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Solid-phase synthesis of photoaffinity probes: highly efficient incorporation of biotin-tag and cross-linking groups

Toshiyuki Kan, Yusuke Tominari, Yuichi Morohashi, Hideaki Natsugari, Taisuke Tomita, Takeshi Iwatsubo and Tohru Fukuyama*

Graduate School of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. E-mail: fukuyama@mol.f.u-tokyo.ac.jp; Fax: +81-3-5802-8694; Tel: +81-3-5841-4777

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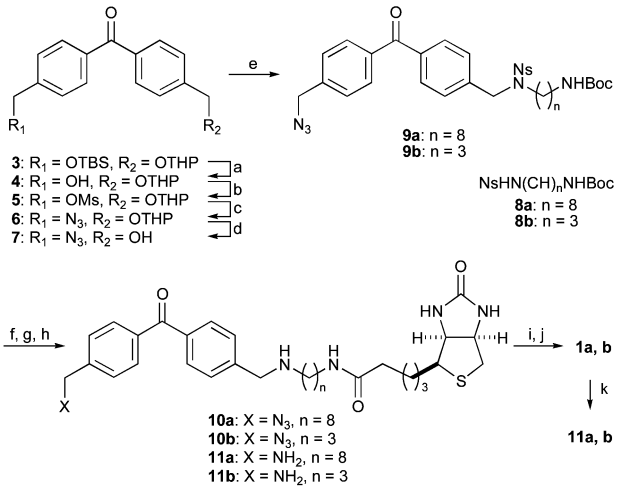
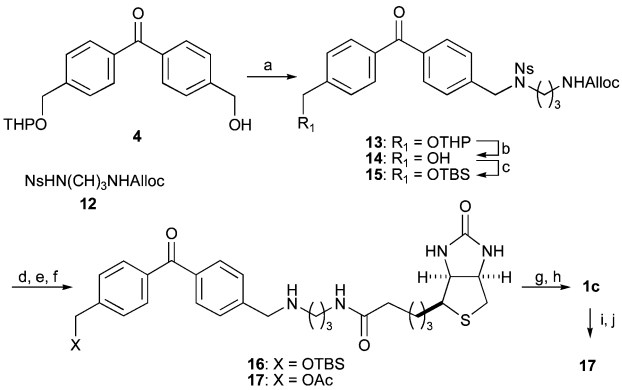
A benzophenone cross-linking group and a biotin-tag hybrid, resin 1a, attached to our novel resin 2 was readily converted to the photoaffnity probe 20 by condensation with the ligand carboxylic acid 19 and cleavage from the resin without purification.

Photoaffinity labelling is a powerful tool for the identification of ligand binding sites and for affinity purification of receptors.[1,2] Ever since avidin-biotin technology enabled non-radioactive detection and affinity isolation of photolabelled targets, the photoaffinity probe attached to a photoactive cross-linking group and a biotin-tag, has become a widely used technique. Although many investigations on the synthesis of such probes have been reported, only a few convenient incorporations of both units have been developed.[3,4] Recently, with the emergence of combinatorial libraries and ease of purification, solid-phase synthesis has attracted much attention. We envisioned that the biotin and the benzophenone photoactive group conjugated with the resins **1a–c** would be valuable intermediates for the synthesis of numerous photoaffinity probes. As shown in Fig 1, the resins **1a–c** would readily provide the probes by acylation with the carboxylic acid of the ligand and subsequent acidic cleavage. The advantage of this solid-phase synthesis is not only the obtainment of probes without tedious purification steps but also the generation of probe libraries. Recently, we developed a novel trityl-type resin **2** and demonstrated its application for an efficient solid phase synthesis of polyamines.[5] The high reactivity of the resin **2** was expected to enable the loading of the secondary amine of **1**. Herein we report the synthesis of the polymer-supported probe intermediates **1a–c** and their application for the synthesis of the photoaffinity probe **20** for investigation of γ-seceretase.

