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HETEROSPIROCYCLIC 3-AMINO-2*H*-AZIRINES AS CONVENIENT BUILDING BLOCKS IN PEPTIDE SYNTHESIS

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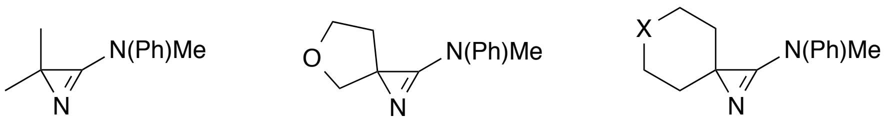
Dedicated to Professor Dr. Tohru Fukuyama on the occasion of his 70th birthday

Abstract – Heterospirocyclic 3-amino-2*H*-azirines with a tetrahydropyran (Thp), tetrahydrothiopyran (Tht), or *N*-protected piperidine (Pip) moiety are useful reagents for the preparation of peptides containing the corresponding six-membered heterocyclic 4-amino-4-carboxylic acid unit. In the present study, tripeptides of the type H-Asp-D-Ala-Xaa-OMe, where Xaa is the heterocyclic amino acid, were prepared according to the 'azirine/oxazolone method'.

INTRODUCTION

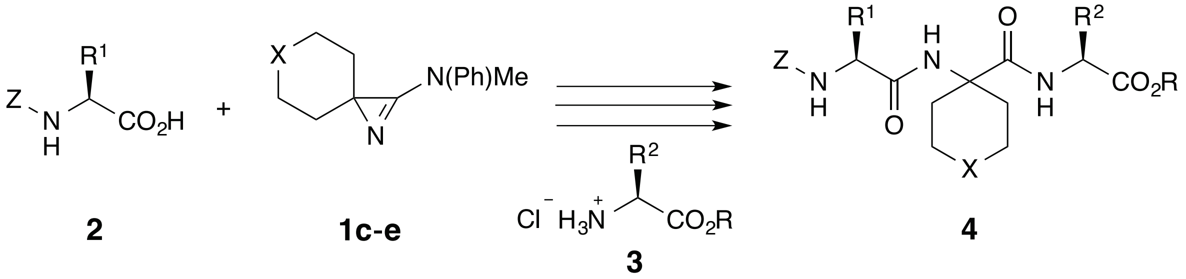
The so-called 'azirine/oxazolone method'[2] is a convenient approach for the synthesis of peptides containing α,α-disubstituted α-amino acids, e.g., α-aminoisobutyric acid (peptaibols).[3] The utility of the 2,2-dimethyl-substituted 3-amino-2*H*-azirine **1a** in the preparation of such peptides has been demonstrated in a long series of publications.[4] A special type of α,α-dialkylated α-amino acids are five- and six-membered heterocyclic n-amino-n-carboxylic acids,[5,6] and their synthons in the method mentioned above are spirocyclic 3-amino-2*H*-azirines **1b**–**e**.[7]



1a **1b** **1c** X = NBoc
1d X = O
1e X = S

Figure 1. 3-Amino-2*H*-azirines as synthons for α-aminoisobutyric acid (Aib, **1a**) and heterocyclic n-amino-n-carboxylic acids (**1b**–**e**)

In a recent publication, we have shown that 3-amino-2*H*-azirines **1c–e** are useful synthons for the preparation of tripeptides of type **4** (Scheme 1), which can be used further for the synthesis of larger peptides.[8] The first reaction step is the azirine coupling with an *N*-protected amino acid **2**, followed by a selective hydrolysis of the terminal amide function and the second coupling with an amino acid ester **3** via a 1,3-oxazol-5-one intermediate.

