

The Application of Quantitative Analytical Constructs for Chemistry Optimization, Monomer Rehearsal and Reactivity Prediction in Solid Phase Library Synthesis

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Abstract: The use of quantitative dual linker analytical constructs for the high-throughput generation of solid phase reaction data is described. This methodology can be applied to chemistry optimization, monomer rehearsal and monomer reactivity prediction as a tool to aid the production of solid phase compound libraries.

Keywords: Analytical methods, combinatorial chemistry, mass spectrometry, solid-phase synthesis.

The synthesis of compound libraries is a standard procedure in the pharmaceutical industry. The automation of such syntheses can provide many hundreds of compounds in a relatively short period of time. Both split-mix [1] and discrete compound libraries share a common development stage in which optimization of their reaction chemistries must be undertaken. Since it is both impractical and laborious to perform a complete rehearsal of every combination of monomers available (complete combinatorial rehearsal), this process often involves the iterative synthesis of smaller arrays of compounds to optimise generic reaction conditions. Our observation is that small array syntheses have in some cases proven problematic because the monomers chosen for array synthesis were either not representative of the reactivity of the diverse set of monomers used in the final library, or certain combinations of monomers were found to be incompatible. We therefore required a technique that could enhance the quality of monomer rehearsal data, assisting in set selection for compound library synthesis.

Solid-phase array syntheses can often be a time consuming process and the compounds produced may also prove difficult to analyze using generic techniques such as Mass Spectroscopy and LC-UV. Methods are available which can address some of the problems associated with the analysis of compounds synthesized on solid supports [2], however our efforts have been primarily focused on a dual-linker analytical construct approach [3]. This methodology comprises two chemically orthogonal linkers, which are joined by an analytical enhancer (AE). Compounds present on the resin can be released through a conventional cleavage (L2), or at the construct linker (L1). Cleavage at the construct linker provides a fragment which is sensitive to

mass spectroscopy [4] and also contains a unique isotopic signature to aid the identification of the compounds present (scheme 1) [5].

This strategy has been successfully implemented for the qualitative analysis of compounds produced on a single resin bead from a split-mix combinatorial library [6].

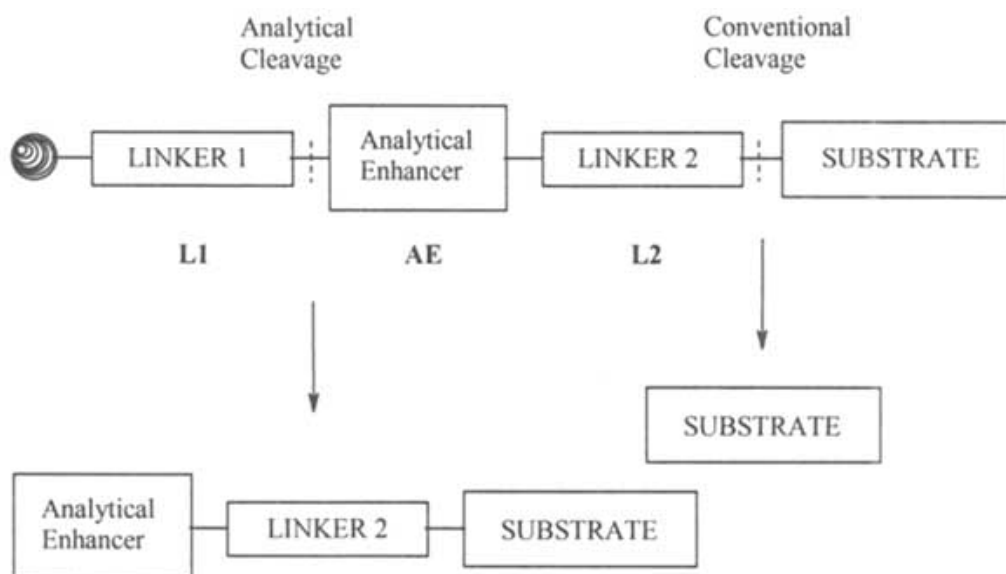
In addition to the qualitative identification of compounds of solid-phase transformations, the application of analytical constructs has now been extended to incorporate atoms or groups that assist the quantification of the components present on the support. Incorporation of an anthracene moiety has been demonstrated, in combination with chromatography (LC-UV), as a method of introducing relative quantification to solid phase reaction sequences (scheme 2) [7]. Analysis of test samples by LC-MS-UV provides data that not only gives characterization through the MS sensitized fragments, but also excellent relative quantification of product mixtures through the ratio of peak areas in the UV spectrum at 387 nm (scheme 2).

