Bioorganic & Medicinal Chemistry Letters 20 (2010) 3158–3160

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Macrocyclic BACE inhibitors: Optimization of a micromolar hit to nanomolar leads

Yifang Huang [a], Eric D. Strobel [a], Chih Y. Ho [a], Charles H. Reynolds [a], Kelly A. Conway [a], Jennifer A. Piesvaux [a], Douglas E. Brenneman [a], George J. Yohrling [a], H. Moore Arnold [a], Daniel Rosenthal [a], Richard S. Alexander [a], Brett A. Tounge [a], Marc Mercken [b], Marc Vandermeeren [b], Michael H. Parker [a], Allen B. Reitz [a], Ellen W. Baxter [a,*]

[a] Research and Early Development, Johnson & Johnson Pharmaceutical Research & Development, LLC, Welsh & McKean Roads, PO Box 0776, Spring House, PA 19477-0776, USA
[b] Research and Early Development, Johnson & Johnson Pharmaceutical Research & Development, LLC, Turnhoutseweg 30, B-2340 Beerse, Belgium

ARTICLE INFO

Article history:
Received 8 December 2009
Revised 18 March 2010
Accepted 26 March 2010
Available online 30 March 2010

Keywords:
BACE
BACE inhibitors
Alzheimer's disease

ABSTRACT

We have identified macrocyclic inhibitors of the aspartic protease BACE, implicated in the etiology of Alzheimer's disease. An X-ray structure of screening hit **1** in the BACE active site revealed a hairpin conformation suggesting that constrained macrocyclic derivatives may also bind there. Several of the analogs we prepared were >100× more potent than **1**, such as **7** (5 nM K_i).

Alzheimer's disease (AD) is a chronic degenerative disorder of the central nervous system resulting in severe cognitive deficits as well as psychiatric symptoms.[1] More than 20 million people suffer from this condition worldwide, and, with the increasing number of elderly, the number of AD patients is expected to increase dramatically. Current therapies are merely palliative and include the cholinesterase inhibitors, donepezil, galanthamine, and rivastigmine, which are used to treat mild and moderate AD and the NMDA antagonist, memantine, employed for moderate to severe AD. The discovery of disease-modifying agents for AD is a desperately unmet need.[2] Agents which interfere with the processing of the amyloid precursor protein (APP) are especially attractive targets. In particular, the aspartic protease, BACE-1 (β-site amyloid cleaving enzyme, memapsin2, Asp2)[3] catalyzes the initial proteolytic cleavage of APP to generate the β-amyloid$_{1-40(42)}$ peptides which have a crucial role in the pathogenesis of AD. The homozygous BACE knockout mouse has significantly reduced levels of β-amyloid while maintaining a normal phenotype[4] although more recent studies may challenge this claim.[5] In addition, BACE-1 gene deletion in the Tg2576 mouse, which overproduces β-amyloid, results in a reduction of the cognitive deficit.[6] BACE-1 inhibitors have been shown to lower β-amyloid levels in the brains of mice[4a,7] and thus show promise in the treatment of AD.

A high throughput screen of our corporate compound collection resulted in the identification of 2-amino-3,4-dihydroquinazoline **1** as a BACE-1 inhibitor with modest, but promising, activity (0.9 μM K_i).[8] Unlike previously reported peptide-like BACE-1 inhibitors, such as OM99-2,[9] which bind to the active site in an extended conformation, surprisingly, **1** adopted a tight hairpin shape (Fig. 1) by X-ray crystallography.[8] This structural information suggested that the two ends of the hit compound could be connected to form a macrocycle which might improve the potency of our series. Utilizing the structure of **1** as a template, a variety of prospective mac-

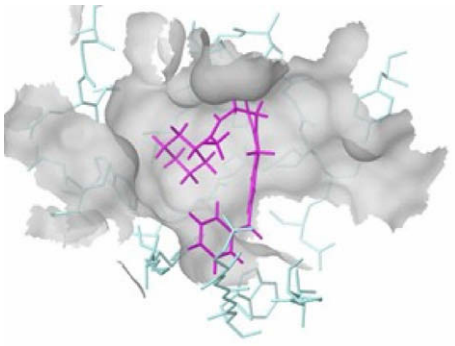
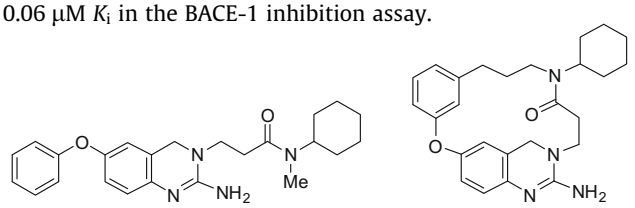


