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# Substrate specificity of native dTDP-D-glucose-4,6-dehydratase: chemo-enzymatic syntheses of artificial and naturally occurring deoxy sugars

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**Abstract**

Incubation of dTDP-glucose with the enzyme dTDP-glucose-4,6-dehydratase [EC 4.2.1.46] from wild type *E. coli B* yielded a mixture of 3- and 4-keto-6-deoxy sugars after work-up. Model experiments with chemically synthesized methyl 6-deoxy-4-keto-glucoside (**9**) revealed that dTDP-6-deoxy-α-D-*ribo*-hexopyran-3-ulose (**3**) is formed by keto–enol tautomerization during the isolation procedure from initially formed dTDP-6-deoxy-α-D-*xylo*-hexopyran-4-ulose (**2**). dTDP-3-deoxyglucose (**4**) and dTDP-3-azido-3-deoxyglucose (**6**) were substrates and showed Michaelis–Menten kinetics (**4**: $K_M = 200$ μM and $V_{max} = 130$ μmol/h mg; **6**: $K_M = 300$ μM and $V_{max} = 90$ μmol/h mg). In 100-mg-scale experiments, both non-natural substrates gave the respective 6-deoxy-4-keto compounds, dTDP-3,6-dideoxy-α-D-*erythro*-hexopyran-4-ulose (**5**) and dTDP-3-azido-3,6-dideoxy-α-D-*xylo*-hexopyran-4-ulose (**7**), in yields ranging from 24 to 40%. © 1996 Elsevier Science Ltd.

*Keywords:* dTDP-sugars; dTDP-D-glucose-4,6-dehydratase [EC 4.2.1.46]; Enzymatic deoxygenation

## 1. Introduction

In bacteria, the pathway leading to L-rhamnose and related dTDP-activated 6-deoxy sugars originates from dTDP-glucose (**1**, cf. Scheme 1). A single-step deoxygenation at

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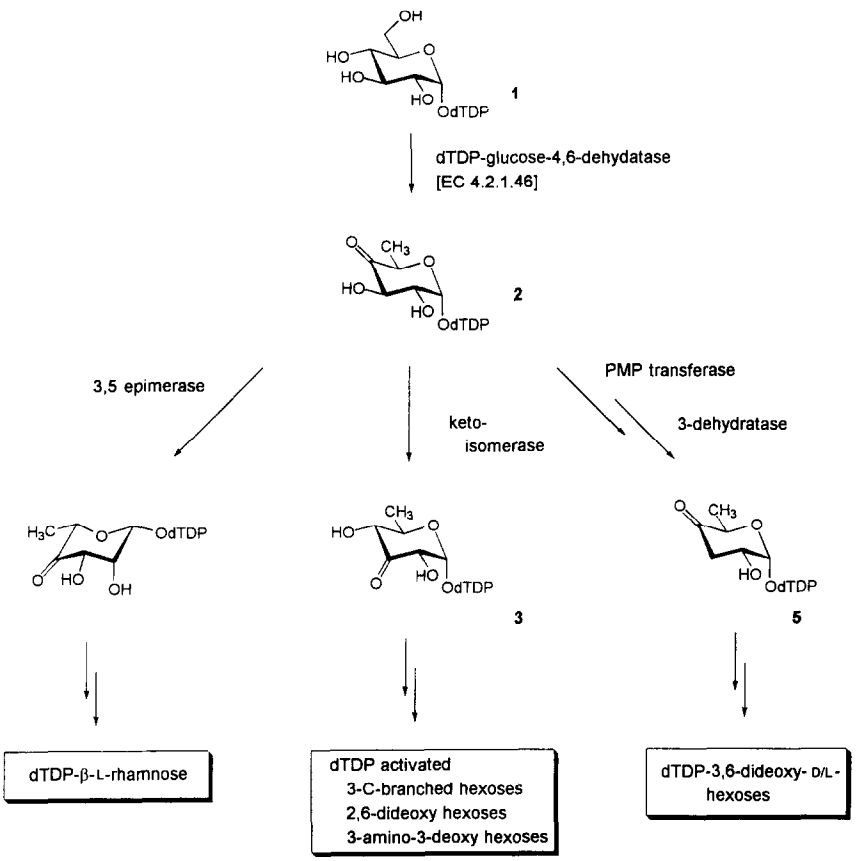



Scheme 1.

