Solution-Phase Synthesis of Mixed Amide Libraries by Simultaneous Addition of Functionalities (SPSAF) to a Diketopiperazine Tetracarboxylic Acid Scaffold Monitored by GC Analysis of Isobutyl Alcohol

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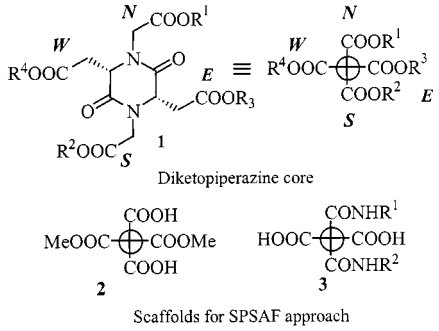
A symmetric diketopiperazine scaffold **2** has been prepared in a very simple two-step procedure from L-aspartic acid dimethyl ester. This product (a tetracarboxylic acid equally protected at the two symmetric positions) has been employed as a template for the synthesis of mixed amide libraries in the solution phase using the SPSAF (simultaneous addition of functionalities) strategy. By judicious choice of the amines employed, it is possible to prepare parallel libraries containing hundreds of products using just a small number of different amines. We have also developed a simple method for monitoring the required conversion of the acid into amides based on an assay of the amount of *i*BuOH (determined by GC) formed during the coupling mediated by isobutyl chloroformate. We have observed that a conversion higher than 90% (*i*BuOH by GC) guarantees correct formation of the desired amides. This indirect method for assessing the conversion in a combinatorial reaction employing mixed reactants (SPSAF) can conveniently be used for the routine determination of libraries prepared in the solution phase. In a broader perspective, the present results contribute as a further step in the development of new and simple systems for monitoring the progress and evolution of combinatorial reactions

Introduction

Combinatorial chemistry has become an important tool for the synthesis of small organic molecules that serve as new lead structures in drug discovery processes or give access to complex entities with novel structural and functional properties.[1–7] The preparation of libraries is generally carried out on a solid support, which obviates the need for a purification step following the multi-step procedure. Recently, solution-phase parallel or recombining syntheses have been considered as practicable alternatives to solid-phase protocols, potentially allowing the preparation of larger quantities of diversomers at low cost.[8–12]

Diversity can be introduced in a molecule by preparing linear oligomers composed of different building blocks coupled in a sequential fashion or by attaching multiple functional groups or residues to a suitable rigid or semi-rigid scaffold. The latter "radial" strategy can be particularly useful in the preparation of products of limited flexibility, where the attached groups can be accommodated inside enzyme or receptor pockets.

Amongst several rigid molecules described in the literature as core units for the generation of families of molecular diversomers,[13–17] we recently designed a diketopiperazine template **1**, fourfold functionalized with carboxyl groups.[18] This product turned out to be a valuable scaffold for the solution-phase preparation of pools of amides using either a parallel or split and mix synthetic protocol (Scheme 1). Diketopiperazines are the smallest cyclic peptides in nature and such structures are present in several synthetic and natural products possessing therapeutic properties.[19–21] Furthermore, diketopiperazines have been shown to be useful starting materials for the rational design of several drugs.[15,22,23]



Scheme 1

In our previous communication,[18] we explored all the possible syntheses of a differently protected diketopiperazine tetracarboxylic acid in order to obtain a product that could be subjected to a combinatorial protocol four times. The rather complex synthesis of such products and some difficulties encountered in achieving the desired coupling at all four positions prompted us to develop a pair of twofold functionalized carboxylic derivatives (**2** and **3**) for the preparation of small libraries in the solution phase using the simultaneous addition of functionalities (SPSAF)[24] approach to increase the molecular diversity of the products derived from our scaffold. The advantages of the SPSAF approach for the preparation of libraries have recently been

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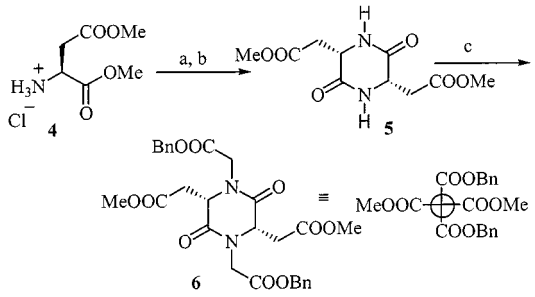
highlighted by an elegant synthesis of polyazapyridinophanes reported by Cook and co-workers.[24] One of the problems associated with the SPSAF procedure is the lack of rapid and reliable analysis methods for verifying the presence of all the predicted diversomers and the formation of undistorted libraries. Although several techniques have been proposed for the validation of medium arrays of mixed compounds, most of them are time-consuming[25] and need to be supported by high-technology instrumentation.[26,27]

We report herein that it is possible to apply the SPSAF procedure to a symmetric diketopiperazine scaffold for the preparation of libraries of mixed amides using the isobutyl chloroformate (IBC) protocol and that the correct formation of the desired product can be monitored by quantitative GC analysis of the isobutyl alcohol (*i*BuOH) formed as a co-product in the coupling reaction.

