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A New Diketopiperazine tetra-Carboxylic Acid as Template for the Homogeneous Phase Synthesis of Chemical Libraries

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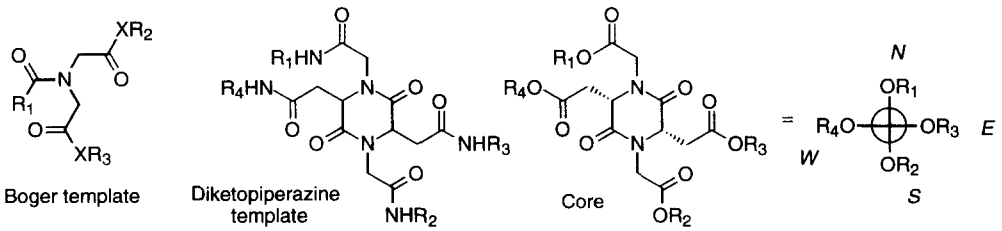
Abstract: The synthesis of a tetra-functionalized template based on a diketopiperazine skeleton is described together with some protocols for the synthesis of families of diversomers using a parallel synthesis approach. © 1997 Elsevier Science Ltd.

Combinatorial chemical libraries are currently employed for the discovery of new products especially in the field of drug development.[1] Protocols for the preparation of libraries using solid-phase synthesis have emerged as the most versatile,[2] while solution-phase syntheses have not been widely considered as a practicable alternative.

Nevertheless the ideal combinatorial library of valuable chemicals would contain considerable amounts of small organic molecules (possibly drug oriented) lending themselves to rapid screening and structure determination.[3]

This target might be prepared by making a rigid core molecule carrying different functional groups, with well defined stereochemistry and stereorientation, that could be subsequently functionalized with different building blocks to generate a library of molecular diversomers. This original idea was first proposed by Nicolau and Hirschmann,[4] Hirschmann[5] and Rebeck Jr.[3] and further exploited by others.[6] Very recently Boger[7] refined this approach proposing a solution-phase strategy leading to libraries of threefold functionalized compounds.

We describe here the synthesis of a tetra-functionalized template based on a diketopiperazine skeleton that can be employed to prepare libraries of small and medium size organic molecules using the parallel or the split and recombine strategy in homogeneous solution.[8]

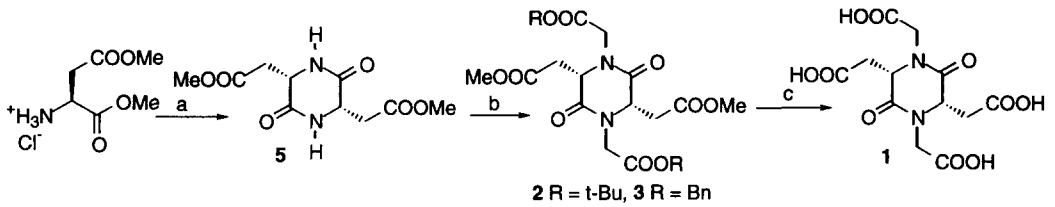


1 $R_1 = R_2 = R_3 = R_4 = H$.
2 $R_1 = R_2$ = t-Bu; $R_3 = R_4$ = Me.
3 $R_1 = R_2$ = Bn; $R_3 = R_4$ = Me.
4 $R_1 = R_2$ = t-Bu; R_3 = Me R_4 = Bn

Scheme 1

The diketopiperazine core should present the conformational constraint required for a ligand as the four carboxylic group should be sufficiently flexible to accommodate the installed groups inside the enzyme or the receptor pockets.[9]

