Pergamon

Tetrahedron Letters, Vol. 38, No. 31, pp. 5473-5476, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)01224-0

Investigations towards the Synthesis of dTDP-2,6-Dideoxy-D-*erythro*-3-hexulose - a Potential Intermediate in the Biosynthesis of Rare Sugars[1]

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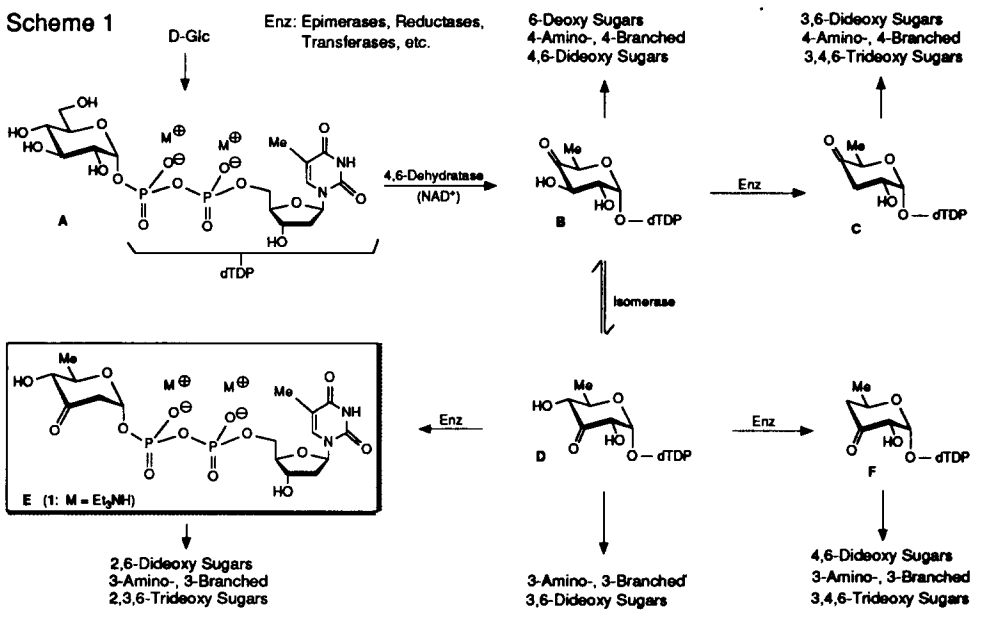
Abstract: The synthesis of the target molecule **1** is based on thexyldimethylsilyl 4-O-acetyl-2,3,6-trideoxy-3-C-methylene-β-D-*erythro*-hexopyranoside (**7**) which is readily obtained via two different routes from tri-O-acetyl-D-glucal (**2**). Replacement of the anomeric silyl group by the diethylphosphite group, then performing a phosphite/phosphate exchange reaction, and finally removal of all protective groups afforded an α/β-mixture of 2,3,6-tri-deoxy-3-C-methylene-D-*erythro*-hexopyranosyl phosphate (**8**); its ozonolysis furnished the corresponding 3-ulose **10**. Treatment of **8** with dTMP-morpholidate in pyridine led to the 3-C-methylene analogue **9**α of the target molecule; ozonolysis of **9**α afforded **1** which – as expected – experienced relatively fast β–elimination under work-up conditions.



Many antibiotics contain deoxy-, aminodeoxy-, and/or C-branched deoxysugar moieties; elucidation of their biosynthesis is still under investigation.[2,3] Starting from dTDP-glucose (Scheme 1, **A**), the first intermediate in the biosynthetic pathway is dTDP-6-deoxy-D-*xylo*-4-hexulose (**B**; instead of dTDP also the

