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A simple photo-affinity labeling protocol

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After photo-crosslinking and proteolysis of a photoaffinity labeled ligand to its receptor, the ester group incorporated in the ligand is cleaved by an amine with specific functionality, which displaces the bulk of the ligand from the peptide fragments, and facilitates subsequent tandem mass spectrometric sequencing.

With the very rapid advancement in analytical methods, the technique of photoaffinity labeling has become an increasingly central tool for investigating 3D interactions between ligands and receptors on a molecular basis.[1] Although photoaffinity labeling was introduced over thirty years ago by Westheimer,[2] the protocol remains basically unchanged. It involves synthesis of radioactive and photo-labeled ligands, incubation, photolysis, proteolysis and sequencing of radiolabeled peptide fragments by amino acid sequencer. However, in the case of membrane-bound receptors, the isolation of cross-linked peptide fragments from a sticky peptide mixture has been a particularly challenging and occasionally impossible task; nevertheless, photoaffinity labeling is the only general method to investigate such ligand–receptor interactions at a molecular level.[1]

Recently we introduced the protocol of using the bifunctional photoaffinity probe (BPP)[3] **1** coupled with tandem mass spectrometry (MS) to facilitate the often tedious photoaffinity studies dealing with membrane-bound receptors (Fig.1).

The ligand-BPP moiety which is bound to biotin through a linker is incubated with the receptor and irradiated with 350 nm light leading to site **A** photolysis and photocrosslinking. This is followed by receptor proteolysis and a second irradiation with 350 nm light, but under mildly basic conditions, upon which site

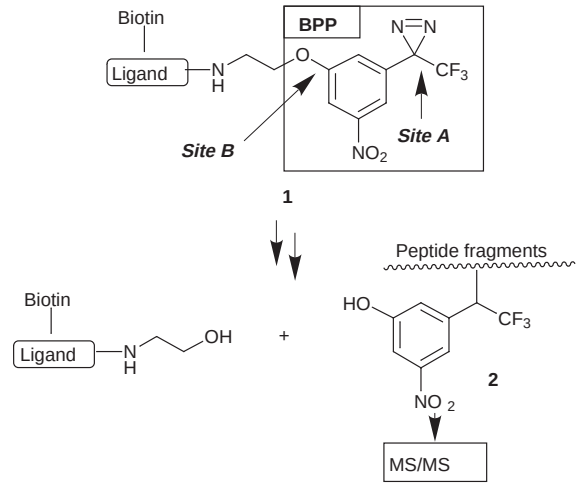

Fig. 1 Bifunctional photoaffinity probe (BPP).

