J|A|C|S
ARTICLES
Published on Web 09/10/2002

Enantiodivergence in Small-Molecule Catalysis of Asymmetric Phosphorylation: Concise Total Syntheses of the Enantiomeric D-*myo*-Inositol-1-phosphate and D-*myo*-Inositol-3-phosphate

Bianca R. Sculimbrene, Adam J. Morgan, and Scott J. Miller*

Contribution from the Department of Chemistry, Merkert Chemistry Center, Boston College, Chestnut Hill, Massachusetts 02467-3860

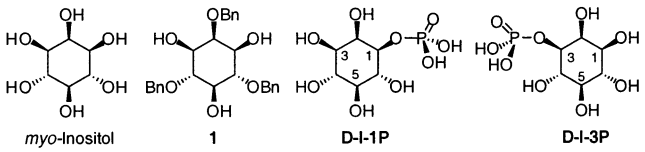
Received June 21, 2002

Abstract: Peptide-based catalysts have been found that catalyze the enantiodivergent phosphorylation of a *meso myo*-inositol-derived triol (**1**). The sequential screening of random peptide libraries, followed by the evaluation of a focused library, led to the identification of two peptides (**2** and **24**) that are complementary in producing enantiomeric D-*myo*-inositol-1-phosphate and D-*myo*-inositol-3-phosphate derivatives. The catalysts were then used to complete efficient total syntheses of both D-I-1P and D-I-3P in optically pure form. Additional information is gleaned from relative rate experiments that unambiguously show the catalysts to afford enantioselection through rate accelerative pathways with respect to simple achiral alkylimidazole catalysts. Furthermore, solvent effect studies show that the two enantiodivergent catalysts exhibit different tolerances of polar media. The systematic discovery of site-selective catalysts establishes a basis for future studies of chiral catalysts that differentiate unique functional groups in polyfunctional molecules.

Introduction

In the field of asymmetric synthesis, a particularly powerful feature of catalytic processes is that catalysts may often be obtained in either enantiomeric form.[1] Thus, the absolute stereochemical identity of a desired reaction product may be selected at the outset of process development through the selection of the appropriate antipode of the catalyst. Less often, although amply precedented, opposite enantioselectivities may be observed through changes in reaction conditions when a unique catalyst is used.[2] In the field of biocatalysis, however, the development of enantiodivergent catalysts is less straightforward. Enzymes are composed almost exclusively of L-amino acids. Fully enantiomeric enzymes, composed of all D-amino acids, are unknown in nature to our knowledge.[3] Nevertheless, an important feature of enzymes is their ability to achieve opposite enantioselectivities through the formation of highly diverse three-dimensional structures, which in turn may provide complementary enantiospecificities.[4]

We have been studying low-molecular weight peptide-based catalysts for a number of transformations with the goal of developing highly selective, readily tuned catalysts for organic synthesis.[5,6] In this context, we are also interested in catalysts that may at once provide high selectivity, wide functional group tolerance, and also site-selectivity. Such catalysts might, in turn, allow for synthetic pathways to polyfunctional targets with a reduced dependence on protecting groups. Because of the interest in all possible stereoisomers of the inositol phosphates, which are important players in cellular signaling events,[7] we have chosen these targets as a testing ground for this objective. Thus, we set out to discover small-molecule peptides that could perform enantiodivergent phosphorylation of a *meso myo*-inositol derivative. In analogy to enzymes, we sought to achieve catalysts that were not enantiomeric, but rather of alternative secondary structure. Reported herein are the results of our experiments that have yielded complementary pentapeptides that are highly enantioselective catalysts in the desymmetrization of a *meso*-inositol substrate **1**.[8] The two catalysts have enabled



concise syntheses of the enantiomeric D-*myo*-inositol-1-phosphate (D-I-1P) and D-*myo*-inositol-3-phosphate (D-I-3P), each in optically pure form.

* Address correspondence to this author. E-mail: scott.miller.1@bc.edu.

(1) For reviews on asymmetric catalysis, see*: Comprehensive Asymmetric Catalysis, Vols. I*−*III*; Jacobsen, E. N., Pfaltz, A., Yamamoto, H., Eds.; Springer-Verlag: Heidelberg, Germany, 1999.