Herein we illustrate the use of analytical constructs to provide high-throughput qualitative and quantitative data for the optimization of reaction chemistry for use in solid phase parallel syntheses. In addition we demonstrate that this strategy, when used in conjunction with computational techniques, may be applied to generate informative reactivity data for a representative set of monomers.

To validate this method a ubiquitous solid phase reaction involving the reductive amination of a resin bound aromatic aldehyde with a set of amines was chosen. The use of a construct resin will reveal all solid supported reaction components and allow their extent of formation to be accurately determined (scheme 3).

This reductive amination reaction highlights three distinct applications of quantitative analytical constructs. Firstly this methodology serves as a monomer rehearsal experiment, whereby monomers producing the highest yielding reactions can be selected for library production.

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Scheme 1. The concept of analytical constructs - Substrate release is obtained through cleavage at linker 2 (L2). Construct cleavage (L1) provides a fragment that is sensitized to ESI⁺ mass spectroscopy and contains a peak split isotopic doublet to aid identification.

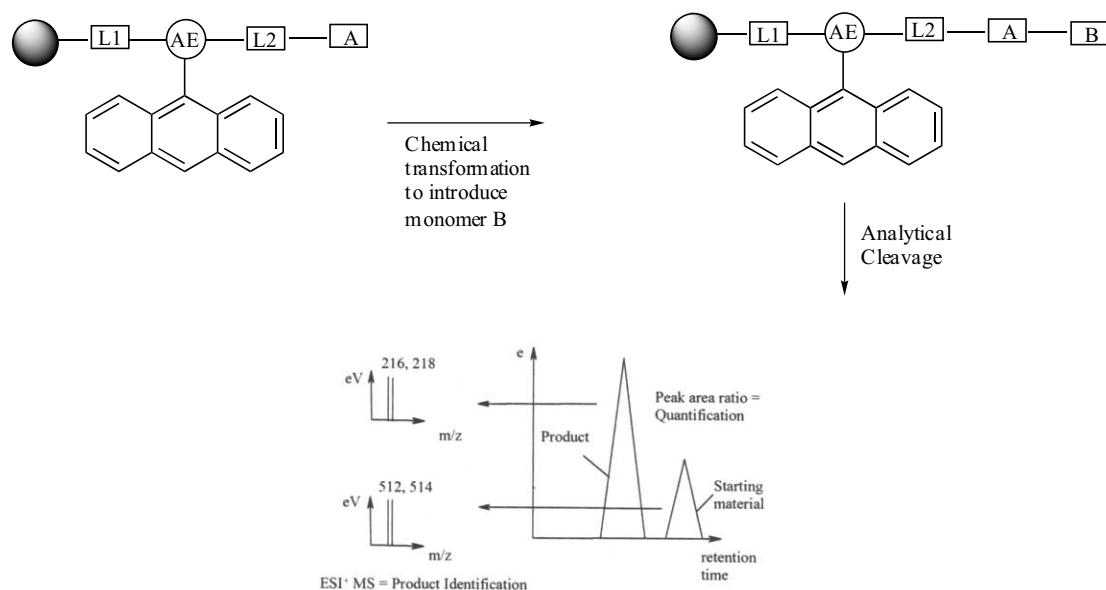
Secondly, the use of a computationally selected amine set representing a wide range of amine reactivity allows exploration of the scope of the reaction and facilitates future work towards reactivity models. Finally a reaction optimization procedure will be illustrated by performing the reaction under a series of different reaction conditions.