Figure 1. High throughput screening hit **1** in the active site of BACE-1.[8]

* Corresponding author. Tel.: +1 215 885 3648.
E-mail address: EllenBaxter@msn.com (E.W. Baxter).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.03.097

rocycles were evaluated computationally.[10] For example, **2** fit the model and was synthesized. Much to our delight, this analog had a 0.06 μM K_i in the BACE-1 inhibition assay.

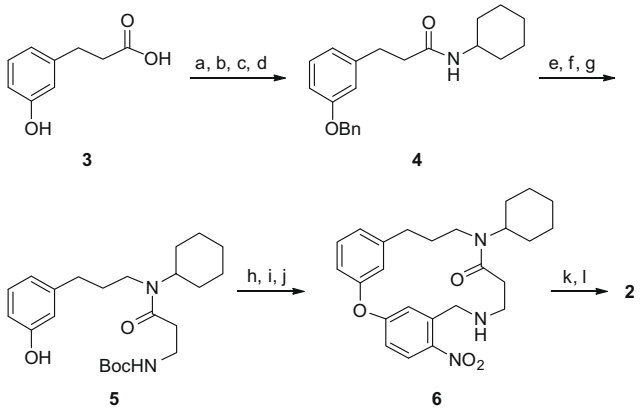


1 2

The synthesis of **2** was initiated with 3-(3-hydroxyphenyl)propionic acid (**3**) (Fig. 2). Reaction of **3** with benzyl bromide provided bis-benzylated material. Selective hydrolysis of the benzyl ester provided the intermediate acid which was converted to amide **4**. The amide was reduced to the corresponding amine which was then coupled to *N*-Boc-β-alanine followed by O-debenzylation to yield **5**. Condensation of **5** with 5-fluoro-2-nitrobenzaldehyde with subsequent removal of the Boc group and intramolecular reductive amination afforded macrocycle **6**. Reduction of the nitro group followed by condensation with cyanogen bromide yielded target **2**.

While macrocycle **2** had promising BACE inhibition, the compound was only weakly active in a cellular assay measuring $A\beta_{1-40}$ secretion in CHO cells transfected with the Swedish familial AD mutant APP (K670N/M671L) with a 0.51 μM IC_{50}. X-ray crystallographic studies of **1** revealed the vacant hydrophobic S_1' pocket. We reasoned that the introduction of a lipophilic substituent that would fill this pocket might improve the potency of this chemical series both against BACE and in cellular assays of Aβ secretion. Molecular modeling indicated that a cyclohexyl group would be an optimal substituent, occupying the S_1' pocket (Fig. 3).

To examine this hypothesis, compound **7** was synthesized (Fig. 4). Reduction of the cyano group in **8** to the corresponding phenethylamine followed by reductive alkylation with cyclohexanone provided **9** which was coupled with γ-amino acid **10** (>99% ee).[8] Subsequent O-debenzylation, condensation with 5-fluoro-2-nitrobenzaldehyde, and Boc-group removal provided late stage intermediate **11**. Macrocyclization was achieved via an intramolecular reductive amination. Reduction of the nitro group revealed the penultimate bis-amine which was reacted with cyanogen bromide to afford target **7**. Macrocycle **7** was a potent BACE inhibitor with a 5 nM K_i in the enzyme assay and a 17 nM IC_{50} in the cellular assay.



