ORGANIC LETTERS

2004
Vol. 6, No. 26
4921−4924

Mono- and Bivalent Ligands Bearing Mannose 6-Phosphate (M6P) Surrogates: Targeting the M6P/ Insulin-Like Growth Factor II Receptor

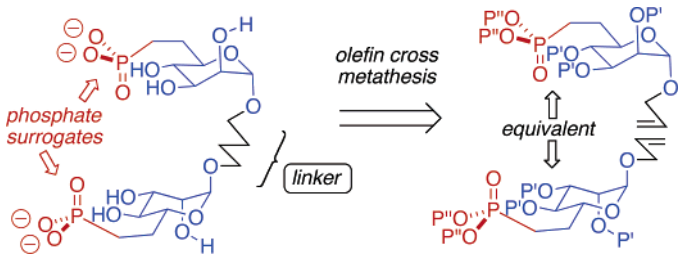
David B. Berkowitz,*,† Gourhari Maiti,† Bradley D. Charette,† Christine D. Dreis,‡ and Richard G. MacDonald*,‡

Department of Chemistry, University of Nebraska, Lincoln, Nebraska 68588-0304, and Department of Biochemistry & Molecular Biology, University of Nebraska Medical Center, Omaha, Nebraska 68198-5870

dbb@unlserve.unl.edu; rgmacdon@unmc.edu

Received October 6, 2004

ABSTRACT



Mannose 6-phosphate mimics locked into the α-configuration and bearing hydrolase-resistant phosphate surrogates were synthesized and evaluated for binding affinity to the mannose 6-phosphate/insulin-like growth factor II receptor (M6P/IGF2R). Affinity increases as the phosphate surrogate is varied in the order malonyl ether < malonate < phosphonate. An alkene cross-metathesis approach to sought-after *bivalent* M6P-bearing ligands is also described. These compounds were designed to map onto biantennary sectors of high-mannose-type oligosaccharides carried by glycoprotein M6P/IGF2R ligands.

Mammalian cells possess two receptors for mannose 6-phosphate (M6P)-decorated glycoproteins; namely, (i) the small 46 kDa cation-dependent mannose 6-phosphate receptor (CD-MPR) and (ii) the large 300 kDa cation-independent, mannose 6-phosphate/insulin-like growth factor II receptor (CI-MPR or M6P/IGF2R).[1] Both serve to mediate the trafficking of hydrolytic Golgi enzymes. In addition to its M6P-binding site, the CI-MPR also possesses a distinct binding site for IGF-II. Its ability to internalize circulating IGF-II has led to the suggestion that the CI-MPR may be a tumor suppressor gene.[2] IGF-II internalization appears to be accelerated through the binding of bi- or multivalent high-affinity ligands to the M6P sites of the receptor.[3]

Consistent with the important role this receptor plays in cell growth and motility, altered structure, function, or expression of the CI-MPR is seen in a number of cancers, including those of the liver,[4] lung,[5] GI tract,[6] and breast.[7] A mouse model shows that CI-MPR deficiency leads to cardiac hyperplasia.[8]

Whereas crystal structures are available for the free CD-MPR, and its complexes with M6P and with pentamannosyl phosphate (PMP),[9] the much larger CI-MPR has proven to be a greater challenge. The CI-MPR possesses 15 homolo-

† University of Nebraska, Lincoln.
‡ University of Nebraska Medical Center.

(1) For a review, see: Ghosh, P.; Dahms, N.; Kornfeld, S. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 202−213.
(2) (a) O'Gorman, D. B.; Weiss, J.; Hettiaratchi, A.; Firth, S. M.; Scott, C. D. *Endocrinology* **2002**, *143*, 4287−4294. (b) Hankins, G. R.; De Souza, A. T.; Bentley, R. C.; Patel, M. R.; Marks, J. R.; Iglehart, J. D.; Jirtle, R. L. *Oncogene* **1996**, *12*, 2003−2009.
(3) York, S. J.; Arneson, L. S.; Gregory, W. T.; Dahms, N. M.; Kornfeld, S. *J. Biol. Chem.* **1999**, *274*, 1164−1171.
(4) Yamada, T.; De Souza, A. T.; Finkelstein, S.; Jirtle, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 10351−10355.
(5) (a) Tsujiuchi, T.; Sasaki, Y.; Tsutsumi, M.; Konishi, Y. *Mol. Carcinog.* **2002**, *36*, 32−37. (b) Kong, F.-M.; Anscher, M. S.; Washington, M. K.; Killian, J. K.; Jirtle, R. L. *Oncogene* **2000**, *19*, 1572−1578.
(6) Pavelic, K.; Kolak, T.; Kapitanovic, S.; Radosevic, S.; Spaventi, S.; Kruslin, B. *J. Pathol.* **2003**, *201*, 430−438.

