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Design and synthesis of a tetracyclic tripeptide mimetic frozen in a polyproline type II (PP2) helix conformation†

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A synthesis of the new tetracyclic scaffold ProM-19, which represents a XPP tripeptide unit frozen in a PPII helix conformation, was developed. As a key building block, *N*-Boc-protected ethyl (1*S*,3*S*,4*R*)-2-aza-bicyclo[2.2.1]hept-5-ene-2-carboxylate was prepared through a diastereoselective aza-Diels–Alder reaction and subsequent hydrogenolytic removal of the chiral *N*-1-phenylethyl substituent under temporary protection of the double bond through dihydroxylation and reconstitution by Corey–Winter olefination. The target compound Boc-[ProM-19]-OMe was then prepared *via* subsequent peptide coupling and Ru-catalyzed ring-closing metathesis steps employing (*S*)-*N*-Boc-allylglycine and *cis*-5-vinyl-proline methyl ester as additional building blocks. In addition, Ac-[2-Cl-Phe]-[Pro]-[ProM-19]-OMe was prepared by solution phase peptide synthesis as a potential ligand for the ena-VASP EVH1 domain.

Introduction

The search for synthetic small-molecule molecules which are able to selectively inhibit or modulate relevant protein–protein interactions represents an important task in biomedical research.[1] In this context, we are interested in molecules capable of replacing natural binding partners of protein domains specialized in the recognition of so-called proline-rich motifs (PRMs).[2] Such specific interactions are involved in a variety of relevant cellular processes, including tyrosine kinase receptor signaling,[3] endocytosis,[4] cytoskeletal restructuring,[5] transcription,[6] and splicing.[7] As a distinctive feature, PRMs adopt a left-handed polyproline type II (PP2) helix secondary structure upon binding to their target domains. This helix type, in addition to the absence of hydrogen bonding, is characterized by a helical pitch of about 9 Å (three residues per turn) with ψ- and ϕ-angles of about 145° and −75°, respectively (Fig. 1).[8]

Following the concept of conformational preorganization, we have previously synthesized tricyclic diproline mimetics, such as ProM-1[9] and ProM-2,[10] (as Pro-Pro equivalents rigidified in a PP2 helix conformation) by introduction of a *Z*-vinylidene bridge between the two pyrrolidine rings (Fig. 2).

We also demonstrated the value of these scaffolds by successfully developing small molecule ligands for the EVH-1

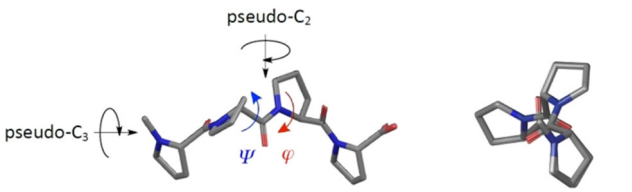

Fig. 1 Section of an idealized polyproline type II (PP2) helix in different perspectives.

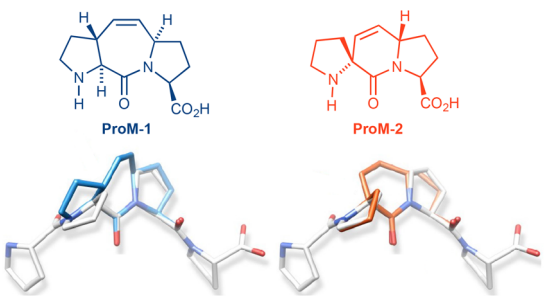

Fig. 2 Top: Structures of ProM-1 and ProM-2. Bottom: Modeling studies show that exchange of the central diproline unit of $(Pro)_4$ (white) by the rigidified units ProM-1 (left) or ProM-2 (right) does not lead to a distortion of the idealized PP2 helix conformation.

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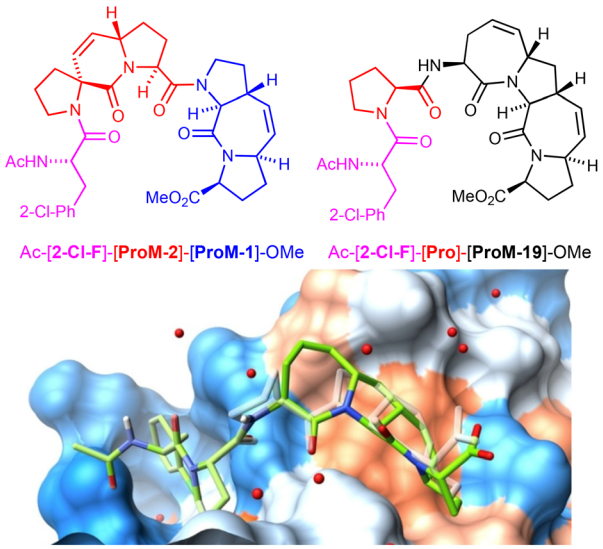


Fig. 3 Top: Structure of the established EVH-1 ligand (left) and the targeted analog containing the tetracyclic tripeptide mimicking unit ProM-19 (right). Bottom: Simulated fit of the latter ligand (shown in green) on the EVH-1 surface in comparison to Ac-[2-Cl-Phe]-[Pro]-[Pro]-[ProM-1]-OMe (shown in white). Red dots indicate water molecules.

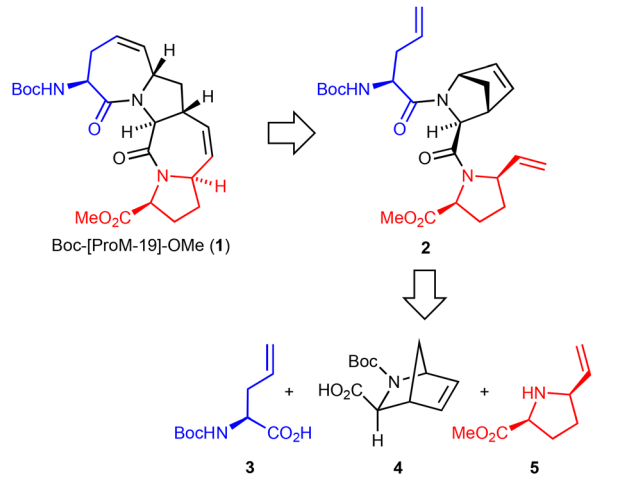
domain.[11] For instance, the compound Ac-[2-Cl-Phe]-[ProM-2]-[ProM-1]-OMe (Fig. 3) was shown to selectively bind to the Ena/VASP EVH1 domain with nanomolar affinity and thereby to impair invasion and extravasation of breast cancer cells.[12]

Having successfully synthesized and applied tricyclic diproline mimetics such as ProM-1 and ProM-2, we now asked ourselves whether it would be possible to further expand our toolbox of geometrically defined scaffolds by synthesizing the tetracyclic system ProM-19 (shown in black in Fig. 3) which was designed as an N-terminally extended analog of ProM-1 representing a tripeptide mimetic rigidified in a PPII helix conformation. However, due the structural complexity of this molecule, its synthesis represented a non-trivial task.

We here describe the stereocontrolled synthesis of the new scaffold ProM-19 (in form of the protected derivative **1**) and of the novel ligand Ac-[2-Cl-Phe]-[Pro]-[ProM-19]-OMe derived thereof, which according to docking simulations would also perfectly fit to the surface of the Ena/Vasp EVH1 domain in the canonical fashion (Fig. 3).[12]

Results and discussion

Our initial strategy for the synthesis of the required tetracyclic unit **1** (Boc-[ProM-19]-OMe) is shown in Scheme 1. We intended to apply a domino ring-closing/ring-opening metathesis (ring rearrangement metathesis)[13] in the key step employing the precursor **2**, which in turn could possibly be assembled from the building blocks **3**, **4** and **5** through amide bond formation.

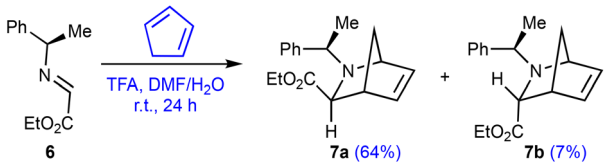


Scheme 1 Retrosynthetic analysis.

