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Solid phase synthesis of 1,6-disubstituted 2,3-diketopiperazines and 1,2-disubstituted piperazines from *N*-acylated amino acids

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Abstract

The parallel synthesis of 1,6-disubstituted 2,3-diketopiperazines and 1,2-disubstituted piperazines from resin-bound reduced *N*-acylated amino acids is described. © 1999 Elsevier Science Ltd. All rights reserved.

The solid phase synthesis of organic compounds has become a standard tool of the drug discovery process due to the speed and versatility of preparing a large number of structurally diverse compounds for combinatorial libraries.[1] Many natural products containing a diketopiperazine structure have been isolated that encompass a wide range of biological activities.[2] Furthermore, a large number of pharmacologically active molecules contain the piperazine ring, including the antipsychotic agents fluophenazine and clozapine, used in the treatment of schizophrenia, the serotonin (5-HT_{1A}) receptor ligands flesinoxan, buspiridone, tandospirone, and ipsapirone, as anxiolytic agents, and the HIV reverse transcriptase inhibitor ateviridine, for the treatment of AIDS.[3] Herein, we describe an efficient method for the solid phase synthesis of 1,6-disubstituted 2,3-diketopiperazines and 1,2-disubstituted piperazines.

Following Boc deprotection and neutralization from a *p*-methylbenzhydrylamine resin-bound Boc protected amino acid, the free amine was *N*-acylated with a variety of commercially available carboxylic acids in the presence of diisopropylcarbodiimide (DIPCDI) and hydroxybenzotriazole (HOBt). The amide bonds were then reduced to generate two secondary amines[4] which, following treatment with oxalyldiimidazole and hydrogen fluoride (HF) cleavage, provided the desired diketopiperazines in good yields and high purity.[5] Prior to HF cleavage, and expanding our 'libraries from libraries' approach,[6d] the resulting resin-bound diketopiperazine was treated with borane in THF to reduce the oxamide moieties to their corresponding amines. Following HF cleavage, the 1,2-disubstituted piperazines were obtained in good purity. We also investigated the synthesis of piperazines by reductive alkylation of the resin-bound diamines in the presence of glyoxal and sodium cyanoborohydride. Following HF cleavage, the desired product was obtained in lower purity (<40%, Scheme 1: g, h) when compared to the method previously described (Scheme 1: e, g).

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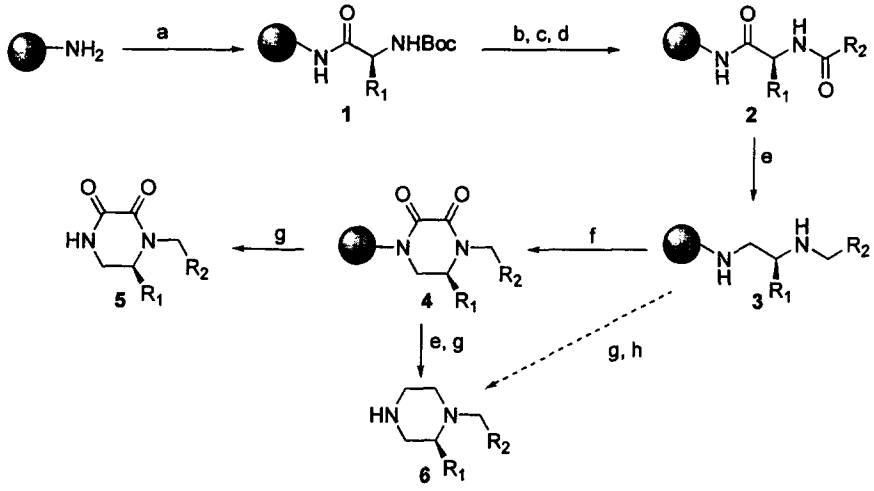

Scheme 1. (a) Boc-Xaa-OH, DIPCDI, HOBt, DMF; (b) 55% TFA in DCM; (c) 5% DIPEA in DCM; (d) R_2COOH, DIPCDI, HOBt, DMF; (e) B_2H_6, THF, 65°C; (f) ImCOCOIm, in DMF; (g) HF/anisole; (h) HCOCHO, $NaBH_3CN$, 1% AcOH in DMF

We initially optimized this approach by the parallel synthesis of 12 different diketopiperazines and piperazines derived from three amino acids (Tyr, Phe and Ala) and four carboxylic acids (phenylacetic acid, acetic acid, cyclohexane carboxylic acid and isobutyric acid). As shown in Table 1, excellent purities were obtained for all cases with yields higher than 80% relative to the initial loading of the resin. Substituents with poor yields and/or purities (<60%) were excluded in the final protocols. Figs. 1 and 2 show the LC-MS spectra of the diketopiperazine **5a** and the corresponding piperazine **6a**, which are representative of the purities obtained. This work exemplifies our ongoing efforts toward the solid-phase

