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Practical synthesis of a new analytical construct: thiopyrimidine safety-catch linker for facile monitoring of solid-phase chemistry

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Abstract

We report here a new analytical construct based on a thiopyrimidine safety-catch linker. This construct adds to the existing portfolio of linkers available for facile monitoring of reactions conducted on solid support. A practical solution phase synthesis of the precursor is described, together with the proof of concept and cleavage protocols. © 2000 Elsevier Science Ltd. All rights reserved.

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Combinatorial techniques are now widely accepted as a powerful strategy in drug discovery programs.[1–4] However, successful as it is proving to be, monitoring of reactions conducted on solid-phase remains problematic.[5] Recently 'analytical constructs' have been successfully introduced to address such problems of analysis.[6,7]

A typical analytical construct (Fig. 1) contains a conventional linker, 'linker 2', allowing the release of substrates in a classical manner, but also an orthogonal linker, 'linker 1', cleavable by a specific reagent in an additional 'analytical mode'. If the latter mode is used, an analytical fragment incorporating the substrate is produced, which is highly sensitised to electrospray mass spectrometry and easily identified by its isotopic label. We have recently reported the use of nitroveratryl-,[6] 2-nitrophenyl sulfonamide-[8] and Dde- based linkers,[8] cleavable by photolysis, thiolysis or hydrazinolysis, respectively. All of these possess a high degree of orthogonality with conventional linkers 2. However, for its general application to solid-phase chemistry, this analytical construct methodology requires a portfolio of linkers 1 compatible with the reaction sequence envisaged.

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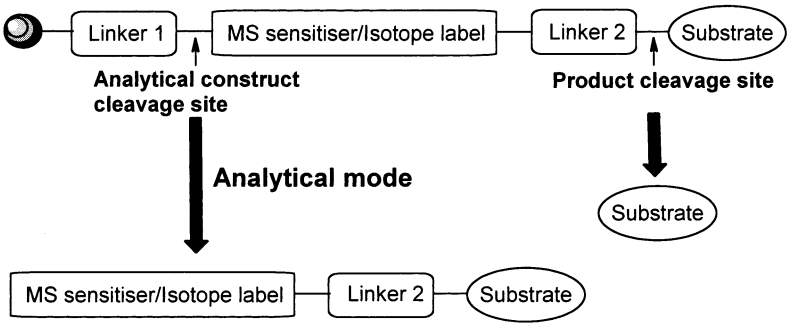


Figure 1. Analytical construct dual linker

Herein we describe a new analytical construct linker and its facile solution phase synthesis. This thiopyrimidine linker (Fig. 2), which exploits a safety-catch mechanism for cleavage,[9] has now been added to our portfolio of linkers dedicated to tackle analysis problems on solid support. The design of this new linker was based on previously reported chemistry developed for the production of pyrimidine libraries.[10–12] Analytical cleavage of the thiopyrimidine linker is effected by nucleophilic displacement using 1-methylpiperazine after activation by OXONE® oxidation of the alkylthio linkage. 1-Methylpiperazine provides, in this particular case, both the cleavage reagent and the sensitiser (Fig. 2).

