Sweet signal transduction

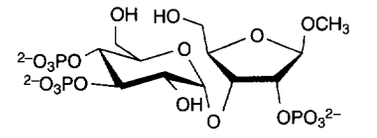
A quick and simple way to make a mimic of a second messenger molecule thought to be involved in manic depression has been devised by UK chemists. They say the compound is almost as potent as the natural compound and may help in the search for new drugs that intervene in brain-cell signalling.

D-*myo*-Inositol (1,4,5)-trisphosphate [Ins(1,4,5)P_3] relays information from chemical transmitters from other cells by triggering calcium release in a target cell. This second signal then affects other cellular events, such as the histamine triple response. Once the task is complete, the enzyme inositol monophosphatase (IMPase) recycles the inositol core. When the recycling process gets out of control, however, too much or too little Ins(1,4,5)P_3 is available, and researchers believe that this can lead to the wild mood swings associated with manic depression, and other disorders.

Lithium salts used to control manic depression have potentially severe side effects, such as kidney damage and thyroid abnormalities. According to Professor Barry Potter (School of Pharmacy & Pharmacology, University of Bath, UK) recent indications that the activity of lithium lies with IMPase open up the possibility of designing lithium surrogates. "The action of lithium is, unusually, uncompetitive," he explains, "design of inhibitory molecules is therefore a very difficult task".

Potter and his coworkers hope to find new ways of controlling the inositol polyphosphate 'cascade' through the design of novel synthetic agents. "An ever-increasing number of disorders are now being linked to malfunctions of cellular signalling pathways," explains Potter "our team has already synthesized a number of potent antagonists and enzyme inhibitors".

With support from the Wellcome Trust, Potter and his team have used the simple sugars glucose and ribose to build an Ins(1,4,5)P_3 mimic based on two microbial compounds known as adenophostins. The adenophostins, according to Potter, are 10–100 times more potent in triggering the inositol trisphosphate receptor than Ins(1,4,5)P_3, and are under study as potential leads. In their design, Potter and his team stripped away various chemical motifs from the adenophostins to come up with a range of potential mimics. One of their compounds, ribophostin, was a basic structure that retained inositol trisphosphate-like activity, [*J. Chem. Soc., Chem. Commun.* (1997) 449]. With an understanding of the chemical features needed to trigger the receptor Potter and his team can now begin looking for new molecules that block the receptor and inhibit the enzymes that work on inositol trisphosphate.



3-*O*-(α-D-glucopyranosyl)-β-D-ribofuranoside-2,3′,4′-trisphosphate

A D-myo-*inositol (1,4,5)-triphosphate mimic.*

Potter says that signal transduction therapy holds much promise, but cautions that not enough is understood about the major pathways of cellular signalling, so predicting *in vivo* responses is not yet possible. He sees increased understanding as an important goal of current research: "Progress in the design of phosphate-based prodrugs is very encouraging for the future, and the ability to start with cheap and readily available chiral sugars, such as here, is providing a new impetus for what is still a relatively young field," he says.

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New combinatorial chemistry

In February, the Society of Chemical Industry (SCI) brought experts from industry and academia together in London for a one-day symposium entitled *New Combinatorial Chemistry*. Novel solid-phase organic chemistry, new linker molecules and strategies for library design were some of the key topics.

The symposium was opened by Dr D. Hollinshead (Zeneca, Alderley Edge, UK), who outlined some general strategies used in combinatorial chemistry before introducing the first speaker of the day, Dr R. Shute (Zeneca), who spoke on compound libraries based on a homocysteine template. Zeneca's solid-phase synthesis group wanted a template that has a low molecular weight (total molecule < 800 Da), can accommodate three different substituents and is non-oligomeric, flexible and highly functionalized (for easy attachment of substituents) for library generation. Homocysteine was found to fit all these criteria and in addition is easily accessible through the cheap, readily available H-Cys-thiolactone (**1**). A method was developed to link this thiolactone to a solid support via the α-carbon, so that the template could be fully derivatized while remaining attached to the solid-support (Figure 1, Scheme 1). Immobilized thiolactone **2** was converted to the desired homocysteines **3** by acylation of the lactone's amine with excess anhydride, followed by ring opening with a primary amine, catalysed by $Hg(CF_3CO_2)_2$, and subsequent alkylation of the sulphur. This sequence of reactions was found to work well in a multi-parallel synthesis set-up, and is suitable for synthesizing pools of compounds.

