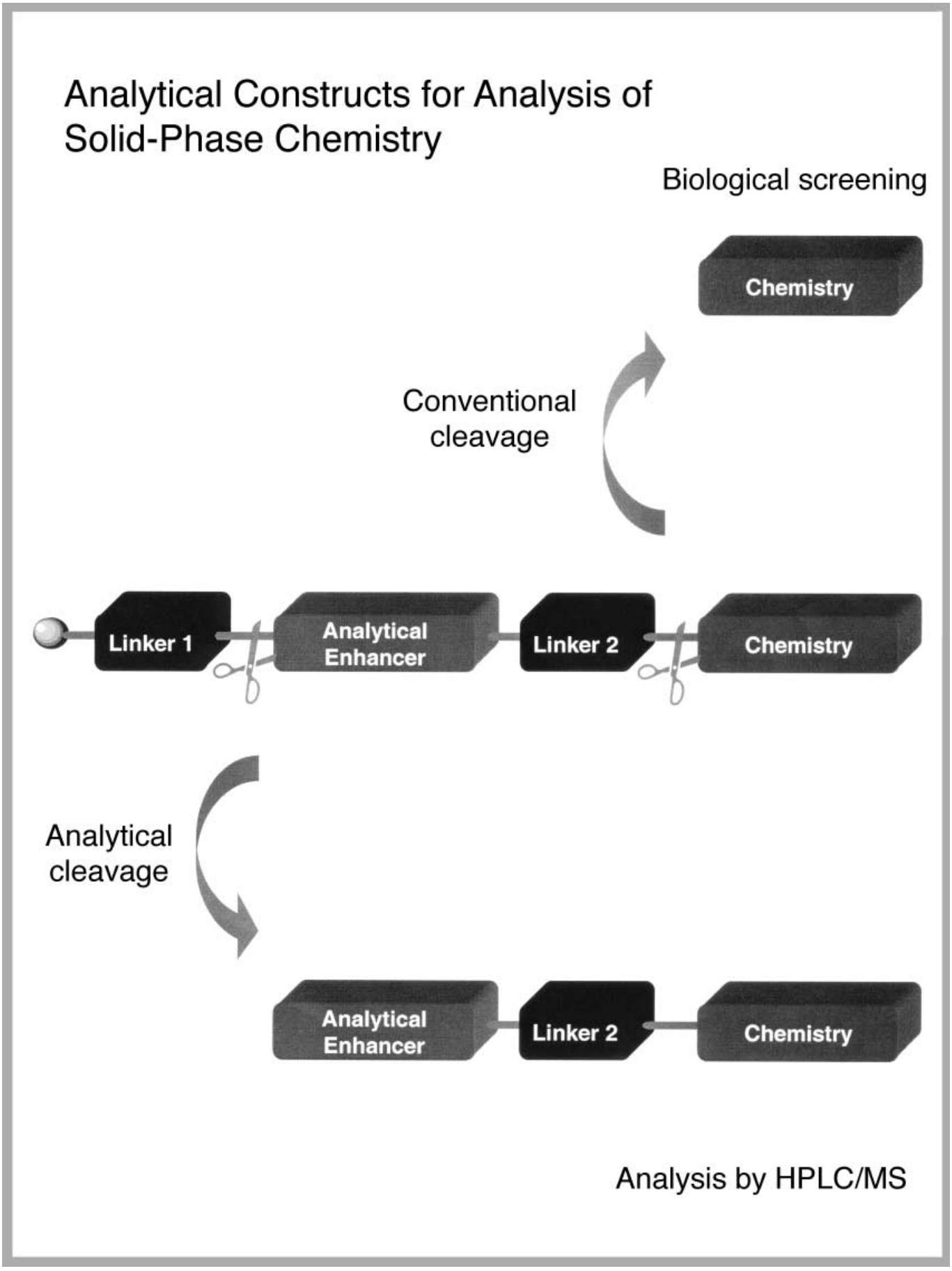

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Analytical Construct Resins for Analysis of Solid-Phase Chemistry

Miles S. Congreve,*[a] Steven V. Ley,[b] and Jan J. Scicinski[a]

Abstract: Rapid and unambiguous analysis of reactions performed on resin supports can be achieved by using "analytical constructs". These resins allow the synthesis of materials using solid-phase methods in the usual manner, but they also contain functionality enabling cleavage of analytically enhanced derivatives of the resin-bound products. This is possible due to the use of two linkers bound in series to the polymer. Cleavage at the first linker yields the products attached to an analytical enhancer that facilitates detection. Orthogonal cleavage at the second linker yields the desired products in the usual manner.

Keywords: combinatorial chemistry · isotopic labeling · mass spectrometry · solid-phase synthesis · UV/Vis spectroscopy

Introduction

Solid-phase library synthesis is now an established method of discovering new lead molecules in pharmaceutical research.[1–3] However, development of new sequences of reactions, often first in solution followed by optimisation of each step on solid phase, is a time consuming process. This is not only because of differences in the course of chemical reactions in solution and on polymer,[4] but also due to difficulties in the analysis of the products bound to the resin. Indeed, although important, the analytical methods available to study resin-bound products are limited by their low throughput and their inherent insensitivity, particularly in the analysis of chemistry on single beads.[5–7]

The power of the "split-mix-pool" strategy for solid-phase combinatorial synthesis has allowed production of huge numbers of chemical entities present as mixtures for biological screening.[8–9] However, such libraries remain largely uncharacterised owing to the complexities of monitoring the chemistry and of analysing either the product mixtures generated or the single compounds cleaved from individual resin beads. Although library-tagging approaches enable the elucidation of library-product structures from single beads, they do not enable a critical appraisal of the success in actually synthesising the material.[10–14] Overall, these problems in both the development of the chemistry in a split-mix-pool format and the subsequent analysis of completed libraries has recently led to a reduced emphasis on solid-phase library production as a strategy for lead discovery in many pharmaceutical organisations.

