Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2803–2808

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

EXPLORATION OF β -TURN SCAFFOLDING MOTIFS AS COMPONENTS OF SIALYL Le^x MIMETICS AND THEIR RELEVANCE TO P-SELECTIN

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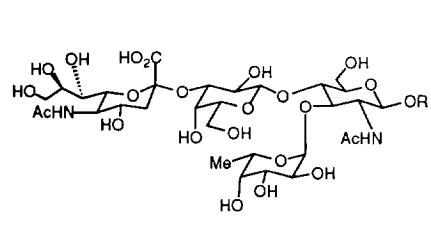
Received 17 July 1998; accepted 28 August 1998

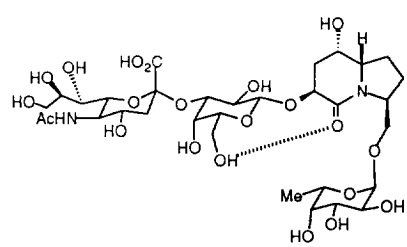
Abstract: Monocyclic and bicyclic lactam units representing β-turn surrogates were incorporated into a sialyl Le^x structure by replacement of the natural sugar components. Low micromolar activity was found in a new P-selectin binding assay.

As a result of the potential therapeutic benefits of discovering discrete structures that are capable of mimicking sLe^x **1** in cell adhesion processes,[1] much effort had been expended in the synthesis of simplified analogs.[2] In spite of the large variety of chemical, structural, and functional modifications of sLe^x itself, and the design of related structures, progress in the discovery of novel structural entities in this area has been relatively modest, mainly due to the complexity of the process of recognition and the apparent specificity of the natural substrate itself.[3]

Figure 1.



1, Sialyl Le^x



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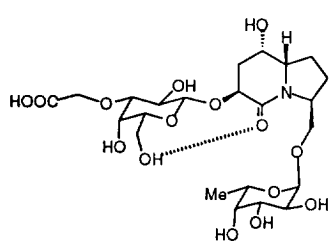

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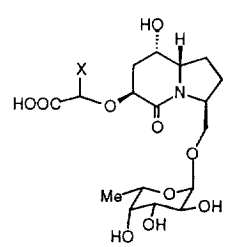
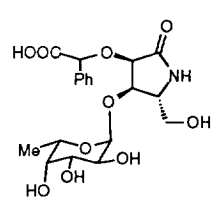


4, X = Ph

5, X = H



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Continuing our interest in the design of glycomimetic motifs related to sLe^x,[4] we report herein the synthesis of analogs and novel entities in which one or more sugar residue has been replaced by a functionally

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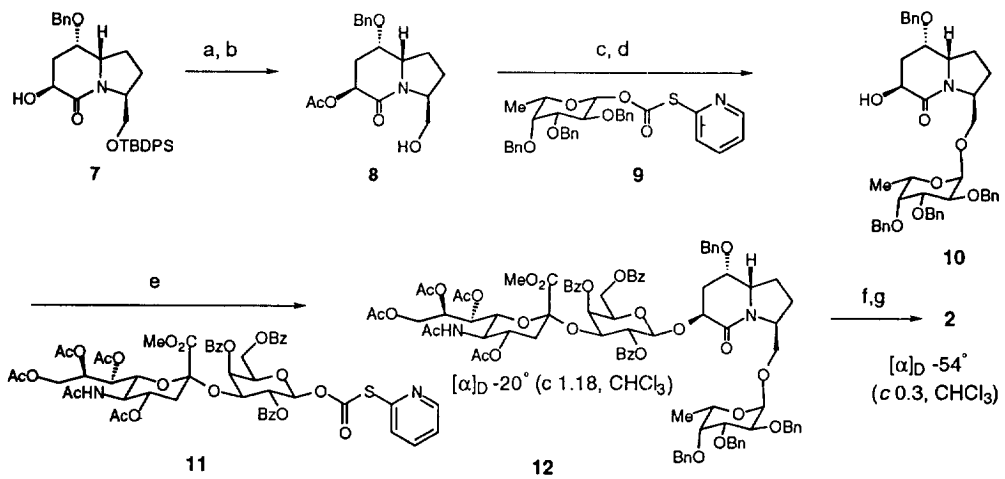
mimetic unit. Specifically, we report on the incorporation of an indolizidinone-type unit as a β-turn-like scaffold,[5] and of a hydroxylated γ-lactam[6] to replace the GlcNAc and the D-gal-GlcNAc disaccharide residues respectively (Figure 1).

