A Novel 2*H*-Azirin-3-amine as a Dipeptide (Aib-Hyp) Synthon

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The synthesis of methyl (2*S*,4*R*)-4-(benzyloxy)-*N*-(2,2-dimethyl-2*H*-azirin-3-yl)prolinate (**10**), a novel 2*H*-azirin-3-amine ('3-amino-2*H*-azirine'), is described (*Scheme 1*). The reaction of methyl (2*S*,4*R*)-*N*-(2-methylpropanoyl)-4-(benzyloxy)prolinate (**7**) with *Lawesson* reagent gave methyl (2*S*,4*R*)-4-(benzyloxy)-*N*-[2-(methylthio)propanoyl]prolinate (**8**) and consecutive treatment with $COCl_2$, 1,4-diazabicyclo[2.2.2]octane (DABCO), and NaN_3 led to **10**. The use of **10** as a building block of the dipeptide Aib-Hyp (Aib = 2-aminoisobutyric acid, Hyp = (2*S*,4*R*)-4-hydroxyproline) is demonstrated by the syntheses of several model peptides (*Scheme 2* and *Table*). The benzyl protecting group of the 4-OH function in Hyp in the model peptides has been removed in good yields.

Introduction. – In the last few years, we have shown that 2*H*-azirin-3-amines ('3-amino-2*H*-azirines') can be used as synthons for α,α-disubstituted glycines (α,α-disubstituted α-amino acids) in peptide synthesis. The procedure for the introduction of such amino acids into peptides is the so-called 'azirine/oxazolone method' [1], which was developed as a convenient preparative access to such peptides. This strategy has been extensively employed in the synthesis of linear oligopeptides [2–5], endothiopeptides [6–8], cyclic peptides [9–12], and cyclic depsipeptides [12–15] containing α,α-disubstituted glycines.

Recently, 2*H*-azirin-3-amines became available that are enantiomerically pure, such as the (*S*)-isovaline (Iva) synthon **1** [16] and the (*S*)-2-(methyl)phenylalanine (Phe(Me)) synthon **2** [4], and that are used to synthesize stereochemically pure peptides. Furthermore, the first representative of a novel type of 2*H*-azirine-3-amine, namely *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate (**3**), has been prepared and found to be suitable as a dipeptide synthon for the sequence Aib-Pro (Aib = 2-aminoisobutyric acid) [17]. It has been used in the synthesis of the peptaibol antibiotics *Trichovirin I 1B* and *I 4A* [17, 18] as well as in the synthesis of endothiopeptides [7]. Moreover, a dipeptide synthon **4** for the sequence 'α-aminoisobutyric acid-*N*-methyl alanine' (Aib-(Me)Ala) [19] and the first example of a heterospirocyclic *N*-(2*H*-azirin-3-yl)-L-prolinate **5** [20], which has been used in the preparation of an analogue of the C-terminal nonapeptide of *Trichovirin I 1B* have been synthesized.

In the present paper, we describe the synthesis of the Aib-Hyp (Hyp = (2*S*,4*R*)-4-hydroxyproline) synthon methyl (2*S*,4*R*)-4-(benzyloxy)-*N*-(2,2-dimethyl-2*H*-azirin-3-yl)prolinate (**10**; *Scheme 1*) that was performed in analogy to the Aib-Pro synthon **4**

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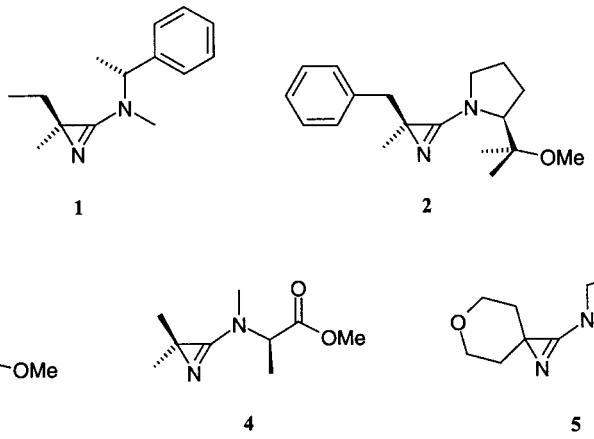
[1]) Part of the planned Ph. D. thesis of *R. A. B.*, Universität Zürich.

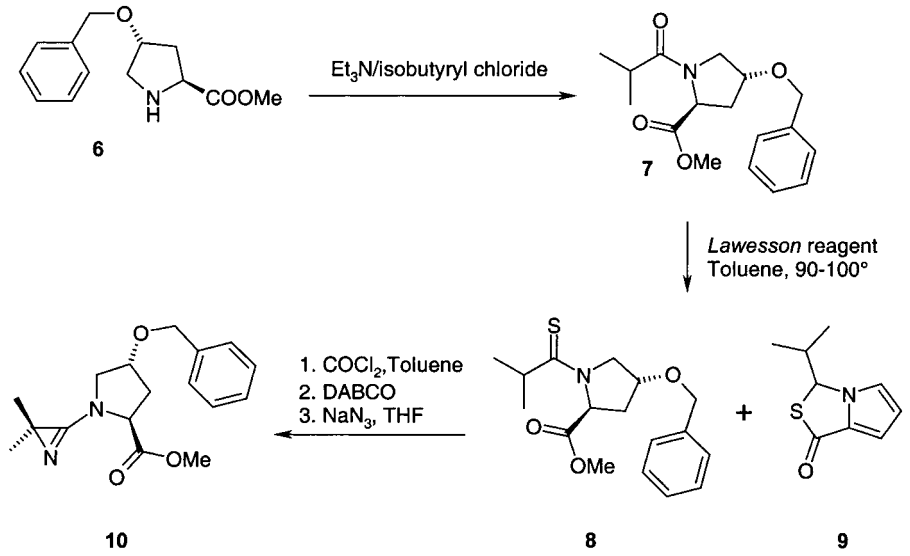
[2]) Part of an undergraduate student program under the guidance of *R. A. B.* and *R. T. N. L.*



[17] and its applicability in the synthesis of model peptides. As several peptaibols, *e.g.*, antiamoebin I [21], emerimicin IV [22], and zervamicin ZIA [23], contain the dipeptide segment Aib-Hyp, this novel azirine is an attractive building block in the synthesis of this class of conformationally restricted, biologically active peptides (*cf.* [24]).

