1. Current literature highlights

1.1. Positional scanning peptide libraries to determine kinase substrate specificity

Combinatorial libraries have been applied for a number of purposes generally related to the discovery of novel pharmacologically active molecules or investigating biological function. One particular valuable use is to map the substrate specificity of enzymes such as proteases and kinases. A recent publication describes a method for the generation of a positional scanning library of peptides that was subsequently used to determine kinase substrate specificity.\(^1\)

The positional scanning library concept was pioneered by Houghton, and has since provide researchers with libraries of up to a trillion peptides, that can be screened to generate optimal peptide motifs without the need for subsequent resynthesis and iterative deconvolution. The scanning libraries are comprised of a number of peptide mixtures where one amino acid is kept fixed in a specific position in each mixture while all other positions are degenerate. By screening the mixtures, preferred residues in each position can be readily ascertained. A critical requirement for such libraries is that every component is similarly represented in the mixture. As amino acids have variable reactivity as dictated by steric hindrance and electronic effects, the concept of an isokinetic mixture has been developed in which equimolar incorporation of all amino acids in the degenerate positions is achieved by varying the proportions of activated amino acids as dictated by their relative reactivity.

In this present study, the stable, non-hygroscopic and commercially available pentafluorophenyl (Pfp) esters were used as the reactive amino acid derivatives. The isokinetic mixture was determined by a screening method in which mixtures of Pfp-amino acids reacted with a resin-bound dimer (Ala-Tyr) and the product ratios were determined by hplc. It was found that Pfp-esters were sufficiently stable in DMF solution and a Design of Experiments (DOE) approach provides the information required to optimise the isokinetic mixture.

As the library was used to determine kinase substrate specificity, the N-terminus biotinylated 16-mers contained an invariant serine residue in the 8th position. Arginine was excluded due to poor stability, and a consistent Ala-Gly spacer was included between the degenerate positions and a Lys–Lys solubility tag to aid Edman degradation. The isokinetic ratios determined indicated that up to 13 equivalents of Ile were required for each equivalent of Asp. All other residues were intermediate, with Val (9.62-fold) and Asn (5.05-fold) requiring the next highest ratios.

The library was synthesised using these ratios, and used to determine the substrate-specificity of bovine protein kinase A (PKA), and the outcome was to confirm the known consensus sequence of R/K–R/K–X–S/T.

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

2.1. Solid-phase synthesis

Ionic liquids and onium salts have been used as soluble supports for peptide synthesis. These new supports combine easy monitoring, high loading capacities, large scale preparation, and homogeneous kinetics characteristics while keeping the advantages of solid-phase synthesis including easy purification and workup.\(^2\)

2.2. Solution-phase synthesis

A straightforward solution-phase synthesis of a small library of symmetrical 1,3-diamidophospholipids has been described using readily available, cheap reagents and introducing a simple phosphoramidate protecting group strategy. The approach requires no stringent exclusion of either air or water, and the reported structures might serve as an interesting and flexible platform for further studies in biophysics and related fields.\(^3\)

A multigram synthesis of pyridyl amine ligands has been reported; these ligands being studied for their potential use in commercial processes. A practical scale-up was hindered by benzotriazole byproducts of a Grignard addition, but with an improved purification to remove these byproducts and a strategic re-ordering of synthetic steps, an array of \(N,N\)-dimethyl-[(6-arylpyrid-2-yl)alkylamines was made efficiently on a large scale.\(^4\)

2.3. Scaffolds and synthons for combinatorial libraries

The synthesis of glycosylated Fmoc amino acids by reaction of mono- and disaccharide peracetates with Fmoc amino acids having free carboxyl groups was rapidly promoted by Lewis acids (SnCl\(_4\), BF\(_3\)·Et\(_2\)O) under microwave irradiation. The products are useful building blocks for the synthesis of glycopeptides.\(^5\)
2.4. Solid-phase supported reagents

A polymer-supported palladium(II) N,N-bis(naphthylidenimino)diethylentriamine complex has been found to be a highly active catalyst for Sonogashira coupling reactions. The reactions are performed under copper- and phosphine-free conditions in an air atmosphere. The palladium catalyst is easily separated, and can be reused several times without significant loss in catalytic activity.7

