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Chemical-enzymatic synthesis of azasugar phosphonic acids as glycosyl phosphate surrogates

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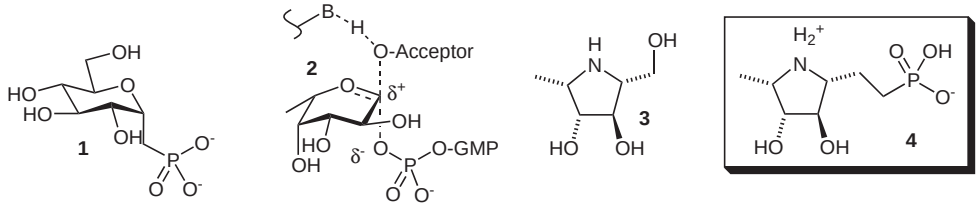
Abstract—Starting from achiral materials two stereoisomeric phosphonylated dihydroxypyrrolidines containing four stereogenic centers were synthesized enantioselectively employing a combination of enzymatic and transition-metal-mediated methods. Both compounds contain features of the transition state of the enzyme-catalyzed fucosyl transfer reaction and represent building blocks of potential inhibitors against this class of enzymes.

Glycosyl phosphates are central intermediates of the primary metabolism and serve as glycosyl donors in the biosynthesis of complex carbohydrate structures. Moreover, they are involved in the regulation of biochemical pathways. Accordingly, much effort has been devoted to the development of glycosyl phosphate mimics[1] as effectors of cellular processes. Substitution of the phosphoester oxygen with a carbon atom leads to structurally very similar, yet hydrolytically stable phosphono analogues such as **1** (Scheme 1). Most glycosyltransferases require glycosyl phosphates or derivatives thereof as glycosyl donors. Like glycoside hydrolysis,[2] glycosyl transfer has been postulated to proceed through transition states exhibiting significant oxocarbenium character (e.g. **2**).[3] Whereas polyhydroxylated pyrrolidines like **3** are potent transition state analogues of glycoside hydrolysis, they are very poor inhibitors of glycosyltransferases.[4] This may be due to the lack of structural elements representing the phosphoester leaving group.

The objective of this study was to develop a variable enantioselective approach towards azasugar phosphonic acids of type **4** combining the functional properties of glycosyl phosphonates like **1** and azasugars like **3**. These compounds represent a minimal structural motif of glycosyltransferase transition state analogues and will serve as stable building blocks for inhibitors against this pharmaceutically relevant[5] class of enzymes.

A retrosynthetic outline is depicted in Scheme 2. The pyrrolidine ring can be formed by diastereoselective reductive amination of a suitable phosphonoazidoketone already containing three stereogenic centers. The latter should be accessible both enantio- and diastereoselectively from an enantiomerically pure α-azidoaldehyde and 3-keto-4-hydroxy butanoic acid **5** using an enzyme-mediated aldol reaction.[6] Of the four dihydroxyacetone phosphate-depending aldolases with complementing stereoselectivity at least two—fructose-1,6-diphosphate aldolase and rhamnulose-1-phosphate



Scheme 1. Methylphosphonate analogue of glucose-1-phosphate (**1**), fucosyltransferase transition state (**2**, GMP = guanosin monophosphate), fucosidase transition state analogue (**3**), azasugar phosphonic acid (**4**).

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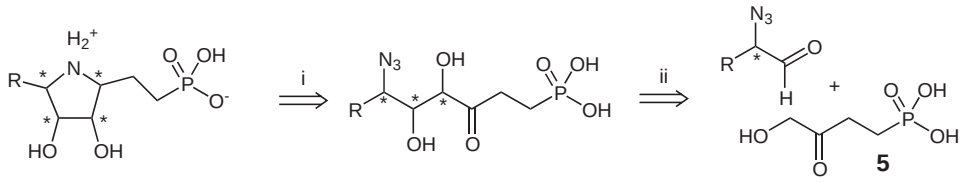

Scheme 2. Retrosynthesis of five-membered azasugar phosphonic acids: (i) reduction; (ii) aldolase reaction.

aldolase[7]—accept **5** instead of the natural substrate DHAP. Thus, a variety of diastereomeric cyclic products should be accessible from a chosen aldehyde substrate.

