Chemical-Enzymatic Synthesis of Iminocyclitol Phosphonic Acids

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Abstract: Fuculose 1-phosphate and fructose-diphosphate aldolases were employed in the chemoenzymatic synthesis of the iminocyclitols **1** and **2** possessing a phosphonic acid moiety. These compounds are useful as core synthons for the development of specific glycosyltransferase inhibitors.

Keywords: aldolase; glycosyltransferase inhibition; iminocyclitol; phosphonic acid

Cell surface carbohydrates play a pivotal role in various molecular recognition processes and are considered to be attractive targets for drug discovery.[1,2] Among many strategies developed to target these molecules, selective inhibition of glycosyltransferases and glycosidases, which are involved in the synthesis and processing of cell-surface oligosaccharides, is of particular interest.[1,3] Although a number of effective inhibitors have been developed to target glycosidases,[3] inhibition of glycosyltransferases still represents a significant challenge.

Glycosyltransferases catalyze the transfer of the sugar moiety from the activated sugar nucleotide donor to a specific hydroxy group of the acceptor sugar. The transition state structure of glycosyltransferase reactions[4] is thought to be similar to that of the glycosidase reaction,[5,6] exhibiting a flattened half-chair conformation with substantial oxocarbenium ion character at the anomeric position (Figure 1, **a** and **b**). Iminocyclitols mimic the partial positive charge in the transition state and are potent inhibitors of glycosidases,[7,8] but are only modest inhibitors of glycosyltransferases.[9,10] Iminocyclitols that incorporate a nucleoside diphosphate mimic are, however, good inhibitors of α-galactosyltransferase.[11] We envision that iminocyclitols that possess a negatively charged moiety which mimics the pyrophosphate of the natural substrate may be important synthons for development of high-affinity inhibitors of glycosyltransferases (Figure 1, **c**). Phosphonic acids are isosteric analogues of naturally occurring phosphates due to

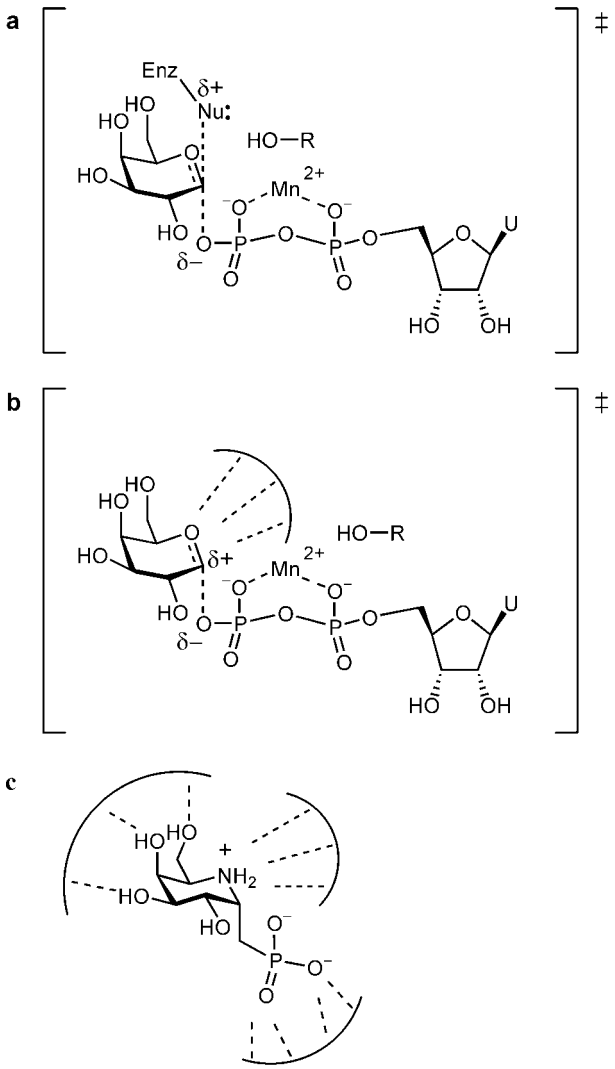

Figure 1. (**a**) Proposed transition state for retaining α-1,3-galactosyltransferase via a double displacement or (**b**) front attack of acceptor; (**c**) iminocyclitol phosphonic acid as a transition state mimic.