Results and Discussion. – *Synthesis of the Aib-Hyp Synthon* **10**. The 2*H*-azirin-3-amine **10** was synthesized from the starting material methyl (2*S*,4*R*)-(benzyloxy)prolinate hydrochloride (**6**; Hyp(Bn)-OMe · HCl), which was treated with isobutyryl chloride in the presence of Et_3N to form methyl (2*S*,4*R*)-4-(benzyloxy)-*N*-(2-methylpropanoyl)prolinate (**7**) in 82% yield (*Scheme 1*). Thionation of **7** with

Scheme 1



Lawesson reagent in toluene (90–100°, 25 min) led to methyl (2*S*,4*R*)-4-(benzyloxy)-*N*-(2-methyl-1-thioxopropyl)prolinate (**8**) in 81% yield. After a longer reaction time (50 min), the unexpected product **9**, formed by initial cyclization and subsequent elimination of the BnO group, was obtained in yields up to 47%.

The reaction of **8** in CH_2Cl_2 with a solution of $COCl_2$ in toluene in the presence of catalytic amounts of DMF, evaporation of the solvent, dissolution of the residue in THF, addition of 1,4-diazabicyclo[2.2.2]octane (DABCO), and filtration led to a THF solution of methyl (2*S*,4*R*)-4-(benzyloxy)-*N*-(1-chloro-2-methylprop-1-en-1-yl)prolinate. Treatment of the latter with NaN_3 for one night gave **10** in 37% yield.

Syntheses of the Model Peptides. With the aim of testing the usefulness of **10** as a dipeptide synthon, the reactions with Z-Leu-OH, Fmoc-Val-OH, Boc-Ala-OH, and Fmoc-Phe-Gly-OH, respectively, in CH_2Cl_2 with 1 equiv. of **10** were carried out at room temperature. After stirring for one night, the expected tri- and tetrapeptides **12** and **13**, respectively, were obtained in good-to-very-good yields (*Scheme 2* and *Table*).

Scheme 2

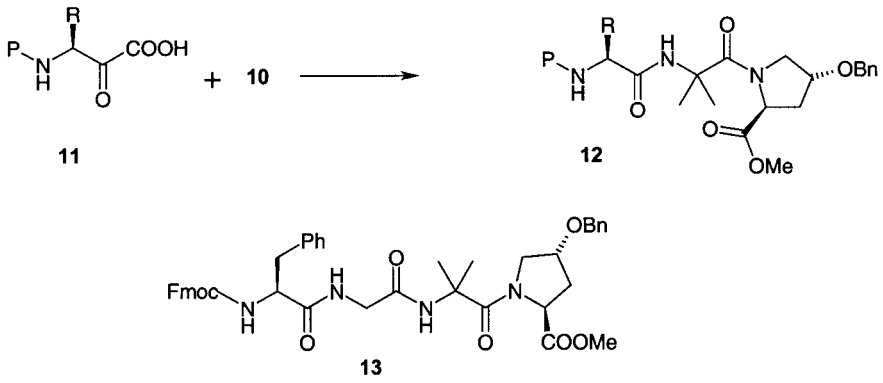
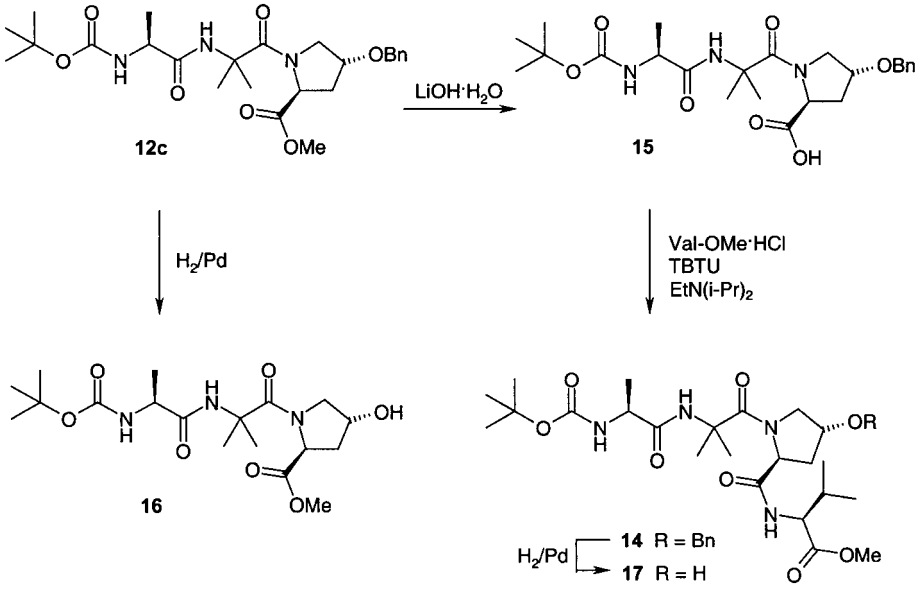


Table. *Syntheses of the Model Peptides* **12** *and* **13**

Amino acid **11**	P	R	Product	Yield [%]
Z-Leu-OH	Z	Me_2CHCH_2	Z-Leu-Aib-Hyp(Bn)-OMe (**12a**)	68
Fmoc-Val-OH	Fmoc	Me_2CH	Fmoc-Val-Aib-Hyp(Bn)-OMe (**12b**)	95
Boc-Ala-OH	Boc	Me	Boc-Ala-Aib-Hyp(Bn)-OMe (**12c**)	88
Fmoc-Phe-Gly-OH	Fmoc-Phe	H	Fmoc-Phe-Gly-Aib-Hyp(Bn)-OMe (**13**)	97

With the intention of establishing the general applicability of the dipeptide synthon **10** in peptide synthesis, the tetrapeptide Boc-Ala-Aib-Hyp(Bn)-Val-OMe (**14**) was prepared. Hydrolysis of the methyl ester **12c** with $LiOH \cdot H_2O$ (*Scheme 3*) led quantitatively to Boc-Ala-Aib-Hyp(Bn)-Val-OH (**15**), which was coupled without further purification with Val-OMe · HCl and the coupling reagent *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) in MeCN in the presence of EtN(i-Pr)$_2$. After chromatographic workup, the tetrapeptide **14** was obtained in 96% yield.

