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SOLID-PHASE SYNTHESIS OF A LIBRARY OF PIPERAZINEDIONES AND DIAZEPINEDIONES VIA KAISER OXIME RESIN

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Abstract: A combinatorial library of piperazinediones has been prepared by automated parallel solid-phase synthesis. The five-step reaction protocol makes use of Kaiser oxime resin, to enable cleavage from the polymeric support concomitant with an intramolecular displacement reaction, under very mild conditions. The methodology was also successfully extended to the preparation of the seven-membered ring homologs, diazepinediones.

Introduction

Piperazinediones (also known as diketopiperazines) have been shown to exhibit a variety of biological activities.[1-3] A common synthesis approach to piperazinediones has been preparation of the appropriate dipeptide, either on solid-phase or in solution, and then cyclization of the dipeptide in solution. Solid-phase mix-and-split syntheses of piperazinedione combinatorial libraries, based on this approach, have been reported.[4,5] The formation of piperazinediones is also a well-known side reaction in solid-phase peptide synthesis, occurring primarily when conformationally-constrained (e.g., proline) or bulky amino acids are attached to the resin, following deprotection of the second amino acid residue.[6,7] Piperazinedione libraries have also been prepared by adaptation of this process;[8-10] in principle, any uncyclized materials should remain attached to the resin. Taylor and coworkers[11] have prepared three simple piperazinediones from Gly, Leu, Val, and Pro, via Kaiser oxime resin. This resin forms an active ester linkage at the point of attachment, which is relatively acid-stable, but is susceptible to cleavage by exposure to nucleophiles. This makes it an attractive support for concomitant cyclization of the dipeptide and cleavage of the piperazinedione product from the resin. In this report, we describe our investigation of the utility and scope of Kaiser oxime for combinatorial synthesis, through the preparation of a library of piperazinediones derived from a structurally diverse collection of amino acid building blocks. As well, we have extended the methodology to a novel preparation of some diazepinedione analogs.

Results and Discussion

Methods: For some of the exploratory and optimization work, solid-phase syntheses were carried out manually, using 6-mL polypropylene filter tubes, and orbital shaking. The large majority of the syntheses were carried out as arrays of 96 via automation, using an Advanced ChemTech Model 496 "Multiple Organic Synthesizer".[12] In our optimized general procedure (Scheme 1), DMF[13] was used as reaction solvent in preference to DCM, to avoid occasional solubility problems; acetic anhydride was added after coupling steps to cap unreacted nucleophilic sites and reduce subsequent side-reactions; and acetic acid was added during the cleavage/cyclization step to enhance the reaction rate.

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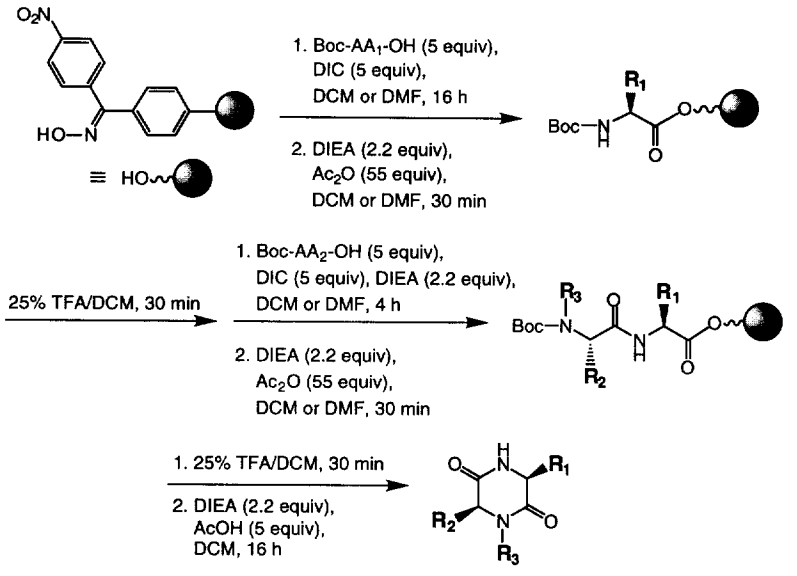