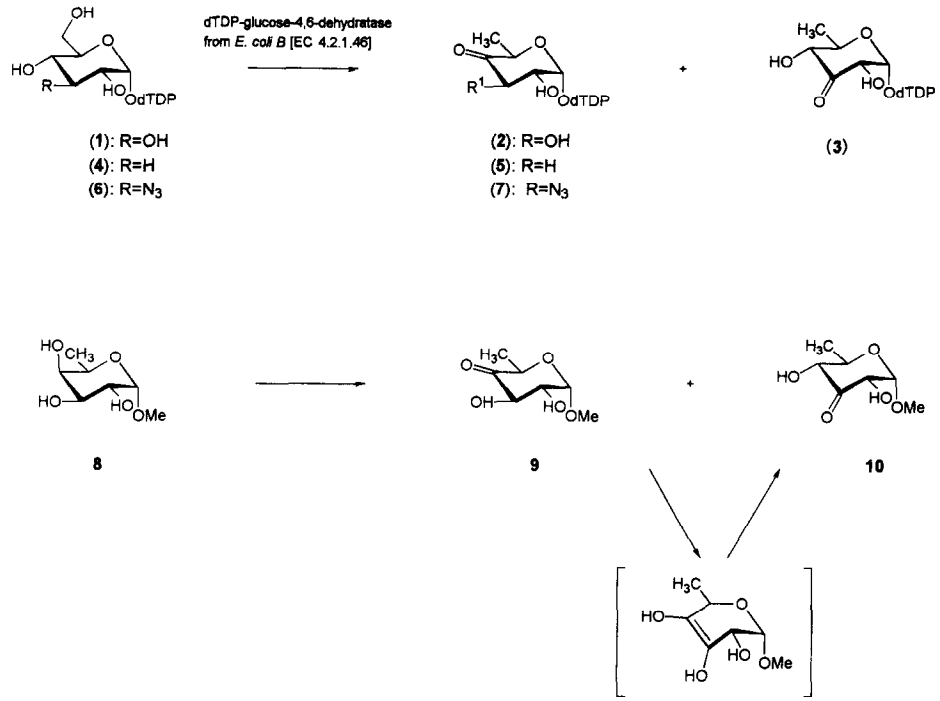
C-6 combined with a subsequent oxidation at C-4 [1] yields dTDP-6-deoxy-α-D-*xylo*-hexopyran-4-ulose (**2**). The enormous synthetic potential of the 4-keto function is reflected by the number of biosynthetical branches originating from **2**: (*1*) epimerization at C-3 and C-5, followed by subsequent reduction gives dTDP-L-rhamnose; (*2*) by keto-enol tautomerization via the 3-keto compound **3**, 3-C-branched sugars, 2-deoxy-, and aminodeoxy-hexoses are synthesized; (*3*) further α-deoxygenation yields 3,6-dideoxy sugars. The isomerase, although frequently postulated to bring about the enol-tautomerization from **2** to **3** [2–7], has hitherto not been isolated, most probably due to its low in vivo activity, and only a part of the gene coding for the C-terminus has yet been identified [8]. We now wish to report on the use of synthetic analogs of dTDP-glucose in the enzymatic deoxygenation, employing native dTDP-glucose-4,6-dehydratase [EC 4.2.1.46]. While working on these problems, the synthesis of dTDP-β-L-rhamnose itself has been reported by others [9] using an expressed p3778 vector under control of the T7 promotor in *E. coli*. With a recombinant enzyme from *Salmonella typhimurium* and a wild type protein from *E. coli B* in raw extracts, we have been able

to prove the formation the dTDP-6-deoxy-4-ulose **2** by NMR [10]. We here report with full experimental detail the observed non-enzymatic isomerization of 4-keto-6-deoxy sugar, the respective control experiments, and the use of non-isomerizable derivatives, where HO-3 has been replaced by hydrogen or azide.

