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Solid-Phase Synthesis of Substituted 2,3-Diketopiperazines from Reduced Polyamides

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Abstract—An efficient method for the solid phase synthesis of 1,6-disubstituted 2,3-diketopiperazine and 1,4,5-trisubstituted 2,3-diketopiperazine derivatives is described. The reduction of resin-bound acylated amino acids or resin-bound acylated dipeptides, followed by treatment with oxalyldiimidazole, affords the corresponding diketopiperazines in good yield and high purity. This is an example of a broader approach to the solid phase synthesis of individual heterocyclic compounds using peptides directly or indirectly as starting materials.

Introduction

Combinatorial chemistry enables the rapid generation of large numbers of diverse compounds, and when combined with high-throughput screening techniques, offers a powerful strategy for the discovery and generation of drug leads. Heterocyclic compounds having a high degree of structural diversity have proven to be broadly and economically useful as therapeutic agents.[1] Diketopiperazines are conformationally constrained scaffolds that are common in nature. Many natural products containing a diketopiperazine structure have been isolated and have been shown to have a wide range of biological activities. Included in such compounds are inhibitors of the mammalian cell cycle,[2] inhibitors of plasminogen activator-1 and topoisomerase.[3] Diketopiperazines have been reported to be useful as ligands to the neurokinin-2 receptor, and competitive antagonists to Substance P at the neurokinin-1 receptor.[4] A number of approaches have been reported for the solid-phase synthesis of diketopiperazine derivatives: Gordon and Steelle developed a strategy for the solid-phase synthesis of diketopiperazines based on reductive amination on the solid support;[5] a similar approach has been published by Krchňák and co-workers for the synthesis of persubstituted 2,5-diketopiperazine,[6] and Scott and co-workers developed an alternative strategy for the synthesis of a similar diketopiperazine library using α-bromocarboxylic acids and a range of amines.[7]

All existing reported approaches for the solid-phase synthesis of diketopiperazines describe only the synthesis of classic 2,5-diketopiperazines.[8] We report here an efficient method for the solid-phase synthesis of 2,3-diketopiperazines, a less studied class of compounds. Our approach is based on the reduction of resin-bound acylated amino acids or resin-bound acylated dipeptides, followed by the treatment of the resulting resin-bound polyamines with oxalyldiimidazole to afford the corresponding 1,6-disubstituted-2,3-diketopiperazine and 1,4,5-trisubstituted-2,3-diketopiperazine derivatives, respectively (Scheme 1).

Results and Discussion

Starting from a *p*-methylbenzhydrylamine (MBHA) resin-bound acylated amino acid, the amides were reduced in the presence of borane–THF complex to afford two secondary amines.[9–11] The treatment of the resulting diamine with oxalyldiimidazole generated the 1,6-disubstituted-2,3-diketopiperazine **3** in good yield and high purity following HF cleavage. We initially examined the feasibility of this synthetic route using five representative L-amino acids (Ser, Tyr, Val, Ala, Phe), and four carboxylic acids (phenylacetic acid, acetic acid, cyclohexyl acetic acid, isobutyric acid). A total of 19 disubstituted diketopiperazines **3** were synthesized as controls (Table 1).

In order to increase the amount of diversity around the diketopiperazine ring, we extended our approach to the solid-phase synthesis of 1,4,6-trisubstituted-2,3-diketopiperazines **6**. Starting from the same resin-bound amino acid **1** and following trityl (Trt) protection of the amino group, a selective *N*-alkylation of the amide linked to the solid support was performed using lithium *t*-butoxide in THF, followed by addition of an alkylating agent in dimethyl sulfoxide (DMSO).[12,13] We have successfully used the following halogenated alkyls for the *N*-alkylation

Keywords: combinatorial chemistry; solid-phase synthesis; 2,3-diketopiperazines.

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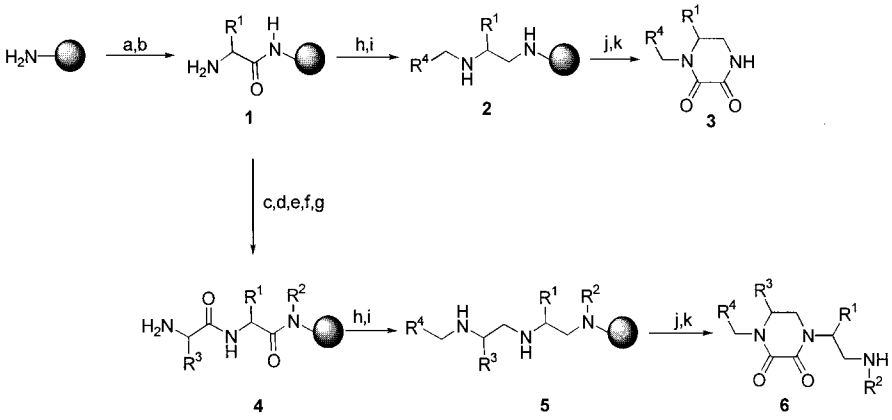
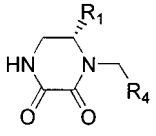


Scheme 1. Solid-phase synthesis of 1,6-disubstituted 2,3-diketopiperazines and 1,4,5-trisubstituted 2,3-diketopiperazines from resin-bound polyamines: (a) Fmoc-Xaa-OH, DIPCDI, HOBt, DMF; (b) 20% piperidine in DMF; (c) Trt-Cl, DIPEA, DCM; (d) R_2-X, ^{t}BuOLi, DMSO; (e) 2% TFA in DCM, DIPEA/DCM; (f) Fmoc-Xaa-OH, DIPCDI, DMF; (g) 20% piperidine in DMF; (h) R^4COOH, DIPCDI, DMF; (i) BH_3–THF, 65°C; (j) oxalyldiimidazole, DMF; (k) HF/anisole.

of amides: methyliodide, ethyliodide, benzylbromide, 2-bromomethylnaphthylene and allylbromide.[12] Following removal of the Trt protecting group with 2% TFA in DCM and neutralization, the second amino acid was added using traditional solid-phase peptide chemistry,[14] and the resulting dipeptide **4** was treated with appropriate carboxylic acids to obtain the acylated dipeptide. The reduction of the amide groups of the resin-bound *N*-acylated dipeptide using diborane in THF at 65°C yielded a single tertiary and two secondary amines **5**.[11,13] Potential racemization during tritylation, amide alkylation and reduction was examined using analytical reverse-phase high performance liquid chromatography (RP-HPLC). Different dipeptides were used as controls for the reactions involved, and all possible diasterioisomers were synthesized. Racemization was monitored by comparing the respective absorbances at 214 nm of two diastereomeric pairs that do not coelute. Different pairs for diastereoisomers that do not coelute were run in different gradients. The lack of diastereomeres in the RP-HPLC and in the ^{1}H NMR suggest little (<2%) or no racemization occurred during the *N*-alkylation.[13] The treatment of the resin-bound polyamine **5** with oxalyldiimidazole in anhydrous DMF, followed by HF cleavage, yielded the desired 1,4,5-trisubstituted-2,3-diketopiperazine **6** (Table 2).

