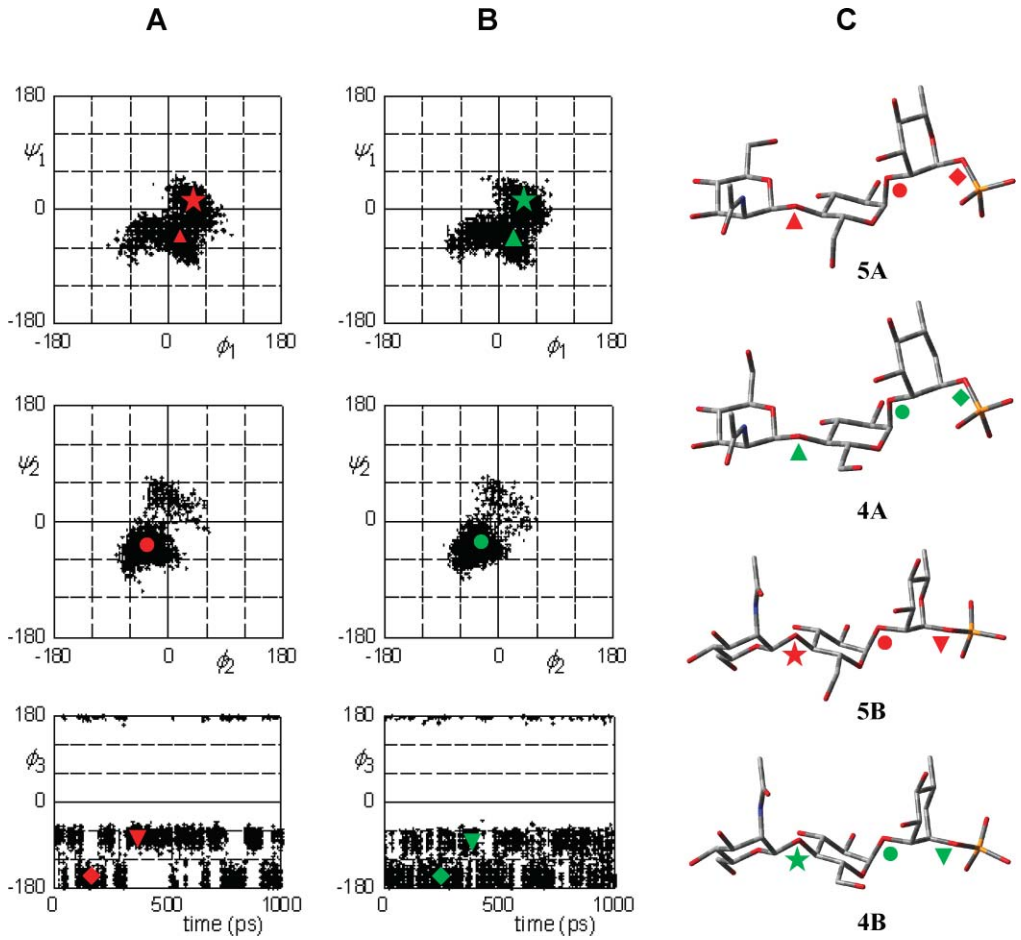
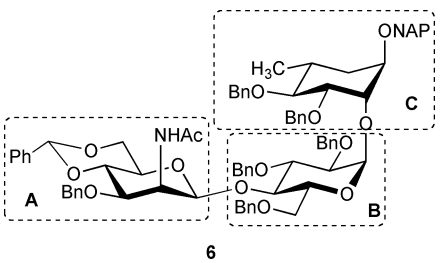



**Fig. 3** $\phi_1/\psi_1$ and $\phi_2/\psi_2$ scatter plots and history of $\phi_3$ from the MD simulations of (**A**) trisaccharide **5** and (**B**) its carba-analogue **4**. (**C**) Significant conformations of compounds **5** and **4** with their glycosidic torsional angles shown on the plots: **5A**, $\phi_1/\psi_1$ 19/−51 (▲), $\phi_2/\psi_2$ −35/−38 (●), $\phi_3$ −168 (◆); **5B**, $\phi_1/\psi_1$ 43/5 (★), $\phi_2/\psi_2$ −36/−37 (●), $\phi_3$ −69 (▼); **4A**, $\phi_1/\psi_1$ 24/−55 (▲), $\phi_2/\psi_2$ −39/−43 (●), $\phi_3$ −166 (◆); **4B**, $\phi_1/\psi_1$ 41/9 (★), $\phi_2/\psi_2$ −33/−37 (●), $\phi_3$ −73 (▼).




6

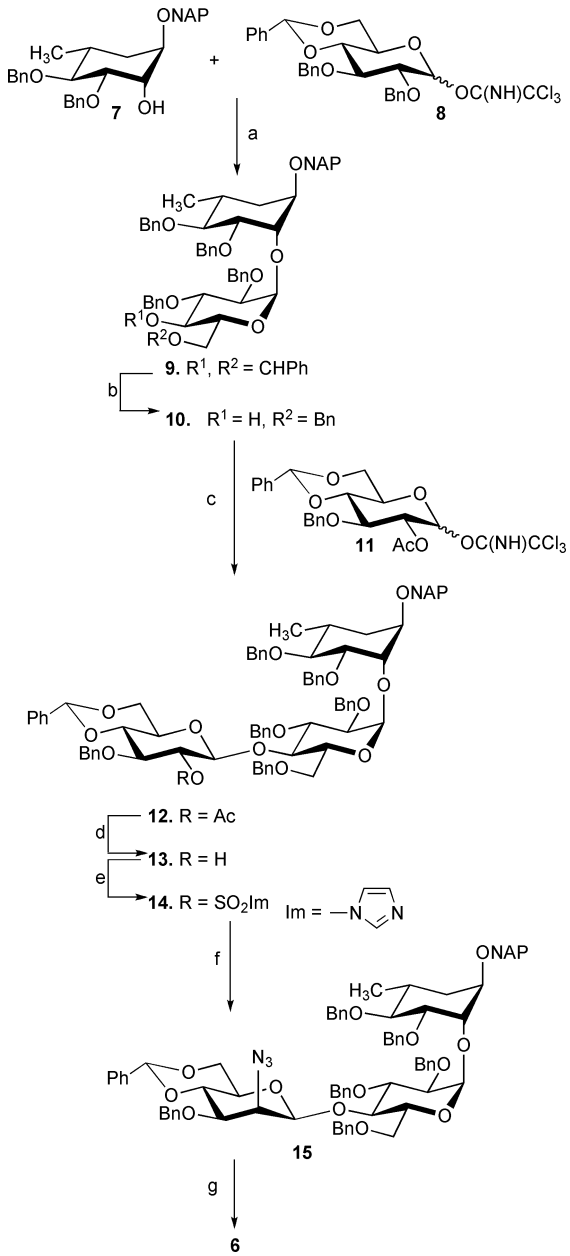
**Fig. 4** The trisaccharide intermediate **6**.

