Supporting Information

Fragment-based Discovery of Non-peptidic BACE-1 Inhibitors using Tethering

Wenjin Yang[†]*, Raymond V. Fucini[‡], Bruce T. Fahr[†], Mike Randal[#], Kenneth E. Lind[†], Melissa B. Lam[#], Wanli Lu[†], Yafan Lu[†], Douglas R. Cary[†], Michael J. Romanowski[#], Dennis Colussi[§], Beth Pietrak[§], Timothy J. Allison[£], Sanjeev K. Munshi[£], David M. Penny[#], Phuongly Pham[†], Jian Sun[†], Anila E. Thomas[‡], Jennifer M. Wilkinson[‡], Jeffrey W. Jacobs[†], Robert S. McDowell[†], Marcus D. Ballinger[‡]*

Departments of [†]Chemistry, [‡]Biochemistry, and [#]Protein Sciences, Sunesis Pharmaceuticals, Incorporated, 341 Oyster Point Boulevard, South San Francisco, California 94080

[§]Department of Alzheimer's Research, Merck Research Laboratories, PO Box 4, West Point, PA 19486

[£]Department of Structural Biology, Merck Research Laboratories, PO Box 4, West Point, PA 19486

*To whom correspondence should be addressed. Phone: 650-266-3645 (WY), 650-837-7981 (MB). E-mail: Sunesiswenjin@yahoo.com, mballing@exelixis.com.
[†]Sunesis Pharmaceuticals, Department of Chemistry.
[‡]Sunesis Pharmaceuticals, Department of Biochemistry.
[#]Sunesis Pharmaceuticals, Department of Protein Sciences.
[§]Merck Research Labs, Department of Alzheimer's Research.

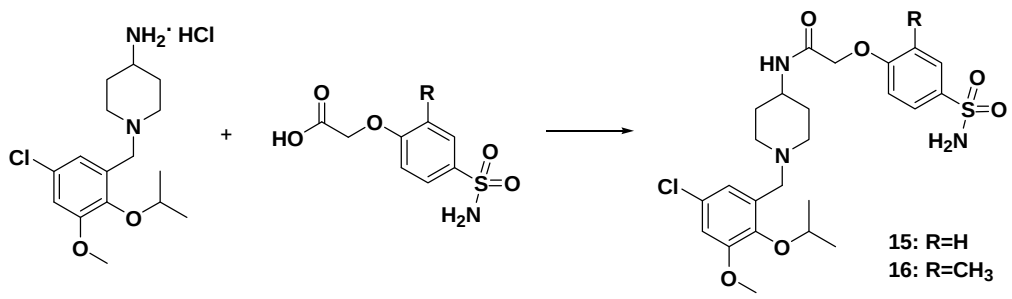
Abbreviations:
HATU, 2-(1H-7-azabenzotriazol-1-yl)--1,1,3,3-tetramethyl uronium hexafluorophosphate; DIEA, diisopropylethylamine; EI-MS, electrospray ionization mass spectrometry; ES (+) MS, positive ion electrospray mass spectrometry; TFA, trifluoroacetic acid; DCM, Dichloromethane.

Chemistry

Monophore Library.

The Sunesis monophore library consisted of a proprietary collection of diverse, low molecular weight fragments selected for their favorable drug-like properties, including clogP, polar surface area, and net hydrogen bond donors and acceptors. These fragments were functionalized with disulfide-containing linkers to permit capture by cysteine-bearing proteins. Most fragments were functionalized with a disulfide tether using amide bond chemistry. The synthetic methods for this library have been described by Erlanson et al(*1*).

General procedure for preparing compound 13 and 14:

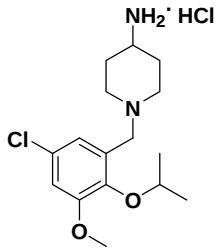


To the mixture of substituted phenoxy-acetic acid compound and HATU was added 1-(5-chloro-2-isopropoxy-3-methoxy-benzyl)-piperidin-4-ylamine, then DIEA. After stirring at room temperature for 3 h, the solvent was evaporated under reduced pressure. The crude product was dissolved in methanol for purification using HPLC (reversed phase) to give final compounds **13** and **14**.

