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Parallel Solid Phase Synthesis of Tetrasubstituted Diethylenetriamines Via Selective Amide Alkylation and Exhaustive Reduction of N-Acylated Dipeptides

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Solid-phase; reduction; alkylation; peptides; polyamines

Abstract: Polyamines are a rapidly developing area of vital importance to biomedical science. Selective N-alkylation followed by N-terminal acylation and the complete reduction of carbonyl amide bonds enables the preparation by parallel solid phase synthesis of a wide range of N^1,N^5,1,4-tetrasubstituted-1,5-diamino-3-azapentane derivatives.

The rapid parallel synthesis of large numbers of individual compounds is now a widely used means to prepare a variety of compounds having a high degree of structural diversity.[1] A solid phase approach for the generation of a large number of N-methyl and N-benzyl substituted diethylenetriamines is described. Multiple amine functionalities are common in drugs active within the central nervous system. Polyamines have also been shown to be ideally suited to bind to and then condense DNA.[2] Although a number of solution phase approaches for the synthesis of polyamines have been reported, only a limited number of solid-phase strategies are available for this synthesis. The parallel synthesis of tertiary amines has been described by Green using an ester or amide tether to polystyrene resin.[3] Recently, Morphy and co-workers reported the synthesis of a range of tertiary amines using a "traceless" linker on a polystyrene resin (REM resin).[4] The solid phase synthesis of primary amines has also been reported by Katritzky and co-workers via nucleophilic addition of organometallic reagents to aldimines on solid support[5], and recently, the solid phase synthesis of unsymmetrical secondary amines as an application for the synthesis of arylethanolamines and arylpropanolamines has been reported by Purandre.[6]

Herein, we report an efficient approach for the solid phase synthesis of polyamines.[7] Following the complete reduction of all amide bonds, we have been able to generate a wide range of N-methyl and N-benzyl trisubstituted diethylenetriamines from N-acylated dipeptides (Scheme 1).

Starting from p-methylbenzhydrylamine (MBHA) resin-bound N-tert-butyloxycarbonyl (Boc) amino acid **1**, the Boc group is removed using a mixture of trifluoroacetic acid and dichloromethane. The resulting amine salt is neutralized, and the resulting primary amine is then protected with triphenylmethyl chloride (TrtCl). The secondary amide **2** is then selectively alkylated in the presence of lithium t-butoxide and an alkylating reagent

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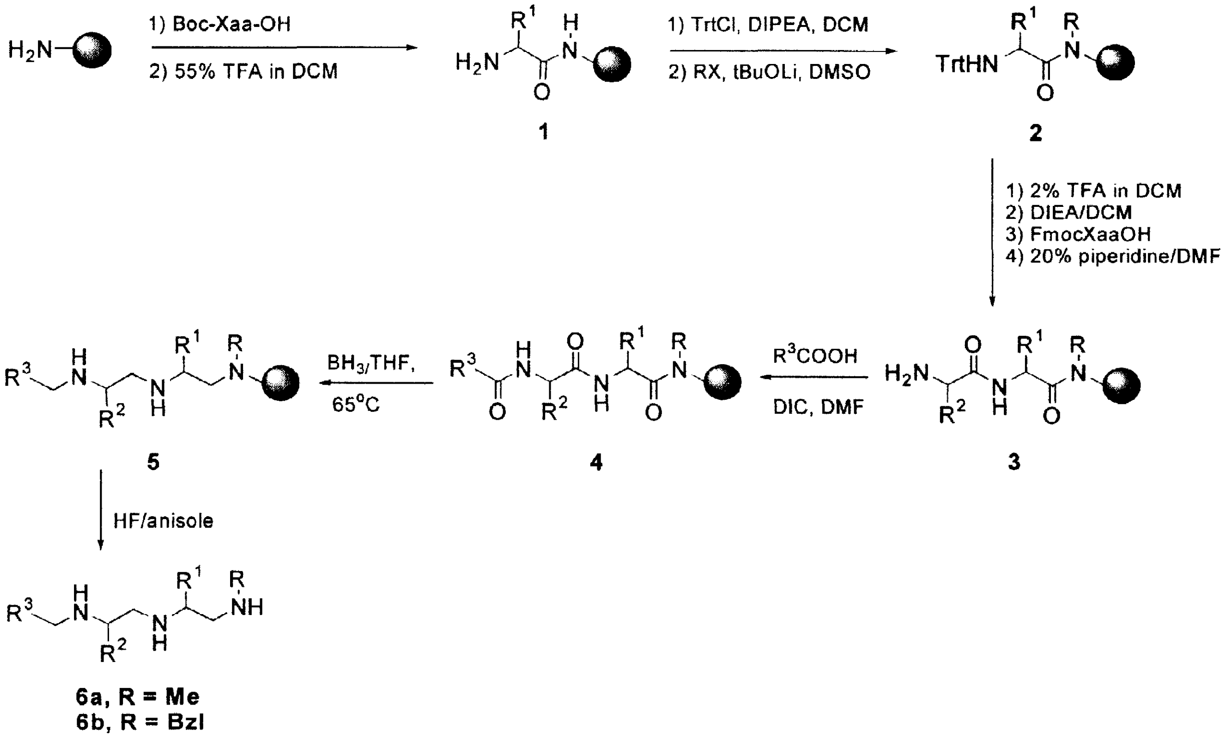