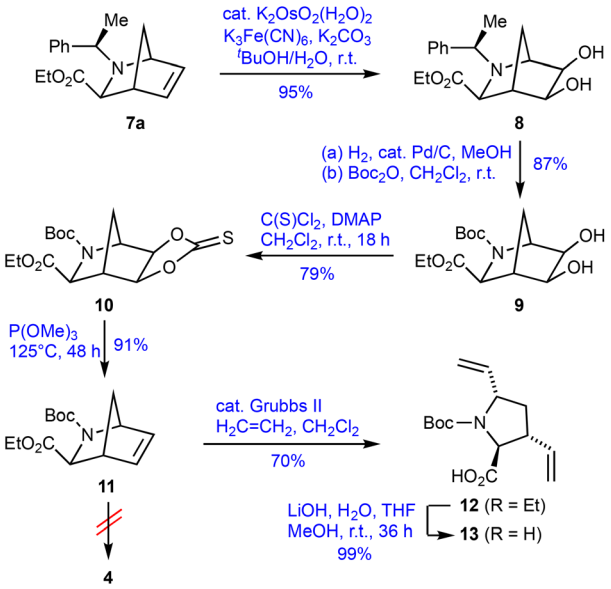
While the protected *cis*-5-vinyl-proline **5** was available in our laboratory[14] and the allyl-glycine derivative **3** could be prepared in enantiomerically pure form *via* known methods,[15] we considered a Diels–Alder approach as a most attractive approach to construct the 2-aza-bicyclo[2.21]heptane ring system of building block **4**.[16]

The synthesis of **4** commenced with an asymmetric aza-Diels–Alder reaction according to Waldmann[16a] (Scheme 2). Best results were obtained when the imine **6**, prepared in quantitative yield from ethyl glyoxylate and (*R*)-1-phenylethylamine (Na_2CO_3, toluene, r.t., 1.5. h), was reacted with cyclopentadiene in the presence of trifluoroacetic acid and catalytic amounts of water.[16b] Under optimized conditions, the reaction proceeded smoothly even on a 10 g scale to give the desired *exo*-product **7a** in 64% isolated yield after chromatographic purification, besides 7% of the *endo*-diastereomer **7b**.

Since direct hydrogenolytic cleavage of the chiral N-substituent was not feasible in the presence of the C=C double bond, we decided to temporarily protect this bond by dihydroxylation.[17] Thus, **7a** was treated with $K_3Fe(CN)_6$ and K_2CO_3 in the presence of 0.025 mol% of K_2OsO_2 to give the *exo*-diol **8** as a single diastereomer in 95% yield (Scheme 3). At this stage, hydrogenolysis of the benzylic C–N bond with Pd/C in MeOH was achieved in good yield, and it was found to be advantageous to pass a hydrogen stream directly through the reaction mixture. After filtration of the product solution through a pad of Celite and removal of the solvent, the crude amine was dissolved in dichloromethane and treated directly



Scheme 2 Synthesis of the 2-aza-bicyclo[2.2.1]heptane derivative **7a** by hetero-Diels–Alder reaction.



Scheme 3 Synthesis of the 3,5-divinylproline derivative **13** as a synthetic equivalent of the aza-bicyclic building block **4**.

with Boc_2O, after which the *N*-Boc-protected diol **9** was obtained in 87% yield (2 steps). This compound showed a remarkable tendency to form massive crystals from CH_2Cl_2 with an edge length of up to 10 mm. Crystallographic analysis confirmed its relative and absolute configuration (Fig. 4).

Having successfully accomplished the exchange of the chiral *N*-phenylethyl substituent by a Boc protecting group, we employed a Corey–Winter reaction[18] to reconstitute the double bond. For this purpose, the diol **9** was first reacted with thiophosgene and DMAP, and the resulting cyclic thiocarbonate **10** was subsequently heated with trimethyl phosphite to afford the expected olefin **11** in 72% yield over two steps. When attempting to saponify the ester function of **11**, this compound surprisingly proved to be highly sensitive towards aqueous alkali, and we were unable to prepare the targeted aza-bicyclic building block **4** with a free carboxylic acid function. For this reason, we converted **9** into the 3,5-divinylproline derivative **12** by Ru-catalyzed ring-opening metathesis in the presence of ethylene.[19] And unlike **11**, the ester function in **12** (obtained in 70% yield) could now be readily hydrolyzed with aqueous LiOH, yielding the acid **13** in quantitative yield, which in terms of the planned strategy (Scheme 1) represents a synthetic equivalent of **4**. Noteworthy, an attempt to achieve the conversion of **9** to **12** through periodate cleavage of the diol[17b] and subsequent Wittig olefination gave rise to a mixture of diastereomers, obviously due to enolization/epimerization at the stage of the dialdehyde.

With the ring-opened acid building block **13** (instead of **4**) in our hands, we continued the synthesis (Scheme 4) by react-

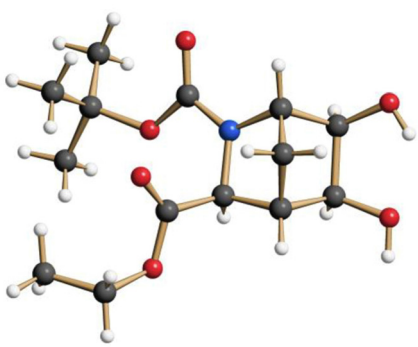
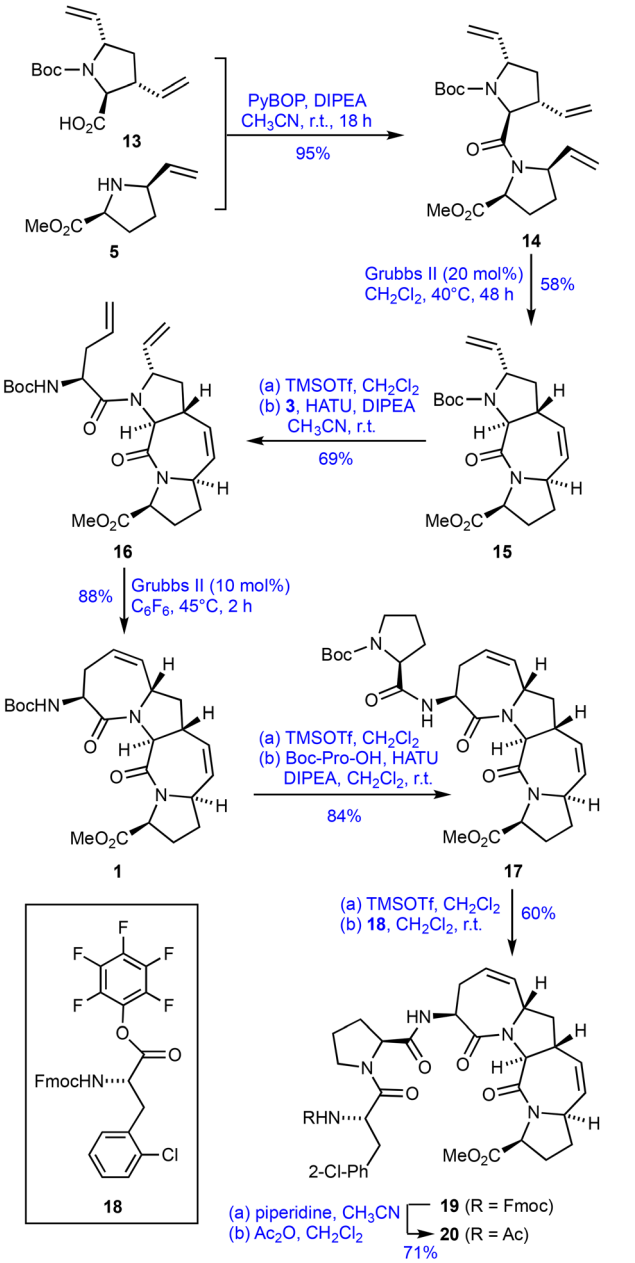


Fig. 4 Structure of diol **9** in the crystalline state.



Scheme 4 Synthesis of the designed tetracyclic tripeptide mimetic Boc-[ProM-19]-OMe (**1**) and its further transformation to the potential EVH-1 ligand **20**.

ing **13** with the known 5-vinylproline ester **5** [14] under proven peptide coupling conditions (PyBOP, DIPEA).[20] Ring closing metathesis of the resulting trivinyldiproline derivative **14** then proved surprisingly difficult. However, repeated addition of small amounts of the Grubbs II catalyst (20 mol% in total) to a dilute solution of **14** in dichloromethane over a period of 48 hours succeeded in achieving a satisfactory conversion, and the tricyclic product **15** was obtained in 58% yield.

The tricycle **15**, which formally represents a vinyl-ProM-1 derivative, was then treated with TMSOTf to cleave off the Boc protecting group and the resulting amine was directly coupled to (*S*)-*N*-Boc-allylglycine (**3**) in the presence of HATU, DIPEA.[21] The subsequent cyclization of **15** through ring-closing metathesis then proceeded smoothly in the presence of the Grubbs II catalyst in hexafluorobenzene to afford the targeted ProM-19 derivative **1** in 61% overall yield from **15** (3 steps).