protecting groups of intermediate **6** are all removable by catalytic hydrogenolysis but possess the required orthogonality to have preferential access to the points of chain elongation for further manipulations (*e.g.* conjugation and oligomerization). It is in fact possible to selectively deprotect either the anomeric position at the reducing end by removal of the 2-naphthylmethyl (NAP) ether, or the 4-OH of mannosamine by regioselective opening of the benzylidene acetal. The disconnection strategy adopted for the synthesis of the trisaccharide consists in the initial formation of the α-linked disaccharide **B–C** by the use of a 4,6-*O*-benzylidene glucosyl donor. It is in fact well established that the stereoselectivity of the reaction of α-glucosylation is a function of the protecting groups on glucose, and 4,6-*O*-benzylidene glucosyl donors, protected with no participating groups at the 2-position, are usually α-selective.[11a,16,17] Mannosamine unit **A** is in turn conjugated to disaccharide **B–C** through β-glucosylation followed by epimerization of β-glucopyranoside to β-mannopyranoside.

Previously obtained carba-L-rhamnose **7** (Scheme 1),[18] suitably protected for later modifications, was glycosylated at position 2 with 4,6-*O*-benzylidene glucosyl trichloroacetimidate donor **8**.[19] The reaction was carried out in dichloromethane under the catalysis of triethylsilyl triflate and gave product **9**, with the desired stereochemistry, in acceptable yield. The α-configuration of the new glycosidic bond was confirmed through $^1$H-NMR analysis by the value of 3.8 Hz for the $J_{1',2'}$ coupling constant. Reductive opening of the benzylidene acetal to the corresponding 6-*O*-benzyl ether was in turn accomplished by treatment of **9** with triethylsilane in the presence of boron trifluoride–diethyl ether complex, which gave the disaccharide acceptor **10** in good yield.[20]

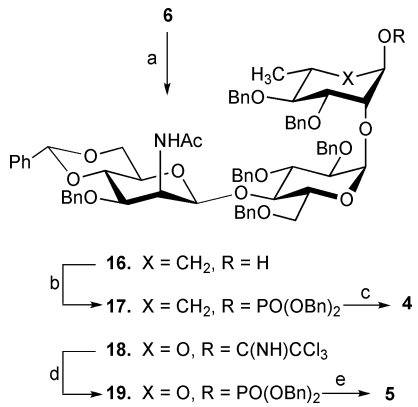
Trimethylsilyl triflate-promoted coupling of donor **11**,[21] with acceptor **10** in dichloromethane at low temperature gave trisaccharide **12** in high yield. The β-configuration of the glycosidic bond was demonstrated by the characteristic 8.2 Hz value of the




**Scheme 1** *Reagents and conditions:* (a) TESOTf, DCM, –20 °C, 65%; (b) $Et_3SiH$, $BF_3{\cdot}Et_2O$, DCM, 0 °C, 85%; (c) TMSOTf, DCM, –20 °C, 90%; (d) MeONa, DCM/MeOH, 88%; (e) $Im_2SO_2$, NaH, DMF, –40 °C, 93%; (f) $NaN_3$, DMF, 110 °C, 70%; (g) $NiCl_2$, $NaBH_4$, DCM/MeOH, 0–10 °C, then $Ac_2O$, 74%.

*trans*-diaxial $J_{1'',2''}$ coupling constant in the $^1$H-NMR spectrum. After removal of the acetate with Zémplen transesterification, the 2-OH of compound **13** was activated in high yield as its imidazylate and subjected to azide displacement with sodium azide to give mannoside **15**. Final conversion of the azido function into the acetamido group, accomplished through reduction followed by acetylation, afforded the desired orthogonally protected intermediate **6**. In order to introduce the phosphate, the NAP group of compound **6** was removed by treatment with DDQ[22] to yield **16** (Scheme 2), which was subjected to phosphorylation using phosphorus trichloride–imidazole, followed by treatment with benzyl alcohol and *in situ* oxidation by *m*-chloroperbenzoic




**Scheme 2** *Reagents and conditions:* (a) DDQ, DCM/MeOH, 0–25 °C, 78%; (b) $PCl_3$, imidazole, TEA, BnOH, *m*CPBA, DCM/$CH_3CN$, 73%; (c) $H_2$, $Pd(OH)_2$, EtOAc/MeOH/$H_2O$, 60%; (d) $HOPO(OBn)_2$, DCM, 87%; (e) see ref. 11b.

acid to give compound **17** in good yield. Final removal of the protecting groups by hydrogenolysis gave the desired carba-analogue trisaccharide **4**.

The natural trisaccharide repeating unit **5**, necessary for the biological evaluation as reference compound, was prepared as described previously.[11b] We wish to report an alternative to the former procedure in the phosphorylation step to give compound **19**, which was obtained after glycosylation between trichloroacetimidate donor **18**[7b] and commercially available dibenzyl phosphate at room temperature.[23] The reaction afforded stereoselectively α-phosphate **19** in high yield and was highly reproducible. Compound **19** was deprotected according to ref. 11b.

## Biology

In order to compare the biological activity of the newly synthesized carba-L-rhamnose trisaccharide **4** with that of the corresponding natural trisaccharide **5** and the native polysaccharide **19F** (positive control), classical competitive ELISA assays were performed. In particular we evaluated the abilities of increasing concentrations ($10^{-8}$–$10^{0}$ mg/mL) of each compound to inhibit the binding between the 19F polysaccharide, coated onto plates, and the anti-19F human polyclonal antibody.

Fig. 5 shows the inhibition curves obtained with the compounds under evaluation. The relative efficacy of each compound was calculated by measuring the maximum effect elicited in this system (relative efficacy), while the concentration that produces the 50% of the maximum effect ($EC_{50}$) was taken as indirect index of its relative potency. Table 1 shows the calculated data. As expected, the chain length of the saccharide molecules seems to be important for their biological activity. The 19F native polysaccharide, indeed, exhibited both an higher efficacy (100% of inhibition at $10^{-2}$ mg/mL) and affinity ($EC_{50} = 8.9 \times 10^{-5}$ mg/mL) than the synthesized compounds, confirming that high molecular weight polysaccharides have a conformational specificity (conformational epitopes) more suitable for antibody binding.[24] Herein we show that even the single repeating unit has inhibitory properties. In fact, the analysis of the results demonstrates that both compound **4** and the corresponding natural trisaccharide **5** are recognized by the anti-19F antibody. The carba-L-rhamnose trisaccharide **4** was slightly more effective than the natural trisaccharide **5**,

**Table 1** Results of the competitive ELISA assays

| Compound | $EC_{50}$ [mg/mL] | Maximum inhibition values [%][a] |
|---|---|---|
| **19F** | $8.9 \times 10^{-5}$ | 100 |
| **4** | $2.7 \times 10^{-2}$ | 48 ± 3 |
| **5** | $5.2 \times 10^{-3}$ | 33 ± 3 |

[a] Measured at 1 mg/mL.

