Efficient Synthesis of Conformationally Constrained Peptidomimetics Containing 2-Oxopiperazines[1]

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The enantioselective synthesis of a series of peptides containing the 3-substituted 2-oxopiperazine system is reported. The method involves a direct diastereoselective alkylation of the *N*-(hydroxyalkyl)-2-oxopiperazine **3**, prepared in three steps from methyl L-leucinate in 38% yield, followed by oxidation of the hydroxyl function. Esterification of the resulting acids **11** and then deprotection and acylation of N-4 afforded tripeptide analogues **16** substituted by a large variety of alkyl side chains at the 3-position of the 2-oxopiperazine.

The determination of the bioactive conformation of peptides, potentially highly flexible molecules, has often been a key step leading to the synthesis of peptide analogues with greater and more specific biological activity. In addition to X-ray crystallography analysis, the determination of peptide bioactive conformations is most frequently achieved using either a combination of computational and sophisticated spectroscopic methods[2] or by the study of multiple selectively conformationally-constrained (non)peptide analogues according to the strategy particularly pioneered by Freidinger.[3] To expand upon the latter approach, several building blocks able to specifically stabilize some parts of the peptide side chains or backbone have been designed.[4] 2-Oxopiperazines **1** constitute an interesting example of these building blocks in which the N_i and N_{i+1} atoms of the peptide backbone are linked by an ethylene bridge and consequently in which the ω_i, ϕ_i, and ψ_i torsion angles in compounds **2b** are restricted compared to the parent peptide **2a** (Figure 1).

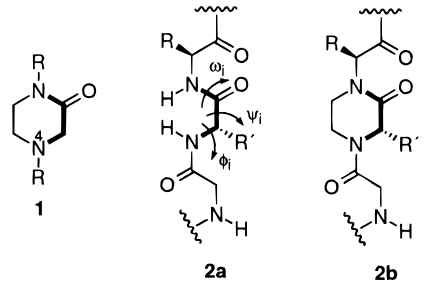

Figure 1. Torsion angles frozen in a 2-oxopiperazine peptide analogue.

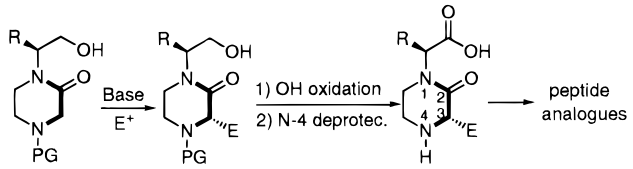

Figure 2. General strategy for obtaining the 3-substituted 2-oxopiperazine containing peptides.

Although enkephalin analogues containing 3-substituted 2-oxopiperazine skeleton clearly display interesting biological activities,[5] very few methods have been available for the synthesis of these kind of conformationally constrained compounds.[6−9] Moreover, in these strategies, 2-oxopiperazines are built invariably by condensation of two amino acids, the side chain of one becoming the C-3 substituent. Such an approach drastically narrows the choice of the potential substituents at this position to the side chains of readily available amino acids. This limitation is severe since it is generally considered that side chains are critical for specific peptide-receptor interactions.

During the course of our studies on the asymmetric synthesis of biologically interesting compounds, we designed a general and highly enantioselective synthesis of 3-substituted 2-oxopiperazines (Figure 2).[10] This method utilized a nonracemic α-substituted β-amino alcohol, whose nitrogen atom is a part of the 2-oxopiperazine, as a chiral inductor. Stereoselective alkylation of the 2-oxopiperazine C-3 atom was accomplished after enolate formation by addition of electrophiles. The observed diastereoselectivity has been explained by a rigid intermediate.[11,12] X-ray crystallography analysis

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(1) Abbreviations used: DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide; HMPA, hexamethylphosphoramide; IBCF, isobutyl chloroformate; NMM, *N*-methylmorpholine; THF, tetrahydrofuran; TBDMSCl, *tert*-butyldimethylsilyl chloride.

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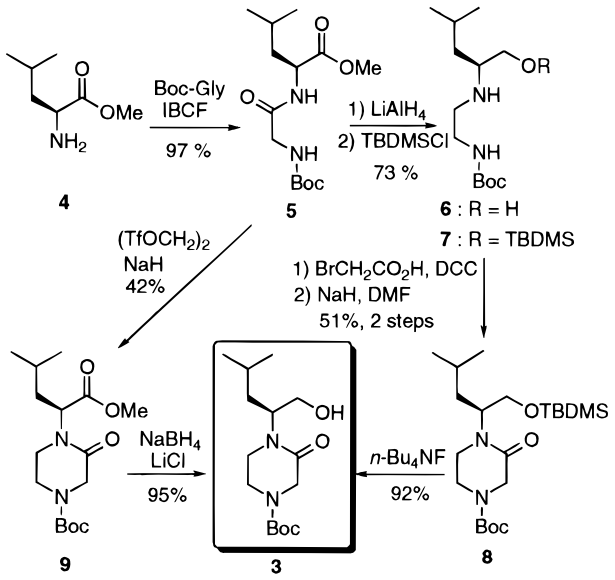
Scheme 1



established that using a natural amino acid to prepare 2-oxopiperazine **3** allowed the creation of a dipeptide analogue with the same C-3 configuration as its unconstrained parent.[10]

Furthermore, these substituted 2-oxopiperazines would contain the two functions required to synthesize oligopeptides since selective regeneration of the acidic function by oxidation of the free hydroxyl and deprotection of N-4 should lead to derivatives suitable for subsequent peptide chemistry (Figure 2).

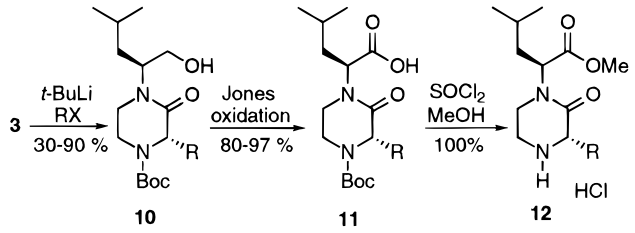
In order to confirm the possible use of **3** for the synthesis of templates suitable for peptide chemistry and widen the scope of our methodology, we describe herein a general procedure and its limitations for the enantiomerically pure synthesis of 3-substituted 2-oxopiperazine derivatives. We also present the first examples of the application of this strategy to the preparation of tripeptide analogues.

Results and Discussion

Synthesis of Oxopiperazine 3. In order to demonstrate the generality of our method and its possible application to peptidomimetic chemistry, we decided to use a proteogenic amino acid as precursor of a chiral inductor. For this reason, L-leucine was chosen instead of D-phenylglycine, as used in all our previous studies. 2-Oxopiperazine **3** could be synthesized in six steps (35−40% overall yield) starting from L-Leu-OMe (**4**) using our established conditions[10] (Scheme 1). This synthesis involved the preparation of the known dipeptide **5**.[13] Selective reduction of amide and ester carbonyl groups furnished **6**, whose alcohol function was protected as the silyl ether (**7**) before a two-step cyclization leading to **3** [$[\alpha]^{20}_D$ −16.5 (c = 0.11, $CHCl_3$)] after deprotection of the hydroxyl group.

In order to make our strategy more attractive, we decided to explore a shorter route for the synthesis of 2-oxopiperazine **3**. We sought a method for the direct formation of **3** from dipeptide **5**. To the best of our knowledge, there was no example of such a reaction conducted on an amidocarbamate compound. Initially, we attempted to condense dipeptide **5** with ethylene sulfate, which we have found to be an efficient 1,2-dielectrophile in this laboratory.[14] Unfortunately, with this compound we did not observe the formation of expected product and recovered the starting material completely. We became aware of a synthesis of 1,2,4-triazinones[15] involving a *N*,*N*-cyclization by use of ethylene glycol bis-triflate.[16] When we reacted ethylene glycol bis-triflate with compound **5**, oxopiperazine **9** was formed in 42% yield (Scheme 1). Compound **3** was obtained in optically pure [$[\alpha]^{20}_D$ −16.1 (c = 0.11, $CHCl_3$)] form after reduction of the ester function in **9** by $NaBH_4$ in the presence of LiCl.[17] Hence, this new pathway allowed the synthesis of **3** in the same yield (40%) as in the first route but in only three steps from Leu-OMe.

