Carbohydrate Synthesis

DOI: 10.1002/anie.200703237

Efficient Synthesis of Nucleoside Diphosphate Glycopyranoses**

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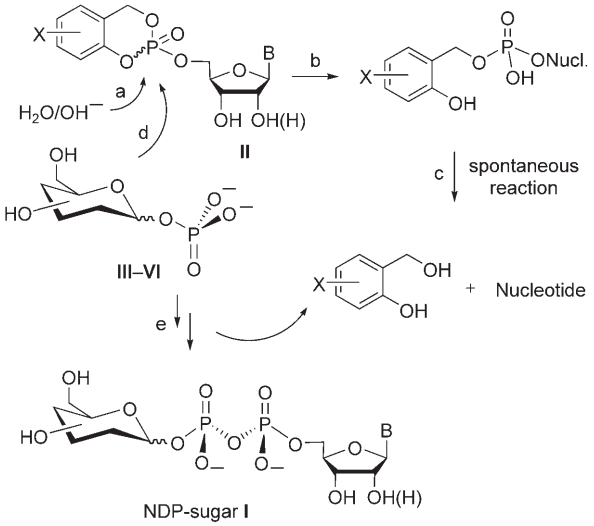
Nucleoside diphosphate pyranoses **1** (NDP sugars; see Scheme 1) play a key role as glycosyl donors in the synthesis of oligo- and polysaccharides.[1,2] Moreover, they serve as precursors of deoxysugars, aminodeoxysugars, chain-branched sugars, uronic acids, as well as glycoconjugates. In biosynthetic pathways the energy-rich linkage between the C1 atom and the β-phosphate is cleaved, and thus the glycosyl part is enzymatically transferred to an oligosaccharide chain, releasing the nucleoside diphosphate (NDP) moiety. For biosynthesis studies of oligosaccharides (for example, of lipopolysaccharides)[3] an efficient access to this important class of compounds is needed. The classical method is the coupling of glycosyl 1-phosphates to nucleotide morpholidates (Moffat–Khorana method).[4,5] However, this reaction normally takes days, and the chemical yields are often low (5–25%). Attempts to improve the reaction yields by using 1*H*-tetrazole as activator[6–9] are often not successful and have failed also in our hands. Instead of the morpholidates, also imidazolides have been used in the past but without improving the yields markedly.[10–12] Alternatively, Hindsgaul and Jakeman published a procedure starting from nucleoside diphosphates and glycopyranosyl bromides.[13,14] However, yields were also found to be low, and often the stereochemistry at the anomeric center could neither be controlled nor stereospecifically formed. Thiem and co-workers reported an enzymatic procedure starting from unprotected sugars that were first phosphorylated and then treated with a nucleoside triphosphate.[15,16] However, this reaction sequence is based on a three-enzyme pathway and depends on the availability of the needed kinases and NDP sugar pyrophosphorylases and on expensive nucleoside triphosphates. The yields obtained have seldom exceeded 30%. Herein, we report on a conceptionally new chemical synthesis of NDP sugars that uses *cyclo*-saligenyl (*cyclo*Sal) nucleosyl phosphate triesters **II** as an active ester (Scheme 1).

Originally, the *cyclo*Sal technique was developed to deliver biologically active nucleotides into cells.[17] The cleavage relies on a nucleophilic attack of the neutral phosphate triester by water or hydroxide and a subsequent selective hydrolysis pathway to yield the nucleotide (pathway a–c; Scheme 1). The technique has been applied successfully to a variety of nucleoside analogues, providing superior antiviral activity.[18–20] However, the same type of compounds may also be used as an active ester for synthetic applications. Here, *cyclo*Sal nucleotides like **II** were treated with glycopyranosyl 1-phosphate salts **III–VI** as nucleophiles with formation of the pyrophosphate bond in NDP sugars (pathway d,e; Scheme 1).



Scheme 1. General principle of the cleavage of *cyclo*Sal phosphate triesters using water/hydroxide or pyranose 1-phosphates. See text for details.