In an effort to overcome some of these difficulties, work in our laboratories has focused on the development of "analytical-construct" resins (Figure 1) in which additional functionality (an "analytical enhancer"), inserted between two orthogonally cleaved linkers (linkers 1 and 2), serves to aid the analysis of resin-bound species. Cleavage at linker 1 releases the resin-bound products linked to the analytical enhancer. The "analytical fragment" released contains both an ionisable group, to aid detection by mass spectrometry (MS) (an "MS sensitiser"), and an isotopic label, to produce a doublet in the mass spectrum to aid interpretation of the data (a "peak splitter"). Alternatively, cleavage at linker 2 yields the unlabelled products in the usual way. These analytical-construct resins represent a new approach for both the quality control of solid-phase combinatorial libraries and for the development of new synthetic sequences on solid support.

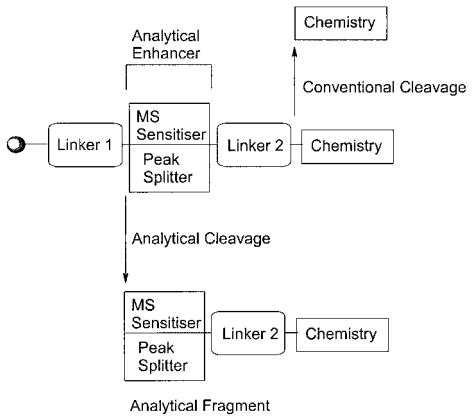

Figure 1. Generic analytical construct design.

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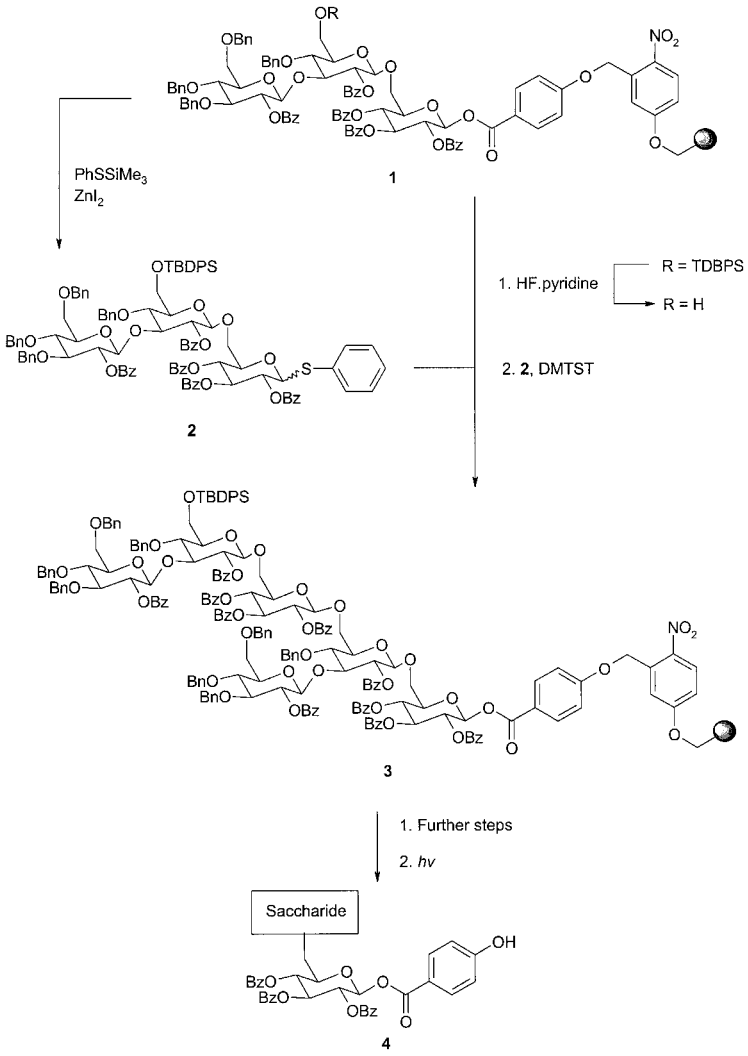
"Multidetachable" Resins for Compound Synthesis

The concept of using two linkers in series on a resin support is well established. Merrifield and Tam first reported "multidetachable" resins as an approach to peptide synthesis as early as 1979.[15–20] Having two linkers that could be cleaved under different conditions allowed synthesis of either the peptide itself or of a functionalised derivative that enabled purification before reattachment to another resin for further synthetic elaboration. A number of similar reports have subsequently appeared.[21–28] Recently, Nicolaou reported the solid-phase synthesis of a dodecasaccharide using a multidetachable resin.[29] Supported substrate **1** served as a precursor for both the donor and acceptor fragments of the synthesis (Scheme 1). Cleavage of a portion of resin **1** with



Scheme 1. Multidetachable linker system for oligosaccharide synthesis.

concomitant conversion to donor **2**, followed by coupling with the silyl-deprotected acceptor resin **1** (R = H) gave hexasaccharide **3**. By using this iterative strategy donor **2** was used to form the corresponding dodecasaccharide in three more coupling stages with resin-bound donors, prior to photolytic cleavage of the nitrobenzyl group to yield the product as its *p*-phenoxybenzoic acid glycoside (**4**).

