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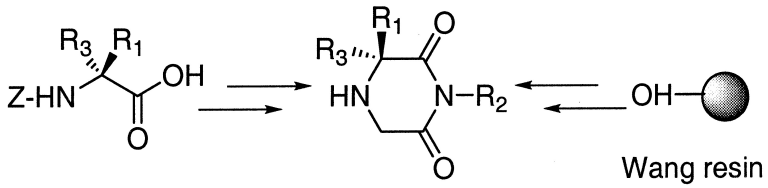

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2,6-Diketopiperazines from Amino Acids, from Solution-Phase to Solid-Phase Organic Synthesis

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A method to prepare 1,3-disubstituted 2,6-diketopiperazines (2,6-DKP) as useful heterocyclic library scaffolds in the search of new leads for drug discovery is described. The method can be used in solution-phase and solid-phase conditions. In the key step of the synthesis, the imido portion of the new molecule is formed in solution through intramolecular cyclization, under basic conditions, of a secondary amide nitrogen on a benzyl ester. A Wang resin carboxylic ester is used as the acylating agent under solid-phase conditions, allowing the cyclization to take place with simultaneous cleavage of the product from the resin ("cyclocleavage"). The synthetic method worked well with several couples of amino acids, independently from their configuration, and was used for the parallel synthesis of a series of fully characterized compounds. The use of iterative conditions in the solid phase (repeated addition of fresh solvent and potassium carbonate to the resin after filtering out the product-containing solution) allowed us to keep diastereoisomer content below the detection limit by HPLC and ^{1}H NMR (200 MHz).

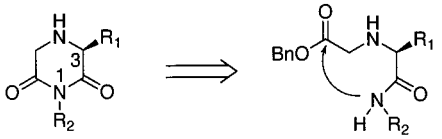
Heterocyclic frameworks are playing an important role as library scaffolds in the search of new lead compounds for drug discovery. Among the heterocyclic templates the 2,3- and 2,5- diketopiperazines (2,3- and 2,5-DKPs) have been extensively described in the literature because they are present in several biologically active compounds.[1] Piperazinone ring systems are also used as conformationally restricted active peptide analogues.[2] In contrast, less attention has been paid to 2,6-diketopiperazines (2,6-DKPs), although their framework is really close to the 2,6-diketo-Δ3-piperazine ring of the Flutimide (an endonuclease inhibitor of influenza virus)[3] and it is present as bis(2,6-diketopiperazine) in the topoisomerase II inhibitor ICRF-154 and related compounds, having promising anticancer efficacy.[4]

To the best of our knowledge, there are few examples of 2,6-DKP syntheses;[5] here a general method to prepare 1,3-substituted 2,6-DKP in solution phase and the validation of the method for the shift to the solid phase will be described. Our initial interest was devoted to the synthesis of the indole-containing heterocycles, but the method is of wider application.

The formation of the heterocyclic ring system was achieved using as a key step the intramolecular cyclization, under basic conditions, of the secondary amide over the acylating benzyl ester of an (alkylcarbamoylmethylamino)-acetic acid benzyl ester as depicted in the Scheme 1.

The approach to 2,6-DKPs by homogeneous solution-phase synthesis is described in Scheme 2. We started from *N*-*tert*-butoxycarbonyl (*N*-Boc) or *N*-benzyloxycarbonyl (*N*-Z) amino acid **1**, which was reacted with a primary amine **2** under standard peptide coupling conditions to form the amide **3**, which at the end gave rise to the imido portion of the heterocycle. Subsequent N deprotection was performed using TFA/dichloromethane (method A) or catalytic hydrogenolysis (method B) for *N*-Boc and *N*-Z amino acids, respectively. The resulting free amine **4** was alkylated with benzyl bromoacetate to form the acyclic intermediate **5**. The optimal conditions found to minimize N dialkylation were to perform the reaction in DMSO using 0.5 equiv of benzyl bromoacetate and 1 equiv of amine **4**, without use of tertiary base.[6] By use of this procedure, the alkylation step gave a crude reaction mixture containing compounds **5** and **7** in a ratio of about 90:10, which after flash chromatography were obtained as pure compounds. Both compounds **5** and **7** were submitted to cyclization by a simple reaction in DMF in the presence of anhydrous K_2CO_3 at 50 °C to form 2,6-DKP **6** and **8**.

Scheme 1



When this procedure is used, the substituents in position 3 of the forming DKP (R_1, R_3) can be chosen from the large collection of side chains of natural and unnatural α-amino acids while the substituent in position 1 (R_2) comes from the large family of primary amines including amino acids. An example of selected compounds obtained by this method is exhibited in Table 1. Our interest was devoted to compounds of type **6**, and no efforts were undertaken for the synthetic optimization of compound **8**. The lower yield of compound **6c** (compared with **6a**,**b**,**d**) was due to the tendency of intermediate methyl ester **4c** to spontaneously cyclize to classical 2,5-DKP **9** by standing at room temperature and during the alkylaton step.

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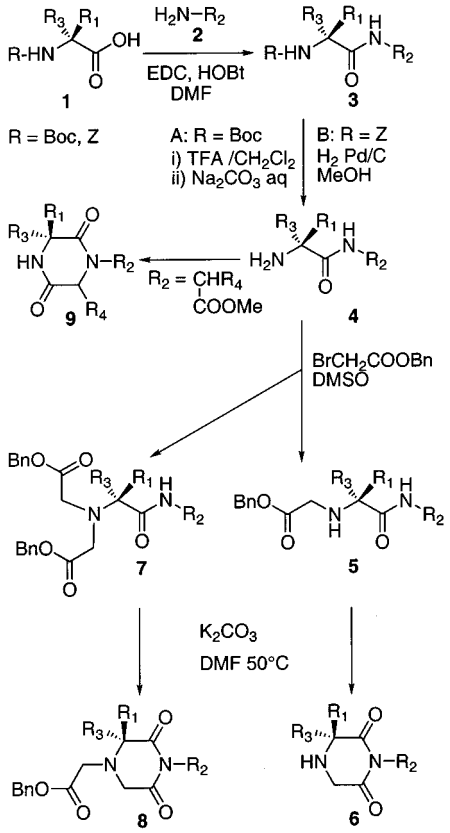
Scheme 2. Homogeneous Phase Synthesis of 2,6-DKPs **6**