Results and Discussion

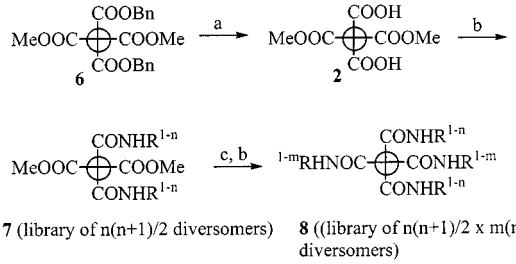
As mentioned above, our diketopiperazine template has four sites to combinatorialize, carboxylic groups that can easily be differentiated during the synthesis, allowing the preparation of *N*/*S*/*E*/*W*[28] differently substituted compounds. Despite this potential, we decided to differentiate only the two orthogonal positions and thus used an *NS*/*EW* scaffold **2** (Scheme 2). Our strategy was to prepare the simplest possible scaffold in a straightforward manner and to postpone the introduction of diversity until the subsequent SPSAF steps.



Scheme 2. (a) NH_3, $CHCl_3$; (b) heating at 65 °C for 5 days (25% yield); (c) $BrCH_2COOBn$, Ag_2O in DMF (65% yield)

The scaffold was synthesized starting from aspartic acid dimethyl ester **4** according to the Fischer procedure.[29] Temperature-controlled slow cyclization followed by crystallization from acetone gave the best yields of the intermediate diketopiperazine **5** (25%). Although low yielding, this procedure was very simple and started from inexpensive materials. The diketopiperazine was subsequently alkylated with benzyl bromoacetate in DMF in the presence of Ag_2O to give ester **6** in 68% yield.

This method provides orthogonal protection of the two pairs of carboxyl groups (*NS*/*EW*), allowing couplings through sequential deprotection to combinatorialize **6** in different modes (Scheme 3). Thus, deprotection at the *NS* position gives the diacid **2**, which can be reacted with *n* different amines (Figure 1) in a parallel mode to give *n* amides **7**. Each amide can then be deprotected at the *EW* position and further coupled with *m* different amines to give a parallel library of $n \times m$ mixed amides.



