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A trifunctional reagent for photoaffinity labeling†

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Abstract

A photolabel, a biotin tag, and a moenomycin ligand have been attached orthogonally to the three functional groups of isoserine to provide compound **13** that is to be used in affinity labeling of penicillin binding protein **1b**. The urethane group in **13** can be cleaved with *n*-butylamine in methanol or water.

Photoaffinity labeling can provide important information about binding sites of enzyme–substrate or enzyme–inhibitor complexes.[1] After photolabeling the enzyme is usually digested enzymatically and the labeled fragments are isolated and submitted to structural analysis. Biotin-labeling is a powerful method for identifying labeled protein fragments and isolating them by affinity chromatography based on the strong interaction of biotin with either avidin or strepavidin.[2] Thus a carbene-generating photoprobe[3] of type **1** has been prepared and used for labeling the acceptor binding site of a galactosyltransferase (Scheme 1).[4] Recently, Nakanishi and co-workers discussed the problem of elucidating the structures of the labeled peptides by tandem MS without separation of the mixture and they proposed that it would be desirable to remove both the ligand and the biotin tag from the peptide before MS sequencing in order to avoid complications in MS analysis. Based on these considerations they designed a bifunctional photoaffinity probe of type **2**. These compounds have two photolabile groupings: (i) the diazirine moiety which generates a carbene intermediate on irradiation at 350 nm and (ii) the alkyl *m*-nitrophenyl ether which on irradiation at 365 nm undergoes a photosubstitution reaction in mildly basic solution to yield the corresponding *m*-nitrophenol. The latter reaction can be used to remove the ligand-biotin part before MS sequencing.[5] We were interested in a compound of type **3** for affinity labeling of the transglycosylase module of penicillin-binding protein **1b**. This enzyme catalyzes one of the last steps of the biosynthesis of bacterial cell wall peptidoglycan.[6]

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† Dedicated with best wishes to Professor Horst Kessler on the occasion of his 60th birthday.

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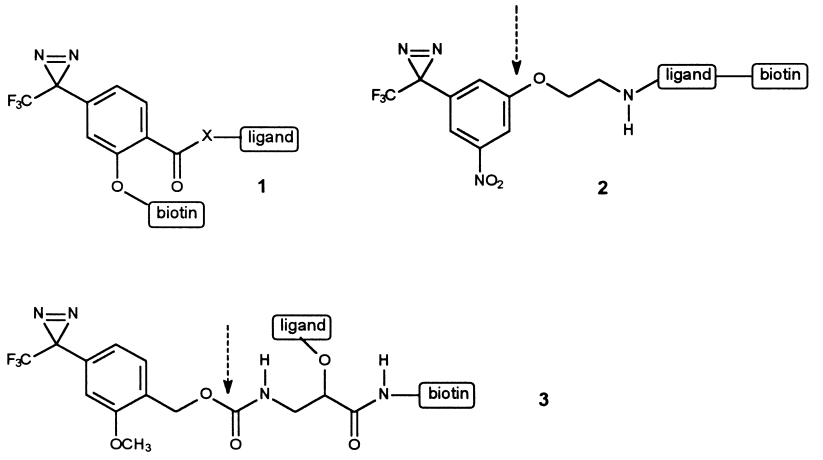
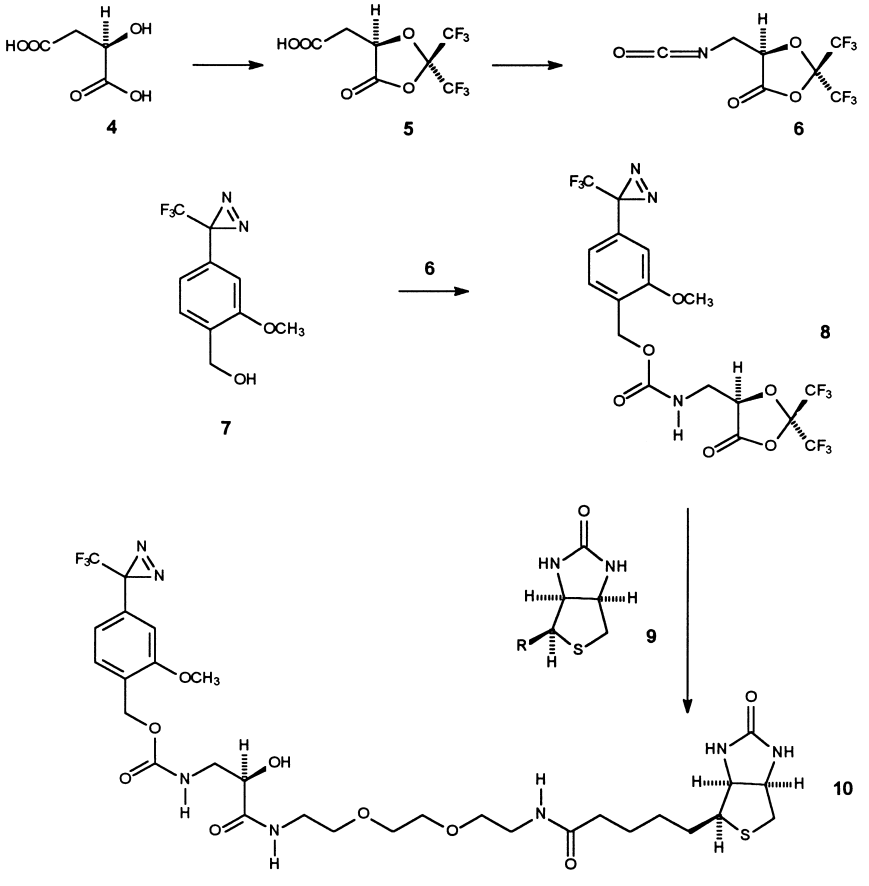


