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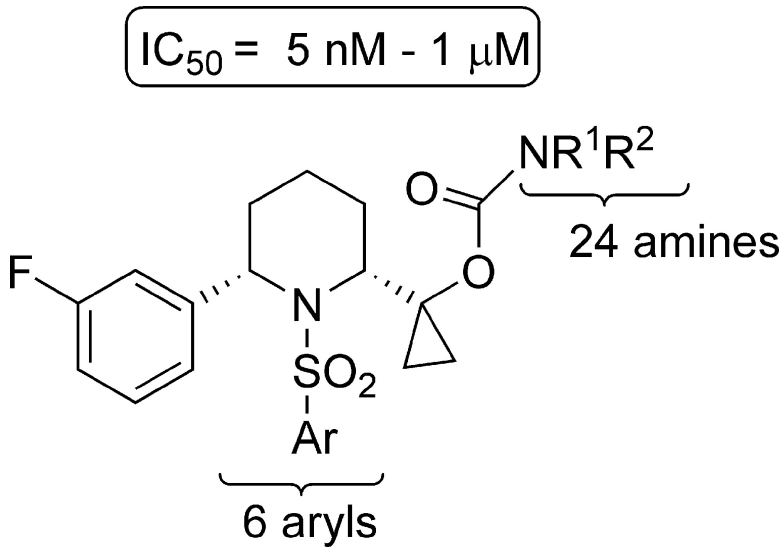

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Solution-Phase Parallel Synthesis of Carbamates as γ-Secretase Inhibitors

Henry A. Vaccaro,† Zhiqiang Zhao,† John W. Clader,† Lixin Song,‡ Giuseppe Terracina,‡ Lili Zhang,‡ and Dmitri A. Pissarnitski*,†

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A novel methodology for parallel liquid-phase synthesis of carbamates suitable for the preparation of sterically hindered molecules is disclosed. The alcohols are converted to 4-nitrophenylcarbonates, followed by the reaction with amines. Side product 4-nitrophenol and the unreacted excess amines are scavenged by appropriately chosen cleanup resins, selected among Amberlyst A26 (hydroxide form) and macroporous sulfonic acid (MP-TsOH) or polystyrene isocyanate (PS-NCO) and polystyrene benzaldehyde (PS-PhCHO) resins. As a part of a medicinal chemistry program directed toward finding γ-secretase inhibitors as prospective drug candidates for Alzheimer's disease, a 6 × 24 library of carbamates was prepared. Out of 144 library members, 133 had a purity for the targeted compound of 80% or better. The prepared compounds were assessed in the γ-secretase inhibition assay and demonstrated activity with IC_{50} values in the range from 1 μM to 5 nM, with the activity of 7 compounds being better than 10 nM.

Introduction

Molecules containing a carbamate functional group are common in the drug discovery process. Carbamates are often incorporated into biologically active molecules with high oral bioavailability and low clearance, as in the case of the urinary incontinence drug solifenacin (**1**)[1] (Figure 1). Alternatively, attachment of the carbamate side chain to a known biologically active molecule may improve its physicochemical properties, which makes carbamates useful as prodrugs. For instance, a carbamate modification of an antitumor agent camptothecin[2] accounts for the improvement of the aqueous solubility of the resulting drug irinotecan (**2**)[3] (Figure 1).

Carbamates remain an active field of drug research for diverse medicinal chemistry programs throughout the world, including antitumor,[4] anti-infective,[5] immunosuppressive,[6] and central nervous system[7] areas.

We became interested in the synthesis of carbamates in the course of a drug discovery program targeted to Alzheimer's disease (AD). This common degenerative brain disorder accounts for up to 70% of all cases of dementia and is the third most-common cause of death in the United States.[8] One of the major pathological hallmarks of AD is abnormal extracellular deposition of β-amyloid peptide (Aβ) in the form of plaques in the brains of AD patients. Although the exact cause of AD is unknown, a large body of evidence suggests[9] that overproduction of Aβ is central to its pathogenesis. Aβ is produced by proteolysis of the larger amyloid precursor protein (APP) by sequential action of β- and γ-secretases. Because γ-secretase plays a central role in the generation of Aβ peptide, inhibition of this enzyme was proposed as a target for the treatment of AD.[10]

Recently, a few cyclic sulfonamides of type **3** (Figure 2), bearing a carbamate side chain, were shown to inhibit γ-secretase in vitro and to reduce amyloid burden in vivo in the animal model of AD.[11] To effectively explore structure–activity relationship (SAR) of Ar and NR^1R^2 groups in **3**, an expeditious parallel synthesis of new analogs of **3** was required. Although several methodologies for parallel solid-[12] and liquid-phase[13] synthesis of carbamates have been reported previously, their practicality for the needs of drug discovery has not been demonstrated. Herein, we disclose a new and practical method for the liquid-phase parallel synthesis of sterically hindered carbamates, exemplified by the synthesis of a targeted library of γ-secretase inhibitors **3** in a 6 × 24 format (Figure 2).

Results and Discussion

Chemistry. Synthesis of the Scaffolds. Synthesis of the chemset **6**{*1–6*} is depicted in Scheme 1. Suzuki coupling of methyl 6-bromopicolinate with 3-fluorophenylboronic acid was conducted using sodium carbonate as the base in a 6:3 mixture of toluene and ethanol as the solvent at 90 °C over a period of 16 h. When subsequent catalytic hydrogenation

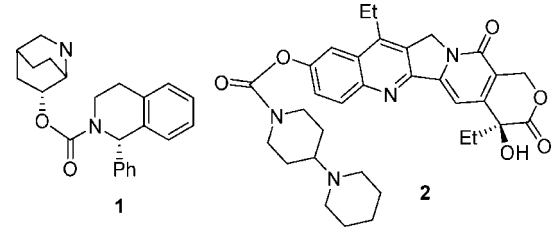

Figure 1. Solifenacin (**1**) and irinotecan (**2**) as examples of carbamate-based marketed drugs.

