# Conformationally Restricted Peptide Mimetics by Ring-Closing Olefin Metathesis

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**Abstract:** Elegant chemical methodology restricting the backbone flexibility of biologically active peptides has attracted growing interest. A practical synthetic strategy is presented to access ten-membered lactam peptide mimetics. Employing a ring-closing olefin metathesis as the key reaction step, the cyclic olefin moiety was obtained with *cis* configuration. Conformational investigations were performed with two model peptides.

**Key words:** peptide mimetics, cyclic olefins, ring-closing metathesis, conformational investigations, medicinal chemistry

In the last few decades, there has been growing interest in the therapeutic use of peptide drugs.[1] Major achievements in solid-phase peptide synthesis, large-scale preparation, and purification techniques have been developed to produce efficiently this intricate substance class in a competitive manner.[2]

However, the use of native peptides is frequently limited by proteolytic degradation, unfavorable pharmacokinetic properties,[1a] or low target selectivity.[3] Therefore, research has focused on the exploration of peptide mimetics that allow disadvantages associated with natural sequences to be circumvented, and, thus, to exploit fully their therapeutic potential in the treatment of severe diseases. Besides the incorporation of structurally modified amino acids, elegant chemical strategies restricting the peptide backbone flexibility have attracted growing interest.[3–5] If the bioactive conformation has been adjusted properly by suitable constraint elements, the number of degrees of conformational freedom and, thus, binding-associated loss of entropy can be decreased, thus increasing target affinity. Moreover, off-target binding may be reduced, metabolic stability will be enhanced, and an increase of lipophilicity originating from hydrocarbon-based constraint elements may ameliorate cell penetration through membrane barriers.

Grubbs' ring-closing olefin metathesis (RCM)[6] has emerged as a versatile tool that facilitates the conformational rigidization of peptides.[7] This highly orthogonal strategy has been exploited to synthesize peptide macrocycles. Replacing disulfide moieties, RCM has become a very useful tool for the formation of 'dicarba' peptides. The synthesis of carba-analogous derivatives of somatostatin led to selective somatostatin sst5 receptor ligands.[8] Grubbs and co-workers were successful in covalently cross-linking two *O*-allylserine residues in the positions *i* and *i+4* of a peptide, thus, inducing the formation of a stable, short helix.[9] Verdine and co-workers developed a 'peptide stapling' strategy in which an all-hydrocarbon cross-link was generated within natural peptides by RCM of inserted α,α-disubstituted nonproteogenic amino acids bearing olefinic side chains.[10] This strategy resulted in a binding affinity to the target protein resulting from the human double minute oncogene (hDM2), that was increased by three orders of magnitude when compared to the wild-type peptide.[10a] Another attractive method to stabilize α-helix structures has been described as an RCM-based 'hydrogen bond surrogate' (HBS) approach. Here, the formation of helices is enforced by completely replacing the H-bonded –HN–C(=O)–HN– motif, which is originally formed by the N-terminal amide carbonyl group of amino acid *i* and the NH of amino acid *i+4*, by using the covalent isostere $–CH_2–C=C–CH_2–N–$ as a constraint element.[11]

Besides its application for cyclopeptide mimetics, RCM has also proved superior in the synthesis of a large number of lactam-bridged dipeptide surrogates. Depending on the size of the ring and the position of the olefin, distinct dihedral angles of the peptide backbone can be adjusted. Figure 1 indicates the positions of the peptide backbone that have been used to attach the alkene-based, conformationally rigidizing elements. Grubbs and co-workers demonstrated that the nitrogen atoms of the first and the following amino acid (N ⇒ N+1) could be connected by RCM to give lactam derivatives of type **I**.[12] Liskamp and co-worker were able to broaden successfully the scope of this concept to amino acids different from glycine.[13] Formal bridging $C_\alpha$ of an amino acid with the backbone nitrogen of the following amino acid ($C_\alpha$ ⇒ N+1) leads to the formation of Freidinger lactams.[14] Upon ring-closing olefin metathesis, monocyclic seven-membered 'dehydro-Freidinger lactams' were synthesized by Grubbs' laboratory (type **IIIa**)[12] and by Piscopio et al.[15] We further exploited the potential of the concept to access eight-, nine- and ten-membered congeners and investigated the resulting conformational properties.[16] Furthermore, RCM was successfully used for the synthesis of β-peptide mimicking analogues.[17] Proline-derived fused lactams of type **IIIb** offer the possibility to adjust gradually the $\Psi_{i+1}$ dihedral angle from 140–170°, a range frequently adopted by prolyl residues in protein cystal structures.[18] Moreover,

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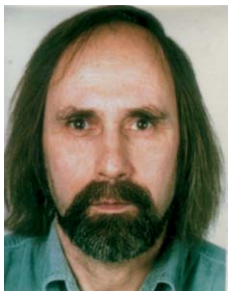
spirocyclic proline derivatives of type **IIIc** were designed and constructed by RCM resulting in highly potent type II β-turn nucleating scaffolds.[19] It is worthy of note that the principle of proline annulation via positions 1 and 5 of the pyrrolidine ring was successfully demonstrated by the groups of Moeller, Wagner and Schmalz.[20,21] The $C_\alpha \Rightarrow C_\alpha$+1 linkage principle is realized in compounds of type **IV**. A highly efficient type VI β-turn mimetic was obtained when the fused proline derivative **IVa** was synthesized via the RCM approach.[22] Nine-membered lactam derivatives of type **IVb** were obtained in racemic form taking advantage of a tandem Ugi reaction/RCM protocol.[23] To complement this, Lubell's group developed an EPC-strategy leading to a complete series of eight-, nine- and ten-membered scaffolds.[24] The laboratory of Katzenellenbogen demonstrated the synthesis of the *trans*-configured azacyclodecenone of type **IVc**, which has been described as the first peptide mimetic that showed type I β-turn inducing properties.[25]

A proline-based tripeptide mimetic following the conjunction principle $C_\alpha \Rightarrow$ N+2 was recently designed in our laboratory.[26] We followed the idea to replace the turn stabilizing side chain of aspartate or asparagine by a covalent

## Biographical Sketches

**Satish Wakchaure** was born in 1979, Sangamner, India. He received his Master's degree in Organic Chemistry from the University of Pune, India in 2005. In 2006, he moved to the National Chemical Laboratory, Pune, India, holding a position as a research assistant. From 2006–2007, he worked as an industrial placement student at GlaxoSmithKline Pharmaceuticals, UK. From 2008 to 2009, he held an appointment at GlaxoSmithKline Pharmaceuticals, Thane, India. He started his Ph.D. in 2010 under the supervision of Prof. Peter Gmeiner at the University of Erlangen-Nürnberg. He is currently focused on the synthesis of peptide mimetics.

**Jürgen Einsiedel** was born in 1969 in Nürnberg. He received his degree in pharmacy (1994) at the University of Erlangen-Nürnberg. His Ph.D. (2000) at the University of Erlangen-Nürnberg concerned the EPC synthesis of dopamine receptor ligands starting from amino acids. He is currently holding a senior scientist position. His research interests are neurotensin receptor ligands, peptide mimetics, and solid-phase-supported peptide synthesis.



**Reiner Waibel** was born in 1951 in Singen. He received his diploma in chemistry in Freiburg in 1979. His Ph.D. work was concerned with isolation and structure elucidation of natural products in the group of Prof. Hans Achenbach, leading to a Ph.D. degree in 1983. He is currently working in the laboratory of Prof. Peter Gmeiner. His scientific interest is mainly focused on structure elucidation of unknown compounds and conformational analysis of peptides and peptide mimetics.

**Peter Gmeiner** was born in 1959 in Vohenstrauß. He received his Ph.D. in 1986 from the University of Munich. From 1987 to 1988 he was a postdoc at the University of California in Berkeley, USA. Peter Gmeiner subsequently returned to Munich as a research associate. Upon receiving his habilitation in 1992, he was appointed as a Professor of Pharmaceutical Chemistry at the University of Bonn. Since October 1996, he has held the chair of Full Professor of Pharmaceutical/Medicinal Chemistry at the University of Erlangen-Nürnberg. Peter Gmeiner's research spans the design, organic synthesis, and pharmacological investigation of bioactive molecules when class I G-protein coupled receptors (GPCRs) are addressed as allosteric target proteins. Within these studies, the synthesis of peptide mimetics is of particular interest.

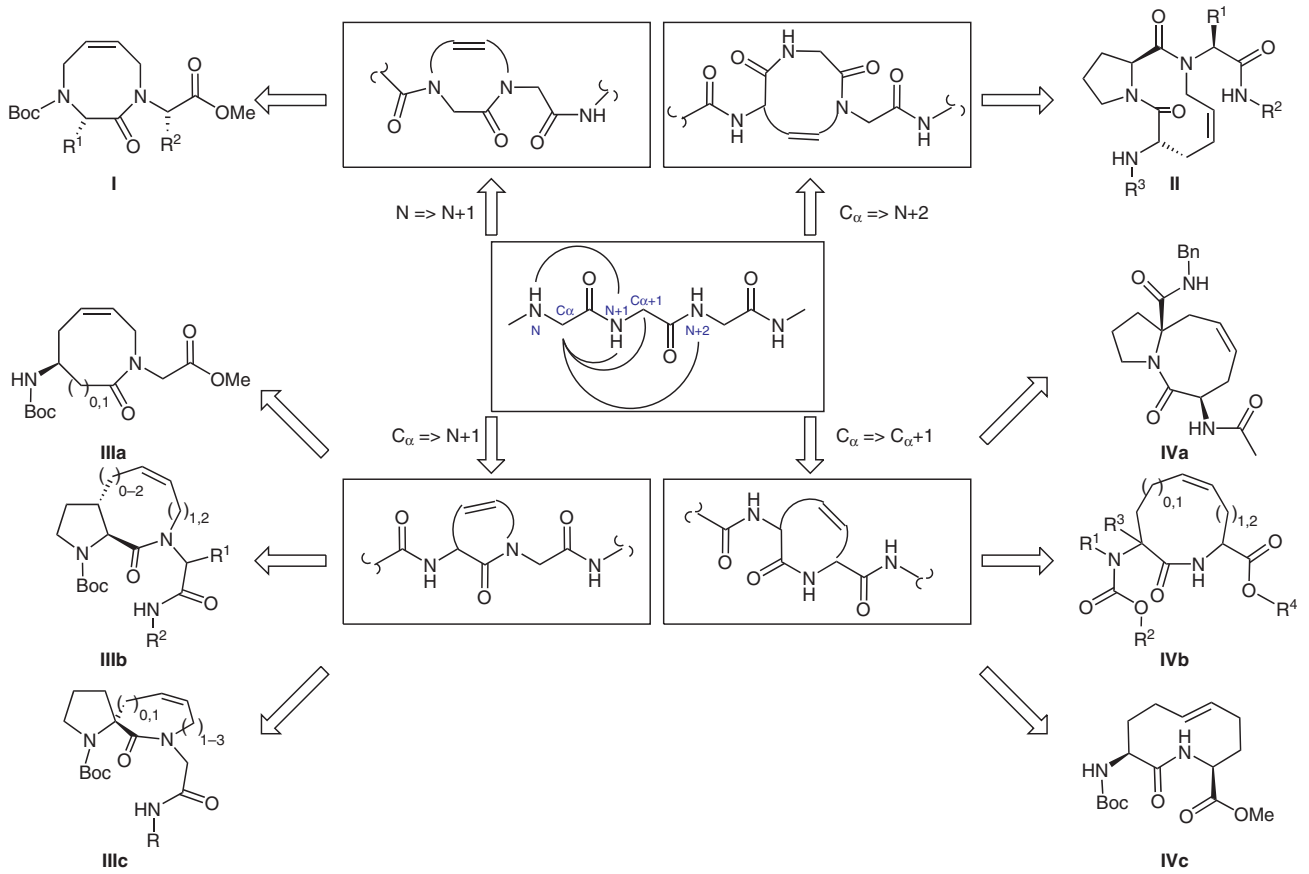



**Figure 1** Secondary-structure-inducing peptide mimetic scaffolds synthesized via Grubbs olefin metathesis

alkene element leading to the *cis*-configured, ten-membered scaffold of type **II**. Hence, a highly efficient type I β-turn mimetic simulating an Asx turn via the HBS principle was realized. Complementary to the concept of J. A. Katzenellenbogen,[25] peptide mimetics of type **II** offer the possibility to present individual amino acid side chains putatively forming attractive contacts to biological receptors.

Based on the conformationally restricted peptide mimetic of type **II**, we envisaged an exploration of the scope and limitations of the synthetic approach when we intended to exchange the reverse-turn nucleator proline by individual open-chain amino acids (Figure 2). We were intrigued by question of whether the resulting diene precursors devoid of the rigid proline still allowed the formation of ten-membered *cis*-alkene ring systems of type **V**.

