Deoxysugars via Microbial Reduction of 5-Acyl-isoxazolines: Application to the Synthesis of 3-Deoxy-D-fructose and Derivatives

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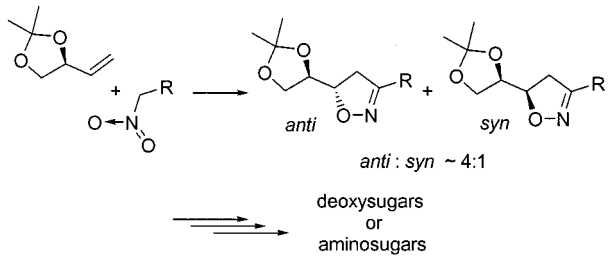
5-Acylisoxazolines **3a**−**d** were obtained by 1,3-dipolar cycloaddition from acetoxymethyl vinyl ketone and nitro precursors. Compounds **3a**−**d** were biotransformed by *Aspergillus niger* into a 1:1 mixture of stereomers of 5-dihydroxyethyl isoxazolines (+)-**4a**−**d** (anti) and (−)-**5a**−**d** (syn). Both stereomers were obtained in good yields and with high optical purities. Carbonyl reduction by *Aspergillus niger* produces alcohols of *R*-configuration thus giving an access to D-sugar analogues: Compound (+)-**4d** was converted to 3-deoxy-D-*erythro*-hexulose and several protected derivatives. Total synthesis of 3-deoxy-D-fructose-6-phosphate was also achieved in two steps and 64% overall yield from (+)-**4d**.

Intoduction

2-Isoxazolines (4,5-dihydroisoxazoles) are easily obtained by 1,3-dipolar cycloaddition to alkenes of nitrile-oxides or silyl-nitronates generated in situ from primary nitro-compounds or chloro-aldoximes. These heterocycles have proven versatile intermediates for the synthesis of different classes of functionalized molecules.[1] Of special interest is complete reduction of isoxazolines to γ-amino alcohols as well as conversion to aldols through reductive N−O bond cleavage followed by hydrolysis of the intermediate imine. These transformations have been used in the construction of carbohydrates, especially aminosugars[2] and deoxysugars.[3] Thus, the use of (*S*)-2-vinyl-1,3-dioxolane as dipolarophile allowed the synthesis of 2-deoxy-D-ribose,[3a] D-lividosamine,[2c] or D-allosamine[2f] via stereoselective hydroxylation at position 4 of the heterocycle. In every case, diastereoselection was observed during the cycloaddition, leading to an approximately 4:1 mixture of anti and syn isomers (Scheme 1).

This anti-directing effect appears as a general feature of nitrile-oxide cycloadditions[4] and makes α-chiral olefins

Scheme 1



valuable substrates to achieve stereoselective synthesis. Alternatively, bioconversion processes have been reported for the preparation of chiral isoxazolines including enzyme kinetic resolutions of ester-substituted isoxazolines[5] or microbial reductions of isoxazolines bearing a carbonyl group.[6] Indeed, bakers' yeast reduction of 5-acetyl-isoxazolines has been shown to lead to both enantiomerically pure diastereomers in excellent yields (Scheme 2).[6a]

This nondiastereoselective approach allows for potential access to all four stereomers through an appropriate choice of the biocatalyst and reaction conditions, giving a unique configuration for the newly formed alcohol.

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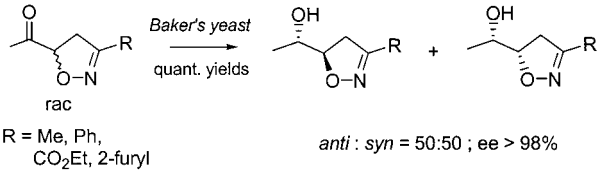
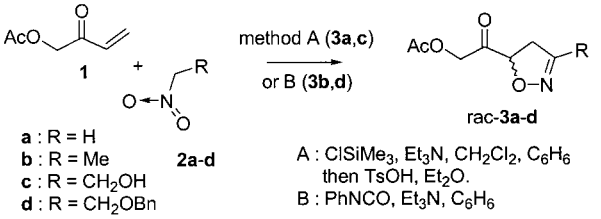
Scheme 2



Scheme 3



In this paper, we describe our first results concerning the microbial reduction of 5-acyl-isoxazolines applied to deoxysugar synthesis. Several chiral molecules were obtained in good yields and with high optical purities using the fungus *Aspergillus niger*. This microorganism produces alcohols of *R* configuration, precursors of D-sugar analogues. This strategy was applied to the synthesis of 3-deoxy-D-fructose (3-deoxy-D-*erythro*-hexulose) and several protected derivatives.

Results and Discussion

Isoxazolines **3a**−**d** were obtained by cycloaddition of 1,3-dipoles on acetoxymethyl vinyl ketone **1** easily prepared from butyne-1,3-diol[7] (Scheme 3).

Mukaiyama's procedure[8] (method B) was employed to generate nitrile-oxides from nitroethane and compound **2d** obtained from nitroethanol and benzyltrichloroacetimidate.[9] Isoxazolines **3b** and **3d** were obtained in 57% and 75% yields, respectively. However, this procedure was not suitable with nitromethane[10] or nitroethanol used as dipole precursor. Silyl-nitronates[3c,7] were generated instead (method A) and 2-silyloxy-isoxazolidines obtained upon cycloaddition were converted into isoxazolines **3a** and **3c** by acid treatment. Although **3a** was thus isolated in 61% yield, this direct synthesis of **3c** from nitroethanol still suffers from a low yield of 38% but in agreement with previously reported results.[11]

Compounds **3b** and **3c** (1 g·L^{-1}) were first reduced by freeze-dried bakers' yeast (20 g·L^{-1}) under nonfermentative conditions. In both cases, reduction was complete within 48 h and accompanied by acetate hydrolysis leading to a 1:1 mixture of diols **4** (anti) and **5** (syn). After chromatographic separation, stereochemical analysis showed these molecules to be nearly racemic. Our findings greatly differ from the highly specific reduction of 5-acetyl-isoxazolines by bakers' yeast[6a] described earlier. However, the added acetoxy group is likely to alter the reduction specificity. Furthermore, acetate hydrolysis observed during the course of the reaction results in the presence of two different substrates for enzymatic reduction. Indeed, reduction of the free ketol derived from **3b** (data not shown) gave quite different results: a 3:1 mixture of (−)-**4b** and (+)-**5b** was obtained with 63% and 91% ee, respectively. These results clearly indicate that reduction of isoxazolines **3** by bakers' yeast is a complex process likely involving more than one reductase.