Scheme 3



The cleavage of the Bn protecting group of the 4-OH function of Hyp in **12c** and **14** was carried out by catalytic hydrogenation in the presence of Pd/C and Pd black[3]), yielding the peptides Boc-Ala-Aib-Hyp-OMe (**16**) and Boc-Ala-Aib-Hyp-Val-OMe (**17**) in 87 and 80% yield, respectively (*Scheme 3*).

In summary, we have shown that methyl (2*S*,4*R*)-4-(benzyloxy)-*N*-(2,2-dimethyl-2*H*-azirin-3-yl)prolinate (**10**) can be used conveniently as a dipeptide synthon for the sequence Aib-Hyp in the 'azirine/oxazolone method'. Coupling may take place with amino acids as well as with peptide acids in good to very good yields. After the azirine coupling, the peptide chain can be elongated by standard peptide chemistry. The BnO group of Hyp can be cleaved by catalytic hydrogenation if Hyp is the terminal amino acid as well as in an internal position.

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Experimental Part

1. *General*. See [6]. M.p.: *Büchi B-540*. Unless otherwise stated, NMR spectra in $CDCl_3$. CI-MS with NH_3.

2. *Methyl (2*S*,4*R*)-4-(Benzyloxy)*-N*-(2,2-dimethyl-2*H*-azirin-3-yl)prolinate* (**10**). 2.1. *Methyl (2*S*,4*R*)-4-(Benzyloxy)*-N*-(2-methylpropanoyl)prolinate* (**7**). To a soln. of (2*S*,4*R*)-Hyp(Bn)-OMe·HCl (**6**; 13.13 g, 48.3 mmol) and Et_3N (20.2 ml, 144.9 mmol) in CH_2Cl_2 (200 ml), isobutyryl chloride (5.1 ml, 48.3 mmol) was added at 0°. The mixture was stirred for 4 h at r.t., the solvent evaporated, and the residue was dissolved in Et_2O.

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[3]) The reaction did not take place when only Pd/C was used.

Then, H_2O was added until the precipitated $Et_3N \cdot HCl$ dissolved. Extraction with Et_2O, drying ($MgSO_4$), evaporation of the solvent, and distillation of the remaining oil yielded 12.08 g (82%) of **7**. Colorless oil. IR (neat): 3480*w*, 3064*m*, 3031*m*, 2972*s*, 2872*m*, 1747*s*, 1651*s*, 1497*m*, 1472*s*, 1429*s*, 1363*s*, 1319*s*, 1265*s*, 1200*s*, 1091*s*, 1027*s*, 952*w*, 915*w*, 890*w*, 855*w*, 802*w*, 742*s*, 699*s*. ^{1}H-NMR: 7.38–7.27 (*m*, 5 arom. H); 4.59–4.47 (*m*, PhCH_2, CH(4)); 4.30–4.27 (*m*, CH(2)); 3.78 (*dd*, $J = 10.8$, 4.9, H_a of CH_2(5)); 3.73 (*s*, MeO); 3.65 (*dd*, $J = 10.8$, 3.0, H_b of CH_2(5)); 2.59 (*sept.*, $J = 6.8$, CH(2′)); 2.42–2.33, 2.11–2.02 (2*m*, CH_2(3)); 1.12 (*t*-like, $J = 6.4$, 2 Me). ^{13}C-NMR: 175.9, 172.9 (2*s*, 2 CO); 137.5 (*s*, 1 arom. C); 128.4, 127.8, 127.4 (3*d*, 5 arom. C); 77.0 (*d*, CH(4)); 71.2 (*t*, PhCH_2); 57.5 (*d*, CH(2)); 52.1 (*t*, CH_2(5)); 52.0 (*q*, MeO); 34.5 (*t*, CH_2(3)); 32.1 (*d*, CH(2′)); 18.6, 18.5 (2*q*, 2 Me). ESI-MS: 633 ($[2M + Na]^+$), 328 ($[M + Na]^+$). Anal. calc. for $C_{17}H_{23}NO_4 \cdot 0.1\ H_2O$ (307.17): C 66.47, H 7.61, N 4.56; found: C 66.40, H 7.47, N 4.47.

2.2. *Methyl (2*S*,4*R*)-4-(Benzyloxy)*-N-*(2-methyl-1-thioxopropyl)prolinate* (**8**). A soln. **7** (991 mg, 3.35 mmol) and *Lawesson* reagent (744 mg, 1.84 mmol) in abs. toluene (20 ml) was heated to 90–100° for 25 min. After cooling to r.t. and evaporation, CC (CH_2Cl_2/hexane 5:2) yielded 728 mg (70%) of **8** as well as 140 mg (14%) of **7**. Yield of **8** calculated with respect to the amount of material consumed: 81%. Yellow oil. IR ($CHCl_3$): 3066*w*, 2976*s*, 2868*m*, 1742*s*, 1602*w*, 1496*w*, 1436*s*, 1384*m*, 1362*s*, 1331*s*, 1268*s*, 1229*s*, 1202*s*, 1180*m*, 1162*m*, 1099*s*, 1027*m*, 976*w*, 919*w*, 886*w*, 699*m*, 668*s*. ^{1}H-NMR: 7.40–7.30 (*m*, 5 arom. H); 5.08 (*dd*, $J = 8.6$, 6.2, CH(2)); 4.59, 4.50 (*AB*, $J = 11.8$, PhCH_2); 4.40–4.3 (*m*, CH(4)); 3.95 (*dd*, $J = 11.9$, 5.2, H_a of CH_2(5)); 3.88 (*dd*, $J = 11.7$, 3.8, H_b of CH_2(5)); 3.73 (*s*, MeO); 2.96 (*sept.*, $J = 6.6$, CH(2′)); 2.50–2.41, 2.25–2.17 (2*m*, CH_2(3)); 1.24, 1.22 (2*d*, $J = 6.6$, 2 Me). ^{13}C-NMR: 210.2 (*s*, CS); 171.1 (*s*, CO); 137.3 (*s*, 1 arom. C); 128.6, 128.1, 127.5 (3*d*, 5 arom. C); 76.2 (*d*, CH(4)); 71.4 (*t*, PhCH_2); 63.6 (*d*, CH(2)); 55.2 (*t*, CH_2(5)); 52.3 (*q*, MeO); 38.7 (*d*, CH(2′)); 34.3 (*t*, CH_2(3)); 22.6, 22.2 (2*q*, 2 Me). CI-MS: 323 (20), 322 (100, $[M + H]^+$). Anal. calc. for $C_{17}H_{23}NO_3S \cdot 0.1\ H_2O$ (323.24): C 63.17, H 7.23, N 4.33, S 9.92; found: C 63.01, H 7.12, N 4.28, S 9.71.

