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LETTERS

Solid-Phase Synthesis of Diketopiperazines, Useful Scaffolds for Combinatorial Chemistry[1]

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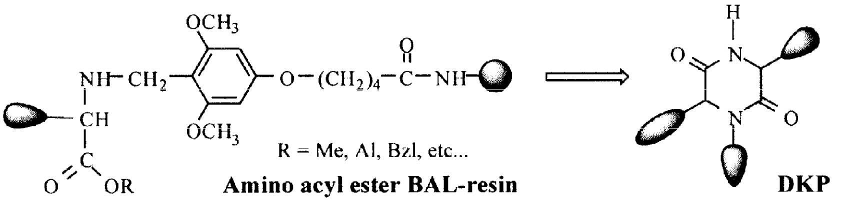
Abstract : Diketopiperazines, which are cyclic dipeptides, are often formed by a side reaction of solid-phase peptide synthesis. Using the new "Backbone Amide Linker," this chemistry can be conveniently harnessed for the intentional preparation of diketopiperazines. These products will be useful scaffolds for combinatorial chemistry, since they incorporate three different points of diversity: both amino acid side-chains and one (of the two) amide bonds. © 1998 Elsevier Science Ltd. All rights reserved.

One current strategy for the design of new drugs consists of the display, on a suitable rigid scaffold, of functional groups that are known to be involved in interactions with the corresponding receptor. To further speed up drug discovery, many modern programs of medicinal chemistry exploit combinatorial methods, i.e., the simultaneous or parallel synthesis of libraries, and their subsequent screening.[3] Preparations of libraries may be conducted in solution or in the solid-phase mode, with the latter often preferred for reasons that are precedented in syntheses of biomolecules such as peptides and oligonucleotides.[4] It is imperative that solid-phase methods be extended to cover preparations of appropriate scaffolds, in ways that at least two reactive points for introducing diversity are included in the synthetic schemes.

Diketopiperazines (2,5-piperazinediones, DKPs), which are the smallest cyclic peptides, are a common motif in several natural products with therapeutic properties. Included are inhibitors of the mammalian cell cycle,[5] of plasminogen activator-1,[6] and of topoisomerase I,[7] as well as competitive antagonists to Substance P at the neurokinin-1 receptor.[8] Furthermore, DKPs have been shown to be useful scaffolds for rational design of several drugs.[9] The present paper reports on a novel and general method for intentional solid-phase DKP synthesis.[10] These DKPs incorporate three different points of diversity: both amino acid side-chains and one (of the two) amide bonds.



DKPs have been observed as unwanted side products, arising from intramolecular aminolysis at the dipeptidyl level, in both solution and solid-phase stepwise peptide synthesis.[11] DKP formation has been established to be either acid[11b,e] or base catalyzed,[11a,c-g] and is strongly dependent on the nature and the

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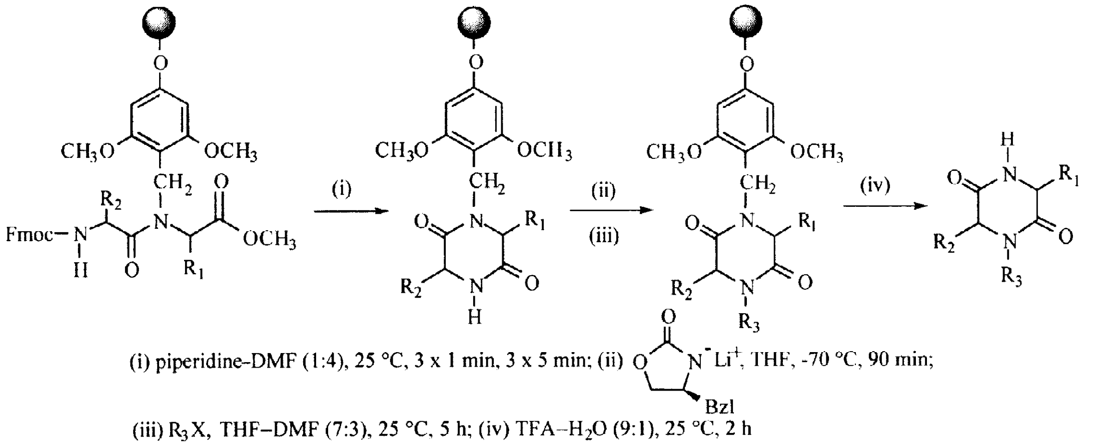
sequence of the amino acids. Thus, the reaction is favoured by the presence, in either the first or second positions, of amino acids (Gly, Pro, or *N*-alkyl) that can easily adopt a *cis*-configuration in the resulting amide bond. Another favorable combination is to have one L- and one D-amino acid in the dipeptide, due to the minimal steric interference between the two side-chains.[4c,11b] Furthermore, rates of DKP formation differ considerably depending on the *C*-carboxyl ester protecting groups, or the structures of the peptide-resin linkages in the solid-phase mode.[11a,e,f,h,i] Several methods which minimize or avoid completely this side reaction are described in the literature.[11b,g,h,12]