Two diastereomers of an azasugar phosphonic acid (**4** and its 5-epimer **13**) containing characteristic features of the fucosyltransferase transition state were chosen as a starting point to demonstrate the viability of our approach. As shown in Scheme 3, both enantiomers of α-azidopropionic aldehyde **10** serving as aldol acceptors were obtained from cinnamoic aldehyde, which was first transformed into racemic alcohol **7**. *R*-**7** was selectively acetylated using Amano lipase AK to give *R*-**8** with >95% ee.[8] After separation by silica gel chromatography the remaining *S*-**7** (>95% ee) was acetylated using acetic anhydride in pyridine. Subsequently, Pd(0)-catalyzed allylic substitution was applied to transform both *S*-**8** and *R*-**8** into the corresponding azides **9** with complete retention of the absolute configuration.[9] Notably, no regioisomer is formed during this reaction. α-Azidoaldehydes **10** were generated from *S*-**9** and *R*-**9**, respectively, by ozonolysis at −78°C in $MeOH/CH_2Cl_2$ followed by reduction with dimethyl sulfide in the presence of water.[10] DHAP analogue **5** was prepared according to Page et al.[11] Although this compound has been demonstrated to be a viable substrate analogue of dihydroxyacetonephosphate,[7] we are aware of only one preparative application.[12]

Commercially available rabbit muscle fructose bisphosphate aldolase (RAMA) catalyzed the aldol reaction of each *S*- and *R*-**10** with 0.7 equiv. of **5**. *S*-**10**, exhibiting the same absolute configuration as the natural substrate D-glycerinaldehyde, was converted at a significantly higher rate than *R*-**10**. Whereas **11** was formed in diastereomerically pure form, **12** contained up to 20% of isomer **11** depending on the reaction time, indicating, that under neutral conditions *R*-**10** undergoes slow isomerization to *S*-**10**, which is converted much faster. After ion exchange chromatography **11** and **12** were obtained in 85 and 67% yield, respectively.[13] The absolute configurations of the two new stereogenic centers of **11** and **12** have not been determined experimentally, but were derived from the stereoselectivity of RAMA.[6] The final hydrogenation step was performed in aqueous solution under atmospheric pressure using 10% Pd on coal. Compounds **4** and **13** were obtained as white solids after gel permeation chromatography on Bio-Gel P2 in 56 and 45% yield, respectively.[14] In both cases the reductive amination proceeded diastereoselectively. The hydrogen atoms are delivered from the face of the hydroxyl group adjacent to the carbonyl carbon of the cyclic imine intermediate as has been observed in the reduction of comparable azidoketones lacking the phosphonyl substituent.[15] This assignment of the absolute configuration is supported by the presence of weak NOE signals involving the hydrogen atom introduced at the imine carbon atom of both **4** and **13** (Scheme 4).

In summary, a flexible enantioselective synthesis of dihydroxylated pyrrolidines bearing an ethylphosphonyl substituent, has been developed. It is based on an aldolase-catalyzed enantio- and diastereoselective C,C-bond formation utilizing an unnatural aldol donor substrate. The coupling of products **4** and **13** to guanosinmonophosphate (GMP) in order to obtain fully functional analogues of the fucosyltransferase transition state **2** is currently in progress.

Acknowledgements

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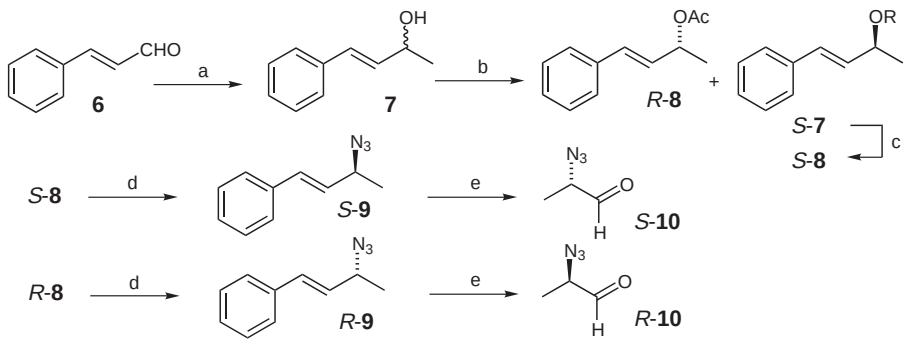
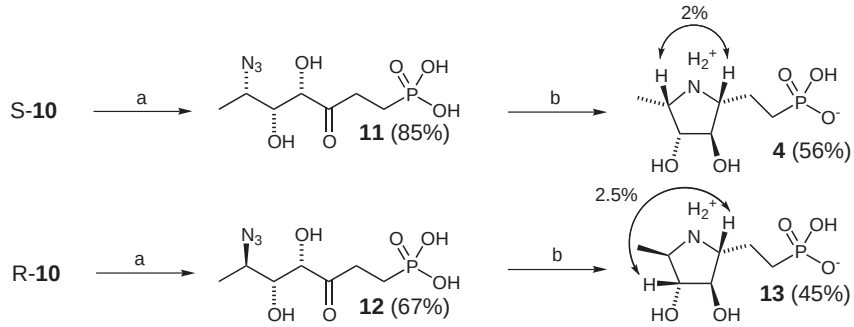


Scheme 3. Enantioselective synthesis of α-azido aldehydes *S*-10 and *R*-10. (a) MeLi, THF (90%); (b) Amano lipase AK, vinyl acetate, hexane; (c) Ac_2O, pyr (95%); (d) NaN_3, 0.01 equiv. $Pd(dppb)_2$, THF–H_2O (2.5:1), 50°C (85%); (e) i. O_3, CH_2Cl_2–MeOH (1:1), −78°C, ii. Me_2S, H_2O (90%).