## 2. Results and discussion

The enzyme was prepared from 15 L of culture broth by a method previously described [11–13] to give 30 U of total enzyme activity and 17 mg protein/mL. The resulting raw extract could be stored for at least 4 weeks at −18 °C without loss of activity. When the natural substrate, dTDP-glucose (**1**), was incubated with the enzyme, unexpectedly two new products were isolated after work-up and subsequent anion-exchange chromatography. By NMR analysis, **2** and **3** were identified as glycosyl phosphates, having the typical $^{3}J_{\text{H-1,P}}$ and $^{4}J_{\text{H-2,P}}$ couplings of 6.8 (6.9) and 3.2 Hz, owing to the 1,2-*trans*-relationship between the anomeric phosphate and H-2. Together with $^{2}J_{\text{C-1,P}}$- and $^{3}J_{\text{C-2,P}}$-hetero coupling constants of 6.6 and 8.4 Hz, respectively, a small vicinal $^{3}J_{1,2}$ coupling evidenced the *α-gluco* configuration. Finally, a successful deoxygenation was indicated for both derivatives by two doublets for H-6″ at 1.04 and 1.08 ppm, respectively. Therefore, it may be assumed that along with the deoxygenation a rearrangement has taken place, induced either chemically or enzymatically. In order to prove the non-enzymatical rearrangement by keto-enol tautomerization and epimerization at C-4, the nucleoside diphosphate sugar was compared to chemically synthesized methyl 4-keto-glycosides. Methyl 6-deoxy-α-D-galactopyranoside (**8**) was oxidized under constant pH (7.0) by bromine in aqueous solution. After 5 h, two new products could be observed and were identified after cellulose chromatography to be 4-keto-glycoside **9** and 3-keto-glycoside **10**. The separated keto-glycosides have been incubated under various conditions, amongst which have been those used for the purification of nucleoside diphosphate sugars (strongly basic ion exchanger) and those of the assay procedure (100 mM NaOH, lit. [11,15]). In both cases, the formation of **10** from **9** was observed and therefore it was possible to chemically simulate the isomerization in vitro as is depicted in Scheme 2. Based on this, an isomerase activity in the enzyme preparation could be ruled out, which implies that during the work-up, presumably during contact with the basic anion exchange, resin compound **3** is obtained by analogy to the isomerization of **9** → **10** by keto-enol tautomerization. Finally, by changing the work-up conditions [10], the formation of **3** could be completely suppressed. Therefore, it may be speculated that enzyme assistance neither in vitro nor in vivo is a prerequisite for the spontaneous isomerization observed here.

The next issue addressed was the substrate tolerance of the dehydratase towards structural analogs, such as both 3-deoxy derivatives **4** and **6**. Should a non-enzymatic enol tautomerization be responsible for the formation of the product mixture, compounds having HO-3 replaced by a hydrogen or an azide should not give rise to multiple-product formation due to the above described isomerization. However, the assay for the enzymatic formation of keto sugars, which has been used for **1** [11,15], gave misleading readings for the product concentrations when derivatives **4** and **6** were reacted. The




Scheme 2.

extinction was dependent on the time span between mixing with NaOH and reading the extinction at 318 nm, with an observed peak at approx. 10 min after enolization. Therefore, the extinction was detected exactly 10 min after NaOH addition against a blank with protein and deoxy thymidine equimolar to the concentration of the respective dTDP-glucose derivative in the corresponding incubation sample. Table 1 shows the $K_M$ and $V_{max}$ values for the *E. coli B* enzyme and dTDP-glucose (**1**), and for both derivatives **4** and **6**. The selectivity of the enzyme with the three different substrates **1**, **4**, and **6**, as expressed by their individual ratios of $V_{max}/K_M$, was 13:3:1.