As a first attempt in the synthesis of diketopiperazine derivatives **6**, we initially optimized the reaction conditions of this synthetic route by the parallel synthesis of 24 individual compounds. We chose benzyl bromide as the R_2

Table 1. RP-HPLC purity and masses found for the prepared diketopiperazines **3**

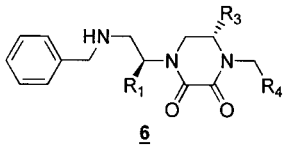


3

Entry	R_1	R_4	HPLC purity (%)[a]	M_W expected	M_W found
3a	CH_2–C_6H_4–OH	CH_2Ph	>95	324.37	325.4 (MH^+)
3b	CH_2–C_6H_4–OH	CH_2–C_6H_{11}	92	316.39	317.3 (MH^+)
3c	CH_2–C_6H_4–OH	$CH(CH_3)_2$	89	276.33	277.2 (MH^+)
3d	CH_2Ph	CH_2Ph	>95	308.37	309.3 (MH^+)
3e	CH_2Ph	CH_3	>95	232.28	233.2 (MH^+)
3f	CH_2Ph	CH_2–C_6H_{11}	>95	300.40	301.2 (MH^+)
3g	CH_2Ph	$CH(CH_3)_2$	92	260.33	261.2 (MH^+)
3h	CH_3	CH_2Ph	>95	232.28	233.2 (MH^+)
3i	CH_3	CH_3	>95	156.18	157.2 (MH^+)
3j	CH_3	CH_2–C_6H_{11}	>95	224.30	225.2 (MH^+)
3k	CH_3	$CH(CH_3)_2$	>95	184.24	185.1 (MH^+)
3l	CH_2OH	CH_2Ph	70	248.28	249.9 (MH^+)
3m	CH_2OH	CH_3	83	172.18	173.2 (MH^+)
3n	CH_2OH	CH_2–C_6H_{11}	87	240.30	241.2 (MH^+)
3o	CH_2OH	$CH(CH_3)_2$	92	200.24	201.9 (MH^+)
3p	$CH(CH_3)_2$	CH_2Ph	90	260.33	261.2 (MH^+)
3q	$CH(CH_3)_2$	CH_3	>95	184.24	185.2 (MH^+)
3r	$CH(CH_3)_2$	CH_2–C_6H_{11}	89	252.35	253.2 (MH^+)
3s	$CH(CH_3)_2$	$CH(CH_3)_2$	93	212.29	213.2 (MH^+)

[a] The products were run on a Vydac C_{18} column, using a 5–95% gradient of 0.05% TFA in ACN in 7 min. The purity was estimated using analytical traces at 214 nm. The yields obtained in all cases were higher than 75% relative to the initial loading of the resin.

Table 2. RP-HPLC purity and masses found for the prepared diketopiperazines **6**



6

Entry	R_1	R_3	R_4	HPLC purity (%)[a]	M_W expected	M_W found
6a	$(CH_2)_4N(CH_3)CH_2Ph$	Ph	CH_2Ph(*m*-Cl) (*o*-Cl)	93	670.28	671.6 (MH^+)
6b	CH_2Ph	CH_3	CH_2Ph(*m*-OMe)	92	485.62	486.6 (MH^+)
6c	$CHCH_2CH(CH_3)_2$	CH_2Ph	CH_2Ph	93	497.30	498.3 (MH^+)
6d	$(CH_2)_4N(CH_3)CH_2Ph$	Ph	Cycloheptyl	94	608.86	609.5 (MH^+)
6e	CH_2OH	CH_2Ph	CH_2Ph	76	471.25	472.2 (MH^+)
6f	CH_2OH	$CH(CH_3)_2$	CH_2Ph	78	423.25	424.2 (MH^+)
6g	CH_3	CH_2Ph	CH_2Ph	>95	455.26	456.3 (MH^+)
6h	$(CH_2)_4N(CH_3)CH_2Ph$	$CH(CH_3)_2$	CH_2Ph(*p*-OMe)	>95	598.82	599.6 (MH^+)
6i	$(CH_2)_4N(CH_3)CH_2Ph$	CH_2Ph	CH_2Ph	>95	616.38	617.6 (MH^+)
6j	$(CH_2)_4N(CH_3)CH_2Ph$	$CH(CH_3)_2$	CH_2Ph	>95	568.38	569.5 (MH^+)
6k	$(CH_2)_4N(CH_3)CH_2Ph$	CH_3	CH_2Ph(*p*-Me)	92	554.77	555.5 (MH+)
6l	$(CH_2)_4N(CH_3)CH_2Ph$	$CH(CH_3)_2$	CH_2Ph(*p*-Me)	>95	582.82	583.7 (MH+)
6m	$(CH_2)_4N(CH_3)CH_2Ph$	$CH(CH_3)CH_2CH_3$	CH_2Ph(*p*-Me)	>95	596.85	597.7 (MH+)
6n	CH_2Ph	$CH(CH_3)_2$	CH_2Ph(*p*-Me)	>95	497.67	498.4 (MH+)
6o	CH_2Ph	$CH(CH_3)CH_2CH_3$	CH_2Ph(*p*-Me)	>95	511.70	512.6 (MH+)
6p	$(CH_2)_4N(CH_3)CH_2Ph$	CH_3	CH_2Ph(*p*-OMe)	95	570.76	571.7 (MH+)
6q	$(CH_2)_4N(CH_3)CH_2Ph$	$CH(CH_3)_2$	CH_2Ph(*p*-OEt)	92	612.84	613.6 (MH+)
6r	$(CH_2)_4N(CH_3)CH_2Ph$	Ph	CH_2Ph(*p*-OEt)	92	646.86	647.9 (MH+)
6s	$(CH_2)_4N(CH_3)CH_2Ph$	Ph	CH_2-adamantyl	84	660.93	662.0 (MH+)
6t	$(CH_2)_4N(CH_3)CH_2Ph$	CH_3	CH_2Ph(*p*-OEt)	94	584.79	585.7 (MH+)
6u	$(CH_2)_4N(CH_3)CH_2Ph$	Ph	CH_2Ph(*p*-Me)	92	632.83	633.6 (MH+)
6v	$(CH_2)_4N(CH_3)CH_2Ph$	$CH(CH_3)CH_2CH_3$	CH_2Ph(*p*-OMe)	>95	626.87	627.7 (MH+)
6w	$(CH_2)_4N(CH_3)CH_2Ph$	Ph	CH_2Ph(*p*-Me)	93	616.83	617.7 (MH+)
6x	CH_2Ph	CH_2Ph	CH_2Ph	>95	531.29	532.2 (MH+)