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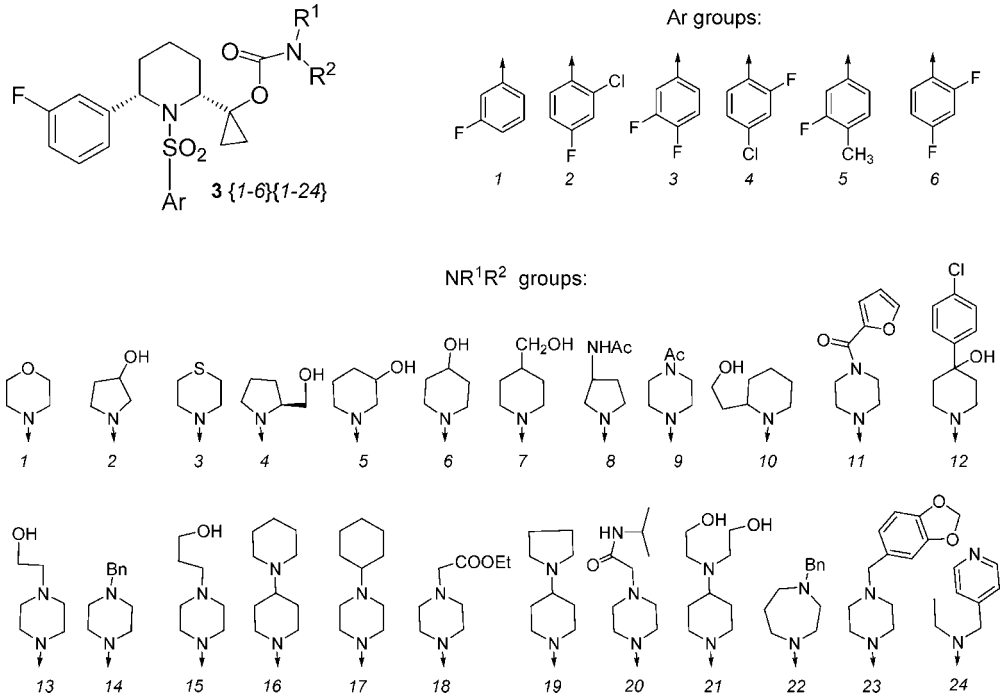
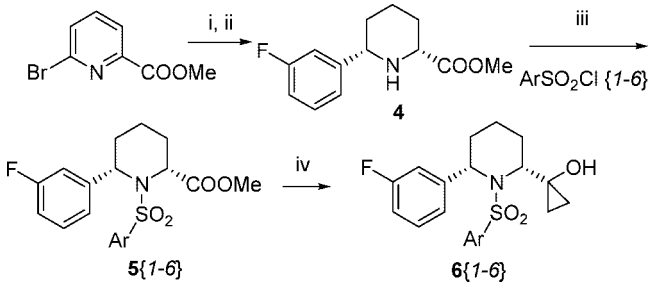


Figure 2. Library format for the synthesis of γ-secretase inhibitors.

Scheme 1. Synthesis of tertiary alcohols **6**{*1–6*}[a]



[a] For the definition of the Ar groups, see Figure 2. Reaction conditions: (i) 3-fluorophenylboronic acid, cat $Pd(PPh_3)_4$, Na_2CO_3, toluene/EtOH = 2:1, 90 °C, 10 h; (ii) H_2 (1 atm), cat PtO_2, MeOH/AcOH = 4:1; (iii) $ArSO_2Cl$, Pyr, 70 °C, overnight; (iv) EtMgBr (3 equiv), $Ti(OPr\text{-}i)_4$ (0.3 equiv), THF, 0 °C.

of the product was conducted at atmospheric pressure (rubber balloon), reduction of the pyridine ring took precedence over hydrogenation of the 3-fluorophenyl ring, selectively providing piperidine **4**. Sulfonylation of relatively hindered **4** with an array of six phenyl-substituted sulfonyl chlorides was achieved at elevated temperatures in pyridine as solvent. After acidic workup, compounds **5**{*1–6*} were purified using prepacked silica gel SPE cartridges. In the next step, tertiary alcohols **6**{*1–6*} were obtained by Kulinkovich cyclopropanation.[14] Slow addition of methylmagnesium bromide to a chilled mixture of the esters **5**{*1–6*} and titanium tetraisopropoxide in THF was essential to ensure good yields (70–80%) in this transformation. Once again, silica gel SPE cartridges were used to purify the products **6** from the highly polar baseline material.

Synthesis of the Carbamate Library 3. In general, carbamates **3** can be accessed by the union of alcohols **6** and amines via a carbonyl linker derived from a synthetic equivalent of phosgene (Figure 3). Initially, we considered the pros and cons of several literature procedures described for the parallel synthesis of carbamates. While *N,N*-disuccinimidyl carbonate (DSC)-mediated synthesis was shown to be compatible with automated synthetic stations, the final compounds had to be purified by normal or reverse-phase chromatography to remove the side product *N*-hydroxysuccinimide.[13a] Experiments with polymer-supported chloroformate resin[13b] failed in our hands to generate synthetically useful yields of **3** for the test reactions, probably because the intrinsic steric hindrance of tertiary alcohols **6** was exacerbated by the polymer support. Although phosgene represents a case of an unencumbered molecule which could overcome steric hindrance in **6**, we were concerned with the high toxicity of this chemical, which could render preparation of a large library unsafe.[15]

Here, we describe the application of 4-nitrophenylchlorocarbonate as a convenient phosgene equivalent suitable for the synthesis of sterically hindered carbamate libraries, as exemplified by the preparation of **3**, with the byproducts of the reaction, including 4-nitrophenol, scavenged by the appropriately chosen commercial resins.

