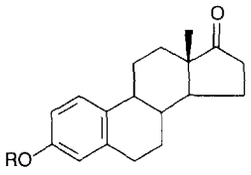
Steroid sulfatase inhibitors

The enzymes involved in oestrogen biosynthesis are attractive drug targets. Hormone-dependent breast cancer in postmenopausal women has been the subject of much research for the development of aromatase inhibitors, several of which are now under clinical investigation. Such inhibitors do not, however, totally reduce oestrogen levels *in vivo*, and one reason lies in the existence of a major separate pathway by which oestrogens can be biosynthesized, via the hydrolysis of the conjugate oestrone 3-sulfate **2** to oestrone **1** by oestrone sulfatase. There are very high levels of **2** in blood and in tumours, and the development of inhibitors of oestrone sulfatase is therefore an exciting new concept and alone or in combination with an aromatase inhibitor, they may form the basis of a new endocrine therapy [Reed, M.J. *et al. Endocrine-Related Cancer* (1996) 3, 1–15]. Inhibition of a related sulfatase, dehydroepiandrosterone sulfatase, which is involved in the formation of another steroid, androstenediol, with potent oestrogenic properties, may offer additional therapeutic benefit.



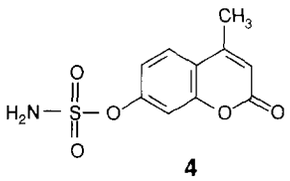
1 R=H

2 R=SO_3^-

3 R=SO_2NH_2

Cancer Research Campaign funded work at the University of Bath, UK, and at Imperial College of Science Technology and Medicine (London, UK) has resulted in the synthesis of the first highly potent oestrone sulfatase inhibitors, the best of which is oestrone 3-*O*-sulfamate **3** [Howarth N.M. *et al. J. Med. Chem.* (1994) 37, 219–221], which acts as a time-dependent irreversible active-site directed inhibitor [Purohit, A. *et al. Biochemistry* (1995) 34, 11508–11514] and which blocks oestrone sulfate hydrolysis *in vivo* by 99% and reduces tumour growth rate [Purohit, A. *et al. Int. J. Cancer* (1995) 63, 106–111].

However, the use of an oestrogen derivative as a therapy in women with oestrogen-dependent tumours may be questionable, because **3** has recently been shown to possess surprising super-oestrogenic properties [Elger, W. *et al. J. Steroid Biochem. Mol. Biol.* (1995) 55, 395–403]. There is also now considerable interest in **3** as a memory-enhancing agent by virtue of its inhibition of dehydroepiandrosterone sulfate hydrolysis [Li *et al. J. Endocrinol.* (1995) 144, Abstr P155] and as an immunomodulator [Rook, G.A.W. *et al. Immunol. Today* (1994) 15, 301–303]. It is therefore clearly desirable to develop nonsteroidal derivatives that act by the same mechanism. The first examples of such inhibitors, such as the coumarin sulfamate **4**, have just been published [Woo, L.W.L. *et al. J. Med. Chem.* (1996) 39, 1349–1351].



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Such inhibitors are potent *in vivo*, but are completely devoid of oestrogenic acitivity. The further development of such compounds should enable the therapeutic use of oestrone sulfatase inhibitors to be broadened, not only for endocrine-related cancers, but also for other conditions such as autoimmune disease.

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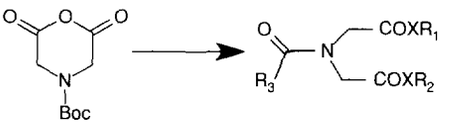
Combinatorial chemistry

Combinatorial solution chemistry

The design and synthesis of combinatorial libraries has generated an enormous revival of interest in solid-phase chemistry. However, solution chemistry for libraries has not been totally ignored. Boger, D.L. and coworkers [*J. Am. Chem. Soc.* (1996) 118, 2567–2573] describe how solution methods can give multi-milligram amounts of highly pure combinatorial products. The template **1** is a densely-functionalized core that has been used to generate a 27-member (3 × 3 × 3) library. At each stage of the synthesis, the released functionality was used to purify the intermediates and final products by simple liquid–liquid extraction. The final compounds were obtained in an average yield of 61% and with an average purity of 95.3%. Using these solution techniques, other libraries of up to 960 components (6 × 8 × 20) have been constructed. As the authors point out, the techniques used here could be applied equally successfully to larger libraries generated as mixtures.



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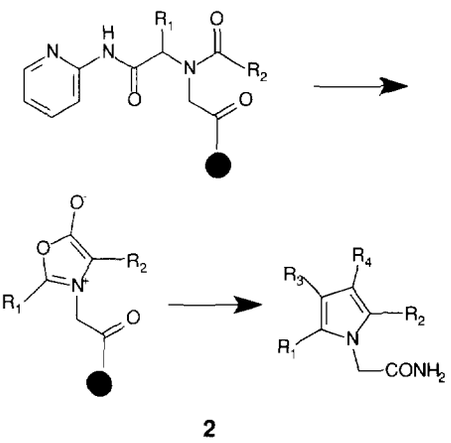
Extended Ugi reaction

Ontogen have published a useful extension of the Ugi reaction on solid-phase. This reaction is the 1,3-dipolar cycloaddition reaction of münchnones **2** with alkynes [Mjalli *et al. Tetrahedron Lett.* (1996) 37, 2943–2946] that could lead to a potential library of 10^8 different tetra- and pentasubstituted pyrroles. The münchnones are generated on solid phase by the cyclization of *N*-acyl-*N*-alkyl-alpha-amino acids or amides produced by the Ugi four-component reaction. The preferred approach to the Ugi product is to use either phenyl or pyridylisocyanide with the amine component immobilized on the solid phase. The reaction of the münchnones with other dipolarophiles will lead to alternative heterocyclic systems, and this is currently under investigation by the authors.



