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Parallel synthesis of DAPT derivatives and their γ-secretase-inhibitory activity

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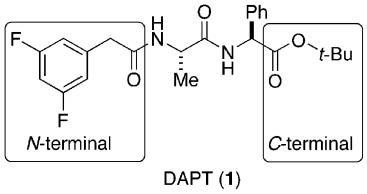
Abstract—Parallel synthesis of the C-terminal-modified DAPT (**1**) derivatives was accomplished utilizing our novel resin **7**. Condensation reaction of the *N*-acylamino acid **10** with the amines **11a–o** proceeded smoothly to give the corresponding amides **6a–o** without any epimerization. Among the analogues, the benzophenonemethyl amide derivative **6o** showed 30 times more potent activity than the original DAPT (**1**).

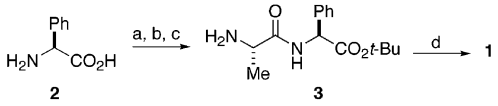
The amyloid-β peptide (Aβ) has been recognized as the pathogenic molecule in Alzheimer's disease (AD).[1] The production of Aβ is executed by a stepwise cleavage of the transmembrane protein amyloid-β precursor protein (βAPP) by a set of membrane-bound proteases termed β-secretase and γ-secretase. The γ-secretase has been identified as an important therapeutic target for AD, and various γ-secretase inhibitors have been investigated.[2] Recently, researchers in the Elan group reported that *N*-[*N*-(3,5-difluorophenylacetyl)-L-alanyl-]-(*S*)-phenylglycine-*t*-butyl ester (DAPT **1**) exhibited an excellent γ-secretase-inhibitory activity in vitro and could lower brain Aβ levels dramatically in APP-transgenic mice.[3] These excellent results prompted us to investigate the structure–activity relationship of DAPT (**1**) derivatives (Fig. 1).



DAPT (**1**)

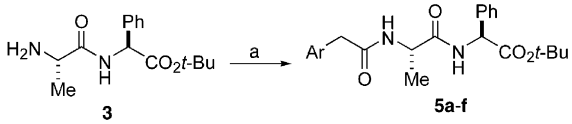
Figure 1. Structure of DAPT (**1**).

Our preliminary structure–activity relationship study was carried out with a partially modified DAPT (**1**) derivative by comparison with **1**. First, a standard sample of the DAPT (**1**) was synthesized from the commercially available L-phenylglycine **2**, as shown in Scheme 1. After conversion to the *t*-butyl ester, condensation with Cbz-L-alanine and catalytic hydrogenolysis provided the primary amine **3**. Coupling of **3** with 3,5-difluorophenylacetic acid was induced by WSCD to provide **1**. As shown in Scheme 2, the N-terminal aromatic ring of **1** was replaced by condensation of **3** with the corresponding commercially available arylacetic acids **4a–f** to



2 **3**

Scheme 1. Reagents and conditions: (a) $HClO_4$, CH_3CO_2t-Bu, rt, 61%; (b) Cbz-L-alanine, DCC, HOBt, Et_3N, CH_2Cl_2, rt; (c) Pd/C, H_2, MeOH, rt, 87% (2 steps); (d) 3,5-$F_2C_6H_3CH_2CO_2H$, WSCD·HCl, CH_2Cl_2, rt, 68%.



3 **5a-f**

a: Ar = *o*-Br-C_6H_6- **d**: Ar = *o*-HO-C_6H_6-
b: Ar = *m*-Br-C_6H_6- **e**: Ar = *m*-HO-C_6H_6-
c: Ar = *p*-Br-C_6H_6- **f**: Ar = *p*-HO-C_6H_6-

Scheme 2. Reagents and conditions: (a) $ArCH_2CO_2H$ (**4a–f**), WSCD·HCl, CH_2Cl_2.

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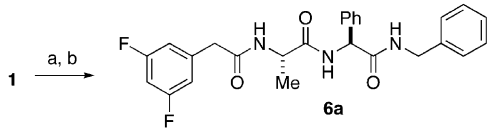
give **5a–f**. Since the analogues **5a–f** had no inhibitory activity of γ-secretase at 10 μM,[4] we decided to change the C-terminal *t*-butyl ester of **1**.