"Multidetachable" Resin Encoding Systems

The use of multidetachable or "dual linker" resins for library encoding was first reported by Geysen, in which the codes were designed to be read by mass spectrometry.[30] In this work, Geysen introduced two important concepts that were later applied to the development of analytical-construct resins. Firstly, the use of a mixture of isotopes of a given functionality (here amino acid residues) between the two linkers introduced an "isotopic label" into the coding design (Figure 2). Up to ten different codes were proposed, consisting of multiple variants of unlabelled and ^{13}C-labelled glycine and alanine. Introduction of the code enabled the identification of the first monomer used in the library synthesis. Geysen also proposed further introduction of one or more reference masses to give the resin a unique mass signature, or "bar code", to unequivocally identify the structure of each library product. Additionally, use of an equimolar mixture of isotopes introduced a doublet separated by a known mass difference into the mass spectrum. This "peak split" gave rise to a clear ion-pair fingerprint, readily identifying material released from the resin and allowing all other extraneous signals to be ignored.

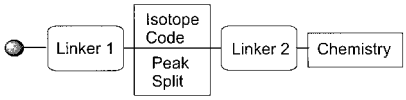


Figure 2. Isotope or mass encoding.

The second important concept introduced by Geysen for the design of mass-spectral codes was that of a "MS sensitiser". Geysen recognised that any useful method would require a reliable mass spectrum of the coding sequence, which would need to be detectable from a single resin bead. In order to achieve this sensitivity he proposed introduction of a charged group to facilitate detection in ESI positive-ion mode, typically an amine moiety.

Development of Dual Linker Analytical Constructs

A commonly used alternative to on-resin analysis is the cleavage of small resin aliquots and subsequent analysis of the resultant solutions by HPLC and LC/MS to identity and assess purity of the products. However, contemporary solid-phase chemistry used in library synthesis generates, by its nature, diverse products, which may not necessarily have common or reliable ionisation characteristics for confident mass spectral analysis.[31]

Geysen sought to address these issues by building on the encoding strategies described earlier, proposing the concept of "analytical constructs" in which the solid-supported substrate and linker is attached to the resin through an analytically enhancing group and a second linker (Figure 3).[32] The analytical enhancer, between the linkers, contained a "MS sensitiser" ensuring reliable ionisation[33–35] of each product (*t*Boc-Lysine) and a "peak split" or sequence code that

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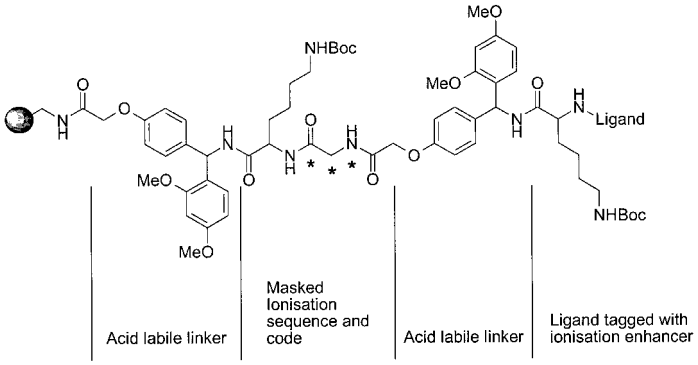

Figure 3. Geysen's dual linker analytical construct for reaction screening.

consisted of a mixture of isotopes of glycine as described above. Exposure of this construct, designed for reaction screening,[30b] to trifluoroacetic acid (TFA), cleaves both Knorr linkers with concomitant deprotection of the *t*Boc-Lysine to produce the charged amine for MS sensitisation. Thus the analytically enhanced sequence code and the ligand tagged with an ionisation enhancer are released into solution for detection by mass spectrometry.

A similar approach to monitoring solid-phase organic chemistry was described by Kent and Carrasco,[36] building on earlier work for monitoring peptide synthesis using a "mass-spectral ladder".[33] In this case, by using a photolabile linker as linker 1, direct analysis from single resin beads was possible by exploiting laser irradiation in MALDI mass spectrometry (Figure 4). The use of a quaternary ammonium ion containing

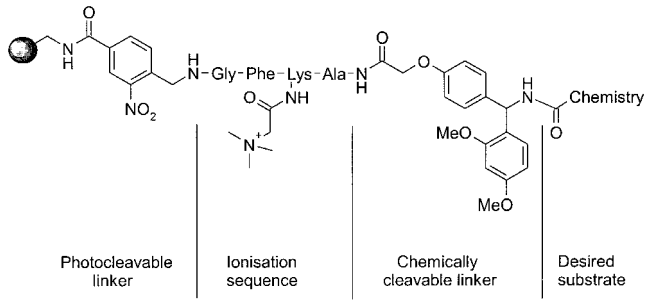

Figure 4. Kent and Carrasco's dual linker analytical construct.

an amino acid side chain in the analytical enhancer served as a MS sensitiser and ensured easy detection of the reaction products. A Rink linker (as linker 2) allowed release of the products in the conventional manner.

