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A Highly Practical RCM Approach towards a Molecular Building Kit of Spirocyclic Reverse Turn Mimics

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Abstract: The development of privileged molecular scaffolds efficiently mimicking reverse turn motifs and thus increasing both binding and selectivity and enabling the elucidation of the bioactive conformation of a natural peptide has attracted remarkable interest. The frequent occurrence of proline in various turn patterns initiated the design of proline-based reverse turn mimetics. As a structural hybridization of a highly potent type VI β-turn inducer **1** with saturated spirocyclic lactams **3** efficiently mimicking type II β turns, we developed a versatile synthetic route towards unsaturated spirocyclic lactams of type **2**, when Seebach's self-reproduction of chirality methodology was combined with a peptide coupling reaction and Grubbs' ring-closing metathesis. By this means, a variety of model peptides with six- up to nine-membered lactam rings were accessible following a uniform pathway. Introduction of suitably protected templates into solid-phase peptide synthesis gave rise to unsaturated spirocyclic analogues of the naturally occurring neuropeptide neurotensin. Spectroscopic investigations as well as DFT calculations on a high level of theory revealed a remarkable dependence of the reverse-turn inducing potency on the ring size. While the secondary structure of the unsaturated spirocyclic ε-lactam **12** closely agrees with the reference γ-lactam **3a**, the unsaturated δ-lactam **11** serves as an extraordinarily potent β-turn inducer which is even superior to β-lactams of type **3b**. The eight-membered unsaturated spirocyclic lactam **13** adopts a conformation almost ideally matching the prerequisites for a canonical type II β turn with the highest stability of the whole series. In contrast, the nine-membered spirolactam **14** represents a scaffold with a high conformational flexibility.

Keywords: metathesis · peptidomimetics · reverse turns · spiro compounds

Introduction

Reverse-turn conformations play a crucial role in biological recognition and signal transduction processes. As an example, over one hundred peptide activated GPCRs bind ligands with turn structure.[1] Thus, the development of privileged molecular scaffolds efficiently mimicking reverse turn motifs is of paramount importance when structural constraints are exploited to increase both binding and selectivity and to elucidate the bioactive conformation of the natural peptide.[2] Considerable efforts have been invested in the development of lactam-bridged β-turn mimics presenting the four amino acids residues i, $i+1$, $i+2$ and $i+3$ in a defined three-dimensional pattern.[3] Employing unsaturated constraint elements, we took advantage of the versatility of Grubbs' ring-closing metathesis, since the availability of a set of similar scaffolds giving rise to a spectrum of reverse turn geometries with individually situated and adaptable side chains is crucial to the success of a bioisosteric replacement.[4] The frequent occurrence of proline in the positions $i+1$ and $i+2$ of β turns of type I/II and VI, respectively, and their ability to trigger biological signals by *cis*/*trans* isomerization attracted special interest to proline-based reverse turn mimetics.[2b,5] Built on (*S*)-proline in position $i+2$, we have recently reported on a rational molecular design of type VI β turn inducing peptide mimetics **1** when Seebach's self-reproduction of chirality and Grubbs' ring-closing metathesis allowed the construction of differently sized lactam bridges and, thus, fine tuning of conformational behavior.[6] To extend our synthetic approach towards type II β-turn inducing model systems, the proline-derived reverse turn nucleating moiety had to be moved into position $i+1$ enabling the formation of an olefin-based lactam bridge to the back-

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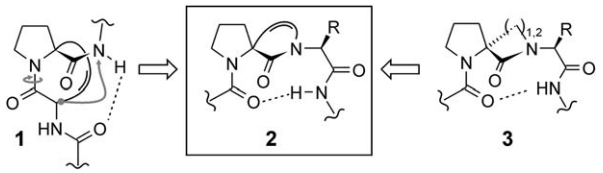
bone nitrogen of Xaa(i+2). Thus, the molecular design of our target scaffold **2** rigidizing the backbone dihedral angles $\phi(i+1)$ and $\psi(i+1)$ involved *cis*/*trans* isomerization and formal migration of the lead compound **1**. The target structure **2** is also the rational consequence of structural hybridization of **1** with the saturated templates of type **3**, being characterized as the most potent type II β-turn inducers, yet (see below).[3,7]



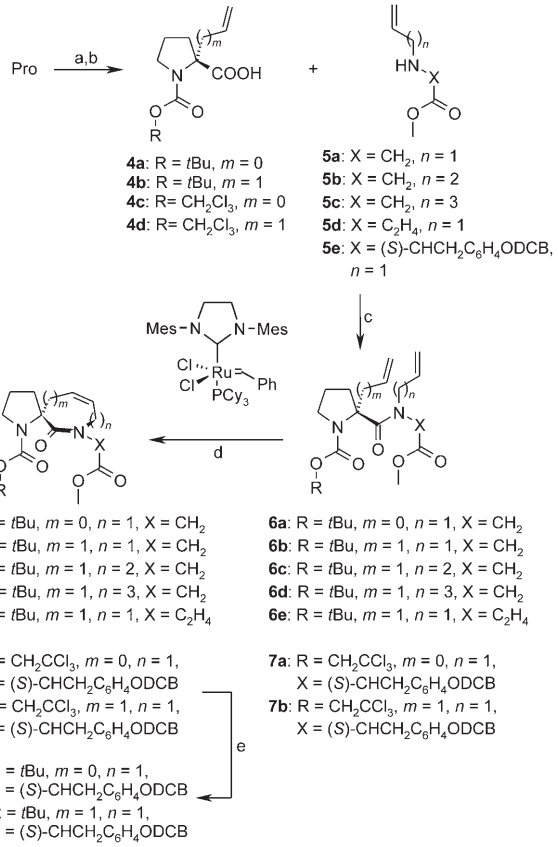
We herein present a practical and high yielding chirospecific synthesis of six- to nine-membered model peptide surrogates, solid-phase supported application towards an artificial neuropeptide analogue and structural characterization which was based on spectroscopic investigations and subsequent DFT-based refinement.

Results and Discussion

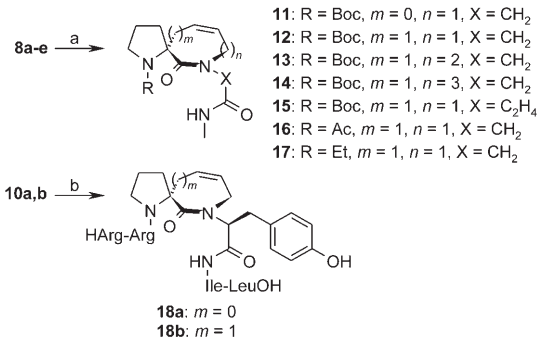
Starting from natural proline, chiral building blocks **4a**–**d**[7a,8] could be readily synthesized by Seebach's self-reproduction of chirality methodology[9] and subsequent introduction of the nitrogen protection groups Boc and Troc, respectively (Scheme 1). Due to the strong steric demand of the reactants, peptide-bond formation between the protected α-alkenyl proline derivatives **4a**,**b** and the glycine, β-alanine and tyrosine derived N-alkenyl amino acids **5a**–**e**[10] turned out to be challenging when most of the newly established coupling methods failed and acid chloride formation had to be circumvented to avoid formation of Leuchs anhydride.[11] Fortunately, ligation of the glycine and β-alanine derived building blocks **5a**–**d** could be accomplished yielding 57–88% of **6a**–**e** when the highly reactive coupling reagent HATU was used in NMP at elevated temperatures for up to three days in a strictly oxygen and moisture free reaction vessel. Facilitating the activation by $SOCl_2$, Troc protection proved to be advantageous for coupling of the tyrosine derived secondary amine **5e** to give **7a**,**b** in 68–74% yield. To promote formation of six- to nine-membered ring systems, the metathesis precursors of type **6** and **7** were subjected to 5 mol% of Grubbs' second generation ruthenium-based catalyst[12] in refluxing dichloromethane. In all cases, we observed smooth RCM resulting in formation of the spirocyclic lactams **8a**–**e** and **9a**,**b**, respectively, in 65–97% yield. Exchange of protecting groups was accomplished almost quantitatively when the N-Troc derivatives **9a**,**b** were converted into the N-Boc protected building blocks **10a**,**b**.

To provide comparable reverse-turn model systems for conformational studies, the molecular scaffolds of type **8** were transformed into the corresponding N-methylamides **11–15** by reaction with methylamine (Scheme 2). For representative structural investigations on the seven-membered system, the Boc group was replaced by an acetyl and ethyl functionality resulting in formation of the peptide surrogates **16** and **17**, respectively. To demonstrate the suitability of our molecular scaffolds for solid phase supported peptide synthesis, the conformationally constrained neurotensin 8–13 analogues[13] of type **18** should be prepared starting from a PAM resin that was preloaded with Boc-Leu. In fact, the Boc protected amino acids isoleucine, N^{ω}-tosylarginine and Pro-Tyr surrogates that were obtained by saponification of **10a**,**b** could be efficiently attached following HATU coupling and TFA deprotection. After cleavage and removal of the side chains using neat HF and HPLC purification, the neurotensin mimics of type **18** were isolated in pure form.

