3-Aminobicyclo[1.1.1]pentane-1-carboxylic Acid Derivatives: Synthesis and Incorporation into Peptides

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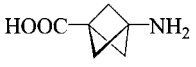
We have developed an efficient synthesis of derivatives of 3-aminobicyclo[1.1.1]pentane-1-carboxylic acid (**7**, **8**, **9**, and **10**) starting from [1.1.1]propellane (**3**). These rigid analogues of γ-aminobutyric acid have been incorporated into linear and cyclic peptides using solution chemistry and solid-phase techniques.

Introduction

The chemistry of bicyclo[1.1.1]pentanes has gained considerable attention over the years and a broad range of differently substituted derivatives have been synthesized.[2] Among them, the recently prepared 2-(3′-substituted bicyclo[1.1.1]pentyl)glycines represent the only examples of amino acids bearing the bicyclo[1.1.1]pentane moiety.[3]

That study has prompted us to report our investigations concerning the synthesis of derivatives of 3-aminobicyclo-[1.1.1]pentane-1-carboxylic acid **1**. This amino acid can be considered as a rigid analogue of 4-aminobutyric acid, which is known to act as a neurotransmitter in the brain.



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Furthermore, unnatural amino acids having special properties, such as rigidity, bulkiness or conformational constraints, are of interest as building blocks for the synthesis of modified peptides.[4]

Results and Discussion

We chose the 1,3-bicyclo[1.1.1]pentane-1-carboxylic acid **6** as the key intermediate for the synthesis of target compound **1**. This substance has been prepared by Michl et al. through a multi-step procedure.[5] Treatment of the dibromocyclopropane **2** with 2.2 equiv. of methyllithium generated the [1.1.1]propellane **3**, which was reacted with biacetyl under irradiation with an UV lamp to afford the diketone **4**. Compound **4** was then subjected to the haloform reaction followed by esterfication of both carboxylic acid functions. Basic hydrolysis of the diester with one equiv. of NaOH led to the selective saponification of one ester group and led to **6**.[5]

An alternative synthesis of the carboxylic acid **6** was carried out by using the addition of a Grignard reagent to the [1.1.1]propellane **3** and subsequent trapping of the intermediate with an electrophile.[6,2b] Specifically, the addition of phenylmagnesium bromide to the central bond of the propellane **3** afforded 3-phenylbicyclo[1.1.1]pentylmagnesium bromide, which could be trapped by methyl chloroformate to give compound **5**. Oxidation of the precursor **5** with $NaIO_4/RuCl_3$ was performed according to standard procedures and gave **6** in 49% yield.[7]

We investigated two different Curtius rearrangement procedures for the conversion of carboxylic acid **6** to the differently protected amino acid ester **7**. According to the Yamada-variation,[8] an isocyanate intermediate is produced upon refluxing one equiv. of acid **6**, diphenyl phosphorazidate (DPPA) and triethylamine. This intermediate is trapped by the alcoholic solvent (Method A) to afford the protected amino acid **7**.

We also applied a modification of this procedure in an attempt to improve the yield.[9] Refluxing acid **6** with DPPA in toluene for 2 h afforded the corresponding isocyanate, which was detected by IR spectroscopy. *tert*-Butyl alcohol was then added and the mixture was heated under reflux for a further 12 h (Method B). After workup, however, the amino acid ester **7** was isolated in lower yield than it was when prepared according to Method A. Attempts to add fluorenylmethanol to the isocyanate, to obtain the Fmoc-

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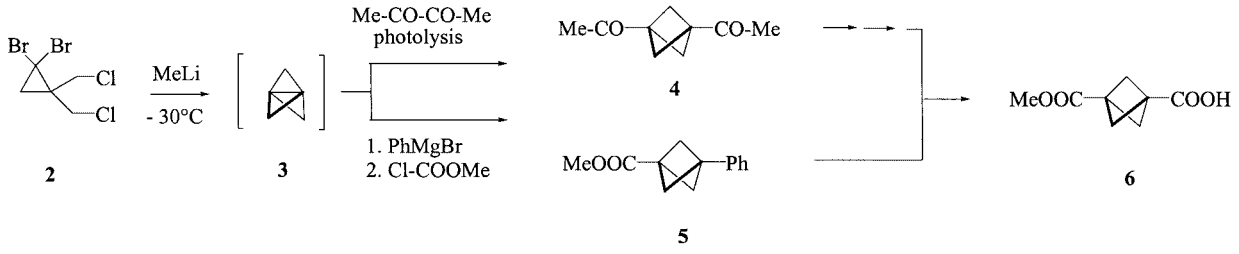
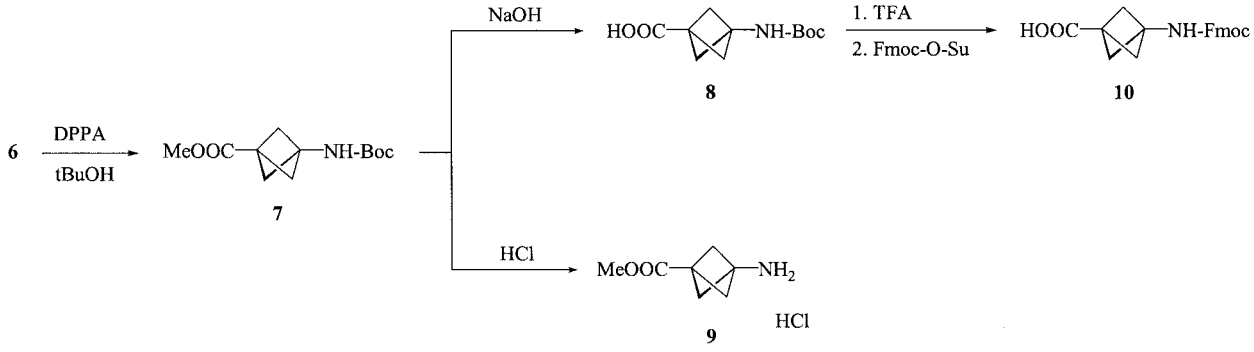