10.1021/ol0479444 CCC: $27.50
Published on Web 12/03/2004

gous extracytoplasmic repeats, each of which resembles the single such domain of the CD-MPR. Of these, there are two known high-affinity M6P binding sites at domains 3 and 9.[10] The receptor is likely a dimer,[3,11] implying that ligands could have accessibility to several M6P binding pockets. Just this year, the first X-ray structures of an M6P-binding CI-MPR fragment, domain 1−3 constructs, with and without bound M6P, were reported.[12]

Native glycoprotein ligands for the M6P site(s) on the CI-MPR bear mannose-rich, N-linked oligosaccharides that are derived from the characteristic triantennary undecasaccharide illustrated in Figure 1. In glycopeptide ligands that bind well

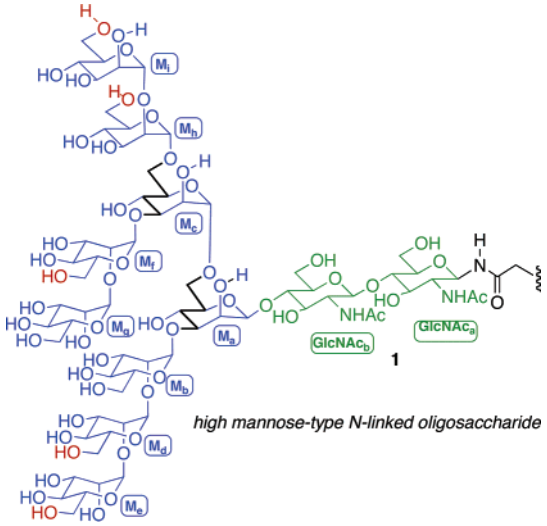


Figure 1. Structure of high-mannose-type oligosaccharides as found in N-linked glycoproteins. Green = *N*-acetylglucosamine; blue = mannose (labeling follows Kornfeld ref 13); red = possible phosphorylation sites).

to the MPRs, the nonamannopyranose run has been phosphorylated and trimmed. In pioneering work, Kornfeld studied the various phosphorylation patterns one typically sees.[13]

It appears that bi- to multivalent M6P-bearing ligands bind more tightly to the CI-MPR than do monovalent ones. Less clear is how many M6P residues are needed and how they are optimally spaced. Kornfeld's early studies involved isolation of native mixtures of mannose-rich oligosaccharides from cellular glycoproteins. These studies revealed that most Asn-linked oligosaccharides carry one or two phosphorylated mannoses, with the latter binding more tightly than the former. Later, elegant work by Hindsgaul revealed that one could gain nearly an order of magnitude in binding to the CI-MPR with pentasaccharides bearing two M6P residues, as opposed to one.[14] However, neither of these studies could reproduce the high relative binding affinities (RBAs $\geq 10^3$ vs M6P) seen with native ligands such as hGUS (human β-glucuronidase) or the synthetic glycoprotein, PMP−BSA (pentamannose phosphate−bovine serum albumin).

This led Bock and co-workers to make a series of glycopeptide ligands.[15] Compound **2a** is the most commonly