Scheme 1. Solid phase synthesis of N-methyl and N-benzyl diethylenetriamine derivatives.

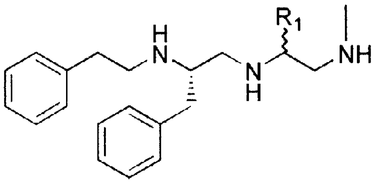
(methyl iodide or benzyl bromide). Potential racemization during N-alkylation has been studied using analytical reverse phase high performance liquid chromatography (RP-HPLC) and capillary electrophoresis. It was found that 0-5% racemization occurred depending on the amino acid involved.

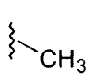
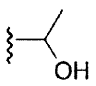
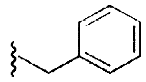
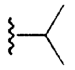
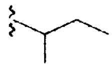
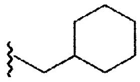
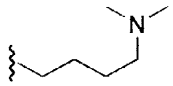
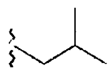
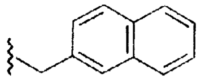
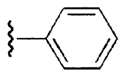
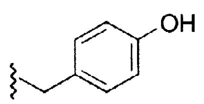
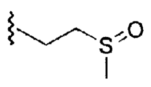
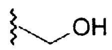
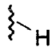
Interestingly, the alkylation of the amide nitrogen of the resin linkage dramatically increases the acid sensitivity of the MBHA resin-bound peptide, excluding the use of Boc-amino acids for the second coupling. Therefore, Fmoc-amino acids were employed in subsequent couplings. Following Fmoc removal and N-acylation of the resin-bound dipeptide **3**, exhaustive reduction of the amide bonds was achieved using borane in tetrahydrofuran[7], with the desired products readily obtained following cleavage of the resin-bound triamine with anhydrous HF. We have successfully used exhaustive alkylation and/or exhaustive reduction of amide groups to generate compounds having completely different physico-chemical properties from the starting peptides (peralkylated peptides, reduced peptides and reduced peralkylated peptides).[8-9]