To investigate the conformational properties of our reverse-turn model system in comparison to the [4.3]- and [4.4]-spirocyclic lead compounds **3b** and **3a**, respectively, IR and NMR spectroscopic studies including variable temperature (VT) and NOESY experiments were performed in



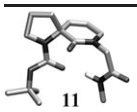
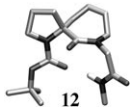
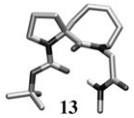
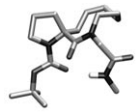
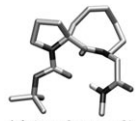
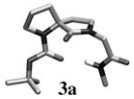
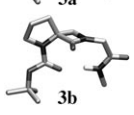
Scheme 1. a) 1) Cl_3CCHO, MeCN (87%); 2) **4a**,**c**: i) LDA, then $HCOOCH_3$, THF, −78 → −40 °C (65%), ii) $MePPh_3Br$, KO*t*Bu, PhMe, 80 °C (58–74%); **4b**,**d**: LDA, then allyl bromide, THF, −78 → −40 °C (69%); b) 1) MeOH, AcCl, 2.5–7 d; 2) **4a**,**b**: i) Na_2CO_3, ii) Boc_2O, CH_2Cl_2, 60–67 h; **4c**,**d**: $Cl_3CCH_2OCOOSu$, DIPEA, CH_2Cl_2, 1.5–2.5 h; 3) NaOH, MeOH, H_2O, 50–60 °C, 1–2.5 h (33–57%); c) **6a**–**e**: HATU, DIPEA, NMP, 85 °C, 1–3 d (57–88%); **7a**,**b**: 1) $SOCl_2$, DMF, $CHCl_3$, toluene, 50–60 °C, 1.25–2 h; 2) **5e**, DIPEA, NMP, 60–65 °C, 1–2.5 h (68–74%); d) [Ru], CH_2Cl_2, reflux, 0.5–6 h (65–97%); e) 1) Zn, HOAc, 3.5–4.5 h, 2) Na_2CO_3, 3) Boc_2O, CH_2Cl_2, 6–13 h (94–95%).



Scheme 2. a) **11**–**15**: CH_3NH_2, EtOH, 0 °C to RT, 1.75–26 h (73–98 %); **16**, **17**: 1) TFA, CH_2Cl_2, 0 °C (to RT), 30 min; 2) **16**: AcCl, DIPEA, CH_2Cl_2, RT, 18 h; **17**: EtBr, Na_2CO_3, DMF, 60 °C, 14 h; 3) CH_3NH_2, EtOH, RT, 2–2.75 h (24–79 %); b) 1) NaOH, H_2O, THF, MeOH, 0 °C, 1.5–2 h (96–99 %); 2) SPPS: resin = Boc-Leu-O-PAM, amino acids = Boc-Ile-OH, Boc-Arg(Tos)-OH, coupling: HATU, DIPEA, NMP/TFA; 3) HF, anisole; 4) HPLC.

2 mM solution thus excluding intermolecular interactions. With the aim to further refine the experimentally derived structures, density functional calculations on a high level of theory (B3LYP/6-311G(d,p)) were performed. This approach facilitated the extraction of diagnostic structural indicators and, in turn, the confirmation of structural properties by comparing DFT-based calculated NMR data with the experimentally obtained signals. Our initial studies were directed to the azepinone **12**. For a type-II β turn, a stable hydrogen bond between the Boc C=O group and the NH will be expected which was clearly indicated by the extensive absorption bands at 3340 cm^{-1} (Table 1). On the other hand, N–H stretching absorptions in the range of 3450 cm^{-1} being diagnostic for non-hydrogen bonded states could not be detected. NMR derived δ(NH) values unambiguously demonstrated the existence of a 10-membered intramolecular hydrogen bond when NH of the N-Boc and N-acetyl derivatives **12** and **16** resonated significantly more downfield (7.63 ppm) than that N-ethylamine **17** (6.38 ppm). Temperature dependent chemical shift changes of ^{1}H NMR signals as a measure for the stability of secondary structures indicate that the unsaturated spirocyclic ε-lactam **12** might form an even more stable intramolecular H-bond than the reference γ-lactam **3a** when $\Delta\delta/\Delta T$ values of −4.8 and −5.6 ppb K^{-1} were observed, respectively. The spectroscopic properties of the homologous β-alanine derivative **15** illustrate that a ring enlargement of the backbone substantially reduces reverse-turn stability ($\Delta\delta/\Delta T$ = 9.9 ppb K^{-1}). HMQC, H,H-COSY and NOESY investigations on the ring geometry of **12** were carried out, revealing a chair-like folding of the ring with the double bond oriented away from the hydrogen bond. DFT calculations revealed a $\psi(i+1)$ of 131.2° indicating a deviation of 11.2° from a canonical type II β turn and a low pseudo-dihedral angle $\beta(C{=}O(i),C^{\alpha}(i+1),C^{\alpha}(i+2),N(i+3))$ of 21.2°, the latter being expected minimal in the case of a β turn. Overall, the secondary structure of the unsaturated spirocyclic ε-lactam **12** closely agrees with the reference γ-lactam **3a**.

Table 1. Analytical data of the model peptides.

Structure[a]	NMR		IR	DFT		
	δ(NH) [ppm][b]	$\Delta\delta/\Delta T$ [ppb K^{-1}][c]	ν(NH) [cm^{-1}][d]	$\psi(i+1)$ [°]	d [Å][e]	β [°][f]
 **11**	7.64	−4.0	3337	128.5	2.03	18.1
 **12**	7.63	−4.8	3340	131.2	2.06	21.2
 **13**	7.29	−4.0	3352	120.8	2.15	4.4
 **14** (conformer 1)				179.7	3.48 2.05[g]	28.5
	6.61	n.d.	3368 3455			
 **14** (conformer 2)				113.3	2.16	−8.6
15	7.38	−9.9	3359 3459			
16	7.63	−4.6	3337			
17	6.38	n.d.	3450 3375			
 **3a**	7.81	−5.6	3337	132.8	2.10	21.2
 **3b**	8.23	−4.6	3338	124.3	2.08	11.8

[a] The least energy conformers obtained by DFT calculations are depicted. [b] 2 mM $CDCl_3$ solution, 300 K. [c] 2 mM $CDCl_3$ solution. [d] 2 mM $CHCl_3$ solution. [e] d(C=O(i)···HN(i+3)). [f] $\beta(C{=}O(i),C^{\alpha}(i+1),C^{\alpha}(i+2),N(i+3))$ pseudo-dihedral angle. [g] d(C=O(i+1)···HN(i+3)).

Formal contraction of the unsaturated constraint element leads to the novel six-membered unsaturated spirolactam **11** showing vicinal coupling constants of the allylic CH_2 position that indicate a fairly planar ring geometry. According to diagnostic IR and NMR data including a low temperature coefficient of $-4.0\ ppb\,K^{-1}$ and the calculated C=O(*i*)···HN-(*i*+3) distance of 2.03 Å, the unsaturated δ-lactam serves as an extraordinarily potent β-turn inducer which is even superior to β-lactams of type **3b**.

On the other hand, both a significant attenuation of the intramolecular hydrogen bond and an increase of stability of the type II β turn were observed for the eight-membered template **13**. To better understand this, careful NMR work was done when diagnostic NOESY data were indicative for a boat-like conformer with the double-bond directed away from the hydrogen bond. After DFT-based geometry optimization, the extraordinary low temperature coefficient of $-4.0\ ppb\,K^{-1}$ could be explained very well when the characteristic torsion angle $\psi(i+1)$ of 120.8° and the pseudo-dihedral angle $\beta = 4.4°$ almost perfectly agreed to the ideal angles of 120 and 0°, respectively. The upfield shift for δ(NH) and higher IR wavenumbers are obviously due to the higher C=O(*i*)···HN(*i*+3) distance of 2.15 Å. Significant NMR line-broadening which could be reduced at low temperature indicated a certain conformational flexibility of the eight-membered ring. It is also worthy of note that the DFT-based predictions of chemical shift values were very close to measured δ values clearly corroborating the NOESY derived boat conformation of the azocinone moiety.

Quite differently, the nine-membered spirolactam **14** represents a scaffold with a high conformational flexibility when the spectroscopic data indicated an equilibrium of H-bonded and -nonbonded structures. Theoretical investigations involving molecular dynamic and subsequent DFT calculations led to the assumption that formation of a γ turn is also energetically favored for **14**.

In conclusion, we were able to establish a uniform synthetic procedure towards a set of type II β-turn mimics displaying complementary properties. In accordance to our studies on type VI β-turn inducers, the eight-membered lactam inducer afforded the most stable reverse turn. Based on an X-ray or NMR derived 3D-structure of a biological target, deviating back-bone geometries and flexibilities of scaffolds of type **2** resulting in alternative displacements and adaptabilities of recognition elements, especially a side chain in position *i*+2, can be exploited for a structure based design of highly specific inhibitors.

Experimental Section

Methods and materials: Reagents and solvents were obtained from commercial sources unless stated otherwise, and were used as received. Reactions were carried out under nitrogen atmosphere except aminolysis, Troc cleavage and ester hydrolysis. Column chromatography was performed using 60 μm silica gel from Merck. For TLC silica gel 60 F_{254} plates from Merck were used (UV, I_2 or ninhydrin detection). Melting temperatures were determined on a Buechi 510 apparatus and are uncorrected. α_D values were measured on a Perkin Elmer 241 polarimeter. NMR data were acquired on a Bruker AM-360, Avance 360 MHz or Avance 600 MHz spectrometer. Chemical shifts are noted in ppm relative to TMS. IR spectroscopy was carried out on a Jasco FT/IR 410 spectrometer. EI-MS, ESI-MS and HRMS were performed on Finnigan MAT TSQ 70, Bruker esquire 2000 and JEOL GCmate II spectrometers, respectively. Peptide synthesis was carried out using an ACT 90 peptide synthesizer from Advanced Chemtech. Preparative HPLC was performed on an Agilent 1100 Series preparative HPLC system with a VWL detector.

