# High yielding selective access to spirocyclopropanated 5-oxopiperazine-2-carboxylates and 1,4-diazepane-2,5-diones from methyl 2-chloro-2-cyclopropylideneacetate†

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The 2-spirocyclopropanated methyl 5-oxopiperazine-2-carboxylate **3** and the 3-spirocyclopropanated 6-chloro-1,4-diazepane-2,5-dione **4** could both be prepared at choice in 93 and 88% yield, respectively, from methyl 2-chloro-2-cyclopropylideneacetate (**1**) in a sequence of Michael addition of 3-benzyloxypropylamine, peptide coupling with *N*-Boc-glycine, Boc-group removal and cyclization. Transformation of the benzyloxypropyl side chain, peptide coupling with *N*-Boc-(*S*)-asparagine, deprotection and repeated cyclization led to the octahydro[2*H*]pyrazino[1,2-*a*]pyrazinetrione scaffold **10** containing a rigidified mimic of a tripeptide with a DGR motif. The overall yield of **11** after deprotection of **10** (a total of 13 steps in 8 distinct operations) was 30%.

## Introduction

Amongst the various previously reported mimics of the biologically highly relevant RGD (Arg-Gly-Asp) tripeptide motif[2] were several conformationally restricted ones with oxopiperazine[3] and 2,5-dioxopiperazine moieties.[4] As has previously been demonstrated, the multifunctional building block methyl 2-chloro-2-cyclopropylideneacetate (**1**),[5] for which a second-generation synthesis has recently been developed,[6] provides an easy access to a large variety of heterocycles[7] including spirocyclopropanated octahydro[2*H*]pyrazino[1,2-*a*]pyrazines[8] with an incorporated oxopiperazine as well as a dioxopiperazine moiety. Since the yields of the oxopiperazinecarboxylates as the precursors to the bicyclic compounds were only 21–22%, due to a predominant formation of a seven-membered lactam in the first cyclization step, we set out to determine conditions for a selective preparation of the six-membered heterocycle. Here we report on the success of this project and an application to the synthesis of a bicyclic and thereby rigidified mimic of a tripeptide containing a retro-RGD motif.

## Results and discussion

As an extremely good Michael acceptor, methyl 2-chloro-2-cyclopropylideneacetate (**1**) is known to rapidly undergo addition of all sorts of nucleophiles including secondary and primary amines, and the products can further react with nucleophilic substitution of the chlorine substituent adjacent to the cyclopropyl group.[7] Thus, 3-benzyloxypropylamine, which can be regarded as a non-functionalized precursor of an arginine analogue, added smoothly onto **1** (Scheme 1). Subsequent peptide coupling employing *N*,*N*′-dicyclohexylcarbodiimide (DCC) with Boc-glycine in THF, without isolation of the unstable Michael adduct, provided the acyclic dipeptide **2** in 88% yield. The NMR characterization of this compound was difficult, due to its dynamic behavior on the NMR timescale at ambient temperature, and the thermal lability of the *tert*-butoxycarbonyl group prevented measurements at an elevated temperature (90 °C). After chromatography and acidic deprotection (trifluoroacetic acid (TFA) or methanolic HCl) of **2**, base-induced cyclization gave, according to $^1$H NMR, a 1 : 1 mixture of the six-membered cyclic α-amino acid ester **3** and the seven-membered 6-chloro-1,4-diazepane-2,5-dione (**4**) in 40% yield. The separation of these two compounds by chromatography on silica gel was difficult because of their nearly identical retention factors, however, when the crude mixture was treated with cold $Et_2O$–MeOH, **4** crystallized and could be filtered off as a colorless solid. The mother liquor contained the oily methyl oxopiperazinecarboxylate **3**, which was easily purified by filtration over silica gel.

In view of the low overall yield and the low selectivity for the formation of either products **3** or **4** as well as the tedious separation, various conditions were screened to find out the best ones for selective formation of either one of the products (Table 1). Deprotection of the dipeptide **2** with trifluoroacetic acid (TFA), aqueous work-up and subjection to basic conditions in a biphasic system ($K_2CO_3$ in $CH_2Cl_2$) predominantly yielded the 6-chloro-1,4-diazepane-2,5-dione **4** (66 *vs.* 29% of **3**). Cyclization in aqueous $NH_3$ at elevated temperature after *N*-deprotection of **2** with trifluoroacetic acid or with methanolic HCl gave even better yields of **4** (70 and 81%, respectively), because extractive work-up was not necessary, and **3** was not formed at all. The best yield of **4** (88%), however, was achieved by deprotection of **2** with methanolic HCl followed by cyclization at 60 °C in methanolic $NH_3$. On the other hand, initial basic hydrolysis of the ester function in the dipeptide **2**, subsequent *N*-deprotection and ensuing cyclization under basic conditions (methanolic $NH_3$) followed by reesterification, gave

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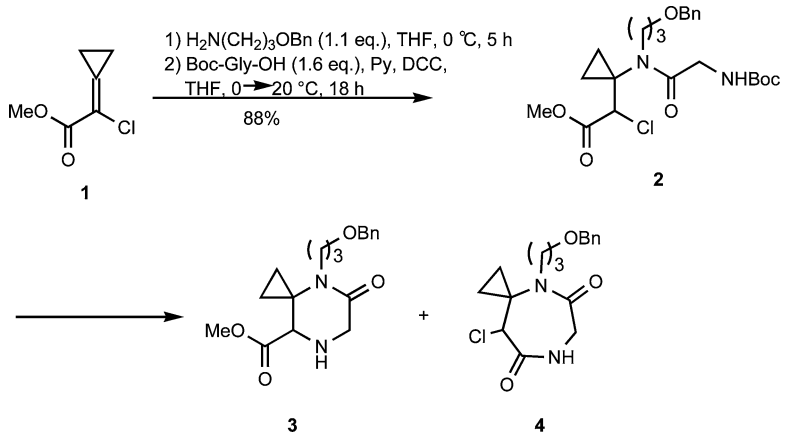



**Scheme 1** Assembly of the 4-oxopiperazinecarboxylate **3** and the cyclopropanated 6-chloro-1,4-diazepane-2,5-dione **4** from methyl 2-chloro-2-cyclopropylideneacetate **1**. For details of the second step see Table 1.

the methyl oxopiperazinecarboxylate **3** selectively and in very high yield (93%). Under these conditions, **4** is not formed at all, because the carboxylate ion is not prone to undergo attack by a nucleophile, yet careful monitoring of the reaction by TLC and NMR is necessary to obtain best yields. Eventually, a similarly good yield of **3** could be achieved by simply stirring the deprotected (methanolic HCl) dipeptide **2** in THF with an excess of triethylamine. Though in this case the diazepane-2,5-dione **4** was also formed in 14% yield, multigram quantities (up to 6 g) of **3** could easily be obtained in 84% yield according to this protocol.

