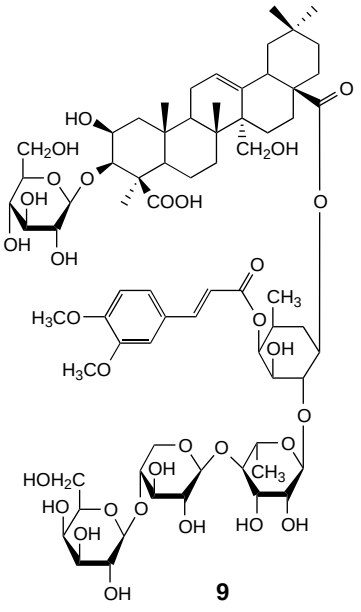

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Although these compounds were shown to inhibit serum glucose level increase in oral glucose-loaded rats, they had no effect on serum glucose levels in either normal rats, intraperitoneal glucose-loaded rats or alloxan-induced diabetic mice. However, the compounds were shown to inhibit gastric emptying in rats and to inhibit glucose uptake across rat small intestine *in vitro*. These compounds, therefore, appear to act by slowing the gastric transit from the stomach to the small intestine and by inhibiting glucose uptake by the intestinal epithelia.

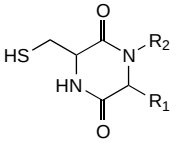
Combinatorial chemistry

Matrix metalloproteinase inhibitors

Many inhibitors of the matrix metalloproteinases (MMPs) have been previously reported, several types being produced in combinatorial libraries. A recent paper describes the design of MMP inhibitors based on diketopiperazines (DKPs) [Szardenings, A.K. *et al. J. Med. Chem.* (1998) 41, 2194–2200]. The synthesis of DKPs on solid-phase has been reported many times, but in the library described, the compounds contain a thiol group designed to chelate the catalytic metal ion.

Two libraries of 684 compounds were prepared in 36 pools of 19 compounds on TentaGel resin and tested against collagenase-1, gelatinase-B, stromelysin-1 and matrilysin. The second of these libraries (**1**) contained either L- or D-cysteine to provide the zinc-chelating group, and varied R_1 (from 19 amino acid precursors) and R_2 (from 18 aldehyde precursors) as potential side chains to S1' and S2' subsites, respectively.

Screening the compound mixtures, and deconvolution of the most active mixtures confirmed that the S1' pocket is the primary specificity determining site, whereas the group that occupied S2' could be varied while maintaining inhibitory activity. Deconvolution of the anisaldehyde-derived mixture revealed that cyclohexylalanine was the preferred residue in R_1 for activity against collagenase-1 and gelatinase-B (**2**).



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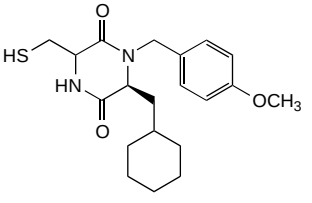

2

Substrate-based protein tyrosine kinase inhibitors

Protein tyrosine kinases (PTKs) are enzymes that phosphorylate specific tyrosine residues in a wide variety of functional proteins and have a role in regulating numerous cellular processes. One key role of PTKs is in the process of tumourogenesis, and this has suggested the potential for inhibitors as antiproliferative drugs.

One bead-one-peptide combinatorial libraries have been previously used to determine the heptapeptide substrate sequence, YIYGSFK-NH_2, for the p60c-src PTK [Lam, K.S. *et al. Int. J. Pept. Protein Res.* (1995) 45, 587–592]. A new paper describes the design and synthesis of inhibitors of this PTK, based on the substrate sequence [Alfaro-Lopez, J. *et al. J. Med. Chem.* (1998) 41, 2252–2260]. Over 70 analogues were prepared with a focus on conformational and topographical constraints, especially through the generation of a cyclic β-turn mimic using an intramolecular disulphide bond. The two most potent structures contained two new nonphosphorylatable tyrosine mimetics giving IC_{50} values of 0.13 and 0.54 μM. These compounds were 420- and 100-fold more active, respectively, than the starting peptide in this study, although they showed poor Lck/Src selectivity. Co-crystallization studies may generate more information about the binding modes of these compounds that may lead to the design of superior inhibitors.

Solid-phase carbohydrate synthesis

Carbohydrates are important mediators of cellular recognition and adhesion, and the initial events in viral and bacterial infections. Consequently, oligosaccharides provide important targets for drug discovery through combinatorial synthesis. However, until very recently there were very few effective ways of making these molecules in an efficient and stereocontrolled fashion. Last year, Nicolaou's group described a reiterative solid-phase approach that depended on the use of thioglycosides as the glycosyl donors [Nicolaou, K.C. *et al. J. Am. Chem. Soc.* (1997) 119, 449–550]. Unfortunately, this methodology was limited by the generation of mixtures of anomers at each cleavage stage and the need to reactivate cleavage fragments before they could be reincorporated into the growing oligosaccharide.