(*R*)-*N*-(2-Allyl-*N*-*tert*-butoxycarbonylprolin-1-yl)-*N*-but-3-enylglycine methyl ester (6c): In a thoroughly dried apparatus (*R*)-*N*-Boc-α-allylproline (**4b**; 100 mg, 0.392 mmol) and HATU (164 mg, 0.431 mmol) were dissolved in NMP (4 mL). After addition of DIPEA (134 μL, 0.783 mmol), the mixture was stirred at room temperature for 15 min, whereupon a solution of *N*-but-3-enylglycine methyl ester (**5b**; 112 mg, 0.782 mmol) in NMP (1 mL) was added. The mixture was heated to 85 °C. After 28 h, another portion of **5b** (56.0 mg, 0.391 mg) in NMP (0.5 mL) was added and stirring was continued for 23 h, until no remaining active ester was detectable by IR spectroscopy of a small sample. After cooling down, brine and water (5 mL each) were added and the mixture was extracted with Et_2O (5×5 mL). The combined organic layers were washed with saturated $NaHCO_3$, 5% aqueous citric acid, brine, and water (5 mL each), dried with $MgSO_4$, concentrated and the residue was purified by column chromatography (hexanes/ethyl acetate 6:1 → 5:1) furnishing **6c** as a colorless oil (128 mg, 86% based on **4b**). $R_f = 0.39$ (hexanes/ethyl acetate 1:1); $[\alpha]_D^{25} = 24.0$ ($c = 0.5$, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$; rotamers and broadened signals were observed): δ = 1.43, 1.45 (2×s, 9H, Boc), 1.89–2.06 (m, 2H, CH_2), 2.14–2.42 (m, 4H, CH_2), 2.74 (dd, 1H, J=14.0, 7.8 Hz, CH_2), 2.91 (dd, 1H, J=14.0, 6.0 Hz, CH_2), 3.13–3.33 (m, 1H, CH_2), 3.41–3.53 (m, 2H, CH_2), 3.59–3.67 (m, 2H, CH_2), 3.73 (s, 3H, OCH_3), 4.33 (d, 0.68H, J=16.7 Hz, C$^\alpha H_2$), 4.45 (d, 0.32H, J=18.1 Hz, C$^\alpha H_2$), 5.03–5.18 (m, 4H, C=CH_2), 5.67–5.84, 5.82 (m and dddd, 2H, J=17.2, 12.4, 4.9, 4.9 Hz, C=dsCH); ^{13}C NMR (90 MHz, $CDCl_3$; rotamers were observed): δ = 21.7, 22.5 (2CH_2), 28.5, 28.6 (2Boc CH_3), 32.6, 32.7, 35.0, 36.0, 40.9, 42.5, 47.8, 48.2, 48.4, 48.7, 49.0, 52.2 (CH_2, OCH_3), 68.2, 68.4 (2C$^\alpha$), 80.0, 80.7 (2Boc C^q), 117.5, 117.9, 118.7, 118.9, 134.3, 134.4, 134.5, 134.7 (*C*=*C*), 153.4, 153.5 (2Boc *C*=O), 170.2, 170.5, 173.8, 173.9 (ester and amide *C*=O); IR (neat): $\tilde{\nu}$ = 2977, 1751, 1695, 1645 cm^{-1}; EIMS: *m*/*z*: 380 [M^+]; elemental analysis calcd (%) for $C_{20}H_{32}N_2O_5$: C 63.14, H 8.48, N 7.36; found: C 62.88, H 8.41, N 7.44.

(*R*)-(1-*tert*-Butoxycarbonyl-6-oxo-1,7-diazaspiro[4.7]dodec-10-en-7-yl)-acetic acid methyl ester (8c): In a thoroughly dried apparatus **6c** (61.7 mg, 0.162 mmol) was dissolved in CH_2Cl_2 (79 mL). A solution of Grubbs′ 2nd generation catalyst (6.7 mg, 4.9 mol%) in CH_2Cl_2 (2 mL) was added while stirring. The solution was heated to reflux for 4 h, then it was allowed to cool down to room temperature. After addition of silica gel the solvent was removed in vacuo and the crude product adsorbed to the silica gel was purified by column chromatography (hexanes/ethyl acetate 2:1 → 1:1), furnishing **8c** as a yellow oil (51.5 mg, 90%). $R_f = 0.27$ (hexanes/ethyl acetate 1:2); $[\alpha]_D^{22} = -61.4$ ($c = 0.5$, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$; rotamers and broadened signals were observed): δ = 1.46 (s, 9H, Boc), 1.75–1.84 (m, 1H, CH_2), 1.86–1.94 (m, 1H, CH_2), 2.00 (ddd, 1H, J=14.7, 7.2, 4.8 Hz, CH_2), 2.35–2.42 (m, 2H, CH_2), 2.54 (ddd, 1H, J=11.4, 6.7, 4.5 Hz, CH_2), 2.66–2.96 (m, 2H, CH_2), 3.39–3.51 (m, 2H, CH_2), 3.56–3.68 (m, 2H, CH_2), 3.69 (s, 3H, OCH_3), 3.96–4.07 (m, 1H, CH_2), 4.30 (d, 0.3H, J=17.0 Hz, C$^\alpha H_2$), 4.57 (d, 0.7H, J=17.0 Hz, C$^\alpha H_2$), 5.38–5.51 (m, 1H, C=CH), 5.79–5.89 (m, 0.3H, C=CH), 5.94–6.04 (m, 0.7H, C=CH); ^{13}C NMR (90 MHz, $CDCl_3$; rotamers were observed): δ = 22.5, 22.6, 27.7 (CH_2), 28.7 (Boc CH_3), 28.8, 40.9, 47.4, 48.7, 49.2, 49.6, 49.9, 52.1 (CH_2 and OCH_3), 70.7 (spiro *C*), 79.2 (Boc C^q), 125.5, 128.6 (*C*=*C*), 154.7, 155.0 (Boc *C*=O), 170.3, 173.5 (ester, lactam *C*=O); IR (neat): $\tilde{\nu}$ = 2974, 1750, 1704, 1687, 1649 cm^{-1}; EIMS: *m*/*z*: 352 [M^+]; elemental analysis calcd (%) for $C_{18}H_{28}N_2O_5 \times 0.25\,H_2O$: C 60.57, H 8.05, N 7.85; found: C 60.79, H 8.16, N 7.66.

(*R*)-(1-*tert*-Butoxycarbonyl-6-oxo-1,7-diazaspiro[4.7]dodec-10-en-7-yl)-acetic acid *N*-methylamide (13): A methylamine solution (8 M in ethanol,

2 mL) was added while stirring to **8c** (19.3 mg, 0.0545 mmol) on an ice bath. After 45 min at 0 °C and 1.75 h at room temperature the solvent was removed in vacuo and the residue was purified by flash chromatography (hexanes/ethyl acetate 1:2 → 0:1), furnishing **13** as a colorless solid (18.4 mg, 96 %). M.p. 69–72 °C; R_f = 0.32 (CH_2Cl_2/methanol 95:5); $[\alpha]_D^{25}$ = 63.8 (c = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, 220 K): δ = 1.46 (s, 9H, Boc), 1.90–1.97 (m, 1H, C$^{3'}$H$^{\alpha}$), 2.01 (ddd, 1H, J = 12.2, 6.8, 3.5 Hz, C$^{4'}$H$^{\alpha}$), 2.06–2.12, 2.08 (m and dd, 2H, J = 14.6, 8.2 Hz, C$^{3'}$H$^{\beta}$, C$^{12'}$H$^{\beta}$), 2.41–2.46 (m, 2H, C$^{9'}$H$_2$), 2.60–2.65 (m, 1H, C$^{4'}$H$^{\beta}$), 2.81 (d, 3H, J = 4.6 Hz, NCH$_3$), 3.10 (ddd, 1H, J = 16.1, 3.1, 3.1 Hz, C$^{8'}$H$^{\beta}$), 3.42, 3.45 (d and dd, 2H, J = 16.8, 14.6, 7.4 Hz, C^{2}H$_2$ and C$^{12'}$H$^{\alpha}$), 3.50 (ddd, 1H, J = 10.7, 7.6, 3.5 Hz, C$^{2'}$H$_2$), 3.56 (ddd, 1H, J = 10.7, 8.9, 7.2 Hz, C$^{2'}$H$_2$), 4.65 (brd, 1H, J = 16.8 Hz, C^{2}H$_2$), 5.16 (bddd, 1H, J = 16.1, 10.7, 5.9 Hz, C$^{8'}$H$^{\alpha}$), 5.57–5.65 (m, 2H, HC$^{10'}$=C$^{11'}$H), 7.56 (q, 1H, J = 4.6 Hz, NH); ^{13}C NMR (90 MHz, $CDCl_3$): δ = 23.5 (C$^{3'}$H$_2$), 26.2 (NCH$_3$), 28.5 (Boc CH$_3$), 28.6 (C$^{9'}$H$_2$), 34.3 (C$^{12'}$H$_2$), 39.0 (C$^{4'}$H$_2$), 46.8 (C$^{8'}$H$_2$), 48.1 (C$^{2'}$H$_2$), 53.2 (C^{2}H$_2$), 70.1 (spiro C), 80.1 (Boc C^{q}), 124.2 (C$^{11'}$H), 128.7 (C$^{10'}$H), 154.9 (Boc C=O), 169.5 (amide C=O), 174.0 (lactam C=O); IR (neat): $\tilde{\nu}$ = 3357, 2974, 2930, 1670, 1633 cm^{-1}; IR (2 mM, $CHCl_3$): $\tilde{\nu}$ = 3352 cm^{-1}; EIMS: m/z: 351 [M^+]; elemental analysis calcd (%) for $C_{18}H_{29}N_3O_4 \times 0.25\,H_2O$: C 60.74, H 8.35, N 11.81; found: C 60.77, H 8.24, N 11.37.