Table 1
Individual 1,6-disubstituted 2,3-diketopiperazines and 1,2-disubstituted piperazines

R_1	R_2	5 (MW found)	6 (MW found)	5 (HPLC purity)	6 (HPLC purity)
$-CH_2-C_6H_4-OH$	$-CH_2-C_6H_5$	325.4 (MH^+)	297.2 (MH^+)	> 95%	>95%
$-CH_2-C_6H_5$	$-CH_2-C_6H_5$	309.3(MH^+)	281.2 (MH^+)	>95%	91%
$-CH_3$	$-CH_2-C_6H_5$	233.2(MH^+)	205.2 (MH^+)	>95%	>95%
$-CH_2-C_6H_4-OH$	$-CH_3$	248.28(MH^+)	221.2 (MH^+)	>95%	>95%
$-CH_2-C_6H_5$	$-CH_3$	233.2(MH^+)	281.2 (MH^+)	>95%	>95%
$-CH_3$	$-CH_3$	157.2(MH^+)	ND	>95%	ND
$-CH_2-C_6H_4-OH$	$-C_6H_{11}$	317.3(MH^+)	289.3 (MH^+)	92%	90%
$-CH_2-C_6H_5$	$-C_6H_{11}$	301.2(MH^+)	273.3 (MH^+)	>95%	90%
$-CH_3$	$-C_6H_{11}$	225.2(MH^+)	197.2 (MH^+)	>95%	92%
$-CH_2-C_6H_4-OH$	$-CH(CH_3)_2$	277.2(MH^+)	249.2 (MH^+)	91%	90%
$-CH_2-C_6H_5$	$-CH(CH_3)_2$	261.2(MH^+)	233.2 (MH^+)	92%	85%
$-CH_3$	$-CH(CH_3)_2$	185.1(MH^+)	157.2 (MH^+)	>95%	90%

The products were run on a Vydac column, gradient 5 to 95% of 0.05% TFA in ACN in 7 min. The purity was estimated on analytical traces at λ= 214 nm. ND: not determined.

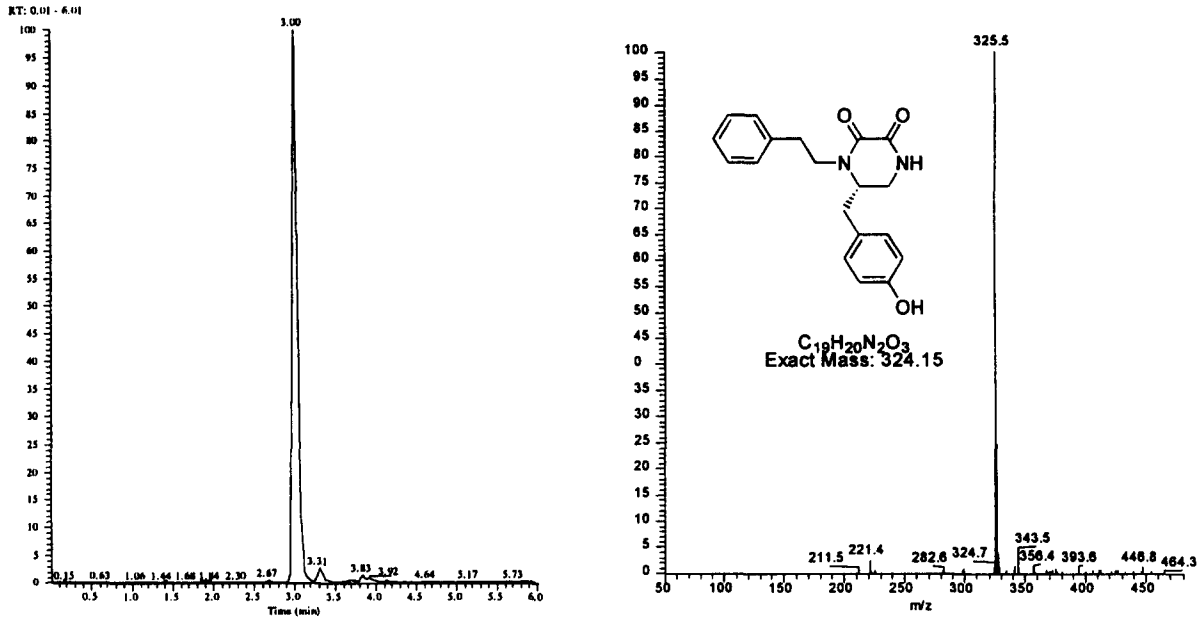

Figure 1. LC-MS of the diketopiperazine **5a** obtained from tyrosine and phenylacetic acid