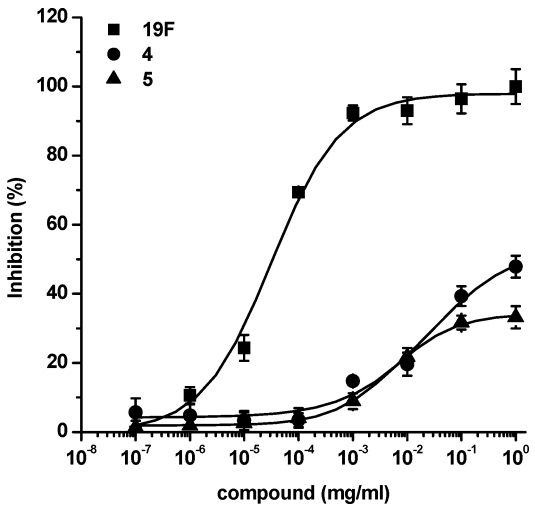



**Fig. 5** Concentration–response curves of saccharides on the inhibition of the binding between the 19F polysaccharide, coated onto the plates, and the anti-19F human polyclonal antibody were evaluated by a competitive ELISA method (see the Experimental section). Values are means of at least four experiments run in triplicate.

though less potent (see Table 1), in inhibiting the binding to the specific antibody. This suggests that the replacement of the pyranose oxygen of L-rhamnose with a methylene group, which confers advantages in term of chemical stability, does not strongly affect the biological properties of the compound.

## Conclusions

In conclusion, we have reported a concise synthesis of compound **4**, the carba-analogue of the CPS repeating unit of *Streptococcus pneumoniae* type 19F, where the residue of L-rhamnose has been replaced with a carba-L-rhamnose. Analogue **4** appears to be a good mimic of the natural compound as supported both by molecular dynamics simulations, which show a conformational behavior nearly identical for the two compounds, and by biological assays, which evidence a slightly different but comparable potency. Our studies support that the trisaccharide carba-analogue is a good candidate for further investigations. Since the biological activity at the level of the repeating unit monomers is comparable, further developments may involve the study of the activity of longer oligomers, in order to eventually find new molecules for the development of synthetic vaccines against pneumococcal infection or address important aspects of the immune response to analogues of antigenic structures. With respect to the natural compound, the carba-analogue offers advantages in terms of chemical stability. Furthermore, from a synthetic point of view, it would be easier to oligomerize the monomeric carba-trisaccharide since the phosphodiester bridge among the repeating units no longer involves an anomeric position, thus reducing the chemical problems associated with the preparation of pure α-linked anomeric phosphates.

## Experimental

### Computational methods

Molecular dynamics (MD) simulations were performed with the HyperChem™ 7.5 software from Hypercube, Inc. employing the $MM^+$ force field. The simulations on compounds **4** and **5** were carried out in vacuum whereas those on their substructures α-L-Rha*p*-1-$OPO_3H_2$, α-D-Glc*p*-(1→2)-α-L-Rha*p*-1-$OPO_3H_2$, and the corresponding carba-analogues were carried out both in vacuum and using explicit water as solvent. For the vacuum simulations, following a 20 ps equilibration of the molecule, the production run was carried out at 300 K for 1000 ps (**4** and **5**) and for 500 ps (substructures). A time step of 1 fs was used in the integration algorithm and the trajectories were saved every 50 fs for further analysis. For the water simulations initial conditions were prepared by placing the mono- and disaccharides in a previously equilibrated cubic water box of approximately 19 and 23 Å, respectively, following minimization and heating. This procedure resulted in systems with the mono and disaccharides and either 198 and 382 TIP3P water molecules, respectively. Following a 20 ps equilibration of the system the production run was carried out for 500 ps at 300 K with a switched cutoff for non-bond interactions set from 5.35 to 9.35 Å and from 7.55 to 11.55 Å for the mono and disaccharides, respectively. Also for water simulations a time step of 1 fs was used in the integration algorithm and the trajectories were saved every 50 fs. Trajectory analyses were carried out on the glycosidic angles of compounds **4** and **5**, defined as $\phi_1$ H1–C1″–O–C4′, $\psi_1$ C1″–O–C4′–H4′, $\phi_2$ H1′–C1′–O–C2, $\psi_2$ C1′–O–C2–H2, $\phi_3$ X–C1–O–P (**5**: X = O; **4**: X = $CH_2$), and on the corresponding torsional angles of their mono- and disaccharide substructures.

### General

All reagents were the highest commercial quality and used without further purification except where noted. Air- and moisture-sensitive liquids and solutions were transferred *via* oven-dried syringe or stainless steel cannula through septa. Organic solutions were concentrated by rotary evaporation below 45 °C at approximately 20 mmHg. All reactions were carried out under anhydrous conditions using oven-dried glassware within an argon atmosphere. Dry solvents and liquid reagents were distilled prior to use. Dichloromethane (DCM) was distilled from calcium hydride; *N*,*N*-dimethylformamide (DMF), acetonitrile ($CH_3CN$), and methanol (MeOH) were dried on activated molecular sieves; triethylamine (TEA) was distilled from calcium hydride, triethylsilane ($Et_3SiH$) was distilled at 20 torr. NaH was washed three times with hexane prior to use. All reactions were monitored by TLC on silica gel 60 F-254 plates (Merck), spots being developed with 5% $H_2SO_4$ in MeOH/$H_2O$ (1:1) or with a phosphomolybdate-based reagent and subsequently heated at 110 °C. Flash column chromatography was performed on silica gel 60 (230–400 mesh, Merck) Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 20 °C. $^1H$, $^{13}C$ and $^{31}P$ NMR spectra were recorded with a Bruker AVANCE-500 spectrometer at a sample

temperature of 298 K. Chemical shifts are reported on the δ (ppm) scale and are relative to TMS as internal reference. $^{31}P$ NMR spectrum for compound **17** was calibrated by using the $H_3PO_4$ signal (δ = 0.0 ppm). Resonances were assigned by means of 2D COSY experiment (standard Bruker pulse program). The mass spectrometric analyses were performed in positive or negative electrospray (ESI-MS). MS spectra were recorded on a Thermo Quest Finnigan LCQ™ Deca ion trap mass spectrometer equipped with a Finnigan ESI interface; data were processed by Finnigan Xcalibur software system.

