Synthesis of Chiral Analogues of FTY720 and its Phosphate

Klaus Hinterding,*[a,1] Sylvain Cottens,[a,1] Rainer Albert,[a,1] Frederic Zecri,[a] Peter Buehlmayer,[a] Carsten Spanka,[a] Volker Brinkmann,[a] Peter Nussbaumer,[b] Peter Ettmayer,[b] Klemens Hoegenauer,[b] Nathanael Gray,[c] Shifeng Pan[c]

[a] Novartis Institutes for Biomedical Research, S507.409, 4002 Basel, Switzerland
Fax +41(61)3246735; E-mail: klaus.hinterding@pharma.novartis.com

[b] Novartis Forschungsinstitut GmbH, Brunner Str. 59, 1235 Wien, Austria

[c] Novartis Institute for Functional Genomics Inc,, 10675 John Jay Hopkins Drive, San Diego CA 92121, USA

Received 16 May 2003

Abstract: Efficient and versatile protocols for the synthesis of chiral analogues of the novel immunomodulator FTY720 and its phosphate are described. These synthetic procedures allow for broad structural variation and deliver essential tools to further elucidate FTY720's novel mechanism of action.

Key words: phosphates, sphingolipids, stereoselective synthesis, drugs, chiral auxiliaries

FTY720 (Figure 1) is a novel immunomodulator which acts via S1P-receptor agonism and is highly effective in animal models of organ transplantation and autoimmunity.[2] Additionally, in a recently completed Phase II clinical trial, the drug has proven efficacious in preventing kidney allograft rejection in humans.[3] Unlike any other immunosuppressant currently on the market, FTY720 does not inhibit T- and B-cell proliferation and activation at therapeutically relevant concentrations; instead it leads to a sequestration of lymphocytes from the periphery into secondary lymphoid organs.[4] According to our current understanding, the phosphorylated molecule FTY720-Phosphate **2** (Figure 1), which is generated in vivo via a sphingosine-kinase, signals as an agonist through four of five sphingosine-1-phosphate (S1P) receptors (formerly known as EDG-receptors).[5]

Chiral analogues of FTY720 and its phosphate played an important role in the initial understanding of FTY720's mode of action.[6] They are also invaluable tools to further clarify the pharmacology of single S1P-receptors.[5] We herein describe experimental details of a practical and versatile stereoselective synthesis of chiral FTY720 analogues **3**, **5**, **7**, **9** and their corresponding phosphates **4**, **6**, **8**, **10**.[7]

The Schöllkopf protocol[8] was chosen as the centerpiece of our synthesis, because a) both Val-Gly- and Val-Ala-derived auxiliaries are commercially available in either *R*- and *S*-configuration[9] and b) the protocol allows for broad structural variations at the quaternary center of the hydrophilic head-group, the central aromatic ring, and the lipophilic side-chain of FTY720 (Figure 1).

Generation of the Quaternary Center

The generation of the quaternary center was accomplished by sequential double alkylation of the Gly-derived Schöllkopf-auxiliary (Scheme 1, steps a and b). Both enantiomeric forms of the target molecules were available by either choosing the appropriate configuration of the auxiliary or by adjusting the sequence of alkylation steps. While, in general, the first alkylation proceeded uneventfully irrespective of the size of the alkylating reagent, the overall yield was higher, if the sterically smaller residue was introduced last. Iodides as alkylating reagents were preferred, but satisfactory results and yields (ca. 65% per alkylation step) were also obtained with bromides.

