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Macrocyclic peptidomimetic inhibitors of β-secretase (BACE): First X-ray structure of a macrocyclic peptidomimetic-BACE complex

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Abstract—The synthesis of novel macrocyclic peptidomimetic inhibitors of the enzyme BACE1 is described. These macrocycles are derived from a hydroxyethylene core structure. Compound **7** was co-crystallized with BACE1 and the X-ray structure of the complex elucidated at 1.6 Å resolution. This molecule inhibits the production of the Aβ peptide in HEK293 cells overexpressing APP751sw.

Alzheimer's disease (AD) is a major neurodegenerative disorder that affects 10% of the population over 65% and 40% over 80 years.[1,2] Symptoms of the disease are behavioral disturbances and loss of cognitive function. AD represents an unmet medical need that adds approximately 100 billion dollars to healthcare costs in the United States alone. In addition, AD also causes great deal of suffering to the patient and places a burden on their families.

Treatments currently available for AD are cholinesterase inhibitors and NMDA-receptor antagonists. The rationale behind the former approach is that these agents increase cholinergic transmission by interfering with degradation of the Ach neurotransmitter. Unfortunately, this approach does not stop the progressive loss of cholinergic neurons, and eventually the treatment becomes ineffective. Treatment with NMDA-receptor antagonists appears to be no more effective in terms of progression of the disease. A better approach would be to develop agents that interfere with the mechanism that leads to neurodegeneration.

AD is characterized by the presence of amyloid plaques that are believed to produce neuronal toxicity and cell death.[3] These plaques are constituted mainly from an insoluble form of Aβ amyloid, a 40–42 amino acid peptide produced in the brain from the amyloid precursor protein (APP). APP is processed by at least three secretases. α-Secretase cleaves APP to form a carboxy terminal 83 amino acid fragment (C83), which is processed by γ-secretase to produce p3, a non amyloidogenic form of Aβ–peptide. Alternatively, APP can be processed initially by β-secretase (BACE1) and the resulting C99 membrane bound C-terminal peptide can be hydrolyzed by γ-secretase to form the 40 amino acid Aβ-peptide ($A\beta_{40}$). Shorter and longer forms of the peptide also are produced, in particular $A\beta_{42}$, which has a high propensity to aggregate and is the principal Aβ species found in amyloid plaques. Notably, the BACE1 enzyme is only found in ER/Golgi or endosome compartments of neurons, whereas several α-secretase candidates and the γ-secretase complex have broad tissue distribution.

Conceptually, the inhibition of either β- or γ-secretase by a small molecule would preclude the formation of Aβ and therefore plaque formation. γ-Secretase inhibitors have been reported to attenuate the formation of Aβ and plaques in animal models. However, the therapeutic index of these inhibitors requires careful monitoring. This is due to the implication of γ-secretase in

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essential functions, including facilitating Notch signaling, which is important for specifying cell fates during development and for regulating differentiation of self-renewing cell types in adults.[4] In contrast, β-secretase KO mice are viable and free of the gross phenotypic changes that are observed, for example with presenilin knock-outs, except for the complete absence of Aβ in their brains.[5]

Beta amyloid cleaving enzyme (BACE1) has recently been identified as the principal β-secretase in neurons.[6] It is a trans-membrane aspartyl protease expressed in neuronal cells that cleaves APP inside vesicles associated with ER/Golgi apparatus and endosomes. Tang et al. reported in 2000 that OM99-2 (Fig. 1), a peptidomimetic based on the hydroxyethylene transition state isostere, was a nanomolar inhibitor of BACE. The Oklahoma group subsequently reported the X-ray structure of the OM99-2-BACE complex showing the main features of the enzyme–inhibitor interactions.[7]

With the goal of designing low molecular weight BACE1 inhibitors,[8] we envisioned that linking two residues of our small peptidomimetic inhibitors[9] would provide a macrocyclic structure that would be more potent, and would have improved absorption properties, relative to the corresponding open chain analog. The linkage of distant residues should produce a decrease in the number of possible low energy conformations, and therefore, if one of the remaining conformations is complementary to the enzyme active site, the binding affinity should be increased. In addition, it is known that cyclic peptides are more resistant to gastric tract degradation adding an additional attractive feature to this class of inhibitors.[10]

Examination of Tang's,[7] and our X-ray data[9] suggested P1–P3 and P1–N2 as suitable points of union due to their proximity, orientation, and localization into the large P1–P3 lipophillic pocket (Fig. 2).