Scheme 3. (a) Deprotection NS; (b) coupling; (c) deprotection EW

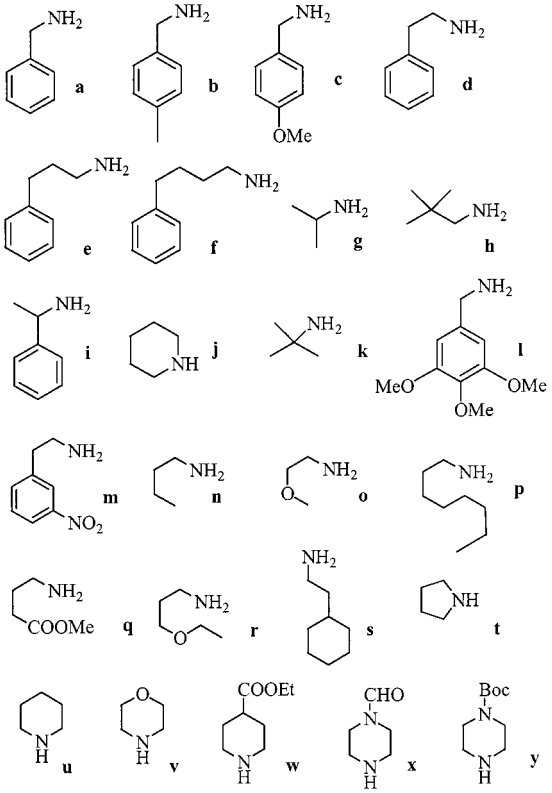


Figure 1. Amines employed

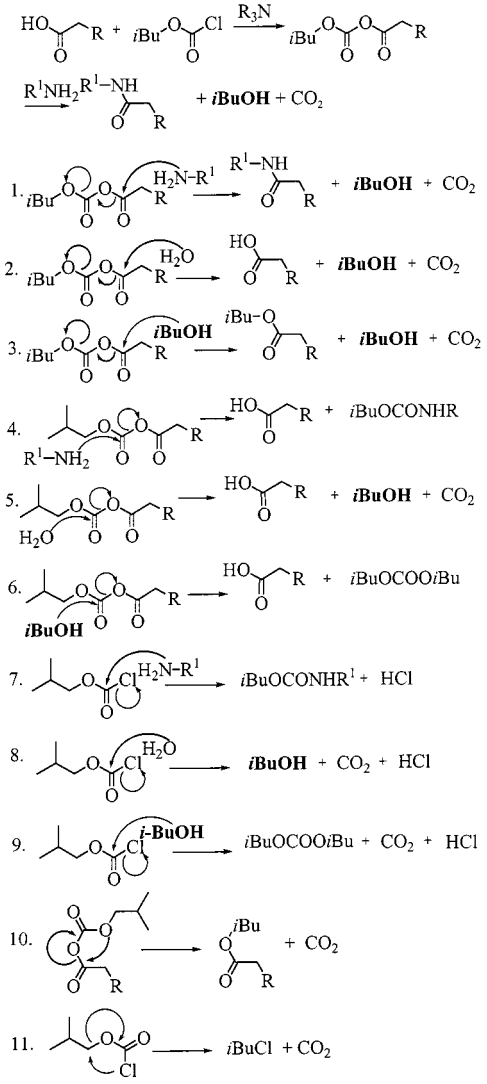
Alternatively, by applying SPSAF the diversity can be further increased within a parallel protocol. If acid **2** is reacted at *NS* with *n* sets of 3 amines, for example, we obtain a parallel library of 6*n* compounds. Thus, after the further deprotection–coupling step at the *EW* position with *m* sets of 3 different amines, we will obtain a library of $36 \times n \times m$. Just with $n = m = 3$, SPSAF will allow the generation a library of 324 different products.

To carry out this procedure, we first had to devise a selective deprotection scheme that would yield the di-protected scaffold required for the SPSAF approach. To this end, we chose to use the benzyl group for *NS* protection and the methyl group for *EW* protection. Thus, removal of the benzyl protecting group was readily accomplished by hydrogenolytic cleavage without affecting the methyl groups, and *EW* deprotection was simply achieved by treatment with KOH in MeOH, leaving the amides on the scaffold.

When using the scaffold **2** in the simultaneous addition of functionalities, it was found that an approximately equal amount of each electrophile was required in order to obtain undistorted libraries.[24] On this basis, the libraries generated by simultaneous reactions of these amines can be expected to contain all the possible compounds in approximately equal amounts depending on the symmetry of the scaffold employed. We then had to develop a rapid and simple method for verifying that acid **2** had indeed been completely converted into the amides **7**.

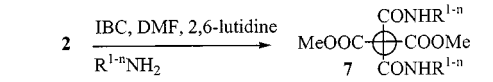
For the coupling reaction, we chose the isobutyl chloroformate (IBC) protocol. It is well known that IBC reacts with carboxylic acids in the presence of a tertiary amine to give the isobutylcarbonic acid mixed anhydride.[30] This intermediate is susceptible to attack by a primary amine, which gives the corresponding amide, carbon dioxide, and *i*BuOH. Thus, the amount of *i*BuOH formed (easily determined by gas chromatographic analysis) may be correlated to the conversion of the carboxylic acids formed in the deprotection step. Using a stoichiometric amount of IBC, detection of the theoretical amount of *i*BuOH would indicate that the reaction had reached quantitative conversion and hence could be stopped.

Scheme 4 depicts all the possible reactions and side-processes involved in this mixed anhydride coupling. We assumed that *i*BuOH was only formed during the correct addition of the amine to the mixed anhydride (entry 1). The other conceivable sources of this alcohol (entries 2, 5, and 8) could be excluded by working under strictly anhydrous conditions. In entry 3, one mol of *i*BuOH is formed from one mol of *i*BuOH without affecting the global balance. Entry 6 can be regarded the sole reaction that could modify the balance of *i*BuOH, although it can be considered highly improbable.[31] Finally, entry 7 can be excluded by employing stoichiometric amounts of IBC.



Scheme 4

To verify the above hypothesis, it was necessary to react the diacid **2** with the individual amines in the presence of IBC and then compare the amount of *i*BuOH formed with the (isolated) yields of the products. Initial attempts, using the most common protocol [IBC in THF or CH_2Cl_2 in the presence of *N*-methylmorpholine as the base and 4-methylbenzylamine (**b**, Figure 1) as the nucleophile] proved unsuccessful. Conversions were not as high as needed for a combinatorial transformation and it was found that *N*-methylmorpholine could overlap with the *i*BuOH peak upon GC analysis. After several attempts, we found that the best results could be achieved by reacting acid **2** with 1 equiv. of IBC in dry DMF at –15 °C in the presence of 2,6-lutidine as the base, followed by reaction with a stoichiometric amount of the amine (Scheme 5). A weighed amount of *tert*-butyl acetate was also added as a GC internal standard. The reaction mixture was directly analysed using a Carbowax capillary column (30 m) and the amount of *i*BuOH formed was determined by comparison of its peak with that of the internal standard. After 1 h at –15 °C and 12 h at room temperature, we found that more than 97% of the theoretical amount of *i*BuOH had been formed. The solvent was evaporated and the residue was redissolved in $CHCl_3$ and subjected to acidic and alkaline workup. After evaporation of the solvent once more, the weight of the crude material corresponded to a yield of 96%. The identity and purity of the product were verified by ^{1}H- and ^{13}C-NMR analysis. Moreover, the HPLC purity was found to be in excess



