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A novel linker for the attachment of alcohols to solid supports

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Abstract

A novel linker for the immobilisation of alcohols on solid support is described. This linker can be cleaved either enzymatically using penicillin amidase, or by very mild acid hydrolysis using 10% TFA.

Keywords: supported reactions; enzymes; alcohols, carbohydrates.

The recent surge of interest in solid-phase organic synthesis has resulted in the need for new solid-phase methodologies, in particular new linkers for functional groups other than amines and carboxylic acids [1]. Enzyme-cleavable linkers are particularly attractive because cleavage might be achieved under mild, neutral and aqueous conditions. Two reports have demonstrated that enzymes can be used to cleave molecules from solid supports using a phosphodiesterase [2] and an endopeptidase (chymotrypsin) [3]. A major drawback of both methods is that the compound released from the solid support retains part of the recognition site of the enzyme, *i.e.* a phosphate ester [2] and a peptide with C-terminal phenylalanine residues [3]. Although both 'tags' could in principle subsequently be cleaved with phosphatases or peptidases respectively, this would introduce several additional synthetic steps into the reaction sequences. Here we describe the design and synthesis of a more general linker, which can be cleaved either with penicillin amidase (EC 3.5.1.11), a commercially available and widely used enzyme [4] or by the use of dilute TFA, thereby complementing the existing range of alcohol linkers.

Penicillin amidase is known to catalyse the hydrolysis of a wide range of amines protected as the corresponding phenylacetyl derivatives and has also been used in peptide synthesis for the cleavage of cysteine protecting groups [5]. Thus, in order to incorporate the enzyme recognition site, the linker **1** was designed as shown in Scheme 1, in which -OR

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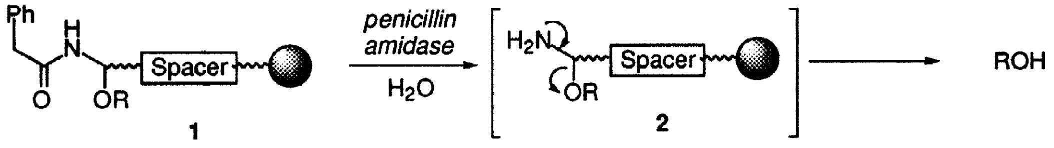
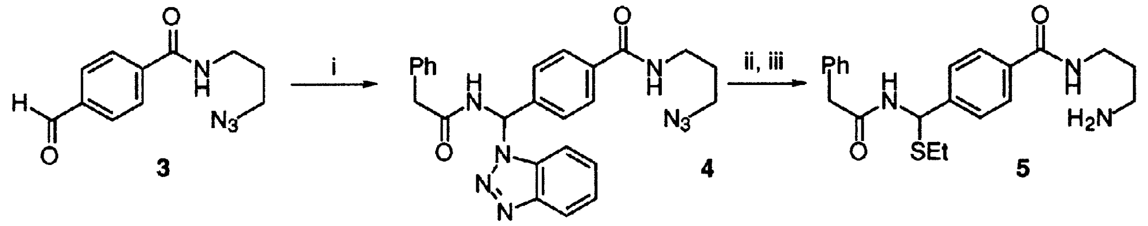
represents the alcohol group. It was envisaged that cleavage would be initiated by hydrolysis of the phenylacetamide moiety, generating the hemiaminal **2** which should easily fragment in aqueous medium releasing the alcohol ROH.



Scheme 1

An activated form of the linker was accessible using methodology developed by Katritzky *et al.* [6-8]. The benzotriazole derivative **4** was prepared in 67% yield by refluxing aldehyde **3** with benzotriazole and phenylacetamide in a Dean-Stark apparatus (Scheme 2). Although the benzotriazole is a good leaving group, and can be replaced by strong nucleophiles, we have found that the thioethyl group is more convenient for our purposes. The latter can be activated with a thiophilic reagent, such as *N*-iodosuccinimide, and is then susceptible to displacement by relatively poor nucleophiles such as secondary alcohols. The thioethyl derivative **5** was easily obtained in excellent yield by reaction of **4** with sodium ethanethiolate followed by reduction of the azide group to afford the amine **5**.



Scheme 2

(i) Phenylacetamide (1 eq), benzotriazole (1 eq), TsOH (0.2eq), toluene, reflux, Dean-Stark, 16h, 67%; (ii) sodium ethanethiolate (10 eq.), THF, RT, 4h, 96%; (iii) triphenylphosphine (1.2 eq), H_2O, THF, RT, 16h, 96%.