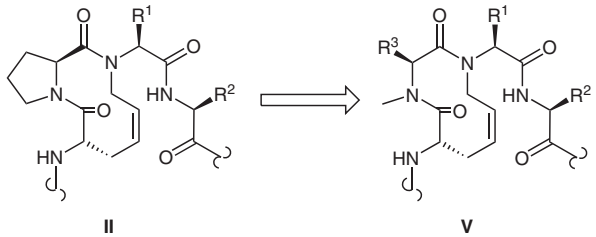
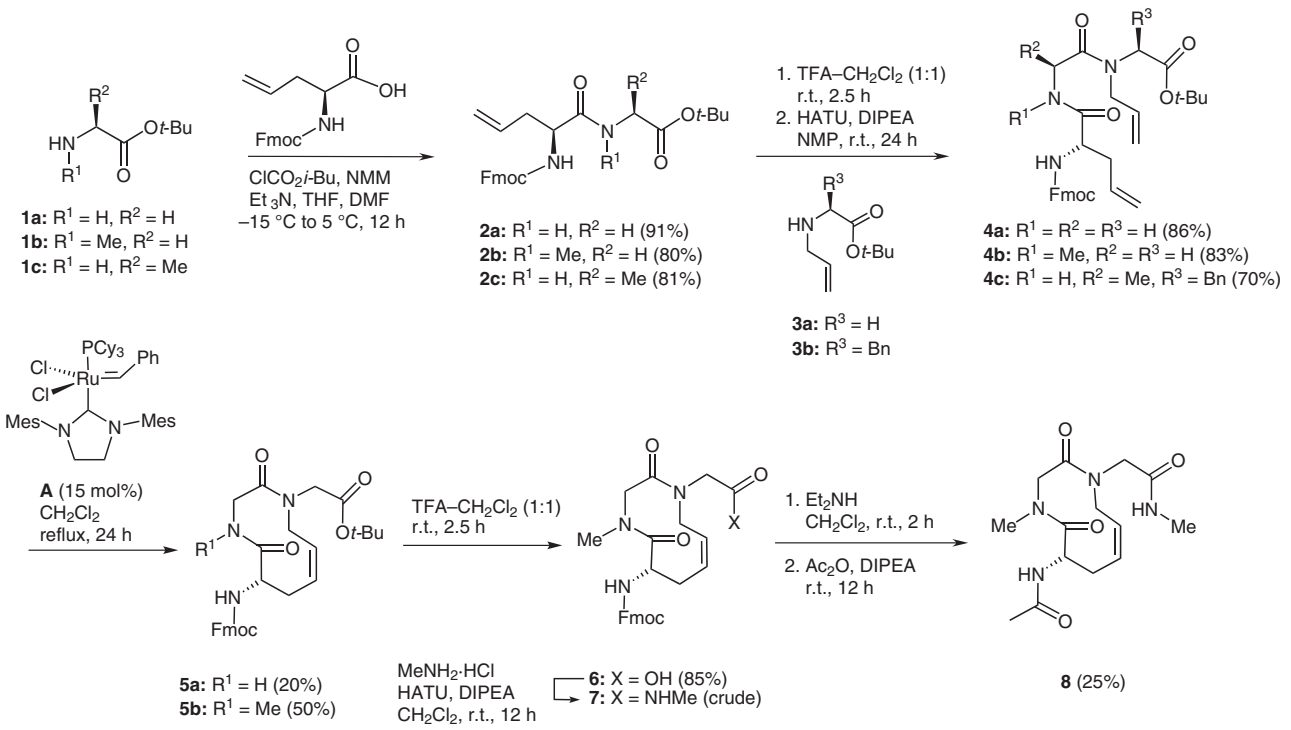



**Figure 2** Substitution of proline leads to peptide mimetics of type **V**

Our initial investigations were directed to the incorporation of glycine and *N*-methylglycine replacing the cyclic amino acid proline (Scheme 1). We employed the *tert*-butyl esters of glycine (**1a**) and *N*-methylglycine (**1b**) and performed acylation with (*S*)-Fmoc-allylglycine, which was activated by using the mixed anhydride method.[27] Thus, we obtained the dipeptides **2a**,**b**, which were readily deprotected with trifluoroacetic acid. Utilizing HATU-promoted activation of **2a**,**b**, we were able to couple the carboxylic acids with *N*-allylglycine *tert*-butyl ester (**3a**)[28] to furnish the dienes **4a**,**b** in high yield. RCM employing the Grubbs second-generation catalyst (**A**) led to the formation of a ten-membered ring resulting in production of the lactams **5a**,**b**. In contrast to the more effective formation of **5b** (50%), the metathesis step to obtain the glycine derivative **5a** only worked with low conversion of the starting material (20%). Removal of the catalyst during workup was done taking advantage of the water-soluble phosphine, tris(hydroxymethyl)phosphine.[29] With the help of $^1$H NMR spectroscopy it was possible to establish the geometry of the double bonds of both cyclic olefins **5a**,**b**, when the *cis* configuration was determined. For lactam **5a**, it was possible to obtain resolved olefin protons employing acetone-$d_6$ as the solvent, allowing the *cis*-specific coupling constant of 10 Hz to be determined. For **5b**, we were able to assess the *cis* configuration at a later stage of the synthesis. To approach a model tetrapeptide mimet-






**Scheme 1** Synthesis of conformationally restricted peptide mimetics

ic, the *tert*-butyl ester derivative **5b** was subjected to acidic cleavage with trifluoroacetic acid to give the carboxylic acid **6**. Applying HATU as the activating reagent, peptide coupling was performed with methylamine hydrochloride to obtain the intermediate **7**. Fmoc deprotection using diethylamine and subsequent acylation with acetic anhydride to gave rise to the model peptide **8**.

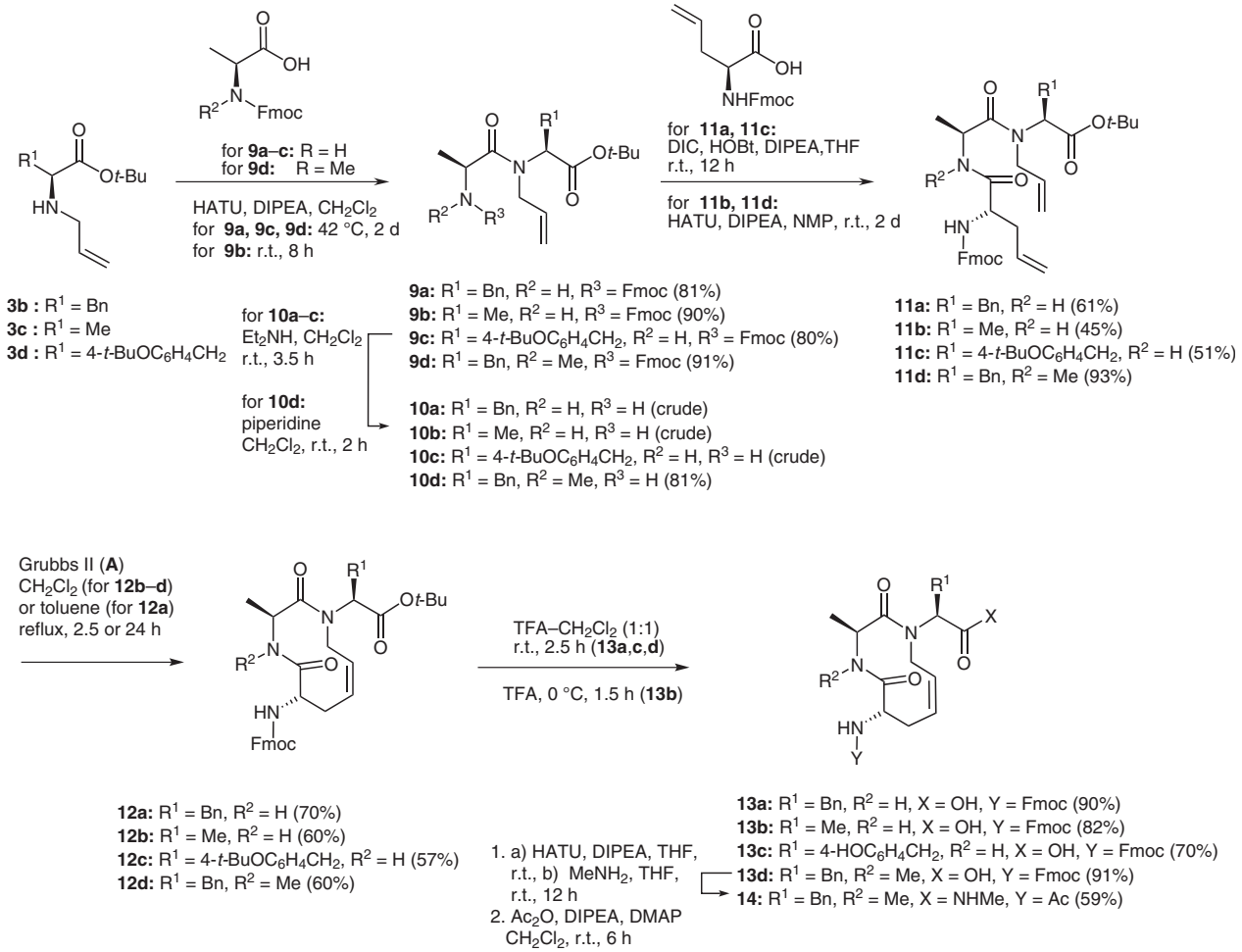
The prolyl residue should be also replaced by alanine as a prototype of an α-substituted amino acid and coupled to phenylalanine. According to the pathway described for the preparation of **8**, peptide coupling with alanine *tert*-butyl ester (**1c**) and (*S*)-Fmoc-allylglycine gave the protected dipeptide **2c**. HATU-promoted coupling of **2c** with *N*-allylphenylalanine *tert*-butyl ester (**3b**)[30] furnished a mixture of diastereomers **4c**. This side reaction could not be suppressed by employing alternative coupling reagents including triphosgene, carbodiimides (DCC or EDC), phosphonium salts like PyBOP, isobutyl chloroformate, or COMU.[31] Obviously, activation of the dipeptide **2c** led to epimerization of the *N*-acylalanine methine via the formation of an oxazolone intermediate.[27] In order to circumvent this problem, we decided to change the reaction sequence for the construction of the precursor diene (Scheme 2).

Thus, starting from the *N*-allylamino acid derivatives **3b**, **3c**,[32] **3d**,[26] HATU-promoted acylation with Fmoc-alanine or Fmoc-*N*-methylalanine at elevated temperatures gave the dipeptides **9a**,[31] and **9c**,**d**. Apparently, higher activation energy is crucial due to the bulkier benzyl residues of the phenylalanine derivatives. This is in accordance with the peptide coupling of the less sterically demanding *N*-allylalanine *tert*-butyl ester, which resulted in the formation of dipeptide **9b**[11] at room temperature.

In the presence of piperidine, the *N*-methyl-derivative **9d** was deprotected; flash chromatography gave **10d**, which was characterized by NMR spectroscopy. Observing a minor fraction of a side product (represented by a second set of proton resonances), we assumed that a minor fraction of the dipeptide had cyclized. Acylation with (*S*)-Fmoc-allylglycine gave the cyclization precursor **11d** in 93% yield. To avoid 'head-to-tail' cyclization, Fmoc deprotection of **9a**–**c** was performed with the volatile diethylamine to give the crude dipeptides **10a**–**c**. Residues of diethylamine were removed by washing with aqueous ammonium chloride solution and the crude material was directly transformed into the cyclization precursors **11a**–**c**.

Ring-closing metathesis of the dienes **11a**–**c** was accomplished employing Grubbs II catalyst (**A**) to afford the expected ten-membered, exclusively *cis*-configured lactams **12a**–**c** in 50–60% yield. Interestingly, for the conversion of the diene **11d** into the lactam **12d**, the reaction time was significantly lower and the cyclization worked with only 5 mol% of catalyst.

Using trifluoroacetic acid, deprotection of the *tert*-butyl ester functions of **12a**–**d** gave the carboxylic acid building blocks **13a**–**d** in 70–91% yield. It is noteworthy that in the case of the tyrosine derivative **12c**, additional cleavage of the *tert*-butyl ether was observed. Conversion of **13d** into the model peptide **14** was accomplished by activation of the carboxylic acid, aminolysis, concomitant Fmoc-cleavage applying a methylamine solution in tetrahydrofuran, and subsequent acetylation of the amine function.




**Scheme 2** Alternative reaction sequence for the synthesis of conformationally restricted peptide mimetics

Detailed NMR spectroscopic studies revealed the configuration and the conformational properties of the Gly-Gly-derived peptide mimetic **8** and its Ala-Phe analogue **14** (Figure 3). Both compounds exhibited very similar NMR spectroscopic properties. The most striking features were the low-field-shifted signals of the H3α protons and the small coupling constants for the coupling of H10β (*N*-allylic methylene protons) and the vicinally positioned olefinic proton (H9) indicating a dihedral angle close to 90°.

A strong NOE between the protons of the *N*-methyl group in position 4 and H6 demonstrated the *s-trans*-configuration of the amide bond connecting N4 and C5. The olefinic double bond was established to be *cis* by significant NOEs between the olefinic protons and diagnostic $^3J$ coupling constants (**8**: $J$ = 10.5 Hz; **14**: $J$ = 11.4 Hz).

Neither for compound **8** nor for **14** could any appreciable amount of amide rotamers be detected. However, the NMR signals of **8** appeared broadened at ambient temperature indicating some conformational flexibility. In contrast, the signals of the alanine derivative **14** appeared only slightly broadened, suggesting a more rigid ring system. NOE measurements (Figure 3) demonstrated very similar structures for both **8** and **14**. A transannular NOE between the protons of the *N*-methyl group in position 4

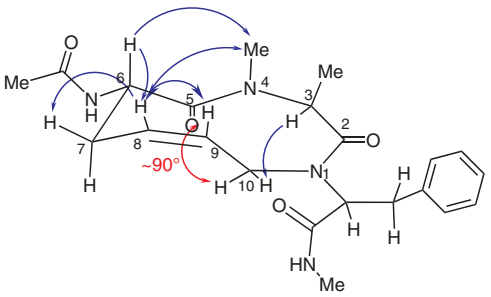



**Figure 3** Conformation of the model peptide **14** experimentally verified by NOE investigations (schematic presentation)

and one of the olefinic protons established their close proximity and a conformation that prevents a hydrogen bond between the C-terminal N–H and the carbonyl oxygen in position 5 and, thus, β-turn formation. Interestingly, similar coupling constants for all resolved ring proton resonances of the *tert*-butyl ester derivatives **12a–d** revealed the same ring folding principle as was shown by the model peptides **8** and **14**.

In conclusion, we were able to successfully extend the RCM-based synthetic strategy to access ten-membered lactam peptide mimetics of type **V**. It was feasible to re-

place the proline unit of scaffold **II** by glycine or alanine and its *N*-methyl analogues, leading to a set of novel building blocks that are suited for the Fmoc-supported incorporation into peptides. Careful NOE studies with the model peptides **8** and **14** revealed conformational properties not allowing a β-turn-like conformation.