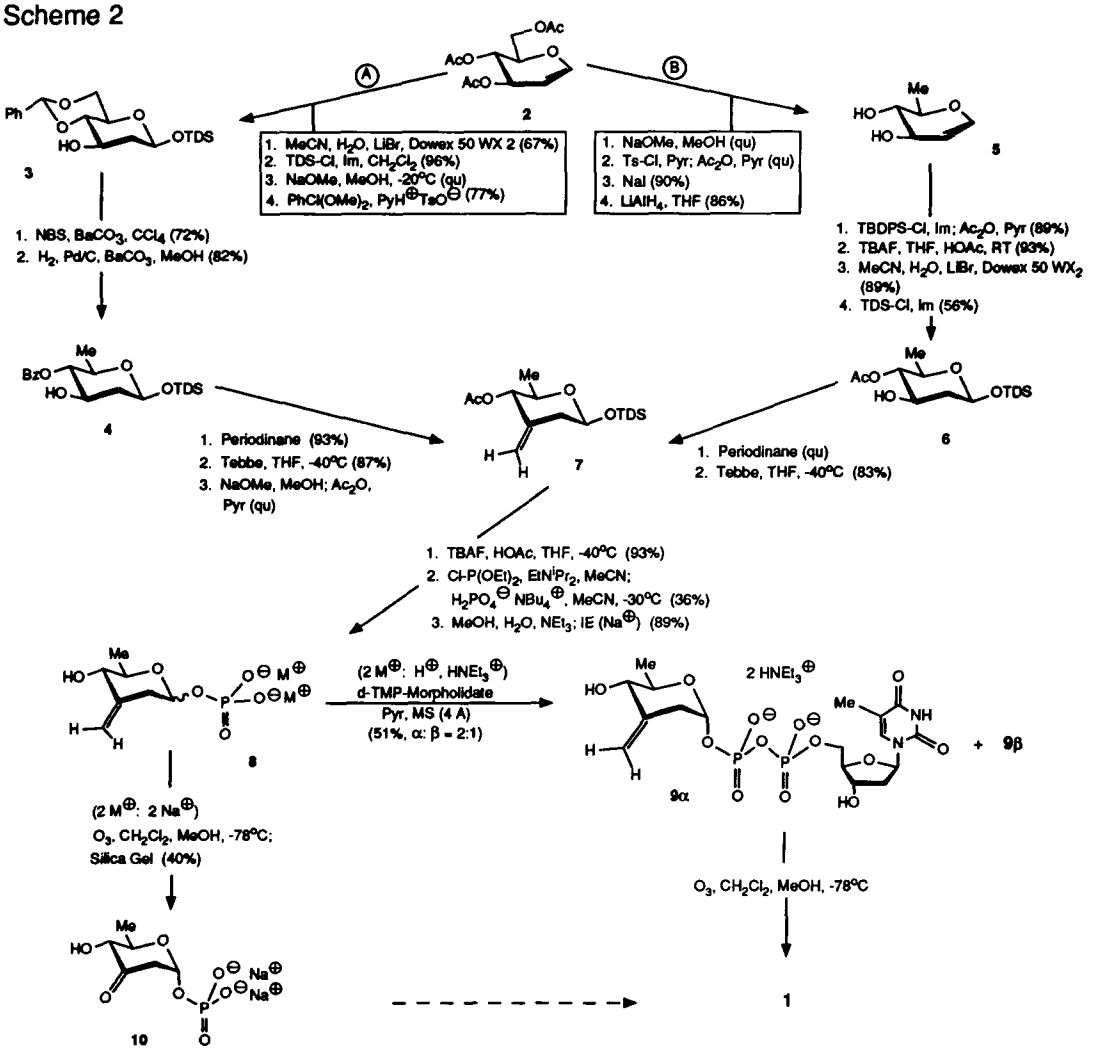
corresponding CDP intermediate is found). **B** is a direct precursor of the commonly occurring 6-deoxysugars;[2-5] via **C** also 3,6-dideoxysugars and derivatives are accessible. For the biosynthesis of 2,6- and 4,6-dideoxysugars an isomerase apparently converts **B** into the corresponding D-*ribo*-3-hexulose **D** which serves as next key intermediate for the preparation of dideoxy-, trideoxy-, aminodeoxy-, and C-branched sugars.[3-6] Elucidation of the biosynthesis of these sugars (e.g., with 4,6-dehydratase- or isomerase-deficient mutants[3] etc.) necessitates the potential intermediates **D**, **E**, and **F** (**E**: 2,6- and **F**: 4,6-dideoxy-D-*erythro*-3-hexulose). Their synthesis is particularly difficult due to the hydrolytic instability of nucleoside diphosphates of deoxysugars[7] and the tendency of 3-uloses to undergo β-elimination of the nucleoside diphosphate residue.[8]