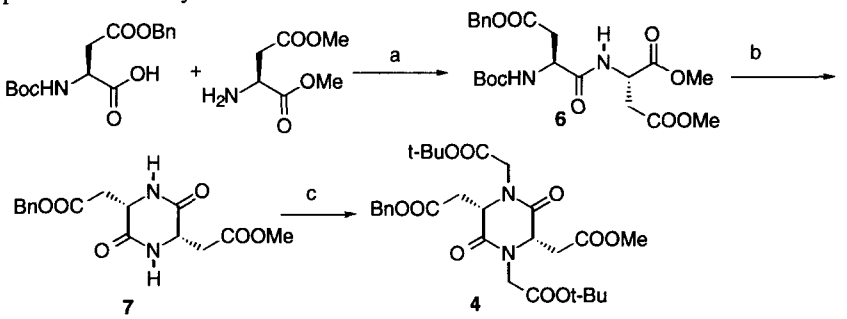
The syntheses of the tetra-acid **1** and the orthogonally protected derivatives **2** and **3** were carried out starting from compound **5**, prepared from aspartic acid dimethyl ester following the Fisher procedure.[10] Temperature controlled slow cyclisation followed by crystallisation from acetone gave the best yields of **5** (25%). Although low yielding, this procedure is recommended as it is simple and starts from inexpensive material. Compound **5** was further alkylated with *tert*-butyl iodoacetate or benzyl bromoacetate in DMF in the presence of Ag_2O to give esters **2** and **3** respectively in 68 and 75% yield.



a. i. NH_3 (gas), $CHCl_3$. ii. 65°C, 5 days. b. $ICH_2COOt\text{-}Bu$ (or $BrCH_2COOBn$), Ag_2O, DMF.
c. HCl in EtOAc followed by KOH in MeOH for **2** or H_2 Pd/C followed by KOH in MeOH for **3**.

Scheme 2

The NS/E/W[11] orthogonally protected compound **4** was prepared starting from differently protected aspartic acid (Scheme 3). The dipeptide **6** was deprotected with $HCl_{(g)}$ in EtOAc and the free base, obtained after treatment of the hydrochloride with an ion exchange resin, cyclised in the absence of solvent at 65°C for 5 days to give product **7** in 45% yield.



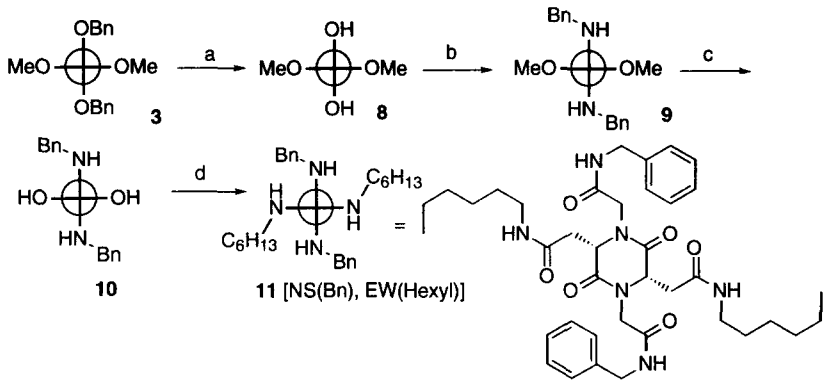
a. i-BuOCOCl, NMM. b. HCl (g) EtOAc, Amberlite IRA-400 (OH) then 65°C 5 days. c. ICH2COOt-Bu, DMF, Ag_2O

Scheme 3

Both these procedures require only one purification by flash chromatography (purification of the tetra-esters **2, 3** or **4**) so that this protocol was successfully applied to a 10 g scale synthesis. Products **1-4** resulted diastereoisomerically (^1H and ^{13}C NMR analysis, 300 MHz) and enantiomerically pure (^1H NMR analysis, 300 MHz in the presence of $Eufod_3$).

Different procedures can be employed to obtain different families of diversomers starting from compounds **1-4.** The uncontrolled reaction of tetra-acid **1** with different amines, although possible, was not attempted for the expected problems of validation of the obtained mixtures.[12]

The homo NS/EW protected esters **2** and **3** were first employed for a two-fold permutational synthesis in the model reaction reported in scheme 4.



