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Solution-phase combinatorial chemistry

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Summary

The use of solution phase techniques has been explored as an alternative to solid-phase chemistry approaches for the preparation of arrays of compounds in the drug discovery process. Solution-phase work is free from some of the constraints of solid-phase approaches but has disadvantages with respect to purification. This article will also illustrate some of the advances made in recent years in solution phase array chemistry including using supported reagents and simple extractive protocols for the effective preparation of high quality samples.

Introduction

While this review focuses on material that has been disclosed in the primary literature, some relevant work presented at recent meetings is included to indicate the importance of emerging technologies. This chapter covers solution phase synthesis of pools of compounds and of discrete samples and the emerging field of fluorous synthesis. The use of liquid-liquid and liquid-solid extraction has been employed in both the preparation of pools and discrete samples and will be discussed at appropriate points.

Articles from two companies have reviewed their approaches to solution phase libraries. Merritt et al. [1] described the evolution of approaches at Glaxo Wellcome. The initial approach to pooled libraries was successful in identifying leads for medicinal chemistry programmes but also identified problems and prompted future efforts to be directed to discrete libraries. Garr et al. [2] reviewed the methods used at Panlabs for preparation and analysis of three classes of compounds for the Optiverse^{TM} Screening Library. A series of solution phase libraries based on 4-aminopiperidine, piperazine and 4-aminobenzylamine were synthesised by acylation, sulphonylation and N-alkylation.

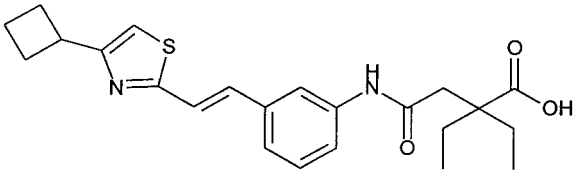

Figure 1. The structure of Ro24-5913.

Solution-phase synthesis of pools of compounds

A number of reports have described such approaches, some of which have served to give an indication of the historical development of the area. The inherent disadvantage of this approach – namely the generation of physical mixtures of compounds and the inability to employ any tagging strategy to assist in the deconvolution – has restricted the pool size to small numbers.

Maehr and Yang [3] confirmed that the Leukotriene D_4 antagonist Ro24-5913 had the optimum structural features for bioactivity using a solution based approach (Figure 1). A library of 700 compounds was prepared from 10 halomethyl ketones, 7 nitro aromatic aldehydes and 10 acyclic anhydrides. The most active compounds were identified by generation and screening of iterative sub-libraries; however, none was more potent than the original lead compound.

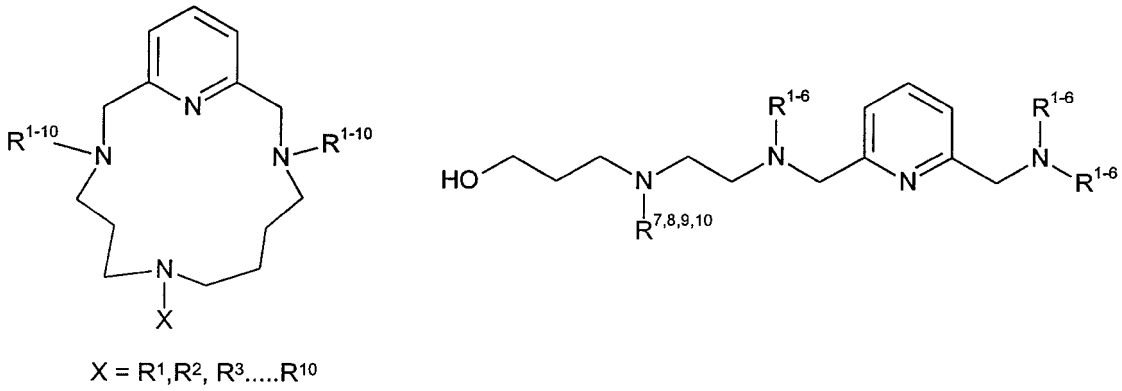

Figure 2. Polyazapyridinophane and pyridinopolyamine scaffolds.

Isis Pharmaceuticals have disclosed a series of libraries based on polyazapyridinophane and linear pyridinopolyamine scaffolds [4,5]. Pools of compounds were prepared by N-alkylation of two of the amino groups contained within the scaffold using a mixture of benzyl halides followed by deprotection and reaction of the third amino group with a single alkylating agent (Figure 2). The libraries were shown to contain compounds having antibacterial activity in the low micromolar range.

The use of rigid scaffolds was also employed by Falorni et al. [6] who prepared a number of small libraries having a diketopiperazine tetra-carboxylic acid template. The diversity elements were introduced via amide bond chemistry.