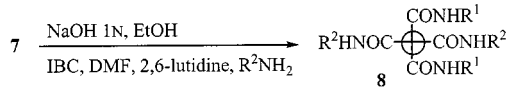
R^1–NH_2	**7** (GC) -- yield[a]	R^1–NH_2	**7** (GC) -- yield[a]
a	**7aa** (99%) -- 97%	**b**	**7bb** (99%) -- 96%
c	**7cc** (99%) -- 97%	**d**	**7dd** (99%)-- 97%
e	**7ee** (99%) -- 98%	**f**	**7ff** (98%) -- 96%
g	**7gg** (97%) -- 96%	**h**	**7hh** (96%) -- 95%
i	**7ii** (94%) -- 93%	**j**	**7jj** (95%) -- 95%

[a] Yields of isolated and fully characterized products. The structure of the amines employed are reported in chart 1.

Scheme 5

of 99%. In order to test our method, we repeated this comparative experiment with a representative set of amines (Figure 1, **a–j**, Scheme 5). In all cases, even the more difficult ones, an error of less than 2% was found.

Next, the amides **7aa**, **7gg**, and **7jj** were separately submitted to alkaline hydrolysis at *EW* with 1 N NaOH. The corresponding acids were isolated as solid products in good yields and each compound was reacted with amines **b**, **e**, and **h** to give the mixed amides **8aabb**, **8aaee**, **8aahh**, **8ggbb**, **8ggee**, **8gghh**, **8jjbb**, **8jjee**, and **8jjhh**. The very high conversions (99–93%) were again monitored by GC analysis of *i*BuOH and the yields of the isolated products matched the conversions thus determined to within ±4% (Scheme 6).

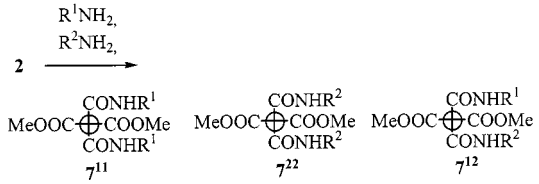


R^2–NH_2	**8** (GC) -- yield[a]	R^2–NH_2	**8** (GC) -- yield[a]
b	**8aabb**(96%) -- 95%	**e**	**8jjee** (99%) -- 96%
b	**8ggbb** (99%) -- 97%	**h**	**8aahh** (99%)-- 97%
b	**8aahh** (99%) -- 98%	**h**	**8gghh**(98%) -- 96%
e	**8aaee** (97%) -- 96%	**h**	**8jjhh** (96%) -- 95%
e	**8ggee** (94%) -- 93%		

Scheme 6

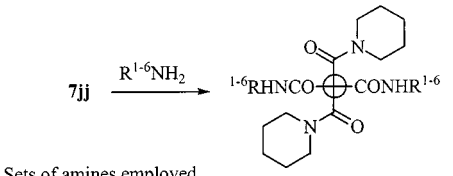
The extension of the method to SPSAF was then verified, again using the *i*BuOH method, and the results were cross-checked with NMR and HPLC/ES-MS analyses of the components of the library. Diacid **2** was reacted with an equimolar mixture of butylamine **n** and octylamine **p** following the same protocol described above (Scheme 7). After 2 h at room temperature, the assay of *i*BuOH corresponded to a conversion of 97%. After acidic and alkaline workup, products **7nn**, **7pp**, and **7np** were recovered as a crude mixture in 96% yield (by weight). The products were then separated by flash chromatography to give yields of 22%, 24%, and 46%, respectively, and were fully characterized. A further set of representative amines was reacted and the products obtained were analysed by HPLC/ES-MS (Scheme 7). We found that when mixtures of amines with analogous steric hindrance were used, the formation of an undistorted library corresponded to a conversion of about 90% (determined by GC of *i*BuOH). When amines of different reactivities were used, we observed a conversion lower than 80% with formation of a library unbalanced in the relative amounts of the diversomers.7

Next, a simple diamide **7jj** was hydrolysed and reacted further with a set of six representative amines (Scheme 8), chosen so that the masses of the components of the library would be suitably different. In all cases, when a conversion higher than 90% (GC of *i*BuOH) was reached, a correct distribution of products in the library was formed. In this case, the GC values were verified by cross-analysis with ES-MS data.