Table 1. Reduction of *rac*-3a−d by *Aspergillus niger*

Substrate	Yield[a] (%) 4+5	ee products (%)[b] (+)-4a-d	(−)-5a-d
rac-3			
3a : R = H	89	>98	97
3b : R = Me	98	>98	>98
3c : R = CH_2OH	67	97	92
3d : R = CH_2OBn	88	96	87

[a] **4** and **5** were always isolated in approximately equal quantities. [b] Determined by HPLC on a chiralcel OJ (**4a**−**d**, **5b**) or by comparison to optical rotations of authentic samples (**5a**, **5c**, and **5d**).

We next turned to other microorganisms. Being primarily interested in an access to the D-series of sugar analogues, we focused our attention on biocatalysts giving an alcohol of *R* configuration. After screening of a few strains, the best results were obtained with the fungus *Aspergillus niger* (ATCC 9142) and are summarized in Table 1. The reductions of **3a**−**d** (1.2−1.6 g·L^{-1}) were performed at 27 °C in water with fresh resting cells (100 g·L^{-1}). Carbonyl reduction and acetate hydrolysis occurred with equivalent rates and were complete within 24 h leading to a 1:1 mixture of diols **4** and **5**.

Absolute configurations were determined by reference to authentic samples of (+)-**4a**−**d** and (−)-**5a**−**d** obtained by cycloaddition from (*S*)-2-vinyl-1,3-dioxolane (see Scheme 1). Here again, our results are different from those concerning the reduction of 5-acetyl-isoxazolines by *Aspergillus niger* which produced the syn isomer with low enantioselectivity.[6b] Reduction of isoxazolines **3** leads to both diastereomers in good yields and high ee, thus offering an access to D-sugar analogues.

As shown in Scheme 4, 3-deoxy-D-fructose **6** and derivatives **7**−**11** have been obtained from diol (+)-**4d** formed with 96% ee after reduction of rac-**3d** by *Aspergillus niger*. Catalytic hydrogenation of (+)-**4d** over Pd on charcoal (10%) was achieved under hydrolytic conditions to give **6** in 80% yield. Though an efficient synthesis of this molecule from D-fructose has already been described in three steps and 15% overall yield,[12] the isoxazoline route constitutes a competitive alternative and allows for access to unusual protection patterns as shown by the synthesis of **8** and **9**. Hydrogenation over Raney Ni in the presence of water and acetic acid thus allowed the conversion of isoxazolines **4d** and **7** to ketols in 88% and 76% yields, respectively, while preserving the benzyl and acetonide protective groups. Finally, isoxazoline (+)-**4d** was used as precursor of 3-deoxy-D-fructose-6-phosphate (**11**), a molecule of special interest for the study or inhibition of enzymatic reactions involving the biologically important D-fructose-6-phosphate. Phosphorylation of (+)-**4d** was accomplished using dibenzylphosphoroiodidate generated from tribenzyl phosphite and iodine.[13]

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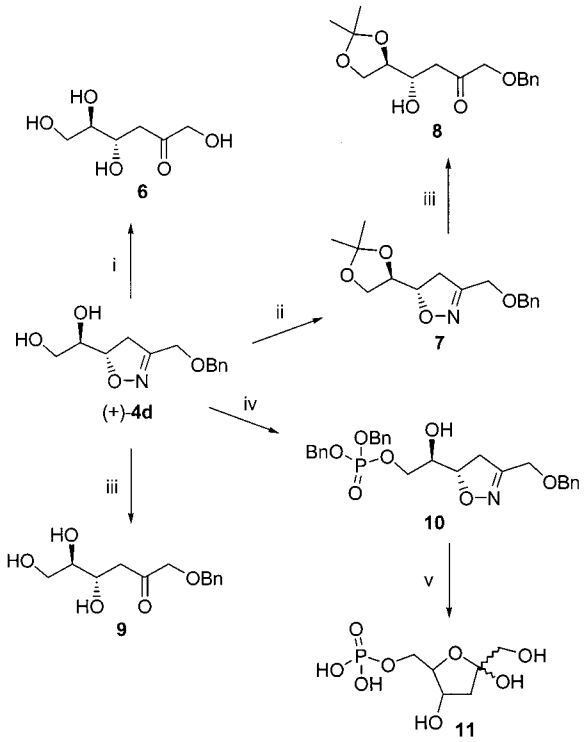
Scheme 4



i : H_2, Pd/C , H_2O, AcOH, MeOH ; ii : $(CH_3)_2C(OMe)_2$, C_6H_6, H^+ ; iii : H_2, Ni, MeOH, H_2O, AcOH ; iv : $P(OBn)_3$, I_2, pyr., CH_2Cl_2 ; v : H_2, Pd/C, H_2O, MeOH.

With 1.5 mol equiv of phosphorylating agent, **10** was isolated in 80% yield. The reaction showed high regioselectivity, as no monophosphorylated regioisomer and only traces of diphosphorylated isoxazoline were detected in the crude material. However, upon purification by column chromatography, partial migration of the dibenzyl phosphate moiety was observed (up to 5%), leading to a mixture of inseparable isomers. Ring opening and complete deprotection was then achieved in one single step by hydrogenation over Pd catalyst. Under these conditions, hydrolysis of the intermediate imine was catalyzed by the rapidly deprotected dihydrogenophosphate group and required no external acid. Although this multistep reaction appeared quite effective, we could not avoid the formation of small quantities of amino derivatives (both diastereomers) via competitive reduction of the intermediate imine formed upon N−O bond cleavage. These impurities were removed by selective adsorption on a cation exchange column (Dowex 50 W, H^+), and **11** was thus isolated in 80% yield, contaminated by less than 5% of the 5-phosphate derivative. NMR analyses (0.5 mol·L^{-1}, D_2O, pH 7.6) showed **11** to be a mixture of furanoses (35% and 36%) and open (29%) forms.