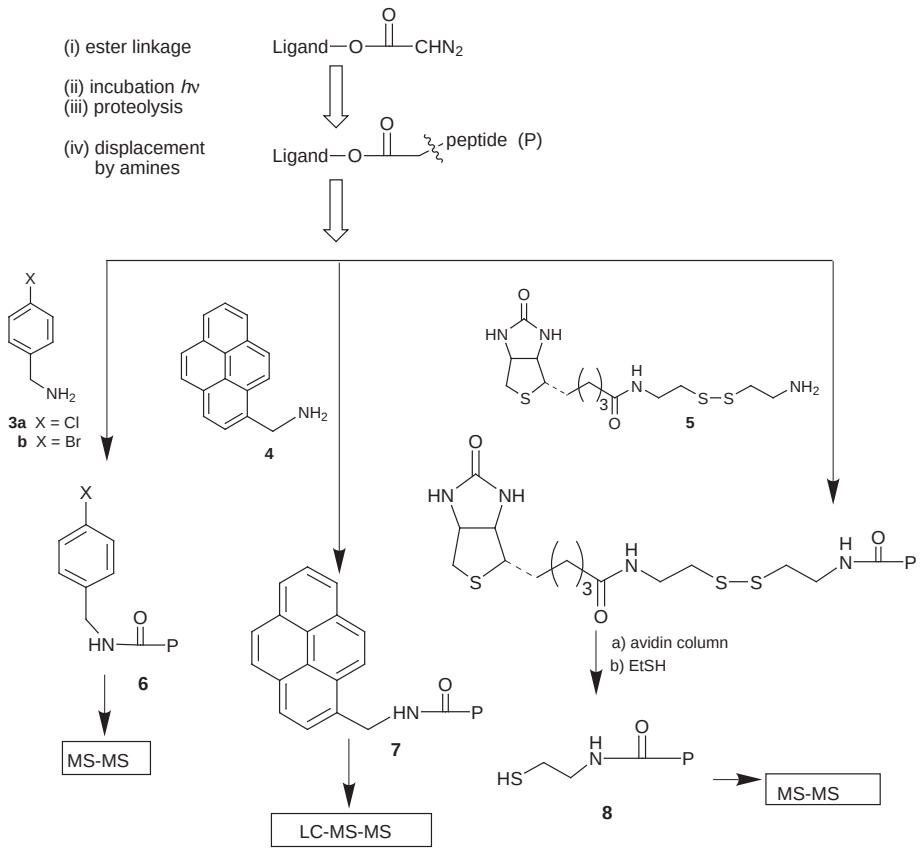

Fig. 2 General scheme of the protocol.

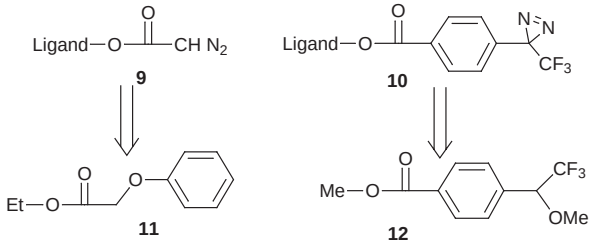

Fig. 3 Model compounds for the aminolysis reaction.

B cleavage occurs to detach the bulk of the ligand from the crosslinked peptide fragment; the ligand moiety and non-crosslinked fragments are separated from crosslinked fragment **2**, which is sequenced by tandem MS. This protocol is currently being applied to a particular ligand–receptor pair.

In the following, we introduce an alternative to further facilitate the photoaffinity crosslinking and sequencing processes. In the case of BPP, the ligand moiety was removed by a second photolysis performed under mild base conditions. In this revised protocol which does not use BPP, the ligand moiety is removed by aminolysis of a labile ester bond, as summarized in Fig. 2: (i) esterification of the hydroxy group with the appropriate photolabel, *e.g.* diazoacetate; (ii) incubation of ligand with its receptor followed by photolysis; (iii) enzymatic and/or chemical cleavage of the receptor; (iv) ester aminolysis and removal of the ligand moiety using amines **3**–**5**†‡ with functionalities for identification and/or purification purposes, *e.g.* bromine or chlorine atoms as tags for MS identification (**3**), fluorescence tag for HPLC detection (**4**), biotin tag for purification with immobilized avidin (**5**); (v) sequencing of tagged peptide fragments by tandem MS (MS/MS).[4–6]

Here, aminolysis performs the dual function of removing the undesired ligand moiety[7–9] and introducing the tag for MS sequencing, as well as purification. Namely, benzylamines **3** carry the isotopic halogen atoms[10] for facile MS identification, pyren-1-ylmethylamine **4** introduces a fluorescent tag[11] for chromatographic detection, if necessary, prior to MS, while biotinylated amine **5** with a cleavable disulfide bond is useful for separation of cross-linked peptide fragment(s) by avidin chromatography.[12,13]

A wide variety of photolabeling probes can be adapted to the protocol either directly or *via* short linkers, *e.g.* ethanolamine and glycolic acid, to modify either the ligand or the photolabile probes before linking. The aminolysis was checked with model compounds **11** and **12**, the products that might be produced from photolabeled ligands **9** and **10** (Fig. 3), respectively.

Ester **11** was converted into the amide upon treatment with 150 equiv.‡ of amines **3a**, **3b**, **4**, and **5**,§ 50° C, 1 day, in yields of 75, 61, 64 and 52%, respectively. Aminolysis of ester **11** with base **3a**, performed in the presence of four peptides Gly-Gly-Phe, Lys-Phe, Gly-Leu-Tyr and Phe-Tyr, resulted in a 62% transformation, demonstrating that peptide bonds are not affected; the sluggish aminolysis of aromatic esters was accelerated upon addition of NaCN,¶ in which case 70% of ester **12**‖ was aminolized overnight by 100 equiv. of amine **3a** in pyridine.** The disulfide bond in the biotinylated amide derived from **5** and **11** was readily cleaved by EtSH to give product **8** (P = phenoxymethyl) without biotin, suited for tandem MS analysis, in quantitative yield.††

The present simple protocol, which provides an alternative to the BPP approach, is currently being applied to an actual ligand–receptor system.