[a] The products were run on a Vydac C_{18} column, using a 5–95% gradient of 0.05% TFA in ACN in 7 min. The purity was estimated using analytical traces at 21 nm. The yields obtained in all cases were higher then 75% relative to the initial loading of the resin.

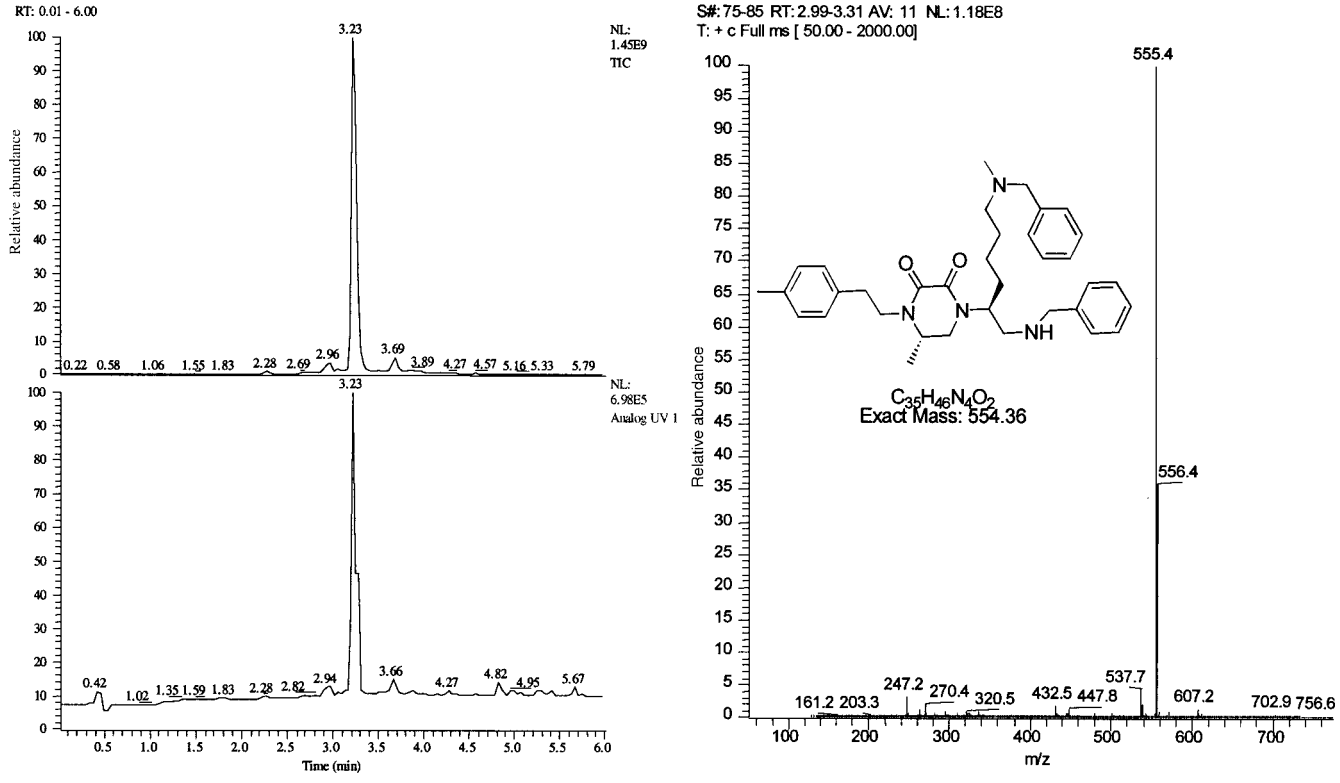


Figure 1. LC-MS of the diketopiperazine **6k** obtained from lysine, alanine and 4-methylphenylacetic acid.