Preferred building blocks for the first alkylation (step a) were benzyl-protected iodides like **12**, since they allowed

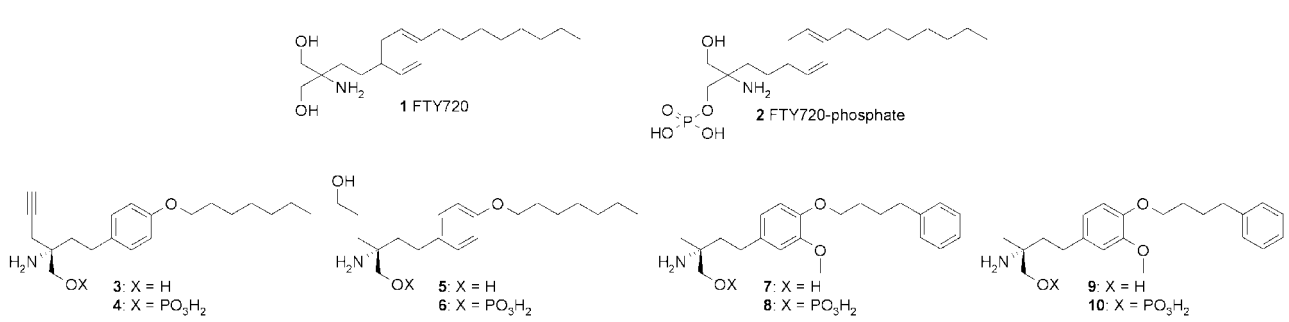
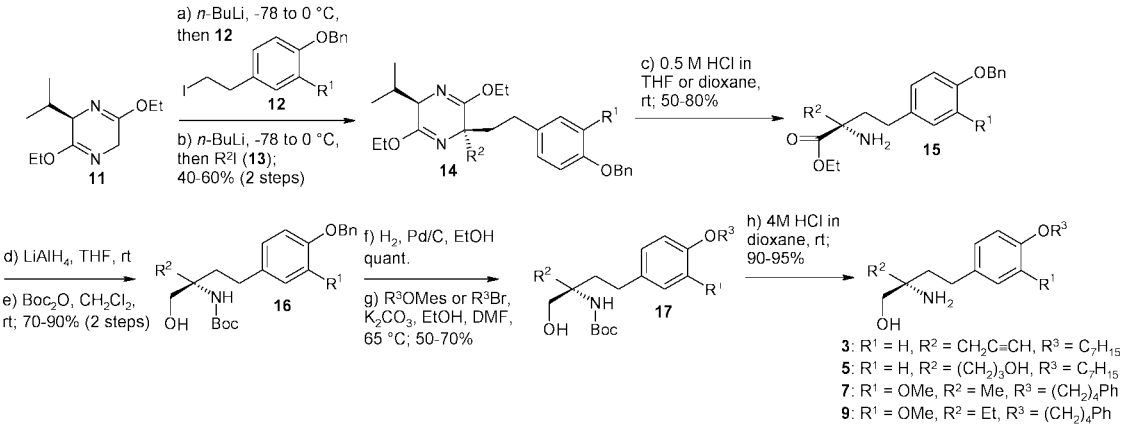


Figure 1 Structures of the immunomodulators FTY720, its phosphate and structural analogues

Synthesis 2003, No. 11, Print: 05 08 2003.
Art Id.1437-210X,E;2003,0,11,1667,1670,ftx,en;C03403ss.pdf.



Scheme 1 Synthesis of FTY720 amino alcohol analogues **3**, **5**, **7**, **9** and hydrolysis of the bislactim ether

for broad structural variation of the lipophilic side-chain after benzylic deprotection (step f) and phenol ether formation (step g).