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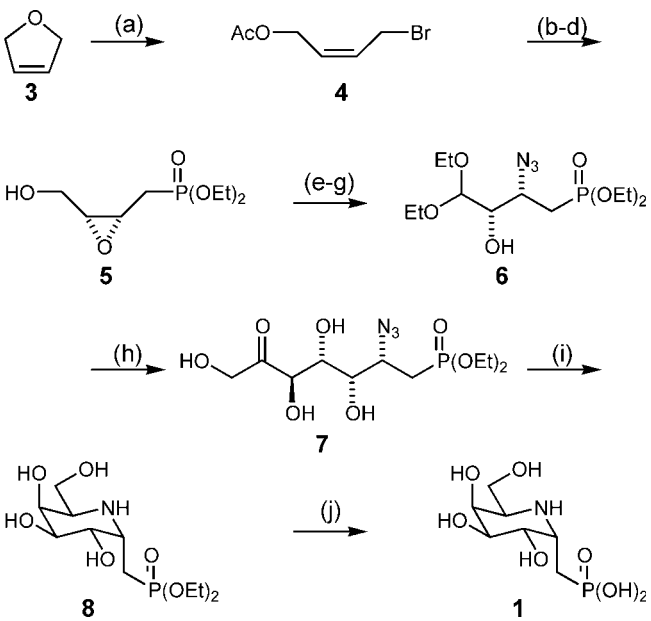
the geometrical and polar similarity and possess stability toward hydrolytic enzymes.[12] Iminocyclitol phosphonic acids are thus interesting targets for synthesis. The preparation of 6-membered iminocyclitol phosphonic acids was, however, not reported previously.[13] We describe here the chemoenzymatic synthesis of iminocyclitol phosphonic acids employing dihydroxyacetone phosphate (DHAP)-dependent aldolases.

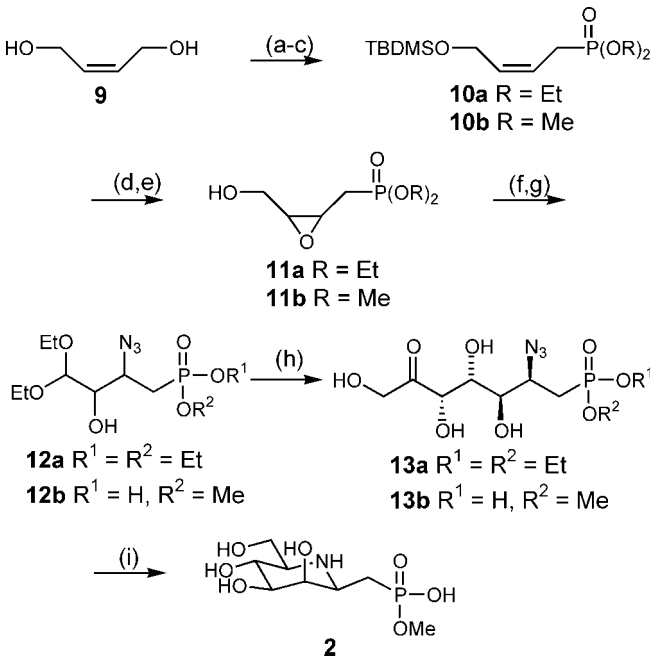
The condensation of DHAP and azido-aldehydes to give azido-sugars catalyzed by fructose diphosphate (FDP) aldolase (EC 4.1.2.13) and fuculose 1-phosphate (Fuc 1-P) aldolase (EC 4.1.2.17) establishes the four stereocenters present in the target iminocyclitols. The azide is then reduced and hydrogenation of the resulting imine from the less hindered face produces the iminocyclitol.[14,15,16] The aldolase/reductive amination approach was successfully employed for the synthesis of 1-deoxymannojirimycin, 1-deoxynorjirimycin,[14,15] and β-L-homofuconojirimycin.[10] As an extension of this strategy, we designed azido phosphonates as acceptor substrates for the synthesis of iminocyclitol phosphonic acids with the D-galactose (**1**) and D-mannose (**2**) configurations.