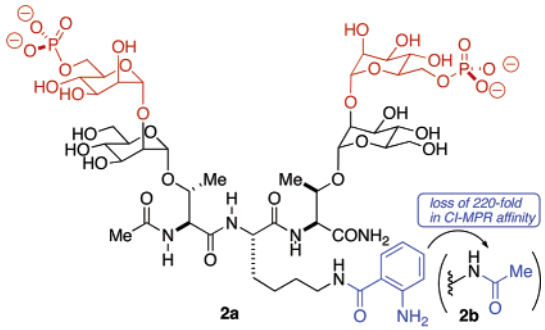


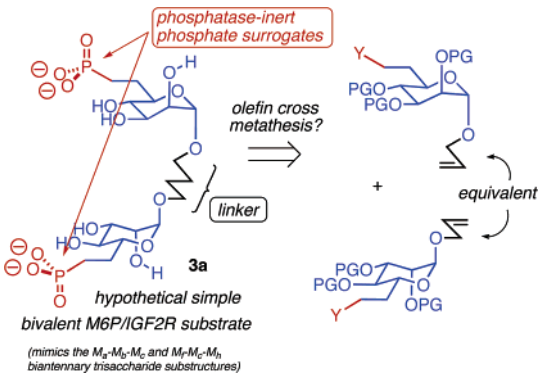
Figure 2. Bivalent M6P-bearing ligand (**2a**) due to Bock and its lower-affinity congener (**2b**).

used bioorganic tool of this sort. It does show the sought-after 3 orders of magnitude in RBA relative to M6P, and for this reason, it has been postulated that this is the first molecular "ruler" that spans two M6P sites on the receptor.[3] However, a closer look at the Bock work[15] reveals that **2b** binds 220-fold less well than **2a**, suggesting that the anthranoyl group in **2a** contributes significantly to binding. Thus, it is not so surprising that **2a** fails to stabilize receptor dimers and to accelerate IGF-II internalization by the CI-MPR, characteristics of the high-affinity multivalent ligand hGUS.[3]

Therefore, we set out to explore new approaches to M6P-bearing model ligands. Our goals were to develop a strategy that would provide for simple mono- and bivalent[16] ligands with variable tether lengths. We would eliminate phosphate esters and amide bonds from our model ligands to build in phosphatase and protease resistance. A cross-metathesis (CM) approach employing allyl (or related) glycosides of M6P-mimics appeared to be viable.[17] As can be seen

(7) Oates, A. J.; Schumaker, L. M.; Jenkins, S. B.; Pearce, A. A.; DaCosta, S. A.; Arun, B.; Ellis, M. J. C. *Breast Cancer Res. Treat.* **1998**, *47*, 269−281.
(8) (a) Ludwig, T.; Eggenschwiler, J.; Fisher, P.; D'Ercole, A. J.; Davenport, M. L.; Efstratiadis, A. *Dev. Biol.* **1996**, *177*, 517−535. (b) Wang, Z.-Q.; Fung, M. R.; Barlow, D. P.; Wagner, E. F. *Nature* **1994**, *372*, 464−467. (c) Lau, M. M. H.; Stewart, C. E. H.; Liu, Z.; Bhatt, H.; Rotwein, P.; Stewart, C. L. *Genes Dev.* **1994**, *8*, 2953−2963.
(9) Olson, L. J.; Zhang, J.; Dahms, N. M.; Kim, J.-J. P. *J. Biol. Chem.* **2002**, *277*, 10156−10161 and refs cited therein.
(10) Hancock, M. K.; Yammani, R. D.; Dahms, N. M. *J. Biol. Chem.* **2002**, *277*, 47205−47212.
(11) (a) Byrd, J. C.; MacDonald, R. G. *J. Biol. Chem.* **2000**, *275*, 18638−18646. (b) Byrd, J. C.; Park, J. H. Y.; Schaffer, B. S.; Garmroudi, F.; MacDonald, R. G. *J. Biol. Chem.* **2000**, *275*, 18647−18656.
(12) (a) Olson, L. J.; Dahms, N. M.; Kim, J.-J. P. *J. Biol. Chem.* **2004**, *279*, 34000−34009. (b) Olson, L. J.; Yammani, R. D.; Dahms, N. M.; Kim, J.-J. P. *EMBO J.* **2004**, *23*, 2019−2028.
(13) (a) Varki, A.; Kornfeld, S. *J. Biol. Chem.* **1980**, *255*, 10847−10858. (b) Varki, A.; Kornfeld, S. *J. Biol. Chem.* **1983**, *258*, 2808−2818. (c) Tong, P. Y.; Gregory, W.; Kornfeld, S. *J. Biol. Chem.* **1989**, *264*, 7962−7969.
(14) Distler, J. J.; Guo, J.; Jourdian, G. W.; Srivastava, O. P.; Hindsgaul, O. *J. Biol. Chem.* **1991**, *266*, 21687−21692.
(15) Franzyk, H.; Christensen, M. K.; Joergensen, R. M.; Meldal, M.; Cordes, H.; Mouritsen, S.; Bock, K. *Bioorg. Med. Chem.* **1997**, 5, 21−40.
(16) For the only other study, of which we are aware, that targets simple tethered bivalent M6P ligands, see: Lehmann, J.; Schweizer, F.; Weitzel, U. P. *Carbohydr. Res.* **1995**, *270*, 181−189.
(17) (a) Chatterjee, A. K.; Morgan, J. P.; Scholl, M.; Grubbs, R. H. *J. Am. Chem. Soc.* **2000**, *122*, 3783−3784. (b) Dominique, R.; Das, S. K.; Roy, R. *Chem. Commun.* **1998**, 2437−2438.