3 4 5 6 2

Figure 2. Reagents and conditions: (a) BnBr, K_2CO_3, MeCN, reflux, 18 h (100%); (b) 1 N NaOH, MeOH, THF, 3 h (96%); (c) $(COCl)_2$, CH_2Cl_2, DMF, 4 h (100%); (d) $C_6H_{11}NH_2$, NEt_3, CH_2Cl_2, 1 h (94%); (e) $LiAlH_4$, THF, reflux, 24 h (72%); (f) $BocHNCH_2CH_2CO_2H$, 1-methylmorpholine, HBTU, DMF, 18 h (96%); (g) H_2 (50 psi), 10% Pd/C, EtOH, 18 h (100%); (h) 5-fluoro-2-nitrobenzaldehyde, K_2CO_3, DMF, 50 °C, 18 h (68%); (i) TFA, CH_2Cl_2, 2 h (98%); (j) (i) CH_2Cl_2, 4A MS, 3 h, (ii) $NaBH(OAc)_3$, 24 h (78%); (k) $SnCl_2$, EtOH, 18 h; (l) BrCN, EtOH, 24 h (23%, two steps).

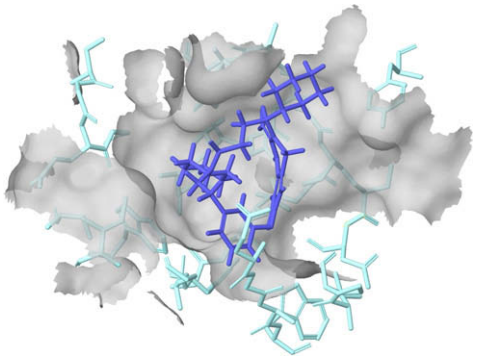
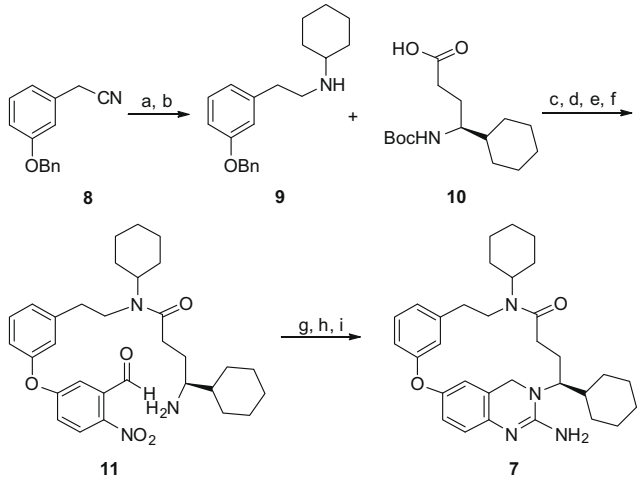


Figure 3. Left: docking of proposed macrocycle **7** in the BACE active site. (Maestro Version 9, Schrodinger, L.L.C).



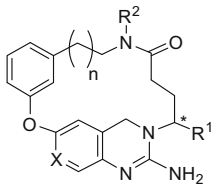
8 9 10 11 7

Figure 4. Reagents and conditions: (a) BH_3–THF, THF, reflux, 18 h (71%); (b) cyclohexanone, $NaBH(OAc)_3$, HOAc, THF, 18 h (92%); (c) **10**, DIPEA, HBTU, DMF, 18 h (100%); (d) H_2 (50 psi), 10% Pd/C, EtOH, 6 h (99%); (e) 5-fluoro-2-nitrobenzaldehyde, K_2CO_3, DMF, 50 °C, 18 h (97%); (f) TFA, CH_2Cl_2, 4 h (78%); (g) (i) CH_2Cl_2, 4A MS, 30 min, (ii) $NaBH(OAc)_3$, 18 h (93%); (h) H_2 (50 psi), 10% Pd/C, THF, EtOH, 6 h; (i) BrCN, EtOH, reflux, 18 h (48%, two steps).

With this exciting result in hand, we embarked on an SAR study of our series (Table 1). Expanding the ring size of **7** by one methylene group as in compound **12** resulted in a sixfold loss in enzyme inhibition. The (*S*)-enantiomer **12** was six times more potent than (*R*)-enantiomer **13** in the BACE assay. Replacement of the cyclohexyl substituent in **7** (K_i = 5 nM) with an *iso*-propyl (K_i = 8 nM) as in **14** resulted in equivalent enzymatic activity, but a fivefold decrease in cellular activity, suggesting that increased lipophilicity of the R_1 substituent is critical for cell penetrance. Replacement of the *N*-cyclohexyl group (compound **7**) by *N*-(4-tetrahydropyranyl) (compound **15**) resulted in an over threefold reduction in both enzymatic and cellular activity. Moreover, where R_2 is 4-tetrahydropyranyl, and R_1 is cyclohexyl (compound **15**) versus the case where R_1 is *iso*-propyl (compound **16**), the BACE K_is are comparable (17 nM and 22 nM), but the cellular IC_{50}s are vastly different (73 nM and 685 nM).