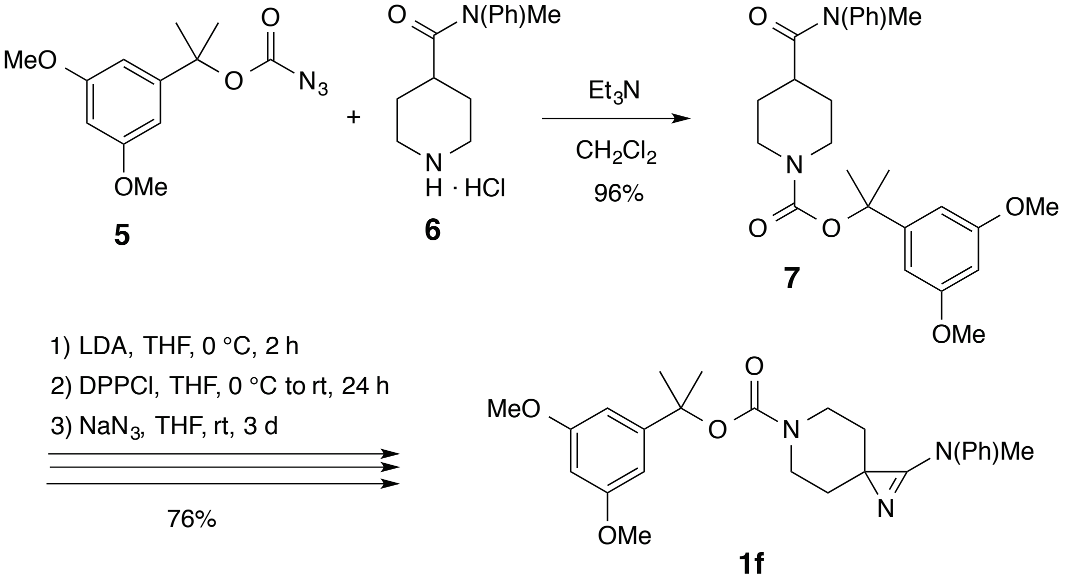


Scheme 1. Synthesis of tripeptides of type **4** (Z = benzyloxycarbonyl)

An interesting type of tripeptides containing α,α-disubstituted α-amino acids are L-aspartyl-D-alanyl-AC$_n$C-OMe derivatives, with AC$_n$C = 1-aminocycloalkane-1-carboxylic acids, which have been studied as potential peptide sweeteners by Goodman and coworkers.[9] For example, it was shown that the taste of the tripeptides with the six-membered and smaller carbocyclic amino acids is sweet, whereas the analogues with larger carbocyclic rings taste bitter. The goal of the present study was the synthesis of analogous tripeptides containing a heterocyclic six-membered 4-amino-4-carboxylic acid by using heterospirocyclic 3-amino-2*H*-azirines of type **1c–e** as amino acid synthons.