A library of β-amino alcohols was prepared by Ganesan et al. [7] by the lithium perchlorate promoted ring opening of epoxides using small pools of primary and secondary amines. The amines were divided into structural classes and representatives of each class were used in multiple pools of four. Each pool of amines was reacted with 80 epoxides to generate the library of >6000 samples.

Application of purification methods has been used to improve the quality of products in a number of examples. Boger et al. have published two papers on the preparation of 'indexed' libraries suitable for probing receptor and protein homo- and hetero-dimerisation events [8,9]. Two different linking strategies were developed; either an olefinic linker introduced via olefin metathesis or a bis-amide linker (Figure 3). The work extends their previous synthesis of libraries using liquid-liquid extraction to effect purification of intermediate stages in the library preparation.

Neuville and Zhu [10] reported the preparation of a library containing eight pools each of six aryl piperazines by nucleophilic substitution followed by deprotection and acylation under Schotten-Baumann conditions. The products were isolated in high purity by removal of excess reagents using liquid-liquid extraction.

The discovery of an antirhinoviral lead from a library of 4000 ureas was disclosed by Kaldor and coworkers [11]. Pools of 10 compounds were prepared by reaction of excess isocyanate with an equimolar mixture of 10 amines. Purification was effected by addition of aminomethylpolystyrene to remove isocyanate impurities followed by filtration and evaporation (Figure 4).

Solution-phase synthesis of discrete compounds

Parallel synthesis is now established as an integral component of lead optimisation methodology and increasing numbers of reports on the use of this approach in medicinal chemistry programmes are appearing in the literature. Jarvest et al. [12] explored the structure activity relationship at the 2-position of benzoxazinones in the search for inhibitors of herpes simplex virus-1 protease. Reaction of anthranilic acids with excess isocyanate or chloroformate afforded the 2-amino or 2-alkoxy substituted derivatives (Figure 5).

Carroll and co-workers [13] reported on the preparation of benzimidazole libraries from anilines and carboxylic acids using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline.

Application of effective parallel purification methods continues to improve the quality of products prepared using solution phase approaches. Sim and Ganesan [14] developed a one-pot three component synthesis of thiohydantoins using the reductive am-