### 2-Naphthylmethyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl-(1→2)-3,4-di-*O*-benzyl-5a-carba-α-L-rhamnopyranoside (9)

A solution of glucosyltrichloroacetimidate[19] **8** (835 mg, 1.408 mmol) and 2-OH carba-rhamnoside[18] **7** (340 mg, 0.704 mmol) in dry DCM (14 mL) was cooled to –20 °C, then triethylsilyl trifluoromethanesulfonate (0.1 M solution in dry DCM, 1.41 mL) was added dropwise. After 45 min the reaction was quenched by the addition of saturated $NaHCO_3$ solution and diluted with DCM. After separation, the aqueous layer was extracted three times with DCM and the combined organic layers were dried and concentrated. The crude product was purified twice by flash chromatography (first hexane/EtOAc 85:15, then toluene/EtOAc 97:3, v/v) to give pure **9** (420 mg, 65%) as an amorphous powder (Found: C, 77.70; H, 6.43. Calc. for $C_{59}H_{60}O_9$: C, 77.61; H, 6.62%); $[\alpha]_D$ +44.5 (*c* 1 in $CHCl_3$); $\delta_H$(500 MHz, $CDCl_3$) 1.07 (d, $J_{5,6}$ = 6.5 Hz, 3 H, 3 H-6), 1.65–1.81 (m, 2 H, 2 H-5a), 1.91–2.03 (m, 1 H, H-5), 3.42 (dd, $J_{3,4} = J_{4,5}$ = 10.0 Hz, 1 H, H-4), 3.52 (dd, $J_{1',2'}$ = 3.8, $J_{2',3'}$ = 9.5 Hz, 1 H, H-2′), 3.56–3.65 (m, 3 H, H-1, H-4′ and H-6′a), 3.86 (dd, $J_{2,3}$ = 2.9, $J_{3,4}$ = 9.5 Hz, 1 H, H-3), 3.96 (dd, $J_{5',6'b}$ = 5.0, $J_{6'a,6'b}$ = 10.5 Hz, 1 H, H-6′b), 4.06 (dd, $J_{2',3'} = J_{3',4'}$ = 9.5 Hz, 1 H, H-3′), 4.09–4.12 (m, 1 H, H-2), 4.29–4.36 (m, 1 H, H-5′), 4.47–5.02 (m, 10 H, 5 $CH_2$Ph), 4.73 (d, $J_{1',2'}$ = 3.5 Hz, 1 H, H-1′), 5.55 (s, 1 H, CHPh), 7.18–7.94 (m, 32 H, arom.); $\delta_C$(125 MHz, $CDCl_3$) 18.2, 31.8, 32.0, 62.6, 69.1, 70.7, 72.5, 73.7, 74.4, 75.0, 75.2, 76.4, 78.4, 79.4, 80.7, 82.6, 82.8, 97.8, 101.2, 125.5–139.0 (40C arom.); ESI-MS (positive-ion mode) *m/z* 935.5 $[M + Na]^+$ (100%), Calcd for $C_{59}H_{60}O_9 + Na^+$ 935.4.

### 2-Naphthylmethyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→2)-3,4-di-*O*-benzyl-5a-carba-α-L-rhamnopyranoside (10)

Compound **9** (311 mg, 0.341 mmol) was dissolved in dry DCM (6.5 mL), and triethylsilane (0.54 mL, 3.410 mmol) was added. The solution was cooled to 0 °C followed by the addition of $BF_3{\cdot}Et_2O$ (0.086 mL, 0.682 mmol). The reaction was stirred at 0 °C for 3 h, then quenched with triethylamine, diluted with DCM and concentrated *in vacuo*. The residue was purified by flash chromatography (hexane/EtOAc 85:15, v/v) to afford compound **10** (265 mg, 85%). (Found: C, 77.61; H, 6.90. Calc. for $C_{59}H_{62}O_9$: C, 77.44; H, 6.83%); $[\alpha]_D$ +48.2 (*c* 1 in $CHCl_3$); $\delta_H$(500 MHz, $CDCl_3$) 1.08 (d, $J_{5,6}$ = 6.5 Hz, 3 H, 3 H-6), 1.66–1.80 (m, 2 H, 2 H-5a), 1.92–2.02 (m, 1 H, H-5), 2.33–2.40 (m, 1 H, OH), 3.28 (dd, $J_{5',6'a}$ = 3.7, $J_{6'a,6'b}$ = 10.5 Hz, 1 H, H-6′a), 3.38 (m, 2 H, H-4 and H- 6′b), 3.49 (dd, $J_{1',2'}$ = 3.5, $J_{2',3'}$ = 9.5 Hz, 1 H, H-2′), 3.56–3.60 (m, 1 H, H-1), 3.72 (dd, $J_{2',3'} = J_{3',4'}$ = 9.5 Hz, 1 H, H-3′), 3.79–3.86 (m, 2 H, H-3 and H- 4′), 4.13–4.16 (m, 1 H, H-2), 4.16–4.21 (m, 1 H, H-5′), 4.33–5.01 (m, 12 H, 6 $CH_2$Ph), 4.78 (d, $J_{1',2'}$ = 3.3 Hz, 1 H, H-1′), 7.15–7.90 (m, 32 H, arom.); $\delta_C$(125 MHz, $CDCl_3$) 18.2, 31.9, 32.0, 69.0, 69.9, 70.8, 70.9, 72.2, 73.0, 73.5, 74.3, 75.0, 75.2, 75.4, 79.8, 80.7, 80.8, 83.0, 96.6, 125.5–128.2 (40 C); ESI-MS (positive-ion mode) *m/z* 937.6 $[M + Na]^+$ (100%), Calcd for $C_{59}H_{62}O_9 + Na^+$ 937.4.

### 2-Naphthylmethyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→2)-3,4-di-*O*-benzyl-5a-carba-α-L-rhamnopyranoside (12)