All chemicals and solvents were obtained from commercial sources and used as received. All reactions carried out under an $N_2$ atmosphere except cleavage of protecting groups. TLC: silica gel 60 F254 aluminum plates (UV, $KMnO_4$, $I_2$, and ninhydrin detection). Flash chromatography: 60 μm silica gel. Solvents were removed by rotary evaporation under reduced pressure. IR spectroscopy: FT/IR spectrophotometer, film on NaCl plates. NMR spectra: Bruker Advance 600 or a Bruker AM 360 at 300 K unless otherwise noted, relative to TMS. HPLC-MS analyses were carried out on an analytical HPLC system with a VWL detector, coupled to a Bruker Esquire 2000–mass spectrometer with electron spray or atmospheric pressure chemical ionization (ESI or APCI, respectively). ESI-TOF high mass accuracy and resolution experiments were performed on a Bruker maXis MS in the laboratories of the Chair of Bioinorganic Chemistry (Prof. Dr. Ivana Ivanović-Burmazović), Department of Pharmacy and Chemistry, Friedrich-Alexander University of Erlangen Nuremberg. Purities of the products were assessed using an Agilent 1200 analytical HPLC equipped with a Zorbax Eclipse XDB-C8 column (4.6 × 150 mm, 5 μm, flow rate: 0.5 mL/min) and a diode array detector employing the following gradient systems (MeOH = A, MeCN = B): M1: MeOH–$H_2O$ + 0.1% TFA; gradient: 0–10 min: 40–70% A, 10–20 min: 70–100% A, 20–25 min: 100% A. M2: MeOH–$H_2O$ + 0.1% $HCO_2H$; gradient: 0–2 min: 30% A, 2–20 min: 30–100% A, 20–25 min: 100% A. M3: MeOH–$H_2O$ + 0.1% $HCO_2H$; gradient: 0–2 min: 5% A, 2–25 min: 5–100% A, 25–27 min: 100% A. M4: MeOH–$H_2O$ + 0.1% $HCO_2H$; gradient: 0–2 min: 30% A, 2–30 min: 30–100% A, 30–32 min: 100% A. M5: MeOH–$H_2O$ + 0.1% $HCO_2H$; gradient: 0–22 min: 30–80% A, 22–25 min: 80–100% A, 25–28 min: 100% A. M6: MeOH–$H_2O$ + 0.1% $HCO_2H$; gradient: 0–20 min: 60–70% A, 20–22 min: 70–100% A, 22–24 min: 100% A. M7: MeOH–$H_2O$ + 0.1% $HCO_2H$; gradient: 0–22 min: 50–100% A, 22–25 min: 100% A. M8: MeOH–$H_2O$ + 0.1% $HCO_2H$; gradient: 0–20 min: 30–100% A, 20–25 min: 100% A. M9: MeOH–$H_2O$ + 0.1% $HCO_2H$; gradient: 0–5 min: 50–70% A, 5–10 min: 70–100% A, 10–15 min: 100% A. M10: MeOH–$H_2O$; gradient: 0–2 min: 50% A, 2–12 min: 50–100% A, 12–15 min: 100% A. A1: MeCN–$H_2O$ + 0.1% TFA; gradient: 0–10 min: 40–70% B, 10–20 min: 70–100% B, 20–25 min: 100% B. A2: MeCN–$H_2O$ + 0.1% $HCO_2H$; gradient: 0–20 min: 30–100% B, 20–25 min: 100% B. A3: MeCN–$H_2O$ + 0.1% $HCO_2H$; gradient: 0–2 min: 5% B, 2–25 min: 5–100% B, 25–27 min: 100% B. A4: MeCN–$H_2O$ + 0.1% $HCO_2H$; gradient: 0–22 min: 50–100% B, 22–25 min: 100% B. A5: MeCN–$H_2O$ + 0.1% $HCO_2H$; gradient: 0–20 min: 30–80% B, 20–23 min: 80–100% B, 23–26 min: 100% B. A6: MeCN–$H_2O$ + 0.1% $HCO_2H$; gradient: 0–25 min: 20–70% B, 25–27 min: 70–100% B, 27–29 min: 100% B.

The following abbreviations are used *N*,*N*′-diisopropylcarbodiimide (DIC), 1-hydroxybenzotriazole (HOBt), 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC), (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP), {[(1-cyano-2-ethoxy-2-oxoethylidene)amino]oxy}(dimethylamino)(morpholino)carbenium hexafluorophosphate (COMU).

### *tert*-Butyl {[(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)pent-4-enoyl]amino}acetate (2a); Typical Procedure

To a cooled soln (–15 °C) of Fmoc-allylglycine (800 mg, 2.37 mmol) and NMM (240 mg, 261 μL, 2.37 mmol) in THF (5 mL), isobutyl chloroformate (388 mg, 368 μL, 2.84 mmol) was added with stirring. After 15 min, a soln of glycine *tert*-butyl ester hydrochloride (**1a**·HCl, 476 mg, 2.84 mmol) and $Et_3N$ (287 mg, 396 μL, 2.84 mmol) in DMF (7 mL) was added and the resulting mixture was stirred for 12 h at 5 °C. After 12 h, the soln was filtered, $H_2O$ (15 mL) was added to the filtrate, and extraction was performed with $Et_2O$ (3 × 30 mL). The combined organic layers were washed with 5% citric acid (2 × 20 mL), $H_2O$ (25 mL), and brine (25 mL), and dried ($Na_2SO_4$). The solvent was removed in vacuo and flash chromatography (*n*-hexane–EtOAc, 60:40) was performed to give **2a** (972 mg, 91%) as a pale yellow foam; HPLC (220 nm) system M1: $t_R$ = 21.1 min (99%), system A1: $t_R$ = 15.8 min (97%).

$[\alpha]_D^{21}$ –9.4 (*c* 0.7, $CHCl_3$).

IR (NaCl): 1735, 1718, 1701, 1667 $cm^{-1}$.

$^1H$ NMR (600 MHz, $CDCl_3$): δ = 7.76 (m, 2 H,), 7.55–7.61 (m, 2 H), 7.39 (m, 2 H), 7.29–7.34 (m, 2 H), 6.44 (br s, 1 H), 5.76 (br s, 1 H), 5.26–5.29 (m, 1 H), 5.13–5.19 (m, 2 H), 4.37–4.48 (m, 2 H), 4.25–4.32 (m, 1 H), 4.21–4.25 (m, 1 H), 3.84–4.00 (m, 2 H), 2.46–2.64 (m, 2 H), 1.47 (s, 9 H).

$^{13}C$ NMR (90 MHz, $CDCl_3$): δ = 170.9, 168.6, 156.0, 143.8, 141.3, 132.7, 127.7, 127.1, 125.0, 120.0, 119.4, 82.5, 67.2, 63.7, 54.2, 47.2, 42.0, 36.8, 28.0.

MS (ESI): *m*/*z* = 473.2 $[M + Na]^+$.

### *tert*-Butyl {[(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)pent-4-enoyl]methylamino}acetate (2b)

Following the typical procedure for **2a** using Fmoc-allylglycine (1000 mg, 2.96 mmol), NMM (299 mg, 325 μL, 2.96 mmol), and isobutyl chloroformate (485 mg, 460 μL, 3.55 mmol) in THF (5 mL) and sarcosine *tert*-butyl ester hydrochloride (**1b**·HCl, 645 mg, 3.55 mmol) and $Et_3N$ (359 mg, 495 μL, 3.55 mmol) in DMF (7 mL). Flash chromatography (*n*-hexane–EtOAc 70:30) furnished **2b** (1100 mg, 80%) as a pale yellow foam; HPLC (220 nm) system M1: $t_R$ = 21.6 min (98%), system A1: $t_R$ = 17.4 min (98%).

$[\alpha]_D^{25}$ +2.6 (*c* 1.9, $CHCl_3$).

IR (NaCl): 1740, 1720, 1650 $cm^{-1}$.

$^1H$ NMR (600 MHz, $CDCl_3$): δ = 7.76 (m, 2 H), 7.56–7.62 (m, 2 H), 7.39 (m, 2 H), 7.31 (m, 2 H), 5.71–5.85 (m, 1 H), 5.63–5.70 (m, 1 H), 5.09–5.20 (m, 2 H), 4.80 (ddd, *J* = 6.9, 6.9, 6.9 Hz, 0.75 H), 4.59 (ddd, *J* = 6.9, 6.9, 6.9 Hz, 0.25 H), 4.35–4.41 (m, 1 H), 4.30 (d, *J* = 17.0 Hz, 0.75 H), 4.28–4.35 (m, 1 H), 4.18–4.25 (m, 1.25 H), 3.93 (d, *J* = 18.1 Hz, 0.25 H), 3.76 (d, *J* = 17.0 Hz, 0.75 H), 3.14 and 3.00 (2 × s, 3 H), 2.58 (ddd, *J* = 13.9, 6.9, 6.9 Hz, 0.75 H), 2.52 (ddd, *J* = 13.9, 6.9, 6.9 Hz, 0.25 H), 2.42 (ddd, *J* = 13.9, 6.9, 6.9 Hz, 0.75 H), 2.37 (ddd, *J* = 13.9, 6.9, 6.9 Hz, 0.25 H), 1.44 and 1.47 (2 × s, 9 H); rotamers were observed.

$^{13}C$ NMR (90 MHz, $CDCl_3$): δ = 172.0, 171.9, 167.9, 167.8, 155.9, 144.1, 144.0, 141.4, 132.7, 132.6, 127.8, 127.2, 125.3, 120.1, 119.1, 119.0, 83.0, 82.2, 67.2, 52.3, 50.6, 50.5, 50.4, 47.3, 37.6, 37.3, 36.5, 35.3, 28.2, 28.1; rotamers were observed.

MS (ESI): *m*/*z* = 487.3 $[M + Na]^+$.

### *tert*-Butyl (*S*)-2-{[(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)pent-4-enoyl]amino}propanoate (2c)

Following the typical procedure for **2a**, using Fmoc-allylglycine (985 mg, 2.92 mmol), NMM (295 mg, 320 μL, 2.92 mmol), and isobutyl chloroformate (478 mg, 454 μL, 3.50 mmol) in THF (5 mL) and alanine *tert*-butyl ester hydrochloride (**1c**·HCl, 636 mg, 3.50 mmol) and $Et_3N$ (354 mg, 488 μL, 3.50 mmol) in DMF (7 mL). After filtration and concentration of the filtrate, $H_2O$ (20 mL) was added and immediate crystallization of the dipeptide was observed. After filtration and subsequent washings with $H_2O$ and dilute aq $NaHCO_3$, the material was dried (1099 mg, 81%). This material was used for the following reactions without further purification; mp 102 °C; HPLC (220 nm) system M1: $t_R$ = 21.7 min (97%), system A1: $t_R$ = 16.9 min (96%).

$[\alpha]_D^{25}$ –4.1 (*c* 0.7, $CHCl_3$).

IR (NaCl): 1732, 1708, 1661, 1540 $cm^{-1}$.

$^1H$ NMR (600 MHz, $CDCl_3$): δ = 7.78 (m, 2 H), 7.58–7.62 (m, 2 H), 7.42 (m, 2 H), 7.31–7.35 (m, 2 H), 6.50 (d, *J* = 7.0 Hz, 2 H), 5.71–5.85 (m, 1 H), 5.33–5.41 (m, 1 H), 5.15–5.21 (m, 2 H), 4.42–4.49 (m, 2 H), 4.35–4.42 (m, 1 H), 4.22–4.30 (m, 2 H), 2.49–2.61 (m, 2 H), 1.47 and 1.48 (2 × s, 9 H); rotamers were observed.

$^{13}C$ NMR (150 MHz, $CDCl_3$): δ = 171.4, 170.3, 156.0, 143.7, 141.3, 132.7, 127.7, 127.1, 125.1, 120.0, 119.3, 82.5, 67.1, 54.1, 49.3, 48.8, 37.2, 27.9, 18.5; rotamers were observed.

MS (ESI): *m*/*z* = 487.3 $[M + Na]^+$.

***tert*-Butyl (*S*)-2-(Allylamino)propanoate (3c)[32]**

Allyl bromide (2.38 g, 1.7 mL, 19.8 mmol) was slowly added to a soln of alanine *tert*-butyl ester hydrochloride (1000 mg, 5.51 mmol) and DIPEA (1.82 g, 2.5 mL, 17.93 mmol) in MeCN (50 mL) at r.t. After 3 h, the mixture was filtered, $H_2O$ (50 mL) was added to the filtrate, and extraction was performed with $Et_2O$ (3 × 50 mL). The combined organic layers were washed with $H_2O$ (50 mL) and brine (50 mL), and dried ($Na_2SO_4$). The solvent was removed in vacuo at low temperature and flash chromatography (*n*-hexane–EtOAc, 70:30) was performed to give **3c** (815 mg, 80%) as a colorless oil; HPLC (220 nm) system M2: $t_R$ = 24.1 min (98%), system A2: $t_R$ = 24.9 min (98%).

$[\alpha]_D^{24}$ –26.2 (*c* 0.5, $CHCl_3$).

IR (NaCl): 3335, 1730, 1644, 1643 $cm^{-1}$.

$^1H$ NMR (600 MHz, $CDCl_3$) δ = 5.88 (dddd, *J* = 17.2, 10.2, 6.0, 6.0 Hz, 1 H), 5.18 (dddd, *J* = 17.2, 3.0, 1.4, 1.4 Hz, 1 H), 5.08 (dddd, *J* = 10.2, 3.0, 1.4, 1.4 Hz, 1 H), 3.26 (dddd, *J* = 13.6, 6.0, 1.4, 1.4 Hz, 1 H), 3.23 (q, *J* = 7.0 Hz, 1 H), 3.15 (dddd, *J* = 13.6, 6.0, 1.4, 1.4 Hz, 1 H), 1.47 (s, 9 H), 1.26 (d, *J* = 7.0 Hz, 3 H).

$^{13}C$ NMR (90 MHz, $CDCl_3$): δ = 175.3, 136.6, 116.4, 81.0, 56.7, 50.7, 28.3, 19.3.

MS (ESI): *m*/*z* = 186.0 $[M + H]^+$.

HRMS (ESI-TOF): *m*/*z* $[M + H]^+$ calcd for $C_{10}H_{20}NO_2$: 186.1494; found: 186.1487.

***tert*-Butyl [Allyl({[(*S*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)pent-4-enoyl]amino}acetyl)amino]acetate (4a); Typical Procedure**

Compound **2a** (1028 mg, 2.28 mmol) was dissolved in $CH_2Cl_2$ (10 mL) and TFA–$CH_2Cl_2$ (1:1, 10 mL) was added. After stirring at r.t. for 3 h, the mixture was concentrated and thoroughly dried in vacuo to give the crude carboxylic acid, which was used in the following reaction without further purification. The carboxylic acid thus obtained and HATU (1300 mg, 3.42 mmol) were dissolved in NMP (15 mL) followed by the addition of DIPEA (589 mg, 780 μL, 4.56 mmol) at r.t. After 15 min of stirring, a soln of *N*-allylglycine *tert*-butyl ester (**3a**, 781 mg, 4.56 mmol) in NMP (5 mL) was added to the mixture. After 24 h, the mixture was diluted with $H_2O$ (25 mL) and extracted with $Et_2O$ (4 × 25 mL). The combined organic layers were washed with aq 5% citric acid (25 mL), $H_2O$ (25 mL), and brine (25 mL), and dried ($Na_2SO_4$), and the solvent was removed in vacuo. The product was purified by flash chromatography (*n*-hexane–EtOAc, 50:50) to give **4a** (1083 mg, 86%) as a colorless oil; HPLC (220 nm) system M1: $t_R$ = 21.6 min (96%), system A2: $t_R$ = 18.7 min (96%).

$[\alpha]_D^{25}$ –9.8 (*c* 0.9, $CHCl_3$).

IR (NaCl): 1954, 1914, 1844, 1739, 1649 $cm^{-1}$.

$^1H$ NMR (600 MHz, $CDCl_3$): δ = 7.76 (m, 2 H), 7.56–7.61 (m, 2 H), 7.39 (m, 2 H), 7.31 (m, 2 H), 7.03 and 7.00 (2 × s, 1 H), 5.67–5.83 (m, 2 H), 5.40 (br s, 1 H), 5.09–5.28 (m, 4 H), 4.30–4.45 (m, 3 H), 4.21–4.25 (m, 1 H), 4.15–4.20 (m, 1 H), 3.98–4.10 (m, 3 H), 3.84–3.95 (m, 2 H), 2.47–2.62 (m, 2 H), 1.47 and 1.46 (2 × s, 9 H); rotamers were observed.