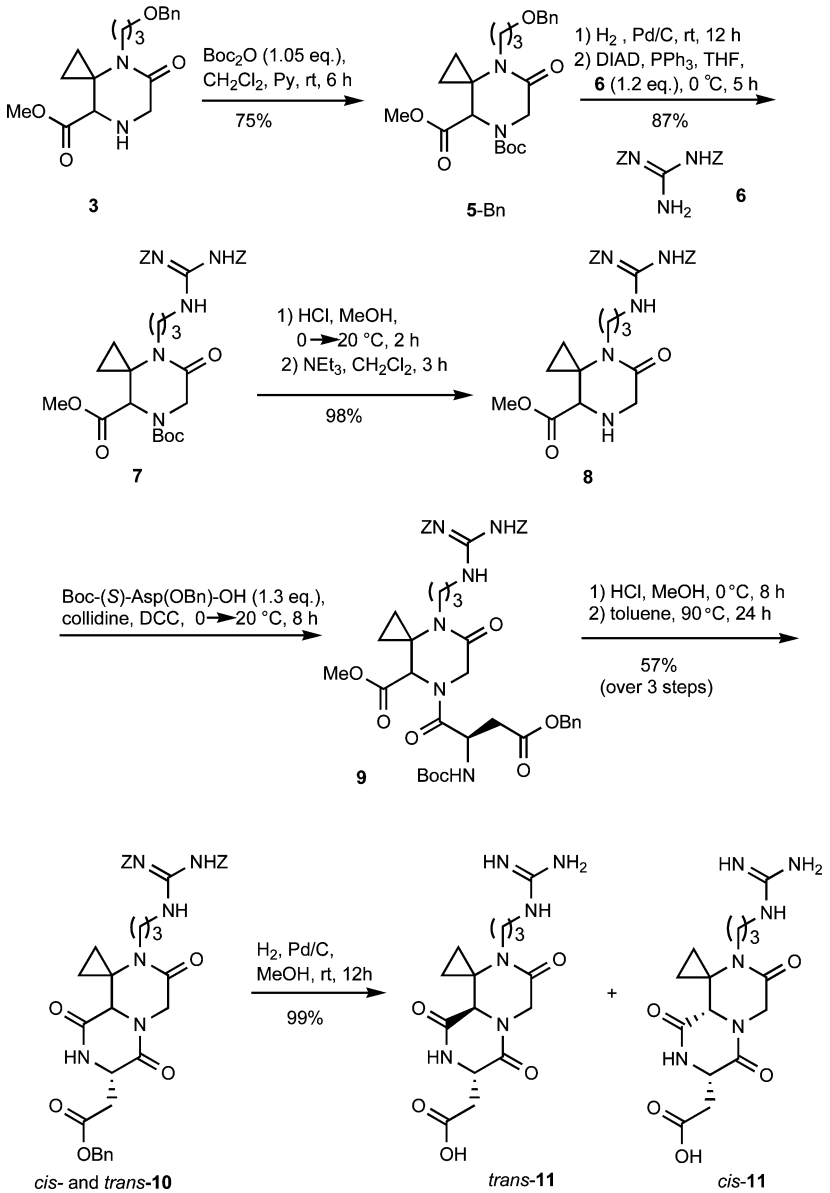
*tert*-Butoxycarbonylation of the secondary amine **3** gave **5**-Bn in 75% yield after chromatographic separation from approximately 14% of **4** remaining from the cyclization step (Scheme 2). Deprotection of the benzyloxy group in **5**-Bn by catalytic hydrogenation in methanol and filtration of the crude mixture over Celite® gave the primary alcohol **5**-H in nearly quantitative yield. The latter was directly used for the Mitsunobu reaction with 1.5 equivalents of the twofold *Z*-protected guanidine **6**[9] in the presence of stoichiometric amounts of triphenylphosphine and diisopropyl azodicarboxylate (DIAD) at 0 °C. The termination of the reaction was indicated by the disappearance of the intensive orange color of DIAD, and the fully protected oxopiperazinecarboxylate **7** was isolated in 87% yield (over two steps and after chromatography). Removal of the *N*-Boc group with methanolic HCl and liberation of the amine with an excess of triethylamine provided **8** in 98% yield.

Standard peptide coupling of **8** with *N*-Boc-(*S*)-Asp-OBn employing DCC and 2,4,6-collidine as a sterically encumbered base to prevent racemization yielded the fully protected tripeptide mimic **9** (Scheme 2), which was also difficult to characterize by NMR spectroscopy because of its dynamic behavior at 20 °C. The latter must be due to several coexisting conformers of this tripeptide, which only slowly (on the $^1$H-NMR timescale) convert into each other. Spectra could not be recorded at elevated temperature because of its thermal instability.

Removal of the *N*-Boc group from **9** was achieved by treatment with an excess of methanolic HCl, and the resulting amino-terminal dipeptide fragment in the deprotected **9** was cyclized by heating the compound in toluene at 90 °C to furnish the fully protected octahydro[2*H*]oxopyrazino[1,2-*a*]pyrazinedione **10** containing the rigidified *retro*-RGD motif with a β-amino acid analogue of arginine. This unseparable 1 : 1 mixture of diastereomers was completely deprotected by catalytic hydrogenation in methanol over palladium on charcoal. The crude mixture was purified by filtration over Celite® to prevent any loss of the DGR tripeptide mimic **11**, which is extremely soluble in water. Thus, the 1 : 1 mixture of *trans*- and *cis*-**11** was obtained as a colorless solid in 99% yield.