Scheme 1

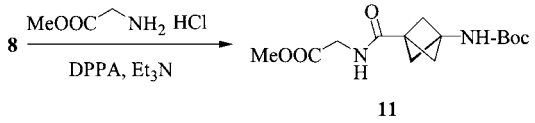


Scheme 2

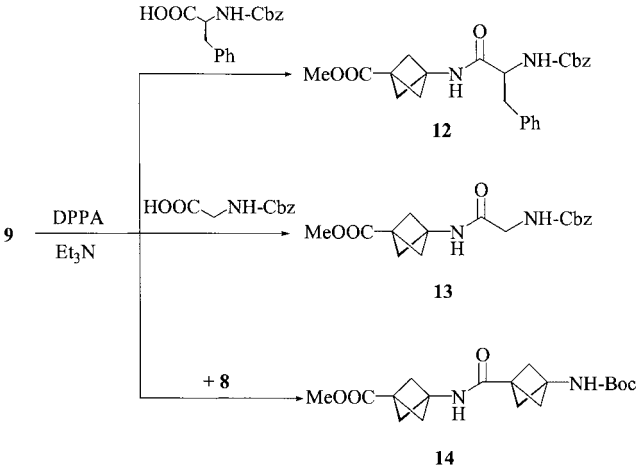
protected amino ester directly, failed and complex product mixtures were obtained.[10] The structure of compound **7** was established by spectroscopic methods and by X-ray analysis. The signals of the methylene groups of the bicyclo[1.1.1]pentane cage at $\delta = 2.13$ ppm are typical for this subunit, as are the chemical shifts of the bridged carbon atoms at $\delta = 35$ and 45 ppm. The X-ray analysis revealed [11] that, at 1.852 Å, the inter-bridgehead distance C1−C3 is in the range found for similar bicyclo[1.1.1]pentanes.[2,12]

The orthogonal protective groups of compound **7** were removed according to standard procedures to give both the N-Boc-protected amino acid **8** and the amino acid ester **9**. Treatment of **8** with TFA and Fmoc protection resulted in the formation of compound **10**, which is a suitable derivative for solid-phase peptide synthesis.

We investigated the formation of dipeptides to get more insight into the reactivity of the sterically demanding monoprotected derivatives **8** and **9**. A series of dipeptides **11**−**14** were synthesized, mostly in high yields, by coupling different amino acids with both the N- and C-termini of the 3-aminobicyclo[1.1.1]pentanecarboxylic acid using DPPA as condensation reagent.



Scheme 3



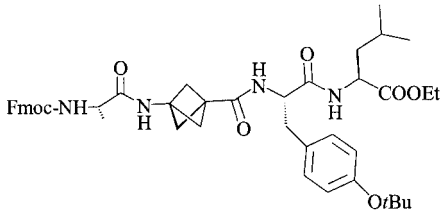
Scheme 4

The lower yield of the bis-bicyclo[1.1.1]pentane dipeptide **14** is possibly a consequence of steric congestion of the bicyclo[1.1.1]pentane cages. Compound **14** is practically insoluble in most organic solvents. Interestingly, we observed two signals having a significant shift difference (almost 0.2 ppm) for the methylene groups in the ^{1}H NMR spectrum.

In the further course of our investigations, we explored the possibility of incorporating the protected 3-aminobicyclo[1.1.1]pentane-1-carboxylic acid **10** into longer peptides.

Liquid-phase chemistry was applied for the synthesis of the tetrapeptide **15**, whereas we used solid-phase chemistry for the preparation of the peptides **16** and **17**. The se-

quences for **16** and **17** are derived from the neuropeptide cholecystokinine (CCK).[13] The CCK-B subreceptor in the brain acts as neurotransmitter and neuromodulator. The shortest CCK fragment that exhibits a high affinity for the CCK-B receptor is the C-terminal CCK-4 having the sequence Trp−Met−Asp−Phe.[14]



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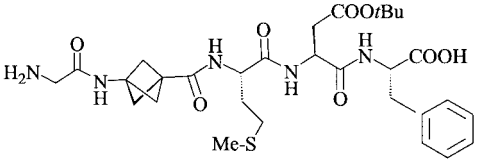