The benzodiazepines olanzapine and clozapine are nowadays manufactured by a three-step process with a final yield below 50%. An approach to an environmentally-friendly intensive process requires the development of multifunctional solid catalyst able to catalyse multistep reactions. A bifunctional metal–acid solid catalyst has been prepared and is able to carry out hydrogenation–cycloisolation–amination reactions in a cascade process.9

2.5. Novel resins, linkers and techniques

A facile solid-phase synthetic approach to the synthesis of 3,4-disubstituted-2-aminothiazolium derivatives has been reported. Functionalised aminomethylphenyl silica gel was used as a ‘volatileisable’ support, and the products were cleaved with 10% HF and obtained in high yields and purities.8

The enzyme-mediated enantioselective hydrolysis of water-soluble polymer-supported carboxylates has been disclosed. The representative monomethoxy poly(ethylene glycol) (MPEG, average MW 5000)-supported substrate was synthesised by immobilisation of (±)-1-phenylethanol onto the modified MPEG (MPEG/NH2) through an carboxylate linker with a succinate spacer. The structure of the spacer between the MPEG moiety and the carboxylate linker strongly affected both reactivity and enantioselectivity, and the substrate bearing a glutarate spacer gave the best result.9

2.6. Library applications

The discovery, synthesis, and preliminary structure–activity relationship (SAR) of a novel class of vasopressin V3 (V1b) receptor antagonists has been described. One compound identified by high throughput screening of a diverse, three million-member compound collection, prepared using ECLIPS™ technology, had good activity in a V3 binding assay (IC50 = 0.20 μM), but less than desirable physicochemical properties. Optimisation of the compound yielded potent analogues with improved drug-like characteristics.10

The alarming increase in infections caused by multiple drug resistant bacteria including methicillin-resistant Staphylococcus aureus has prompted a desperate search for new antimicrobials. The parallel solid-phase synthesis of analogues of the cationic anti-microbial peptide gramicidin S (GS) has been described using an amino acid side chain attachment strategy. The ornithine (Orn) residue was replaced by different aromatic D-amino acids. The antibacterial activity of these analogues against several clinically-important drug-resistant Gram-positive and Gram-negative pathogens has been reported.11

The parallel synthesis of two natural cyclopeptides, isolated from the seeds of Triola G, Gerauer M, Goermer K, Brunsveld L, Waldmann H. The structural features of both natural products and synthetic compounds. Analysis of a prototype library based upon nonacetic acid has led to the discovery of triazole-containing nonacetic acid analogues, a new structural class of antibiotic that exhibits bactericidal activity against drug resistant, Gram-positive pathogens including Staphylococcus aureus and Enterococcus faecalis.13

A chemical lead optimisation campaign directed at VU0238429, the first M2,-preferring positive allosteric modulator (PAM), discovered through analogue work analogous to VU0019498, a pan G M3, M1, M2, M3, PAM has been recently published. An iterative parallel synthesis approach was employed to incorporate basic heterocycles to improve physicochemical properties.14

EGF receptor-binding peptides have been found by a peptide screening method using fifteen fluorescent amino acids as fluorescent tags. Of 225 peptides prepared in a library, an 8-mer peptide containing a dipeptide unit, Y–F, which was the strongest binding peptide to the EGF receptor has been identified.15

Structure-activity relationship studies have been conducted on HIV integrase inhibitory peptides which were found by the screening of an overlapping peptide library derived from HIV-1 gene products. An alanine-scan was also performed on the lead compound for the identification of the amino acid residues responsible for the inhibitory activity. The results indicated the importance of an α-helical structure for the expression of inhibitory activity, and presented a binding model of integrase and the lead compound.16

References


Further reading

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


Dobele M, Vanderheiden S, Jung N, Brase S. Synthesis of aryl fluorides on a solid support and in solution by utilizing a fluorinated solvent. Angewandte Chemie (International ed. in English) 2010;49(43):5986–8.


Rostamizadeh S, Ghaieni HR, Aryan R, Amani A-M. One-pot synthesis of 3,5-disubstituted 1,2,4-oxadiazoles directly from nitrile and hydroxylamine hydrochloride under solvent-free conditions using potassium fluoride as catalyst and solid support. *Synthetic Communications* 2010;40(20):3084–92.