Hydrolysis of the Bislactim Ether

For Me-substituted chiral centers, the hydrolysis of *R*-Val-derived bislactim ethers **14** (step c) proceeded in good yields of ca. 60–80% by stirring a *homogenous* solution of starting material in mixtures of either dioxane or THF with trifluoroacetic acid or aqueous HCl overnight. The final concentration of acid was adjusted to 0.1–0.5 M. Increasing the steric bulk at the quaternary center only slightly, e.g. by substitution of ethyl for methyl, already required significantly longer reaction times of 5–7 days. Nevertheless, satisfactory yields (50–60%) were still obtained, if trifluoroacetic acid was used at 0.5–0.7 M in acetonitrile. The use of higher concentrations of various acids (HCl, TFA, HBr) in different solvents (dioxane, THF, EtOH) did not result in a reduction of reaction times but in an increased formation of amido ester and diketopiperazine side-products and thereby lower yields.[10] It is of importance to note that the configuration of the asymmetric centers in bislactim ethers **14** had a significant influence on the optimal conditions of hydrolysis: in the case of the *S*-Val stereoisomer of **14** a 1:1 mixture of TFA–water was used to obtain a good yield of 72% after stirring at room temperature for 64 hours.

Completion of Amino Alcohol Synthesis

After reduction of the ethyl ester functionality (step d), the amino-group was protected as its Boc-derivative (step e). Benzylic deprotection via hydrogenolysis (step f) was followed by the incorporation of the lipophilic side chain via phenol alkylation using bromides or mesylates in the presence of either K_2CO_3 or Cs_2CO_3 (step g). The use of iodides in this alkylation step led to predominant elimination and alkene formation. Uneventful Boc-deprotection completed the synthetic sequence to deliver amino alcohols **3**, **5**, **7** and **9**.

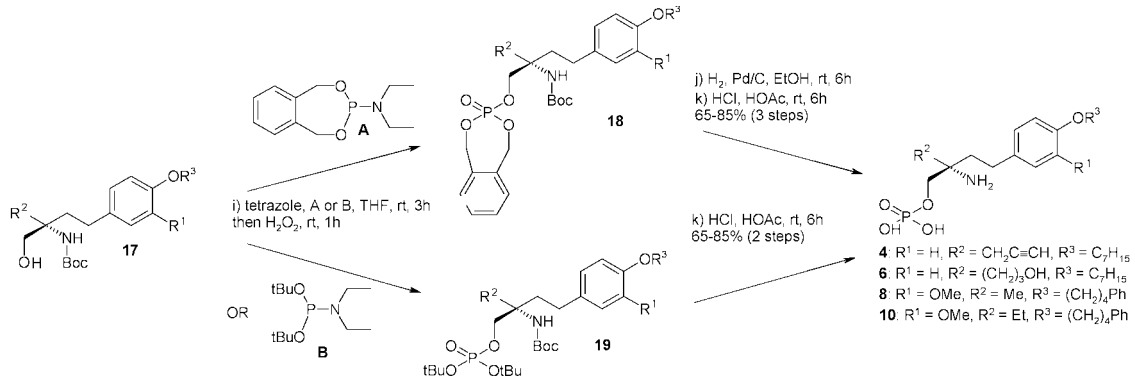
Synthesis of phosphates

Due to the difficult physicochemical properties of aminophosphates, in particular their low solubility, and due to lack of commercially available sphingosine kinases, their biochemical synthesis and isolation is tedious. We therefore developed straightforward chemical approaches for the preparation of phosphates **4**, **6**, **8**, **10**, which do not require a purification step of the final products (Scheme 2). The nature and lability of substituents R^2 and R^3 determined whether trivalent phosphorylation agent **A**,[11] which is deprotected via hydrogenation, or **B**,[12] which is deprotected via acid treatment, was used. Both methods delivered the final products **4**, **6**, **8**, **10** with similar high yields of 65–80% over all steps starting from Boc-protected amino alcohols **17**. The only chromatographic purification of this sequence was performed after oxidation on fully protected phosphates **18** and **19**. While the use of **A** required an additional deprotection step (hydrogenolysis, step j) its reaction products **18** where somewhat more stable than the ones of **B** (**19**) under standard work-up and chromatography conditions.[13]

Critical steps of the reaction sequence are exemplified by the synthesis of amino alcohol **9** and phosphate **10**.