Should the non-enzymatic epimerization account for the formation of **2** and **3**, single reaction products could be expected in both HO-3 modified dTDP-sugars **4** and **6**.

Table 1
$K_M$ and $V_{max}$ values for the *E. coli B* enzyme and dTDP-glucose (**1**), and for both derivatives **4** and **6**

| | **1** | *E. coli B* [a] | *S. erythrea* [b] | **4** | **6** |
|---|---|---|---|---|---|
| $K_M$ ($\mu$M) | 80 | 70 | 33 | 200±35 | 350±60 |
| $V_{max}$ ($\mu$mol/hmg) | 260 | 420 | 23 | 130±22 | 90±15 |
| $V_{max}/K_M$ (mL/smg) | 0.90 | 1.67 | 0.19 | ≈0.18 | ≈0.07 |

[a] According to lit. [11,15] for substrate **1**.

[b] According to Hutchinson et al. [21], this enzyme is $NAD^+$ dependent, measured by radioactive assay. All other compounds as described in the Experimental section.

Apparently, dTDP-3-deoxy-D-*ribo*-hexopyranose (**4**) gave, after a 3 days incubation with the enzyme and conventional work-up procedure, 4-ulose **5**. The site of deoxygenation again is C-6, clearly assigned by a doublet at 1.09 ppm in the proton, and a singlet at 12.2 ppm in the carbon spectrum. The newly formed carbonyl function is represented by two signals: the hydrated one in $D_2O$ at 93.2 ppm and the carbonyl signal in $Me_2SO$ at 203.1 ppm. Thus, by employing substrate **4**, the physiologically occurring 3-deoxy intermediate **5** could be synthesized in vitro in one step from **4**. The biosynthesis in contrast is proposed to depart from **2** in two steps: Schiff-base formation with pyridoxamine phosphate, followed by a radical deoxygenation [14]. Finally, the enzymatic conversion of **6** was addressed. Similar reaction conditions gave, after 4 days of incubation, a deoxygenated product, having similar characteristic NMR data as discussed above, from which the structure of **7** could undoubtedly be assigned (cf. Tables 2 and 3).

Following the described protocol, three examples of a preparative enzymatic deoxygenation of sugar nucleotides using wild type enzyme could be demonstrated. This provided an access to three nucleoside diphosphate-activated deoxy sugars **2**, **3**, and **5**, which are intermediates in the biosynthesis of bacterial deoxy sugars. Additionally, an artificial structure was prepared, azido sugar **7**. Further investigations presently under way, on the substrate specificities of bacterial enzymes of the deoxy sugar pathway, will help to provide routes towards modified deoxy sugar glycosyl donors.

## 3. Experimental

*General procedures.*—$^1$H NMR spectra were recorded with Bruker AM-300 or AM-400 spectrometers at the frequencies indicated, employing standard pulse angles. If necessary, coupled protons were assigned by $^1$H,$^1$H-COSY and carbons by $^{13}$C,$^1$H correlated spectroscopy. All reactions were monitored by TLC on silica gel plates ($GF_{254}$, E. Merck) and detected by either UV-absorption or charring with 5% $H_2SO_4$ in EtOH and subsequent heating to 500 °C. Column chromatography was performed on Silica Gel 60 (230–400 mesh, E. Merck) with solvents listed below. Samples were desalted by Sephadex G10 size-exclusion chromatography (column size 160 × 2 cm), the eluate was monitored by UV-detection. Diphosphates were purified by ion-exchange chromatography (Dowex 1-X2, $Cl^-$, 80 × 1.5 cm) and eluted by a linear 0 → 0.8 M LiCl gradient.