Scheme 2



a : R = H
b : R = CH_3
c : R = CH_2Ph
d : R = $CH_2CH(CH_3)_2$
e : R = CH_2(*o*-BrC_6H_4)
f : R = $CH_2CH{=}CH_2$
g : R = CH_2CO_2t-Bu
h : R = CH_2CO_2Et
i : R = $(CH_2)_2OCOMe$

Diastereoselective Alkylation of Oxopiperazine 3. In the stereoselective alkylation of C-3 in **3**, amide enolate was formed first in THF using *t*-BuLi (2 equiv)/HMPA as a base (Scheme 2). This enolate was reacted separately with nine different electrophiles, giving **10a**−**i**. Alkylation with methyl iodide furnished **10b** in 90% yield (de > 96% *vide infra*) together with 5% of the C-3 dimethylated derivative. Dialkylation occurred only in this case. Compounds **10c**,**e**,**f** were prepared in 75−80% yield (de > 96%) using benzyl bromide, 2-bromobenzyl bromide, and allyl bromide as electrophiles, respectively. Consistent with previous observations on a closely related compound,[12] a significantly lower alkylation yield was obtained when isobutyl bromide was used as the electrophile and **10d** was isolated in only 30% yield (de 99%). It is noteworthy that while compounds **10b**−**d** could have been synthesized by condensation of natural amino acids, compounds **10e**,**f** could not, yet they were easily obtained by our strategy.

Most of these electrophiles had been studied in the phenylglycinol series;[10,12] therefore, we decided to evaluate more functionalized electrophiles and particularly bromo ester derivatives. Only **10g** and **10h** could be easily obtained by alkylation of **3** with *tert*-butyl bromoacetate and ethyl bromoacetate (67% and 66% yield, 78% and 75% de, respectively). The alkylation reaction with 2-bromoethyl acetate afforded **10i** in only 10% yield together with several other compounds, two of which were identified as **13** (20% yield) and the transesterified

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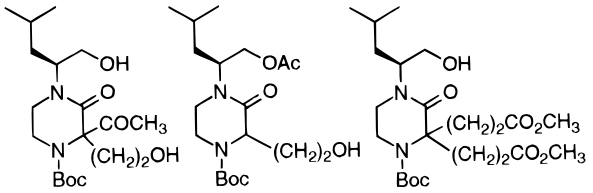
Table 1. Chemical Shifts (δ, ppm) of Leu α-C*H* of 10b−h and Some of Their C-3 Diastereoisomers in $CDCl_3$

products	10b	10c	10d	10e	10f	10g	10h
(*S*) C-3	4.54	4.70	4.47	4.58	4.60	4.16	4.35
(*R*) C-3			5.15			4.94	4.85

derivative **14** (10% yield). When methyl 3-bromopropionate was used as the electrophile, **15** was obtained in 45% yield.



13 **14** **15**

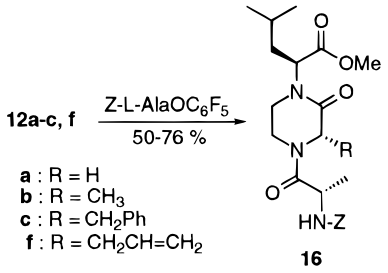
Oxidation of the primary alcohol function of **3** and **10b**−**f** using Jones reagent afforded the corresponding acids (**11a**−**f**) in 80−97% yield. Ester formation with concomitant N-4 Boc removal ($SOCl_2$/MeOH) furnished amino esters **12a**−**f** as hydrochloride salts in quantitative yields.

Determination of the Optical Purities of 10 and 12. The optical integrity of **10** and **12** was first studied by ^1H and ^{13}C NMR spectroscopy. For compounds **10b**−**f**, only one diastereomer was observed in each NMR spectrum, establishing the diastereomeric excesses of these alcohols to be at least 96%. Compound **12d** was a known product whose former preparation required drastic conditions.[7] Despite promising NMR results with **12d**, the optical rotation of **12d** prepared by our method conflicted slightly with the literature [**12d** $[\alpha]^{20}_D$ −57 (*c* = 0.1, EtOH) (lit.[7] $[\alpha]^{20}_D$ −67 (*c* = 0.97, EtOH))]. Accordingly, we questioned the accuracy of our NMR measurements. Thus, we prepared a partially C-3-epimerized mixture of **10d** (*t*-BuLi/THF, 4 h then H_2O) and were able to demonstrate by HPLC that the optical purity of the previously obtained sample of **10d** was at least 99%. Because the transformations of **10d** to **12d** should not promote racemization, we believe alkylated compounds **10b**−**f** as well as **12b**−**f** are formed with high degrees of optical purity.

X-ray crystallographic analysis of **10b**[18] confirmed the expected *S*-absolute configuration of both asymmetric centers. We used the chemical shift of α-Leu CH as a diagnostic tool to determine the absolute configuration of the 3-position of **10c**−**h**. Hence, in deuteriochloroform, this signal was constantly observed at $4.2 < \delta < 4.7$ when the stereochemistry of C-3 was *S* and at $4.8 < \delta < 5.2$ in the case of the C-3 *R* configuration (Table 1). Consequently, the C-3 *S* configuration is assigned for **10c**−**h**. Finally, these results also confirmed the importance of the chiral inductor configuration over its side chain nature, since similar diastereomeric excesses are observed with D-phenylglycinol or L-leucine derivatives.

Preparation of Tripeptide Analogues 16. Because of the great number of diastereomerically pure C-3-substituted 2-oxopiperazine derivatives accessible by this method, we were eager to demonstrate the possible incorporation of our building block into a peptide. Because elongation of the peptide chain from the C-terminal end should be classically realized starting from compounds **11**, we only studied the reactivity of N-4 toward acylation, its sterically hindered nature leading possibly to lower reactivity. Activation of the carboxyl group of Z-Ala by IBCF and coupling with **12a** afforded **16a** in 48% yield; analogous coupling with **12f** furnished **16f** in only 27% (Scheme 3). Using more reactive coupling intermediates led to improved yields. Formation of the pentafluorophenol ester of Z-Ala (DCC, C_6F_5OH, CH_2Cl_2) and condensation with **12a**−**c** and **12f** afforded **16a**−**c** and **16f**, respectively, in 50−75% yield.

Scheme 3



Conclusion

In summary, the synthetic pathway described above provides a general entry into a new series of interesting peptidomimetics. Contrasting results have been observed for alkylation reactions using bromo esters, but analogues expected from these electrophiles should be available from **11f.** The ease of synthesis of enantiomerically pure 2-oxopiperazines **11** combined with the ability to vary the C-3 positions with a large number of side chains should widen the use of this class of conformationally constrained peptidomimetics in the determination of numerous structure−activity relationships. Studies concerning the influence of the different substituents (at N-4, C-3, or the C-terminal end) on the conformation of the 2-oxopiperazine system are currently in progress and will be reported in due time.

Experimental Section

^1H and ^{13}C NMR spectra were recorded at 300.13 and 75.47 MHz. IR spectra were recorded on a 1600-FTIR instrument. Mass spectra were obtained in chemical ionization mode by direct insertion (ionizing gas NH_3). Dichloromethane, ethanol, and methanol were dried over CaH_2 and distilled prior to use. Et_2O and THF were dried over sodium/benzophenone (then lithium aluminum hydride in the case of THF) before distillation. All alkylation and condensation reactions were carried out under nitrogen atmosphere. Thin layer chromatography was performed on precoated silica gel plates (60-F_{254}, 0.2 mm) and revealed by heating with phosphomolybdic acid. Silica gel (grade 60, 230−400 mesh) was used for column chromatography. Diastereomeric excesses (de) were determined by HPLC using a NOVAPAK 18 mm column eluted with a MeOH/H_2O system. Elemental analyses were performed by Laboratoire de microanalyse du CNRS, ICSN, Gif-sur-Yvette.