'Privileged structure' libraries

The 'privileged structure' library concept was discussed by Dr Tony Baxter of Oxford Diversity (Abingdon, UK), the combinatorial chemistry division of Oxford Asymmetry. Privileged structures

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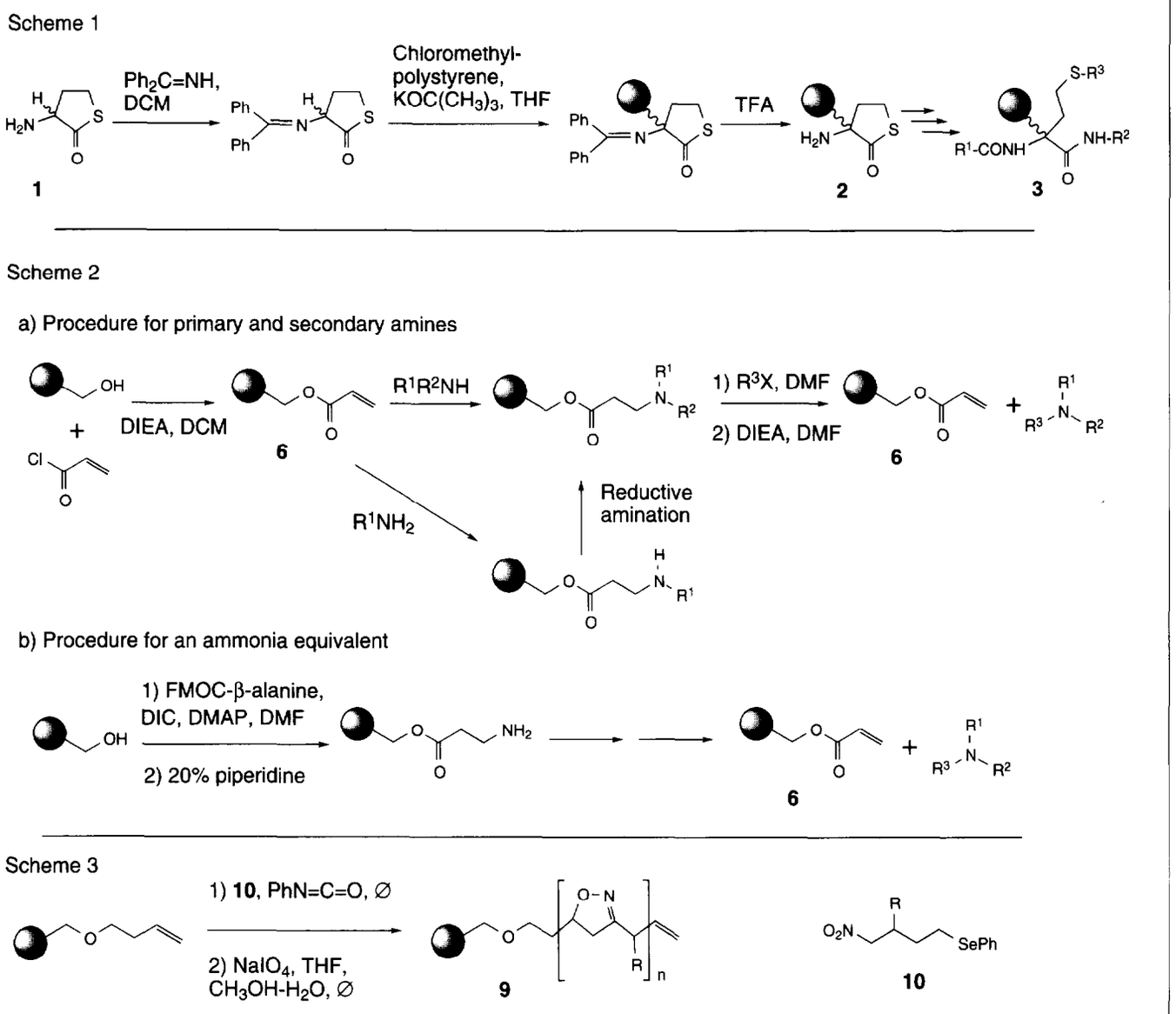

Figure 1. *Scheme 1: Solid-phase synthesis of a homocysteine library starting from H-Cys-thiolactone, which is linked to the resin via the α-carbon. Scheme 2: Synthesis of tertiary amines using the REM-resin. Scheme 3: Example of a solid-phase 1,3-dipolar addition reaction. DCM, dichloromethane; DIC, 2-dimethylaminoisopropyl chloride hydrochloride; DIEA, diethylisopropylamine; DMAP, 4-dimethylaminopyridine; DMF, dimethylformamide; THF, tetrahydrofuran; TFA, trifluoroacetic acid.*

are conformationally restricted templates that are resistant to hydrophobic collapse and have at least three sites of diversity. An example of a privileged structure used by Oxford Diversity to prepare a library is benzoxazepinone **4** (Figure 2). Using solution-phase chemistry early in the synthesis and automated solid-phase chemistry for subsequent steps (1,260 reactions in total, of which 960 were automated), 480 compounds based on this template were prepared. Analysis showed that 75% of the products were over 50% pure. This template is now used to prepare several thousand compounds for lead discovery.