Ninety-seven different N-benzyl-triamines **6a** (R = Bzl) and 97 different N-methyl triamines **6b** (R = Me) were synthesized using the "T-bag" method of parallel synthesis[1] (Tables 1-4). The individual building blocks were varied while the other two positions remained fixed (Scheme 1, with 29 different functionalities derived from amino acids at R_1, 28 different functionalities derived from amino acids at R_2 and 40 different functionalities derived from carboxylic acids at R_3[10]). These individual compounds served as controls to determine whether the individual building blocks used at each of the variable positions could be successfully incorporated into the synthesis. Modifications to the amino acid side chains during the N-alkylation and reduction steps have been studied. During N-alkylation with methyl iodide and benzyl bromide, the protected N^{ε}-amine of lysine was alkylated, and the Boc protecting group was reduced during the reduction step to yield the corresponding N^{ε},N^{ε}-dimethyl and N^{ε}-benzyl, N^{ε}-methyl-polyamines, respectively.[11] The library synthesis, along with the screening results, will be reported elsewhere.[12]

Recently, the resin-bound polyamine **5** was used as a template for the solid phase synthesis of a range of heterocyclic compounds, such as cyclic ureas and cyclic thioureas.[13] In Fig. 1, the LC-MS spectra of some individual diethylenetriamines representative of the purities obtained for all of the compounds prepared are shown. This work exemplifies our ongoing efforts toward the solid phase synthesis of individual acyclic and heterocyclic compounds and combinatorial libraries using short peptides as starting material.[7,9,13-15]

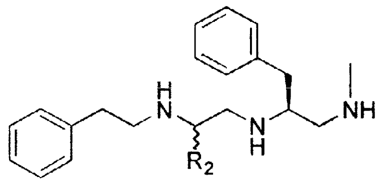
Table 1[17]. RP-HPLC Purity and Masses Found for Compounds Synthesized in which R^1 was Varied[a].

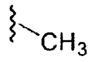
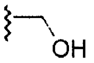
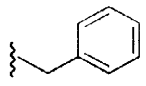
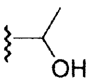
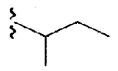
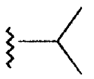
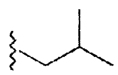
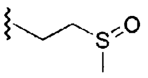
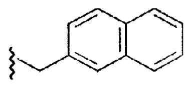
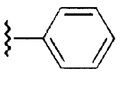
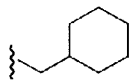
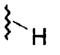
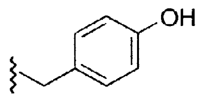


	R^1	HPLC Purity	Masses (found)		R^1	HPLC Purity	Masses (found)
1		85%	326.2 $(M+H)^+$	**9**		>95%	356.2 $(M+H)^+$
2		>95%	402.2 $(M+H)^+$	**10**		92%	354.2 $(M+H)^+$
3		87%	368.2 $(M+H)^+$	**11**		90%	408.3 $(M+H)^+$
4		90%	411.2 $(M+H)^+$	**12**		91%	354.2 $(M+H)^+$
5		94%	368.2 $(M+H)^+$	**13**		>95%	452.2 $(M+H)^+$
6		94%	368.2 $(M+H)^+$	**14**		65%	388.2 $(M+H)^+$ 368.2 $(M+H)^+$-20
7		85%	418.2 $(M+H)^+$	**15**		78%	418.2 $(M+H)^+$ 386.2 $(M+H)^+$-32
8		91%	342.2 $(M+H)^+$	**16**		83%	312.2 $(M+H)^+$ 326.2 $(M+H)^+$+14

[a]For **1-13**, R^1 is derived from the corresponding L-amino acids [9] (similar purities were found for the diastereomers derived from the D-amino acids); for **14** and **15**, R^1 is derived from corresponding L-amino acids; and for **16**, R^1 is derived from glycine (a total of 29 compounds).

Table 2[17]. RP-HPLC Purity and Masses Found for Compounds Synthesized in which R^2 was Varied[a].