Transformation of the C-terminal *t*-butyl ester group of **1** to the corresponding amide would be a desirable alternative, due to its stability under various conditions.[5] After removal of the *t*-butyl ester of **1**, coupling with benzylamine under mixed-anhydride conditions provided **6a**[6] (Scheme 3). Since the inhibitory activity of **6a** was comparable to that of **1**, we selected **6a** as the lead compound, and planned to synthesize various analogues at the C-terminal benzyl amide of **6a**.



Scheme 3. Reagents and conditions: (a) TFA, 92%; (b) PivCl, Et_3N; $PhCH_2NH_2$, CH_2Cl_2, 0 °C, 72%.

Solid-phase synthesis has emerged as a powerful tool for the construction of diverse libraries. Furthermore, the method has the added advantage of being a convenient way of purifying insoluble hydrophobic peptides. Thus, the synthesis of various DAPT derivatives by solid-phase would be attractive. However, the usual solid-phase peptide synthesis of binding the C-terminal carboxylic acid to the resin makes the preparation of various derivatives at the C-terminal amide difficult. Recently, we developed a trityl-type resin 7, which reacted readily with amines, and we demonstrated its application for an efficient solid-phase synthesis of polyamines.[7] The high utility of the resin **7** was expected to promote the synthesis of diverse amide derivatives at the C-terminal of **1** by loading with the N-terminal amine (Fig. 2).

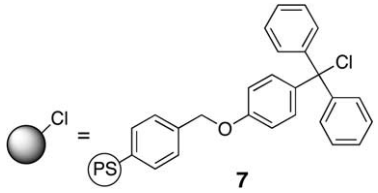
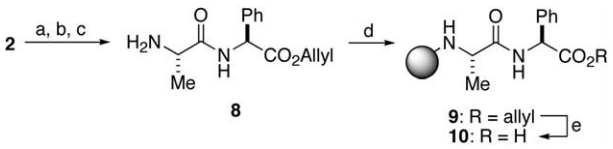


Figure 2. Structure of the resin **7**.

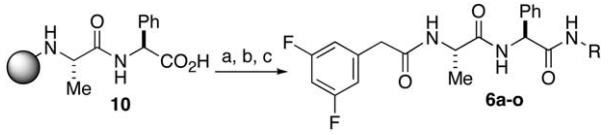
Preparation of the loading precursor **8** started with L-phenylglycine **2**. After protection as the allyl ester, condensation with Boc-L-alanine and removal of the Boc group under acidic conditions gave the amine **8**. Attachment of the primary amine **8** to the resin **7** was induced by *i*-Pr_2NEt, and subsequent deprotection of the allyl ester by the palladium-catalyst afforded **10**, as shown in Scheme 4.

It is well known that activation and acylation of *N*-acyl-amino acid often causes epimerization at the α-position. However, no epimerization was observed in the amide bond formation of the acid derivative **10** with amines.[8] Presumably the bulky polymer support prevented the formation of the oxazolone intermediate and/or the



Scheme 4. Reagents and conditions: (a) allyl alcohol, TsOH, benzene, Dean–Stark trap, reflux, 89%; (b) Boc-L-alanine, WSCD·HCl, HOBt, Et_3N, CH_2Cl_2, rt, 70%; (c) 20% TFA–CH_2Cl_2, 69%; (d) resin **7**, *i*-Pr_2NEt, CH_2Cl_2; MeOH; (e) $Pd(PPh_3)_4$, pyrrolidine, DMF.

excess HOBt accelerated the amide formation. Parallel synthesis of the DAPT analogues **6a–o** was accomplished by condensation of **10** with the various primary amines **11a–o**, cleavage from the resin by 1% TFA in CH_2Cl_2 and subsequent incorporation of 3,5-difluorophenylacetic acid unit by its pentafluorophenyl activated ester (Scheme 5). This simple protocol also has the advantage of providing sufficiently pure samples by only washing with Et_2O. The results of the parallel synthesis of DAPT derivatives are summarized in Table 1 and Figure 3.



Scheme 5. Reagents and conditions: (a) RNH_2 (**11a–o**), DCC (3 equiv), HOBt (1.5 equiv), CH_2Cl_2 or NMP; (b) 1% TFA–CH_2Cl_2; (c) 3,5-$F_2C_6H_3CH_2CO_2Pfp$, *i*-Pr_2NEt, CH_2Cl_2; washed with Et_2O. Pfp = pentafluorophenyl.

