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A short route to nucleoside diphosphate activated D- and L-hexoses

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Abstract—Leloir transferases utilise nucleoside diphosphate sugars, which are notoriously difficult to synthesise and handle. Starting off from D- or L-configurated glycals, a facile synthesis of nucleotide sugars by epoxidation and direct coupling with uridine diphosphate is described. © 2001 Published by Elsevier Science Ltd.

One, if not the greatest obstacle in using Leloir transferases for glycosylation reactions is their absolute requirement for activated sugars. The syntheses of these are non-trivial and their handling is requiring profound preparative skills. Contributions, in which the natural pathway towards activated sugars has been exploited are nowadays classical examples of the elegant combination of biochemistry and carbohydrate synthesis.[1]

The advantages, however, of chemical syntheses are embedded in a broader applicability since activated sugars can be envisaged, which are complementing the repertoire of hexoses abundant in mammalian systems. This is paid for by a more cumbersome preparative procedure, the synthesis of a glycosyl phosphate, which is subsequently coupled to a nucleotide to yield the diphosphate.[2] Yields are generally quite disappointing at this final step, which unfortunately also marks the end point of the synthetic assembly line. We were prompted by this dilemma to embark on the direct coupling of glycosyl donors with a nucleoside diphosphate. This idea has a short history in the carbohydrate community, which is examplified by contributions of Hindsgaul,[3] Hanessian,[4] and Schmidt.[5] These groups have approached the problem by reacting their respectively favoured glycosyl donors with nucleoside diphosphates or nucleotides, as in the latter case.

Here, we present a very general layout for the synthesis of nucleoside diphosphate activation, which was inspired by reports on the regioselective attack of 1,2-anhydro sugars using protic acids, such as carboxylates,[6] phosphate mono- and diesters.[6,7] Such reagents gave rise to high α/β-selectivities at good overall yields, depending on the used solvent.

Starting off from easily obtainable D-glucal (**1**), D-galactal (**2**), and L-rhamnal (**7**)[8] the respective α-configurated 1,2-anhydro sugars **3**, **4** and **8** were obtained quantitatively according to the standard procedures[9] (Scheme 1).

Special attention had to be paid to the preparation of the diphosphate, which was converted to the free acid by passing through a column of DOWEX-50 (H^+) and titrated with tetrabutyl ammonium hydroxide solution (40% w/v) to pH 6. Contrary to the procedure reported earlier,[3b] pH in our examples showed to be the parameter to which utmost attention had to be given. At pH 7 all charges of the diphosphate were screened by tetrabutyl ammonium counterions, hence the coupling yields were dramatically decreased. The other corner of the operating window was marked by pH 5. Here, initially product formation was observed by MS, but extensive decomposition into cyclic hexosyl 1,2-phosphates and UMP was taking place.

Thus, the lyophilised TBA salt[10] (UDP×1.7 TBA, titrated to pH 6) was dispersed in anhydrous dichloromethane (approx. 3 ml/mmol) and added to the solution of the glycosyl donor. In a stream of argon, the reaction mixture was concentrated to yield a syrup, which was stirred for not more than 20 h. After this period the reaction was complete and worked up by removing the organic solvent, filtration through Celite

