Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 1, pp. 47-50, 1995

0960-894X/95 $9.50+0.00

0960-894X(94)00455-2

REDUCTIVE ALKYLATION ON A SOLID PHASE: SYNTHESIS OF A PIPERAZINEDIONE COMBINATORIAL LIBRARY

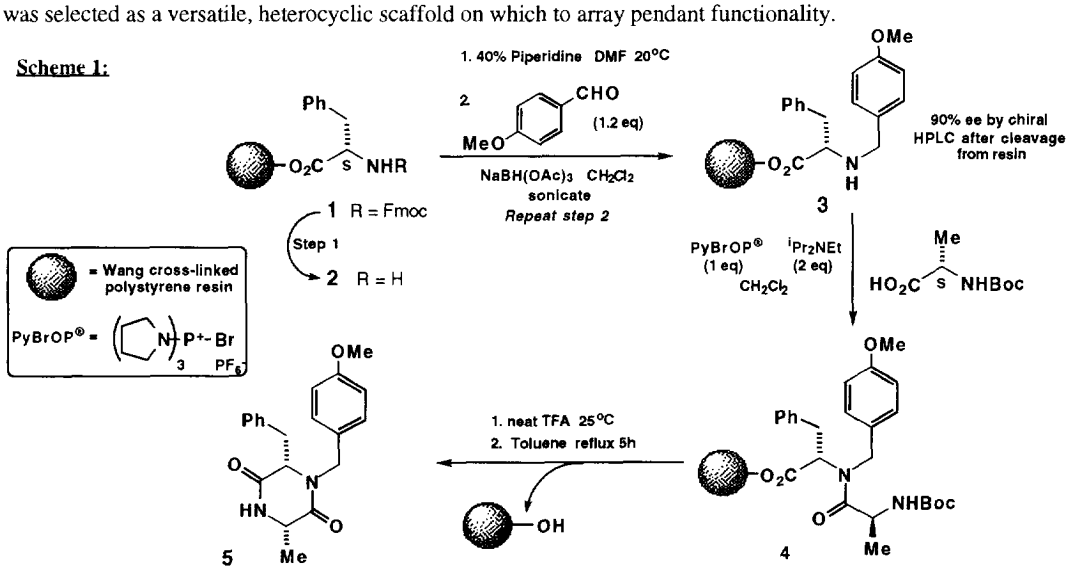
David W. Gordon and **John Steele***

Discovery Chemistry Department, Pfizer Central Research, Sandwich, Kent CT13 9NJ, UK

Abstract: *The synthesis of a prototype trisubstituted piperazinedione combinatorial library of 1,000 compounds has been achieved from three precursor sets - two sets of ten α-amino acids and one set of ten aldehydes. A sodium triacetoxyborohydride-mediated reductive alkylation was crucial to the success of the multi-step synthesis on resin. This protocol represents a new method to augment compound files rapidly with novel heterocyclic entities for high-speed screening.*

Combinatorial synthesis of libraries containing linear peptide and non-peptide structures is widely recognised as a valuable new tool for lead discovery in the search for new therapeutics. Of the methods for achieving the synthesis of large numbers of library components, one of the most flexible is the resin-based mix and split technology, first described by Furka and co-workers in 1988[1] and subsequently developed further by several other groups.[2,3] The speed and convenience of screening compound mixtures is a powerful aspect of this technology. In general, this technique has been restricted to peptide-type coupling reactions,[2] although solid-phase chemistry for the assembly of some heterocycles as individual compounds is now becoming established.[4] We describe here the solid-phase synthesis of a prototype combinatorial library of 1,000 piperazinediones (diketopiperazines or DKPs) **5**, each containing three centres of molecular diversity. The rigid DKP template was selected as a versatile, heterocyclic scaffold on which to array pendant functionality.