*dTDP-6-deoxy-α-*D-xylo-*hexopyran-4-ulose and dTDP-6-deoxy-α-*D-ribo-*hexopyran-3-ulose* (**2** *and* **3**).—The raw extract from *E. coli B* cells (15 g cells, harvested in late log phase) was prepared following the literature procedure and kept at − 18 °C [13]. In a sterile container, flushed permanently with nitrogen or handled under a laminar flow bench, dTDP-glucose disodium salt (**1**) (50 mg, 82 μmol) was dissolved in TRIS/HCl buffer (1 mL, 50 mM, 0.5 mM DTT, pH 7.5) to which the raw extract (5 U, estimated according to lit. [15]) was added. The reaction volume was filled up to a total volume of 10 mL with bidistilled water and the reaction mixture was shaken gently at 37 °C. After 2 days, when TLC showed no further product formation (solvent *A*: 2-propanol–EtOH–$H_2O$ = 5:3:2, 5% HOAc, 2% $NEt_3$; or solvent *B*: 2-propanol–$H_2O$–$NH_3$ in water =

Table 2
$^1$H NMR data

| | **2** | | **3** | | **4** | | **5** | |
|---|---|---|---|---|---|---|---|---|
| | $^1$H NMR (ppm) | mult; $^3J$ (Hz), [$J_{het}$ (Hz)] | $^1$H NMR (ppm) | mult; $^3J$ (Hz), [$J_{het}$ (Hz)] | $^1$H NMR (ppm) | mult; $^3J$ (Hz), [$J_{het}$ (Hz)] | $^1$H NMR (ppm) | mult; $^3J$ (Hz), [$J_{het}$ (Hz)] |
| *Hexose* | | | | | | | | |
| **1″** | 5.42 | dd, 3.7, [6.8] | 5.45 | dd, 4.1, [6.9] | 5.54 | dd, 3.1, [6.5] | 5.38 | dd, 3.1, [6.5] |
| **2″** | 3.40 | ddd, 10.2, [3.2] | 4.20 | d br, ≈ 1.0 [a], [3.2] | 3.82–3.51 | m, 3 H | 3.74 | $m_c$ |
| **3″** | 3.67 | d | – | – | 2.34 + 2.15 | ddd/ddd, 12.6 [a], 10.5, 5.3 | 2.47 + 2.25 | dd/dd, 12.6 [b], 11.6, 5.5 |
| **4″** | – | | 3.68 | d, 9.2 | see H-2″ | | – | |
| **5″** | 3.90 | q, 6.5 | 3.44 | dq, 6.3 | 4.00–3.90 | m, 2 H | 4.02 | q, 6.6 |
| **6a″** [a] | 1.04 | d | 1.08 | d | see H-5″ | | 1.09 | d |
| **6b″** | | | | | see H-2″ | | | |
| *Ribose* | | | | | | | | |
| **1′** | 6.20 | dd | 6.21 | dd | 6.22 | dd | 6.22 | dd |
| **2a′** | 2.31–2.27 | dd + m, 2 H | 2.31–2.28 | dd + m | 2.35–2.30 | m, 2 H | 2.35–2.30 | m, 2 H |
| **2b′** | see H-2a′ | | | see H-2a′ | see H-2a′ | | see H-2a′ | |
| **3′** | 4.42 | mc | 4.45 | m | 4.68 | m | 4.68 | m |
| **4′** | 4.00–3.90 | m | 4.00–3.98 | m | 4.08–3.90 | | 4.08–3.90 | m |
| **5a′** | see H-4′ | | see H-4′ | | see H-4′ | | see H-4′ | |
| **5b′** | see H-4′ | | see H-4′ | | see H-4′ | | see H-4′ | |
| *Base* | | | | | | | | |
| **6** | 7.84 | s | 7.71 | s | 7.72 | s | 7.72 | s |
| **$CH_3$** | 1.70 | s | 1.84 | s | 1.84 | s | 1.84 | s |
| **$OCH_3$** | | | | | | | | |