A potential drawback with the above approaches is the presence of either a side-chain-protected or permanently charged amine functionality in the analytical enhancer, limiting the range of chemistry that can be performed with these resins. To address this limitation, McKeown et al. designed a construct in which the MS-sensitising amine is released upon cleavage of the first linker, a nitroveratryl derivative (Figure 5).[37] The utility of the construct was demonstrated by comparison of MS analysis of conventionally synthesised capped dipeptides with their analytically enhanced derivatives assembled on the construct resin (Figure 6). The underivatised materials could not be readily detected by MS (Figure 6, top) despite being shown to be present in excellent purity by ^{1}H NMR spectroscopy. The construct fragments, however, were easily detected as characteristic ion pairs, derived from a mixture of isotopes of nitrogen atoms in the enhancer. Detection of the products was also possible down to the level of a single bead (Figure 6, bottom).

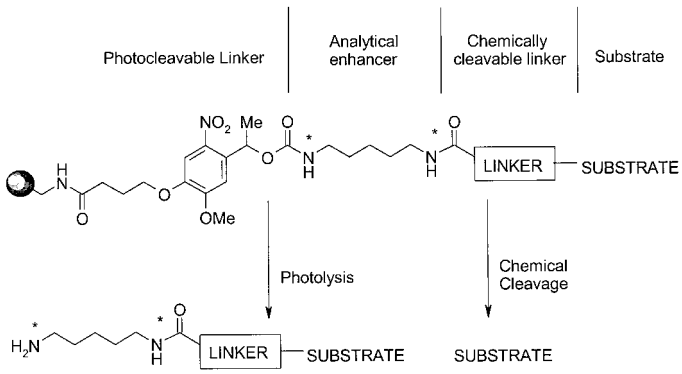

Figure 5. Analytical construct with peak splitter and MS sensitiser. * = 50% labelled with $2 \times {}^{15}$N. Resin = ArgoGel™.

Split-Mix-Pool Library Analysis

In an extension of this approach, a construct for analysis of library products produced by using "split-mix-pool" methodology has been described.[38] The construct (Figure 7) consisted of a pyrimidine safety-catch linker as linker 1,[39] an analytical enhancer containing a peak splitter and an acid cleavable linker as linker 2. A 4^3 library was synthesised in IRORI™ Kans[40–41] and the products then analysed by cleavage of single beads (at linker 1) releasing each analytical fragment. For comparison, single beads were also cleaved conventionally (at linker 2) and analysed by MS. The remaining beads were then cleaved at linker 2 and the presence of products confirmed by ^{1}H NMR spectroscopy. In each case, single-bead cleavage at the pyrimidine linker (Route a, Figure 7) unambiguously identified the library products. In comparison, from conventional single-bead cleavage (Route b, Figure 7) only 25 of the 64 products could be confidently identified. Figure 8 illustrates one example where the library member did not ionise (a), but was readily detected as its analytical fragment (b).

UV Chromophore-Containing Analytical Constructs

Although the relative quantification of product mixtures derived from similar scaffolds by MS-techniques has been reported,[42] mass spectrometry is not routinely used for the quantitative analysis of more diverse molecules. A limitation of the above analytical-construct systems, therefore, is that they do not facilitate accurate determination of the relative proportions of mixtures of products. To overcome this problem, analytical constructs have recently been developed