N-[1-(5-Chloro-2-isopropoxy-3-methoxy-benzyl)-piperidin-4-yl]-2-(4-sulfamoyl-phenoxy)-acetamide 13. ^{1}H NMR (400 MHz, METHANOL-D4) δ ppm 1.31 (d, *J*=5.87 Hz, 6 H) 1.82 (m, 2 H) 2.14 (d, *J*=13.21 Hz, 2 H) 3.20 (t, *J*=12.72 Hz, 2 H) 3.54 (d, *J*=11.74 Hz, 2 H) 3.91 (s, 3 H) 4.06 (m, 1 H) 4.31 (s, 2 H) 4.62 (s, 2 H) 4.77 (m, 1 H) 7.13 (s, 3 H) 7.22 (s, 1 H) 7.86 (d, *J*=8.80 Hz, 2 H). EI-MS *m/z*: 526 $(M + H)^+$.

N-[1-(5-Chloro-2-isopropoxy-3-methoxy-benzyl)-piperidin-4-yl]-2-(2-methyl-4-sulfamoyl-phenoxy)-acetamide 14. ^{1}H NMR (400 MHz, METHANOL-D4) δ ppm 1.28 (d, *J*=6.36 Hz, 6 H) 1.82 (d, *J*=15.16 Hz, 2 H) 2.07 (m, 2 H) 2.33 (m, 3 H) 3.17 (t, *J*=12.47 Hz, 2 H) 3.51 (d, *J*=12.23 Hz, 2 H) 3.75 (m, 1 H) 3.88 (s, 3 H) 4.04 (m, 1 H) 4.30 (s, 2 H) 4.63 (s, 2 H) 4.73 (m, 1 H) 6.91 (d, *J*=8.31 Hz, 1 H) 7.18 (m, 2 H). EI-MS *m/z*: 540 $(M + H)^+$.

Preparation of 1-(5-Chloro-2-isopropoxy-3-methoxy-benzyl)-piperidin-4-ylamine:



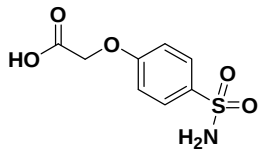
To an ice-cold mixture of 5-chloro-2-hydroxy-3-methoxy-benzaldehyde (10.0 g, 54.4 mmol) in 110.0 mL DMF was added 60% NaH in mineral oil (2.87g, 71.8 mmol) in portions. After stirring for 5 minutes to reach room temperature, 2-iodo-propane (7.1 mL, 71.1 mmol) was added. The reaction mixture was stirred at 65 °C for 12 h, then diluted with water, extracted with ethyl ether, washed with water, 1 N NaOH and brine, dried with $MgSO_4$ and filtered. The solvent was removed under reduced pressure to give 5-chloro-2-isopropoxy-3-methoxy-benzaldehyde (8.75 g, 70.4%) as a yellow solid that was used for the next step without purification. ES (+) MS m/e = 251 (M+23).

To the mixture of 5-chloro-2-isopropoxy-3-methoxy-benzaldehyde (4.58 g, 20.05 mmol) and piperidin-4-yl-carbamic acid *tert*-butyl ester (4.50 g, 22.46 mmol) in 50.0 mL

methanol was added sodium triacetoxy-borohydride (14.78 g, 69.74 mmol). After stirring at room temperature for 3 h, the reaction mixture was diluted with water, and extracted with ethyl acetate. The ethyl acetate layer was washed with water and brine, dried with $MgSO_4$ and filtered. The solvent was removed under reduced pressure to provide [1-(5-Chloro-2-isopropoxy-3-methoxy-benzyl)-piperidin-4-yl]-carbamic acid *tert*-butyl ester (6.772 g, 73.0%) as colorless oil that was used for next step without purification. ES (+) MS m/e = 413 (M+1).

