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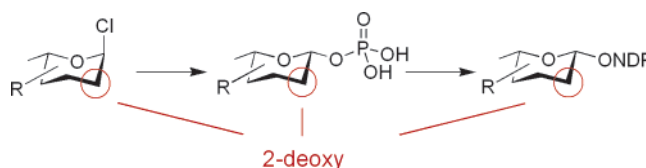
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ABSTRACT



β -2-Deoxy sugar nucleotides are substrates used by a variety of glycosyltransferases (Gtfs). We have developed a chemical route to synthesize β -2-deoxy sugar phosphates that starts from α -glycosyl chlorides. Our approach reliably provides access to a range of NDP β -2-deoxy sugars essential for studying glycosyltransferases involved in the synthesis of biologically active natural products.

Deoxy sugars are essential constituents of a variety of biologically active natural products that include vancomycin, erythromycin, and daunomycin (Figure 1).¹ As a consequence, considerable effort has been directed toward developing methods that will attach deoxy sugars to various natural product aglycones. Enzymes are proving to be efficient tools for glycosylating these aglycones, particularly since the relaxed substrate specificity of many glycosyltransferases (Gtfs) in terms of both glycosyl donors and glycosyl acceptors makes it possible to prepare analogues.^{2–4} One limitation of enzymatic glycosylation methods is the difficulty sometimes encountered in obtaining the required NDP deoxy sugar⁵ donors. Although enzymatic methods for making NDP sugars have been investigated,⁶ chemical methods still represent the most flexible approach. However, the β -linked 2-deoxy L-sugar donors, which are used by many

natural product Gtfs, are especially difficult to synthesize chemically because intermediates can be unstable and the desired β -anomers are thermodynamically disfavored.^{1a,7,8} Here, we report the first stereoselective method for preparing 2-deoxy β -L-glycosyl phosphates from α -glycosyl chlorides. Such phosphates can easily be transformed to the corre-

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(2) For recent reviews, see: (a) Walsh, C. T. *ChemBioChem* **2002**, *3*, 124–134. (b) Mendez, C.; Salas, J. A. *Trends Biotech.* **2001**, *19*, 449–456.

(3) For recent examples of syntheses of analogues using an in vivo approach, see: (a) Hoffmeister, D.; Dräger, G.; Ichinose, K.; Rohr, J.; Bechthold, A. *J. Am. Chem. Soc.* **2003**, *125*, 4678–4679. (b) Remsing, L. L.; Gonzalez, A. M.; Nur-e-Alam, M.; Fernandez-Lozano, M. J.; Brana, A. F.; Rix, U.; Oliveira, M. A.; Mendez, C.; Salas, J. A.; Rohr, J. *J. Am. Chem. Soc.* **2003**, *125*, 5745–5753.

(4) Examples for the chemoenzymatic synthesis of analogues using purified glycosyltransferases: (a) Losey, H. C.; Peczu, M. W.; Chen, Z.; Eggert, U. S.; Dong, S. D.; Pelczer, I.; Kahne, D.; Walsh, C. T. *Biochemistry* **2001**, *40*, 4745–4755. (b) Losey, H. C.; Jiang, J.; Biggins, J. B.; Oberthür, M.; Ye, X.-Y.; Dong, S. D.; Kahne, D.; Thorson, J. S.; Walsh, C. T. *Chem. Biol.* **2002**, *9*, 1305–1314. (c) Fu, X.; Albermann, C.; Jiang, J.; Liao, J.; Zhang, C.; Thorson, J. S. *Nature Biotechnol.* **2003**, *21*, 1467–1469. (d) Albermann, C.; Soriano, A.; Jiang, J.; Vollmer, H.; Biggins, J. B.; Barton, W. A.; Lesniak, J.; Nikolov, D. B.; Thorson, J. S. *Org. Lett.* **2003**, *5*, 933–936.

(5) NDP = nucleotide diphosphoryl; TDP = thymidine diphosphoryl; TMP = thymidine monophosphoryl; UDP = uridine diphosphoryl.

(6) Recently, two TDP β -2-deoxy-L-sugars were synthesized enzymatically: (a) TDP *epi*-vancosamine: Chen, H.; Thomas, M. G.; Hubbard, B. K.; Losey, H. C.; Walsh, C. T.; Burkart, M. D. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 11942–11947. (b) TDP L-olivose: Amann, S.; Dräger, G.; Rupprath, C.; Kirschning, A.; Elling, L. *Carbohydr. Res.* **2001**, *335*, 23–32.