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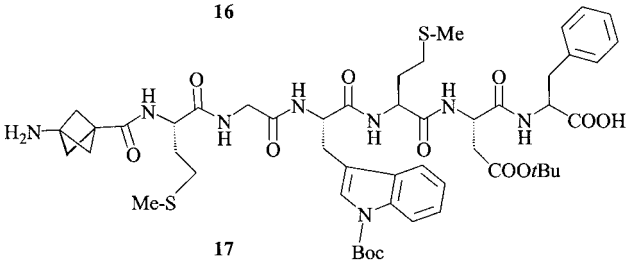

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Scheme 5

In summary, we have described the synthesis of some C- and N-terminal protected derivatives of 3-aminobicyclo-[1.1.1]pentanecarboxylic acid (**1**). These compounds represent rigid derivatives of 4-aminobutyric acid and can easily be incorporated into peptides by classical coupling methods as well as by solid-phase techniques.

Experimental Section

Melting points are uncorrected. NMR: Bruker AC-300, DPX-300; spectra were recorded in [D_6]DMSO if not stated otherwise. MS: Finnegan MAT 90. HRMS: Finnegan MAT 95. Elemental analysis: CHNS-932 Analyser (Leco). TentaGel-S-Trt-(*L*)-Phe-Fmoc resin used in the synthesis of peptides **16** and **17** was purchased at Fa. PepChem, Tübingen, Germany. Protected amino acids and coupling reagents are from Nova Biochem.

Abbreviations: DIEA = *N*,*N*-diisopropylethylamine; DPPA = diphenylphosphoryl azide; HBTU = *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; Fmo*c* = 9-fluorenylmethoxycarbonyl; DIC = Diisopropylcarbodiimide; HOAt = 1-Hydroxy-7-azabenzotriazole.

Compounds **2**,[15] **3**[16] and **6**[5] were prepared according to literature procedures.

Methyl 1-Phenylbicyclo[1.1.1]pentane-3-carboxylate (5): Phenylmagnesium bromide (100 mL, 0.107 mol, 1.07 M in diethyl ether) was added at 0 °C to a solution of [1.1.1]propellane (**3**) in diethyl ether,[16] which was prepared from **2** (40.5 g, 0.15 mol) and MeLi (232 mL, 0.33 mol, 1.42 M in diethyl ether). The mixture was stirred for 4 days at room temperature before being added dropwise within 5 h to a solution of methyl chloroformate (9.92 g, 0.105 mol) in diethoxymethane (100 mL). The mixture was warmed to room temperature and then stirred for a further 12 h. The reaction mixture was quenched by the addition of HCl (2 N, 100 mL) and the organic phase was washed with brine (3 × 80 mL). The combined aqueous phases were extracted with diethyl ether. After drying the combined organic phases with $MgSO_4$, the solvent was evaporated under reduced pressure and the residue was distilled (b.p. 115 °C/10^{-3} Torr) to afford **5** as a yellowish oil. [9.7 g, 32% (from **2**)]. The NMR spectra are in accordance with literature data.[7]

3-(Methoxycarbonyl)bicyclo[1.1.1]pentane-1-carboxylic Acid (6): Ester **5** (1.01 g, 5 mmol), $NaIO_4$ (19.25 g, 90 mmol) and $RuCl_3{\cdot}H_2O$ (41% Ru, 37 mg, 0.15 mmol) were added to a mixture of CCl_4 (30 mL), MeCN (30 mL) and water (45 mL). After stirring the mixture for 48 h, a further charge of $RuCl_3{\cdot}H_2O$ (20 mg) was added and the reaction was stirred for a further 48 h. The reaction mixture was diluted with water (90 mL) and was extracted with dichloromethane (1 × 150 mL, 1 × 100 mL, 3 × 50 mL). The solvents of the combined organic phases were evaporated almost completely, the residue was dissolved in diethyl ether (200 mL) and charcoal was added. After drying ($MgSO_4$) and filtration through a pad of silica gel, followed by evaporation of the solvent, the crude product **6** was obtained. Crystallization from heptane/chloroform gave the pure product **6** (338 mg, 49%). M.p. 138−139 °C (ref.[7] 139.5−140 °C). The NMR spectra are in accordance with literature data.[7]

Methyl 3-(*tert*-Butoxycarbonylamino)bicyclo[1.1.1]pentane-1-carboxylate (7): Method A: DPPA (22.0 mL, 28.1 g, 102 mmol) was added dropwise to a solution of carboxylic acid **6** (17.3 g, 102 mmol) and triethylamine (14.2 mL, 10.4 g, 102 mmol) in dry *t*BuOH (320 mL). The solution was stirred at room temperature for 4 h and then heated under reflux for 24 h. The solvent was evaporated under reduced pressure and the residue was extracted with *tert*-butyl methyl ether (3 × 50 mL). The solution was washed with aqueous sodium hydrogencarbonate solution and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure afforded amino acid derivative **7** as colourless crystals (20.3 g, 83%).