Conclusion

The biotransformation of acylisoxazolines **3a**−**d** by *Aspergillus niger* gives both diastereomers (+)-**4a**−**d** and (−)-**5a**−**d** in good yields and high enantiomeric excess.

As exemplified in the case of (+)-**4d**, these molecules are useful intermediates for the construction of several D-sugar analogues. The strategy described is applicable to the synthesis of aminosugar derivatives through appropriate remodeling of isoxazolines **4** and **5.** Finally, microbial reductions producing an alcohol of *S* configuration would allow for access to the L series of carbohydrates. Work is in progress.

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Experimental Section[14]

2-Oxobut-3-enyl acetate (1) was prepared in a yield of ca. 65% from butyne-1,4-diol according to the modified procedure of Andersen.[6]

1-Benzyloxy-2-nitroethane (2d). To a stirred solution of 2-nitroethanol (6.0 g, 66 mmol) and benzyl-trichloroacetimidate (18.3 g, 72.5 mmol) in cyclohexane−CH_2Cl_2 (2:1, 200 mL) was added triflic acid (300 μL, 3.3 mmol). After 1 h at room temperature, the mixture was filtered, washed with aq saturated $NaHCO_3$ (50 mL) and with water (50 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash chromatography (eluent, cyclohexane−EtOAc, 4:1, v/v) to give **2d** (11.3 g, 94%) as a colorless liquid: IR (neat film) 1556, 1377, 1215, 1117 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.35 (5H, m), 4.58 (2H, s), 4.55 (2H, t, J = 5.0 Hz), 3.95 (2H, t, J = 5.0 Hz)); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.1, 128.6, 128.1, 127.8, 75.2, 73.5, 65.4.

2-(4,5-Dihydroisoxazol-5-yl)-2-oxoethyl Acetate (3a). Method A. To a stirred solution of **1** (1.28 g, 10 mmol), nitromethane, (1.1 mL, 20 mmol) and Et_3N (4.16 mL, 30 mmol) in C_6H_6−CH_2Cl_2 (1:1, 80 mL) was added dropwise chlorotrimethylsilane (3.8 mL, 30 mmol). The mixture was heated to 60 °C for 2 h. The solution was cooled, filtered, and concentrated under reduced pressure. The residue was dissolved in Et_2O (150 mL), and after filtration, *p*-TsOH (0.42 g, 2.2 mmol) was added to the solution. After 1 h stirring, the solution was washed with aq saturated $NaHCO_3$ (25 mL), and the aqueous layer was extracted with EtOAc (2 × 25 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Purification by flash chromatography (eluent, cyclohexane−EtOAc, 1:1) gave **3a** (1.5 g, 61%) as a colorless liquid: IR (neat film) 1750, 1737, 1607, 1234, 1070, 825 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.19 (1H, s), 4.96 (1H, dd, J = 6.5, 8.5 Hz), 4.90 (2H, m), 3.27 (2H, m), 2.11 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.4, 170.2, 146.3, 80.2, 66.4, 38.6, 20.3. Anal. Calcd for $C_7H_9NO_4$: C, 49.12; H, 5.30; N, 8.18. Found: C, 48.90; H, 5.38; N, 8.28.

2-(3-Methyl-4,5-dihydroisoxazol-5-yl)-2-oxoethyl Acetate (3b). Mukaiyama's Procedure: Method B. To a solution of nitroethane (5.4 mL, 75 mmol) and Et_3N (0.35 mL, 2.5 mmol) in dry C_6H_6 (50 mL) was added dropwise a mixture of **1** (6.4 g, 50 mmol), phenyl isocyanate (8.1 mL, 75 mmol), and Et_3N (0.7 mL, 5 mmol) in C_6H_6 (100 mL). After 1 h stirring, addition of phenyl isocyanate (2.7 mL, 25 mmol) and Et_3N (0.35 mL, 2.5 mmol) was repeated. After 24 h, water (20 mL) was added, and the mixture was stirred for an additional 2 h. The precipitated material was removed by filtration and washed with cyclohexane (50 mL). The aqueous layer was extracted

(14) **General**: IR spectra were determined on a Perkin-Elmer 881 spectrometer, and the resonances are expressed in frequency units (ν cm^{-1}). NMR spectra were recorded at 400 MHz for ^1H and 100 MHz for ^{13}C on a Bruker AC 400 spectrometer. $CHCl_3$ (δ = 7.27), $CDCl_3$ (δ = 77.1) was used as the respective internal standard expressed in ppm. Optical rotations were determined on a JASCO polarimeter. HPLC experiments for enantiomeric excess determination were performed with a chiralcel OJ column and monitored at 220 nm [eluents, hexane−^{i}PrOH, 9:1 (**4d**, **7**), hexane−EtOH, 97:3 (**4b**), 98:2 (**5b**), 94:6 (**4c**), hexane−MeOH, 97:3 (**4a**)]. Column chromatographies were performed on Merck Kieselgel 60 (0.040−0.063 mm) and commercial Kieselgel 60 F254 plates were used for thin-layer chromatography. *Aspergillus niger* ATCC 9142 was laboratory-grown in the following medium: yeast extract (5 g), soyoptim (Roquette, 5 g), glucose (20 g), NaCl (5 g), KH_2PO_4 (5 g), and H_2O (1 L), preculture 24 h and culture 24 h. (*S*)-But-3-ene-1,2-diol was purchased from Interchim, France. All solvents were distilled before use following usual procedures. Satisfactory analytical data (± 0.3%) were obtained for all new compounds at the Service Central d'Analyse du CNRS, Solaize, France.

with CH_2Cl_2 (2 × 25 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Purification by flash chromatography (eluent, cyclohexane−EtOAc, 1:1) gave **3b** (5.3 g, 57%) as a pale yellow oil: IR (neat film) 1750, 1737, 1633, 1234, 1071, 849 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.95 (1H, dd, J = 7.2, 10.8 Hz), 4.90 (2H, m), 3.18 (2H, m), 2.10 (3H, s), 1.96 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.0, 170.1, 155.9, 82.0, 66.5, 41.5, 20.3, 12.6. Anal. Calcd for $C_8H_{11}NO_4$: C, 51.89; H, 5.99; N, 7.56. Found: C, 51.78; H, 6.13; N, 7.51.