	R^2	HPLC Purity	Masses (found)		R^2	HPLC Purity	Masses (found)
17		92%	326.2 (M+H)$^+$	25		85%	342.2 (M+H)$^+$ 326.2 (M+H)$^+$-16
18		>95%	402.2 (M+H)$^+$	26		85%	356.2 (M+H)$^+$
19		>95%	368.2 (M+H)$^+$	27		>95%	354.2 (M+H)$^+$
20		>95%	368.2 (M+H)$^+$	28		>95%	368.2 (M+H)$^+$
21		>95%	354.2 (M+H)$^+$	29		83%	418.2 (M+H)$^+$
22		>95%	452.2 (M+H)$^+$	30		>95%	388.2 (M+H)$^+$ 368.2 (M+H)$^+$-20
23		>95%	408.2 (M+H)$^+$	31		92%	418.2 (M+H)$^+$ 386.2 (M+H)$^+$-32
24		>95%	418.2 (M+H)$^+$				

[a] For **17-28**, R^2 is derived from the corresponding L-amino acids[9] (similar purities were found for the diastereomers derived from the D-amino acids); for **29** and **30**, R^2 is derived from the corresponding L-amino acids; and for **31**, R^2 is derived from glycine (a total of 27 compounds).

Table 3[17]. RP-HPLC Purity and Mass Found for Compound Synthesized when R^2 was Derived from β-alanine.

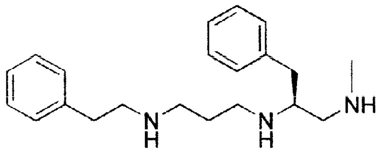
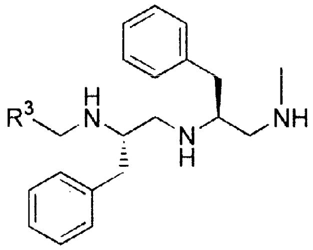
Structure	HPLC Purity	Mass (found)
	89%	326.2 (M+H)$^+$

Table 4[17]. RP-HPLC Purity and Masses Found for Compounds Synthesized in which R^3 was Varied[a].



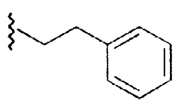
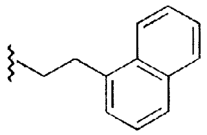
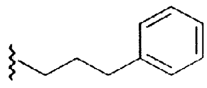
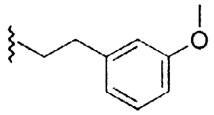
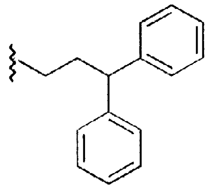
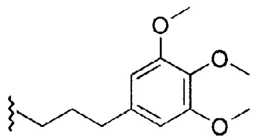
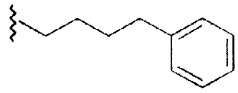
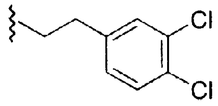
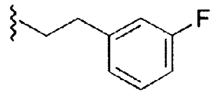
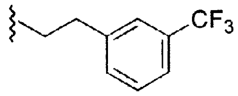
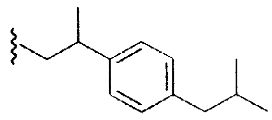
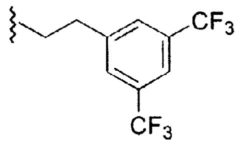
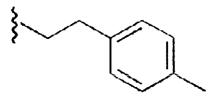
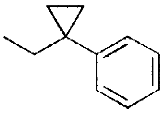
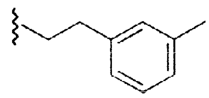
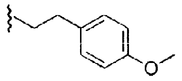
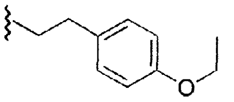
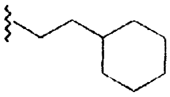
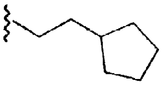
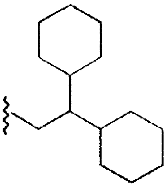
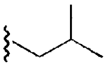
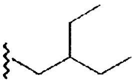
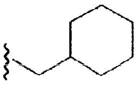
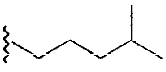
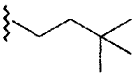
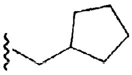
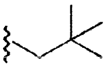
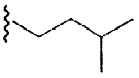
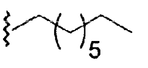
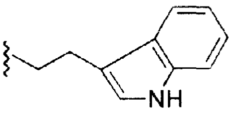
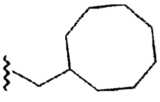
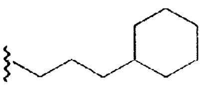
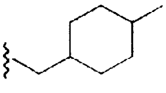
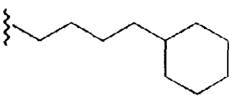
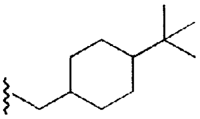
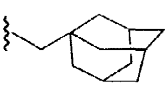
	R^3	HPLC Purity	Masses (found)		R^3	HPLC Purity	Masses (found)
33		>95%	402.2 $(M+H)^+$	**41**		92%	452.2 $(M+H)^+$
34		>95%	416.2 $(M+H)^+$	**42**		>95%	432.2 $(M+H)^+$
35		93%	492.3 $(M+H)^+$	**43**		93%	506.2 $(M+H)^+$
36		>95%	430.2 $(M+H)^+$	**44**		89%	470.2 $(M+H)^+$ 472.1 $(M+H)^+$+2
37		>95%	420.2 $(M+H)^+$	**45**		>95%	470.2 $(M+H)^+$
38		>95%	472.3 $(M+H)^+$	**46**		>95%	538.2 $(M+H)^+$
39		>95%	416.2 $(M+H)^+$	**47**		>95%	428.2 $(M+H)^+$
40		>95%	416.2 $(M+H)^+$	**48**		92%	432.2 $(M+H)^+$