Method B: A solution of carboxylic acid **6** (6.87 g, 40 mmol), triethylamine (6.1 mL, 4.4 g, 44 mmol) and DPPA (8.65 mL, 11.0 g, 40 mmol) in toluene (200 mL) was stirred for 30 min at ambient temperature and then under reflux for 2 h. After formation of the intermediate isocyanate (monitored by IR spectroscopy), dry *tert*-butyl alcohol (8.88 g, 120 mmol) was added. The solution was heated under reflux for 24 h and then the solvent was evaporated under reduced pressure. The residue was extracted with *tert*-butyl methyl ether (3 × 20 mL). The solution was washed with aqueous sodium hydrogencarbonate solution and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure afforded the amino acid derivative **7** (4.03 g, 48%) as colourless crystals. M.p. 123 °C. IR (KBr): $\tilde{\nu}$ = 3442, 1730, 1685, 1653 cm^{-1}. ^1H NMR (400 MHz): δ = 1.37 (s, 9 H), 2.13 (s, 6 H), 3.60 (s, 3 H) ppm. ^{13}C NMR (400 MHz): δ = 28.1 (CH_3), 34.9 (C), 45.2 (C), 51.4 (CH_3), 53.5 (CH_2), 77.9 (C), 154.4 (C), 169.4 (C) ppm. MS (79 eV): *m*/*z* = 168 (4), 154 (14), 57 (100), 41 (22). $C_{12}H_{19}NO_4$ (241.28): calcd. C 59.73, H 7.94, N 5.80; found C 59.67, H 7.91, N 5.60.

3-(*tert*-Butoxycarbonylamino)bicyclo[1.1.1]pentane-1-carboxylic Acid (8): NaOH (2.40 g, 60 mmol) in MeOH (40 mL) was added dropwise within 10 min to a solution of **7** (11.2 g, 46.2 mmol) in MeOH (200 mL). The mixture was heated under reflux for 3 h. After evaporation of the solvent under reduced pressure, the residue was dissolved in water. The aqueous phase was extracted three times with dichloromethane and then acidified with dilute HCl (pH 6). The combined organic phases were dried ($MgSO_4$), and the solvents were evaporated under reduced pressure. The residue was recrystallised from diethyl ether to yield carboxylic acid **8** (9.30 g, 88%). M.p. 172 °C (decomp.). IR (KBr): $\tilde{\nu}$ = 3600−3200, 2987, 1698, 1645 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.46 (s, 9 H), 2.30 (s, 6 H) ppm. ^{13}C NMR (400 MHz): δ = 28.1 (CH_3), 35.2 (C), 45.0 (C), 53.3 (CH_2), 77.7 (C), 154.5 (C), 170.8 (C) ppm. MS (79 eV): *m*/*z* = 127 (4), 126 (5), 57 (100), 41 (23). $C_{11}H_{17}NO_4$ (227.26): calcd. C 58.14, H 7.54, N 6.16; found C 58.44, H 7.48, N 6.22.

Methyl 3-Aminobicyclo[1.1.1]pentane-1-carboxylate Hydrochloride (9): Gaseous HCl was bubbled for 1 h through a solution of the Boc-protected amino acid **7** (21.4 g, 88.4 mmol) in dry methanol (370 mL) at −78 °C. The temperature was maintained below −50 °C. The solution was warmed to room temperature and evaporation of the solvents under reduced pressure afforded the spectroscopically pure amino acid **9** (15.4 g, 98%). For further purification, compound **9** was recrystallised from methanol. M.p. 209 °C (decomp.) IR (KBr): $\tilde{\nu}$ = 3016, 2990, 1739, 1630 cm^{-1}. ^1H NMR (400 MHz): δ = 2.25 (s, 6 H), 3.63 (s, 3 H, COOMe) ppm. ^{13}C NMR (100 MHz): δ = 34.7 (C), 43.1 (C), 51.7 (CH_3), 52.6 (CH_2), 168.0 (C) ppm. MS (70 eV): *m*/*z* = 163 (12), 82 (100). $C_7H_{12}ClNO_2$ (177.63): calcd. C 47.33, H 6.81, N 7.89, Cl 19.96; found C 47.55, H 6.77, N 7.95, Cl 19.93.