Second Alkylation of Schöllkopf-Auxiliary (Step b); (2*R*,5*R*)-2-[2-(4-Benzyloxy-3-methoxyphenyl)ethyl]-3,6-diethoxy-2-ethyl-5-isopropyl-2,5-dihydropyrazine (14; R^1 = OMe, R^2 = Et); Typical Procedure

To a solution of monoalkylated Schöllkopf-adduct (2*R*,5*R*)-2-[2-(4-benzyloxy-3-methoxyphenyl)ethyl]-3,6-diethoxy-5-isopropyl-2,5-dihydropyrazine (**14**; R^1 = OMe, R^2 = H; 1.5 g, 3.3 mmol) in THF (4 mL) was added a solution of *n*-BuLi in hexanes (1.6 M) at –75 °C. After stirring for 30 min at this temperature, a solution of EtI (618 mg, 4.0 mmol) in THF (1 mL) was added dropwise. The stirring was continued at –75 °C for 30 min, before warming to 0 °C and stirring for another 60 min. Quenching with sat. aq $NaHCO_3$ was followed by extraction with EtOAc (3 × 15 mL), drying of the



Scheme 2 Synthesis of FTY720 phosphate analogues **4**, **6**, **8**, **10**

organic phase ($MgSO_4$) and evaporation of the solvent. Chromatography on silica gel using hexanes–EtOAc (99:1 to 95:5) yielded the desired product as a colorless oil (984 mg, 62%).

Hydrolysis of Double-Alkylated Bislactim Ether (Step c); (*R*)-2-Amino-4-(4-benzyloxy-3-methoxyphenyl)-2-ethylbutyric Acid Ethyl Ester (15; R^1 = OMe, R^2 = Et); Typical Procedure

A homogenous solution of (2*R*,5*R*)-2-[2-(4-benzyloxy-3-methoxyphenyl)ethyl]-3,6-diethoxy-2-ethyl-5-isopropyl-2,5-dihydropyrazine (**14**, R^1 = OMe, R^2 = Et, 984 mg, 2.0 mmol) in MeCN (43 mL) and 2 M trifluoroacetic acid in H_2O (21 mL) was stirred for 5 d at r.t. After addition of aq sat. $NaHCO_3$ (1.5 ml), the reaction mixture was extracted with EtOAc (3 × 20 mL). Drying ($MgSO_4$) and evaporation of the solvent was followed by chromatography on silica gel using EtOAc–hexanes (1:2) to yield the desired ethyl ester as a colorless oil (395 mg, 52%).

Completion of Amino Alcohol Synthesis (Steps d–h); (*R*)-2-Amino-2-ethyl-4-[3-methoxy-4-(4-phenylbutoxy)phenyl]butan-1-ol (9); Typical Procedure

To a solution of ester **15** (R^1 = OMe, R^2 = Et, 395 mg, 1.1 mmol) in anhyd THF (10 ml) was added a solution of $LiAlH_4$ in THF (1 M, 1.7 mmol, 1.7 mL). Stirring at r.t. for 1 h was followed by quenching the mixture with aq sat. Na_2SO_4 solution and extraction with EtOAc (3 × 15 mL). After drying (Na_2SO_4) and evaporation of the solvent, the crude material (385 mg) was dissolved in CH_2Cl_2 (15 mL) and treated with Boc_2O (1.7 mmol, 371 mg) at r.t. and the mixture was stirred for 16 h. Evaporation of the solvent and chromatography using EtOAc–hexanes (2:3) afforded alcohol **16** (R^1 = OMe, R^2 = Et, 1.0 mmol, 402 mg) in 95% yield. The alcohol **16** was dissolved in EtOH (25 mL) and stirred together with 10% Pd/C (90 mg) for 1 h at r.t. under an atmosphere of H_2. Filtration over Celite and evaporation of the solvent yielded the phenol in quantitative yield as an oil, which solidified. A mixture of this phenol (313 mg, 1.0 mmol), K_2CO_3 (415 mg, 3.0 mmol), phenylbutyl bromide (426 mg, 2.0 mmol) in EtOH (9 mL) and DMF (3 mL) was stirred at 55 °C for 16 h. Addition of EtOAc (50 mL) was followed by washing with H_2O (25 mL) and brine (25 mL). Drying (Na_2SO_4) and evaporation of solvent was followed by chromatography on silica gel using Et_2O–hexanes (4:1) to give Boc-protected product **17** (R^1 = OMe, R^2 = Et, 0.7 mmol, 330 mg) in 72% yield as white solid. Stirring Boc-urethane **17** (0.5 mmol, 230 mg) for 16 h at r.t. in dioxane containing 4 M HCl (5 mL), yielded the desired amino alcohol **9** (0.4 mmol, 158 mg) as a white solid after precipitation with Et_2O, filtration and drying.