Linking positions P1–P3 has recently been described in BACE1 inhibitors for structures derived from an ethanolamine core;[11] also, other macrocycles derived from a hydroxyethylene core structure have been synthesized based on the linkage of positions P2–P3.[12]

The strategy developed to build the macrocyclic structures was to modify our lead inhibitor **1** by Grubbs cyclization of alkene moieties at P1 and P3 or between P1 and the nitrogen of the P2 amino acid. It was determined by iterative docking studies[13] that the best P1–P3 linkage was a six-atom long saturated chain that forms a 13-membered ring (including the inhibitor backbone). For the P1–N2 connection, the five-carbon saturated linker was predicted to be optimal, leading to a 10-membered ring.

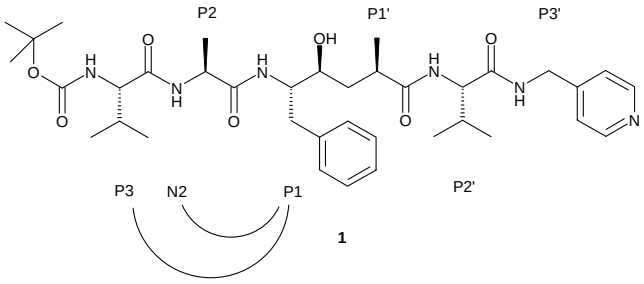


Figure 2.

The synthesis of the macrocyclic peptidomimetics was performed as depicted in Scheme 1. *N*-Boc serine methyl ester was converted into the iodoserine derivative by treatment with iodine and imidazole. Conversion of the alkyl iodide into the corresponding alkylzinc reagent followed by alkylation with allyl chloride[14] afforded, after hydrolysis, intermediate **8**.

Compound **8** was transformed into the pivotal lactone **9** by reduction to *N*-Boc homoallylglycinal,[15] alkylation with the Zn–Cu reagent obtained from 3-iodopropionic acid ethyl ester,[16] thermal lactonization and, for P1′ = Me, stereoselective methylation with LHDMS and methyl iodide.

The C-terminal fragment **10** was prepared by standard EDCI coupling of *N*-Boc alanine and 4-aminomethyl pyridine or benzylamine followed by cleavage of the Boc group under acidic conditions. The N-terminal fragment **11**, used to prepare compounds **2**–**5**, was prepared by EDCI coupling of **8** and alanine methyl ester, followed by ester hydrolysis.

The N-terminal fragment **12** used to build macrocyles **6**–**7** was prepared starting with L-alanine. The *p*-nitrobenzenesulfonyl group was introduced as an amino protecting group to facilitate the N–H alkylation with allyl bromide in the presence of potassium carbonate. Deprotection of the *N*-allylsulfonamide by treatment with thiophenol and potassium carbonate provided *N*-allyl-Ala-(OMe).[17] The coupling of this amino acid with