[1-(5-Chloro-2-isopropoxy-3-methoxy-benzyl)-piperidin-4-yl]-carbamic acid *tert*-butyl ester was dissolved in 25% TFA in DCM and stirred at room temperature for 2 h. The solvent was evaporated under vacuo, and the amine residue converted to an HCl salt using 4M HCl in dioxane to yield 1-(5-Chloro-2-isopropoxy-3-methoxy-benzyl)-piperidin-4-ylamine-bis HCl. (1.5 g, 16.4 mmol) as a white solid. EI-MS *m/z*: 313 $(M + H)^+$.

Preparation of (4-Sulfamoyl-phenoxy)-acetic acid:



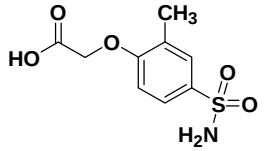
To an ice-cold solution of phenoxy-acetic acid methyl ester (1.0 g, 6.0 mmol) in 40.0 mL DCM, was added chlorosulfonic acid (4.0mL, 60.0 mmol) drop wise. The reaction mixture was stirred at 0 °C for 1 h, and then at room temperature for 12 h. The reaction mixture was quenched slowly with ice water and extracted with DCM. The DCM layer was washed with water and brine, dried with $MgSO_4$ and filtered. Solvent was removed under reduced pressure to provide (4-chlorosulfonyl-phenoxy)-acetic acid methyl ester (1.12 g, 70.0%) as a semi-solid which was used for next step without purification. ^1H NMR (400 MHz, CHLOROFORM-D) δ ppm 3.83 (s, 3 H) 4.74 (s, 2 H) 7.04 (d, *J*=8.31 Hz, 2 H) 7.98 (d, *J*=8.80 Hz, 2 H).

To the mixture of 4-chlorosulfonyl-phenoxy)-acetic acid methyl ester (0.1325 g, 0.5 mmol) in 2.5 mL THF was added concentrated ammonium hydroxide (0.10 mL, 1.0 mmol). After stirring at room temperature for 12 h, water was added to the resulting

mixture. White solid was formed. The white solid was washed and collected by filtration to yield (4-sulfamoyl-phenoxy)-acetic acid methyl ester (0.061 g, 50.0%).

The mixture of (4-sulfamoyl-phenoxy)-acetic acid methyl ester (0.061 g, 0.5 mmol) in 0.5 mL methanol was added 2.0 mL of 1.0 M LiOH. After heating at 60 °C for 2h upon completion, the reaction mixture was acidified with 12 N HCl and extracted with ethyl acetate. The ethyl acetate layer was washed with water and brine, dried with $MgSO_4$ and filtered. The solvent was removed under reduced pressure to provide (4-sulfamoyl-phenoxy)-acetic acid (0.031 g, 52.0%) as a white solid. ES (+) MS m/e = 215 (M-16).

Preparation of the starting materials: (2-Methyl-4-sulfamoyl-phenoxy)-acetic acid:



To an ice cold solution of (2-methylphenoxy) acetic acid (3.77 g, 22.7 mmol) in 50.0 mL of 1:9 v/v methanol/benzene, 3.0 mL of 2.0 M (trimethylsilyl) diazomethane in hexanes was added slowly. After stirring to reach room temperature (3 h), the resulting mixture was quenched with acetic acid until the solution no longer had a trace of yellow color, and then diluted with ethyl ether. The ethyl ether layer was washed with water and brine, dried with $MgSO_4$ and filtered. Solvent was removed under reduced pressure to provide (2-methylphenoxy) acetic acid methyl ester (4.14g, 100.0 %) as colorless oil, which was used for the next step without purification. ES (+) MS m/e = 203 (M+23).

