An Efficient Transformation of (–)-Quinic Acid into Carba-L-rhamnose

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Abstract: A novel stereoselective synthesis of carba-L-rhamnose from (–)-quinic acid is described. The title compound is obtained with an appropriate pattern of protecting groups to be used for the preparation of *Streptococcus pneumoniae* type 19F capsular polysaccharide repeating unit.

Key words: carba-L-rhamnose, stereoselective synthesis, capsular polysaccharide, quinic acid, shikimic acid

Pneumococcal invasive infections are still a major problem[1] both in developed and developing countries[2] causing meningitis, otitis, septicemia and pneumonia. It would be therefore desirable to have access to vaccines to protect population against these pathogens.

Among the different pathogens responsible for such infections, *Streptococcus pneumoniae*[3] is one of the most virulent. *S. pneumoniae* belongs to the class of encapsulated bacteria, and antibodies directed against the capsular polysaccharides (CPS) mediate the resistance to infections from these pathogens, making these polymers useful for vaccine preparations, especially using conjugate vaccines able to induce T-cell dependent response.[4]

We are currently involved in a project devoted to the development of glycoconjugated vaccines through synthetic methods. We focused our attention on *S. pneumoniae* 19F whose CPS is composed of trisaccharide repeating units [→4-β-D-ManpNAc-(1→4)-α-D-Glcp-(1→2)-α-LRhap-1→; Figure 1, a] joined by phosphodiester bridges.

The polysaccharide, however, due to the presence of the phosphate on the anomeric position is somewhat labile, making the preservation of a vaccine during the storage more problematic. Moreover, the presence of the phosphodiester bridge makes the chemical synthesis of oligomer of the repeating unit very difficult, not only because of the lability of the anomeric phosphate, but also for the possibility to form two anomers for each phosphodiester bridge.

For these reasons, we decided to synthesize analogs of the natural repeating units, which are able to avoid the above-mentioned problems. It has to be highlighted that it is quite uncommon to use antigen mimics for vaccine preparations. In the case of analogs, which are able to mimic precisely enough the natural antigen it is reasonable to expect that the induced response would be able to protect the organism against bacterial infections.

In order to obtain a stable analog of the anomeric phosphate, we considered the synthesis of a carba sugar analog, namely carba-L-rhamnose, as it should give a stable phosphate at the former anomeric position, without problems with diastereomeric mixture formation. Moreover, it is possible to consider the possibility to use automated techniques similar to those for the synthesis of oligonucleotides in order to obtain oligomers with different chain length (Figure 1).

To the best of our knowledge, only one example of the synthesis of carba-L-rhamnose appeared in the literature.[5] The paper describes the formation of carbasugars ex-

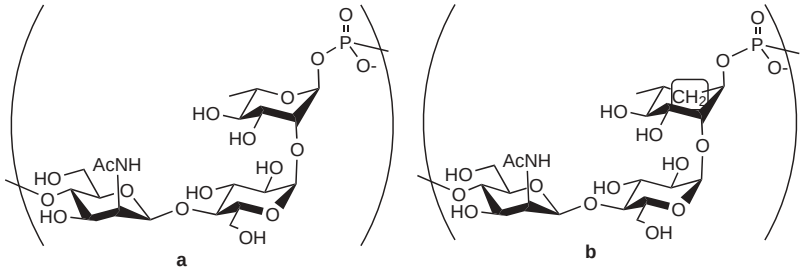

Figure 1 Structure of *S. pneumoniae* 19F repeating unit (a) and its carba-L-rhamnose containing analog (b).

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ploiting a radical cyclization of iodohept-1-enitols derived from aldoses. Although the approach is attractive, the aim of such work was the study of the radical cyclization, and not the preparation of a specific compound. In particular, the carba-L-rhamnose was obtained together with a carba-D-gulo compound, starting from a galacto derivative. Moreover, for the synthesis of the trisaccharidic repeating unit, we needed a proper pattern of protecting groups.

Looking at the structure and, particularly, at the stereochemistry of carba-L-rhamnose, it can be envisaged that such compound shares many features with (–)-shikimic acid (Figure 2, a).