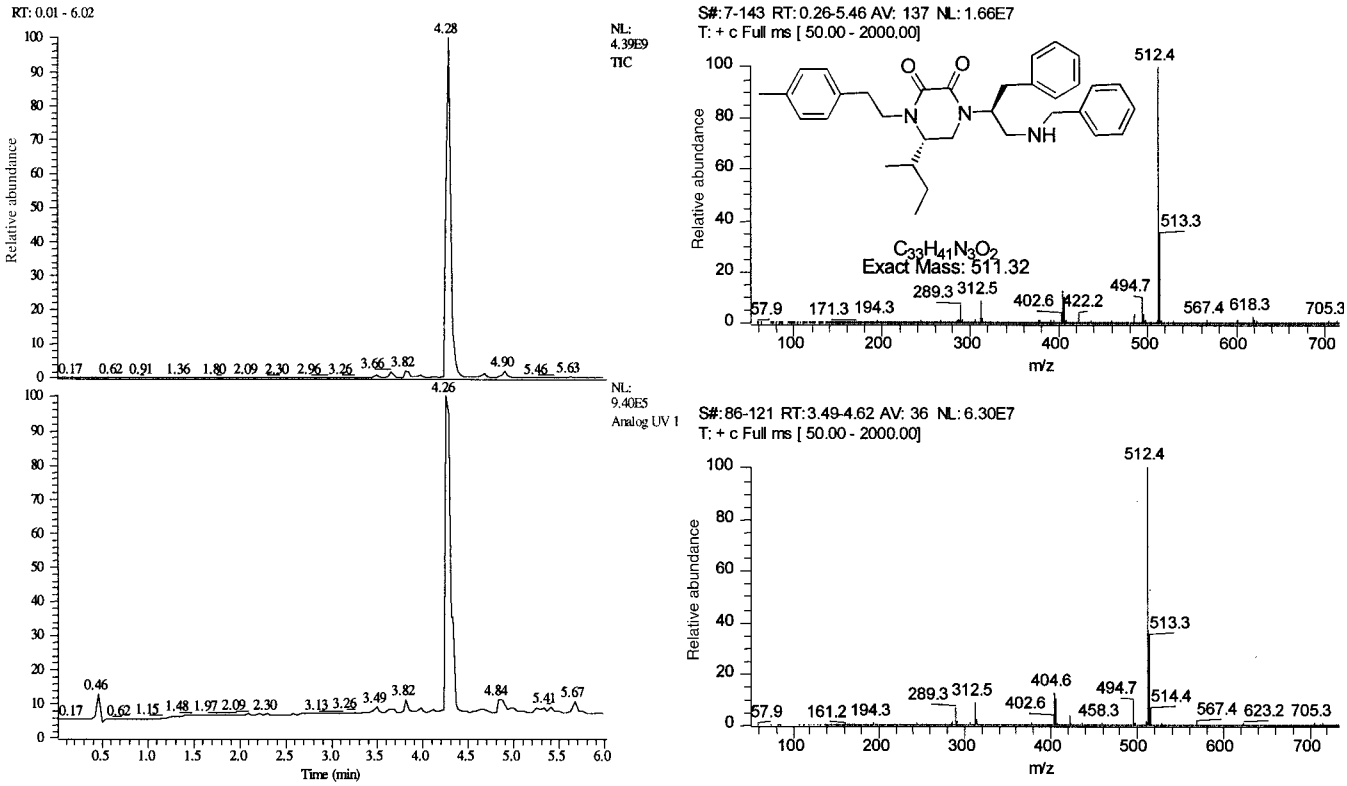


Figure 2. LC-MS of the diketopiperazine **6o** obtained from phenylalanine, isoleucine and 4-methylphenylacetic acid.

alkylating group, five different L-amino acids (Ser, Ala, Phe, Lys and Leu) for R five amino acids for R_3 (Val, Phe, Ile, Ala and Phenylglycine) and eight carboxylic acids for R_4 (phenylacetic acid, *p*-tolyl acetic acid, *p*-methoxyphenylacetic acid, *p*-ethoxyphenylacetic acid, adamantane acetic acid, *m*-methoxyphenylacetic acid, cycloheptyl carboxylic acid and 3,4-dichlorophenylacetic acid). As shown in Table 2, excellent purities were obtained for all cases. During *N*-alkylation with Bzl-Br, the protected N^{ϵ}-amine of lysine was also *N*-benzylated; during the reduction step, the Boc protecting group was reduced to the *N*-methyl.[12,13] Figs. 1 and 2 show the LC-MS spectra of the diketopiperazines **6k** and **l**, which are representatives of the purities obtained for all cases.

Modifications occurring in the functionalized amino acid side chains during the *N*-alkylation and reduction steps have been reported.[15] Expanding our optimization and control compound set, we examined more than 40 amino acids at the first and second positions of diversity, and 60 carboxylic acids at the third position. These individual control compounds, in which the individual building blocks were varied while the other two positions remained fixed, were synthesized in order to determine the effect of the reaction conditions on each of the building blocks. The amino acids Arg(Pmc), Trp(Boc), and His(Trt) which yielded aggregation and/or polymerized products were excluded since they led to the desired product in less than 50%. Excellent yields were obtained for the remaining amino acids with average HPLC purities higher than 80%. We also observed that following amide reduction electron rich substituted benzoic acids (such as *p*-methoxy-benzoic acid), which yield *N*-benzyl derivatives, are completely cleaved due to the required exposure for six hours to HF cleavage conditions. These carboxylic acids were therefore excluded from our libraries as well as the nitro aromatic carboxylic acids due to the partial reduction of the nitro group during borane treatment. The majority of the aromatic carboxylic acids chosen for the library synthesis are derivatives of phenylacetic acid. Using the information from these control experiments and following the selection of the appropriate compounds for each of the three positions of diversity, a trisubstituted 2,3-diketopiperazine library is being prepared. The synthesis of this library and its use in screening for the identification of highly active compounds will be reported elsewhere. We have also successfully used the strategy described to generate cyclic urea and cyclic thiourea libraries by treating the generated resin-bound polyamine **5** with carbonyldiimidazole and thiocarbonyldiimidazole, respectively.[15]

Using the 'libraries from libraries' concept,[16] this laboratory has reported previously a range of combinatorial libraries prepared on the solid-phase, then cleaved and utilized in solution.[12,13,17] Such libraries incorporate a wide range of functionalities, including hydrogen bonding donors and acceptors, positive and negative charges, functionalities of differing chiralities, hydrophobic and hydrophilic substituents, etc.

Conclusion

This work is part of our ongoing efforts in the solid-phase synthesis of individual small molecule and heterocyclic compounds and subsequent combinatorial libraries using

amino acids and peptides as the starting material.[17] *N*-acylated amino acids and *N*-acylated dipeptides have been successfully used for the generation of a variety of 1,6-disubstituted 2,3-diketopiperazines and 1,4,5-trisubstituted 2,3-diketopiperazines, respectively, in high purity. The general nature of this approach permits not only large numbers of individual diketopiperazines to be prepared, but also mixture-based combinatorial libraries.[17]

Experimental

Fmoc-amino acid derivatives and HOBt were purchased from Calbiochem-Novabiochem Corp. (San Diego, CA), Bachem Bioscience Inc. (Philadelphia, PA), and Bachem California (Torrance, CA). MBHA resin (1% divinylbenzene, 100–200 mesh, 1 mmol/g substitution) was purchased from Peninsula Laboratories, Inc. (Belmont, CA). *N*,*N*′-Diisopropylcarbodiimide (DIPCDI) 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). Analytical RP-HPLC was performed on a Beckman System Gold instrument (Fullerton, CA). Samples were analyzed using a Vydac 218TP54 C_{18} column (0.46×25 cm).