In the first stage of the library synthesis, alcohols **6**{*1–6*} were converted into corresponding 4-nitrophenylcarbonates **7**{*1–6*} by reaction with 4-nitrophenylchlorocarbonate. Although an excess of the reagent was needed for full consumption of the starting material, the virtue of derivatives

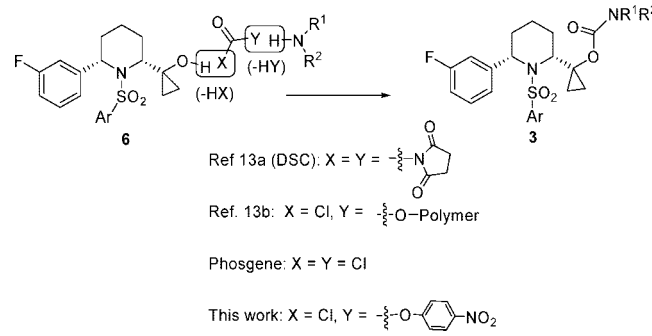
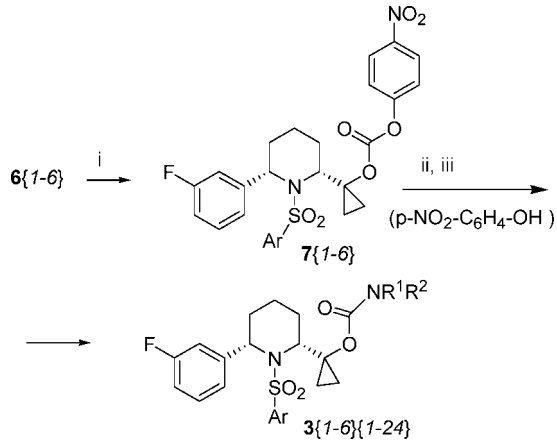


Figure 3. Methods for the formation of a carbamate linker.

Scheme 2. Conversion of tertiary alcohols **6**{*1–6*} into the final targets **3**[a]



[a] For the definition of HNR^1R^2, see Figure 2. Reaction conditions: (i) 4-nitrophenylchlorocarbonate (2 equiv), pyridine (3 equiv), CH_3CN/THF=1:3, 1–3 days; (ii) HNR^1R^2, DCE, 60 °C, 2.5 days; (iii) Amberlyst A26 basic resin, MP–TsOH or PS–isocyanate and PS–benzaldehyde resins.

Table 1. Scavenging of 4-Nitrophenol with Resins

resin	manufacturer	remaining 4-nitrophenol (%)
PS–DIEA	Argonaut/Biotage	18.3
TBD–Me	Argonaut/Biotage	2.6
PS–trisamine	Argonaut/Biotage	4.2
Amberlyst A26	Alfa Aesar	<0.2

7 was the combination of their high reactivity toward amines with substantial chemical stability. This allowed for their separation from excess of 4-nitrophenylchlorocarbonate by either crystallization or chromatography. Purified compounds **7** could then be stored for weeks at room temperature without any signs of decomposition and smoothly underwent coupling with the amines {*1–24*} depicted in Scheme 2. In test runs, TLC analysis of the crude reaction mixtures indicated full consumption of the starting carbonates **7**{*1–6*} after overnight heating in 1,2-dichloroethane (DCE). Other solvents, such as THF or DMF, were also investigated. Although the use of DMF as the solvent resulted in shorter reaction times, the products were often contaminated by dimethylaminocarbonate (**3** with NR^1R^2=NMe_2), where the dimethylamino group originated via decomposition of DMF.

LCMS analysis of selected library members synthesized in DCE indicated that the only admixtures present were the unreacted excess of the amines HNR^1R^2 and the expected side product of the reaction 4-nitrophenol.

For more than 30 years, functionalized polystyrene resins have been known to be effective in the removal of weak acids such as phenols from solutions.[16] At the outset, we screened several commercial resins to evaluate their ability to remove 4-nitrophenol dissolved in DCE, emulating the conditions projected for the crude reaction mixtures. A stock solution of 4-nitrophenol and anisole (internal standard) in DCE was prepared, with the concentration of each reagent equal to 5 mg/mL. After a 1.0 mL aliquot of the stock solution was shaken with 50 mg of the various resins (Table 1) for 5 h, the solutions were analyzed by HPLC with UV detection at 254 nm, and the remaining amount of 4-nitrophenol was quantified. Amberlyst A26 basic resin was identified, using this model system, as having the best performance in the removal of 4-nitrophenol (Table 1).

On the basis of the results of this validation study, Amberlyst A26 basic resin[17] was chosen for the scavenging of 4-nitrophenol from the library members. Removal of the excess unreacted amines {*1–12*} (Figure 2) could be accomplished by absorption on macroporous sulfonic acid (MP–TsOH resin). Since each cleanup step involved an extended period of shaking of the crude reaction products with the resin, the overall process could be significantly accelerated by *simultaneous* application of both the basic and the acidic resins. This feat of simultaneous addition of both acid and base to the same reaction vessel is possible because the polymeric support of the cleanup reagents prevents them from reacting with each other.