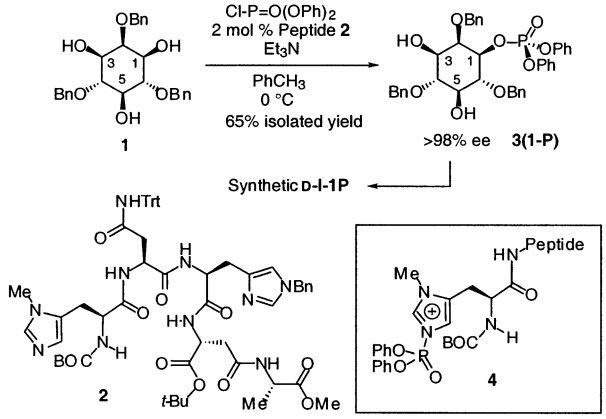
(2) For an excellent review of enantiodivergence in asymmetric synthesis, see: Sibi, M. P.; Liu, M. *Curr. Org. Chem.* **2001**, *5*, 719.

(3) For a synthetic example, see: Milton, R. C. L.; Milton, S. C. F.; Kent, S. B. H. *Science* **1992**, *256*, 1445.

(4) (a) For a "Directed Evolution" approach to modified enzymes with reversed enantioselectivity, see: Zha, D.; Wilensek, S.; Hermes, M.; Jaeger, K.-E.; Reetz, M. T. *J. Chem. Soc., Chem. Commun.* **2001**, 2664. (b) See also: Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Elsevier Science Ltd.: Oxford, U.K., 1994.

(5) Acylation: (a) Copeland, G. T.; Miller, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 6496. (b) Jarvo, E. R.; Copeland, G. T.; Papaioannou, N.; Bonitatebus, P. J., Jr.; Miller, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 11638. Azidation: (c) Guerin, D. J.; Miller, S. J. *J. Am. Chem. Soc.* **2002**, *124*, 2134. (d) Horstmann, T. E.; Guerin, D. J.; Miller, S. J. *Angew. Chem., Int. Ed.* **2000**, *39*, 3635.

Scheme 1

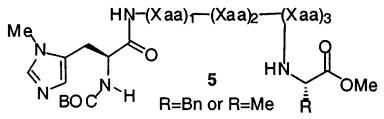


Results and Discussion

Discovery of Enantiodivergent Peptide Catalysts. While the "ideal" synthesis of D-I-3P (or D-I-3P) could rely on a one-step synthesis of the target monophosphate from free *myo*-inositol, we chose triol **1** as a test substrate, since it (a) exhibits excellent solubility in the organic solvents where the peptide-based catalysts we have studied function best[9] and (b) triol **1** contains three, rather than six, potential sites of derivatization. In previously communicated results, we reported that pentapeptide **2** is an effective catalyst for the desymmetrization of 2,4,6-tribenzyl-*myo*-inositol (**1**) through enantioselective phosphorylation (Scheme 1).[10] When 2 mol % catalyst, diphenylchlorophosphate (DPCP) as the phosphorylating reagent, and triethylamine as an HCl scavenger are employed, a highly selective reaction is observed at 0 °C (>98% ee for 3(1-P), 65% isolated yield). Using this catalyst, we completed a concise total synthesis of D-*myo*-inositol-1-phosphate. Because the catalysis proceeds through a putative phosphoryl imidazolium ion (**4**),[11] we referred to the peptide as a kinase mimic, in analogy to the histidine-dependent class of kinases that participates in cell-signaling pathways.[12,13]

Our approach to the discovery of enantiodivergent phosphorylation catalysts employed screening a combination of random and focused libraries. Our initial screen (Figure 1A) of small-peptide catalysts for asymmetric phosphorylation of substrate **1** was based on 39 peptides (tetra- through octapeptides) that contained the nucleophilic residue L-π(Me)-histidine. Within the library were a number of catalysts that selectively phosphorylated the 1-position to give 3(1-P), as well as some that were selective for the enantiotopic 3-position, albeit in lower selectivity. The members of the initial 39-member library were chosen randomly, largely on the basis of sequences we had prepared that were soluble in organic solvents. In addition, they were intended to be highly diverse, since we were searching for leads in the absence of any type of "lead information" on stereoselectivity (*vide infra*). Because such a high percentage of the library exhibited some degree of enantioselectivity, we suspected that expanding the screen to include other unrelated sequences might afford structure-selectivity relationships that could suggest sequences that were particularly suited to phosphorylation of the 3-position. The results of the expanded screen are shown in Figure 1B. As in our previous studies, unpurified peptides were screened at room temperature.