**Table 1** Optimization of the deprotection–cyclization of the dipeptide **2** to either yield the 5-oxopiperazine-2-carboxylate **3** or the cyclopropanated 6-chloro-1,4-diazepane-2,5-dione **4** (see Scheme 2)

| Entry | Conditions | Yield **3** (%) | Yield **4** (%) |
|---|---|---|---|
| 1 | (1) TFA, $CH_2Cl_2$; (2) $NaHCO_3$, DMF, 20 °C | 20 | 20 |
| 2 | (1) TFA, $CH_2Cl_2$; (2) aq. $K_2CO_3$, $CH_2Cl_2$ | 29 | 66 |
| 3 | (1) TFA, $CH_2Cl_2$; (2) aq. $NH_3$, 60 °C | — | 70 |
| 4 | (1) HCl, MeOH; (2) aq. $NH_3$, 60 °C | — | 81 |
| 5 | (1) HCl, MeOH; (2) $NH_3$, MeOH, 60 °C | — | **88** |
| 6 | (1) TFA, $CH_2Cl_2$; (2) $Na_2CO_3$, DMF, 25 °C | 49 | < 2 |
| 7 | (1) NaOH, MeOH, 20 °C, 8 h; (2) MeOH, HCl, 0 °C, 4 h; (3) MeOH, $NH_3$, 0 → 20 °C, 10 h; (4) MeOH, cat. conc. $H_2SO_4$, 8 h | **93** | — |
| 8 | (1) HCl, MeOH; (2) $NEt_3$, THF | 84 | 14 |




**Scheme 2** Conversion of the oxopiperazinecarboxylate **3** to the spirocyclopropanated octahydro[2*H*]oxopyrazino[1,2-α]pyrazinedione **11**.

## Conclusion

The overall achievement constitutes a 13-step synthesis (8 distinct operations) of a conformationally rigidified peptidomimetic with the *retro*-RGD sequence (DGR) from methyl 2-chloro-2-cyclopropylideneacetate (**1**) in 30% overall yield. The three-membered ring in the intermediate **2** formed by Michael addition of a primary amine to **1** and subsequent peptide condensation with *N*-Boc-glycine, due to its Thorpe–Ingold effect,[10] is instrumental in favoring the cyclization to an oxopiperazinecarboxylate **3**. The seven-membered 6-chloro-1,4-diazepane-2,5-dione **4**, which can alternatively be obtained with high selectivity from **1**, 3-benzyloxypropylamine and *N*-Boc-protected glycine, also offers itself as a multifunctional scaffold for various pharmacophores and as a precursor to other interesting heterocycles.[11]

## Experimental section

### General remarks

All reagents were used as purchased from commercial suppliers without further purification. All reactions in non-aqueous solvents were carried out using standard Schlenk techniques under an atmosphere of dry nitrogen. Solvents were purified and dried according to conventional methods prior to use; diethyl ether ($Et_2O$) and tetrahydrofuran (THF) were freshly distilled from sodium–benzophenone. Solvents are abbreviated as follows: Py = pyridine, EtOAc = ethyl acetate, MeOH = methanol, *n*BuOH = *n*-butanol, TFA = trifluoroacetic acid. $^1$H- and $^{13}$C-NMR spectra were recorded at ambient temperature on a Bruker AM 250 or a Varian UNITY-300 instrument. Chemical shifts $\delta$ are given in

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ppm relative to residual resonances of solvents ($^1$H: 7.26 ppm for chloroform; $^{13}$C: 77.0 ppm for $CDCl_3$), coupling constants $J$ are given in Hertz. Characterization of the multiplicity of signals: s = singlet, br s = broad singlet, d = doublet, t = triplet, m = multiplet. The multiplicities of signals were determined by the DEPT (Bruker) or the APT (Varian) technique. IR: Bruker IFS 66. MS: Finnigan MAT 95. Chromatography: Separations were carried out on Merck Silica 60 (0.063–0.200 mm, 70–230 mesh ASTM). The dimensions of the columns are given as "diameter × height of the silica gel column". TLC: Macherey–Nagel, TLC plates Alugram® Sil G/UV$_{254}$. Detection under UV light at 254 nm, development with MOPS (10% molybdophosphoric acid, solution in ethanol) or ninhydrin (100 mL *n*-BuOH, 3 mL glacial acetic acid, 100 mg ninhydrin) reagent. Melting points: apparatus according to Dr Tottoli (Büchi); the values are uncorrected. Elemental analyses: Mikroanalytisches Laboratorium des Instituts für Organische und Biomolekulare Chemie der Universität Göttingen.

**Methyl {1-[(3-Benzyloxypropyl)-(2-*tert*-butoxycarbonylamino-acetyl)amino]cyclopropyl}chloroacetate (2).** To a solution of 3-benzyloxypropylamine[12] (13.2 g, 79.9 mmol) in THF (100 mL) was added dropwise at 0 °C a solution of **1**[6] (10.6 g, 72.3 mmol) in THF (50 mL), and the mixture was stirred at this temperature for 5 h. At 0 °C, pyridine (9.64 mL, 119 mmol), *N*-*tert*-butoxycarbonylglycine (20.9 g, 119 mmol) as well as a solution of DCC (24.7 g, 119 mmol) in THF (70 mL) were added, and the mixture was stirred with rewarming to 20 °C for 18 h. The precipitate was filtered off, washed with THF (2 × 50 mL), and the solvent was removed under reduced pressure. The residue was taken up in $CH_2Cl_2$ (300 mL), the mixture extracted with cold (0 °C) 1 N HCl (100 mL), water (100 mL) and sat. $NaHCO_3$ solution (3 × 100 mL), the organic extracts were dried over $Na_2SO_4$, the solvent was removed under reduced pressure and the residue subjected to chromatography on 800 g of silica gel (10 × 28 cm, pentane–ethyl acetate 6 : 4; $R_f$ = 0.38, ninhydrin) to yield 29.9 g (88%) of **2** as an oil. IR (film): $\nu$ = 3451 cm$^{-1}$ (NH), 3288, 3245, 3045, 1770, 1675 (CO), 1410, 1399, 702, 688. $^1$H NMR (300 MHz, $C_2D_2Cl_4$, 120 °C): $\delta$ = 0.87–1.42 (m, cPr-H, $CH_2$, 6 H), 1.45 (s, *t*Bu-H, $CH_2$, 11 H), 1.92 (unresolved quintet, $CH_2$, 2 H), 3.53 (m, $CH_2$, 2 H), 3.81 (s, $OCH_3$, 3 H), 4.02 (br s, CHCl, 1 H), 4.47 (m, NHC=O, 1 H), 7.26–7.45 (m, aryl-H, 5 H). $^{13}$C NMR (75.5 MHz, $C_2D_2Cl_4$, 120 °C): $\delta$ = 20.1 ($CH_2$, cPr-C), 23.7 ($CH_2$, cPr-C), 28.3 ($CH_3$, *t*Bu-C), 29.2 ($CH_2$), 42.0 (C, cPr-C), 43.2 ($CH_2$), 47.8 ($CH_2$), 52.2 ($CH_3$), 64.8 (CH), 67.6 ($CH_2$), 72.8 (C, *t*Bu-C) 79.5 ($CH_2$), 126.4 (CH, aryl-C), 128.3 (CH, aryl-C), 138.1 (C, aryl-C), 168.3 (CO), 168.5 (CO), 171.8 (CO). MS (70 eV, DCI); *m*/*z* (%): 486 (75) [M + $NH_4^+$], 469 (35), 452 (30), 435 (10), 163 (65), 123 (100), 106 (90). $C_{23}H_{33}ClN_2O_6$ (469.0): calcd. C 58.91, H 7.09, N 5.97, Cl 7.56; found C 58.60, H 6.83, N 5.85, Cl 7.35%.