In the case of amines {*13–24*}, MP–TsOH resin could not be used for the cleanup because the presence of a basic nitrogen atom in the products **3**{*1–6*, *13–24*} would have resulted in the retention of the product together with the excess amines on the resin. Therefore, polystyrene isocyanate resin (PS–NCO) and polystyrene benzaldehyde (PS–PhCHO) resin were used in place of MP–TsOH resin.

After resin cleanup, the purity of the samples was assessed by LCMS. While 11 library members out of the 144 failed to reach the purity criterion of 80%, the purity of 102 members exceeded the 90% level, and the remaining 31 members had a purity between 80 and 90%. Starting from 10 mg of carbonates **7**, the yields of the 136 members exceeded 2 mg, providing useful amounts for biological testing. The median yield of the products equaled 7.9 mg (or 83% chemical yield). Full characterization of purities and yields of the library members is given in the Supporting Information.

Biological activity. In vitro screening of the library was performed in the membrane preparation of γ-secretase[18] and demonstrated inhibitory activity with IC_{50} values in the range from 1 μM to 5 nM. As evident from Figure 4, amines *21, 19, 17*, and *13* provided the most-active compounds, and the three most-active aryl groups were *3, 5*, and *1*. The data for the seven most-active compounds (IC_{50} < 10 nM) are listed in Table 2.

Conclusions

We developed a practical liquid-phase method suitable for the synthesis of libraries of sterically hindered carbamates, which was exemplified by the synthesis of a targeted library of γ-secretase inhibitors. Six tertiary alcohols were converted to corresponding 4-nitrophenylcarbamates prior to the addition of a set of 24 amines. We demonstrated that the side product of the reaction 4-nitrophenol could be removed from the reaction mixture by Amberlyst A26 basic resin. Depending on the expected basicity of the target, the excess of amine reagent was removed either by MP–TsOH or by PS–isocyanate and PS–benzaldehyde resin. Out of 144 library members, 133 had a purity for the targeted compound of

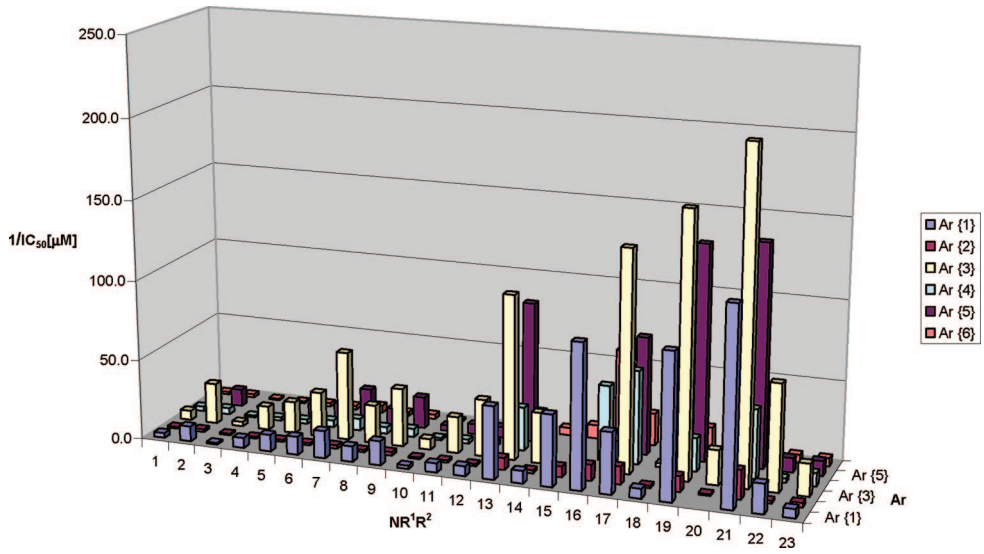


Figure 4. Biological activity of selected library members.

Table 2. Data for the Most-Active Compounds in the γ-Secretase Assay

entry	Ar	NR^1R^2	IC_{50} (nM)
1	*3*	*21*	4.9
2	*3*	*19*	6.1
3	*5*	*21*	7.2
4	*3*	*17*	7.3
5	*5*	*19*	7.5
6	*1*	*21*	8.2
7	*3*	*13*	9.8

80% or better. Biological testing in the γ-secretase assay identified seven compounds with $IC_{50} < 10$ nM.

Experimental Section

General Methods. Anhydrous solvents were purchased from Aldrich in Sure-Seal bottles, and chemicals from commercial sources were used without further purification. Reactions were routinely run under a dry nitrogen blanket using standard techniques. Synthesis of the library was performed in a 96-well format, in 2 mL glass reactors from Kimble–Kontes (Kombi-Screen, part 041145–0701) that was sealed with a Teflon-coated silicone septa. The plates were heated in Kem-Lab heated reactors (J-Kem Scientific, catalog no. KLS-DW-120H) with a digital reaction controller (J-Kem Scientific, catalog no. KLS-150). The progress of reactions was monitored using silica gel plates (Analtech, 250 μM), which were visualized under UV light or with ceric ammonium nitrate stain. The reaction solutions were transferred to Ex-Bloks (Exelixis) via a Tecan liquid-handler containing scavenger resins purchased from Argonaut Technologies (now part of Biotage) and Alfa Aesar. Phenomenex prepacked silica gel cartridges were used for solid-phase extractions. Depending on the scale, evaporation of solvents was performed on a rotary vacuum evaporator (Buchi) or SpeedVac concentrator (Thermo Savant, now a part of ThermoFisher; consisting of a SC210A concentrator, a RVT4104 refrigerated vapor trap, a OFP400 vacuum pump, and a DVG50 digital vacuum gauge).