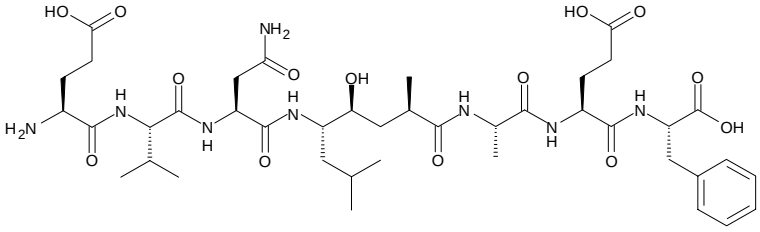
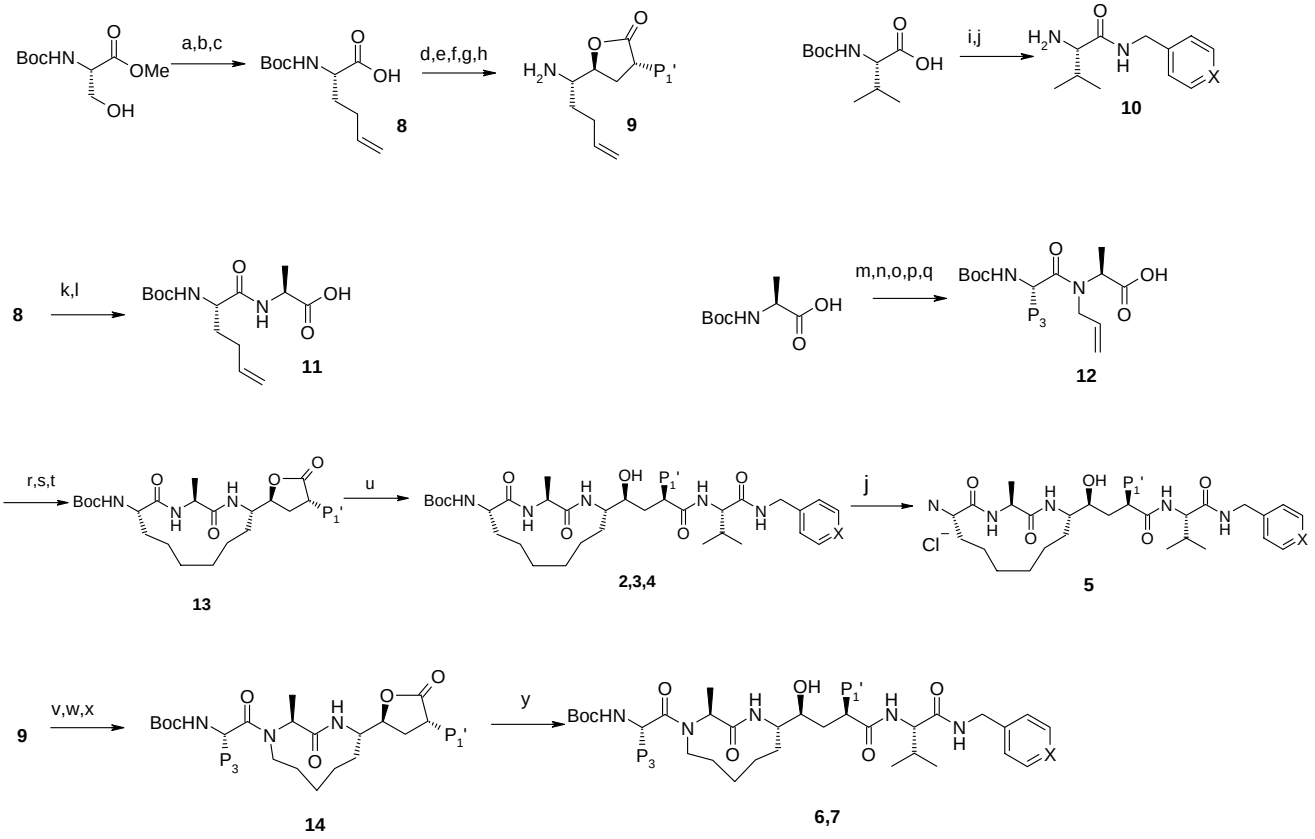


Figure 1. OM-99-2.



Scheme 1. Reagents: (a) I_2/PPh_3/imidazole; (b) Zn/CuCN.LiCl/Allyl Chloride; (c) Cs_2CO_3; (d) NMM/*i*-BuOCOCl/MeNHOMe.HCl; (e) $LiALH_4$; (f) iodopropionic acid ethyl ester/Zn-Cu/DMA/Ti(IV); (g) acetic acid/110 °C; (h) for P1 = Me LHMDS/MeI; (i) 2-aminomethylpyridine or benzylamine/EDCI/HOBT/TEA/DMAP; (j) HCl; (k) alanine methyl ester/EDCI/HOBT/TEA/DMAP; (l) Cs_2CO_3; (m) *p*-nitro-benzenesulfonyl chloride/TEA; (n) K_2CO_3/Allyl bromide; (o) thiophenol/K_2CO_3; (p) BocILE or BocVAL/PyBrop/HOAT/DIPEA; (q) Cs_2CO_3; (r) **11**/EDCI/HOBT/TEA/DMAP; (s) bis(tricyclohexylphosphine)benzylideneruthenium (IV)dichloride; (t) H_2; (u) 2-hydroxypyridine/**10**; (v) **12**/EDCI/HOBT/TEA/DMAP; (w) bis(tricyclohexylphosphine)benzylideneruthenium(IV)dichloride; (x) H_2; (y) 2-hydroxypyridine/**10**.