**Methyl 4-(3-benzyloxypropyl)-5-oxo-4,7-diazaspiro[2.5]octane-8-carboxylate (3).**

*Method A.* To a solution of **2** (5.92 g, 12.6 mmol) in MeOH (70 mL) was added 1 M aq. NaOH (15 mL), and the emulsion was stirred at 20 °C for 8 h. All volatiles were removed *in vacuo*, the aq. phase was extracted with $CH_2Cl_2$ (3 × 50 mL), the combined organic phases were dried over $Na_2SO_4$, the solvent was removed *in vacuo*, and the residue was stirred in methanolic HCl (20 mL, 92 mmol, 4.60 M) at 0 °C for 4 h. All volatiles were removed *in vacuo*, and the residue was stirred in methanolic $NH_3$ (25 mL, 50 mmol, 2 M) at 0 °C with rewarming to 20 °C for 10 h. All volatiles were removed *in vacuo*, the residue was dissolved in MeOH (100 mL), conc. $H_2SO_4$ (3 mL) was added, and the mixture was stirred for 8 h. Removal of the solvent *in vacuo*, uptake of the residue in $CH_2Cl_2$ (200 mL), extraction with sat. $NaHCO_3$ solution (3 × 30 mL), drying of the organic phase over $Na_2SO_4$, removal of the solvents *in vacuo* and chromatographic purification on 200 g of silica gel (5 × 30 cm, $CH_2Cl_2$–MeOH 95 : 5, $R_f$ = 0.33, ninhydrin) yielded 3.90 g (93%) of **3** as an oil. IR (film): $\nu$ = 3340 cm$^{-1}$ (NH), 3040, 2912, 2835, 1750 (CO), 1705 (CO), 1660 (CO), 859, 756. $^1$H NMR (250 MHz, $CDCl_3$): $\delta$ = 0.75–0.96 (m, cPr-H, 1 H), 0.97–1.05 (m, cPr-H, 1 H), 1.20–1.30 (m, cPr-H, 1 H), 1.34–1.44 (m, cPr-H, 1 H), 1.57–1.70 (m, 2 $CH_2$, 4 H), 2.10–2.18 (br s, NH, 1 H), 3.18 (s, CH, 1 H), 3.45 (m, $CH_2$, 2 H), 3.58 (s, $NCH_2C$=O, 2 H), 3.68 (s, $OCH_3$, 3 H), 4.49 (s, $CH_2Bn$, 2 H), 7.25–7.33 (s, aryl-H, 5 H). $^{13}$C NMR (62.9 MHz, $CDCl_3$): $\delta$ = 8.8 ($CH_2$, cPr-C), 15.2 ($CH_2$, cPr-C), 28.9 ($CH_2$), 38.8 ($CH_2$), 40.0 (C, cPr-C), 47.9 ($CH_2$), 52.6 ($CH_3$), 62.9 (CH), 67.5 ($CH_2$), 72.8 ($CH_2$, OBn), 127.36 (CH, aryl-C), 127.40 (CH, aryl-C), 138.1 (C, aryl-C), 172.1 (CO), 172.5 (CO). MS (70 eV, EI); *m*/*z* (%): 332 (10) [M$^+$], 273 (10), 241 (25), 209 (10), 167 (20), 91 (100) [$C_7H_7^+$]. $C_{18}H_{24}N_2O_4$ (332.4): calcd. C 65.04, H 7.28, N 8.43; found C 64.90, H 7.34, N 8.80%.

*Method B.* To a solution of **2** (3.68 g, 7.85 mmol) in $CH_2Cl_2$ (20 mL) was added dropwise at 0 °C TFA (6.50 mL, 87.5 mmol), and the mixture was stirred with rewarming to 20 °C for 18 h. It was then cooled again to 0 °C and poured into a vigorously stirred mixture of DMF (40 mL) and $NaHCO_3$ (6.00 g, 71.4 mmol). The suspension was stirred at 20 °C for 24 h, diluted with $CH_2Cl_2$ (200 mL), the organic phase was extracted with water (2 × 100 mL) and brine (100 mL), the aq. phase was re-extracted with $CH_2Cl_2$ (2 × 50 mL), and the combined organic phases were dried over $Na_2SO_4$. Removal of the solvent *in vacuo* and crystallization of the residue from MeOH–$Et_2O$ at –20 °C yielded 527 mg (20%) of **4** as a colorless solid, mp 103–106 °C. Concentration of the mother liquor *in vacuo* and chromatographic purification of the residue on 50 g of silica gel (2 × 30 cm, dichloromethane–methanol 95 : 5, ninhydrin) yielded 521 mg (20%) of **3** as a colorless oil.