Starting from **4a**,**b** and from **5a**,**c**,**d**, respectively, compounds **11**, **12**, **14**, and **15** were prepared analogously:

(*R*)-(1-*tert*-Butoxycarbonyl-6-oxo-1,7-diazaspiro[4.5]dec-9-en-7-yl)acetic acid *N*-methylamide (11): Colorless oil; R_f = 0.22 (CH_2Cl_2/methanol 95:5); $[\alpha]_D^{22}$ = 47.8 (c = 0.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.44 (s, 9H, Boc), 1.85–1.95 (m, 2H, CH_2), 2.09–2.15 (m, 1H, CH_2), 2.43–2.48 (m, 1H, CH_2), 3.52 (d, 3H, J = 4.7 Hz, NCH_3), 3.27 (d, 1H, J = 16.8 Hz, C$^{2}H_2$), 3.52 (ddd, 1H, J = 10.5, 7.1, 4.7 Hz, C$^{2'}H_2$), 3.60 (ddd, 1H, J = 10.5, 7.3, 7.3 Hz, C$^{2'}H_2$), 3.87 (ddd, 1H, J = 18.1, 3.0, 2.1 Hz, C$^{8'}H_2$), 4.09 (ddd, 1H, J = 18.1, 3.0, 2.1 Hz, C$^{8'}H_2$), 5.02 (d, 1H, J = 16.8 Hz, C$^{2}H_2$), 5.64 (ddd, 1H, J = 10.2, 2.1, 2.1 Hz, C=CH), 5.80 (ddd, 1H, J = 10.2, 3.0, 3.0 Hz, C=CH), 7.67 (brs, 1H, NH); ^{13}C NMR (90 MHz, $CDCl_3$): δ = 23.9, 26.3 (CH_2, NCH_3), 28.7 (Boc CH_3), 39.4, 48.3, 49.8 (CH_2), 62.2 (spiro C), 80.6 (Boc C^{q}), 120.7, 128.7 (C=C), 155.1 (Boc C=O), 168.6, 170.6 (amide, lactam C=O); IR (neat): $\tilde{\nu}$ = 3338, 2975, 1753, 1670 (b), 1657 cm^{-1} (shoulder); IR (2 mM, $CHCl_3$): $\tilde{\nu}$ = 3337 cm^{-1}; EIMS: m/z: 223 [M^+−Boc], [M^+] not observed, elemental analysis calcd (%) for $C_{16}H_{25}N_3O_4$: C 59.43, H 7.79, N 12.99; found: C 59.49, H 7.76, N 12.98.

(*R*)-(1-*tert*-Butoxycarbonyl-6-oxo-1,7-diazaspiro[4.6]undec-9-en-7-yl)acetic acid *N*-methylamide (12): Colorless gum; R_f = 0.18 (CH_2Cl_2/methanol 95:5); $[\alpha]_D^{24}$ = 27.5 (c = 0.2, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.46 (s, 9H, Boc), 1.84 (ddd, 1H, J = 11.9, 7.4, 3.4 Hz, C$^{4'}H^{\alpha}$), 1.87–1.94 (m, 1H, C$^{3'}H_2$), 2.00–2.05 (m, 1H, C$^{3'}H_2$), 2.12 (dd, 1H, J = 14.2, 7.7 Hz, C$^{11'}H^{\beta}$), 2.24–2.29 (m, 1H, C$^{4'}H^{\beta}$), 2.82 (d, 3H, J = 4.5 Hz, NCH_3), 3.36, 3.37, 3.36–3.40 (dd, d, m, 3H, J = 14.7, 7.6, 16.8 Hz, C$^{8'}H^{\beta}$, C$^{2}H_2$, C$^{11}H^{\alpha}$), 3.50–3.57 (m, 2H, C$^{2'}H_2$), 4.56 (ddd, 1H, J = 14.7, 6.4, 1.5 Hz, C$^{8'}H^{\alpha}$), 5.09 (d, 1H, J = 16.8 Hz, C$^{2}H_2$), 6.11–6.15 (m, 1H, C$^{10'}H$), 6.24–6.28 (m, 1H, C$^{9'}H$), 7.69 (brss, 1H, NH); ^{13}C NMR (90 MHz, $CDCl_3$): δ = 23.4 (C$^{3'}H_2$), 26.2 (NCH_3), 28.5 (Boc CH_3), 32.5 (C$^{11'}H_2$), 37.4 (C$^{4'}H_2$), 45.7 (C$^{8'}H_2$), 48.4 (C$^{2'}H_2$), 55.2 (C$^{2}H_2$), 69.5 (spiro C), 80.3 (Boc C^{q}), 131.0 (C$^{9'}$H), 132.5 (C$^{10'}$H), 155.2 (Boc C=O), 169.3 (amide C=O), 175.2 (lactam C=O); IR (neat): $\tilde{\nu}$ = 3344, 2978, 2881, 1668, 1635 cm^{-1}; IR (2 mM, $CHCl_3$): $\tilde{\nu}$ = 3340 cm^{-1}; ESI-MS: m/z: 337.1 [M^+]; elemental analysis calcd (%) for $C_{17}H_{27}N_3O_4 \times H_2O$: C 57.45, H 8.2, N 11.82; found: C 57.48, H 7.87, N 11.84.

(*R*)-(1-*tert*-Butoxycarbonyl-6-oxo-1,7-diazaspiro[4.8]tridec-11-en-7-yl)-acetic acid *N*-methylamide (14): Colorless crystals; M.p. 176 °C; R_f = 0.17 (CH_2Cl_2/methanol 95:5); $[\alpha]_D^{25}$ = −70.2 (c = 0.5, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$; strongly broadened signals were observed): δ = 1.47 (s, 9H, Boc), 1.67–1.92 (m, 3H, CH_2), 2.04–2.64 (m, 6H, CH_2), 2.77 (d, 3H, J = 4.6 Hz, NCH_3), 2.94–3.12 (m, 1H, CH_2), 3.23–3.94 (m, 5H, CH_2), 4.05–4.25 (m, 1H, CH_2), 5.68 (ddd, 1H, J = 9.8, 9.8, 7.2 Hz, C=CH), 5.87–6.15 (m, 1H, C=CH), 6.63 (brs, 1H, NH); ^{13}C NMR (90 MHz, $CDCl_3$; rotamers were observed): δ = 22.6, 22.7, 23.2, 26.3, 26.4 (CH_2, NCH_3), 28.6, 28.7 (Boc CH_3), 35.9, 37.3, 45.8, 48.3, 52.1, 52.3 (CH_2), 71.3, 71.7 (spiro C), 79.6, 79.7 (Boc C^{q}), 128.7, 129.0, 131.1, 132.0 (C=C), 155.4, 155.6 (Boc C=O), 170.4, 175.3 (amide and lactam C=O); IR (neat): $\tilde{\nu}$ = 3336, 2971, 2932, 1686, 1637 cm^{-1}; IR (2 mM, $CHCl_3$): $\tilde{\nu}$ = 3455 (w), 3368 (s) cm^{-1}; EIMS: m/z: 365 [M^+]; elemental analysis calcd (%) for $C_{19}H_{31}N_3O_4$: C 62.44, H 8.55, N 11.50; found: C 62.39, H 8.63, N 11.48.

(*R*)-3-(1-*tert*-Butoxycarbonyl-6-oxo-1,7-diazaspiro[4.6]undec-9-en-7-yl)-propionic acid *N*-methylamide (15): Colorless gum; R_f = 0.18 (CH_2Cl_2/methanol 95:5); $[\alpha]_D^{24}$ = 32.0 (c = 0.5, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$): δ = 1.46 (s, 9H, Boc), 1.78–1.90 (m, 2H, CH_2), 1.95–2.09 (m, 2H, CH_2), 2.18–2.24 (m, 2H, CH_2), 2.65 (ddd, 1H, J = 13.2, 11.3, 4.2 Hz, CH_2), 2.81 (d, 3H, J = 4.6 Hz, NCH_3), 3.17 (ddd, 1H, J = 13.5, 11.3, 2.7 Hz, CH_2), 3.36–3.59 (m, 4H, CH_2), 4.25 (ddd, 1H, J = 13.5, 4.2, 4.2 Hz, CH_2), 4.38 (ddd, 1H, J = 15.0, 6.1, 1.7 Hz, CH_2), 6.05 (dddd, 1H, J = 9.4, 7.7, 5.5, 1.7 Hz, C=CH), 6.12–6.20 (m, 1H, C=CH), 7.37 (brs, 1H, NH); ^{13}C NMR (90 MHz, $CDCl_3$): δ = 23.5, 26.6 (CH_2, NCH_3), 28.7 (Boc CH_3), 32.8, 36.0, 37.6, 47.5, 48.6, 51.4 (CH_2), 69.8 (spiro C), 80.0 (Boc C^{q}), 131.0, 132.1 (C=C), 155.0 (Boc C=O), 173.6, 174.8 (amide, lactam C=O); IR (neat): $\tilde{\nu}$ = 3338, 2976, 1691, 1671, 1655, 1633 cm^{-1}; IR (2 mM, $CHCl_3$): $\tilde{\nu}$ = 3459 (w), 3358 (s) cm^{-1}; EIMS: m/z: 351 [M^+].