$^{13}C$ NMR (150 MHz, $CDCl_3$): δ = 170.84, 170.79, 168.3, 168.1, 167.8, 167.5, 155.9, 143.9, 143.7, 141.3, 132.7, 132.2, 131.5, 127.7, 127.1, 125.1, 119.9, 119.3, 118.7, 118.2, 83.1, 82.2, 67.1, 54.2, 50.3, 49.8, 48.4, 48.0, 47.1, 41.14, 41.11, 37.2, 28.02, 27.98; rotamers were observed.

MS (ESI): *m*/*z* = 570.3 $[M + Na]^+$.

HRMS (ESI-TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{31}H_{37}N_3NaO_6$: 570.2580; found: 570.2574.

***tert*-Butyl [Allyl({[(*S*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)pent-4-enoyl]methylamino}acetyl)amino]acetate (4b)**

Following the typical procedure for **4a**. Cleavage of the *tert*-butyl ester: **2b** (1250 mg, 2.69 mmol), $CH_2Cl_2$ (10 mL), and TFA–$CH_2Cl_2$ (1:1, 10 mL). Peptide coupling: crude carboxylic acid derivative, *N*-allylglycine *tert*-butyl ester (**3a**, 921 mg, 5.38 mmol), HATU (1534 mg, 4.04 mmol), and DIPEA (695 mg, 921 μL, 5.38 mmol) in NMP (25 mL). Flash chromatography (*n*-hexane–EtOAc, 70:30) afforded **4b** (1254 mg, 83%) as a colorless oil; HPLC (220 nm) system M2: $t_R$ = 22.6 min (99%), system A2: $t_R$ = 19.4 min (99%).

$[\alpha]_D^{25}$ +3.0 (*c* 1.8, $CHCl_3$).

IR (NaCl): 1789, 1700, 1735, 1685, 1654 $cm^{-1}$.

$^1H$ NMR (600 MHz, $CDCl_3$): δ = 7.75 (m, 2 H), 7.52–7.62 (m, 2 H), 7.39 (m, 2 H), 7.31 (m, 2 H), 5.70–5.90 (m, 2 H), 5.51–5.57 and 5.64–5.70 (m, 1 H), 5.07–5.34 (m, 4 H), 4.78–4.85 (m, 0.8 H), 4.40–4.67 (m, 1.2 H), 4.35–4.41 (m, 1 H), 4.26–4.35 (m, 1 H), 4.19–4.24 (m, 1 H), 4.03–4.16 (m, 1 H), 3.81–4.03 (m, 4 H), 3.20, 3.17, 3.00, and 2.99 (4 × s, 3 H), 1.48, 1.46, and 1.45 (4 × s, 9 H); rotamers were observed.

$^{13}C$ NMR (90 MHz, $CDCl_3$): δ = 172.4, 172.0, 168.3, 168.2, 168.0, 167.9, 155.9, 155.8, 144.0, 143.9, 141.3, 132.6, 132.1, 127.7, 127.1, 125.3, 120.0, 118.9, 118.5, 117.9, 83.0, 82.1, 67.2, 50.8, 50.6, 50.1, 49.4, 49.2, 48.3, 47.3, 37.4, 36.8, 28.2, 28.1; rotamers were observed.

MS (ESI): *m*/*z* = 584.4 $[M + Na]^+$.

HRMS (ESI-TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{32}H_{39}N_3NaO_6$: 584.2737; found: 584.2728.

***tert*-Butyl (*S*)-2-(Allyl{(*S*)-2-[(*S*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)pent-4-enoylamino]propanoyl}amino)-3-phenylpropanoate (4c)**

Following the typical procedure for **4a**. Cleavage of the *tert*-butyl ester: **2c** (682 mg, 1.47 mmol), $CH_2Cl_2$ (5 mL), and TFA–$CH_2Cl_2$ (1:1, 5 mL). Peptide coupling: crude carboxylic acid derivative, *N*-allyl-(*S*)-phenylalanine *tert*-butyl ester (**3b**, 578 mg, 2.21 mmol), HATU (840 mg, 2.21 mmol), and DIPEA (286 mg, 378 μL, 2.21 mmol) in NMP (15 mL). After stirring for 24 h at 40 °C, a second portion of **3a** (192 mg, 0.735 mmol) and HATU (280 mg, 0.735 mmol) in NMP (5 mL) were added to the mixture and stirring was continued at 40 °C for a further 24 h. Flash chromatography (*n*-hexane–EtOAc, 50:50) afforded a mixture of diastereomers of **4c** (670 mg, 70%) as a pale yellow oil. Since this reaction affords a mixture of diastereomers, NMR data is given in the protocol that furnished **4c** as a pure isomer (see **11a**); HPLC (220 nm) system M1: $t_R$ (peak 1) = 23.5 min (42%) and $t_R$ (peak 2) = 23.7 min (58%), system A1: $t_R$ (peak 1) = 20.6 min (43%) and $t_R$ (peak 2) = 20.8 min (57%).

IR (NaCl): 1729, 1643, 1517 $cm^{-1}$.

MS (ESI): *m*/*z* = 674.4 $[M + Na]^+$.

***tert*-Butyl [(*Z*)-(*S*)-6-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-2,5-dioxo-3,4,5,6,7,10-hexahydro-1,4-diazecin-1(2*H*)-yl]acetate (5a); Typical Procedure**

To a soln of diene **4a** (215 mg, 0.37 mmol) in $CH_2Cl_2$ (120 mL) a soln of Grubbs II catalyst (**A**; 33.4 mg, 0.039 mmol, 10 mol%) in $CH_2Cl_2$ (10 mL) was added. After refluxing for 6 h, a second portion of Grubbs II catalyst (**A**; 16.7 mg, 0.019 mmol, 5 mol%) in $CH_2Cl_2$ (5 mL) was added and reflux was continued. After 24 h, the solvent

was removed in vacuo and flash chromatography (*n*-hexane–EtOAc 50:50) was performed. Removal of the residual ruthenium complex was done by dissolving the purified material and $P(CH_2OH)_3$ (287 mg, 2.32 mmol) in $CH_2Cl_2$ (10 mL), adding silica gel (50 mg) and stirring for 15 min. Subsequent filtration furnished **5a** (44.2 mg, 20%) as a pale yellow oil; HPLC (254 nm) system M2: $t_R$ = 21.0 min (97%), system A2: $t_R$ = 15.9 min (99%).

$[\alpha]_D^{24}$ –8.3 (*c* 0.9, $CHCl_3$).

IR (NaCl): 1736, 1720, 1671, 1655 $cm^{-1}$.

$^1H$ NMR (600 MHz, $CDCl_3$): δ = 7.74–7.79 (m, 2 H, $Ar_{Fmoc}$), 7.53–7.60 (m, 2 H, $Ar_{Fmoc}$), 7.37–7.45 (m, 2 H, $Ar_{Fmoc}$), 7.29–7.35 (m, 2 H, $Ar_{Fmoc}$), 6.22 (br s, 1 H, $NH_{Fmoc}$ or H4), 5.67–5.90 (m, 3 H, H9/H8, H3), [in acetone-$d_6$, resonances of the olefin protons are the following: 5.84 (ddd, *J* = 10.0, 9.5, 9.5 Hz, 1 H, H9), 5.73 (ddd, *J* = 10.0, 8.5, 6.2 Hz, 1 H, H8)], 5.05–5.21 (m, 1 H, H4 or $NH_{Fmoc}$), 4.34–4.46 (m, 2 H, Fmoc-$CH_2$), 4.19–4.25 (m, 1 H, $CH_{Fmoc}$), 3.88–4.13 (m, 4 H, H3′, $NCH_2CO$, H6), 3.67–3.78 (m, 1 H, H10), 3.44–3.53 (m, 1 H, H10′), 2.39–2.53 (m, 2 H, H7/H7′), 1.48 (s, 9 H, *t*-Bu).

MS (ESI): *m*/*z* = 542.2 $[M + Na]^+$.

HRMS (ESI-TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{29}H_{33}N_3NaO_6$: 542.2267; found: 542.2262.

### *tert*-Butyl [(*Z*)-(*S*)-6-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-4-methyl-2,5-dioxo-3,4,5,6,7,10-hexahydro-1,4-diazecin-1(2*H*)-yl]acetate (5b)

Following the typical procedure for **5a** using diene **4b** (200 mg, 0.36 mmol) in $CH_2Cl_2$ (120 mL) and a soln of Grubbs II catalyst (**A**; 36.1 mg, 0.054 mmol, 15 mol%) in $CH_2Cl_2$ (30 mL). Flash chromatography (*n*-hexane–EtOAc, 50:50) and removal of residual ruthenium complex using $P(CH_2OH)_3$ (268 mg, 2.20 mmol) and silica gel (36 mg) in $CH_2Cl_2$ (10 mL) afforded **5b** (95 mg, 50%) as a pale yellow oil; HPLC (220 nm) system M1: $t_R$ = 20.1 min (99%), system A1: $t_R$ = 14.1 min (96%).

$[\alpha]_D^{25}$ –10.4 (*c* 2.5, $CHCl_3$).

IR (NaCl): 1740, 1722, 1649 $cm^{-1}$.

$^1H$ NMR (600 MHz, $CDCl_3$): δ = 7.77 (d, *J* = 7.5 Hz, 2 H, $Ar_{Fmoc}$), 7.59 (dd, *J* = 7.5, 1.8 Hz, 2 H, $Ar_{Fmoc}$), 7.40 (d, *J* = 7.5 Hz, 2 H, $Ar_{Fmoc}$), 7.32 (d, *J* = 7.5 Hz, 2 H, $Ar_{Fmoc}$), 5.80 (d, *J* = 8.4 Hz, 1 H, $NH_{Fmoc}$), 5.65–5.75 (m, 2 H, H9/H8), 5.61 (d, *J* = 15.3 Hz, 1 H, H3), 4.70 (ddd, *J* = 11.6, 8.4, 2.6 Hz, 1 H, H6), 4.40 (dd, *J* = 10.6, 7.4 Hz, 1 H, Fmoc-$CH_2$), 4.37 (dd, *J* = 10.6, 7.4 Hz, 1 H, Fmoc-$CH_2$), 4.22 (dd, *J* = 7.4, 7.4 Hz, 1 H, $CH_{Fmoc}$), 4.14 (dd, *J* = 15.5, 10.8 Hz, 1 H, H10α), 4.02 (d, *J* = 17.0 Hz, 1 H, $NCH_2CO$), 3.94 (d, *J* = 17.0 Hz, 1 H, $NCH_2CO$), 3.46–3.49 (m, 1 H, H10β), 3.44 (d, *J* = 15.3 Hz, 1 H, H3′), 3.09 and 3.03 (2 × s, 3 H, $NCH_3$), 2.58 (ddd, *J* = 12.6, 11.6, 8.4 Hz, 1 H, H7), 2.41 (ddd, *J* = 12.6, 7.8, 2.6 Hz, 1 H, H7′), 1.48 (s, 9 H).

$^{13}C$ NMR (150 MHz, $CDCl_3$): δ = 173.1, 168.6, 168.0, 155.6, 144.0, 143.8, 141.5, 141.4, 130.0, 127.9, 127.2, 125.2, 124.0, 120.2, 82.5, 82.3, 67.24, 67.19, 51.3, 51.1, 49.0, 48.1, 47.5, 47.3, 36.7, 33.9, 28.2.

MS (APCI): *m*/*z* = 534.3 $[M + H]^+$.

HRMS (ESI-TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{30}H_{35}N_3NaO_6$: 556.2423; found: 556.2418.

### [(*Z*)-(*S*)-6-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-4-methyl-2,5-dioxo-3,4,5,6,7,10-hexahydro-1,4-diazecin-1(2*H*)-yl]acetic Acid (6)

The cleavage of the *tert*-butyl ester was performed using **5b** (112 mg, 0.21 mmol) dissolved in $CH_2Cl_2$ (1 mL) and employing TFA–$CH_2Cl_2$ (1:1, 2 mL). The crude carboxylic acid derivative thus obtained was purified by flash chromatography ($CH_2Cl_2$–MeOH–AcOH, 75:20:5) to afford **6** (85 mg, 85%) as a pale yellow oil; HPLC (220 nm) system M9: $t_R$ = 11.3 min (95%), system A5: $t_R$ = 12.4 min (96%).

$[\alpha]_D^{26}$ –26.6 (*c* 0.5, MeOH).

IR (NaCl): 3354, 2927, 1686, 1639 $cm^{-1}$.

$^1H$ NMR (600 MHz, $CDCl_3$, 240 K): δ = 7.81 (m, 2 H), 7.68 (m, 2 H), 7.40 (m, 2 H), 7.32 (m, 2 H), 5.58–5.80 (m, 3 H), 4.52 (dd, *J* = 12.5, 2.5 Hz, 1 H), 4.33 (dd, *J* = 10.5, 7.0 Hz, 1 H), 4.31 (dd, *J* = 10.5, 7.0 Hz, 1 H), 4.18–4.26 (m, 2 H), 4.05 (dd, *J* = 16.0, 10.5 Hz, 1 H), 3.75 (br d, *J* = 16.2 Hz, 1 H), 3.56 (br d, *J* = 16.2 Hz, 1 H), 3.34 (d, *J* = 15.5 Hz, 1 H), 2.58 (ddd, *J* = 13.0, 12.5, 8.5 Hz, 1 H), 2.26 (ddd, *J* = 13.0, 8.0, 2.5 Hz, 1 H), 3.09 (s, 3 H).

MS (ESI): *m*/*z* = 500.2 $[M + Na]^+$.

HRMS (ESI-TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{26}H_{27}N_3NaO_6$: 500.1798; found: 500.1778

### 2-[(*Z*)-(*S*)-6-(Acetylamino)-4-methyl-2,5-dioxo-3,4,5,6,7,10-hexahydro-1,4-diazecin-1(2*H*)-yl]-*N*-methylacetamide (8)

To a suspension of **6** (100 mg, 0.20 mmol) and HATU (160 mg, 0.42 mmol) in $CH_2Cl_2$ (5 mL), DIPEA (27.1 mg, 36 μL, 0.21 mmol) was added. After stirring for 15 min at r.t., a soln of $MeNH_2$·HCl (14.3 mg, 0.21 mmol) and DIPEA (72 μL, 0.42 mmol) in $CH_2Cl_2$ (5 mL) was added. After 1 h, a second portion of $MeNH_2$·HCl (14.3 mg, 0.21 mmol) and DIPEA (36 μL, 0.21 mmol) in $CH_2Cl_2$ (5 mL) was added. The mixture was stirred for 8 h at r.t. and concentrated to obtain the crude *N*-methylacetamide **7**. Subsequently, a soln of 15% $Et_2NH$ in $CH_2Cl_2$ (2 mL) was added to the residue. After stirring for 2 h at r.t., addition of a soln of $Ac_2O$ (216 mg, 0.2 mL, 2.1 mmol) and DIPEA (271 mg, 0.36 mL, 2.1 mmol) in $CH_2Cl_2$ (4 mL) was performed and stirring was continued for 12 h at r.t. The mixture was poured into aq $NH_4Cl$ soln (3 mL) and extracted with EtOAc (3 mL). The aqueous layer was concentrated without heating and the resulting solid was purified by using flash chromatography ($CH_2Cl_2$–MeOH–25% $NH_4OH$, 90:5:5) to furnish **8** (16.2 mg, 25%) as a pale yellow oil; HPLC (220 nm) system M10: $t_R$ = 5.9 min (95%), system A5: $t_R$ = 12.4 min (96%).