A solution of 2-*O*-acetyl-glucosyltrichloroacetimidate[21] **11** (375 mg, 0.688 mmol) and 4′-*O*-deprotected disaccharide **10** (252 mg, 0.275 mmol) in dry DCM (10 mL) was cooled to –20 °C, then trimethylsilyl trifluoromethanesulfonate (0.1 M solution in dry DCM, 1.38 mL) was added. After 2 h, the reaction mixture was quenched with triethylamine and the solvent evaporated. The crude product was purified by flash chromatography (toluene/acetone 96:4, v/v) to give **12** (320 mg, 90%) as colourless oil (Found: C, 74.89; H, 6.34. Calc. for $C_{81}H_{84}O_{15}$: C, 74.98; H, 6.53%); $[\alpha]_D$ +30.7 (*c* 1 in $CHCl_3$); $\delta_H$(500 MHz, $CDCl_3$) 1.08 (d, $J_{5,6}$ = 6.5 Hz, 3 H, 3 H-6), 1.66–1.79 (m, 5 H, $OCOCH_3$ and 2 H-5a), 1.92–2.10 (m, 1 H, H-5), 3.11 (ddd, $J_{5'',6''b}$ = 5.0, $J_{4'',5''} = J_{5'',6''a}$ = 10.0 Hz, 1 H, H-5″), 3.19 (dd, $J_{5',6'a}$ = 1.5, $J_{6'a,6'b}$ = 11.0 Hz, 1 H, H-6′a), 3.36–3.49 (m, 4 H, H-4, H-2′, H-3″ and H-6″a), 3.52 (dd, $J_{5',6'b}$ = 1.7, $J_{6'a,6'b}$ = 11.0 Hz, 1 H, H-6′b), 3.58–3.61 (m, 1 H, H-1), 3.64 (dd, $J_{3'',4''} = J_{4'',5''}$ = 10.0 Hz, 1 H, H-4″), 3.77 (dd, $J_{2',3'} = J_{3',4'}$ = 9.5 Hz, 1 H, H-3′), 3.85 (dd, $J_{2,3}$ = 2.6, $J_{3,4}$ = 9.6 Hz, 1 H, H-3), 3.92 (dd, $J_{3',4'} = J_{4',5'}$ = 9.5 Hz, 1 H, H-4′), 4.04–4.11 (m, 2 H, H-2 and H-5′), 4.15–4.21 (m, 2 H, H-6″b and CH*H*Ph), 4.31 (d, $J_{1'',2''}$ = 8.2 Hz, 1 H, H-1″), 4.45–5.03 (m, 13 H, $CH_2$Ph), 4.72 (d, 1 H, $J_{1',2'}$ = 3.5 Hz, H-1′), 4.91 (dd, $J_{1'',2''} = J_{2'',3''}$ = 8.2 Hz, 1 H, H-2″), 5.50 (s, 1 H, CHPh), 7.10–7.90 (m, 42H, arom.); $\delta_C$(125 MHz, $CDCl_3$) 18.2, 20.6, 31.8, 32.2, 65.9, 67.1, 68.7, 70.1, 70.8, 72.5, 73.2, 73.4, 73.5, 73.9, 74.8, 75.1, 76.7, 77.1, 77.2, 78.5, 79.1, 79.7, 81.4, 81.7, 82.8, 97.8, 100.6, 101.2, 125.5–139.4 (52C arom.), 168.9. ESI-MS (positive-ion mode) *m/z* 1319.9 $[M + Na]^+$ (100%), Calcd for $C_{81}H_{84}O_{15}$+ $Na^+$ 1319.6.

### 2-Naphthylmethyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→2)-3,4-di-*O*-benzyl-5a-carba-α-L-rhamnopyranoside (13)

To a stirred solution of **12** (293 mg, 0.226 mm) in dry DCM (4.5 mL) sodium methoxide in dry methanol (0.5 M solution, 0.23 mL) was added. The reaction was stirred for 60 h at room temperature, then it was neutralized with an ion exchange resin (Dowex 50 × 8, $H^+$ form), filtered and concentrated. The crude product was subjected to flash chromatography (toluene/acetone 97:3, v/v) to give pure **13** (250 mg, 88%) as colourless oil (Found: C, 75.71; H, 6.78. Calc. for $C_{79}H_{82}O_{14}$: C, 75.58; H, 6.58%); $[\alpha]_D$ +42.8 (*c* 1 in $CHCl_3$); $\delta_H$(500 MHz, $CDCl_3$) 1.10 (d, $J_{5,6}$ = 6.5 Hz, 3 H, 3 H-6), 1.68–1.81 (m, 2 H, 2 H-5a), 1.95–2.05 (m, 1 H, H-5), 3.05–3.11 (m, 2 H, H-5″ and OH), 3.19 (dd, $J_{5',6'a}$ = 1.8, $J_{6'a,6'b}$ = 11.6 Hz, 1 H, H-6′a), 3.41–3.62 (m, 7 H, H-1, H-4, H-2′, H-2″, H-3″, H-4″ and H-6″a), 3.65 (dd, $J_{5',6'b}$ = 2.7, $J_{6'a,6'b}$ = 11.6 Hz, 1 H, H-6′b), 3.85 (dd, $J_{2,3}$ = 3.0, $J_{3,4}$ = 9.5 Hz, 1 H, H-3), 3.91 (dd, $J_{2',3'} = J_{3',4'}$ = 9.5 Hz, 1 H, H-3′), 3.96–4.01 (m, 2 H, H-4′ and H-6″b), 4.13–4.15 (m, 1 H, H-2), 4.21–4.26 (m, 1 H, H-5′),

4.31–5.02 (m, 14 H, $CH_2Ph$), 4.53 (d, $J_{1'',2''} = 8.0$ Hz, 1 H, H-1″), 4.75 (d, $J_{1',2'} = 3.6$ Hz, 1 H, H-1′), 5.49 (s, 1H, CHPh), 7.10–7.90 (m, 42 H, arom.); $\delta_C$(125 MHz, $CDCl_3$) 18.2, 31.9, 32.0, 66.3, 68.2, 68.7, 69.8, 70.9, 72.4, 73.4, 73.7, 74.4, 74.5, 74.9, 75.2, 75.6, 75.9, 77.3, 79.8, 80.3, 80.6, 80.9, 81.2, 83.1, 96.7, 101.2, 103.8, 125.3–139.3 (52 C arom.); ESI-MS (positive-ion mode) *m/z* 1277.7 [M + Na]$^+$ (100%), Calcd for $C_{79}H_{82}O_{14}$ + $Na^+$ 1277.6.

### 2-Naphthylmethyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(*N*-imidazole-1-sulfonyl)-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→2)-3,4-di-*O*-benzyl-5a-carba-α-L-rhamnopyranoside (14)

