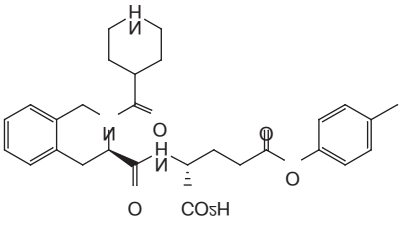
used the very high affinity between avidin and biotin ($K_a = 10^{15}$ M$^{-1}$) by initially tethering tryptophan to biotin through a hydrophilic linker.

The first step of the two-step screening process involved incubation of the biotinylated tryptophan with beads containing approximately 2.5 million different pentapeptide sequences constructed from a set of 19 amino acids. The second step was incubation of the beads with fluorescein-labelled streptavidin. Only those beads containing peptide sequences that had already bound tryptophan would have biotin exposed, which was therefore available to bind to streptavidin. By pouring the beads onto a plate and examining them under UV light, beads that contained the avidin–biotin complex were detected by their fluorescence and were selected for peptide sequencing. From the beads isolated, peptide sequencing revealed the consensus sequences Tyr/His-Gly-Gly-Tyr and His-Pro-Gly-His.

### A protease inhibitor library

The flagellated protozoan, *Trypanosoma cruzi,* is the causative agent of Chagas disease, a debilitating and incurable illness prevalent in Latin America. As the parasite requires access to a broad range of tissue types by penetration through the fibrous extracellular matrix, it has been postulated that specific proteases are expressed to aid this process. Indeed, a 80 kDa protease has been identified, and a recent publication describes the use of combinatorial chemistry in identifying new inhibitors of this protease [Vendeville, S. *et al.* (1999) *Chem. Pharm. Bull.* 47, 194–198].

Two orthoganol, self-deciphering peptide libraries, each of 15625 tripeptides in 125 mixtures, were synthesized in solid-phase from 23 D-amino acids and 2 non-chiral amino acids. Following HF(hydrogen fluoride)-catalyzed cleavage from the solid support, purification and then analysis, the highest level of protease inhibition observed originated from the cleavage by-product, H-Ipe-D-Tic-D-Glu(*S*-paratolyl)-OH (**2**).



**5**

This compound has an $IC_{50}$ value of 12 μM and introduces a new class of structures for further investigation of the role of this protease in the biology of *T. cruzi.* The compound and close analogues are being studied for their selectivity over other prolyl endopeptidases.

*Nick Terrett*
*Discovery Chemistry*
*Pfizer Central Research*
*Sandwich, UK*
*fax: +44 1304 655419*
*e-mail:*
*nick_terrett@sandwich.pfizer.com*

## Targeting protein–protein interactions: the HIV protease

The retroviral genome is translated as polyprotein fusions that require further processing by proteases. As protease function is necessary for the proper maturation of infectious virions, inhibition of the dimeric HIV-encoded protease immediately offered an attractive therapeutic target. This was aided by the viral enzyme's homology to aspartyl proteases such as renin, while its unique substrate specificity boded well for selective inhibitors. The subsequent development of drugs based on transition-state analogues [reviewed by Vacca, J.P. and Condra, J.H. (1997) *Drug Discovery Today* 2, 261–272] has been a great success for rational and structure-based drug discovery, and has revolutionized the treatment of HIV patients by combination therapy.

Another means of targeting the HIV protease is by disrupting its assembly into a dimer (which occurs with a nanomolar dissociation constant). Because the active site is composed of an aspartate residue from each monomer, dimerization is essential for enzyme activity. The dimerization interface is largely formed by interdigitation of N- and C-terminal residues in a four-stranded, antiparallel β-sheet. Attempts at blocking dimerization are much less developed than active-site occupancy by substrate or transition-state mimetics. One advantage of the dimerization strategy is the heavily conserved nature of the interface, involving cooperative interactions between the N- and C-terminal of each monomer. Thus, viral resistance may be harder to develop by single point mutations relative to active site inhibitors.

J. Chmielewski's group has extensively studied [reviewed in *Synlett* (1998) 1040–1044] tethered peptides based on the protease terminal sequences as mimics of the interface. These peptides are inhibitors of protease activity at micromolar concentrations, and act by affecting the monomer–dimer equilibrium as shown by size-exclusion chromatography, protein crosslinking, and fluorescence spectroscopy. As an alternative approach, H. Schramm and coworkers [*Biochem. Biophys. Res. Commun.* (1996) 227, 484–488; *AIDS* (1998) 12, 682–685] have screened crystallographic databases for potential compounds that bind to the dimerization interface. Several triterpenoids and steroids were identified with micromolar activity.

Extensive screening of natural product extracts was largely unsuccessful in generating non-peptide leads for protease inhibition. A recent paper by Daniel Rich's group [*J. Am. Chem. Soc.* (1998) 120, 8893–8894], however,

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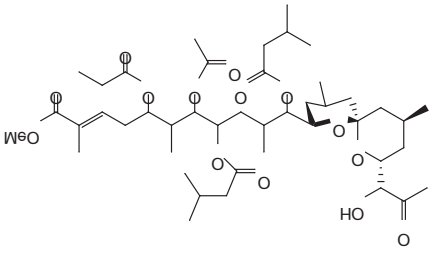
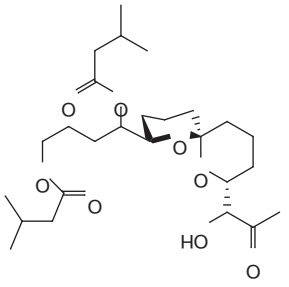
shows how such compounds can unexpectedly lead to the dimerization approach. Molecular surgery on didemnaketal A (**1**), an ascidian-derived HIV protease inhibitor ($IC_{50}$ 2 μM) led to the

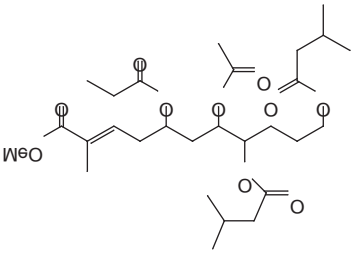


**1**

production of the simplified spiroketal, compound (**2**). Four diastereomeric pairs of (**2**) (the absolute configuration of didemnaketal is unknown) were made by multistep synthesis but proved to be inactive. This indicated that the natural product's acyclic fragment was more important, and hence, all eight di-



**5**



**3**

astereomers of (**3**) were then synthesized. Of these, seven were inhibitors at micromolar concentrations. The most potent diastereomer (5*S*, 7*R*, 8*S*), $K_i$ = 2.1 μM, was shown by kinetic analysis to be a reversible and noncompetitive inhibitor. This data, coupled with the fact that pentaester (**3**) does not contain the typical functional groups found in active site inhibitors, suggests that its mechanism of action is rather by affecting protease dimerization.

Targeting protein–protein interactions is a long-standing holy grail of drug discovery. The described examples show that active compounds can be discovered by this principle in the context of the HIV protease, although it is important that the mechanism is proven conclusively to avoid artifacts because of weak active-site inhibition. It remains to be seen if such leads can be optimized to the level of potency and stability desired for therapeutic use.

*A. Ganesan*
*Department of Chemistry*
*University of Southampton*
*Southampton, UK*
*e-mail: a.ganesan@usa.net*