Scheme 1. Piperazinedione Synthesis[13]

Library Design and Analysis: Our library synthesis made use of 75 structurally diverse *N*-Boc-α-amino acids; this describes a virtual library of $(n^2 + n)/2 = 2{,}850$ possible piperazinediones. This set of amino acid building blocks included both D and L isomers, natural and unnatural amino acids, and *N*-methyl derivatives. More than 1,000 different syntheses were carried out, and all reaction products were characterized for purity and identity by HPLC/UV/ELSD and LC/MS analyses.[14] Approximately 600 piperazinediones were considered to meet acceptable criteria for biological screening; all of these samples contained the desired product as the major component, and most of these had >70% purity based on HPLC/ELSD. Yields of dried crude products, determined by using a robotic vial-handling weigh system, were generally 25–50%. Representative products were purified and fully characterized by NMR and MS. Some examples of product analyses are provided in Table 1.

Observations: According to our investigation of more than 1,000 examples, automated synthesis via Kaiser oxime resin successfully provided the desired product for a wide variety of amino acid building blocks, but the method was not without some limitations. Some general observations regarding the piperazinedione synthesis can be made; however, it should be noted that these generalizations may be skewed by the particular combinations of AA_1 and AA_2 that were investigated in our studies. The success of piperazinedione product formation appeared to be independent of amino acid chirality, but was strongly dependent on the side-chain group, as follows:

Effect of the first amino acid in the sequence (AA_1, Scheme 1): generally good results were obtained when Val, Ile, Leu, Phe, Trp, Ser(Bzl), Thr(Bzl), Cys(Bzl), Tyr(Bzl), Lys(Z), Lys(For), Asp(Bzl), or Glu(Bzl) was used as AA_1 in the reaction sequence; however, poor results were often obtained for Ala, Cha, Asn, Gln, Arg(NO_2), Arg(Z,Z), and constrained amino acids (i.e., Pro, Pip, Thz, Tic, Oic). Some of these cases may be due to premature cyclization and cleavage from the resin upon removal of the *N*-Boc protecting group from the second amino acid residue, followed by loss of product during the resin washing steps.[6,7]

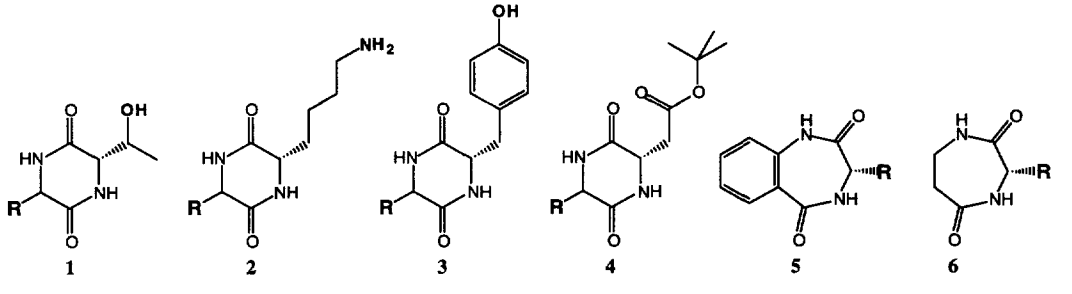
Table 1. Examples of Analyses of Piperazinedione and Diazepinedione Products

AA_1	AA_2	Cyclic product HPLC retention time (min)[a]	Purity(%)[a]
Cys(Acm)	Trp	4.5	92
D-Leu	Trp	6.2	96
D-Leu	MePhe	6.7	99
Met(O_2)	MePhe	4.6	92
Phe(NO_2)	MeLeu	7.5	99
Phg	Tic	7.4	73
D-Met	Oic	6.0	91
D-Phe	Thr(*O*-*t*Bu)[b]	3.6	42
D-Phe	Tyr(*O*-*t*Bu)[b]	4.9	83
Met	Tyr(*O*-*t*Bu)[b]	3.5	70
Glu(Bzl)	Tyr(*O*-*t*Bu)[b]	6.0	80
Cha	Lys(ε-*N*-Boc)[b]	5.4	74
Asp(Bzl)	2-Abz[c,d]	7.6	99
Trp	β-Ala[c]	6.3	72
Tyr(Bzl)	β-Ala[c]	7.2	59