2.3. *2-(1-Methylethyl)-3-thia-1-azabicyclo[3.3.0]octa-5,7-dien-4-one* (*=1-(1-Methylethyl)-1*H*-pyrrolo[1,2-*c*]thiazol-3-one*, **9**). The same mixture as described in *Sect. 2.2* was stirred for 50 min at 90–100°. Evaporation and CC (CH_2Cl_2/hexane 5:2) yielded 21% of **8** as well as 47% of **9**.

Data for **9**: Yellow oil. IR (neat): 3359*w*, 3121*w*, 2966*m*, 2874*w*, 1693*s*, 1521*m*, 1460*m*, 1378*s*, 1306*w*, 1213*w*, 1065*m*, 1021*m*, 956*w*, 931*m*, 867*s*, 835*m*, 743*s*, 683*w*. ^{1}H-NMR: 7.08–7.07, 6.65–6.63, 6.54–6.52 (3*m*, 3 arom. H); 5.74 (*d*, $J = 3.7$, Me_2CHC*H*); 2.56–2.46 (*m*, Me_2C*H*CH); 1.08, 0.73 (2*d*, $J = 6.8$, *Me*$_2$CH). ^{13}C-NMR: 181.3 (*s*, CO); 131.0 (*s*, C(5)); 123.3, 116.6, 106.7 (3*d*, C(6), C(7), C(8)); 68.9 (*d*, Me_2CH*C*H); 34.4 (*d*, Me_2*C*HCH); 19.1, 14.1 (2*q*, 2 Me). CI-MS: 199 (100, $[M + NH_4]^+$), 182 (15, $[M + H]^+$), 181 (10, $M^{+\bullet}$).

2.4. *Methyl (2*S*,4*R*)-4-(Benzyloxy)*-N-*(1-chloro-2-methylprop-1-en-1-yl)prolinate*. To a stirred soln. of **8** (0.81 g, 2.52 mmol) in CH_2Cl_2 (10 ml) and 3 drops of DMF, cooled to 0°, was added a soln. of $COCl_2$ (2M in toluene, 1.3 ml, 2.6 mmol). After 40 min, an additional amount of $COCl_2$ (2M in toluene, 0.60 ml, 1.2 mmol) was added, and the mixture was stirred for another 90 min. Then, the solvent was evaporated, the residue was dissolved in abs. THF (20 ml), and 1,4-diazabicyclo[2.2.2]octane (DABCO; 0.288 g, 2.567 mmol) was added. After 20 min stirring at r.t., the mixture was filtered under N_2, and the residue was washed with THF. The pale yellow soln. was used directly in the subsequent reaction.

2.5. *Methyl (2*S*,4*R*)-4-(Benzyloxy)*-N-*(2,2-dimethyl-2*H*-azirin-3-yl)prolinate* (**10**). To the soln. from the above experiment was added NaN_3 (0.460 g, 7.076 mmol). After stirring overnight, the mixture was filtered through a *Celite* pad, washed with Et_2O, and evaporated. The residue was dissolved in AcOEt, the soln. washed with sat. aq. $NaHCO_3$ soln. and brine, and dried ($MgSO_4$). Evaporation and two consecutive CC (AcOEt/hexane 9:1 and AcOEt/hexane 1:1) gave 284 mg (37%) of **10**. Colorless oil. IR ($CHCl_3$): 3015*m*, 2950*m*, 2871*w*, 1768*s*, 1671*m*, 1495*w*, 1454*m*, 1438*m*, 1370*m*, 1266*m*, 1232*m*, 1178*m*, 1096*m*, 1027*w*, 908*w*, 851*w*, 698*w*. ^{1}H-NMR: 7.37–7.28 (*m*, 5 arom. H); 4.56–4.44 (*m*, PhCH_2, CH(2)); 4.31–4.24 (*m*, CH(4)); 3.79–3.71 (*m*, CH_2(5)); 3.75 (*s*, MeO); 2.50–2.45, 2.29–2.20 (2*m*, CH_2(3)); 1.33, 1.28 (2*s*, 2 Me). ^{13}C-NMR: 172.3 (*s*, CO); 166.3 (*s*, C(3′)); 137.5 (*s*, 1 arom. C); 128.5, 127.9, 127.6 (3*d*, 5 arom. C); 76.6 (*d*, CH(4)); 71.2 (*t*, PhCH_2); 59.6 (*d*, CH(2)); 52.5 (*q*, MeO); 39.8 (*s*, C(2′)); 36.3 (2*t*, CH_2(3), CH_2(5)); 25.1 (2*q*, 2 Me). ESI-MS: 627 ($[2M + Na]^+$), 605 ($[2M + H]^+$), 335 ($[M + MeOH + H]^+$), 325 ($[M + Na]^+$), 303 ($[M + H]^+$). Anal. calc. for $C_{17}H_{22}N_2O_3 \cdot 0.5\ H_2O$ (311.39): C 65.57, H 7.45, N 9.00; found: C 65.52, H 7.52, N 8.93.