N-Boc valine or *N*-Boc isoleucine was not possible under our standard EDCI coupling conditions. However, the coupling could be achieved in high yield using PyBroP/HOAT to afford **12**.[17]

Both **11** and **12** were coupled with **9** to produce the diene intermediates, which were submitted to Grubbs cyclication. The macrocyclization occurred in 50–70% yield, and the resulting cycloalkenes were hydrogenated in quantitative yield to produce **13** and **14**, respectively. Lactones **13** and **14** were opened with **10** by heating a melted mixture of the two compounds in the presence of 2-hydroxypyridine[18] to produce the BACE inhibitors **2**, **3**, **4**, **6** and **7**. BACE inhibitor **5** was prepared by cleavage of the Boc group of compound **3** under acidic conditions.

Compounds **1**–**7** were tested for the inhibition of BACE1 both in an in vitro FRET and a cellular assay,[9] and the results are shown in Table 1. For the enzymatic assay, IC_{50} values were determined using 20 nM purified recombinant human BACE1:Fc. The assay contains 100 μM ELGY-9 (an aminobenzoate-based FRET peptide containing Swedish mutation at the β-secretase cleavage site) in 50 mM ammonium acetate, pH 4.6, 1 mg/ml BSA, and 1 mM Triton X-100. The assay is run at room temperature for 4 h. An increase in the relative fluorescence of the reaction mixture is used to determine enzyme activity, with an umbilliferone excitation/emission filter.

The whole cell assay was performed in order to measure the cellular permeability of the compounds. Endogenous BACE1 is expressed in HEK293 cells. This cell line over expresses the APP751swe protein. The assay is conducted with 35,000 cells per well in a 96-well plate format in DMEM containing 10% FBS. Compounds are incubated with cells for 4 h at 37 °C and 5% CO_2. The conditioned media are removed from the culture wells.

The Aβ ELISA (using monoclonal antibodies 266 and biotinylated-3D6 as capture and reporting antibodies, respectively) is used to measure the amount of Aβ (total) peptide produced during the incubation time.

We started our investigation by preparing the P1–P3 macrocyclic analogs. Compound **2** shows weak enzymatic activity and no cellular potency. In order to improve these parameters, we reproduced the previously reported SAR in the open chain analogs.[9] Thus, introducing a methyl group at P1′ (compound **3**) improves 35-fold the enzymatic activity but still no cellular

Table 1. In vitro values

Compound	P3	P1′	X at P3′	BACE FRET IC_{50} (μM)[a]	HEK293 Aβ inhibition IC_{50} (μM)[a]
1	$CHMe_2$	Me	N	0.082 (±0.023)	2.831 (±0.129)
2	—	H	H	9.704 (±1.413)	>100
3	—	Me	H	0.285 (±0.016)	>100
4	—	Me	N	4.925 (±0.169)	>100
5	—	Me	H	2.397 (±0.089)	>100
6	$CHMe_2$	Me	N	0.260 (±0.002)	5.305 (±0.191)
7	(*S*)-MeCHEt (Ile)	Me	N	0.065 (±0.039)	0.880 (±0.042)

[a] Values are means of three experiments, standard deviation is given in parentheses.

potency was obtained. It is interesting to compare compound **3** with literature compounds such as BOC-Val-Met-Leu*Ala-Val-NHBn, an acyclic analog (with the Leu*Ala hydroxyethylene isostere) described by Ghosh et al.[19], which was reported to have a K_i of 2.5 μM against recombinant BACE1 enzyme. Part of the potency of this compound was attributed to occupation of P2 with the Met side chain, yet despite the absence of this interaction compound **3** shows at least equivalent activity, even allowing for assay differences, consistent with the hypothesis that the reduction in degrees of freedom should produce an increase in inherent affinity.

With the aim of improving cellular penetration and/or solubility we introduced a basic site either at P3′ (compound **4**) or at P3 (compound **5**). Unfortunately, in contrast to our findings in the open chain analog series, none of these changes improved cellular activity. These results suggest that this type of P1–P3 cyclization is not optimal for obtaining whole cell BACE1 inhibitors.

Then we turned our attention to the P1–N2 cyclization. These analogs could provide advantages in terms of elimination of one hydrogen bond donor (N2–H), better occupancy of the S3 site, and reduced flexibility of the cycle by means of reducing the ring size. In order to test these hypotheses, we prepared compounds **6** and **7** according to the route shown in Scheme 1. Compound **6** improves enzymatic potency compared with **4** and shows, for the first time, moderate cellular activity. In order to further improve of potency, the Val residue at the P3 position was replaced with Ile[9] (compound **7**). This new compound shows a notable improvement in enzymatic and whole cell potency (4- and 60-fold, respectively) over compound **6** (see Table 1).