For the preparation of the iminocyclitol phosphonic acids derived from mannose and galactose, the phosphonate group was introduced prior to the aldolase reaction. In the case of the galactose derivative, the required aldehyde phosphonate was readily prepared starting from 2,5-dihydrofuran (**3**) (Scheme 1). Reaction of **3** with acetyl bromide (AcBr) at room temperature provides 1-bromo-4-*O*-acetoxy-2-*cis*-butene (**4**) in high yield. Reaction with triethyl phosphite, acid-catalyzed deprotection of the hydroxy group, and Sharpless asymmetric epoxidation[17] gives the epoxy alcohol **5**. Swern oxidation, protection of the resultant aldehyde as the diethyl acetal and regioselective epoxide opening with diethylaluminum azide[18] (generated *in situ*) gives the azido acetal **6**. Deprotection of the diethyl acetal and fuculose-1-phosphate aldolase-catalyzed aldol condensation[19] with DHAP,[20] followed by dephosphorylation with acid phosphatase furnishes azido-sugar **7**. Hydrogenation provides the iminocyclitol phosphonate **8** as the major product. Deprotection of the diethyl phosphonate moiety with bromotrimethylsilane results in the phosphonic acid **1**.[21]

For the synthesis of the iminocyclitol phosphonate with the mannose configuration, the aldehyde-phosphonate was prepared starting from *cis*-butene-1,4-diol (**9**) (Scheme 2). Diol **9** was monoprotected as the *tert*-butyldimethylsilyl ether, the free hydroxy group was converted to the iodide which reacted with triethyl phosphite or trimethyl phosphite to give phosphonates **10a** and **10b**, respectively. Removal of the silyl ethers in **10a** and **10b** was accomplished with tet-



Scheme 1. Reagents and conditions: (a) AcBr, 94%; (b) $P(EtO)_3$, NaI, 88%; (c) EtOH, *p*-TsOH, 91%; (d) (–)-DIPT, $Ti(Oi\text{-}Pr)_4$, cumene hydroperoxide, 84%; (e) $(COCl)_2$, DMSO, Et_3N; (f) $(EtO)_3CH$, *p*-TsOH, 65% over two steps; (g) Et_2AlCl, LiN_3, 70%; (h) (1) TFA; (2) DHAP, FucA, pH 6.7; (3) acid phosphatase, pH 4.8, 30% based on DHAP; (i) H_2 (50 psi), Pd/C, 35%; (j) (1) TMSBr; (2) H_2O, THF, 79%.



Scheme 2. (a) TBDMSCl, Et_3N, 90%; (b) PPh_3, I_2, imidazole, 94%; (c) $P(OEt)_3$, 77% (**10a**); $P(OMe)_3$, NaI, 92% (**10b**); (d) TBAF/AcOH, 94% (**11a**); AcOH, 100% (**11b**); (e) *m*CPBA, 60 % (**11a**); 63% (**11b**); (f) $(COCl)_2$, DMSO, Et_3N (**12a**); Dess-Martin (**12b**); (f) $(EtO)_3CH$, *p*-TsOH, over two steps, 65% (**12a**); 74% (**12b**); (g) NaN_3, NH_4Cl, 57% (**12a**); 98% (**12b**); (h) (1) 0.1N HCl, 53 °C; (2) DHAP, FDP aldolase, pH 6.7; (3) acid phosphatase, pH 4.8, 85% ; (i) H_2 (50 psi), Pd/C.

rabutylammonium fluoride(TBAF)/acetic acid and acetic acid, respectively. Epoxidation with 4-chloroperoxybenzoic acid (*m*CPBA) provided epoxy alcohols **11a** and **11b**. Dess-Martin oxidation of the hydroxy group, protection of the resulting aldehyde as the diethyl acetal and regioselective epoxide opening with sodium azide (NaN_3) yielded azido acetals **12a** and **12b**. Under the reaction conditions (NaN_3/ammonium chloride), partial deprotection to the monoethyl phosphonic acid was observed in the case of the **12a** while only the monomethyl phosphonic acid **12b** was obtained. Deprotection of the diethyl acetal of **12a** and FDP aldolase-catalyzed condensation with DHAP gave azido sugar phosphonate **13a** in 20 – 36% yield (based on DHAP). On the other hand, condensation of DHAP with the aldehyde obtained from **12b** proceeded with excellent yield (85% based on DHAP) and diastereoselectivity. Dephosphorylation catalyzed by acid phosphatase gave azido-sugar phosphonate **13b**. The higher yield and diastereoselectivity of the reaction with aldehyde derived from **12b** could be a result of monomethyl phosphonic acid functionality mimicking the phosphate moiety present in the natural substrate of the FDP aldolase, D-glyceraldehyde-3-phosphate.[22] Although no detailed studies on the substituent R^1 were conducted, it appeared that the enzyme prefers a small size. Hydrogenation of **13b** in the presence of palladium on carbon furnished the expected iminocyclitol phosphonic acid **2** as the major product.