The final conversion of **1** into ligand **20** (Ac-[2-Cl-Phe]-[Pro]-[ProM-19]-OMe) commenced with the removal of the Boc group (TMSOTf) and HATU-mediated coupling with *N*-Boc-proline. After renewed removal of the Boc protecting group, the 2-chlorophenylalanine unit was attached employing the Fmoc-protected pentafluorophenyl ester **18** as a reagent.[22] Noteworthy, the corresponding *N*-acetylated reagent could not be employed due to epimerization of the stereocenter – probably *via* an azlactone intermediate.[23] However, the exchange of the Fmoc against an *N*-acetyl group was smoothly accomplished at the very end of the sequence by reacting the coupling product **19** subsequently with piperidine and acetic anhydride. This way, the devised the potential EVH-1 ligand **20** was obtained in satisfying overall yield as shown in Scheme 4. The biological investigation of **20** is not yet finished and the results will be reported separately in the context of a broader study.

Conclusions

In conclusion, we have elaborated an efficient stereoselective synthesis of the novel tetracyclic scaffold ProM-19 (in form of the protected derivative **1**) which was designed as a conformationally defined XPP tripeptide mimetic locked in a polyproline type 2 (PPII) helix conformation. We also demonstrated the applicability of **1** in solution phase peptide synthesis. In the course of the work, a practical asymmetric synthesis of the bridged bicyclic chiral building block **11** was elaborated. Further studies are now required to explore whether ProM-19-based ligands offer advantages over those derived from previously developed PPII helix-inducing diproline equivalents,[9,10,14,24,25] due to the higher degree of structural preorganization within the tetracyclic core structure of ProM-19.

Experimental

(*R*,*E*)-Ethyl 2-((1-phenylethyl)imino)acetate (6)

To a stirred solution of (*R*)-1-phenylethylamine (7.4 g, 61.1 mmol, 1.0 eq.) in 100 mL of dry toluene were added 31.2 g of anhydrous Na_2SO_4. Then, a solution of 12.5 g (61.2 mmol, 1.95 eq.) of ethyl glyoxylate in 15 mL of toluene was added dropwise and the mixture was stirred for 1.5 h at room temp. The solid was filtered off and the residue was washed with toluene. The organic phase was concentrated under reduced pressure and the residue dried in vacuum (oil pump). The product **6** (12.54 g, 61.1 mmol, 99%) was obtained as a slightly yellowish oil, which was used directly in the subsequent aza-Diels–Alder reaction. **^{1}H NMR** (300 MHz, $CDCl_3$): δ (ppm) = 7.73 (d, J = 1.0 Hz, 1H, H-2), 7.39–7.08 (m, 5H, ar-H), 4.61 (q, J = 6.7 Hz, 1H, Ph-CH), 4.33 (q, J = 7.1 Hz, 2H, OCH_2), 1.62 (d, J = 6.7 Hz, 3H, CH_3), 1.34 (t, J = 7.1 Hz, 3H, CH_3).

(1*S*,3*S*,4*R*)-Ethyl 2-((*R*)-1-phenylethyl)-2-azabicyclo[2.2.1]-hept-5-ene-3-carboxylate (7a) and its C-3-epimer (7b)

A solution of 12.54 g of the imine **6** (61.10 mmol, 1.00 eq.) in 100 mL of DMF was cooled to 0 °C before 5.0 mL of TFA (64.9 mmol, 1.05 eq.), 13.0 mL of freshly distilled cyclopentadiene (157 mmol, 2.60 eq.) and 33 μL of water (1.83 mmol, 0.03 eq.) were added. The mixture was then stirred under light exclusion for 24 h at room temperature. Then, 50 mL of a sat. aqueous $NaHCO_3$ solution were added and the pH adjusted to 8 by addition of solid Na_2CO_3 before the mixture was extracted with 4 × 100 mL of MTBE. The combined organic phases were dried with $MgSO_4$ and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography on silica (EtOAc/CyHex = 1 : 8) to afford 10.68 g (39.35 mmol, 64%) of **7a** (39.35 mmol, 64%) and 1.22 g of **7b** (4.48 mmol, 7%), both as yellowish oils. **Data for 7a: TLC**: R_f = 0.21 (CyHex/EtOAc = 10 : 1). **^{1}H NMR** (300 MHz, $CDCl_3$): δ (ppm) = 7.36–7.09 (m, 5H, H-Ar), 6.46–6.34 (m, 1H, H-5), 6.26 (dd, J = 5.6, 1.6 Hz, 1H, H-6), 4.29 (s, 1H, H-1), 3.80 (m, 2H, OCH_2), 3.03 (q, J = 6.5 Hz, 1H, H-8), 2.89 (s, 1H, H-4), 2.20 (s, 1H, H-3), 2.13 (d, J = 8.3 Hz, 1H, H-7), 1.40 (d, J = 6.6 Hz, 4H, H-7, CH_3), 0.94 (t, J = 7.1 Hz, 3H, CH_3). **^{13}C NMR** (75 MHz, $CDCl_3$): δ (ppm) = 174.3 (C═O), 144.9 (ar-C), 136.4 (C-5), 132.9 (C-6), 128.0 (ar-C), 127.9 (ar-C), 127.0 (ar-C), 65.0 (C-3), 63.9 (benz-C), 62.6 (C-1), 60.2 (OCH_2), 49.1 (C-4), 45.3 (C-7), 22.5 (CH_3), 14.3; 14.0 (CH_3). **IR (FT-ATR):** $\tilde{\nu}$ (cm^{-1}): 2976 (m), 1743 (s), 1723 (m), 1454 (m), 1376 (m), 1193 (m), 1163 (s), 1108 (m), 1058 (m), 1034 (m), 701 (s). **GC-MS:** m/z = 271 $[M]^+$ (1), 204 (9), 176 (21), 160 (16), 131 (15), 105 (100), 91 (11), 77 (16), 51 (5). $[\alpha]^{20}$ (c = 0.63, $CHCl_3$): −328.8° (365 nm), −178.7° (436 nm), −94.7° (546 nm), −81.8° (579 nm), −78.3° (589 nm). **Data for 7b: TLC:** R_f = 0.39 (CyHex/EtOAc = 10 : 1). **^{1}H NMR** (300 MHz, $CDCl_3$): δ (ppm) = 7.49–7.08 (m, 5H, ar-H), 6.40 (ddd, J = 5.6, 3.1, 1.2 Hz, 1H, H-5), 6.03 (dd, J = 5.6, 2.0 Hz, 1H, H-6), 4.24 (q, J = 7.1 Hz, 2H, OCH_2), 3.53 (td, J = 3.1, 1.4 Hz, 1H, H-1), 3.13–3.07 (m, 1H, H-4), 3.03 (q, J = 6.5 Hz, 1H, benz-H), 2.46 (s, 1H, H-3), 1.92 (dt, J = 8.2, 1.7 Hz, 1H, H-7), 1.31 (t, J = 7.1 Hz, 3H, CH_3), 1.23 (ψd, J = 6.4 Hz, 4H, CH_3 + H-7). **^{13}C NMR** (75 MHz, $CDCl_3$): δ (ppm) = 174.6 (C═O), 144.9 (ar-C), 135.8 (d, C-5), 133.6 (d, C-6), 128.2 (ar-H); 127.4 (ar-H), 126.9 (ar-H), 64.2 (C-3), 63.4 (d, benz-C), 63.3 (C-1), 60.5 (OCH_2), 49.5 (C-4), 45.6 (C-7), 23.7 (CH_3), 14.2 (CH_3). **IR (FT-ATR):** $\tilde{\nu}$ (cm^{-1}): 2977 (m), 1779 (w), 1747 (s), 1723 (m), 1454 (m), 1191 (m), 1172 (s),

1164 (s), 1109 (m), 1053 (m), 1028 (m), 702 (s). **GC-MS:** m/z = 271 $[M]^+$ (1), 204 (10), 176 (20), 160 (16), 131 (14), 105 (100), 91 (11), 77 (15), 51 (5). $[\alpha]^{20}$ (c = 0.29, $CHCl_3$) = 406.3° (365 nm), 230.8° (436 nm), 126.0° (546 nm), 109.0° (579 nm), 124.3° (589 nm).