NaH (45 mg, 1.87 mmol) was added to a stirred solution of **13** (240 mg, 0.191 mmol) in dry DMF (3.9 mL) at room temperature. After 1 h, the suspension was cooled to –40 °C and 1,1′-sulfonyldiimidazole (379 mg, 1.91 mmol) in dry DMF (1 mL) was added. After 5 h the reaction mixture was quenched with MeOH and allowed to warm to room temperature, then diluted with DCM. The mixture was washed with $H_2O$, then the aqueous layer was extracted three times with DCM. The combined organic layers were dried over $Na_2SO_4$, filtered and evaporated. Flash chromatography (toluene/acetone 96:4, v/v) of the crude product gave **14** (246 mg, 93%) as colourless oil (Found: C, 71.01; H, 6.28; N, 2.05; S, 2.42. Calc. for $C_{82}H_{84}N_2O_{16}S$: C, 71.08; H, 6.11; N, 2.02; S, 2.31%); $[\alpha]_D$ +27.8 (*c* 1 in $CHCl_3$); $\delta_H$(500 MHz, $CDCl_3$) 1.10 (d, $J_{5,6} = 6.5$ Hz, 3 H, 3 H-6), 1.72–1.77 (m, 2 H, 2 H-5a), 1.93–2.04 (m, 1 H, H-5), 2.93–3.00 (m, 1 H, H-5″), 3.24 (br d, $J_{6'a,6'b} = 11.0$ Hz, 1 H, H-6′a), 3.34 (dd, $J_{2'',3''} = J_{3'',4''} = 9.0$ Hz, 1 H, H-3″), 3.40–3.61 (m, 6 H, H-1, H-4, H-2′, H-6′b, H-4″ and H-6″a,), 3.74 (dd, $J_{2',3'} = J_{3',4'} = 9.5$ Hz, 1 H, H-3′), 3.87 (dd, $J_{2,3} = 2.5$, $J_{3,4} = 9.5$ Hz, 1 H, H-3), 3.93 (dd, $J_{3',4'} = J_{4',5'} = 9.5$ Hz, 1 H, H-4′), 4.10–4.15 (m, 2 H, H-2 and C*H*HPh), 4.20–4.26 (m, 3 H, H-5′, H-1″ and H-6″b), 4.36 (dd, $J_{1'',2''} = 8.2$, $J_{2'',3''} = 9.0$ Hz, 1 H, H-2″), 4.44–5.10 (m, 13 H, $CH_2Ph$), 4.73 m, 1 H, H-1′), 5.45 (s, 1 H, CHPh), 7.00–7.02 (m, 1 H, imidazole), 7.08–7.10 (m, 1 H, imidazole), 7.15–7.90 (m, 43 H, arom. and imidazole); $\delta_C$(125 MHz, $CDCl_3$) 18.2, 32.0, 32.2, 65.7, 67.4, 68.5, 69.3, 70.8, 72.7, 73.4, 73.5, 74.2, 74.7, 75.1, 75.2, 75.8, 76.8, 77.2, 79.2, 79.4, 80.8, 82.0, 83.2, 84.9, 97.6, 98.5, 101.4, 118.3–139.3 (55 C arom.); ESI-MS (positive-ion mode) *m/z* 1407.4 [M + Na]$^+$ (100%), Calcd for $C_{82}H_{84}N_2O_{16}S$ + $Na^+$ 1407.5.

### 2-Naphthylmethyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→2)-3,4-di-*O*-benzyl-5a-carba-α-L-rhamnopyranoside (15)

To a stirred solution of **14** (240 mg, 0.173 mmol) in dry DMF (3.5 mL), sodium azide (112 mg, 1.73 mmol) was added and the resulting solution was heated at 110 °C. After 5 h, the reaction was allowed to cool to room temperature and then diluted with $H_2O$ and DCM. After separation, the aqueous phase was extracted twice with DCM and twice with EtOAc, and the combined organic layers were dried over $Na_2SO_4$, filtered and evaporated. The crude product was purified by flash chromatography (toluene/acetone 96:4, v/v) to give **15** (155 mg, 70%) as colourless oil (Found: C, 74.22; H, 6.58; N, 3.21. Calc. for $C_{79}H_{81}N_3O_{13}$: C, 74.10; H, 6.38; N, 3.28%); $[\alpha]_D$ +20.2 (*c* 1 in $CHCl_3$); $\delta_H$(500 MHz, $CDCl_3$) 1.10 (d, $J_{5,6} = 6.5$ Hz, 3 H, 3 H-6), 1.69–1.83 (m, 2 H, 2 H-5a), 1.95–2.05 (m, 1 H, H-5), 2.92–2.99 (m, 1 H, H-5″), 3.01–3.11 (m, 2 H, 2 H-6′), 3.31 (dd, $J_{2'',3''} = 3.5$, $J_{3'',4''} = 9.5$ Hz, 1 H, H-3″), 3.42–3.48 (m, 2 H, H-4 and H-2′), 3.53–3.63 (m, 3 H, H-1, H-2″ and H-6″a), 3.84–3.93 (m, 4 H, H-3, H-3′, H-4′ and H-4″), 4.01 (dd, $J_{5,6''b} = 4.8$, $J_{6''a,6''b} = 10.5$ Hz, 1 H, H-6″b), 4.08 (d, $J_{gem} = 12.2$ Hz, 1 H, C*H*HPh), 4.13–4.16 (m, 1 H, H-2), 4.20–4.25 (m, 1 H, H-5′), 4.37 (d, $J_{1'',2''} = 1$ Hz, 1 H, H-1″), 4.46–5.05 (m, 13 H, $CH_2Ph$), 4.72 (d, $J_{1',2'} = 3.5$ Hz, 1 H, H-1′), 5.52 (s, 1 H, CHPh), 7.15–7.90 (m, 42 H, arom.); $\delta_C$(125 MHz, $CDCl_3$) 18.1, 32.0, 32.1, 63.6, 67.1, 67.9, 68.4, 69.3, 70.9, 72.3, 72.6, 73.3, 73.5, 74.5, 75.1, 75.4, 75.6, 76.3, 77.5, 78.5, 79.4, 80.1, 81.0, 83.2, 96.6, 100.4, 101.5, 125.5–139.3 (52 C arom.); ESI-MS (positive-ion mode) *m/z* 1302.6 [M + Na]$^+$ (100%), Calcd for $C_{79}H_{81}N_3O_{13}$ + $Na^+$ 1302.6.

### 2-Naphthylmethyl 2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→2)-3,4-di-*O*-benzyl-5a-carba-α-L-rhamnopyranoside (6)