Introduction of a 4-carboxycyclohexyl substituent as R_2 resulted in a fourfold loss in enzyme activity and a 18-fold loss in cellular potency for the *cis* analog **17** (K_i = 20 nM) while the corresponding *trans* isomer **18** was inactive. Additionally, modifications of the core dihydroquinazoline were explored. Introduction of a 7-fluoro (analog **19**, K_i = 8 nM) or 7-methoxy substituent (analog **20**, K_i = 12 nM) resulted in a slight decrease in BACE activity, and a twofold reduction in cellular potency. Replacement of the

Table 1
Structure–activity relationships



Compd	Configuration	R^1	R^2	*n*	X	BACE K_i (nM)	Cellular $A\beta_{1-40}$ IC_{50} (nM)
7	(*S*)	Cyclohexyl	Cyclohexyl	1	CH	5	17
12	(*S*)	Cyclohexyl	Cyclohexyl	2	CH	31	34
13	(*R*)	Cyclohexyl	Cyclohexyl	2	CH	186	660
14	(*S*)	*iso*-Propyl	Cyclohexyl	1	CH	8	90
15	(*S*)	Cyclohexyl	4-Tetrahydropyranyl	1	CH	17	73
16	(*S*)	*iso*-Propyl	4-Tetrahydropyranyl	1	CH	22	685
17	(*S*)	Cyclohexyl	*cis*-4-Carboxycyclohexyl	1	CH	20	310
18	(*S*)	Cyclohexyl	*trans*-4-Carboxycyclohexyl	1	CH	>1000	>1000
19	(*S*)	Cyclohexyl	Cyclohexyl	1	CF	8	30
20	(*S*)	Cyclohexyl	Cyclohexyl	1	C(OMe)	12	36
21	(*S*)	Cyclohexyl	Cyclohexyl	1	N	5	7

7-carbon atom with nitrogen (analog **21**) maintained BACE activity (K_i = 5 nM), but improved cellular activity to an IC_{50} of 7 nM. A number of these macrocyclic analogs were tested in vivo, and β-amyloid lowering was detected in plasma.[11] However, upon intravenous, intraperitoneal, or oral administration, only trace levels of compound were detected in the brain, and no lowering of β-amyloid in the brain was observed. Preliminary experiments suggest that P-glycoprotein efflux may prevent brain penetration of these compounds. In conclusion, structural biology is a powerful tool in the design of potent, non-peptide BACE inhibitors, but a continuing challenge is the discovery of compounds which reduce β-amyloid levels in the brain upon oral administration.

Acknowledgments

We thank Chris Teleha, Scott Ballentine, Craig Diamond, and Laura Reany for the large scale synthesis of several key intermediates.