Inspection of molecular models and a study of available data[7] on the conformation of sLe^x led us to consider compounds **2-6** as plausible mimetics. In particular, we were intrigued by the possibility of maintaining an orientation of the D-gal unit in **2** and **3** that approximated the natural disposition of the D-gal-GlcNAc disaccharide residue, due to an anticipated H-bonding between the C-6 hydroxymethyl group and the indolizidinone carbonyl as shown in Figure 1. To the best of our knowledge, the incorporation of β-turn-like motifs in sLe^x mimetics has not been addressed. The choice of the indolizidinone and γ-lactam scaffolds was based on the expectation that the α-fucosyl and other residues shown in structures **2-6** would have an orientation similar to that found in sLe^x itself.

The indolizidinone motif **7** previously synthesized from L-pyroglutamic acid[8] was α-fucosylated with the newly introduced thiocarbonate activated donor **9**,[9] and the resulting glycoside was deacetylated to give **10**, which we used as a versatile scaffold for introducing diversity at the sialyl-D-gal site in the original sLe^x (Scheme 1).

Scheme 1.

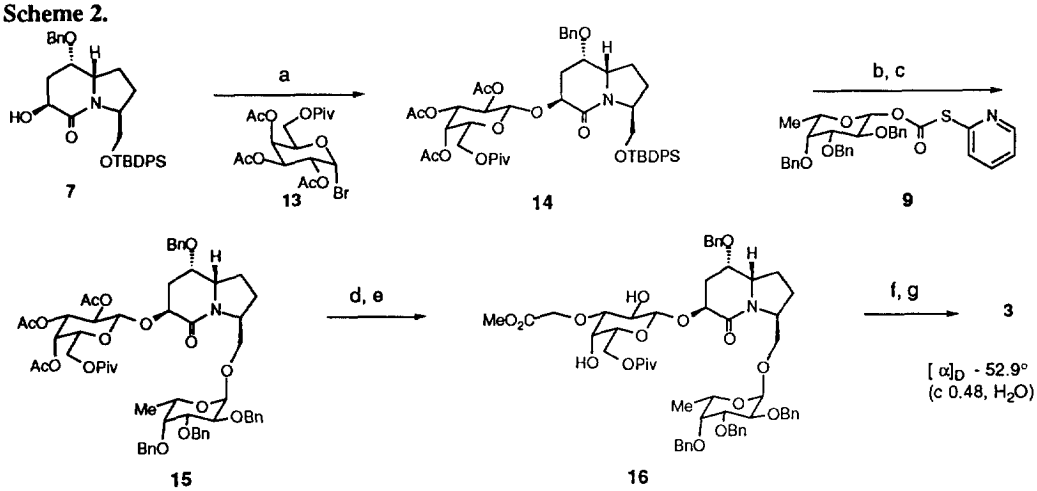


a. Ac_2O, Py, rt, 1 h, 90% ; b. HF, Py, 0 °C, 4 h, 78%; c. $CuBr_2$, Bu_4NBr, CH_2Cl_2, rt, 16 h, 70%; d. NaOMe, rt, 1 h, 91%; e $CuBr_2$, Bu_4NBr, CH_2Cl_2, rt, 24 h, 83%; f. $Pd(OH)_2/C$, H_2, MeOH, rt, 12 h, 80%; g. NaOMe, rt, 5 h, then Dowex-50 (H^+), rt, 2 h, quant.