Except as noted, the synthesis of each of these examples was carried out via automation, and the major component in the product sample was the desired piperazinedione or diazepinedione, based on an assessment of HPLC/UV/ELSD and LC/MS analyses.[14] AA_1 and AA_2 refer to the building blocks used, as in Scheme 1. [a]Based on HPLC/ELSD analysis; [b]the major cyclic product lacks the side-chain *t*Bu or Boc protecting group, as anticipated; [c]the product is a diazepinedione; [d]prepared by manual solid-phase synthesis (20% isolated yield).

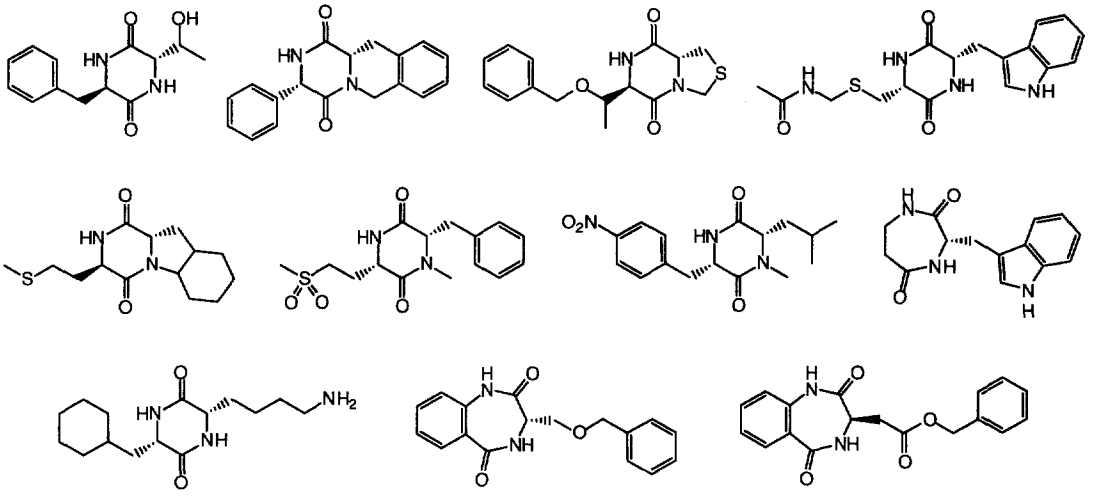
Effect of the second amino acid in the sequence (AA_2, Scheme 1): best results were obtained with Phg, Tyr(Bzl), Glu(Bzl), Tic, Oic, and, in particular, any *N*-methyl-amino acid as the second amino acid; poor results were obtained with Asn, Gln, Idc, Thz, and His(Bzl).

Use of Acid-labile Protecting Groups: As a variation of our piperazinedione synthesis, amino acids such as Asp(β-*O*-*t*Bu), Thr(*O*-*t*Bu), Lys(ε-*N*-Boc), and Tyr(*O*-*t*Bu) were incorporated in the second coupling step. It was considered that the side-chain acid-labile protecting groups in these cases would be cleaved in the penultimate step, and that subsequent cyclization to form the piperazinedione would occur in preference to any reaction through the side-chain functionality. This approach was generally successful, and several products of the type **1** – **3** shown below were obtained (Table 1). Interestingly, in the case of Asp(β-*O*-*t*Bu), the side-chain ester remained mostly intact, providing products of type **4**.