3. *Syntheses of Model Peptides. General Procedure* (*GP*). To a soln. of the amino or peptide acid (1 equiv.) in dry CH_2Cl_2, **10** (*ca.* 1 equiv.) was added, and the mixture was stirred overnight. After evaporation, the residue was purified by CC (SiO_2).

3.1. *Methyl* N*-[(Benzyloxy)carbonyl]-(*S*)-leucyl-dimethylglycyl-(2*S*,4*R*)-4-(benzyloxy)prolinate* (Z-Leu-Aib-Hyp(Bn)-OMe, **12a**). According to the *GP*, Z-Leu-OH (92 mg, 0.347 mmol) in CH_2Cl_2 (5 ml) and **10** (101 mg, 0.334 mmol), CC (AcOEt/hexane 5:4): 128 mg (68%) of **12a**. Colorless, thick oil, which solidified under h.v. IR (KBr): 3298*s*, 3033*m*, 2958*s*, 1717*s*, 1661*s*, 1622*s*, 1528*s*, 1455*s*, 1413*s*, 1368*s*, 1262*s*, 1208*s*, 1114*s*,

1044*s*, 923*w*, 855*w*, 813*w*, 783*w*, 735*m*, 697*s*, 625*m*. ^{1}H-NMR: 7.29–7.19 (*m*, 10 arom. H); 7.05 (br. *s*, NH); 5.16 (*d*, *J* = 8.5, NH); 5.00 (*s*, PhC*H*$_2$(Z)); 4.55 (*dd*, *J* = 7.0, 8.3, CH(α)(Hyp)); 4.43, 4.37 (*AB*, *J* = 11.7, PhC*H*$_2$(Hyp)); 4.13–4.03 (*m*, CH(γ)(Hyp), CH(α)(Leu)); 3.68–3.58 (*m*, CH$_2$(δ)(Hyp)); 3.66 (*s*, MeO); 2.28–2.19, 1.96–1.85 (2*m*, CH$_2$(β)(Hyp)); 1.56–1.49 (*m*, CH(γ)(Leu)); 1.53, 1.48 (2*s*, 2 Me(Aib)); 1.44–1.31 (*m*, CH$_2$(β)(Leu)); 0.83 (*d*, *J* = 6.2, 2 Me(Leu)). ^{13}C-NMR: 171.8, 171.5, 169.6 (3*s*, 3 CO); 155.3 (*s*, CO(carbamate)); 136.5, 135.3 (2*s*, 2 arom. C); 127.6, 127.5, 127.2, 127.1, 127.0, 126.6 (6*d*, 10 arom. C); 77.4 (*d*, CH(γ)(Hyp)); 70.3 (*t*, PhCH$_2$(Hyp)); 66.0 (*t*, PhCH$_2$(Z)); 58.6 (*d*, CH(α)(Hyp)); 55.8 (*s*, C(α)(Aib)); 52.7 (*d*, CH(α)(Leu)); 52.1 (*t*, CH$_2$(δ)(Hyp)); 51.3 (*q*, MeO); 40.4 (*t*, CH$_2$(β)(Leu)); 32.1 (*t*, CH$_2$(β)(Hyp)); 23.7 (*d*, CH(γ)(Leu)); 22.5, 22.3, 21.9, 20.9 (4*q*, 2 Me(Aib), 2 Me(Leu)). ESI-MS: 590 ([*M* + Na]$^+$), 568 ($M^{+\cdot}$).

3.2. *Methyl* N-*{[(9*H*-Fluoren-9-yl)methoxy]carbonyl}-(*S*)-valyl-dimethylglycyl-(2*S*,4*R*)-4-(benzyloxy)prolinate* (Fmoc-Val-Aib-Hyp(Bn)-OMe, **12b**). According to the *GP*, Fmoc-Val-OH (139 mg, 0.41 mmol) in CH_2Cl_2 (5 ml) and **10** (124 mg, 0.41 mmol), CC (AcOEt/hexane 1:1): 250 mg (95%) of **12b**. Colorless, thick oil, which solidified under h.v. IR (KBr): 3381*m*, 3314*s*, 2962*m*, 2884*m*, 1721*s*, 1684*s*, 1614*s*, 1524*s*, 1469*s*, 1451*s*, 1420*s*, 1382*m*, 1360*m*, 1288*m*, 1242*s*, 1208*s*, 1180*s*, 1030*m*, 999*m*, 859*w*, 810*w*, 790*w*, 760*m*, 734*s*, 697*m*, 621*w*. ^{1}H-NMR: 7.76–7.22 (*m*, 13 arom. H); 7.11 (br. *s*, NH); 5.40 (*d*, *J* = 8.7, NH); 4.63 (*t*-like, *J* = 7.2, CH(α)(Hyp)); 4.50–4.38 (*m*, PhC*H*$_2$(Hyp), CH$_2$O(Fmoc)); 4.22–4.17 (*m*, CH(Fmoc), CH(γ)(Hyp)); 3.97–3.94 (*m*, CH(α)(Val)); 3.76–3.71 (*m*, CH$_2$(δ)(Hyp)); 3.73 (*s*, MeO); 2.29–2.25, 2.07–1.94 (2*m*, CH$_2$(β)(Hyp), CH(β)(Val)); 1.66, 1.61 (2*s*, 2 Me(Aib)); 0.93, 0.88 (2*d*, *J* = 6.6, 2 Me(Val)). ^{13}C-NMR: 172.6, 172.5 (2*s*, CO(Hyp), CO(Aib)); 169.3 (*s*, CO(Val)); 156.3 (*s*, CO(carbamate)); 143.9, 141.2, 137.3 (3*s*, 5 arom. C); 128.4, 127.9, 127.6, 127.1, 126.9, 125.0, 119.8 (7*d*, 13 arom. C); 77.4 (*d*, CH(γ)(Hyp)); 71.2 (*t*, PhC*H*$_2$(Hyp)); 66.7 (*t*, CH$_2$O(Fmoc)); 60.3 (*d*, CH(α)-(Val)); 59.6 (*d*, CH(α)(Hyp)); 56.9 (*s*, C(α)(Aib)); 53.0 (*t*, CH$_2$(δ)(Hyp)); 52.2 (*q*, MeO); 47.1 (*d*, CH(Fmoc)); 33.1 (*t*, CH$_2$(β)(Hyp)); 31.2 (*d*, CH(β)(Val)); 22.9, 22.8 (2*q*, 2 Me(Aib)); 19.0, 17.7 (2*q*, 2 Me(Val)). ESI-MS: 664 ([*M* + Na]$^+$). Anal. calc. for $C_{17}H_{22}N_2O_3$ (641.77): C 69.25, H 6.75, N 6.55; found: C 69.16, H 7.04, N 6.49.

