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A Simple Multiple Release System for Combinatorial Library and Peptide Analysis.

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Abstract: A method which allows the portion wise release of compounds from an insoluble support is described. The synthesis of the multiple release system is exceptionally simple and easy to modify to allow the quantities of compound released to be controlled and will have wide applicability in the screening and analysis of combinatorial libraries.

Combinatorial chemistry is causing a revolution in both drug discovery and organic chemistry[1]. Methods that generate a diverse library of oligomers, either on a solid support or in solution and that allow the rapid identification of a binding ligand to an enzyme or receptor are rapidly being developed. Library generation taking place by a (-division-synthesis-mixing-) strategy is such that each distinct "bead" of the support will contain only a single compound.[2]

Screening techniques for active compounds are of crucial importance for the success of the combinatorial concept. Two main methods predominate[1]. The direct screening of resin-bound libraries for members which inhibit enzymes or receptors is fast and well established. This approach does suffer from several drawbacks: (i) Enzyme/receptor accessibility to the immobilised compounds may be limited by the bulk of the solid support, although the development of new resins (eg TentaGel)[3] has done much to overcome this problem. (ii) Compounds will be identified which bind, yet do not inhibit, the enzyme or receptor of interest. This necessitates a second screening procedure to identify inhibitory binding. (iii) One end of the library is inaccessible.[4]
An alternative approach is the release of the library into solution ready for direct biological testing. However, this complicates the analysis since mixtures of compounds are tested and identification is no longer a matter of analysing the peptide or other oligomer physically attached to an identified resin bead. Several methods have been devised to overcome this problem. Iterative deconvolution of mixed compounds by batch testing is one approach.[5] This process requires the assay to be precise and limits the library size, especially in cases where several family members might have similar biological activities. Perhaps the most elegant approach is the process of multiple release [6] such that portions of the compound attached to the solid support are released under chemically defined conditions allowing activity to be pinpointed to a single bead by a release/divide/release strategy. Presently, however, this method uses a complex linker scaffold and the compounds released have modified C-termini.

We now report a method of multiple release which is easily accessible, simple to use, which can be tailored to alter the quantities of compound released during the screening process and is expandable to include a wide variety of different available linkers (see scheme 1). The aminomethyl resin[7] **1** was treated with a mixture of