Peptides for both the initial and expanded screens (peptides 1−136) were selected on the basis of sequences that we had previously prepared in our laboratory. In addition to sequences that were biased to form β-turns and β-hairpins in organic solvents,[14,15] we wished to include others that were highly diverse, since we were not sure at the outset what sequences would be appropriate. To achieve diverse sequences, we turned to a randomization algorithm that would afford sequences that were in principle unrelated.[16] We chose pentapeptide **5** as the core structure and then assigned each of the 16 amino acid monomers a letter. The algorithm then delivered 80 random sets



of three-letter combinations. These were inserted into structure **5**, and the members were synthesized for screening.

The results of the expanded screen were striking in that the distribution of catalysts that were selective for the enantiotopic 1- and 3-positions was nearly statistical. For example, in the expanded screen, two new sequences (**6** and **7**) afforded 3(3-P) in >55% ee; likewise, two new sequences were selective for the enantiotopic 3(1-P) in >55% ee. It is important to note that, in desymmetrization reactions such as these, the extent to which the selective formation of the product occurs is related to the overall conversion of the reaction.[18,19] Mindful of this issue, we are cautious not to overinterpret small differences in the ee of the isolated 3(3-P). The conditions of the screen were intended to produce the monophosphate at about 70% conversion. Nevertheless, inspection of Figure 1A and B reveals that the

(6) For other representative metal-free peptide and peptide-like enantioselective catalysts, see: (a) Sigman, M. S.; Jacobsen, E. N. *J. Am. Chem. Soc.* **1998**, *120*, 4901. (b) Iyer, M. S.; Gigstad, K. M.; Namdev, N. D.; Lipton, M. *J. Am. Chem. Soc.* **1996**, *118*, 4910. (c) For a review, see: Jarvo, E. R.; Miller, S. J. *Tetrahedron* **2002**, *58*, 2481.

(7) (a) Agranoff, B. W.; Fisher, S. K. In *Inositol Phosphates and Derivatives: Synthesis, Biochemistry and Therapeutic Potential*; Reitz, A. B., Ed.; ACS Symposium Series 463; American Chemical Society: Washington, DC, 1991; p 20. (b) Billington, D. C. *The Inositol Phosphates: Chemical Synthesis and Biological Significance*; VCH: New York, 1993.

(8) We have observed enantiodivergent peptide catalysis for acyl transfer. See: Copeland, G. T.; Jarvo, E. R.; Miller, S. J. *J. Org. Chem.* **1998**, *63*, 6784.

(9) There are often advantages to employing enzymes in organic solvents also. See: Klibanov, A. M. *Nature* **2001**, *409*, 241−246.

(10) Sculimbrene, B. R.; Miller, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 10125.

(11) For the spectroscopic observation of the NMI−DPCP adduct, see: Mal'tseva, T. V.; Ivanova, E. M.; Korobeinicheva, I. K. *Izv. Sib. Otd. Akad. Nauk. SSSR, Ser. Khim. Nauk.* **1985**, 112−116.

(12) Pirrung, M. C. *Chem. Biol.* **1999**, *6*, R167.

(13) For several examples of His-dependent kinases, see: (a) Bilwes, A. M.; Alex, L. A.; Crane, B. R.; Simon, M. I. *Cell* **1999**, *96*, 131. (b) Mizuguchi, H.; Cook, P. F.; Tai, C. H.; Hasemann, C. A.; Uyeda, K. *J. Biol. Chem.* **1999**, *274*, 2166. (c) Fraser, M. E.; James, M. N.; Bridger, W. A.; Wolodko, W. T. *J. Mol. Biol.* **1999**, *285*, 1633.

(14) (a) Gellman, S. H. *Curr. Opin. Chem. Biol.* **1998**, *2*, 717. (b) Venkatraman, J.; Shankaramma, S. C.; Balaram, P. *Chem. Rev.* **2001**, *101*, 3131.

(15) β-Turns and β-hairpins have been found to exhibit enantioselectivity in a number of other catalytic processes. For example, see: (a) Reference 5. (b) Gilbertson, S. R.; Collibee, S. E.; Agarkov, A. *J. Am. Chem. Soc.* **2000**, *122*, 6522.