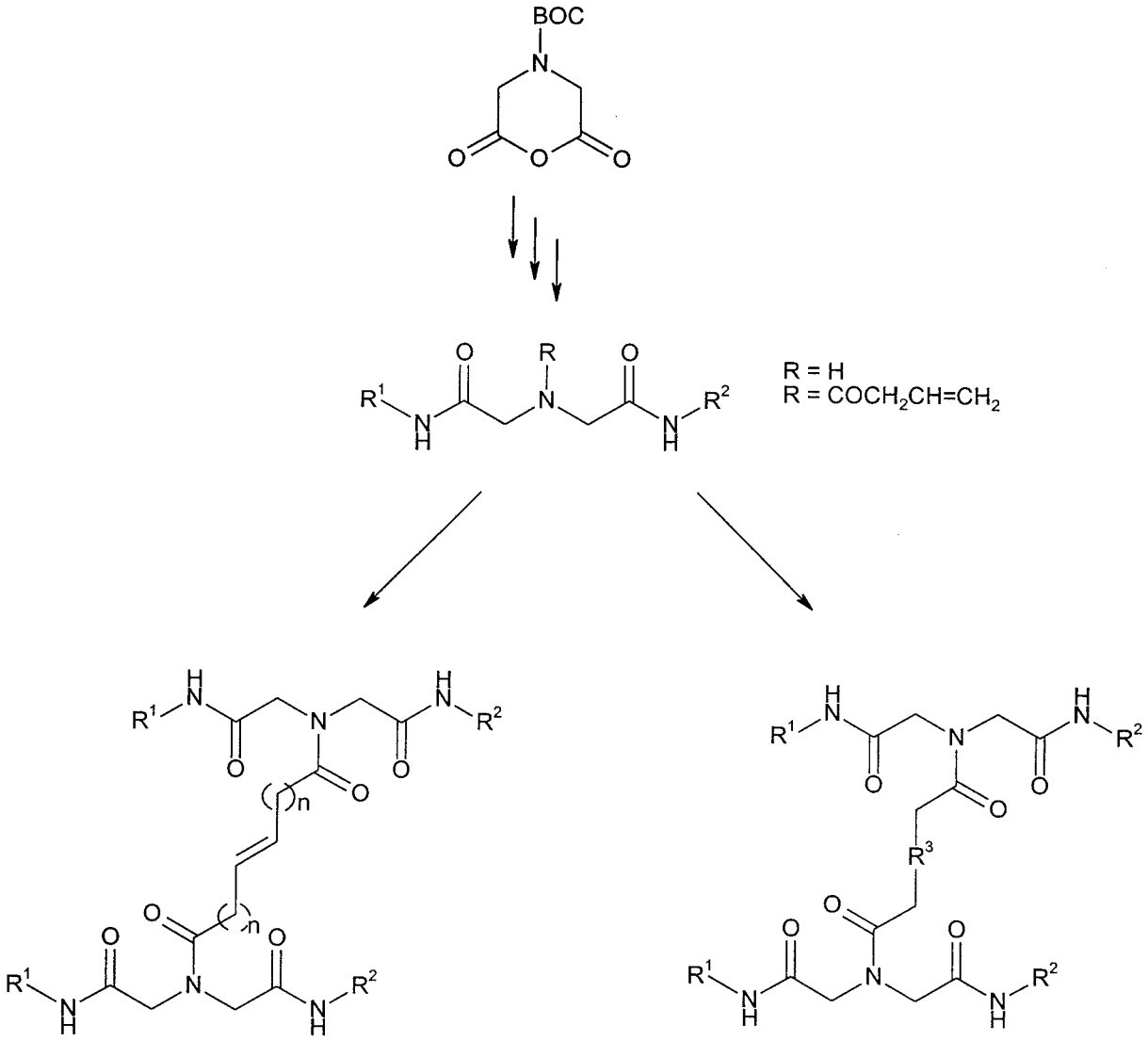

Figure 3. Preparation of libraries varying in length of linking tether.

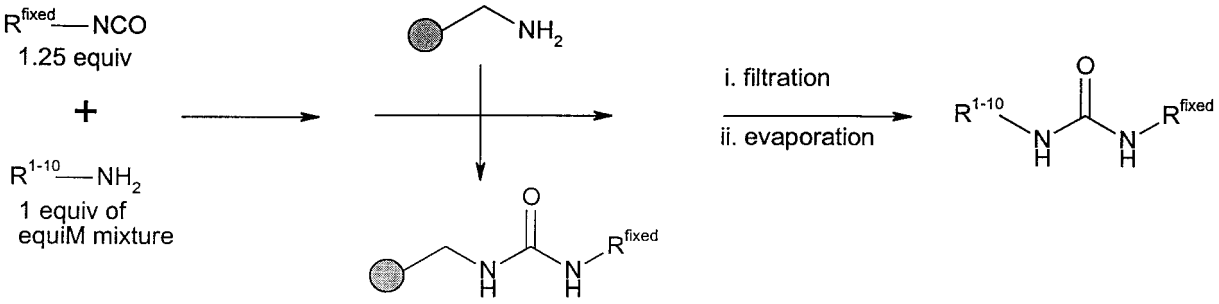

Figure 4. Use of solid supported 'covalent scavenger'.

ination of α-amino esters with aromatic aldehydes and sodium triacetoxyborohydride followed by the reaction with an isocyanate in the presence of triethylamine (Figure 6). The thiohydantions were isolated

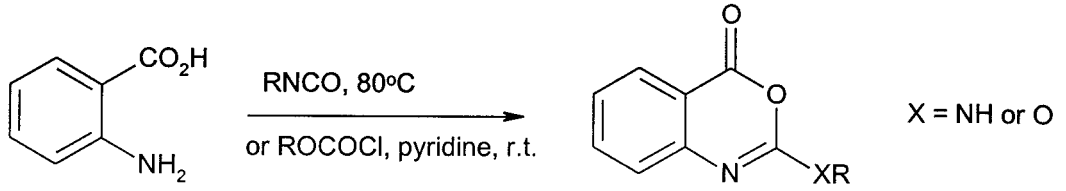

Figure 5. Synthesis of 2-hetero atom substituted benzoxazinones.

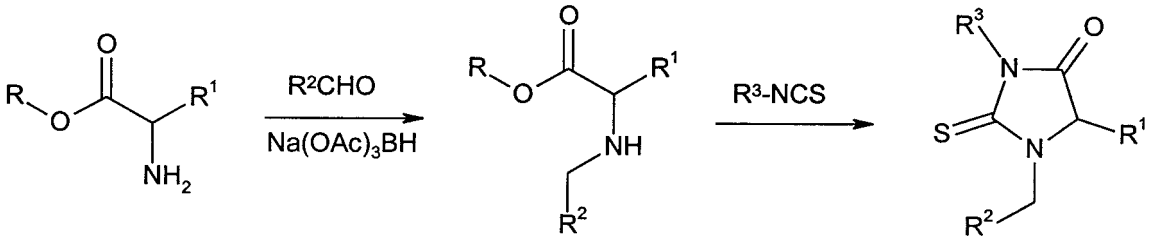

Figure 6. Synthesis of thiohydantoins.

by an aqueous work-up protocol which incorporated the addition of glycine to convert unreacted reagents into water soluble materials. The methodology was used in the preparation of an array of 600 discrete compounds.