Table 1. Yields of DAPT derivatives **6a–o** and their γ-secretase inhibitory activity compared with DAPT

Compd	R =	Yield (%)[a]	Activity[b]
6a	CH_2Ph	57	1
6b	*n*-C_4H_9	26	0.3
6c	$CHPh_2$	26	1
6d	$CH_2C_6H_4$-*o*-OMe	27	0.2
6e	$CH_2C_6H_4$-*m*-OMe	43	0.1
6f	$CH_2C_6H_4$-*p*-OMe	12	0.4
6g	$CH_2C_6H_4$-*o*-Cl	42	0.1
6h	$CH_2C_6H_4$-*m*-Cl	25	0.1
6i	$CH_2C_6H_4$-*p*-Cl	26	0.6
6j	$CH_2C_6H_4$-*o*-Br	32	0.3
6k	$CH_2C_6H_4$-*m*-Br	19	0.1
6l	$CH_2C_6H_4$-*p*-Br	36	0.6
6m	$CH_2C_6H_4$-*p*-F	17	0.1
6n	$CH_2C_6H_4$-*p*-CO_2Me	28	0.6
6o[9]	$CH_2C_6H_4$-*p*-COPh	48	30

[a] Overall yield from **10** in 3 steps and characterization of all compounds (**6a–o**) were performed by ^1H NMR and HR-MS.[10]

[b] The IC_{50} value of DAPT (**1**) for inhibition of Aβ 40 was 0.05 μM. The relative activity was determined by dividing the IC_{50} of DAPT by that of each compound.[4]

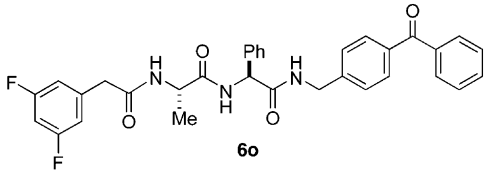


Figure 3. Structure of **6o**.

The results of the inhibitory activity of **6a–o** are also given in Table 1. As expected, the benzyl **6a** and the benzhydryl amide **6c** possessed similar activity to the original DAPT (**1**). Among the derivatives **6a–o**, the benzophenonemethyl (*p*-benzoylbenzyl) amide derivative **6o**[10] showed the highest activity (30 times more potent than **1**). This result suggested that the C-terminal benzophenone group would play a key role in the activity. Furthermore, **6o** could be used as a probe for elucidation of γ-secretase, since benzophenones have been used extensively as photocross-linking agents.[11] Recently, photoaffinity labeling has become a powerful tool for identification of substrate binding sites of membrane proteins. Utilizing the probe **6o** to collect detailed structure information for the γ-secretase ultimately would allow the discovery of novel drugs.

Further application of this protocol for the synthesis of various DAPT (**1**) derivatives and the photoaffinity labeling experiment with derivatives of **6o** are currently under investigation in our laboratories.[12]