Typical procedure for the individual synthesis of 1,6-disubstituted 2,3-diketopiperazine (3)

Solid phase syntheses were carried out using the 'teabag' method, in which the resin is contained within sealed polypropylene mesh packets.[18] The completeness of amino acid coupling and *N*-acylation were verified using the ninhydrin test.[19]

(1) *Amino acid coupling and* N-*acylation:* 100 mg *p*-methylbenzydrylamine (MBHA) resin (0.1 mol-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 (Fmoc-Xaa-OH, 6 equiv.) was coupled using the conventional reagents hydroxybenzotriazole (HOBt, 6 equiv.) and diisopropylcarbodiimide (DIPCDI, 6 equiv.) in anhydrous DMF for 60 min. Following removal of the Fmoc group with 20% piperidine in DMF (2×10 min) and washing with DMF (8×), 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. The resin packet (1 mol-equiv. resin, 100 mg of starting resin, 0.2 mol-equiv. carbonyl) and boric acid (15-fold excess over each amide bond) were added to each tube. Trimethyl borate (15-fold excess over each amide bond) was added, followed by 1 M BH_3–THF (40-fold excess over each amide bond). 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 methanol (2×) and DMF (6×) and dried. The completeness of the reaction was verified by cleavage and analysis following reduction.

(3) *Disubstituted diketopiperazine formation:* The cyclization occurred following treatment of the resin-bound reduced acylated amino acid overnight with 5-fold 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.

1-Phenethyl-6(*S*)-*p*-hydroxybenzyltetrahydro-2,3-diketopiperazine (3a). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.43 (d, J=4.5 Hz, 1H), 7.32–6.66 (m, 9H), 3.88 (m, 1H), 3.76 (m, 1H), 3.23 (dd, J=13.1, 2.4 Hz) 2.89 (m, 1H), 2.88 (m, 2H), 2.82 (m, 2H), 2.63 (dd, J=13.3, 9.5 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): 157.4, 157, 156.1, 138.9, 130.1, 128.7, 128.3, 127.3, 126.3, 115.2, 56.9, 54.9, 47.5. ES-MS calcd for $C_{19}H_{20}N_2O_3$: 324.373, found: 325.402 (MH^+).

1-Ethyl-6(*S*)-benzyltetrahydro-2,3-diketopiperazine (3e). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.45 (d, J=3.8 Hz, 1H), 7.34–7.23 (m, 5H), 3.72 (m, 1H), 3.64 (dd, J=13.3, 6.9 Hz, 1H), 3.45 (dd, J=13.2, 3.3 Hz), 2.99 (dd, J=13.3, 5.1 Hz, 1H), 2.95 (dd, J=14.4, 5.4 Hz, 1H), 2.88 (dd, J=13.5, 6.92 Hz, 1H), 2.81 (dd, J=13.1, 9.7 Hz, 1H), 1.08 (t, J=6.9 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): 157.6, 156.8, 137.4, 129.2, 128.5, 126.6, 55.8, 40.5, 40.2, 36.7, 13.1. ES-MS calcd for $C_{13}H_{16}N_2O_2$: 232.284, found: 233.207 (MH^+).

1-Isobutyl-6(*S*)-methyltetrahydro-2,3-diketopiperazine (3k). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.47 (d, J=4.1 Hz, 1H), 3.62 (m, 1H), 3.61 (m, 1H), 3.53 (dd, J=13.4, 7.8 Hz, 1H), 3.06 (m, 1H), 2.70 (dd, J=13.1, 7.2 Hz, 1H), 1.93 (m, 1H), 1.22 (d, J=7.1 Hz, 3H), 0.88 (d, J=6.7 Hz, 3H), 0.82 (d, J=6.7 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): 157.5, 157.4, 51.6, 50.6, 43.1, 26.5, 19.9, 19.9, 16.7. ES-MS calcd for $C_9H_{16}N_2O_2$: 184.243, found: 185.105 (MH^+).

1-Ethyl-6(*S*)-isopropyltetrahydro-2,3-diketopiperazine (3q). ^{1}H NMR (500 MHz, DMSO-d_6) 8.34 (d, J=3.9 Hz, 1H), 3.81 (m, 1H), 3.46 (m, 1H), 3.28 (m, 2H), 2.89 (m, 1H), 1.96 (m, 1H), 1.09 (t, J=6.9 Hz, 1H), 0.96 (d, J=6.7 Hz, 3H), 0.88 (d, J=6.7 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): 157.5, 157.4, 51.6, 50.6, 43.1, 26.5, 19.9, 19.9, 16.7. ES-MS calcd for $C_9H_{16}N_2O_2$: 184.245, found: 185.206 (MH^+).

1-Cyclohexylmethyl-6(*S*)-*p*-hydroxybenzyltetrahydro-2,3-diketopiperazine (3b) ^{1}H NMR (500 MHz, DMSO-d_6) 8.45 (d, J=5.2 Hz, 1H), 7.01 (d, J=8.1 Hz, 2H), 6.69 (d, J=8.5 Hz, 2H), 3.57 (dd, J=7.3, 13.3 Hz, 1H), 3.48 (m, 2H), 2.96 (dd, J=5.6, 12.1 Hz), 1H), 2.85 (dd, J=4.8, 13.3 Hz, 1H), 2.65 (dd, J=9.3, 13.2 Hz, 1H), 2.57 (dd, J=6.9, 13.1 Hz, 1H), 1.60 (m, 6H), 1.14 (m, 3H), 0.94 (m, 1H), 0.85 (m, 1H). ES-MS calcd for $C_{18}H_{24}N_2O_3$: 316.398, found: 317.301 (MH^+).

1-Isobutyl-6(*S*)-*p*-hydroxybenzyltetrahydro-2,3-diketopiperazine (3c). ^{1}H NMR (500 MHz, DMSO-d_6) 8.46 (d, *J*=5.2 Hz, 1H), 7.01 (d, *J*=8.2 Hz, 2H), 6.69 (d, *J*=8.5 Hz, 2H), 3.53 (m, 3H), 2.96 (dd, *J*=5.6, 12.7 Hz, 1H), 2.86 (dd, *J*=4.9, 13.4 Hz, 1H), 2.66 (dd, *J*=9.3, 13.4 Hz, 1H), 2.56 (dd, *J*=7.1, 13.1 Hz, 1H), 1.96 (m, 1H), 0.87 (d, *J*=6.3 Hz, 3H), 0.81 (d, *J*=6.8 Hz, 3H). ES-MS calcd for $C_{15}H_{20}N_2O_3$: 276.332, found: 277.207 (MH^+).