3.3. *Methyl* N-*[(*tert*-Butoxy)carbonyl]-(*S*)-alanyl-dimethylglycyl-(2*S*,4*R*)-4-(benzyloxy)prolinate* (Boc-Ala-Aib-Hyp(Bn)-OMe, **12c**). According to the *GP*, Boc-Ala-OH (78 mg, 0.41 mmol) in CH_2Cl_2 (5 ml) and **10** (123 mg, 0.41 mmol), CC (CH_2Cl_2, 1.25% MeOH): 177 mg (88%) of **12c**. Colorless solid. M.p. 207–209°. IR (KBr): 3368*s*, 3285*s*, 3062*w*, 2979*m*, 2935*m*, 2795*w*, 1730*s*, 1713*s*, 1683*s*, 1611*s*, 1546*s*, 1525*s*, 1500*m*, 1471*m*, 1456*m*, 1425*s*, 1376*s*, 1356*s*, 1299*m*, 1263*m*, 1242*s*, 1212*s*, 1173*s*, 1138*m*, 1114*m*, 1068*m*, 1036*m*, 1018*m*, 946*w*, 862*w*, 807*w*, 791*w*, 735*m*, 695*w*, 646*m*, 628*m*. ^{1}H-NMR ((D$_6$)DMSO): 8.03 (br. *s*, NH); 7.34–7.27 (*m*, 13 arom. H); 6.74 (br. *s*, NH); 4.48 (*s*, PhC*H*$_2$(Hyp)); 4.32–4.26, 4.21–4.13 (2*m*, CH(α)(Hyp), CH(γ)(Hyp)); 4.08–3.97, 3.82–3.74 (2*m*, CH$_2$(δ)(Hyp); 3.62 (*d*, *J* = 7.7, CH(α)(Ala)); 3.59 (*s*, MeO); 2.12–1.88 (*m*, CH$_2$(β)(Hyp)); 1.33 (*s*, Me$_3$C); 1.33, 1.31 (2*s*, 2 Me(Aib)); 1.17 (*d*, *J* = 7.1, Me(Ala)). ^{13}C-NMR ((D$_6$)DMSO): 172.3, 171.5 (2*s*, 3 CO); 154.8 (*s*, CO(carbamate)); 138.0 (*s*, 1 arom. C); 128.0, 127.3 (2*d*, 5 arom. C); 77.9 (*s*, Me$_3$*C*); 76.4 (*d*, CH(γ)(Hyp)); 70.0 (*t*, PhCH$_2$(Hyp)); 58.7 (*d*, CH(α)(Hyp)); 55.2 (*s*, C(α)(Aib)); 51.5 (*t*, CH$_2$(δ)(Hyp)); 51.4 (*q*, MeO); 49.4 (*d*, CH(α)(Ala)); 32.6 (*t*, CH$_2$(β)(Hyp)); 27.9 (*q*, *Me*$_3$C); 24.7, 24.2 (2*q*, 2 Me(Aib)); 18.1 (*q*, Me(Ala)). CI-MS: 727 (50, [2*M* − 255]$^+$), 492 (95, [*M* + 1]$^+$), 236 (100, [*M* − 255]$^+$). Anal. calc. for $C_{25}H_{37}N_3O_7 \cdot 0.33\ H_2O$ (497.60): C 60.34, H 7.63, N 8.44; found: C 60.45, H 7.58, N 8.30.

3.4. *Methyl* N-*{[(9*H*-Fluoren-9-yl)methoxy]carbonyl}-(*S*)-phenylalanyl-glycyl-dimethylglycyl-(2*S*,4*R*)-4-(benzyloxy)prolinate* (Fmoc-Phe-Gly-Aib-Hyp(Bn)-OMe, **13**). According to the *GP*, Fmoc-Phe-Gly-OH (120 mg, 0.27 mmol) in CH_2Cl_2 (5 ml) and **10** (82 mg, 0.27 mmol), CC (AcOEt): 195 mg (97%) of **13**. Colorless enamel. IR (KBr): 3297*m*, 3363*m*, 3030*m*, 2945*m*, 1744*s*, 1649*s*, 1622*s*, 1522*s*, 1452*s*, 1410*s*, 1363*m*, 1333*m*, 1259*s*, 1206*s*, 1177*m*, 1032*m*, 945*w*, 914*w*, 856*w*, 741*s*, 700*m*, 647*w*, 621*w*. ^{1}H-NMR: 7.74–7.21 (*m*, 18 arom. H, 2 NH); 5.81 (*d*, NH); 4.63 (*t*-like, *J* = 7.3, CH(α)(Hyp)); 4.51–4.08 (*m*, PhC*H*$_2$(Hyp), CH$_2$O(Fmoc), CH(α)(Phe), CH(Fmoc), CH(γ)(Hyp)); 3.98–3.68 (*m*, CH$_2$(δ)(Hyp), CH$_2$(α)(Gly)); 3.64 (*s*, MeO); 3.22–3.01 (*m*, CH$_2$(β)(Phe)); 2.24–2.02, 1.95–1.88 (2*m*, CH$_2$(β)(Hyp)); 1.52, 1.51 (2*s*, 2 Me(Aib)). ^{13}C-NMR: 172.9, 172.3, 171.8, 167.7 (4*s*, 4 CO); 156.3 (*s*, CO(carbamate)); 143.6, 141.2, 137.6, 136.7 (4*s*, 6 arom. C); 129.1, 128.6, 128.3, 127.7, 127.5, 127.0, 126.9, 124.9, 119.9 (9*d*, 18 arom. C); 77.3 (*d*, CH(γ)(Hyp)); 71.2 (*t*, PhC*H*$_2$(Hyp)); 66.1 (*t*, CH$_2$O(Fmoc)); 59.5 (*d*, CH(α)(Hyp)); 56.8 (*s*, C(α)(Aib)); 56.6 (*d*, CH(α)(Phe)); 52.8 (*t*, CH$_2$(δ)(Hyp)); 52.0 (*q*, MeO); 47.0 (*d*, CH(Fmoc)); 43.3 (*t*, CH$_2$(α)(Gly)); 37.4 (*t*, CH$_2$(β)(Phe)); 33.3 (*t*, CH$_2$(β)(Hyp)); 24.5, 23.9 (2*q*, 2 Me(Aib)). ESI-MS: 769 ([*M* + Na]$^+$). Anal. calc. for $C_{43}H_{46}N_4O_8 \cdot 0.75\ H_2O$ (760.38): C 67.92, H 6.30, N 7.37; found: C 67.79, H 6.24, N 7.14.