Monomethoxy aldehyde linkers are traditionally employed for releasing amides in solid phase synthesis. A reductive amination reaction is performed as the initial resin loading step and the secondary amine produced elaborated before acidic cleavage of the final compound. This first vital reductive amination step cannot be analyzed directly in a quantitative fashion, as the secondary amine generated

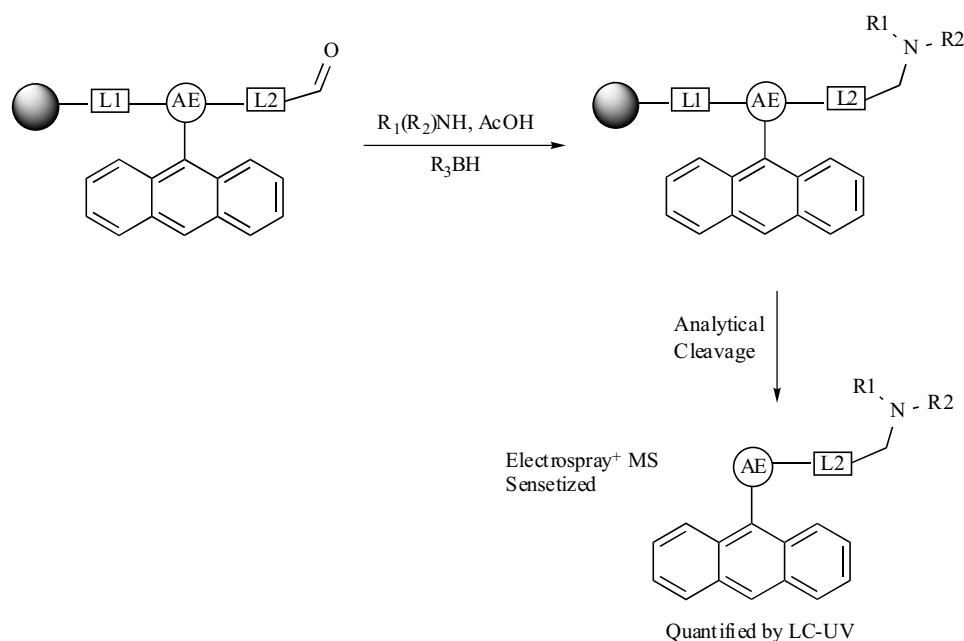
cannot be released from the resin with acid. Previously to monitor this transformation it has been necessary to perform an acylation to render the fragment acid labile. The use of an analytical construct linker approach, however, facilitates the direct study of this reaction.

In addition to acting as an acid labile linker, this substrate was chosen to simultaneously represent a typical aldehyde monomer, for example in the reductive amination of secondary amines to provide the corresponding tertiary amines on resin.

In order to investigate the effects of electronic and steric properties of the reaction, a set of amines was



Scheme 2. Concept of analytical constructs with quantification handle. Incorporation of an anthracene moiety, which exhibits a strong and remote UV absorbance at 387 nm, facilitates quantification of reaction products.



Scheme 3. Reductive amination of a construct tethered aldehyde resin. Following reductive amination of a set of amines analytical cleavage at **L1** provides fragments that can be identified and quantified.

computationally selected. The criteria used for selecting the set of monomers have previously been shown to correlate with their proton affinity [8]. Amines with diverse values in the calculated properties of the amine nitrogen atom were therefore selected, yielding a set of primary, secondary, and aromatic amines. Due to their different properties the amines were expected to exhibit a wide reactivity range in reductive amination reactions*. The reactions were performed using four different reduction conditions to illustrate how constructs can be used for a library reaction optimization experiment.

The monomethoxy benzaldehyde♦ linker was coupled to nitrosulfonamide construct resin (**1**) to produce the desired solid supported substrate (**2**) for reductive amination studies[11]. A set of 84 amines was condensed as discrete compounds with the aldehyde resin (**2**). The resin was mixed with each amine in the presence of acetic acid for 1 hour before the addition of the appropriate reducing agent. After 24 h the wells were drained and the resins washed before treatment with a solution of mercaptoethanol in methanolic sodium methoxide to effect cleavage of the construct linker **L2** (scheme 4).

