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Note

Synthesis of 2-acetamido-3-*O*-acetyl-2-deoxy-D-mannose phosphoramidites

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The bacterium *Neisseria meningitidis*, group A, produces an antiphagocytic capsule [1] composed of α-(1 → 6)-linked *N*-acetylmannosamine phosphate polymers, in which a portion of the 3-hydroxyl groups are acetylated [2]. *Neisseria meningitidis* groups A, B, C, W-135, and Y cause meningococcal meningitidis, a world-wide health problem [3]. The meningococcal groups A, C, W-135, and Y polysaccharides are components of the currently licensed vaccine against meningococcal disease above the age of 2 years [4]. This vaccine is not recommended for use in infants. A useful approach to making effective vaccines against encapsulated bacteria is to conjugate a poly- or oligo-saccharide derived from the capsule to a protein carrier and thereby modify the mechanism of immunity [5]. Because the mechanism of this modification and its dependence on the size of the conjugated saccharide is not understood, we wanted to prepare a series of glycoconjugates in which the structure and size of the oligosaccharide was tightly controlled. Making monodisperse oligosaccharides by random degradation of polysaccharide results in low yields and does not afford significant control over oligosaccharide structure. Noting that DNA and the capsule of *N. meningitidis* group A are both phosphodiester-linked polysaccharides, we decided to adapt the phosphoramidite methodology which is commonly used for DNA synthesis [6] to the preparation of α-(1 → 6)-linked 2-acetamido-3-*O*-acetyl-2-deoxy-D-mannose phosphate oligomers. The

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first requirement of this approach is the synthesis of a suitable monomer. This paper describes the synthesis of such a suitably protected phosphoramidite derivative of 2-acetamido-3-*O*-acetyl-2-deoxy-D-mannose.

The overall scheme for synthesis is outlined in Scheme 1. The anomeric position was simultaneously activated and anomeric control achieved by making the oxazoline **2**. The oxazoline of mannosamine was prepared in 75% yield by treating a mixture of pentaacetate anomers **1** with triflic acid [7]. Opening the oxazoline ring with 4-methoxybenzyl alcohol produced the 1-protected glycoside **3**. The 4-methoxybenzyl group was chosen for temporary protection of the 1-position because it can be removed in the presence of a benzyl group [8] used to protect position 4. Positions 3, 4, and 6 were deprotected with sodium methoxide to give **4**.

Compound **5** was obtained by protecting the 4 and 6 positions as the benzylidene acetal [9]. Not only did this protecting group allow the selective introduction of a 3-*O*-acetyl group (**6**), but its partial removal allowed semipermanent protection of the 4-position as the benzyl ether.