As starting materials, 5-nitro-*cyclo*Sal-3′-*O*-acetylthymidine **2** and peracetylated glycopyranosyl phosphates **3–6** were prepared (see Scheme 3). Thus, thymidine was protected by silylation with *tert*-butyldimethylsilylchloride (TBDMS-Cl) at the 5-position. The product was treated with acetic anhydride to yield fully protected thymidine in 92% yield. Finally, the TBDMS group was cleaved by $(n\mathrm{Bu})_4\mathrm{NF}$ to give 3′-*O*-acetylthymidine (3′-OAcT) in 96% yield. This material was converted into target triester **2** by reaction with 5-nitro-*cyclo*Sal phosphorochloridite **7**, which was prepared as reported before starting from 2-hydroxymethyl-4-nitrophenol (**8**).[21] Alcohol **8** was prepared from the salicylic acid derivative **9** by borane reduction in THF (86% yield). The intermediate phosphite was subsequently oxidized using oxone ($2\,KHSO_5{\cdot}KHSO_4{\cdot}K_2SO_4$) to the *cyclo*Sal phosphate triester (>90% yield; Scheme 2). The product was pure enough to be used as a raw material in the following reactions with glycosyl 1-phosphates. However, when oxone was replaced by the originally used *tert*-butylhydroperoxide, the

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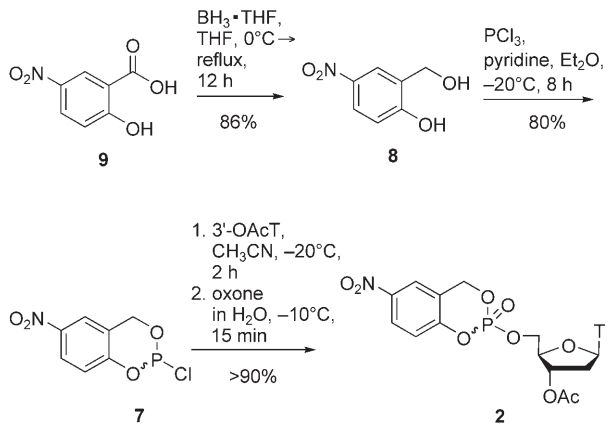
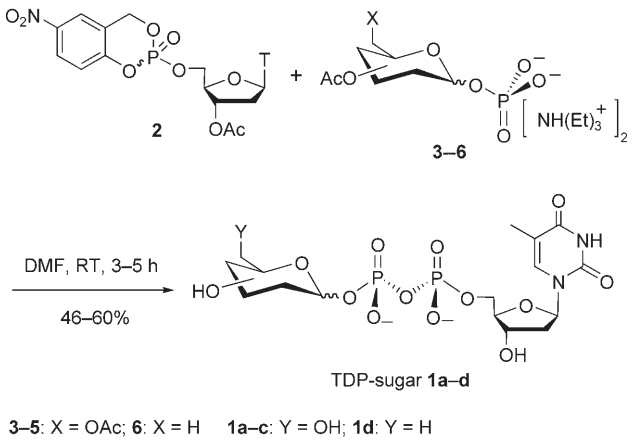
[**] This work has been accomplished in the network of the SFB 470/A8. We are grateful for financial support from the Deutsche Forschungsgemeinschaft.



Scheme 2. Synthesis of 5-nitro-*cyclo*Sal-(3′-OAc)-thymidine monophosphate.



3–5: X = OAc; **6**: X = H **1a–c**: Y = OH; **1d**: Y = H

Scheme 3. Synthesis of the NDP sugars starting from the *cyclo*Sal phosphate triester.

yields were found to be significantly lower, and sometimes the reaction failed completely. Alternatively, also an iodine/pyridine/THF/water solution as used in oligonucleotide synthesizers can be used.