As shown in Scheme 1, the synthesis of the probe precursors of **10a,b** started with the benzophenone derivative **3**.[6] Thus, the azide group was incorporated into **3** by mesylation and subsequent azide displacement. After acidic hydrolysis of the THP group, coupling of the benzophenone derivative **7** and the diamine linkers **8a,b** was accomplished by alkylation of the 2-nitrobenzenesulfonamide (Ns-strategy).[7] Upon treatment of **7** and **8a,b** with DEAD and triphenylphosphine, the coupling reaction proceeded smoothly to afford **9a,b** respectively. Since **8a,b** was synthesized by a selective mono-protection of symmetrical diamines, several lengths of diamine linkers were readily available.[5b,c] After removal of the Boc group, condensa-



Scheme 1 *Reagents and conditions*: (a) TBAF, THF, rt, 95%. (b) MsCl, Et_3N, CH_2Cl_2, rt, 95%; (c) NaN_3, DMF, rt, 93%; (d) CSA, MeOH, rt, 94%; (e) **8a** or **8b**, DEAD, PPh_3, toluene–THF, rt; (f) TFA, CH_2Cl_2, rt; (g) (+)-Bio-OPfp, i-Pr_2NEt, 1,4-dioxane, rt; glycine, aq. NaOH, rt; (h) PhSH, Cs_2CO_3, CH_3CN, 50 °C, 37% for **10a**, 40% for **10b** (4 steps); (i) **2** (5 eq.), DMAP, DMF–i-Pr_2NEt, rt; MeOH, rt; (j) $SnCl_2$, PhSH, Et_3N, THF, rt; (k) 1% TFA–CH_2Cl_2, rt.



Scheme 2 *Reagents and conditions*: a) **12**, DEAD, PPh_3, toluene–THF, rt, 70%; (b) CSA, MeOH, rt, 99%; (c) TBSCl, imidazole, DMF, rt, 81%; (d) $Pd_2(dba)_3$, PPh_3, pyrrolidine, CH_2Cl_2, rt, 57%; (e) (+)-Bio-OPfp, i-Pr_2NEt, 1,4-dioxane, rt, 85%; glycine, aq. NaOH, rt; (f) PhSH, Cs_2CO_3, CH_3CN, 50 °C, 80%; (g) **2** (5 eq), DMAP, DMF–i-Pr_2NEt, rt; (h) TBAF, THF, rt; (i)Ac_2O, pyridine, rt; (j) 1% TFA–CH_2Cl_2, rt.