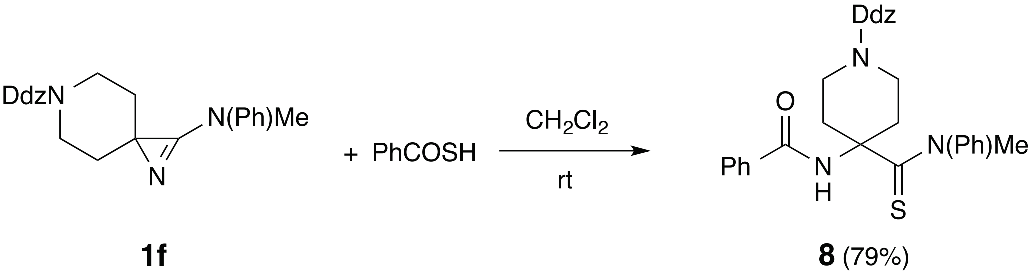
RESULTS AND DISCUSSION

Whereas the syntheses of the heterospirocyclic 3-amino-2*H*-azirines **1c–e** were carried out under standard conditions,[7c] the preparation of analogues of **1c** with a benzyloxycarbonyl (Z) or allyloxycarbonyl (Alloc) protecting group failed. With the intention to have in hand also a synthon for 4-aminopiperidine-4-carboxylic acid with a benzyloxycarbonyl-type of protecting group, we prepared the 3’,5’-dimethoxy-1,1-dimethylbenzyloxycarbonyl (Ddz)-protected azirine **1f** (Scheme 2). Thus, the reaction of the azide **5**[10] with *N*-methyl-*N*-phenylpiperidine-4-carboxamide hydrochloride (**6**) in CH_2Cl_2/Et_3N at room temperature gave the Ddz-protected amide **7** in 96% yield. Stepwise reaction of the latter with LDA, diphenyl chlorophosphate (DPPCl), and NaN_3 according to our previously described protocol[7b,11] led to the desired azirine **1f** in 76% yield.



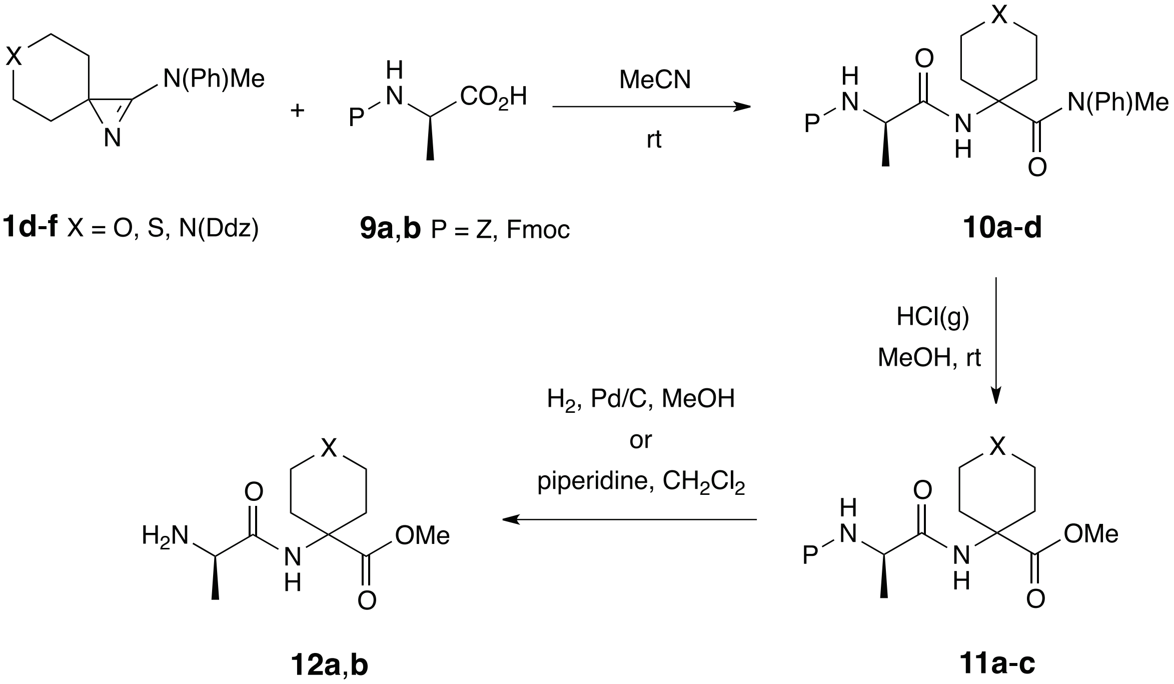
Scheme 2. Synthesis of the Ddz-protected heterospirocyclic 3-amino-2*H*-azirine **1f**

Similar to other 2*H*-azirines of type **1**, the new derivative **1f** is a stable compound, which can be stored in the refrigerator for long time. Furthermore, its reactivity towards acidic compounds is analogous to that of **1a**–**e**. For example, the reaction with thiobenzoic acid in CH_2Cl_2 at room temperature[11] gave the 4-(benzoylamino)piperidine-4-thioamide **8** in 79% yield (Scheme 3).