Table 4. (continued)

	R^3	HPLC Purity	Masses (found)		R^3	HPLC Purity	Masses (found)
49		>95%	446.2 $(M+H)^+$	**61**		>95%	408.2 $(M+H)^+$
50		>95%	326.2 $(M+H)^+$	**62**		>95%	394.2 $(M+H)^+$
51		>95%	354.2 $(M+H)^+$	**63**		55%	490.4 $(M+H)^+$ 298.2^a
52		>95%	354.2 $(M+H)^+$	**64**		>95%	382.2 $(M+H)^+$
53		>95%	394.2 $(M+H)^+$	**65**		>95%	382.2 $(M+H)^+$
54		>95%	366.2 $(M+H)^+$	**66**		94%	382.3 $(M+H)^+$
55		>95%	408.2 $(M+H)^+$	**67**		>95%	368.2 $(M+H)^+$
56		>95%	368.2 $(M+H)^+$	**68**		89%	396.2 $(M+H)^+$
57		84%	441.2 $(M+H)^+$ 538.2 $(M+H)^+$+97	**69**		>95%	408.2 $(M+H)^+$
58		>95%	422.3 $(M+H)^+$	**70**		>95%	408.3 $(M+H)^+$
59		>95%	436.2 $(M+H)^+$	**71**		>95%	450.3 $(M+H)^+$
60		92%	420.3 $(M+H)^+$	**72**		92%	420.3 $(M+H)^+$

[a] Second molecular ion is due to incomplete coupling of the carboxylic acid from which R^3 was derived.