Amine **5** was then coupled to a variety of solid supports in reasonable to excellent yields (Scheme 3)[1]. A number of Tentagel® and PEGA® resins were investigated because of their compatibility with aqueous reaction conditions and because they had been reported to be suitable for enzyme-catalysed reactions [9]. Coupling yields were between 75% (TentaGel®-COOH) and 100% (PEGA®-COOH). Polystyrene was also successfully derivatised in quantitative yield by activating the resin as the acylfluoride[2] and should be useful when the

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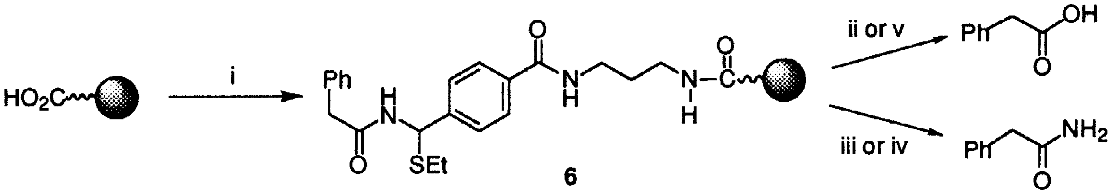
[1] **Typical procedure for the coupling of the linker 5 to solid supports** (for Tentagel and PEGA resins): A mixture of the resin (100 mg; loading 0.2 mmol/g), the linker 5 (50 mg, 0.13 mmol), TBTU (77 mg, 0.24 mmol), HOBt (32 mg, 0.24 mmol) and N,N-diisopropylethylamine (31 mg, 0.24 mmol) in DMF (2ml) was shaken for 16h at 25 °C. The resin was washed twice with 10 ml aliquots of THF, DMF, DMF:MeOH (1:1), DMF,THF and CH_2Cl_2 and dried.

[2] **Typical procedure for the coupling of the linker 5 to solid supports** (for polystyrene resin): Cyanuric fluoride (0.837 g, 6.2 mmol) was added (with caution) to a suspension of carboxypolystyrene resin (1.00 g; loading 1.24 mmol/g), and pyridine (0.200 ml, 2.48 mmol) in dichloromethane (5 ml) and the mixture was agitated on a blood rotator for 16h at 25 °C. The resin was washed with 10 ml aliquots of THF, DMF, THF and CH_2Cl_2 and dried in a vacuum oven (ν_{max} 1805 cm^{-1}). N,N-Diisopropylethylamine (0.432 ml, 2.48 mmol) was added to a suspension of the linker 5 (2.40 g, 6.23 mmol) and the acyl fluoride resin (1.00 g,1.24 mmol/g) in DMF (5ml) and was agitated on a blood rotator for 16h at 25 °C. The resin was washed twice with 10 ml aliquots of THF, DMF, DMF:MeOH (1:1), DMF,THF and CH_2Cl_2 and dried.

linker is used for non-aqueous chemistry, since the loading is generally higher (1.24 mmol/g).



Scheme 3
(i) 5 (6.5 eq), 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (12 eq), HOBt (12 eq), DIEA (12 eq), DMF, 16h, 25°C; (ii) 2M NaOH; (iii) 2M HCl; (iv) TFA:DCM:H_2O 9:10:1; (v) penicillin amidase.

The loading values of the linker on the resin were determined by hydrolytic cleavage[3] of the phenylacetamide group using either strong acid or base and quantitative determination by HPLC of the amount of phenylacetamide or phenylacetic acid released, respectively[4]. These loading values were then used to determine the efficiency of cleavage of the linker **6** using penicillin amidase[5]. It was found that penicillin amidase was indeed able to effect cleavage of **6**, although the yield of enzyme cleavage was strongly dependent on the resin used, ranging from 25% for PEGA® to a maximum yield of 50% for TentaGel®. We are currently attempting to improve these yields by varying the spacer arm of the linker and also by using tailor-made resins that are more compatible with enzyme catalysed reactions [10]. In view of the susceptibility of the linker to cleavage under mild acid conditions (aqueous TFA)[6], this protocol was used in subsequent experiments involving attachment of a range of alcohols.

The thioethyl group in **6** was activated by treatment with *N*-iodosuccinimide followed by displacement with a variety of alcohols **7** to **11** (Scheme 4)[7]. Fmoc-protected serine methylester **7** was found to couple in excellent yield to the polystyrene-linker and could also be cleaved quantitatively, as judged by HPLC analysis after acid release. Fmoc analysis of the resin bound **7** was in good agreement with the values derived from cleavage reactions. Secondary alcohols **8** and **9** gave good yields of coupling. These could possibly be improved by double-coupling methods, since analysis of phenylacetamide loading revealed that some of the linker had remained intact on the resin.

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[3] **Chemical cleavage of the linker from solid support and determination of the loading:** (for Tentagel and PEGA resins): A suspension of the resin (2 mg) in 2M HCl (300 μl) or 2M NaOH (300 μl) was shaken for 16h at 25 °C. An aliquot of the supernatant solution was neutralised and analysed by HPLC by comparison with standard solutions.