(1*S*,3*S*,4*S*,5*S*,6*R*) Ethyl 5,6-dihydroxy-2-((*R*)-1-phenylethyl)-2-aza-bicyclo[2.2.1]heptane-3-carboxylate (8)

To a solution of 5.5 g of **7a** (20.3 mmol, 1.0 eq.) in 120 mL of *t*BuOH/H_2O (1 : 1) were added 8.4 g of K_2CO_3 (60.8 mmol, 3.0 eq.), 20.0 g of $K_3Fe(CN)_6$ (60.8 mmol, 3.0 eq.), and 37 mg of $K_2OsO_2 \times 2H_2O$ (0.005 mmol). The mixture was stirred at room temp. until TLC control indicated full conversion. After addition of water (150 mL) the mixture was extracted with MTBE (3 × 150 mL) and the combined organic layers were washed with sat. aqueous NaCl solution. After removing the solvent under reduced pressure, the residue (yellowish oil) was purified by flash column chromatography on silica gel (EtOAc/CyHex = 1.5 : 1) and dried in an oil pump vacuum to give 5.88 g of diol **8** (19.3 mmol, 95%) as a colorless oil. **TLC:** R_f = 0.23 (CyHex/EtOAc = 3 : 2). **^{1}H NMR** (300 MHz, $CDCl_3$): δ (ppm) = 7.35–7.12 (m, 5H, ar-H), 4.31 (d, J = 5.7 Hz, 1H, H-6), 3.85 (d, J = 5.7 Hz, 1H, H-5), 3.76–3.62 (m, 2H, OCH_2), 3.60–3.54 (m, 2H, H-1, H-8), 2.50 (s, 1H, H-3), 2.25 (s, 1H, H-1), 1.96 (d, J = 10.7 Hz, 1H, H-7), 1.80 (d, J = 10.6 Hz, 1H, H-7), 1.45 (d, J = 6.5 Hz, 3H, benz-CH_3), 0.93 (t, J = 7.1 Hz, 3H, CH_3). **^{13}C NMR** (75 MHz, $CDCl_3$): δ (ppm) = 173.5 (C═O), 143.9 (ar-C), 128.1 (ar-C); 127.9 (ar-C); 127.4 (ar-C), 73.3 (C-5), 67.3 (C-6), 65.6 (C-3), 61.7 (C-1), 60.4 (OCH_2), 60.2 (benz-C), 48.9 (C-4), 29.6 (C-7), 22.3 (benz-CH_3), 13.9 (CH_3). **IR (FT-ATR):** $\tilde{\nu}$ (cm^{-1}): 3410 (br), 1739 (s), 1199 (s), 1178 (s), 1144 (m), 1082 (s), 1058 (m), 1031 (s). **GC-MS:** m/z = 375 $[M]^+$ (17), 290 (13), 244 (74), 232 (54), 214 (4), 184 (22), 172 (37), 140 (36), 105 (100), 79 (15), 68 (34). $[\alpha]^{20}$ (c = 0.63, $CHCl_3$) = 16.2° (365 nm), 11.8° (436 nm), 7.8° (546 nm), 7.4° (579 nm), 7.3° (589 nm).

2-(*tert*-Butyl) 3-ethyl (1*S*,3*S*,4*S*,5*S*,6*R*)-5,6-dihydroxy-2-aza-bicyclo[2.2.1]heptane-2,3-dicarboxylate (9)

To a solution of diol **8** (7.5 g, 24.56 mmol, 1.0 eq.) in 75 mL of dry methanol was added 750 mg of Pd/C (10% Pd) under an atmosphere of argon. Then, the flask was flushed with hydrogen and a slow stream of hydrogen was constantly passed through the stirred suspension using a steel cannula and a balloon. After TLC control indicated complete conversion (1–5 d) the mixture was filtered through a pad of Celite and the solvent was removed under reduced pressure. The crude product was dissolved in 75 mL of CH_2Cl_2 and 8.1 g of Boc_2O (36.8 mmol, 1.5 eq.) were added. The mixture was stirred for 24 h at room temp. Then, all volatiles were removed under reduced pressure and the residue was purified by flash column chromatography on silica (CH_2Cl_2/MeOH = 30 : 1) to give 6.4 g of **9** (21.2 mmol, 87%) as a colorless crystalline solid. **TLC:** R_f = 0.30 (CH_2Cl_2/MeOH = 20 : 1). **^{1}H NMR** (600 MHz, $CDCl_3$, mixture of rotamers): δ (ppm) = 4.34 (d, J = 4.7 Hz, 0.7H, OH_{rot1}), 4.23–4.15 (m, 2H, OCH_2), 4.13 (s, 0.7H, H-1_{rot1}), 4.05 (s, 0.3H, H-1_{rot2}), 4.00–3.98 (m, 0.3H, H-5_{rot2}), 3.95 (t, J = 5.1 Hz, 0.7H, H-6_{rot1}), 3.92 (t, J = 4.9 Hz, 0.7H, H-5_{rot1}), 3.87–3.86 (m, 0.3H, H-6_{rot2}), 3.70 (s, 0.3H, H-3_{rot2}), 3.64 (d, J = 4.7 Hz, 0.7H, -OH_{rot1}), 3.60 (s, 0.7H, H-3_{rot1}), 3.27 (d, J = 4.7 Hz, 0.3H, OH_{rot2}), 3.21 (d, J = 5.1 Hz, 0.3H, OH_{rot2}), 2.57–2.55 (m, 1H, H-4), 1.86–1.79 (m, 1H, H-7), 1.82 (br, 1H, H-7), 1.46 (s, 2.70H, *tert*-Bu_{rot2}), 1.38 (s, 6.3H, *tert*-Bu_{rot1}), 1.28 and 1.26 (2 × t, J = 7.1, 3H, Me). **^{13}C NMR** (151 MHz, $CDCl_3$): δ (ppm) = 170.4/170.3 (C═O_{ester}), 153.8/153.5 (C═O_{Boc}), 80.9/80.6 (O$\underline{C}$Me$_3$), 73.0/72.6 (C-5), 72.2/71.0 (C-6), 61.3/61.2 (OCH_2), 60.2/59.7 (C-3), 60.2/59.2 (C-1), 48.1/47.6 (C-4), 28.7/28.4 (C-7), 28.2/28.0 (C($\underline{C}$H$_3$)$_3$), 14.3/14.1 (CH_3). **IR (FT-ATR):** $\tilde{\nu}$ (cm^{-1}): 3397 (br), 1749 (m), 1701 (s), 1677 (s), 1404 (s), 1368 (m), 1160 (s). **GC-MS:** m/z = 301 $[M]^+$ (2), 245 (3), 228 (7), 200 (16), 184 (10), 172 (14), 165 (4), 154 (4), 140 (100), 128 (20), 110 (18), 96 (6), 80 (10), 68 (67), 57 (70), 41 (36). $[\alpha]^{20}$ (c = 0.57, $CHCl_3$) = −165.5° (365 nm), −102.5° (436 nm), −59.1° (546 nm), −51.5° (579 nm), −55.5° (589 nm). **M.p.:** 147.5–148.5 °C (from CH_2Cl_2/MeOH).

2-(*tert*-Butyl) 3-ethyl (1*S*,3*S*,4*S*,5*S*,6*R*)-5,6-thiooxodioxolo-2-aza-bicyclo[2.2.1]heptane-2,3-dicarboxylate (10)

A solution of diol **9** (6.40 g, 21.2 mmol, 1.0 eq.) and 6.23 g of DMAP (50.98 mmol, 2.4 eq.) in 106 mL of dry CH_2Cl_2 was cooled to 0 °C before 1.95 mL of thiophosgene (25.5 mmol, 1.2 eq.) were added dropwise and the mixture was stirred for 18 h at room temp. For workup, the mixture was diluted with 50 mL of CH_2Cl_2 and washed with 1 N HCl aqueous solution (2 × 50 mL). The aqueous layer was back-extracted with 50 mL CH_2Cl_2. The combined organic solutions were dried with $MgSO_4$ and the solvent was removed under reduced pressure. The crude product obtained was purified by flash column chromatography on silica (CH_2Cl_2/MeOH = 100 : 1) to afford 5.75 g of thiocarbonate **10** (16.8 mmol, 79%) as a yellowish solid. **TLC:** R_f = 0.29 (CyHex/EtOAc = 3 : 1). **^{1}H NMR** (600 MHz, $CDCl_3$, mixture of rotamers): δ (ppm) = 5.02–4.99 (m, 1H, H-5), 4.94 (m, H-6_{rot1}), 4.84 (m, 0.5H, H-6_{rot2}), 4.62 (s, 0.5H, H-1_{rot1}), 4.5 (s, 0.48H, H-1_{rot2}), 4.28–4.15 (m, 2H, OCH_2), 3.75 (s, 0.5H, H-3_{rot2}), 3.66 (s, 0.5H, H-3_{rot1}), 3.06–3.02 (m, 1H, H-4), 2.19 (m, 1H, H-7), 1.72 (dd, J = 12.0, 5.7 Hz, 1H, H-7), 1.47 (s, 4.5H, *tert*-Bu), 1.40 (s, 4.5H, *tert*-Bu), 1.33–1.25 (m, 3H, CH_3). **^{13}C NMR** (151 MHz, $CDCl_3$): δ (ppm) = 191.0/190.8 (C═S), 169.0/168.7 (C═O_{ester}), 152.9/152.3 (C═O_{Boc}), 85.2/85.1 (C-6), 83.4/83.3 (C-5), 81.9/81.7 ($\underline{C}$Me$_3$), 62.0/61.9 (OCH_2), 58.1/57.7 (d, C-3), 57.9/56.7 (C-1), 46.2/45.4 (C-4), 29.7/27.4 (C-7), 28.2/28.1 (C($\underline{C}$H$_3$)$_3$), 14.2/14.1 (CH_3). **IR (FT-ATR):** $\tilde{\nu}$ (cm^{-1}): 1748 (m), 1703 (s), 1395 (s), 1367 (m), 1346 (m), 1296 (s), 1161 (s), 1119 (m); **GC-MS**: same as for compound **11** due to rapid thermal fragmentation. $[\alpha]^{20}$ (c = 0.505, $CHCl_3$) = −192.9° (365 nm), −105.1° (436 nm), −59.4° (546 nm), −51.7° (579 nm), −55.4° (589 nm). **M.p.:** 71.0–72.0 °C (from CH_2Cl_2/MeOH).