Treatment of **10** with the 2-pyridylthiocarbonate activated sialyl-D-gal residue[9] **11** afforded an excellent yield of the pseudotetrasaccharide**12**. Removal of the protective groups led to the indolizidinone sLe^x mimetic **2**. Alternatively, formation of the β-galactoside **14** was achieved under standard conditions (Scheme 2). Desilylation followed by α-fucosylation[9] led to the pseudotrisaccharide **15** after separation from a minor amount of the undesired β-fucoside (~5/1 α/β). Partial deesterification, stannylidene formation, and site-selective alkylation with methyl bromoacetate led to the corresponding ether analog **16**. Removal of the protective groups in the usual manner led to the desired pseudotrisaccharide **3** in which the sialyl residue is replaced by a glycolic acid unit.[10]

We next addressed the synthesis of simpler α-fucosyl glycosides in which the sialyl-D-gal-GlcNAc residue was replaced by the indolizidinone scaffold with an acidic appendage (Scheme 3). Thus, the α-fucoside **10**

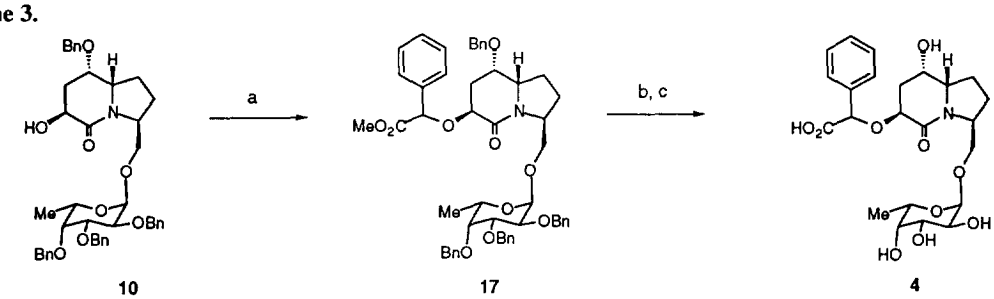
Scheme 2.



a. AgOTf, tetramethylurea, -78 °C, 2 h, 60%; b. HF, Py, rt, 4 h, 75%; c. $CuBr_2$, Bu_4NBr, CH_2Cl_2, rt, 16 h, 71%; d. NaOMe, 0 °C, 5 h, 80%; e. Bu_2SnO, then methyl bromoacetate, 100 °C, 12 h, 50%; f. $Pd(OH)_2/C$, H_2, MeOH, rt, 12 h, quant; g. MeONa, MeOH, rt, 5 h, then 0.1 N NaOH, then Dowex-50 (H^+), quant.

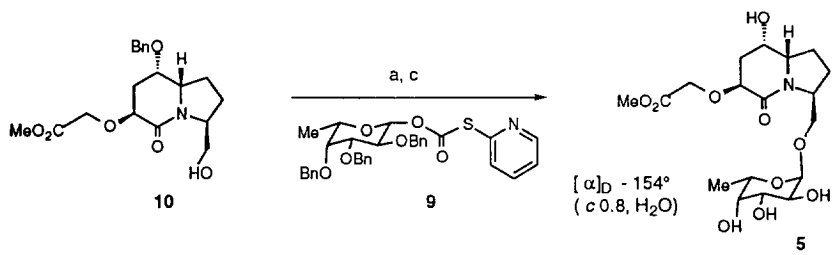
was treated with methyl α-diazophenylacetate in the presence of rhodium diacetate[11] to afford a 3/1 diastereomeric mixture of mandelate ethers **17** that could be chromatographically separated. The mixture of ethers was subjected to a two-step deprotection protocol to afford the mimetic **4** as a mixture of diastereomers. In order to have an appreciation for the importance of the functional requirements at the acidic appendage in **4**, which was included to simulate the sialyl residue, we also prepared the glycolate ether **5** (Scheme 4). Alkylation of **7** under standard conditions, followed by desilylation led to the alcohol **10**, which was subjected to sequential α-fucosylation and deprotection to give **5**.

Scheme 3.



a. Methyl α-diazo phenylacetate, $Rh_2(OAc)_4$, rt, 12 h, 93%; b. $Pd(OH)_2/C$, H_2, MeOH, rt, 12h, quant.; c. 0.1 N NaOH, rt, 3 h then Dowex-50 (H^+), quant.