Scheme 3. Reaction of 3-amino-2*H*-azirine **1f** with thiobenzoic acid

The reaction of the heterospirocyclic 3-amino-2*H*-azirines **1d**–**f** with Z- or Fmoc-protected D-alanine (D-Ala) under the standard conditions of the 'azirine coupling' (MeCN, rt) led to the enantiomerically pure (NMR) dipeptide amides **10a**–**d** in 85–95% yield (Scheme 4, Table 1). In analogy to the selective hydrolysis of peptide amides of type **10**, treatment of **10a**,**b** with HCl gas in MeOH at rt to ca. 60 °C gave the dipeptide methyl esters **11a**,**b** in excellent yields without loss of stereochemical purity. In the case of the piperidine derivative **10c**, the Ddz-protecting group was also removed, and the methyl ester **11c** with the free NH group in the heterocycle was obtained in 68% yield. Subsequent deprotection of the terminal amino group of **11a**,**b** by hydrogenolysis or treatment with piperidine in CH_2Cl_2, respectively, yielded the desired dipeptide methyl esters **12a**,**b** in good yields.



Scheme 4. Synthesis of dipeptide esters **12** via ‘azirine coupling’ (see also Table 1)

Table 1. Synthesis of H-Asp-D-Ala-Xaa-OMe tripeptides **15** via the ‘azirine/oxazolone method’

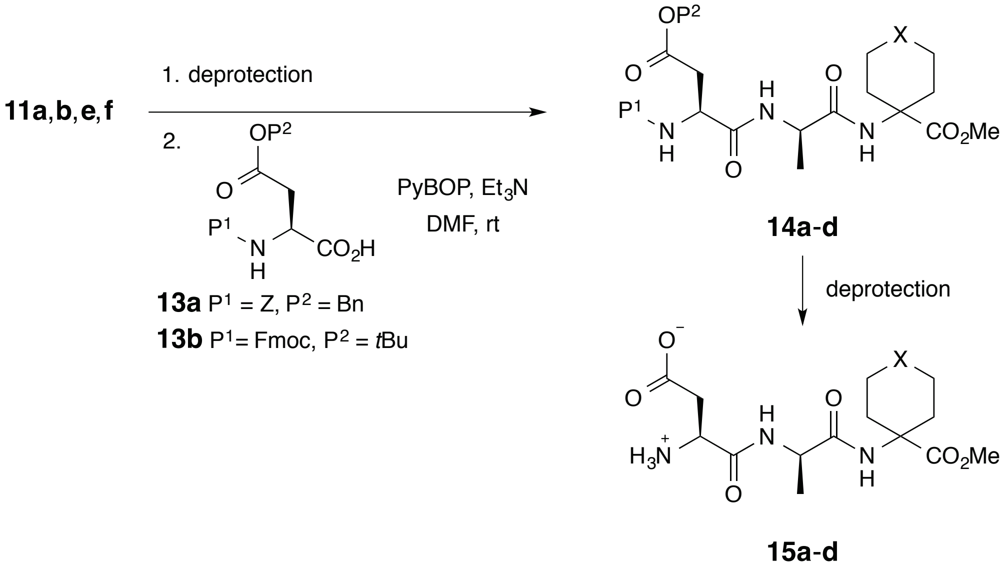
1	X	9	P	10	Yield (%)[a]	11	Yield (%)[a]	12	Yield (%)[a]	14	Yield (%)[a]	15	Yield (%)[a]
d	O	**a**	Z	**a**	92	**a**	92	**a**	81	**a**	80	**a**	98
e	S	**b**	Fmoc	**b**	87	**b**	94	**b**	76	**b**	92	**b**	ca. 90[j]
f	N(Ddz)	**a**	Z	**c**	95	**c**[b]	68			-		-	
						d[c]	87			-		-	
						e[d]	70	-		**c**[f]	84	**c**[h]	quant.
f	N(Ddz)	**b**	Fmoc	**d**	85	**f**[e]	76	-		**d**[g]	44	**d**[i]	ca. 90[j]