Dr Baxter also discussed Oxford Diversity's polymer resins and linker molecules. He mentioned that the company prepares its own resins, to ensure that they have the right specifications and are free from contamination. Resin synthesis is complex and time-consuming, so resin is recycled whenever possible. The company has developed a 'clean-break linker' based on silicon (**5**; Figure 2), which delivers highly pure products and is particularly suited to use in automated systems.

Single-bead encoded libraries

A bead or template used for library synthesis can be tagged with a

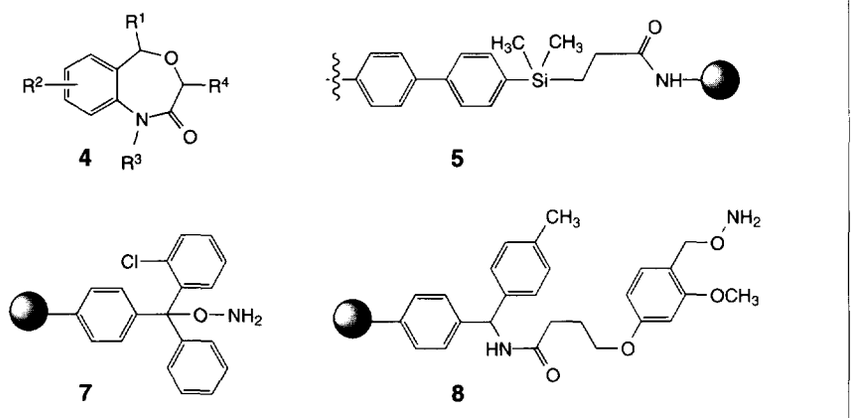

Figure 2. *Oxford Diversity's benzoxazepinone* ***4*** *and 'clean-break' linker* ***5****, and British Biotechnology's novel hydroxamic acid linkers* ***7*** *and* ***8****.*

distinctive small molecule, such as a haloaromatic or a secondary amine, every time it undergoes a reaction. In this way the chemical reaction history of a bead, and therefore the molecular structure of the product it carries, can be recorded. The advantages of combinatorial libraries encoded in this way were reviewed by Dr Mark Gallop (Affymax, Palo Alto, CA, USA). He argued that mass spectroscopy is not always effective in identifying reaction products on beads, because compounds are not always efficiently ionized and the presence of nearly equal-weight compounds in the library can make identification very difficult. The use of tags that can be easily identified by HPLC can overcome these difficulties, and so ensure that only the active compounds in a pool are resynthesized.

Dr Gallop then proceeded to describe some tools for handling single beads that have been developed at Affymax. Robots in the company have been equipped with capillary tubes that can pick up the beads by suction and transfer them to 96-well plates. Each well of these plates is fitted with a plastic cup that has a 80 μm hole in the bottom – wide enough to let through solvents, but too narrow for beads, which have an average diameter of 150 μm. This set-up was designed to facilitate the removal and analysis of tags; after cleavage the tags can be collected at the solvent in the bottom of the well, and a clean bead is left in the cup. The cups can be broken off, which ensures easy handling of the single beads. Scientists at Affymax are also working on an 864-well format for this system.

Novel linker

Dr David Rees (Organon, Newhouse, UK) discussed a novel type of linker that has been developed at Organon, the REM resin (**6**). This resin has been functionalized with acrylate linkers, which can be **re**generated and are attached via a **M**ichael addition, hence 'REM'. The resin has been used to synthesize a range of tertiary amines from secondary amines, using the Michael addition mentioned above, followed by an alkylation and Hofmann elimination (Figure 1, Scheme 2a). The resin can also be employed to prepare tertiary amines from primary amines or an ammonia equivalent by adding a reductive amination step (Figure 1; Scheme 2a and 2b). The purity of the tertiary amines was generally around 98%, and yields ranged from 50 to 80%. Using D- or L-proline as the starting material, tertiary amines with an enantiomeric excess of up to 96% could be obtained. The yield and purity of the products stayed the same through four cycles of the resin. The main drawback of the method is that R^3 has to be very reactive for the alkylation step to proceed, limiting the range of amines that can be prepared this way.

2-Aminothiazoles

The possibilities of solution-phase parallel synthesis were highlighted by Dr D. Judd (Glaxo Wellcome, Stevenage, UK). He discussed the synthesis of four solution-phase libraries based on 2-aminothiazoles [see also *Drug Discovery Today* (1997) 1, 458–460]. The 2-aminothiazole template was chosen because it is a very common pharmacophore; since 1985, 95 papers on this compound have appeared in the *Journal of Medicinal Chemistry*, and about 1% of the structures in the *World Drug Index* are 2-aminothiazoles. All syntheses were carried out in 4 × 5 arrays of 1 dram (3.7 ml) glass vials open to the atmosphere. Reagents were dispensed as 0.25 ml, 0.1 M aliquots, so that 25 mmol of single compound would be obtained under optimal conditions.