$[\alpha]_D^{26}$ –42.8 (*c* 0.2, $CHCl_3$).

IR (NaCl): 1750, 1700, 1674, 1670 $cm^{-1}$.

$^1H$ NMR (600 MHz, $CDCl_3$, 240 K): δ = 6.62 (d, *J* = 7.7 Hz, 1 H, N*H*Ac), 6.41 (q, *J* = 4.5 Hz, 1 H, N*H*$CH_3$), 5.71–5.80 (m, 2 H, H8/H9), 5.67 (d, *J* = 16.0 Hz, 1 H, H3), 4.94 (ddd, *J* = 12.0, 7.7, 3.3 Hz, 1 H, H6), 4.30 (d, *J* = 14.8 Hz, 1 H, $NCH_2CO$), 4.07 (dd, *J* = 16.3, 10.1 Hz, 1 H, H10α), 3.77 (d, *J* = 14.8 Hz, 1 H, $NCH_2CO$), 3.64 (d, *J* = 16.3 Hz, 1 H, H10β), 3.49 (d, *J* = 16.0 Hz, 1 H, H3), 3.06 (s, 3 H, $NCH_3$), 2.83 (d, *J* = 4.5 Hz, 1 H, NHC*H*$_3$), 2.51 (ddd, *J* = 12.6, 12.0, 8.2 Hz, 1 H, H7), 2.43 (ddd, *J* = 12.6, 7.7, 3.2 Hz, 1 H, H7′), 2.05 (s, 3 H, $COCH_3$); a minor rotamer was additionally observed.

$^1H$ NMR (600 MHz, acetone-$d_6$, 250K): δ = 7.65 (d, *J* = 7.1 Hz, 1 H, N*H*Ac), 7.42 (br s, 1 H, N*H*$CH_3$), 5.80 (ddd, *J* = 11.3, 10.5, 2.0 Hz, 1 H, H9), 5.73 (ddd, *J* = 10.5, 9.5, 9.5 Hz, 1 H, H8), 5.53 (d, *J* = 15.5 Hz, 1 H, H3), 4.71 (ddd, *J* = 12.2, 7.1, 2.7 Hz, 1 H, H6), 4.28 (d, *J* = 16.4 Hz, 1 H, $NCH_2CO$), 4.05 (dd, *J* = 16.2, 11.3 Hz, 1 H, H10α), 3.75 (d, *J* = 16.4 Hz, 1 H, $NCH_2CO$), 3.56 (d, *J* = 16.2 Hz, 1 H, H10β), 3.26 (d, *J* = 15.5 Hz, 1 H, H3′), 3.10 (s, 3 H, $NCH_3$), 2.67 (d, *J* = 4.7 Hz, 3 H, NHC*H*$_3$), 2.24 (ddd, *J* = 12.8, 9.5, 2.7 Hz, 1 H, H7′), 2.52 (ddd, *J* = 12.8, 12.2, 9.5 Hz, 1 H, H7), 1.92 (s, 3 H, $COCH_3$).

HRMS (ESI-TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{14}H_{22}N_4NaO_4$: 333.1539; found: 333.1529.

### *tert*-Butyl (*S*)-2-[(*S*)-*N*-Allyl-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propanamido]-3-phenylpropanoate (9a);[31] Typical Procedure

To a soln of Fmoc-alanine (1630 mg, 5.22 mmol) and HATU (2980 mg, 7.83 mmol) in $CH_2Cl_2$ (150 mL), DIPEA (675 mg, 890 μL, 5.22 mmol) was added at r.t. After 25 min of stirring, a soln of **3b** (2050 mg, 7.83 mmol) in $CH_2Cl_2$ (30 mL) was added and stirring was continued at 42 °C for 2 d. Thereafter, the mixture was washed with

$H_2O$ (3 × 50 mL) and brine (2 × 50 mL), dried ($Na_2SO_4$), and concentrated in vacuo. Flash chromatography (*n*-hexane–EtOAc, 60:40) afforded **9a** (2114 mg, 81%) as a pale yellow foam; HPLC (220 nm) system M1: $t_R$ = 23.6 min (98%), system A1: $t_R$ = 21.5 min (96%)

$[\alpha]_D^{25}$ –72.7 (*c* 1.1, $CHCl_3$).

IR (NaCl): 1729, 1650, 1498 $cm^{-1}$.

$^1H$ NMR (360 MHz, $CDCl_3$): δ = 7.76 (d, *J* = 7.5 Hz, 2 H), 7.59 (d, *J* = 7.5 Hz, 2 H), 7.40 (dd, *J* = 7.5, 7.5 Hz, 2 H), 7.31 (dd, *J* = 7.5, 7.5 Hz, 2 H), 7.13–7.30 (m, 6 H), 5.68 (d, *J* = 7.9 Hz, 1 H), 5.59 (dddd, *J* = 17.3, 10.5, 6.1, 5.0 Hz, 1 H), 5.15 (d, *J* = 17.3 Hz, 1 H), 5.13 (d, *J* = 10.5 Hz, 1 H), 4.57 (dq, *J* = 7.9, 6.8 Hz, 1 H), 4.32–4.38 (m, 2 H), 4.20–4.25 (m, 1 H), 4.19 (dd, *J* = 10.1, 5.2 Hz, 1 H), 3.81 (dd, *J* = 16.8, 5.0 Hz, 1 H), 3.32 (dd, *J* = 14.3, 5.2 Hz, 1 H), 3.23 (dd, *J* = 14.3, 10.1 Hz, 1 H), 1.42 and 1.37 (2 × s, 9 H), 1.31 and 1.01 (2 × d, each *J* = 6.8 Hz, 3 H); a minor rotamer was additionally observed.

$^{13}C$ NMR (90 MHz, $CDCl_3$): δ = 172.7, 169.3, 155.6, 144.1, 144.0, 141.5, 138.2, 133.1, 129.5, 128.6, 127.8, 127.2, 126.9, 125.3, 120.1, 118.6, 81.8, 67.1, 62.3, 51.9, 47.34, 47.29, 34.9, 28.1, 27.9, 19.5.

MS (ESI): *m*/*z* = 577.3 $[M + Na]^+$.

***tert*-Butyl (*S*)-2-[(*S*)-*N*-Allyl-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propanamido]propanoate (9b)[11]**

Following the typical procedure for **9a** using Fmoc-alanine (1008 mg, 3.24 mmol), HATU (1232 mg, 3.24 mmol), and DIPEA (419 mg, 555 μL, 3.24 mmol) in $CH_2Cl_2$ (100 mL) and **3c** (300 mg, 1.62 mmol) in $CH_2Cl_2$ (15 mL) for 8 h at r.t. Washing of the mixture with aq $NaHCO_3$ (2 × 25 mL), $H_2O$ (3 × 50 mL), and brine (2 × 50 mL). Flash chromatography (*n*-hexane–EtOAc, 60:40) yielded **9b** (698 mg, 90%) as a pale yellow foam; HPLC (220 nm) system M2: $t_R$ = 22.9 min (99%), system A2: $t_R$ = 20.4 min (99%).

$[\alpha]_D^{25}$ –33.3 (*c* 0.6, $CHCl_3$).

IR (NaCl): 1650, 1510, 1526 $cm^{-1}$.

$^1H$ NMR (600 MHz, $CDCl_3$): δ = 7.76 (d, *J* = 7.5 Hz, 2 H), 7.57–7.62 (m, 2 H), 7.40 (dd, *J* = 7.5, 7.5 Hz, 2 H), 7.28–7.33 (m, 2 H), 5.96 (d, *J* = 7.5 Hz, 0.2 H), 5.91 and 5.82 (2 × dddd, *J* = 17.5, 10.0, 5.3, 5.3 Hz, 1 H), 5.72 (d, *J* = 7.5 Hz, 0.8 H), 5.28 (d, *J* = 17.5 Hz, 0.8 H), 5.26 (d, *J* = 10.0 Hz, 0.8 H), 5.15 (d, *J* = 17.5 Hz, 0.2 H), 5.13 (d, *J* = 10.0 Hz, 0.2 H), 4.77 (q, *J* = 7.2 Hz, 0.8 H), 4.73 and 4.62 (2 × dq, *J* = 7.5, 6.8 Hz, 1 H), 4.42 (q, *J* = 7.2 Hz, 0.2 H), 4.31–4.38 (m, 2 H), 4.18–4.24 (m, 1.2 H), 4.04 (dd, *J* = 17.9, 5.3 Hz, 0.8 H), 3.88 (dd, *J* = 17.9, 5.3 Hz, 0.8 H), 3.68 (dd, *J* = 15.9, 5.3 Hz, 0.2 H), 1.47 (d, *J* = 6.8 Hz, 0.6 H), 1.44 and 1.42 (2 × s, 9 H), 1.39 and 1.38 (2 × d, each *J* = 7.2 and 6.8 Hz, respectively, 5.4 H); a minor rotamer was additionally observed.

$^{13}C$ NMR (150 MHz, $CDCl_3$): δ = 173.3, 170.7, 155.6, 155.4, 144.1, 144.0, 141.4, 134.0, 127.8, 127.2, 125.3, 120.1, 117.8, 82.9, 81.7, 67.1, 55.7, 54.5, 48.5, 47.5, 47.4, 47.3, 46.0, 28.1, 28.0, 20.0, 19.5, 15.7, 14.8.

MS (ESI): *m*/*z* = 501.3 $[M + Na]^+$.

***tert*-Butyl (*S*)-2-[(*S*)-*N*-Allyl-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)propanamido]-3-(4-*tert*-butoxyphenyl)propanoate (9c)**

Following the typical procedure for **9a** using Fmoc-alanine (150 mg, 0.48 mmol), HATU (183 mg, 0.48 mmol), and DIPEA (62 mg, 82 μL, 0.48 mmol) in $CH_2Cl_2$ (50 mL) and **3d** (80 mg, 0.24 mmol) in $CH_2Cl_2$ (10 mL) for 24 h at 42 °C. The mixture was washed with aq $NaHCO_3$ (2 × 20 mL), $H_2O$ (20 mL), and brine (20 mL). Flash chromatography (*n*-hexane–EtOAc, 60:40) yielded **9c** (225 mg, 80%) as a pale yellow foam; HPLC (220 nm) system M1: $t_R$ = 24.2 min (99%), system A1: $t_R$ = 23.1 min (98%).

$[\alpha]_D^{24}$ –85.0 (*c* 0.2, $CHCl_3$).

IR (NaCl): 1727, 1650, 1646, 1608 $cm^{-1}$.

$^1H$ NMR (600 MHz, $CDCl_3$): δ = 7.73–7.78 (m, 2 H), 7.57–7.61 (m, 2 H), 7.38–7.42 (m, 2 H), 7.29–7.32 (m, 2 H), 7.02–7.06 (m, 2 H), 6.90–6.94 (m, 2 H), 5.73 (d, *J* = 7.6 Hz, 1 H), 5.53 (dddd, *J* = 17.8, 10.5, 6.2, 5.0 Hz, 1 H), 5.12 (d, *J* = 17.8 Hz, 1 H), 5.11 (d, *J* = 10.5 Hz, 1 H), 4.54 (dq, *J* = 7.6, 6.8 Hz, 1 H), 4.31–4.36 (m, 2 H), 4.19–4.24 (m, 1 H), 4.00–4.05 (m, 1 H), 3.78 (dd, *J* = 16.8, 5.0 Hz, 1 H), 3.18–3.29 (m, 3 H), 1.42 (s, 9 H), 1.32 (s, 9 H), 1.30 (d, *J* = 6.8 Hz, 3 H); a minor rotamer was additionally observed.

$^{13}C$ NMR (90 MHz, $CDCl_3$) δ = 172.5, 169.1, 155.5, 154.0, 143.9, 143.8, 141.3, 133.0, 132.9, 129.8, 127.7, 127.1, 125.2, 124.4, 119.9, 118.8, 81.6, 78.4, 66.9, 62.5, 52.0, 47.2, 47.1, 33.9, 28.8, 27.8, 19.4.

MS (ESI): *m*/*z* = 649.4 $[M + Na]^+$.

***tert*-Butyl (*S*)-2-{(*S*)-*N*-Allyl-2-[(9*H*-Fluoren-9-ylmethoxycarbonyl)methylamino]propanamido}-3-phenylpropanoate (9d)**

Following the typical procedure for **9a** using Fmoc-*N*-methylalanine (1075 mg, 3.31 mmol), HATU (2517 mg, 6.62 mmol), and DIPEA (428 mg, 567 μL, 3.31 mmol) in $CH_2Cl_2$ (100 mL) and **3b** (1300 mg, 4.96 mmol) in $CH_2Cl_2$ (20 mL). Flash chromatography (*n*-hexane–EtOAc, 70:30) furnished **9d** (1710 mg, 91%) as a pale yellow foam.

$[\alpha]_D^{24}$ –124.5 (*c* 1.8, $CHCl_3$).

IR (NaCl): 1733, 1695, 1655, 1597 $cm^{-1}$.

$^1H$ NMR (360 MHz, $CDCl_3$): δ = 7.70–7.83 (m, 2 H), 7.51–7.62 (m, 2 H), 7.36–7.43 (m, 2 H), 7.28–7.33 (m, 2 H), 7.11–7.34 (m, 5 H), 5.88 (dddd, *J* = 17.0, 10.9, 5.7, 5.7 Hz, 0.3 H), 5.50 (dddd, *J* = 17.5, 10.3, 7.0, 4.4 Hz, 0.7 H), 5.20 (dd, *J* = 17.0, 1.4 Hz, 0.3 H), 5.13 (dd, *J* = 10.3, 1.4 Hz, 0.3 H), 5.07 (d, *J* = 17.5 Hz, 0.7 H), 5.04 (q, *J* = 6.8 Hz, 0.7 H), 4.98 (d, *J* = 10.3 Hz, 0.7 H), 4.53 (q, *J* = 6.8 Hz, 0.3 H), 4.32–4.44 (m, 2 H), 4.16–4.23 (m, 1 H), 4.09 (dd, *J* = 10.0, 5.4 Hz, 1 H), 3.87 (dd, *J* = 16.7, 4.4 Hz, 1 H), 3.28 (dd, *J* = 14.0, 5.4 Hz, 1 H), 3.23 (dd, *J* = 14.0, 10.0 Hz, 1 H), 3.18 (dd, *J* = 16.7, 7.0 Hz, 1 H), 2.79 and 2.73 (2 × s, 3 H), 1.44 and 1.41 (2 × s, 9 H), 1.28 and 0.84 (2 × d, each *J* = 6.8 Hz, 3 H); rotamers were observed.

