1. Current literature highlights

1.1. Cysteine protease inhibitors

Cysteine proteases are important therapeutic targets because of their role in a number of diseases, such as tumour growth (cathepsin B) and osteoporosis (cathepsin K). Cysteine proteases catalyse the hydrolysis of amide bonds in peptides and proteins through nucleophilic attack by an active-site cysteine thiol on the amide carbonyl. Most inhibitors of cysteine proteases exploit this mechanism and contain electrophilic functionality such as carbonyl groups, or Michael acceptors that react with the active-site cysteine residue. In an effort to discover cathepsin B inhibitors, a cathepsin implicated in cancer, a library of mercaptomethyl ketones was prepared. 1

A library of 2016 compounds was synthesised on solid phase. Screening was undertaken against cathepsin B at a concentration of 1 μM, and of the compounds screened, 110 library members inhibited >50% of the enzyme activity upon a five minute incubation with cathepsin B (as determined by rates of cleavage of the fluorescent substrate Cbz-Phe-Arg-AMC). When rescreened at 333 nM, 18 were found to cause >50% inhibition of the enzyme. One of the most potent compounds was (i) which exhibited a $K_i$ of 2 nM. In addition to delivering potent inhibitors against cathepsin B, the 2016 membered library should be a rich source of inhibitors against other cysteine proteases.

1.2. Homocysteine S-methyltransferase

The challenge of functional genomics and proteomics is to translate sequencing data into a precise understanding of protein function in cells, tissues, and whole organisms. Small ligands that specifically interact with proteins can be very effective tools in defining proteome function. Given the high number of proteins in mammalian organisms, high-throughput screening procedures have been developed to assist in this complicated task. The potential drawback of these methods is that the full range of proteins that interact with specific ligands may not be discovered if the screening is performed with only a limited set of proteins. Consequently, approaches that study the effects of ligands in whole cells are becoming increasingly important. In order to discover novel protein-ligand interactions, a recent publication discloses a method based on affinity capture principles coupled to combinatorial chemistry. 2

Phosphinic pseudopeptides have been shown to function as transition state analogues of zinc metalloproteases and these compounds are highly potent inhibitors of this enzyme class. Given the ability of the phosphoryl group to interact with zinc atoms, phosphinic peptides may also interact with other zinc metalloenzymes. This proposal was tested by preparing affinity columns harbouring 361 different phosphinic peptides and used them

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to isolate all interacting proteins from crude rat liver homogenates. By applying a deconvolution process, the authors were able to identify the ligand within the phosphinate peptide library that had the highest affinity and specificity towards one newly discovered protein target, betaine:homocysteine S-methyltransferase (BHMT). The deconvolution process suggested the synthesis of 19 different phosphinic pseudopeptides of general formula Ac-Val-DLDL-Ala-\[PO_2/C\_0\_2-CH_2]-DLDL-Leu-X_0aa-NH_2. The ability of these pseudopeptides to inhibit human recombinant BHMT at 100 \( \mu M \) concentration gave (ii) as one of the most potent compounds, with 80% inhibition of human recombinant BHMT. These phosphinic pseudopeptide inhibitors of BHMT may be promising tools for studying the physiological function of BHMT.

Val-Phe-\[PO_2-CH_3\]-Leu-His-NH_2

(ii)

2. A summary of the papers in this month’s issue

2.1. Solid-phase synthesis

A new strategy employing an Ugi four-component reaction and a microwave-assisted intramolecular Heck cyclisation in a sequential fashion to access an array of N-containing heterocycles has been reported. The route was also amenable to solid-phase chemistry demonstrating potential for library generation. Indane-derived bis(oxazolines) have been synthesised on solid support in two steps and 93% overall yield starting from commercially available substrates. This ligand is as effective as tert-butyl bis(oxazoline) in hetero Diels–Alder reaction both in solution and on polymer support. Peptide coupling reagents have been demonstrated to be versatile reagents for the formation of aliphatic isothiocyanates and thioureas on solid phase from the corresponding solid-phase anchored aliphatic primary amines. The preparation of an array of benzimidazoles and benzothiazoles from polymer-bound esters has been described. Polymer-bound esters were treated with 2-aminothiophenols or 1,2-phenylenediamines in the presence of a Lewis acid to afford the corresponding benzothiazole or benzimidazole cleavage products.