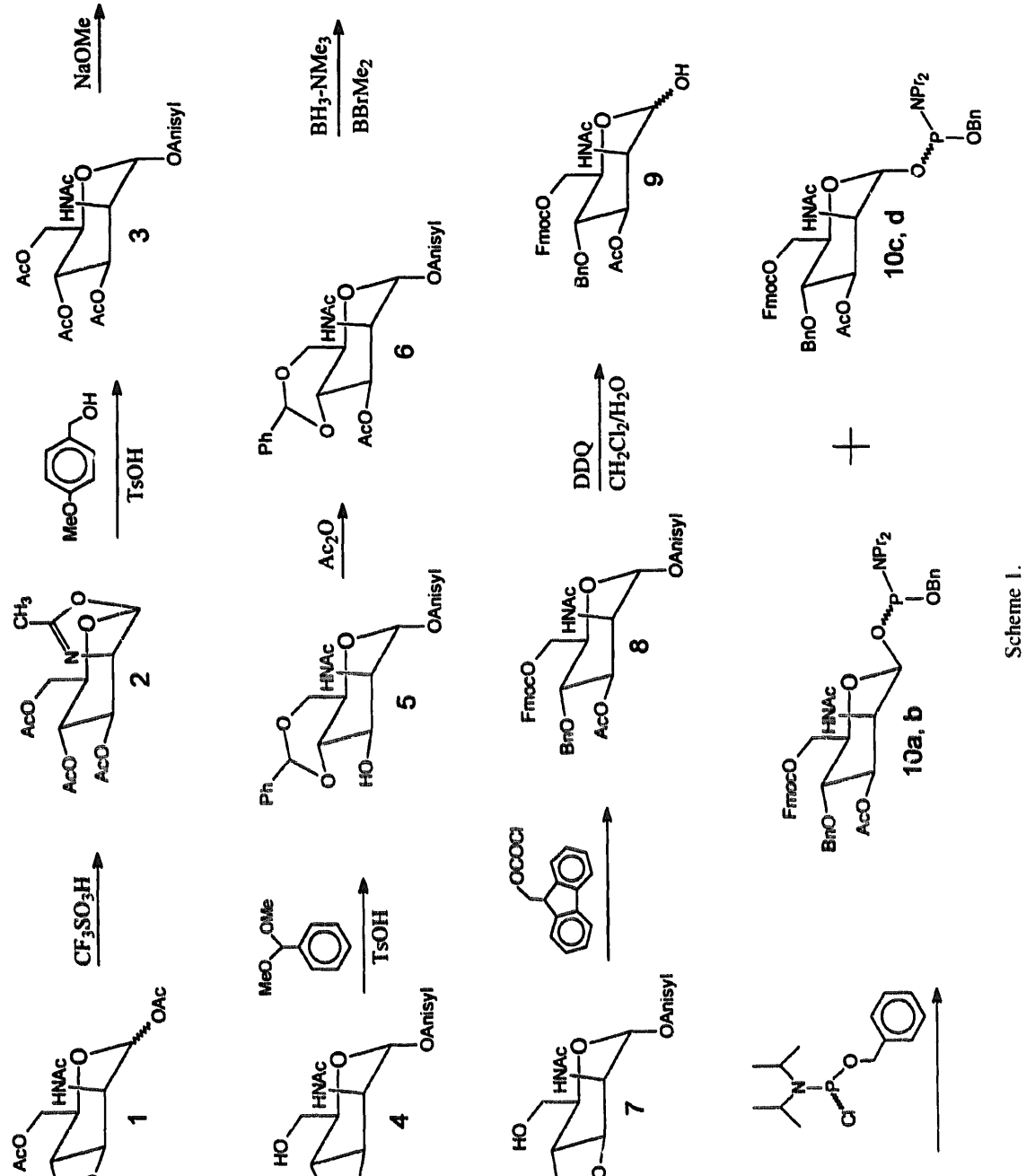
Treatment of **6** with a mixture of borane–trimethylamine complex and dimethylboron bromide [10] at dry ice temperature afforded the desired 4-benzyl derivative **7** in 73% yield and produced only trace amounts of the 6-benzyl isomer.

Fluorenemethyloxycarbonyl (Fmoc) was chosen as a protecting group because of its ease of removal under conditions compatible with the acid labile target molecules. The Fmoc group was introduced quantitatively in the 6-position of compound **7** by treatment with 9-fluorenylmethyl chloroformate in pyridine [11]. This temporary protecting group is required for the acceptor position in oligosaccharide synthesis in a fashion similar to the trityl group in oligonucleotide synthesis.

Treatment of compound **8** with 1,8-dichloro-4,5-dicyanobenzoquinone (DDQ) in dichloromethane–water [8] for 5 h gave compound **9** as a mixture of anomers in 74% yield. The anomers were not separable by HPLC either on silica gel or C-18 columns, therefore phosphoramidites were prepared using the anomeric mixture.

Reaction of **9** with β-cyanoethyl *N,N*-diisopropylchlorophosphoramidite gave the expected four isomers (two anomers and two enantiomers at phosphorus) in good yield (not shown). Small amounts of the four isomers were readily separated by HPLC on silica gel (Fig. 1, bottom). Because all four isomers were available, it is possible to unambiguously determine the anomeric configuration by NOE. These experiments showed that the anomers eluted in the order β, α, β, α. No attempt was made to determine the stereochemistry at phosphorus. Although the stereochemistry at phosphorus is irrelevant because the phosphorus in the final product will be achiral, it was necessary to separate the anomers. Separation was practical on an analytical scale, but attempts to separate preparatively useful amounts using a 2.5 × 35 cm column were unsuccessful due to peak tailing.

Because the elution order of the cyanoethylphosphoramidites made a routine isolation of pure α anomer impractical, we decided to explore the possibility of changing the elution order by changing the phosphorus protecting group. A phosphityliting reagent in which the 2-cyanoethyl moiety was replaced by a benzyl protecting group was prepared by reaction of dichlorobenzyl phosphite with diisopropylamine. This reagent, benzyl *N,N*-diisopropylchlorophosphoramidite [12,13], was treated with compound **9** to give


CF3SO3H
TsOH
NaOMe
Ac2O
BH3-NMe3
BBrMe2
DDQ
CH2Cl2/H2O
TsOH
1
2
3
4
5
6
7
8
9
10a, b
10c, d

Scheme 1.