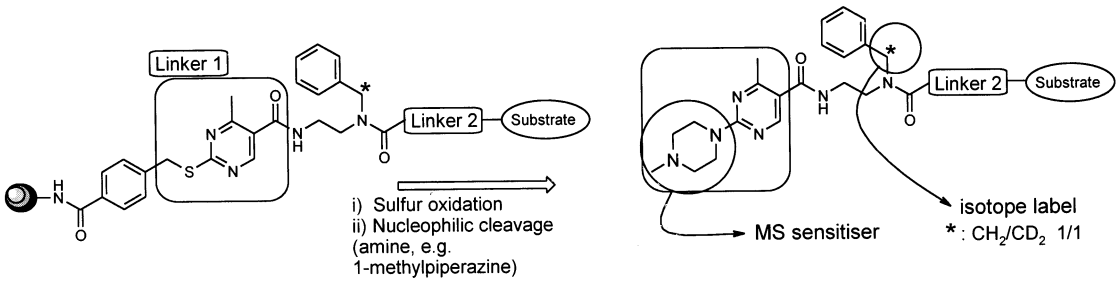


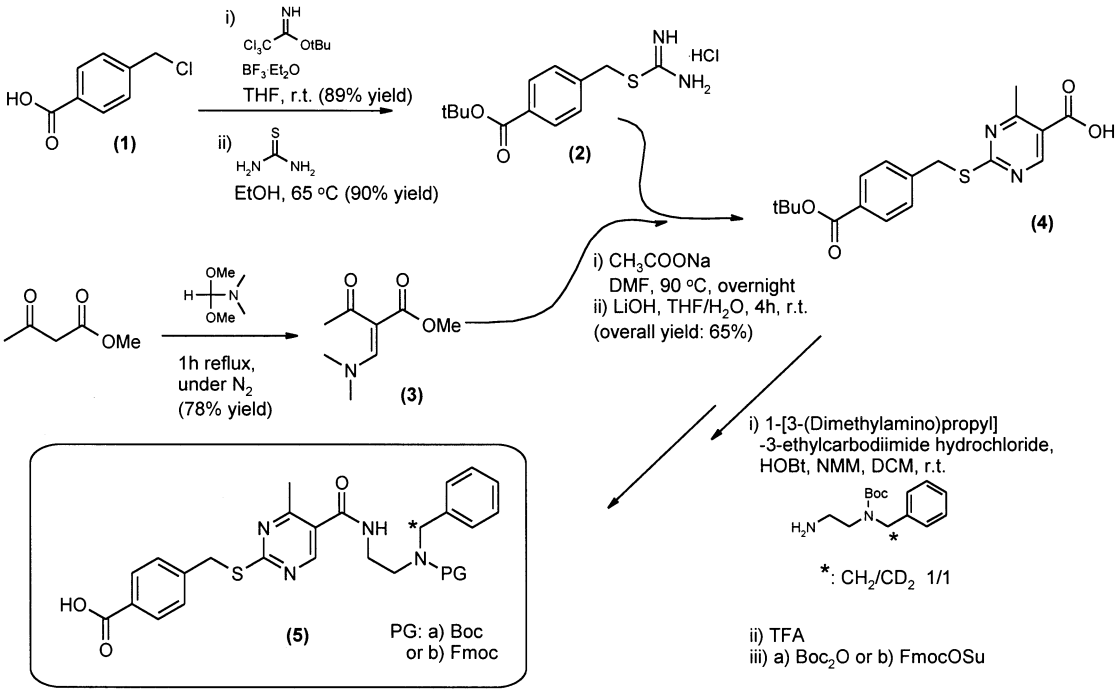
Figure 2. Thiopyrimidine analytical construct

1. Synthesis of thiopyrimidine construct precursor

While in principle the thiopyrimidine construct could be synthesised directly on resin, a solution phase approach is preferred for its preparation for two reasons. Firstly, the linker can be purified and characterised before its attachment to the resin, eliminating any impurity which could arise from a stepwise construction on the solid support. Secondly, the versatile intermediate obtained can be loaded onto any resin of choice.

4-(Chloromethyl)benzoic acid (**1**) was protected using *tert*-butyl 2,2,2-trichloroacetimidate[13] in the presence of a catalytic amount of boron trifluoride diethyl etherate (Scheme 1). The trichloroacetamide by-product was simply removed by passing the mixture through a plug of silica. Thiourea alkylation[12] provided the required thiouronium salt (**2**), which required no further purification. Activated diketoderivative (**3**) was obtained by Knoevenagel condensation of methyl acetoacetate and *N*,*N*-dimethylformamide dimethyl acetal. Cyclocondensation of

thiouronium salt (**2**) with diketoderivative (**3**)[14] provided, after saponification, the expected alkylthiopyrimidine (**4**). Coupling of the labelled amine, removal of protecting groups by TFA and protection of the amine with either Boc or Fmoc group finally afforded protected linker (**5**). It is noteworthy that (**5**) can be obtained in good overall yield and excellent purity with primarily aqueous/organic extraction techniques and no difficult chromatography. In addition we have readily prepared 200 g of intermediate alkylthiopyrimidine (**4**) by scaling up the previous procedure.