(*R*)-(1-Acetyl-6-oxo-1,7-diazaspiro[4.6]undec-9-en-7-yl)acetic acid methyl ester: Compound **8b** (39.6 mg, 0.117 mmol) was dissolved in CH_2Cl_2 (1 mL) and treated with TFA/CH_2Cl_2 (1:1, 1 mL) while cooling with an ice bath. After 30 min the solvent was removed, the residue was re-dissolved in CH_2Cl_2 and evaporated to dryness. After addition of CH_2Cl_2 (1 mL) and DIPEA (60.0 μL, 0.350 mmol), the mixture was cooled with an ice bath and treated with acetyl chloride in CH_2Cl_2 (10 %, 100 μL, 0.141 mmol). The mixture was stirred at room temperature for 3.5 h, whereupon another portion of acetyl chloride solution (10 %, 50 μL, 0.0703 mmol) was added and stirring was continued for 14.5 h. Subsequently, the mixture was diluted with CH_2Cl_2 (3 mL), washed with aqueous HCl (1 N, 2 mL), and the aqueous phase was re-extracted with CH_2Cl_2 (3×2 mL). The combined organic layers were dried with $MgSO_4$, evaporated to dryness and the residue was purified by column chromatography (CH_2Cl_2/methanol 98:2 → 95:5), furnishing the title compound as an almost colorless viscous oil (30.0 mg, 91 %). R_f = 0.20 (CH_2Cl_2/methanol 95:5); $[\alpha]_D^{24}$ = −59.6 (c = 0.25, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$): δ = 1.87–2.10, 2.05 (m and s, 7H, CH_2 and acetyl CH_3), 2.23–2.35 (m, 1H, CH_2), 3.51–3.83, 3.61, 3.72 (m, d, s, 8H, J = 17.4 Hz, CH_2, NCH_2, C$^{2}H_2$, OCH_3), 4.30 (dd, 1H, J = 15.4, 5.1 Hz, NCH_2), 4.78 (d, 1H, J = 17.4 Hz, C$^{2}H_2$), 5.98–6.08 (m, 2H, HC=CH); ^{13}C NMR (90 MHz, $CDCl_3$): δ = 23.69, 23.73 (CH_2, acetyl CH_3), 33.1, 36.7, 48.1, 49.7 (CH_2), 52.1, 52.6 (OCH_3, CH_2), 70.4 (spiro C), 128.5, 130.8 (C=C), 169.6, 170.4 (ester and amide C=O), 174.6 (lactam C=O); IR (neat): $\tilde{\nu}$ = 2952, 1634 cm^{-1}; EIMS: m/z: 280 [M^+].

(*R*)-(1-Acetyl-6-oxo-1,7-diazaspiro[4.6]undec-9-en-7-yl)acetid acid *N*-methylamide (16): Starting from (*R*)-(1-acetyl-6-oxo-1,7-diazaspiro-[4.6]undec-9-en-7-yl)acetic acid methyl ester, the compound was prepared analogously to **13** to yield a colorless oil that partially solidifies upon standing. R_f = 0.37 (CH_2Cl_2/methanol 9:1); $[\alpha]_D^{23}$ = 43.6 (c = 0.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.91 (ddd, 1H, J = 12.5, 7.0, 3.4 Hz, C$^{4'}H^{\alpha}$), 2.01–2.08 (m, 1H, C$^{3'}H^{\alpha}$), 2.09 (dd, s, 4H, J = 14.1, 7.6 Hz, C$^{11'}H^{\beta}$, acetyl CH_3), 2.17 (m, 1H, C$^{3'}H^{\beta}$), 2.33 (dddd, 1H, J = 12.5, 10.6, 7.4, 1.7 Hz, C$^{4'}H^{\beta}$), 2.83 (d, 3H, J = 4.9 Hz, NCH_3), 3.38, 3.39 (dd, d, J = 14.7, 7.6, 16.8 Hz, C$^{8'}H^{\beta}$, C$^{2}H_2$), 3.46 (dddd, 1H, J = 14.1, 5.8, 1.7, 1.7 Hz, C$^{11'}H^{\alpha}$), 3.65 (ddd, 1H, J = 9.9, 7.6, 2.8 Hz, C$^{2'}H^{\alpha}$), 3.70 (ddd, 1H, J = 9.9, 9.6, 6.8 Hz, C$^{2'}H^{\beta}$), 4.61 (ddd, 1H, J = 14.7, 6.4, 1.9 Hz, C$^{8'}H^{\alpha}$), 5.04 (d, 1H, J = 16.8 Hz, C$^{2}H_2$), 6.13–6.17 (m, 1H, C=C$^{10'}H$), 6.28–6.33 (m, 1H, C=C$^{9'}H$), 7.69 (brs, 1H, NH); ^{13}C NMR (90 MHz, $CDCl_3$): δ = 23.3 (acetyl CH_3), 24.1 (C$^{3'}H_2$), 26.4 (NCH_3), 31.7 (C$^{11'}H_2$), 37.1 (C$^{4'}H_2$), 45.8 (C$^{8'}H_2$), 49.6 (C$^{2'}H_2$), 55.4 (C$^{2}H_2$), 70.3 (spiro C), 131.6 (C=C$^{9'}$), 132.3 (C=C$^{10'}$), 169.2, 170.0 (2×amide C=O), 174.7 (lactam C=O); IR (neat): $\tilde{\nu}$ = 3324, 2951, 1671, 1624 cm^{-1}; IR (2 mM, $CHCl_3$): $\tilde{\nu}$ = 3337 cm^{-1}; EIMS: m/z: 279 [M^+]; elemental analysis calcd (%) for $C_{14}H_{21}N_3O_3 \times 0.25\,H_2O$: C 58.32, H 7.69, N 14.57; found: C 58.45, H 7.39, N 14.67.

(*R*)-(1-Ethyl-6-oxo-1,7-diazaspiro[4.6]undec-9-en-7-yl)acetic acid methyl ester: Compound **8b** (42.0 mg, 0.124 mmol) was dissolved in CH_2Cl_2 (1 mL) and treated with TFA (0.5 mL) while cooling with an ice bath. After stirring for 5 min at this temperature and 30 min at room temperature, the solvent was removed and the residue was dried thoroughly.

After dissolving the crude product in DMF (2 mL) and transferring the solution to a sealed tube, Na_2CO_3 (39.0 mg, 0.368 mmol) and ethyl bromide (28.0 μL, 0.375 mmol) were added and the mixture was heated to 80 °C for 2.25 h and 60 °C for 14 h under nitrogen. After cooling down, water and saturated aqueous Na_2CO_3 were added and the mixture was extracted with Et_2O (4×). The combined organic layers were dried with $MgSO_4$, concentrated and the residue was purified by column chromatography (CH_2Cl_2/methanol/dimethylethylamine 95:4:1), furnishing the title compound as a colorless oil (11.1 mg, 34 %). R_f = 0.26 (CH_2Cl_2/methanol 2:1); $[\alpha]_D^{24} = -16.5$ (c = 0.2, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$; broadened signals were observed): δ = 1.12 (dd, 3H, J = 7.1, 7.1 Hz, CH_2CH_3), 1.74–1.93 (m, 3H, CH_2), 2.22 (br d, 1H, J = 17.7 Hz, CH_2CH_3), 2.31–2.38 (m, 1H, CH_2), 2.44–2.53 (m, 1H, CH_2), 2.61 (br d, 1H, J = 17.7 Hz, CH_2CH_3), 2.76–2.86 (m, 2H, CH_2), 3.00–3.07 (m, 1H, CH_2), 3.94–4.04, 4.00 (m, d, 2H, J = 17.4 Hz, CH_2, C^2H_2), 4.07–4.15 (m, 1H, CH_2), 4.29 (d, 1H, J = 17.4 Hz, C^2H_2), 5.72–5.79 (m, 1H, C=CH), 5.82–5.88 (m, 1H, C=CH); ^{13}C NMR (90 MHz, $CDCl_3$): δ = 14.3 (CH_2CH_3), 21.3, 32.1, 33.5 (CH_2), 44.4, 50.0, 50.5 (NCH_2), 52.3, 52.8 (OCH_3, CH_2), 78.0 (spiro C), 125.2, 128.7 (C=C), 170.0, 175.4 (C=O); IR (neat): $\tilde{\nu}$ = 2962, 1749, 1645 cm^{-1}; EIMS: m/z: 266 [M^+].

(*R*)-(1-Ethyl-6-oxo-1,7-diazaspiro[4.6]undec-9-en-7-yl)acetic acid *N*-methylamide (17): Starting from (*R*)-(1-ethyl-6-oxo-1,7-diazaspiro-[4.6]undec-9-en-7-yl)acetic acid methyl ester, the compound was prepared analogously to **13** to yield a pale yellow oil. R_f = 0.12 (CH_2Cl_2/methanol 1:1); $[\alpha]_D^{24} = -151$ (c = 0.1, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$, 2 mM; broadened signals were observed): δ = 1.08–1.23 (m, 3H, CH_2CH_3), 1.80–2.06 (m, 3H, CH_2), 2.26 (br d, 1H, J = 16.8 Hz, CH_2CH_3), 2.32–2.51 (m, 2H, CH_2), 2.58 (br d, 1H, J = 16.8 Hz, CH_2CH_3), 2.68–3.23, 2.78 (m, d, 6H, J = 4.3 Hz, CH_2, NCH_3), 3.79 (d, 1H, J = 15.3 Hz, C^2H_2), 3.93–4.32, 4.28 (m, br d, 3H, J = 15.3 Hz, CH_2, C^2H_2), 5.77–5.90 (m, 2H, HC=CH), 6.38 (br s, 1H, NH); IR (neat): $\tilde{\nu}$ = 3411, 3321, 2925, 1668 (shoulder), 1647 cm^{-1}; IR (2 mM, $CHCl_3$): $\tilde{\nu}$ = 3450 (s), 3375 (w) cm^{-1}; EIMS: m/z: 265 [M^+]; HRMS: m/z: calcd for $C_{14}H_{23}N_3O_2$: 265.1790; found: 265.1790.