To an ice-cold solution of (2-methylphenoxy) acetic acid methyl ester (3.76 g, 20.9 mmol) in 100.0 mL DCM, was added chlorosulfonic acid (10.0 mL, 150.0 mmol) drop wise. The reaction mixture was stirred at 0 °C for 1 h and then room temperature for 12 h. The reaction mixture was quenched slowly with ice water and extracted with DCM. The DCM layer was washed with water and brine, dried with $MgSO_4$ and filtered. The solvent was removed under reduced pressure to provide (4-chlorosulfonyl-2-methyl-phenoxy)-acetic acid methyl ester (4.22 g, 72.4%) as a semi-solid, which was used for the next step without purification.

To a solution of (4-chlorosulfonyl-2-methyl-phenoxy)-acetic acid methyl ester (0.5574 g, 5.0 mmol) in 10.0 mL THF was added concentrated NH_4OH (0.540 mL, 8.0 mmol). After stirring at room temperature for 3 h, the reaction mixture was quenched with 1 N HCl and extracted with ethyl acetate. The ethyl acetate layer was washed with water and brine, dried with $MgSO_4$ and filtered. The solvent was removed under reduced pressure to provide (2-methyl-4-sulfamoyl-phenoxy)-acetic acid methyl ester (0.420 g, 80.0 %) as a white solid, which was used for the next step without purification. ES (+) MS m/e = 243 (M-16).

The mixture of (2-methyl-4-sulfamoyl-phenoxy)-acetic acid methyl ester (0.420 g, 1.60 mmol) in 5.0 mL methanol was added 3.2 mL of 1.0 N NaOH. After heating at 60 $^{\circ}$C for 2h to complete the reaction, the mixture was acidified with 1 N HCl and extracted with ethyl acetate. The ethyl acetate layer was washed with water and brine, dried with $MgSO_4$ and filtered. Solvent was removed under reduced pressure to provide (2-methyl-4-sulfamoyl-phenoxy)-acetic acid (0.6303 g, 91.9%) as a white solid. ES (+) MS m/e = 268 (M+23).

Computational chemistry.

Replacements for the benzyl group were chosen from a list of ~12,000 commercially available aldehydes. The core piperidine structure (without disulfide bond) was enumerated with all aldehyde substituents and docked into the active site of the V332C mutant structure using ICM software (Molsoft). Light restraints (1 kcal/mol) were placed on the heavy atoms of the piperidine core to constrain to the known position from the crystal structure. The top 1000 resulting poses were fully enumerated and covalently attached to the V332C residue. These poses were then subjected to 3 rounds of minimization in which the atoms of the monophore and protein were allowed to move, while the constraints were lowered (1, 0.1, 0.01 kcal/mol respectively). A final set of 200

compounds with low energy poses was chosen such that they provided a diverse sampling of molecular properties (MW, # donors, # acceptors, # rotatable bonds). Choices of substituents to replace the thiol–containing linker fragments were also filtered by constrained docking using ICM.

Cloning, expression, and purification of BACE-1 protein variants.

BACE-1 cysteine mutants were generated in the background of an enzyme variant capable of cleaving off its prodomain under acidic conditions(*2*). For some constructs, a variant containing additional surface mutations K75A and E77A was used as a template(*3*). Briefly, a T7-driven expression plasmid, encoding pro-BACE-1 starting at amino acid 9p (numbering based on Hong, et al(*4*); Genbank accession number NP_036236), was used as a DNA template for introduction of ELNL processing-site mutations in place of the wildtype LPLR-encoding sequence at residues 29p to 32p with the QuikChange Site-Directed Mutagenesis Kit from Stratagene as recommended by the manufacturer. The processing-site mutant BACE-1 constructs were transformed into BL21 Star *E. coli* cells (Invitrogen), cells were grown at 37ºC to an OD600 = 0.8, and protein expression was induced with 1 mM IPTG for 3 hrs. Cell pellets, harvested by centrifugation, were resuspended in 10 mM Tris-HCl pH 8.0 and 1 mM EDTA and lysed with a microfluidizer (two passages). Insoluble inclusion bodies containing BACE-1 were isolated by centrifugation and washed twice by resuspension in PBS (10 mM NaH_2PO_4 pH 7.4, 150 mM NaCl) supplemented with 0.5% Triton X-100. Washed inclusion body pellets were solubilized in 50 mM CAPS pH 10, 8 M urea, 1 mM EDTA, and 100 mM β-mercaptoethanol, and remaining insoluble debris removed by centrifugation at 18,000 rpm for 30 min. BACE-1 was refolded by slow injection of the