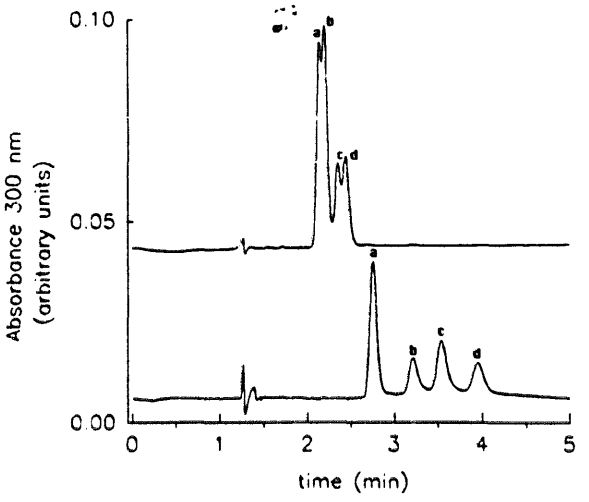


Fig. 1. Silica gel HPLC chromatograms of benzyl-protected phosphoramidites, **10a–d** (top) and cyanoethyl-protected phosphoramidites (bottom). Peaks are lettered according to elution order.

another series of phosphoramidites. This reaction gave products **10a–d** in good yield. The reaction mixture was purified, on a 2.5 × 35 cm silica gel HPLC column to give mixtures of **10a, b** and **10c, d**. These were further separated on a milligram scale and, although the resolution of **10a** from **10b** and **10c** from **10d** were relatively poor (Fig. 1, top), NOE-difference experiments showed that **10a** and **10b** were in the β configuration and **10c** and **10d** were in the α configuration.

1. Experimental

General methods.—*N*-Acetylmannosamine was purchased from Sigma, all other chemicals were from Aldrich. ^{1}H NMR spectra were recorded at 300 MHz and 300 K using a General Electric GN-300 spectrometer. Tetramethylsilane (internal) and 85% H_3PO_4 (external) were the references. NMR assignments were made on the basis of decoupling or COSY experiments as required. Optical rotations were determined with a Polyscience SR6 polarimeter. Melting points were determined with a Büchi model 510 apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA. Column chromatography was performed on 130–270 mesh silica gel. Silica gel TLC plates were developed with 10:10:1 toluene–EtOAc–MeOH and visualized by charring with 5% H_2SO_4 in EtOH or by UV light, as appropriate.

*2-Methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-β-*D*-mannopyrano)-[2,1:4',5']-2-oxazoline* (**2**).—To a solution of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-mannopyranose (**1**) (24.5 g, 63 mmol) in CH_2Cl_2 (100 mL) at room temperature was added trifluoromethanesulfonic acid (12.8 mL, 3 equiv). After 40 min the dark solution was cooled in ice and Et_3N (35 mL) was slowly added. The mixture was diluted with more CH_2Cl_2 and washed with water, 1 M sodium phosphate buffer pH 6.6, and thrice more with water. The organic layer was dried ($MgSO_4$) and evaporated to obtain a crystalline mass.

The product was recrystallized from benzene–hexane to obtain **2** (15.5 g, 75%). A further crop was obtained by concentrating and recrystallizing the mother liquor (0.5 g, 2%); mp 125–127 °C (lit [14] 132–133°); R_f 45; $[\alpha]_D$ −38.9 (*c* 3.6, MeOH). ^{1}H NMR ($CDCl_3$): δ 5.79 (d, 1 H, *J* 5.9 Hz, H-1), 4.40 (ddd, 1 H, *J* 5.9, 1.8, 5.5 Hz, H-2), 2.13 (d, 3 H, *J* 1.8 Hz, 2'-CH3). Anal. Calcd for $C_{14}H_{19}NO_8$: C, 51.06; H, 5.85; N, 4.25. Found: C, 51.15; H, 5.80; N, 4.30.