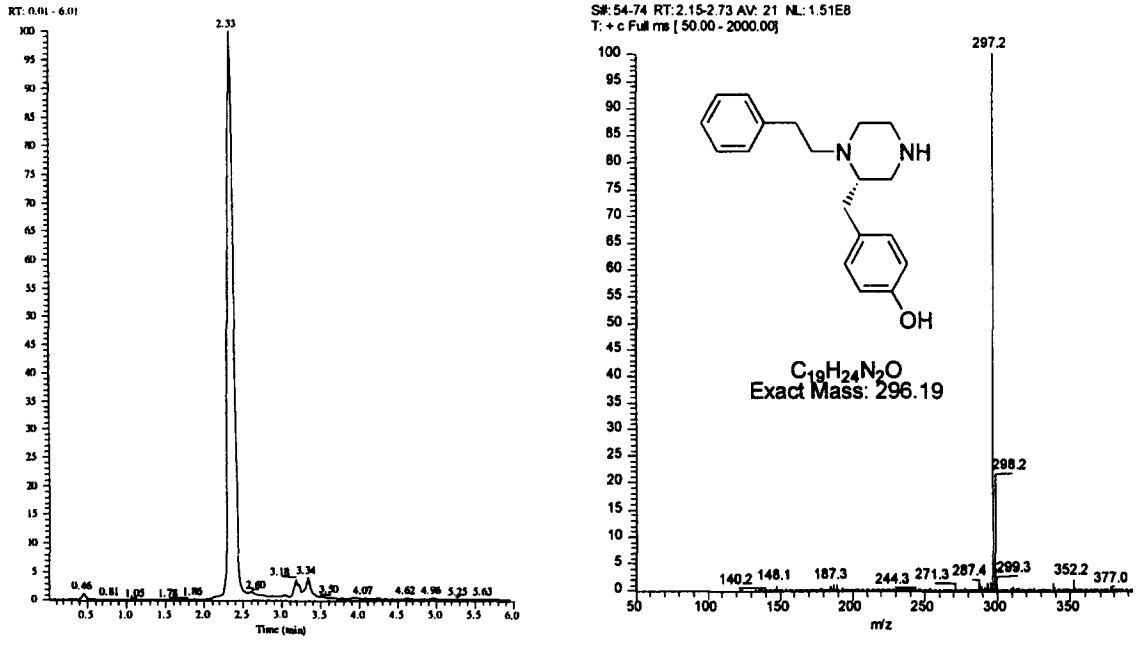

Figure 2. LC-MS of the piperazine **6a** obtained from tyrosine and phenylacetic acid

synthesis of individual acyclic and heterocyclic compounds and combinatorial libraries using resin-bound amino acids and/or short peptides as starting material.[6]

Acknowledgements

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5. General procedure for the solid phase synthesis of 1,6-disubstituted 2,3-diketopiperazines and 1,2-disubstituted piperazines. (1) Amino acid coupling and *N*-acylation: 100 mg *p*-methylbenzydrylamine (MBHA) resin (1 molar equiv./g, 100–200 mesh) was contained within a sealed polypropylene mesh packet. Reactions were carried out in 10 ml polyethylene bottles. Following neutralization with 5% diisopropylethylamine (DIPEA) in dichloromethane (DCM), the resin was washed with DCM. The first amino acid (Boc-Xaa-OH, 6 equiv.) was coupled using the conventional reagents diisopropylcarbodiimide (DIPCDI, 6 equiv.) and hydroxybenzotriazole (HOBt, 6 equiv.) in anhydrous DMF for 60 min. Following removal of the Boc group with 55% TFA in DCM and washing with DCM (5×) and 5% DIPEA in DCM (3×), the amino acid was *N*-acylated with a carboxylic acid (10 equiv.) in the presence of DIPCDI (10 equiv.) and HOBt (10 equiv.) overnight in anhydrous DMF. (2) Exhaustive reduction of the amide groups: The reduction was performed in 50 ml Kimax tubes under nitrogen. Boric acid (40×) and trimethyl borate (40×) were added, followed by 1 M BH_3–THF (40×). The tubes were heated at 65°C for 72 h, followed by quenching with MeOH. The resin was then washed with methanol (4×) and the borane disproportionated by treatment with piperidine at 65°C overnight. The resin was then washed with DMF (6×) and methanol (2×) and dried. (3) Disubstituted diketopiperazine formation: The cyclization occurred following treatment of the resin-bound reduced *N*-acylated amino acid overnight with fivefold excess of oxalyldiimidazole (0.1 M) in anhydrous DMF. Following cleavage from the resin with anhydrous HF in the presence of anisole at 0°C for 90 min, the desired product was extracted with acetonitrile:water (50:50) and lyophilized. (4) Piperazine formation: The resin-bound diketopiperazine was treated in the same conditions described above for the reduction of the amide bonds. Following cleavage from the resin with anhydrous HF in the presence of anisole at 0°C for 7 h, the desired product was extracted with acetonitrile:water (50:50) and lyophilized.
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