urea-solubilized protein to between 50 and 100 volumes of rapidly stirring 10 mM Na_2CO_3 pH 10, followed by incubation at room temperature for 3-5 days. When BACE-1 enzymatic activity no longer increased over time, the pH of the refolding solution was adjusted to 8.0 by addition of Tris-HCl to a final 10-mM concentration. The protein was purified by ion-exchange chromatography on Q-Sepharose using a 40-min linear gradient between 50 mM and 1.0 M NaCl in 10 mM Tris-HCl pH 8.0. BACE-1 fractions were acidified to pH 4.5 with sodium citrate which resulted in enzyme activation and N-terminal autoprocessing. Once autoprocessing was complete, the proteins were further purified directly by ion-exchange chromatography on S-Sepharose. Purified enzyme was dialyzed at 4°C against 20 mM Tris pH 7.5, 0.15 M NaCl, and stored at 4°C. Protein concentrations were determined by measuring absorbance at 280 nm, using $\varepsilon_{280}^{1\%} = 0.74$.

LCMS Detection of tethering screen hits

A screening sample was injected (Gilson 215 autosampler) with a flow rate of 0.7 ml/min onto a Phenomenex Jupiter C5, 300 Angstrom, 5 μm, 50X2 mm column in 50% Solvent A (water/0.05% TFA)/50% Solvent B (CH_3CN/0.05% TFA) and subjected to a linear gradient to 5% Solvent A (water/0.05% TFA)/95% Solvent B (CH_3CN/0.05% TFA) over 0.75 minutes, followed by a linear gradient to 95% Solvent A / 5% Solvent B over 0.25 minutes and then held for 1 min at 95% Solvent A. Spectra were deconvoluted using the Excalibur software package (Finnigan), and peak areas used to quantitate the fraction of protein conjugated to bound cystamine and monophore compounds. Each of the 11 BACE-1 cysteine mutants yielded mass spectra with sensitivity sufficient to detect >10% modification of proteins by monophores. Primary hits (>10% modification of the

protein by a specific monophore thiol) were confirmed in secondary assays versus resynthesized, discrete compounds.

Crystallization, data collection, and structure determination.

Although monophore disulfides could often also yield completely conjugated cysteine variants, methanethiosulfonate (MTS) monophore dervatives, which are highly reactive toward free thiols, generally produced virtually homogenously labeled protein preparations suitable for structural studies. MTS derivatives were prepared from monophore compounds using published methods(*5*).

In order to prepare homogeneously modified BACE-1 cysteine mutant-monophore conjugates, 6xHis tag-cleaved proteins were dialyzed overnight against a reaction buffer composed of 20 mM Tris-HCl pH 7.5, 30 mM NaCl, 5 mM $MgCl_2$, and incubated with 1 mM MTS-monophore derivative at 1.5-4.5 mg/ml final concentration for 0.5 hr at room temperature. The extent of the modification was evaluated with electrospray ionization mass spectrometry (Finnigan) and the complexes were purified by gel filtration. Reactions yielding > 90% labeled protein were dialyzed overnight into 20 mM Tris pH 7.5, 0.15 M NaCl, and concentrated to 6-8 mg/ml.