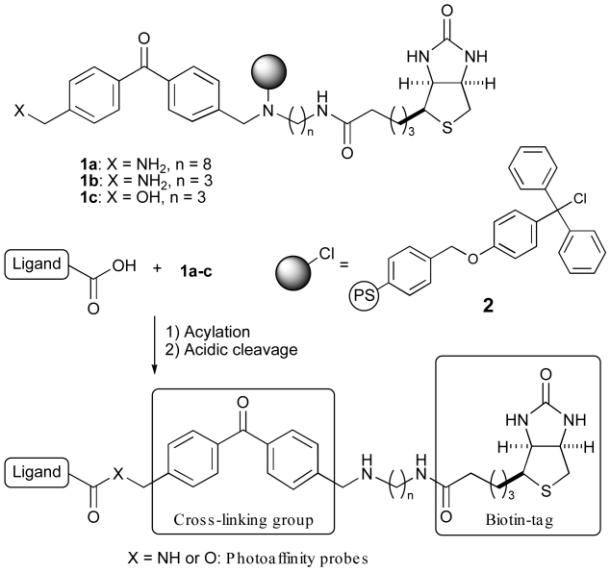


Fig. 1

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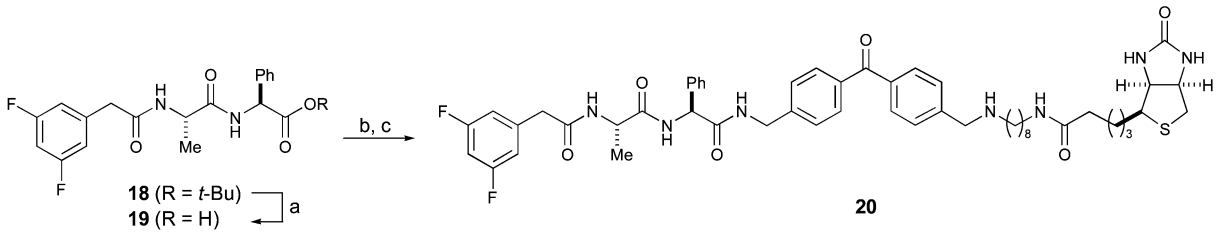

Scheme 3 *Reagents and conditions*: (a) TFA, rt, 92%; (b) **1a**, DCC, HOBt, i-Pr_2NEt, NMP, rt; (c) 1% TFA–CH_2Cl_2, rt (77%, 2 steps).

tion of the (+)-biotin pentafluorophenyl ester (Bio-OPfp) and deprotection of the Ns group gave the secondary amines **10a,b**. Attachment of **10a,b** to the resin **2** was induced by i-Pr_2NEt, and subsequent reduction of the azide group with $SnCl_2$, PhSH and Et_3N afforded the amines **1a,b**. Confirmation of the structures of **1a,b** was determined by examination of **11a,b**† after cleavage from the resin. As shown in Scheme 2, the alcohol type probe precursor **1c** was synthesized by a similar procedure with **1a,b**. Condensation of the alcohol **4** and the sulfonamide **12**.[5c] under Mitsunobu conditions and switching from the THP group to TBS group afforded **15**. After removal of the Alloc-group, installation of the biotin unit and removal of the Ns-group gave the secondary amine **16**. Loading **16** onto the resin **2** and deprotecting the TBS group provided **1c**, the structure of which was also confirmed by conversion to **17**.†

As shown in Scheme 3, the utility of the probe precursor **1a** was demonstrated by synthesis of the photoaffinity probe **20** for investigation of the γ-secretase. The γ-secretase may be involved in the generation of an amyloid β-peptide (Aβ), which is implicated in Alzheimers' disease (AD).[8] Since the γ-secretase inhibitor may be important as a therapeutic agent for AD, numerous inhibitors have been investigated. Recently, researchers at Elan reported that *N*-[*N*-3,5-difluorophenylacetyl-L-alanyl-]-*S*-phenylglycine-*tert*-butyl ester (DAPT **18**) exhibited excellent inhibitory activity toward γ-secretase.[9] Our preliminary investigation of the structure–activity relationship of DAPT proved that modification of the C-terminal of DAPT **18** to a benzyl amide maintained its activity.[10] Thus, incorporation of the labelling moiety into the C-terminal of **18** seemed appropriate. Attachment of the carboxylic acid **19** readily derived from **18** with the resin **1a** was performed in the presence of DCC and HOBt. Cleavage from the resin under acidic conditions (1% TFA–CH_2Cl_2) afforded **20** in high purity without any purification. The cross-linking experiment of the probe **20** with γ-secretase was carried out in our laboratories, the results of which will be reported elsewhere.

In conclusion, the polymer-supported probe precursors **1a–c** may be powerful tools for the preparation of photoaffinity probes. It should be noted that the hydroxyl group of **1c** was readily converted to the corresponding halide, which could be reacted with several nucleophiles such as amines and thiols. This solid-phase synthetic strategy has the advantage of not only facilitating purification but also of generating a library of probes. Indeed, by attaching several lengths of linkers and cross linking groups (azides and diazirines) to the resin, this protocol would conceivably generate numerous probes. Further investigation on the development of solid-phase synthetic strategies are underway in our labolatories.