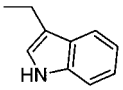
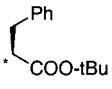
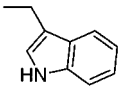
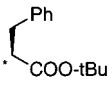
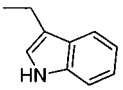
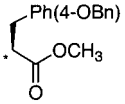
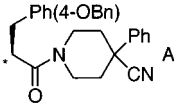
The potential epimerization at C-3 of DKPs (for $R_1 \neq H$, $R_3 = H$; and $R_1 = H$, $R_3 \neq H$) during cyclization reaction was investigated by HPLC and ^{1}H NMR. The resulting 2,6-DKPs (**6** and **8**) were found to be configurationally unstable under the cyclization conditions, and the extent of epimerization was mainly related to the reaction time or, better, to the time that the formed DKP remains in the reaction media. The epimerization problem forced us to use again the chromatographic purification of final products in order to obtain diastereomerically pure compounds.

The above-described method proved to be useful for the solution-phase parallel synthesis of new substituted 2,6-DKPs, but the need for a careful purification of intermediates and products makes it unsuitable for fully automated procedures required to obtain libraries of compounds.

For these reasons, taking into account the information acquired during the homogeneous-phase synthesis, we planned a solid-phase organic synthesis (SPOS) in order to evaluate if an easily automatable procedure could be found for the construction of 2,6-DKP libraries. To extend the homogeneous-phase method to the solid phase, we simply observed the resemblance between the benzyl alcohol and the functional group linkage of the Wang resin. This observation convinced us to use a Wang resin carboxylic ester as an acylating agent in the same way as the benzyl esters **5**.[7] By this route, the cyclization can succeed with simultaneous cleavage from the resin of the forming products (cyclocleavage).[8]

Scheme 3 describes the SPOS of the 2,6-DKP. To better use the advantage offered by the solid-phase support, a procedure that provided for the larger number of synthetic steps over the resin was planned, limiting the manipulations

Table 1. Homogeneous-Phase Synthesis of 2,6-DKPs[a]

Compd.	R_1	R_3	R_2	method	yield[b] %
6a		H		B	32
8a		H		B	5[c]
6b		H	—CH_2Bn	B	36
6c	H	H		A	15
6d	H	H		A	23

[a] The asterisk (*) represents the point of attachment. [b] Overall yield from **1**. [c] Byproduct in the synthesis of **6a**.

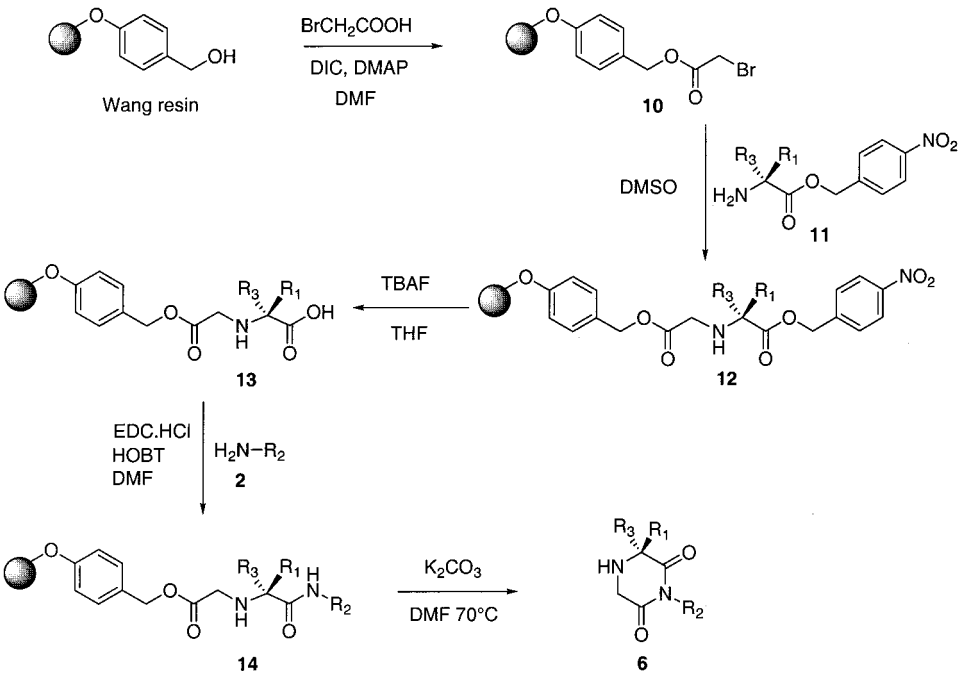
of the intermediates in the homogeneous phase to the preparation of amino acid *p*-nitrobenzyl esters **11**[9] and to the eventual transformations of R_2 groups of the primary amine.

The Wang resin was reacted with bromoacetic acid in the presence of DIC and DMAP in DMF to give the bromoacetic Wang resin ester **10**.[10] Nucleophilic displacement of the bromine atom was achieved by reaction with amino acid *p*-nitrobenzyl ester **11**, the resulting ester was deprotected using tetrabutylammonium fluoride (TBAF) in THF solution, applying the method of Rinehart et al. for the homogeneous phase.[11] This was revealed as a critical step having an estimated yield of 70%, checked by resin weight.[12] The resulting free acids **13** were condensed with primary amines or amino acid derivatives, and the final cyclization was performed, as for the homogeneous-phase reaction, using K_2CO_3 in DMF, but the reaction appeared to require a little higher temperature to complete the conversion: 70 vs 50 °C. The resulting DKPs were obtained by simple evaporation of DMF after filtration from the resin, and the crude products were purified by filtration over silica gel mainly to remove the potassium salts.[13]

The HPLC chromatograms, checked at 220 nm, showed a purity of the obtained 2,6-DKPs spanning from 80% to more than 95%, and these 2,6-DKPs were analyzed by MS, HPLC, and ^{1}H NMR, the selected data being shown in Table 2. Each compound was submitted to the cyclocleavage step in DMF/K_2CO_3 at 70 °C for 4 h except for compound **6f**, which was reacted for 8 h. Thus, the major extent of racemization of the C-3 position in the 2,6-DKP of **6f** was ascribable to the longer reaction time. Although the side chains R_2 were chiral, the amino acid racemization test[14] showed that the exocyclic side chains are configurationally more stable with respect to the endocyclic C-3 and no appreciable racemization was noted after 4 h of reaction time.