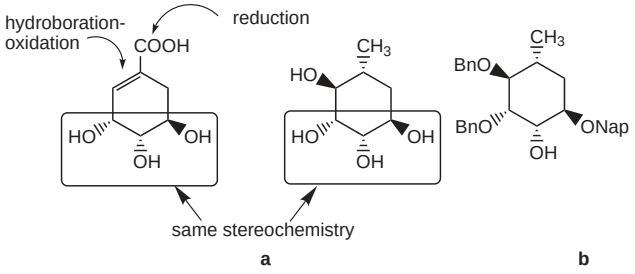
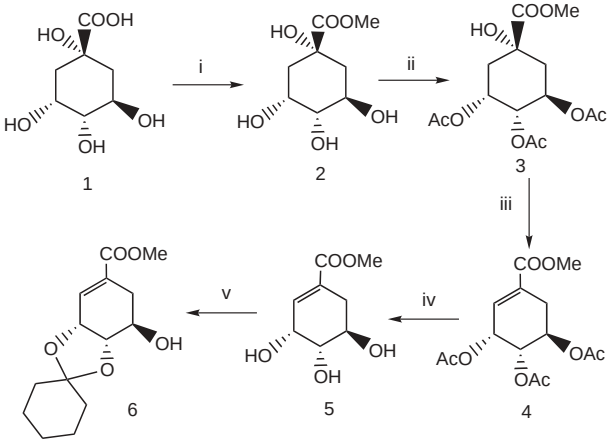


Figure 2 (a) Structural analogies between (–)-shikimic acid and carba-L-rhamnose; (b) protected carba-L-rhamnose for incorporation in *S. p.* 19F repeating unit.

In particular, the three hydroxyl groups have the same stereochemistry as those in carba-L-rhamnose. Moreover, the carboxylic acid could be reduced to a methyl group and the hydroboration–oxidation of the double bond should allow the installation of the missing hydroxyl group and the required stereochemistry at the two newly formed stereocenters. Finally, an appropriate pattern of protecting groups should be envisaged, in order to synthesize the *Streptococcus pneumoniae* 19F repeating unit (Figure 2, b).



Scheme 1 i) MeOH, H_2SO_4, r.t.; ii) AcCl, pyridine, CH_2Cl_2, –20 °C; iii) $SOCl_2$, 0 °C to r.t., 76% over three steps; iv) MeOH, H_2SO_4, r.t.; v) cyclohexanone, dioxane PTS, 60 °C, 70% over two steps.

We decided to use benzyl ethers as permanent protecting groups for the hydroxyls in positions 3 and 4, to introduce a naphthylmethyl group,[6] orthogonal to benzyls, in position 1, and to leave the hydroxyl group at position 2 ready to be glycosylated for the synthesis of the above-mentioned trisaccharidic repeating unit.

As (–)-shikimic acid is quite expensive, it was replaced by (–)-quinic acid (**1**) as starting material, which can be easily converted to (–)-shikimic acid, also allowing the introduction of desired protecting groups. In a preliminary attempt, following a literature procedure,[7] (–)-quinic acid was converted to methyl ester **2**, acetylated, dehydrated to give compound **4**, and, after further protecting group manipulation, converted into 3,4-*iso*propylidene derivative. However, the product obtained after reduction of the carboxylic ester **6** proved to be too water-soluble making necessary the introduction of a more hydrophobic protecting group.

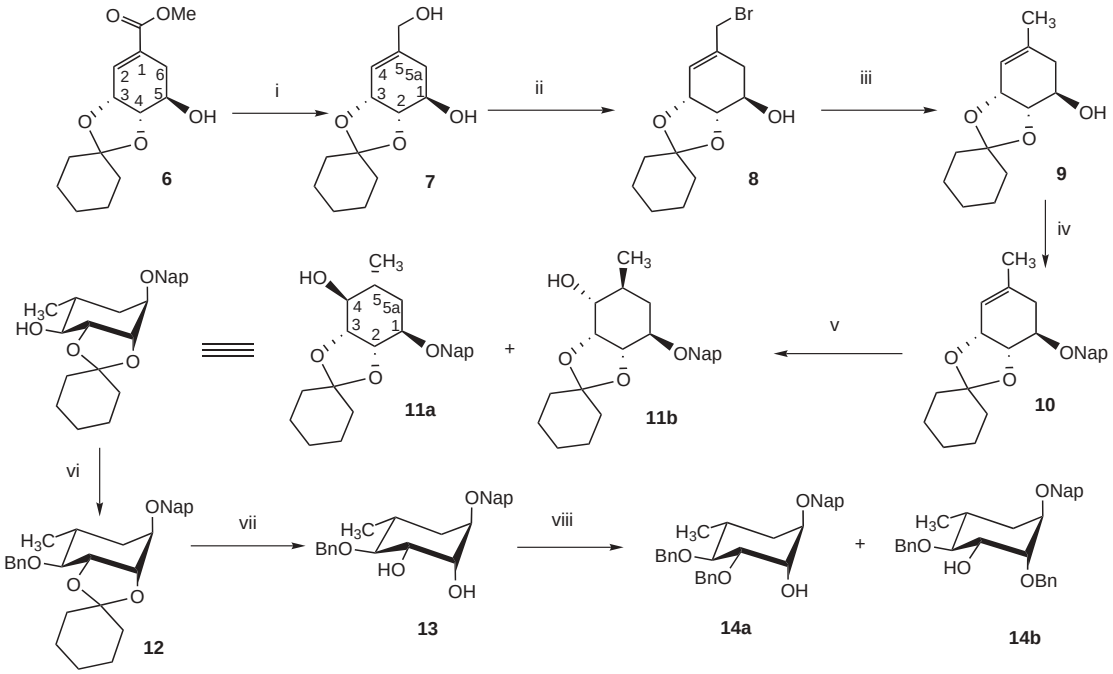
Moreover, although the authors do not comment on the direction of the elimination reaction, in our hands, the expected (–)-shikimic ester **4** was accompanied by a substantial amount of the 4-*epi* derivative [ratio (–)-shikimic: (–)-4-*epi*-shikimic 85:15], which was not separable by chromatography.