(7) (a) Niggemann, J.; Lindhorst, T. K.; Walford, M.; Laupichler, L.; Sajus, H.; Thiem, J. *Carbohydr. Res.* **1993**, *246*, 173–183. (b) Uchiyama, T.; Hinds, O. J. *Carbohydr. Chem.* **1998**, *17*, 1181–1190. (c) Müller, T.; Schmidt, R. R.; *Tetrahedron Lett.* **1997**, *38*, 5473–5476. (d) Komatsu, H.; Awano, H. *J. Org. Chem.* **2002**, *67*, 5419–5421.

(8) This problem is also encountered in the construction of β -2-deoxy O-glycosides. For approaches to overcome this problem, see for example: Marzabadi, C. H.; Franck, R. W. *Tetrahedron* **2000**, *56*, 8385–8417.

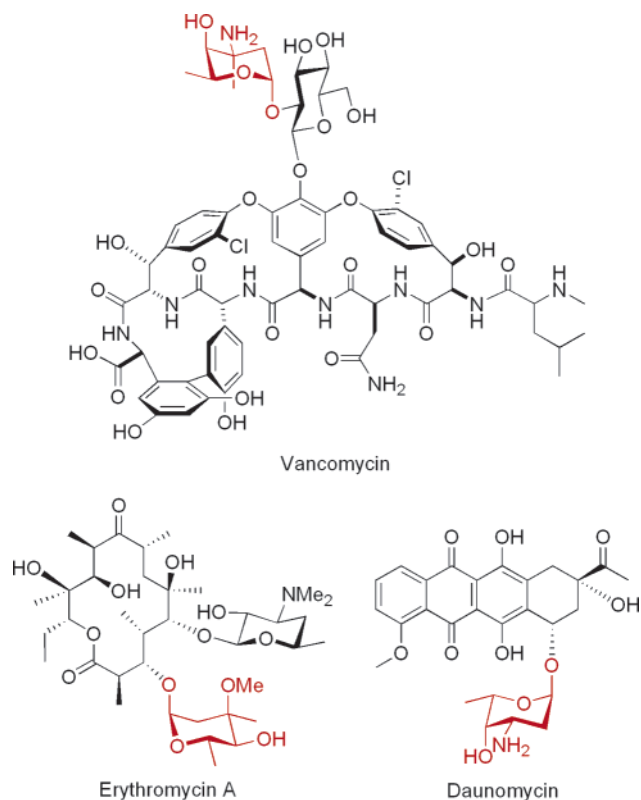
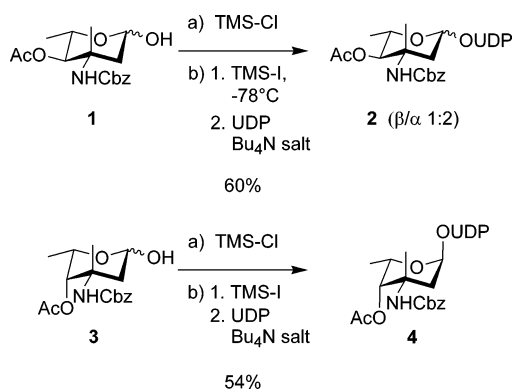


Figure 1. Deoxy sugar-containing natural products.

sponding TDP 2-deoxy sugars,⁵ which are substrates for Gtfs in the biosynthesis of various glycosylated natural products.

As part of our ongoing investigations into the substrate selectivity of various glycosyltransferases, we desired an efficient method for the preparation of β -2-deoxy sugar nucleotides. We were able to obtain one substrate, UDP *L*-*epi*-vancosamine,⁵ as an α/β -mixture^{4a} using an approach similar to that reported by Hindsgaul.^{7b} This procedure involved coupling of the protected *epi*-vancosamine lactol **1**, via the anomeric iodide, with the tetrabutylammonium salt of UDP (Scheme 1). Although this method generated the

Scheme 1. Synthesis of Protected UDP *epi*-Vancosamine **2** and UDP Vancosamine **4**