(2*S*)-6-(*tert*-Butoxycarbonyl)-2-isobutyl-3,6-diazahexan-1-ol (6). To an ice-cooled solution of dipeptide **5** (16.4 g, 54.3 mmol) in dry Et_2O (300 mL) was added $LiAlH_4$ (2.05 g, 54.0 mmol). The mixture was stirred for 1 h at rt, and then additional $LiAlH_4$ (5.39 g, 142.0 mmol) was added to the reaction. Stirring was continued for 30 h, and then a 15% aqueous solution of NaOH (2.4 mL) was added dropwise to the reaction cooled at −10 °C. One hour later, water (6 mL) was added, and the suspension was stirred overnight at rt. The white precipitate was filtered and then washed with CH_2Cl_2. The filtrate was dried over $MgSO_4$, evaporated, and

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dissolved in Et_2O. The insoluble material was discarded and the solution concentrated yielding, in 75% yield, **6** as a viscous oil that was rapidly used for the next step. The hydrochloride salt was isolated as a white hygroscopic solid: $[\alpha]^{19}_D$ +13.3 (c = 0.15, MeOH, HCl salt); ^{1}H NMR (free base) δ ($CDCl_3$) 5.25 (bs, 1H), 3.52 (dd, 1H, J = 10.8, 3.5 Hz), 3.19 (dd, 1H, J = 10.8, 6.6 Hz), 3.12 (m, 2H), 2.78 (m, 1H), 2.68 (m, 2H), 2.10 (m, 2H, OH, NH), 1.60 (m, 1H), 1.41 (s), 1.31 (m, 1H), 1.20 (m, 1H), 0.87 (d, 6H, J = 6.5 Hz); ^{13}C NMR (free base) δ ($CDCl_3$) 156.5, 79.7, 63.2, 56.6, 46.3, 40.8, 28.2, 24.8, 22.9, 22.5; IR (free base) (film) 3333, 1711, 1173 cm^{-1}; MS m/z 261 (MH^+, 100), 205 (79), 175 (80), 118 (26). Anal. Calcd for $C_{13}H_{28}N_2O_3 \cdot H_2O$: C, 56.11; H, 10.79; N, 10.07. Found: C, 56.57; H, 10.49; N, 8.81.

(2*S*)-6-(*tert*-Butoxycarbonyl)-2-isobutyl-1-[(*tert*-butyldimethylsilyl)oxy]-3,6-diazahexane (7). To an ice-cooled solution of **6** (2.53 g, 9.73 mmol) were added imidazole (1.32 g, 19.4 mmol) in DMF (8 mL) and a solution of *tert*-butyldimethylsilyl chloride (1.760 g, 11.7 mmol) in DMF (5 mL). The solution was stirred for 3 h at 0 °C. The reaction was quenched by addition of H_2O (15 mL) and then made alkaline by addition of a saturated aqueous solution of Na_2CO_3 (60 mL). The aqueous phase was extracted with Et_2O (2 × 100 mL), which was further washed with H_2O (3 × 50 mL), dried over $MgSO_4$, and concentrated. The viscous oil (98% yield) could be used in such a state or the hydrochloride salt (70% yield) could be crystallized from Et_2O: mp 187 °C (Et_2O); $[\alpha]^{19}_D$ +3.9 (c = 0.13, MeOH, HCl salt); ^{1}H NMR (free base) δ ($CDCl_3$) 5.16 (bs, 1H), 3.56 (dd, 1H, J = 10.1, 4.0 Hz), 3.37 (dd, 1H, J = 10.1, 6.3 Hz), 3.30 (bs, 1H), 3.16 (m, 2H), 2.69 (m, 2H), 2.58 (m, 1H), 1.58 (m, 1H), 1.37 (s), 1.18 (m, 2H), 0.84 (m, 15H), 0.00 (s, 6H); ^{13}C NMR (free base) δ ($CDCl_3$) 156.1, 79.0, 64.5, 56.7, 45.9, 40.4, 40.4, 28.3, 25.8, 24.9, 23.0, 22.7, 18.2, 1.0; IR (free base) (film) 3333, 1708, 1253, 1173, 1096 cm^{-1}; MS m/z 375 (MH^+, 100), 319 (24), 244 (6), 173 (7). Anal. Calcd for $C_{19}H_{42}N_2O_3Si \cdot HCl$: C, 55.54; H, 10.47; N, 6.21. Found: C, 55.69; H, 10.71; N, 6.49.

(1′*S*)-4-(*tert*-Butoxycarbonyl)-1-[1′-isobutyl-2′-[(*tert*-butyldimethylsilyl)oxy]ethyl]-2-oxopiperazine (8). A solution of bromoacetic acid (5.64 g, 40.6 mmol) and DCC (4.04 g, 19.6 mmol) in CH_2Cl_2 (30 mL) was stirred at rt for 45 min. The white precipitate was removed and the filtrate poured into a solution of **7** (3.58 g, 9.58 mmol) and NMM (3 mL, 27.3 mmol) in CH_2Cl_2 (15 mL) cooled at −15 °C. The reaction was stirred for 2.5 h and then diluted with CH_2Cl_2 (100 mL) and quenched by addition of H_2O (125 mL). The organic phase was collected at rt and the aqueous phase extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were washed with H_2O (3 × 100 mL), dried over $MgSO_4$, and concentrated to afford a viscous oil that was purified by silica gel chromatography (CH_2Cl_2) (yield 73%). To this oil (3.46 g, 7 mmol), dissolved in 80 mL of THF/DMF (1:1) and cooled at 0 °C, was added NaH (0.50 g, 21 mmol). The mixture was stirred for 4 h at rt and then quenched by careful addition of H_2O (400 mL). The solution was extracted with EtOAc (3 × 100 mL), combined organic phases were washed with H_2O (4 × 100 mL), and **8** was directly isolated by crystallization from EtOH 80% (70% yield) or column chromatography (MeOH, CH_2Cl_2) (90% yield): mp 110−112 °C (EtOH); $[\alpha]^{19}_D$ −23.1 (c = 0.1, $CHCl_3$); ^{1}H NMR δ ($CDCl_3$) 4.60 (m, 1H), 4.01 (s, 2H), 3.58 (m, 2H), 3.50 (m, 2H), 3.33 (m, 1H), 3.22 (m, 1H), 1.46 (m, 2H), 1.41 (s, 9H), 1.21 (m, 1H), 0.87 (d, 6H, J = 6.4 Hz), 0.81 (s, 9H), 0.02 (s, 6H); ^{13}C NMR δ ($CDCl_3$) 166.0, 153.8, 80.5, 63.9, 52.4, 47.9, 41.7, 40.4, 36.4, 28.3, 25.7, 24.9, 23.2, 22.2, 18.0, 1.41, 1.30; IR (Nujol) 1693, 1651, 1252, 1179, 1100 cm^{-1}; MS m/z 415 (MH^+, 100), 359 (24), 301 (36), 245 (14), 225 (7). Anal. Calcd for $C_{21}H_{42}N_2O_4Si$: C, 60.87; H, 10.14; N, 6.76. Found: C, 61.14; H, 9.86; N, 6.91.

Methyl (2*S*)-2-Isobutyl-2-[4′-(*tert*-butoxycarbonyl)-2′-oxopiperazin-1′-yl]acetate (9). To a solution of **5** (1.00 g, 3.3 mmol) in Et_2O (200 mL) was added a 55% oily suspension of NaH (0.15 g, 4.0 mmol) in mineral oil at 0 °C. The suspension was stirred for 15 min, and then a solution of ethylene glycol ditriflate (1.30 g, 4.0 mmol) in Et_2O (15 mL) was slowly added. The mixture was stirred for 1 h at 0 °C, and then a second portion of a 55% oily suspension of NaH (0.21 g, 5.7 mmol) was added. The reaction was stirred for 1 h at 0 °C and 15 h at rt and then poured onto a 1 N HCl solution (15 mL)/ice. The phases were separated, the aqueous phase was extracted with Et_2O (1 × 100 mL), and the organic phases were dried and concentrated. The oily residue purified by silica gel column chromatography [EtOAc/C_6H_{12} (1:2)] yielded an oil in 42% yield that crystallized from cyclohexane: mp 80−81 °C (cyclohexane); $[\alpha]^{20}_D$ −31.3 (c = 0.08, $CHCl_3$); ^{1}H NMR δ ($CDCl_3$) 5.28 (dd, 1H, J = 9.8, 6.2 Hz), 4.05 (AB syst., 2H), 3.66 (m, 1H), 3.63 (s, 3H), 3.46 (m, 1H), 3.31 (m, 2H), 1.65 (m, 2H), 1.40 (m, 1H), 1.39 (s, 9H), 0.88 (d, 3H, J = 6.4 Hz), 0.86 (d, 3H, J = 6.4 Hz); ^{13}C NMR δ ($CDCl_3$) 171.7, 166.3, 153.7, 80.6, 53.4, 52.1, 47.7, 42.3, 40.1, 36.7, 28.2, 24.8, 23.0, 21.1; IR (film) 1743, 1698, 1660, 1170 cm^{-1}; MS m/z 329 (MH^+, 58), 273 (100). Anal. Calcd for $C_{16}H_{28}N_2O_5$: C, 58.54; H, 8.54; N, 8.54. Found: C, 58.14; H, 8.64; N, 8.05.