Scheme 4. Aldolase-catalyzed synthesis of azasugar phosphonates **4** and **13** (NOE indicated by arrows). (a) RAMA, 3 equiv. **5**, H_2O, pH 7.0; (b) H_2, 10% Pd-C, H_2O, pH 1.

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10. After the TLC indicated complete reduction of the ozonide organic solvents were removed under vacuum and the resulting aqueous aldehyde solution was extracted twice with hexane to remove benzaldehyde, which is formed as by-product. The aldehyde is configurationally stable for several days at 4°C in aqueous solution as determined by time-dependent optical rotation measurement.
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13. Reactions were performed at pH 7 on 1 mmol scales using 400 U/mmol and 1000 U/mmol RAMA for 2 hours and 36 hours for the synthesis of **11** and **12**, respectively. Ion-exchange chromatography was performed on Dowex 1-2 (Fluka) using diluted HCl for elution.
14. Selected spectroscopic data: **11**: ^{1}H NMR (500 MHz, D_2O): $\delta=4.45$ (d, 1H, $J=2.0$ Hz), 4.02 (dd, 1H, $J=8.0$, 2.0 Hz), 3.66 (dq, 1H, $J=8.0$, 6.5 Hz), 2.90–2.66 (m, 2H), 1.68 (dt, 2H, $J=17.5$, 8.0 Hz), 1.26 (d, 3H, $J=6.5$ Hz); ^{31}P NMR (202.5 MHz, D_2O): $\delta=25.2$; ^{13}C NMR (125.8 MHz, D_2O): $\delta=213.9$ (Cq, d, $J=14.3$ Hz), 76.9 (CH), 74.7 (CH), 59.9 (CH), 33.8 (CH_2, br), 22.0 (d, CH_2, $J=131.4$ Hz), 15.2 (CH_3); **12**: ^{1}H NMR (500 MHz, D_2O): $\delta=4.50$ (sbr, 1H), 3.92 (dbr, 1H, $J=8.5$ Hz), 3.66 (dq, 1H, $J=6.5$, 7.0 Hz), 2.80–2.70 (m, 2H), 1.83 (dt, 2H, $J=17.0$, 8.0 Hz), 1.19 (d, 3H, $J=7.0$ Hz); ^{31}P NMR (202.5 MHz, D_2O): $\delta=23.0$; ^{13}C NMR (125.8 MHz, D_2O): $\delta=212.3$ (d, Cq, $J=14.6$ Hz), 76.8 (CH), 73.7 (CH), 57.6 (CH), 33.3 (CH_2, br), 21.9 (d, CH_2, $J=134$ Hz), 15.2 (CH_3); **4**: ^{1}H NMR (500 MHz, D_2O): $\delta=4.05$ (sbr, 2H), 3.78 (dq, 1H, $J=7.0$, 3.0 Hz), 3.40 (tbr, $J=7.0$ Hz), 2.08–1.99 (m, 2H), 1.66–1.50 (m, 2H), 1.31 (d, 3H, $J=7.0$ Hz); ^{31}P NMR (202.5 MHz, D_2O): $\delta=17.70$; ^{13}C NMR (125.8 MHz, D_2O): $\delta=78.8$ (CH), 76.3 (CH), 66.8 (d, CH, $J=16.2$ Hz), 58.0 (CH), 25.6 (d, CH_2, $J=10.0$ Hz), 25.2 (d, CH_2, $J=131$ Hz), 10.1 (CH_3); FAB: m/z ($M+H^+$); calcd 226, obsd 226; **13**: ^{1}H NMR (500 MHz, D_2O): $\delta=3.89$ (dd, 1H, $J=7.0$, 7.0 Hz), 3.79 (dd, $J=7.0$, 7.0 Hz), 3.46 (dq, 1H, $J=7.0$, 7.0 Hz), 3.40 (dt, 1H, $J=7.0$, 7.0 Hz), 2.10–1.80 (m, 2H), 1.70–1.62 (m, 2H), 1.37 (d, 3H, $J=7.0$ Hz); ^{31}P NMR (202.5 MHz, D_2O): $\delta=17.56$; ^{13}C NMR (125.8 MHz, D_2O): $\delta=79.3$ (CH), 78.0 (CH), 61.9 (d, CH, $J=13.2$ Hz), 56.5 (CH), 24.8 (d, CH_2, $J=4.0$ Hz), 24.6 (d, CH_2, $J=131$ Hz), 14.6 (CH_3); FAB: m/z ($M+H^+$); calcd 226, obsd 226.
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