2-(3-Hydroxymethyl-4,5-dihydroisoxazol-5-yl)-2-oxoethyl Acetate (3c). 3c was prepared according to method A from **1** (1.88 g, 14.7 mmol), nitroethanol (1.6 mL, 22.3 mmol), Et_3N (6.1 mL, 43.7 mmol), and Me_3SiCl (5.6 mL, 44.1 mmol). Purification by flash chromatography (eluent CH_2Cl_2−MeOH, 97:3) gave **3c** (1.13 g, 38%) as a pale yellow oil: IR (neat film) 3347, 1747, 1737, 1633, 1235, 1065, 855 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.08 (1H, dd, J = 6.0, 11.5 Hz), 4.89 (2H, m), 4.33 (2H, m), 3.34 (2H, m), 2.75 (1H, s), 2.16 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.7, 170.6, 159.5, 82.2, 66.5, 57.2, 48.5 38.2. Anal. Calcd for $C_8H_{11}NO_5$: C, 47.76; H, 5.51; N, 6.96. Found: C, 47.55; H, 5.59; N, 6.93.

2-(3-Benzyloxymethyl-4,5-dihydroisoxazol-5-yl)-2-oxoethyl Acetate (3d). 3d was prepared according to method B from **1** (5 g, 39 mmol) and **2d** (6.4 g, 35.3 mmol). Purification by flash chromatography (eluent, cyclohexane−Et_2O, 1:1) gave **3d** (7.7 g, 75%) as a pale yellow oil: IR (neat film) 1750, 1738, 1232, 1074, 856 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.34 (5H, m), 5.05 (1H, dd, J = 6.5, 12.0 Hz), 4.96 (2H, m), 4.52 (2H, m), 4.30 (2H, s), 3.34 (2H, m), 2.16 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.3, 170.1, 157.1, 137.0, 128.5, 128.0, 127.9, 82.2, 72.8, 66.4, 63.8, 38.4, 20.3. Anal. Calcd for $C_{15}H_{17}NO_5$: C, 61.84; H, 5.88; N, 4.81. Found: C, 61.51; H, 5.87; N, 4.91.

General Procedure for the Reduction of 3a−d with *Aspergillus niger*. The reactions were performed in 500 mL conical flasks containing each **3a−d** (60−80 mg) dissolved in ethanol (1 mL), *Aspergillus niger* wet cells (5 g), and water (50 mL). After incubation at 27 °C for 24 h on a rotating table set at 200 rpm, the mixture was filtered, and the cells were carefully washed with water (50 mL). The filtrate was concentrated to dryness under reduced pressure. The residue was triturated with MeOH (20 mL), insoluble material was removed by filtration, and the solution was concentrated under reduced pressure. Products were purified by flash chromatography as described below.

(5*S*)-5-[(1*R*)-1,2-Dihydroxyethyl]-4,5-dihydroisoxazole (4a) and (5*R*)-5-[(1*R*)-1,2-Dihydroxyethyl]- 4,5-dihydroisoxazole (5a). From **3a** (240 mg, 1.4 mmol) according to general procedure. Flash chromatography (eluent, CH_2Cl_2−MeOH, 9:1) afforded a 1:1 mixture of **4a** and **5a** (163 mg, 89%) as a colorless liquid. Anal. Calcd for $C_5H_9NO_3$: C, 45.79; H, 6.92; N, 10.68. Found: C, 46.10; H, 7.11; N, 10.47. **4a** and **5a** were further separated by a second column chromatography (eluent Et_2O−MeOH−AcOH, 93:5:2). **4a** was obtained as a white solid (mp 75 °C): IR ($CHCl_3$) 3686, 3596, 3435, 1603, 1276, 1074, 840 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.42 (1H, t, J = 2.0 Hz), 5.10 (2H, s), 4.68 (1H, ddd, J = 5.5, 8.5, 11.0 Hz), 3.77 (3H, m), 3.23 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.5, 80.1, 73.4, 64.5, 37.6; $[\alpha]^{25}_D$ = +93.2 (*c* 1, MeOH); ee > 98%. **5a** was obtained as a white solid (mp 103 °C): IR ($CHCl_3$) 3686, 3565, 3441, 1603, 1280, 1102, 840 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.42 (1H, t, J = 1.5 Hz), 5.09 (2H, s), 4.76 (1H, ddd, J = 3.0, 8.5, 11.5 Hz), 3.80 (3H, m), 3.27 (1H, ddd, J = 1.5, 11.0, 18.0 Hz), 3.18 (1H, ddd, J = 2.0, 8.5, 18.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.5, 79.8, 74.4, 64.4, 38.5; $[\alpha]^{25}_D$ = −150.2 (*c* 1, MeOH); ee = 97%.