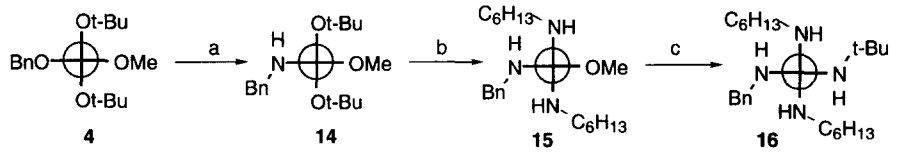
a. H_2, Pd/C 10%, MeOH. b. EtOCOCl, NMM, $BnNH_2$. c. NaOH 2N in MeOH followed by HCl 37%.
d. *i*-BuOCOC l, NMM, $C_6H_{13}NH_2$.

Scheme 4

The best order of events was: deprotection at NS, coupling with amines, deprotection at EW and coupling with a second sets of amines. The problems arose from finding the best reaction conditions to realise a high throughput organic synthesis.

Diacid **8** was obtained in higher yields and purity removing the benzyl ester **3** using H_2/Pd on charcoal. The crude NS acid **8** was reacted with 3 eq of benzylamine using the mixed anhydride technique (with ethyl chloroformate) in THF to give diamide **9** which was directly saponified to diacid **10** which crystallyzed directly in the reaction medium (aqueous) and could be recovered in high yield by simple filtration.[13] This step of purification of the diacid is indispensable to separate **10** from by-products of the first coupling. Diacid **10** reacted with isobutyl chloroformate in dry DMF at -10°C and after 30' at this temperature, 3 eq of hexylamine were added. After stirring for 12 h at room temperature, DMF was removed under vacuum, substituted with $CHCl_3$ and the organic layer purified by subsequent washing with acidic and basic solutions. Finally product **11** was isolated by simple evaporation of the chloroform. This procedure afforded pure **11** (^{1}H NMR analysis, 300 MHz) in high yield following a "robot-like" procedure.[14]

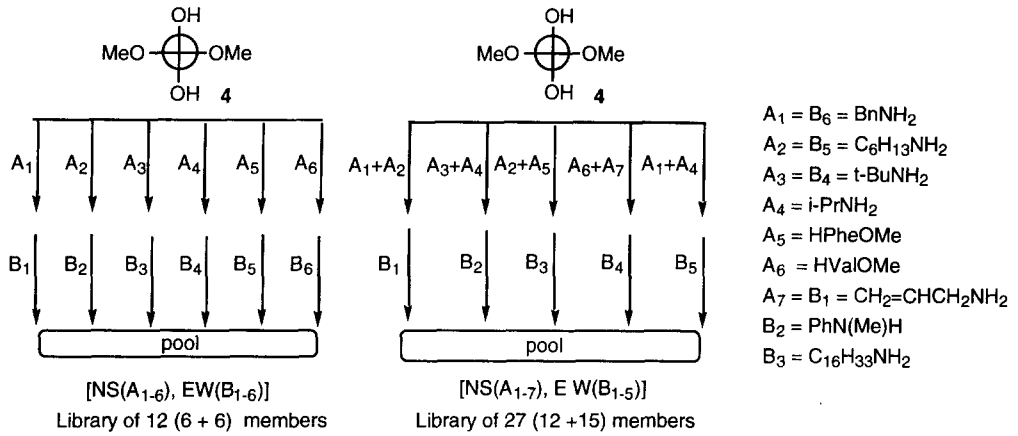
A model reaction was also carried out on template **4** as described in scheme 5. We found that the best approach was: W deprotection with H_2 and Pd/C, coupling with a nucleophile in the presence of ethyl chloroformate, followed by NS deprotection in acidic medium and coupling with an amine always in the presence of ethyl chloroformate and finally E deprotection and coupling in the presence of isobutyl chloroformate in DMF.



a. *i.* H_2, Pd/C, MeOH. *ii.* EtOCOCl, NMM, THF, -15°C, $BnNH_2$. b. *i.* HCl/EtAc *ii.* EtOCOCl, NMM, DMF, -15°C, $BnNH_2$. c. *i.* NaOH 2N, MeOH . *ii.* HCl 37%. *iii.* i-BuOCOCl, NMM, DMF, -15°C, t-$BuNH_2$

Scheme 5

We apply this procedure to a simple parallel synthesis of a 6 + 6 library and to a more diversified library of 27 individual components, after a full mix of the two steps, reacting the diacid **4** with two different amines. (Scheme 6) Validation of the libraries were done by simple TLC and NMR techniques before pool.



