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The attachment and cleavage of phenols from solid supports and their single bead mass spectral analysis

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Abstract

The attachment of simple phenols to chloromethyl polystyrene, and their rapid cleavage at room temperature is described. Further, phenols from single resin beads can be unequivocally identified by Fourier transform ion cyclotron resonance mass spectrometry of the dansylates prepared in situ.

Keywords: phenol cleavage; single bead analysis; FTICR mass spectrometry.

Linkers used in solid phase organic chemistry should be stable to the chemistry used in the library construction, but should usually be easily cleaved on demand.[1] However, there are occasions when it is useful to have highly inert linkages to resin which can only be cleaved under more forcing conditions, e.g. to attach tagging molecules. The attachment of groups to the benzylic position of the chloromethyl polystyrene resin is analogous to the solution-phase benzyl protection of those groups. A range of chemistry has been developed for the deprotection of benzyl groups in solution,[2] but relatively little of this chemistry has been applied to the solid phase. We report herein the use of a thioanisole-based system for the facile removal of phenols from a solid support, and their identification using Fourier transform ion cyclotron resonance mass spectrometry (FTICRMS). The use of GC-MS in the identification of phenols cleaved from solid support with strong acid has been described in the patent literature.[3]

1. Phenol attachment to resin

The attachment of phenols to chloromethyl resin (resin used was 1.6 meq. 250–300 μm chloromethyl polystyrene) using the conditions reported by Merrifield,[4] proceeds very well using

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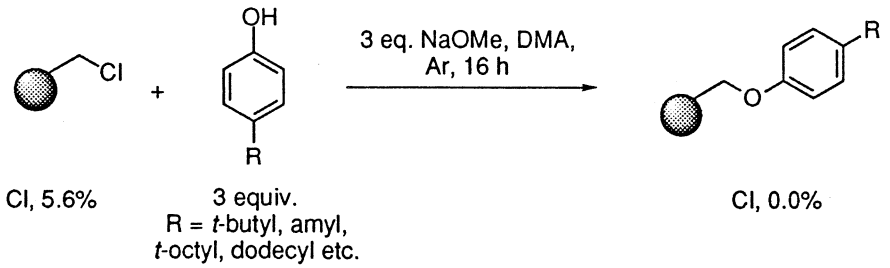
three equivalents of phenol in dimethylacetamide (DMA) at 50°C with sodium methoxide as base, as judged by chlorine analysis of the resin (there is negligible competition from methoxide). Typical phenols used are the alkyl phenols shown in Scheme 1. The use of a high boiling point solvent like DMA allows the reaction vessel to be sealed to ensure dryness, whilst not developing a hazardous vapour pressure at 50°C.



Scheme 1. General method for attachment of phenols to chloromethyl resin

A competition reaction was performed between 4-hydroxybenzyl alcohol (the Wang linker) and 4-amylphenol, in order to test how well the hydrophilic Wang linker reacted when compared with the more hydrophobic alkyl phenol. The reaction mixture consisted of a total of three equivalents of varying ratios of the two phenols. Incorporation of the Wang linker onto the resin was determined by subsequent derivatisation with Fmoc-leucine, followed by cleavage of the Fmoc group with piperidine in DMF, and analysis of the supernatant at 290 nm. As expected, the hydrophobic phenol reacted faster than the hydrophilic hydroxybenzyl alcohol. The level of Fmoc attached to the resin, and hence the level of incorporation of the Wang linker, increases more slowly than its concentration in the reagent mixture, implying that the reaction between the resin and alkyl phenol is faster (Fig. 1). This observation has important implications for functionalising beads with different ratios of different phenols.

