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Isoserine-based biotinylated photoaffinity probes that interact with *penicillin-binding protein 1b*†

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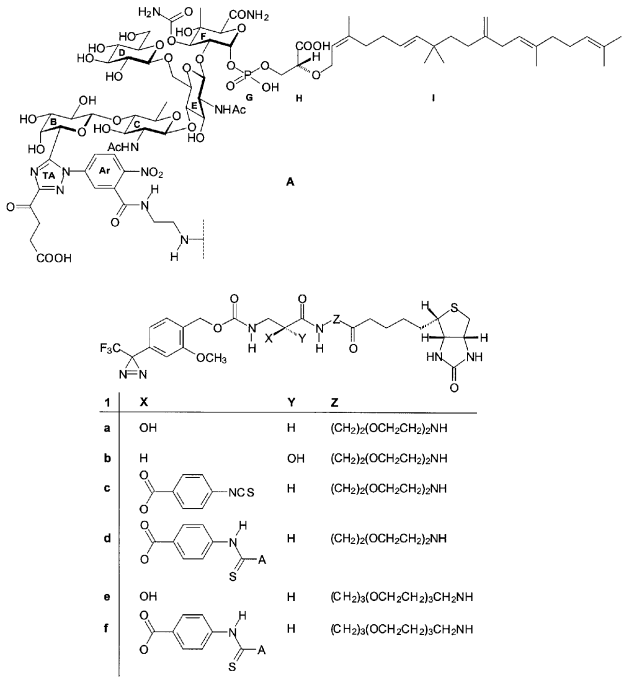
The photolytic decomposition of trifunctional carbene generating photoaffinity probes in methanolic solution was studied, a cleavage reaction with butylamine in water, the conjugation with a ligand (moenomycin), and experiments that demonstrate that the fully armed probes interact with *penicillin-binding protein* 1b.

Photoaffinity labeling has been demonstrated to be an efficient method for studying the interactions of biologically significant ligands with their target macromolecules.[1] We have recently described the synthesis of photoaffinity label **1a**[2] and the advantages of a trifunctional scaffold such as the isoserine unit in **1a** (obtained from (*S*)-malic acid) which can accommodate the photophore (here an aryl trifluoromethyl diazirine), the biotin tag, and the ligand attachment site *independently*.[3] Any desired ligand can be coupled to the free OH group of **1a** by a suitable method. It is the aim of the present Communication to discuss (i) the photochemistry of this system, (ii) to demonstrate that before MS sequencing of labeled peptides the ligand and the biotin tag can be removed by cleavage of the urethane group, and (iii) that **1a** with a suitable ligand can indeed be used to label a protein specifically.

The description of photolabels **1b** and **1e** is also included. **1b** (see ESI†) has been obtained (starting from (*R*)-malic acid) exactly as described for **1a**, and **1e** was prepared similarly (from (*S*)-malic acid) but with a different linker Z (Scheme 1).

Photochemical experiments at an analytical scale (0.76 mmol L^{-1} in methanol, mercury high pressure lamp HBO 100 W, monochromator (λ_{irr} = 366 nm) indicate (see the isosbestic point in Fig. 1) that **1b** is cleanly converted into the methoxy derivative **2a**. Similar results have been obtained for **1e**. The molar absorption coefficients ε at 366 nm of **1b** and **1e** (methanolic solution) have been determined to be 357.3 and 352.6 mol^{-1} L cm^{-1}, respectively. The quantum yields for the decomposition of **1b** and **1e** were measured making use of the potassium ferrioxalate actinometry and were found to be 5.6×10^{-2}. The overall rate constants for the photochemical decomposition of **1b** and **1e** on irradiation at 366 nm in methanolic solution were then calculated to be 4.8×10^{-4} and 4.7×10^{-4} s^{-1}, respectively (see Fig. S1, ESI†). On a preparative scale, 366 nm irradiation of **1b** and **1e** in methanol (0.7 mM) at 10 °C under argon furnished the methoxy compounds **2a** and **2b** in 62 and 70%, respectively (according to the ^{19}F NMR spectra of the reaction mixtures). After chromatographic purification, both **2a** and **2b** were isolated and fully characterized by NMR and MS.

It has been pointed out by Nakanishi,[4] that it would be useful to remove the ligand and the biotin tag from labeled peptides before MS sequencing in order to avoid complications in the MS analysis. Such a specific cleavage site is the urethane grouping of the photolabels **1a**, **1b** and **1e**. **2a** was treated with a 25% aqueous solution of *n*-butylamine in water at 20 °C for 71 h and after work-up and chromatographic separations **3** (48%), **4a** (34%), **4b** (30%) and **5** (31%) were isolated (Scheme 2). The lower yield of **3** compared to the counterparts is due to losses during the separation procedure. The result shows that it will be possible to remove the ligand and the biotin from labeled



Scheme 1

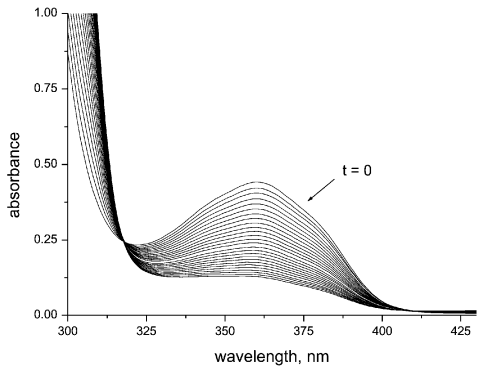


Fig. 1 Time course of the photodecomposition of **1b**. UV/VIS spectra of the solution photolyzed in 1 min intervals ($I_0 = 7.2 \times 10^{-8}$ E s^{-1}, λ_{irr} = 366 nm). For conditions, see text.