Sets of amine	% of conversion (GC)	7^{11} 7^{22}, 7^{12} yield (HPLC)
a, b	93	21, 20, 45
b, c	95	20, 22, 46
a, d	90	22, 21, 43
k, g	89	21, 19, 34
k, h	91	20, 18, 44
h, e	78	16, 27, 21
k, b	70	17, 16, 20
n, p	96	22, 24, 46

Scheme 7



Sets of amines employed

Set A: **a, b, c, f, l, m.**

Set B: **n, o, p, q, r, s.**

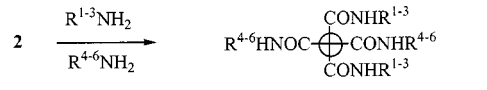
Set C: **t, u, v, w, x, y.**

8 (library of 21 diversomers)

Scheme 8

As a conclusion of this work, we set out to assess whether it was possible to use scaffold **2** as a template on which different groups could be arranged, thereby generating small libraries in the solution phase, using just GC analysis of the *i*BuOH formed as a validation tool.

Thus, **2** was first reacted with a set of 3 amines [benzylamine (**a**), *p*-methoxybenzylamine (**c**), and 2-phenylethylamine (**d**), Scheme 9]. GC analysis of *i*BuOH showed a conversion in excess of 98% after 2 h at room temperature. The products were extracted with $CHCl_3$ to give a 92% recovered yield (by weight) of crude material, which was hydrolysed with 1 M NaOH in EtOH. After acidic workup, the acids were extracted with $CHCl_3$. Working with a mixture of different acid amides, we found that a complete recovery of the products could only be achieved by carrying out a continuous extraction with hot $CHCl_3$ for 12 h. In this way, we recovered more than 92% (by weight) of the acids. Finally, reaction with amines **m**, **n**, and **o** led to a conversion of 92% to give a library of 36 compounds, which was analysed by ES-MS. The distribution of the molecular peaks was in good agreement with the proposed structures and the expected relative amounts. Other small libraries were prepared according to this protocol using sets of amines similar in shape and reactivity. With this criterion met, we invariably obtained conversions higher than 90% and undistorted libraries.



8 (library of 45 diversomers)

Amines employed:
$R^{1-3}NH_2$: **a, c, d**
$R^{4-6}NH_2$: **m, n, o.**

Scheme 9

Conclusions

The formation of the libraries described here using the SPSAF approach demonstrates the versatility of the diketopiperazine scaffold. This precursor can easily be prepared (two-step procedure) and gives rise to stable products, mainly solids, that can readily be purified and worked-up. The symmetry of the system restricts the number of compounds that can be formed upon reaction with a mixture of amines, allowing successful application of SPSAF.

Moreover, we have found that it is possible to monitor the formation of undistorted libraries by quantitative GC analysis of the amount of *i*BuOH formed during the IBC-mediated coupling. We have observed that a conversion in excess of 90% (GC of *i*BuOH) guarantees correct formation of the desired amides. The present result demonstrates that it is possible to gain access to simple libraries in the solution phase using only a simple apparatus (such as a gas chromatograph) to monitor the reaction. This indirect method for assessing the conversion in a combinatorial reaction can be conveniently used for the routine determination of libraries prepared in the solution phase using our scaffold and the protocol described here. Moreover, although not yet investigated, we feel that this approach would probably also be applicable to solid-phase syntheses. Extension of the above work is currently being directed towards the use of amino acids as amines for the preparation of larger multi-component architectures, further functionalized in order to obtain dendrimeric-type structures. In a broader perspective, the present results contribute as a further step in the development of new and simple systems for monitoring the evolution of combinatorial reactions.

Experimental Section

General: DMF and IBC were dried by standing over 4 Å molecular sieves for 12 h. All the amines employed were distilled from KOH prior to use. All reactions were carried out under nitrogen. – Gas chromatographic analyses were carried out using a Carbowax 20 M 30 m capillary column. Oven program: 45 °C for 2 min, then 20 °C/min up to 200 °C; carrier gas He (8 mL/min). The amount of *i*BuOH formed was determined by comparison of its peak area with those of the internal standards *tert*-butyl acetate and 1-butyl alcohol, corrected for the FID relative responses. – HPLC analysis was carried out using a C-18 column ODS3-Pl.5–25017 eluting with a gradient of MeCN/H_2O containing 0.1% TFA from 25:75 to 75:25 over 30 min; UV cell at 254 nm. – ES-MS spectra were recorded using a Micromass Platform LC with injection from the HPLC system. – ^{1}H- and ^{13}C-NMR spectra were recorded at 300 and 75 MHz, respectively.