References and notes

1. (a) Goedert, M.; Spillantini, M. G. *Science* **2006**, *314*, 777; (b) Roberson, E. D.; Mucke, L. *Science* **2006**, *314*, 781.
2. (a) Giacobini, E.; Becker, R. E. *J. Alzheimer Dis.* **2007**, *12*, 37; (b) Robichaud, A. J. *Curr. Top. Med. Chem.* **2006**, *6*, 553; (c) Churcher, I. *Curr. Top. Med. Chem.* **2006**, *6*, 579; (d) Catalano, S. M.; Dodson, E. C.; Henze, D. A.; Joyce, J. G.; Krafft, G. A.; Kinney, G. G. *Curr. Top. Med. Chem.* **2006**, *6*, 597.
3. (a) Silvestri, R. *Med. Res. Rev.* **2009**, *29*, 295; (b) Stockley, J. H.; O'Neill, C. *Cellu. Mol. Life Sci.* **2008**, *65*, 3265; (c) Ghosh, A. K.; Gemma, S.; Tang, J. *Neurotherapeutics* **2008**, *5*, 399; (d) Venugopal, C.; Demos, C. M.; Rao, K. S. J.; Pappolla, M. A.; Sambamurti, K. C. N. S. *Neurol. Disord. Drug Targets* **2008**, *7*, 278; (e) Olson, R. E.; Marcin, L. R. *Annu. Rep. Med. Chem.* **2007**, *42*, 27; (f) Hills, I. D.; Vacca, J. P. *Curr. Opin. Drug Discovery Dev.* **2007**, *10*, 383.
4. (a) Nishitomi, K.; Sakaguchi, G.; Horikoshi, Y.; Gray, A. J.; Maeda, M.; Hirata-Fukae, C.; Becker, A. G.; Hosono, M.; Sakaguchi, I.; Minami, S. S.; Nakajima, Y.; Li, H.-F.; Takeyama, C.; Kihara, T.; Ota, A.; Wong, P. C.; Aisen, P. S.; Kato, A.; Kinoshita, N.; Matsuoka, Y. *Neurochemistry* **2006**, *99*, 1555; (b) Luo, Y.; Bolon, B.; Damore, M. A.; Fitzpatrick, D.; Liu, H.; Zhang, J.; Yan, Q.; Vassar, R.; Citron, M. *Neurobiol. Dis.* **2003**, *14*, 81. and references cited therein.
5. (a) Hu, X.; Hicks, C. W.; He, W.; Wong, P.; Macklin, W. B.; Trapp, B. D.; Yan, R. *Nat. Neurosci.* **2006**, *9*, 1520; (b) Laird, F. M.; Cai, H.; Savonenko, A. V.; Farah, M. H.; He, K.; Melnikova, T.; Wen, H.; Chiang, H.-C.; Xu, G.; Koliatsos, V. E.; Borchelt, D. R.; Price, D. L.; Lee, H.-K.; Wong, P. C. *J. Neurosci.* **2005**, *25*, 11693; (c) Dominguez, D.; Tournoy, J.; Hartmann, D.; Huth, T.; Cryns, K.; Deforce, S.; Serneels, L.; Camacho, I. E.; Marjaux, E.; Craessaerts, K.; Roebroeck, A. J. M.; Schwake, M.; D'Hooge, R.; Bach, P.; Kalinke, U.; Moechars, D.; Alzheimer, C.; Reiss, K.; Saftig, P.; De Strooper, B. *J. Biol. Chem.* **2005**, *280*, 30797.
6. Ohno, M.; Sametsky, E. A.; Younkin, L. H.; Oakley, H.; Younkin, S. G.; Citron, M.; Vassar, R.; Disterhoft, J. F. *Neuron* **2004**, *41*, 27.
7. (a) Sankaranarayanan, S.; Holahan, M. A.; Colussi, D.; Crouthamel, M.-C.; Devanarayan, V.; Ellis, J.; Espeseth, A.; Gates, A. T.; Graham, S. L.; Gregro, A. R.; Hazuda, D.; Hochman, J. H.; Holloway, K.; Jin, L.; Kahana, J.; Lai, M.-T.; Lineberger, J.; McGaughey, G.; Moore, K. P.; Nantermet, P.; Pietrak, B.; Price, E. A.; Rajapakse, H.; Stauffer, S.; Steinbeiser, M. A.; Seabrook, G.; Selnick, H. G.; Shi, X.-P.; Stanton, M. G.; Swestock, J.; Tugusheva, K. S.; Tyler, K. X.; Vacca, J. P.; Wong, J.; Wu, G.; Xu, M.; Cook, J. J.; Simon, A. J. *J. Pharmacol. Exp. Ther.