Scheme 1. Solution phase route to thiopyrimidine linker

2. Proof of concept: linker stability and ESI-MS analysis

To validate the use of thiopyrimidine based analytical construct we prepared a model substrate on a resin incorporating our new construct (Fig. 3).[15] The reaction sequence consisted of coupling the acid labile aldehyde linker to the analytical construct resin, followed by attachment of 4-methylbenzylamine to the resin by reductive amination, coupling of *N*-α-Fmoc-*N*-α-methyl-*L*-alanine and finally capping with 4-nitrobenzoic acid to afford resin (**6**) (Fig. 3).

The resin was then treated with OXONE® to oxidise the sulfur atom (we found electron rich linkers and substrates unstable to *m*-CPBA) and cleavage with 1-methylpiperazine affords the analytical fragment (**7**) (Fig. 3). An easily identified doublet, exhibiting a peak split of 2, is by far the main signal in the spectrum, the signal-to-noise ratio being very high, even at this single bead level.

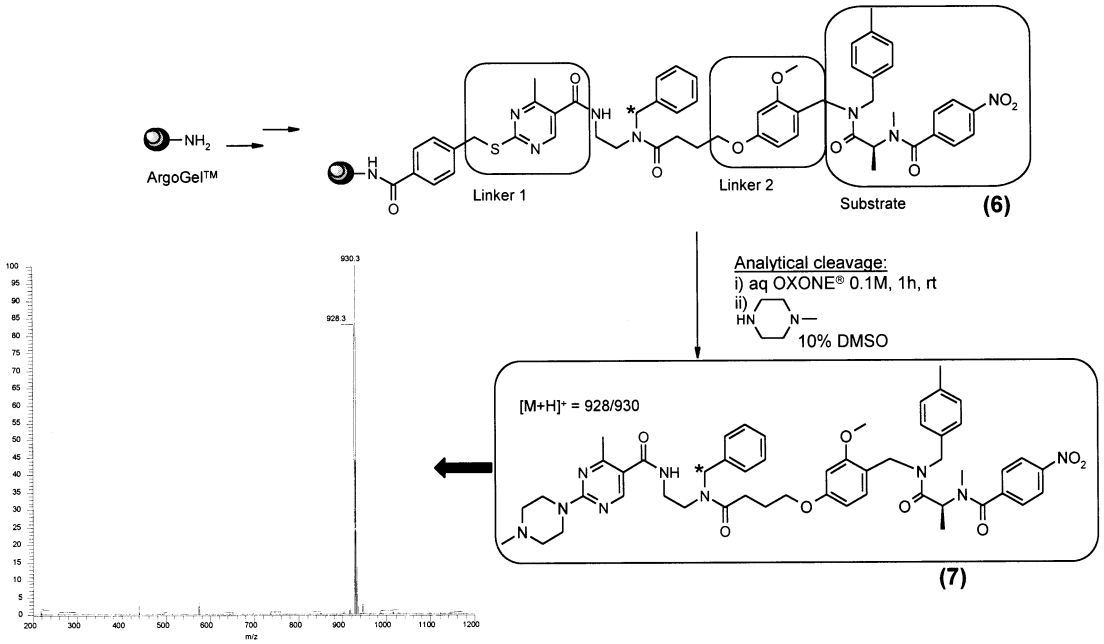


Figure 3. Analytical fragment release and ESI-MS spectrum at the single bead level of a model substrate

In summary, we have developed a new analytical construct 'linker 1' and designed a practical route for its synthesis. This linker represents a highly versatile building block now available to us in two protected forms and bulk quantities to couple to any solid support and subsequently to any conventional linker 2. Importantly, the preparation of the construct resin is rapid and straightforward (cf. well-behaved coupling of standard linkers to resin by simple amide bond formation). The new thiopyrimidine-based linker reported here provides further flexibility/orthogonality to the application of analytical constructs and is now part of the portfolio of linkers dedicated to such a modular approach. We have recently used the present linker for the synthesis and successful single bead level analysis of a 64 member model library.[15] Further applications of this strategy are in progress and represent significant steps towards solving our current solid phase analysis problems at the single bead level.

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