$^{13}C$ NMR (150 MHz, $CDCl_3$): δ = 171.0, 169.2, 155.9, 144.0, 143.9, 143.80, 143.78, 141.4, 138.2, 136.8, 134.4, 133.6, 129.5, 129.3, 128.6, 128.5, 127.7, 127.1, 127.0, 126.9, 126.6, 125.05, 125.02, 124.99, 124.93, 120.04, 119.99, 117.7, 116.9, 82.3, 81.5, 68.0, 67.7, 62.1, 61.8, 51.5, 51.2, 50.9, 50.2, 47.4, 47.3, 35.8, 34.7, 29.1, 28.7, 28.0, 27.9, 14.8, 14.7.

MS (ESI): *m*/*z* = 591.3 $[M + Na]^+$.

***tert*-Butyl (*S*)-2-{Allyl[(*S*)-2-(methylamino)propanoyl]amino}-3-phenylpropanoate (10d)**

To a soln of **9d** (500 mg, 0.88 mmol) in $CH_2Cl_2$ (9 mL), piperidine (1.6 mL) was added. After stirring for 2 h at r.t., the solvent was removed under reduced pressure at low temperature. The residue was purified by flash chromatography ($CH_2Cl_2$–MeOH, 96:4) furnishing **10d** (247 mg, 81%) as a pale yellow oil; HPLC (220 nm) system M2: $t_R$ = 15.6 min (98%), system A2: $t_R$ = 9.3 min (98%).

$[\alpha]_D^{22}$ –64.6 (*c* 0.6, $CHCl_3$).

IR (NaCl): 1732, 1651, 1597 $cm^{-1}$.

$^1H$ NMR (360 MHz, $CDCl_3$): δ = 7.23–7.31 (m, 2 H), 7.19–7.23 (m, 1 H), 7.15–7.19 (m, 2 H), 5.61 (dddd, *J* = 17.2, 10.4, 5.7, 5.2 Hz, 1 H), 5.20 (dddd, *J* = 17.2, 1.5, 1.5, 1.5 Hz, 1 H), 5.11 (dddd, *J* = 10.4, 1.5, 1.5, 1.5 Hz, 1 H), 4.26 (dd, *J* = 10.7, 5.0 Hz, 1 H), 3.76 (dd, *J* = 17.4, 5.2, 1.5, 1.5 Hz, 1 H), 3.33 (dd, *J* = 14.2, 5.0 Hz, 1 H), 3.27 (q, *J* = 7.0 Hz, 1 H), 3.23 (dd, *J* = 14.2, 10.7 Hz, 1 H), 3.14 (dd, *J* = 17.4, 5.7, 1.5, 1.5 Hz, 1 H), 2.21 (s, 3 H), 1.46 (s, 9 H), 1.18 (d, *J* = 7.0 Hz, 3 H).

$^{13}C$ NMR (150 MHz, $CDCl_3$): δ = 176.0, 169.5, 138.2, 133.7, 129.3, 128.4, 126.6, 117.6, 81.6, 62.4, 55.4, 51.2, 34.8, 28.0, 19.7.

MS (ESI): *m*/*z* = 347.2 $[M + H]^+$.

***tert*-Butyl (*S*)-2-(Allyl{(*S*)-2-[(*S*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)pent-4-enoylamino]propanoyl}amino)-3-phenylpropanoate (11a); Typical Procedure**

Compound **9a** (102 mg, 0.18 mmol) was dissolved in a soln of 32% $Et_2NH$ in $CH_2Cl_2$ (5 mL). After stirring for 3.5 h at r.t., $CH_2Cl_2$ (20 mL) was added and the soln was washed with aq sat. $NH_4Cl$ soln (3 × 20 mL), dried ($Na_2SO_4$), and concentrated in vacuo at low temperature. The resulting material **10a** was dried in vacuo and used in the following coupling procedure without further purification. To a soln of Fmoc-allylglycine (152 mg, 0.45 mmol) and HOBt (61 mg, 0.45 mmol) in THF (3 mL), DIPEA (58 mg, 77 μL, 0.45 mmol) and DIC (57 mg, 70 μL, 0.45 mmol) and, after 25 min a soln of the crude amine **10a** in THF (6 mL) were added. After 12 h, the soln was diluted with $H_2O$ (20 mL) and extracted with $Et_2O$ (4 × 20 mL). The combined organic layers were washed with $H_2O$ (20 mL) and brine (20 mL), dried ($Na_2SO_4$), and concentrated in vacuo. Flash chromatography (*n*-hexane–EtOAc, 70:30) gave **11a** (72 mg, 61%) as a pale yellow oil; HPLC (220 nm) system M2: $t_R$ = 23.8 min (96%), system A2: $t_R$ = 21.9 min (98%).

$[\alpha]_D^{22}$ –80.3 (*c* 1.1, $CHCl_3$).

IR (NaCl): 1729, 1644, 1526 $cm^{-1}$.

$^1H$ NMR (360 MHz, $CDCl_3$): δ = 7.73–7.79 (m, 2 H), 7.55–7.62 (m, 2 H), 7.37–7.43 (m, 2 H), 7.29–7.35 (m, 2 H), 7.24–7.29 (m, 2 H), 7.19–7.24 (m, 1 H), 7.10–7.18 (m, 2 H), 6.86 (d, *J* = 6.9 Hz, 1 H), 5.63–5.80 (m, 1 H), 5.48–5.62 (m, 1 H), 5.38 (d, *J* = 7.5 Hz, 1 H), 5.08–5.19 (m, 4 H), 4.73 (dq, *J* = 7.9, 6.8 Hz, 1 H), 4.44 (dd, *J* = 10.5, 7.5 Hz, 1 H), 4.31–4.38 (m, 1 H), 4.21–4.30 (m, 1 H), 4.18 (dd, *J* = 10.2, 5.1 Hz, 1 H), 3.81 (dd, *J* = 16.4, 5.0 Hz, 1 H), 3.31 (dd, *J* = 14.2, 5.1 Hz, 1 H), 3.25 (dd, *J* = 16.4, 6.0 Hz, 1 H), 3.20 (dd, *J* = 14.2, 10.2 Hz, 1 H), 2.44–2.58 (m, 2 H), 1.43 and 1.40 (2 × s, 9 H), 1.31 and 1.00 (2 × d, each *J* = 6.9 Hz, 3 H); a minor rotamer was additionally observed.

$^{13}C$ NMR (150 MHz, $CDCl_3$): δ = 173.1, 172.4, 169.8, 169.0, 155.8, 143.9, 143.8, 141.3, 137.9, 136.8, 133.6, 132.8, 132.6, 129.3, 128.8, 128.5, 127.7, 127.1, 126.7, 125.1, 120.0, 119.4, 118.6, 117.3, 83.0, 81.7, 67.1, 62.3, 62.1, 54.2, 51.7, 47.2, 45.9, 37.4, 34.5, 27.9, 27.8, 19.1, 19.0; a minor rotamer was additionally observed.

MS (ESI): *m*/*z* = 674.4 $[M + Na]^+$.

HRMS (ESI-TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{39}H_{45}N_3NaO_6$: 674.3206; found: 674.3201.

***tert*-Butyl (*S*)-2-(Allyl{(*S*)-2-[(*S*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)pent-4-enoylamino]propanoyl}amino)propanoate (11b)**

Following the typical procedure for **11a**. Fmoc deprotection: **9b** (100 mg, 0.21 mmol), 32% $Et_2NH$ in $CH_2Cl_2$ (5 mL), 3 h at r.t.; workup used $CH_2Cl_2$ (10 mL) and aq sat. $NH_4Cl$ (3 × 15 mL). Coupling: Fmoc-allylglycine (192 mg, 0.57 mmol), DIC (72 mg, 88 μL, 0.57 mmol), HOBt (77 mg, 0.57 mmol), DIPEA (74 mg, 98 μL, 0.57 mmol), and the crude primary amine **10b** in THF (10 mL). Flash chromatography (*n*-hexane–EtOAc, 65:35) gave **11b** (54 mg, 45%) as a pale yellow foam; HPLC (220 nm) system M2: $t_R$ = 23.0 min (99%), system A2: $t_R$ = 19.9 min (99%).

$[\alpha]_D^{23}$ –83.8 (*c* 0.3, $CHCl_3$).

IR (NaCl): 1732, 1638, 1540 $cm^{-1}$.

$^1H$ NMR (600 MHz, $CDCl_3$): δ = 7.74–7.81 (m, 2 H), 7.56–7.61 (m, 2 H), 7.37–7.42 (m, 2 H), 7.29–7.34 (m, 2 H), 6.84 (d, *J* = 6.4 Hz, 1 H), 5.85–5.93 (m, 1 H), 5.67–5.77 (m, 1 H), 5.34–5.40 (m, 1 H), 5.21–5.28 (m, 1.6 H), 5.10–5.17 (m, 2.4 H), 4.73–4.83 (m, 2 H), 4.39–4.45 (m, 1 H), 4.31–4.38 (m, 1 H), 4.19–4.29 (m, 2 H), 3.99–4.06 (m, 1 H), 3.82–3.90 (m, 1 H), 2.42–2.58 (m, 1 H), 1.43 (s, 9 H), 1.36 (2 × d, each *J* = 6.4 Hz, 6 H); a minor rotamer was additionally observed.

$^{13}C$ NMR (90 MHz, $CDCl_3$): δ = 172.8, 170.5, 169.9, 155.9, 143.9, 143.8, 141.4, 133.8, 132.6, 127.7, 127.1, 125.1, 120.0, 119.3, 117.6, 81.7, 67.2, 54.3, 48.2, 47.2, 46.2, 37.4, 28.0, 19.2, 14.6; a minor rotamer was additionally observed.

MS (ESI): m/z = 598.4 $[M + Na]^+$.

HRMS (ESI-TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{33}H_{41}N_3NaO_6$: 598.2893; found: 598.2890.

***tert*-Butyl (*S*)-2-(Allyl{(*S*)-2-[(*S*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)pent-4-enoylamino]propanoyl}amino)-3-(4-*tert*-butyloxyphenyl)propanoate (11c)**

Following the typical procedure for **11a**. Fmoc deprotection: **9c** (105 mg, 0.17 mmol), 32% $Et_2NH$ in $CH_2Cl_2$ (5 mL), 3 h at r.t.; workup $CH_2Cl_2$ (10 mL) and aq sat. $NH_4Cl$ (3 × 15 mL). Coupling: Fmoc-allylglycine (145.0 mg, 0.43 mmol), DIC (54 mg, 72 μL, 0.43 mmol), HOBt (58 mg, 0.43 mmol), DIPEA (56 mg, 74 μL, 0.43 mmol), and the crude primary amine **10c** in THF (8 mL). Flash chromatography (*n*-hexane–EtOAc, 70:30) gave **11c** (59 mg, 51%) as a pale yellow foam; HPLC (220 nm) system M1: $t_R$ = 24.0 min (99%), system A1: $t_R$ = 22.2 min (94%).

$[\alpha]_D^{23}$ –51.9 (*c* 0.6, $CHCl_3$).

IR (NaCl): 1730, 1643, 1550 $cm^{-1}$.

$^1H$ NMR (600 MHz, $CDCl_3$): δ = 7.73–7.79 (m, 2 H), 7.55–7.62 (m, 2 H), 7.38–7.43 (m, 2 H), 7.29–7.35 (m, 2 H), 7.00–7.05 (m, 2 H), 6.87–6.95 (m, 3 H), 5.67–5.78 (m, 1 H), 5.46–5.55 (m, 1 H), 5.38 (d, *J* = 7.6 Hz, 1 H), 5.04–5.19 (m, 4 H), 4.68–4.75 (m, 1 H), 4.40–4.47 (m, 1 H), 4.30–4.37 (m, 1 H), 4.19–4.28 (m, 1 H), 4.00–4.08 (m, 1 H), 3.72–3.81 (m, 1 H), 3.14–3.34 (m, 3 H), 2.44–2.58 (m, 2 H), 1.41 and 1.38 (2 × s, 9 H), 1.32 and 1.38 (2 × s, 9 H), 1.28 (d, *J* = 6.8 Hz, 3 H); a minor rotamer was additionally observed.

$^{13}C$ NMR (150 MHz, $CDCl_3$): δ = 172.0, 169.8, 169.0, 155.9, 154.1, 143.9, 143.8, 141.3, 132.9, 132.8, 132.6, 129.8, 127.7, 127.1, 125.1, 124.4, 120.0, 119.4, 118.6, 81.7, 78.5, 67.1, 62.3, 54.3, 51.9, 47.2, 45.9, 37.3, 33.9, 28.8, 27.9, 27.8, 19.1; a minor rotamer was additionally observed.

MS (ESI): *m*/*z* = 746.5 $[M + Na]^+$.

HRMS (ESI-TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{43}H_{53}N_3NaO_7$: 746.3781; found: 746.3776.

***tert*-Butyl (*S*)-2-[Allyl-((*S*)-2-{[(*S*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)pent-4-enoyl]methylamino}propanoyl)amino]-3-phenylpropanoate (11d)**

Following the typical procedure for **4a** using Fmoc-allylglycine (1110.0 mg, 3.28 mmol), HATU (2076.0 mg, 5.46 mmol), DIPEA (424 mg, 561 μL, 3.28 mmol), and the secondary amine **10d** (945.0 mg, 2.73 mmol) in NMP (100 mL) for2 d at r.t. Flash chromatography (*n*-hexane–EtOAc, 60:30) gave **11d** (1690 mg, 93%) as a pale yellow foam; HPLC (220 nm) system M1: $t_R$ = 23.8 min (99%), system A1: $t_R$ = 21.8 min (99%).

$[\alpha]_D^{22}$ –110.8 (*c* 1.0, $CHCl_3$).

IR (NaCl): 1726, 1680 $cm^{-1}$.