Phosphate 10 [R^1 = OMe, R^2 = Et, R^3 = $(CH_2)_4Ph$] ; Typical Procedure

To a solution of Boc-protected amino alcohol **17** [R^1 = OMe, R^2 = Et, R^3 = $(CH_2)_4Ph$, 0.2 mmol, 100 mg] and tetrazole (recrystallized from toluene, 0.6 mmol, 42 mg) in anhyd THF (2 mL) was added phosphoamidite **A** (0.3 mmol, 72 mg), dissolved in anhyd THF (0.5 mL). After stirring at r.t. under argon for 1 h, H_2O_2 was added (30%, 240 μL, ca. 2 mmol) and the stirring was continued for 30 min. Quenching with aq sat. $Na_2S_2O_3$ and extraction with EtOAc (3 × 10 mL) was followed by drying (Na_2SO_4), evaporation of solvent and chromatography on silica gel using Et_2O–CH_2Cl_2 (1:1) to yield the fully protected phosphate **18** [R^1 = OMe, R^2 = Et, R^3 = $(CH_2)_4Ph$, 118 mg] as a colorless oil. This intermediate was dissolved in MeOH (10 mL) and stirred together with Pd/C (10%, 50 mg) for 2 h under an atmosphere of H_2. After filtration over Celite and washing with MeOH and CH_2Cl_2, the solvent was removed in vacuo and the residue dissolved in AcOH (2 mL) and HCl (37%, 0.5 mL). After 16 h at r.t., the solvent was removed by lyophilization to yield the desired phosphate **10** [R^1 = OMe, R^2 = Et, R^3 = $(CH_2)_4Ph$, 72 mg, 80%] as a white solid.

Spectral Data of Selected Final Products

(*S*)-2-Amino-2-[2-(4-heptyloxyphenyl)ethyl]pent-4-yn-1-ol (3)

^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.90 (t, *J* = 7 Hz, 3 H, CH_3), 1.23–1.45 [m, 8 H, $(CH_2)_4$], 1.72 (p, *J* = 7 Hz, 2 H, OCH_2CH_2), 1.86 (dd, *J* = 7, 11 Hz, 2 H, C_q-CH_2), 2.54–2.65 (m, 4 H, C_q-CH_2, $PhCH_2$), 3.16 (s, 1 H, C≡CH), 3.57 (d, *J* = 6 Hz, 2 H, CH_2OH), 3.94 (t, *J* = 7 Hz, 2 H, OCH_2), 5.65 (t, *J* = 6 Hz, 1 H, OH), 6.88 (d, *J* = 8 Hz, 2 H_{arom}), 7.12 (d, *J* = 8 Hz, 2 H_{arom}), 8.00–8.20 (br, 3 H, NH_3^+).

MS (ES+): *m*/*z* = 318.5 $(MH)^+$.