* **2009**, *328*, 131; (b) Meredith, J. E., Jr.; Thompson, L. A.; Toyn, J. H.; Marcin, L.; Barten, D. M.; Marcinkeviciene, J.; Kopcho, L.; Kim, Y.; Lin, A.; Guss, V.; Burton, C.; Iben, L.; Polson, C.; Cantone, J.; Ford, M.; Drexler, D.; Fiedler, T.; Lentz, K. A.; Grace, J. E., Jr.; Kolb, J.; Corsa, J.; Pierdomenico, M.; Jones, K.; Olsen, R. E.; Macor, J. E.; Albright, C. F. *J. Pharmacol. Exp. Ther.* **2008**, *326*, 502; (c) Barrow, J. C.; Stauffer, S. R.; Rittle, K. E.; Ngo, P. L.; Yang, Z.; Selnick, H. G.; Graham, S. L.; Munshi, S.; McGaughey, G. B.; Holloway, M. K.; Simon, A. J.; Price, E. A.; Sankaranarayanan, S.; Colussi, D.; Tugusheva, K.; Lai, M.-T.; Espeseth, A. S.; Xu, M.; Huang, Q.; Wolfe, A.; Pietrak, B.; Zuck, P.; Levorse, D. A.; Hazuda, D.; Vacca, J. P. *J. Med. Chem.* **2008**, *51*, 6259; (d) Ghosh, A. K.; Kumaragurubaran, N.; Hong, L.; Kulkarni, S.; Xu, X.; Miller, H. B.; Reddy, D. S.; Weerasena, V.; Turner, R.; Chang, W.; Koelsch, G.; Tang, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1031; (e) Stanton, M. G.; Stauffer, S. R.; Gregro, A. R.; Steinbeiser, M.; Nantermet, P.; Sankaranarayanan, S.; Price, E. A.; Wu, G.; Crouthamel, M.-C.; Ellis, J.; Lai, M.-T.; Espeseth, A. S.; Shi, X.-P.; Jin, L.; Colussi, D.; Pietrak, B.; Huang, Q.; Xu, M.; Simon, A. J.; Graham, S. L.; Vacca, J. P.; Selnick, H. *J. Med. Chem.* **2007**, *50*, 3431; (f) Ghosh, A. K.; Kumaragurubaran, N.; Hong, L.; Kulkarni, S. S.; Xu, X.; Chang, W.; Weerasena, V.; Turner, R.; Koelsch, G.; Bilcer, G.; Tang, J. *J. Med. Chem.* **2007**, *50*, 2399; (g) Hussain, I.; Hawkins, J.; Harrison, D.; Hille, C.; Wayne, G.; Cutler, L.; Buck, T.; Walter, D.; Demont, E.; Howes, C.; Naylor, A.; Jeffrey, P.; Gonzalez, M. I.; Dingwall, C.; Michel, A.; Redshaw, S.; Davis, J. B. *J. Neurochem.* **2007**, *100*, 802; (h) Stachel, S. J.; Coburn, C. A.; Sankaranarayanan, S.; Price, E. A.; Pietrak, B. L.; Huang, Q.; Lineberger, J.; Espeseth, A. S.; Jin, L.; Ellis, J.; Holloway, M. K.; Munshi, S.; Allison, T.; Hazuda, D.; Simon, A. J.; Graham, S. L.; Vacca, J. P. *J. Med. Chem.* **2006**, *49*, 6147; (i) Asai, M.; Hattori, C.; Iwata, N.; Saido, T. C.; Sasagawa, N.; Szabó, B.; Hashimoto, Y.; Maruyama, K.; Tanuma, S.; Kiso, Y.; Ishiura, S. *J. Neurochem.* **2006**, *96*, 533; (j) Chang, W.-P.; Koelsch, G.; Wong, S.; Downs, D.; Da, H.; Weerasena, V.; Gordon, B.; Devasamudram, T.; Bilcer, G.; Ghosh, A. K.; Tang, J. *J. Neurochem.* **2004**, *89*, 1409.
8. Baxter, E. W.; Conway, K. A.; Kennis, L.; Bischoff, F.; Mercken, M. H.; De Winter, H. L.; Reynolds, C. H.; Tounge, B. A.; Luo, C.; Scott, M. K.; Huang, Y.; Braeken, M.; Pieters, S. M. A.; Berthelot, D. J. C.; Masure, S.; Bruinzeel, W. D.; Jordan, A. D.; Parker, M. H.; Boyd, R. E.; Qu, J.; Qu, J.; Qu, J.; Brenneman, D. E.; Reitz, A. B. *J. Med. Chem.* **2007**, *50*, 4261.
9. Ghosh, A. K.; Shin, D.; Downs, D.; Koelsch, G.; Lin, X.; Ermolieff, J.; Tang, J. *J. Am. Chem. Soc.* **2000**, *122*, 3522.
10. Jorgensen, W. L.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1988**, *110*, 1657.
11. When macrocycle **7** was dosed orally in non-transgenic mice, a statistically significant effect on Aβ lowering in plasma was observed: 23%, 22%, and 13%, at 50 mg/kg, 25 mg/kg, and 10 mg/kg dose, respectively, in 10% solutol.