To a stirred solution of **15** (140 mg, 0.109 mmol) in DCM/MeOH 1:1.5 (5 mL) cooled at 0 °C $NiCl_2 \cdot 6H_2O$ (156 mg, 0.656 mmol) and $NaBH_4$ (41 mg, 1.09 mmol) were added. The colour of the solution changed from green to black. The reaction mixture was maintained at 10 °C for 2 h, then $Ac_2O$ (0.11 mL) was added. After 1 h the reaction was quenched by the addition of saturated aqueous $NaHCO_3$ and diluted with DCM. After separation, the aqueous phase was extracted twice with DCM, twice with EtOAc and twice with $CHCl_3$. The combined organic layers were dried over $Na_2SO_4$, filtered and evaporated. Flash chromatography (hexane/AcOEt 7:3, v/v) of the crude product gave pure **6** (104 mg, 74%) as colourless oil (Found: C, 74.84; H, 6.56; N, 1.20. Calc. for $C_{81}H_{85}NO_{14}$: C, 75.04; H, 6.61; N, 1.08%); $[\alpha]_D^{25}$ +16.1 (*c* 1 in $CHCl_3$); $\delta_H$(500 MHz, $CDCl_3$) 1.10 (d, $J_{5,6} = 6.5$ Hz, 3 H, 3 H-6), 1.67–1.81 (m, 5 H, 2 H-5a and $COCH_3$), 1.96–2.06 (m, 1 H, H-5), 3.03–3.16 (m, 2 H, H-6′a and H-5″), 3.27–3.34 (m, 2 H, H-6′b and H-3″), 3.46 (dd, $J_{3,4} = J_{4,5} = 9.8$ Hz, 1 H, H-4), 3.49 (dd, $J_{1',2'} = 3.5$, $J_{2',3'} = 9.5$ Hz, 1 H, H-2′), 3.52–3.60 (m, 2 H, H-1 and H-4″), 3.65 (dd, $J_{5'',6''a} = J_{6''a,6''b} = 10.3$ Hz, 1 H, H-6″a), 3.81 (dd, $J_{2',3'} = J_{3',4'} = 9.5$ Hz, 1 H, H-3′), 3.86 (dd, $J_{2,3} = 2.8$, $J_{3,4} = 9.8$ Hz, 1 H, H-3), 3.99 (dd, $J_{3',4'} = J_{4',5'} = 9.5$ Hz, 1 H, H-4′), 4.10 (dd, $J_{5'',6''b} = 4.7$, $J_{6''a,6''b} = 10.3$ Hz, 1 H, H-6″b), 4.13–4.20 (m, 2 H, H-2 and H-5′), 4.25 (d, $J_{gem} = 12.0$ Hz, 1 H, C*H*HPh), 4.43–4.78 (m, 13 H, H-1′, H-1″, H-2″ and $CH_2Ph$), 4.82–5.05 (m, 3 H, $CH_2Ph$), 5.49–5.53 (m, 2 H, CHPh and NH), 7.05–7.95 (m, 42 H, arom.); $\delta_C$(125 MHz, $CDCl_3$) 18.2, 23.2, 29.7, 31.9, 50.6, 67.1, 67.9, 68.7, 69.5, 70.8, 71.1, 72.4, 73.3, 73.4, 74.4, 74.7, 75.0, 75.8, 75.9 (2C), 78.5, 79.6, 80.5, 80.7, 82.8, 96.8, 99.8, 101.7, 125.5–139.5 (52C arom.), 170.4; ESI-MS (positive-ion mode) *m/z* 1318.8 [M + Na]$^+$ (100%), Calcd for $C_{81}H_{85}NO_{14}$ + $Na^+$ 1318.6.

### 2-Acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→2)-3,4-di-*O*-benzyl-5a-carba-α-L-rhamnopyranose (16)

To a solution of **6** (90 mg, 0.069 mmol) in DCM/MeOH 2:1 (4.5 mL) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (48 mg, 0.208 mmol) was added at 0 °C. The reaction was allowed to warm to room temperature and, after 3 h, saturated aqueous $NaHCO_3$ was added. The reaction was diluted with DCM and washed

three times with saturated aq. $NaHCO_3$. The aqueous layers were then extracted three times with DCM. The combined organic layers were dried over $Na_2SO_4$, filtered and evaporated. The crude product was purified by flash chromatography (hexane/EtOAc from 6:4 to 1:1 v/v) to give **16** (63 mg, 78%) as colourless oil (Found: C, 72.89; H, 6.83; N, 1.30. Calc. for $C_{70}H_{77}NO_{14}$: C, 72.71; H, 6.71; N, 1.21%); $[\alpha]_D$ +16.3 (*c* 1 in $CHCl_3$); $\delta_H$(500 MHz, $CDCl_3$) 1.12 (d, $J_{5,6}$ = 7.0 Hz, 3 H, 3 H-6), 1.60–1.87 (m, 6 H, 2 H-5a, $COCH_3$ and OH), 2.02–2.11 (m, 1 H, H-5), 3.05–3.12 (m, 1H, H-5″), 3.23–3.28 (m, 1 H, H-6′a), 3.36 (dd, $J_{2'',3''}$ = 4.2, $J_{3'',4''}$ = 9.5 Hz, 1 H, H-3″), 3.39–3.44 (m, 1 H, H-6′b), 3.49–3.59 (m, 3 H, H-4, H-2′ and H-4″), 3.63 (dd, $J_{5'',6''a}$ = $J_{6''a,6''b}$ = 10.3 Hz, 1 H, H-6″a), 3.85–3.93 (m, 3 H, H-2, H-3 and H-3′), 3.99 (dd, $J_{3',4'}$ = $J_{4',5'}$ = 9.5 Hz, 1 H, H-4′), 4.01–4.12 (m, 4 H, H-1, H-4′, H-5′, H-6″b), 4.32 (d, $J_{gem}$ = 12.2 Hz, 1 H, C*H*HPh), 4.49–4.89 (m, 12 H, H-1″, H-2″ and $CH_2Ph$), 4.91 (d, $J_{1',2'}$ = 3.5 Hz, 1 H, H-1′), 4.97 (d, $J_{gem}$ = 12.0 Hz, 1 H, C*H*HPh), 5.47–5.54 (m, 2 H, CHPh and NH), 7.10–7.55 (m, 35 H, arom.); $\delta_C$(125 MHz, $CDCl_3$) 18.0, 23.2, 31.5, 34.5, 50.5, 66.9, 67.1, 68.0, 68.7, 70.0, 71.2, 72.6, 73.5, 73.6, 73.7, 74.8, 75.9, 76.1, 77.2, 78.6, 79.6, 80.4, 80.6, 81.0, 98.4, 99.8, 101.6, 126.1–139.4 (42C arom.), 170.3; ESI-MS (positive-ion mode) *m*/*z* 1178.8 $[M + Na]^+$ (100%), Calcd for $C_{70}H_{77}NO_{14} + Na^+$ 1178.5.

### 2-Acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→2)-3,4-di-*O*-benzyl-5a-carba-α-L-rhamnopyranosyl dibenzyl phosphate (17)