Samples were analyzed by LC-UV mass spectroscopy and the ratio of components in the UV spectrum at 387 nm monitored. Each component from the UV spectrum was then characterized from the corresponding MS peak split doublet.

The clarity of the LC-UV and MS spectra obtained is exemplified in the spectra below (fig. 1).

These spectra illustrate the incomplete reductive amination of a selection of amines with *tetra*-butylammonium borohydride. The desired product peaks are

clearly visible and display mass spectra consistent with the products. In addition to providing the product yield, this method can be used to generate valuable data on other species formed in the reaction. This spectrum also shows the presence of remaining aldehyde (r.t. = 6.1 min.) and corresponding alcohol product (r.t. = 5.8 min.), formed through reduction of the aldehyde. This information would be inaccessible using a conventional acylation/cleavage protocol to monitor this reaction.

It was also demonstrated that sampling of the resin throughout the experiment could be used to provide kinetic information on the course of the reductions. We believe the use of analytical constructs could provide a general method for providing kinetic information on reaction pathways [10].

The product yields from the complete set of reductive amination experiments are displayed pictorially below (fig. 2).

This graph clearly illustrates the range of yields obtained from this study. This is an informative data set not only for monomer selection, but it also provides a balanced data set for reactivity prediction (complete tabulated results are provided in the supporting information). Further analysis of the data reveals product distribution of each of the reaction components e.g. for secondary amines (fig. 3).

The aim of these experiments was to demonstrate the utility of quantitative analytical constructs and not to perform a detailed analysis of the reactivity amines chosen for reductive amination. However, the following general trends were identified: Primary amines produced higher yielding reactions than secondary amines, which in turn were better than anilines. Sterically hindered primary amines exhibited higher yielding reactions when tetrabutylammonium borohydride was used as the reducing agent. Primary amines directly attached to alkyl rings were

*. The computational parameters used in the selection of this amine set and the subsequent reactivity modelling are beyond the scope of this article and will be the subject of a separate publication.

♦ Free acid commercially available from Peakdale Fine Chemicals Ltd.