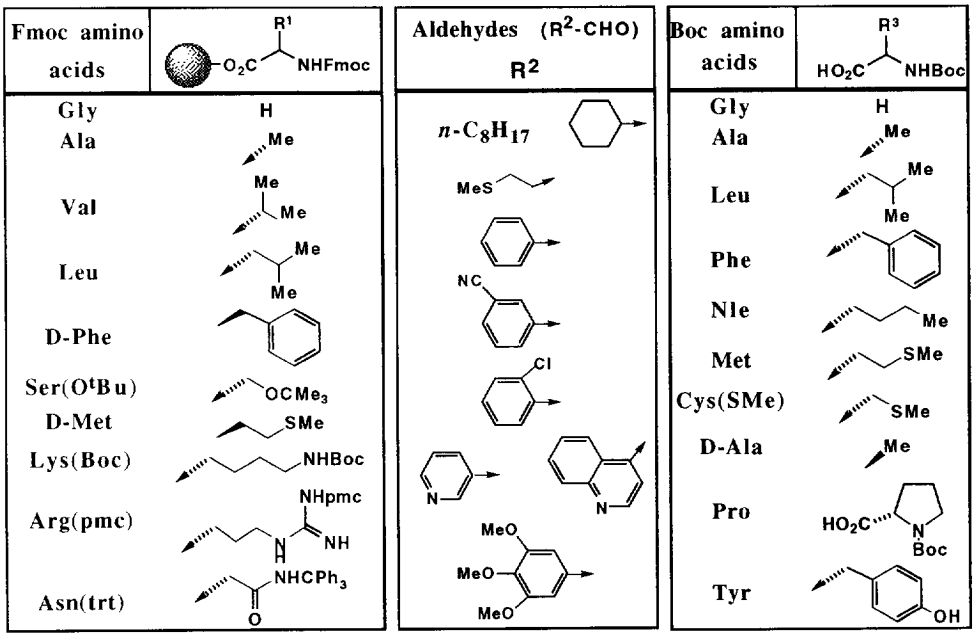
Scheme 1:



Scheme 1 contains the synthetic protocol for a representative DKP derived from Fmoc-Phe-Wang resin **1**, Boc-Ala and *p*-methoxybenzaldehyde.

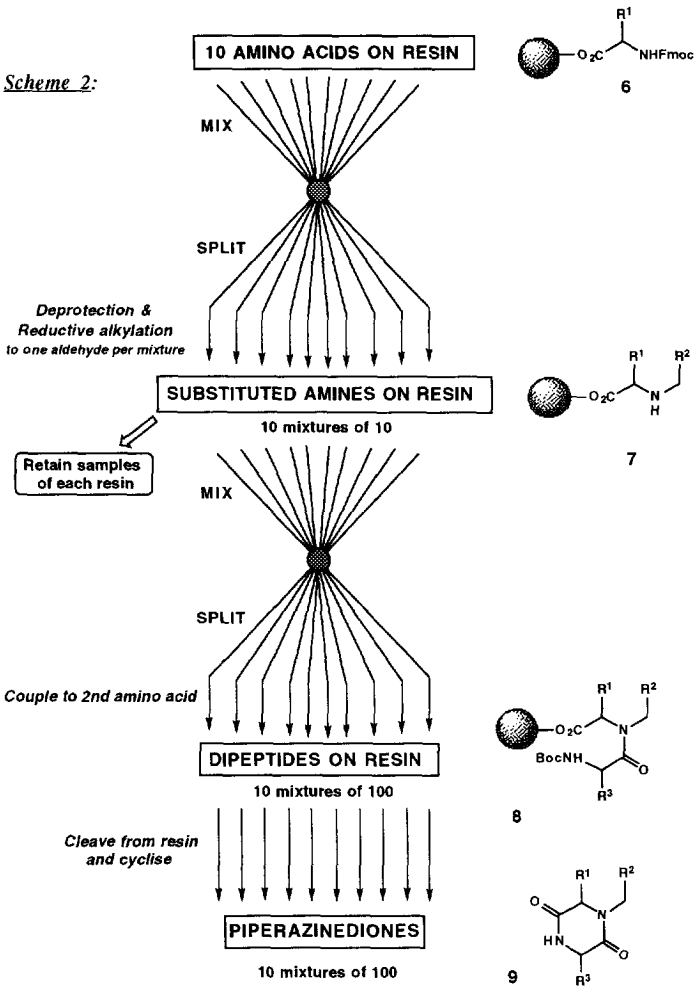
The reductive alkylation of peptides on MBH[5] and Merrifield[6] resins using sodium cyanoborohydride in acidified DMF is a well-established process but pH sensitivity and potential reagent toxicity made this an unattractive option for the present work. We have instead used an efficient sodium triacetoxyborohydride-facilitated alkylation of amino acids such as **2** on Wang resin by a range of aldehydes, affording an array of novel, resin-bound secondary amines including **3**. Yields for the general process **2**→**3** are typically 85-95%, although partial racemisation of some homochiral amino acids (notably Phe as shown) was observed. This reductive alkylation has proved compatible with a wide range of natural and synthetic α-amino acids and is tolerant of almost any aldehyde, although in a potential 'worst case' combination of a hindered amino acid (e.g. Val, see later) and an electronically deactivated aldehyde, isolated yields of ~20% were obtained, the balance of recovered material being the unreacted amino acid. Aliphatic aldehydes also gave minor but significant amounts of *bis* alkylation (1-10%), generating tertiary amines. Coupling of a relatively unreactive secondary amine (eg **3**) to a further Boc-protected amino acid proved to be a greater chemical challenge. After examination of a range of activated ester coupling techniques, this transformation was best achieved in our hands using a PyBrOP mediated double-coupling protocol, generating an amide (eg **4**). A double coupling was found to be essential to achieve consistent yields of >90%. The optimal method of assembling target DKPs such as **5** employed a TFA-mediated Boc deprotection and concomitant resin cleavage followed by a short reflux of the evaporated filtrate in toluene to induce cyclisation. Without the thermolysis in toluene, no DKP formation was observed in any of our work. In the specific case given in ***Scheme 1***, the overall yield for synthesis of the pure DKP **5** was 42% based on resin-bound amino acid with trace amounts of bis-alkylated Phe recovered as the only identifiable by-product. Chiral HPLC analysis[7] of a sample of the reductive alkylation product **3** after resin cleavage indicated slight racemisation, presumably occurring prior to reduction of the intermediate imine.