Hydroxamic acid MMP inhibitors

Dr Mark Whittaker (British Biotechnology, Oxford, UK) presented a nice example of how combinatorial chemistry can be applied to lead optimization. He described the synthesis of several libraries of potential matrix metalloproteinase (MMP) inhibitors, based on British Biotechnology's clinical candidates batimastat (BB94) and marimastat (BB2516). X-ray studies of a MMP–batimastat complex have shown that the hydroxamate moiety in batimastat is very important for activity as it chelates the MMP's zinc atom [see also *Drug Discovery Today* (1996) 1, 16–26]. In solution, this hydroxamate is synthesized in two steps from a carboxylic acid. Whittaker and coworkers developed a method of functionalizing resin with a hydroxamate group, which is transferred to the product upon cleavage [*Tetrahedron Lett.* (1996) 37, 8045–8048 and (1997) 38, 321–322]. This enabled the group to carry out automated solid-phase synthesis of a library of 500 hydroxamic acid tripeptides (as 10 mixtures of 50 compounds) and a library of sulphonamides with a hydroxamic acid moiety (as 25 single compounds). No compounds with greater MMP inhibitor activity than the clinical candidates were identified in either library.

Dr Whittaker also described two O-hydroxylamine resins, **7** and **8** (Figure 2), that had been developed to improve the yield of the cleavage reaction, and increase the purity of the hydroxamic acid product. Resin **7** can be cleaved with 2% TFA and triethylsilane in dichloromethane, and resin **8** with 1% TFA and triisopropylsilane in dichloromethane.

Using resin **8** instead of the hydroxylamine Wang resin improved yields by up to 20%.

New solid-phase chemistry

The arsenal of reactions suitable for solid-phase synthesis is still only a fraction of that available for solution-phase chemistry, but it is growing rapidly. In the last presentation of the day, Professor Mark Kurth (University of California, Davis, CA, USA) demonstrated how 1,3-dipolar additions can be employed in solid-phase synthesis. He prepared a series of poly-isoxazolines (**9**) from selenonitrates (**10**; Figure 1, Scheme 3). Phenyl isocyanate was used to convert the nitrate to a nitryl oxide, which reacts with the double bond to form the isoxazoline ring. The selenide group is subsequently removed with sodium periodate, and the resulting aldehyde cleaved to give an olefin, so that the reaction can be repeated. Examples of isoxazoles, isoxazolines and poly-isoxazolines that had been synthesized using this solid-phase 1,3-dipolar cycloaddition were shown.

The SCI is organizing a two-day symposium on *Challenges and Issues in High-Throughput Screening* on 6–8 July in Manchester (UK). For more information contact the SCI Conference Secretariat, tel: +44 171 235 3681, fax: +44 171 235 7743 or e-mail: conferences@chemind.demon.co.uk.

Henriëtte Willems

Book review

Cancer Therapeutics: Experimental and Clinical Agents

edited by B.A. Teicher, Humana Press, 1997. $125 (xii + 451 pages) ISBN 0 896 03460 7

Cancer Therapeutics: Experimental and Clinical Agents covers nearly 100 years of scientific and clinical investigation to treat or cure the many diseases called cancer. In 19 chapters from 29 contributors it successfully traces the stepwise advances that have led to the current treatments and the discoveries that point to future promise.

The book is divided into two equal parts – 'Cytotoxic Agents: Old and New' and 'Newer Strategies and Targets' – that chronicle the past achievements, highlight current therapeutic challenges, and summarize new therapeutic opportunities. It does a fine job of introducing many existing classes of agents in Part I, including the nitrogen mustards, phosphoramide and related mustards, nitrosoureas, platinum complexes, anthracyclines, Topo I and II inhibitors, the taxoids, some DNA groove binders, bis-naphthalmides and enediynes, but should not be considered comprehensive. Many classical agents are not treated in detail (e.g. mitomycins, vinca alkaloids, bleomycins), in spite of exciting recent developments in the basic science underlying their properties.

Part II focuses on agents and strategies for controlling growth, inhibiting growth, activating or deactivating stromal or malignant cells, or altering intra- or intercellular signaling cascades. It contains reviews of matrix metalloproteinase inhibitors, interferons and other cytokines, angiogenesis inhibitors, antisense oligonucleotides, growth factors and their inhibitors, immunoconjugates, *ras* targeted agents, and gene therapy. This section does a superb job of summarizing the current status of the newer opportunities for the treatment of cancer, especially for those who may be unfamiliar with the rapidly developing science.

Both sections are well referenced (typically up to 1994, occasionally 1995) and well composed, though a bit light on the representation of chemical structures. It makes for easy reading for those new to the field, yet it is valuable to those who have been daily engaged in the field for years.

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