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SOLUTION-PHASE SYNTHESIS OF A β-AMINO ALCOHOL COMBINATORIAL LIBRARY

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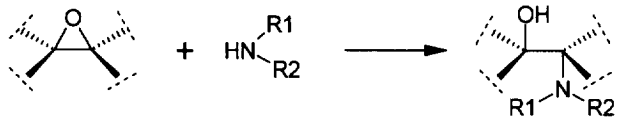
Abstract: A β-amino alcohol library was prepared in solution by the lithium perchlorate promoted ring-opening of epoxides by primary and secondary amines. For increased efficiency in synthesis and screening, each reaction contained one epoxide and a pool of four amines. © 1997 Elsevier Science Ltd.

The preparation of chemical libraries by combinatorial methods was first accomplished over a decade ago. Initial efforts were focused on oligomeric biopolymers, notably peptides and nucleotides, for which high-yielding automated synthetic protocols were available. More recently, attention has shifted[1] to the production of "drug-like" small molecules for lead discovery and optimisation. Such libraries were usually prepared by solid-phase techniques, although solution-phase chemistry[2] is a viable option for short synthetic sequences. Here, we report one such example, the construction of a pooled β-amino alcohol[3] library based on the nucleophilic ring opening of epoxides by amines (eq. 1).



eq. (1)

Classical conditions[4] for this reaction involve high temperature and a large excess of the amine. More stoichiometric ratios of amine to epoxide are possible with the use of activating agents. We investigated two such reagents, alumina[5] and lithium perchlorate,[6] in model studies. Lithium perchlorate generally gave higher yields coupled with easier reaction workup. Unlike alumina, lithium perchlorate also did not require anhydrous solvent and successfully promoted the reaction of aromatic amines with epoxides.[7]

We next tested the suitability of the conditions for pooled reactions. A set of 400 commercially available primary and secondary amines was divided into 80 pools of five according to structural class and reactivity. Each of these pools was reacted with two epoxides - styrene oxide and methyl *cis*-9,10-epoxyoctadecanoate (obtained by the epoxidation of methyl oleate)- at room temperature and 45 °C. The reaction mixtures were monitored by a combination of TLC, HPLC, and LC-MS. In the majority of reactions,

products derived from several or all of the five amines were identified. With unsymmetrical epoxides, variable amounts[8] of the two regioisomeric products were observed. In those cases where products were not formed, increasing the temperature to 60 °C and reaction time to 3 days usually enabled successful reaction. The amines which were still unreactive under these conditions were sterically hindered [e.g. *ortho*-substituted anilines] or electronically deactivated [e.g. α-amino acid esters, anilines with electron-withdrawing groups]. Based on these results, 320 suitable amines were selected and grouped into 80 pools of four (Table 1).

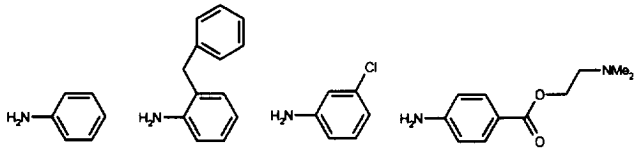
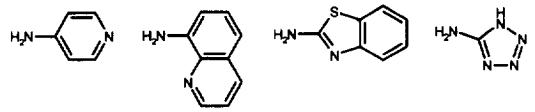
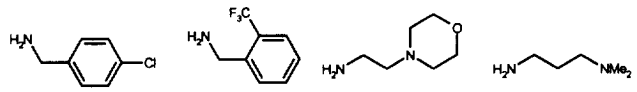
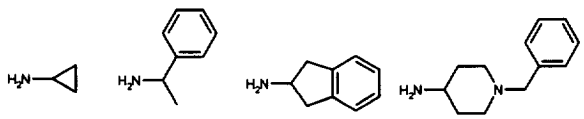
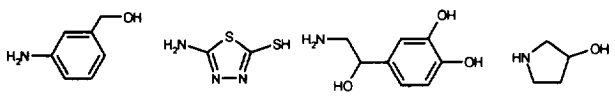
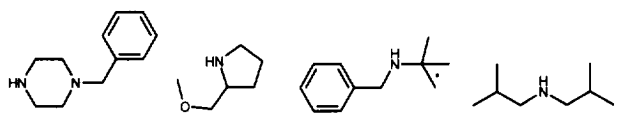
Amine Class	Number	Examples
Anilines	64	
Heteroaromatic	48	
Primary, unhindered	64	
Primary, α-branched	28	
Polynucleophilic	80	
Secondary	36	

Table 1. Classification of the 320 amines according to structural similarity, with representative examples from each class.