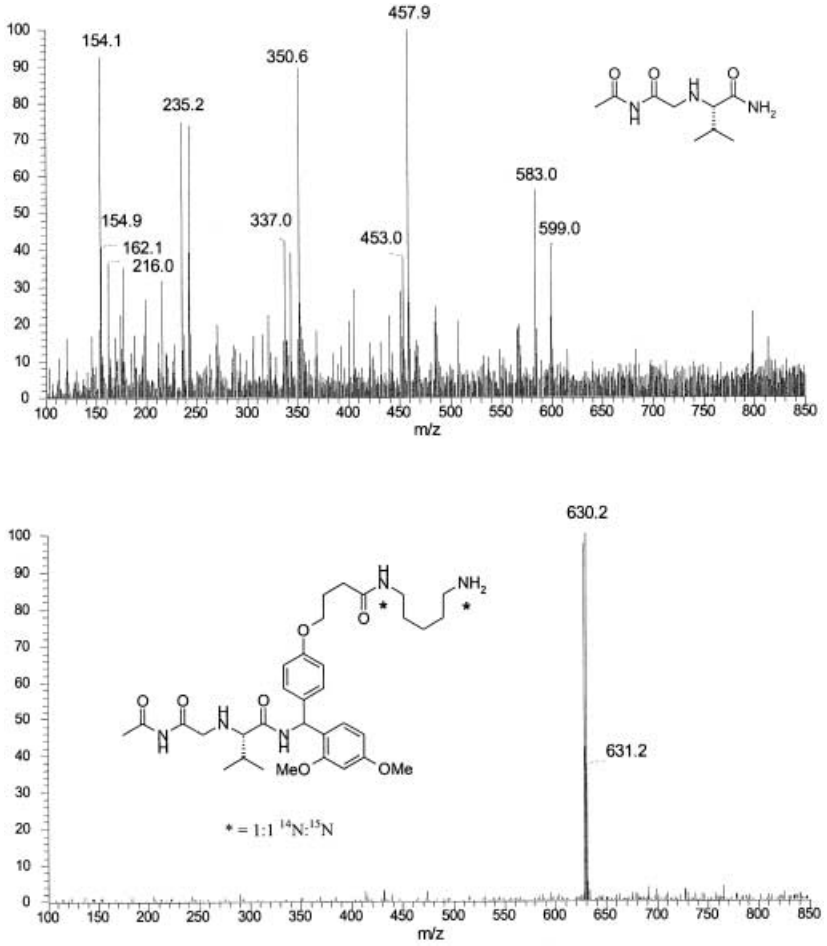

Figure 6. Electrospray MS of Ac-Gly-Val prepared on the analytical construct. Top: Conventional synthesis and cleavage ($[M+H]^+ = 216$). Bottom: Construct resin and photolytic cleavage from a single bead ($[M+H]^+ = 628/630$).

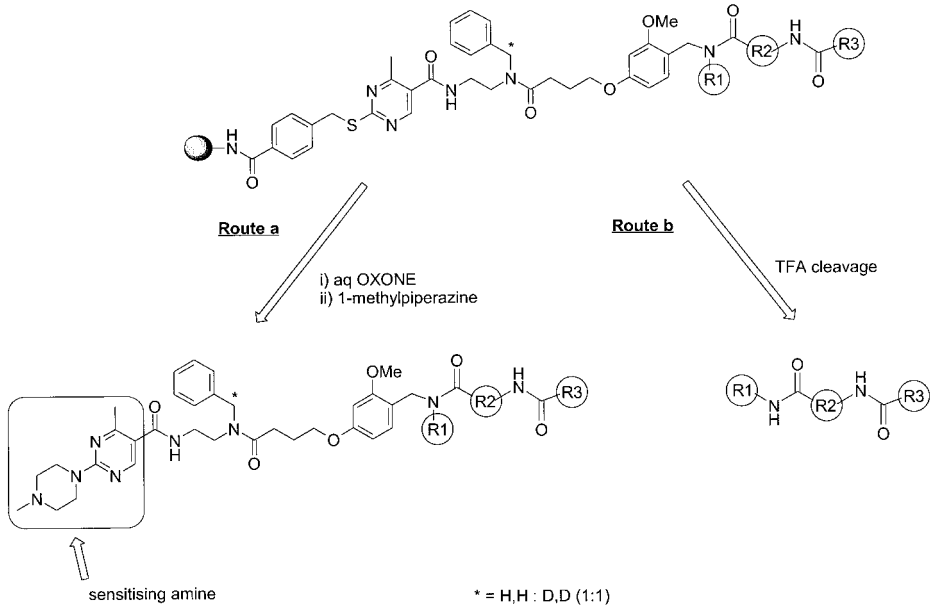

Figure 7. Route a: Analytical fragment release. Route b: Classical cleavage for biological screening.

that incorporate a UV chromophore (dansyl (**A**) or anthryl (**B**), Figure 9) to enable more facile detection and relative quantification of product mixtures.[43–45] Both chromophores have characteristic UV spectra and facilitate detection of products at the single-bead level. The characteristic UV spectra enable analysis of mixtures at a "remote" wavelength (for dansyl at 339 nm and for anthryl at 386 nm) at which generally only materials incorporating the chromophore, and hence derived from the resin sample, will be visible, allowing rapid interpretation of the data. A possible advantage of the anthryl-containing construct over the dansyl-containing system is that the MS-sensitising group is released only upon analytical cleavage in the former, whereas in the latter the basic amino-group is unprotected and could conceivably limit the range of chemistry possible with the resin.

The utility of the anthracene construct system has been demonstrated by analysis after each step in the synthesis of the fibrinogen antagonist **7**[46] (Scheme 2). Using the construct **5**, it possible to readily establish conversions during each chemical transformation. Additionaly, assessment of purity and assignment of by-products using standard LC-MS analysis was found to be straightforward.[45]

Study of the Properties of Linkers

A particularly useful application of analytical constructs is in the study of the properties of linkers. Murray et al.[47] used constructs **8** and **9** to study the relative stabilities to common reagents of the 2-nitrobenzenesulfonamide[48] and 4,4-dimethyl-2,6-dioxocyclohex-1-ylidene (Dde)[49] linkers, respectively