*4-Methoxybenzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-*D*-mannopyranose* (**3**).—A solution of compound **2** (16.5 g, 50 mmol), 4-methoxybenzyl alcohol (12.5 mL, 100 mmol) and benzene (300 mL) was placed in a simple distillation apparatus and boiled until 5 mL of benzene was collected. *p*-Toluenesulfonic acid (0.25 g), which had been dried by melting in vacuum, was added and distillation was continued for 5 min. The solution was cooled, washed with 1 M sodium phosphate, pH 7, dried (Na_2SO_4) and evaporated. The resulting syrup was chromatographed on silica gel using 10% acetone–CH_2Cl_2 to afford 16.4 g (70%) of **3** as a syrup; R_f 0.48; $[\alpha]_D$ +65.5 (*c* 3.3 MeOH). ^{1}H NMR ($CDCl_3$): δ 4.79 (d, 1 H, *J* 1.2 Hz, H-1), 4.62 (ddd, 1 H, *J* 1.2, 9.2, 4.5 Hz), 5.65 (d, 1 H, *J* 9.2 Hz, NH). Anal. Calcd for $C_{22}H_{29}NO_{10}$: C, 56.53; H, 6.25; N, 3.00. Found: C, 56.35; H, 6.41; N, 2.90.

*4-Methoxybenzyl 2-acetamido-2-deoxy-α-*D*-mannopyranose* (**4**).—Compound **3** (16.1 g, 34 mmol) was treated with NaOMe (2 g, 37 mmol) dissolved in MeOH (150 mL). After 20 min 40 g of Dowex-1 (H^+) was added. The solution was evaporated and purified on a silica gel column using a gradient of 5% MeOH to 10% MeOH in CH_2Cl_2. Evaporation afforded 11 g (95%) of colorless syrup; R_f 0.03; $[\alpha]_D$ +148° (*c* 0.3, MeOH). ^{1}H NMR (Me_2SO-d_6): δ 4.61 (d, 1 H, *J* 1.4 Hz, H-1), 4.74 (d, 1 H, *J* 4.9 Hz, 3-OH), 4.81 (d, 1 H, *J* 5.1 Hz, 4-OH), 4.48 (t, 1 H, *J* 5.9 Hz, 6-OH). Anal. Calcd for $C_{16}H_{23}NO_7$: C, 56.30; H, 6.79; N, 4.10. Found: C, 56.31; H 6.86; N 4.05.

*4-Methoxybenzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-*D*-mannopyranose* (**5**).—Compound **4** (9.5 g, 28 mmol) was dissolved in 100 mL DMF and treated with benzaldehyde dimethyl acetal (6 mL, 1.2 equiv) and *p*-toluenesulfonic acid (65 mg). The mixture was refluxed under aspirator vacuum for 1 h [9]. After cooling, 5 mL of saturated aq $NaHCO_3$ was added and the solution was evaporated. The residue was resuspended in 300 mL CH_2Cl_2 and filtered. The filtrate was evaporated to ~ 100 mL and ~ 1–2 volumes of ether were added. The resulting crystals were removed and washed with ether to obtain 10.0 g (82%) of compound **5**; mp 205–205.5 °C; R_f 0.34; $[\alpha]_D$ +84.4° (*c* 1.8, MeOH). ^{1}H NMR: δ 3.66 (t, 1 H, *J* 9.9 Hz, H-4), 4.26 (dd, 1 H, *J* 4.6, 10.0 Hz, H-6), 5.59 (s, 1 H, Ph-CH). Anal Calcd for $C_{23}H_{27}NO_7$: C, 64.32; H, 6.34; N, 3.26. Found: C, 64.42; H, 6.40; N, 3.26.

*4-Methoxybenzyl 2-acetamido-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-α-*D*-mannopyranose* (**6**).—A solution of compound **5** (9.8 g, 23 mmol), dimethylaminopyridine (100 mg), and Ac_2O (3.6 mL, 1.5 equiv) in 100 mL pyridine was stirred for 15 min. Methanol (10 mL) was added and the solution was evaporated. The residue was dissolved in CH_2Cl_2 and washed with dilute HCl, twice with brine, saturated $NaHCO_3$ and water. The organic layer was dried (Na_2SO_4) and evaporated to yield 10.5 g (96%) of colorless foam; mp 209–209.5 °C; R_f 0.45; $[\alpha]_D$ +62° (*c* 7.8, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 5.41 (dd, 1 H, *J* 4.5, 10.5 Hz, H-3). Anal. Calcd for $C_{23}H_{29}NO_8$: C, 63.68; H, 6.20; N, 2.97. Found: C, 63.58; H, 6.21; N, 3.07.