After successful synthesis of **D**,[9,10] we report here our efforts to the synthesis of **E** as triethylammonium salt (= compound **1**) which – as 2-deoxy-3-uloside – is the most labile compound amongst the intermediates in Scheme 1. The synthetic strategy is based on removal of all protective group(s) at the glycosyl monophosphate stage and introduction of the 3-oxo function by ozonolysis of the corresponding methylene group in the penultimate or last reaction step, in order to avoid β-elimination during the various chemical transformations. For the introduction of a methylene group at C-3 and deoxygenation at C-6 tri-O-acetyl-D-glucal (**2**, Scheme 2) was selected as starting material; it was first transformed into the 2-deoxyglucose derivative (route (A)) by water addition in aqueous acetonitrile in the presence of Dowex 50 WX 2 as catalyst, following a known procedure.[11] 1-O-Silylation with thexyldimethylsilyl chloride (TDS-Cl) in the presence of imidazole and then removal of all O-acetyl groups (Zemplén conditions: NaOMe, MeOH)[12], and 4,6-O-benzylidenation with benzaldehyde dimethylacetal and pyridinium p-toluenesulfonate ($PyrH^+$ $p\text{-}TsO^-$) as catalyst afforded 2-deoxy-glucopyranoside **3**.[13] Regioselective opening of the benzylidene residue with N-bromosuccinimide (NBS) in the presence of $BaCO_3$ (method of Hanessian et al.)[14] afforded the 4-O-benzoyl-6-bromo derivative which on hydrogenation afforded 4-O-benzoyl-2,6-dideoxy-glucopyranoside **4**. Oxidation with periodinane furnished the 3-ulose which gave with Tebbe's reagent[15] the 3-C-methylene derivative. Replacement of the 4-O-benzoyl group by the more readily removable acetyl group under standard conditions afforded the desired 2,6-dideoxy-3-C-methylene derivative **7**[16] in good overall yield.

A second approach to **7** (route (B)) essentially followed literature procedures regarding the synthesis of 6-deoxyglucal (**5**).[17] Selective protection of the 3-hydroxy group in **5** could be performed, as expected[18] with *tert*-butyldiphenylsilyl chloride (TBDPS-Cl) in the presence of imidazole; ensuing acetylation gave the corresponding 4-O-acetyl derivative. Water addition to the CC-double bond as described above and then 1-O-silylation with TDS-Cl/imidazole furnished 4-O-acetyl-2,6-dideoxy-hexoside **6**; oxidation with periodinane led to the 3-ulose which on reaction with Tebbe's reagent[15] provided **7** in comparable overall yield.

Synthesis of the target molecule from **7** required first selective removal of the 1-O-silyl group; this could be performed by treatment with tetrabutylammonium fluoride (TBAF) in THF/HOAc at -40°C in high yield. Introduction of the phosphate residue was finally successfully carried out by a phosphite/phosphate exchange reaction;[19,20] various other procedures failed[20] due to low stability of the desired phosphate. To this aim, reaction with diethyl phosphochloridite in the presence of Hünig's base was performed; ensuing reaction with tetrabutylammonium dihydrogenphosphate in acetonitrile at -30°C gave a 1:1 α/β-mixture of the 4-O-acetyl group containing phosphates; the anomers could not be separated at this stage. Their treatment with triethylamine in $MeOH/H_2O$ enabled removal of the 4-O-acetyl group; β-elimination of the phosphate group was not observed under these conditions. Exchange of the triethylammonium ions for sodium ions gave 2,3,6-trideoxy-3-C-methylene-D-hexopyranosyl phosphate **8**. Ozonolysis in CH_2Cl_2/MeOH at -78°C and then

Scheme 2



purification over silica gel (eluents: $CHCl_3/MeOH/H_2O$ = 12:8:1) led to separation of the α-anomeric 3-ulose phosphate **10**, which could be fully characterized[16] because the disodium salt was expectedly[21] quite stable.

Two routes were investigated for the synthesis of target molecule **1**. First, attempts to attach the thymidinephosphate moiety to **10** via the morpholidate procedure[9,22] (reaction of the triethylammonium salt of **10** with thymidine phosphomorpholidate in pyridine) in order to directly yield **1**, led only to the phosphate elimination product of **10**. Therefore, the morpholidate procedure was employed to the triethylammonium salt of **8** furnishing the desired thymidine diphosphate sugars **9α** and **9β** which could be separated by preparative HPLC (3% CH_3CN in 0.025 M Et_3NH^+ HCO_3^- buffer; t_R (**9α**) = 72 min, t_R (**9β**) = 45 min) and fully characterized by ^1H-, ^{31}P-NMR and FAB-MS data.[16] Compound **9α** is of interest for biological studies as a potential competitive inhibitor of **1**. Ozonolysis of **9α** was performed in CH_2Cl_2/MeOH at -78°C; HPLC analysis (3% CH_3CN in 0.025 M Et_3NH^+ HCO_3^- buffer) indicated formation of the target molecule **1** [t_R = 11 min; ^1H NMR (250 MHz, D_2O): δ 5.78, ddd, $J_{1",P}$ = 7.1, $J_{1",2"e}$ = 4.4, $J_{1",2"a}$ < 1 Hz. 1 H, H-1")]; however,

isolation of pure **1** was thus far not possible because thymidine diphosphate elimination turned out to be a fast reaction. Yet, for immediate biological studies **1** can be made available via the outlined synthetic strategy.