For library synthesis, aliquots containing 0.05 mmol of an epoxide and 0.1 mmol of $LiClO_4$ dissolved in acetonitrile were dispensed into 80 vials. Each of these was reacted with a pool containing 0.015 mmol each of four amines. Reactions were usually carried out for four days at 55 °C. At this stage, random samples were analysed by MS for product formation, and heating was continued in cases of incomplete reaction. Of over 80 epoxides tried, most gave satisfactory results.[9] Figure 1a shows a typical MS profile where all four expected products are seen. With highly reactive amine pools, we also observed 2:1 adducts arising from further reaction of the β-amino alcohol with a second molecule of epoxide [Figure 1b].

We have prepared a library of over 6,000 samples using the above procedure. Taking into account the

possibility of regioisomeric products with unsymmetrical epoxides, the library potentially contains over 40,000 β-amino alcohols. Workup of reaction mixtures simply consisted of removal of acetonitrile, and resuspension in aqueous DMSO at a final concentration of 1 mM based on the starting epoxide. Ten microliter aliquots of the aqueous solutions were transferred to microtiter plate wells for bioassay. We had previously determined that lithium perchlorate did not interfere in a variety of enzyme, ligand-binding, and cell-based assays even at much higher concentrations than present in our samples.

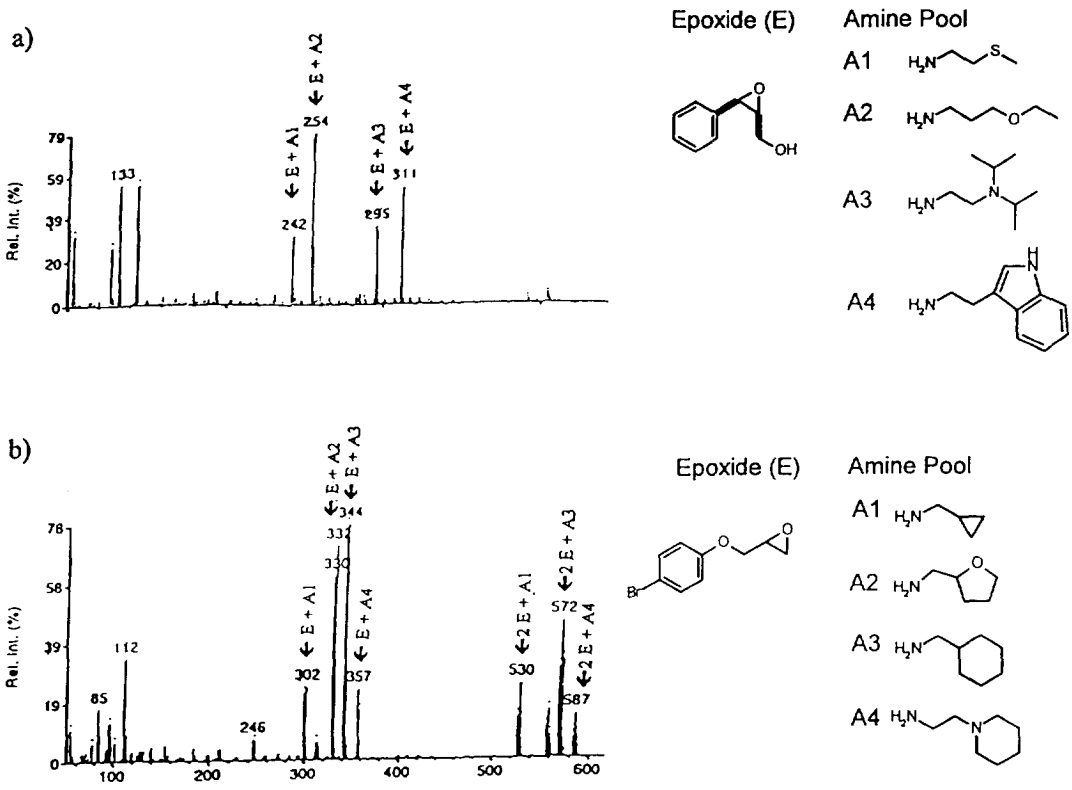


Figure 1. MS of two typical library samples. Mass spectra were determined in positive ion mode using a Perkin-Elmer Sciex III with atmospheric pressure chemical ionization.