(1′*S*)-4-(*tert*-Butoxycarbonyl)-1-(1′-isobutyl-2′-hydroxyethyl)-2-oxopiperazine (3). From Compound 8. To a cold solution (0 °C) of compound **8** (0.42 g, 1 mmol) in THF (13 mL) was added 1.84 mL of a 1.0 M solution of Bu_4NF in THF. The reaction was stirred for 2 h at rt, and then H_2O (20 mL) was added. The two phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (2 × 30 mL). The combined organic phases were washed with H_2O (4 × 30 mL), dried, and concentrated, affording after silica gel column chromatography (eluting with a gradient of MeOH in CH_2Cl_2) **3** as a white solid crystallized from EtOAc (yield: 92%).

From Compound 9. A solution of compound **9** (0.33 g, 1 mmol) in THF (7 mL) was cooled at 0 °C. Then LiCl (0.08 g, 2 mmol), $NaBH_4$ (0.08 g, 2 mmol), and absolute EtOH (14 mL) were consecutively added. The reaction was allowed to warm at rt and stirred for 10 h, and then the reaction was adjusted to pH 4 by addition of a 10% aqueous solution of citric acid. The solution was concentrated, and the residue was dissolved in H_2O (60 mL) and extracted with CH_2Cl_2 (3 × 40 mL). The organic phases were combined, dried over $MgSO_4$, and concentrated, and compound **3** was obtained in 95% yield after silica gel column chromatography (EtOAc, C_6H_{12}) and crystallization from EtOAc: mp 108−112 °C (EtOAc); $[\alpha]^{19}_D$ −16.5 (c = 0.1, $CHCl_3$); ^{1}H NMR δ ($CDCl_3$) 4.61 (m, 1H), 4.05 (AB syst, 2H), 3.65 (dd, 1H, J = 11.7, 4.1 Hz), 3.56 (m, 3H), 3.24 (m, 2H), 1.86 (bs, 1H), 1.44 (m, 2H), 1.43 (s, 9H), 1.22 (m, 1H), 0.89 (d, 6H, J = 6.4 Hz); ^{13}C NMR δ ($CDCl_3$) 167.1, 153.7, 80.6, 63.1, 53.9, 47.8, 41.3, 40.3, 36.3, 28.3, 24.9, 23.2, 22.1; IR (Nujol) 3324, 1697, 1639, 1186 cm^{-1}; MS m/z 301 (MH^+, 100), 245 (59), 225 (4), 214 (6), 201 (9), 152 (7). Anal. Calcd for $C_{15}H_{28}N_2O_4 \cdot 1/4H_2O$: C, 59.11; H, 9.36; N, 9.19. Found: C, 59.23; H, 9.23; N, 9.05.

General Procedures. Preparation of Alkylated Compounds 10b−i. A solution of piperazin-2-one **3** (0.3 mmol) in THF (4 mL) and HMPA (1 mmol) was cooled at −78 °C under nitrogen atmosphere. A pentane solution of *t*-BuLi (0.6 mmol) was carefully added, the flow of nitrogen removed, and the reaction stirred 10 min. The electrophile (1 mmol) was then slowly added and the reaction stirred at −78 °C for 2−7 h. At the end of this period, a saturated aqueous solution of ammonium chloride (10 mL) was poured and the reaction mixture allowed to warm to rt. The two phases were separated and the aqueous phase extracted by CH_2Cl_2 (3 × 5 mL). The organic phases were combined, dried over $MgSO_4$, and concentrated under reduced pressure. Purification of the alkylated compounds was achieved by silica gel column chromatography (EtOAc, cyclohexane).

Preparation of Acids 11. To a solution of alcohol (0.3 mmol) in acetone (1.5 mL) cooled at 0 °C was added Jones reagent (0.45 mL). The solution was stirred for 30 min at rt and then cooled at 0 °C, and 2-propanol (6 mL) was added. The solution was stirred for 30 min more at rt. The solvent was removed *in vacuo*, and then H_2O (10 mL) was added and the aqueous phase was extracted with EtOAc (3 × 5 mL). The organic phase was washed with water (3 × 8 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The acids obtained in 80−97% yield were used in the next step without purification.

Preparation of *N*-Deprotected Esters 12. A solution of acid **11** (1 mmol) in MeOH (5 mL) was cooled in an ice bath. $SOCl_2$ (0.4 mL, 5.5 mmol) was carefully added, and the reaction was stirred overnight at rt. The solution was evaporated and the excess of HCl eliminated by successive addition/evaporation of MeOH. Compounds **12** were obtained in quantitative yield.

Preparation of Peptide Analogues 16. To a solution of Z-Ala (0.580 g, 2.6 mmol) in CH_2Cl_2 (10 mL) was added DCC (0.268 g, 1.3 mmol). The solution was stirred for 30 min and the white precipitate filtered off the reaction. The filtrate was poured, at 0 °C, into a solution of pentafluorophenol (0.24 g, 1.3 mmol) and DMAP (cat.) in CH_2Cl_2 (3 mL). The reaction was stirred for 1.5 h at 0 °C and then diluted with CH_2Cl_2 (20 mL) and successively washed with 1 N HCl (2 × 10 mL) and saturated Na_2CO_3 (2 × 15 mL). The dried organic phase was concentrated and the white precipitate recrystallized from Et_2O in 85% yield. The crystals (0.09 g, 2.45 mmol) were dissolved in DMF (1 mL) and poured into a solution of **12** (2.04 mmol) and NMM (6.0 mmol) in DMF (3 mL). The reaction was stirred for 15−35 h at 35 °C and then diluted with CH_2Cl_2 (3 mL) and quenched by addition of 1 N HCl (7.5 mL). The organic phase was collected, the aqueous phase was re-extracted with CH_2Cl_2 (2 × 3 mL), the combined organic phases were washed with a 1 N HCl solution (3 × 4 mL), dried, and concentrated, and compound **16** was purified by silica gel column chromatography [EtOAc/C_6H_{12} (2:1)].

(1′*S*,3*S*)-4-(*tert*-Butoxycarbonyl)-1-(1′-isobutyl-2′-hydroxyethyl)-3-methyl-2-oxopiperazine (10b) was prepared according to the general procedure in 90% yield: mp 124 °C (cyclohexane); $[\alpha]^{20}_D$ +61.6 (c = 0.13, $CHCl_3$); ^{1}H NMR δ ($CDCl_3$) 4.54 (m, 2H), 3.95 (m, 1H), 3.64 (dd, 1H, J = 11.6, 4.0 Hz), 3.54 (m, 1H), 3.30 (m, 1H), 3.21−3.08 (m, 2H), 2.74 (bs, 1H), 1.58−1.16 (m, 3H), 1.42 (s, 9H), 1.37 (d, 3H, J = 6.9 Hz), 0.88 (d, 6H, J = 5.7 Hz); ^{13}C NMR δ ($CDCl_3$) 170.4, 153.5, 80.4, 63.0, 53.8, 53.2, 41.4, 37.3, 36.3, 28.3, 24.9, 23.2, 22.0, 17.8; IR (Nujol) 3334, 1695, 1634, 1170 cm^{-1}; MS m/z 315 (MH^+, 2), 283 (6), 259 (18), 241 (5), 227 (100), 213 (24), 183 (5), 171 (2), 158 (18), 155 (19), 139 (7), 113 (7). Anal. Calcd for $C_{16}H_{30}N_2O_4$: C, 61.15; H, 9.55; N, 8.92. Found: C, 60.98; H, 9.28; N, 8.81.