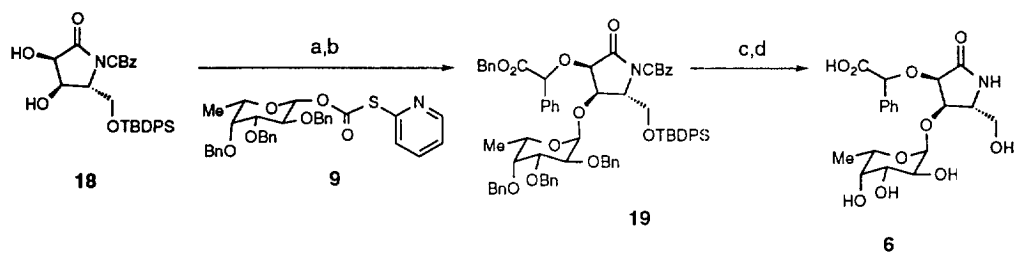
Scheme 4.



a. $CuBr_2$, Bu_4NBr, CH_2Cl_2, rt, 16 h, 70%; b. $Pd(OH)_2/C$, H_2, MeOH, rt, 12 h, 80%; c. 0.1N NaOH, rt, 2 h, 90%; then Dowex-50 (H^+).

Finally, we also prepared a simpler lactam scaffold analog **6** as illustrated in Scheme 5. Thus, α-fucosylation of the lactam diol[12] **18** was selective (3/1) at the C-3 hydroxy group. Etherification at the C-4 hydroxy group via the α-diazo ester protocol gave the protected pseudodisaccharide **19** which was deprotected to afford the desired mimetic **6** as a mixture of diastereomers at the mandelate ether carbon atom.

Scheme 5.



a. $CuBr_2$, Bu_4NBr, CH_2Cl_2, rt, 16 h, 60%; b. benzyl α-diazophenylacetate, $Rh_2(OAc)_4$, rt, 12 h, 70%; c. TBAF, AcOH, rt, 6 h, 90%; d. $Pd(OH)_2/C$, H_2, MeOH, rt, 12 h, 85%.

E and P Selectin binding: In spite of their favorable molecular juxtaposition with the natural substrate, the conformationally constrained lactam motifs used as replacements of each unit except the α-fucosyl residue in sLe^x were found to be devoid of E-selectin binding activity (IC_{50} >10 μM). Analysis[13] of the conformational preferences of the analogs **2-6** using the systematic multiple minimum (SUMM) method[14] implemented in Macromodel[15] revealed that the inactive mimic **2**, taken as a representative model, did not have any low energy conformers near the bioactive conformational space found for sLe^x.[4]

The binding of indolizidinone sLe^x mimetics to E- and P-selectin was measured by competitive binding assays.[16] Whereas compounds **2, 3**, and **5** did not show any affinity for E-selectin, their activity for P-selectin[17] was in the low μM range while that of sLe^x was in the mM range. The compounds are currently being further evaluated in *in vitro* and *in vivo* models and the results will be published in due course. Although less is known regarding the points of interaction of sLe^x and its analogs with the P-selectins,[18] the results with the prototypical scaffold analogs reported in this Letter are indeed encouraging. Promising data in the literature[2,19] with simplified sLe^x structures particularly in the case of E-selectins, are steps in the right direction, but the issues of biological results and their pertinence are complicated by the intrinsically weak binding affinities and the lack of uniformly acceptable and general testing protocols applicable in laboratories at different geographic locations.

Acknowledgments. We thank NSERCC for financial assistance through the Medicinal Chemistry Chair Program.

References and Notes.

