Pergamon

Tetrahedron: *Asymmetry* 10 (1999) 3139–3145

TETRAHEDRON: *ASYMMETRY*

Facile approach towards phosphorylated azasugars as potential glycosyl phosphate mimics

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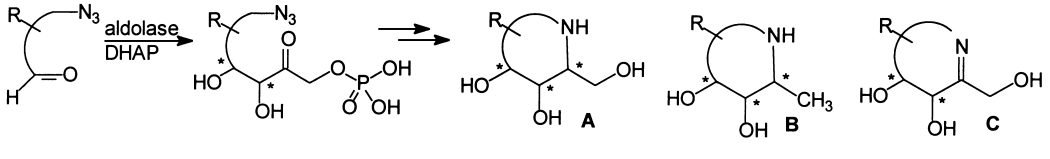
Received 8 June 1999; accepted 6 August 1999

Abstract

A sequence of two chemoselective reductions enables the stereoselective synthesis of phosphorylated azasugars starting from products of dihydroxyacetonephosphate-dependent aldolases.

1. Introduction

As apparent transition state analogs of glycoside-processing enzymes both polyhydroxylated piperidines and pyrrolidines represent important bioactive compounds.[1] One of the most versatile approaches towards such azasugars combines enantioselective aldolase-catalyzed C–C bond formations with diastereoselective reductive amination processes.[2] Aldolase products have been reductively transformed into three different types of cyclic products (A–C in Scheme 1) depending on the chemistry employed. Enzyme-catalyzed removal of the phosphate group followed by catalytic hydrogenation gives rise to cyclic amines A containing a hydroxymethyl group, whereas methyl-substituted cyclic amines B are obtained upon direct hydrogenation of aldol products due to accompanying reductive dephosphorylation. Recently,[3] it has been demonstrated that cyclic imines **C** are accessible when the reduction of the dephosphorylated aldolase products is performed at low pH (Fig. 1).



Scheme 1. Azasugars resulting from reductive treatment of products of DHAP-depending aldolases

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PII: S0957-4166(99)00322-5

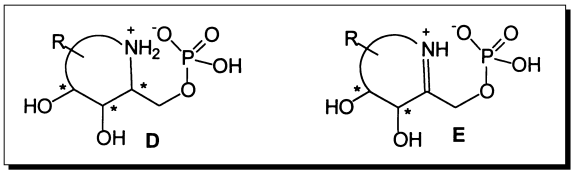


Figure 1.

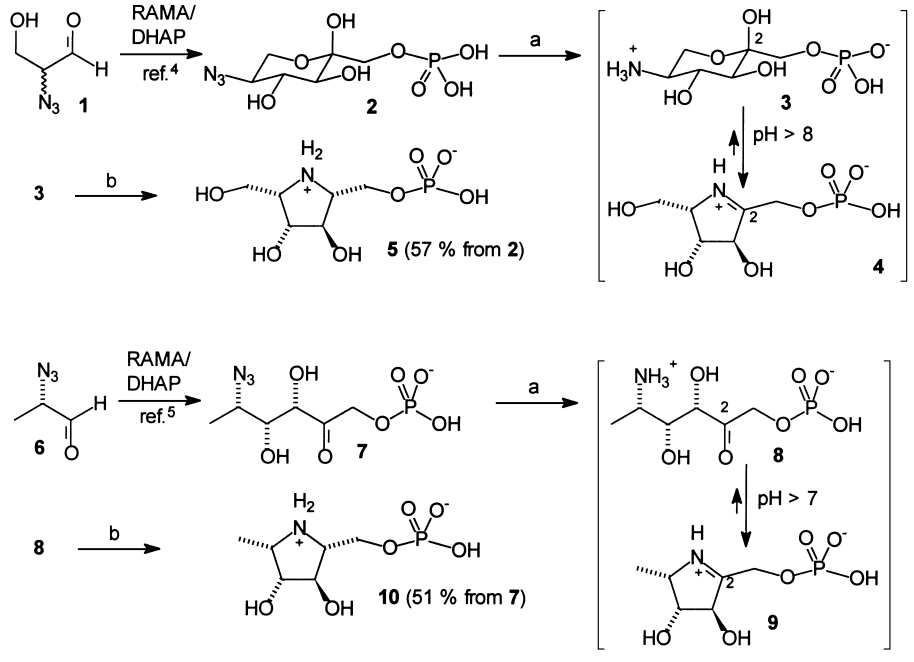
Herein, we present a facile approach for the conversion of aldolase products into phosphorylated cyclic amines and imines of type D and E, respectively. Both five- and six-membered cycles are accessible. The products can be regarded as nitrogen-containing non-isosteric analogs of glycosyl phosphates, which are key intermediates in the biosynthesis of complex carbohydrates and glycoconjugates.