(1′*S*,3*S*)-4-(*tert*-Butoxycarbonyl)-1-(1′-isobutyl-2′-hydroxyethyl)-3-(phenylmethyl)-2-oxopiperazine (10c) was prepared according to the general procedure in 76% yield: mp 115 °C (cyclohexane); $[\alpha]^{20}_D$ +48.8 (c = 0.21, $CHCl_3$); ^{1}H NMR δ ($CDCl_3$) 7.3−7.1 (m, 5H), 4.70 (m, 2H), 3.98 (m, 1H), 3.65 (m, 1H), 3.52 (m, 1H), 3.22 (bs., 2H), 2.99 (dd, 1H, J = 7.4 4.0 Hz), 2.87 (m, 1H), 2.55 (m, 1H), 2.15 (bs, 1H), 1.41−1.18 (m, 3H), 1.34 (s, 9H), 0.97 (d, 3H, J = 6.1 Hz), 0.89 (d, 3H, J = 6.1 Hz); ^{13}C NMR δ ($CDCl_3$) 168.9, 153.6, 137.4, 129.8, 128.3, 126.6, 80.5, 63.3, 58.7, 54.2, 41.2, 37.9, 37.9, 36.2, 28.1, 24.6, 23.3, 22.0; IR (Nujol) 3384, 1700, 1615, 1168 cm^{-1}; MS m/z 391 (MH^+, 100), 335 (15), 299 (13), 199 (41). Anal. Calcd for $C_{22}H_{34}N_2O_4$: C, 67.69; H, 8.72; N, 7.18. Found: C, 67.27; H, 8.35; N, 7.14.

(1′*S*,3*S*)-4-(*tert*-Butoxycarbonyl)-3-isobutyl-1-(1′-isobutyl-2′-hydroxyethyl)-2-oxopiperazine (10d) was prepared according to the general procedure in 30% yield: mp 120 °C (cyclohexane); $[\alpha]^{20}_D$ +29.3 (c = 0.08, $CHCl_3$); ^{1}H NMR δ ($CDCl_3$) 4.47 (m, 2H), 4.02 (m, 1H), 3.61 (m, 2H), 3.34 (m, 1H), 3.21 (m, 1H), 3.11 (dt, 1H, J = 11.7, 3.2 Hz), 2.47 (bs, 1H), 1.60 (m, 4H), 1.43 (s, 9H), 1.28 (m, 2H), 0.91 (m, 12H); ^{13}C NMR δ ($CDCl_3$) 170.5, 154.0, 80.7, 63.3, 56.1, 54.2, 41.8, 41.4, 37.3, 36.3, 28.3, 24.9, 24.8, 23.2, 23.0, 22.2, 22.0; IR (Nujol) 3464, 1672, 1642, 1166 cm^{-1}; MS m/z 357 (MH^+, 2), 300 (29), 283 (7), 269 (100), 255 (23), 244 (95), 227 (6), 213 (13), 201 (45), 199 (28), 197 (48), 181 (8), 113 (7), 99 (8). Anal. Calcd for $C_{19}H_{36}N_2O_4 \cdot 1/4H_2O$: C, 63.24; H, 10.12; N, 7.77. Found: C, 63.18; H, 9.64; N, 7.84.

(1′*S*,3*S*)-3-[(2-Bromophenyl)methyl]-4-(*tert*-butoxycarbonyl)-1-(1′-isobutyl-2′-hydroxyethyl)-2-oxopiperazine (10e) was prepared according to the general procedure in 75% yield: mp 138 °C (cyclohexane); $[\alpha]^{20}_D$ −2.1 (c = 0.09, $CHCl_3$); ^{1}H NMR δ ($CDCl_3$) 7.47 (d, 1H, J = 7.8 Hz), 7.14 (m, 2H), 7.05 (m, 1H), 4.82 (d, 1H, J = 7.3 Hz), 4.58 (m, 1H), 4.16 (m, 1H), 3.63 (m, 1H), 3.48 (m, 2H), 3.30 (m, 2H), 3.04 (m, 2H), 2.94 (bs, 1H), 1.45−1.22 (m, 3H), 1.10 (s, 9H), 0.89 (m, 6H); ^{13}C NMR δ ($CDCl_3$) 168.8, 153.4, 137.4, 132.7, 131.9, 128.3, 127.3, 125.3, 80.5, 63.2, 57.7, 54.5, 41.7, 37.7, 37.1, 36.3, 28.2, 24.8, 23.2, 22.1; IR (film) 3447, 1695, 1637, 1166 cm^{-1}; MS m/z 471 (MH^+, 98), 469 (MH^+, 100), 415 (66), 413 (67), 389 (12), 299 (5), 199 (52). Anal. Calcd for $C_{22}H_{33}N_2O_4Br \cdot 1/3H_2O$: C, 55.58; H, 6.95; N, 5.89. Found: C, 55.28; H, 6.88; N, 5.87.

(1′*S*,3*S*)-3-Allyl-4-(*tert*-butoxycarbonyl)-1-(1′-isobutyl-2′-hydroxyethyl)-2-oxopiperazine (10f) was prepared according to the general procedure in 80% yield: mp 85 °C (cyclohexane); $[\alpha]^{20}_D$ +64.0 (c = 0.1, $CHCl_3$); ^{1}H NMR δ ($CDCl_3$) 5.73 (m, 1H), 5.02 (d, 1H, J = 15.6 Hz), 4.98 (d, 1H, J = 8.5 Hz), 4.60 (m, 1H), 4.52 (m, 1H), 4.02 (m, 1H), 3.61 (m, 1H), 3.50 (m, 1H), 3.28 (m, 1H), 3.18 (m, 1H), 3.09 (m, 1H), 2.90 (bs, 1H), 2.57 (m, 2H), 1.40 (m, 2H), 1.40 (s, 9H), 1.19 (m, 1H), 0.87 (d, 6H, J = 5.0 Hz); ^{13}C NMR δ ($CDCl_3$) 169.1, 153.7, 133.9, 118.0, 80.6, 63.2, 57.0, 54.0, 41.3, 37.9, 37.4, 36.3, 28.2, 24.8, 23.2, 21.9; IR (film) 3444, 1695, 1644, 1171 cm^{-1}; MS m/z (MNH_4^+, 7), 341 (MH^+, 100), 285 (38), 241 (6), 229 (9). Anal. Calcd for $C_{18}H_{32}N_2O_4 \cdot 1/6H_2O$: C, 62.97; H, 9.32; N, 8.16. Found: C, 63.06; H, 8.84; N, 8.02.

***tert*-Butyl 2-[(1′*S*,3*S*)-4-(*tert*-Butoxycarbonyl)-1-(1′-isobutyl-2′-hydroxyethyl)-2-oxopiperazin-3-yl]acetate (10g)** was prepared as an oil according to the general procedure in 67% yield: de 78% (HPLC): ^{1}H NMR of the major diastereomer δ (CD_3OD, 55 °C) 4.51 (m, 1H), 4.42 (m, 1H), 3.85 (m, 1H), 3.37 (d, 2H, J = 6.9 Hz), 3.16 (m, 3H), 2.65 (dd, 1H, J = 15.3, 5.9 Hz), 2.57 (dd, 1H, J = 15.3, 5.6 Hz), 1.50−0.95 (m, 3H), 1.28 (s, 9H), 1.25 (s, 9H), 0.74 (d, 6H, J = 6.5 Hz); ^{13}C NMR δ (CD_3OD, 55 °C) 171.4, 169.8, 154.4, 82.2, 63.3, 55.8, 55.6, 42.5, 40.4, 39.6, 37.9, 28.6, 28.4, 25.9, 23.6, 22.6; IR (film) 3418, 1728, 1699, 1652, 1152 cm^{-1}; MS m/z 415 (MH^+, 78), 359 (100), 303 (41), 271 (9), 180 (25). Anal. Calcd for $C_{21}H_{38}N_2O_6$: C, 60.87; H, 9.18; N, 6.76. Found: C, 60.78; H, 9.18; N, 6.57.