(*R*)-*N*-(2,2,2-Trichloroethoxycarbonyl)-2-allylproline methyl ester: Acetyl chloride (8.60 mL, 121 mmol) was added slowly to methanol (50 mL) while thoroughly stirring on an ice bath. After 5 min a solution of (2*R*,5*R*)-5-(2-propenyl)-2-trichloromethyl-1-aza-3-oxabicyclo-[3.3.0]octane-4-one[9b] (3.44 g, 12.1 mmol) in methanol (10 mL) was added and the ice bath was removed. After stirring at room temperature for 4.5 d, the solvent was removed in vacuo, the residue was re-dissolved in methanol and evaporated to dryness. Hydrochloric acid (1 N, 20 mL) was added and the mixture was washed with diethyl ether (2×10 mL). The pH of the mixture was gradually adjusted to 12 by addition of aqueous NaOH (2 N) and extracted with diethyl ether (4×10 mL). The combined organic layers were dried with $MgSO_4$, evaporated at 700 mbar and added to an ice-cold methanolic HCl solution, freshly prepared from acetyl chloride (2 mL, 28.1 mmol) and methanol (10 mL). The solution was evaporated almost to dryness and the hydrochloride of (*S*)-2-allylproline methyl ester was precipitated by addition of diethyl ether and dried thoroughly (2.30 g, ≈92 %, crude).

A fraction of the crude product (2.00 g, ≈9.72 mmol) was dispersed in CH_2Cl_2 (20 mL) and treated cautiously with DIPEA (5.00 mL, 29.2 mmol), followed by a solution of succinimidyl-(2,2,2-trichloroethyl)-carbonate (Troc-OSu; 3.67 g, 12.6 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred at room temperature for 1.5 h, whereupon the solvent was removed and the residue was purified by twofold column chromatography (hexanes/ethyl acetate 4:1), furnishing the title compound as a pale yellow oil (2.40 g, 66 %). R_f = 0.22 (hexanes/ethyl acetate 4:1); $[\alpha]_D^{20}$ = 25.1 (c = 1.0, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$; rotamers were observed): δ = 1.77–1.99 (m, 2H, C^4H_2), 2.04–2.26 (m, 2H, C^3H_2), 2.67, 2.69 (2 dddd, 1H, J = 14.3, 8.4, 0.9, 0.9 Hz; 14.3, 8.5, 0.9, 0.9 Hz, allyl CH_2), 3.12 (dddd, 1H, J = 14.3, 6.4, 1.4, 1.4 Hz, allyl CH_2), 3.48, 3.53 (2 ddd, 1H, J = 10.8, 7.4, 7.4 Hz; 10.6, 7.3, 7.3 Hz, NCH_2), 3.72, 3.73 (2 s, 3H, OCH_3), 3.76–3.83 (m, 1H, NCH_2), 4.69, 4.73, 4.75, 4.77 (4 d, 2H, J = 11.9, 12.0, 11.9, 12.0 Hz, Cl_3CCH_2), 5.12–5.19 (m, 2H, C=CH_2), 5.67–5.81 (m, 1H, CH=C); ^{13}C NMR (90 MHz, $CDCl_3$; rotamers were observed): δ = 22.5, 23.0, 35.6, 37.3, 37.8, 39.1, 48.5, 49.6, 52.5, 52.7 (CH_2, OCH_3), 67.7, 68.4 (C^2), 74.6, 75.3 (Cl_3CCH_2), 95.0, 95.8 (Cl_3C), 119.5, 119.6 (C=CH_2), 132.6, 132.7 (C=CH_2), 152.4 (carbamate C=O), 174.0, 174.7 (ester C=O); IR (neat): $\tilde{\nu}$ = 3079, 2954, 2883, 1744, 1720, 1639 cm^{-1}; EIMS: m/z: 345, 343, 347 [M^+]; elemental analysis calcd (%) for $C_{12}H_{16}Cl_3NO_4 \times 0.25\,H_2O$: C 41.28, H 4.76, N 4.01; found: C 41.22, H 4.94, N 3.90.

(*R*)-*N*-(2,2,2-Trichloroethoxycarbonyl)-2-allylproline (4d): (*R*)-*N*-(2,2,2-Trichloroethoxycarbonyl)-2-allylproline methyl ester (2.20 g, 6.38 mmol) was dissolved in methanol (30 mL), and aqueous NaOH (2 N, 30 mL) was added while stirring thoroughly. After heating the mixture to 50 °C for 2.5 h, water (5 mL) was added and methanol was evaporated under reduced pressure (40 °C, 95 mbar). The remaining solution was washed with diethyl ether (2×20 mL), adjusted to pH < 1 by addition of hydrochloric acid (5 N) and extracted with diethyl ether (2×10 mL). The combined organic layers were dried with $MgSO_4$, concentrated and purified by column chromatography (CH_2Cl_2/HCOOH 95:5), furnishing **4d** as a pale yellow viscous oil (1.72 g, 81 %). R_f = 0.24 (CH_2Cl_2/HCOOH 95:5); $[\alpha]_D^{22}$ = 21.8 (c = 1.0, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$; rotamers were observed): δ = 1.84–2.03 (m, 2H, C^4H_2), 2.11–2.32 (m, 2H, C^3H_2), 2.64–2.72 (m, 1H, allyl CH_2), 3.07–3.16 (m, 1H, allyl CH_2), 3.48, 3.53 (2 ddd, 1H, J = 10.6, 7.4, 7.4 Hz; 10.6, 7.5, 7.5 Hz, NCH_2), 3.78, 3.80 (2 ddd, 1H, J = 10.6, 7.2, 4.9 Hz; 10.6, 7.4, 4.9 Hz, NCH_2), 4.73, 4.77, 4.80, 4.80 (4 d, 2H, J = 11.9, 11.0, 11.9, 11.0 Hz, Cl_3CCH_2), 5.14–5.20 (m, 2H, C=CH_2), 5.65–5.81 (m, 1H, CH=C), 10.50 (br s, 1H, $COOH$); ^{13}C NMR (90 MHz, $CDCl_3$; rotamers were observed): δ = 22.5, 22.9, 35.5, 37.3, 37.5, 38.8, 48.8, 49.6 (CH_2), 67.4, 68.6 (C^2), 74.9, 75.3 (Cl_3CCH_2), 95.0, 95.5 (Cl_3C), 119.9, 120.0 (C=CH_2), 132.0, 132.3 (C=CH_2), 152.3, 153.1 (carbamate C=O), 178.0, 179.8 ($COOH$); IR (neat):$\tilde{\nu}$ = 3077, 2980, 2883, 1718 cm^{-1}; EIMS: m/z: 331, 329, 333 [M^+]; elemental analysis calcd (%) for $C_{11}H_{14}Cl_3NO_4 \times 0.5\,H_2O$: C 38.90, H 4.45, N 4.12; found: C 39.08, H 4.27, N 4.01.

(*S*)-*N*-Allyl-*O*-(2,6-dichlorobenzyl)tyrosine methyl ester: To a solution of (*S*)-*O*-(2,6-dichlorobenzyl)tyrosine methyl ester[10d] (2.00 g, 5.12 mmol) in DMF (20 mL) was added DIPEA (1.75 mL, 10.2 mmol), followed by allyl bromide (1.08 mL, 12.8 mmol) while stirring at room temperature. After 2 h, water (20 mL) was added and the mixture was extracted with Et_2O (3×20 mL).[10e] The combined organic layers were washed with brine (20 mL), dried with $MgSO_4$, concentrated, and the residue was purified by flash chromatography (hexanes/ethyl acetate 9:1 → 3:1), yielding the title compound as a pale yellow oil (1.62 g, 80 %). R_f = 0.18 (hexanes/ethyl acetate 2:1); $[\alpha]_D^{24}$ = 16.1 (c = 2.0, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$): δ = 1.58 (br s, 1H, NH), 2.92 (d, 2H, J = 6.7 Hz, C^3H_2), 3.12 (dddd, 1H, J = 13.9, 6.0, 1.3, 1.3 Hz, allyl CH_2), 3.27 (dddd, 1H, J = 13.9, 6.0, 1.3, 1.3 Hz, allyl CH_2), 3.53 (t, 1H, J = 6.7 Hz, C^2H), 3.66 (s, 3H, OCH_3), 5.07 (dddd, 1H, J = 10.2, 1.9, 1.3, 1.3 Hz, CH=CH_2), 5.13 (dddd, 1H, J = 17.2, 1.9, 1.3, 1.3 Hz, CH=CH_2), 5.25 (s, 2H, OCH_2), 5.82 (dddd, 1H, J = 17.2, 10.2, 6.0, 6.0 Hz, CH=CH_2), 6.93–6.97 (m, 2H, CH^{aryl}), 7.10–7.14 (m, 2H, CH^{aryl}), 7.22–7.26 (m, 1H, CH^{aryl}), 7.35–7.37 (m, 2H, CH^{aryl}); ^{13}C NMR (90 MHz, $CDCl_3$): δ = 38.9 (CH_2), 50.6 (OCH_3), 51.6 (CH_2), 62.1 ($C^{\alpha}H$), 65.3 (OCH_2), 115.0, 116.4, 128.5, 129.9, 130.2, 130.4, 132.2, 136.1, 137.0, 157.8 (C^{aryl}, C=C), 175.0 (C=O); IR (neat): $\tilde{\nu}$ = 3332, 3076, 2948, 2840, 1736, 1643 cm^{-1}; EIMS: m/z: 393, 395 [M^+]; elemental analysis calcd (%) for $C_{20}H_{21}Cl_2NO_3$: C 60.92, H 5.37, N 3.55; found: C 60.79, H 5.33, N 3.51.