A solution of $PCl_3$ (0.036 mL, 0.41 mmol) in dry DCM (6 mL) was added at 0° C to a solution of imidazole (85 mg, 1.25 mmol) in dry DCM (6 mL). After stirring at 0 °C for 10 min, TEA (0.17 mL, 1.25 mmol) was added and the mixture was stirred for further 10 min at 0 °C. A solution of **16** (60 mg, 0.052 mmol) in dry DCM/$CH_3CN$ 1:1 (3 mL) was then added and, after stirring for 30 min at 0 °C, a solution of benzyl alcohol (0.13 mL, 1.25 mmol) in dry DCM (6 mL) was added and the reaction mixture was allowed to warm to room temperature. After 1 h the solution was cooled again to 0 °C and *m*-chloroperbenzoic acid (90 mg, 0.52 mmol) was added. After an additional hour the reaction was quenched with saturated aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$. The aqueous layer was extracted three times with DCM, then the combined organics were dried over $Na_2SO_4$, filtered and evaporated. Flash chromatography (hexane/AcOEt from 7:3 to 1:1, v/v) of the crude product gave **17** (54 mg, 73%) as colourless oil (Found: C, 71.04; H, 6.52; N, 1.11. Calc. for $C_{84}H_{90}NO_{17}P$: C, 71.22; H, 6.40; N, 0.99%); $[\alpha]_D$ +25.4 (*c* 1 in $CHCl_3$); $\delta_H$(500 MHz, $CDCl_3$) 1.00 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 3 H-6), 1.50–1.88 (m, 6 H, H-5, 2 H-5a and $COCH_3$), 3.04–3.14 (m, 2 H, H-5″ and H-6′a), 3.29–3.42 (m, 3 H, H-4, H-6′b and H-3″), 3.50 (dd, $J_{1',2'}$ = 3.5, $J_{2',3'}$ = 9.5 Hz, 1 H, H-2′), 3.55 (dd, $J_{3'',4''}$ = $J_{4'',5''}$ = 9.5 Hz, 1 H, H-4″), 3.61–3.72 (m, 2 H, H-3 and H-6″a), 3.76 (d, $J_{2',3'}$ = $J_{3',4'}$ = 9.5 Hz, 1 H, H-3′), 4.01 (dd, $J_{3',4'}$ = $J_{4',5'}$ = 9.5 Hz, 1 H, H-4′), 4.06–4.17 (m, 3 H, H-2, H-5′ and H-6″b), 4.27 (d, $J_{gem}$ = 12.0 Hz, 1 H, C*H*HPh), 4.48–4.70 (m, 10 H, H-1, H-1″, H-2″ and $CH_2Ph$), 4.71–5.06 (m, 8 H, $CH_2Ph$), 4.78 (d, $J_{1',2'}$ = 3.5 Hz, 1 H, H-1′), 5.48–5.54 (m, 2 H, CHPh and NH), 7.10–7.55 (m, 45 H, arom.); $\delta_C$(125 MHz, $CDCl_3$) 17.8, 23.2, 31.5, 32.9, 50.6, 67.2, 67.9, 68.7, 69.4 (d, $J_{C,P}$ = 5.5 Hz), 69.4 (d, $J_{C,P}$ = 5.6 Hz), 69.8, 71.2, 72.2, 73.4 (2C), 74.1 (d, $J_{C,P}$ = 6.0 Hz), 74.6, 75.0, 75.8, 75.9 (2 C), 78.6, 79.3, 78.0, 80.5, 82.2, 97.2, 99.8, 101.6, 125.8–139.4 (54C arom.), 170.3; $\delta_P$(202 MHz, $CDCl_3$) -1.70; ESI-MS (positive-ion mode) *m*/*z* 1438.7 (83%), 1439.6 (100%) 1440.4 (40%) $[M + Na]^+$, Calcd for $C_{84}H_{90}NO_{17}P + Na^+$ 1438.6.

### 2-Acetamido-2-deoxy-β-D-mannopyranosyl-(1→4)-α-D-glucopyranosyl-(1→2)-5a-carba-α-L-rhamnopyranosyl dihydrogen phosphate (4)

To a solution of compound **17** (50 mg, 0.035 mmol) in EtOAc/MeOH/$H_2O$ = 1:1:1 (3 mL), $Pd(OH)_2/C$ (1:5, $w/w_{substrate}$) and 3 drops of acetic acid were added. The mixture was shaken under hydrogen atmosphere for 48 h and then filtered over Celite. After evaporation of the solvent, the crude was purified by flash chromatography (EtOAc/*i*PrOH/$H_2O$ = 1:1:1) to give compound **4** (13 mg, 60%) as an amorphous white solid. (Found: C, 41.44; H, 6.47; N, 2.38. Calc. for $C_{21}H_{38}NO_{17}P$: C, 41.52; H, 6.30; N, 2.31%); $[\alpha]_D$ +11.9 (*c* 1 in $H_2O$); $\delta_H$(500 MHz, $D_2O$) 0.94 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 3 H-6), 1.52–1.60 (m, 1 H, 1 H-5a), 1.70–1.79 (m, 2 H, H-5 and 1 H-5a), 2.00 (s, 3 H, $COCH_3$), 3.30 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, 1 H, H-4), 3.35–3.41 (m, 1 H, H-5″), 3.45 (dd, $J_{3'',4''}$ = $J_{4'',5''}$ = 9.7 Hz, 1 H, H-4″), 3.52 (dd, $J_{1',2'}$ = 3.5, $J_{2',3'}$ = 9.7 Hz, 1 H, H-2′), 3.59–3.69 (m, 2 H, H-4′ and H-6′a), 3.70–3.78 (m, 4 H, H-3, H-6′b, H-3″ and H-6″a), 3.81 (dd, $J_{2',3'}$ = $J_{3',4'}$ = 9.7 Hz, H-3′), 3.86 (dd, $J_{5'',6''}$ = 2.0, $J_{6''a,6''b}$ = 12.2 Hz, 1 H, H-6″b), 3.95–4.01 (m, 2 H, H-2 and H-5′), 4.25–4.30 (m, 1 H, H-1), 4.46–4.50 (m, 1 H, H-2″), 4.82 (d, $J_{1'',2''}$ = 1.2 Hz, 1 H, H-1″), 4.94 (d, $J_{1',2'}$ = 3.5 Hz, 1 H, H-1′); $\delta_C$(125 MHz, $D_2O$) 17.0, 22.0, 31.6, 33.0, 53.3, 59.8, 60.3, 66.6, 69.7 (*br* s, C-1), 70.0, 71.0, 71.1, 71.6, 72.0, 75.0, 76.5, 78.8, 96.6, 99.3, 175.4; ESI-MS (negative-ion mode) *m*/*z* 606.3 $[M-1]^-$, Calcd for $C_{21}H_{38}NO_{17}P$ 607.19.

### 2-Acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl dibenzyl phosphate (19)

Trichloroacetimidate donor **18**[11b] (40 mg, 0.031 mmol) and dibenzylphosphate (9 mg, 0.031 mmol) were dissolved in DCM (0.5 mL) and allowed to react at room temperature for 1 h. The solvent was removed under reduced pressure and the crude purified by flash chromatography (toluene/acetone = 9:1) to give compound **19** (38 mg, 88%) as a colourless oil. Physical data were in agreement with those reported in ref. 11b.

### Competitive ELISA assay

96-Well flat-bottomed plates were incubated overnight at 4–8 °C with a mixture of *S. pneumoniae* 19F CPS (Sanofi-Aventis, France) (1 mg/mL) and methylated human serum albumin (1 mg/mL). A solution of foetal calf serum (5%) in phosphate-buffered saline supplemented with Brij-35 (0.1%) and sodium azide (0.05%) was applied to the plates for blocking of nonspecific binding sites. The plates were incubated overnight at 4–8 °C with a solution (1:200) of rabbit anti-19F, used as reference serum (Statens Serum Institut, Artillerivej, Denmark). When compounds **4** and **5** were tested, they were added to each well immediately before the addition of the reference serum. The plates were then incubated with alkaline phosphatase conjugate goat anti-rabbit IgG (Sigma-Aldrich, Milan, Italy), stained with *p*-nitrophenylphosphate, and

the absorbance was measured at 405 nm with an Ultramark microplate reader (Bio-Rad Laboratories S.r.l., Milan, Italy).

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