2. Results and discussion

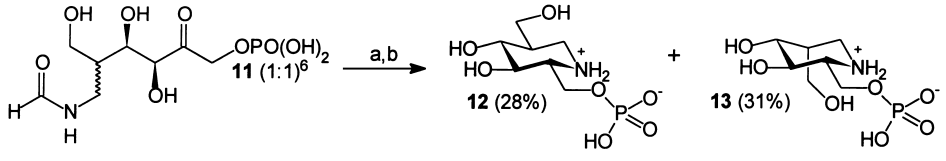
Scheme 2 illustrates the synthesis of phosphorylazasugar **5**. Upon reaction of 2.5 equivalents of azidoaldehyde **1** with DHAP in the presence of rabbit muscle aldolase (RAMA) under thermodynamic control **2** is obtained as the exclusive product.[4] When **2** is hydrogenated on Pd–C in 0.5 M HCl, ammonium hemiketal **3** can be trapped in high yield. When the pH of the aqueous solution of **3** is raised, an equilibrium between **3** and cyclic phosphorylimine **4** is established. At pH >8, **4** is the predominating isomer as judged from the chemical shift of C-2 (178.6 ppm for **4** vs. 97.6 ppm for **3**). In contrast to hemiketal **3** the imine is unstable as indicated by ^{1}H NMR. Whereas further catalytic hydrogenation of **3** results in the formation of a cyclic amine of type B due to reductive dephosphorylation, we found that the phosphoryl function is retained when $NaBH_3CN$ is employed as the reducing agent, thus, yielding phosphorylazasugar **5**. The reductive amination proceeds with high diastereoselectivity. The identity of **5** was unambigiously established by enzymatic dephosphorylation. ^{1}H and ^{13}C NMR spectra of the resulting type A imino sugar are indistinguishable from those reported by Wong and co-workers.[4] Notably, both reduction using $NaBH_3CN$ and catalytic hydrogenation on Pd–C led to products with the 2*R* configuration.

The synthesis of **10** followed the same procedures. RAMA-catalyzed reaction of enantiomerically pure aldehyde **6** with DHAP resulted in the formation of **7**,[5] which was catalytically hydrogenated under acidic conditions. In contrast to **3**, the lacking hydroxyl group in **8** prevents stabilization of the carbonyl function via intramolecular hemiketal formation. Nevertheless, **8** was succesfully trapped in more than 80% yield, thus, demonstrating that selective azide reduction is not limited to aldol products of type **2**. When the pH is raised to 7.0, cyclic imine **9** is the predominating isomer as judged from the chemical shift of C-2 (177.8 ppm for **9** vs. 208.3 ppm for **8**). Reductive amination using $NaBH_3CN$ at pH 6–7 diastereoselectively yields phosphorylazasugar **10**. The absolute configuration of **10** was again confirmed by enzymatic dephosphorylation to the corresponding type A imino sugar.[5]

The synthesis of two six-membered phosphorylazasugars starting from an *N*-formyl-containing aldol product is depicted in Scheme 3. The RAMA-catalyzed synthesis of **11** yielded a mixture of two diastereomers.[6] The *N*-formyl group was efficiently removed by treatment with 1 M HCl at 35°C for 24 h. Subsequent reductive amination ($NaBH_3CN$, pH 6–7) yielded a mixture of phosphorylazasugars **12** and **13**, which were separated by semipreparative HPLC. The stereochemistry of both isomers was assigned based on their vicinal coupling constants assuming chair conformation.