(2*S*,2′*R*)-*N*-Allyl-*O*-(2,6-dichlorobenzyl)-*N*-(1-[2,2,2-trichloroethoxycarbonyl]-2-allylpyrrolidin-2-ylcarbonyl)tyrosine methyl ester (7b): Compound **4d** (510 mg, 1.54 mmol) was dissolved in $CHCl_3$ (5 mL) and toluene (10 mL). After addition of DMF (10 μL), thionyl chloride (550 μL, 7.56 mmol) was added dropwise while stirring. After heating the mixture to 60 °C for 1.25 h, the solvents were evaporated and the residue was dried thoroughly. Subsequently, the crude material was dissolved in NMP (10 mL). After addition of a solution of *N*-allyl-*O*-(2,6-dichlorobenzyl)-tyrosine methyl ester (608 mg, 1.54 mmol) in NMP (5 mL) and DIPEA (790 μL, 4.61 mmol), the mixture was heated to 65 °C for 2.5 h. After cooling down, saturated aqueous Na_2CO_3 (5 mL) and water (10 mL) were added and the mixture was extracted with diethyl ether (1×10, 4×5 mL). The combined organic layers were washed with aqueous citric acid (5 %, 10 mL), dried with $MgSO_4$, evaporated and the residue was purified by twofold column chromatography (1. hexanes/ethyl acetate/

HCOOH 78:20:2, 2. hexanes/ethyl acetate 3:1), furnishing **7b** as a colorless gum (744 mg, 68%). R_f = 0.43 (hexanes/ethyl acetate1:1); $[\alpha]_D^{19}$ = −57.8 (c = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$; broadened signals were observed): δ = 1.88–2.08 (m, 2H, CH_2), 2.10–2.29 (m, 2H, CH_2), 2.74 (dd, 1H, J=13.6, 7.7 Hz, C$^3H_2$), 2.97–3.22, 3.19 (m, dd, 2H, J=13.6, 6.5 Hz, CH_2, C$^3H_2$), 3.33–3.72, 3.49, 3.60, 3.70 (m, dd, dd, s, 7H, J=13.9, 4.4; 11.2, 7.8 Hz, CH_2, OCH_3), 3.74–4.00 (m, 2H, CH_2), 4.58 (d, 1H, J= 12.0 Hz, Cl_3CCH_2), 4.91 (d, 1H, J=12.0 Hz, Cl_3CCH_2), 5.08–5.17 (m, 4H, C=CH_2), 5.25 (s, 2H, OCH_2), 5.49–5.73 (m, 1H, CH=C), 5.81 (dddd, 1H, J=17.1, 11.1, 6.1, 6.1 Hz, CH=C), 6.91–6.97 (m, 2H, CH^{aryl}), 7.16–7.24 (m, 3H, CH^{aryl}), 7.35–7.38 (m, 2H, CH^{aryl}); ^{13}C NMR (90 MHz, $CDCl_3$; broadened signals were observed): δ = 22.6, 34.6, 36.0, 40.4, 48.0, 49.3, 52.3 (CH_2, OCH_3), 65.6, 69.6, 69.9 (OCH_2, C^{2}H, C$^{2'}$), 74.9 (Cl_3CCH_2), 95.9 (Cl_3C), 115.1, 115.3, 119.3, 128.7, 130.5, 130.9, 132.4, 132.5, 133.4, 134.0, 137.2 (C^{aryl}, C=C), 151.9, 157.8 (C^{aryl}, carbamate C=O), 171.3, 171.8 (ester, amide C=O); IR (neat): $\tilde{\nu}$ = 2951, 1739 (shoulder), 1719, 1644 cm^{-1}; EIMS: m/z: 704, 706, 708 [M^+]; elemental analysis calcd (%) for $C_{31}H_{33}Cl_5N_2O_6$: C 52.67, H 4.71, N 3.96; found: C 52.51, H 4.74, N 3.89.

(2*S*,5′*R*)-3-[4-(2,6-Dichlorobenzyloxy)phenyl]-2-(1-[2,2,2-trichloroethoxycarbonyl]-6-oxo-1,7-diazaspiro[4.6]undec-9-en-7-yl)propionic acid methyl ester (9b): Prepared from **7b** (500 mg, 0.707 mmol) in CH_2Cl_2 (135 mL) and Grubbs' 2nd generation catalyst (30.0 mg, 5 mol%) in CH_2Cl_2 (5 mL) as described for **8c** (reaction time: 30 min). Column chromatography (hexanes/ethyl acetate 2:1) furnished **9b** as a colorless solid (464 mg, 97%). M.p. 54–57°C; R_f = 0.37 (hexanes/ethyl acetate 1:1); $[\alpha]_D^{20}$ = −62.8 (c = 0.5, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$; rotamers and broadened signals were observed): δ = 1.83–2.20 (m, 4H, CH_2), 2.28 (dddd, 1H, J=13.2, 7.9, 7.9 Hz, CH_2), 2.97–3.09 (m, 1H, CH_2), 3.28 (dd, 1H, J=14.3, 6.6 Hz, C$^3H_2$), 3.55–3.74, 3.70 (m, s, 7H, CH_2, OCH_3), 4.04 (ddd, 1H, J=16.6, 7.2, 1.1 Hz, CH_2), 4.18 (d, 0.35H, J=11.8 Hz, Cl_3CCH_2), 4.57 (d, 0.65H, J=11.9 Hz, Cl_3CCH_2), 4.88 (d, 0.65H, J= 11.9 Hz, Cl_3CCH_2), 5.08–5.18 (m, 1.35H, C^2H, Cl_3CCH_2), 5.25 (s, 2H, OCH_2), 5.62–5.72 (m, 1H, C=CH), 5.82–5.90 (m, 1H, C=CH), 6.93–6.96 (m, 2H, CH^{aryl}), 7.11–7.17 (m, 2H, CH^{aryl}), 7.21–7.25 (m, 1H, CH^{aryl}), 7.34–7.37 (m, 2H, CH^{aryl}); ^{13}C NMR (90 MHz, $CDCl_3$; rotamers were observed): δ = 22.4, 23.1, 33.6, 34.5, 34.7, 35.0, 36.7, 37.7, 44.8, 48.4, 49.6, 52.2, 52.3 (CH_2, OCH_3), 62.4, 62.6, 65.5, 65.6, 70.1, 70.4 (C$_2$H, OCH_2, spiro C), 74.8, 74.9 (Cl_3CCH_2), 96.0 (CCl_3), 115.3, 126.6, 127.7, 128.6, 130.27, 130.33, 130.4, 130.5, 132.5, 137.2 (C^{aryl}, C=C), 152.9, 153.4, 157.9 (C^{aryl}, carbamate C=O), 171.5, 171.7, 174.0, 174.4 (ester and lactam C=O); IR (neat): $\tilde{\nu}$ = 2952, 1737, 1719, 1633 cm^{-1}; EIMS: m/z: 676 [M^+]; elemental analysis calcd (%) for $C_{29}H_{29}Cl_5N_2O_6$: C 51.31, H 4.31, N 4.13; found: C 51.50, H 4.32, N 3.95.

(2*S*,5′*R*)-3-[4-(2,6-Dichlorobenzyloxy)phenyl]-2-(1-*tert*-butoxycarbonyl-6-oxo-1,7-diazaspiro[4.6]undec-9-en-7-yl)propionic acid methyl ester (10b): Compound **9b** (300 mg, 0.442 mmol) was dissolved in acetic acid (10 mL). Zinc dust (600 mg in 6 portions) was added during 2.5 h while stirring vigorously. After complete addition, stirring was continued for 1 h, whereupon the mixture was filtered through Celite and the residue was rinsed thoroughly with acetic acid. The filtrate was concentrated in vacuo, dispersed in water (5 mL) and treated with saturated aqueous Na_2CO_3 (5 mL). The mixture was extracted with diethyl ether (1×5, 4×3 mL), the combined organic layers were dried with $MgSO_4$, evaporated to dryness and dried thoroughly. Subsequently, the residue was dissolved in a solution of Boc_2O (156 mg, 0.715 mmol) in CH_2Cl_2 (5 mL). After stirring for 6 h, another portion of Boc_2O (312 mg, 1.43 mmol) in CH_2Cl_2 (2 mL) was added and stirring was continued for 13 h, whereupon the solvent was removed and the residue was purified by column chromatography (hexanes/ethyl acetate 2:1), furnishing **10b** as a colorless solid (251 mg, 94%). M.p. 58–60°C; R_f = 0.31 (hexanes/ethyl acetate 1:1); $[\alpha]_D^{24}$ = −80.4 (c = 0.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, 330 K; rotamers and broadened signals were observed): δ = 1.41 (s, 9H, Boc CH_3), 1.74–2.10, 2.06 (m, dd, 4H, J=15.3, 7.0 Hz, CH_2), 2.21 (ddd, 1H, J=12.7, 7.6, 7.6 Hz, CH_2), 3.04 (dd, 1H, J=13.4, 8.1 Hz, CH_2), 3.25 (dd, 1H, J=14.4, 6.4 Hz, CH_2), 3.37–3.73, 3.58, 3.68 (m, ddd, s, 7H, J=10.4, 7.4, 7.4 Hz, CH_2, OCH_3), 3.88–4.47 (m, 1H, CH_2), 5.04–5.13 (m, 1H, C^2H), 5.24 (s, 2H, OCH_2), 5.68–5.75 (m, 1H, C=CH), 5.84–5.91 (m, 1H, C=CH), 6.91–6.94 (m, 2H, CH^{aryl}), 7.15–7.18 (m, 2H, CH^{aryl}), 7.20–7.23 (m, 1H, CH^{aryl}), 7.33–7.36 (m, 2H, CH^{aryl}); ^{13}C NMR (90 MHz, $CDCl_3$; rotamers and broadened signals were observed): δ = 22.3, 23.1 (CH_2), 28.6 (Boc CH_3), 33.7, 34.7, 37.6, 44.9, 48.7, 52.2 (CH_2, OCH_3), 62.7, 63.4, 65.4, 69.6 (OCH_2, C^{2}H, spiro C), 79.5 (Boc C^q), 115.2, 125.8, 128.6, 129.5, 130.5, 130.8, 131.3, 132.4, 137.1 (C^{aryl}, C=C), 154.6, 157.8 (Boc C=O, C^{aryl}), 171.7, 175.1 (ester, lactam C=O); IR (neat): $\tilde{\nu}$ = 2976, 1739, 1688, 1633 cm^{-1}; EIMS: m/z: 602 [M^+]; elemental analysis calcd (%) for $C_{31}H_{36}Cl_2N_2O_6$: C 61.69, H 6.01, N 4.64; found: C 61.68, H 6.03, N 4.51.