D-Glucose, D-mannose, D-galactose and 6-deoxy-D-gulose[22] were peracetylated by standard conditions and then converted into the 1-phosphate **3–6** by two different methods. In the case of the α-configured glycosyl 1-phosphates **3(α)–6(α)**, pentaacetylglycopyranoses were deprotected at the anomeric center by hydrazinium acetate[23] and subsequently treated with dibenzyl(diisopropylamino)phosphine and 1*H*-tetrazole to give the phosphite intermediate,[24] which was oxidized by *m*-chloroperbenzoic acid at 0 °C to give dibenzylphosphate triesters in 31–70 % overall yield. For formation of the β-configured counterparts, pentaacetylglycopyranoses were converted into the glycopyranosyl bromides (HBr, HOAc), which were then treated with dibenzylphosphite in the presence of Ag_2CO_3 at 0 °C.[25] This reaction led to the exclusive formation of the β-D-glycopyranosyl phosphates in 81–98 % yield. In case of D-mannose even this reaction sequence led to the formation of the α-configured phosphate triester. Subsequently, the phosphate moieties were deprotected by hydrogenolysis using Pd/C, H_2, and NEt_3, which gave α- or β-configured D-glucose- (**3(α)** and **3(β)**), D-galactose- (**4(α)** and **4(β)**), D-mannose- (**5(α)**), and D-6-deoxygulose phosphates (**6(α)** and **6(β)**) as their triethylammonium salts in 73–95 % yield.

The highest yields for the coupling of 5-nitro-*cyclo*Sal nucleotide **2** and the glycosyl 1-phosphates **3–6** were obtained using DMF as solvent. After 3–5 h at room temperature the *cyclo*Sal phosphate triester was completely consumed, and a single product was formed in this highly stereospecific reaction in all cases (Scheme 3). In contrast, with pyridine as solvent, very long reaction times were required and *cyclo*Sal triester **2** slowly decomposed.

Prior to purification, the acetyl groups in the crude product were cleaved by a 1:7:3 mixture of NEt_3/MeOH/H_2O. This deprotection *before* purification made the subsequent isolation by chromatography decisively easier. TDP sugars **1a–d** were finally purified on a glass column filled with RP-18 silica gel. Usually, two passages were sufficient to isolate the product in high purity. Yields after purification were found to be between 46 % and 60 % (Table 1). As expected, the spectroscopic characterization proved that the anomeric configuration was unchanged in the product compared to the glycopyranosyl 1-phosphates.

Table 1: Yields of the prepared dTDP pyranoses.

	D-Glc		D-Man		D-Gal		6d-D-Gul	
	1a(α)	**1a(β)**	**1b(α)**	**1b(β)**	**1c(α)**	**1c(β)**	**1d(α)**	**1d(β)**
yield [%]	60	40	49	–	58	46	–	57

Instead of using the triethylammonium salt of the sugar phosphates, also more lipophilic counterions (e.g. $(n\text{Bu})_4N^+$) were investigated. Although the solubility of the salts was improved and the reaction works as well, the purification of the NDP sugars was extremely difficult.

To compare our novel method with one of the classical methods, the synthesis of TDP D-glucose **1a(α)** and **1a(β)** was repeated using the morpholidate protocol with either pyridine or DMF as solvent. Although some of the starting morpholidate was still present, the reaction was stopped after five days. After deprotection and purification the target compound **1a(α,β)** was isolated in only 10 % yield. Thus, our disclosed novel method allows a fast and efficient preparation of anomerically defined NDP glycopyranoses.

Using this new procedure, hitherto unknown TDP D-6-deoxygulopyranoses **1d(β)** were prepared and will be used now for biosynthetic studies of lipopolysaccharides. Moreover, the presented method has also been applied to the synthesis of NDP sugars with uridine, 2′-deoxyguanosine, and cytidine as nucleosides.[26]

In summary, this report confirms for the first time that the *cyclo*Sal technique not only can be used as a nucleotide delivery system (pronucleotides) but is also applicable as a new approach for the synthesis of biomolecule conjugates linked by a pyrophosphate group.