2-(*tert*-Butyl) 3-ethyl (1*S*,3*S*,4*R*)-2-azabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate (11)

A solution of 5.70 g (16.6 mmol, 1.0 eq.) of thiocarbonate **10** in 30 mL of $P(OMe)_3$ was refluxed under argon (at 125 °C oil bath temp.) for 2 d. After cooling to room temp. the flask was connected to a cold trap (cooled with liquid N_2) and an oil pump

vacuum was applied until all excess $P(OMe)_3$ had condensed into the external cold trap (to allow proper disposal). The remaining crude product was purified by flash column chromatography on silica gel (CyHex : EtOAc, 7 : 1) to give 4.04 g of olefin **11** (15.11 mmol, 91%) as a clear oil. **TLC**: R_f = 0.53 (CyHex/EtOAc, 3 : 1). **^{1}H NMR** (500 MHz, $CDCl_3$; mixture of rotamers): δ (ppm) = 6.47 (br, 0.6H, H-5_{rot1}), 6.37–6.35 (m, 1.5H, H-5_{rot2}/H-6), 4.77 (s, 0.6H, H-1_{rot1}), 4.64 (s, 0.5H, H-1_{rot2}), 4.19 (m, 2H, H-12), 3.46 (s, 0.5H, H-3_{rot2}), 3.37 (s, 0.6H, H-3_{rot1}), 3.25 (s, 1H, H-4), 1.96 (dt, J = 8.6, 1.8 Hz, 1H, H-7), 1.45–1.43 (m, 1H, H-7), 1.41–1.36 (m, 9H, H-10), 1.26 (t, J = 7.2 Hz, 3H, H-13). **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers): δ (ppm) = 171.6 (s, C=O_{ester}), 155.7/154.6 (q, C=O_{Boc}), 137.1 (d, C-5); 136.8/136.2 (d, C-6), 79.8 (s, $\underline{C}Me_3$), 61.8/60.3 (d, C-1), 61.1/61.0 (t, O-CH_2), 59.0 (d, C-3), 48.9/48.2 (d, C-4), 45.4/45.1 (t, C-7), 28.3 (q, C($\underline{C}H_3)_3$), 14.2/14.1 (q, CH_3). **IR (FT-ATR):** $\tilde{\nu}$ (cm^{-1}): 1748 (m), 1697 (s), 1388 (s), 1365 (s), 1159 (s), 1122 (s). **GC-MS**: m/z = 267 $[M]^+$ (7), 211 (6), 194 (4), 167 (10), 151 (21), 138 (82), 102 (18), 94 (100), 67 (15), 57 (59), 41 (16). $[\alpha]^{20}$ (c = 0.96, $CHCl_3$) = −781.0° (365 nm), −461.7° (436 nm), −257.3° (546 nm), −223.6° (579 nm), −217.2° (589 nm).

1-(*tert*-Butyl) 2-ethyl (2*S*,3*R*,5*S*)-3,5-divinyl-pyrrolidin-1,2-dicarboxylate (12)

A solution of 800 mg of olefin **11** (2.99 mmol, 1.00 eq.) in 250 mL of dry CH_2Cl_2 was prepared under argon and ethene was passed through the solution (from a balloon as reservoir). After 5 min, 127 mg of Grubbs II catalyst (0.15 mmol, 0.05 eq.) were added and a constant small flow of ethene was passed through the solution for 4 h. The solution was then concentrated under reduced pressure and the residue was purified by flash column chromatography on silica gel (CyHex : EtOAc, 5 : 1) to give **12** (613 mg, 2.08 mmol, 70%) as a clear oil. **TLC**: R_f = 0.34 (CyHex/EtOAc, 5 : 1). **^{1}H NMR** (500 MHz, $CDCl_3$; mixture of rotamers): δ (ppm) = 5.85–5.76 (m, 1.6H, H-12, H-14_{rot1}), 5.70 (ddd, J = 17.4, 10.2, 7.4 Hz, 0.4H, H-14_{rot2}), 5.16–4.96 (m, 4H, H-13, H-15), 4.41 (q, J = 7.0 Hz, 0.6H, H-5_{rot1}), 4.31 (q, J = 7.1 Hz, 0.4H, H-5_{rot2}), 4.23–4.07 (m, 2.4H, H-2_{rot1}, H-10), 4.04 (d, J = 5.2 Hz, 0.6H, H-2_{rot2}), 2.78 (p, J = 6.8 Hz, 1H, H-3), 2.36–2.26 (m, 1H, H-4), 1.66–1.59 (m, 1H, H-4), 1.36 (m, 9H, H-8), 1.22 (dt, J = 11.1, 7.1 Hz, 3H, H-11). **^{13}C NMR** (125 MHz, $CDCl_3$): δ (ppm) = 172.3; 171.9 (s, C=O_{ester}), 154.3; 153.4 (s, C=O_{Boc}), 140.17 139.4 (d, –CH= at C-5), 138.1 (d, –CH= at C-3), 116.0/115.9 (t, =CH_2), 114.5/114.2 (t, =CH_2), 80.1; 80.0 (s, $\underline{C}Me_3$), 65.5/65.1 (d, C-2), 60.8 (t, O-CH_2), 60.7/60.6 (d, C-5), 46.7/45.7 (d, C-3), 38.2/37.6 (t, C-4), 28.1/28.1 (q, C($\underline{C}H_3)_3$), 14.2/14.1 (q, CH_3). **IR (FT-ATR:** $\tilde{\nu}$ (cm^{-1}): 1744 (s), 1694 (s), 1365 (s), 1254 (m), 1184 (s). **GC-MS**: m/z = 295 $[M]^+$ (1), 239 (3), 222 (17), 194 (58), 166 (98), 148 (2), 122 (100), 105 (8), 94 (8), 77 (8), 67 (16), 57 (74), 41 (39). $[\alpha]^{20}$ (c = 0.39, $CHCl_3$) = 48.9° (365 nm), 21.0° (436 nm), 8.1° (546 nm), 6.1° (579 nm), 4.6° (589 nm).