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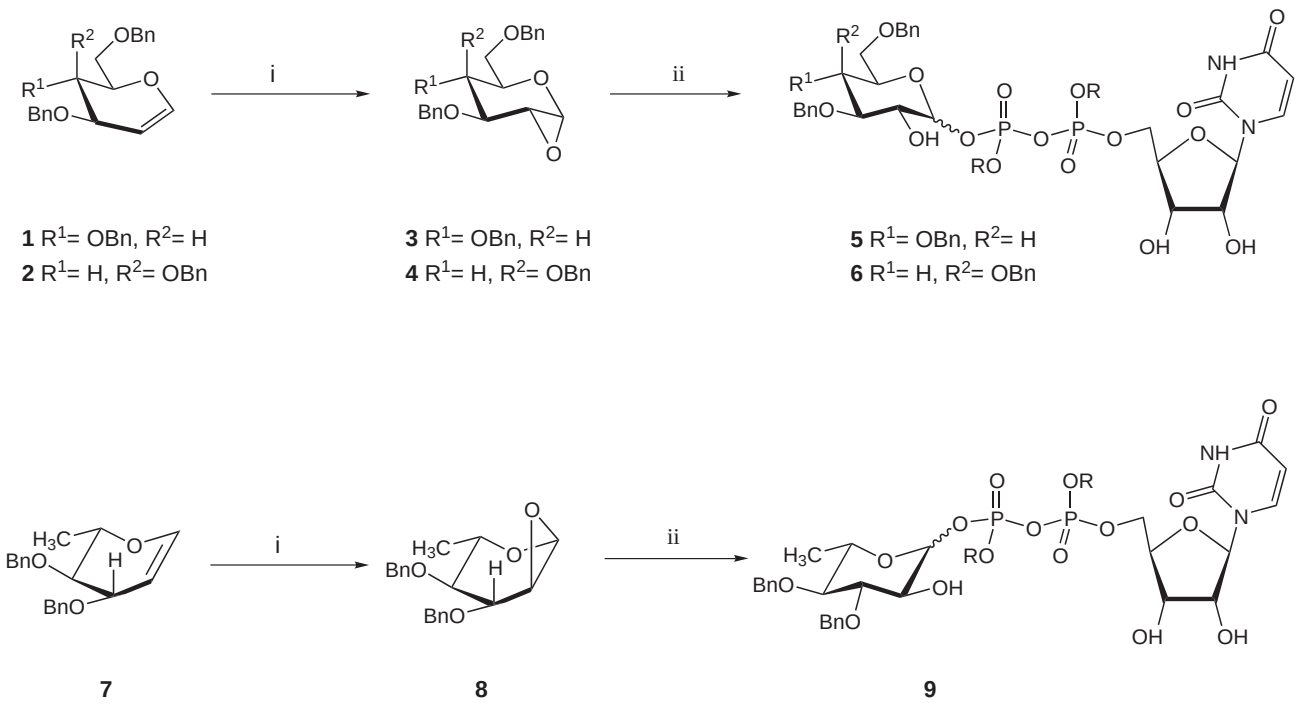

Scheme 1. Syntheses of nucleotide sugars starting from D- and L-glycal precursors. (i) DMDO, CH_2Cl_2, 0°C, 1 h, quant.; (ii) UDP TBA salt, CH_2Cl_2, rt, 20 h (40–60%).

with buffered solution (30 mM NH_4HCO_3) and purified by ion-exchange chromatography using DEAE cellulose (HCO_3^- form, gradient elution 30 mM→400 mM NH_4HCO_3).

After pooling of the appropriate fractions and lyophilisation, UDP 3,4,6-tri-*O*-benzyl-D-glucose and -galactose, **5** and **6**, were isolated as white powders. Interestingly, L-gluco configurated **9** could not be isolated from excess UDP as such, but instead had to be treated with alkaline phosphatase (pH 7.8, 37°C) prior to ion-exchange chromatography. All benzyl ethers **5**, **6**, and **9** gave clean analytical and spectroscopic analyses; the isolated yields were typically 40–60%, the α/β ratio varied around 1:1.[11] (typical data of D- and L-sugars are given in Ref.[13]). The benzylated UDP hexoses were stable upon storage and could be deblocked by hydrogenation as was described earlier.[3b]

For the D-series it has been shown that enzymatic transformations by Leloir transferases are not hampered by the presence of anomeric mixtures.[3b] In the L-hexosyl phosphate series, β-configurated glycosyl donors are substrates for the respective L-sugar transferases, the stereochemistry of which is difficult to obtain by conventional methodology.[12]

In summary, this method comprises of a very fast and simple reagent-free procedure for the synthesis of stable α- and β-configurated UDP sugars, which can easily be extended to functionalised glycals of either the D- or L-series. Further investigations are presently under way to exploit this method for the synthesis of libraries of nucleoside diphosphates.