[a] Yield of isolated product. [b] The dipeptide ester with the deprotected piperidine ring (X = NH) was obtained. [c] X = N(Fmoc), P = Z; obtained from **11c**. [d] X = N(Boc), P = Z; obtained from **11d**. [e] X = N(Z), P = Fmoc; obtained from **10d**. [f] X = N(Boc), P^1 = Z, P^2 = Bn. [g] X = N(Z), P^1 = Fmoc, P^2 = *t*Bu. [h] X = N(Boc). [i] X = N(Z). [j] Not obtained in pure form.

With the aim of obtaining dipeptide methyl esters of type **11** with a *N*-protected piperidine moiety, **11c** was treated with Fmoc-chloride and Et_3N in CH_2Cl_2 at rt to give Z-D-Ala-Pip(Fmoc)-OMe (**11d**) in 87% yield (Table 1). The same product could be prepared from **10c** in an analogous manner without isolation of the intermediate **11c** (76% yield). Furthermore, the exchange of the Fmoc against the Boc protecting group was performed by subsequent treatment of **11d** with piperidine in CH_2Cl_2 followed by

Boc-anhydride $(Boc)_2O$)/Et_3N to give Z-D-Ala-Pip(Boc)-OMe (**11e**). Similarly, Fmoc-D-Ala-Pip(Z)-OMe (**11f**) was obtained from **10d** via acid-catalyzed methanolysis and treatment of the crude product with Et_3N and Z-chloride (76% yield).

Unfortunately, the isolated deprotected dipeptides **12a**,**b** were not soluble in suitable solvents for peptide coupling. For this reason, the coupling with the diprotected aspartic acid derivatives Z-Asp(OBn)-OH (**13a**) and Fmoc-Asp(O*t*Bu)-OH (**13b**), respectively, were carried out with the crude materials **12a**,**b**. Thus, **11a** was deprotected hydrogenolytically in DMF/MeOH, the catalyst was removed by filtration and MeOH evaporated. Then, **13a**, Et_3N, and PyBOP as the coupling reagent were added, and after 16 h the tripeptide **14a** was obtained in 80% yield (Scheme 5, Table 1). In an analogous manner, **11e** was coupled with **13a** to give the fully protected Z-Asp(OBn)-D-Ala-Pip(Boc)-OMe (**14c**) in 84% yield. Both tripeptides were transformed into the desired zwitterionic analogues **15a**,**c** via hydrogenolytic deprotection.



Scheme 5. Synthesis of tripeptide methyl esters **15** (H-Asp-D-Ala-Xaa-OMe; see also Table 1)

The Fmoc-protected dipeptides **11b**,**f** were deprotected by treatment with piperidine in DMSO at rt. After evaporation of piperidine, **13b** and PyBOP were added, and after stirring at rt for 16 h and chromatographic purification, **14b** and **14d** were isolated in 92 and 44% yield, respectively. The deprotection to give **15b**,**d** was achieved in two steps: removal of the Fmoc group with piperidine in CH_2Cl_2 at rt and subsequent cleavage of the *tert*-butyl ester by treatment with trifluoroacetic acid (TFA). Whereas the partially deprotected tripeptides H-Asp(O*t*Bu)-D-Ala-Tht-OMe and H-Asp(O*t*Bu)-D-Ala-Pip(Z)-OMe were obtained in pure forms, the desired zwitterionic compounds **15b**,**d** were still contaminated with small amounts of impurities, which could not be removed.