^{1}H NMR spectra of the synthetic intermediates and final products were obtained with Varian Mercury Plus 400 MHz and Bruker Avance 500 MHz spectrometers in $CDCl_3$ using residual $CHCl_3$ as an internal standard. Characterization of the purity and identity of the library members was carried out by liquid chromatography–mass spectrometry (LC-MS) using a Shimadzu LC-10AD HPLC module coupled with API 150EX Sciex mass spectrometer under positive electrospray ionization with simultaneous UV detection at 254 nM. The HPLC method used an analytical Phenomenex Gemini C_{18} column (4.6 × 50 mm, 5.0 μM); eluent A was water with 0.5% TFA, and eluent B was acetonitrile with 0.5% TFA. Gradient elution from 10% B to 100% B was used over 7 min, followed by a 2.5 min hold at 100% B, at a flow rate of 1.0 mL/min.

Purification of the compounds from the library was done by a Waters Prep LC-MS system consisting of a Waters Delta 600 solvent delivery system, a Waters 2700 sample manager with LC Packing 1/1000 splitter, a Waters Reagent Manager makeup pump (1 mL/min), a Waters 996 photodiode array detector, a Micromass ZQ mass spectrometer, and Waters Fraction Collector II. Waters Sunfire C_{18}, 5 μm, 19 × 50 mm columns were used with a flow rate of 20 mL/min and gradient elution from 5 to 95% MeCN/water (0.1% formic acid) in 7 min, with a hold at 95% MeCN/water for 3 min

Assessment of Performance of Resins for the Scavenging of 4-Nitrophenol. A mixture of 30 mg of 4-nitrophenol and 30 mg of anisole (internal standard) was dissolved in DCE, bringing the total volume of the solution to 6.0 mL. Then 1.0 mL of the stock solution was placed in a 2 dram glass vial, treated with 50 mg of the resin (Table 1), capped, and shaken gently over a period of 5 h. A 25 μL aliquot of the testing solution, free of resin beads, was withdrawn and diluted with 1.0 mL of methanol, prior to the HPLC analysis. The peak areas of 4-nitrophenol and anisole were integrated, and the remaining amount of 4-nitrophenol was estimated using the untreated stock solution as the control.

Methyl 6-Bromopicolinate. To a solution of 40.0 g (0.198 mol) of 6-bromopicolinc acid in 500 mL of methanol at 0 °C was added dropwise 67.8 mL (0.790 mol) of thionyl chloride. After the addition was complete, the flask was equipped with a reflux condenser and heated at 70 °C

overnight. The mixture was cooled and concentrated. The residue was partitioned between 500 mL of dichloromethane and 500 mL of saturated sodium bicarbonate. The organic phase was dried over sodium sulfate, and the solvent was evaporated. The residue was purified by flash chromatography using a gradient of 0–20% of ethyl acetate in hexanes as solvent to furnish 31.05 g (73%) of the title compound. ^{1}H NMR: δ 8.06 (1 H, dd, J = 8.5, 0.8 Hz), 7.82 (1 H, t, J = 7.8 Hz), 7.53 (1 H, dd, J = 8.1, 0.9 Hz), 4.00 (3 H, s). ESI-MS: m/z 296.1, 298.1 ($M^+ + 1$).

Methyl *cis*-6-(3-Fluorophenyl)Piperidine-2-carboxylate (4). (i) A mixture of 31.05 g (0.143 mol) of methyl 6-bromopicolinate, 26.1 g (0.186 mol) of 3-fluorophenylboronic acid, 16.0 g (13.8 mmol) of tetrakis(triphenylphosphine)palladium(0), 31.8 g (0.30 mol) of sodium bicarbonate, 400 mL of toluene, and 200 mL of ethanol was stirred at 90 °C with a reflux condenser under a nitrogen atmosphere. The mixture was cooled, filtered through Celite, and partitioned between ethyl acetate and water. The organic phase was washed with brine, dried over magnesium sulfate and concentrated. The product was purified by flash chromatography using 15% ethyl acetate in hexanes as solvent to furnish 20.0 g (66%) of methyl 6-(3-fluorophenyl)picolinate. ^{1}H NMR: δ 8.08 (1 H, m), 7.95–7.86 (2 H, ser. m.), 7.83–7.76 (2 H, ser. m.), 7.45 (1 H, m), 7.13 (1 H, m), 4.03 (3 H, s). ESI-MS: m/z = 232.1 ($M^+ + 1$).

(ii) A solution of 14.4 g (62.3 mmol) of methyl 6-(3-fluorophenyl)picolinate in a mixture of 540 mL of methanol and 135 mL of acetic acid was hydrogenated over 1.5 g of platinum oxide using a rubber balloon. The progress of the reaction was monitored by TLC (20% of ethyl acetate in hexanes as solvent) and mass spectrometry. The reaction was judged complete after a period of 8 h, at which moment the reaction flask was purged with nitrogen, the catalyst was removed by filtration, and the solvent was evaporated. The residue was redissolved in 250 mL of dichloromethane and washed with 250 mL of saturated sodium bicarbonate. The organic phase was dried over sodium sulfate and evaporated to furnish 9.5 g (64%) of the title compound (**4**). ^{1}H NMR: δ 7.32–3.23 (1 H, m), 7.18–7.09 (2 H, ser. m.), 6.94 (1 H, m), 3.74 (3 H, s), 3.66 (1 H, m), 3.50 (1 H, m), 2.12–2.04 (1 H, ser. m.), 2.04–1.95 (1 H, ser. m.), 1.82–1.73 (1 H, ser. m.), 1.61–1.39 (4 H, ser. m.). ESI-MS: m/z 238.1 ($M^+ + 1$).