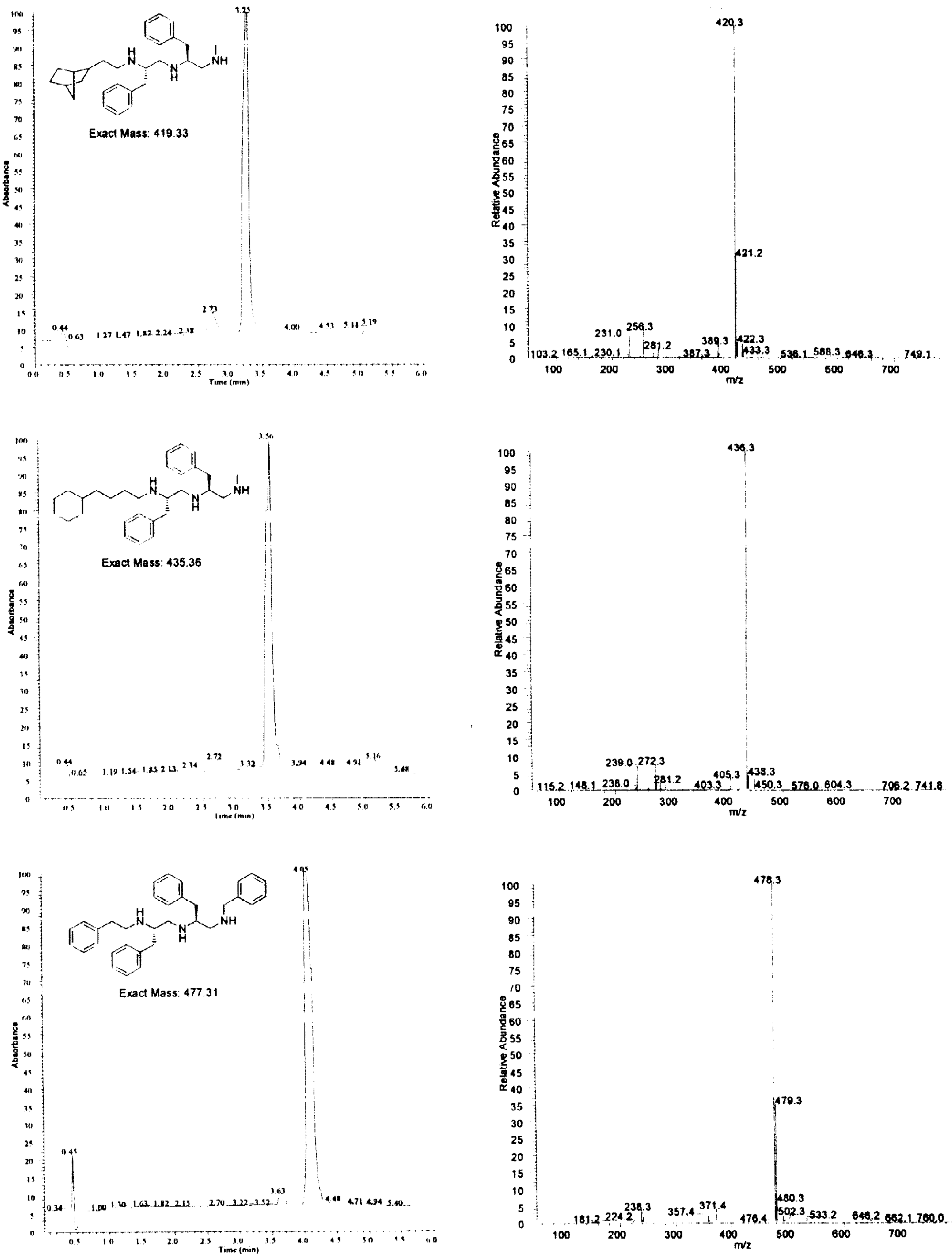


Fig. 1. Representative LC-MS of individual triamines.

EXPERIMENTAL

Boc-and Fmoc-amino acid derivatives and hydroxybenzotriazole (HOBt) were purchased from Calbiochem-Novabiochem Corp. (San Diego, CA), and Bachem Biosciences, Inc. (Philadelphia, PA). MBHA resin, 1% divinylbenzene, 100-200 mesh, 0.81 meq/g substitution, was purchased from Peninsula Laboratories, Inc (Belmont, CA). N,N'-Diisopropylcarbodiimide (DIC) was purchased from Chem Impex International (Wood Dale, IL), trifluoroacetic acid from Halocarbon (River Edge, NJ) and hydrogen fluoride from Air Products (San Marcos, CA). All other reagents and anhydrous solvents (DMSO and THF) were purchased from Aldrich Chemical Company (Milwaukee, WI).