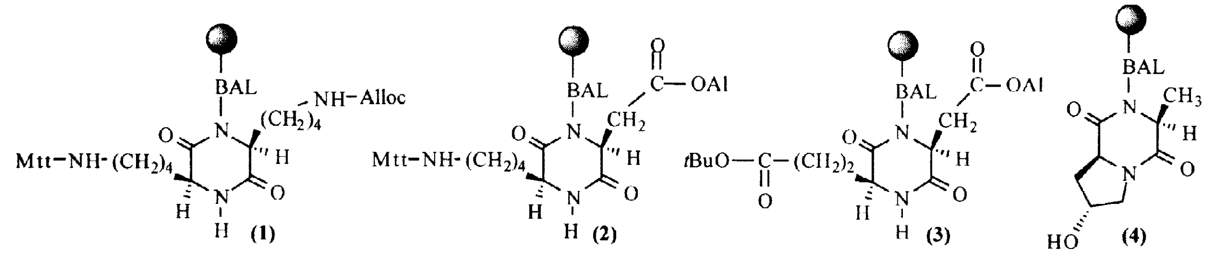
We have described recently the Backbone Amide Linker (BAL) approach for fluorenylmethoxycarbonyl (Fmoc) solid-phase peptide synthesis, whereby the growing peptide is anchored through a backbone nitrogen instead of the *C*-terminal carboxyl.[13] In working with BAL-anchored amino acyl allyl and *n*-alkyl esters towards the goal of preparing cyclic and *n*-alkyl ester peptides, respectively, we observed an almost quantitative DKP side reaction during Fmoc group removal at the dipeptidyl level.[14,15] The DKPs thus formed remain covalently attached to the solid support, and can be released later by trifluoroacetic acid (TFA). However, in the present work we take intentional advantage of the facile formation of DKPs to prepare resin-bound as well as soluble DKPs.



(i) piperidine-DMF (1:4), 25 °C, 3 x 1 min, 3 x 5 min; (ii) $N^{-}Li^{+}$, THF, -70 °C, 90 min; (iii) R_3X, THF–DMF (7:3), 25 °C, 5 h; (iv) TFA–H_2O (9:1), 25 °C, 2 h

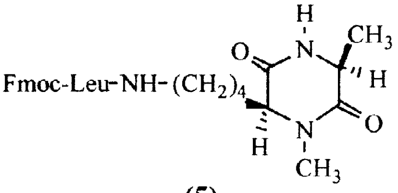
Our strategy starts by addition of 5-(4-formyl-3,5-dimethoxyphenoxy)valeric acid (PALdehyde)[16] to an amino-functionalized solid support (PS or PEG-PS) *via* HATU/DIEA coupling,[17] followed by on-resin reductive amination using the appropriate amino acid methyl ester hydrochloride salts[18] and $NaBH_3CN$ in DMF.[19] Acylation of the secondary α-amino group attached to the BAL-resin was found to be slower than comparable reactions of primary amines. High yields for acylation were obtained by applying HATU/DIEA in CH_2Cl_2-DMF (9:1).[20] Next, treatment of the Fmoc-dipeptidyl BAL-resin with piperidine–DMF (1:4)[21] led to essentially quantitative formation of DKP-BAL-resin.[22] More diversity could be introduced by alkylation of the DKP amide bond;[23] such a step should be performed before any further manipulations of amino acid side-chains that involve the formation of an additional amide bond (e.g., by acylation of an ε-amino function to provide compound **5** shown on the next page). Finally, DKPs were liberated into solution by treatment with TFA–H_2O (9:1).