Experimental Section

Compound **2** (100 mg, 0.2 mmol) and the corresponding glycopyranosyl 1-phosphate **3–6** (1.2 equiv) were dissolved in dry DMF (5 mL), and the solution was stirred for 3–5 h at room temperature (conversion monitored by TLC). The solvent was evaporated under reduced pressure, and the residue was dissolved in H_2O and extracted with CH_2Cl_2. The aqueous phase was lyophilized and subsequently treated with NEt_3 (0.7 mL), MeOH (5 mL), and H_2O (2 mL). The mixture was maintained at room temperature for 12 h. After lyophilization, the crude product was purified on on RP-18 column using H_2O or a H_2O/MeCN gradient as eluent. Spectroscopic characterization is exemplified as given for compounds **1c(α)** and **1c(β). 1c(α)**: ^{1}H NMR (400 MHz, D_2O): $\delta = 1.29$ (t, 18H, $^3J_{HH} =$ 7.4 Hz, $2 \times CH_3$-NEt_3), 1.94 (d, 3H, $^4J_{HH} = 1.0$ Hz, thymine-CH_3), 2.37–2.44 (m, 2H, H-2′), 3.21 (q, 12H, $^3J_{HH} = 7.4$ Hz, $2 \times CH_2$-NEt_3), 3.71–3.83 (m, 3H, H-5, H-6), 3.93 (dd, 1H, $^3J_{HH} = 10.3$ Hz, $^3J_{HH} =$ 3.0 Hz, H-3), 4.04 (d, 1H, $^3J_{HH} = 3.0$ Hz, H-4), 4.19–4.20 (m, 4H, H-2, H-4′, H-5′), 4.63 (m, 1H, H-3′), 5.65 (dd, 1H, $^3J_{HP} = 7.3$ Hz, $^3J_{HH} =$ 3.8 Hz, H-1), 6.36 (dd, 1H, $^3J_{HH} = 6.5$ Hz, $^3J_{HH} = 6.5$ Hz, H-1′), 7.76 ppm (d, 1H, $^4J_{HH} = 1.0$ Hz, thymine-CH_3); ^{31}P NMR (162 MHz, D_2O): $\delta = -11.20$ (d, $J_{PP} = 20.9$ Hz, P_β), −12.66 ppm (d, $J_{PP} = 20.9$ Hz, P_α); HR-MS (ESI) calcd for $C_{16}H_{25}N_2O_{16}P_2$ [*M*+H]: 563.0685; found: 563.0671. **1c(β)**: ^{1}H NMR (400 MHz, D_2O): $\delta = 1.23$ (t, 18H, $^3J_{HH} =$ 7.3 Hz, $2 \times CH_3$-NEt_3), 1.88 (s, 3H, $^4J_{HH} = 1.0$ Hz, thymine-CH_3), 2.27–2.39 (m, 2H, H-2′), 3.15 (q, 12H, $^3J_{HH} = 7.3$ Hz, $2 \times CH_2$-NEt_3), 3.57 (dd, 1H, $^3J_{HH} = 7.6$ Hz, $^2J_{HH} = 10.3$ Hz, H-6), 3.63–3.67 (m, 1H, H-4), 3.70 (dd, 1H, $^3J_{HH} = 3.0$ Hz, $^2J_{HH} = 10.3$ Hz, H-6), 3.74–3.80 (m, 2H, H-3, H-5), 3.87 (d, 1H, $^3J_{HH} = 3.3$ Hz, H-2), 4.13–4.14 (m, 3H, H-4′, H-5′), 4.58–4.60 (m, 1H, H-3′), 4.91 (dd, 1H, $^3J_{HP} = 7.6$ Hz, $^3J_{HH} = 7.6$ Hz, H-1), 6.31 (dd, 1H, $^3J_{HH} = 6.6$ Hz, $^3J_{HH} = 6.6$ Hz, H-1′), 7.71 ppm (d, 1H, $^4J_{HH} = 1.0$ Hz, thymine-CH_3); ^{31}P NMR (162 MHz, D_2O): $\delta =$ −11.75 (d, $J_{PP} = 20.7$ Hz, P_β), −13.24 ppm (d, $J_{PP} = 20.7$ Hz, P_α); HR-MS (ESI) calcd for $C_{16}H_{25}N_2O_{16}P_2$ [*M*+H]: 563.0685; found: 563.0673.

Received: July 19, 2007
Revised: August 30, 2007
Published online: November 21, 2007

Keywords: carbohydrates · glycoconjugates · glycophosphotransferases · glycosylation · nucleotides

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