Ethyl 2-[(1′*S*,3*S*)-4-(*tert*-Butoxycarbonyl)-1-(1′-isobutyl-2′-hydroxyethyl)-2-oxopiperazin-3-yl]acetate (10h) was prepared as an oil according to the general procedure in 66% yield: de 75% (NMR): ^{1}H NMR of the major diastereomer δ ($CDCl_3$, 55 °C) 4.66 (t, 1H, J = 5.1 Hz), 4.28 (m, 1H), 4.11 (q, 2H, J = 7.1 Hz), 4.09 (m, 1H), 3.72 (dd, 1H, J = 11.6, 8.6 Hz), 3.62 (dd, 1H, J = 11.6, 4.5 Hz), 3.41 (m, 2H), 3.27 (m, 1H), 3.04 (dd, 1H, J = 15.7, 5.2 Hz), 2.85 (dd, 1H, J = 15.7, 5.1 Hz), 1.63 (m, 2H), 1.46 (s, 9H), 1.30 (m, 1H), 1.23 (t, 3H, J = 7.2 Hz), 0.92 (d, 3H, J = 6.4 Hz), 0.91 (d, 3H, J = 6.4 Hz); ^{13}C NMR δ ($CDCl_3$) 172.2, 168.0, 153.5, 80.9, 63.0, 60.7, 57.1, 53.9, 43.9, 38.9, 37.5, 36.5, 28.2, 24.7, 23.2, 22.0, 14.0; IR (film) 3443, 1731, 1696, 1651, 1170 cm^{-1}; MS m/z 387 (MH^+, 59), 331 (100), 287 (26). Anal. Calcd for $C_{19}H_{34}N_2O_6$: C, 59.07; H, 8.81; N, 7.25. Found: C, 59.23; H, 8.56; N, 7.08.

(2*S*)-2-Isobutyl-2-[4′-(*tert*-butoxycarbonyl)-2′-oxopiperazin-1′-yl]acetic acid (11a) was prepared in 80% yield according to the general procedure: $[\alpha]^{20}_D$ −39.8 (c = 0.09, $CHCl_3$); ^{1}H NMR δ ($CDCl_3$) 7.10 (bs, 1H), 5.24 (m, 1H), 4.10 (AB syst., 2H), 3.72 (m, 1H), 3.58−3.28 (m, 3H), 1.72 (m, 2H), 1.42 (s, 9H), 1.42 (m, 1H), 0.90 (m, 6H); ^{13}C NMR δ ($CDCl_3$) 174.7, 167.0, 153.8, 80.9, 54.1, 47.7, 42.8, 41.0, 36.7, 28.4, 24.9, 23.1, 21.2; IR (Nujol) 2900, 1740, 1694, 1613, 1166 cm^{-1}; MS m/z 332 (MNH_4^+, 100), 315 (MH^+, 85), 276 (30), 271 (15), 259 (70). Anal. Calcd for $C_{15}H_{26}N_2O_5$: C, 57.32; H, 8.28; N, 8.92. Found: C, 57.24; H, 8.18; N, 8.74.

(2*S*,3′*S*)-2-Isobutyl-2-[4′-(*tert*-butoxycarbonyl)-3′-methyl-2′-oxopiperazin-1′-yl]acetic acid (11b) was prepared in 87% yield according to the general procedure: mp 139 °C (EtOAc); $[\alpha]^{20}_D$ +29.8 (c = 0.13, $CHCl_3$); ^{1}H NMR δ ($CDCl_3$) 5.14 (m, 1H), 4.53 (d, 1H, J = 6.6 Hz), 3.91 (d, 1H, J = 12.3 Hz), 3.42 (m, 1H), 3.22 (m, 2H), 1.71 (m, 2H), 1.60 (m, 1H) 1.41 (s, 9H), 1.36 (d, 3H, J = 6.7 Hz), 0.89 (m, 6H); ^{13}C NMR δ ($CDCl_3$) 174.5, 170.4, 153.7, 80.7, 54.3, 53.3, 42.4, 38.1, 36.7, 28.3, 25.0, 23.1, 21.2 18.0; IR (Nujol) 3000, 1740, 1702, 1596, 1173 cm^{-1}; MS m/z 329 (MNH_4^+, 1), 328 (MH^+, 1), 273 (21), 272 (19), 128 (21), 127 (89), 198 (14), 183 (16), 181 (26), 172 (32), 155 (30), 139 (36), 113 (100). Anal. Calcd for $C_{16}H_{28}N_2O_5$: C, 58.54; H, 8.54; N, 8.53. Found: C, 58.41; H, 8.38; N, 8.58.

(2*S*,3′*S*)-2-Isobutyl-2-[4′-(*tert*-butoxycarbonyl)-3′-(phenylmethyl)-2′-oxopiperazin-1′-yl]acetic acid (11c) was prepared in 89% yield according to the general procedure: $[\alpha]^{20}_D$ +34.7 (*c* = 0.07, $CHCl_3$); ^{1}H NMR δ ($CDCl_3$) 7.19−7.09 (m, 5H), 5.24 (dd, 1H, *J* = 10.6, 5.2 Hz), 4.74 (m, 1H), 4.15 (bs, 1H), 3.98 (m, 1H), 3.29 (m, 1H), 3.20 (bs, 2H), 2.85 (m, 1H), 2.63 (m, 1H), 1.70−1.19 (m, 3H), 1.33 (s, 9H), 0.93 (d, 3H, *J* = 6.5 Hz), 0.88 (d, 3H, *J* = 6.5 Hz); ^{13}C NMR δ ($CDCl_3$) 174.2, 169.0, 153.8, 137.5, 129.8, 128.3, 126.7, 80.8, 58.7, 54.3, 42.1, 37.7, 36.4, 28.1, 24.6, 23.1, 21.3; IR (Nujol) 3060, 1738, 1658, 1643 cm^{-1}; MS *m*/*z* 422 (MNH_4^+, 15), 405 (MH^+, 100), 361 (14), 349 (78), 313 (13), 291 (18), 269 (4), 257 (3), 213 (52). Anal. Calcd for $C_{22}H_{32}N_2O_5 \cdot 1/4H_2O$: C, 64.63; H, 7.95; N, 6.85. Found: C, 64.74; H, 7.88; N, 6.75.

(2*S*,3′*S*)-2-Isobutyl-2-[4′-(*tert*-butoxycarbonyl)-3′-isobutyl-2′-oxopiperazin-1′-yl]acetic acid (11d) was prepared in 89% yield according to the general procedure: $[\alpha]^{20}_D$ +15.0 (*c* = 0.08, $CHCl_3$); ^{1}H NMR δ ($CDCl_3$) 5.09 (dd, 1H, *J* = 9.5, 6.2 Hz), 4.59 (m, 1H), 4.01 (m, 1H), 3.39 (m, 1H), 3.20 (m, 2H), 1.68, (m, 4H), 1.45 (m, 2H), 1.44 (s, 9H), 0.88 (m, 12H); ^{13}C NMR δ ($CDCl_3$) 174.8, 170.5, 154.3, 81.3, 56.2, 54.9, 42.7, 41.9, 37.7, 36.8, 28.4, 25.1, 24.7, 23.2, 23.0, 22.4, 21.4; IR (Nujol) 3100, 1740, 1666, 1644, 1180 cm^{-1}; MS *m*/*z* 388 (MNH_4^+, 68), 371 (MH^+, 100), 327 (60), 315 (44), 274 (26), 271 (16), 257 (25), 229 (7), 201 (7), 152 (10). Anal. Calcd for $C_{19}H_{34}N_2O_5$: C, 61.62; H, 9.19; N, 7.56. Found: C, 61.74; H, 9.05; N, 7.42.