Scheme 2. Chemical-enzymatic synthesis of five-membered phosphorylazasugars **5** and **10**. Reagents and conditions: (a) H_2/Pd–C, 0.5 M HCl, 15 min; (b) 2 equiv. $NaBH_3CN$, pH 6–7, 4°C to 25°C, 12 h



Scheme 3. Synthesis of six-membered phosphorylazasugars **12** and **13**. Reagents and conditions: (a) 0.55 M HCl, 12 h, 35°C; (b) 2 equiv. $NaBH_3CN$, pH 6–7, 4°C to 25°C, 12 h

3. Conclusion

In summary, a procedure for the conversion of phosphoryl-containing aldolase products into phosphorylazasugars has been developed. Its application to the synthesis of various compounds of the pyrrolidine and piperidine type, respectively, has been demonstrated. In contrast to comparable aldolase applications the dihydroxyacetonephosphate moiety is almost completely retained in the final products. Notably, a wide range of stereoisomers should be accessible by choosing aldolases with the appropriate stereoselectivities.[2] Until recently, only very few reports on phosphorylated azasugars have been given to the best of our knowledge,[8–10] although these compounds may be regarded as nitrogen-containing analogs of glycosyl phosphates, which are ubiquitous intermediates in the biosynthesis of complex carbohydrates or partial structures thereof. We are currently exploring the potential of phosphorylazasugars as inhibitors of glycosyl transferring enzymes.

4. Experimental

All chemicals and solvents used were of the highest available purity. The following stationary phases were used for chromatography. TLC: aluminum foils Merck 60 F 254 (elution with isopropanol:25%

ammonium hydroxide:water 6:3:2), flash chromatography: 0.040–0.063 mm Merck silica. Semipreparative HPLC on a LiChrospher®-diol phase (8×250 mm) under elution with mixtures of MeOH and MTBE containing 0.1% of trifluoroacetic acid in conjunction with a light scattering detector. The following spectrometers were used to record physical data. NMR: Bruker DRX 500 (^{1}H: 500.1 MHz, ^{13}C: 125.8 MHz, ^{31}P: 202.5 MHz) at 25°C against TMS as external standard (^{1}H, ^{13}C NMR). Data of proton-decoupled ^{31}P spectra were referenced against 85% phosphoric acid, which served as internal standard. FAB-MS: Finnigan MAT 95 SQ using a glycerol matrix.

Notably, **5**, **10**, **12** and **13** retain small amounts (<10%) of inorganic material even after extensive purification.

4.1. Aldolase reactions

Aldolase-catalyzed syntheses of **2**,[4] **7**[5] and **11**[6] were performed according to published procedures using rabbit muscle aldolase (Fluka, 16–25 U/mg) and DHAP obtained by acid hydrolysis of dihydroxyacetone phosphate dimer bis(ethylketal) (Fluka).[7]

2: 54% Yield; ^{13}C NMR (D_2O): δ 96.96 (d, *J*=7.8 Hz, Cq), 71.76 (CH), 70.19 (CH), 65.78 (d, *J*=4.8 Hz, CH_2), 60.52 (CH), 59.57 (CH_2).

7: 52% Yield; ^{13}C NMR (D_2O): δ 210.6 (d, *J*=6.8 Hz, C=O), 75.49 (CH), 74.87 (CH), 68.51 (d, *J*=4.5 Hz, CH_2), 59.41 (CH), 15.17 (CH_3).