(5*S*)-5-[(1*R*)-1,2-Dihydroxyethyl]-3-methyl-4,5-dihydroisoxazole (4b) and (5*R*)-5-[(1*R*)-1,2-Dihydroxyethyl]-3-methyl-4,5-dihydroisoxazole (5b). From **3b** (360 mg, 1.9 mmol) according to general procedure. Flash chromatography (eluent, CH_2Cl_2−MeOH, 9:1) afforded a 1:1 mixture of **4b** and **5b** (276 mg, 98%) as a colorless liquid. Anal. Calcd for $C_6H_{11}NO_3$: C, 49.65; H, 7.64; N, 9.65. Found: C, 49.76; H, 7.76; N, 9.52. **4a** and **5a** were further separated by a second column chromatography (eluent Et_2O−MeOH−AcOH, 93:5:2). **4b** was obtained as a white solid (mp 40 °C): IR ($CHCl_3$) 3585, 3412, 1634, 1047, 868 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 5.10 (2H, s), 4.72 (1H, ddd, J = 5.5, 8.5, 10.5 Hz), 3.82 (1H, ddd, J = 4.5, 6.0, 10.0 Hz), 3.78 (1H, dd, J = 4.0, 11.5 Hz), 3.70 (1H, dd, J = 5.5, 11.5 Hz), 3.20 (2H, m), 2.16 (3H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 158.2, 82.0, 73.6, 64.5, 40.6, 13.2; $[\alpha]^{25}_D$ = +112.4 (*c* 2.9, MeOH); ee > 98%. **5b** was obtained as a colorless oil: IR (neat film) 3369, 1637, 1046, 869 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.60 (1H, ddd, J = 4.0, 8.0, 12.0 Hz), 3.67 (3H, m), 3.35 (2H, s), 3.02 (1H, dd, J = 10.5, 17.0 Hz), 2.92 (1H, dd, J = 8.0, 17.0 Hz), 1.98 (3H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 158.1, 81.7, 74.4, 64.4, 41.4, 13.1; $[\alpha]^{25}_D$ = −153.6 (*c* 1.5, MeOH); ee > 98%.

(5*S*)-5-[(1*R*)-1,2-Dihydroxyethyl]-3-hydroxymethyl-4,5-dihydroisoxazole (4c) and (5*R*)-5-[(1*R*)-1,2-Dihydroxyethyl]-3-hydroxymethyl-4,5-dihydroisoxazole (5c). From **3c** (390 mg, 1.93 mmol) according to general procedure. Flash chromatography (eluent, CH_2Cl_2−MeOH, 8:2) afforded a 1:1 mixture of **4c** and **5c** (208 mg, 67%) as a colorless liquid. Anal. Calcd for $C_6H_{11}NO_4$: C, 44.72; H, 6.88; N, 8.69. Found: C, 44.62; H, 6.97; N, 8.47. **4c** and **5c** were further separated by a second column chromatography (eluent Et_2O−MeOH−AcOH, 88:10:2). **4c** was obtained as a white solid (mp 47 °C): ^1H NMR (400 MHz, CD_3OD) δ 5.08 (3H, s), 4.78 (1H, ddd, J = 6.0, 8.5, 10.5 Hz), 4.49 (2H, s), 3.85 (1H, ddd, J = 4.5, 6.0, 10.5 Hz), 3.80 (1H, dd, J = 4.5, 11.5 Hz), 3.73 (1H, dd, J = 6.0, 11.5 Hz), 3.29 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 161.2, 82.4, 73.5, 64.5, 58.2, 37.2; $[\alpha]^{25}_D$ = +120.1 (*c* 1.2, MeOH); ee = 97%. **5c** was obtained as a colorless oil: IR (neat film) 3350, 1652, 1046, 881 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 5.16 (3H, s), 4.85 (1H, ddd, J = 2.5, 9.0, 11.0 Hz), 4.49 (2H, s), 3.81 (3H, m), 3.33 (1H, dd, J = 11.0, 17.0 Hz), 3.23 (1H, dd, J = 8.5, 17.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 161.2, 82.2, 74.4, 64.3, 58.2, 37.9; $[\alpha]^{25}_D$ = −135.1 (*c* 1.9, MeOH); ee = 92%.

(5*S*)-5-[(1*R*)-1,2-Dihydroxyethyl]-3-benzyloxymethyl-4,5-dihydroisoxazole (4d) and (5*R*)-5-[(1*R*)-1,2-Dihydroxyethyl]-3-benzyloxymethyl-4,5-dihydroisoxazole (5d). From **3d** (600 mg, 2.04 mmol) according to general procedure. Flash chromatography (eluent, CH_2Cl_2−MeOH, 9:1) afforded a 1:1 mixture of **4d** and **5d** (451 mg, 88%) as a colorless liquid. Anal. Calcd for $C_{13}H_{17}NO_4$: C, 62.14; H, 6.82; N, 5.57. Found: C, 62.11; H, 6.86; N, 5.51. **4d** and **5d** were further separated by a second column chromatography (eluent Et_2O−MeOH−AcOH, 97:1:2). **4d** was obtained as a white solid (mp 45 °C): IR (CCl_4) 3602, 1216, 1092, 878 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.33 (5H, m), 4.57 (1H, ddd, J = 5.0, 8.0, 11.0 Hz), 4.53 (2H, s), 4.27 (2H, s), 3.81 (1H, m), 3.70 (1H, m), 3.70 (2H, s), 3.58 (1H, m), 3.13 (1H, dd, J = 8.0, 17.5 Hz), 3.02 (1H, dd, J = 11.0, 17.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.4, 137.2, 128.6, 128.1, 128.0, 80.9, 72.9, 71.9, 64.5, 63.2, 36.4; $[\alpha]^{25}_D$ = +81.1 (*c* 1, MeOH); ee = 96%. **5d** was isolated as a white solid (mp 59 °C): IR ($CHCl_3$) 3562, 3451, 1627, 1097, 877 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.33 (5H, m), 4.65 (1H, ddd, J = 4.0, 8.0, 11.5 Hz), 4.52 (2H, m), 4.27 (2H, s), 3.70 (2H, m), 3.63 (1H, m), 3.22 (1H, d, J = 6.0 Hz), 3.12 (1H, dd, J = 11.0, 17.5 Hz), 3.03 (1H, dd, J = 8.0, 17.5 Hz), 2.93 (1H, t, J = 5.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.3, 137.2, 128.5, 128.0, 127.9, 80.8, 73.0, 72.7, 64.3, 63.6, 37.5; $[\alpha]^{25}_D$ = −104.0 (*c* 1.9, MeOH); ee = 87%.