Merritt et al. [1] and others [15] have disclosed a number of techniques for parallel phase separation which facilitates the aqueous washing of crude products. The initial approach used a commercially available hydrophobic membrane in a polypropylene cartridge to separate a chlorinated solvent from an aqueous phase. An alternative liquid solid extraction involving an absorbent packing has also been used by workers at Arris Pharmaceuticals in the preparation of triazine libraries [16].

A complementary protocol, appropriately termed the 'lollipop' method, for the separation involving solvent less dense than the aqueous phase was also disclosed [1]. The technique involves cooling the biphasic mixture in the presence of an array of pins, after the freezing process the solidified aqueous phase is removed attached to the pins.

There has been the anticipated increase in reports of the use of supported reagents to effect functional group transformations or remove excess reagents and by-products from crude solution phase reaction products. Combinatorial approaches using polymer supported reagents have been recently reviewed by Kaldor and Siegel [17].

Tartar and co-workers [18] reported the synthesis of polymer supported 1-hydroxybenzotriazole (Figure 7). Reaction of the reagent with a carboxylic acid in the presence of an activating agent afforded the polymer bound activated ester which was reacted with amines to liberate the amide in solution.

Supported electrophilic, nucleophilic or ionic reagents used to remove impurities from solution have been termed scavenger reagents, polymer supported quenching reagents (PSQ) or complementary molecular reactivity/molecular recognition polymer (CMR/R polymer). Use of such reagents provides a versatile counterpart to the approach described above. Booth and Hodges [19] utilised a high loading amine resin derived from chloromethylpolystyrene and tris(2-aminoethyl)amine in the preparation of ureas, thioureas, sulphonamides and amides.

Gayo and Suto [20] employed basic and acidic ion-exchange resins to remove excess reagents in the formation of amides and ureas, respectively. The combination of the ion exchange resin and solvent were optimised simultaneously in a parallel approach, demonstrating the further important application to reaction development.

An extension of the approach to additional chemical reactions has been reported by other groups. Flynn and co-workers [21] have developed some protocols for the Moffatt oxidation of secondary alcohol to ketones (Figure 8) and the addition of organometallic reagents to aldehydes. The same group has also developed the use of tetrafluorophthalic anhydride to assist in the purification of reaction mixtures [22].

Parlow et al. [23] used supported reagents and scavengers in two approaches to the optimisation of a lead pyrazole-5-carboxamide (Figure 9) investigating

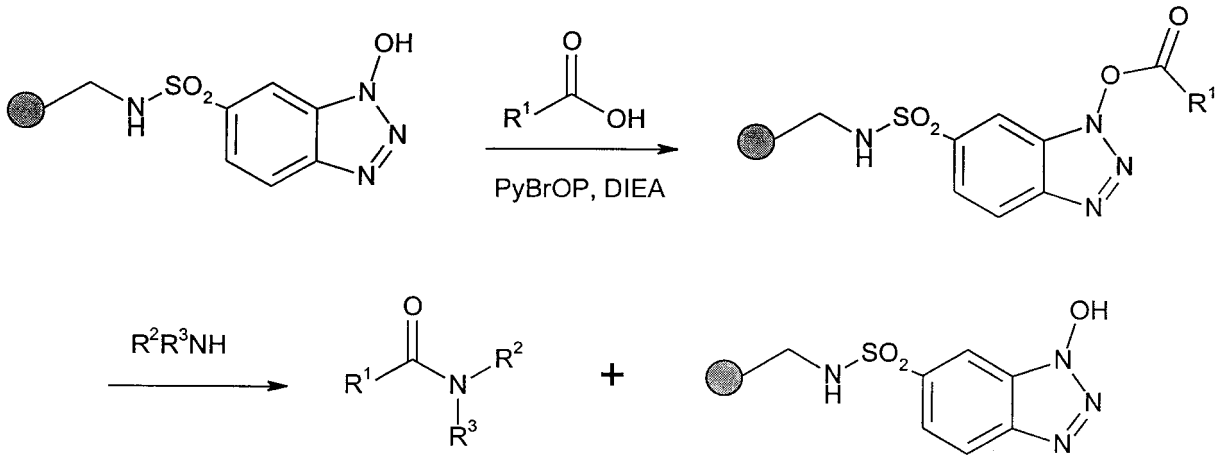

Figure 7. Use of polymer supported 1-hydroxybenzotriazole.