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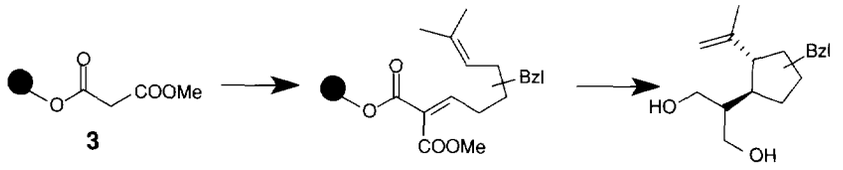

Stereoselective cycloalkane formation

Tietze, L.F. and Steinmetz, A. [*Angew. Chem. Int. Ed. Engl.* (1996) 35, 651–652] have demonstrated a two-component domino reaction leading to cyclopentane and cyclohexanes. A malonate functionalized polymer **3** was condensed with an aldehyde as a prelude to an ene rearrangement catalysed by zinc bromide. Following cleavage from the resin by diisobutylaluminium hydride reduction, this Knoevenagel–ene process generated cycloalkanes with high stereoselectivity.

Optimizing library design

In order to discover novel drug discovery leads, many companies are employing combinatorial chemistry to synthesize huge numbers of compounds for high-throughput biological assay. An alternative to this process is the iterative synthesis of fewer compounds, but using the results of each round of screening to dictate the compounds prepared in the subsequent rounds. The difficult question is how to use the results to design the next library. One way around this issue is to use computational genetic algorithms to design the structures. A recent paper from a group at Sterling-Winthrop [Singh, J. *et al. J. Am. Chem. Soc.* (1996) 118, 1669–1676] describes the strategies of selection, crossover and mutation used to optimize affinity and selectivity of peptides for stromelysin. The synthesis of 300 compounds through five generations was sufficient to identify peptides with improved activity.

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Emerging molecular targets

Human adenovirus protease

The three-dimensional structure of the human adenovirus protease has just been resolved to 2.6 Å by Dr. Walter F. Mangel and coworkers at Brookhaven National Laboratory (Upton, NY, USA). The human adenovirus causes acute upper respiratory, eye and intestinal tract infections. Such infections are common in children and are a leading cause of death in developing nations.

The new structure and previous studies suggest at least three different sites on the protease where drugs might act to inhibit proteolytic activity. One is the active site where the enzyme cleaves the viral precursor proteins to produce the protein species needed to form the assembled viron. The other two sites are binding regions for cofactors, which are essential for enzymatic activity, an unusual requirement for a viral protease. One cofactor is a short peptide whose binding site has been determined. The other cofactor is the viral DNA whose binding site remains to be determined [Ding, J. *et al. EMBO J.* (1996) 15, 1778–1783].

Mangel's group suggest that the presence of three sites of attack on the enzyme makes it highly likely that an inhibitory compound could be discovered. Moreover, simultaneous administration of compounds that bind at each site may overcome the problems of resistance that are common to antiviral agents. Thus, it is important to determine the site at which the viral DNA binds to the enzyme. The researchers are currently attempting to grow crystals in the presence of the viral DNA in order to answer this important question. In the meantime, the human adenovirus protease might prove to be an excellent candidate for high-throughput screening or rational drug design programs.

Proton-pumping ATPase and male contraception

An acidic environment in the vas deferens and epididymis is essential for the maturation of sperm and for maintaining their immobility and quiescent state prior to ejaculation. Previous studies implicated a carbonic anhydrase and a Na^+/H^+ exchanger in maintenance of this environment. However, Dr Sylvie Breton and coworkers at Harvard Medical School (Boston, MA, USA) and Woods Hole Marine Biology Laboratory (Woods Hole, MA, USA) report that high concentrations of a proton-pumping ATPase also line the luminal surfaces of the vas deferens and epididymis and suggest that the enzyme is important in maintaining this acidic environment. They also propose that the ATPase may be an effective target for a male contraceptive [*Nat. Med.* (1996) 2, 470–472]. A rise in pH resulting from inhibition of the ATPase could arrest the development of the sperm and trigger premature mobility, resulting in decreased fertility.

In the same issue, Dr Malcolm Potts (University of California, Berkeley, CA, USA) provides a discussion of the considerable political hurdles involved in the development of an oral male contraceptive [*Nat. Med.* (1996) 2, 398–399].

Egr-1 and vascular injury

The development of vascular occlusive lesions as a result of vascular injury is a complex process of vascular remodeling involving the coordinated effects of numerous growth factors that stimulate endothelial and smooth muscle cells. Such factors include platelet-derived growth factor A and B, human transforming growth factor-β1, tissue factor and urokinase-type plasminogen activator. Each of these growth factors may be induced in endothelial cells upon activation of specific transcription factors, but the key elements that regulate their coordinated expression upon vascular injury are not well understood.

Dr Levon M. Khachigian and coworkers from Brigham and Women's Hospital (Boston, MA, USA) report that the expression of these growth factors upon vascular injury may be coordinated by the transcription factor Erg-1 [*Science* (1996) 271, 1427–1431]. The authors found that expression of Erg-1 preceded the expression of each of the growth factors. Moreover, the gene for each growth factor has a binding region for Erg-1 in its promoter. If the authors are correct in their contention that the expression of Erg-1 is the trigger that leads to complex process of vascular remodeling, Erg-1 may emerge as an important target for the development of new compounds to combat vascular diseases.

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