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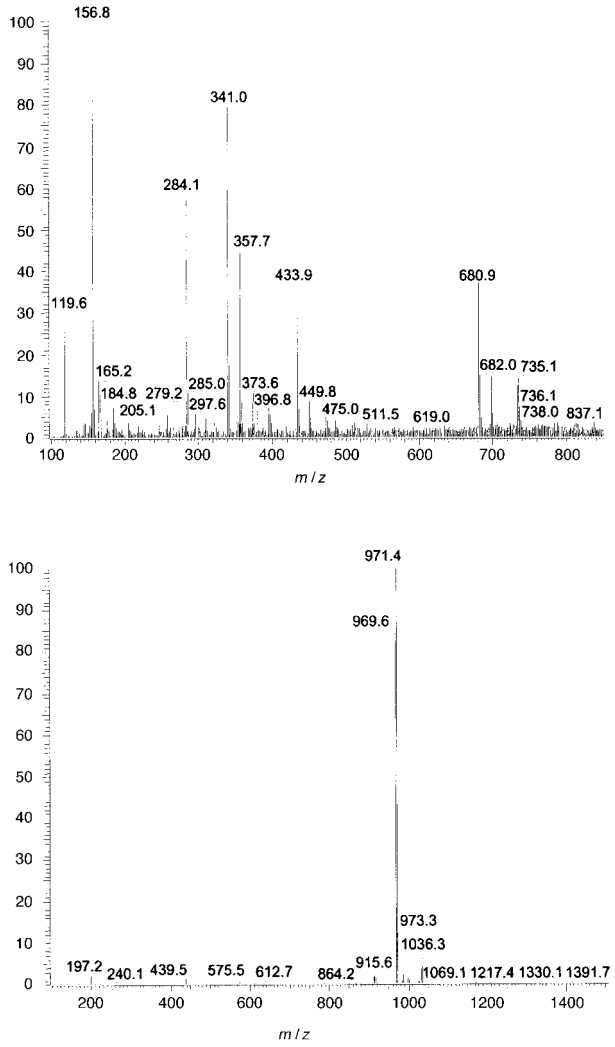


Figure 8. Electrospray MS of the library component corresponding to a typical library sample. Top: Conventional cleavage ($[M+H]^+ = 341$). Bottom : Analytical construct fragment release ($[M+H]^+ = 969.5$).

(Scheme 3). Treatment of resins **8** and **9** with a range of commonly used reagents prior to release (with TFA) of the analytical fragments **10** and **11** enabled the degree to which the linkers had been modified by the reagents to be assessed. This rapid and straightforward survey allowed limitations in the use of the two linkers to be readily identified. For example, the Dde group was found to be reduced by sodium borohydride, whilst the sulfonamide linker was unexpectedly cleaved by fluoride ions.

Analytical constructs have also been exploited in the de novo design of linkers. In work directed towards the preparation of libraries of small heterocycles derived from amino acids, Berst et al. employed a construct approach to aid the development of a latent aryl hydrazine "safety-catch" linker. Using the construct greatly facilitated the optimisation of the crucial linker loading and cleavage steps.[50]

Study of Reaction Kinetics on Solid Phase

The ease and sensitivity of analytical-construct analysis for in situ monitoring of chemical reactions on the solid support

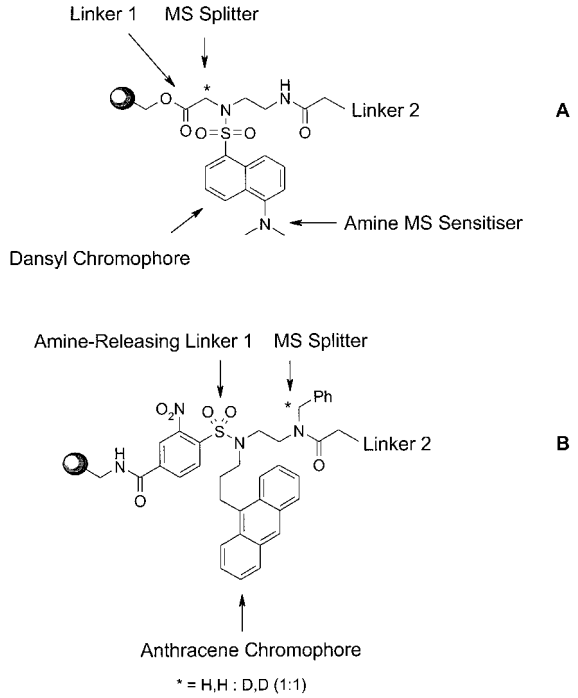
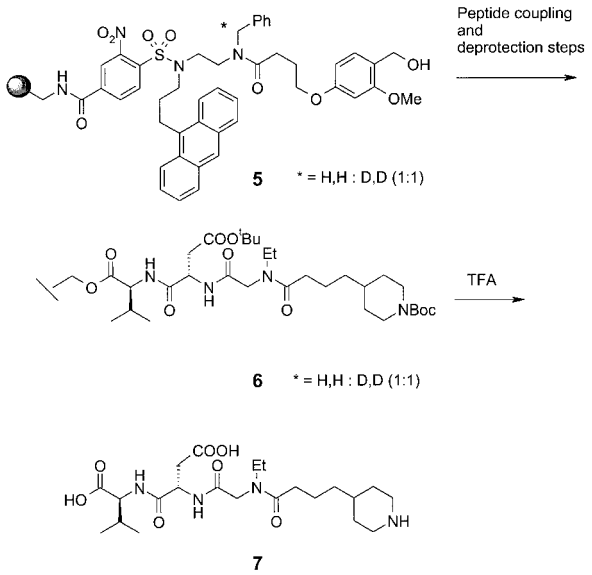
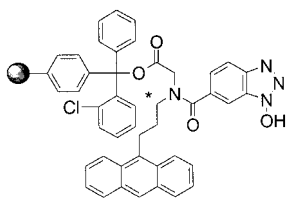