Synthesis of 4a and 5a from (*S*)-But-3-ene-1,2-diol. A mixture of (*S*)-but-3-ene-1,2-diol (0.25 mL, 3 mmol), dimethoxypropane (0.55 mL, 4.5 mmol), and amberlyst 15 (H^+, 100 mg) in C_6H_6 (20 mL) was heated to 55−60 °C in a distillation apparatus. After 1 h, azeotropic distillation of MeOH was complete, and the reaction mixture was cooled and filtered. To the solution of vinyldioxolane in C_6H_6 (15 mL) thus obtained were added CH_2Cl_2 (15 mL), nitromethane (0.325 mL, 6 mmol), Et_3N (1.25 mL, 9 mmol), Me_3SiCl (1.14 mL, 9 mmol), and the mixture was heated to reflux. After 90 h, addition of nitromethane (0.325 mL, 6 mmol), Et_3N (1.25 mL, 9 mmol), and Me_3SiCl (1.14 mL, 9 mmol) was repeated, and reflux was continued until total disappearance of vinyldioxolane (140 h). The mixture was cooled and filtered, and the solution was concentrated under reduced pressure. The residue was dissolved in MeOH (20 mL), and *p*-TsOH (200 mg, 1.05 mmol)

was added. The solution was stirred 24 h at room temperature, neutralized with Et_3N, and concentrated in vacuo. The residue was purified by flash chromatography as already described to give a 3:1 mixture of **4a** and **5a** (156 mg, 39%). **4a**: $[\alpha]^{25}_D$ = +93.8 (*c* 0.5, MeOH); ee > 98%; **5a**: $[\alpha]^{25}_D$ = −154.5 (*c* 0.4, MeOH).

Synthesis of 4b and 5b from (*S*)-But-3-ene-1,2-diol. 4b and **5b** were prepared according to method B from (S)-2,2-dimethyl-4-vinyl-1,3-dioxolane prepared as previously described (**4a** and **5a**) from (*S*)-but-3-ene-1,2-diol (240 μL, 2.84 mmol). To the solution of olefin in C_6H_6 (25 mL) were added phenyl isocyanate (0.92 mL, 8.5 mmol), Et_3N (66 μL, 0.47 mmol), and nitroethane (300 μL, 4.26 mmol), and the mixture was stirred at room temperature. Additions of phenyl isocyanate (130 μL, 1.18 mmol) and Et_3N (14 μL, 0.1 mmol) were repeated at 2 h and 48 h, and stirring was continued until total disappearance of the alcene (72 h). After addition of water (10 mL), the mixture was further stirred for 2 h and then filtered. The precipitate was washed with cyclohexane (50 mL), and the aqueous layer was extracted with cyclohexane (3 × 25 mL) after saturation with NaCl. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in MeOH (20 mL), and, after addition of amberlyst 15 (H^+, 100 mg), the solution was stirred at room temperature for 24 h and concentrated in vacuo. The residue was purified by flash chromatography as already described to give a 3.7:1 mixture of **4b** and **5b** (211 mg, 51%). **4b**: $[\alpha]^{25}_D$ = +112.2 (*c* 1, MeOH); ee > 98%. **5b**: $[\alpha]^{25}_D$ = −153.0 (*c* 1.5, MeOH); ee > 98%.

Synthesis of 4c and 5c from (S)-But-3-ene-1,2-diol. 4c and **5c** were prepared from (*S*)-but-3-ene-1,2-diol (238 μL, 2.83 mmol) according to method B as described for **4b** and **5b** using tetrahydropyranyl ether of 2-nitroethanol[15] (0.74 g, 4.25 mmol) as the dipole precursor. Addition of PhNCO and Et_3N were repeated at 2, 48, and 64 h and vinyldioxolane was not detected after 4 days at room temperature. Flash chromatography afforded a 2.9:1 mixture of **4c** and **5c** (181 mg, 40%). **4c**: $[\alpha]^{25}_D$ = +129.7 (*c* 3.3, MeOH); ee > 98%. **5c**: $[\alpha]^{25}_D$ = −147.7 (*c* 1.6, MeOH).

Synthesis of 4d and 5d from (*S*)-But-3-ene-1,2-diol. 4d and **5d** were prepared from (*S*)-but-3-ene-1,2-diol (170 μL, 2 mmol) according to method B as described for **4c** and **5c** using **2d** (0.78 g, 4.3 mmol) as the dipole precursor. Vinyldioxolane was not detected after 6 days stirring at room temperature. Purification by flash chromatography as already described afforded a 3.5:1 mixture of **4d** and **5d** (270 mg, 54%). **4d**: $[\alpha]^{25}_D$ = +84.5 (*c* 1, MeOH); ee > 98%; **5d**: $[\alpha]^{25}_D$ = −119.5 (*c* 1.7, MeOH).