3.5. *Methyl* N-*[(*tert*-Butoxy)carbonyl]-(*S*)-alanyl-dimethylglycyl-(2*S*,4*R*)-4-(benzyloxy)prolyl-(*S*)-valinate* (Boc-Ala-Aib-Hyp(Bn)-Val-OMe, **14**). To a soln. of **12c** (78 mg, 0.16 mmol) in THF/MeOH/H_2O 3:1:1 (5 ml) was added LiOH · H_2O (20 mg, 0.48 mmol), and the mixture was stirred at r.t. After 20 h, the mixture was transferred into a separatory funnel, diluted with 5% $KHSO_4$ soln., and extracted with CH_2Cl_2 (3×). The combined org. phase was dried ($MgSO_4$) and concentrated *in vacuo*, yielding 76 mg (quant.) of Boc-Ala-Aib-

Hyp-Val-OH (**15**) as a crude product. To a soln. of Val-OMe · HCl (30 mg, 0.176 mmol), **15** (76 mg, 0.16 mmol), and *O*-(benzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU, 54 mg, 0.168 mmol) in MeCN (4 ml) was added EtN(i-Pr)$_2$ (0.08 ml, 0.48 mmol), and the mixture was stirred at r.t. until **15** was completely consumed (TLC). After 20 h, the soln. was concentrated *in vacuo*. CC (AcOEt/hexane 2:1 → AcOEt) gave 90 mg (96%) of **14**. Colorless, thick oil, which solidified under h.v. IR (KBr): 3297*s*, 2977*s*, 2936*m*, 2876*m*, 1741*s*, 1711*s*, 1658*s*, 1536*s*, 1469*s*, 1454*s*, 1409*m*, 1366*s*, 1312*m*, 1248*s*, 1169*s*, 1094*m*, 1072*s*, 1027*m*, 901*w*, 856*w*, 821*w*, 738*m*, 698*w*, 611*w*. ^{1}H-NMR ((D$_6$)DMSO): 8.12 (br. *s*, NH); 7.89 (*d*, *J* = 8.0, NH); 7.36–7.27 (*m*, 5 arom. H); 6.81 (br. *s*, NH); 4.48 (*s*, PhC*H*$_2$(Hyp)); 4.12–4.01, 3.83–3.70 (2*m*, CH(*α*)(Hyp), CH(*γ*)(Hyp)), CH$_2$(*δ*)(Hyp)); 3.54–3.42, 3.39–3.29 (2*m*, CH(*α*)(Ala), CH(*α*)(Val)); 3.60 (*s*, MeO); 2.18–2.05, 1.93–1.79 (2*m*, CH$_2$(*β*)(Hyp), CH(*β*)(Val)); 1.37 (*s*, Me$_3$C); 1.37, 1.33 (2*s*, 2 Me(Aib)); 1.19 (*d*, *J* = 7.1, Me(Ala)); 0.90, 0.87 (2*d*, *J* = 6.8, 2 Me(Val)). ^{13}C-NMR ((D$_6$)DMSO): 172.1, 171.7, 171.5, 171.4 (4*s*, 4 CO); 154.9 (*s*, CO(carbamate)); 138.1 (*s*, 1 arom. C); 128.1, 127.2 (2*d*, 5 arom. C); 78.0 (*s*, Me$_3$*C*); 76.6 (*d*, CH(*γ*)(Hyp)); 69.7 (*t*, Ph*C*H$_2$(Hyp)); 59.4, 57.5 (2*d*, CH(*α*)(Hyp), CH(*α*)(Val)); 55.5 (*s*, C(*α*)(Aib)); 52.4 (*t*, CH$_2$(*δ*)(Hyp)); 51.2 (*q*, MeO); 49.7 (*d*, CH(*α*)(Ala)); 33.7 (*t*, CH$_2$(*β*)(Hyp)); 29.4 (*d*, CH(*β*)(Val)); 27.9 (*q*, *Me*$_3$C); 25.0, 24.4 (2*q*, 2 Me(Aib)); 18.8, 18.4 (2*q*, 2 Me(Val)); 18.1 (*q*, Me(Ala)). ESI-MS: 613 ([*M* + Na]$^+$). Anal. calc. for C$_{30}$H$_{46}$N$_4$O$_8$ · H$_2$O (608.74): C 59.19, H 7.95, N 9.20; found: C 59.31, H 8.05, N 9.15.