$^1H$ NMR (600 MHz, $CDCl_3$): δ = 7.73–7.78 (m, 2 H), 7.53–7.60 (m, 2 H), 7.37–7.41 (m, 2 H), 7.20–7.35 (m, 5 H), 7.09–7.16 (m, 2 H), 5.66–5.75 (m, 1 H), 5.45–5.56 (m, 2 H), 5.38 (q, *J* = 6.9 Hz, 1 H), 5.06–5.24 (m, 4 H), 4.66 (ddd, *J* = 8.0, 8.0, 5.2 Hz, 1 H), 4.36–4.42 (m, 1 H), 4.29–4.34 (m, 1 H), 4.26 (dd, *J* = 10.3, 5.2 Hz, 1 H), 4.19–4.28 (m, 1 H), 4.18–4.22 (m, 1 H), 3.76 (dd, *J* = 16.6, 4.8 Hz, 1 H), 3.32 (dd, *J* = 16.6, 7.3 Hz, 1 H), 3.30 (dd, *J* = 14.1, 5.2 Hz, 1 H), 3.24 (dd, *J* = 14.1, 10.3 Hz, 1 H), 3.72–3.81 (m), 2.42–2.48 (m, 1 H), 2.20–2.28 (m, 2 H), 1.46 and 1.45 (2 × s, 9 H), 1.28 and 0.79 (2 × d, *J* = 6.8 Hz, 3 H); a minor rotamer was additionally observed.

$^{13}C$ NMR (150 MHz, $CDCl_3$): δ = 171.2, 170.8, 169.2, 155.8, 143.9, 143.8, 141.3, 134.3, 133.6, 132.7, 132.4, 129.7, 129.3, 128.7, 128.5, 127.7, 127.1, 125.2, 125.1, 120.0, 119.0, 118.3, 81.7, 67.0, 65.9, 61.3, 53.4, 51.0, 49.3, 48.8, 47.8, 47.2, 37.2, 36.3, 33.9, 30.3, 28.0, 15.3, 14.7; a minor rotamer was additionally observed.

MS (ESI): *m*/*z* = 688.4 $[M + Na]^+$.

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{40}H_{47}N_3NaO_6$: 688.3363; found: 688.3357.

***tert*-Butyl (*S*)-2-[(*Z*)-(3*S*,6*S*)-6-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-2,5-dioxo-3,4,5,6,7,10-hexahydro-1,4-diazecin-1(2*H*)-yl]-3-phenylpropanoate (12a); Typical Procedure**

To a soln of diene **11a** (70 mg, 0.11 mmol) in toluene (120 mL) a soln of Grubbs II catalyst (**A**; 4.7 mg, 0.05 mmol, 5 mol% ) in toluene (10 mL) was added. After refluxing for 1 h, a second portion of **A** (4.7 mg, 0.05 mmol, 5 mol%) was added and reflux was continued under constant $N_2$ flow through the mixture. After 2.5 h, the solvent was removed in vacuo and flash chromatography (*n*-hexane–EtOAc, 50:50) afforded **12a** (46.8 mg, 70%) as a pale yellow oil; HPLC (220 nm) system M4: $t_R$ = 31.0 min (96%), system A2: $t_R$ = 20.0 min (95%).

$[\alpha]_D^{22}$ –75.0 (*c* 1.0, $CHCl_3$).

IR (NaCl): 1725, 1649, 1534 cm$^{-1}$.

$^1$H NMR (600 MHz, $CDCl_3$): δ = 7.70–7.78 (m, 2 H, $Ar_{Fmoc}$), 7.52–7.62 (m, 2 H, $Ar_{Fmoc}$), 7.36–7.42 (m, 2 H, $Ar_{Fmoc}$), 7.27–7.33 (m, 4 H, $Ar_{Fmoc}$, $Ar_{Phe}$), 7.19–7.25 (m, 1 H, $Ar_{Phe}$), 7.05–7.15 (m, 2 H, $Ar_{Phe}$), 6.16 (d, *J* = 7.8 Hz, 1 H, $NH_{Fmoc}$), 5.86 (d, *J* = 7.2 Hz, 1 H, H4), 5.83 (ddd, *J* = 11.3, 10.8, 2.2 Hz, 1 H, H9), 5.72 (ddd, *J* = 10.8, 10.2, 8.0 Hz, 1 H, H8), 4.74 (dq, *J* = 7.2, 6.7 Hz, 1 H, H3), 4.34–4.41 (m, 2 H, Fmoc-$CH_2$), 4.18–4.23 (m, 1 H, $CH_{Fmoc}$), 3.85 (ddd, *J* = 10.2, 7.8, 2.8 Hz, 1 H, H6), 3.60 (dd, *J* = 10.5, 4.4 Hz, 1 H, $CH_{Phe}$), 3.54 (dd, *J* = 16.2, 11.3 Hz, 1 H, H10α), 3.37 (dd, *J* = 13.8, 10.5 Hz, 1 H, Phe-$CH_2$), 3.31 (dd, *J* = 13.8, 4.4 Hz, 1 H, Phe-$CH_2$), 2.58 (d, *J* = 16.2 Hz, 1 H, H10β), 2.25–2.36 (m, 2 H, H7/H7′), 1.49 (s, 9 H, *t*-Bu), 1.40 (d, *J* = 6.7 Hz, 3 H, Ala-$CH_3$); a minor rotamer was additionally observed.

$^{13}$C NMR (150 MHz, $CDCl_3$): δ = 170.4, 169.3, 168.7, 155.2, 143.9, 143.7, 141.33, 141.31, 138.3, 131.2, 129.1, 128.8, 127.8, 127.7, 126.8, 125.3, 125.1, 120.1, 82.0, 67.1, 66.3, 53.2, 49.0, 48.1, 47.2, 34.5, 32.8, 28.0, 17.9; a minor rotamer was additionally observed.

MS (ESI): *m*/*z* = 646.4 [M + Na]$^+$.

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{37}H_{41}N_3NaO_6$: 646.2893; found: 646.2888.

***tert*-Butyl (*S*)-2-[(*Z*)-(3*S*,6*S*)-6-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-2,5-dioxo-3,4,5,6,7,10-hexahydro-1,4-diazecin-1(2*H*)-yl)]propanoate (12b)**

Following the typical procedure for **5a** using diene **11b** (80 mg, 0.14 mmol) in $CH_2Cl_2$ (200 mL) and a soln of Grubbs II catalyst (**A**; 6.0 mg, 0.007 mmol, 5 mol%) in $CH_2Cl_2$ (10 mL). After 2 h a second portion of **A** (6.0 mg, 0.007 mmol, 5 mol%) in $CH_2Cl_2$ (10 mL) was added. Flash chromatography (*n*-hexane–EtOAc, 30:70) afforded **12b** (48.1 mg, 60%) as brown oil; HPLC (220 nm) system M8: $t_R$ = 21 min (94%), system A2: $t_R$ = 17 min (98%).

$[\alpha]_D^{22}$ –2.4 (*c* 0.6, $CHCl_3$).

IR (NaCl): 3326, 2934, 1715, 1644 cm$^{-1}$.

$^1$H NMR (600 MHz, $CDCl_3$): δ = 7.77 (d, *J* = 7.5 Hz, 2 H, $Ar_{Fmoc}$), 7.60 (d, *J* = 7.5 Hz, 2 H, $Ar_{Fmoc}$), 7.41 (dd, *J* = 7.5, 7.5 Hz, 2 H, $Ar_{Fmoc}$), 7.32 (dd, *J* = 7.5, 7.5 Hz, 2 H, $Ar_{Fmoc}$), 6.18 (d, *J* = 8.8 Hz, 1 H, H4), 5.93 (d, *J* = 7.7 Hz, 1 H, $NH_{Fmoc}$), 5.90 (ddd, *J* = 11.4, 11.4, 2.8 Hz, 1 H, H9), 5.83 (ddd, *J* = 11.4, 10.0, 7.8 Hz, 1 H, H8), 4.96 (dq, *J* = 8.8, 6.8 Hz, 1 H, H3), 4.36–4.42 (m, 2 H, Fmoc-$CH_2$), 4.20–4.24 (m, 1 H, $CH_{Fmoc}$), 3.97 (dd, *J* = 16.1, 11.4 Hz, 1 H, H10α), 3.96 (q, *J* = 6.8 Hz, 1 H, $CH_{Ala}$), 3.93 (ddd, *J* = 10.6, 7.7, 2.8 Hz, 1 H, H6), 3.51 (d, *J* = 16.1 Hz, 1 H, H10β), 2.49 (ddd, *J* = 13.3, 10.6, 10.4 Hz, 1 H, H7), 2.44 (ddd, *J* = 13.3, 7.8, 2.8 Hz, 1 H, H7′), 1.47 (d, *J* = 6.8 Hz, 3 H, Ala-$CH_3$), 1.46 (s, 9 H, *t*-Bu), 1.38 (d, *J* = 6.8 Hz, 3 H, Ala-$CH_3$); a minor rotamer was additionally observed.

$^{13}$C NMR (150 MHz, $CDCl_3$): δ = 170.2, 169.9, 169.5, 155.3, 143.9, 143.7, 141.4, 132.2, 127.8, 127.1, 125.8, 125.1, 120.1, 81.6, 67.1, 58.8, 53.4, 48.2, 47.2, 46.6, 32.9, 28.0, 18.1, 14.3.

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{31}H_{37}N_3NaO_6$: 570.2580; found: 570.2566.

***tert*-Butyl (*S*)-3-(4-*tert*-Butoxyphenyl)-2-[(*Z*)-(3*S*,6*S*)-6-(9*H*-fluoren-9-ylmethoxycarbonylamino)-3-methyl-2,5-dioxo-3,4,5,6,7,10-hexahydro-1,4-diazecin-1(2*H*)-yl]propanoate (12c)**

Following the typical procedure for **5a** using diene **11c** (100 mg, 0.14 mmol) in $CH_2Cl_2$ (125 mL) and a soln of Grubbs II catalyst (**A**; 18.2 mg, 0.021 mmol, 15 mol%) in $CH_2Cl_2$ (25 mL). Flash chromatography (*n*-hexane–EtOAc, 50:50) afforded **12c** (55 mg, 57%) as a brown oil; HPLC (220 nm) system M2: $t_R$ = 23.8 min (99%), system A2: $t_R$ = 21.8 min (99%).

$[\alpha]_D^{24}$ –83.8 (*c* 1.4, $CHCl_3$).

IR (NaCl): 1734, 1700, 1600 cm$^{-1}$.

$^1$H NMR (600 MHz, $CDCl_3$): δ = 7.74–7.78 (m, 2 H, $Ar_{Fmoc}$), 7.56–7.61 (m, 2 H, $Ar_{Fmoc}$), 7.37–7.42 (m, 2 H, $Ar_{Fmoc}$), 7.28–7.35 (m, 2 H, $Ar_{Fmoc}$), 6.97–7.06 (m, 2 H, $Ar_{Tyr}$), 6.87–6.96 (m, 2 H, $Ar_{Tyr}$), 6.26 (d, *J* = 7.8 Hz, 1 H, $NH_{Fmoc}$), 5.87 (d, *J* = 7.6 Hz, 1 H, H4), 5.78–5.84 (m, 1 H, H9 or H8), 5.68–5.75 (m, 1 H, H8 or H9), 4.73 (dq, *J* = 7.6, 6.7 Hz, 1 H, H3), 4.33–4.41 (m, 2 H, Fmoc-$CH_2$), 4.18–4.23 (m, 1 H, $CH_{Fmoc}$), 3.85 (ddd, *J* = 10.1, 7.8, 2.1 Hz, 1 H, H6), 3.64 (dd, *J* = 10.6, 4.5 Hz, 1 H, CH Tyr), 3.52 (dd, *J* = 16.1, 11.3 Hz, 1 H, H10α), 3.32 (dd, *J* = 13.9, 10.6 Hz, 1 H, Tyr-$CH_2$), 3.26 (dd, *J* = 13.9, 4.5 Hz, 1 H, Tyr-$CH_2$), 2.56 (d, *J* = 16.1 Hz, 1 H, H10β), 2.23–2.37 (m, 2 H, H7/H7′), 1.48 (s, 9 H, Boc-*t*-Bu), 1.39 (d, *J* = 6.7 Hz, 3 H, Ala-$CH_3$), 1.34 (s, 9 H, O*t*-Bu); a minor rotamer was additionally observed.

$^{13}$C NMR (90 MHz, $CDCl_3$): δ = 170.3, 169.3, 168.8, 155.2, 154.1, 143.8, 143.7, 141.3, 133.2, 131.3, 131.2, 129.5, 127.7, 127.1, 125.3, 125.1, 124.5, 119.9, 82.1, 78.6, 67.2, 66.6, 53.4, 49.0, 48.2, 47.3, 33.9, 32.9, 29.0, 28.2, 18.1.

MS (ESI): *m*/*z* = 718.5 [M + Na]$^+$.

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{41}H_{49}N_3NaO_7$: 718.3468; found: 718.3463.

***tert*-Butyl (*S*)-2-[(*Z*)-(3*S*,6*S*)-6-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3,4-dimethyl-2,5-dioxo-3,4,5,6,7,10-hexahydro-1,4-diazecin-1(2*H*)-yl]-3-phenylpropanoate (12d)**

Following the typical procedure for **5a** using diene **11d** (320 mg, 0.48 mmol) in $CH_2Cl_2$ (200 mL) and a soln of Grubbs II catalyst (**A**; 20.0 mg, 0.024 mmol, 5 mol%) in $CH_2Cl_2$ (25 mL) with reflux for 8 h. Flash chromatography (*n*-hexane–EtOAc, 50:50) afforded **12d** (183 mg, 60%) as a brown oil; HPLC (220 nm) system M2: $t_R$ = 23.1 min (98%), system A2: $t_R$ = 20.8 min (97%).

$[\alpha]_D^{22}$ –97.5 (*c* 0.4, $CHCl_3$).

IR (NaCl): 1728, 1647, 1600 cm$^{-1}$.

$^1$H NMR (600 MHz, $CDCl_3$): δ = 7.70–7.78 (m, 2 H, $Ar_{Fmoc}$), 7.54–7.60 (m, 2 H, $Ar_{Fmoc}$), 7.37–7.41 (m, 2 H, $Ar_{Fmoc}$), 7.28–7.33 (m, 4 H, $Ar_{Fmoc}$, $Ar_{Phe}$), 7.21–7.25 (m, 1 H, $Ar_{Phe}$), 7.05–7.13 (m, 2 H, $Ar_{Phe}$), 5.77 (d, *J* = 8.3 Hz, 1 H, $NH_{Fmoc}$), 5.72 (ddd, *J* = 11.4, 10.8, 1.3 Hz, 1 H, H9), 5.63 (ddd, *J* = 10.8, 10.2, 8.0 Hz, 1 H, H8), 5.28 (q, *J* = 6.8 Hz, 1 H, H3), 4.60 (ddd, *J* = 11.3, 8.3, 2.8 Hz, 1 H, H6), 4.34–4.40 (m, 2 H, Fmoc-$CH_2$), 4.18–4.23 (m, 1 H, $CH_{Fmoc}$), 3.65 (dd, *J* = 10.3, 5.0 Hz, 1 H, $CH_{Phe}$), 3.53 (dd, *J* = 16.4, 11.4 Hz, 1 H, H10α), 3.34 (dd, *J* = 13.9, 10.3 Hz, 1 H, Phe-$CH_2$), 3.29 (dd, *J* = 13.9, 5.0 Hz, 1 H, Phe-$CH_2$), 2.89 (s, 3 H, $NCH_3$), 2.52 (d, *J* = 16.4 Hz, 1 H, H10β), 2.40 (ddd, *J* = 12.7, 11.3, 10.2 Hz, 1 H, H7), 2.29 (ddd, *J* = 12.7, 8.0, 2.8 Hz, 1 H, H7′), 1.53 and 1.49 (2 × s, 9 H, *t*-Bu), 1.35 (d, *J* = 6.8 Hz, 3 H, Ala-$CH_3$); a minor rotamer was additionally observed.