CONCLUSIONS

The heterospirocyclic 3-amino-2*H*-azirines **1d–f** were shown to be convenient starting materials for the synthesis of peptides containing saturated six-membered heterocyclic 4-amino-4-carboxylic acids. In addition to the previously described **1d** and **1e** containing a tetrahydropyran and tetrahydrothiopyran ring, respectively, the new synthon **1f**, a 1,6-diazaspiro[2.5]oct-1-ene derivative with a Ddz-protected piperidine moiety, was used for the 'azirine coupling' with *N*-protected D-alanine to give the corresponding dipeptide amides in high yields. The Ddz protecting group can be removed easily under acidic conditions allowing the smooth exchange of the protecting group. The terminal amide function of the dipeptides with a heterocyclic α-amino carboxylic acid was transformed into the corresponding methyl ester via selective methanolysis. Deprotection of the *N*-terminus and coupling with asparagine derivatives leads to the fully protected tripeptide esters of type P^1-Asp(P^2)-D-Ala-Xaa-OMe. The deprotection of the examples with hydrogenolytically removable groups was achieved successfully to give the zwitterionic tripeptide esters, which are analogues of those with a carbocyclic α-amino carboxylic acid. It is worth mentioning that the taste of tripeptide **15a** with a tetrahydropyran moiety is sweet, similar to the cyclohexane analogue,[9] whereas that of **15c** with a Boc-protected piperidine ring is bitter.

EXPERIMENTAL

General remarks. Melting points were determined using a Mettler FP5 apparatus, and they are uncorrected. Thin layer chromatography (TLC): Merck silica gel 60 F_{254} plates (0.25 mm); column chromatography (CC): silica gel Merck 60 (0.040–0.063 mm. The IR spectra were recorded on a Perkin-Elmer 297 or Perkin-Elmer 781 spectrophotometer in $CDCl_3$ or in KBr; absorptions in cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra were measured on a Bruker ARX-300, AM-400 or AMX-600 instrument (300/75.4, 400/100.6, and 600/150.9 MHz, resp.) in $CDCl_3$ with TMS as internal standard. Chemical shifts (δ) are given in ppm and coupling constants *J* in Hz. Mass spectra (MS) were recorded on a Finnigan SSQ-700 (CI, NH_3, 150 eV) or Finnigan TSQ-700 (ESI) instrument. Optical rotations [α_D]: Zeiss LEP-A_2 polarimeter, in MeOH at 20–22 °C.

Starting materials. The synthesis of *N*-methyl-*N*-phenyl-6-oxa-1-azaspiro[2.5]oct-1-en-2-amine (**1d**) and *N*-methyl-*N*-phenyl-6-thia-1-azaspiro[2.5]oct-1-en-2-amine (**1e**) has been described previously.[7b] The amino acid derivatives Z- and Fmoc-D-Ala (**9a**,**b**) and Z, Bn- and Fmoc, *t*Bu-protected Asp as well as all used reagents were commercially available. Reported yields refer to isolated pure products.

Abbreviations. AcOEt, ethyl acetate; D-Ala, D-alanine; Alloc, allyloxycarbonyl; Asp, aspartic acid; Bn, benzyl; Boc, *tert*-butyloxycarbonyl; CC, column chromatography; Ddz, 3',5'-dimethoxy-1,1-dimethylbenzyloxycarbonyl; DMF, *N,N*-dimethylformamide; DPPCl, diphenyl chlorophosphate; Et_2O, diethyl ether; Fmoc, fluorenylmethyloxycarbonyl; LDA, lithium diisopropylamide; MeCN, acetonitrile; Pip, 4-aminopiperidine-4-carboxylic acid; PyBOP, [(benzotriazol-1-yl)oxy]tripyrrolidinophosphonium hexafluorophosphate; THF, tetrahydrofuran; Thp, 4-aminotetrahydropyran-4-carboxylic acid; Tht, 4-aminotetrahydrothiopyran-4-carboxylic acid; Z, benzyloxycarbonyl.