11: 1:1 mixture of diastereomers (54% combined yield); ^{13}C NMR (D_2O): δ 164.46, 164.38, 97.01 (d, *J*=3.5 Hz Cq), 71.52 (CH), 70.17 (CH), 69.22 (CH), 67.52 (CH), 63.61 (d, *J*=6.9 Hz, CH_2), 61.05 (CH_2), 59.31 (CH_2), 42.46 (CH), 41.05 (CH), 35.82 (CH_2), 33.26 (CH_2).

*4.2. Phosphoric acid mono-(5*S*-amino-2*R*,3*S*,4*R*-trihydroxy-tetrahydro-pyran-2-ylmethyl) ester **3***

A solution of aldolase product **2** (142 mg, 0.5 mmol) in 5 mL of 0.5 M HCl was hydrogenated under vigorous stirring in the presence of 50 mg of 10% Pd on coal for 15 min. At this time all starting material was consumed as indicated by TLC. The catalyst was removed by centrifugation. Although the resulting product solution is stable for at least three days at room temperature, rapid decomposition was observed during concentration and chromatography. Therefore, 0.6 mL of the acidic solution was mixed with 0.05 mL of acetone-d_6 and directly subjected to spectroscopic analysis. The use of 0.01 mL acetonitrile as an internal standard revealed a conversion of >80%. ^{1}H NMR (H_2O/acetone-d_6): δ 3.9 (dd, 1H, *J*=10.5, 6.0 Hz, H-1), 3.79 (d, 2H, *J*=9.5 Hz, H-$6_{ax,eq}$), 3.77 (dd, 1H, *J*=9.5, 8.5 Hz, H-4), 3.73 (dd, 1H, *J*=10.5, 5.5 Hz, H-1), 3.46 (d, *J*=9.5 Hz, H-3), 3.19 (dt, *J*=8.5, 9.5 Hz, H-5); ^{13}C NMR (H_2O/acetone-d_6): δ 97.58 (d, $J_{C\text{-}2,P}$=9.5 Hz, C-2), 70.89 (C-3), 70.06 (C-4), 66.68 (d, $J_{C\text{-}1,P}$=5.0 Hz, C-1), 58.80 (C-6), 51.73 (C-5); ^{31}P (H_2O/acetone-d_6): δ -0.34.

*4.3. Phosphoric acid mono-(3*R*,4*R*-dihydroxy-5*S*-hydroxymethyl-4,5-dihydro-3H-pyrrol-2-ylmethyl) ester **4***

To an NMR tube containing a solution of **3** in a mixture of water and acetone-d_6 (see above) was carefully added 3 M NaOH until the pH had reached 8.5. NMR analysis revealed the formation of **4** as the predominating species. Decomposition takes place in the range of several hours as indicated by comparison of appropriate signals with the methyl group of the internal standard acetonitrile. ^{1}H NMR (H_2O/acetone-d_6): δ 4.62 (d, 1H, *J* =6.0 Hz, H-3), 4.20 (dd, 1H, *J*=6.0, 5.5 Hz, H-4), 4.01–3.96 (m, 1H,

H-5), 3.70–3.60 (m, 2H, H-6); ^{13}C NMR (H_2O/acetone-d_6): δ 178.61 (d, $J_{C-2,P}$ =7.0 Hz, C-2), 80.86 (C-3), 77.12 (C-4), 71.26 (C-5), 61.65 (d, $J_{C-1,P}$ =3.5 Hz, C-1); ^{31}P (H_2O/acetone-d_6): δ −4.55.