(2*S*,3*R*,5*S*)-1-(*tert*-Butoxycarbonyl)-3,5-divinyl-pyrrolidin-2-carboxylic acid (13)

To a solution of 613 mg of ethyl ester **12** (2.08 mmol, 1.0 eq.) in 20 mL of THF/MeOH (3 : 1) were added 4 mL of a 2.5 N LiOH solution (10 mmol, 10.0 eq.) and the mixture was stirred for 36 h at room temperature. Then, 10 mL of CH_2Cl_2 was added and the mixture was concentrated under reduced pressure. The aqueous phase was brought to pH = 1 with aqueous 1 N HCl solution and extracted with CH_2Cl_2 (4 × 25 mL). The combined organic layers were dried over $MgSO_4$ and all volatiles were removed under reduced pressure to yield 556 mg of carboxylic acid **13** (2.08 mmol, 100%) as a colorless highly viscous oil. **TLC**: R_f = 0.51 (CyHex/EtOAc/HOAc, 100 : 100 : 5). **^{1}H NMR** (600 MHz, $CDCl_3$; mixture of rotamers): δ (ppm) = 9.96 (br, 1H, COOH), 5.86 (ddd, J = 17.4, 10.3, 7.6 Hz, 1H, H-10), 5.86–5.80 (m, 0.6 H, H-10_{rot2}), 5.75 (ddd, J = 17.6, 10.2, 7.4 Hz, 0.4H, H-10_{rot1}), 5.22–4.99 (m, 4H, H-11, H-13), 4.45 (q, J = 7.1 Hz, 0.6H, H-5rot2), 4.34 (q, J = 7.2 Hz, 0.4H, H-5_{rot1}), 4.23 (d, J = 4.7 Hz, 0.4H, H-2_{rot1}), 4.11 (d, J = 5.3 Hz, 0.6H, H-2_{rot2}), 2.99–2.88 (m, 1H, H-3), 2.41 (dt, J = 14.1, 7.7 Hz, 0.4H, H-4_{rot1}), 2.35 (dt, J = 13.8, 7.4 Hz, 0.6H, H-4_{rot2}), 1.79–1.64 (m, 1H, H-4), 1.41; 1.40 (2 × s, 9H, *t*Bu). **^{13}C NMR** (151 MHz, $CDCl_3$): δ (ppm) = 178.6; 177.0 (s, C=O_{acid}), 155.0; 153.4 (s, C=O_{Boc}), 139.9; 139.1 (d, =CH), 138.0; 137.8 (d, =CH), 116.5; 116.3 (t, =CH_2), 114.9; 114.6 (t, =CH_2), 80.8 (s, $\underline{C}Me_3$), 65.5; 65.0 (d, C-2), 60.9 (d, C-5), 46.7; 45.4 (d, C-3), 38.3; 37.8 (t, C-4), 28.2 (q, C($\underline{C}H_3)_3$). **IR (FT-ATR):** $\tilde{\nu}$ (cm^{-1}): 3054 (br), 2979 (m), 1745 (m), 1712 (s), 1693 (s), 1645 (m), 1392 (s), 1367 (s), 1307 (m), 1254 (m), 1164 (s). **HRMS (ESI)**: calcd for [M + Na]$^+$ 290.1363; found 290.1363. $[\alpha]^{20}$ (c = 0.895, $CHCl_3$) = +33.9° (365 nm), +14.8° (436 nm), +5.7° (546 nm), +4.5° (579 nm), +3.9° (589 nm).

tert-Butyl (2*S*,3*R*,5*S*)-2-((2*S*,5*R*)-2-(methoxycarbonyl)-5-vinyl-pyrrolidin-1-carbonyl)-3,5-divinylpyrrolidin-1-carboxylate (14)

In an inert Schlenk flask, 1.85 g of acid **13** (6.92 mmol, 1.0 eq.) was dissolved in 25 mL of dry acetonitrile and 1.07 g of amine **5** (6.92 mmol, 1.0 eq.) was added. Subsequently, 4.68 g of PyBOP (9.00 mmol, 1.3 eq.) and 3.54 mL of DIPEA (20.8 mmol, 3.0 eq.) were added successively. The reaction mixture was stirred for 18 h at room temp. before dilution with 50 mL of water and extraction three times with 80 mL of MTBE each. The combined organic phases were dried over $MgSO_4$ and freed from solvent under reduced pressure. The crude product obtained was subsequently purified by flash column chromatography on silica gel (CyHex : EtOAc, 2 : 3). 1.68 g of dipeptide **14** (4.16 mmol, 95%) was obtained in the form of a yellowish oil. **TLC**: R_f = 0.36 (CyHex/EtOAc, 2 : 3). **^{1}H NMR** (600 MHz, $CDCl_3$; mixture of rotamers): δ (ppm) = 5.95–5.88 (m, 1H), 5.87–5.75 (m, 1H,), 5.82–5.74 (m, 1H), 5.45 (m, 1H), 5.13 (m, 1H), 5.02 (m, 1H), 5.02 (m, 1H), 4.97 (m, 2H), 4.90–4.84 (t, 0.65H), 4.57–4.55 (m, 0.7H), 4.52–4.47 (m, 1H), 4.41–4.38 (m, 0.65H), 4.34 (m, 0.65H), 4.30 (m), 3.74; 3.72 (2 × s, 3H), 2.85–2.79 (m, 1H), 2.73–2.64 (m, 1H), 2.27–2.11 (m, 2H), 2.01–1.80 (m, 2H), 1.64–1.60 (m, 1H), 1.39; 1.37 (2 × s, 9H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ (ppm) = 172.8/172.6; 172.5/172.2, 154.9/153.9, 141.0/140.5, 139.9/139.7, 138.6/138.2, 117.2/116.7, 115.1, 114.1/113.8, 79.8, 63.5/63.2, 61.2/61.0, 60.8, 60.7, 60.0/59.8, 52.2/52.0 46.7/45.7, 37.5/36.2, 32.9/32.6, 28.5/28.3/28.2, 26.9/26.9. For the assignment of NMR signals, see the ESI.† **IR**

(FT-ATR): $\tilde{\nu}$ (cm^{-1}): 2978 (m), 1750 (s), 1709 (s), 1686 (s), 1657 (s), 1422 (s), 1389 (s), 1366 (s), 1201 (s), 1172 (s). **GC-MS:** *m*/*z* = 404 $[M]^+$ (1), 331 (5), 303 (85), 277 (1), 236 (7), 222 (7), 194 (4), 166 (87), 156 (12), 154 (22), 122 (100), 96 (23), 79 (18), 67 (18), 57 (56), 41 (43). $[\alpha]^{20}$ (*c* = 0.51, $CHCl_3$) = 42.2° (365 nm), 21.6° (436 nm), 10.0° (546 nm), 8.3° (579 nm), 6.8° (589 nm). For the assignments of NMR signals, see the ESI.†

1-(*tert*-Butyl) 8-methyl (2*S*,3a*R*,5a*R*,8*S*,10a*S*)-10-oxo-2-vinyl-3,3a,5a,6,7,8,10,10a-octahydrodipyrrolo[1,2-*a*:3′,2′-*e*]azepin-1,8(2*H*)-dicarboxylate (15)

Under an atmosphere of argon, 532 mg of dipeptide **14** (1.32 mmol, 1.0 eq.) were dissolved in 250 mL of absolute CH_2Cl_2 (distilled from P_4O_{10} and filtered through a pad of basic alumina (activity level 1). Then 56 mg (5 mol%) of Grubbs II catalyst was added and the mixture was heated to 40 °C for 48 h. During this time, additional batches of Grubbs II catalyst (typically 22 mg, 2 mol%) were added every 2 h until a total amount of 20 mol% was reached. For work-up, the reaction solution was concentrated under reduced pressure and the crude product purified by flash column chromatography on silica gel (CyHex : EtOAc, 1 : 3) to yield 288 mg of **15** (0.77 mmol, 58%) as a grayish solid. **TLC:** R_f = 0.22 (CyHex/EtOAc, 1 : 1). **^{1}H NMR** (500 MHz, $CDCl_3$; mixture of rotamers): δ (ppm) 5.85–5.74 (m, 1.25H), 5.66 (ddd, *J* = 17.0, 10.1, 8.3 Hz, 0.75H), 5.55 (ddd, *J* = 11.2, 2.9, 1.6 Hz, 1H), 5.26–4.97 (m, 2H), 4.80 (dd, *J* = 7.8, 2.8 Hz, 0.75H), 4.73–4.62 (m, 1.25H), 4.50 (dd, *J* = 25.5, 10.6 Hz, 1H), 4.32 (ddd, *J* = 10.5, 8.1, 5.6 Hz, 0.25H), 4.25 (ddd, *J* = 10.3, 8.3, 5.7 Hz, 0.75H), 3.69 (s, 0.75H), 3.68 (s, 2.25H), 2.99–2.85 (m, 1H), 2.39–2.25 (m, 1H), 2.28–2.17 (m, 1H), 2.11–1.97 (m, 2H), 1.96–1.80 (m, 1H), 1.59–1.46 (m, 1H), 1.41 (s, 6H), 1.38 (s, 2H). **^{13}C NMR** (100 MHz, $CDCl_3$; mixture of rotamers): δ (ppm) = 172.5; 172.3, 169.7/169.1, 154.2/153.0, 140.0/138.8, 129.6/129.3, 128.9/128.6, 115.1/114.7, 80.0/79.6, 63.2/62.8, 61.8/61.5, 59.5/59.4, 57.1, 52.3/52.1, 39.7/39.5, 39.2/39.1, 33.1/33.0, 28.3/28.1, 27.2/27.0. **IR (FT-ATR):** $\tilde{\nu}$ (cm^{-1}): 1701 (s), 1658 (s), 1427 (m), 1401 (m), 1384 (m), 1364 (m), 1319 (m), (s), 1167 (s). **GC-MS:** *m*/*z* = 376 $[M]^+$ (1), 320 (38), 303 (34), 276 (100), 261 (24), 247 (34), 207 (20), 189 (27), 175 (12), 134 (20), 120 (27), 108 (19), 94 (22), 80 (19), 57 (42), 41 (73). **HRMS (ESI):** calcd for $[M + H]^+$ 377.2071; found 377.2077, calcd for $[M + Na]^+$ 399.1890; found 399.1890. $[\alpha]^{20}$ (*c* = 0.65, MeOH) = −448.3° (436 nm), −266.6° (546 nm), −234.0° (579 nm), −224.8° (589 nm). **M.p.:** 166–167 °C (CH_2Cl_2). For the assignments of NMR signals, see the ESI.†