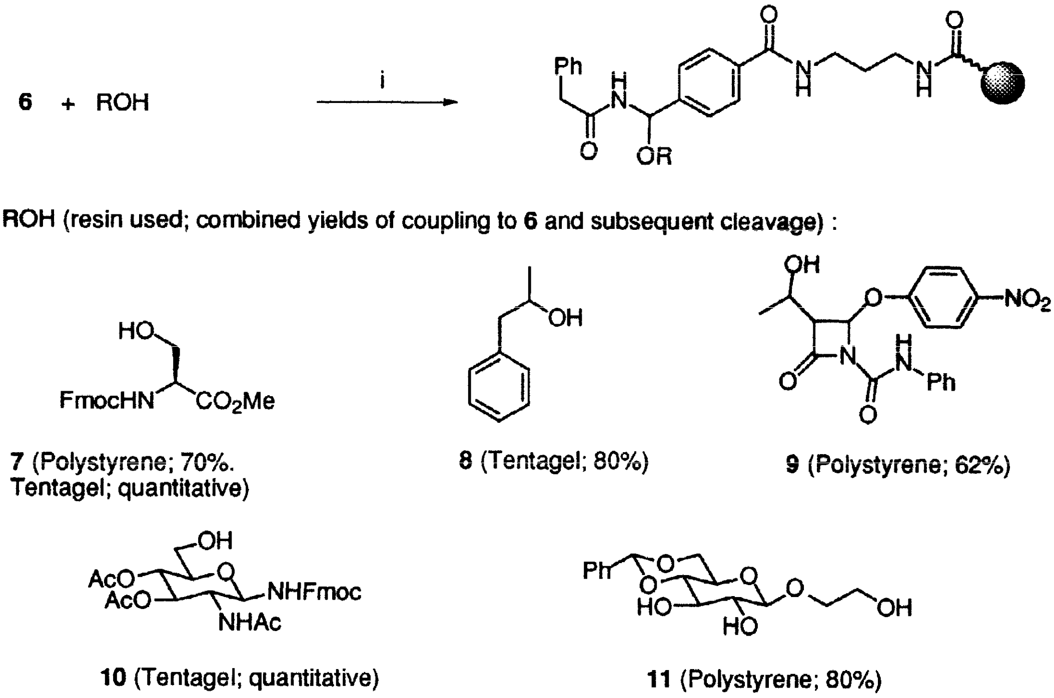
[4] **HPLC-analysis:** HPLC was carried out on a Waters HPLC system with a Phenomenex Sphereclone (5μm) ODS2 column (250 mm x 4.6 mm) using a gradient of two eluents (25 mM potassium phosphate buffer pH 6.5; acetonitrile).

[5] **Enzymatic cleavage of the linker from solid support:** A solution of penicillin amidase (700 units) in 0.1M potassium phosphate buffer (pH 7.5; 0.4 ml) was added to the resin (2 mg) and the mixture was shaken for 16h at 25 °C. The solution was removed, treated with 2 M HCl (0.5 ml) and extracted with CH_2Cl_2 (2 x 5 ml). The combined organic layers were concentrated, the remaining residue was dissolved in 300 μl methanol and analysed by HPLC.

[6] **Cleavage of linker using mild acid conditions** (for polystyrene resins): A suspension of the resin (10 mg, 0.01 mmol) in CH_2Cl_2:TFA:H_2O (10:9:1 v/v, 4ml) was agitated for 3h. An aliquot (300 μl) was removed, concentrated under reduced pressure, dissolved into the same volume of MeOH and analysed by HPLC.

[7] **Typical procedure for the coupling of alcohols to 6:** A suspension of the resin bound linker **6** (50 mg, 0.05 mmol), alcohol (**7-11**) (0.25 mmol) and 4 Å molecular sieves in CH_2Cl_2 (2ml) was agitated on a blood rotator for 30 min. *N*-Iodosuccinimide (28 mg, 0.13 mmol) (and in the case of alcohols **8**, **10** and **11** 4μmol of triflic acid) was added and the suspension agitated for a further 16h. The resin was washed with twice with 3 ml aliquots of THF, DMF, DMF:MeOH (1:1), DMF, THF, CH_2Cl_2 and was separated from molecular sieves by decantation.

Since we were particularly interested in applying the linker to solid phase carbohydrate synthesis, the protected glucosides **10** and **11** were coupled to linker **6**. Yields of coupling were markedly improved to 75% and 80% respectively, by adding catalytic amounts of triflic acid (0.125 eq) to the NIS solution.



Scheme 4
(i) ROH, *N*-Iodosuccinimide, CH_2Cl_2, RT, 16h.

In summary, we have developed an efficient synthesis of the linker **6** and have shown that a variety of alcohols can be coupled under mild conditions. The linker can be cleaved by penicillin amidase, although yields are currently restricted to 50%, or alternatively by quantitative mild acid cleavage. Both attachment to, and cleavage from, the linker can be achieved under much milder conditions than methodology based on dihydropyran-functionalised resins [11]. It should therefore be particularly useful for more acid labile compounds, such as carbohydrates and acid-labile protecting groups.

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