(2*S*,5′*R*)-3-[4-(2,6-Dichlorobenzyloxy)phenyl]-2-(1-*tert*-butoxycarbonyl-6-oxo-1,7-diazaspiro[4.6]undec-9-en-7-yl)propionic acid: Compound **10b** (150 mg, 0.249 mmol) was dissolved in methanol (5 mL) and THF (1 mL) and cooled with an ice bath. After addition of aqueous NaOH (2N, 5 mL), a precipitate formed which was re-dissolved by addition of THF (2 mL). After stirring for 1.5 h at this temperature, methanol and THF were evaporated (40°C, 90 mbar), the remaining solution was diluted with water, acidified to pH 4 with aqueous citric acid (5%) and extracted with diethyl ether (3×5 mL). The combined organic layers were washed with water (5 mL), dried with $MgSO_4$, concentrated and the product was dried thoroughly in vacuo, furnishing the title compound as a colorless solid (145 mg, 99%). M.p. 94–101°C; R_f = 0.15 (CH_2Cl_2/methanol/HCOOH 95:3:2); $[\alpha]_D^{22}$ = −150 (c = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, [D_5]pyridine, 380 K; broadened signals were observed): δ = 1.50 (s, 9H, Boc CH_3), 1.61–1.69 (m, 1H, CH_2), 1.77–1.83 (m, 1H, CH_2), 1.98 (ddd, 1H, J=12.9, 6.4, 6.4 Hz, CH_2), 2.12 (dd, 1H, J=16.1, 5.5 Hz, CH_2), 2.35 (ddd, 1H, J=12.9, 7.4, 7.4 Hz, CH_2), 3.24 (dd, 1H, J=13.8, 7.7 Hz, CH_2), 3.58–3.63, 3.55, 3.65 (m, ddd, dd, 3H, J=10.2, 7.3, 5.0 Hz; 10.2, 7.7 Hz, CH_2), 3.72 (br d, 1H, J=16.1 Hz, CH_2), 4.00–4.17 (m, 2H, CH_2), 5.32 (s, 2H, OCH_2), 5.68–5.86 (m, 3H, C^2H, HC=CH), 7.05–7.13 (m, 3H, CH^{aryl}), 7.28–7.31 (m, 2H, CH^{aryl}), 7.38–7.42 (m, 2H, CH^{aryl}); ^{13}C NMR (90 MHz, $CDCl_3$; broadened signals were observed): δ = 23.3 (CH_2), 28.6 (Boc CH_3), 32.2, 34.2, 37.1, 48.2, 48.6 (CH_2), 65.45, 65.55, 69.5 (OCH_2, C^{2}H, spiro C), 81.1 (Boc C^q), 115.3, 128.5, 130.2, 130.4, 130.5, 130.9, 131.2, 132.3, 137.0 (C^{aryl} and C=C), 155.7, 157.7 (Boc C=O, C^{aryl}), 171.3, 175.5 (lactam C=O, COOH); IR (neat): $\tilde{\nu}$ = 2975, 1733, 1685, 1653 cm^{-1}; APCI-MS: m/z: 589 [M^++H]; elemental analysis calcd (%) for $C_{30}H_{34}Cl_2N_2O_6$: C 61.12, H 5.81, N 4.75; found: C 61.08, H 6.00, N 4.61.

(2*S*,5′*R*)-3-[4-(2,6-Dichlorobenzyloxy)phenyl]-2-(1-*tert*-butoxycarbonyl-6-oxo-1,7-diazaspiro[4.5]dec-9-en-7-yl)propionic acid: Prepared from (*S*)-*N*-allyl-*O*-(2,6-dichlorobenzyl)tyrosine methyl ester and (2*R*,5*R*)-4-oxo-2-trichloromethyl-5-vinyl-1-aza-3-oxabicyclo[3.3.0]octane[7a] via **4c** analogously to **10b**, followed by ester hydrolysis as described above. Colorless solid (141 mg, 20% over eight steps). M.p. 124–130°C; R_f = 0.26 (CH_2Cl_2/methanol/HCOOH 93:2:5); $[\alpha]_D^{22}$ = −90.3 (c = 1.0, $CHCl_3$); ^{1}H NMR (360 MHz, [D_5]pyridine, 380 K): δ = 1.43 (s, 9H, Boc CH_3), 1.63–1.73 (m, 1H, CH_2), 1.75–1.83 (m, 1H, CH_2), 2.04–2.16 (m, 1H, CH_2), 2.41 (ddd, 1H, J=12.4, 7.0. 5.2 Hz, CH_2), 3.30 (dd, 1H, J=14.6, 8.5 Hz, CH_2), 3.53 (ddd, 1H, J=10.2, 7.2, 7.2 Hz, CH_2), 3.65 (dd, 1H, J= 14.6, 6.6 Hz, CH_2), 3.78–3.86 (m, 1H, CH_2), 4.04–4.08 (m, 2H, CH_2), 5.31 (s, 2H, OCH_2), 5.56–5.60 (m, 1H, C^2H), 5.63 (ddd, 1H, J=10.0, 3.2, 3.2 Hz, C=CH), 5.72 (ddd, 1H, J=10.0, 1.3, 1.3 Hz, C=CH), 7.04–7.08 (m, 2H, CH^{aryl}), 7.11–7.13 (m, 1H, CH^{aryl}), 7.27–7.30 (m, 2H, CH^{aryl}), 7.40–7.44 (m, 2H, CH^{aryl}); ^{13}C NMR (90 MHz, $CDCl_3$; rotamers and strongly broadened signals were observed): δ = 22.8, 23.6 (CH_2), 28.0, 28.5 (Boc CH_3), 28.2, 29.7, 33.6, 38.6, 48.1 (CH_2), 62.5, 63.3, 65.4, 65.5, 66.1 (OCH_2, C^{2}H, spiro C), 80.0, 81.2 (Boc C^q), 115.0, 115.4, 121.0, 127.5, 128.5, 129.6, 129.9, 130.0, 130.4, 130.9, 131.0, 132.3, 137.0 (C^{aryl}, C=C), 153.7, 154.9, 157.6, 157.8 (Boc C=O, C^{aryl}), 170.6, 171.0 (lactam C=O, COOH); IR (neat): $\tilde{\nu}$ = 3361, 2925, 1736, 1693, 1656 cm^{-1}; EIMS: m/z: 575, 574 [M^+]; elemental analysis calcd (%) for $C_{29}H_{32}Cl_2N_2O_6$: C 60.53, H 5.60, N 4.87; found: C 60.40, H 5.78, N 4.86.

Peptide synthesis: Commercially available PAM (4-hydroxymethyl)phenylacetamide) resin preloaded with Boc-Leu was deprotected using TFA/CH_2Cl_2/indole (50:50:0.1; 20 min), followed by neutralization with 10% DIPEA in CH_2Cl_2 followed by several washes with CH_2Cl_2. Boc-Ile-OH, the carboxylic acids derived from **10a**,**b**, and Boc-Arg(Tos)-OH were coupled according to the following procedure: HATU (3–5 equiv) and the carboxylic acid (3–5 equiv) were dissolved in NMP (least volume possible). After addition of DIPEA (6–10 equiv) the mixture was added to

the resin and agitated for 8–16 h, followed by several CH_2Cl_2 washes. If possible, complete acylation was monitored with the Kaiser Test. When the test indicated incomplete coupling or when Boc-Arg(Tos)-OH was coupled to the secondary amino group of the spirocyclic templates, respectively, the procedure was repeated. After deprotection with TFA (20 min), the next coupling cycle was started. Upon completion, the N-termini were deblocked (TFA) and the HF cleavage from the resin using anisole as the scavenger (HF/anisole 9:1, 2 h, 0 °C;) was performed. HF was evaporated and the resin was washed with *tert*-butyl methyl ether. The pure peptides were obtained by extraction of the resin with acetic acid, followed by lyophilization and purification via preparative HPLC (gradient elution: 5–35 % CH_3CN + 0.1 % TFA/H_2O + 0.1 % TFA) on a ZORBAX 300SB-C18 PrepHT (21.2×250 mm, 7 μm) column.

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