BACE-1-monophore conjugates were subjected to multiple crystallization screens (Hampton Research and Emerald BioSystems). Modified protein was mixed 1:1 (v/v) with mother liquor and incubated at 20°C. Crystallization conditions are listed in Supporting Information Table S1. All crystals for data collection were cryoprotected in mother liquor supplemented with 20% (v/v) glycerol for approximately 1 min and rapid immersion in liquid nitrogen.

Diffraction data were collected under standard cryogenic conditions a Rigaku RU-3R rotating-anode generator and an R-AXIS IV detector (hits **1**, **2**, **4** and **14**), MicroMax-007 HF (hit **13**), Beamline 7.1 at the Stanford Synchrotron Research Laboratory (hit **10**), and Beamline 5.0.1 at the Advanced Light Source (hit **3**), and were processed and scaled with *CrystalClear* from Rigaku/Molecular Structure Corporation (*6*). The structures were determined from single-wavelength native diffraction experiments by molecular replacement with *AMoRe* (*7*) using a search model from a previously determined structure (PDB code 2P8H) (*8*). The refinement of the initial solutions with *REFMAC* (*9-11*) yielded experimental electron-density maps suitable for model building with *O* (*12*). Residues not visible in the electron-density maps were omitted from refinement of the final atomic models. *PROCHECK* (*13*) revealed good stereochemistry (see Table 2 for a summary of X-ray data and refinement statistics). All proteins and small-molecule inhibitors in the figures were rendered with *PyMOL* (*14*).

Table S1. Crystallization conditions for BACE-1 complexes and monophore conjugates.

Conjugate			Crystallization	
Complex (PDB ID code)	Concentration (mg/ml)	Buffer	Buffer	Temperature (°C)
V332C+Hit **1** (2ZJH)	7	20 mM Tris pH 7.5, 0.15 M NaCl	0.1 M Bis-Tris pH 6.5, 0.1 M NaCl, 1.5 M $(NH_4)_2SO_4$	20
T329C+Hit **2** (2ZJI)	7	20 mM Tris pH 7.5, 0.15 M NaCl	2.0 M sodium formate pH 7.0	20
T231C+Hit **3** (2ZJJ)	7	20 mM Tris pH 7.5, 0.15 M NaCl	0.1 M imidazole pH 8.0, 0.2 M $Ca(OAC)_2$, 10% w/v PEG 8,000	20
T72C+Hit **4** (2ZJK)	7	20 mM Tris pH 7.5, 0.15 M NaCl	0.1 M Bis-Tris pH 5.5, 0.2 M $Li_2SO_4 \cdot H_2O$, 25% w/v PEG 3,350	20
V332C+Hit **10** (2ZJL)	7	20 mM Tris pH 7.5, 0.15 M NaCl	0.1 M $(CH_3)_2AsO_2Na \cdot 3H_2O$ pH 6.5, 1.26 M $(NH_4)_2SO_4$	20
K75A, E77A+Hit **13** (2ZJM)	6	20 mM Tris pH 7.5, 0.15 M NaCl	0.1 M HEPES pH 7.5, 1.5 M Li_2SO_4, Merck L'671 + soak with Hit **13**(1)	20
K75A, E77A+Hit **14** (2ZJN)	6	20 mM Tris pH 7.5, 0.15 M NaCl	0.1 M HEPES pH 7.5, 1.5 M Li_2SO_4, Merck L'671 + soak with Hit **14**(1)	20

(1) Crystals were soaked for several days in 0.1 M $NaH_2(C_3H_5O(COO)_3)$ pH 5.0 and 1.5 M Li_2SO_4 supplemented with 0.5 mM inhibitor.