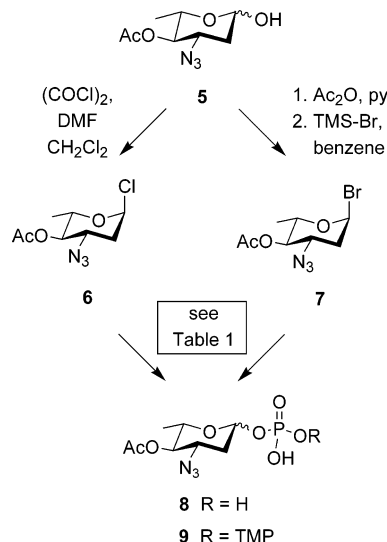


UDP *epi*-vancosamine derivative **2** in only a 1:2 β/α -ratio, we obtained after deprotection sufficient material for the initial characterization of Gtfs involved in the biosynthesis of vancomycin and chloroeremomycin.^{4a}

Unfortunately, this method proved to be unreliable, giving significantly lower β/α -ratios in closely related systems. For example, when vancosamine lactol **3**, which differs from **1** only in the stereochemistry at C-4, was subjected to these reactions, only the α -isomer **4** was obtained (Scheme 1). Therefore, we began to explore alternative methods to make β -2-deoxy sugar nucleotides.

We speculated that the low β -selectivities obtained using the in situ-generated α -glycosyl iodides resulted from a substitution reaction that proceeded predominantly via an $\text{S}_{\text{N}}1$ pathway.⁹ More stable anomeric leaving groups, while still being reactive enough to be replaced by suitable nucleophiles, might therefore shift the substitution toward an $\text{S}_{\text{N}}2$ pathway, leading to higher β -selectivities starting from an α -configured donor. To address this possibility, we prepared the *L*-acosamine (4-*epi*-daunosamine) derivative **5** (Scheme 2),

Scheme 2. Model System for β -Phosphate Formation



which is easily obtained from 3,4-di-*O*-acetyl-*L*-rhamnal.¹⁰ *L*-Acosamine is a model system for a 2,6-dideoxy sugar found in many glycosylated natural products. Efforts to generate the anomeric phosphate from the corresponding anomeric trichloroacetimidate or phosphite failed due to the instability of these glycosyl donors. However, α -chloride **6**, which was obtained from lactol **5** (oxalyl chloride, DMF),¹¹ and α -bromide **7**, generated via the anomeric acetate using TMS-Br,¹² proved to be stable, enabling us to investigate a variety of reaction conditions for the generation of β -phosphates.

(9) Coupling is conducted at pH > 7; therefore, no acid-catalyzed product equilibration should occur.

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Table 1. Effect of Solvent and Nucleophile on Glycosyl Phosphate Formation

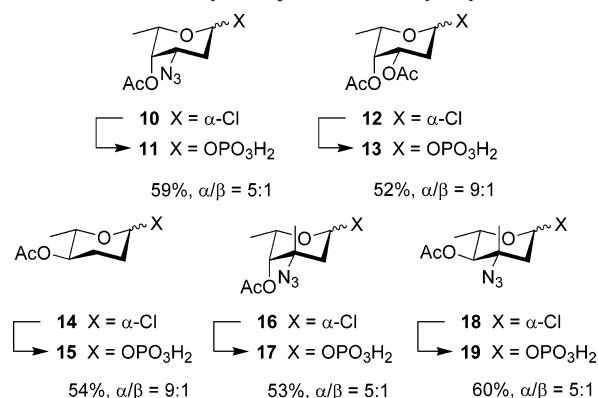
entry	donor	product	solvent	nucleophile ^a	β/α^b (yield)
1	6 ^c	8	CH ₂ Cl ₂	TBAP	2:1 (56%)
2	7 ^d	8	CH ₂ Cl ₂	TBAP	1:2 (51%)
3	6 ^c	8	toluene	TBAP	2:1 (48%)
4	6 ^c	8	CH ₃ NO ₂	TBAP	2:1 (54%)
5	6 ^c	8	CH ₃ CN	TBAP	5:1 (58%)
6	7 ^d	8	CH ₃ CN	TBAP	4:1 (52%)
7	6 ^c	9	CH ₃ CN	TDP Bu ₄ N-salt	2:1 (54%)

^a TBAP = Bu₄NH₂PO₄. ^b β/α -ratios were determined by ¹H NMR. ^c DIPEA (pH > 7), 3 Å molecular sieves, 0 °C → rt, 3 h. ^d DIPEA (pH > 7), 3 Å molecular sieves, -30 °C → rt, 3 h.