Methyl (2*S*,5*S*)-(5-Methoxycarbonylmethyl-3,6-dioxopiperazin-2-yl)-acetate (5): A suspension of L-aspartic acid dimethyl ester hydrochloride (35.0 g, 177 mmol) in $CHCl_3$ (350 mL) was cooled to 0 °C, whereupon dry NH_3 was passed through it until a copious precipitate was deposited. This solid was removed by filtration through Celite and the filtrate was diluted with $CHCl_3$ (100 mL), washed with H_2O (50 mL) and satd. brine (25 mL), and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure left an oil (27.1 g, 188 mmol), which was then heated at 62–65 °C under an atmosphere of N_2 for 5 days. The residue was taken up in acetone (50 mL) and the resulting solution was refluxed for 2 h. After cooling to room temperature, the solid was collected by filtration affording pure diketopiperazine **5** (5.48 g, 24%) as a white solid; m.p. 236–238 °C. – $[\alpha]_D^{25}$ = –37.4 (*c* = 1.0, DMSO). – ^{1}H NMR ([D$_6$]DMSO): δ = 8.22 (br. s, 2 H, NH), 4.28–4.36 (m, 2 H, CH), 3.60 (s, 6 H, CH_3), 2.68–2.73 (m, 4 H, CH_2). – $C_{10}H_{14}N_2O_6$: calcd. C 46.51, H 5.46, N 10.85; found C 46.48, H 5.43, N 10.80.

Methyl (2*S*,5*S*)-[1,4-Bis(benzyloxycarbonylmethyl)-5-methoxycarbonylmethyl-3,6-dioxopiperazin-2-yl]acetate (6): In a two-necked flask under nitrogen, compound **5** (2.5 g, 9.7 mmol), $BrCH_2COOBn$ (18.3 g, 122 mmol), Ag_2O (18.5 g, 79.8 mmol), and DMF (30 mL) were mixed with stirring at room temperature. The resulting mixture was then heated at 40 °C for 48 h in a thermostatted bath. It was subsequently filtered through a Celite pad and the filtrate was concentrated to dryness under reduced pressure. Purification of the residue by flash chromatography (AcOEt/petroleum ether, 1:1) gave benzyl ester **2** (4.1 g, 76%) as a white solid; m.p. 77–79 °C. – $[\alpha]_D$ = –14.2 (*c* = 1.0, $CHCl_3$). – ^{1}H NMR ($CDCl_3$): δ = 7.25–7.40 (m, 10 H, arom. H), 5.12–5.38 (m, 4 H, CH_2), 4.40–4.55 (m, 2 H, CH), 4.10–4.28 (m, 4 H, CH_2), 3.61 (s, 6 H, CH_3), 2.80–3.10 (m, 4 H, CH_2). – ^{13}C NMR ($CDCl_3$): δ = 173.8, 172.2, 141.5, 141.3, 141.1, 128.7, 128.5, 128.3, 127.3, 127.2, 127.1, 126.9, 71.7, 71.5, 52.4, 52.2, 50.4, 50.3, 47.6, 47.4, 32.8, 32.7. – $C_{28}H_{30}N_2O_{10}$: calcd. C 60.64, H 5.45, N 5.05; found C 59.84, H 5.37, N 5.15.

(2*S*,5*S*)-[4-Carboxymethyl-2,5-bis(methoxycarbonylmethyl)-3,6-dioxopiperazin-1-yl]acetic Acid (2): A mixture of dibenzyl ester **2** (4.1 g, 7.4 mmol) and 10% Pd/C (0.4 g) in absolute methanol (100 mL) was stirred under H_2 atmosphere for 12 h at room temperature. The catalyst was removed by filtration through a Celite pad, and the filtrate was concentrated to dryness under reduced pressure to give pure **2** (2.77 g, 99% yield) (TLC: EtOH/H_2O/aq. NH_3, 10:1.2:1.6, R_f = 0.65) as a white crystalline solid; m.p. 171–173 °C. – $[\alpha]_D$ = +35.7 (*c* = 1.0, H_2O). – ^{1}H NMR ([D$_6$]DMSO/D_2O): δ = 4.41 (m, 2 H, CH), 3.90–4.16 (m, 4 H, CH_2), 3.58 (s, 6 H, CH_3), 2.83–3.01 (m, 4 H, CH_2). – ^{13}C NMR ([D$_6$]DMSO): δ = 176.4, 173.8, 172.1, 52.4, 50.5, 49.3, 32.3. – MS; *m*/*z* (relative intensity): 376.10 [M^+ + 2 H] (10), 314.15 [M^+ – 2CO_2] (57), 226.17 (33). – $C_{14}H_{18}N_2O_{10}$ (374.30): calcd. C 44.92, H 4.85, N 7.48; found C 45.1, H 4.88, N 7.52.