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10. UDP trisodium salt (100 mg, 0.2 mmol) was dissolved in water (1 ml) and the solution was passed through DOWEX 50W X8 (H^+). Fractions containing protonated UDP were detected by UV absorption, pooled and titrated using aqueous $Bu_4N(OH)$ (40% w/v) to pH 6. The resulting solution was lyophilised to yield UDP Bu_4N salt as a white powder.
11. Benzylated UDP sugars were obtained as a white powders upon lyophilisation, **5** (~50%, α/β ratio 1:1), **6** (~64%, α/β ratio 1:0.75), **9** (47%, α/β ratio 0.8:1).
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13. Selected physical data of compounds **5** and **9**: compound **5**: ^1H NMR (600 MHz, D_2O): $\delta = 7.77$ (d, 1H, H-6α),

7.74 (d, 1-H, H-6β), 7.15–7.30 (m, 26H, arom.), 6.87–6.95 (m, 4H, arom.), 5.85 (m, 1H, H-1′α), 5.83 (d, 1H, H-1′β), 5.80 (d, 1H, H-5α), 5.72 (d, 1H, H-5β), 5.45 (dd, 1H, H-1″α), 4.87 (dd∼t, 1H, H-1″β), 4.74–4.80 (2*d, 2H, -CH_2, benzyl), 4.58–4.60 (2*d, 2H, -CH_2, benzyl), 4.42–4.50 (m, 2H, H-3′α and β), 4.24–4.46 (4*d, 8H, -CH_2, benzyl), 4.10 (dd, 1H, H-2′α), 4.14–4.20 (m, 2H, H-4′α and β), 4.11–4.19 (m, 1H, H-2′β), 4.05–4.10 (m, 4H, H-5′a and H5′b α and β), 3.87 (m, 1H, H-5″α), 3.77 (dd∼t, 1H, H-3″α), 3.60 (m, 4H, H-6″a and H-6″b α and β), 3.54 (dd, 1H, H-2″α), 3.53 (m, 1H, H-3″β), 3.45 (m, 2H, H-4″a and H-4″b α and β), 3.39 (dd∼t, 1H, H-2″β) ppm; $J_{5,6}=8.0$, $J_{1',2'}=4.3$, $J_{1''\alpha,2''\alpha}=3.2$, $J_{1''\alpha,P}=7.0$, $J_{2''\alpha,3''\alpha}=8.9$, $J_{3''\alpha,4''\alpha}=9.4$, $J_{4''\alpha,5''\alpha}=10.4$, $J_{1''\beta,2''\beta}=7.9$, $J_{1''\beta,P}=7.9$, $J_{2''\beta,3''\beta}=8.9$ Hz; ^{13}C NMR (90 MHz, D_2O): $\delta=168.94$, 168.77 (C-4, α/β), 154.63, 154.30 (C-2, α/β), 144.52, 144.20 (C-6, α/β), 139.5–140.3 (arom.), 131.1–131.7 (arom.), 105.56, 105.34 (C-5, α/β), 100.81 (C-1″β, $J_{P\text{-}O\text{-}C}=6.7$ Hz), 98.66 (C-1″α, $J_{P\text{-}O\text{-}C}=6.7$ Hz), 91.49, 91.19 (C-1′, α/β), 86.28, 85.81, 85.76, 84.76 (C-3″, C-4′, α/β), 79.44 (C-2″β, $J_{P\text{-}O\text{-}C\text{-}C}=7.8$ Hz), 77.77 (C-2″α, $J_{P\text{-}O\text{-}C\text{-}C}=7.8$ Hz), 78.60, 78.06, 77.11, 76.87, 