| | **6** | | **7** | | **9** [d] | | **10** [d] | |
|---|---|---|---|---|---|---|---|---|
| | $^1$H NMR (ppm) | mult; $^3J$ (Hz), [$J_{het}$ (Hz)] | $^1$H NMR (ppm) | mult; $^3J$ (Hz), [$J_{het}$ (Hz)] | $^1$H NMR (ppm) | mult; $^3J$ (Hz) | $^1$H NMR (ppm) | mult; $^3J$ (Hz) |
| *Hexose* | | | | | | | | |
| **1″** | 5.58 | dd, 3.0, [6.6] | 5.43 | dd, 3.8, [7.0] | 4.72 | d, 3.6 | 4.88 | d, 4.5 |
| **2″** | 3.44 | m, 2 H | 3.40 | *m* | 3.54 | dd, 10.2 | 4.31 | dd, 1.5 [c] |
| **3″** | see H-2″ | | 3.42 | d | 4.30 | d | – | |
| **4″** | 3.90–3.55 | m | – | | – | | 3.78 | dd, 9.5 |
| **5″** | 4.02 | ≈ ddd, 5.4, 11.0 | 4.21 | q, 6.4 | 4.23 | q, 6.6 | 3.61 | dq, 6.0 |
| **6a″** [a] | see H-4″ | 12.5 [f] | 1.12 | d | 1.11 | d | 1.29 | d |
| **6b″** | see H-4″ | | – | | | | | |
| *Ribose* | | | | | | | | |
| **1′** | 6.30 | dd | 6.31 | dd | | | | |
| **2a′** | 2.35–2.30 | m, 2 H | 2.35–2.30 | m, 2 H | | | | |
| **2b′** | see H-2a′ | | see H-2a′ | | | | | |
| **3′** | 4.44 | *m* | 4.49 | m | | | | |
| **4′** | 4.00–3.92 | m | 4.00–3.88 | m | | | | |
| **5a′** | see H-4′ | | see H-4′ | | | | | |
| **5b′** | see H-4′ | | see H-4′ | | | | | |
| *Base* | | | | | | | | |
| **6** | 7.70 | s | 7.72 | s | | | | |
| **$CH_3$** | 1.77 | s | 1.84 | s | | | | |
| **$OCH_3$** | | | | | 3.41 | | 3.28 | s |

[a] $^4J_{2,4}$.
[b] $^2J_{3a,3b}$.
[c] This row contains data for H-6a″; in case of 6-deoxy sugars it contains data for H-6″.
[d] Spectra recorded in MeOD, all others in $D_2O$.
[e] $^4J_{2,4}$.
[f] $^2J_{6a,6b}$.