(5*S*)-5-[(1*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl]-3-benzyloxy-4,5-dihydroisoxazole (*anti*-7) and (5*R*)-5-[(1*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl]-3-benzyloxy-4,5-dihydroisoxazole (*syn*-7). A 1:1 mixture of **4d** and **5d** (2 g, 8 mmol) was obtained upon reduction of **3d** by *Aspergillus niger*, followed by column chromatography (eluent, CH_2Cl_2−MeOH, 9:1). The mixture of isomers was dissolved in C_6H_6 (50 mL), and 2,2-dimethoxypropane (1.5 mL, 12.2 mmol) and amberlyst 15 (H^+, 500 mg) were added. The solution was heated at 90 °C in a distillation apparatus. After 2 h, azeotropic distillation of MeOH was complete. The reaction mixture was cooled, filtered, and concentrated under reduced pressure. Isomers *anti*- and *syn*-**7** were separated by flash chromatography (eluent, Et_2O−cyclohexane, 1:1). Isoxazoline *anti*-***7*** (1.03 g, 42%) was obtained as a colorless liquid: IR (neat film) 1625, 1212, 1074 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.33 (5H, m), 4.54 (1H, ddd, *J* = 6.5, 7.0, 10.5 Hz), 4.54 (2H, s), 4.30 (2H, s), 4.12 (1H, dd, *J* = 6.5, 8.5 Hz), 4.05 (1H, ddd, *J* = 5.0, 6.5 Hz), 3.88 (1H, dd, *J* = 5.0, 8.5 Hz), 3.16 (1H, dd, *J* = 10.5, 17.5 Hz), 3.07 (1H, dd, *J* = 7.0, 17.5 Hz), 1.43 (3H, s), 1.35 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.7, 137.3, 128.6, 128.1, 128.0, 109.8, 80.9, 76.1, 72.8, 67.2, 64.5, 38.0, 26.8, 25.2. Anal. Calcd for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.26; N, 4.81. Found: C, 65.79; H, 7.22; N, 5.02; $[\alpha]^{25}_D$ = +72.3 (*c* 2.4, $CHCl_3$); ee = 96%. Isoxazoline *syn*-***7*** (1.13 g, 46%) was obtained as a colorless liquid: IR (neat film) 1626, 1213, 1076 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.33 (5H, m), 4.67 (1H, ddd, *J* = 4.5, 7.5, 11.0 Hz), 4.53 (2H, m), 4.31 (2H, s), 4.23 (1H, ddd, *J* = 4.5, 6.5 Hz), 4.06 (1H, dd, *J* = 6.5, 8.5 Hz), 3.85 (1H, dd, *J* = 6.5, 8.5 Hz), 3.11 (1H, dd, *J* = 11.0, 17.5 Hz), 2.97 (1H, dd, *J* = 7.5, 17.5 Hz), 1.44 (3H, s), 1.37 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.4, 137.4, 128.6, 128.1, 128.0, 110.1, 79.9, 76.7, 72.6, 65.4, 64.4, 37.2, 26.3, 25.4. Anal. Calcd for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.26; N, 4.81. Found: C, 65.98; H, 7.29; N, 5.02; $[\alpha]^{25}_D$ = −89.4 (*c* 1.3, $CHCl_3$); ee = 87%.

1-*O*-Benzyl-3-deoxy-5,6-*O*-isopropylidene-D-*threo*-hexulose (8). To a solution of isoxazoline *anti*-**7** (90 mg, 0.31 mmol) in 10:5:1 CH_2Cl_2−MeOH−H_2O (10 mL) were added AcOH (36 μL, 0.62 mmol) and a spatula tip of Raney Ni (estimated to 100 mg). The reaction was placed under hydrogen using a balloon. The mixture was stirred vigorously for 2 h. After addition of $NaHCO_3$ (100 mg), the mixture was stirred for 10 min, filtered through Celite, and concentrated under reduced pressure. The residue was purified by flash chromatography (eluent, CH_2Cl_2−MeOH, 98:2) to give **8** (69 mg, 76%) as a colorless liquid: IR (neat film) 3461, 1724, 1212, 1065 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.33 (5H, m), 4.60 (2H, s), 4.10 (2H, s), 4.09 (1H, m), 4.03 (1H, m), 3.95 (2H, m), 3.02 (1H, d, *J* = 4.0 Hz), 2.85 (1H, dd, *J* = 2.5, 17.5 Hz), 2.66 (1H, dd, *J* = 8.5, 17.5 Hz), 1.40 (3H, s), 1.34 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.2, 136.9, 128.6, 128.1, 128.0, 109.5, 77.6, 75.2, 73.4, 68.7, 66.7, 42.3, 26.6, 25.1. Anal. Calcd for $C_{16}H_{22}O_5$: C, 65.29; H, 7.53. Found: C, 65.00; H, 7.61; $[\alpha]^{25}_D$ = −20.8 (*c* 2.6, $CHCl_3$).

3-Deoxy-D-*threo*-hexulose (6). To a solution of **4d** (100 mg, 0.4 mmol) in 9:1 MeOH−H_2O (10 mL) were added AcOH (68 μL, 1.2 mmol) and 10% Pd/C (100 mg). The reaction was placed under hydrogen using a balloon and stirred vigorously for 20 h. After addition of $NaHCO_3$ (100 mg), the mixture was stirred for 10 min, filtered through Celite, and concentrated under reduced pressure. The residue was purified by flash chromatography (eluent, CH_2Cl_2−MeOH, 8:2) to give **6** (48 mg, 73%) as a semisolid substance which was not recrystallized. NMR spectra showed the presence of five forms in equilibrium: pyranoses (39% and 9%), furanoses (27% and 20%), and open form (5%): ^1H NMR (400 MHz, CD_3OD) δ 4.53 (m), 4.39 (m), 4.31−4.05 (m), 3.93−3.54 (m), 2.93 (dd, *J* = 3.5, 15.5 Hz), 2.80 (dd, *J* = 9.0, 16.0 Hz), 2.60 (dd, *J* = 7.5, 13.5 Hz), 2.37 (dd, *J* = 7.0, 13.5 Hz), 2.31 (dd, *J* = 7.0, 13.5 Hz), 2.18 (dd, *J* = 4.0, 14.5 Hz), 2.10 (dd, *J* = 12.3, 12.4 Hz), 2.05 (dd, *J* = 4.0, 13.5 Hz), 2.05 (dd, *J* = 3.0, 14.5 Hz), 1.70 (dd, *J* = 5.0, 12.5 Hz).^{13}C NMR (100 MHz, CD_3OD) δ 212.2, 107.3, 107.1, 98.7, 97.7, 88.6, 87.6, 76.1, 73.1, 73.0, 69.9, 69.6, 69.4, 69.3, 68.7, 68.1, 67.5, 67.1, 66.9, 65.3, 64.4, 63.2, 60.7, 43.8, 43.5, 43.4, 36.3, 34.5; $[\alpha]^{25}_D$ = −41.0 (*c* 1.0, water) [lit.[9] −44 (*c* 1.0, water)].