Table 1 shows the stereochemical outcome of glycosylation of tetrabutylammonium dihydrogen phosphate (Bu₄NH₂-PO₄, TBAP) with chloride **6** and bromide **7**. A correlation between increased β -selectivity (2:1 vs 1:2) in the formation of phosphate **8** and increasing stability of the donor (Cl > Br) was observed in dichloromethane (entries 1 and 2). Variation of the solvent polarity did not have a significant effect on the stereochemical outcome, as evidenced by the fact that nitromethane (entry 4) gave comparable results to dichloromethane and toluene (entry 3). However, when acetonitrile, which is similar in polarity to nitromethane, was used as a solvent, the β/α -ratios improved to 5:1 (chloride **6**) and 4:1 (bromide **7**) (entries 5 and 6). It has previously been observed that nitriles favor the β -anomer in many glycosylation reactions, perhaps because nitrile-containing solvents participate in the reaction, forming an α -nitrilium ion so that attack from the β -face is favored.¹³ On the basis of these model studies, we selected anomeric chlorides as our donors of choice for further work both because they gave better β -selectivities and were easier to generate and handle than the corresponding anomeric bromides.

Having established favorable donor and solvent conditions, we next examined the use of different phosphate nucleophiles.¹⁴ Obviously, the most straightforward approach to sugar nucleotides would be to couple the anomeric chloride directly to TDP. However, when chloride **6** was coupled with the tetrabutylammonium salt of TDP, the corresponding protected TDP sugar **9** could be isolated, but a decrease in β -selectivity from 5:1 to 2:1 was observed (Table 1, entry 7). Therefore, we decided that better stereochemical control could be achieved by coupling the glycosyl phosphate to an activated nucleotide monophosphate rather than attempting to install the NDP group in a single step. Under optimized conditions, coupling of chloride **6** with Bu₄NH₂PO₄ in acetonitrile gave phosphate **8** (β/α = 5:1) in 58% yield.¹⁵

To explore the utility of the conditions established above, we prepared the α -chloro derivatives of the five β -2-deoxy

Scheme 3. 2-Deoxy Phosphates from Glycosyl Chlorides^a

^a Reaction conditions: Bu₄NH₂PO₄, CH₃CN, 0 °C to rt.

sugars shown in Scheme 3 (see Supporting Information). Compounds **10** and **12** are precursors of sugar moieties found in the anthracycline group of antibiotics, e.g., daunomycin and aclacinomycin A, while **14** is part of tetrocarcin A. Chlorides **16** and **18** are precursors of *epi*-vancosamine and vancosamine, respectively, which are constituents of the glycopeptide antibiotics chloroeremomycin and vancomycin, respectively. Each of these 2-deoxy glycosyl chlorides were subjected to phosphorylation, and the β -phosphate was the predominant product in all cases, with β/α -ratios ranging from 5:1 to 9:1.¹⁶ Hence, the method reliably produces the desired β -anomer for a range of 2-deoxy sugars with high selectivity.

Phosphates **8** (L-acosamine), **11** (L-daunosamine), **13** (2-deoxy L-fucose), **17** (L-vancosamine), and **19** (L-*epi*-vancosamine) were prepared on a larger scale and converted to the corresponding TDP derivatives by coupling with TMP morpholidate⁵ using the conditions developed by Wong (Scheme 4).¹⁷ The undesired α -isomers could usually be removed at this stage by careful separation using preparative reversed-phase HPLC. Deprotection and, for azido-containing