In summary, fuculose 1-phosphate and fructose-diphosphate aldolases have been demonstrated to be useful catalysts to provide efficient chemoenzymatic routes to iminocyclitols possessing the phosphonic acid moiety. These compounds are difficult to prepare by other means. Although the yields for the aldolase reactions are not high, the reactions involve only a few steps, and improvement of yields can be achieved with use of negatively charged azido phosphonates with a small substituent on the monophosphonate ester group. Other stereoisomers may be prepared with different aldolases. These iminocyclitol phosphonates are useful as core synthons for the development of specific glycosyltransferase inhibitors.

Experimental Section

General Remarks

The reagents used were purchased from Aldrich or Sigma. High resolution mass spectra (HRMS) were recorded on a VG ZAB-ZSE instrument with fast atom bombardment (FAB). ^{1}H NMR spectra were obtained at 400 MHz, ^{13}C NMR at 100 MHz and ^{31}P NMR at 162 MHz on a Bruker AMX 400. Silica gel 60 (230 – 240 mesh) from Mallinckrodt was used for flash column chromatography.

Azidosugar Phosphonate (7)

A solution of acetal **6** (50 mg, 0.147 mmol) in CH_2Cl_2:H_2O (730 μL:4 μL) was cooled to 0 °C and trifluoroacetic acid (70 μL) was then added. After 6 h, Amberlite IRA-400 resin (HO^- form) was added until pH was 4. The resin was removed by filtration and H_2O (540 μL) was added and the CH_2Cl_2 was removed under vacuum. DHAP (200 μL of 0.5 M solution in H_2O, 1 mmol) was added and the pH of the mixture was adjusted to 6.7 with 1.0 N NaOH. Fuc 1-P aldolase (2 units) was added to the mixture and the resulting mixture was stirred at room temperature for 48 h. The pH was adjusted to 4.7 with 1 N NaOH. Acid phosphatase (340 units) was added and the mixture was incubated at 37 °C for 24 h. The reaction mixture was neutralized to pH 7 with 4.0 N NaOH and concentrated. The residue was extracted with MeOH, the extracts were filtered and the filtrate concentrated under vacuum. Purification by flash column (silica, gradient, EtOAc:MeOH:0.002% $CaCl_2$ = 12:1:1 to 6:1:1) gave azido sugar **7** as an oil; yield: 10.6 mg (30% based on DHAP). 1H NMR (D_2O, 400 MHz): δ = 4.14 (t, 1H, *J* = 4.8 Hz), 4.10 – 3.98 (m, 5H), 3.95 – 3.88 (m, 1H), 3.50 – 3.35 (m, 2H), 2.20 – 2.07 (m, 1H), 2.03 – 1.88 (m, 1H), 1.20 (t, 6H, *J* = 7.0 Hz); 13C NMR (D_2O, 100 MHz): δ = 103.0, 82.47, 82.26, 70.54 (d, *J* = 5.8 Hz), 63.76 (d, *J* = 6.6 Hz), 62.30, 58.7 (d, *J* = 6.2 Hz), 26.07 (d, *J* = 143.3 Hz), 15.62 (d, *J* = 6.1 Hz). HRMS (FAB): *m/z* calcd. for $C_{11}H_{22}O_8N_3P$ + Na^+ (M + Na^+): 378.1042; found: 378.1054.

Iminocyclitol Phosphonate (1)

To a solution of iminocycitol phosphonate **8** (15.4 mg, 0.05 mmol) in CH_2Cl_2 (2 mL) were added triethylamine (56 μL, 0.4 mmol), followed by bromotrimethylsilane (40 μL, 0.3 mmol). The resulting solution was stirred for 12 h, then concentrated under vacuum. THF/H_2O (1:1, 1 mL) was added and the reaction mixture was stirred for 3 h, then concentrated and checked by ^{31}P NMR for complete deprotection. The product was purified using QAE Sephadex A-25 anion exchange resin (bicarbonate form) (gradient, H_2O to 100 mM ammonium bicarbonate) to give **1** after repeated coevaporations with water to remove the ammonium bicarbonate; yield: 10.1 mg (79%). ^{1}H NMR (D_2O, 400 MHz): δ = 3.91 (br s, 1H), 3.86 (br s, 1H), 3.76–3.58 (m, 3H), 2.88 (br q, 1H, *J* = 8.2 Hz), 2.77 (t, 1H, *J* = 6.5 Hz), 1.62 (dd, 2H, *J* = 16.7, 6.8 Hz); ^{13}C NMR (D_2O, 100 MHz): δ = 77.73 (d, *J* = 8.7 Hz), 72.68, 71.55, 64.19, 61.57, 57.52, 35.33 (d, *J* = 127.1 Hz); ^{31}P NMR ($CDCl_3$, 162 MHz): δ = 19.42; LRMS (ESI): *m/z* calcd. for $C_7H_{16}NO_7P$ – H^+ (M – H^+): 256; found: 256.