4.4. Phosphoric acid mono-(3R,4R-dihydroxy-5S-hydroxymethyl-pyrrolidin-2R-ylmethyl) ester ***5***

The solution of **3** (2.5 mL) was cooled to 4°C and the pH was adjusted to 6–7 by addition of 3 M NaOH. $NaBH_3CN$ (31 mg, 0.5 mmol) was added and the resulting solution was stirred at 4°C for 3 h maintaining a pH of 6–7. It was then allowed to stand at room temperature overnight. Excess $NaBH_3CN$ was destroyed by acidification to pH 2–3 using 6 M HCl. After neutralization with 3 M NaOH 1 g of coarse silica gel (0.063–0.2 mm, Merck) was added and the solvent was evaporated at 45°C in vacuo. The adsorbed material was subjected to silica gel chromatography under elution with isopropanol:25% NH_4OH:H_2O (10:3:2–3:3:2) giving 34 mg (0.14 mmol) of **5** (57% yield from **2**). $[\alpha]_D^{25}$ +9.9 (c=0.2, H_2O); ^{1}H NMR (D_2O): δ 4.24 (dd, 1H, *J*=4.0, 2.5 Hz, H-4), 4.15 (dd, 1H, *J*=4.0, 3.0Hz, H-3), 4.09–3.99 (m, 2H, H-1), 3.94 (dd, 1H, *J*=12.0, 5.0 Hz, H-6), 3.87 (dd, 1H, *J*=12.0, 6.0 Hz, H-6), 3.79–3.74 (m, 1H, H-5), 3.62–3.57 (m, 1H, H-2); ^{13}C NMR (D_2O): δ 76.2 (C-3), 75.4 (C-4), 65.79 (d, $J_{C-2,P}$=6.1 Hz, C-2), 62.60 (C-5), 61.79 (d, $J_{C-1,P}$=3.6 Hz, C-1), 57.95 (C-6); ^{31}P (D_2O): δ −4.92; IR (KBr) ν 3194 (vs, broad), 2517 (m), 1634 (m), 1458 (m), 1402 (s), 1179 (s) 1117 (vs, broad), 778 (m) cm^{-1}; HRMS ($C_6H_{15}NPO_7$, MH^+): calcd 244.0586, obsd 244.0586.

4.5. Phosphoric acid mono-(5S-amino-3S,4R-dihydroxy-2-oxo-hexyl) ester ***8***

A solution of aldolase product **7** (135 mg, 0.5 mmol) in 5 mL of 0.5 M HCl was hydrogenated under vigorous stirring in the presence of 50 mg of 10% Pd on coal for 15 min. At this time all starting material was consumed as indicated by TLC. The catalyst was removed by centrifugation. Since the product was found to be similarily unstable as **3** (see above), 0.6 mL of the acidic solution was mixed with 0.05 mL of acetone-d_6 and directly subjected to spectroscopic analysis. The use of 0.01 mL acetonitrile as internal standard revealed a conversion of >80%. ^{13}C NMR (H_2O/acetone-d_6): δ 208.05 (d, $J_{C-2,P}$=7.5 Hz, C-2), 75.96 (CH-O), 71.61 (CH-O), 69.15 (d, $J_{C-1,P}$=4.0 Hz, C-1), 50.02 (C-5), 14.94 (C-6); ^{31}P (H_2O/acetone-d_6): δ −0.467.

4.6. Phosphoric acid mono-(3R,4R-dihydroxy-5S-methyl-4,5-dihydro-3H-pyrrol-2-ylmethyl) ester ***9***

To an NMR tube containing a solution of **8** in a mixture of water and acetone-d_6 (see above) was carefully added 3 M NaOH until the pH had reached 7.0. NMR analysis revealed the formation of **9** as the predominating species. ^{13}C NMR (H_2O/acetone-d_6, 288 K): δ 177.8 (d, $J_{C-2,P}$=7.0 Hz, C-2), 80.871 (CH-O), 77.56 (CH-O), 66.54 (C-5), 62.18 (d, $J_{C-1,P}$=4.0 Hz, C-1), 13.64 (C-6); ^{31}P (H_2O/acetone-d_6): δ −1.25.