76.72, 76.69 (C-4″, α/β, -CH_2, benzyl), 75.79, 75.08 (C-3′, α/β), 73.79, 72.59, 72.27, 72.19 (C-5″, C-6″, α/β), 70.41, 70.04 (C-2′, α/β), 67.83, 67.61 (C-5′, α/β, $J_{P\text{-}O\text{-}C}=6.7$ Hz) ppm; ^{31}P NMR (242.5 MHz, D_2O): $\delta=-10.57$ (2*d, $P_{1,\alpha/\beta}$), -12.43 (2*d, $P_{2,\alpha/\beta}$) ppm; $J_{P\text{-}O\text{-}P}=19.6$ Hz; MS (ESI-MS, nanospray) (m/z) 835 $[M]^-$, 417 $[M]^{2-}$. Compound **9**: ^{1}H NMR (600 MHz, D_2O): $\delta=7.81$ (d, 1-H, H-6β), 7.77 (d, 1H, H-6α), 7.16–7.33 (m, 20H, arom.), 5.83 (d, 1H, H-1′β), 5.82 (d, 1H, H-5β), 5.78 (d, 1H, H-1′α), 5.76 (d, 1H, H-5α), 5.39 (dd, 1H, H-1″α), 4.85 (dd∼t, 1H, H-1″β), 4.40–4.80 (8*d, 8H, -CH_2, benzyl), 4.52–4.60 (m, 2H, H-3′α and β), 4.22 (m, 1H, H-2′β), 4.16 (m, 1H, H-2′α), 4.03–4.21 (m, 6H, H-4′, H-5′a, H-5′b, α and β), 3.89 (m, 1H, H-5″α), 3.73 (dd∼t, 1H, H-3″α), 3.53 (m, 1H, H-2″α), 3.50 (dd∼t, 1H, H-3″β), 3.44 (m, 1H, H-5″β), 3.37 (dd∼t, 1H, H-2″β), 3.12–3.20 (2*dd, 2H, H-4″α and β), 1.18 (d, 3H, -CH_3 β), 1.15 (d, 3H, -CH_3 α) ppm; $J_{5,6}=8.2$, $J_{1',2'}=4.2$, $J_{1''\alpha,2''\alpha}=2.8$, $J_{1''\alpha,P}=7.8$, $J_{2''\alpha,3''\alpha}=9.7$, $J_{3''\alpha,4''\alpha}=9.4$, $J_{4''\alpha,5''\alpha}=9.4$, $J_{5''\alpha,CH3}=5.7$, $J_{1''\beta,2''\beta}=8.0$, $J_{1''\beta,P}=7.9$, $J_{2''\beta,3''\beta}=9.1$, $J_{3''\beta,4''\beta}=9.4$, $J_{4''\beta,5''\beta}=9.4$, $J_{5''\beta,CH3}=5.7$ Hz; ^{13}C NMR (90 MHz, D_2O): $\delta=188.60$, 188.56 (C-2, α/β), 169.02, 168.90 (C-4, α/β), 144.54, 144.42 (C-6, α/β), 139.8–140.3 (arom.), 131.2–131.7 (arom.), 105.48, 105.57 (C-5, α/β), 100.57 (C-1″β), 98.73 (C-1″α), 91.27, 91.22 (C-1′, α/β), 86.17, 86.02, 85.40, 85.21 (C-3″, C-4′, α/β), 78.58 (C-2″β), 77.29 (C-2″α, $J_{P\text{-}O\text{-}C\text{-}C}=7.5$ Hz), 78.09, 76.67, 74.35, 72.54, 72.42, 72.24 (C-3′, C-4″, C-5″, α/β, -CH_2, benzyl), 71.12 (C-2′, α/β), 67.83, (C-5′, α/β, $J_{P\text{-}O\text{-}C}=4.1$ Hz), 19.90 (C-6″, α/β) ppm; MS (ESI-MS, nanospray) (m/z) 729 $[M]^-$, 364 $[M]^{2-}$. Upon hydrogenolysis (cf. lit. 3b), compounds **5** and **6** were identical in all spectroscopic aspects to commercial samples of UDP glucose and UDP galactose (Sigma).