4. *Deprotection of the OH Group*. 4.1. *Methyl* N-*[(*tert-*Butoxy)carbonyl]-(*S*)-alanyl-dimethylglycyl-(2*S*,4*R*)-4-hydroxyprolinate* (Boc-Ala-Aib-Hyp-OMe, **16**). To a soln. of **12c** (83 mg, 0.17 mmol) in MeOH/AcOH 10:1 (5 ml) were added Pd/C (9 mg) and Pd black (15 mg), and the soln. was stirred under H$_2$ overnight. After filtration through a *Celite* pad, CC (AcOEt), and drying (MgSO$_4$), 59 mg (87%) of **16** were obtained. Colorless solid. M.p. 203–204°. IR (KBr): 3440*m*, 3291*s*, 2983*m*, 2954*m*, 2937*m*, 1739*s*, 1710*s*, 1677*s*, 1538*s*, 1453*m*, 1429*s*, 1391*m*, 1364*s*, 1313*m*, 1278*m*, 1248*s*, 1176*s*, 1106*m*, 1086*m*, 1070*m*, 1115*m*, 954*w*, 880*w*, 854*w*, 756*w*, 642*w*, 623*w*. ^{1}H-NMR: 7.27 (br. *s*, NH); 5.58 (br. *s*, NH); 4.64 (*t*-like, *J* = 8.0, CH(*α*)(Hyp)); 4.47 (br. *s*, CH(*γ*)(Hyp)); 4.17–4.05, 3.99–3.84 (2*m*, CH$_2$(*δ*)(Hyp)); 3.71 (*s*, MeO); 3.62 (*d*, *J* = 7.7, CH(*α*)(Ala)); 2.31–2.18, 1.97–1.84 (2*m*, CH$_2$(*β*)(Hyp)); 1.53, 1.52 (2*s*, 2 Me(Aib)); 1.44 (*s*, Me$_3$C); 1.32 (*d*, *J* = 7.9, Me(Ala)). ^{13}C-NMR: 173.1, 171.7 (2*s*, 3 CO); 156.1 (*s*, CO(carbamate)); 80.0 (*s*, Me$_3$*C*); 70.6 (*d*, CH(*γ*)(Hyp)); 59.2 (*d*, CH(*α*)(Hyp)); 56.4 (*s*, C(*α*)(Aib)); 55.4 (*t*, CH$_2$(*δ*)(Hyp)); 52.0 (*q*, MeO); 50.0 (*d*, CH(*α*)(Ala)); 35.9 (*t*, CH$_2$(*β*)(Hyp)); 28.2 (*q*, *Me*$_3$C); 24.9, 24.3 (2*q*, 2 Me(Aib)); 17.7 (*q*, Me(Ala)). CI-MS: 547 (50, [*M* + 146 + 1]$^+$), 402 (100, [*M* + 1]$^+$), 146 (90). Anal. calc. for C$_{25}$H$_{37}$N$_3$O$_7$ · 0.25 H$_2$O (405.96): C 53.26, H 7.82, N 10.35; found: C 53.46, H 7.71, N 9.97.

4.2. *Methyl* N-*[(*tert-*Butoxy)carbonyl]-(*S*)-alanyl-dimethylglycyl-(2*S*,4*R*)-4-hydroxyprolyl-(*S*)-valinate* (Boc-Ala-Aib-Hyp-Val-OMe, **17**). To a soln. of **14** (60 mg, 0.102 mmol) in MeOH/AcOH 10:1 (5 ml) were added Pd/C (10 mg) and Pd black (25 mg), and the soln. was stirred under H$_2$ during 2 d. After filtration through a *Celite* pad, CC (AcOEt), and drying (MgSO$_4$), 41 mg (80%) of **17** were obtained. Colorless enamel. IR (KBr): 3304*s*, 2978*s*, 2937*m*, 2878*w*, 1741*s*, 1659*s*, 1544*s*, 1470*m*, 1415*s*, 1393*m*, 1367*s*, 1313*m*, 1249*s*, 1207*m*, 1170*s*, 1071*m*, 1025*m*, 964*w*, 921*w*, 855*w*, 802*w*, 778*w*, 757*w*, 732*w*, 645*w*, 606*w*. ^{1}H-NMR: 7.63 (br. *d*, *J* = 7.8, NH); 7.30 (br. *s*, NH); 5.59 (br. *s*, NH); 4.79 (*t*-like, *J* = 8.1, CH(*α*)(Hyp)); 4.41 (br. *s*, CH(*γ*)(Hyp)); 4.33 (*dd*, *J* = 7.9, 6.0, CH(*α*)(Ala)); 4.17–4.08, 4.00–3.93 (2*m*, CH$_2$(*δ*)(Hyp)); 3.70 (*s*, MeO); 3.62 (*dd*, *J* = 11.3, 7.7, CH(*α*)(Val)); 2.26–2.17, 2.06–1.97 (2*m*, CH$_2$(*β*)(Hyp), CH(*β*)(Val)); 1.52, 1.49 (2*s*, 2 Me(Aib)); 1.44 (*s*, Me$_3$C); 1.27 (*d*, *J* = 7.2, Me(Ala)); 0.95, 0.94 (2*d*, *J* = 6.8, 2 Me(Val)). ^{13}C-NMR: 172.5, 172.2, 172.0, 171.9 (4*s*, 4 CO); 156.0 (*s*, CO(carbamate)); 80.3 (*s*, Me$_3$*C*); 70.5 (*d*, CH(*γ*)(Hyp)); 60.3, 57.8 (2*d*, CH(*α*)(Hyp), CH(*α*)(Val)); 56.5 (*s*, C(*α*)(Aib)); 56.0 (*t*, CH$_2$(*δ*)(Hyp)); 51.7 (*q*, MeO); 49.7 (*d*, CH(*α*)(Ala)); 36.3 (*t*, CH$_2$(*β*)(Hyp)); 30.3 (*d*, CH(*β*)(Val)); 28.2 (*q*, *Me*$_3$C); 25.1 (*q*, 2 Me(Aib)); 18.9, 18.3 (2*q*, 2 Me(Val)); 17.4 (*q*, Me(Ala)). CI-MS: 502 (100, [*M* + 1]$^+$), 245 (70).

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