3-[(9-Fluorenylmethoxycarbonyl)amino]bicyclo[1.1.1]pentane-1-carboxylic Acid (10): 3-(*N*-Boc-amino)bicyclo[1.1.1]pentane-1-carboxylic acid (**8**, 2.01 g, 8.8 mmol) was treated with TFA (95% in water; 5 mL) for 1 h followed by coevaporation of the excess of TFA with heptane. The crude 3-aminobicyclo[1.1.1]pentane-1-carboxylic acid trifluoroacetate was dissolved in a mixture of acetone/water (20 mL, 1:1) and $Na_2CO_3 \cdot 10H_2O$ (2.52 g) was added. The pH was adjusted to 9 by addition of a saturated Na_2CO_3 solution. Fmoc-*O*-succinimide (2.97 g, 8.8 mmol) was added over a period of 30 min. The mixture was stirred for 12 h and the value of the pH was maintained all this time at 9 by further addition of a few drops of saturated Na_2CO_3 solution. The mixture was diluted with ethyl acetate (50 mL) and acidified carefully with 6 N HCl. The phases were separated and the organic phase was washed with brine (3 × 30 mL) and dried with $MgSO_4$. After evaporation of the solvent under reduced pressure, the crude product **10** (2.83 g, 92%) was obtained. Compound **10** was further purified by recrystallisation from acetonitrile. M.p. 233−235 °C. ^1H NMR (300 MHz): δ = 2.09 (s, 6 H), 2.50 (s, 1 H), 3.34 (m, 2 H), 4.27 (m, 1 H), 7.33−7.90 (m, 8 H), 12.37 (s, 1 H) ppm. ^{13}C NMR (75 MHz): δ = 30.7 (C), 39.8 (C), 46.7 (CH), 53.4 (CH_2), 65.2 (CH_2), 120.2 (CH), 125.2 (CH), 127.1 (CH), 127.7 (CH), 140.9 (C), 144.0 (C), 155.2 (C), 170.8 (C) ppm. MS (70 eV): *m*/*z* = 349 (4) [M^+], 178 (100), 165 (14). HRMS (70 eV): calcd. 349.13124, found 349.13114 $C_{21}H_{19}NO_4$ (349.37): calcd. C 72.24, H 5.49, N 4.01; found C 72.05, H 5.61, N 4.01.

Synthesis of Dipeptides 11−14

General Procedure: DPPA (1.08 mL, 1.38 g, 5.00 mmol) dissolved in DMF (15 mL) was added within 10 min to an ice-cooled solution of the carboxyl component (4.5 mmol) and the amino compound (4.6 mmol) in DMF (20 mL). Subsequently, triethylamine (1.40 mL, 1.01 g, 10 mmol) dissolved in DMF (15 mL) was added dropwise within 10 min to the reaction mixture. The mixture was warmed to room temp. and stirred for 12 h before a mixture of ethyl acetate and benzene (2:1, 200 mL) was added. The solution was washed with 2 N HCl (2 × 50 mL), water (2 × 50 mL) and brine (50 mL). (In the case of the Boc-protected dipeptide **14**, 5% aqueous citric acid was used instead of 2 N HCl.) The organic phase was dried ($MgSO_4$). After evaporation of the organic solvents under reduced pressure, the dipeptides **11−14** were obtained as colorless crystals.

Methyl {[3-(*tert*-Butoxycarbonylamino)bicyclo[1.1.1]pent-1-yl]-carbonyl}glycinate (11): Yield: 89%. M.p. 160 °C (MeOH). IR (KBr): $\tilde{\nu}$ = 3323, 2981, 1691 cm^{-1}. ^1H NMR (400 MHz): δ = 1.38 (s, 9 H), 2.06 (s, 6 H), 3.62 (s, 3 H), 3.77 (d, *J* = 6 Hz, 2 H) ppm. ^{13}C NMR (100 MHz): δ = 28.6 (CH_3), 37.1 (C), 40.9 (CH_2), 45.2 (C), 52.1 (CH_3), 53.6 (CH_2), 78.3 (C), 155.0 (C), 169.6 (C), 170.7 (C) ppm. MS (70 eV): *m*/*z* = 299 ([M^+ + 1], 1), 109 (100). $C_{14}H_{22}N_2O_5$ (298.34): calcd. C 56.36, H 7.42, N 9.39; found C 56.23, H 7.39, N 9.12.

Compound 12: Yield: 83%. M.p. 117 °C (diethyl ether/pentane). IR (KBr): $\tilde{\nu}$ = 3305, 2922, 1666 cm^{-1}. ^1H NMR (400 MHz): δ = 2.23 (s, 6 H), 2.70−2.97 (m, 2 H), 3.61 (s, 3 H), 4.12 (m, 1 H), 4.95 (m, 2 H), 7.18−7.68 (m, 10 H) ppm. ^{13}C NMR (100 MHz): δ = 35.5 (C), 37.5 (CH_2), 45.2 (C), 51.4 (CH_3), 53.7 (CH_2), 56.1 (CH), 65.1 (CH_2), 126.1 (CH), 127.4 (CH), 127.6 (CH), 127.9 (CH), 128.2 (CH), 129.1 (CH), 136.9 (C), 137.8 (C), 155.7 (C), 169.2 (C), 171.8 (C) ppm. HRMS: calcd. 422.1842; found 422.1803. $C_{24}H_{26}N_2O_5$ (422.48): calcd. C 68.23, H 6.20, N 6.63; found C 67.91, H 6.18, N 6.85.

Compound 13: Yield: 94%. M.p. 137 °C (MeOH). IR (KBr): $\tilde{\nu}$ = 3600−3300, 2926, 1670 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ = 2.35 (s, 6 H), 3.68 (s, 3 H), 3.75 (m, 2 H), 5.13 (s, 2 H), 7.26−7.36 (m, 5 H) ppm. ^{13}C NMR (100 MHz): δ = 36.0 (CH_3), 43.8 (CH_2), 45.7 (C), 51.9 (CH_3), 54.3 (CH_2), 65.9 (CH_2), 128.2 (CH), 128.2 (CH), 128.8 (CH), 137.5 (C), 156.9 (C), 169.7(C), 170.0 (C) ppm. MS (70 eV): *m*/*z* = 332 (2) [M^+], 301 (5), 91 (100). $C_{17}H_{20}N_2O_5$ (332.36): calcd. C 61.44, H 6.06, N 8.43; found C 61.31, H 5.88, N 8.46.