1-*O*-Benzyl-3-deoxy-D-*threo*-hexulose (9). A solution of **4d** (70 mg, 0.28 mmol) in 9:1 MeOH−H_2O (10 mL) was treated as described for the synthesis of **8**. Purification by flash chromatography (eluent, CH_2Cl_2−MeOH, 9:1) afforded **9** (62 mg, 88%) as a white solid (mp 88 °C): NMR spectra showed the presence of five forms in equilibrium:^1H NMR (400 MHz, $CDCl_3$) δ 7.32 (5H, m), 4.58 (2H, m), 4.35 (m), 4.24 (m), 4.02 (m), 3.88−3.50 (m), 3.35 (m), 2.66 (m), 2.32 (dd, *J* = 8.0, 14.0 Hz), 2.25 (dd, *J* = 7.0, 14.0 Hz), 2.09 (dd, *J* = 5.0, 14.0 Hz), 2.04 (dd, *J* = 4.0, 14.5 Hz), 1.92 (d, *J* = 14.0 Hz), 1.79^{-1}.65 (m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.1, 137.5, 137.4, 137.2, 137.1, 128.6, 128.57, 128.51, 128.1, 128.06, 128.02, 127.9, 106.1, 105.6, 96.7, 95.8, 88.3, 88.1, 75.4, 74.7, 74.1, 74.0, 73.8, 73.6, 73.4, 72.7, 72.4, 68.5, 67.8, 66.5, 65.3, 63.9, 63.4, 63.1, 62.7, 59.4, 44.5, 42.5, 42.1, 35.5, 34.2, 29.7. Anal. Calcd for $C_{13}H_{18}O_5$: C, 61.41; H, 7.13. Found: C, 61.44; H, 7.12; $[\alpha]^{25}_D$ = +1.4 (*c* 3.0, $CHCl_3$, after 6 h).

Dibenzyl 2(*R*)-2-(3-Benzyloxymethyl-4,5-dihydroisoxazol-5-yl)-2-hydroxyethyl Phosphate (10). I_2 (0.76 g, 3 mmol) was added to a solution of tribenzyl phosphite (1.12 g, 3.18 mmol) in EtOH-free CH_2Cl_2 (20 mL) at −20 °C. After 20 min, the clear colorless solution was allowed to warm to 25 °C. It was then added dropwise over a period of 1 h to a solution of **4d** (0.5 g, 2 mmol) and pyridine (0.64 mL, 8 mmol)

(15) Kozikowski, A. P.; Adamczyk, M. *J. Org. Chem.* **1983**, *48*, 366.

in CH_2Cl_2 (20 mL) at −30 °C. The solution was then allowed to warm to room temperature, filtered, and concentrated under reduced pressure. The residue was diluted with Et_2O (20 mL) and water (10 mL). The organic layer was washed with aq $KHSO_4$ 0.3 M (3 × 10 mL), aq saturated $NaHCO_3$ (10 mL), and brine (10 mL) and dried over $MgSO_4$. After concentration, the crude compound was chromatographed over silica gel (eluent, cyclohexane−EtOAc, 4:6) to give **10** (0.74 g, 72%) as a colorless oil which crystallized slowly after few weeks at −20 °C (mp 48 °C): IR (neat film) 3370, 1263, 1018, 879 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.33 (15H, m), 5.05 (4H, m),4.51 (1H, m), 4.50 (2H, s), 4.25 (2H, s), 4.18 (1H, d, J = 5.0 Hz), 4.12 (2H, m), 3.76 (1H, m), 3.14 (1H, dd, J = 7.5, 17.5 Hz), 3.02 (1H, dd, J = 10.5, 17.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.8, 137.1, 135.4 (d, J = 6.0 Hz), 128.53, 128.49, 128.4, 128.3, 127.9, 79.5, 72.5, 70.2 (d, J = 6.0 Hz), 69.5 (d, J = 5.0 Hz), 68.7 (d, J = 6.0 Hz), 64.2, 36.9. Anal. Calcd for $C_{27}H_{30}NO_7P$: C, 63.40; H, 5.91; N, 2.74; P, 6.05. Found: C, 63.61; H, 5.90; N, 3.00; P, 6.14; $[\alpha]^{25}_D$ = +40.9 (*c* 1.5, $CHCl_3$).

3-Deoxy-D-threo-hexulose-6-phosphate Disodium Salt (11). To a solution of **10** (300 mg, 0.5 mmol) in 9:1 MeOH−H_2O (10 mL) was added 10% Pd/C (100 mg). The reaction was placed under hydrogen using a balloon and stirred vigorously for 18h. The catalyst was removed by filtration through Celite and the solution concentrated under reduced pressure. The residue was diluted with H_2O (1 mL), applied onto a 10 × 1 cm column of Dowex 50W (H^+, 200−400 mesh), and eluted with H_2O. Acidic fractions were combined and adjusted to pH 7.6 with 1 M NaOH. After lyophylization, **11** (136 mg, 80%) was isolated as a white solid. NMR spectra showed the presence of three forms in equilibrium: ^1H NMR (400 MHz, D_2O) δ 4.61−3.54 (m), 2.88 (dd, J = 3.5, 16.0 Hz), 2.74 (dd, J = 9.0, 16.0 Hz), 2.55 (dd, J = 7.0, 14.0 Hz), 2.38 (dd, J = 7.0, 13.5 Hz), 2.23 (dd, J = 7.0, 13.5 Hz), 2.00 (dd, J = 3.5, 14.0 Hz); ^{13}C NMR (100 MHz, D_2O) δ 214.9, 108.7, 108.3, 88.1 (d, J = 8.0 Hz), 87.7 (d, J = 8.0 Hz), 76.4 (d, J = 5.5 Hz), 74.3, 74.0, 70.6, 70.1, 68.1, 67.8, 67.4 (d, J = 3.5 Hz), 67.3 (d, J = 3.5 Hz), 66.6 (d, J = 3.5 Hz), 44.3, 44.1, 43.7; $[\alpha]^{25}_D$ = +3.4 (*c* 1, H_2O, after 3 h).

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