The synthetic method works well with several couples of amino acids, independently from their configuration. The problems arose when more hindered amino acids were used,

Scheme 3. Solid-Phase Synthesis of 2,6-DKPs **6**



for example, in the cases of **6u** and **6v** no 2,6-DKPs were obtained. In the case of compound **6u** the use of a longer reaction time (48 h) or higher temperature (up to 80 °C) confirmed that intermediates **14u** and **5u** did not cyclize neither using solid-phase nor solution-phase methodology. For compound **6v**, a greater problem was ascribable to the low loading level obtained in the synthesis of intermediate **12v**.

The 2,6-DKPs unsubstituted at position C-3 (i.e., **6s**) were sensitive to hydrolysis and alcoholysis of the imide group, however the problem was surmounted using anhydrous conditions and avoiding the use of MeOH during the resin washing. The synthesis of C-3 doubly alkylated 2,6-DKPs was particularly favorable, in fact in these cases the C-3 epimerization cannot occur and the cyclization step resulted in fast and clean reactions, probably by intervention of *gem* dialkyl effect.[15]

It is noteworthy that in the crude reaction product, the HPLC analysis and the ^{1}H NMR spectroscopy did not show the presence of a doubly alkylated compound of type **8** or that related to it, a fact in accordance with the observation that substrates on a polymer support can react as in dilute solution. Furthermore, the solid-phase synthesis also allowed the use of amine **2**, where R_2 is a carboxymethyl ester, without the risk of the side reaction that gave rise to 2,5-DKPs (as in the homogeneous phase), demonstrating a decreased reactivity of amine **12** in the solid phase rather than in the solution phase.

Conclusion

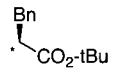
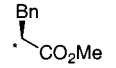
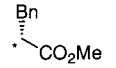
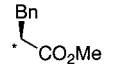
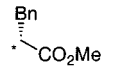
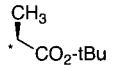
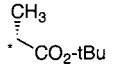
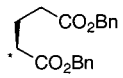
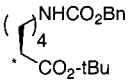
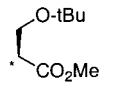
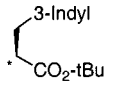
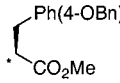
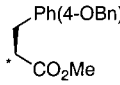
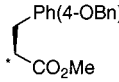
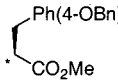
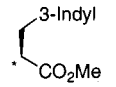
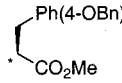
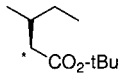
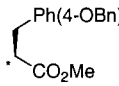
A general method to prepare 1,3-disubstituted 2,6-diketopiperazines both by homogeneous phase or by SPOS was presented. The positive results, during solid-phase synthesis, in avoiding N dialkylation and 2,5-DKPs formation are in contrast with the stereochemical purity of the final 2,6-DKPs because epimerization took place at the C-3 ring carbon. This is due to the fact that cyclization in the solid phase is slower than in solution phase and the forming DKPs reside for a greater time at higher temperature under the basic epimerization conditions. To overcome the problem, the solution containing the formed DKP was filtered out, fresh solvent and K_2CO_3 were added to the resin, and the entire procedure was repeated four to five times during the cyclocleavage reaction time. The eluate was collected and evaporated to obtain DKPs with a diastereoisomers content under the detection threshold limit by HPLC and ^{1}H NMR (200 MHz). Although this methodology was easy to automate, we considered it to be more expeditious to obtain 2,6-DKPs that are diastereoisomerically impure in order to perform preliminary biological tests of a larger number of compounds at the same time. In this way, in the cases in which we had observed some biological activity we could use the standard method in the homogeneous phase to synthesize the corresponding stereochemically pure compounds.

The more interesting comparison between the two alternative synthetic approaches is that solid-phase methodology can be automated more easily than solution-phase methodology, and considering the global reaction time to prepare the final products, we found the SPOS to be 4 times faster than the homogeneous-phase method.

Experimental Section

Wang resin (100−200 mesh, 1% cross-linked; loading of 0.5−1.2 mmol/g) and Boc-protected amino acids were purchased from Novabiochem. Amino acid ester derivatives were purchased from Bachem. Other unspecified reagents were purchased from SAF group. Reactions in the solid phase were performed in PTFE tubes using Quest 210 apparatus manufactured by Argonaut Technologies. Reactions in solution phase were performed in glass tubes using a Carousel reaction station manufactured by Radleys.

Table 2. Selected Compounds **6** Obtained by SPOS[a]

entry	R_1	R_3	R_2	yield[b] %	HPLC[c] purity%	dr[d]
a	CH_2-3-Indyl	H		10	96	96/4
e	CH_2-3-Indyl	H		5	91	90/10
f	CH_2-3-Indyl	H		17	87	60/40[e]
g	H	CH_2-3-Indyl		12	95	85/15
h	H	CH_2-3-Indyl		4	95	90/10
i	H	CH_2-3-Indyl		12	94	88/12
j	H	CH_2-3-Indyl		3	94	n.d.[f]
k	CH_2-3-Indyl	H		10	94	n.d.[f]
l	CH_2-3-Indyl	H		7	95	97/3
m	CH_2-3-Indyl	H		6	95	75/25
n	H	CH_2-3-Indyl		4	87	80/20
o	H	CH_2-Ph(4-OBn)		12	87	93/7
p	CH2-^{i}Pr	H		2	89	n.d.[f]
q				4	99	–
r	CH_3	CH_3		7	95	–
s	H	H		5	95	–
t	CH_2-3-Indyl CH_3	CH_3 CH_2-3-Indyl		2	90	50/50
u	CH_2-3-Indyl	H		–		
v	^{i}Pr	H		–		

[a] Indyl = indolyl. The asterisk (*) represents the point of attachment. [b] Isolated yields; calculated from **1** using the substitution level of the starting resin. [c] Determined using relative peak areas with monitoring at 220 nm, expressed as the sum of diastereomeric peaks. [d] Referenced to C-3 position of 2,6 DKP; determined by ^{1}H NMR integral signals of CH(N1) of the R_2 substituent. The higher number is related to the expected diastereoisomer. [e] The cyclocleavage time was twice those of the other compounds in the table. [f] Not determined. Neither HPLC nor ^{1}H NMR allowed dr determination.