Phosphoric Acid Mono-{(*S*)-2-amino-2-[2-(4-heptyloxyphenyl)ethyl]pent-4-ynyl} Ester (4)

^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.90 (t, *J* = 7 Hz, 3 H, CH_3), 1.25–1.45 [m, 8 H, $(CH_2)_4$], 1.72 (p, *J* = 7 Hz, 2 H, OCH_2CH_2), 1.91 (m, 2 H, C_q-CH_2), 2.58 (m, 2 H, C_q-$CH_2C{\equiv}CH$), 2.67 (m, 2 H, $PhCH_2$), 3.19 (s, 1 H, CCH), 3.93 (m, 4 H, $PhOCH_2$, CH_2OP), 6.86 (d, *J* = 8 Hz, 2 H_{arom}), 7.13 (d, *J* = 8 Hz, 2 H_{arom}).

MS (ES+): *m*/*z* = 398.3 $(MH)^+$, 795.3 $(2M + H)^+$.

MS (ES–): *m*/*z* = 396.3 $(M - H)^-$, 397.3 (M^-), 793.3 $(2M - H)^-$.

Phosphoric Acid Mono-{(*S*)-2-amino-2-[2-(4-heptyloxyphenyl)ethyl]-5-hydroxypentyl} Ester (6)

^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.90 (t, *J* = 7 Hz, 3 H, CH_3), 1.25–1.53 [m, 10 H, CH_2, $(CH_2)_4$], 1.65–1.85 (m, 6 H, 2 × C_q-CH_2, OCH_2CH_2), 2.53 (m, 2 H, $PhCH_2$), 3.43 (m, 2 H, CH_2OH), 3.88 (d, *J* = 7 Hz, 2 H, CH_2OP), 3.94 (t, *J* = 7 Hz, 2 H, $PhOCH_2$), 6.86 (d, *J* = 8 Hz, 2 H_{arom}), 7.12 (d, *J* = 8 Hz, 2 H_{arom}).

MS (ES+): *m*/*z* = 418.3 $(MH)^+$, 873.4 $(2 M + K)^+$.

MS (ES–): *m*/*z* = 416.4 $(M - H)^-$, 417.3 (M^-), 833.5 $(2 M - H)^-$.

(*R*)-2-Amino-4-[3-methoxy-4-(4-phenyl-butoxy)-phenyl]-2-methyl-butan-1-ol (7)

^{1}H-NMR (400 MHz, DMSO): δ = 0.98 (s, 3 H, CH_3), 1.52 (q, *J* = 7 Hz, C_q-CH_2), 1.74 [m, 4 H, $(CH_2)_2$], 2.53 (m, 2 H, PhC*H*$_2$), 2.68 (m, 2 H, Ph-CH_2), 3.18 (d, *J* = 6 Hz, 2 H, C*H*$_2$OH), 3.78 (s, 3H, OCH_3), 3.94 (s, 2 H, OCH_2), 4.57 (t, *J* = 6 Hz, 1 H, OH), 6.69 (d, *J* = 8 Hz, 1 H_{arom}), 6.80 (s, 1 H_{arom}), 6.84 (d, *J* = 8 Hz, 1 H_{arom}), 7.15–7.35 (m, 5 H, 5 H_{arom}).

MS (ES+): 358.3 $(MH)^+$

(*R*)-2-Amino-2-ethyl-4-[3-methoxy-4-(4-phenylbutoxy)phenyl]butan-1-ol (9)

^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.85 (t, *J* = 9 Hz, 3 H, CH_3), 1.35 (dq, *J* = 9, 5 Hz, 2 H, C*H*$_2$CH$_3$), 1.50 (t, *J* = 8 Hz, 2 H, C_q-CH_2), 1.71 (m, 4 H, $OCH_2CH_2CH_2$) 2.48 (m, 2 H, PhC*H*$_2$), 2.65 (m, 2 H, PhC*H*$_2$), 3.20 (s, 2 H, C*H*$_2$OH), 3.76 (s, 3 H, OCH_3), 3.93 (t, *J* = 7 Hz, 2 H, PhOC*H*$_2$), 4.50 (br, 1 H, OH), 6.67 (d, *J* = 8 Hz, 1 H_{arom}), 6.78 (s, 1 H_{arom}), 6.83 (d, *J* = 8 Hz, 1 H_{arom}), 7.18–7.33 (m, 5 H_{arom}).