1-(*tert*-Butyl)-8-methyl-(2S,3a*R*,5a*R*,8*S*,10a*S*)-10-oxo-2-vinyl-3,3a,5a,6,7,8,10,10a-octahydrodipyrrolo[1,2-*a*:3′,2′-*e*]azepin-1,8(2*H*)-dicarboxylate (16)

A solution of 390 mg of tricycle **15** (1.04 mmol, 1.0 eq.) in 7 mL of dry CH_2Cl_2 was cooled to 0 °C before 1.6 mL of TFA (20.7 mmol, 20 eq.) were added dropwise. The mixture was stirred at room temp. for 1.5 h before all volatiles were removed under oil pump vacuum. The residue obtained was taken up twice in 3 mL of CH_2Cl_2 followed by solvent removal in vacuum. Finally, the residue was dissolved in 4 mL of CH_2Cl_2 and little solid Na_2CO_3 was added to neutralize any remaining acid. In a second flask, 336 mg of Boc-L-allylglycine-OH (**3**) (1.56 mmol, 1.5 eq.) were dissolved in 5 mL of dry acetonitrile before HATU (692 mg, 1.82 mmol, 1.75 eq.) and DIPEA (1.55 mmol, 1.5 eq.) were added. To this mixture was then transferred at room temp. by means of a syringe needle the above-prepared CH_2Cl_2 solution of the deprotected amine (rinsing the Na_2CO_3 residue with 2 mL of CH_2Cl_2). After addition of 265 μL of DIPEA (1.55 mmol, 1.5 eq.) and 10 mg of solid Na_2CO_3 the mixture was stirred for 18 h at room temp. before it was filtered through a short pad of Celite and rinsed with 50 mL of CH_2Cl_2/MeOH (30 : 1). Then, the solvents were removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 30 : 1) to yield 338 mg of **16** (0.71 mmol, 69%) as a yellow foam. **TLC:** R_f = 0.53 (EtOAc). **^{1}H NMR** (500 MHz, $CDCl_3$, data for main rotamer): δ (ppm) = 5.97–5.84 (m, 1.4H), 5.79 (dt, *J* = 11.2, 2.2 Hz, 1H), 5.75–5.64 (m, 0.6H), 5.63–5.54 (m, 1H), 5.43–5.22 (m, 2H), 5.18–5.04 (m, 2H), 4.93–4.89 (m, 1H), 4.78–4.76 (m, 1H), 4.74–4.70 (m, 2H), 4.42–4.37 (m, 1H), 3.74–3.67 (m, 3H), 2.99–2.91 (m, 1H), 2.55–2.50 (m, 1H), 2.41–2.31 (m, 3H), 2.11–2.00 (m, 2H), 1.91–1.80 (m, 1H), 1.70–1.65 (m, 1H). **^{13}C NMR** (125 MHz, $CDCl_3$, data for main rotamer): δ (ppm) = 172.3, 172.1, 168.4, 155.5, 137.9, 133.5, 130.0, 128.1, 118.2, 79.0, 63.9, 62.9, 59.3, 57.2, 52.3, 51.7, 40.9, 38.1, 37.1, 32.8, 28.4, 27.2. **IR (FT-ATR):** $\tilde{\nu}$ (cm^{-1}): 3335 (br), 3977 (w), 1705 (m), 1673 (m), 1645 (m), 1501 (w), 1434 (m), 1164 (s). **HRMS(ESI):** calcd for $[M + H]^+$ 474.2598; found 474.2601, calcd for $[M + Na]^+$ 496.2418; found 496.2418. $[\alpha]^{20}$ (*c* = 0.68, $CHCl_3$) = −241.1° (436 nm), −138.0° (546 nm), −120.8° (579 nm), −115.8° (589 nm). For the assignments of NMR signals, see the ESI.†

Boc-[ProM-19]-OMe (1)

Under an atmosphere of argon, a solution of 338 mg of **16** (0.71 mmol, 1.0 eq.) in 50 mL of dry hexafluorobenzene was heated to 45 °C before 10 mol% of Grubbs II catalyst (dissolved in hexafluorobenzene) was slowly added over 2 h. After stirring for another 2 h the solvent was removed under reduced pressure and the residue purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 30 : 1). The product was purified once again by flash column chromatography (CH_2Cl_2/MeOH, 25 : 1). To remove traces of Ru, the obtained gray foam was dissolved in 5 mL of a mixture of CH_2Cl_2/MeOH, 20 : 1 and stirred with Quadrasil AP for 1 h. After filtration and rinsing with 20 mL of CH_2Cl_2/MeOH (20 : 1) the combined organic solutions were concentrated under reduced pressure and the product was dried in oil pump vacuum to give 277 mg of pure **1** (0.61 mmol, 88%) as a still slightly grayish foam. **TLC:** R_f = 0.21 (CH_2Cl_2/MeOH, 20 : 1). **^{1}H NMR** (500 MHz, $CDCl_3$): δ (ppm) = 5.86–5.79 (m, 2H), 5.70–5.65 (m, 1H), 5.61–5.56 (m, 1H), 4.78–4.67 (m, 3H), 4.64–4.59 (m, 1H), 4.34–4.29 (m, 1H), 2.99–2.90 (m, 1H), 2.73 (br, 1H), 2.61–2.54 (1H), 2.42 (dt, *J* = 11.6, 5.7 Hz, 1H), 2.36–2.29 (m, 1H), 2.17–2.00 (m, 2H), 1.95–1.80 (m, 1H), 1.72–1.63 (m, 1H), 1.45 (s, 10H). **^{13}C-NMR** (125 MHz, $CDCl_3$): δ

(ppm) = 172.5, 170.8, 168.7, 155.4, 130.0, 128.8, 128.0, 79.7, 64.6, 59.4, 57.5, 57.3, 52.3, 40.1, 37.5, 33.0, 29.2, 28.3, 27.0. **IR (FT-ATR):** $\tilde{\nu}$ (cm^{-1}): 3410 (br), 1651 (s), 1433 (s), 1168 (s). **HRMS (ESI):** calcd for $[M + Na]^+$ 446.2286; found 446.2289, calcd for $[M + Na]^+$ 468.2105; found 468.2104. $[\alpha]^{20}$ (c = 0.5, $CHCl_3$) = −217.1° (436 nm), −128.9° (546 nm), −113.6° (579 nm), −109.5° (589 nm). For the assignments of NMR signals, see the ESI.†

Peptide 17

Under an atmosphere of argon, a solution of 50 mg of **1** (112 µmol, 1.0 eq.) in 5.0 mL of dry CH_2Cl_2 was cooled to 0 °C before 20 µL of TMSOTf (112 µmol, 1.0 eq.) were added. Stirring was continued at 0 °C until TLC control indicated complete cleavage of the Boc protecting group. Then, 1 mL of sat. aqueous $NaHCO_3$ was added and the mixture was extracted four times with 5 mL of CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure to a volume of *ca.* 2 mL. In a separate flask, 36 mg of *N*-Boc-proline (168 µmol, 1.5 eq.) were dissolved under argon in 3 mL of CH_2Cl_2 before 64 mg of HATU (168 µmol, 1.5 eq.) and 48 µL of DIPEA (280 µmol, 2.5 eq.) were added and stirring was continued for 1 h at room temp. To the resulting solution of the active ester was then dropwise added the amine prepared above and the mixture was stirred for 18 h at room temp. Then, the solvent was removed under reduced pressure and the residue was purified by flash column chromatography (CH_2Cl_2/MeOH, 20 : 1) to give 51 mg of the peptide **17** (94 µmol, 84%) as a yellowish foam. (Note: According to ^{1}H NMR, the product was contaminated with some (≤5%) tetramethylurea which could not be completely separated off even after multiple chromatography.) **TLC**: R_f = 0.26 (CH_2Cl_2/MeOH, 20 : 1). **^{1}H NMR** (500 MHz, $CDCl_3$, mixture of rotamers): δ (ppm) = 7.67 (d, J = 7.6 Hz, 1H), 6.04–5.55 (m, 4H), 4.82–4.15 (m, 6H), 3.69 (s, 3H), 3.54–3.22 (m, 2H), 2.98–2.90 (m, 1H), 2.71–1.61 (m, 12H), 1.42 (s, 9H). **IR (FT-ATR):** $\tilde{\nu}$ (cm^{-1}): 3484 (br), 3413 (br), 3322 (br), 1678 (s), 1666 (s), 1645 (s), 1513 (s), 1433 (m), 1392 (s), 1365 (m), 1198 (m), 1163 (s). **HRMS(ESI):** calcd for $[M + H]^+$ 543.2813; found: 543.2816, calcd for $[M + Na]^+$ 565.2633; found 565.2626. $[\alpha]^{20}$ (c = 0.575, $CHCl_3$) = −278.5° (436 nm), −161.2° (546 nm), −141.2° (579 nm), −136.0° (589 nm). For the assignments of NMR signals, see the ESI.†