Diazepinediones: As a further study of the utility of oxime resin for combinatorial library synthesis, we extended the synthesis protocol to the preparation of the seven-membered ring analogs, benzodiazepinediones (**5**). Eight derivatives **5** were successfully prepared by manual solid-phase synthesis using *N*-Boc-2-aminobenzoic acid as Boc-AA_2-OH (Scheme 1), from 12 examples attempted (Table 1). Interestingly, three examples of diazepinediones (**6**) were also successfully obtained by using an unconstrained β-amino acid (β-alanine) as AA_2, and L-Trp, L-Tyr(Bzl), and L-Lys(Z) as AA_1 (Table 1). Alternative methods for the solid-phase synthesis of benzodiazepinediones (**5**) have been recently described in the literature,[15–17] whereas β-alanine-derived diazepinediones (**6**) appear to be almost unprecedented.[18]

Conclusions: A combinatorial library of several hundred piperazinediones was successfully prepared via parallel solid-phase synthesis, primarily via automation. The library synthesis is distinguished from other reported piperazinedione library syntheses in that Kaiser oxime resin was utilized, allowing cyclization to occur concomitant with cleavage from the resin under very mild conditions. The reaction protocol was also successfully applied to the preparation of a small set of the diazepinedione homologs.



Examples of Library Members Prepared

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13. Abbreviations: DMF, *N,N*-dimethyl-formamide; DCM, dichloromethane; DIC, diisopropylcarbodiimide; DIEA, diisopropyl-ethylamine; TFA, trifluoroacetic acid; Boc-AA1-OH, *N-tert*-butoxycarbonyl-(amino acid #1); Bzl, benzyl; Z, carbobenzyloxy; For, formyl; Ser(Bzl), *O*-benzyl serine; Asp(Bzl), aspartic acid β-benzyl ester; Cha, cyclohexylalanine; Arg(NO_2), *N*γ-nitro-arginine; Pip, pipecolic acid (homoproline); Thz, thiazolidine-4-carboxylic acid (thioproline); Tic, tetrahydro-isoquinoline-carboxylic acid; Oic, octahydroindole-2-carboxylic acid; Phg, phenylglycine; Idc, indoline-2-carboxylic acid; Asp(β-*O*-tBu), aspartic acid β-*tert*-butyl ester; Thr(*O*-*t*Bu), *O-tert*-butyl-threonine; Lys(ε-*N*-Boc), *N*ε-*tert*-butoxycarbonyl-lysine; Tyr(*O*-*t*Bu), *O-tert*-butyl-tyrosine; Cys(Acm), *S*-acetamidomethyl-cysteine; MePhe, *N*-methyl-phenylalanine; Met(O_2), methionine sulfone; Phe(NO_2), *p*-nitro-phenylalanine; 2-Abz, 2-aminobenzoic acid.

14. HPLC/UV/ELSD analyses employed Perkin–Elmer Pecosphere 3X3C C-18 reverse-phase cartridge columns (0.46 × 3.3 cm), at 3.0 mL/min, with an elution gradient of 0–70% aqueous acetonitrile (containing 0.1% TFA) over 7 min, then 70–100% over 0.5 min, then 100% for 2.5 min, with a 5-min equilibration period; UV detection was at 200 nm, and a Sedere Sedex Model 55 was used for evaporative

light-scattering detection (ELSD). In general, the purity based on UV detection (200 nm) was somewhat less than that based on ELSD; however, ELSD is established to be a more "universal" detector, giving near-equivalent response to a variety of compounds (Kibbey, C. E. *Mol. Diversity* **1995**, *1*, 247). A Finnigan LCQ was used for LC/MS analyses.

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18. To our knowledge, only two examples of **6** have been previously reported: (a) **6**, R = H: Hodge, C. A.; Fernandez, C. H.; Jadhav, P. K.; Lam, P. Y.-S. PCT Int. Appl. WO 9 422 840, 1994; (b) **6**, R = CH_2Ph: Day, R. A.; Tharp, R. L.; Madis, M. E.; Wallace, J. A.; Silanee, A.; Hurt, P.; Mastruserio, N. *Pept. Res.* **1990**, *3*, 169.