Table 3
$^{13}C$ NMR data

| | **2** | | **3** | | **4** | | **5** | | **6** | | **7** | | **9** | **10** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $^{13}C$ NMR (ppm) | *J*-hetero (Hz) | $^{13}C$ NMR (ppm) | *J*-hetero (Hz) | $^{13}C$ NMR (ppm) | *J*-hetero (Hz) | $^{13}C$ NMR (ppm) | *J*-hetero (Hz) | $^{13}C$ NMR (ppm) | *J*-hetero (Hz) | $^{13}C$ NMR (ppm) | *J*-hetero (Hz) | $^{13}C$ NMR (ppm) | $^{13}C$ NMR (ppm) |
| *Hexose* | | | | | | | | | | | | | | |
| **1″** | 95.1 | 6.6 | 94.9 | 6.6 | 96.0 | 6.4 | 95.7 | 6.4 | 95.2 | 6.4 | 95.7 | 6.4 | 100.0 | 101.0 |
| **2″** | 71.8 | 8.4 | 72.0 | 8.4 | 86.8 | 8.4 | 69.1 | 8.5 | 84.2 | 8.0 | 82.3 | 8.1 | 75.7 | 73.5 |
| **3″** | 73.2 | | 93.7 | | 28.1 | | 32.4 | | 56.2 | | 60.2 | | 76.1 | 203.8 |
| **4″** | 94.3 | | 77.4 | | 68.6 | | 93.2; [203.1 [a]] | | 67.1 | | 94.1; [201.4 [a]] | | 204.7 | 76.2 |
| **5″** | 70.2 | | 70.1 | | 69.9 | | 69.8 | | 69.5 | | 71.4 | | 68.3 | 69.4 |
| **6″** | 12.0 | | 11.3 | | n.o. | | 12.2 | | n.o. | | 13.6 | | 12.8 | 16.2 |
| *Ribose* | | | | | | | | | | | | | | |
| **1′** | 85.6 | | 85.3 | | 85.3 | | 85.6 | | 83.2 | | 82.4 | | | |
| **2a′** | 39.0 | | 38.7 | | 38.7 | | 38.4 | | 37.5 | | 38.0 | | | |
| **2b′** | – | | – | | – | | – | | – | | – | | | |
| **3′** | 71.0 | | 71.2 | | 71.5 | | 71.0 | | 70.2 | | 70.0 | | | |
| **4′** | 85.9 | 9.6 [b] | 84.4 | 9.6 [b] | 83.2 | n.o. [c] | 81.2 | n.o. | 81.0 | n.o. | 82.6 | n.o. | | |
| **5a′** | 65.4 | 4.8 [d] | 65.3 | 6.0 [d] | 65.7 | 4.6 [d] | 65.9 | 4.5 [d] | 65.0 | n.o. | 64.7 | n.o. | | |
| **5b′** | – | | | | – | | – | | – | | – | | | |
| *Base* | | | | | | | | | | | | | | |
| **2** | 152.4 | | 151.7 | | 151.8 | | 153.1 | | 154.2 | | 154.9 | | | |
| **4** | 166.0 | | 166.1 | | 166.9 | | 167.7 | | 168.3 | | 167.5 | | | |
| **5** | 112.2 | | 113.0 | | 112.0 | | 112.4 | | 114.0 | | 113.2 | | | |
| **6** | 137.7 | | 137.4 | | 136.7 | | 138.4 | | 137.2 | | 136.3 | | | |
| **$CH_3$** | 12.0 | | 12.0 | | 11.8 | | 11.6 | | 12.2 | | 13.6 | | | |
| **$OCH_3$** | | | | | | | | | | | | | 55.2 | 53.0 |

[a] Non-hydrated C = O.
[b] $^3J_{C\text{-}4,P}$.
[c] n.o. = not observed or assigned due to a broad signal or overlap.
[d] $^2J_{C\text{-}5,P}$.

7:3:1), the reaction was stopped by denaturing the enzyme for 30 s in a boiling water bath. The mixture was then frozen, and concentrated to approx. 3 mL by lyophilization. The concentrate was applied to Sephadex G10 (160 × 2 cm) to give high molecular fractions and UV-active sugar nucleotide fractions. These were pooled, lyophilized, and fractionated by ion-exchange chromatography (Dowex 1-X2, $Cl^-$, 80 × 1.5 cm, gradient 0 → 0.8 M LiCl, 800 mL); diphosphates were eluted at 0.4–0.5 M salt. The appropriate fractions were pooled, concentrated to 1–2 mL by lyophilization, and desalted by Sephadex G10 chromatography. After lyophilization of the product fractions, the dilithium salts of **2** and **3** (3:2 according to $^1$H NMR, 26 mg, 54%) were obtained as white foams. For $^1$H and $^{13}$C NMR ($D_2O$) data see Tables 2 and 3, respectively.

*dTDP-3,6-dideoxy-α-*D-erythro-*hexopyran-4-ulose* (**5**).—dTDP-3-deoxy-α-D-*ribo*-hexopyranose (**4**, 50 mg, 80 μmol), which was prepared from chemically synthesized dilithium salt [17–19] by passing through a column of Dowex W50 ($Na^+$) at 4 °C and subsequent lyophilization, was dissolved in TRIS/HCl buffer (500 μL, 50 mM, 0.5 mM DTT, pH 7.5) to which the raw extract (10 U, measured according to lit. [11,15] with **1** as substrate) was added. Further processing is similar to the deoxygenation described for **1**. Isolation after 3 days yielded **5** (20 mg, 41%). For $^1$H and $^{13}$C NMR data see Tables 2 and 3, respectively.