*Method C.* A solution of **2** (9.60 g, 20.5 mmol) was stirred in methanolic HCl (80 mL, 100 mmol, 1.25 M) at 0 °C for 4 h. All volatiles were removed *in vacuo*, the residue was dissolved in THF (200 mL), triethylamine (10.0 mL, 72.1 mmol) was added, and the suspension was stirred at 20 °C for 15 h. The precipitate was filtered off, washed with THF (2 × 50 mL), and all volatiles were removed *in vacuo*. Chromatographic purification of the residue on 100 g of silica gel (4 × 30 cm, $CH_2Cl_2$–MeOH 95 : 5, ninhydrin) yielded 5.72 g (84%) of **3** as a colorless oil, along with *ca.* 14% of **4**. This mixture was used in the next step without further purification.

**4-(3-Benzyloxypropyl)-9-chloro-4,7-diazaspiro[2.6]nonane-5,8-dione (4).** Compound **2** (3.24 g, 6.91 mmol) was dissolved in methanolic HCl (70 mL, 63.7 mmol, 0.91 M) at 0 °C, and the mixture was stirred with rewarming to 20 °C for 11 h. All volatiles were removed *in vacuo*, the residue was dissolved in methanolic $NH_3$ (80 mL, 373 mmol, 4.66 M, pH ~ 10), and the mixture heated at 60 °C for 4.5 h. The solvents were removed *in vacuo*, and the residue subjected to chromatography on 70 g of silica gel (23 × 3 cm, $CH_2Cl_2$–MeOH 95 : 5, ninhydrin, $R_f$ = 0.30) to furnish the crude product as an oil, which crystallized upon treatment with MeOH–$Et_2O$ at –20 °C to yield 2.04 g (88%) of **4** as a colorless

solid, mp 103–106 °C. IR (KBr): $\nu = 3250$ cm$^{-1}$ (NH), 3175, 3056, 2837, 2801, 1652 (CO), 801, 745. $^1$H NMR (250 MHz, $CDCl_3$): $\delta =$ 1.11–1.23 (m, cPr-H, 1 H), 1.23–1.31 (m, cPr-H, 1 H), 1.35–1.44 (m, cPr-H, 1 H), 1.60–1.70 (m, cPr-H, 1 H), 1.92 (m, $CH_2$, 2 H), 3.30–3.46 (m, $CH_2$, $NCH_2$, 4 H), 3.68–3.90 (m, $NCH_2CO$, CHCl, 3 H), 4.41 (s, $CH_2Ph$, 2 H), 7.24–7.35 (s, aryl-H, 5 H), 7.67 (s, NH, 1 H). $^{13}$C NMR (62.9 MHz, $CDCl_3$): $\delta = 15.1$ ($CH_2$, cPr-C), 19.9 ($CH_2$, cPr-C), 29.3 ($CH_2$), 42.0 (C, cPr-C), 47.5 ($CH_2$), 47.8 ($CH_2$), 64.7 (CH), 67.7 ($CH_2$), 73.0 ($CH_2$), 127.7 (CH, aryl-C), 128.4 (CH, aryl-C), 138.1 (C, aryl-C), 168.1 (CO), 169.9 (CO). MS (70 eV, DCI); *m/z* (%): 301 (25) [$(M - Cl)^+$], 195 (30), 91 (100) [$C_7H_7^+$]. $C_{17}H_{21}ClN_2O_3$ (336.8): calcd. C 60.62, H 6.28, N 8.32; found C 60.71, H 6.38, N 8.55%.

**Methyl 4-(3-benzyloxypropyl)-7-*tert*-butoxycarbonyl-5-oxo-4,7-diazaspiro[2.5]octane-8-carboxylate (5-Bn).** To a solution of **3** (5.00 g, 15.0 mmol) in $CH_2Cl_2$ (100 mL) were added at 20 °C pyridine (1.34 mL, 16.5 mmol) and $Boc_2O$ (3.45 g, 15.8 mmol), and the mixture was stirred at this temperature for 6 h. Extraction with cold (0 °C) 1 N HCl (30 mL), water (30 mL) and sat. $NaHCO_3$ solution (30 mL), drying of the organic phase over $Na_2SO_4$, removal of all volatiles *in vacuo* and chromatographic purification of the residue on 40 g of silica gel (3 × 20 cm, $Et_2O$, $R_f = 0.49$) yielded 4.87 g (75%) of **5**-Bn as an oil. IR (film): $\nu = 3327$ cm$^{-1}$, 3088, 3063, 2930, 2830, 1750 (CO), 1682 (CO), 894, 741. $^1$H NMR (250 MHz, $CDCl_3$): $\delta =$ 0.70–0.85 (m, cPr-H, 1 H), 0.90–1.00 (m, cPr-H, 1 H), 1.20–1.35 (m, cPr-H, 2 H), 1.37 (s, *t*Bu, 4.3 H), 1.45 (s, *t*Bu, 4.7 H), 1.50–1.70 (m, 1 H), 2.75–3.00 (m, 1 H), 3.35–3.40 (m, 2 H), 3.61 (s, $CH_2N$, 2 H), 3.70 (s, $CH_3O$, 3 H), 3.83 (s, 1 H), 3.90–4.08 (m, 1 H), 4.33–4.55 (m, 1 H), 4.45 (s, 2 H), 7.27–7.38 (m, 5 H). $^{13}$C NMR (62.9 MHz, $CDCl_3$): $\delta =$ 9.6 ($CH_2$, cPr-C), 13.1 ($CH_2$, cPr-C), 28.1 ($CH_3$, *t*Bu), 28.2 ($CH_3$, *t*Bu), 28.9 ($CH_2$), 39.1 ($CH_2$), 40.1 (C, cPr-C), 47.8 ($CH_2$), 52.3 ($CH_3$), 61.8 (C), 63.3 (CH), 67.5 ($CH_2$), 73.0 ($CH_2$), 127.6 (CH), 128.3 (CH), 138.2 (CH), 163.2 (C), 168.4 (CO), 169.0 (CO), 170.1 (CO), 170.5 (CO). MS (ESI); *m/z* (%): 887 (100) [2 M + $Na^+$], 455 (40) [M + $Na^+$]. HRMS (ESI): calcd. for $C_{23}H_{33}N_2O_6$ [M + $H^+$] 433.2339; found 433.2333.