1-Phenethyl-6(*S*)-benzyltetrahydro-2,3-diketopiperazine (3d). ^{1}H NMR (500 MHz, DMSO-d_6) 8.45 (d, *J*=5.1 Hz, 2H), 7.30 (m, 5H), 7.21 (m, 5H), 3.87 (m, 1H), 3.50 (m, 1H), 3.26 (dd, *J*=4.1, 13.17 Hz, 1H), 3.01 (m, 1H), 2.94 (dd, *J*=5.5, 13.3 Hz, 1H), 2.81 (m, 4H). ES-MS calcd for $C_{19}H_{20}N_2O_2$: 308.371, found: 309.306 (MH^+).

1-Cyclohexylmethyl-6(*S*)-*p*-hydroxybenzyltetrahydro-2,3-diketopiperazine (3f). ^{1}H NMR (500 MHz, DMSO-d_6) 8.47 (d, *J*=5.1 Hz, 1H), 7.32 (m, 2H), 7.24 (m, 3H), 3.56 (m, 3H), 3.01 (dd, *J*=5.1, 13.3 Hz, 1H), 2.94 (dd, *J*=5.9, 13.7, Hz, 1H), 2.79 (dd, *J*=9.5, 13.1 Hz, 1H), 2.57 (dd, *J*=7.1, 13.4 Hz, 1H), 1.65 (m, 6H), 0.93 (m, 1H), 0.83 (m, 1H). ES-MS calcd for $C_{18}H_{24}N_2O_2$: 300.402, found: 301.208 (MH^+).

1-Isobutyl-6(*S*)-benzyltetrahydro-2,3-diketopiperazine (3g). ^{1}H NMR (500 MHz, DMSO-d_6) 8.49 (d, *J*=5.18 Hz, 1H), 7.33 (m, 3H), 7.24 (m, 2H), 3.63 (m, 1H), 3.54 (m, 2H), 2.99 (dd, *J*=5.3 Hz, 1H), 2.94 (dd, *J*=5.9, 13.9 Hz, 1H), 2.79 (dd, *J*=9.4, 13.1 Hz, 1H), 2.56 (dd, *J*=7.1, 13.4 Hz, 1H), 1.05 (m, 1H), 0.87 (d, *J*=6.7 Hz, 3H), 0.81 (d, *J*=6.8 Hz, 3H). ES-MS calcd for $C_{15}H_{20}N_2O_2$: 260.334, found: 261.207 (MH^+).

1-Phenethyl-6(*S*)-methyltetrahydro-2,3-diketopiperazine (3h). ^{1}H NMR (500 MHz, DMSO-d_6) 8.43 (d, *J*=3.9 Hz, 1H), 7.29 (m, 2H), 7.24 (m, 3H), 3.86 (ddd, *J*=5.95, 5.49, 8.6 Hz, 1H), 3.47 (m, 1H), 3.39 (dd, *J*=4.1, 12.9 Hz, 1H), 3.16 (m, 1H), 2.9 (m, 2H), 1.17 (d, *J*=6.7 Hz, 3H). ES-MS calcd for $C_{13}H_{16}N_2O_2$: 232.283, found: 233.209 (MH^+).

1-Ethyl-6(*S*)-methyltetrahydro-2,3-diketopiperazine (3i). ^{1}H NMR (500 MHz, DMSO-d_6) 8.43 (1s, 1H), 3.68 (m, 1H), 3.57 (m, 2H), 3.04 (m, 2H), 1.23 (d, *J*=6.7 Hz, 3H), 1.09 (t, *J*=6.9 Hz, 3H). ES-MS calcd for $C_7H_{12}N_2O_2$: 156.187, found: 157.204 (MH^+).

1-Cyclohexylmethyl-6(*S*)-methyltetrahydro-2,3-diketopiperazine (3j). ^{1}H NMR (500 MHz, DMSO-d_6) 8.45 (d, *J*=4.0 Hz, 1H), 3.60 (m, m, 3H), 3.04 (dd, *J*=5.6, 11.1 Hz, 1H), 2.72 (dd, *J*=6.8, 13.4 Hz, 1H), 1.63 (m, 6H), 1.21 (d, *J*=8.6 Hz, 3H), 1.14 (m, 3H), 0.93 (m, 1H), 0.88 (m, 1H). ES-MS calcd for $C_{12}H_{20}N_2O_2$: 224.301, found: 225.287 (MH^+).

1-Phenethyl-6(*S*)-hydroxymethyltetrahydro-2,3-diketopiperazine (31). ^{1}H NMR (500 MHz, DMSO-d_6) 8.33 (d, *J*=4.7 Hz, 1H), 7.30 (m, 5H), 5.12 (m, 1H), 3.93 (m, 1H), 3.32 (m, 2H), 3.18 (m, 3H), 2.84 (m, 2H). ES-MS calcd for $C_{13}H_{16}N_2O_3$: 248.28, found: 249.20 (MH^+).

1-Methyl-6(*S*)-hydroxymethyltetrahydro-2,3-diketopiperazine (3m). ^{1}H NMR (500 MHz, DMSO-d_6) 8.34 (d, *J*=4.35 Hz, 1H), 5.13 (1s, 1H), 3.67 (m, 2H), 3.50 (m, 3H), 3.08 (m, 2H), 1.10 (m, 3H). ES-MS calcd for $C_7H_{12}N_2O_3$: 172.186, found: 173.203 (MH^+).

1-Cyclohexylmethyl-6(*S*)-hydroxymethyltetrahydro-2,3-diketopiperazine (3n). ^{1}H NMR (500 MHz, DMSO-d_6) 8.35 (d, *J*=4.8 Hz, 1H), 5.12 (s, 1H), 3.63 (dd, *J*=7.2, 13.4 Hz, 1H), 3.57 (m, 2H), 3.42 (m, 3H), 2.74 (dd, *J*=6.9, 13.1 Hz, 1H), 1.63 (m, 6H), 1.19 (m, 3H), 1.16 (m, 1H), 0.88 (m, 1H). ES-MS calcd for $C_{12}H_{20}N_2O_3$: 240.301, found: 241.208 (MH^+).