Methyl 3-{-*N*-[3′-*N*-(*tert*-Butoxycarbonyl)aminobicyclo[1.1.1]pent-1′-yl-carbonyl]amino}bicyclo[1.1.1]pentane-1-carboxylate (14): Yield: 37%. M.p. 285 °C (dec., $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3321, 2921, 1690 cm^{-1}. ^1H NMR (400 MHz): δ = 1.37 (s, 9 H), 2.02 (s, 6 H), 2.21 (s, 6 H), 3.60 (s, 3 H) ppm. ^{13}C NMR (100 MHz): δ = 28.1 (CH_3), 35.6 (C), 44.2 (C), 44.6 (C), 45.2 (C), 51.0 (CH_3), 53.0 (CH_2), 53.8 (CH_2), 154.4 (C), 163.8 (C), 169.2 (C) ppm. $C_{18}H_{26}N_2O_5$ (350.41): calcd. C 61.70, H 7.48, N 7.99; found C 61.48, H 7.57, N 8.27.

Synthesis of Peptide Fmoc-L-Ala-Bcp-L-Tyr(O*t*Bu)-L-Leu-OMe 15 by Liquid-Phase Chemistry. General Procedure: Deprotection − Removal of the Fmoc Group: The protected peptide was dissolved in 20% piperidine in DMF ($c \approx 0.2$ M) and the reaction mixture was stirred for 15 min. The course of the reaction was monitored by TLC. The volatiles were evaporated under reduced pressure and the residue was used in the next coupling steps as the amine component.

Coupling Procedure: The amino and the carboxyl components, as well as the coupling reagent, were dissolved in DMF in 3-necked flask under an Ar atmosphere at 0 °C. A solution of Hünig's base dissolved in DMF was then added dropwise within 10 min. The

mixture was stirred at 0 °C for 2 h and then warmed to room temperature and stirred for a further 12 h. Purification: The reaction mixture was dissolved in ethyl acetate/benzene (2:1, 30 mL) and washed with 5% aqueous citric acid (2 × 10 mL), water (2 × 10 mL), saturated $NaHCO_3$ solution (2 × 10 mL), and brine (2 × 10 mL). After drying the organic phase ($MgSO_4$) and evaporating the solvent under reduced pressure, the product was purified by chromatography.

Synthesis of Dipeptide Fmoc-NH-L-Tyr(O*t*Bu)-L-Leu-OMe: According to the general procedure, *N*-Fmoc-*O*-*t*Bu-L-tyrosine (0.504 g, 1.10 mmol), methyl L-leucinate (0.222 g, 1.23 mmol), DPPA (0.240 mL, 0.306 g, 1.11 mmol), and DIEA (0.420 mL, 0.31 g, 2.40 mmol) were reacted in DMF (20 mL). After the evaporation of the solvent under reduced pressure, the dipeptide were obtained as a colourless foam (0.609 g, 94.6%). The substance was used in the next step without further purification. R_f (petroleum ether/EtOAc, 1:4) = 0.83. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.80 [d, *J* = 5.6 Hz, 6 H, Leu-CH(CH_3)$_2$], 1.25 [s, 9 H, Tyr-C(CH_3)$_3$], 1.30−1.70 [m, 3 H, Leu-CH_2, Leu-C*H*(CH_3)$_2$], 2.95 (d, 2 H, Tyr-CH_2), 3.58 (s, 3 H, Leu-OCH_3), 4.10−4.55 (m, 5 H, Fmoc-C*H*, Fmoc-CH_2, Leu-C*H*, Tyr-C*H*), 5.30 (s, 1 H, Leu-N*H*), 6.05 (s, 1 H, Tyr N*H*), 6.82−7.70 (m, 12 H, Fmoc C$H_{arom.}$, Tyr-C$H_{arom.}$) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 21.9, 22.7 [Leu-CH(*C*H$_3$)$_2$], 24.7 [Leu-*C*H(CH$_3$)$_2$], 28.8 [Tyr-C(*C*H$_3$)$_3$], 37.8 (Tyr-*C*H$_2$), 41.5 (Leu-*C*H$_2$), 47.1 (Tyr-*C*H), 50.9 (Leu-*C*H), 52.3 (Leu-O*C*H$_3$), 55.0 (Fmoc-*C*H), 67.1 (Fmoc-*C*H$_2$), 78.4 [Tyr-*C*(CH$_3$)$_3$], 120.0, 124.3, 125.0, 127.1, 127.7, 129.9 (Fmoc-*C*H$_{arom.}$, Tyr-*C*H$_{arom.}$), 130.5, 141.3, 143.7, 143.8 (Fmoc-*C*$_{arom.}$, Tyr-*C*$_{arom.}$), 154.5 (Fmoc-O*C*O), 170.5 (Tyr-*C*ON), 172.7 (Leu-*C*OOMe) ppm. HRMS ($C_{35}H_{42}N_2O_6$) calcd. 586.3043; found 586.3059.