We then decided to prepare the cyclohexylidene derivative **6**, by a modified literature procedure[8] in dioxane at 60 °C instead of toluene at reflux. A 0.1 M solution of compound **5** in dioxane was treated with 3 molar equivalents of cyclohexanone and a catalytic amount (about 10%) of *p*-toluensulfonic acid for 3 hours. It was now possible to eliminate the undesired isomer by careful flash chromatography (Scheme 1).

The carboxylic ester **6** was reduced to the corresponding alcohol **7** with DIBALH[9] and the primary allylic hydroxyl group was selectively replaced for a bromide using triphenylphosphine and carbon tetrabromide[10] giving compound **8** in excellent yield (Scheme 2).

Removal of bromine with lithium triethylborohydride[11] and protection of the free hydroxyl group as naphthylmethyl ether (Nap) in phase-transfer conditions using 18-crown-6 as catalyst,[12] afforded smoothly compound **10**,[13] ready for the hydroboration–oxidation reaction.

Hydroboration reaction was initially attempted using $BH_3{\cdot}SMe_2$ complex,[14] followed by conventional oxidation with hydrogen peroxide and sodium hydroxide. Although the reaction worked well, the ratio between the diastereomeric products **11a** and **11b** was 87:13 (from ^{1}H NMR).[15] We then decided to turn to 9-BBN, which should be more stereoselective.[16] In fact, although it was necessary to work at the reflux of THF, the reaction gave the desired product **11** in high yield and excellent stereoselection (**11a**/**11b** >97:3, determined by ^{1}H NMR). Protection of the newly formed hydroxyl group as benzyl ether in the same conditions previously used for the introduction of the Nap ether and conventional deprotection of the cyclohexylidene acetal with trifluoroacetic acid gave the diol **13**. To introduce regioselectively the benzyl protecting



Scheme 2 i) DIBALH, THF, 0 °C, 78%; ii) Ph_3P, CBr_4, *syn*-collidine, r.t., 93%; iii) SuperHydride®, THF, 0 °C to r.t., 92%; iv) NapBr, KOH, 18-crown-6, THF, r.t., 85%; v) 9-BBN, reflux, then NaOH, H_2O_2, 0 °C to r.t.; 90% (**11a**/**11b** 97:3); vi) BnBr, KOH, 18-crown-6, THF, r.t., 89%; vii) 60% aq TFA, CH_2Cl_2, r.t., 87%; viii) BnBr, Bu_2SnO, Bu_4NBr, reflux, then 50 °C, 96% (**14a**/**14b** 93:7).

group on the 3-OH we exploited the stannylene technique, which should allow the selective introduction of the alkylating agent on the equatorial OH group of a 1,2-*cis*-diol.[17] As expected, the reaction gave a 93:7 mixture of the properly protected carba-L-rhamnose **14a** ready for further glycosylation reaction, together with the 3-OH free compound **14b**. The benzylation position of **14a** and **14b** was determined after acetylation reaction of the products, on the basis of the downfield shift of the proton in position 2 or 3, respectively.