2.2. Solution-phase synthesis

A novel acid fluoride for use in the liquid-phase synthesis of substituted benzimidazoles, benzoxazoles and benzothiazoles has been developed. Its synthetic utility was exemplified by preparation of a structurally diverse set of aromatic heterocycles. An efficient, microwave-assisted method for the liquid-phase combinatorial synthesis of 1,3-disubstituted hydantoins has been developed. The desired products were then liberated from the soluble matrix in modest yield and high purity. A general procedure for the synthesis of new substituted thiaisatoic anhydrides or 6- or 7-aryl-1H-thieno[3,2-d][1,3]oxazine-2,4-diones under microwave heating conditions in high yields has been reported and applied to a library synthesis. A range of techniques have been demonstrated for the solution-phase synthesis of esters within an EOF-based borosilicate glass micro reactor, manipulating reagent flow using electroosmotic flow.

2.3. Solid-phase supported reagents

Novel polymer supported IBX esters and amides have been prepared in two steps, and tested as oxidants by the conversion of a series of alcohols to the corresponding aldehydes or ketones. A polymer-supported diphenylphosphoryl azide has been prepared, and has been shown to be a useful reagent due to its lower toxicity, moisture tolerance and ease of workup after reaction.

2.4. Novel resins and linkers

A novel improved controlled pore glass (CPG) support based on the 2-(hydroxymethyl)-6-nitrobenzoyl (HMNB) protecting group has been developed for the synthesis of 3’-aminoalkylated oligonucleotides. High surface area silica pellets have been shown to be excellent supports for the preparation of silica-supported reagents. Linker chemistry and ligand synthesis can be carried out on these materials and reactions can be monitored by solid state \( ^{13} \)C NMR studies on individual pellets. A novel technology utilizing \( \alpha \)-diazo functionalised solid phase resins to isolate phosphorylated peptides from non-phosphorylated substrates has been reported. Employing a cleavable carbohydrate-peptide linker, a new strategy for single-bead analysis of multivalent cyclic neoglycopeptides based on Edman degradation has been described. Carbohydrates are detached from the cyclopeptide templates before single-bead analysis, allowing for micro sequencing under routine conditions. Triazine-based antibiotics have been prepared by the attachment of cyanuric chloride onto a Marshall-type safety catch linker, followed by successive aromatic nucleophilic substitutions, linker activation and nucleo-
phlic cleavage. High-loading dendrimer beads allowed the release of sufficient amount of compound from a single bead to give clear inhibition.\textsuperscript{17}

Four \textit{N}-Fmoc protected polyoxyethylene-based amino acid type linkers have been designed and synthesised for peptide derivatisation on solid phase. Three of them were obtained in a crystalline form.\textsuperscript{18}

### 2.5. Library applications

Linear and cyclic olefin peptides containing the substrate sequence for human T-cell leukemia virus type-1 (HTLV-1) have been efficiently synthesized on a solid support using the Horner–Emmons reaction. The linear olefin peptide was cleaved by HTLV-1 protease at the scissile site, whereas the cyclic olefin peptide functions as a competitive inhibitor rather than a substrate.\textsuperscript{19}

SPOT synthesis—a well established method for the rapid preparation of peptide arrays—has recently been extended to the assembly of \textit{N}-alkylglycine (peptoid) library arrays. The potential of this method for the rapid identification of novel nonpeptidic protein ligands was demonstrated by synthesis and screening of a library consisting of 8000 peptoids and peptomers allowing the identification of micromolar ligands for the monoclonal antibody Tab-2.\textsuperscript{20}

A combinatorial series of \textit{N}-substituted glycine oligomers (peptoids) and peptide-peptoid hybrids have been synthesized on solid-phase and were pharmacologically characterized at the mouse melanocortin receptors (MC1R, MC3R–MC5R) for agonist activity.\textsuperscript{21}

### References


### Further Reading

Papers on combinatorial chemistry or solid-phase synthesis from other journals