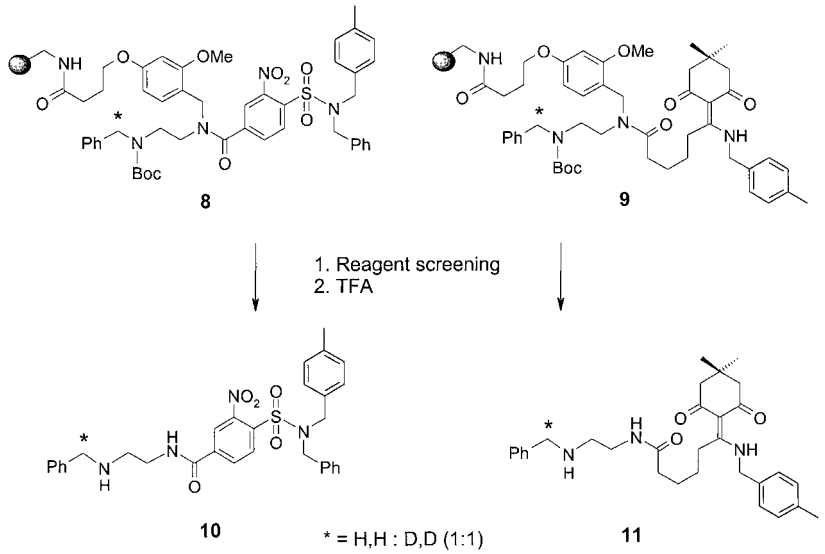
Figure 9. UV chromophore containing analytical constructs.



Scheme 2. Synthesis of a representative molecule using an analytical construct to monitor the chemical steps.

enables the study of the kinetics of reactions that occurr on the resin. The *automated* study of the loading and cleavage kinetics of a 1-hydroxybenzotriazole linker was undertaken using an analytical construct **12**, which incorporated the





Scheme 3. Analytical constructs used for reaction scanning.

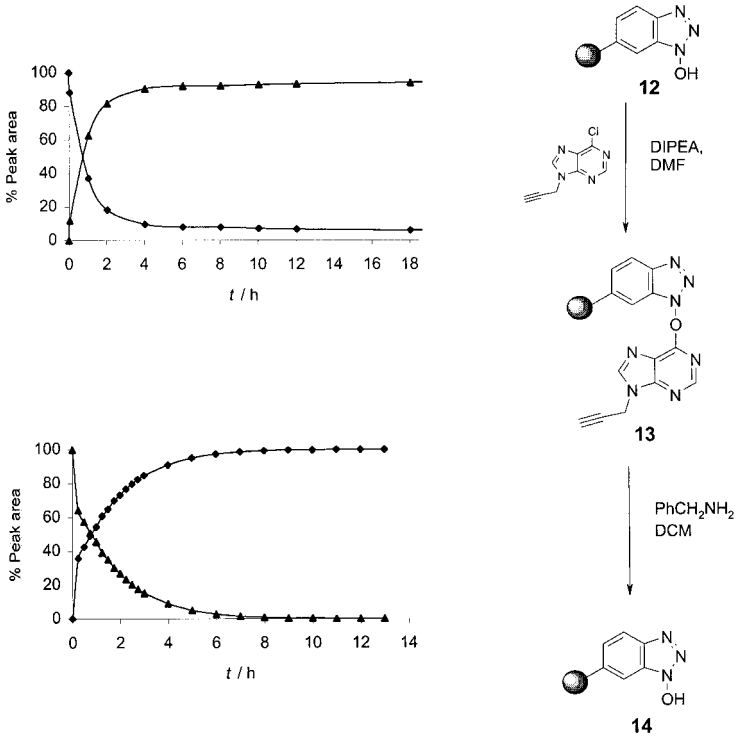


Figure 10. Kinetic data obtained by automated cleavage of the analytical construct showing relative peak areas at 386 nm of the analytical fragments corresponding to the loading of the chloropurine onto the 1-hydroxybenzotriazole construct (top); the displacement of the purine with 2% benzylamine in dichloromethane (bottom). Both reactions were performed at 25 °C.

anthryl chromophore to quantify the conversion of starting materials to products in each reaction (Figure 10).[51] Additionally, the incorporation of the anthryl chromophore in the analytical construct allowed resin cleavage and analysis without the need for prior resin washing, since the absorbances due to the reaction medium were minimal at the analysis wavelength (386 nm). Analytical cleavage of the chlorotrityl linker (linker 1) released a carboxylic acid as the ionisable functionality to act as the MS sensitiser in ESI negative-ion mode. Loading of a subsituted chloropurine was measured by using automated resin sampling and quenching into a cleavage solution and indicated 95% conversion after 8 h at room temperature. The displacement of the purine from the resin with benzylamine was similarly studied, showing complete conversion in 10 h.

Reporter Resins

An additional application of analytical constructs is the concept of using the resins as a reporting system for chemistry being carried out on conventional resins. This is achieved by using a small proportion of the construct resin mixed with a conventional resin for any given reaction sequence. The construct resin can then be used to indicate the outcome of the reactions by sampling the mixed resins and selectively cleaving the analytical fragment from the construct beads. In order to exemplify this approach, a series of model library compounds was prepared (Scheme 4).[52] The chemical sequence was successfully followed by analytical cleavage and HPLC analysis (shown) of the resultant analytical fragments, by using the reporter resin as 5% of the total resin mixture. MAS ^{1}H NMR spectra of the resin-bound intermediates confirmed that the chemistry on the reporter resin was generally predictive of the chemistry occurring on the conventional resin. The reporter resin system was found to be predictive of the final quality of the library products, thus giving further evidence that the reporter is representative of the whole.