4.7. Phosphoric acid mono-(3R,4R-dihydroxy-5S-methyl-pyrrolidin-2R-ylmethyl) ester ***10***

The solution of **8** (2.5 mL) was treated with 31 mg (0.5 mmol) $NaBH_3CN$ exactly following the procedure used for the synthesis of **5**. The reaction product was adsorbed onto coarse silica as described above. Silica gel chromatography using isopropanol:25% NH_4OH:H_2O (12:3:2–6:3:2) afforded 29 mg (0.13 mmol) of **10** (51% yield from **2**). $[\alpha]_D^{25}$ +29.2 (c=0.5, H_2O); ^{1}H NMR (D_2O): δ 4.20–4.10 (m, 3H, H-3, H-4, H-1), 4.03 (dtbr, 1H, 12.0, 8.0 Hz, H-1), 3.90 (dq, 1H, *J*=4.0, 6.0 Hz, H-5), 3.70 (dt, 1H, 9.0, 4.0 Hz, H-2), 1.42 (d, 3H, *J*=6.0 Hz, H-6); ^{13}C NMR (D_2O): δ 76.08 (C-3), 75.57 (C-4), 65.94 (d,

$J_{C\text{-}2,P}$=6.0 Hz, C-2), 61.54 (d, $J_{C\text{-}1,P}$=4.5 Hz, C-1), 57.69 (C-5), 10.33 (C-6); ^{31}P (D_2O): δ −3.89; IR (KBr) ν 3190 (vs, broad), 2505 (m), 1633 (m), 1452 (m), 1401 (s), 1176 (s) 1105 (vs, broad), 801 (m) cm^{-1}; HRMS ($C_6H_{15}NPO_6$, MH^+): calcd 228.0637, obsd 228.0631.

*4.8. Phosphoric acid mono-(3R,4R-dihydroxy-5R-hydroxymethyl-piperidin-2R-ylmethyl) ester **12** and phosphoric acid mono-(3R,4R-dihydroxy-5S-hydroxymethyl-piperidin-2R-ylmethyl) ester **13***