**Methyl 4-(3-hydroxypropyl)-7-*tert*-butoxycarbonyl-5-oxo-4,7-diazaspiro[2.5]octane-8-carboxylate (5-H).** To a prehydrogenated suspension of Pd/C (500 mg, 10% Pd/C, ~5 mol%) in MeOH (100 mL) was added a solution of **5**-Bn (4.45 g, 10.3 mmol) in MeOH (100 mL), and the suspension was stirred at 20 °C for 12 h. Filtration over Celite®, washing of the solids with MeOH (2 × 50 mL), and removal of the volatiles from the filtrate *in vacuo* yielded 3.45 g (98%) of **5**-H as an oil (diethyl ether, $R_f = 0.10$, ninhydrin). IR (KBr): $\nu = 3433$ cm$^{-1}$, 3095, 2952, 2933, 1749 (CO), 1697 (CO), 1394, 1205, 1168, 1008, 951, 745. $^1$H NMR (300 MHz, 125 °C, $C_2D_2Cl_4$): $\delta =$ 0.60–0.80 (m, cPr-H, 1 H), 0.85–0.97 (m, cPr-H, 1 H), 1.20–1.30 (m, cPr-H, 1 H), 1.30 (s, *t*Bu, 4.5 H), 1.36–1.44 (m, cPr-H, 1 H), 1.38 (s, *t*Bu, 4.5 H), 1.62–1.80 (m, $CH_2$, 2 H), 2.85–3.15 (m, 1 H), 3.33 (s, $CH_2$, 2 H) 3.45 (m, $CH_2$, 2 H), 3.54 (s, 1 H), 3.64 (s, $OCH_3$, 3 H), 3.86–4.10 (m, 1 H), 4.20–4.45 (m, 1 H). $^{13}$C NMR (62.9 MHz, 125 °C, $C_2D_2Cl_4$): $\delta =$ 9.7 ($CH_2$, cPr-C), 12.8 ($CH_2$, cPr-C), 27.96 ($CH_3$, *t*Bu), 28.1 ($CH_3$, *t*Bu), 31.3 ($CH_2$), 38.3 ($CH_2$), 39.3 (C, cPr-C), 47.4 ($CH_2$), 52.5 ($OCH_3$), 57.9 ($CH_2$), 61.1 (CH), 169.8 (CO), 170.0 (CO), 170.6 (CO). MS (70 eV, EI); *m/z* (%): 342 (1) [$M^+$], 286 (14), 227 (17), 183 (8), 57 (100), 41 (24). $C_{16}H_{26}N_2O_6$ (342.4): calcd. C 56.13, H 7.65, N 8.18; found C 56.08, H 7.61, N 8.14%.

**Bis(benzyloxycarbonyl)guanidine (6).** A solution of *N*,*N*'-bis(benzyloxycarbonyl)-*S*-methylisothiourea[13] (2.06 g, 5.75 mmol) in methanolic $NH_3$ (30 mL, 140 mmol, 4.66 M) was stirred at 20 °C for 3 h. After 30 min, the colorless precipitate was filtered off and dried *in vacuo* to yield 1.15 g (61%) of **6** as a solid, mp 146–148 °C. $^1$H NMR (250 MHz, $CDCl_3$): $\delta =$ 5.08 (s, $CH_2$, 4 H), 7.24–7.41 (br s, aryl-H, 10 H), 8.23–9.60 (br s, $NH_2$, NH, 3 H). $^{13}$C NMR (62.9 MHz, $CDCl_3$): $\delta = 67.3$ ($CH_2$,), 128.0 (CH, aryl-C), 128.3 (CH, aryl-C), 128.5 (CH, aryl-C), 135.6 (C, $C_{ipso}$), 158.9 (C, N=C–N), 165.7 (C, C=O). The additional spectroscopic data were identical with those reported in the literature.[9]

**Methyl 4-{3-[bis(benzyloxycarbonyl)guanidino]propyl}-7-*tert*-butoxycarbonyl-5-oxo-4,7-diazaspiro[2.5]octane-8-carboxylate (7).** To a suspension of **6** (3.75 g, 11.5 mmol) and triphenylphosphine (3.02 g, 11.5 mmol) in THF (100 mL) was added at 0 °C a solution of **5**-H (3.25 g, 9.50 mmol) in THF (100 mL), and then slowly within 30 min, DIAD (2.23 mL, 11.5 mmol). The mixture was stirred until the orange color had disappeared (5 h), water (10 drops) was added, all volatiles were removed *in vacuo* and the residue purified by chromatography on 100 g of silica gel [3 × 20 cm, pentane–$Et_2O$ 1 : 1 → 0 : 1, ninhydrin, $R_f = 0.27$ (pentane–$Et_2O$ 2 : 1)] to yield 5.47 g (87%) of **7** as a foam. IR (KBr): $\nu = 3393$ cm$^{-1}$ (NH), 3033, 2977, 2952, 1750 (CO), 1717 (CO), 1611 (CO), 1511, 1391, 1251, 1098, 1010, 910, 808, 733. $^1$H NMR (300 MHz, $CDCl_3$): $\delta =$ 0.72–0.88 (m, cPr-H, 2 H), 0.98–1.18 (m, cPr-H, 1 H), 1.28–1.34 (m, cPr-H, 1 H), 1.37 (s, *t*Bu, 4.4 H), 1.45 (s, *t*Bu, 4.6 H), 1.50–1.80 (m, $CH_2$, 2 H), 2.86–3.12 (m, 1 H), 3.20–3.40 (m, 1 H), 3.50 (s, 1 H), 3.56 (s, 3 H), 3.72 (s, 1 H), 3.84–4.02 (m, 1 H), 4.26–4.52 (m, 1 H), 5.05–5.15 (m, 1 H), 5.09–5.11 (m, 2 H), 5.19–5.21 (m, 2 H), 7.28–7.50 (m, aryl-H, 10 H), 9.14–9.40 (m, 2 H). $^{13}$C NMR (125.7 MHz, $CDCl_3$): $\delta =$ 9.6 ($CH_2$, cPr-C), 13.5 ($CH_2$, cPr-C), 28.0 ($CH_2$), 28.1 ($CH_3$, *t*Bu), 28.2 ($CH_3$, *t*Bu), 39.9 (C, cPr-C), 40.4 ($CH_2$), 46.8 (C), 47.8 ($CH_2$), 52.3 ($CH_3$), 61.9 (CH), 66.9 ($CH_2$), 69.0 (2 × $CH_2$), 128.01 (CH, aryl-C), 128.03 (CH, aryl-C), 128.36 (CH, aryl-C), 128.42 (CH, aryl-C), 128.7 (CH, aryl-C), 128.8 (CH, aryl-C), 134.5 (C, aryl-C), 136.7 (C, aryl-C), 155.7 (CN), 160.2 (CO), 163.8 (CO), 168.4 (CO), 172.1 (CO), 170.0 (CO). MS (ESI); *m/z* (%): 1325 (100) [2 M + Na]$^+$, 1303 (6) [2 M + $H^+$], 674 (40) [M + $Na^+$], 652 (26) [M + $H^+$]. HRMS (ESI): calcd. for $C_{33}H_{42}N_5O_9$ [M + $H^+$] 652.2983; found 652.2977.