Scheme 1. Design and Retrosynthetic Analysis of a Bivalent M6P-Presenting Ligand[a]

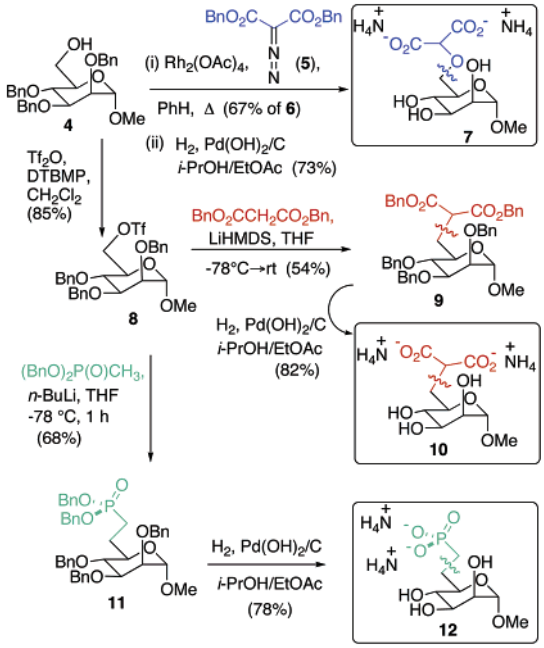


[a] Y = Phosphate surrogate functionality or leaving group precursor.

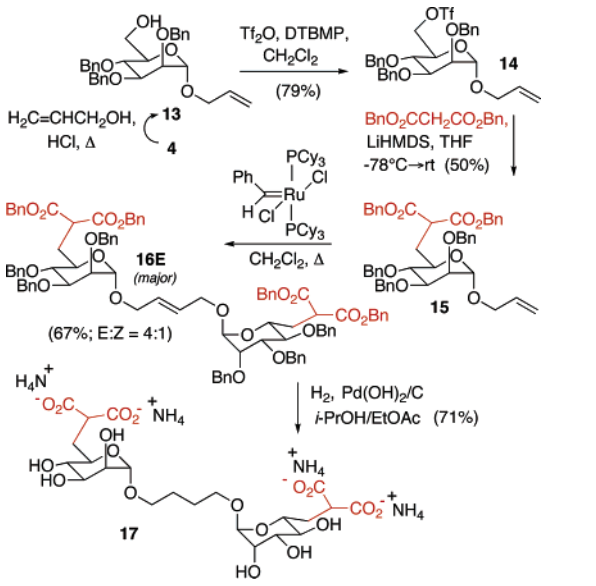
(Scheme 1 and Figure 1), this approach has the further advantage of providing direct access to bivalent ligands that, in terms of spacing, map nicely onto trimannose regions of the high-mannose oligosaccharide. Such model compounds could thus serve as independent probes for specific biantennary bisphosphorylation binding motifs.

Synthetically, the success of such an approach would require either carrying a relatively polar phosphate surrogate functionality into the CM reaction or its installation post-CM. Given that we had in mind to exploit our sugar triflate chemistry, as this had proven to be a versatile method for introducing functionality at the 6-position of hexopyranoses,[18] the latter approach raised the intriguing notion of synthesizing and handling bis-sugar triflates.