$^{13}$C NMR (150 MHz, $CDCl_3$): δ = 172.9, 169.0, 168.6, 155.4, 143.8, 143.7, 141.3, 138.3, 130.0, 129.1, 128.7, 127.7, 127.1, 126.7, 125.1, 123.5, 120.0, 81.8, 67.0, 66.1, 51.7, 49.0, 48.9, 47.1, 34.5, 34.2, 32.0, 28.1, 13.6.

MS (ESI): *m*/*z* = 660.4 [M + Na]$^+$.

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{38}H_{43}N_3NaO_6$: 660.3050; found: 660.3042.

**(*S*)-2-[(*Z*)-(3*S*,6*S*)-6-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-2,5-dioxo-3,4,5,6,7,10-hexahydro-1,4-diazecin-1(2*H*)-yl)]-3-phenylpropanoic Acid (13a); Typical Procedure**

Acidic ester cleavage was performed by dissolving **12a** (50 mg, 0.09 mmol) in $CH_2Cl_2$ (0.2 mL) and employing TFA–$CH_2Cl_2$ (1:3, 1.3 mL) After stirring at r.t. for 3 h, the mixture was concentrated and flash chromatography ($CH_2Cl_2$–MeOH–AcOH, 74:25:1) gave the carboxylic acid **13a** (41.2 mg, 90%) as a yellow foam; HPLC (220 nm) system M7: $t_R$ = 14.8 min (95%), system A6: $t_R$ = 16.3 min (95%).

$[\alpha]_D^{26}$ +13.0 (*c* 1.7, DMSO).

IR (NaCl): 3319, 1689, 1654, 1617, cm$^{-1}$.

$^1$H NMR (600 MHz, $CD_3OD$): δ = 7.77–7.82 (m, 2 H), 7.61–7.68 (m, 2 H), 7.36–7.41 (m, 2 H), 7.33–7.36 (m, 2 H), 7.28–7.35 (m, 6 H), 7.22–7.27 (m, 1 H), 5.73–5.86 (m, 1 H), 5.50–5.58 (m, 1 H), 4.36–4.46 (m, 2 H), 4.27–4.33 (m, 1 H), 4.18–4.25 (m, 2 H), 3.76–3.83 (m, 1 H), 3.50–3.69 (m, 2 H), 3.23–3.28 (m, 1 H), 3.12–3.18 (m, 1 H), 2.49–2.61 (m, 1 H), 2.35–2.44 (m, 1 H), 1.38–1.40 (m, 3 H); a minor rotamer was additionally observed.

HRMS (ESI-TOF): *m*/*z* [M – H]$^-$ calcd for $C_{33}H_{32}N_3O_6$: 566.2291; found: 566.2278.

**(*S*)-2-[(*Z*)-(3*S*,6*S*)-6-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-2,5-dioxo-3,4,5,6,7,10-hexahydro-1,4-diazecin-1(2*H*)-yl]propanoic Acid (13b)**

Acidic ester cleavage was performed with **12b** (30.3 mg, 0.055 mmol) using TFA (1.0 mL) After stirring at 0 °C for 1.5 h, the mixture was concentrated and flash chromatography ($CH_2Cl_2$–MeOH–$H_2O$, 93:6:1) to give the carboxylic acid **13b** (22.1 mg, 82%) as a yellow foam; HPLC (220 nm) system M6: $t_R$ = 13.4 min (98%), system A5: $t_R$ = 13.0 min (96%).

$[\alpha]_D^{26}$ –15.2 (*c* 0.4, MeOH).

IR (NaCl): 3326, 2934, 1715, 1644 cm$^{-1}$.

$^1$H NMR (600 MHz, $CD_3OD$, 240 K): δ = 7.76–7.84 (m, 2 H), 7.62–7.75 (m, 2 H), 7.37–7.43 (m, 2 H), 7.29–7.35 (m, 2 H), 5.87 (ddd, *J* = 11.5, 11.5, 3.0 Hz, 1 H), 5.72 (ddd, *J* = 11.5, 10.5, 9.6 Hz, 1 H), 4.86 (q, *J* = 7.0 Hz, 1 H), 4.28–4.35 (m, 2 H), 4.17–4.25 (m, 1 H), 4.00 (q, *J* = 7.0 Hz, 1 H), 3.84 (dd, *J* = 16.0, 11.5 Hz, 1 H), 3.73 (dd, *J* = 12.0, 1.6 Hz, 1 H), 3.55 (br d, *J* = 16.0 Hz, 1 H), 2.50 (ddd, *J* = 12.5, 12.0, 9.6 Hz, 1 H), 2.20 (ddd, *J* = 12.5, 10.5, 1.6 Hz, 1 H), 1.41 (d, *J* = 7.0 Hz, 3 H), 1.24 (d, *J* = 7.0 Hz, 3 H).

MS (APCI): *m*/*z* = 492.3 [M + H]$^+$.

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{27}H_{29}N_3NaO_6$ 514.1954; found: 514.1943.

**(*S*)-2-[(*Z*)-(3*S*,6*S*)-6-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-2,5-dioxo-3,4,5,6,7,10-hexahydro-1,4-diazecin-1(2*H*)-yl]-3-(4-hydroxyphenyl)propanoic Acid (13c)**

Following the typical procedure for **13a** using **12c** (50 mg, 0.072 mmol) in $CH_2Cl_2$ (0.7 mL) and TFA–$CH_2Cl_2$ (1:1, 1.4 mL) with stirring at r.t. for 3 h. The mixture was concentrated and thoroughly dried in vacuo to give the crude carboxylic acid **13c** (29.3 mg, 70%) as a yellow foam; HPLC (220 nm) system M7: $t_R$ = 15.7 min (94%), system A5: $t_R$ = 13.8 min (97%).

$[\alpha]_D^{26}$ –66.7 (*c* 1.2, MeOH).

IR (NaCl): 3498, 1683, 1646, 1515 cm$^{-1}$.

$^1$H NMR (600 MHz, $CD_3OD$): δ = 7.76–7.81 (m, 2 H), 7.57–7.68 (m, 2 H), 7.35–7.41 (m, 2 H), 7.27–7.33 (m, 2 H), 6.91–6.98 (m, 2 H), 6.67–6.73 (m, 2 H), 5.72–5.83 (m, 1 H), 5.56–5.65 (m, 1 H), 4.60–4.67 (m, 1 H), 4.32–4.39 (m, 2 H), 4.17–4.23 (m, 1 H), 3.65–3.96 (m, 2 H), 3.38–3.48 (m, 1 H), 3.17–3.29 (m, 2 H), 2.49–2.77 (m, 1 H), 2.28–2.38 (m, 1 H), 2.06–2.23 (m, 1 H), 1.27–1.33 (m, 3 H); a minor rotamer was additionally observed.

MS (ESI): *m*/*z* = 606.3 [M + Na]$^+$.

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{33}H_{33}N_3NaO_7$: 606.2216; found: 606.2115.

**(*S*)-2-[(*Z*)-(3*S*,6*S*)-6-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3,4-dimethyl-2,5-dioxo-3,4,5,6,7,10-hexahydro-1,4-diazecin-1(2*H*)-yl]-3-phenylpropanoic Acid (13d)**

Following the typical procedure for **13a** using **12d** (733 mg, 1.15 mmol) in $CH_2Cl_2$ (20 mL) and TFA–$CH_2Cl_2$ (1:1, 16 mL) with stirring at r.t. for 3 h. The mixture was concentrated and thoroughly dried in vacuo to give the crude carboxylic acid **12d** (610 mg, 91%) as a white solid; mp 225 °C; HPLC (220 nm) system M7: $t_R$ = 19.0 min (97%), system A4: $t_R$ = 10.4 min (96%).

$[\alpha]_D^{26}$ –128.2 (*c* 1, DMSO).

IR (NaCl): 3315, 1726, 1706, 1649, 1613 cm$^{-1}$.

$^1$H NMR (600 MHz, $CDCl_3$): δ = 7.73–7.78 (m, 2 H), 7.53–7.60 (m, 2 H), 7.38–7.42 (m, 2 H), 7.29–7.34 (m, 4 H), 7.24–7.28 (m, 1 H), 7.12–7.15 (m, 2 H), 5.81 (d, *J* = 8.4 Hz, 1 H), 5.60–5.70 (m, 2 H), 5.33 (q, *J* = 6.7 Hz, 1 H), 4.70 (ddd, *J* = 11.7, 8.4, 2.9 Hz, 1 H), 4.34–4.41 (m, 2 H), 4.19–4.22 (m, 1 H), 3.79 (dd, *J* = 10.7, 4.3 Hz, 1 H), 3.58 (dd, *J* = 16.4, 10.5 Hz, 1 H, H10α), 3.44 (dd, *J* = 13.9, 10.7 Hz, 1 H), 3.38 (dd, *J* = 13.9, 4.3 Hz, 1 H), 2.87 (s, 3 H), 2.57 (d, *J* = 16.4 Hz, 1 H), 2.41 (ddd, *J* = 12.7, 11.7, 7.8 Hz, 1 H), 2.29 (ddd, *J* = 12.7, 7.6, 2.9 Hz, 1 H), 1.36 (d, *J* = 6.8 Hz, 3 H); a minor rotamer was additionally observed.

$^{13}$C NMR (90 MHz, DMSO-$d_6$): δ = 173.6, 171.3, 168.8, 155.5, 143.8, 140.7, 138.5, 129.3, 129.2, 127.6, 127.1, 126.3, 125.3, 123.3, 120.1, 65.6, 63.4, 50.5, 48.7, 48.2, 46.6, 33.9, 31.3, 30.7, 13.6.

HRMS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{34}H_{35}N_3NaO_6$: 604.2423; found: 604.2425.

**(*S*)-2-[(*Z*)-(3*S*,6*S*)-6-(Acetylamino)-3,4-dimethyl-2,5-dioxo-3,4,5,6,7,10-hexahydro-1,4-diazecin-1(2*H*)-yl]-*N*-methyl-3-phenylpropanamide (14)**

To a soln of carboxylic acid **13d** (100 mg, 0.17 mmol) and HATU (130 mg, 0.34 mmol) in THF (4.0 mL), DIPEA (44 mg, 58 μL, 0.34 mmol) was added. After stirring for 30 min at r.t., 2 M $MeNH_2$ in THF soln (127 μL, 0.51 mmol) was added. After 12 h, a second portion of a 2 M $MeNH_2$ in THF soln (2.0 mL) was added and stirring was continued for 1 h at r.t. The mixture was concentrated in vacuo and the crude secondary amine was disolved in $CH_2Cl_2$ (2.0 mL). N-Acetylation was performed by successively adding DIPEA (659 mg, 873 μL, 5.1 mmol), DMAP (0.1 mg, 0.005 mmol), and $Ac_2O$ (521 mg, 482 μL, 5.1 mmol) and stirring for 12 h at r.t. The mixture was diluted with aq sat. $NH_4Cl$ soln (3.0 mL) and extracted with $CH_2Cl_2$ (3 × 5.0 mL). The combined organic layers were dried ($Na_2SO_4$) and the solvent was removed in vacuo. The product was purified by flash chromatography ($CH_2Cl_2$–MeOH–$NH_4OH$ 25%, 90:5:5) to give **14** (42.1 mg, 59%) as a pale yellow oil; HPLC (220 nm) system M5: $t_R$ = 14.6 min (98%), system A3: $t_R$ = 14.5 min (95%).

$[\alpha]_D^{22}$ –99.6 (*c* 1.2, $CHCl_3$).

IR (NaCl): 2945, 1633, 1541 cm$^{-1}$.

$^1$H NMR (600 MHz, $CDCl_3$): δ = 7.28–7.33 (m, 2 H, $Ar_{Phe}$), 7.23–7.27 (m, 1 H, $Ar_{Phe}$), 7.13–7.17 (m, 2 H, $Ar_{Phe}$), 6.46 (d and br s, *J* = 7.8 Hz, 2 H, $NH_{Ac}$ and N*H*$CH_3$), 5.78 (ddd, *J* = 11.5, 11.4, 2.7 Hz, 1 H, H9), 5.66 (ddd, *J* = 11.4, 10.6, 8.1 Hz, 1 H, H8), 5.28 (q, *J* = 6.8 Hz, 1 H, H3), 4.83 (ddd, *J* = 11.2, 7.8, 3.1 Hz, 1 H, H6), 4.15 (br s, 1 H, $CH_{Phe}$), 3.60 (dd, *J* = 16.3, 11.5 Hz, 1 H, H10α), 3.45 (dd, *J* = 13.2, 9.8 Hz, 1 H, Phe-$CH_2$), 3.21 (dd, *J* = 13.2, 6.0 Hz, 1 H, Phe-$CH_2$), 2.96 (d, *J* = 16.4 Hz, 1 H, H10β), 2.84 (s, 3 H, $NCH_3$), 2.82 (d, *J* = 4.9 Hz, 3 H, NHC*H*$_3$), 2.37 (ddd, *J* = 12.5, 11.2, 10.6 Hz, 1 H, H7), 2.30 (ddd, *J* = 12.5, 8.1, 3.1 Hz, 1 H, H7′), 1.99 (s, 3 H, $COCH_3$), 1.33 (d, *J* = 6.8 Hz, 3 H, Ala-$CH_3$); a minor rotamer was additionally observed.

$^{13}$C NMR (150 MHz, $CDCl_3$): δ = 173.3, 170.74, 170.73, 169.2, 137.1, 130.3, 128.9, 127.1, 124.1, 66.9, 52.2, 48.9, 47.4, 34.8, 33.8, 31.9, 26.5, 23.3, 13.7; a minor rotamer was additionally observed.

MS (APCI): *m*/*z* = 415.4 $[M + H]^+$.

HRMS (ESI-TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{22}H_{30}N_4O_4Na$: 437.2165; found: 437.2156.

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**Supporting Information** for this article is available online at http://www.thieme-connect.com/ejournals/toc/synthesis.

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