*dTDP-3-azido-3,6-dideoxy-α-*D-xylo-*hexopyran-4-ulose* (**7**).—Enzymatically synthesized dTDP-3-azido-3-deoxy-α-D-*ribo*-hexopyranose disodium salt [20] (**6**, 100 mg, 158 mmol) was dissolved in TRIS/HCl buffer (2 mL, 50 mM, 0.5 mM DTT, pH 7.5) to which the raw extract (10 U, measured according to lit. [11,15] with **1** as substrate) was added. Further processing was similar to the deoxygenation described for **1**. Isolation after 4 days yielded **7** (23 mg, 24%). For $^1$H and $^{13}$C NMR data see Tables 2 and 3, respectively.

*Methyl-6-deoxy-α-*D-xylo-*hexopyran-4-uloside* (**9**) *and methyl-6-deoxy-α-*D-ribo-*hexopyran-3-uloside* (**10**).—In an automatic titrator (Schott TR 154/TA 01), an aq soln of bromine (430 mg, 2.69 mmol, 2.4 equiv in 27 mL) was neutralized with NaOH (1.0 N). To this, methyl-α-D-fucopyranoside (**8**, from 6-deoxy-D-galactopyranoside, Sigma) was added and the solution was stirred for 5 h at room temp at pH 7.0. The reaction was quenched by passing a stream of nitrogen through the solution, which then was lyophilized. The residue was extracted several times with 2-propanol. The extract consisted of a mixture of **9** (55%), **10** (5%), and starting material, as was judged by $^1$H NMR. Cellulose chromatography (column size 12 × 3 cm, EtOAc–MeOH 7:1) yielded **9** (70 mg, 35%) after precipitation from $CH_2Cl_2$ with *n*-hexane, $[\alpha]_D^{20}$ +202° (*c* 1.2, $CH_2Cl_2$), $[\alpha]_D^{20}$ +164° (*c* 1.5, $H_2O$); lit. [16]: $[\alpha]_D^{20}$ −160° for L-enantiomer. For $^1$H and $^{13}$C NMR (MeOD) data see Tables 2 and 3, respectively. Anal. Calcd for $C_7H_{12}O_5$ (176.2): C, 47.73; H, 6.87. Found: C, 47.54; H, 7.09. By silica gel chromatography (toluene–acetone 2:1) a small fraction of **10** (20%) could be isolated as pure material. mp 90 °C. $[\alpha]_D^{20}$ +171° (*c* 1.3, $CHCl_3$). For $^1$H NMR and $^{13}$C NMR (MeOD) data see Tables 2 and 3, respectively. Anal. Calcd for $C_7H_{12}O_5$ (176.2): C, 47.73; H, 6.87. Found: C, 47.80; H, 6.94.

*Enzymatic assay.*—Samples were taken in triplicate at 20, 40, 80, 150, and 200 mM for **1** and 80, 150, 300, and 400 mM substrate concentrations for **4** and **6**. Assay conditions for **1** were as described in lit. [11,15] by adjusting the NaOH concentration to

100 mM and reading the extinction at 318 nm after 15 min. For compounds **4** and **6** the extinction was unstable after mixing, with a gradual decrease after 10 min. The extinction was therefore read after exactly 10 min against a blank, containing equimolar concentrations of thymidine and similar enzyme activity. Initial rate constants were calculated from mmol/min plots by differentiation of their second-order polynomial fits for $t = 0$. Kinetic constants $K_M$ and $V_{max}$ were estimated from Hanes plots generated by the "Hyper"-software utility [22].

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