Mass spectra were recordered using a Finnigan LCQ ion trap mass spectrometer equipped with a standard ESI ion source. Ionization conditions were the following: ESI in the positive-ion mode; nebulizer voltage, 4.2 kV; capillary voltage, 3 V; tube lens offset, 30 V; capillary temperature, 220 °C. The nebulizing gas was nitrogen with a sheath gas flow rate of 70 (arbitrary units). Scan modes were full-scan MS and MS/MS product ion scans of the protonated quasi-molecular ions at 30% relative collision energy. Sample introduction was by infusion at 5 μL/min through the built-in syringe pump. The sample (about 50 μg/mL) was dissolved in 1:1 acetonitrile/10 mM ammonium acetate.

^{1}H NMR spectra were recorded on a Varian Gemini or Bruker Avance spectrometer at 200 or 500 MHz, ^{13}C NMR spectra were recorded on a Gemini instrument at 50 MHz using the solvent $CDCl_3$, DMSO-d_6, or CD_3OD, reporting the chemical shift in ppm relative to TMS, DMSO, or MeOH.

HPLC analyses were performed on a Hewlett-Packard 1100 liquid chromatography system using a photodiode array detector and precolumn Widepore C_{18} ODS, 4 mm × 3 mm with a 5 μm Luna column, C_8(2), 100 Å, 4.6 mm × 250 mm, flow of 1 mL/min, and a gradient of water, 0.1%TFA/acetonitrile, 0.1% TFA from 80−20% to 14−86% over 22 ft and 6 ft (isocratic, method A) or from 80−20% to 20−80% over 20 min (method B). Peak areas were integrated at 220 and 270 nm.

Infrared spectra were recordered on a Perkin-Elmer FTIR 1710 spectrometer using the KBr pellet method on the washed and dried resin.

General Procedure for the Preparation in the Homogeneous Phase of 2,6-Diketopiperazines. General Synthesis of 3. To a solution of N-protected amino acid **1** (10 mmol), amine **2** (10 mmol), and hydroxybenzotriazole (4.1 g, 30 mmol) in dry DMF (70 mL) was added EDC·HCl (6 g, 12 mmol). The reaction was stirred for 6 h at ambient temperature and then was worked up by partitioning between ethyl acetate and water (70/70 mL). The ethyl acetate layer was separated and washed in succession with water (70 mL), 5% aqueous $NaHSO_4$ (70 mL), and 5% aqueous $NaHCO_3$ (70 mL). Drying of the organic phase over sodium sulfate and removal of solvent under reduced pressure produced an oil that was washed or solidified on treatment with petroleum ether (40−60 °C)/diethyl ether (1/1, v/v) to give the compound in 90% average yield.

General Synthesis of 4. Method A. To a solution of compound **3** (R = Boc, 3 mmol) and ethane 1,2-dithiol (0.1 mL) in dichloromethane (20 mL), cooled to 5 °C in an ice−water bath, TFA (10 mL) was added. After being stirred for 1 h at 5 °C and 3 h at room temperature, the mixture was evaporated under vacuum to dryness. The residue was then partitioned in ethyl acetate and water-saturated Na_2CO_3 (80/20 mL). The two phases were separated, and the water solution was washed with freshly ethyl acetate to completely remove compound **4**. The combined organic phases were dried over sodium sulfate and evaporated under reduced pressure to give the free amine **4** in quantitative yield.

General Synthesis of 4. Method B. Compound **3** (R = Z, 1.7 mmol) was dissolved in MeOH (35 mL), and 10% Pd/C (0.12 g) was added. The mixture was then hydrogenated under atmospheric pressure for 2−5 h. The catalyst was then filtered off and washed with additional portions of MeOH. The combined filtrates were evaporated under reduced pressure to give compound **4** in quantitative yield.

Example for 5 and 7: Synthesis of 5a. H-Trp-Phe-OtBu (1.38 g, 3.4 mmol) was dissolved in DMSO (50 mL), and benzyl bromoacetate (0. 27 mL, 1.7 mmol) was added. After being stirred for 5 h at room temperature, the reaction mixture was diluted with ethyl acetate (50 mL) and subsequently washed with water (2 × 50 mL). The organic phase was dried over Na_2SO_4 and evaporated to dryness. The resulting yellow oil was purified by flash chromatography, eluating with cyclohexane/ethyl acetate 7:3 to give 490 mg (yield 52%) of compound **5a** (Rf = 0.22) and 70 mg (yield 6%) of byproduct **7a** (R_f = 0.35).

5a. MH^+ *m/z* 557.0. ^{1}H NMR, 200 MHz ($CDCl_3$) δ: 8.32 (1H, bs), 7.39−6.80 (14H, m), 4.92 and 4.89 (2H, ABq, J = 12.2 Hz), 4.80−4.60 (1H, m), 3.46−3.05 (4H, m), 3.04−2.68 (3H, m), 2.18(1H, bs), 1.32(9H, s).

7b. MH^+ *m/z* 704.0. ^{1}H NMR, 200 MHz (DMSO-d_6) δ: 10.76 (1H, bs), 8.30 (1H, d, J = 8.5 Hz), 6.87−7.50 (20H, m), 5.05 (4H, s), 4.25−4.40 (1H, m), 3.78−2.70 (9H, m, overlapped to H_2O signal), 1.27 (9H, s).