1-Isobutyl-6(*S*)-hydroxymethyltetrahydro-2,3-diketopiperazine (3o). ^{1}H NMR (500 MHz, DMSO-d_6) 8.36 (d, *J*=4.7 Hz, 1H), 3.61 (m, 4H), 3.54 (m, 2H), 3.31 (dd, *J*=4.9, 12.9 Hz, 1H), 2.73 (dd, *J*=7.1, 12.9 Hz, 1H), 1.94 (m, 1H), 0.89 (d, *J*=6.8, 3H), 0.84 (d, *J*=6.7 Hz, 3H). ES-MS calcd for $C_9H_{16}N_2O_3$: 200.241, found: 201.109 (MH^+).

1-Phenethyl-6(*S*)-isopropyltetrahydro-2,3-diketopiperazine (3p). ^{1}H NMR (500 MHz, DMSO-d_6) 8.32 (d, *J*=4.36 Hz, 1H), 7.30 (m, 2H), 7.22 (m, 3H), 4.05 (m, 1H), 3.12 (m, 4H), 2.88 (m, 1H), 2.83 (m, 1H), 1.91 (d, *J*=6.86 Hz, 3H), 0.84 (d, *J*=6.80 Hz, 3H). ES-MS calcd for $C_{15}H_{20}N_2O_2$: 260.33, found: 261.20 (MH^+).

1-Cyclohexylmethyl-6(*S*)-isopropyltetrahydro-2,3-diketopiperazine (3r). ^{1}H NMR (500 MHz, DMSO-d_6) 8.36 (d, *J*=5.10 Hz, 1H), 3.76 (dd, *J*=6.76, 13.40 Hz, 1H), 3.57 (dd, *J*=4.18, 13.74 Hz, 1H), 3.28 (dd, *J*=5.89, 13.76 Hz, 1H), 3.17 (dd, *J*=3.89, 7.53 Hz, 1H), 2.60 (dd, *J*=7.39, 13.01 Hz, 1H), 1.95 (m, 1H), 1.67 (m, 4H), 1.59 (m, 2H), 1.14 (m, 3H), 0.93 (d, *J*=6.87 Hz, 3H), 0.87 (d, *J*=6.77 Hz, 3H). ES-MS calcd for $C_{14}H_{24}N_2O_2$: 252.35, found: 253.20 (MH^+).

1-Isobutyl-6(*S*)-isopropyltetrahydro-2,3-diketopiperazine (3s). ^{1}H NMR (500 MHz, DMSO-d_6) 8.39 (d, *J*=4.88 Hz, 1H), 3.74 (dd, *J*=6.95, 12.88 Hz, 1H), 3.56 (dd, *J*=4.41, 13.69 Hz, 1H), 3.29 (dd, *J*=5.86, 13.73 Hz, 1H), 3.20 (m, 1H), 2.59 (dd, *J*=7.81, 12.96 Hz, 1H), 1.97 (m, 2H), 0.93 (d, *J*=6.87 Hz, 3H), 0.89 (d, *J*=7.6 Hz, 3H), 0.88 (d, *J*=7.14 Hz, 3H), 0.82 (d, *J*=6.38 Hz, 3H). ES-MS calcd for $C_{11}H_{20}N_2O_2$: 212.29, found: 213.20 (MH^+).

Typical procedure for the individual synthesis of 1,4,5-trisubstituted 2,3-diketopiperazine (6)

(1) *Amino acid coupling and selective* N-*alkylation:* The first amino acid was coupled in the same conditions as described before. Following removal of the protecting group with 20% piperidine in DMF (1×10 min) and wash with DMF (8×), the mesh packet was shaken overnight in a solution of trityl chloride in DCM/DMF (9:1) in the presence of DIPEA. Completeness of the trityl coupling was verified using the bromophenol blue color test.[20] *N*-alkylation was performed by treatment of the resin packet with 0.5 M lithium *t*-butoxide in THF (20 equiv.) for 10 min at room temperature. Excess base was removed by cannulation, followed by addition of the individual alkylating agent (20 equiv.) in anhydrous DMSO. The solution was vigorously shaken for 2 h at room temperature (this operation was repeated three times).

(2) N-*Acylated dipeptide:* Upon removal of the trityl from the α-amino group with 2% TFA in DCM (2×10 min), the resin packet was washed, neutralized with a solution of 5% DIEA in DCM, and the second amino acid (Fmoc-Xaa-OH) coupled in the same conditions as described above.[†] Following removal of the Fmoc group, the dipeptide was *N*-acylated with a carboxylic acid (10 equiv.) overnight in the presence of DIPCDI (10 equiv.) and HOBt (10 equiv.) in anhydrous DMF.

(3) *Exhaustive reduction of the amide groups:* The reduction was performed in the same conditions as described above (the characterization and the purities of the intermediate diethylenetriamines have been reported).[13]

(4) *Trisubstituted diketopiperazine formation:* The cyclization occurred following treatment of the reduced acylated dipeptide overnight with 5-fold 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 7 h,[21] the desired product was extracted with acetonitrile/water (50:50) and lyophilized.

For the library synthesis, the selected R_1 and R_3 groups were derived from Gly, Phg, Met(O) and the D- and L- forms of the following amino acids: Ala, Phe, Ile, Leu, Nva, Ser(tBu), Thr(tBu), Val, Tyr(tBu), Nle, Cha and Nal. In addition R_1 included the D- and L- forms of Lys(Boc). R_2 included also β-Ala.

The selected R_3 were derived from the following carboxylic acids: 1-phenyl-1-cyclopropane carboxylic acid, *m*-tolylacetic acid, 3-fluorophenylacetic acid, (α,α,α)-trifluoro-*m*-tolylacetic acid, *p*-tolylacetic acid, 3-methoxyphenylacetic acid, 4-methoxyphenylacetic acid, 4-ethoxyphenylacetic acid, 4-isobutyl-α-methylphenylacetic acid, 3,4-dichlorophenylacetic acid, 3,5-bis(trifluoromethyl)-phenylacetic acid, phenylacetic acid, hydrocinnamic acid, 4-phenylbutyric acid, butyric 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.