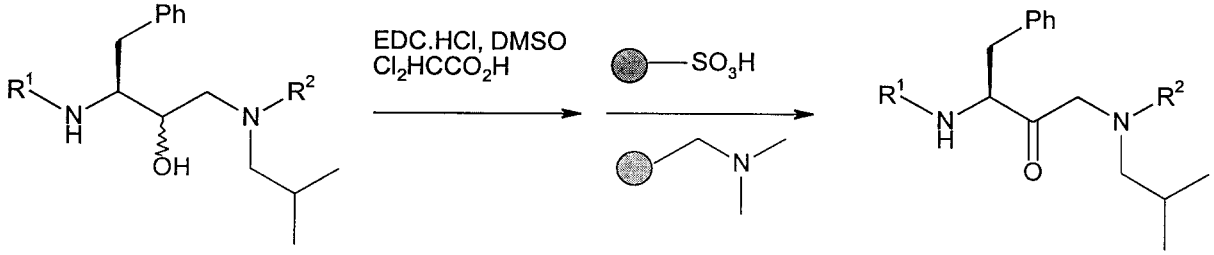

Figure 8. Use of amine encoded carbodiimide in Moffatt oxidations.

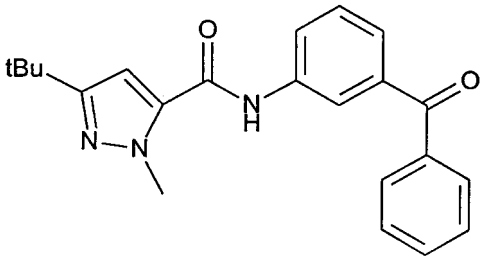

Figure 9. Pyrazole-5-carboxamide optimised by parallel synthesis.

variation in both the heterocyclic and aniline portions of the molecule.

Solid phase extraction (SPE) using a variety of absorbents is now a widely used technique allowing the rapid and facile purification of small molecules. Lawrence et al. [24] prepared an amide library of 225 discrete piperidine analogues using a carbodiimide mediated coupling followed by automated SPE extraction using a bonded silica functionalised with ethylbenzenesulfonic acid (SCX) (Figure 10). Two procedures were developed for the synthesis of neutral products: either a dual SPE protocol using an anionic exchange column followed by a cationic exchange column or modification of the carbodiimide reagents to one containing a basic centre.

A similar protocol was used by Siegel and coworkers [25] in the purification of products from reductive aminations, epoxide openings using amines and urea formation and by Chucholowski et al. [26] in the synthesis of thiazole libraries. A library of 48 ethanolamines in an 8×6 array prepared by the monoalkylation of amines with epoxides (Figure 11) was disclosed by Shuker et al. [27]. The synthetic protocol involved the in situ silylation of the amine with bis-(trimethylsilyl)acetamide, reaction with excess epoxide followed by SCX ion-exchange chromatography.

Fluorous synthesis

The potential of the application of fluorous synthesis as a means of purifying products via phase separation is now beginning to be realised. Curran and co-workers [28] have reported on a number of different fluorous strategies that could be used in solution phase work. In the strategy that has been exploited in the

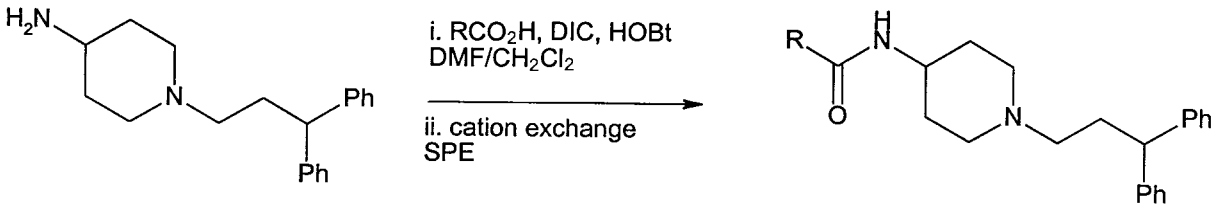

Figure 10. Use of solid phase extraction in purification of amides.