Example for 6 and 8: Synthesis of 6a. To a solution of compound **5a** (0,1 g, 0.18 mmol) in dry DMF (5 mL), finely scratched anhydrous potassium carbonate was added (0.025 g, 0.18 mmol), and the mixture was stirred for 3 h at 50 °C. The reaction mixture was diluted with 5% aqueous $NaHCO_3$ (15 mL) and extracted with ethyl acetate. The organic phase was washed with water and brine and dried over sodium sulfate, then evaporated under reduced pressure. The resulting oil was purified by flash chromatography using cyclohexane/ethyl acetate 1:1, obtaining 55 mg (yield 68%) of compound **6a** (R_f = 0.3) as a pale-yellow wax.

Analytical Data for the 2,6 DKPs of Table 1. 2-(*S*)-[3-(*S*)-(1*H*-Indol-3-ylmethyl)-2,6-dioxopiperazin-1-yl]-3-phenylpropionic Acid *tert*-Butyl Ester (6a). MH^+ *m/z* 448.0. ^{1}H NMR, 200 MHz ($CDCl_3$) δ: 8.23 (1H, bs), 7.65 (1H, d, J = 7.4 Hz), 7.41 (1H, d, J = 7.6 Hz), 7.32−7.05 (7H, m), 6.93 (1H, d, J = 2.3 Hz), 5.58 (1H, dd, J = 5.6, 10.5 Hz), 3.75−3.60 (2H, m), 3.60−3.45 (1H, m), 3.45−3.20 (3H, m), 2.98 (1H, dd, J = 8.2, 18.6 Hz), 1.9 (1H, bs), 1.54 (9H, s). ^{13}C NMR, 50 MHz ($CDCl_3$) δ:172.2, 170.4, 168.2, 137.7, 136.3, 129.3, 128.5, 126.5, 127.6, 122.3, 119.7, 118.7, 111.2, 110.6, 82.1, 59.1, 53.8, 48.9, 34.6, 28.0, 26.0. HPLC (method B): t_R = 18.9 min.

2(*S*)-[4-Ethoxycarbonylmethyl-3(*R*)-(1*H*-indol-3-ylmethyl)-2,6-dioxopiperazin-1-yl]-3-phenylpropionic Acid *tert*-Butyl Ester (8a). MH^+ *m/z* 596.0. ^{1}H NMR, 500 MHz ($CDCl_3$) δ: 7.82 (1H, bs), 7.50 (1H, d, J = 7.8 Hz), 7.39−7.29 (6H, m), 7.24−7.06 (7H, m), 6.99 (1H, bs), 5.58 (1H, dd, J = 6.0, 11.1 Hz), 5.03 and 5.01 (2H, ABq, J = 12.1 Hz) 3.85−3.72 (2H, m), 3.58−3.25 (3H, m), 3.21−3.01 (2H, m), 2.97−2.86 (2H, m), 1.45 (9H, s). ^{13}C NMR, 50 MHz ($CDCl_3$) δ: 171.5, 161.4, 169.3, 168.1, 137.3, 135.8, 135.3, 129.2, 129.4, 128.6, 128.5, 128.3, 127.0, 126.8, 123.1, 122.0, 119.5, 118.3, 111.1, 110.6, 82.3, 66.6, 63.9, 54.8, 53.4, 52.0, 34.5, 28.0, 24.9. HPLC (method B): t_R = 18.6 min.

3(*S*)-(1*H*-Indol-3-ylmethyl)-1-phenethylpiperazine-2,6-dione (6b). MH^+ *m/z* 347.1. ^{1}H NMR, 500 MHz (DMSO-d_6) δ: 10.90 (1H, bs), 7.54 (1H, d, J = 7.9 Hz), 7.35 (1H, d, J = 8.0 Hz), 7.31−7.24 (2H, m), 7.24−7.13 (4H, m), 7.06 (1H, t, J = 7.0 Hz), 6.98 (1H, t, J = 7.0 Hz), 3.88−3.79 (2H, m), 3.74 (1H, dt, J = 4.1, 8.5 Hz), 3.58 and 3.51 (2H, AB part of an ABX system, J = 5.3, 8.5, 17.4 Hz), 3.34−3.22 (1H, m, overlapped to H_2O signal), 3.00 (1H, dd, J = 8.5, 14.8 Hz), 2.79−2.72 (1H, m), 2.72−2.65 (2H, m). HPLC (method A): t_R = 13.9 min.

3-(4-Benzyloxy-phenyl)-2(*S*)-(2,6-dioxopiperazin-1-yl)-propionic Acid Methyl Ester (6c). MH^+ *m/z* 383.1. ^{1}H NMR, 200 MHz ($CDCl_3$) δ: 7.20−7.45 (5H, m), 6.90−7.18 (2H, m), 6.85−6.90 (2H, m), 5.51 (1H, dd, J = 6.1, 10.7), 5.03 (2H, s), 3.74 (3H, s), 3.65−3.12 (6H, m), 2.20 (1H, bs). HPLC (method B): t_R = 14.2 min.

1-[3-(4-Benzyloxyphenyl)-2(*S*)-(2,6-dioxopiperazin-1-yl)-propionyl]-4-phenyl-4-cyanopiperidine (6d). MH^+ *m/z* 537.2. ^{1}H NMR, 200 MHz ($CDCl_3$) δ: 7.52−7.27 (10H, m), 7.15−6.90 (2H, m), 6.95−6.80 (2H, m), 5.75−5.45 (1H, m), 5.04 (2H, s), 3.85−2.90 (10H, m), 2.25−1.60 (5H, m, overlapped to H_2O signal). HPLC (method B): t_R = 16.6 min.

General Procedure for the Preparation in the Solid Phase of 2,6-Diketopiperazines. The example is related to the compound with R_1 = CH_2−3-indolyl.