A 1:1 diastereomeric mixture of **11** (144 mg, 0.5 mmol) was dissolved in 5 mL of 0.55 M HCl and maintained at 35°C overnight. TLC indicated complete removal of the *N*-formyl group. The solution was then cooled to 4°C and the pH was adjusted to 6–7. Under stirring 62 mg (1 mmol) of $NaBH_3CN$ was added. Keeping the pH constant the solution was stirred at 4°C for 3 h and at room temperature overnight. The product mixture was treated as described in the synthesis of **5**. Chromatography on silica using isopropanol:25% NH_4OH:H_2O (12:3:2–6:3:2) as the eluent afforded 76 mg of a mixture of **11** and **12** (59% combined yield) as judged from the ^{1}H NMR spectrum. 10 mg of each diastereomer was isolated by semipreparative HPLC and spectroscopically characterized; **12**: $[\alpha]_D^{25}$ +33.7 (c=0.15, H_2O); ^{1}H NMR (D_2O): δ 4.28–4.20 (m, 2H, H-1), 3.85 (dd, 1H, *J*=12.0, 3.5 Hz, H-7), 3.77 (dd, 1H, *J*=12.0, 6.0 Hz, H-7), 3.70 (t, 1H, *J*=9.5 Hz, H-3), 3.59 (t, 1H, *J*=9.5 Hz, H-4), 3.55 (dd, 1H, *J*=12.5, 4.5 Hz, H-6_{eq}), 3.38–3.31 (m, 1H, H-2), 3.07 (t, 1H, *J*=12.5 Hz, H-6_{ax}), 2.09–1.98 (m, 1H, H-5); ^{13}C NMR (D_2O): δ 71.00 (C-4), 68.91 (C-3), 61.29 (d, $J_{C\text{-}1,P}$=4.0 Hz, C-1), 59.03 (C-7), 58.83 (d, $J_{C\text{-}2,P}$=8.0 Hz, C-2), 44.39 (C-6), 40.85 (C-5); ^{31}P (D_2O): δ −0.84; IR (KBr) ν 3176 (vs, broad), 2529 (m), 1650 (m), 1452 (m), 1400 (s), 1175 (s) 1118 (vs, broad), 931 (m) cm^{-1}; HRMS ($C_7H_{17}NPO_7$, MH^+): calcd 258.0742, obsd 258.0746; **13**: $[\alpha]_D^{25}$ −4.5 (c=0.15, H_2O); ^{1}H NMR (D_2O): δ 4.30 (dt, 1H, *J*=12.0, 6.0 Hz, H-1), 4.19–4.15 (ddd, 1H, *J*=12.0, 5.5, 3.0 Hz, H-1), 4.04 (t, 1H, *J*=8.0 Hz, H-3), 3.99 (dd, 1H, *J*=8.0, 5.0 Hz, H-4), 3.94 (dd, 1H, *J*=11.0, 5.0 Hz, H-7), 3.86 (dd, 1H, *J*=11.0, 4.5 Hz, H-7), 3.59 (dd, 1H, *J*=13.0, 4.5 Hz, H-6), 3.46–3.41 (m, 1H, H-2), 3.31 (dd, 1H, *J*=13.0, 4.0 Hz, H-6), 2.41 (qbr, 1H, *J*=4,5 Hz, H-5); ^{13}C NMR (D_2O): δ 70.03 (C-4), 66.15 (C-3), 60.83 (d, $J_{C\text{-}1,P}$=4.5 Hz, C-1), 59.00 (d, $J_{C\text{-}2,P}$=8.5 Hz, C-2), 58.79 (C-7), 42.74 (C-6), 37.02 (C-5); ^{31}P (D_2O): δ −0.92; IR (KBr) ν 3174 (vs, broad), 2520 (m), 1645 (m), 1455 (m), 1402 (s), 1176 (s) 1107 (vs, broad), 925 (m) cm^{-1}; HRMS ($C_7H_{17}NPO_7$, MH^+): calcd 258.0742, obsd 258.0748.

4.9. Enzymatic dephosphorylation reactions

A sample of **5** or **10** (20 mg) was dissolved in 0.5 mL of 0.1 M sodium acetate buffer (pH 4.8). After adding 50 units of acid phosphatase (Sigma) the solution was stirred for 3 days at 37°C. The solvent was evaporated in vacuo and the residue was purified by flash chromatography using mixtures of CH_2Cl_2, methanol and aqueous NH_4OH to give the following products.

Dephosphorylation product of **5**:[4] ^{1}H NMR (D_2O): δ 3.93 (dd, 1H, *J*=5.0, 3.0 Hz), 3.68 (dd, 1H, *J*=5.5, 3.0 Hz), 3.60 (dd, 1H, 11.5, 6.0 Hz), 3.55 (dd, 1H, *J*=12.0, 5.0 Hz), 3.50–3.43 (m, 2H), 3.13 (dt, 1H, *J*=5.0, 5.5 Hz), 2.82 (dt, 1H, *J*=5.5, 5.0 Hz); ^{13}C NMR (D_2O): δ 76.42, 74.93, 67.17, 63.47, 59.65, 57.28.

Dephosphorylation product of **10**:[5] ^{1}H NMR (CD_3OD): δ 3.85 (dd, 1H, *J*=3.5, 1.5 Hz), 3.70–3.60 (m, 3H); 3.20 (dq, 1H, *J*=4.0, 6.5 Hz), 2.93 (dt, 1H, *J*=3.5, 4.0 Hz); 1.15 (d, 1H, 6.5 Hz); ^{13}C NMR (CD_3OD): δ 81.00, 80.40, 70.45, 63.27, 59.65, 13.53.

Acknowledgements

Financial support from Fonds der Chemischen Industrie is gratefully acknowledged.

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