(16) (a) Urbaniak, G. C.; Plous, S. *Research Randomizer*, version 2.1 [Internet-based computer program]; Middletown, CT. Retrieved most recently June 19, 2002, from http://www.randomizer.org. (b) The library was initially prepared to screen asymmetric acylations. See: Jarvo, E. R.; Evans, C. A.; Copeland, G. T.; Miller, S. J. *J. Org. Chem.* **2001**, *66*, 5522.

(17) A comprehensive list of the sequences and the screening results are provided in the Supporting Information.

(18) The extent to which the bis(phosphorylated) product is formed serves as a correction mechanism for enantioselectivity. See: Schreiber, S. L.; Schreiber, T. S.; Smith, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 1525.

(19) For reviews of enantioselective desymmetrization, see: (a) Poss, C. S.; Schreiber, S. L. *Acc. Chem. Res.* **1994**, *27*, 9. (b) Willis, M. C. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1765.

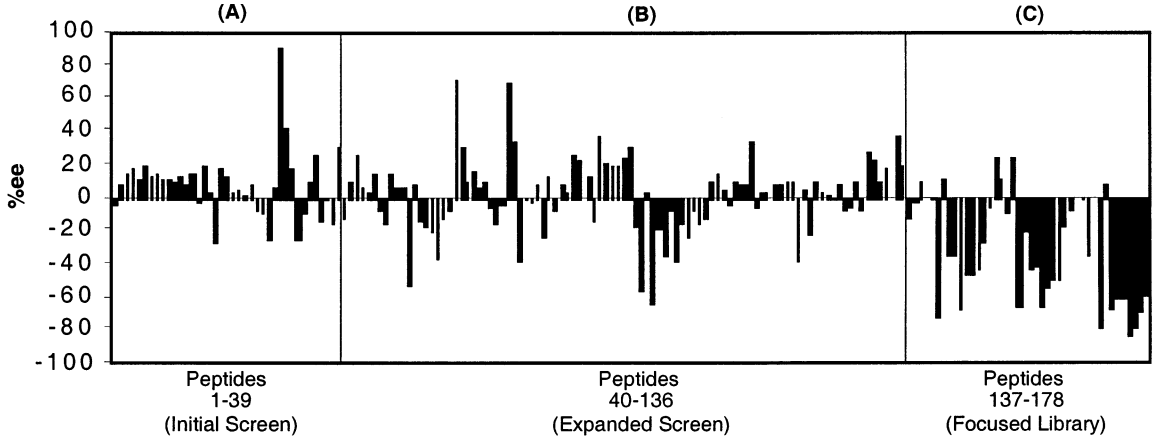
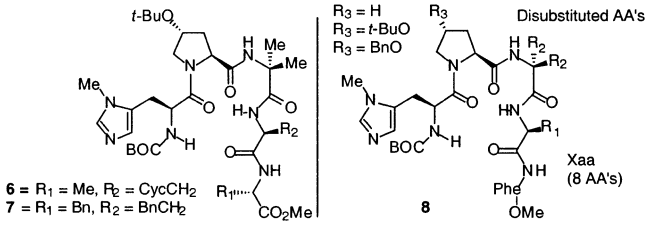


Figure 1. Screening data for enantioselective phosphorylation of triol **1**.

less selective members of the library also distributed themselves into enantiodivergent groups (83 selective for 3(1-P), 51 selective for 3(3-P)). In the context of the selective catalysis for 3(3-P), we focused on peptides **6** and **7**, those moderately selective for 3(3-P). Interestingly, both were in the β-turn family, whereas peptide **2** had its origin in the random library. We then designed a focused library (**8**) around these leads.

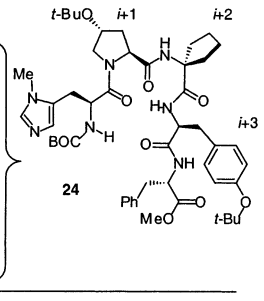


We chose to investigate the role of the *tert*-butoxy group of the L-Hyp residue (R_3) by exchanging it with L-Pro and (Bn)-Hyp in a 42-member library. Likewise, we sought to explore the role of geminal substitution in the *i*+2 position by varying Aib with spirocyclic versions. Finally, in the *i*+3 position, we explored 8 other residues to achieve a 42-member library. The screening data are shown in Figure 1C and Table 1.