Peptide 19

Under an atmosphere of argon, 50 mg of peptide **17** (92 µmol, 1.0 eq.) were dissolved in 2.5 mL of dry CH_2Cl_2. After cooling the solution to 0 °C, 17 µL of TMSOTf (92 µmol, 1.0 eq.) were added and stirring was continued to 0 °C until complete conversion was detected by TLC. Then, 1 mL of sat. aqueous $NaHCO_3$ was added and the mixture was extracted four times with 5 mL of to CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. To the amine residue was then added a solution of 135 mg of Fmoc-L-2-Cl-Phe-OPfp (**18**) (230 µmol, 2.5 eq.) in 5 mL of dry CH_2Cl_2 dropwise and the mixture was stirred at room temp. for 18 h. Then, the solvent was removed under reduced pressure and the crude product purified by flash column chromatography on silica (CH_2Cl_2/MeOH, 20 : 1) to yield 47 mg of **19** (55 µmol, 60%) as a colorless foam. **TLC**: R_f = 0.26 (CH_2Cl_2/MeOH, 20 : 1). **^{1}H NMR** (500 MHz, $CDCl_3$, mixture of rotamers): δ (ppm) = 7.74–7.72 (m, 2H), 7.53–7.11 (m, 8.3H), 4.89 (td, J = 9.1, 5.3 Hz, 0.7H), 4.82–4.49 (m, 6.3H), 5.90–5.67 (m, 3.7H), 5.61–5.54 (m, 1H), 4.89 (td, J = 9.1, 5.3 Hz, 0.7H), 4.75–4.50 (m, 6.3H), 4.26 (dd, J = 10.5, 7.2 Hz, 1H), 4.15 (dd, J = 10.5, 7.4 Hz, 1H), 4.11–4.03 (m, 1H), 3.68 (s, 3H), 3.71–3.44 (m, 0.3H), 3.53–3.44 (m, 0.7H), 3.31 (dd, J = 13.3, 6.7 Hz, 0.3H), 3.20 (dd, J = 13.8, 5.3 Hz, 0.7H),3.08 (dd, J = 13.1, 7.7 Hz, 0.3H), 2.99 (dd, J = 13.8, 9.3 Hz, 0.7H), 2.96 (br, 1H), 2.67–2.55 (m, 2H), 2.47 (dt, J = 11.6, 5.7 Hz, 1H), 2.41–1.81 (m, 8H),1.69 (q, J = 12.2 Hz, 1H). **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers): δ (ppm) = 172.4, 171.2, 170.5, 170.1, 168.4, 155.6, 143.8/143.7, 141.1, 134.4/133.9, 131.9, 129.9/129.6/129.5/128.6/127.9, 127.6, 126.9, 126.8, 125.1, 125.0, 119.9, 66.9, 64.5, 60.0, 59.3, 57.3, 57.0, 53.6, 52.2, 51.9, 47.4, 47.0, 40.1, 37.4, 37.0, 32.9, 29.1, 27.5, 27.0, 25.0. **IR (FT-ATR):** $\tilde{\nu}$ (cm^{-1}): 3292 (br), 1643 (s), 1515 (m), 711 (s). **HRMS(ESI):** calcd for $[M + H]^+$ 846.3264; found: 846.3278, calcd for $[M + Na]^+$ 868.3083; found 868.3092. $[\alpha]^{20}$ (c = 0.51, $CHCl_3$) = −356.4° (365 nm), −213.3° (436 nm) −121.6° (546 nm), −106.2° (579 nm), −102.0 (589 nm). For the assignments of NMR signals, see the ESI.†

Ac-[2-Cl-Phe]-[Pro]-[ProM-19]-OMe (20)

Under an atmosphere of argon, 45 mg of peptide **19** (53.2 µmol, 1.0 eq.) were dissolved in 3.0 mL of dry acetonitrile before 0.21 mL of piperidine (2.12 mmol, 40 eq.) were added. After stirring the mixture for 1 h at room temperature the solvent was removed under reduced pressure. The residue was dissolved three times in 3.00 mL of dry CH_2Cl_2 and re-concentrated under reduced pressure. The resulting crude amine was taken up in 3 mL of dry CH_2Cl_2 and 0.20 mL of Ac_2O (2.12 mmol, 40 eq.) were added. The mixture was stirred for 4 h at room temp. before the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH, 20 : 1 → 10 : 1) to give 25 mg of the target ligand **20** (37.5 µmol, 71%) as a colorless foam. **TLC**: R_f = 0.70 (CH_2Cl_2/MeOH, 10 : 1). **^{1}H NMR** (500 MHz, $CDCl_3$, mixture of rotamers): δ (ppm) = 7.62 (d, J = 9.6 Hz, 0.3H), 7.43 (d, J = 7.2 Hz, 0.7 H), 7.37–7.32 (m, 0.7H), 7.23–7.15 (m, 2.6H), 7.15–7.08 (m, 0.7H), 6.98 (d, J = 8.5 Hz, 0.3H), 6.36 (d, J = 8.5 Hz, 0.7H), 5.90–5.79 (m, 2H), 5.78–5.68 (m, 1H), 5.65–5.55 (m, 1H), 5.09 (td, J = 8.7, 5.5 Hz, 0.7H), 5.01 (td, J = 9.7, 5.0 Hz, 0.3H), 4.80–4.67 (m, 4H), 4.61 (td, J = 7.2, 3.3 Hz, 0.7H), 4.56–4.48 (m, 1H), 4.28 (d, J = 8.1 Hz, 0.3H), 3.72 (q, J = 8.3 Hz, 0.7H), 3.67 (s, 2.1H), 3.59 (s, 0.9H), 3.56 (m, 0.6H), 3.50–3.40 (m, 1H), 3.15 (dd, J = 13.9, 5.5 Hz, 0.7H), 3.05–2.91 (m, 1.7H), 2.80 (dd, J = 13.6, 9.7 Hz, 0.3H), 2.70–2.52 (m, 2H), 2.48 (m, 1H), 2.41–2.27 (m, 2H), 2.25–1.80 (m, 8.1H), 1.77–1.63 (m, 1.9H). **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers): δ (ppm) = 172.4/171.8, 171.3/171.3, 170.5/170.4, 171.1/170.1, 170.1/169.5, 169.1/168.4, 135.4/134.0, 134.4/134.4, 132.0/131.7, 130.0/129.8, 129.5/129.5/129.3/128.6/128.1/127.9/127.8/127.5/

126.8/126.2, 64.7/64.5, 60.7/60.1, 59.6/59.3, 57.7/57.4/57.1, 53.7/52.7, 52.4/52.2, 51.0/50.2, 47.5, 40.3/40.1, 37.4/37.3, 36.4/35.9, 32.9/32.8, 29.7/29.1, 27.5, 27.0, 25.0, 23.0, 22.2. **IR (FT-ATR):** $\tilde{\nu}$ (cm^{-1}): 3298 (br), 1434 (s). **HRMS(ESI):** calcd for $[M + H]^+$ 666.2689; found 666.2692, calcd for $[M + Na]^+$ 688.2508; found 688.2508. $[\alpha]^{20}$ (c = 0.7, $CHCl_3$) = −493.1° (436 nm), −280.2° (546 nm), −244.1° (579 nm), −234.6° (589 nm). For the assignments of NMR signals, see the ESI.†

Author contributions

Chemical syntheses and analyses were conducted by M. T. K. and B. M. K. X-ray crystallographic analyses were contributed by J.-M. N. The conceptualization of the project was performed by R. K. and H.-G. S. Based on a draft by M. T. K., the manuscript was written and polished mainly by H.-G. S. and B. M. K. All authors discussed the results and approved the final version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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