Methyl (2*S*,5*S*)-{5-Methoxycarbonylmethyl-3,6-dioxo-1,4-bis[(phenylmethylcarbamoyl)methyl]piperazin-2-yl}acetate (7aa): Waxy solid (97.3%). – GC (99.7%). – ^{1}H NMR ($CDCl_3$): δ = 7.34–7.14 (m, 10 H, Ar), 7.13–6.97 (m, 2 H, NH), 4.51–4.38 (m, 2 H, CH), 4.36–4.23 (m, 6 H, CH_2), 3.78 (d, 2 H, ^3J = 16 Hz, CH_2), 3.50 (s, 6 H, CH_3), 3.12–2.91 (m, 4 H, CH_2). – ^{1}H NMR (300 MHz, 50 °C, $CDCl_3$): δ = 7.34–7.14 (m, 10 H), 6.99–6.86 (br. s, 2 H), 4.51–4.38 (m, 2 H), 4.37–4.31 (m, 4 H), 4.28 (d, *J* = 16 Hz, 2 H), 3.78 (d,

J = 16 Hz, 2 H), 3.50 (s, 6 H), 3.12–2.91 (m, 4 H). – ^{13}C NMR ($CDCl_3$): δ = 171.7, 167.9, 166.2, 139.1, 130.1, 129.3, 127.5, 58.8, 52.4, 49.7, 43.5, 40.3. – MS; *m*/*z* (relative intensity): 553.58 [M^+ + H] (100). – $C_{28}H_{32}N_4O_8$ (552.58): calcd. C 60.86, H 5.84, N 10.14; found C 60.97, H 5.99, N 10.21.

Methyl (2*S*,5*S*)-{5-Methoxycarbonylmethyl-3,6-dioxo-1,4-bis[(2-phenylethylcarbamoyl)methyl]piperazin-2-yl}acetate (7dd): Waxy solid (97.1%). – GC (99.3%). – ^{1}H NMR (50 °C, $CDCl_3$): δ = 7.40–7.14 (m, 10 H, Ar), 6.84–6.70 (br. s, 2 H, NH), 4.62–4.45 (m, 2 H, CH), 4.23 (d, ^3J = 16.0 Hz, 2 H, CH_2), 4.18 (d, ^3J = 6.8 Hz, 2 H), 3.91 (d, ^3J = 16 Hz, 2 H, CH_2), 3.72 (s, 6 H, CH_3), 3.63–3.49 (m, 2 H, CH_2), 3.14–2.90 (m, 4 H, CH_2), 2.87 (dd, J_1 = 7.3 Hz, J_2 = 16.1 Hz, 4 H, CH_2). – ^{13}C NMR ($CDCl_3$): δ = 171.0, 164.6, 163.2, 137.2, 126.2, 125.8, 123.7, 58.6, 54.4, 45.1, 41.9, 35.2, 32.4. – MS; *m*/*z* (relative intensity): 581.26 [M^+ + H] (100). – $C_{30}H_{36}N_4O_8$ (580.63): calcd. C 62.06, H 6.25, N 9.65; found C 61.57, H 6.19, N 9.60.

Methyl (2*S*,5*S*)-{5-Methoxycarbonylmethyl-3,6-dioxo-1,4-bis[(4-phenylbutylcarbamoyl)methyl]piperazin-2-yl}acetate (7ff): Solid (95.8%); m.p. 134–135 °C. – GC (98.3%). – ^{1}H NMR ([D$_6$]DMSO): δ = 8.15–7.87 (m, 2 H, NH), 7.26–7.12 (m, 10 H, Ar), 4.58–4.49 (m, 2 H, CH), 4.40 (d, ^3J = 16.2 Hz, 2 H, CH_2), 3.76 (d, ^3J = 16.2 Hz, 2 H, CH_2), 3.40 (s, 6 H, CH_3), 3.37–3.21 (m, 8 H, CH_2), 3.18 (d, ^3J = 6.1 Hz, 2 H, CH_2), 3.14 (d, ^3J = 6.1 Hz, 2 H, CH_2), 2.70–2.61 (m, ^3J = 7.8 Hz, 4 H, CH_2). – ^{13}C NMR ([D$_6$]DMSO): δ = 169.7, 164.3, 163.0, 138.4, 125.5, 123.7, 55.6, 46.7, 36.8, 34.2, 30.1, 27.8. – MS; *m*/*z* (relative intensity); 636.3 [M^+ + H] (100). – $C_{34}H_{44}N_4O_8$ (636.74): calcd. C 64.13, H 6.97, N 8.80; found C 64.35, H 6.87, N 8.71.

Methyl (2*S*,5*S*)-{5-Methoxycarbonylmethyl-3,6-dioxo-1,4-bis[2,2-(dimethyl)propylcarbamoylmethyl]piperazin-2-yl}acetate (7hh): Waxy solid (95%). – GC (96.3%). – ^{1}H NMR ([D$_6$]DMSO): δ = 5.90 and 5.56 (m, 4 H, CH_2), 5.04 (m, 2 H, NH), 4.28–4.02 (m, 4 H, CH_2), 3.73 (s, 6 H, CH_3), 3.25–3.10 (m, 4 H, CH_2), 2.83 (m, 2 H, CH), 1.04 (br. s, 18 H, CH_3). – ^{13}C NMR ([D$_6$]DMSO): δ = 171.9, 170.7, 168.8, 58.8, 52.8, 51.1, 46.7, 31.9, 29.9, 24.4. – MS; *m*/*z* (relative intensity): 513.8 [M^+ + H] (100). – $C_{24}H_{40}N_4O_8$ (512.60): calcd. C 56.23, H 7.87, N 10.93; found C 56.31, H 7.78, N 10.90.