Overwhelmingly, the focused library included peptides that were selective for the enantiotopic 3(3-P). The following trends emerged (Table 1). The five-membered spirocyclic residue in the *i*+2 position contributes to a selective catalyst (peptides **10**, **15**, **20**). Furthermore, a preference for *t*-ButTyr at the *i*+3 position emerged (peptides **13**, **18**, **23**). These results taken together suggested that the five-membered ring in combination

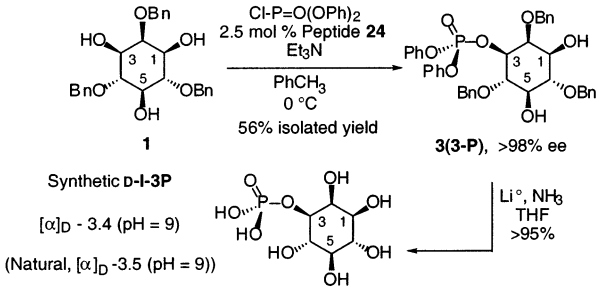
Table 1. Selected Data from Focused Screen 3[a]

Catalyst	*i*+1	*i*+2	*i*+3	3(3-P) ee
7	*t*-BuHyp	Aib	Hfe	65
10	"	spiro-5	"	84
11	"	spiro-6	"	78
12	"	spiro-7	"	36
13	"	Aib	*t*-BuTyr	79
14	BnHyp	Aib	Hfe	55
15	"	spiro-5	"	66
16	"	spiro-6	"	42
17	"	spiro-7	"	10
18	"	Aib	*t*-BuTyr	66
19	Pro	Aib	Hfe	47
20	"	spiro-5	"	68
21	"	spiro-6	"	35
22	"	spiro-7	"	0
23	"	Aib	*t*-BuTyr	72



[a] The screen was performed with unpurified catalysts (2.5 mol %, 25 °C), and runs were uniformly quenched after 6 h. See Supporting Information for details.

Scheme 2



with *t*-ButTyr at the *i*+3 position could yield a superior catalyst. Since our focused library did not include catalyst **24**, we prepared it by independent synthesis. Catalyst **24** is indeed highly selective for the formation of 3(3-P), affording the product in 94% ee at 70% conversion under the unoptimized conditions of the screen.

With an effective catalyst in hand, we were able to complete a concise total synthesis of D-I-3P (Scheme 2) in analogy to our synthesis of enantiomeric D-I-1P. Thus, desymmetrization of triol **1** on a 0.5-gram scale proceeds to give 3(3-P) with total enantiopurity (>98% ee, 56% isolated yield) when catalyst **24** is used under optimized conditions (2.5 mol %, 0 °C, 4 h). Phosphate 3(3-P) is then deprotected in one step to give synthetic D-I-3P whose characteristics match the literature values.[20]

Relative Rates versus NMI and DMAP. The mechanistic aspects of the enantioselective phosphorylation catalysts are currently of interest to our laboratory. In our previous studies of enantioselective peptide-based acylation catalysis, we have documented that the basis of the selectivity is due to enantiomer-specific rate acceleration.[5] To determine if the enantioselectivities exhibited by peptides **2** and **24** could be likewise due to rate acceleration in comparison to the case of *N*-methylimidazole (NMI), we performed a direct comparison of NMI, *N*,*N*-(dimethylamino)pyridine (DMAP), and peptides **2** and **24**. The results of the head-to-head comparison are shown in Figure 2. The data reveal that the catalytic peptides are indeed more active as catalysts than the simple NMI core structure. Furthermore, each peptide is substantially more active than DMAP. Also, noteworthy is that the two peptides exhibit comparable relative activities, consistent with the fact that each is highly selective, albeit in opposite directions in terms of enantiospecificity. While

(20) Billington, D. C.; Baker, R.; Kulagowski, J. J.; Mawer, J. M. *J. Chem. Soc., Chem. Commun.* **1987**, 314.