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Notes and references

† Pyren-1-ylmethylamine hydrochloride and 4-bromobenzylamine hydrochloride are commercially available (Aldrich). The free pyren-1-ylmethylamine and 4-bromobenzylamine were obtained by passing their hydrochlorides through an ion exchange column (OH^- type). If pyren-1-ylmethylamine were brominated, the fluorophore will serve as a MS tag as well.

‡ Although the amino group in Lys in the peptide would be deactivated by protonation or hydrogen bonding, in principle, it can aminolyze the ester. Therefore, excess amine is required, which also accelerates the reaction.

§ Amine **5** was made by the coupling of cystamine and (+)-biotin 4-nitrophenyl ester.

¶ NaCN is a very mild catalyst. It is likely that the attack of cyanide anion occurs only on the ester bond without damage of the peptide fragment (ref. 14).

‖ Ester **12** was readily prepared from the photoaffinity labeling probe 4-(1-azi-2,2,2-trifluoroethyl)benzoic acid (ref. 15) by photolysis and then methylation.

** The aminolysis works in a wide spectrum of polar solvents, such as water, EtOH, MeOH, pyridine, *etc.*

†† *Selected data* for amides: Amide from **3a** and **11**: *m/z* (EI) found: 275.0721 [$(M)^+$ $C_{15}H_{14}O_2N_3{}^{35}Cl$], calc.: 275.0713. Amide from **3a** and **12**: *m/z* (EI) found: 357.0739 [$(M)^+$ $C_{17}H_{15}O_2N^{35}ClF_3$], calc.: 357.0743. Amide from **3b** and **11**: *m/z* (FAB) found: 319.0210 [$(M + 1)^+$ $C_{15}H_{14}O_2NBr^{79}$], calc.: 319.0208. Amide from **4** and **11**: *m/z* (EI) found: 365.1422 [$(M)^+$ $C_{25}H_{19}O_2N$], calc.: 365.1416. Amide from **5** and **11**: *m/z* (FAB) found: 513.1688 [$(M + 1)^+$ $C_{22}H_{33}O_4N_4S_3$], calc.: 513.1664.

1 F. Kotzyba-Hibert, I. Kapfer and M. Goeldner, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 1296 (Review).
2 A. Singh, E. R. Thorton and F. H. Westheimer, *J. Biol. Chem.*, 1962, **237**, 3006.
3 K. Fang, M. Hashimoto, S. Jockusch, J. N. Turro and K. Nakanishi, *J. Am. Chem. Soc.*, 1998, **120**, 8543.
4 R. Orlando, P. T. M. Kenny, C. Moquin-Pattey, K. Lerro and K. Nakanishi, *Org. Mass Spectrom.*, 1993, **28**, 1395.
5 X. Yu, Z. Wu and C. Fenselau, *Biochemistry*, 1995, **34**, 3377.
6 L. E. Ball, J. E. Oatis Jr., K. Dharmasiri, M. Busman, J. Wang, L. B. Cowden, A. Galijatovic, N. Chen, R. K. Crouch and D. R. Knapp, *Protein Sci.*, 1998, **7**, 758.
7 H. K. Bell, *Tetrahedron Lett.*, 1986, **27**, 2263.
8 T. Hogberg, P. Strom, M. Ebner and S. Ramsby, *J. Org. Chem.*, 1987, **52**, 2033.
9 E. M. Suh and Y. Kishi, *J. Am. Chem. Soc.*, 1994, **116**, 11205.
10 H. Y. Li, S. Matsunaga and N. Fusetani, *J. Med. Chem.*, 1995, **38**, 338.
11 C. V. Kumar, A. Buranaprapuk, G. J. Opiteck, M. B. Moyer, S. Jockusch and N. J. Turro, *Proc. Natl. Acad. Sci. U.S.A.*, 1998, **95**, 10361.
12 Y. Hatanaka, M. Hashimoto and Y. Kanaoka, *J. Am. Chem. Soc.*, 1998, **120**, 453.
13 M. A. Shogren-Knaak and B. Imperiali, *Tetrahedron Lett.*, 1998, **39**, 8241.
14 S. Hunig and R. Schaller, *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 36.
15 M. Nassal, *Liebigs Ann. Chem.*, 1983, 1510.

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