4-Methoxybenzyl 2-acetamido-3-O-acetyl-4-benzyl-2-deoxy-α-D-mannopyranose (**7**). —Compound **6** (10.7 g, 23 mmol) was dissolved in CH_2Cl_2 (200 mL) under argon and cooled in dry ice–acetone. A solution of borane–trimethylamine complex (6.6 g, 4 equiv) dissolved in toluene (55 mL) was added, followed by bromodimethylborane (5.6 mL, 2.5 equiv). After stirring for 30 min, 200 mL of 0.5 M sodium phosphate buffer, pH 7.3, was added and the solution was allowed to warm to room temperature. The organic layer was washed with a $NaHCO_3$ solution, twice with water, dried ($MgSO_4$) and evaporated. The residue was chromatographed on silica gel using a gradient of 10% acetone–CH_2Cl_2 to 40% acetone–CH_2Cl_2. Evaporating appropriate fractions afforded 7.8 g (73%) of oil; R_f 0.26, $[\alpha]_D$ +55° (*c* 6.7, MeOH). ^{1}H NMR (benzene-d_6): δ 3.78 (br dt, 1 H, H-6), 3.68 (br d, 1 H, H-6'), 2.52 (br dd, 1 H, *J* 6.1, 3.6 Hz, 6-OH), 4.79, 4.69, 4.79 (doublets, 2 H, *J* 11.5 Hz, Ph-CH_2). Anal. Calcd for $C_{25}H_{31}NO_8$: C, 63.41; H, 6.60; N, 2.96. Found: C, 63.26; H, 6.69; N, 2.91.

4-Methoxybenzyl 2-acetamido-3-O-acetyl-4-O-benzyl-2-deoxy-6-O-(9-fluorenylmethoxycarbonyl)-α-D-mannopyranose (**8**).—Compound **7** (7.6 g, 16 mmol) dissolved in pyridine (100 mL) was cooled to 0 °C and treated with 9-fluorenylmethyl chloroformate (6.3 g, 1.5 equiv). After 2 h on ice the bulk of the pyridine was evaporated and the residue dissolved in CH_2Cl_2. This was washed with dilute HCl, saturated $NaHCO_3$, water, dried ($MgSO_4$) and evaporated. After chromatography on silica gel using a gradient of 2% acetone–CH_2Cl_2 to 30% acetone–CH_2Cl_2, appropriate fractions were evaporated to yield 11.3 g (100%) of colorless syrup, R_f 0.62, $[\alpha]_D$ +58.5° (*c* 1.1, MeOH). ^{1}H NMR ($CDCl_3$): δ 4.41 (dd, 1 H, *J* 3.9, 11.6 Hz, H-6), 4.35 (dd, 1 H, *J* 2.3, 11.6 Hz, H-6'). Anal. Calcd for $C_{40}H_{41}NO_{10}$: C, 69.05; H, 5.99; N, 2.01. Found: C, 68.79; H, 5.99; N, 1.98.

2-Acetamido-3-O-acetyl-4-O-benzyl-2-deoxy-6-O-(9-fluorenylmethoxycarbonyl)-D-mannopyranose (**9**).—Compound **8** (1.8 g) was stirred with 25 mL CH_2Cl_2, 1 mL water, and DDQ (1.2 g, 2 equiv). After 2 h an additional 1.2 g of DDQ and 1 mL of water were added and stirring was continued 3 h more. The mixture was washed with saturated $NaHCO_3$, and twice with water. After drying ($MgSO_4$) and evaporating, the residue was chromatographed on silica gel using a gradient of 5% acetone–CH_2Cl_2 to 30% acetone–CH_2Cl_2. Evaporation of appropriate fractions afforded 130 mg (7%) of starting material and 1.1 g (74%) of the two anomers of compound **9** as an off-white foam; R_f 0.42, $[\alpha]_D$ +32° (*c* 1.4, MeOH). ^{1}H NMR (benzene-d_6): δ 5.17 (br s, H-1, major anomer), 4.89 (br s, H-1, minor anomer), 4.39 (dt, *J* 9.6, ~3 Hz, H-5, major isomer), 3.52 (dt, *J* 9.7, ~3 Hz, H-5 minor). Anal. Calcd for $C_{32}H_{33}NO_9$: C, 66.77; H, 5.78; N, 2.43. Found: C, 66.50; H, 5.85; N, 2.37.