† Electronic supplementary information (ESI) available: spectroscopic characterization for all new compounds. Fig. S1: decrease in absorbance at 366 nm as a function of irradiation time for **1b**. See http://www.rsc.org/suppdata/cc/b2/b204007g/

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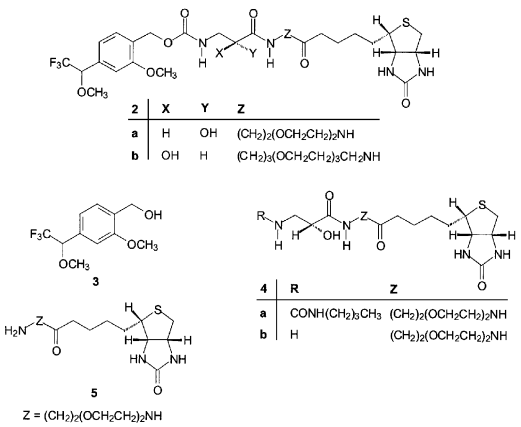

Scheme 2

peptides by cleaving the urethane grouping. As expected, **3** was racemic (CHIRALCEL HPLC, heptane–propan-2-ol 9 : 1).

The free OH group of both **1a** and **1e** was used to attach moenomycin as a ligand. Thus, on reaction of **1a** with 4-isocyanatobenzoic acid, the hydrolytically not very stable ester **1c** was formed which on reaction with the moenomycin A-derived amine **A-H**[5] furnished the stable conjugate **1d**. In an analogous fashion from **1e** the conjugate **1f** was prepared. **1d** and **1f** were fully characterized by high resolution MS (ESI ICR) and ^{1}H and ^{13}C NMR spectroscopy. We have prepared **1d** and **1f** with the aim of labeling the transglycosylase domain of *penicillin binding protein* **1b**[6] (PBP **1b**). Moenomycin A has been shown to inhibit the transglycosylase reaction (one of the final steps in peptidoglycan biosynthesis which is catalyzed by PBP **1b**) very efficiently.[7] Both **1d** and **1f** were found to be antibiotically active against a number of *Staphylococcus aureus* strains (MIC 6×10^{-7} and 4×10^{-7} mol L^{-1}, respectively)[8] and should thus be suitable to photolabel the binding site of moenomycin A at PBP *1b*. In the event, when a crude preparation of PBP **1b** (obtained from *E. coli* JM109/pJP13,[9] as described recently[10]) was incubated with **1d** and then irradiated at 366 nm the SDS PAGE band corresponding to PBP **1b** after blotting to a PVDF membrane clearly showed the presence of biotin after incubation with the streptavidine alkaline phosphatase conjugate and colorimetric analysis. The same result was obtained when a pure sample of PBP **1b** was crosslinked with **1d**. Labeling of PBP **1b** was not observed in the presence of an excess of moenomycin A which indicates that the binding of the crosslinking reagent is specific and can be suppressed competitively by moenomycin A. We have also performed *surface plasmon resonance* (SPR)[11] experiments to provide evidence for a specific interaction between PBP **1b** and **1d**.[12] Thus, the biotin residue in **1d** was used for immobilisation of the moenomycin derivative to a streptavidin sensor surface (SA sensor chip, Biacore). Purified PBP **1b** was injected as soluble analyte and the *SPR* response resulting from the interaction between PBP **1b** and the immobilized **1d** was detected (Fig. 2). Although **1d** is a moenomycin derivative with decreased antibiotic activity, the *SPR* experiments showed specific affinity of **1d** to PBP **1b**. Furthermore, PBP **1b** was pre-incubated with moenomycin A allowing complex formation and the sample was then injected onto the same sensor chip. The competition resulted in decreasing of PBP **1b** binding response to **1d**. Competitive elution of PBP **1b** bound to **1d** with moenomycin A was also quantitative.

In conclusion we have (i) prepared the three generally applicable photoaffinity labels **1a**, **1b**, and **1e**, (ii) studied their photolytic decomposition in methanolic solution, (iii) described the reaction of **2a** with butylamine in water, and (iv) have shown that conjugate **1d** is capable of photolabeling PBP **1b**.[13]

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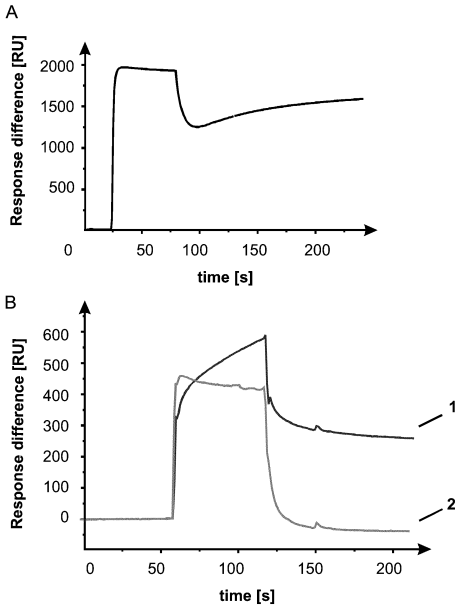

Fig. 2 (A) Immobilization of **1a** to a streptavidin sensor surface (SA sensor chip, Biacore). (flow buffer: HBS/EP, pH 7.4, flow 5 μL min^{-1}, injection time: 60 s). (B) Purified PBP **1b** was injected in the presence and in the absence of moenomycin A. (1) PBP **1b** (750 nM) without preincubation with moenomycin A; (2) PBP **1b** (750 nM) after incubation with 100 μM moenomycin A (flow buffer: 10 mM Tris maleate, pH 6.8, 150 mM NaCl, 0.1 mM $MgCl_2$, 1% Triton X-100, flow 15 μL min^{-1}, injection time: 60 s).

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