Scheme 6

A possible improvement of the diversity could be realised by saponification of methyl ester of amino acid derivatives [NS(A_5), EW(B_5)] and [NS(A_6), EW(B_6)] to give a diacid that can be further functionalized with amines to duplicate the number of products obtained by this route.

We have prepared a simple polyfunctionalized scaffold for liquid-phase synthesis of libraries of small (and medium size) organic molecules in 0.1 g and more scale, employing simple starting material, cheap reagents and a very simple protocol of manipulation that can be, eventually, automated. Studies directed towards finding a validation method for a mixing approach as the use of this scaffold for synthesis of dendrimer-like structures are currently underway in our laboratory.

References and notes

1. a) Mitchison, T.J. *Chem. Biol.* **1994**, *1*, 3. b) Terret, N.K.; Gardner, M.; Gordon, D.W.; Kobylecki, R.J.; Steele, J. *Tetrahedron* **1995**, *51*, 8135. c) Barret, R.W.; Dower W.J.; Fodor, S.P.A.; Gordon, E.M.; Gallop, M.A. *J. Med. Chem* **1994**, *37*, 1233 and 1385. Rohr, J. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 881.
2. Burgess, K. Liaw, A.I.; Wang, N. *J. Med. Chem.* **1994**, *37*, 2985. Floyd, D.; Lewis, C.N.; Whittaker, M.; *Chem. Brit.* **1996**, *32*, 31.
3. Carrel, T.; Wintner, E. A.; Sutherland, A.J. Rebek Jr. J.; Dunayevskiy, Y.M.; *Chem Biol.* **1995**, *2*, 171.
4. Nicolau, K.C.; Salvino, J.M.; Raynor, K.; Pietranico, S.; Reisine, T.; Freidinger, R.M.; Hirschmann; R. *Peptides*, **1989**, 881.
5. Hirschmann, R.; Sprengeler, P.A.; Kawasaki, T.; Leahy, J.W.; Shakespeare, W.C., Smith, A.B. III *Tetrahedron* **1993**, *49*, 3665.
6. Martin, E.J.; Blaney, J.M.; Siani, M.A.; Spellmeyer, D.C.; Wong, A.K.; Moos, W.H. *J.Med. Chem.* **1995**, *38*, 1431. Williard, R.; Jammalamadaka, V.; Zava,, D.; Benz, C.C.; Hunt, C.A.; KushnerP.J.; Scanlan, T.S. *Chem. Biol.* **1995**, *2*, 45.
7. Cheng, S.; Comer, D.D.; Williams, J.P.; Myers, P.L; Boger, D.L. *J. Am. Chem. Soc.* **1996**, *118*, 2567.
8. Although possible in principle, in this communication we do not report on the application of this template to a split and recombine strategy.
9. Our diketopiperazine template should permit the creation of a *population of semirigid molecular arrays, comprising structural families that collectively sample as completely as possible all regions of conformational space* (E.M. Gordon in ref. 1c).
10. Fisher, E.; Koenigs, E.; *Chem. Ber.* **1907**, *40*, 2048
11. The four carboxylic groups of template were named as the four cardinal points and their symbol is used through this communication
12. The authors don't have the facility of a MS-MS high resolution spectrometer employed in similar cases.
13. The purification can be accomplished also in a sealed vial by aspiration of the solvent using a syringe and subsequent cycles of washing and syringe aspiration.
14. We noted sometimes that, at the TLC analysis, traces of by-products were present. Even with major changes of the procedure we were not able to avoid their incidental formation.

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