Outlook

Dual-linker analytical constructs for the analysis of reactions carried out on solid supports provide a new way to approach resin-based chemistries. Analytical constructs have been shown to be useful for the development of new linkers, the rapid monitoring and optimisation of new chemical reactions and library chemistries, and in the quality control of either discrete or split-mix-pool production libraries. More recently, analytical constructs have found an application in a systematic screening approach to the identification of functional group reactivity.[53]

The many advantages of this approach to the analysis of reactions on resins are currently limited by the need to synthesise the analytical resins and that these resins may contain functionality that is not always compatible with the reagents and reaction conditions to be used in the synthesis. The former issue might be addressed by the possible future

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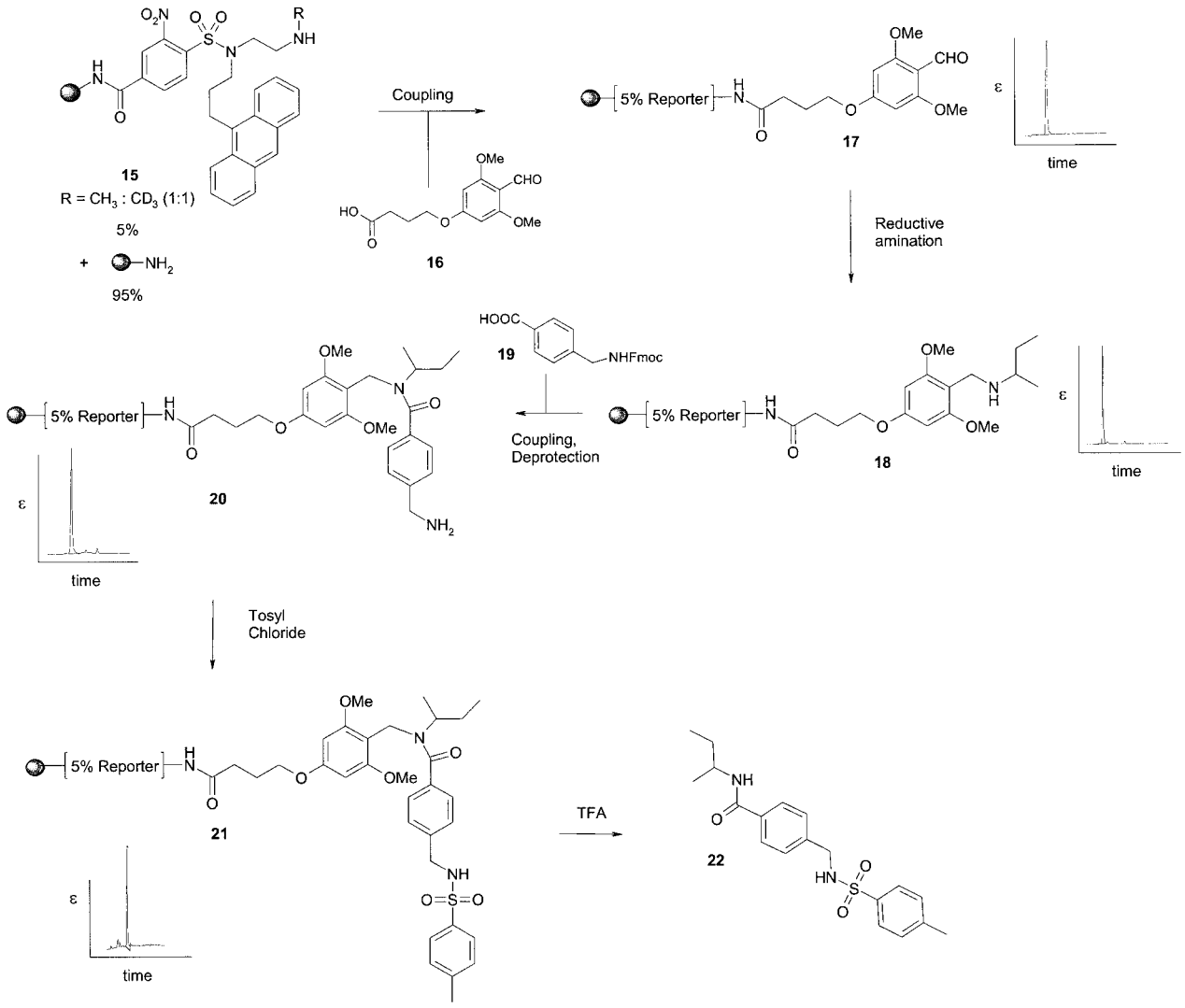

Scheme 4. A reaction sequence analysed using 5% of a reporter resin.

commercial availability of the resins and by use of the resins in the "reporter-bead" mode to maximise their cost effectiveness. The identification of a broader range of analytical resins that offer wider compatibility with reagents typically used in solid-phase synthesis is currently under investigation in our laboratory. The outcome of these studies and the further application of analytical constructs in rapid parallel screening of reaction conditions for the optimisation of resin-supported chemistry will be the subject of subsequent publications.

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