The monomeric components used for the prototype piperazinedione library are given in the ***Tables*** below:



To assemble a prototype combinatorial library of 1,000 piperazinediones, the protocol in ***Scheme 2*** was used:[8]

Scheme 2:



Ten Fmoc-protected amino acids **6** on Wang resin were intimately mixed and then distributed as equivalent mixtures into ten reactor vessels. Each mixture was deprotected using piperidine and reductively alkylated with a single aldehyde. Samples of the resultant secondary amines on resin **7** (~5% by wt) were retained for analysis and subsequent synthetic follow-up of any biologically active mixtures. The remaining resins were mixed again and redistributed into ten further mixtures. Each mixture was coupled to a single amino acid, then the resultant products **8** were cleaved and cyclised as described earlier for the single DKP synthesis to afford ten mixtures, each containing 100 DKPs **9** - overall constructing **a total of 1,000 compounds from an initial pool of 27 contributing units.**

The comprehensive analysis of multicomponent mixtures presents a significant problem. Clearly, some of the 1,000 components may be under-represented as a consequence of two or more consecutive low yields. The aim of our analysis has been to fully validate the chemistry, then characterise intermediate stages in the library synthesis to generate confidence that most (>95%) of the expected components are likely to be present. For the DKP library, this was achieved by assembly and full characterisation of a range of individual, pure DKPs including representative syntheses of potential 'worst-case' products such as **9** (R^1=^{i}Pr, R^3=^{i}Bu, R^2=3,4,5-trimethoxyphenyl - 24% overall). For the intermediate ten-component mixtures of secondary amino acids (prepared by TFA digestion of **7**), HPLC-MS combined with MS-MS was used to unambiguously confirm the presence of 96 of the expected 100 components (ref 9 contains the experimental protocol used for this analysis). Spectroscopic methods are still under development for the effective analysis of 100-component mixtures.

Acknowledgements: We are indebted to Dr. Adrian Wright, Mr. George Perkins and Mr. Keith Burton of the Physical Sciences group at Sandwich for HPLC and MS analytical data.