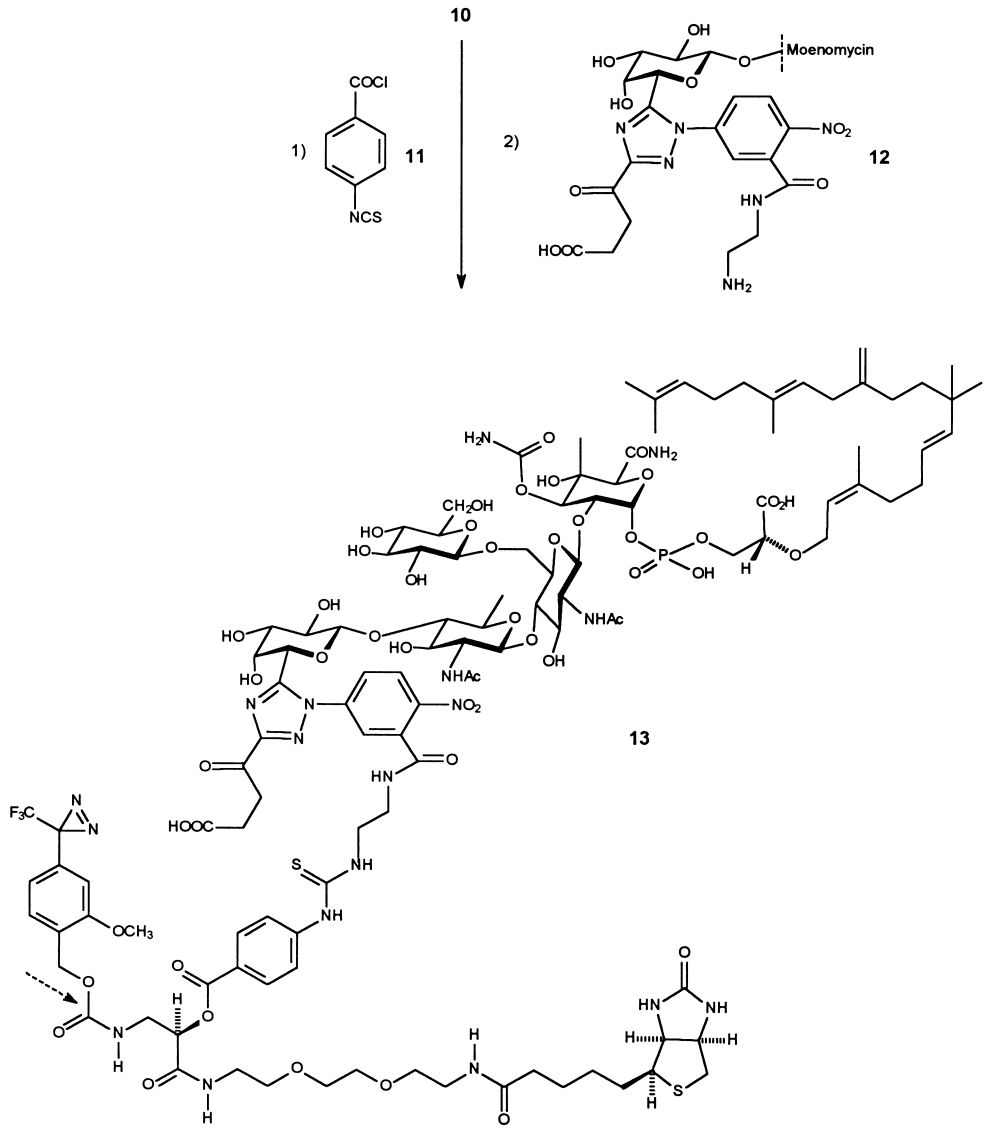
Scheme 1.