With these results in hand, and in order to gain insight into the binding mode, an X-ray structure of the complex between BACE1 and compound **7** was obtained.

The crystal structure of BACE1 complexed with **7** has been refined at 1.6 Å resolution to an *R*-factor of 0.211 (R_{free} = 0.227) using data collected at the Advanced Photon Source beamline 17ID. Crystallographic methods have been published previously.[9]

The structure of compound **7** (Fig. 3) superimposed with that of OM99-2 (PDB entry 1FKN) exhibits a binding mode in common with previously determined peptide based inhibitors.[9]

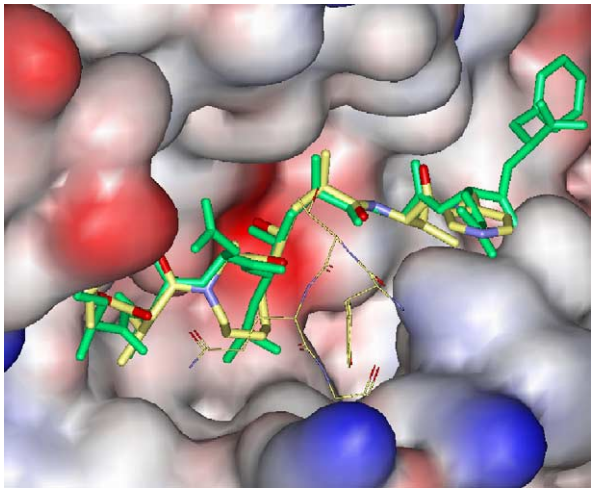


Figure 3. The structure of 7 is shown superimposed with that of OM99-2 (PDB entry 1FKN). OM99-2 is colored green, while C, N, and O atoms in **7** are colored yellow, blue, and red, respectively. An electrostatic surface is shown for the BACE active site, with the surface around flap residues 71–74 (thin lines) omitted for clarity.

The binding mode for compound **7** reveals a very close superposition of the main chain peptide groups on OM99-2 between the P3 and the P3′ groups. The side chains of the two inhibitors also show a very close overlap in the BACE1 active site. Thus, the macrocycle maintains a nearly identical peptide H-bond potential and side-chain interactions with enzyme subsites S3–S3′; this was the objective that the macrocycle was designed to accomplish. However, there are a number of subtle but notable differences in binding, including the loss of the P2 side-chain H-bond potential and the loss of a water mediated H-bond between the P3/P2 amide NH and the Gln73 main chain O atom in compound **7**. There is also a nonoptimal occupancy of the S1 subsite by the macrocycle methylene groups in compound **7** relative to the Phe side chain of compound **1** and the Leu side chain of OM99-2. This nonoptimal occupancy, including the absence of the phenyl group, may explain a major part of the lack of inherent increase in potency that would be expected from the gains in binding energy that would be predicted to be associated with the reduced degrees of freedom in the macrocyclic compound.

It is also interesting to note that the P2′ Val side chain of **7** penetrates deeper into the S2′ subsite than the corresponding Ala residue of OM99-2, and the C-terminal pyridyl ring occupies a very similar position to the P3′

Glu side chain of OM99-2. These factors appear to be less important for increasing potency of **7** relative to **1**.

The design and synthesis of **2**–**7**, macrocyclic analogs of the BACE1 inhibitor **1**, provide further insight into the preferred binding mode for **1** obtained from the crystal structure of BACE1 complexed with **7**. The X-ray structure showed that compound **7**, obtained by cyclization between N2-P1, was able to maintain the backbone structure of OM99-2, and at the same time illustrates the importance of an efficient occupancy of the S1 binding site in terms of potency. Also, the work demonstrates that the N2–H in the peptidic structure is not a required feature for binding to BACE1. Continued work on the improvement of macrocyclic BACE1 inhibitors will be published in due course.