Azidosugar Phosphonate (13b)

A solution of acetal **12b** (0.418 g, 1.42 mmol) in 0.1 N HCl (10.0 mL) was heated at 53 °C. After 6 h, the solution was cooled to room temperature and the pH was adjusted to 6.7 with 5 N NaOH. DHAP (1.9 mL of a 0.375 M solution, 0.712 mmol) was added and the pH of the mixture was readjusted to 6.7. FDP aldolase (182 units, from rabbit muscle) was added to the mixture and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was then neutralized to pH 7 and $BaCl_2 \cdot 2\,H_2O$ (1.08 g,

4.44 mmol) in H_2O (5 mL) was added. The cloudy mixture was kept at 0 °C for 1 h, and the precipitate formed was removed by centrifugation. The supernatant was added to two volumes of acetone and kept at 0 °C for 1.5 h. The precipitate was collected by centrifugation. H_2O (30 mL) was added to the precipitate, followed by cation exchange resin (AG50X-8, H^+). After the mixture was stirred for 15 min, resin was filtered off and the pH of the filtrate was adjusted to pH 4.7 with 5 N NaOH. Acid phosphatase (127 units) was added and the mixture was incubated at 37 °C for 16 h. $BaCl_2 \cdot H_2O$ (0.2 g) in H_2O (2 mL) was added and the solid formed was removed by centrifugation. The supernatant was neutralized to pH 7 with 5.0 N NaOH and concentrated under vacuum. The residue was extracted with CH_2Cl_2:MeOH (1:2) and the extracts were concentrated under vacuum. The residue obtained was lyophilized to give compound **13b** as a pale yellowish solid; yield: 198 mg (85%, based on DHAP). ^{1}H NMR (D_2O, 400 MHz): δ = 4.21 (dd, *J* = 8.1, 8.1 Hz, 1H, H-4), 4.10 (d, *J* = 8.1 Hz, 1H, H-3), 3.80 – 3.55 (m, 7H, H-1a, H-1b, H-5, H-6, POC*H*$_3$), 2.10 – 1.96 (m, 2H, H-7a, H-7b); ^{13}C NMR (D_2O, 100 MHz): δ = 100.12, 84.85 (δ, *J* = 15.1 Hz), 77.58 (d, *J* = 17.4 Hz), 64.82, 61.58 (d, *J* = 4.2 Hz), 54.17, 51.32, 28.67 (d, *J* = 136.1 Hz); ^{31}P NMR (D_2O, 162 MHz): δ = 25.59; HRMS (FAB): *m/z* calcd. for $C_8H_{16}N_5O_8P + Na^+$ (M + Na^+): 336.0573; found: 336.0564.

Iminocyclitol Phosphonate (2)

A mixture of **13b** (180 mg, 0.575 mmol) and 10% palladium on carbon (50 mg) in EtOH (20 mL) was hydrogenated at 50 psi for 24 h. Filtration and concentration gave the crude product as a yellowish solid. An analytical sample of **2** was obtained as a glassy solid by purification with flash chromatography (silica, 2-propanol:H_2O:NH_4OH, 80:5:0.125, then MeOH:NH_4OH, 50:0.5). However, this purification caused the loss of a large amount of material. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.15 (br, s, 1H), 4.02 (dd, *J* = 11.9, 2.3 Hz, 1H), 3.82 – 3.68 (m, 2H), 3.60 – 3.49 (m, 5H), 3.20 (dt, *J* = 10.3, 2.6 Hz, 1H), 2.12 (ddd, *J* = 22.3, 16.5, 6.6 Hz, 1H), 1.98 (ddd, *J* = 24.7, 16.5, 8.3 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 75.58, 71.50 (δ, *J* = 8.4 Hz), 68.52, 62.61, 61.02, 57.64, 53.85, 27.49 (d, *J* = 131.6 Hz); ^{31}P NMR ($CDCl_3$, 162 MHz): δ = 22.65; HRMS (FAB): *m/z* calcd. for $C_8H_{18}NO_7P + H^+$ (M + H^+): 272.0899; found: 272.0904.