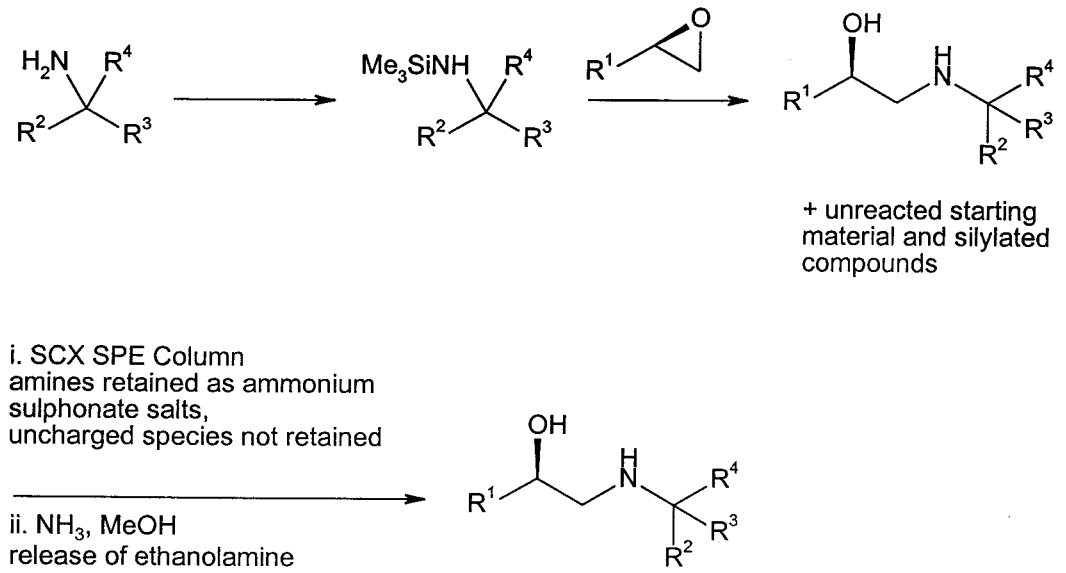

Figure 11. Synthesis of ethanolamines by epoxide opening.

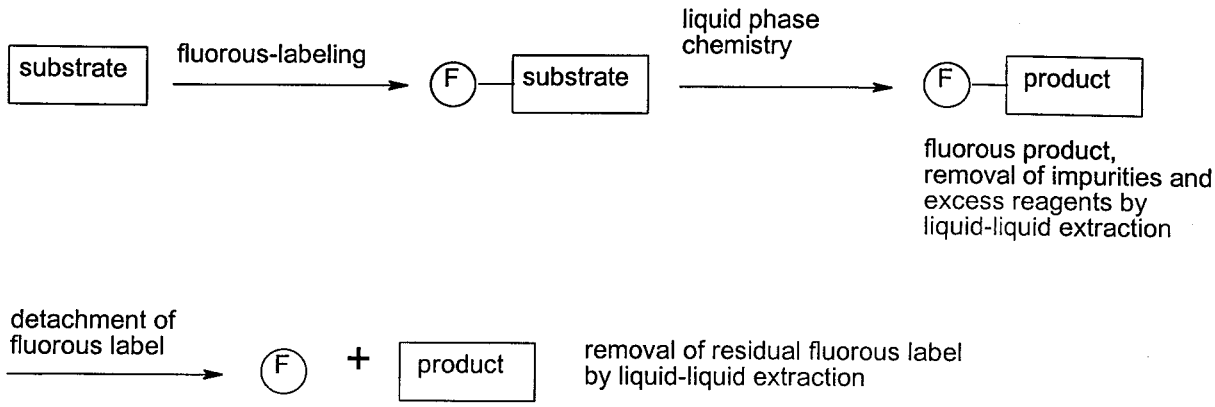

Figure 12. Fluorous synthesis.

preparation of combinatorial libraries an organic substrate has been converted into a fluorous compound by attachment of a 'fluorous label'. Reactions were then conducted and the product purified by a three-phase liquid extraction using fluorous solvents. On completion of the synthetic sequence the target molecule was released from the fluorous label (Figure 12).

Isoxazolines and isoxazoles have been prepared in good yield and excellent purity by reaction of excess nitrile oxide and fluorous labelled silyl ether and silyl propargyl ether respectively [29] (Figure 13). Fluorous variants of the Ugi and Biginelli reaction have also been developed [30].

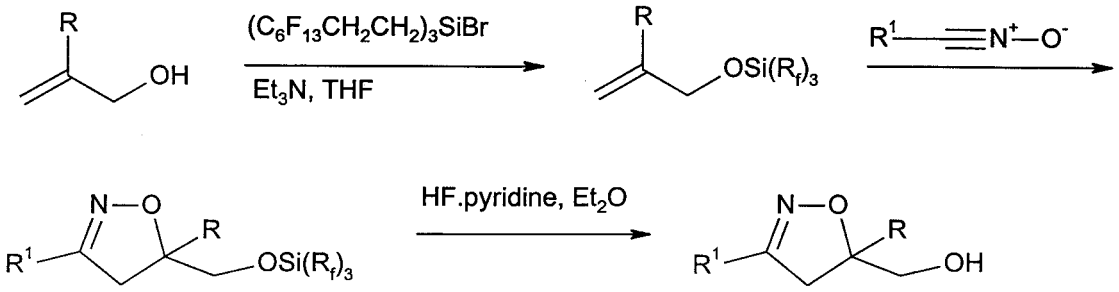

Figure 13. Synthesis of isoxazolines by fluorous synthesis.