References and Notes

1. a) Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. *Abstr. 14th Int. Congr. Biochem., Prague, Czechoslovakia,* **1988**, *5*, 47.
   b) Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. *Int. J. Peptide Protein Res.*, **1991**, *37*, 487.
2. Early publications that describe the use of combinatorial mix and split synthesis include: Houghten, R.A.; Pinilla, C.; Blondelle, S.E.; Appel, J.R.; Dooley, C.T.; Cuervo, J.H. *Nature*, **1991**, *354*, 84. Owens, R.A.; Gesellchen, P.D.; Houchins, B.J.; DiMarchi, R.D. *Biochem. Biophys. Res. Comm.*, **1991**, *181*, 402. Hortin, G.L.; Staatz, W.D.; Santoro, S.A. *Biochem. International*, **1992**, *26*, 731.
3. For two useful recent surveys of library methods, see:
   a) Gallop, M.A.; Barrett, R.W.; Dower, W.J.; Fodor, S.P.A.; Gordon, E.M. *J. Med. Chem.*, **1994**, *37*, 1233 *and* 1385 (two parts).
   b) Pavia, M.R.; Sawyer, T.K.; Moos, W.H. *Bioorg. Med. Chem. Lett.*, **1993**, *3*, 387 and subsequent articles in that issue.
4. For example: Bunin, B.A.; Ellman, J.A. *J. Am. Chem. Soc.*, **1992**, *114*, 10997 (benzodiazepinones) and DeWitt, S.H.; Kiely, J.S.; Stankovic, C.J.; Schroeder, M.C.; Reynolds Cody, D.M.; Pavia, M.R. *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 6909 (hydantoins).
5. Coy, D.H.; Hocart, S.J.; Sasaki, Y. *Tetrahedron*, **1988**, *44*, 835. Hocart, S.J.; Nekola, M.V.; Coy, D.H. *J. Med. Chem.*, **1987**, *30*, 739.
6. Tourwé, D.; Piron, J.; Defreyn, P.; Van Binst, G. *Tetrahedron Lett.*, **1993**, *34*, 5499.
7. HPLC analytical separation achieved using a reverse phase ES-OVM 4.6mm x 16cm column, eluting 97:3 phosphate buffer (pH6.6): MeCN at 1ml/min. UV detection at 270nM.
8. Experimental details for the library assembly are as follows:
   Deprotection: Fmoc amino acids on resin (Wang cross-linked polystyrene, 0.2mmol each; weights varied with resin loading density) were combined, intimately mixed and stirred in 40%v/v piperidine/DMF (40ml) for 20 minutes. The resin was drained, washed (5xDMF), dried and the entire deprotection repeated. ***Reductive alkylation:*** This dry resin was evenly distributed into each of ten round-bottomed flasks and each suspended in CH_2Cl_2 (0.5ml). To each was added one of the ten aldehydes (0.24mmol) in CH_2Cl_2 (0.5ml). Each flask was sonicated in an ultrasound bath for 20 minutes followed by addition of a pre-sonicated solution of sodium triacetoxyborohydride (0.28mmol) in dichloromethane (0.5ml). All reactors were sonicated for five minutes then stirred vigorously for 16h. Each resin was filtered, washed (H_2O, aqueous $NaHCO_3$, H_2O, THF: 3x2ml each), dried and the reductive alkylation procedure repeated. 10% by weight of each dry resin-bound secondary amine intermediate was retained for future analysis and iterative follow-up. ***Coupling:*** All remaining resin was intimately mixed and evenly distributed to each of ten flasks. Each was suspended in CH_2Cl_2 (3ml) and treated with a solution of one of the ten Boc-amino acid components (0.2mmol) in CH_2Cl_2 (0.5ml and DMF, if necessary, to achieve complete solubility), followed by a solution of PyBrOP (0.2mmol) in CH_2Cl_2 (0.5ml) and finally diisopropylamine (0.4mmol). Each reaction was stirred for 24h then filtered, washed (DMF, H_2O, THF), dried and the coupling process repeated. ***Resin Cleavage:*** Each resin was suspended in trifluoroacetic acid (TFA, 1ml) for 3h with occasional agitation, then filtered and washed with CH_2Cl_2 (5ml). Each filtrate was concentrated, the residue dissolved in toluene and concentrated once more to remove any residual TFA. ***DKP cyclisation:*** The residues were dissolved in toluene (10ml) and stirred under reflux for 5h, then evaporated to dryness and the resultant compound mixtures dissolved in DMSO prior to automated screening.
9. **LC/MS analysis.** Putative ten-component mixture samples were analysed by capillary LC-MS using a 150 x 0.32mm capillary column containing hypersil ODS 5µm packing, operating at a flow rate of 3 µl/min. Separations were achieved using a water/acetonitrile/trifluoroacetic acid gradient, increasing from 25% to 50% acetonitrile over 30 min. The mobile phase contained 10% glycerol as a matrix for the Continuous Flow Liquid Secondary Ionisation Mass Spectrometry (CF-LSIMS) interface. All of the eluent was transferred directly to the CF-LSIMS interface on a Kratos Concept-1S mass spectrometer scanning from 725 to 150 amu in 3 seconds. **Direct MS analysis** of the mixtures without prior separation was achieved by chemical ionisation on a VG Trio-3 triple quadrupole instrument using ammonia as the reagent gas. Component structures were confirmed by daughter ion MS/MS using argon as the collision gas.

(*Received in Belgium* 7 *October* 1994; *accepted* 25 *November* 1994)