Synthesis of Tripeptide Fmoc-Bcp-L-Tyr(O*t*Bu)-L-Leu-OMe: The dipeptide Fmoc-L-Tyr(O*t*Bu)-L-Leu-OMe (0.507 g, 0.863 mmol) was deprotected according to the general procedure. The deprotected amino acid, 3-(Fmoc-amino)bicyclo[1.1.1]pentane-1-carboxylic acid (**10**, 0.295 g, 0.845 mmol), HBTU (0.330 g, 0.871 mmol) and DIEA (0.15 mL, 0.11 g, 0.86 mmol) were reacted as described above. After workup and chromatography (petroleum ether/ethyl acetate, 1:4), the tripeptide was obtained as a colourless foam (0.405 g, 69%). R_f = 0.51 (petroleum ether/ethyl acetate, 1:4). $[\alpha]_D$ = −20.5 (*c* = 1, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.81 [d, *J* = 5.7 Hz, 6 H, Leu-CH(CH_3)$_2$], 1.24 [s, 9 H, Tyr-C(CH_3)$_3$], 1.35−1.70 [m, 3 H, Leu-CH_2, Leu-C*H*(CH$_3$)$_2$], 2.15 (s, 6 H, Bcp-CH_2), 2.94 (d, *J* = 6.9 Hz, 2 H, Tyr-CH_2), 3.64 (s, 3 H, Leu-OCH_3), 4.15 (m, 1 H, Tyr-C*H*), 4.20−4.65 (m, 4 H, Fmoc-C*H*, Fmoc CH_2, Leu-C*H*), 6.80−7.80 (m, 12 H, Fmoc C$H_{arom.}$, Tyr-C$H_{arom.}$) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 21.9, 22.7 [Leu-CH(*C*H$_3$)$_2$], 24.8 [Leu-*C*H(CH$_3$)$_2$], 28.8 [Tyr-C(*C*H$_3$)$_3$], 37.5 (Tyr-*C*H$_2$), 41.2 (Leu-*C*H$_2$), 45.0 (Bcp-*C*-3), 47.1 (Leu-O*C*H$_3$), 50.9 (Leu-*C*H), 52.3 (Tyr-*C*H), 53.4 (Bcp-*C*H$_2$), 54.0 (Fmoc-*C*H), 66.5 (Fmoc-C*H*$_2$), 78.4 [Tyr-*C*(CH$_3$)$_3$], 120.0, 124.3, 125.0, 127.1, 127.8, 129.9 (Fmoc-C*H*$_{arom.}$, Tyr-C*H*$_{arom.}$), 133.1, 141.3, 143.8, 143.8 (Fmoc-*C*$_{arom.}$, Tyr-*C*$_{arom.}$), 154.4 (Fmoc-O*C*O), 169.0 (Bcp-*C*ON), 170.0 (Tyr-*C*ON), 172.7 (Leu-*C*OOMe) ppm. HRMS ($C_{41}H_{49}N_3O_7$) calcd. 695.3568; found 695.3569.

Synthesis of Tetrapeptide Fmoc-L-Ala-Bcp-L-Tyr(O*t*Bu)-L-Leu-OMe (15): The tripeptide Fmoc-Bcp-L-Tyr(O*t*Bu)-L-Leu-OMe (0.507 g, 0.720 mmol) was deprotected according to the general procedure. The free amino component, Fmoc-L-alanine hydrate (0.254 g, 0.772 mmol), HBTU (0.320 g, 0.844 mmol), and DIEA (0.13 mL, 0.096 g, 0.77 mmol) were reacted as described above. After workup and chromatography (petroleum ether/ethyl acetate, 1:4) tetrapeptide **15** was obtained (0.120 g, 0.582 mmol, 69%) as a colourless foam. R_f = 0.33 (petroleum ether/ethyl acetate, 1:4). $[\alpha]_D$ = −33.8 (*c* = 1, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.78 [d, *J* = 5.2 Hz, 6 H, Leu-CH(CH_3)$_2$], 1.24 [s, 9 H, Tyr-C(CH_3)$_3$], 1.27 (d, *J* = 6.9 Hz, 3 H, Ala-CH_3), 1.35−1.70 [m, 3 H, Leu-CH_2, Leu-C*H*(CH$_3$)$_2$], 2.18 (s, 6 H, Bcp-CH_2), 2.95 (d, *J* = 6.8 Hz, 2 H, Tyr-CH_2), 3.62 (s, 3 H, Leu-OCH_3), 4.15 (t, 1 H, Tyr-C*H*), 4.20−4.60 (m, 5 H, Fmoc-C*H*, Fmoc-CH_2, Leu-C*H*, Ala-C*H*), 6.80−7.80 (m, 12 H, Fmoc C$H_{arom.}$, Tyr-C$H_{arom.}$) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 18.7 (Ala-*C*H$_3$), 21.9, 22.6 [Leu-CH(*C*H$_3$)$_2$], 24.8 (Leu-*C*H(CH$_3$)$_2$], 28.8 [Tyr-C(*C*H$_3$)$_3$], 37.5 (Tyr-*C*H$_2$), 41.1 (Leu-*C*H$_2$), 45.0 (Bcp-*C*-3), 47.0 (Tyr-*C*H), 50.0 (Ala-*C*H), 50.9 (Leu-*C*H), 52.3 (Leu-O*C*H$_3$), 53.7 (Bcp-*C*H$_2$), 54.0 (Fmoc-*C*H), 67.1 (Fmoc-C*H*$_2$), 78.4 [Tyr-*C*(CH$_3$)$_3$], 120.0, 124.3, 125.0, 127.1, 127.8, 129.9 (Fmoc-C*H*$_{arom.}$, Tyr-C*H*$_{arom.}$), 131.2, 141.3, 143.6, 143.7 (Fmoc-*C*$_{arom.}$, Tyr-*C*$_{arom.}$), 154.4 (Fmoc-O*C*O), 169.0 (Bcp-*C*ON), 170.0 (Tyr-*C*ON), 170.2 (Ala-*C*ON), 172.7 (Leu-*C*OOMe) ppm. HRMS ($C_{45}H_{55}N_4O_8$ = M + 1): calcd. 767.4019, found 767.4029.