General Procedure for the Solid Phase Synthesis of an Individual Triamine

1. Selective N-alkylation. 100 mg p-methylbenzhydrylamine (MBHA) resin (0.81 meq/g, 100-200 mesh) was contained within a sealed polypropylene mesh packet.[1] Reactions were carried out in a 10 ml polyethylene bottle. Following neutralization with 5% diisopropylethylamine (DIEA) in dichloromethane (DCM), the resin was washed with DCM. The first amino acid (Boc-Xaa-OH) was coupled using the conventional reagents HOBt and DIC. Following removal of the protecting group with 55% TFA in DCM and neutralization with a solution of 5% DIEA in DCM, the mesh packet was shaken overnight in a solution of trityl chloride in DCM/DMF (9:1) in the presence of DIEA. Completeness of the trityl coupling was verified using the bromophenol blue color test.[18] N-alkylation was performed by treatment of the resin packet with 1 M lithium t-butoxide in THF. Excess base was removed by cannulation, followed by addition of the individual alkylating agent in DMSO. The solution was vigorously shaken for 2 h at room temperature.

2. N-Aacylated dipeptide synthesis. Upon removal of the trityl group with 2%TFA in DCM (2 x 10 min), the packet was washed, neutralized and the second amino acid (Fmoc-Xaa-OH) was coupled. Following removal of the Fmoc group, the dipeptide was individually acylated with a carboxylic acid in the presence of DIC and HOBt.

3. Exhaustive amide bond reduction. The reductions were performed in 50 ml kimax tubes under nitrogen. Boric acid (40x) and trimethylborate (40x) were added [7,19-21], followed by 1M BH_3-THF (40x). The tubes were heated at 65°C for 72 h. The solution was decanted and quenched with MeOH. Following washing, the resin was treated overnight with piperidine at 65°C. Following cleavage from the resin with anhydrous HF in the presence of anisole [22] for 6 h at 0°C, the desired product was extracted and lyophilized.

Analysis of Selected Compounds

N^1-phenethyl-N^5-benzyl-1(S),4(S)-dibenzyl-1,5-diamino-3-azapentane: ^{1}H-NMR (500 MHZ, DMSO-d_6) δ 2.52 (m, 2H); 2.66 (dd, J= 13.9, 28.6 Hz, 2H); 2.81 (m, 2H); 2.91 (m, 2H); 3.08 (m, 1H); 3.14 (d, J= 3.76 Hz, 1H); 3.16 (d, J= 3.7 Hz, 1H); 3.24 (m, 2H); 3.46 (m, 1H); 4.15 (d, J= 13.08 Hz, 1H); 4.24 (d, J= 13.08 Hz, 1H), 7.05-7.46 (m, 20 H). ^{13}C-NMR: 31.83; 33.76; 38.03; 42.92; 45.52; 49.27; 49.43; 55.44; 58.54; 126.34; 126.80; 126.93; 128.44; 128.60; 128.66; 128.97; 129.03; 129.25; 129.88; 131.75; 136.45; 137.07; 137.86. MS (ESI) *m/z* 478.3 found ($[M+H]^+$), 477.3 calculated.

N^1-ethyl-N^5-methyl-1(S),4(S)-dibenzyl-1,5-diamino-3-azapentane: ^{1}H-NMR (500 MHZ, DMSO-d_6) δ 1.14 (m, 3H); 1.89 (s, 3H); 2.72 (m, 4H); 2.83 (m, 2H); 2.88 (m, 4H), 3.50 (m, 1H), 3.69 (m, 1H), 7.10-7.32 (m, 10 H). ^{13}C-NMR: 12.65; 21.26; 35.57; 39.17; 44.71; 51.74; 55.92; 58.22; 126.22; 126.44; 128.38; 128.45; 129.11;

137.82; 138.18. MS (ESI) *m/z* 326.2 found ([M+H]$^+$), 325.3 calculated.