Methyl (2*S*,5*S*)-[5-Methoxycarbonylmethyl-3,6-dioxo-1,4-bis(2-oxo-2-piperidin-1-yl-ethyl)piperazin-2-yl]acetate (7jj): Waxy solid (94.9%). – GC (95.5%). – ^{1}H NMR (50 °C, $CDCl_3$): δ = 4.64–4.51 (m, 2 H, CH), 4.47 (d, ^3J = 4.5 Hz, 2 H, CH_2), 4.15 (d, J = 4.5 Hz, 2 H, CH_2), 3.70 (s, 6 H, CH_3), 3.47–3.25 (m, 8 H, CH_2), 3.23–2.95 (m, 4 H, CH_2), 1.75–1.44 (m, 12 H, CH_2). – ^{13}C NMR ($CDCl_3$): δ = 171.4, 168.6, 165.2, 56.6, 54.4, 32.5, 45.1, 41.9, 25.9, 25.1. – MS; *m*/*z* (relative intensity): 508.56 [M^+ + H] (100). – $C_{24}H_{36}N_4O_8$ (508.56): calcd. C 56.68, H 7.13, N 11.02; found C 56.71, H 7.19, N 11.06.

Library 7bc: (GC: 95%) 230.3 mg. – ^{1}H NMR (300 MHz, 50 °C, $CDCl_3$): δ = 7.21–7.02 (m, 6 H, Ar), 6.87–6.74 (m, 4 H, CH and NH), 4.50–4.41 (m, 2 H, CH), 4.36–4.19 (m, 6 H, CH_2), 3.84 (d, J = 16 Hz, 2 H, CH), 3.74 (s, 3 H, CH_3), 3.66 (d, J = 16 Hz, 2 H, CH), 3.53 (s, 6 H, CH_3), 3.14–2.93 (m, 4 H, CH_2), 2.28 (s, 3 H, CH_3). – MS; *m*/*z*: 613.25, 597.25, 581.26.

Library 7ab: (GC: 93%) 291.6 mg. – ^{1}H NMR (50 °C, $CDCl_3$): δ = 7.36–7.13 (m, 7 H, Ar), 7.11–7.02 (m, 4 H, Ar), 4.50–4.41 (m, 2 H, CH), 4.39–4.19 (m, 6 H, CH_2), 3.80 (d, J = 17.0 Hz, 2 H, CH), 3.52 (s, 6 H, CH_3), 3.14–2.93 (m, 4 H, CH_2), 2.28 (s, 3 H, CH_3). – MS; *m*/*z*: 581.26, 567.245, 553.22.

Library 7ad: (GC: 99%). – MS; *m*/*z*: 613.25, 583.24, 553.23.

Libraries 7kb and 7he: (GC < 85%). Only the products **7bb** and **7ee**, respectively, could be characterized.

Library 8 – Set A: (GC: 98.1%). – MS; *m*/*z*: 733.29, 702.26, 685.30, 673.27, 671.23, 657.27, 654.27, 643.26, 642.24, 626.24, 625.28, 623.30, 613.25, 612.23, 609.29, 597.25, 595.27, 583.24, 581.26, 567.24, 553.22.

Library 8 – Set B: (GC: 98.6%). – MS; *m*/*z*: 593.35, 569.31, 559.26, 545.28, 541.28, 531.23, 529.25, 515.27, 501.25, 489.21, 485.26, 583.29, 573.24, 553.32, 543.26, 539.30, 529.28, 517.15, 513.29, 499.27, 487.24.

Library 8 – Set C: (GC: 99.4%). – MS; *m*/*z*: 655.29, 611.26, 589.25, 582.27, 568.26, 567.24, 540.23, 538.25, 524.23, 513.21, 511.24, 509.26, 497.22, 495.24, 481.23.

Library *NS-EW* 8(acdmno): The procedure adopted was analogous to that described above, with the sole modification that the extraction with chloroform (as described for the preparation of compound **8aabb**) was substituted by a continuous extraction with hot chloroform for 12 h. All the other steps were as before. – MS; *m*/*z* (peaks in brackets were not completely resolved): 630.74, 644.33, 658.34, 660.32, 673.75, 674.34, 687.33, 690.78, 698.85, 701.35, 702.41, 703.33, 716.43, (716.78), 717.34, 718.40, 730.81, 731.88, 732.42, 733.81, 744.83, 745.41, 746.33, 746.97, 748.90, 750.43, 760.35, (760.48), 761.41, 775.42, (776.50, 776.73), 777.48, 790.49, 791.93, 807.03.

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