Scheme 2.

For our purposes **2** seemed to have a number of disadvantages. The synthesis of the aryl trifluoromethyl diazirine moiety takes more than 15 steps. Furthermore, it seemed almost impossible to couple the biotin tag to the moenomycin ligand without losing the binding capacity to the enzyme.[6] We devised, therefore, the new photolabeling system **3** based on a trifunctional isoserine scaffold which can accommodate the aryl trifluoromethyl diazirine, the biotin tag, and the ligand independently at its three different functional groups (see **3**). The cleavage site is the urethane grouping that can be cleaved with butylamine in methanol or water (vide infra).

Based on these considerations we decided to prepare compound **10**, the synthesis of which makes use of the protection/activation strategy for α-amino and α-hydroxy acids that was developed by Burger and co-workers.[7]



Scheme 3.

Thus, treatment of malic acid with hexafluoroacetone gave **5**, the azide of which was submitted to a Curtius rearrangement to provide **6** as described previously[7] (Scheme 2). Reaction of **6** with Hatanaka's affinity label **7**[8] in $CHCl_3$ solution and subsequent purification by FC (petroleum ether:ethyl acetate, 4:1) furnished urethane **8** in 71% yield (based on **7**). Reaction of **8** with the commercial biotin derivative **9** (for *R* see formula **10**, 1 equiv.) in 1:4 dimethoxyethane:water (15 h at 20°C) occurred as expected, i.e. by amide formation and concomitant loss of the protecting group to provide **10** in 50% yield after lyophilization and FC (CH_2Cl_2:methanol, 4:1). The ^1H and ^{13}C NMR spectra of **10** have fully been assigned using H,H COSY, ^{13}C,^1H HMBC and ^{13}C,^1H HMQC. The ^{13}C chemical shifts ($CDCl_3$) around the CF_3 groups are as follows: $\delta = 122.57$ ($^1J_{C,F} = 275$ Hz, CF_3), 29.66 ($^2J_{C,F} = 40.5$ Hz, *C*-CF_3).

Compound **10** is a general reagent to which any ligand can be attached in a suitable way via the free OH group. We chose the bifunctional linker 4-isothiocyanato benzoyl chloride (**11**) to couple the moenomycin-derived amine **12**[6] (the rest of the moenomycin structure is apparent from formula **13**) to **10** (Scheme 3). Thus, a mixture of **10**, **11**, and Steglich's base in dry pyridine was stirred at –15°C for 72 h. To the reaction mixture a solution of **12** (0.8 equiv.) in dry DMF was added and the mixture was stirred at 20°C for 55 h. Then water was added and solvents were removed by lyophilization. Subsequent FC (1-propanol:water 4:1) provided **13** in 41% yield [^{19}F NMR: $\delta = 12.84$ (external CF_3COOH), ^{31}P NMR: $\delta = -2.19$, external H_3PO_4)].

Treatment of **7** and **13** with butylamine in methanol (reflux) gave identical products as indicated by TLC (petroleum ether:ethyl acetate, 2:1), and the same observation was made when the reaction was performed in water (70°C). These results indicate, that indeed the urethane grouping can be cleaved under these conditions to release both the ligand and the biotin moiety. Work is in progress now to make use of compound **13** in the sense that was discussed in the introductory section.

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