Scheme 2. Installation of Phosphate Surrogate Functionalities upon an α-Mannopyranosyl Core



Scheme 3. Bivalent M6P-Type Ligand via a Triflate Displacement/Alkene Cross-Metathesis Sequence



Three phosphate surrogates were chosen, all diacids, (i) a malonyl ether, (ii) a C2′-linked malonate, and (iii) a phosphonate.[19] To streamline the synthesis, a divergent strategy was employed whereby methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (**4**) served as the precursor to all target compounds. On one hand, Rh-mediated carbene insertion chemistry produced **6**. On the other hand, conversion of **4** to the primary triflate **8**, followed by anionic displacements, yielded the target malonate **9** and phosphonate **11**.

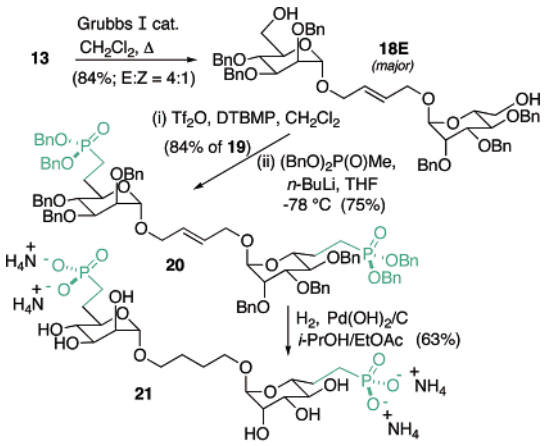
Schemes 3 and 4 illustrate the successful application of a CM approach to first generation bivalent M6P-mimetic ligands. The requisite alkene was installed by methyl to allyl glycoside interconversion under acidic conditions from **4**. Pleasingly, the α-anomeric stereochemistry was cleanly obtained, probably due to a combination of the usual anomeric effect and the Δ2 effect in mannose. Using the Grubbs I catalyst, the cross-metathesis reaction proceeded efficiently, whether the 6-position carried a free alcohol (67%) or a protected malonate (84%). For the bivalent phosphonate, not only could we cleanly obtain the bis-triflate **19**, but its double displacement with dibenzyl lithiomethylphosphonate proceeded very efficiently (75% = 87% per triflate displacement!).

Relative binding affinities to the CI-MPR were determined by ligand displacement assays vs both the synthetic ligand PMP−BSA and native receptor ligand hGUS (Table 1). Analysis of the resulting displacement curves (see Supporting

(18) (a) Shen, Q.; Sloss, D. G.; Berkowitz, D. B. *Synth. Commun.* **1994**, *24*, 1519−1530. (b) Berkowitz, D. B.; Eggen, M.; Shen, Q.; Shoemaker, R. K. *J. Org. Chem.* **1996**, *61*, 4666−4675. (c) Berkowitz, D. B.; Bose, M.; Asher, N. G. *Org. Lett.* **2001**, *3*, 2009−2012.

(19) Compound **12** has been independently synthesized by others and reported to show affinity comparable to M6P (both elute the receptor at 10 mM concentrations) for the CI-MPR via an affinity chromatography assay: (a) Khanjin, N. A.; Montero, J.-L. *Tetrahedron Lett.* **2002**, *43*, 4017−4020. (b) Vidil, C.; Morère, A.; Garcia, M.; Barragan, V.; Hamdaoui, B.; Rochefort, H.; Montero, J.-L. *Eur. J. Org. Chem.* **1937**, *2*, 447−450.

Scheme 4 Bivalent M6P-Type Ligand via the Reverse Sequence: Alkene Cross-Metathesis/Bistriflate Displacement



Information) allows for rather precise quantitation of both IC_{50} and RBA. Several observations emerge from the data. First, the RBAs obtained are quite consistent regardless of radioligand, lending confidence in the data. Second, surveying the phosphate surrogate functionalities examined, one sees a significant SAR, with affinity increasing in roughly order of magnitude steps as one goes from the malonyl ether (**7**) to the malonate (**10**) to the phosphonate (**12**). The deleterious effect of the insertion of the C6-oxygen (i.e., **10**→**7**) is reminiscent of observations made by Frost in examining binding to DHQ synthase.[20]

Given that crystallographic coordinates are now available describing the conformation of a domain 1−3 construct that binds M6P,[12a] attempts were made to examine this same set of M6P analogues, *in silico*, using AutoDock.[21] While the superiority of the phosphonate functionality is reproduced (Table 2), neither of these computations sees the malonate ether effect, nor do they predict the magnitude of the SAR seen. Perhaps, this is an indication that binding to repeat 9 is important.