Supporting Bromoacetic Acid on Wang Resin. Preparation of 10.[10] The Wang resin (5.0 g, 3.8 mmol, loading 0.76 mmol/g) was suspended in DMF (76 mL) and treated with bromoacetic acid (2.64 g, 19 mmol) and 4-(dimethylamino)pyridine (45 mg, 0.38 mmol). Then DIC (3 mL, 19 mmol) was added and the reaction mixture was stirred for 3 h. The mixture was filtered and the resin washed with DCM (2 × 25 mL), DMF (3 × 25 mL), 2-propanol (3 × 25 mL), and DCM (3 × 25 mL). The resin was then dried under vacuum until the weight was constant (5.46 g, 100% yield), and the supported bromoacetic ester was characterized by FTIR (cm^{-1}): 1737 C=O.

2(*S*)-Amino-3-(1*H*-indol-3-yl)propionic Acid 4-Nitrobenzyl Ester (Example for the Preparation of 11). To a solution of Boc-Trp-OH (15.5 g, 51 mmol) and triethylamine (7.4 mL, 54 mmol) in DCM (150 mL) at 0 °C was added *p*-nitrobenzyl chloroformate (11.0 g, 51 mmol). After the mixture was stirred for 10 min at 0 °C, DMAP (0.625 g, 5.1 mmol) was added and the resulting solution was stirred at 0

°C for 1 h. The reaction mixture was washed with 5% aqueous $NaHCO_3$ (80 mL), 0.1 M HCl (60 mL), and saturated NaCl (100 mL). The organic solution was dried over anhydrous $MgSO_4$ and evaporated to dryness.

The residue 2(*S*)-*tert*-butoxycarbonylamino-3-(1*H*-indol-3-yl)propionic acid 4-nitrobenzyl ester (21 g) was characterized by ^{1}H NMR 200 MHz (CD_3OD) δ: 8.85 (1H, bs), 8.12−8.00 (1H, m), 7.58−7.48 (1H, m), 7.38−7.30 (1H, m), 7.22−6.95 (5H, m), 6.95−6.88 (1H, bs), 5.22−5.12 (1H, m), 5.08 (2H, s), 4.80−4.62 (1H, m), 3.40−3.15 (2H, m), 1.45 (9H, s).

A solution of 4 M HCl in dioxane (127 mL, 510 mmol) was added dropwise at 0 °C to a solution of the compound coming from the previous reaction in AcOEt (200 mL). The mixture was stirred for 1.5 h at 0 °C and for 1 h at room temperature. The white precipitate was filtered, washed with Et_2O, and dried under vacuum (yield: 14.3 g, 38 mmol, 74%). ^{1}H NMR 200 MHz (DMSO-d_6) δ: 11.05 (1H, m), 8.55 (3H, bs), 8.15−8.00 (2H, m), 7.60−6.82 (7H, m), 5.18 (2H, bs), 4.55−4.20 (1H, m), 3.45−3.10 (2H, m). ^{13}C NMR (DMSO-d_6) δ: 169.0, 147.0, 142.4, 136.1, 128.4, 126.1, 124.7, 123.2, 121.0, 118.5, 117.9, 111.4, 106.4, 65.48, 52.62, 26.2.

Supported 2(*S*)-Amino-3-(1*H*-indol-3-yl)propionic Acid 4-Nitrobenzyl Ester (Example for the Preparation of 12). To a solution of **11** (14.3 g, 38 mmol) in DMSO (40 mL) was added triethylamine (5.3 mL, 38 mmol), and the precipitated salt was filtered off. The solution was added to the resin **10** (5.46 g, considered as 3.8 mmol), and the reaction mixture was stirred for 20 h at room temperature. The resin was filtered and washed with DCM (2 × 25 mL), DMF (3 × 25 mL), 2-propanol (3 × 25 mL), and DCM (3 × 25 mL). The resin was then dried under vacuum until a constant weight was reached (6.39 g, yield 95%). The supported 2(*S*)-amino-3-(1*H*-indol-2-yl)propionic acid 4-nitrobenzyl ester **12** was characterized by FTIR (cm^{-1}): 3420 N−H, 1741 C═O.

Supported 2(*S*)-Amino-3-(1*H*-indol-3-yl)propionic Acid (Example for the Preparation of 13). The resin **12** (6.39 g, 3.6 mmol) was treated with a solution of TBAF (1M in THF, 40 mL, 40 mmol), and then the mixture was stirred for 1 h. The resin was filtered and washed with DCM (2 × 25 mL), DMF (3 × 25 mL), 2-propanol (3 × 25 mL), and DCM (3 × 25 mL). The resin was then dried under vacuum until a constant weight was reached (5.73 g, yield 70% from starting Wang resin) and was characterized by FTIR (cm^{-1}): 1739 C═O, 1600 C═O.

Supported 14. The resin **13** (0.280 g, 0.2 mmol) was treated with a solution of the amine (0.8 mmol) and DIPEA (0.14 mL, 0.8 mmol) in DMF (1.5 mL). Then a solution of HOBt (0.216 g, 1.6 mmol) in DMF (1 mL) was added. After 5 min EDC·HCl (0.230 g, 1.2 mmol) was added. The reaction mixture was stirred for 16 h.

The resin was filtered and then washed with DCM (2 × 25 mL), DMF (3 × 25 mL), 2-propanol (3 × 25 mL), and DCM (3 × 25 mL) and dried under N_2. FTIR (cm^{-1}): 1736 C═O, 1672 C═O.

Cyclocleavage. The resin was suspended in DMF (2.5 mL) and treated with solid K_2CO_3. The mixture was heated to 70 °C for 4 h. The solution was recovered by filtration in glass tubes. After evaporation of the solvent the residue was purified over 3.5 g of silica gel contained in an 8 cm × 1.8 cm polypropylene syringe eluting with cyclohexanes/ethyl acetate (2:1 or 1:1 or 1:2 v/v), affording the products described in Table 2.

Analytical Data for the 2,6-DKPs of Table 2. Compound 6a. See data in Table 1.