N^1-m-trifluoromethyl-phenethyl-N^5-methyl-1(S),4(S)-dibenzyl-1,5-diamino-3-azapentane: ^{1}HNMR (500 MHZ, DMSO-d_6) 2.57 (s, 3H); 2.72 (m, 2H); 2.88 (m, 2H); 2.91 (m, 6H); 3.50 (m, 2H), 3.72 (m, 2H), 7.20-7.58 (m, 14 H). ^{13}C-NMR: 21.09; 36.95; 37.93; 51.39; 55.53; 58.61; 123.30; 126.31; 126.42; 128.94; 129.14; 129.37; 137.93; 172.05. MS (ESI) *m/z* 470.2 found ([M+H]$^+$), 469.3 calculated.

N^1-phenylpropyl-N^5-benzyl-1(S),4(S)-dibenzyl-1,5-diamino-3-azapentane: ^{1}H-NMR (500 MHZ, DMSO-d_6) δ 1.05 (m, 3H); 2.83 (m, 2H); 2.90 (m, 2H); 3.01 (m, 3H); 3.15 (dd, J= 4.54, 17.21 Hz, 2H); 3.23 (m, 2H); 3.56 (m, 1H); 4.19 (d, J= 13.17 Hz, 1H); 4.25 (d, J= 13.17 Hz); 7-20-7.53 (m, 15 H). ^{13}C-NMR: 16.75; 31.82; 34.06; 43.25; 46.59; 50.45; 59.25; 126.85; 127.06; 128.64; 128.73; 129.08; 129.27; 129.93; 131.80; 136.26; 137.01. MS (ESI) *m/z* 416.2 found ([M+H]$^+$), 415.3 calculated.

N^1-phenethyl-N^5-benzyl-1(S)-isobutyl-4(S)-benzyl-1,5-diamino-3-azapentane: ^{1}H-NMR (500 MHZ, DMSO-d_6) δ 0.89 (d, J= 6.27 Hz, 3H); 0.82 (d, J= 6.35 Hz, 3H); 1.46 (m, 2H); 1.56 (m, 1H); 2.55 (dd, J= 7.03, 12.74 Hz, 2H); 2.67 (dd, J= 15.30, 27.87 Hz, 2H); 2.91 (m, 2H); 2.96 (m, 2H); 3.11 (m, 2H); 3.14 (m, 3H); 3.18 (m, 1H); 7.31-7.20 (m, 15 H). ^{13}C-NMR: 21.14; 23.35; 24.04; 31.54; 36.20; 38.24; 42.02; 44.10; 45.24; 55.49; 57.09; 126.30; 126.65; 128.08; 128.48; 128.51; 128.59; 128.71; 128.76; 128.92; 129.11; 129.24; 137.56; 138.43. MS (ESI) *m/z* 444.4 found ([M+H]$^+$), 443.4 calculated.

ACKNOWLEDGMENTS

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acid, heptanoic acid, isobutyric acid, isovaleric acid, 4-methylvaleric acid, trimethylacetic acid, tert-butylacetic acid, cyclohexanecarboxylic acid, cyclohexylacetic acid, cycloheptanecarboxylic acid, acetic acid, cyclobutanecarboxylic acid, cyclopentanecarboxylic acid, cyclohexanepropionic acid, 4-methyl-1-cyclohexanecarboxylic acid, 4-tert-butyl-cyclohexanecarboxylic acid, 1-adamantaneacetic acid, 3,3-diphenylpropionic acid, dicyclohexylacetic acid, indole-3-acetic acid, 1-naphthylacetic acid, 3-(3,4,5)-trimethoxyphenylpropionic acid, 2-norbornaneacetic acid, cyclopentylacetic acid, 2-ethylbutyric acid.

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