Sulfonamide 5{*1*}. A mixture of 1.50 g (6.32 mmol) of *cis*-methyl 6-(3-fluorophenyl)piperidine-2-carboxylate (**4**) and 3.70 g (18.96 mmol) of 3-fluorobenzenesulfonyl chloride in 50 mL of pyridine was heated at 90 °C over a period of 2 d. The reaction mixture was cooled, diluted with 300 mL of dichloromethane, and washed with 3 × 200 mL of 1 M hydrochloric acid. The organic phase was dried over sodium sulfate, concentrated, and passed through a 20 g silica gel cartridge using 15% of ethyl acetate in hexanes as solvent to furnish 2.1 g (83%) of **5**{*1*}. ^{1}H NMR: δ 7.65 (1 H, m), 7.58–7.46 (2 H, ser. m.), 7.36–7.10 (4 H, ser. m.), 6.90 (1 H, m), 5.06 (1 H, m), 4.72 (1 H, m), 3.39 (3 H, s), 2.10 (2 H, m), 1.93 (1 H, m), 1.60–1.41 (3 H, ser. m.). ESI-MS: m/z 396.1 ($M^+ + 1$).

Sulfonamides 5{*2–6*}. Sulfonamides **5**{*2–6*} were prepared in a manner analogous to that described for **5**{*1*}.

Sulfonamide 5{*2*}. ^{1}H NMR: δ 7.98 (1 H, dd, J = 5.9, 8.9 Hz), 7.30–7.18 (4 H, m), 7.03 (1 H, m), 6.90 (1 H, m), 5.05 (1 H, m), 4.88 (1 H, m), 3.39 (3 H, s), 2.24–2.10 (2 H, m), 2.0–1.83 (1 H, m), 1.70–1.47 (3 H, m). ESI-MS: m/z 430.1 ($M^+ + 1$).

Sulfonamide 5{*3*}. ^{1}H NMR: δ 7.70–7.54 (2 H, m), 7.73–7.06 (4 H, m), 6.90 (1 H, m), 5.0 (1 H, m), 4.72 (1 H, m), 3.45 (3 H, m), 2.17–2.01 (2 H, m), 2.00–1.84 (1 H, m), 1.67–1.50 (3 H, m). ESI-MS: m/z 414.1 ($M^+ + 1$).

Sulfonamide 5{*4*}. ^{1}H NMR: δ 7.76 (1 H, t, J = 7.7 Hz), 7.32–7.21 (5 H, ser. m.), 6.97–6.91 (1 H, m), 5.1 (1 H, m), 4.79 (1 H, m), 3.37 (3 H, s), 2.21–2.1 (2 H, m), 2.0–1.82 (1 H, m), 1.62–1.42 (3 H, m). ESI-MS: m/z 430.1 ($M^+ + 1$).

Sulfonamide 5{*5*}. ^{1}H NMR: δ 7.58–7.46 (2 H, m), 7.37–7.30 (1 H, m), 7.26–7.12 (3 H, ser. m.), 6.94–6.86 (1 H, m), 5.05 (1 H, m), 4.70 (1 H, m), 3.38 (3 H, s), 2.37 (3 H, s), 2.17–2.00 (2 H, m), 1.97–1.85 (1 H, m), 1.55–1.50 (3 H, m). ESI-MS: m/z 410.2 ($M^+ + 1$).

Sulfonamide 5{*6*}. ^{1}H NMR: δ 7.84 (1 H, dd, J = 8.4, 14.4 Hz), 7.31–7.20 (3 H, m), 7.72–6.84 (3 H, m), 5.10 (1 H, m), 4.80 (1 H, m), 3.38 (3 H, s), 2.25–2.10 (2 H, m), 2.01–1.86 (1 H, m), 1.64–1.46 (3 H, m). ESI-MS: m/z 414.2 ($M^+ + 1$).

Alcohol 6{*1*}. To a mixture of 2.1 g (5.3 mmol)of sulfonamide **5**{*1*} and 0.47 mL (1.59 mmol) of titanium tetraisopropoxide in 20.0 mL of THF at 0 °C was added over a period of 40 min 5.3 mL (15.9 mmol) of 3 M solution of ethylmagnesium bromide in ether. After the addition was complete, the reaction was stirred for 2 additional min, quenched with 6.0 mL of 1 M HCl, diluted with 50 mL of water, and extracted with 3 × 50 mL of ethyl acetate. The combined organic phase was washed with brine, dried over magnesium sulfate, and concentrated. The residue was passed through a 20 g silica gel cartridge using 0–40% of ethyl acetate in hexanes to furnish 1.35 g (64%) of **6**{*1*}. ^{1}H NMR: δ 7.66 (1 H, m), 7.60–7.51 (2 H, ser. m.), 7.50–7.36 (3 H, ser. m.), 7.31 (1 H, m), 7.00 (1 H, m), 5.17 (1 H, d, J = 4.4 Hz), 3.52 (1 H, d, J = 7.5 Hz), 2.23 (1 H, m), 2.07–1.84 (2 H, ser. m.), 1.48–1.16 (4 H, ser. m.), 0.73–0.63 (2 H, ser. m.), 0.59–0.49 (2 H, ser. m.). ESI-MS: m/z 394.1 ($M^+ + 1$).

Alcohols 6{*2–6*}. Alcohols **6**{*2–6*} were prepared in a manner analogous to that for **6**{*1*}.