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References and notes

1. Janus, C. *CNS Drugs* **2003**, *17*, 457.
2. Evans, D. A.; Funkenstein, H. H.; Albert, M. S., et al. *JAMA* **1989**, *262*, 2551.
3. (a) Selkoe, D. J. *Physiol. Rev.* **2001**, *81*, 741; (b) Selkoe, D. J. *Nature* **1999**, *99*, A23.
4. Hartmann, D.; Tournoy, J.; Saftig, P.; Annaert, W.; De Strooper, B. J. *Mol. Neurosci.* **2001**, *17*, 171.
5. Luo, Y.; Bolon, B.; Khan, S., et al. *Nat. Neuroscience* **2001**, *4*, 231.
6. Vassar, R. et al. *Science* **1999**, *286*, 734.
7. Hong, L.; Koelsch, G.; Lin, X.; Wu, S.; Terzyan, S.; Ghsoh, A. K.; Zhang, X. C.; Tang, J. *Science* **2000**, *290*, 150.
8. For a review on this subject, see: John, V.; Beck, J. P.; Bienkowski, M. J.; Sinha, S.; Heinrikson, R. I. *J. Med. Chem.* **2003**, *46*, 4625.
9. (a) Lamar, J.; Hu, J.; Bueno, A. B.; Yang, H.; Guo, D.; Copp, J. D.; McGee, J.; Gitter, B.; Timm, D.; May, P.; McCarthy, J.; Chen, S. *Biorg. Med. Chem. Lett.* **2004**, *14*, 239; (b) Lamar, J.; Hu, J.; Yang, H.; Khon, T. D.; Copp, J. D.; McGee, J.; Timm, D.; Erikson, J.; May, P.; McCarthy, J.; Chen, S. *Biorg. Med. Chem. Lett.* **2004**, *14*, 245.
10. Tyndall, J. D. A.; Reid, R. C.; Tyssen, D. P.; Jardine, D. K.; Todd, B.; Passmore, M.; March, D. R.; Pattenden, L. K.; Bergman, D. A.; Alewood, P. F.; Birch, C. J.; Martin, J. L.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 3495.
11. (a) Cumming, J. N.; Iserloh, U.; Kennedy, E. M. *Curr. Opin. Drug Discov. Dev.* **2004**, *7*(4), 536(b) ELAN PHARMACEUTICALS/PHARMACIA & UPJOHN. Macrocycles useful in the treatment of Alzheimer's disease. WO-02100399 (2002).
12. Ghosh, A. K.; Davasamudram, T.; Hong, L.; DeZutter, C.; Xu, X.; Weerasena, V.; Koelsch, G.; Bilcer, G.; Tang, J. *J. Biorg. Med. Chem. Lett.* **2005**, *15*, 15.
13. Docking analysis carried out with in-house algorithm, CDOCKER, to the BACE/OM99-2 X-ray structure (Ref. 7). See: (a) Vieth, M.; Hirst, J. D.; Kolinski, A.; Brooks, C. L., III *J. Comp. Chem.* **1998**, *19*, 1612; (b) Vieth, M.; Hirst, J. D.; Dominy, B. N.; Daigler, H.; Brooks, C. L., III *J. Comp. Chem.* **1998**, *19*, 1623; (c) Vieth, M.; Cummins, D. J. *J. Med. Chem.* **2000**, *43*, 3020; (d) Wu, G.; Robertson, D. H.; Brooks, C. L., III; Vieth, M. *J. Comp. Chem.* **2003**, *24*, 1549; (e) Erickson, J. A.; Jalaie, M.; Robertson, D. H.; Lewis, R. A.; Vieth, M. *J. Med. Chem.* **2004**, *47*, 45.
14. Dunn, M. J.; Jackson, R.; Pietruszka, J.; Turner, D. *J. Org. Chem.* **1995**, *60*, 2210.
15. Brady, S. F.; Sisko, J. T.; Stauffer, K. J.; Colton, C. D.; Qiu, H.; Lewis, S. D.; Assunta, S. Ng.; Shafer, J. A.; Bogusky, M. J.; Veber, D. F.; Nutt, R. F. *Biorg. Med. Chem.* **1995**, *3*, 1063.
16. Decamp, A. E.; Kawaguchi, A. T.; Volante, R. P.; Shinkai, I. *Tetrahedron Lett.* **1991**, *32*, 1867.
17. Reichwein, J. F.; Liskamp, R. M. *Tetrahedron Lett.* **1998**, *39*, 1243.
18. Craparo, H. G. et al. *Arch. Pharm. Med. Chem.* **1996**, *329*, 273.
19. Ghosh, A. K. et al. *J. Med. Chem.* **2001**, *44*, 2865.