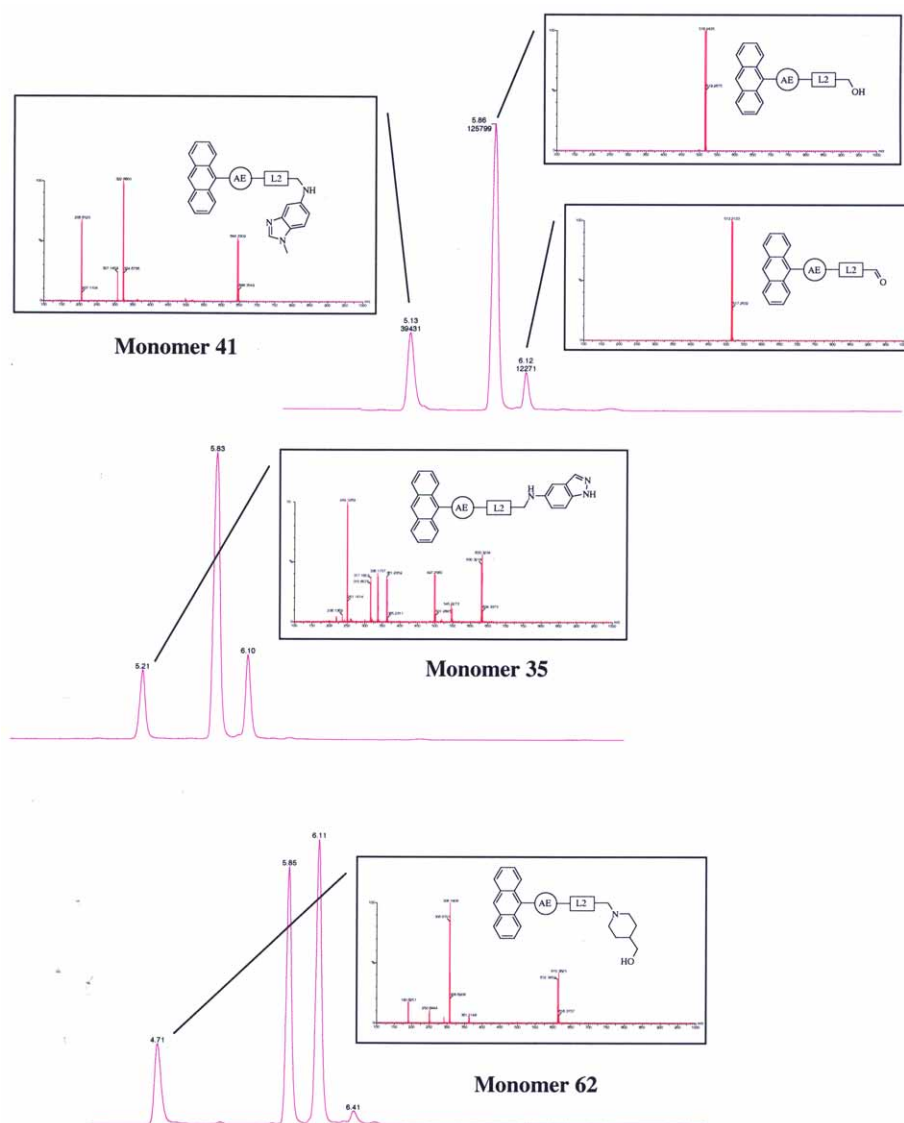


Fig. 1. Representative LC-UV-MS spectra for reaction analysis at 387 nm.