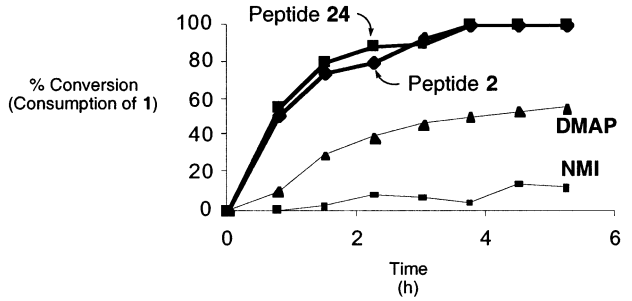


Figure 2. Kinetic comparison of peptide-based catalysts **2** and **24** with DMAP and NMI. Reactions were run at 0 °C with 2.5 mol % of each catalyst. The data shown are the average of two runs. The conversions were determined by ^{1}H NMR.

the exact basis of the specific rate acceleration is not known at this time, it appears that the peptide secondary structures play a key kinetic role in assembling catalysts that are not only selective but also highly active.

Solvent Effects. While the two catalysts are both highly active and selective, there are substantial differences between the two. In anticipation of future studies of other polyol substrates that are not soluble in nonpolar solvents, we have been investigating short peptide sequences that will afford high selectivity and activity in solvents of higher dielectric constant. These experiments have unearthed key differences between peptides **2** and **24** (Table 2). For example, when the desymmetrization of substrate **1** is performed in solvents of varying polarities, pentapeptide **2** shows a relatively robust profile. Enantioselectivity in the product is observed at 95% ee or higher, whether the reaction is conducted in toluene, Et_2O, CH_2Cl_2, THF, or CH_3CN (entries 1−5). Only in DMF is the course of the reaction dramatically altered, and no product formation is observed (entry 6). On the other hand, pentapeptide **24** exhibits significant sensitivity to the nature of the medium. Whereas high selectivity is observed in toluene (>98% ee, entry 1), the product exhibits eroded optical purity in Et_2O (80% ee, entry 2), CH_2Cl_2 (37% ee, entry 3), and THF (35% ee, entry 4). With peptide **24**, no reaction is observed in CH_3CN (entry 5). We speculate that the differential behavior may be due to the different capacity of each peptide to maintain optimal conformations during the course of the catalytic reactions. Nonpolar media are well established to promote robust secondary structures to short peptide sequences.[21] These results support the notion that certain sequences (e.g., **2**) may be particularly suited to a conformational stability, while others may not (e.g., **24**). Enantioselective phosphorylation catalysts that function over a broad range of solvent polarities may be particularly suited to future applications involving substrates that are truly polyfunctional and that may exhibit poor solubility in low-dielectric solvents such as toluene.

(21) Haque, T. S.; Little, J. C.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 6975.

Table 2. Effect of Solvent on Catalytic Desymmetrization of Triol **1**[a]

entry	solvent	peptide **2** (D-I-1-P) (% ee)	peptide **24** (D-I-3-P) (% ee)
1	toluene	>98	>98
2	Et_2O	98	80
3	CH_2Cl_2	98	37
4	THF	95	35
5	CH_3CN	96	no rxn
6	DMF	no rxn	

[a] All reactions were run at 25 °C for 6−8 h.

Conclusion

Through screening a combination of random and focused libraries, we have discovered small-molecule peptides that can select for either the 1-position or the enantiotopic 3-position during catalytic phosphorylation of an inositol derivative. Because each catalyst contains the same chirality of the nucleophilic His residue, the enantiodivergence is perhaps due to different transition state secondary structures. We are now in pursuit of these conformations in order to understand the basis of the enantiodivergence. Furthermore, we are pursuing additional catalysts that allow for enantio- and regioselective functionalization of other polyfunctionalized molecules. The ability to rapidly identify catalysts for enantio- and site-selective phosphorylation of a triol substrate may serve as the basis for a general approach to the discovery of catalysts for site-specific modification of these and other polyfunctional substrates.[22]

Acknowledgment. This research is supported by the NIH (GM-57595). We are also grateful to the NSF, DuPont, Eli Lilly, and Merck for research support. S.J.M. is a Fellow of the Alfred P. Sloan Foundation and a Camille Dreyfus Teacher-Scholar.

Supporting Information Available: Experimental procedures and product characterization for all new compounds synthesized. This material is available free of charge via the Internet at http://pubs.acs.org.

JA027402M

(22) Gani, D. *Nature* **2001**, *414*, 703.