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References and Notes

[1] P. Sears, C.-H. Wong, *Cell. Mol. Life Sci.* **1998**, *54*, 223–252; P. Sears, C.-H. Wong, *Angew. Chem., Int. Ed.* **1999**, *38*, 2301–2324.
[2] A. Varki, *Glycobiology* **1993**, *3*, 97–130.
[3] B. Winchester, G. W. J. Fleet, *J. Carbohydr. Chem.* **2000**, *19*, 471–483; T. D. Heightman, A. T. Vasella, *Angew. Chem.* **1999**, *111*, 794–815; T. D. Heightman, A. T. Vasella, *Angew. Chem., Int. Ed.* **1999**, *38*, 750–770; M. Bols, *Acc. Chem. Res.* **1998**, *31*, 1–8.
[4] B. W. Murray, S. Takayama, J. Schultz, C.-H. Wong, *Biochemistry* **1996**, *35*, 11183–11195.
[5] C. S. Rye, S. G. Withers, *Curr. Opin. Chem. Biol.* **2000**, *4*, 573–580.
[6] M. L. Sinnott, *Chem. Rev.* **1990**, *90*, 1171–1202.
[7] S. Takayama, R. Martin, J. Wu, K. Laslo, G. Siuzdak, C.-H. Wong, *J. Am. Chem. Soc.* **1997**, *119*, 8146–8151.
[8] B. Winchester, G. W. J. Fleet, *Glycobiology* **1992**, *2*, 199; M. Bols *Acc. Chem. Res.* **1998**, *31*, 1–8.
[9] S. Takayama, S. J. Chung, Y. Igarashi, Y. Ichikawa, A. Sepp, R. I. Lechler, J. Wu, T. Hayashi, G. Siuzdak, C.-H. Wong, *Bioorg. Med. Chem.* **1999**, *7*, 401–409.
[10] L. Qiao, B. W. Murray, M. Shimazaki, J. Schultz, C.-H. Wong, *J. Am. Chem. Soc.* **1996**, *118*, 7653–7662.
[11] Y. J. Kim, M. Ichikawa, Y. Ichikawa, *J. Am. Chem. Soc.* **1999**, *121*, 5829–5830.
[12] R. Engel, *Chem. Rev.* **1977**, *77*, 349–367.
[13] For the synthesis of 5-membered iminocyclitol phosphonates, see: J.-B. Behr, C. M. Evina, N. Phung, G. Guillerm, *J. Chem. Soc., Perkin Trans. 1* **1997**, 1597–1599; C. Bouix, P. Bisseret, J. Eustache, *Tetrahedron Lett.* **1998**, *39*, 825–828; C. Bouix, P. Bisseret, J. Eustache, *Tetrahedron Lett.* **2000**, *41*, 3269; I. Gautier-Lefebvre, J.-B. Behr, G. Guillerm, N. S. Ryder, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1483–1486.
[14] T. Ziegler, A. Straub, F. Effenberger, *Angew. Chem.* **1988**, *100*, 737–738; T. Ziegler, A. Straub, F. Effenberger, *Angew. Chem., Int. Ed.* **1988**, *27*, 716–717.
[15] R. L. Pederson, M. J. Kim, C. H. Wong, *Tetrahedron Lett.* **1988**, *29*, 4645–4648.
[16] G. C. Look, C. H. Fotsch, C. H. Wong, *Acc. Chem. Res.* **1993**, *26*, 182–190.
[17] T. Katsuki, K. B. Sharpless, *J. Am. Chem. Soc.* **1980**, *102*, 5974–5976.
[18] F. Benedetti, F. Berti, S. Norbedo, *Tetrahedron Lett.* **1998**, *39*, 7971–7974.
[19] E. Garcia-Junceda, G.-J. Shen, T. Sugai, C.-H. Wong, *Bioorg. Med. Chem.* **1995**, *3*, 945–953.
[20] S.-H. Jung, J.-H. Jeong, P. Miller, C.-H. Wong, *J. Org. Chem.* **1994**, *59*, 7182–7184.
[21] L. Qiao, J. C. Vederas, *J. Org. Chem.* **1993**, *58*, 3480–3482.
[22] C.-H. Wong, G. M. Whitesides in *Enzymes in Synthetic Organic Chemistry, Vol. 12*, Elsevier Science, New York, **1994**.