Conclusions

Solution phase combinatorial chemistry continues to provide an important technique particularly to the medicinal chemist engaged in lead optimisation work. We anticipate that next year will see further development and application of purification technologies which will allow more complex chemistries to be employed. Although work on fluorous techniques has, currently, only been exploited by the original workers, the development of a solid phase extraction with fluorous reverse phase silica [31] and a soluble fluorous phase polymer support [32] indicates the opportunity for further innovative application of the strategy to solution phase approaches.

References

1. Bailey, N., Cooper, A.W.J., Deal, M.J., Dean, A.W., Gore, A.L., Hawes, M.C., Judd, D.B., Merritt, A.T., Storer, R., Travers, S. and Watson, S.P., *Solution-phase combinatorial chemistry in lead discovery*, Chimia, 51 (1997) 832–837.
2. Garr, C.D., Peterson, J.R., Schultz, L., Oliver, A.R., Underiner, T.L., Cramer, R.D., Ferguson, A.M., Lawless, M.S. and Patterson, D.E., *Solution phase synthesis of chemical libraries for lead discovery*, J. Biomol. Screening, 1 (1996) 179–186.
3. Maehr, H. and Yang, R., *Structure optimization of a leukotriene D_4 antagonist by combinatorial chemistry in solution*, Bioorg. Med. Chem., 5 (1997) 493–496.
4. An, H., Cummins, L.L., Griffey, R.H., Bharadwaj, R., Haly, B.D., Fraser, A.S., Wilson-Lingardo, L., Risen, L.M., Wyatt, J.R. and Cook, P.D., *Solution phase combinatorial chemistry. Discovery of novel polyazapyridinophanes with potent antibacterial activity by a solution phase simultaneous addition of functionalities approach*, J. Am. Chem. Soc., 119 (1997) 3696–3708.
5. An, H., Haly, B.D., Fraser, A.S., Guinosso, C.J. and Cook, P.D., *Solution phase combinatorial chemistry. Synthesis of novel linear pyridinopolyamine libraries with potent antibacterial activity*, J. Org. Chem., 62 (1997) 5156–5164.
6. Falorni, M., Giacomelli, G., Nieddu, F. and Taddei, M., *A new diketopiperazine tetra-carboxylic acid as template for the homogeneous phase synthesis of chemical libraries*, Tetrahedron Lett., 38 (1997) 4663–4666.
7. Chng, B.L. and Ganesan, A., *Solution-phase synthesis of a β-amino alcohol combinatorial library*, Bioorg. Med. Chem. Lett., 7 (1997) 1511–1514.
8. Boger, D.L., Chai, W., Ozer, R.S. and Andersson, C.-M., *Solution-phase combinatorial synthesis via the olefin metathesis reaction*, Bioorg. Med. Chem. Lett., 7 (1997) 463–468.
9. Boger, D.L., Ozer, R.S. and Andersson, C.-M., *Generation of targeted C_2-symmetrical compound libraries by solution-phase combinatorial chemistry*, Bioorg. Med. Chem. Lett., 7 (1997) 1903–1908.
10. Neuville, L. and Zhu, J., *Solution phase combinatorial synthesis of arylpiperazines*, Tetrahedron Lett., 38 (1997) 4091–4094.
11. Kaldor, S.W., Fritz, J.E., Tang, J. and McKinney, E.R., *Discovery of antirhinoviral leads by screening a combinatorial library of ureas prepared using covalent scavengers*, Bioorg. Med. Chem. Lett., 6 (1996) 3041–3044.
12. Jarvest, R.L., Parratt, M.J., Debouck, C.M., Gorniak, J.G., Jennings, L.J., Serafinowska, H.T. and Strickler, J.E., *Inhibition of HSV-1 protease by benzoxazinones*, Bioorg. Med. Chem. Lett., 6 (1996) 2463–2466.
13. Thomas, J.B., Fall, M.J., Cooper, J.B., Burgess, J.P. and Carroll, F.I., *Rapid in-plate generation of benzimidazole libraries and amide formation using EEDQ*, Tetrahedron Lett., 38 (1997) 5099–5102.
14. Sim, M.M. and Ganesan, A., *Solution-phase synthesis of a combinatorial thiohydantoin library*, J. Org. Chem., 62 (1997) 3230–3235.
15. Bush, M., Hamilton, T., Robbins, J. and DeWitt, S., *Phase separation devices for high-throughput synthesis, isolation and purification*, CHI Exploiting Molecular Diversity: Small Molecule Libraries for Drug Discovery, Coronado, CA, February 3–5, 1997.
16. Plunkett, M., *Chemistry Development for Library Synthesis*, 3rd IBC 'Nuts and Bolts' Forum Combinatorial Technologies, Coronado, CA, October 23–24, 1997.
17. Kaldor, S.W. and Siegel, M.G., *Combinatorial chemistry using polymer-supported reagents*, Curr. Opin. Chem. Biol., 1 (1997) 101–106.
18. Pop, I.E., Deprez, B.P. and Tartar, A.L., *Versatile acylation of N-nucleophiles using a new polymer supported 1-hydroxybenzotriazole*, J. Org. Chem., 62 (1997) 2594–2603.
19. Booth, R.J. and Hodges, J.C., *Polymer-supported quenching reagents for parallel purification*, J. Am. Chem. Soc., 119 (1997) 4882–4886.
20. Gayo, L.M. and Suto, M.J., *Ion-exchange resins for solution-phase parallel synthesis of chemical libraries*, Tetrahedron Lett., 38 (1997) 513–516.
21. Flynn, D.L., Crich, J.Z., Devraj, R.V., Hockermann, S.L., Par-