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16. Selected NMR data of new compounds [^{1}H NMR (250 MHz, $CDCl_3$, D_2O); ^{13}C-NMR (150.9 MHz, $CDCl_3$); ^{31}P NMR (161.7 MHz, D_2O); FAB MS (negative mode, matrix: DMF/glycerol (1:1), H_2O/TEG (1:1)]: **7**: δ_H 1.21 (d, $J_{5,6}$ = 6.2 Hz, 3 H, H-6), 2.33 (dddd, $J_{methylene,2a}$ = 1.0 Hz, $J_{methylene',2a}$ = 1.2 Hz, $J_{1,2a}$ = 8.9 Hz, $J_{2a,2e}$ = 13.4 Hz, 1 H, H-2$_a$), 2.52 (dd, $J_{1,2e}$ = 2.5 Hz, $J_{2a,2e}$ = 13.4 Hz, 1 H, H-2$_e$), 4.68 (dd, $J_{1,2a}$ = 8.9 Hz, $J_{1,2e}$ = 2.5 Hz, 1 H, H-1), 4.72-4.83 (m, 2 H, methylene-H). – δ_C = 20.12 (s, 1 C, C-6), 43.36 (s, 1 C, C-2), 96.59 (s, 1 C, C-1), 107.95 (s, 1 C, methylene-C), 145.67 (s, 1 C, C-3). – **9**α: δ_H = 1.03-1.09 (m, 3 H, H-6"), 2.39-2.48 (m, 2 H, H-2$_a$", H-2$_e$"), 4.69-4.91 (m, 2 H, methylene-H), 5.43 (m, 1 H, H-1"), 6.16 (dd, $J_{1',2'a}$ = 6.8 Hz, $J_{1',2'e}$ = 6.9 Hz, 1 H, H-1'). – δ_P = -13.27 (d, $J_{P,P'}$ = 20.0 Hz, 1 P), -11.30 (d, $J_{P,P'}$ = 20.0 Hz, 1 P). – m/z (%) = 527(72) [M+H$^+$]$^-$. **9**β: δ_H = 1.11 (d, $J_{5",6"}$ = 6.2 Hz, 3 H, H-6"), 2.62 (dd, $J_{1",2e"}$ = 1.8 Hz, $J_{2a",2e"}$ = 13.8 Hz, 1 H, H-2e"), 2.85 (dd, $J_{1",2a"}$ = 6.8 Hz, $J_{2a",2e"}$ = 13.8 Hz, 1 H, H-2a"), 4.79-4.87 (m, 2 H, methylene-H), 4.94 (ddd, $J_{1",P}$ = 8.2 Hz, $J_{1",2a"}$ = 6.8 Hz, $J_{1",2e"}$ = 1.8 Hz, 1 H, H-1"), 6.15 (dd, $J_{1',2a'}$ = 6.7 Hz, $J_{1',2e'}$ = 7.2 Hz, 1 H, H-1'). – δ_P = -1374 (d, $J_{P,P'}$ = 22.5 Hz, 1 P), -11.27 (d, $J_{P,P'}$ = 22.5 Hz, 1 P).- m/z (%) = 527(72) [M+H$^+$]$^-$. **10**: δ_H = 1.22 (d, $J_{5,6}$ = 5.2 Hz, 3 H, H-6), 2.46 (dd, $J_{1,2a}$ < 1 H, $J_{2a,2e}$ = 14.3 Hz, 1 H, H-2$_a$), 2.84 (dd, $J_{1,2e}$ = 4.3 Hz, $J_{2a,2e}$ = 14.3 Hz, 1 H, H-2$_e$), 5.67 (ddd, $J_{1,P}$ = 6.7 Hz, $J_{1,2a}$ < 1 Hz, $J_{1,2e}$ = 4.3 Hz, 1 H, H-1). – δ_P = 1.11 (s, P_α). – m/z (%) = 225(9) [M+H$^+$]$^-$.
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(*Received in Germany* 20 *May* 1997; *accepted* 16 *June* 1997)