1. For selected reviews, see Lasky, L. A. *Ann. Rev. Biochem.* **1995**, *64*, 113; Bevilacqua, M-P. *Ann. Rev. Immunol.* **1993**, *11*, 767; Musser, J. H. *Ann. Rev. Med. Chem.* **1992**, *27*, 301 and references cited therein.
2. For a recent review, see Simanek, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong C.-H. *Chem. Rev.* **1998**, *98*, 833; Banteli, R.; Ernst, B. *Tetrahedron Lett.* **1997**, *38*, 4059; see also, Tsukida, T.; Hiramatsu, Y.; Tsujishita, H.; Kiyoi, T.; Yoshida, M.; Kurokawa, K.; Moriyama, H.; Ohmoto, H.; Wado, Y.; Saito, T.; Kondo, H.; *J. Med. Chem.* **1997**, *40*, 3534; Kolb, H. C. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2629; Ohmoto, H.; Nakamura, K.; Inoue, T.; Kondo, N.; Inoue, Y.; Yoshino, K.; Kondo, H.; Ishida, H.; Kiso, M.; Hasegawa, A. *J. Med. Chem.* **1996**, *39*, 1339; Wang, R.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 5427; Lin, C.-C.; Shimazaki, M.; Heck, M.-P.; Aoki, S.; Wang, R.; Kimura, T.; Ritzen, H.; Takayama, S.; Wu, S.-H.; Weitz-Schmidt, G.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 6826; Bamford, M. J.; Bird, M.; Gore, P. M.; Holms, D. S.; Priest, R.; Prodger, J. C.; Saez, V. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 239; Toepfer, A.; Kretzschmar, G.; Bartnik, E. *Tetrahedron. Lett.* **1995**, *36*, 9161; Kaila, N.; Yu, H.-A.; Xiang, Y. *Tetrahedron Lett.* **1995**, *36*, 5503.
3. Phillips, M. L.; Nudelman, E.; Gaeta, F. C. A.; Perez, M.; Singhal, A. K.; Hakomori, S. I. *Science* **1990**, *250*, 1130; Tyrell, D.; James. P.; Rao, N.; Foxall, C.; Abbas, S.; Dasgupta, F.; Nashed, M.; Hasegawa. A.; Kiso, M.; Asa, D.; Kidd, J.; Brandly, B. K. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10372; Ramphal, J. Y.; Hiroshige, M.; Lou, B.; Gaudino, J. J.; Hayashi, M.; Chen, S. M.; Chiang, L. C.; Gaeta, F. C. A.; De Frees, S. *J. Med. Chem.* **1996**, *39*, 1359.
4. Hanessian, S.; Reddy, G. V.; Huynh, H. K.; Pan, J.; Pedatella, S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2729; Hanessian, S.; Prabhanjan, H. *Synlett* **1994**, 868.
5. For recent reviews see Hanessian, S.; McNaughton-Smith, G.; Lombart, H.-G.; Lubell, W-D. *Tetrahedron* **1997**, *53*, 12789; Gante, G. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1699; Adang, A. D. P.; Hermkens, P. H. H.; Linders, J. T. M.; Ottenheijm, H. C. J.; van Stavern, C. J. *Rec. Trav. Chim. Pays-Bas* **1994**, *113*, 63; Rizo, G.; Giersach, L. M. *Ann. Rev. Biochem.* **1992**, *61*, 387, and the references cited therein.
6. For a recent example of the incorporation of a heterocycle unit in the sLe^x structure, see Huang, H.; Wong, C.-H. *J. Org. Chem.* **1995**, *60*, 3100.
7. (a) Janke, N.; Kolb, H.C.; Blommers, M. J. J.; Magnani, J. L.; Ernst, B. *Angew. Chem. Int. Ed.* Engl. **1997**, *36*, 2603; (b) Thoma, G.; Schwarzenbach, F.; Duthaler, R. O. *J. Org. Chem.* **1996**, *61*, 514; (c) Scheffer, K.; Ernst, B.; Katopodis, A.; Magnani, J. L.; Wang, W. T.; Weisemann, R.; Peters, T. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1841; (d) Cooke , R. M.; Hale, R. S.; Lister, S. G.; Shah, G.; Weir, M. *Biochemistry* **1994**, *33*, 10591; (e) Liu, Y.-C.; Hummel, C. W.; Huang, C.-H.; Ichikawa, Y.; Nicolaou, K. C.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *144*, 5452.
8. Hanessian, S.; McNaughton-Smith, G. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1567; Hanessian, S.; Huynh, H. K.; Balaux, E. unpublished results.
9. Lou, B.; Huynh, H. K.; Hanessian, S. In *Preparative Carbohydrate Chemistry;* Hanessian, S., Ed.; Dekker: New York, **1996**, p 431.