low, J.L., South, M.S. and Woodward, S., *Chemical library purification strategies based on the principles of complementary molecular reactivity and molecular recognition*, J. Am. Chem. Soc., 119 (1997) 4874–4881.
22. Parlow, J.J., Naing, W., South, M.S. and Flynn, D.L., *In situ chemical tagging: Tetrafluorophthalic anhydride as a 'sequestration enabling reagent' (SER) in the purification of solution-phase combinatorial libraries*, Tetrahedron Lett., 38 (1997) 7959–7962.
23. Parlow, J.J., Mischke, D.A. and Woodward, S.S., *Utility of complementary molecular reactivity and molecular recognition (CMR/R) technology and polymer supported reagents in solution-phase synthesis of heterocyclic carboxamides*, J. Org. Chem., 62 (1997) 5908–5919.
24. Lawrence, R.M., Biller, S.A., Fryszman, O.M. and Poss, M.A., *Automated synthesis and purification of amides: Exploitation of automated solid phase extraction in organic synthesis*, Synthesis, (1997) 553–558.
25. Siegel, M.G., Hahn, P.J., Dressman, B.A., Fritz, J.E., Grunwell, J.R. and Kaldor, S.W., *Rapid purification of small molecules libraries by ion exchange chromatography*, Tetrahedron Lett., 38 (1997) 3357–3360.
26. Chucholowski, A., Masquelin, T., Obrecht, D., Stadlwieser, J. and Villalgordo, J.M., *Novel solution- and solid-phase strategies for the parallel and combinatorial synthesis of small molecular weight compound libraries*, Chimia, 50 (1996) 525–530.
27. Shuker, A.J., Siegel, M.G., Matthews, D.P. and Weigel, L.O., *The application of high-throughput synthesis and purification to the preparation of ethanolamines*, Tetrahedron Lett., 38 (1997) 6149–6152.
28. Studer, A., Hadida, S., Ferritto, R., Kim, S.-Y., Jeger, P., Wipf, P. and Curran, D.P., *Fluorous synthesis: A fluorous-phase strategy for improving separation efficiency in organic synthesis*, Science, 275 (1997) 823–826.
29. Studer, A. and Curran, D.P., *A strategic alternative to solid phase synthesis: Preparation of a small isoxazoline library by 'fluorous synthesis'*, Tetrahedron, 53 (1997) 6681–6696.
30. Studer, A., Jeger, P., Wipf, P. and Curran, D.P., *Fluorous synthesis: Fluorous protocols for the Ugi and Biginelli multicomponent condensations*, J. Org. Chem., 62 (1997) 2917–2924.
31. Curran, D.P., Hadida, S. and He, M., *Thermal allylation of aldehydes with fluorous allylstannane. Separation of organic and fluorous products by solid phase extraction with fluorous reverse phase silica gel*, J. Org. Chem., 62 (1997) 6714–6715.
32. Bergbreiter, D.E. and Franchina, J.G., *A soluble fluorous phase polymer support*, J. Chem. Soc., Chem. Commun., (1997) 1531–1532.