A small amount of compound **11a** was also deprotected to obtain an analytical sample of free carba-L-rhamnose whose optical rotation was consistent with that described in the literature.[5]

Carba-L-rhamnose either unprotected or with a proper protecting group pattern was obtained in a concise and high yield way starting from quinic acid. Work is in progress to synthesize the *Streptococcus pneumoniae* 19F repeating unit containing the carbocyclic analog of the rhamnopyranosidic fragment.

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(13) All new compounds have been characterized by proton and carbon NMR, exact mass, and/or elemental analysis. Spectroscopic data for selected compounds: **5a-Carba-2,3-*O*-cyclohexylidene-1-*O*-naphtylmetyl-α-L-rhamno-pyranoside (11a)**: ^1H NMR (300 MHz, $CDCl_3$): δ = 1.05 (d, 3 H, $J_{Me,5}$ = 6.0 Hz, Me), 1.30–1.80 (m, 12 H,

H-5a and 5 CH_2), 1.80–2.00 (m, 1 H, H-5), 3.30 (dd, 1 H, $J_{3,4}$ = 7.7 Hz, $J_{4,5}$ = 9.9 Hz, H-4), 3.90 (br m, 1 H, H-1), 4.05 (dd, 1 H, $J_{2,3}$ = 5.8 Hz, $J_{3,4}$ = 7.7 Hz, H-3), 4.29 (br m, 1 H, H-2), 4.72 and 4.78 (ABq, 2 H, CH_2-Nap), 7.20–7.90 (m, 7 H, Ar H). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 18.21 (q, CH_3), 23.81 (t), 24.17 (t), 25.13 (t), 30.06 (d, C-5), 32.60 (t), 35.41 (t), 38.10 (t), 71.32 (t), 74.15 (d), 76.71 (d), 78.12 (d), 80.22 (d), 109.74 (s), 125.99 (d), 126.24 (d, 2 C), 127.79 (d), 127.96 (d), 128.31 (d, 2 C), 133.09 (s), 133.37 (s), 135.77 (s). $[\alpha]_D^{25}$ +15.7 (*c* = 1, $CHCl_3$). Mp 99.0–101.0 °C.

5a-Carba-1-naphtylmetyl-3,4-dibenzyl-α-L-rhamnopyranoside (14a,b): ^{1}H NMR (300 MHz, $CDCl_3$): 1.10 (d, 3 H, $J_{6,5}$ = 6.6 Hz, H-6), 1.62 (dt, 1 H, $J_{5aA,5aB}$ = $J_{5aA,5}$ = 14.5 Hz, $J_{5aA,1}$ = 2.5 Hz, H-5aA), 1.86 (dt, 1 H, $J_{5aA,5aB}$ = 14.5 Hz, $J_{5aB,5}$ = $J_{5aB,1}$ = 2.7 Hz, H-5aB), 1.97–2.17 (m, 1 H, H-5), 2.58 (br s, 1 H, -OH), 3.39 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.1 Hz, H-4), 3.80 (br m, 1 H, H-1), 3.86 (dd, 1 H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.1 Hz, H-3), 4.19 (br t, 1 H, H-2), 4.55–5.00 (m, 6 H, 3 ABq, CH_2-Nap and CH_2-Bn), 7.10–8.00 (m, 17 H, Ar H). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 18.34 (q, C-6), 31.78 (d, C-5), 32.05 (t, C-5a), 69.74 (d), 71.20 (t), 72.69 (t), 75.43 (t), 76.16 (d), 82.33 (d), 83.32 (d), 125.71 (d), 125.96 (d), 126.15 (d), 126.25 (d), 127.64 (d), 127.83 (d), 127.92 (d), 127.99 (d), 128.02 (d, 2 C), 128.13 (d, 2 C), 128.24 (d), 128.47 (d, 2 C), 128.60 (d, 2 C), 133.08 (s), 133.40 (s), 136.20 (s), 138.51 (s), 139.14 (s). $[\alpha]_D^{25}$ –18.3 (*c* = 1, $CHCl_3$).

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(15) The stereochemistry of compounds **11a** and **11b** was inferred from the coupling constants of the signal of H-C(4) in ^{1}H NMR spectrum. In fact, H-C(4) appeared as a doublet of doublets for both compounds, but in **11a** the ^3J were 7.7 Hz and 9.9 Hz, while in compound **11b** the constants were 10.8 Hz and 3.2 Hz. Furthermore, H-C(4) in compound **13** appeared as a triplet with a ^3J of 9.9 Hz, clearly indicating the axial orientation of H-C(4) in a chair conformation of **13**.

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