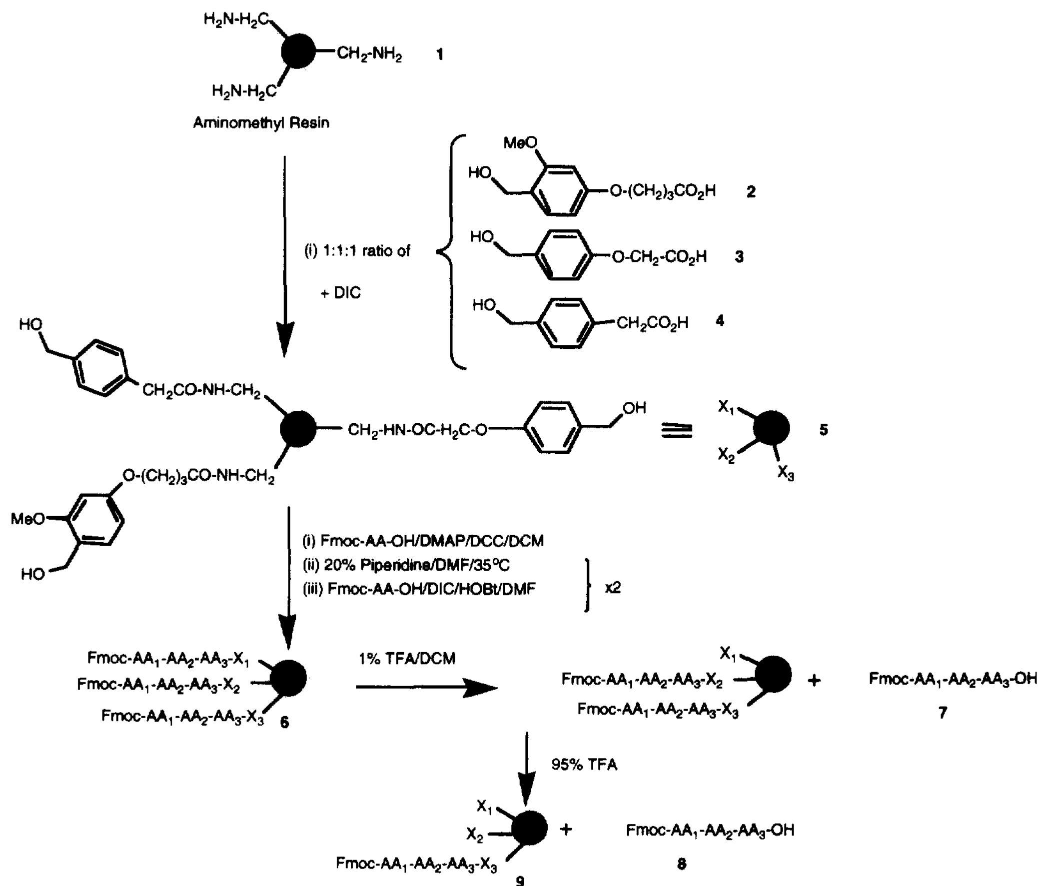
three commercially available linkers, 4-hydroxymethylphenoxyacetic acid **3**, 4-hydroxymethyl-3-methoxy-phenoxybutanoic acid **2** and 4-hydroxymethylphenylacetic acid **4**, di-isopropylcarbodiimide and hydroxybenzotriazole in DMF[8] to generated a multifunctionalised resin **5**. The hydroxymethyl groups of the three linkers were then functionalised and Fmoc peptide chemistry carried out in the normal manner to generate a small model tri-peptide Fmoc-Gly-Lys(boc)-Ala-linker-resin **6**.[9] MALDI TOF MS analysis carried out on the resin as reported,[10] gave the expected molecular ion MH^+ 497.8. Portions of the peptide were cleaved and analysed by the following sequence of conditions:[11]

(i) Linker **2**. Peptide **7** cleaved by 1% TFA

(ii) Linker **3**. Peptide **8** cleaved by 95% TFA[12]

(iii) Linker **4**. Peptide attached to resin **9** for amino acid analysis/direct resin screening or removal of the Fmoc group for sequence analysis and compound identification (e.g. Edman or MS analysis).[13]

The two portions of peptide **7** & **8** released by the cleavage conditions (i) and (ii), both gave the expected analytical data and were released in a ratio of approximately 1:1 [14] Edman sequencing following Fmoc removal from **9** gave the expected peptide sequence.



Scheme 1

In conclusion we have demonstrated an exceptionally simple method for the multiple release of compounds from a solid support which also leaves a portion of the library on the bead for screening/analysis purposes if necessary. In the present case we utilised two acid labile linkers and a third non-cleavable (coding) strand although a forth dimension of orthogonality could be provided by the use of a trityl based linker. This technique could be easily extended to include the release of primary amides using the range of linkers available,[15] while a whole range of other linkers would be similarly incorporated for example photolabile linkers such as the 4-(2-hydroxymethyl)-3-nitro-benzoic acid[16] or enzyme cleavable variants. The system is exceptionally simple to produce and by altering the ratios of the linkers or by stepwise couplings the ratios of the various linkers could be altered for specific screening purposes and compound identification.[13] This method should be readily expandible to compound libraries.

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11. Cleavage conditions. Peptide (7) was cleaved from the resin (6) by treatment with 1% TFA in DCM for 10mins at room temperature. The Lys(Boc) side chain protecting group was removed by 20% TFA/DCM before precipitation of the peptide with diethylether and RP-HPLC performed using a C-18 ODS analytical column, eluting with a linear gradient of H_2O/0.1% TFA —> MeCN/0.1% TFA. Peptide (8) was cleaved by treatment with 95% TFA in DCM for 10mins. The TFA peptide solution was then treated as above. A

single identical peak was observed in all cases as judged by HPLC and FAB MS analysis (497 MH+). ^{1}H nmr data were consistent with the expected peptide. Fmoc removal and Edman sequencing of (9) gave the expected amino acid sequence.

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