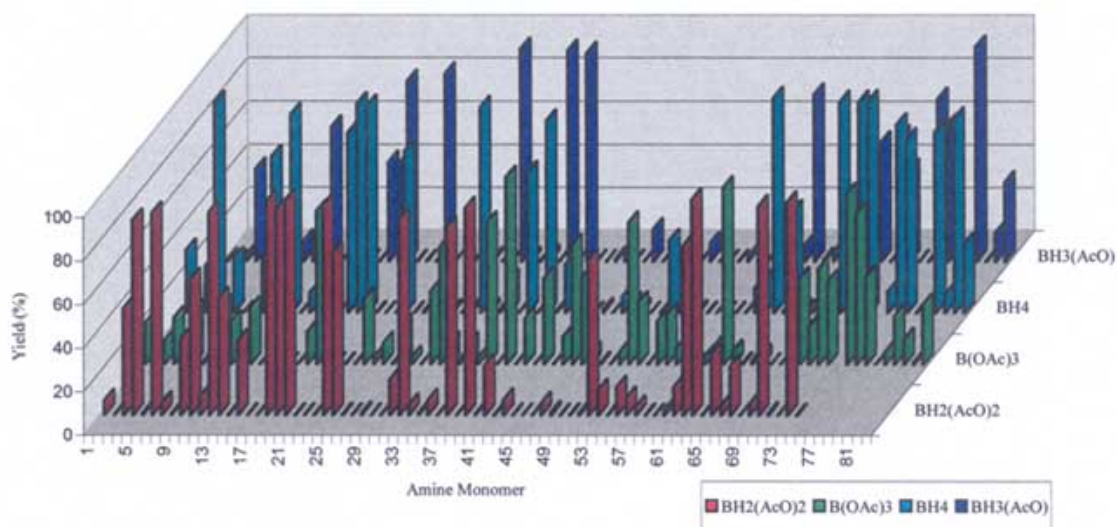
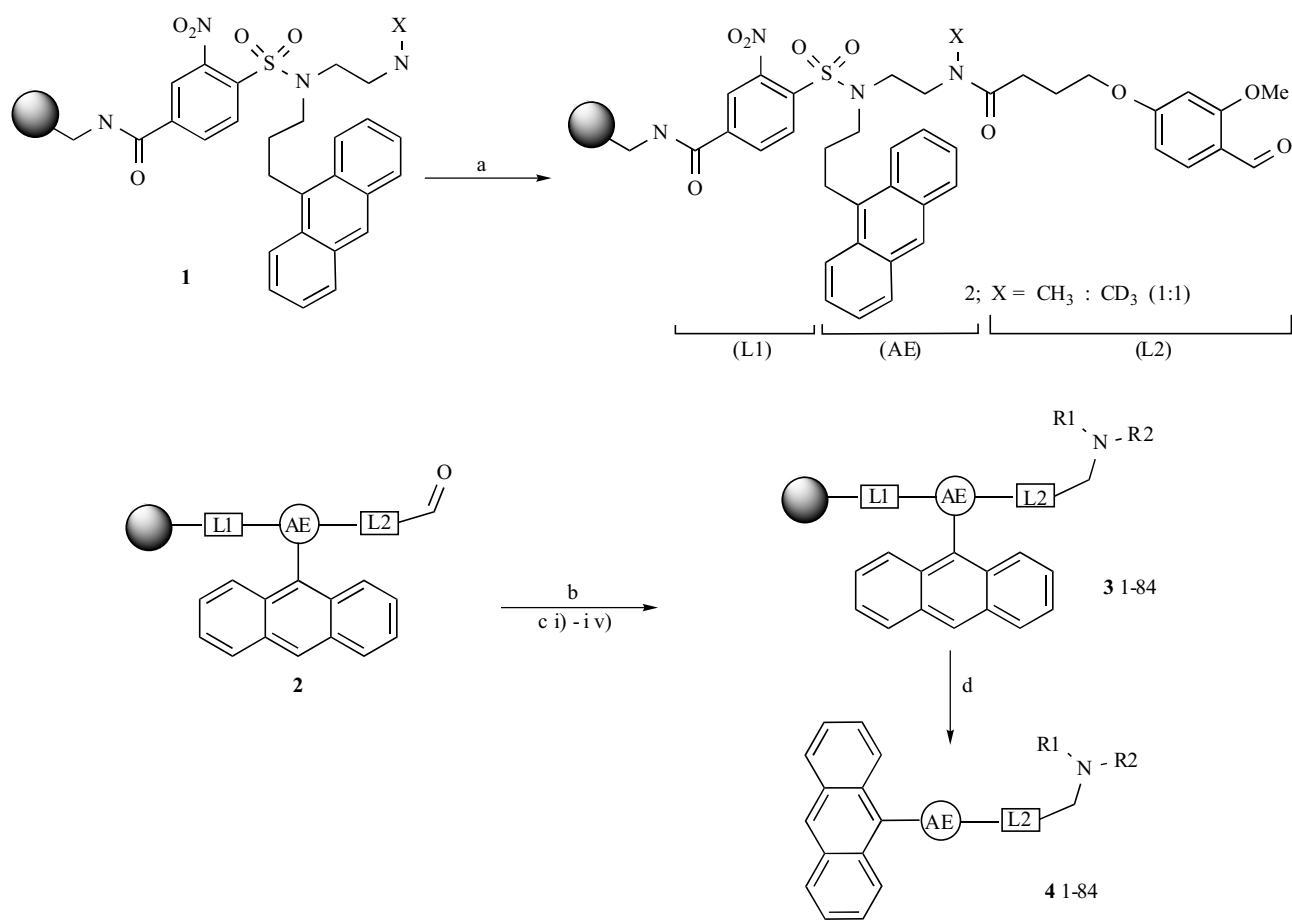


Fig. 2. Reductive amination of aldehyde resin (2) with an amine set in the presence of borohydride reducing agents.