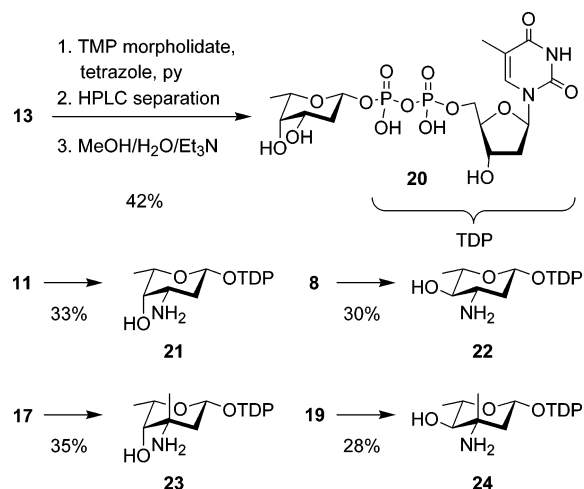
(15) **Synthesis of L-Acosaminy Phosphate 8.** To a solution of lactol **5** (216 mg, 1.0 mmol) in CH₂Cl₂ (10 mL) was added freshly activated molecular sieves (3 Å, 1.0 g), and the mixture was stirred at room temperature for 30 min under argon. Then, a catalytic amount of DMF and oxalyl chloride (2 M in CH₂Cl₂, 0.6 mL, 1.2 mmol) was added, and stirring was continued for 1 h. The mixture was filtered, evaporated without heating, and coevaporated with toluene (three times). The crude glycosyl chloride was dissolved in CH₃CN (10 mL) under argon, and the pH was adjusted to 9 by the dropwise addition of DIPEA. After the addition of freshly activated molecular sieves (3 Å, 1.0 g), the mixture was cooled (ice bath), and Bu₄NH₂PO₄ (0.4 M in CH₃CN, 5.0 mL, 2 mmol) was added. The mixture was stirred for 3 h while warming to room temperature and then filtered and evaporated. The residue was purified by reversed-phase HPLC (5 min with H₂O/0.1% NH₄HCO₃ and then a linear gradient to 100% MeOH/0.1% NH₄-HCO₃ over 50 min; flow rate = 45 mL/min). Evaporation of the product-containing fractions (*R*_f = 0.30, CH₂Cl₂/MeOH/H₂O/Et₃N 8:4:0.2:0.3) gave the ammonium salt of **8** as a white powder upon lyophilization (180 mg, 58%, β/α 5:1, based on ¹H NMR). ¹H NMR (500 MHz, CD₃OD): δ 5.55 (m, 1 H, 1-H_α), 5.21 (ddd, *J*_{1,P} = 8.0, *J*_{1,2a} = 2.2, *J*_{1,2b} = 9.6 Hz, 1 H, 1-H_β). For complete NMR data, see Supporting Information.

(16) When a daunosaminy chloride carrying a 3-NHCbz group instead of the 3-N₃ function was used in the coupling reaction, a decrease in β -selectivity was observed. Therefore, azido derivatives were used for all nitrogen-containing 2-deoxy sugars.

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(13) Schmidt, R. R.; Behrendt, M.; Toepfer, A. *Synlett* **1990**, 694–696.
(14) When protected phosphates, e.g., dibenzyl phosphate, were used in the coupling reaction, the products proved to be very labile and difficult to purify. Consistent with this observation, phosphoramidite coupling of lactol **6** ((1) *i*Pr₂NP(OBn)₂, tetrazole; (2) *m*CPBA) did not generate the dibenzyl phosphate.

Scheme 4. Synthesis of TDP 2-Deoxy Sugars **20–24**^a



^a Reaction conditions for **21–24**: (1) TMP morpholidate, tetrazole, pyridine. (2) HPLC separation. (3) H₂/Pd–C, MeOH. (4) MeOH/H₂O/Et₃N or NaOMe/MeOH.

compounds, reduction thereafter produced the 2-deoxy glycosyl TDP donors **20–24** (see Supporting Information and Scheme 4).

In summary, we have developed a practical chemical method for obtaining the β -anomers of TDP 2-deoxy sugar donors stereoselectively. This method reliably provides access to substrates essential for studying a variety of glycosyltransferases, including Gtfs involved in anthracycline¹⁸ and glycopeptide biosynthesis.¹⁹ Access to epimers and other derivatives of these glycosyl donor substrates will permit further characterization of the specificity of these Gtfs. More importantly, perhaps, our work will allow chemoenzymatic methods to be more thoroughly explored for generating novel sugar-containing natural products.

Acknowledgment. This work was supported by National Institutes of Health Grants 66174 and 69721 (to D.K.)

Supporting Information Available: Experimental procedures and spectral characterization of phosphates **11**, **13**, **15**, **17**, and **19** and TDP sugars **20–24**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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