Synthesis of H$_2$N-Gly-Bcp-L-Met-L-Asp(O*t*Bu)-L-Phe-COOH (16) by Solid-Phase Synthesis: CP-L-Phe-Fmoc resin (1.50 g, 0.900 mmol) was placed in a column fitted at the bottom with a special tap. The tap had the function of controlling an inlet stream of nitrogen and the outlet of solvents and reaction washings. DMF was added to the resin, which was left to swell under a continuous stream of nitrogen. The following deprotection and coupling procedures were performed for each of the four coupling steps.

Deprotection − Removal of the Fmoc Group: A solution of piperidine in DMF (20%, 5 mL) was added to the resin free of solvents. After passage of a stream of nitrogen in the column for 5 min, the resulting solution was drained off. The residue was washed with DMF (2 × 20 mL); each time a stream of nitrogen was passed through the column for 2 min.

Coupling Procedure: A solution of HBTU (3.0 equiv.), Fmoc-protected amino acid (3.0 equiv.) and DIEA (6.0 equiv.) dissolved in DMF (6 mL; see Table 1) was added to the deprotected N-terminus resin. A stream of nitrogen was passed through the resulting mixture for 1 h. After filtration, the resin was washed three times with DMF (3 mL) and once with dichloromethane (3 mL). Each coupling reaction was carried out twice.

Removal of the peptide from the resin was effected by treatment with a mixture of acetic acid, methanol and dichloromethane (5:1:4) within 2 h (about 10 mL/g resin). The resin was filtered off and washed with the same mixture and dichloromethane (10 mL). The combined organic solvents were diluted with heptane (300 mL, to remove the acetic acid azeotropically). The solvents were then evaporated almost completely under reduced pressure. A further charge of heptane (25 mL) was added and the solvent was evaporated again. The residue was dissolved in water and subsequently lyophilized. A grey solid was formed, which was further purified by preparative HPLC chromatography to give the pentapeptide **16**. HRMS ($C_{30}H_{44}N_5SO_8$ = [M^+ + 1]): calcd. 634.2910, found 634.2909.

Synthesis of H$_2$N-Bcp-L-Met-Gly-L-Trp(Boc)-L-Met-L-Asp(O*t*Bu)-L-Phe-COOH (17) by Automated Solid-Phase Synthesis: The peptide synthesis was performed on a Syro II synthesizer (MultiSynTec, Witten, Germany) by using Fmoc strategy and HBTU/DIEA coupling protocols. We used TentaGel-S-Trt-(L)-Phe-Fmoc resin (100 mg; Rapp Polymere, Tübingen, Germany) with a capacity of 0.47 mmol/g. The Fmoc group was deprotected using 20% piperidine in DMF. The final cleavage from the resin was carried out using a mixture of acetic acid, methanol and dichloromethane

Table 1. Reactions

Fmoc-protected amino acid	Mol. mass [g/mol]	Amount amino acid [g/mmol]	Amount HBTU [g]	Amount DIEA [ml]
L-Asp(*O*-*t*Bu) OH	411	1.14/2.77	1.11	0.939
L-Methionine-OH	371	1.00 /2.70	1.12	0.942
Bcp-OH	349	0.942/2.70	1.11	0.940
Glycin-OH	247.32	0.667/2.70	1.14	0.940

(5:1:4) within 2 h (about 10 mL/g resin).The further work up was the same as described for compound **16**. The crude peptide was purified by reverse-phase chromatography on a Shimadzu LC 8 preparative HPLC (300 × 40 mm column; VYDAC 218TPB1520; The Separations Group, Hesperia, CA, USA) using a gradient from 20 to 80% B [A: TFA (1 mL) in water (1000 mL); B: TFA (2 mL) in acetonitrile (800 mL) and water (200 mL)].The flow rate was 70 mL/min. HRMS ($C_{51}H_{70}N_8S_2O_{12}$ + Na): calcd. 1073.4452, found 1073.4435.

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