2-(*S*)-[3-(*S*)-(1*H*-Indol-3-ylmethyl)-2,6-dioxopiperazin-1-yl]-3-phenylpropionic Acid Methyl Ester (6e) and 2-(*R*)-[3-(*R*)-(1*H*-Indol-3-ylmethyl)-2,6-dioxopiperazin-1-yl]-3-phenylpropionic Acid Methyl Ester (6h). MH^+ *m/z* 406.1. ^{1}H NMR, 200 MHz ($CDCl_3$) δ: 8.01 (1H, bs), 7.60 (1H, d, J = 7.3 Hz), 7.42−7.06 (8H, m), 6.92−6.85 (1H, m), 5.58 (1H, dd, J = 5.9, 10.4 Hz), 3.72 and 3.80−3.15 (9H, s overlapped to m), 3.06−2.85 (2H, m). HPLC (method A): t_R = 15.6 min.

2-(*R*)-[3-(*S*)-(1*H*-Indol-3-ylmethyl)-2,6-dioxopiperazin-1-yl]-3-phenylpropionic Acid Methyl Ester (6f). MH^+ *m/z* 406.0. ^{1}H NMR 200 MHz ($CDCl_3$) δ: 8.12 (0.6H, m), 8.02 (0.4H, m, 3R diastereoisomer), 7.70−7.54 (1H, m), 7.42−6.81 (8H, m), 6.72−6.60 (1H, m), 5.58 (0.4H, dd, J = 5.7, 10.7 Hz, 3R diastereoisomer), 5.45 (0.6H, dd, J = 5.2, 10.9 Hz), 3.80−2.85 (10H, m). HPLC (method A): t_R = 15.5 min.

2-(*S*)-[3-(*R*)-(1*H*-Indol-3-ylmethyl)-2,6-dioxopiperazin-1-yl]-3-phenylpropionic Acid Methyl Ester (6g). MH^+ *m/z* 406.1. ^{1}H NMR, 200 MHz ($CDCl_3$) δ: 8.26 (1H, bs), 7.70−7.54 (1H, m), 7.42−6.81 (8H, m), 6.72−6.60 (1H, m), 5.58 (0.15 H, dd, J = 5.5, 10.5 Hz, 3S diastereoisomer), 5.46 (0.85 H, dd, J = 5.5, 10.8 Hz), 3.73 and 3.85−2.98 (10H, s overlapped to m), 1.83 (1H, bs). HPLC (method A): t_R = 15.5 min.

2-(*S*)-[3-(*S*)-(1*H*-Indol-3-ylmethyl)-2,6-dioxopiperazin-1-yl]propionic Acid *tert*-Butyl Ester (6i). MH^+ *m/z* 371.9. ^{1}H NMR, 500 MHz ($CDCl_3$) δ: 8.30 (1H, bs), 7.55 (1H, d, J = 8 Hz), 7.37 (1H, d, J = 8 Hz), 7.30−7.08 (3H, m), 5.18 (1H, q, J = 7.2 Hz), 3.85−3.65 (2H, m), 3.65−3.42 (2H, m), 3.40−3.20 (1H, m), 1.45 (3H, d, J = 7.2 Hz), 1.43 (9H, s). HPLC (method A): t_R = 14.4 min.

2-(*R*)-[3-(*R*)-(1*H*-Indol-3-ylmethyl)-2,6-dioxopiperazin-1-yl]propionic Acid *tert*-Butyl Ester (6j). MH^+ *m/z* 372.1. ^{1}H NMR, 500 MHz ($CDCl_3$) δ: 8.12 (1H, bs), 7.61 (1H, d, J = 8.6 Hz), 7.45−7.05 (4H, m), 5.21 (1H, q, J = 7.1 Hz), 3.85−3.60 (2H, m), 3.64−3.42 (2H, m), 3.32−3.16 (1H, m), 1.46 (3H, d, J = 7.1 Hz), 1.44 (9H, s). HPLC (method A): t_R = 15.7 min.

2-(*S*)-[3-(*S*)-(1*H*-Indol-3-ylmethyl)-2,6-dioxopiperazin-1-yl]pentanedioic Acid Dibenzyl Ester (6k). MH^+ *m/z* 554.1. ^{1}H NMR, 500 MHz ($CDCl_3$) δ: 7.88 (1H, bs), 7.55 (1H, d, J = 8 Hz), 7.45−7.05 (13H, m), 7.00−6.90 (1H, m), 5.30−5.20 (1H, m), 5.10 (2H, s), 5.06 (2H, s), 3.78 (1H, bs), 3.72−3.15 (5, m), 2.60−2.40 (1H, m), 2.35−2.08 (3H, m). HPLC (method A): t_R = 19.1 min.

6-Benzyloxycarbonylamino-2-(*S*)-[3-(*S*)-(1*H*-indol-3-ylmethyl)-2,6-dioxopiperazin-1-yl]hexanoic Acid *tert*-Butyl Ester (6l). MH^+ *m/z* 563.1. ^{1}H NMR, 500 MHz ($CDCl_3$) δ: 9.09 (1H, bs), 7.65 (1H, d, J = 4 Hz), 7.32−7.28 (6H, m), 7.25−7.08 (2H, m), 6.95 (1H, s), 5.30−5.02 and 5.14 (3H,

m overlapped to s), 4.70 (1H, bs), 3.90−3.25 (5H, m), 3.20−3.00 (2H, m), 1.43 and 1.41 (9H, s), 2.1−0.7 (6H, m). HPLC (method A): t_R = 18.1 min.

3-*tert*-Butoxy-2-(*S*)-[3-(*S*)-(1*H*-indol-3-ylmethyl)-2,6-dioxopiperazin-1-yl]propionic Acid Methyl Ester (6m). MH^+ *m/z* 401.9. ^1H NMR, 500 MHz ($CDCl_3$) δ: 8.07 (0.25H, bs, 3R diastereoisomer), 7.74 (0.75H, bs), 7.68 (0.25H, d, J = 8 Hz, 3R diastereoisomer), 7.50 (0.75H, d, J = 8 Hz), 7.38−7.10 (4H, m), 5.49 (0.75H, dd, J = 5.0, 9.8 Hz), 5.44 (0.25H, dd, J = 5.0, 9.4 Hz, 3R diastereoisomer), 4.04−3.95 (1H, m), 3.68 and 3.93−3.49 (8H, s overlapped to m), 3.37−3.06 (1H, m), 1.14−1.07 (9H, m). HPLC (method A): t_R = 14.2 min.