**Methyl 4-{3-[bis(benzyloxycarbonyl)guanidino]propyl}-5-oxo-4,7-diazaspiro[2.5]octane-8-carboxylate (8).** Gaseous HCl was bubbled through a solution of **7** (3.76 g, 5.77 mmol) in MeOH (100 mL) kept at 0 °C, and the mixture was stirred at 20 °C for 2 h. All volatiles were removed *in vacuo*, the residue was dissolved in $CH_2Cl_2$ (100 mL), triethylamine (1.60 ml, 11.5 mmol) was added, and the suspension was stirred for 3 h. Removal of all volatiles *in vacuo*, re-uptake in THF (100 mL), collection of the precipitate on a filter, washing with THF (2 × 50 mL), removal of the solvent from the filtrates *in vacuo* and chromatographic purification of the residue on 40 g of silica gel (3 × 20 cm, $CH_2Cl_2$–MeOH 10 : 1, ninhydrin, $R_f = 0.47$) yielded 3.12 g (98%) of **8** as an oil. IR (film): $\nu = 3390$ cm$^{-1}$ (NH), 3033, 2952, 1721 (CO), 1650 (CO), 1611, 1511, 1437, 1409, 1379, 1255, 1102, 1007, 911, 807, 733. $^1$H NMR (250 MHz, $CDCl_3$): $\delta =$ 0.72–0.87 (m, cPr-H, 2 H), 1.03–1.15 (m, cPr-H, 1 H), 1.20–1.30 (m, cPr-H, 1 H), 1.50–1.70 (m, 2 H), 2.82–3.05 (m, 1 H), 3.07 (s, 1 H), 3.25–3.35 (m, 2 H), 3.46–3.54


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(m, 2 H), 3.59 (s, $OCH_3$, 3 H), 3.60–3.92 (m, 2 H), 5.08–5.12 (m, $CH_2Ph$, 2 H), 5.21 (s, $CH_2Ph$, 2 H), 7.28–7.40 (m, aryl-H, 10 H), 9.18–9.42 (m, NH, 2 H). $^{13}C$ NMR (75.5 MHz, $CDCl_3$, DEPT): $\delta = 8.64$ ($CH_2$, cPr-C), 15.4 ($CH_2$, cPr-C), 28.2 ($CH_2$), 39.5 ($CH_2$), 40.3 (C, cPr-C), 42.5 ($CH_2$, $NCH_2$), 48.0 ($CH_2$, $OCH_2$), 52.4 ($CH_3$, $CO_2CH_3$), 62.8 (CH), 66.8 ($CH_2Ph$), 68.9 ($CH_2Ph$), 127.8 (CH, aryl-C), 128.0 (CH, aryl-C), 128.30 (CH, aryl-C), 128.34 (CH, aryl-C), 128.7 (CH, aryl-C), 134.5 (C), 136.7 (C), 155.6 (C=N), 160.2 (C, $CO_2Bn$), 163.7 (C, $CO_2Bn$), 172.6 (C, NCO), 172.8 (C, CO). MS (70 eV, DCI); *m/z* (%): 552 (22) [M + $H^+$], 342 (15), 296 (17), 279 (100), 222 (18), 208 (28), 126 (45), 104 (14). $C_{28}H_{33}N_5O_7$ (551.6): calcd C 60.97, H 6.03, N 12.70; found C 61.15, H 6.16, N 12.92%.