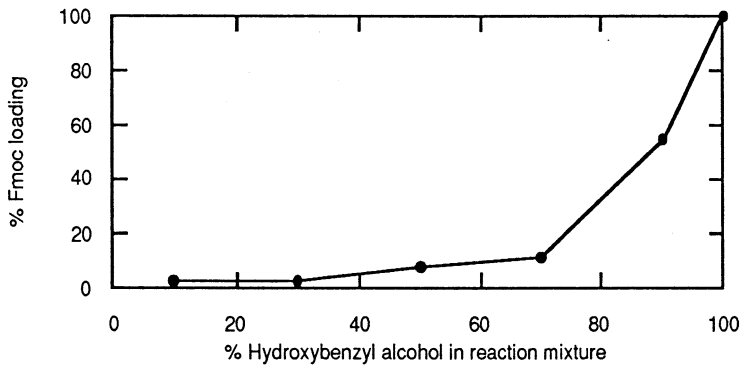
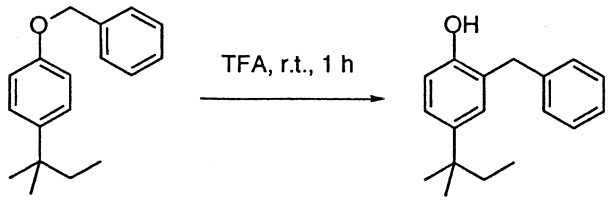


Figure 1. Graph showing percentage of Fmoc loading, corresponding to incorporation of Wang linker, as a function of the composition of the 4-hydroxybenzyl alcohol: 4-amylphenol mixture in the competition resin loading reaction

2. Cleavage of phenols from resin

The effectiveness of the TFA cleavage conditions usually used for the cleavage of the Wang linker was initially investigated using a solution phase model compound (**1**, Scheme 2) constructed by simple benzylation of 4-amylphenol. When this compound was stirred in TFA, starting material was consumed within an hour at room temperature, and as judged by TLC the phenol was apparently released. Upon closer inspection, the product of the reaction was in fact the rearranged product **2** (which gave the same R_F on TLC). The apparent ease of this reaction suggested that, whether this rearrangement were intra- or intermolecular, the *release* of phenol from the solid support would be hindered.

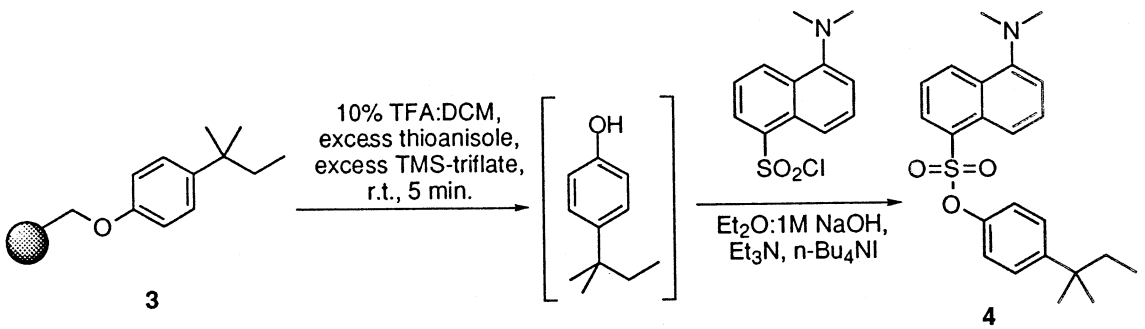


1 **2**, 63% yield

Scheme 2. TFA-induced rearrangement of resin model benzyl ether

Attempts to release phenols from resin by hydrogenolysis with palladium acetate and hydrogen in DMF at room temperature and elevated temperatures failed. This was disappointing, since a number of palladium-catalysed processes have been performed on resin,[5] and palladium acetate has been reported to allow the release of peptides from solid support.[6] It was assumed that the problem was the diffusion of hydrogen into the matrix of the support. When **1** was subjected to catalytic transfer hydrogenation[7,8] with cyclohexene and palladium acetate at 60°C, pure phenol was released. When similar conditions were applied to the resin-bound phenols, no reaction was observed. Neither was there any success with other hydrogen sources such as ammonium formate and cyclohexadiene.