Alcohol 6{*2*}. ^{1}H NMR: δ 8.11 (1 H, dd, J = 5.9, 8.7 Hz), 7.55–7.58 (4 H, ser. m.), 7.11 (1 H, m), 7.00 (1 H, m), 5.06 (1 H, br), 3.70 (1 H, d, J = 7 Hz), 2.25 (1 H, d, J = 13 Hz), 2.0–1.87 (2 H, m), 1.76–1.63 (1 H, m), 1.48–1.32 (2 H, m), 1.28–1.20 (1 H, m), 0.79 (1 H, m), 0.71–0.63 (1 H, m), 0.61–0.51 (2 H, m). ESI-MS: m/z 428.1 ($M^+ + 1$).

Alcohol 6{*3*}. ^{1}H NMR: δ 7.69–7.62 (2 H, m), 7.48–7.31 (4 H, m), 7.02 (1 H, m), 5.16 (1 H, br), 3.50 (1 H, d, J = 7.4 Hz), 2.26 (1 H, d, J = 13 Hz), 2.8–1.87 (2 H, m), 1.48–1.12 (4 H, m), 0.71–0.63 (2 H, m), 0.61–0.51 (2 H, m). ESI-MS: m/z 412.1 ($M^+ + 1$).

Alcohol 6{*4*}. ^{1}H NMR: δ 7.85 (1 H, t, J = 8.5 Hz), 7.53–7.46 (1 H,), 7.45–7.37 (2 H,), 7.33–7.25 (2 H,), 7.00 (1 H, t, J = 8 Hz), 5.09 (1 H, br), 3.60 (1 H, d, J = 7.5 Hz),

2.27 (1 H, d, J = 14.2 Hz), 2.04–1.88 (2 H, m), 1.69–1.51 (1 H, m), 1.47–1.34 (1 H, m), 1.28–1.17 (2 H, m), 0.74–0.62 (2 H, m), 0.61–0.51 (2 H, m). ESI-MS: m/z 428.1 ($M^+ + 1$).

Alcohol 6{*5*}. ^{1}H NMR: δ 5.57–7.42 (3 H, m), 7.42–7.32 (3 H, m), 6.98 (1 H, t, J = 8.1 Hz), 5.15 (1 H, br), 3.50 (1 H, d, J = 7.47 Hz), 2.36 (3 H, s), 2.19 (1 H, d, J = 14.2 Hz), 2.04–1.88 (3 H, m), 1.42–1.17 (3 H, m), 0.73 (1 H, m), 0.69–0.59 (1 H, m), 0.57–0.48 (2 H, m). ESI-MS: m/z 408.1 ($M^+ + 1$).

Alcohol 6{*6*}. ^{1}H NMR: δ 7.94 (1 H, q, J = 8 Hz), 7.53–7.48 (1 H, m), 7.45–7.38 (2 H, m), 7.06–6.98 (3 H, m), 5.09 (1 H, br), 3.60 (1 H, d, J = 7.5 Hz), 2.28 (1 H, d, J = 13.5 Hz), 2.09–1.92 (2 H, m), 1.66–1.19 (4 H, ser. m.), 0.74–0.62 (2 H, m), 0.61–0.51 (2 H, m). ESI-MS: m/z 412.1 ($M^+ + 1$).

Carbonate 7{*1*}. To a mixture of 1.35 g (3.43 mmol) of alcohol **6**{*1*} and 0.56 mL (6.87 mmol) of pyridine in 5.0 mL of acetonitrile and 10.0 mL of THF was added, in one lot, 1.38 g (6.87 mmol) of 4-nitrophenylchlorocarbonate. The heterogeneous mixture was stirred over 3 days, diluted with 100 mL of dichloromethane, washed with 100 mL of 1 M hydrochloric acid and brine, dried over sodium sulfate, and concentrated. The residue was passed through a silica gel cartridge (20 g) using 30% ethyl acetate in hexanes as solvent to furnish 1.65 g (84%) of compound **7**{*1*}. ^{1}H NMR: δ 8.33–8.23 (2 H, m), 7.77–7.69 (1 H, m), 7.68–7.46 (4 H, ser. m.), 7.43–7.28 (4 H, ser. m.), 6.97 (1 H, m), 5.21 (1 H, br.), 5.06 (1 H, d, J = 6.5 Hz), 2.17 (1 H, d, J = 14.8 Hz), 1.54–1.17 (5 H, ser. m.), 1.1–0.84 (2 H, ser. m.), 0.34–0.18 (2 H, ser. m.).

Carbonates 7{*2–6*}. Carbonates **7**{*2–6*} were prepared in a manner analagous to that for **7**{*1*}.

Carbonate7{*2*}. ^{1}H NMR: δ 8.27 (2 H, d, J = 8.3 Hz), 8.17 (1 H, dd, J = 5.1, 8.9 Hz), 7.52–7.31 (6 H, ser m), 7.14 (1 H, t, J = 9 Hz), 6.99 (1 H, t, J = 9 Hz), 5.23 (1 H, d, J = 8.2 Hz), 5.09 (1 H, s), 2.22 (1 H, d, J = 10.7 Hz), 1.80–1.20 (5 H, ser m), 0.95 (2 H, m), 0.29 (2 H, m).

Carbonate7{*3*}. ^{1}H NMR: δ 8.29 (2 H, d, J = 9.3 Hz), 7.81–7.68 (2 H, ser. m.), 7.49 (2 H, d, J = 9.3 Hz), 7.41–7.30 (4 H, ser. m.), 6.99 (1 H, t, J = 7.5 Hz), 5.20 (1 H, s), 5.06 (1 H, d, J = 7 Hz), 2.21 (1 H, d, J = 14.9 Hz), 1.50–1.20 (5 H, ser. m.), 0.92 (2 H, m), 0.28 (2 H, m).