Representative resin-bound DKPs synthesized using this strategy include some with orthogonally protected side-chains.[24] Thus, DKP (**1**) contains two Lys with ε-amino groups protected by allyloxycarbonyl (Alloc) and 4-methyltrityl (Mtt) groups, respectively. DKP (**2**) contains a carboxyl group from an Asp residue protected by an allyl (Al) group, and an amino function protected with Mtt. DKP (**3**) contains two carboxyl groups from Asp and Glu residues protected by Al and *t*-butyl (*t*-Bu) groups, respectively. Finally, DKP (**4**) contains a hydroxyproline moiety, which confers more rigidity to the scaffold. Resins **1-4** were cleaved with TFA to give the corresponding DKPs in solution (retaining acid-stable allyl protection); these were characterized further by analytical HPLC[25] and showed the correct mass upon MALDI-TOF.

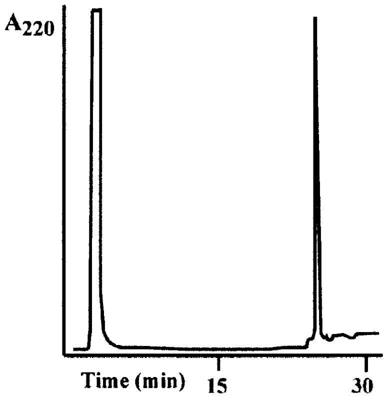


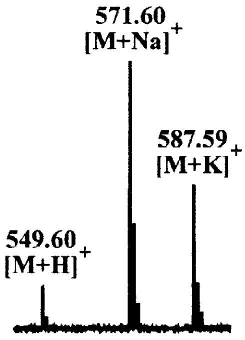
(1) (2) (3) (4)

As a further example, DKP (**5**) was synthesized incorporating Ala and Lys(Mtt) at the first and second positions, respectively. After cyclization, the amide bond was alkylated with CH_3I,[23] the Mtt group was removed selectively with TFA-CH_2Cl_2 (1:99), and Fmoc-Leu-OH was added using HATU/DIEA coupling. TFA cleavage released material with excellent HPLC[25] and MALDI-TOF characteristics, as shown below.



(5)





In conclusion, a strategy has been developed using backbone amide (BAL) anchoring which allows the preparation of rigid DKP scaffolds bearing two or three points of diversity. BAL is compatible with several orthogonal protecting groups for additional elaboration of side-chains and display of a wide range of pendant functionalities. DKP combinatorial libraries prepared will be suitable for both on- and off-resin screening.