(2*S*,3′*S*)-2-Isobutyl-2-[4′-(*tert*-butoxycarbonyl)-3′-[(2-bromophenyl)methyl]-2′-oxopiperazin-1′-yl]acetic acid (11e) was prepared in 88% yield according to the general procedure: $[\alpha]^{20}_D$ −20.6 (*c* = 0.09, $CHCl_3$); ^{1}H NMR δ ($CDCl_3$) 7.48 (d, 1H, *J* = 7.4 Hz), 7.14 (m, 2H), 7.05 (m, 1H), 6.50 (bs, 1H), 5.13 (m, 1H), 4.87 (m, 1H), 4.16 (m, 1H), 3.46 (m, 2H), 3.12 (m, 3H), 1.72 (m, 2H), 1.31 (m, 1H), 1.10 (s, 9H), 0.91 (d, 6H, *J* = 6.3 Hz); ^{13}C NMR δ ($CDCl_3$) 174.1, 168.6, 153.6, 137.2, 132.7, 131.9, 128.4, 127.3, 125.3, 80.7, 57.7, 54.8, 42.6, 37.7, 37.4, 36.7, 27.8, 24.9, 23.1, 21.5; IR (CH_2Cl_2) 3000, 1731−1630 cm^{-1}; MS *m*/*z* 502 (MNH_4^+, 15), 500 (MNH_4^+, 15), 485 (MH^+, 40), 485 (MH^+, 40), 471 (10), 469 (10), 429 (27), 427 (27), 385 (57), 383 (58), 332 (20), 303 (22), 276 (26), 230 (18), 213 (61), 187 (20) 167 (17), 102 (100). Anal. Calcd for $C_{22}H_{31}N_2O_5Br$: C, 54.66; H, 6.42; N, 5.79. Found: C, 54.54; H, 6.62; N, 5.58.

(2*S*,3′*S*)-2-Isobutyl-2-[4′-(*tert*-butoxycarbonyl)-3′-allyl-2′-oxopiperazin-1′-yl]acetic acid (11f) was prepared in 97% yield according to the general procedure: $[\alpha]^{20}_D$ +37.7 (*c* = 0.09, $CHCl_3$); ^{1}H NMR δ ($CDCl_3$) 8.10 (bs, 1H), 5.73 (m, 1H), 5.19 (m, 1H), 5.03 (d, 1H, *J* = 12.9 Hz), 4.98 (d, 1H, *J* = 9.7 Hz), 4.58 (m, 1H), 3.98 (m, 1H), 3.42 (m, 1H), 3.20 (m, 2H), 2.57 (m, 2H), 1.70, (m, 2H), 1.45 (m, 1H), 1.41 (s, 9H), 0.98 (d, 3H, *J* = 6.6 Hz), 0.88 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR δ ($CDCl_3$) 174.8, 169.1, 153.9, 133.5, 118.2, 80.9, 57.0, 54.3, 42.3, 37.4, 36.5, 28.2, 24.8, 23.2, 21.1; IR (Nujol) 3000, 1725, 1694, 1641, 1604, 1173 cm^{-1}; MS *m*/*z* 372 (MNH_4^+, 50), 355 (MH^+, 100), 311 (47), 299 (48), 255 (11), 241 (20), 213 (7). Anal. Calcd for $C_{18}H_{30}N_2O_5 \cdot 1/5H_2O$: C, 60.40; H, 8.38; N, 7.82. Found: C, 60.42; H, 8.49; N, 7.66.

Methyl (2*S*)-2-isobutyl-2-(2′-oxopiperazin-1′-yl)acetate, hydrochloride (12a): $[\alpha]^{20}_D$ −23.2 (*c* = 0.18, EtOH abs); ^{1}H NMR (free base) δ ($CDCl_3$) 5.28 (t, 1H, *J* = 7.7 Hz), 3.63 (s, 3H), 3.52 (s, 2H), 3.22 (m, 2H), 3.03 (t, 2H, *J* = 5.3 Hz), 2.06 (bs, 1H), 1.65 (t, 2H, *J* = 7.0 Hz), 1.45 (m, 1H), 0.88 (d, 3H, *J* = 6.4 Hz), 0.86 (d, 3H, *J* = 6.3 Hz); ^{13}C NMR (free base) δ ($CDCl_3$) 171.8, 168.1, 53.1, 52.0, 50.0, 43.8, 42.9, 36.3, 24.6, 23.0, 21.1; IR (salt, CH_2Cl_2) 2950, 1740, 1666 cm^{-1}; MS *m*/*z* 246 (MNH_4^+, 17), 229 (MH^+, 100), 96 (8). Anal. Calcd for $C_{11}H_{21}N_2O_3Cl \cdot 3/2H_2O$: C, 47.80; H, 8.15; N, 10.11. Found: C, 47.64; H, 7.86; N, 10.06.

Methyl (2*S*,3′*S*)-2-isobutyl-2-(3′-methyl-2′-oxopiperazin-1′-yl)acetate, hydrochloride (12b): $[\alpha]^{20}_D$ −43.4 (*c* = 0.08, EtOH abs); ^{1}H NMR δ ($CDCl_3$) 10.50 (bs, 2H), 5.27 (m, 1H), 4.00−3.30 (m, 5H), 3.70 (s, 3H), 1.70 (m, 6H), 0.92 (d, 3H, *J* = 5.5 Hz), 0.88 (d, 3H, *J* = 5.4 Hz); ^{13}C NMR δ ($CDCl_3$) 171.4, 165.3, 54.1, 53.2, 52.5, 40.3, 39.8, 36.6, 24.7, 23.0, 21.1, 16.0; IR (CH_2Cl_2) 2732, 1734, 1654 cm^{-1}; MS *m*/*z* 260 (MNH_4^+, 15), 243 (MH^+, 100). Anal. Calcd for $C_{12}H_{23}N_2O_3Cl \cdot 3/2H_2O$: C, 47.13; H, 8.51; N, 9.16. Found: C, 47.22; H, 8.11; N, 8.96.

Methyl (2*S*,3′*S*)-2-isobutyl-2-[3′-(phenylmethyl)-2′-oxopiperazin-1′-yl]acetate, hydrochloride (12c): $[\alpha]^{20}_D$ −84.3 (*c* = 0.07, EtOH abs); ^{1}H NMR δ ($CDCl_3$) 7.30 (m, 5H), 5.30 (m, 1H), 4.30−2.80 (m, 10H), 1.70 (m, 2H), 1.40 (m, 1H), 0.90 (m, 6H); ^{13}C NMR δ ($CDCl_3$) 171.3, 164.3, 134.4, 130.1, 129.1, 127.8, 58.1, 54.2, 52.9, 40.6, 36.7, 36.4, 24.7, 23.3, 21.2; IR (CH_2Cl_2) 2700, 1738, 1660 cm^{-1}; MS *m*/*z* 336 (MNH_4^+, 2), 319 (MH^+, 100). Anal. Calcd for $C_{18}H_{27}N_2O_3Cl \cdot H_2O$: C, 57.99; H, 7.78; N, 7.51. Found: C, 58.24; H, 7.19; N, 7.26.

Methyl (2*S*,3′*S*)-2-isobutyl-2-(3′-isobutyl-2′-oxopiperazin-1′-yl)acetate, hydrochloride (12d): $[\alpha]^{20}_D$ −56.9 (*c* = 0.1, EtOH abs); ^{1}H NMR δ ($CDCl_3$) 10.68 (bs, 1H), 10.27 (bs, 1H), 5.25 (m, 1H), 3.88 (m, 2H), 3.68 (s, 3H), 3.68 (m, 1H), 3.52 (m, 1H), 3.28 (m, 1H), 1.98, (m, 3H), 1.68 (m, 2H), 1.55 (m, 1H), 0.98 (m, 6H), 0.92 (d, 3H, *J* = 6.0 Hz), 0.87 (d, 3H, *J* = 7.4 Hz); ^{13}C NMR δ ($CDCl_3$) 171.6, 165.3, 55.5, 54.3, 52.5, 40.8, 39.9, 39.9, 36.7, 24.9, 24.4, 23.1, 22.8, 21.2, 21.0; IR (film) 2630, 1739, 1660 cm^{-1}; MS *m*/*z* 285 (MH^+, 100). Anal. Calcd for $C_{15}H_{29}N_2O_3Cl \cdot 3/2H_2O$: C, 51.79; H, 9.20; N, 8.06. Found: C, 52.26; H, 8.65; N, 7.54.