Samples from this library gave an average hit rate of 1 % in our panel of assays. Structure-activity trends were immediately apparent from the modular way in which samples were dispensed. Thus, all 80 samples from a single epoxide are clustered in one microtiter plate, while samples from a given amine pool are in the same well position in each plate. Deconvolution of hits consisted of reaction of the epoxide with each of the four amines individually followed by retesting of the products. Non-additive effects were observed in a few cases- for example, a pool of phenolic adrenaline derivatives and another of benzimidazoles inhibited two enzyme assays non-specifically. Usually, however, the initial activity in the pool could be replicated after deconvolution. Compounds derived from 5-amino-1,3,4-thidiazole-2-thiol, for instance, were

consistently picked up in a fucosyltransferase assay. The parent amine itself also inhibited the enzyme at the micromolar level. Further studies showed this was probably due to disulfide bond formation with the enzyme, as the activity was lost when the assay was repeated in the presence of dithiothreitol. Interestingly, other thiol-containing amines in our library did not inhibit the enzyme.

In summary, we have developed an expeditious route to milligram quantities of a broad variety of β-amino alcohols using commercially available epoxides and amines. The pool size was deliberately kept conservative in order to minimise problems of differing amine reactivity and also to simplify deconvolution. The use of solution-phase chemistry enables us to employ many monofunctional epoxides and amines which do not possess a "handle" for resin attachment.

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References and Notes

1. For excellent reviews, see *inter alia*: Balkenhohl, F.; von dem Bussche-Hünnefeld, C.; Lansky, A.; Zechel, C. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2289; *Acc. Chem. Res.* **1996**, *29*, issue #3; *Bioorg. Med. Chem.* **1996**, *4*, issue #5.

2. For examples, see: Smith, P. W.; Lai, J. Y. Q.; Whittington, A. R.; Cox, B.; Houston, J. G.; Stylli, C. H.; Banks, M. N.; Tiller, P. R. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2821; Carell, T.; Wintner, E. A.; Sutherland, A. J.; Rebek, J.; Dunayevskiy, Y. M.; Vouros, P. *Chem. & Biol.* **1995**, *2*, 171; Pirrung, M. C.; Chen, J. *J. Am. Chem. Soc.* **1995**, *117*, 1240; Cheng, S.; Comer, D. D.; Williams, J. P.; Myers, P. L.; Boger, D. L.; *J. Am. Chem. Soc.* **1996**, *118*, 2567; Storer, R. *Drug Discovery Today* **1996**, *1*, 248; Bailey, N.; Dean, A. W.; Judd, D. B.; Middlemiss, D.; Storer, R.; Watson, S. P. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1409.

3. The β-amino alcohol functional group is present in many pharmacologically active molecules.

4. For a review, see: Smith, J. G. *Synthesis* **1984**, 629.

5. Posner, G. H.; Rogers, D. Z. *J. Am. Chem. Soc.* **1977**, *99*, 8208.

6. Chini, M.; Crotti, P.; Macchia, F. *J. Org. Chem.* **1991**, *56*, 5939.

7. Recently, lanthanide triflates were also reported to be efficient catalysts for aminolysis of epoxides: Chini, M.; Crotti, P.; Favero, L.; Macchia, F.; Pineschi, M. *Tetrahedron Lett.* **1994**, *35*, 433; Meguro, M.; Asao, N.; Yamamoto, Y. *J. Chem. Soc. Perkin Trans I* **1994**, 2597.

8. With acetonitrile as solvent, ring-opening by an S_N1 pathway is favoured.

9. Notable exceptions were epoxides derived from medium-sized cycloalkenes e.g. cyclooctene oxide and caryophyllene oxide. Similar observations on lack of reactivity were noted in reference 5.

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