1-{1(*S*)-[(Benzylamino)methyl]-5-[benzyl(methyl)amino]-pentyl}-5(*S*)-benzyl-4-phenethyltetrahydro-2,3-diketopiperazine (6i). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.13–7.6 (m, 20H), 4.37 (m, 1H), 4.15–4.23 (m, 2H), 3.67–3.83 (m, 8H), 3.35 (m, 1H), 2.94–3.09 (m, 4H), 2.67–2.84 (m, 4H), 1.58 (m, 2H), 1.16 (m, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): 157.58, 155.97, 138.69, 137.53, 131.41, 131.15, 130.21, 129.89, 129.62, 129.17, 129.04, 128.91, 128.73, 128.68, 128.39, 126.79, 126.36, 58.39, 55.32, 54.34, 50.62, 47.94, 47.65, 37.16, 33.00, 28.42, 22.92. ES-MS calcd for $C_{40}H_{48}N_4O_2$: 616.34, found: 617.60 (MH^+).

1-{1(*S*)-[Hydroxymethyl]-1(*S*)-[benzylaminomethyl)]-ethyl}-5(*S*)-benzyl-4-phenethyltetrahydro-2,3-diketopiperazine (6e). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.24–7.36 (m, 15H), 5.13 (m, 1H), 4.57 (d, *J*=14.5 Hz, 1H), 4.46 (d, *J*=14.5 Hz, 1H), 4.12 (dd, *J*=8.7, 14.8 Hz, 1H), 3.92 (m, 1H), 3.51 (m, 2H), 3.18–3,26 (m, 5H), 3.08 (dd, *J*=3.8, 14.8 Hz, 1H), 2.88 (m, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): 158.47, 156.14, 136.92, 136.13, 129.10, 128.72, 128.64, 128.55, 128.23, 127.56, 127.08, 126.88, 60.06, 56.18, 54.57, 49.92, 46.20, 44.96, 44.72, 42.50, 36.22, 34.57, 31.73. ES-MS calcd for $C_{29}H_{33}N_3O_3$: 471.25, found: 472.2 (MH^+).

1-{1(*S*)-[(Benzylamino)methyl]-5-[benzyl(methyl)amino]-pentyl}-5(*S*)-isopropyl-4-phenethyltetrahydro-2,3-diketopiperazine (6n). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.08–7.40 (m, 14H), 4.92 (m, 1H), 4.17 (m 1H), 4.05 (m, 1H), 3.98 (m, 2H), 3.69 (d, *J*=5.38 Hz, 2H), 3.06 (m, 1H), 2.96–2.88 (m, 5H), 2.77 (m, 2H), 2.26 (1s, 3H), 1.62 (m, 1H), 0.78 (m, 12H). ^{13}C NMR (125 MHz, DMSO-d_6): 157.85, 156.26, 136.78, 135.67, 135.31, 132.23, 129.87, 128.99, 128.89, 128.83, 128.74, 128.57, 128.50, 126.73, 59.33, 50.40, 49.95, 35.00, 32.89, 29.74, 20.66, 19.61, 19.18. ES-MS calcd for $C_{32}H_{39}N_3O_3$: 497.67, found: 498.4 (MH^+).

1-[1(*S*)-(Benzyl)-2-(benzylamine)]-5(*S*)-methyl-3-*m*-methoxyphenethyltetrahydro-2,3-diketopiperazine (6t). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.42–7.19 (m, 11H), 6.85–6.78 (m, 3H), 5.15 (m, 1H), 4.20 (m, 2H), 3.73 (s, 3H), 3.70 (m, 2H), 3.16 (m, 3H), 2.97 (m, 4H), 2.80 (m, 3H), (m, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): 159.35, 157.89, 156.03, 140.38, 136.59, 134.68, 131.79, 129.97, 129.48, 129.05, 128.79, 128.48, 126.71, 120.89, 120.124, 114.239, 113.77, 113.63, 111.87, 54.97, 50.44, 49.47, 46.79, 46.32, 43.13, 42.97, 42.01, 34.70, 33.57, 17.05. ES-MS calcd for $C_{30}H_{35}N_3O_3$: 485.62, found: 486.4 (MH^+).

1-{1-[(Benzylamino)methyl]-5(*S*)-[benzyl(methyl)amino]-pentyl}-5(*S*)-methyl-4-*p*-methoxyphenethyltetrahydro-2,3-diketopiperazine (6p). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.41–6.86 (m, 14H), 4.72 (m, 1H), 3.72 (s, 3H), 3.70 (m, 2H), 3.17 (m, 3H), 3.08 (m, 1H), 3.00 (m, 4H), 2.86–2.76 (m, 5H), 2.52 (s, 3H), 1.249 (m, 2H), 1.23 (m, 2H), 1.18 (m, 2H), 1.14 (m, 3H). ES-MS calcd for $C_{35}H_{46}N_3O_3$: 570.76, found: 471.7 (MH^+).

1-{1(*S*)-[(Benzylamino)methyl]-5-[benzyl(methyl)amino]-pentyl}-5(*S*)-isopropyl-4-*p*-methoxyphenethyltetrahydro-2,3-diketopiperazine (6q). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.53–6.81 (m, 14H), 4.72 (m, 1H), 3.97 (dd, *J*=6.56, 13.30 Hz, 2H), 3.69 (d, *J*=5.25 Hz, 2H), 3.35 (m, 2H), 3.14 (m, 2H), 3.06 (m, 3H), 2.99 (m, 4H), 2.78 (m, 2H), 2.59 (s, 3H), 1.77 (m, 1H), 1.69 (m, 2H), 1.45 (m, 2H), 1.29 (t, *J*=6.68 Hz, 3H), 1.22 (m, 2H), 0.89 (d, *J*=5.00 Hz, 3H), 0.84 (d, *J*=5.00 Hz, 3H). ES-MS calcd for $C_{38}H_{52}N_4O_3$: 612.84, found: 613.7 (MH^+).

[†] It was found that the alkylation of the amide resin linkage dramatically increases the acid sensitivity of the MBHA resin-bound peptide. The *N*-alkylated MBHA resin could be cleaved with 70% TFA in DCM in 30 min. This cleavage can be avoided through the subsequent use of Fmoc chemistry.

Supporting information

Listing of: (a) ^{1}H NMR spectra of all compounds; (b) the LC-MS for compounds **6a**–**x**; and (c) HPLCs of diastereoisomers that do not coelute.

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