Acknowledgements

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References and notes

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2. For a recent review on secretases, see: Wolfe, M. S. *Nature. Rev. Drug. Discov.* **2002**, *1*, 859 and references therein.
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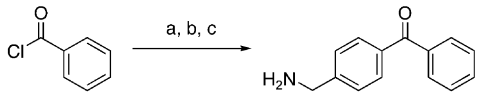
4. Takahashi, Y.; Hayashi, I.; Tominari, Y.; Rikimaru, K.; Morohashi, Y.; Kan, T.; Natsugari, H.; Fukuyama, T.; Tomita, T.; Iwatsubo, T. Inhibitory potencies of DAPT derivatives on γ-secretase activity were analyzed by cell-based assays. Cultured cells overexpressing β-APP were cultured at confluency in DMEM containing 10 mM butyric acid to drive protein expression in the presence of various concentrations of compounds for 16–18 h. Cultured media were collected and subjected to BNT77/BA27 or BNT77/BC05 ELISAs. The detailed data for γ-secretase inhibition activity will be reported elsewhere. For the detailed assay procedures, see: *J. Biol. Chem.* **2003**, *278*, 18664.
5. Methyl ester derivatives of **1** were prepared in a similar manner with those described in Scheme 1. The benzyl ester was synthesized by transesterification ($Ti(O\text{-}i\text{-}Pr)_4$, BnOH) of the methyl ester. These analogues were shown to be less active than **1**.
6. Spectroscopic data for **6a**: white solid; $[\alpha]_D^{24}$ +75.6° ($c = 0.03$, CH_3OH); IR (film) 3282, 1633, 1596, 1539, 1497, 1456, 1387, 1357, 1230, 1118, 996, 855, 728, 697, 728 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.75 (t, 1H, $J = 5.9$ Hz), 8.48 (d, 1H, $J = 7.8$ Hz), 8.39 (d, 1H, $J = 7.6$ Hz), 7.39 (d, 2H, $J = 6.6$ Hz), 7.35–7.19 (m, 6H), 7.12 (d, 2H, $J = 7.1$ Hz), 7.11–7.05 (m, 1H), 6.97 (d, 2H, $J = 6.6$ Hz), 5.44 (d, 1H, $J = 7.8$ Hz), 4.44–4.40 (m, 1H), 4.26 (d, 2H, $J = 5.9$ Hz), 3.50 (s, 2H), 1.21 (d, 3H, $J = 10.0$ Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.8, 169.7, 168.9, 162.1 (dd, $J = 244.4$, 13.2 Hz), 140.7 (t, $J = 10.3$ Hz), 139.0, 138.5, 128.2, 128.2, 127.6, 127.4, 127.1, 18.5, 112.2 (dd, $J = 6.1$ Hz), 101.9 (t, $J = 25.3$), 56.3, 48.1, 42.0, 41.3, 18.0; HRMS (FAB) calcd for $C_{26}H_{25}F_2N_3O_3$ $(M+H)^+$ 466.1942, found 466.1959.
7. (a) Hidai, Y.; Kan, T.; Fukuyama, T. *Tetrahedron Lett.* **1999**, *41*, 4711. (b) Hidai, Y.; Kan, T.; Fukuyama, T. *Chem. Pharm. Bull.* **2000**, *48*, 1570. (c) Kan, T.; Kobayashi, H.; Fukuyama, T. *Synlett* **2002**, 1338.
8. Since the diastereomers in the amide derivatives **6a–o** was not observed in the ^1H NMR spectra, the epimerization did not occur during the condensation.
9. Since the benzophenonemethyl (*p*-benzoylbenzyl) amine was not commercially available, it was prepared from benzoyl chloride in the following three-step sequence: (a) $AlCl_3$, toluene; (b) NBS, $(PhCO_2)_2$, CCl_4, 60 °C, 53% (2 steps); (c) liq. NH_3, THF–MeOH, −33 °C, 83%.



10. Spectroscopic data for **6o**: white crystalline (mp = 191 °C), $[\alpha]_D^{21}$ +25.5° ($c = 0.32$, CH_3OH), IR (film) 3285, 3068, 1647, 1597, 1499, 1469, 1318, 1280, 1243, 1116, 1007, 983, 939, 836, 701; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.87 (t, 1H, $J = 5.8$ Hz), 8.51 (d, 1H, $J = 7.6$ Hz), 8.39 (d, 1H, $J = 7.3$ Hz), 7.68 (d, 2H, $J = 7.1$ Hz), 7.67–7.49 (m, 1H), 7.66 (d, 2H, $J = 7.6$ Hz), 7.62 (d, 2H, $J = 8.0$ Hz), 7.40 (d, 2H, $J = 7.1$ Hz), 7.34–7.28 (m, 5H), 7.06 (t, 1H, $J = 9.3$ Hz), 6.97 (d, 2H, $J = 6.6$ Hz), 5.46 (d, 1H, $J = 7.6$ Hz), 4.46–4.41 (m, 1H), 4.37 (d, 2H, $J = 5.8$ Hz), 3.50 (s, 2H), 1.23 (d, 3H, $J = 7.1$ Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 195.4, 171.9, 169.9, 168.9, 162.1 (dd, $J = 243.9$, 14.4 Hz), 144.2, 140.6 (t, $J = 9.9$ Hz), 138.3, 137.1, 135.5, 132.6, 129.7, 129.5, 128.6, 128.3, 127.7, 127.1, 126.9, 112.2 (dd, $J = 17.7$, 6.6 Hz), 101.8 (t, $J = 25.7$ Hz), 56.4, 48.1, 41.8, 41.3, 18.0; HRMS (FAB) calcd for $C_{33}H_{29}F_2N_3O_4$ 569.2126, found 569.2109.
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