MS (ES+): *m*/*z* = 372.3 (MH^+).

Phosphoric Acid Mono-{(*R*)-2-amino-2-ethyl-4-[3-methoxy-4-(4-phenylbutoxy)phenyl]butyl} Ester (10)

^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.92 (t, *J* = 8 Hz, 3 H, CH_3), 1.65–1.85 (m, 8 H, CH_2), 2.55 (m, 2 H, PhC*H*$_2$), 2.65 (m, 2 H, PhC*H*$_2$), 3.75 (s, 3 H, OCH_3), 3.88 (d, *J* = 7 Hz, 2 H, CH_2OP), 3.94 (t, *J* = 7 Hz, 2 H, PhOC*H*$_2$), 6.70 (d, *J* = 8 Hz, 1 H_{arom}), 6.85 (m, 2 H_{arom}), 7.18–7.33 (m, 5 H_{arom}).

MS (ES–): *m*/*z* = 450.5 (M – H).

References

(1) These authors contributed equally to this study.

(2) Brinkmann, V.; Lynch, K. R. *Curr. Opin. Immunol.* **2002**, *14*, 569.

(3) Tedesco, T.; Kahan, B.; Mourad, G.; Vanrenterghem, Y.; Grinyo, J.; Weimar, W.; Pellet, P.; Chodoff, L.; Sablinski, T. *Am. J. Transplant.* **2001**, *1*, S243.

(4) Chiba, K.; Yanagawa, Y.; Yasubuchi, Y.; Kataoka, H.; Kawaguchi, T.; Ohtsuki, M.; Hoshino, Y. *J. Immunol.* **1998**, *160*, 5037.

(5) (a) Brinkmann, V.; Davis, M. D.; Heise, C. E.; Albert, R.; Cottens, S.; Hof, R.; Bruns, C.; Prieschl, E.; Baumruker, T.; Hiestand, P.; Foster, C. A.; Zollinger, M.; Lynch, K. R. *J. Biol. Chem.* **2002**, *277*, 21453. (b) Mandala, S.; Hajdu, R.; Bergstrom, J.; Quakenbush, E.; Xie, J.; Milligan, J.; Thornton, R.; Shei, G. J.; Card, D.; Keohane, C.; Rosenbach, M.; Hale, J.; Lynch, C. L.; Rupprecht, K.; Parsons, W.; Rosen, H. *Science* **2002**, *296*, 346.

(6) Kiuchi, M.; Adachi, K.; Kohara, T.; Minoguchi, M.; Hanano, T.; Aoki, Y.; Mishina, T.; Arita, M.; Nakao, N.; Ohtsuki, M.; Hoshino, Y.; Teshima, K.; Chiba, K.; Sasaki, S.; Fujita, T. *J. Med. Chem.* **2000**, *43*, 2946.

(7) Hinterding, K.; Albert, R.; Cottens, S. *Tetrahedron Lett.* **2002**, *43*, 8095.

(8) (a) Schöllkopf, U. *Pure Appl. Chem.* **1983**, *55*, 1799. (b) Schöllkopf, U.; Groth, U.; Deng, C. *Synthesis* **1981**, 737.

(9) Commercially available as OMe-ethers from Merck and Fluka.

(10) Schöllkopf, U.; Westphalen, K. O.; Schroeder, J.; Horn, K. *Liebigs Ann. Chem.* **1988**, 781.

(11) Watanabe, Y.; Komoda, Y.; Ebisuya, K.; Ozaki, S. *Tetrahedron Lett.* **1990**, *31*, 255.

(12) Perich, J. W.; Johns, R. B. *Synthesis* **1988**, 142.

(13) See, e.g.: Burger, A.; Tritsch, D.; Biellmann, J. F. *Carbohydr. Res.* **2001**, *332*, 141.