Methyl (2*S*,3′*S*)-2-isobutyl-2-(3′-allyl-2′-oxopiperazin-1′-yl)acetate, hydrochloride (12f): $[\alpha]^{20}_D$ −56.6 (*c* = 0.07, EtOH abs); ^{1}H NMR δ ($CDCl_3$) 10.85 (bs, 1H), 10.10 (bs, 1H), 5.92 (m, 1H), 5.30 (m, 3H), 4.00−3.30 (m, 5H), 3.67 (s, 3H), 2.89 (m, 2H), 1.67, (m, 2H), 1.52 (m, 1H), 0.91 (d, 3H, *J* = 5.9 Hz), 0.85 (d, 3H, *J* = 5.1 Hz); ^{13}C NMR δ ($CDCl_3$) 171.5, 164.0, 130.5, 122.0, 56.8, 54.1, 52.5, 40.9, 39.8, 36.6, 34.7, 24.7, 23.1, 20.9; IR (CH_2Cl_2) 2743, 1740, 1654 cm^{-1}; MS *m*/*z* 269 (MH^+, 100). Anal. Calcd for $C_{14}H_{25}N_2O_3Cl \cdot H_2O$: C, 52.09; H, 8.37; N, 8.68. Found: C, 51.54; H, 8.29; N, 8.20.

Methyl (2*S*)-2-isobutyl-2-[4′-(Z-Ala)-2′-oxopiperazin-1′-yl]acetate (16a) was prepared according to the general procedure in 76% yield: $[\alpha]^{20}_D$ −26.6 (*c* = 0.9, $CHCl_3$); ^{1}H NMR δ ($CDCl_3$, 55 °C) 7.30 (m, 5H), 5.66 (bs, 1H), 5.30 (m, 1H), 5.05 (s, 2H), 4.60 (m, 1H), 4.25 (m, 2H), 3.87 (m, 1H), 3.66 (s, 3H), 3.66 (m, 1H), 3.38 (m, 1H), 3.28 (m, 1H), 1.70 (m, 2H), 1.45 (m, 1H), 1.29 (d, 3H, *J* = 6.8 Hz), 0.90 (d, 3H, *J* = 6.6 Hz), 0.89 (d, 3H, *J* = 6.5 Hz); ^{13}C NMR δ ($CDCl_3$, 55 °C) 171.9, 171.2, 165.8, 155.6, 136.2, 128.5, 128.2, 128.0, 66.9, 53.9, 52.2, 46.9, 46.6, 42.5, 42.2, 37.1, 25.2, 23.0, 21.4, 18.9; IR (CH_2Cl_2) 3318, 1734, 1713, 1652, 1177 cm^{-1}; MS *m*/*z* 451 (MNH_4^+), 65), 434 (MH^+, 41), 300 (100), 229 (19). Anal. Calcd for $C_{22}H_{31}N_3O_6$: C, 60.97; H, 7.16; N, 9.70. Found: C, 60.92; H, 7.18; N, 9.65.

Methyl (2*S*)-2-isobutyl-2-[4′-(Z-Ala)-3′-methyl-2′-oxopiperazin-1′-yl]acetate (16b) was prepared according to the general procedure in 63% yield: $[\alpha]^{20}_D$ +30.0 (*c* = 0.14, $CHCl_3$); ^{1}H NMR δ ($CDCl_3$) 7.30 (s, 5H), 5.78 (d, 1H, *J* = 7.6 Hz, 1H), 5.30 (dd, 1H, *J* = 10.0, 5.4 Hz), 5.05 (s, 2H), 4.84 (q, 1H, *J* = 7.0 Hz), 4.60 (m, 1H), 3.80 (m, 1H), 3.66 (s, 3H), 3.66 (m, 1H), 3.49 (m, 1H), 3.30 (m, 1H), 1.70 (m, 2H), 1.38 (m, 1H), 1.38 (d, 3H, *J* = 7.0 Hz), 1.30 (d, 3H, *J* = 6.7 Hz), 0.92 (d, 3H, *J* = 6.8 Hz), 0.91 (d, 3H, *J* = 6.7 Hz); ^{13}C NMR δ ($CDCl_3$) 171.8, 170.4, 169.4, 155.5, 136.2, 128.4, 128.0, 127.9, 66.7, 53.4, 52.8, 52.3, 46.9, 41.7, 40.7, 37.0, 25.0, 23.1, 21.1, 19.0, 17.5; IR (film) 3295, 1736, 1716, 1652 cm^{-1}; MS *m*/*z* 465 (MNH_4^+, 41), 448 (MH^+, 100), 314 (33). Anal. Calcd for $C_{23}H_{33}N_3O_6$: C, 61.74; H, 7.38; N, 9.39. Found: C, 61.19; H, 7.46; N, 9.11.

Methyl (2*S*)-2-isobutyl-2-[4′-(Z-Ala)-3′-(phenylmethyl)-2′-oxopiperazin-1′-yl]acetate (16c) was prepared according to the general procedure in 50% yield: $[\alpha]^{20}_D$ +66.7 (*c* = 0.12, $CHCl_3$); ^{1}H NMR δ ($CDCl_3$) 7.35−7.05 (m, 10H), 5.67 (d, 1H, *J* = 8.4 Hz, 1H), 5.39 (dd, 1H, *J* = 11.0, 5.0 Hz), 5.11 (s, 2H), 5.04 (m, 1H), 4.57 (m, 1H), 3.65 (s, 3H), 3.59 (m, 1H), 3.40 (dd, 1H, *J* = 13.6, 3.7 Hz), 3.28 (m, 1H), 3.22 (dd, 1H, *J* = 13.6, 5.8 Hz), 2.79 (m, 1H), 2.57 (m, 1H), 1.59 (m, 3H), 1.28 (d, 3H, *J* = 6.8 Hz), 0.98 (d, 3H, *J* = 6.4 Hz), 0.87 (d, 3H, *J* = 6.6 Hz); ^{13}C NMR δ ($CDCl_3$) 171.6, 170.5, 167.7, 155.4, 136.7, 129.7, 128.7, 128.3, 128.2, 128.0, 127.8, 128.8, 66.7, 57.7, 53.3, 52.1, 46.7, 41.5, 41.3, 36.5, 36.4, 24.4, 23.1, 21.2, 18.7, 17.5; IR (CH_2Cl_2) 3307, 1736, 1718, 1654 cm^{-1}; MS *m*/*z* 541 (MNH_4^+, 67), 524 (MH^+, 100). Anal. Calcd for $C_{29}H_{37}N_3O_6$: C, 66.54; H, 7.07; N, 8.03. Found: C, 66.13; H, 7.13; N, 8.12.

Methyl (2*S*)-2-isobutyl-2-[4′-(Z-Ala)-3′-allyl-2′-oxopiperazin-1′-yl]acetate (16f) was prepared according to the general procedure in 60% yield: $[\alpha]^{20}_D$ +36.3 (*c* = 0.10, $CHCl_3$);

^{1}H NMR δ ($CDCl_3$) 7.32 (m, 5H), 5.71 (m, 1H), 5.65 (m, 1H), 5.32 (m, 1H), 5.07 (s, 2H), 5.02 (d, 1H, J = 8.9 Hz), 5.01 (d, 1H, J = 11.8 Hz), 4.92 (m, 1H), 4.62 (m, 1H), 3.84 (m, 1H), 3.67 (s, 3H), 3.67 (m, 1H), 3.47 (m, 1H), 3.31 (m, 1H), 2.65 (m, 2H), 1.69 (m, 2H), 1.39 (m, 1H), 1.31 (d, 3H, J = 6.8 Hz), 0.94 (d, 3H, J = 6.6 Hz), 0.90 (d, 3H, J = 6.5 Hz); ^{13}C NMR δ ($CDCl_3$) 171.8, 170.0, 168.0, 155.6, 137.6, 133.2, 128.5, 128.2, 127.9, 118.5, 66.9, 56.3, 53.5, 52.3, 46.9, 41.8, 41.5, 36.9, 25.0, 23.3, 21.1, 19.0; IR (film) 3318, 1742, 1712, 1653, 1635, 1174 cm^{-1}; MS m/z 491 (MNH_4^+, 7), 474 (MH^+, 12), 340 (50), 327 (100), 237 (24), 211 (7), 181 (12), 108 (9). Anal. Calcd for $C_{25}H_{35}N_3O_6 \cdot H_2O$: C, 61.10; H, 7.53; N, 8.55. Found: C, 61.44; H, 7.24; N, 8.25.

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