Benzyl N,N-*diisopropylchlorophosphoramidite*.—The dichlorobenzylphosphite obtained from treatment of benzyl alcohol with 74 g (0.54 mmol) of PCl_3 [11] was dissolved in 50 mL of CH_2Cl_2. A solution of 15.6 g (0.15 mmol) of diisopropylamine in 20 mL CH_2Cl_2 was added dropwise. The resulting slurry was filtered and the solvent was removed. The oil was centrifuged to remove additional precipitate and distilled in a short-path apparatus at a pressure below 1 mm Hg at a bath temperature of 120–125 °C. (Caution: phosphoramidites have the reputation of exploding when heated.) A colorless oil (7.0 g, 5% from PCl_3) was obtained. ^{1}H NMR (CD_3CN): δ 7.35 (m, 5 H, Ph), 4.89 (d, 2 H, $POCH_2$), 3.83 (m, 2 H, NC*H* CH_3), 1.22 (d, 12 H, NCHCH_3); ^{31}P NMR

(CD_3CN): δ 181.1 (br s). Anal. Calcd for $C_{13}H_{21}ClNOP$: C, 57.04; H, 7.73; Cl, 12.95; N, 5.12. Found: C, 57.21; H, 7.80; Cl, 12.82; N, 5.18.

2-Acetamido-3-O-acetyl-4-O-benzyl-2-deoxy-6(9-fluorenylmethyeneoxycarbonyl)-D-mannopyranosyl N,N-diisopropyl-benzylphosphoramidite (compounds **10**).—Compound **9** (100 mg, 0.17 mmol) dissolved in CH_2Cl_2 (3 mL) was treated with diisopropylethylamine (200 μL, 6 equiv) followed by benzyl *N,N*-diisopropylchlorophosphoramidite (100 μL, 2.2 equiv). After 60 min, 15 μL of MeOH was added and mixture was purified in two injections on a 25 × 350 mm silica gel Dynamax HPLC column (Rainin Instruments, Woburn, MA). The solvent was hexane–EtOAc–Et_3N (60:40:0.5). Evaporation, at a pressure of ~ 0.5 mmHg, of the first pair of peaks afforded 43 mg (30%) of **10a, b** (β) as a colorless oil; R_f 0.75. Evaporation of the second pair of peaks gave 57 mg (40%) of **10c, d** (α) as a colorless oil; R_f 0.70. Elemental analysis was not possible because of the instability of these compounds. A few milligrams of **10a, b** and **10c, d** were each rechromatographed as above to obtain samples of each diastereomer for NMR analysis. **10a**: ^{1}H NMR (C_6D_6): δ 4.97 (dd, 1 H, *J* 1.8, 8.9 Hz, H-1), 5.22 (dd, 1 H, *J* 3.9, 9.4 Hz, H-3), 3.23 (ddd, 1 H, *J* 9.4, 4.0, 2.7 Hz, H-5); ^{31}P NMR (C_6D_6): δ 151.4. **10b**: ^{1}H NMR (C_6D_6): δ 5.09 (dd, 1 H, *J* 1.8, 9.3 Hz, H-1), 5.24 (dd, 1 H, *J* 3.8, 9.6 Hz, H-3), 3.30 (dt, 1 H, *J* 9.6, 3.0 Hz, H-5); ^{31}P NMR (C_6D_6): δ 153.9. **10c**: ^{1}H NMR (C_6D_6): δ 5.44 (dd, 1 H, *J* 1.8, 8.7 Hz, H-1), 5.86 (dd, 1 H, *J* 4.1, 9.8 Hz, H-3), 4.38 (ddd, 1 H, *J* 9.8, 3.6, 2.1 Hz, H-5); ^{31}P NMR (C_6D_6): d 152.9. **10d**: ^{1}H NMR (C_6D_6): δ 5.36 (dd, 1 H, *J* 1.8, 9.5 Hz, H-1), 5.88 (dd, 1 H, *J* 9.8, 4.0 Hz, H-3), 4.38 (ddd, 1 H, *J* 9.8, 4.6, 2.2 Hz, H-5); ^{31}P NMR (C_6D_6): δ 148.8.

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