A recent paper from the same group describes modifications to the chemistry that overcome both of these problems [Nicolaou, K.C. *et al. Angew. Chem., Int. Ed. Engl.* (1998) 37, 1559–1561]. The oligosaccharides were

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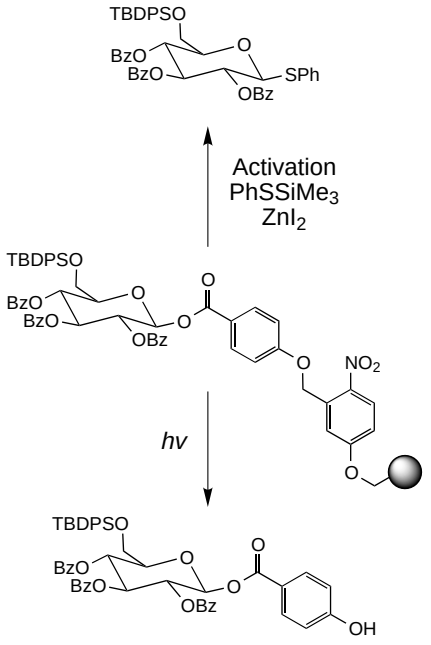

prepared on Merrifield resin attached to a photolabile linker. A spacer group between the anomeric centre and the linker allows a final mild cleavage of the products with retention of anomeric stereochemistry. However, intermediates can also be prepared on resin and cleaved with concomitant activation with $PhSSiMe_3$ to generate thioglycoside intermediates used for further elaboration of resin-bound oligosaccharides. This chemistry has been used in the synthesis of a branched dodecasaccharide related to the phytoalexin elicitor family.

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Simplified characterization of combinatorial libraries

While high-throughput screening and combinatorial chemistry are high-throughput solutions to previously rate-limiting steps, other lead optimization techniques remain labour intensive and of low throughput, placing a tremendous strain on discovery chemistry resources. Because of the sheer number of hits, the characterization of combinatorial libraries is difficult and sometimes tedious. Robust analytical methods that can provide rapid and simple determination of purity, identity and quantity of a synthesized compound are essential to ensure a rapid transition of lead compounds into development.

Until recently accurate quantification of the active component in a sample has not been possible, however, the advent of a novel nitrogen-specific HPLC detector enables the generation of exact potency measurements for any nitrogen-containing compound. Used in tandem with LC/MS, the new chemiluminescence nitrogen detector (CLND) can effectively and efficiently quantitate and characterize complex libraries and samples.

This profile focuses on one such solution – the Antek Model 8060 Nitrogen Specific HPLC Detector, an equimolar nitrogen HPLC detector, which provides true equimolar response for all nitrogen-containing samples allowing a single standard to quantitate multiple and complex samples without the need to re-calibrate. The instrument utilizes the accuracy and precision of its proven Pyro-chemiluminescence® technology to deliver equimolar response for all nitrogen-bearing compounds. Chemiluminescence is the clean, fast, interference-free method for determining all bound nitrogen in a variety of phases. The sample for analysis is injected directly from the HPLC system into the CLND where it is completely combusted in the presence of oxygen. Nitrogen from the sample forms nitric oxide, which is subsequently reacted with ozone to form nitric dioxide in its excited state. Once this species returns to ground state, the release of a photon is detected with a photomultiplier tube (Fig. 1). The measured intensity of chemiluminescence is directly proportional to the nitrogen present.

When characterizing crude products of a synthesis, methods based on traditional detectors, such as UV, can be misleading and time-consuming as the detector response is dependent on absorbance and the chromophores used and also requires purified references for every compound of interest. For detection by UV, the compound must contain a chromophore (Fig. 2). The Model 8060-CLND does not discriminate between compounds on this basis – it gives an equimolar response to nitrogen only, eliminating the need for derivatization and allowing quantitation of all nitrogen-containing compounds. It is the only HPLC detector capable of quantitating an unknown without having to use that unknown as a standard.

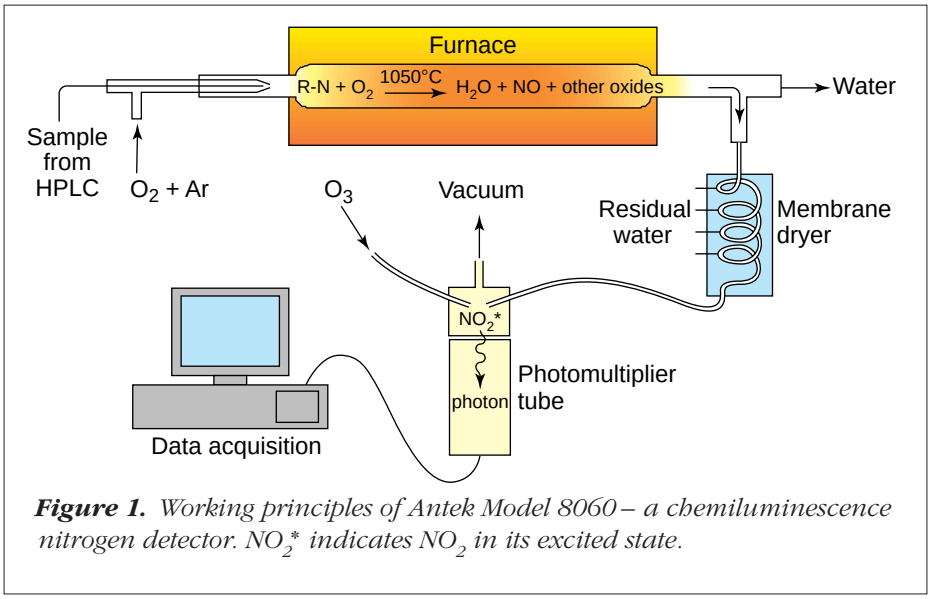

Figure 1. *Working principles of Antek Model 8060 – a chemiluminescence nitrogen detector. NO_2^* indicates NO_2 in its excited state.*

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