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References and Notes

1. Abbreviations used: AAA, amino acid analysis; Ac_2O, acetic anhydride; Al, allyl; Alloc, allyloxycarbonyl; BAL, backbone amide linker; Bu, butyl; Bzl, benzyl; Ddz, 2-(3,5-dimethoxyphenyl)propyl[2]oxycarbonyl; DIEA, *N,N*-diisopropylethylamine; DKP, diketopiperazine, (2,5-piperazinedione); DMF, *N,N*-dimethylformamide; Fmoc, 9-fluorenylmethoxycarbonyl; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HPLC, high performance liquid chromatography; IRAA, internal reference amino acid; Mtt, 4-methyltrityl; MALDI-TOF, matrix assisted laser desorption ionization, time of flight; PALdehyde, 5-(4-formyl-3,5-dimethoxyphenoxy)valeric acid; PEG-PS, polyethylene glycol-polystyrene graft; PS, polystyrene; PyAOP, 7-azabenzotriazol-1-yl-oxytris(pyrrolidino)phosphonium hexafluorophosphate; TFA, trifluoroacetic acid; THF, tetrahydrofuran; Trt, triphenylmethyl (trityl). Amino acid symbols denote L-configuration unless otherwise noted.
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10. Solid-phase strategies for DKP synthesis have been described previously, but are not as versatile as the strategy shown in the present work. Thus, our strategy allows use of any combination of amino acids, unlike a scheme that starts by anchoring Asp or Glu to an appropriate resin: Krchnák, V.; Weichsel, A.S.; Cabel, D.; Lebl, M. in ref. 4c pp. 99-117, nor is the presence of an *N*-alkylamino acid mandatory to favor the cyclization as described by: Gordon, D.W.; Steele, J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 47-50; Szardenings, A.K.; Burkoth, T.S.; Lu, H.H.; Tien, D.W.; Campbell, D.A. *Tetrahedron* **1997**, *53*, 6573-6593.
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14. Built into the BAL approach (see text structure), the anchored first amino acid ester is *N*-alkylated and therefore more likely to form a DKP at the dipeptidyl ester stage.
15. DKP formation in the BAL methodology can be circumvented by (i) incorporation of the second residue as its N^{α}-Trt or Ddz derivative; (ii) selective removal of Trt or Ddz with dilute TFA solutions; and (iii) incorporation of the third residue as its N^{α}-Fmoc derivative under *in situ* neutralization/coupling conditions mediated by PyAOP in DMF in the presence of DIEA (see ref. 12c and 13).
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17. PALdehyde (4 equiv) and HATU (4 equiv) (Carpino, L.A.; El-Faham, A.; Minor, C.A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201-203) were dissolved in DMF, then DIEA (8 equiv) was added, and after 1 min preactivation, this solution was added to amino-functionalized PS or PEG-PS-resin also containing an IRAA. Coupling was allowed to proceed at 25 °C for 2 h, at which time the resin was negative to the Kaiser ninhydrin test.
18. Methyl ester of all amino acids are commercially available. The same chemistry was also successful for other esters; in particular the rates and yields of DKP formation (step i) were shown to be very similar for methyl, allyl, and benzyl esters.
19. Mixtures of amino acid methyl ester hydrochlorides (10 equiv) and $NaBH_3CN$ (10 equiv) in DMF were added to the PALdehyde-IRAA-resins (1 equiv). Reactions were allowed to proceed for 1 h at 25 °C, after which resins were washed with CH_2Cl_2 and MeOH, and finally dried. These conditions assure complete integrity of the amino acyl chiral center.
20. Fmoc-AA-OHs (10 equiv), HATU (10 equiv), and DIEA (20 equiv) in CH_2Cl_2–DMF (9:1) were added to amino acyl ester BAL-resins for 2 h at 25 °C. After washing with DMF and CH_2Cl_2, couplings were repeated with fresh reagents, again 2 h. The resultant resins were washed with DMF and CH_2Cl_2, then treated with Ac_2O–DMF (1:9, 20 min), and washed with DMF.
21. Treatments with piperidine–DMF (1:4) were followed by washes with DMF. At this stage, the Kaiser ninhydrin test was in all cases negative.
22. Yields were determined to be greater than 95%, as follows: Upon completion of the DKP-forming reaction, a third Fmoc-amino acid was coupled, and resin was subjected to acid hydrolysis followed by AAA. The ratio between this third amino acid and the initial two gave the amount of free amine, and could then be used to calculate the overall yield of DKP formation.
23. This transformation was carried out under Ar atmosphere in a screw-cap tube with a Teflon-lined cap, a sintered glass frit, a stopcock, and a jacket where acetone at -70 °C was circulating for the metalation step. Lithiated oxazolidinone in THF, freshly prepared from 5-phenylmethyl-2-oxazolidinone (10 equiv) plus 2.0 M *n*-BuLi in hexane (10 equiv), was added to the DKP-BAL-resins. After 90 min at -70 °C, the alkylating agent (15 equiv) was added, followed by DMF to reach a final solvent ratio of THF–DMF (7:3). The resin was allowed to warm to 25 °C and after 5 h was filtered and washed with THF, THF-H_2O (1:1), THF, and CH_2Cl_2 (Bunin, B.A.; Ellman, J.A. *J. Am. Chem. Soc.* **1992**, *114*, 10997-10998).
24. An *orthogonal* system is defined as a set of completely independent classes of protecting groups, such that each class of groups can be removed in any order and in the presence of all other class (Barany, G.; Merrifield, R.B. *J. Am. Chem. Soc.* **1977**, *99*, 7363-7365; and ref. 11f).
25. Analytical HPLC was performed using Nucleosil C_{18} reversed-phase columns (0.46 x 25 cm) with linear gradients of 0.1% TFA in CH_3CN and 0.1% aqueous TFA, from 1:9 to 1:0, over 30 min, run at 1.0 mL/min. UV detection was at 220 nm. All DKPs studied showed purity similar to the example of DKP (**5**).