Scheme 4. a) Monomethoxy benzaldehyde linker (5 eq.), PyBOP (5 eq.), DIPEA (10 eq.), DMF, 3 h. b) RNHR'₁₋₈₄, AcOH (20 eq.), NMP. c) i) Bu₄NH₄BH₄ (50 eq.), 24 h. ii) Bu₄NH₄BH₄ (20 eq.). iii) Bu₄NH₄BH₄ (20 eq.), AcOH (20 eq.). iv) NaBH(OAc)₃ (20 eq.). d) To each reaction was added 30 μ L of a solution of mercaptoethanol (40 μ l), in NaOMe (0.5 M in MeOH, 1 mL).

observed to give the highest yields in the presence of two equivalents of acetic acid. Cyclic secondary amines gave better results than acyclic. For aromatic amines the yields were particularly poor, making identification of reactivity trends difficult; the low yield in some cases could be assigned to poor solubility of the aniline in the reaction

solvent. Reactivity trends can be inferred, to an extent, from the perceived stability of the intermediate imine / iminium species. However, even this simple chemical transformation involves a complex reaction pathway and such discussion is best reserved for subsequent publications dedicated to this topic [11].

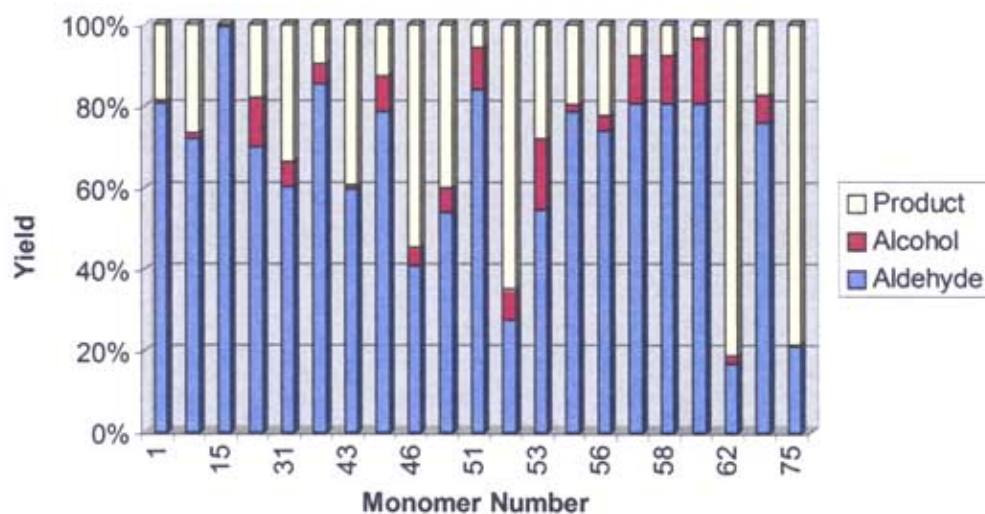


Fig 3. Product distribution for secondary amines used in Bu₄NBH₄ reductive amination.

In conclusion, the use of quantitative analytical constructs for facile, high throughput monomer rehearsal has been demonstrated. The ease of interpretation of the spectral data produced using this technique has far reaching implications not only for the development and optimization of chemistries, but also in efficient monomer rehearsals. The quantitative reactivity data is likely to be very valuable in generating computational reactivity models that could be used to extrapolate experimental results to new monomer candidates. Such predictions, in addition to considering the properties of the biological target, should assist in selecting appropriate monomers for library generation.

EXPERIMENTAL SECTION

Purity of materials on the solid support was assessed by cleavage at L1 and analysis using Liquid Chromatography - Mass Spectra (LCMS) recorded on a Micromass LCT orthogonal accelerating Time-of-Flight (oa-TOF MS) instrument using Positive Ion Electrospray (ESI,+ve). *Column:* Waters Xterra, MSC₁₈, 2.1 x 150mm, 3.5 μ m. *Eluent A:* 0.1% (v/v) Formic acid (aq) *B:* 100% (v/v) Acetonitrile + 0.1% (v/v) Formic acid (aq). *Flow rate:* 0.400 ml min⁻¹. *Detection:* UV Diode Array (DAD) 190-600nm *Method:* 100% A for 2 min. then gradient 0-100% B in A, over 16.0 min, held at 100% B for 2.0 min. then gradient to 100% A over 8 min and held for a further 2 min. Run time 30 min.

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