Table 1. Relative Binding Affinities for the M6P/IGF2R

	radioligand			
	PMP−BSA		hGUS	
ligand	IC_{50} (n)[a]	RBA[b]	IC_{50} (n)[a]	RBA[b]
M6P	0.049 ± 0.015 (6)	1.0	0.033 ± 0.005 (6)	1.0
G6P	>10 (6)	na[c]	>10 (6)	na[c]
7	2.14 ± 1.19 (4)	0.024	1.06 ± 0.22 (5)	0.041
10	0.166 ± 0.090 (4)	0.30	0.126 ± 0.032 (4)	0.35
12	0.025 ± 0.008 (5)	1.82	0.015 ± 0.007 (4)	3.52
17	0.145 ± 0.083 (4)	0.39	0.151 ± 0.019 (6)	0.226
21	0.020 ± 0.005 (6)	2.79	0.014 ± 0.003 (4)	3.16

[a] The mM concentration of each compound that displaced 50% of the indicated radioligand tracers in a binding assay utilizing bovine soluble M6P/IGF2R immobilized on Sepharose 4B was calculated. Values in parentheses denote the number of replicate experiments performed yielding mean ± SEM for each compound. [b] Relative binding affinity was calculated by dividing the IC_{50} value for each compound by that of mannose 6-phosphate (M6P). [c] Not applicable. Values for RBA of the negative control, glucose 6-phosphate (G6P), cannot be calculated from these data.

Table 2. Docking to CI-MPR Repeats 1−3

	AutoDock-predicted $\Delta G_{binding}$ (kcal/mol)				
conformation[a]	M6P	G6P	7	10	12
1[b]	−7.02	−6.83	−6.74	−6.82	−7.13
2[c]	−7.14	−6.51	−6.74	−6.48	−7.18

[a] Conformation to which the ligand set was docked. [b] Conformation 1 denotes the coordinates of chain A from pdb 1SZ0. [c] Conformation 2 denotes the receptor coordinates obtained upon performing a 500 ps molecular dynamics simulation, with 1SZ0 and a terminally phosphorylated α-1,2-linked mannobiose sugar (see Supporting Information for more details).

Significantly, our RBA results with the model bivalent ligands, **17** and **21** (Table 1), show little to no effect of bivalency here, suggesting that they cannot access two M6P binding pockets. This is notable since these ligands map onto specific trimannose sectors of a canonical N-linked high-mannose glycosylation site (Figure 1 and Scheme 1). Specifically, if one considers the three most prevalent patterns of bisphosphorylation seen by Kornfeld in a classic early study (Figure 3),[13a] our results suggest that pattern "I" (at least insofar as it implies an $M_c-M_f-M_h$ biantennary bisphosphate), is not a high-affinity binding element for the CI-MPR. This illustrates how this new bioorganic "measuring tool" might be used to explore other potential bivalent binding modes for mannose-rich glycoprotein ligands to this receptor and others.

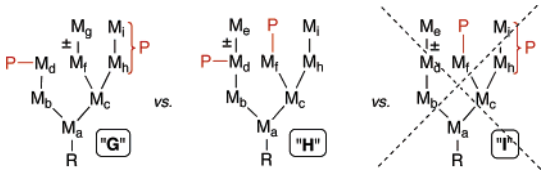


Figure 3. Three possible bis-M6P-presenting motifs for mannose-rich glycoproteins from mouse lymphocyte (ref 13a).

Acknowledgment. The authors thank the Nebraska State Department of Health for a seed grant (D.B.B.) and the NIH (CA 91885; RGM) for support. D.B.B. thanks the Alfred P. Sloan Foundation for a fellowship. B.D.C. acknowledges a UCARE Undergraduate Research Fellowship. Mohua Bose is thanked for early studies. This research was facilitated by shared instrumentation grants for NMR (NIH SIG-1-510-RR-06301, NSF CHE-0091975, NSF MRI-0079750) and GC/MS (NSF CHE-9300831).

Supporting Information Available: NMR spectra, characterization data, and experimental procedures; binding curves and protocols for the radioligand displacement assay; and a description of docking methods and docked images. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0479444

(20) Tian, R.; Montchamp, J.-L.; Frost, J. W. *J. Org. Chem.* **1996**, *61*, 7373−7381.
(21) Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639−1662.