Attention was therefore turned to the use of thioanisole in combination with a Lewis acid to cleave the *O*-benzyl bond nucleophilically.[9,10] When the model compound **1** was treated with excess (approx. 10 equiv.) thioanisole and TMS–triflate in 10% DCM:TFA at room temperature, immediate cleavage was observed with no trace of starting material by TLC after five minutes. When the same conditions were applied to the support-bound amylphenol (**3**, Scheme 3), release of phenol from the support began immediately, giving, aside from the reagents, clean phenol.



3 **4**

Scheme 3. Release and derivatisation of amylphenol from resin using thioanisole cleavage and dansylation

3. Phenol identification

High resolution mass spectrometry was used to identify the cleaved phenols unambiguously. Phenols, and in particular alkyl phenols, do not fly well under electrospray ionisation conditions. Consequently they were derivatised to their dansylates, which fly very well owing to the ease of protonation of the nitrogen atom. A rapid biphasic procedure was developed for the derivatisation (to form **4**, Scheme 3), which is simpler than has previously been reported.[11] This method is sensitive enough to be employed on single beads. In detail the protocol involves initially adding a solution of 10% TFA in DCM (250 μL) containing TMS–triflate (4.5 mM) and thioanisole (7 mM) to the bead. The suspension is allowed to stand, with occasional agitation, for 30 min, then the supernatant is removed under a stream of nitrogen. Diethyl ether (0.5 mL) containing dansyl chloride (15 μM) and triethylamine (5.7 mM) is added to the residue, followed by aqueous sodium hydroxide (1 M, 0.5 mL) containing tetra-*n*-butyl ammonium iodide (0.25 mM). This biphasic solution is stirred vigorously for 3 h, then the organic phase is separated, and concentrated under a stream of nitrogen, and resuspended in acetonitrile containing 0.1% TFA (250 μL). This solution is then filtered and injected into a Bruker BioApex™ 47e FTICR mass spectrometer, using 150 V capillary voltage, 8 scans, 60 μL h^{-1} injection rate, and 10 V cone at a gas temperature of 300°C.

When a single bead of **3** was treated with this cleavage-derivatisation protocol, and analysed using FTICRMS, excellent signal-to-noise was seen in the spectrum (Fig. 2). The use of this instrument allowed the unequivocal identification of this dansylate with a mass error of 5 ppm. We have applied the method to a range of phenols on solid phase, and the method gives excellent reproducibility.

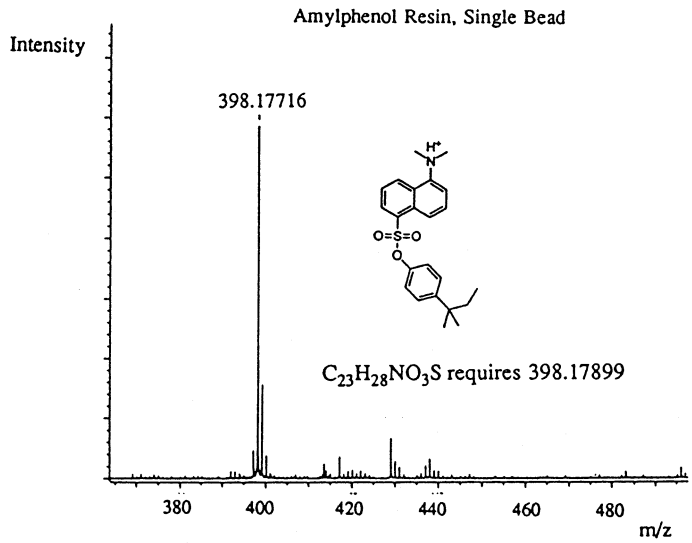


Figure 2. Mass spectrum obtained for the dansylate of amylphenol cleaved from a single resin bead

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