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Notes and references

† Spectroscopic data for **11a–11c**: **11a**: ^{1}H NMR (DMSO-d_6, 400 MHz): δ9.07 (br s, 1H), 8.40 (br s,1H), 7.77–7.75 (m, 4H), 7.68–7.63 (m, 4H), 6.45 (br s, 1H), 4.30–4.25 (m, 3H), 4.17–4.10 (m, 3H), 3.12–3.06 (m, 1H), 2.99–2.97 (m, 2H), 2.94 (br s, 2H), 2.80 (dd, 1H, *J* = 12.6, 12.2 Hz), 2.57 (d, 1H, *J* = 12.2 Hz), 2.01 (t, 2H, *J* = 7.3 Hz), 1.62–1.24 (m, 18H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 194.9, 171.9, 162.8, 138.9, 137.2, 136.8, 136.8, 130.0, 129.8, 128.9, 128.9, 61.1, 59.3, 55.5, 49.6, 48.6, 46.8, 41.9, 39.9, 39.9, 38.3, 35.2, 29.2, 28.5, 28.2, 28.1, 26.3, 25.9, 25.4; HRMS (FAB): calcd for $C_{33}H_{48}N_5O_3S$ (M + H)$^+$: 594.3477, found: 594.3470. **11b**: ^{1}H NMR (DMSO-d_6, 400 MHz): δ8.87 (br s, 1H), 8.30 (br s, 1H), 7.77 (d, 4H, *J* = 8.3 Hz), 7.68–7.63 (m, 4H), 6.39 (br s, 1H), 4.30–4.25 (m, 3H), 4.17–4.10 (m, 3H), 3.12–3.93 (m, 3H), 2.93 (br s, 2H), 2.80 (dd, 1H, *J* = 12.2, 5.1 Hz), 2.56 (d, 1H, *J* = 12.2 Hz), 2.07 (t, 2H, *J* = 7.8 Hz), 1.76–1.74 (m, 2H), 1.51–1.08 (m, 8H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 195.1, 173.0, 163.1, 140.9, 137.6, 136.7, 136.5, 130.2, 130.0, 129.1, 128.5, 61.4, 59.5, 55.6, 55.1, 53.3, 49.8, 48.7, 35.7, 35.3, 28.4, 28.2, 26.2, 25.4; HRMS (FAB): calcd for $C_{28}H_{38}N_5O_3S$ (M + H)$^+$: 524.2695, found: 524.2708. **17**: ^{1}H NMR (DMSO-d_6, 400 MHz): δ8.85 (br s, 1H), 7.95 (br s, 1H), 7.75 (d, 2H, *J* = 8.1 Hz), 7.68 (d, 2H, *J* = 8.3 Hz), 7.61 (d, 2H, *J* = 8.3 Hz), 7.50 (d, 2H, *J* = 8.1 Hz), 6.36 (br s, 1H), 5.14 (s, 2H), 4.26–4.19 (m, 3H), 4.08–4.05 (m, 1H), 3.09–3.04 (m, 3H), 2.88 (br s, 2H), 2.75 (dd, 1H, *J* = 12.4, 5.1 Hz), 2.51 (d, 1H, *J* = 12.4 Hz), 2.05 (s, 3H), 2.02 (t, 2H, *J* = 7.4 Hz), 1.71 (t, 2H, *J* = 7.1 Hz), 1.52–1.22 (m, 8H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 195.0, 172.7, 170.4, 162.7, 145.4, 141.3, 136.5, 136.2, 130.3, 129.9, 129.8, 127.7, 64.8, 61.0, 59.2, 55.4, 49.7, 48.5, 44.8, 39.8, 35.5, 35.1, 28.3, 28.0, 26.1, 25.2, 20.7; HRMS (FAB): calcd for $C_{30}H_{39}N_4O_5S$ (M + H)$^+$: 567.2641, found: 567.2655.

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10 The determination of the structure of the DAPT derivatives and γ-secretase inhibition activity will be reported elsewhere.