**Benzyl spiro[cyclopropane[1,9](8-{3-[bis(benzyloxycarbonyl)-guanidino]propyl}-1,4,7-trioxooctahydro[2*H*]pyrazino[1,2-*a*]pyrazin-3-yl)]acetate (10).** To a solution of **8** (4.45 g, 8.07 mmol) and Boc-(*S*)-Asp-(4-OBn)-OH (3.39 g, 10.5 mmol) in $CH_2Cl_2$ (30 mL) were added at 0 °C 2,4,6-collidine (1.40 mL, 10.5 mmol) and DCC (2.01 g, 9.70 mmol), and the mixture was stirred with rewarming to 20 °C for 8 h. Addition of $CH_2Cl_2$ (80 mL), extraction of the organic phase with cold (0 °C) 1 N HCl (30 mL), water (30 mL) and sat. $NaHCO_3$ solution (2 × 30 mL), drying of the organic phase over $Na_2SO_4$, removal of the solvent *in vacuo* and filtration of the residue over 50 g of silica gel (3 × 15 cm, $CH_2Cl_2$–MeOH 10 : 1, ninhydrin, $R_f = 0.75$) yielded **9** with a dipeptide fragment. This compound was dissolved in $CH_2Cl_2$ (10 mL), and methanolic HCl (50.0 mL, 75 mmol, 1.5 M) was added at 0 °C, the mixture was stirred for 8 h, and all volatiles were removed *in vacuo*. The residue was taken up in $CH_2Cl_2$ (200 mL), the solution neutralized with sat. $NaHCO_3$ solution, the phases were separated, the aq. phase extracted with $CH_2Cl_2$ (3 × 50 mL), and the combined organic phases were dried over $Na_2SO_4$. Removal of the solvent, suspension of the residue in toluene (10 mL), heating at 90 °C for 24 h, removal of the solvent *in vacuo* and chromatographic purification of the residue on 40 g of silica gel (3 × 30 cm, $CH_2Cl_2$–MeOH 50 : 1; MOPS, $R_f = 0.27$) yielded 3.35 g (57%) of **10** as a foam [unseparable mixture of two diastereomers, ratio 1 : 1 (according to NMR)]. IR (KBr): $\nu = 3333$ $cm^{-1}$ (NH), 3032, 2923, 2835, 1752 (CO), 1710 (CO), 1668 (CO). $^1H$ NMR ($C_2D_2Cl_4$, 300 MHz, 125 °C): $\delta = 0.99$–1.66 (m, 2 cPr-H, 4 H), 1.74–1.98 (m, $CH_2$, 2 H), 2.90–3.12 (m, 4 H), 3.18–3.98 (m, 6 H), 4.28–4.44 (m, 4 H), 4.74 (d, $^3J = 9$ Hz, C$H_2$CH, 2 H), 7.24–7.48 (m, aryl-H, 15 H), 9.00–9.30 (br m, 3H). $^{13}C$ NMR (75.5 MHz, $C_2D_2Cl_4$, 125 °C): $\delta = 8.9$ ($CH_2$, cPr-C), 9.3 ($CH_2$, cPr-C), 11.0 ($CH_2$, cPr-C), 12.4 ($CH_2$, cPr-C), 28.21 ($CH_2$), 28.26 ($CH_2$), 28.99 ($CH_2$), 29.04 ($CH_2$), 35.9 ($CH_2$), 36.3 ($CH_2$), 39.1 ($CH_2$), 39.6 ($CH_2$), 40.31 ($CH_2$), 40.39 ($CH_2$), 42.8 (C, cPr-C), 43.0 (C, cPr-C), 51.1 (CH), 51.6 (CH), 60.7 (CH), 61.5 (CH), 66.6 ($CH_2$), 66.7 ($CH_2$), 67.8 ($CH_2$), 68.70 ($CH_2$), 68.72 ($CH_2$), 127.1 (CH, aryl-C), 127.2 (CH, aryl-C), 127.25 (CH, aryl-C), 127.27 (CH, aryl-C), 127.7 (CH, aryl-C), 127.8 (CH, aryl-C), 127.97 (CH, aryl-C), 127.98 (CH, aryl-C), 128.1 (CH, aryl-C), 128.2 (CH, aryl-C), 128.3 (CH, aryl-C), 128.4 (CH, aryl-C), 128.6 (CH, aryl-C), 129.18 (CH, aryl-C), 129.22 (CH, aryl-C), 133.8 (CH, aryl-C), 135.1 (C, aryl-C), 135.2 (C, aryl-C), 138.38 (C, aryl-C), 138.41 (C, aryl-C), 162.9 (CO), 163.4 (CO), 164.2 (CO), 164.9 (CO), 166.9 (CO), 168.2 (CO), 169.2 (CO), 169.4 (CO). MS (EI, 70 eV); *m/z* (%): 725.0 (5) [$M^+$], 505.0 (12), 414.0 (16), 399.0 (8), 91 (100). HRMS (ESI): calcd. for $C_{38}H_{41}N_6O_9$ [M + $H^+$] 725.2935; found 725.2928.

**Spiro[cyclopropane[1,9]8-(3-guanidinopropyl)-1,4,7-trioxo-octahydro[2*H*]pyrazino[1,2-*a*]pyrazin-3-yl]acetic acid (*cis*- and *trans*-11).** A suspension of **10** (1.34 g, 2.63 mmol) and Pd/C (335 mg, 10% Pd/C, ~5 mol%) in MeOH (20 mL) was stirred under an atmosphere of $H_2$ (balloon) at 20 °C for 12 h and the catalyst removed by filtration over Celite®. Removal of the solvent *in vacuo* yielded 737 mg (99%) of *cis*- and *trans*-**11** as a foam (mixture of two diastereomers 1.1 : 1 [according to NMR]). IR (KBr): $\nu = 3326$ $cm^{-1}$ (NH), 3187, 2929, 2850, 1718 (CO), 1668 (CO), 1626 (CO), 1435, 1224. $^1H$ NMR ($CD_3OD$, 300 MHz): $\delta = 0.99$–1.40 (m, 2 cPr-H, 4 H), 1.58–1.73 (m, $CH_2$, 4 H), 2.62–3.00 (m, $CH_2$, 2 H), 3.03–3.19 (m, 1 H), 3.34–3.40 (m, 1 H), 3.54–3.88 (m, 1 H), 3.97–4.10 (m, 1 H), 4.14–4.50 (m, 1 H), 4.66–4.88 (m, 1 H). $^{13}C$ NMR (125.7 MHz, $CD_3OD$): $\delta = 9.9$ ($CH_2$, cPr-C), 10.2 ($CH_2$, cPr-C), 13.5 ($CH_2$, cPr-C), 14.9 ($CH_2$, cPr-C), 26.1 ($CH_2$), 26.4 ($CH_2$), 26.7 ($CH_2$), 27.0 ($CH_2$), 29.0 ($CH_2$), 29.1 ($CH_2$), 30.1 ($CH_2$), 30.3 ($CH_2$), 39.0 ($CH_2$), 39.9 ($CH_2$), 40.4 ($CH_2$), 40.5 ($CH_2$), 46.4 (C, cPr-C), 46.9 (C, cPr-C), 52.4 (CH), 52.6 (CH), 54.1 (CH), 54.4 (CH), 158.60 (C), 158.63 (C), 167.0 (CO), 168.8 (CO), 169.8 (CO), 171.3 (CO), 172.8 (CO), 175.9 (CO). MS (ESI); *m/z* (%), positive peaks: 1099 (12) [3 M + $H^+$], 733 (22) [2 M + $H^+$], 367 (100) [M + $H^+$]; negative peaks: 365 (100) [(M − H)$^-$]. $C_{15}H_{22}N_6O_5$ (366.4): calcd. C 49.17, H 6.05, N 22.94; found C 50.43, H 6.21, N 23.55%.

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