Carbonate7{*4*}. ^{1}H NMR: δ 8.27 (2 H, d, J = 8.6 Hz), 7.91 (1 H, t, J = 7.9 Hz), 7.48 (2 H, d, J = 8.6 Hz), 4.48–7.25 (5 H, ser. m.), 6.98 (1 H, t, J = 9.1 Hz), 5.16–5.09 (2 H, ser. m.), 2.24 (1 H, d, J = 11.5 Hz), 1.68–1.24 (5 H, ser. m.), 0.95 (2 H, m), 0.31 (2 H, m).

Carbonate7{*5*}. ^{1}H NMR: δ 8.28 (2 H, d, J = 9.2 Hz), 7.59 (2 H, t, J = 8.9 Hz), 7.50 (2 H, d, J = 9.2 Hz), 7.43–7.30 (4 H, ser. m.), 6.98 (1 H, t, J = 8.5 Hz), 5.2 (1 H, br), 5.05 (1 H, d, J = 6.7 Hz), 2.38 (3 H, s), 2.17 (1 H, d, J = 11 Hz), 1.46–0.18 (5 H, ser. m.), 0.92 (2 H, m), 0.26 (2 H, m).

Carbonate7{*6*}. ^{1}H NMR: δ 8.27 (2 H, d, J = 9.2 Hz), 7.99 (1 H, q, J = 6.6 Hz), 7.49 (2 H, d, J = 9.2 Hz), 7.47–7.31 (3 H, ser. m.), 7.09–6.95 (3 H, ser. m.), 5.14–5.09 (2 H, ser. m.), 2.23 (1 H, d, J = 11.5 Hz), 1.67–1.20 (5 H, ser. m.), 0.95 (2 H, m), 0.30 (2 H, m).

Synthesis of Library 3. Set-up of Reactions. We used two 96-well format, 2 mL glass reactors (Kimble–Kontes, catalog no. 041145–0701) for this library, separating the reactions with the monobasic amines {*1–12*} and the bisbasic amines {*13–24*}. Stock solutions (50 mL) of compounds **7**{*1–6*} were prepared in DCE with a concentration of 10 mg/mL. Then 1 M stock solutions of the 24 different amines were prepared in either DCE or MeOH (1 mL). For each of the six stock solutions of compounds **7**{*1–6*}, the stock solutions were added, using an 8-channel pipetter, to 12 reaction vessels of two 96-well format, glass reactor blocks [for a total of 144 (72 × 2) wells at 1.0 mL per well (0.017–0.018 mmol of **7**, depending on molecular weight of each representative)]. Then, each well was treated, using a Tecan liquid handler, with 54 μL (0.054 mmol) of one of each of the twelve amine stock solutions. The reaction mixtures were then sealed with a Teflon-coated silicone septa cover seal, and both plates were placed in Kem-Lab heated reactor blocks. The top was clamped down, and the block was heated at 60 °C over a period of 2.5 days. After the mixtures were cooled to room temperature, no more starting **7** was seen in selected reactions by TLC analysis (elution with 20% ethyl acetate in hexanes).

Purification. In case of the monobasic amines {*1–12*}, the reaction mixtures were transferred by a Tecan liquid-handler into an Ex-Blok containing 50 mg of Amberlyst A26 resin (Alfa Aesar, catalog no. A17956) that was previously washed several times with MeOH and then DCM, dried in vacuo, and stored in amber bottles before use and 140 mg of MP–TsOH resin (Argonaut, catlog no. 800462, 1.29 mmol/g). The Ex-Blok was then sealed with a 96-well polypropylene cap mat by hydraulic press. The cap mat was then kept in place by a plastic-top sealer plate, and the Ex-Blok was placed on its side and shaken gently overnight. The bottom of the Ex-Blok was then broken with a hydraulic press, allowing the products to be filtered into a 96-well format, deep well plate (2 mL). After the Ex-Blok was further washed with DCE (0.5 mL, via an 8-channel pipetter), each of the combined product solutions were transferred, via a Tecan liquid handler, into preweighted 2 dram barcoded glass vials. The solvent was then removed by a Thermo Savant SpeedVac concentrator.

In case of bisbasic amines {*13–24*}, essentially the same procedure was used, except that 140 mg of PS–NCO resin (Argonaut, catalog no. 801410, 1.53 mmol/g) and 110 mg PS–benzaldehyde resin (Argonaut, catalog no. 800363, 1.50 mmol/g) were used in place of MP–TsOH resin.

Biological Assay. The library members were dissolved in DMSO with a final concentration of 2 mM prior to serial dilutions for the in vitro testing in the membrane-based γ-secretase inhibition assay, as previously described.[18] Briefly, cell membranes were prepared from HEK 293 cells expressing the C-terminal 99 amino acids of APP carrying the London mutation. To measure γ-secretase activity, the membranes were incubated at 37 °C for 1 h in 50 μL of buffer containing 20 mM Hepes (pH 7.0), 150 mM of NaCl, and 2 mM EDTA. At the end of the incubation, Aβ_{40} was measured using an electrochemiluminescence (ECL)-based immunoassay using G2-10 antibody, which recognizes the

specific terminal structure of Aβ_{40}. The ECL signal was measured using an M384 instrument (BioVeris Corp.) according to the manufacturer's instructions. The IC_{50} data presented in this work were the means of 2–3 independent measurements.

Supporting Information Available. Table of LCMS characterization of all library members including purity, yields, and ^{1}H NMR and LCMS spectra of 20 randomly selected library members. This material is available free of charge via the Internet at http://pubs.acs.org.

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