2(*S*)-[3(*R*)-(1*H*-Indol-3-ylmethyl)-2,6-dioxopiperazin-1-yl]-3-(1*H*-Indol-3-yl)propionic Acid *tert*-Butyl Ester (6n). MH^+ *m/z* 487.0. ^1H NMR, 500 MHz ($CDCl_3$) δ: 7.75 (1H, bs), 7.65−6.98 (9H, m, overlapped to $CHCl_3$ signal), 6.92−6.85 (1H, m), 6.65−6.58 (1H, m), 5.54 (1H, t, J = 8.0 Hz), 3.60−3.21 (7H, m), 1.48 (9H, s). HPLC (method A): t_R = 17.8 min.

2(*S*)-[3(*S*)-(4-Benzyloxybenzyl)-2,6-dioxopiperazin-1-yl]-3-(4-benzyloxy-phenyl)propionic Acid Methyl Ester (6o). MH^+ *m/z* 579.1. ^1H NMR, 500 MHz ($CDCl_3$) δ: 7.50−7.26 (10H, m), 7.12−6.95 (4H, m), 6.95−6.72 (4H, m), 5.50 (1H, dd, J = 6.5, 10.2 Hz), 5.02 (2H, s), 5.01 (2H, s), 3.71 (3H, s), 3.70−2.98 (6H, m), 2.76−2.57 (1H, m). HPLC (method A): t_R = 22.3 min.

3-(4-Benzyloxyphenyl)-2(*S*)-(3(*S*)-isobutyl-2,6-dioxopiperazin-1-yl)propionic Acid Methyl Ester (6p). MH^+ *m/z* 439.1. ^1H NMR, 500 MHz ($CDCl_3$) δ: 7.45−7.28 (5H, m), 7.05−6.90 (2H, m), 6.90−6.75 (2H, m), 5.50 (1H, dd, J = 5.5, 10.6 Hz), 5.02 (2H, s), 3.73 (3H, s), 3.49 (2H, d), 3.43−3.19 (3H, m), 1.39−1.32 (1H, m), 0.93 (3H, d, J = 6.4 Hz), 0.89 (3H, d, J = 6.2 Hz). HPLC (method A): t_R = 18.7 min.

3-(4-Benzyloxyphenyl)-2(*S*)-(3,5-dioxo-1,4-diazaspiro-[5.5]undec-4-yl)propionic Acid Methyl Ester (6q). MH^+ *m/z* 451.1. ^1H NMR, 200 MHz ($CDCl_3$) δ: 7.45−7.28 (5H, m), 7.05−6.90 (2H, m), 6.78−6.90 (2H, m), 5.53 (1H, dd, J = 6.1, 10.6 Hz), 5.03 (2H, s), 3.73 (3H, s), 3.61−3.16 (4H, m), 1.90−1.10 (11H, bm). HPLC (method A): t_R = 18.9 min.

3-(4-Benzyloxyphenyl)-2(*S*)-(3,3-dimethyl-2,6-dioxopiperazin-1-yl)propionic Acid Methyl Ester (6r). MH^+ *m/z* 411.1. ^1H NMR, 500 MHz ($CDCl_3$) δ: 7.28−7.45 (5H, m), 7.05−6.90 (2H, m), 6.90−6.78 (2H, m), 5.53 (1H, dd, J = 6.1, 10.6 Hz), 5.03 (2H, s), 3.73 (3H, s), 3.60−3.15 (4H, m), 1.08 (3H,s), 1.22 (3H, s). HPLC (method A): t_R = 15.1 min.

2(*S*)-(2,6-Dioxopiperazin-1-yl)-3-(1*H*-indol-3-yl)propionic Acid Methyl Ester (6s). MH^+ *m/z* 526.1. ^1H NMR, 500 MHz ($CDCl_3$) δ: 8.05 (1H, bs), 7.52−7.45 (1H, m), 7.38−7.28 (1H, m), 7.22−7.01 (3H, m), 5.54 (1H, dd, J = 7, 8 Hz), 3.76 (3H, s), 3.60−3.53 (2H, m), 3.44 and 3.32 (4H, ABq, J = 18 Hz). HPLC (method B): t_R 9.5 min.

3-(4-Benzyloxyphenyl)-2(*S*)-[3(*R*,*S*)-(1*H*-indol-3-ylmethyl)-3(*R*,*S*)-methyl)-2,6-dioxopiperazin-1-yl]propionic Acid Methyl Ester (6t). MH^+ *m/z* 316.1. ^1H NMR, 500 MHz ($CDCl_3$) δ: 8.11 (1H, bs), 8.03 (1H, bs), 7.71 (1H, d, J = 7.9 Hz), 7.59 (1H, d, J = 7.9 Hz), 7.50−7.08 (18H, m), 6.98 (2H, d, J = 8.5 Hz)), 6.82 (2H, d, J = 8.5 Hz), 6.50−6.35 (4H, m), 5.56 (1H, dd, J = 5.3, 11 Hz), 5.39 (1H, dd, J = 5.1, 11 Hz), 5.05 (2, s), 4.89 and 4.85 (2H, ABq, J = 12 Hz), 3.69 (3H, s), 3.67 (3H, s), 3.60−3.47 (4H, m), 3.47−3.18 (6H, m), 3.01 (1H, dd, J = 11, 14 Hz) 2.89 (1H, d, J = 14 Hz), 2.82 (1H, d, J = 14.6 Hz), 1.28 (3H, s), 1.25 (3H, s). HPLC (method A): t_R = 19.6 and 19.8 min.

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Supporting Information Available. Proton NMR spectra of the compounds described in Table 2. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) The difficulty in determining the quantity of potassium salts in the final compounds prior to submission to pharmacological tests obliged us to use silica gel filtration, with a dramatic dropping in weight with respect to that of the crude 2,6-DKPs. This fact, together with the low yield of the deprotection step of the *p*-nitrobenzyl ester, was responsible for the poor percentage yield of the SPOS method.
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