10. For related replacements, see Liu, A.; Dillon, K.; Campbell, R. M.; Cox, D. C.; Huryn, D. M. *Tetrahedron Lett.* **1996**, *37*, 3785; Prodger, J. C.; Bamford, M. J.; Gore, P. M.; Holmes, D. S.; Saez, V.; Ward, P. *Tetrahedron. Lett.* **1995**, *36*, 2339; Ragan, J. A.; Cooper, K.; *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2563 Yuen, C. T.; Lawson, A. M.; Chai, W.; Larkin, M.; Stoll, M. S.; Stuart, A. C.; Sullivan, F. Y.; Ahern, T. J.; Feizi, T. *Biochemistry* **1992**, *31*, 9126.
11. For pertinent references, see Adams, J.; Spero, D. M. *Tetrahedron* **1991**, *47*, 1769.
12. Woo, K.-C.; Jones, K. *Tetrahedron Lett.* **1991**, *32*, 6949.
13. Kolb, H. C.; Ernst, B. *Chem. Eur. J.* **1997**, *3*, 1571; Kolb, H. C.; Ernst, B. *Pure Appl. Chem.* **1997**, *69*, 1879.
14. Goodman, J. M.; Still, W. C. *J. Comput. Chem.* **1991**, *12*, 1110.
15. F. Mohamadi; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Caufield, C.; Chang, G.; Hendrickson, T.;. Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.
16. Cell-free E-selectin binding assay: Wells in a microtiter plate (pale 1, Falcon probind) are coated with E-selectin/hlg chimera by incubation of 100 mL of the purified chimeric protein at a concentration of 200 ng/well in 50 mM Tris, 0.15 M NaCl, 2 mM $CaCCl_2$, pH 7.4 (Tris-Ca^{2+}). After 2 h, 100 mL of a 1:1 mixture of 1% BSA in Tris-Ca^{2+} and Stabilcoat are added to each well and incubated at 22 °C to block nonspecific binding. During this incubation, inhibitory test compounds, diluted in Tris-Ca^{2+}, 1% BSA, are titrated by a 2-fold serial dilution in a second U-shaped bottom low-bind microtiter plate (plate 2, Costar, Inc.). An equal volume of a preformed complex of a biotinylated sialyl Lewis^a polymer **4** and horseradish peroxidase-labeled streptavidin (KPL, Gathersburg, MD) at 1 mg/mL in Tris-Ca^{2+}, 1% BSA is added to each well. After 2 h at 22 °C, plate 1 is washed with tris-Ca^{2+} and 100 mL/well are transferred from plate 2 to plate 1. The binding reaction is allowed to proceed for 2 h at 22 °C while rocking. Plate 1 is then washed with Tris-Ca^{2+}, and 100 mL of TMB substrate reagent (KPL, Gaithersburg, MD) is added to each well. After 3 min, the colorimetric reaction is stopped by adding 100 mL/well of 1 M H_3PO_4 and the optical density is determined at 450 nm; Cell-free P-selectin binding assay is based on the sameprotocol.
17. Wilkins, P. P.; McEver, R. P.; Cummings, R. D. *J. Biol. Chem.* **1996**, *271*, 18732.
18. See for example, Alon, R.; Hammer, D. A.; Springer, T. A. *Nature* **1995**, *374*, 539.
19. For pertinent references, see Hiramatsu, Y.; Moriyama, H.; Kiyoi, T.; Tsukida, T.; Inoue, Y.; Kondo, H. *J. Med. Chem.* **1998**, *41*, 2302; Marron, T. G.; Woltering, T. J.; Weitz-Schmidt, G.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 9037; Wy, S.-H.; Shimazaki, M.; Lin, C. C.; More, W. J.; Weitz-Schmidt, G.; Wong, C.-H. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 88; Himura, K.; Kajimoto, T.; Weitz-Schmidt, G.; Ollmann, I.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 9265; Wang, R.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 5427; Dupré, B.; Bui, H.; Scott, I. L.; Market, R. V.; Keller, K. M.; Beck, B. J.; Kogan, T. P. *Bioorg. Med.Chem. Lett.* **1996**, *6*, 569; Allanson, N. M.; Davidson, A. H.; Floyd, C. D.; Martin, F. M. *Tetrahedron: Asymmetry* **1994**, *5*, 2061; see also ref 10.