Table S2. Refinement statistics for BACE-1 complex structures

PDB ID	2ZJH	2ZJI	2ZJJ	2ZJK	2ZJL	2ZJM	2ZJN
Variant	Val332→Cys	Thr329→Cys	Lys75→Ala Glu77→Ala Thr231→Cys	Thr72→Cys	Val332→Cys	Lys75→Ala Glu77→Ala	None
Compound	**1**	**2**	**3**	**4**	**10**	**13**	**14**
Space group	$P6_122$	$P6_122$	$P2_12_12_1$	$C2$	$P6_122$	$C222_1$	$P6_122$
Cell constants	a=b=103.6 Å c=168.7 Å	a=b=103.3 Å c=168.0 Å	a=47.2 Å b=93.3 Å c=100.3 Å	a=233.1 Å b=108.5 Å c=64.1 Å β=103.3 °	a=b=104.1 Å c=168.0 Å	a=103.6 Å b=127.8 Å c=76.6 Å	a=b=104.0 Å c=167.1 Å
X-ray source	Rigaku RU-3R	Rigaku RU-3R	ALS 5.0.1	Rigaku RU-3R	SSRL 7-1	Rigaku MicroMax-007 HF	Rigaku RU-3R
Wavelength (Å)	1.54	1.54	0.98	1.54	1.08	1.54	1.54
Resolution (Å)	20-2.6	20-2.3	20-2.2	20-2.8	20-2.1	50-1.9	20-2.7
Number of observations	84887	142477		101873	108584	452425	50480
Number of reflections	16879	24184	18114	37401	31523	40419	15191
Completeness (%)[a]	99.0 (99.8)	100.0 (99.9)		97.8 (98.6)	98.4 (99.5)	100.0 (100.0)	99.7 (99.5)
Mean $I/(\sigma I)$ [a]	10.8 (3.7)	10.8 (4.3)		6.0 (1.7)	11.3 (3.6)	28.0 (5.0)	9.3 (3.7)
R-merge on I [a, b]	0.111 (0.432)	0.078 (0.357)		0.104 (0.555)	0.065 (0.339)	0.094 (0.454)	0.097 (0.328)
Cutoff criteria	I<-3σ(I)	I<-3σ(I)	I<-3σ(I)	I<-3σ(I)	I<-3σ(I)	I<-3σ(I)	I<-3σ(I)
Model and refinement statistics							
Resolution range (Å)	20-2.6	20-2.3	20-2.2	20-3.0	20-2.1	50-1.9	20-2.7
Number of reflections[c]	15848 (844)	22764 (1244)	15750 (877)	28202 (1471)	29497 (1583)	37835 (3802)	14385 (748)
Completeness (%)	97.8	99.3	71.8	95.3	97.0	96.8	99.3
Cutoff criterion	|F|>0.0	|F|>0.0	|F|>0.0	|F|>0.0	|F|>0.0	|F|>0.0	|F|>0.0
Number of residues	356	370	370	1111	344	377	367
Number of water molecules	94	224	164	0	249	260	52
R.m.s.d. bond lengths (Å)	0.006	0.006	0.006	0.006	0.006	0.006	0.006
R.m.s.d. bond angles (°)	0.964	0.883	0.873	0.916	0.946	1.300	0.881
Luzzati error (Å)	0.372	0.330	0.336	0.477	0.351	0.208	0.385
Correlation factor[d]	0.861	0.895	0.901	0.853	0.897	0.936	0.856
$\mathbf{R_{cryst}}$ [e]	22.7	23.4	22.9	24.6	25.8	20.8	22.2
$\mathbf{R_{free}}$	26.9	26.4	26.9	29.3	29.0	23.1	26.0
Ramachandran plot statistics[f]							
Most favored	262 (87.3%)	278 (88.5%)	280 (88.9%)	825 (87.7%)	252 (87.2%)	278 (86.1%)	277 (89.1%)
Additional allowed	36 (12.0%)	35 (11.1%)	34 (10.8%)	111 (11.8%)	35 (12.1%)	43 (13.3%)	32 (10.3%)
Generously allowed	1 (0.3%)	0 (0%)	0 (0.0%)	2 (0.2%)	1 (0.3%)	1 (0.3%)	1 (0.3%)
Disallowed	1 (0.3%)	1 (0.3%)	1 (0.3%)	3 (0.3%)	1 (0.3%)	1 (0.3%)	1 (0.3%)
Overall G-factor[g]	0.1	0.1	0.2	0.1	0.1	0.3	0.2

References

1. Erlanson, D. A., Braisted, A. C., Raphael, D. R., Randal, M., Stroud, R. M., Gordon, E. M., and Wells, J. A. (2000) Site-directed ligand discovery, *Proc Natl Acad Sci U S A 97*, 9367-9372.
2. Ballinger, M. D., and Randal, M. (2005) Constructs for homogeneously processed preparations of beta site APP-cleaving enzyme. PCT/US2004/021816.
3. Coburn, C. A., Stachel, S. J., Li, Y. M., Rush, D. M., Steele, T. G., Chen-Dodson, E., Holloway, M. K., Xu, M., Huang, Q., Lai, M. T., DiMuzio, J., Crouthamel, M. C., Shi, X. P., Sardana, V., Chen, Z., Munshi, S., Kuo, L., Makara, G. M., Annis, D. A., Tadikonda, P. K., Nash, H. M., Vacca, J. P., and Wang, T. (2004) Identification of a small molecule nonpeptide active site beta-secretase inhibitor that displays a nontraditional binding mode for aspartyl proteases, *J Med Chem 47*, 6117-6119.

4. Hong, L., Koelsch, G., Lin, X., Wu, S., Terzyan, S., Ghosh, A. K., Zhang, X. C., and Tang, J. (2000) Structure of the protease domain of memapsin 2 (beta-secretase) complexed with inhibitor, *Science 290*, 150-153.
5. Johnson, J. L., Cusack, B., Hughes, T. F., McCullough, E. H., Fauq, A., Romanovskis, P., Spatola, A. F., and Rosenberry, T. L. (2003) Inhibitors tethered near the acetylcholinesterase active site serve as molecular rulers of the peripheral and acylation sites, *J Biol Chem 278*, 38948-38955.
6. Pflugrath, J. W. (1999) The finer things in X-ray diffraction data collection, *Acta Crystallogr. D 55*, 1718-1725.
7. Navaza, J. (2001) Implementation of molecular replacement in AMoRe, *Acta Crystallogr. D 57*, 1367-1372.
8. Barrow, J. C., Rittle, K. E., Ngo, P. L., Selnick, H. G., Graham, S. L., Pitzenberger, S. M., McGaughey, G. B., Colussi, D., Lai, M. T., Huang, Q., Tugusheva, K., Espeseth, A. S., Simon, A. J., Munshi, S. K., and Vacca, J. P. (2007) Design and Synthesis of 2,3,5-Substituted Imidazolidin-4-one Inhibitors of BACE-1, *ChemMedChem 2*, 995-999.
9. Murshudov, G. N., Vagin, A. A., and Dodson, E. J. (1997) Refinement of macromolecular structures by the maximum-likelihood method, *Acta Crystallogr. D 53*, 240-255.
10. Murshudov, G. N., Vagin, A. A., Lebedev, A., Wilson, K. S., and Dodson, E. J. (1999) Efficient anisotropic refinement of macromolecular structures using FFT, *Acta Crystallogr. D 55*, 247-255.
11. Pannu, N. S., Murshudov, G. N., Dodson, E. J., and Read, R. J. (1998) Incorporation of prior phase information strengthens maximum-likelihood structure refinement, *Acta Crystallogr. D 54*, 1285-1294.
12. Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) Improved methods for building protein models in electron density maps and the location of errors in these models, *Acta Crystallogr. A 47*, 110-119.
13. Laskowski, R. A., MacArthur, M. W., Moss, D. S., and Thronton, J. M. (1993) PROCHECK: a program to check the stereochemical quality of protein structures., *J. Appl. Crystallogr. D 26*, 283-291.
14. DeLano, W. L. (2002) *The PyMOL User's Manual*, DeLano Scientific, San Carlos, CA, USA.