Synthesis of 6-[(3',5'-dimethoxy-1,1-dimethylbenzyloxy)carbonyl]-2-(*N*-methyl-*N*-phenylamino)-1,6-diazaspiro[2.5]oct-1-ene (1f). **1-[(3',5'-Dimethoxy-1,1-dimethylbenzyloxy)carbonyl]piperidine-4-(*N*-methyl-*N*-phenylcarboxamide) (7).** To a stirred solution of 9.86 g (38.7 mmol) piperidine-4-(*N*-methyl-*N*-phenylcarboxamide) hydrochloride (**6**) in CH_2Cl_2 (100 mL) at 0 °C were added Et_3N (12 mL, 86 mmol) and (3',5'-dimethoxy-1,1-dimethylbenzyloxy)carbonyl azide[10] (**5**; 11.3 g, 42.6 mmol). After 1 h at rt, the mixture was washed with aqueous Na_2CO_3 solution (10%, 2×), and the aqueous solutions were extracted with CH_2Cl_2. The combined organic phases were dried (Na_2SO_4) and the solvent evaporated. CC (hexane/AcOEt 1:2) and recrystallization from CH_2Cl_2 gave 16.5 g (96%) of **7**. Colorless crystals; mp 125–126 °C. IR ($CHCl_3$): 3460*w*, 3020*w*, 3000*m*, 2950*m*, 2860*w*, 2840*w*, 1680*s*, 1645*s*, 1600*s*, 1495*m*, 1470*m*, 1455*m*, 1430*s*, 1390*m*, 1350*m*, 1310*m*, 1300*m*, 1285*m*, 1270*m*, 1220*m*, 1160*s*, 1135*m*, 1070*m*, 1050*m*, 1025*m*, 965*w*, 935*w*, 890*w*, 845*w*, 835*w*. ^{1}H-NMR ((D_6)DMSO, 85 °C): 7.47–7.26, 6.44–6.43, 6.36–6.34 (3*m*, 8 arom. H); 3.97–3.68 (*dt*-like, 2 H of $-CH_2NCH_2-$); 3.73 (*s*, 2 MeO); 3.16 (*s*, MeN); 2.62–2.40 (*m*, 2 H of $-CH_2NCH_2-$, CH(4)); 1.66 (*s*, Me_2C); 1.61–1.44 (*m*, $-CH_2CCH_2-$). ^{13}C-NMR ((D_6)DMSO, 85 °C): 173.1 (*s*, C=O); 160.6 (*s*, 2 arom. COMe); 152.6, 148.4, 143.5 (3*s*, OCON, 2 arom. C); 129.1, 126.9, 126.6, 102.6, 98.2 (5*d*, 8 arom. CH); 79.9 (*s*, Me_2CO); 54.7 (*q*, 2 MeO); 42.4 (*t*, $-CH_2NCH_2-$); 37.8 (*d*, CH(4)); 36.6 (*q*, MeN); 28.2 (*q*, Me_2C); 27.7 (*t*, $-CH_2CCH_2-$). ESI-MS: 463 (100, $[M+Na]^+$), 241 (36). Anal. Calcd for $C_{25}H_{32}N_2O_5$ (440.54): C 68.16, H 7.32, N 6.36. Found: C 68.18, H 7.32, N 6.40.

6-[(3',5'-Dimethoxy-1,1-dimethylbenzyloxy)carbonyl]-2-(*N*-methyl-*N*-phenylamino)-1,6-diazaspiro-[2.5]oct-1-ene (1f). To a solution of **7** (7.00 g, 15.9 mmol) in THF (40 mL) at 0 °C under Ar atmosphere was added a 1.5M solution of LDA in cyclohexane (9.5 mL, 19 mmol), and the mixture was stirred at 0 °C for 90 min. Then, DPPCl (4.72 g, 17.6 mmol) was added by means of a syringe at 0 °C, the solution stirred at 0 °C for 30 min and at rt for 24 h. The formed precipitate was filtered of under Ar atmosphere, the filtrate was added to 3.12 g (47.9 mmol) NaN_3 and the mixture stirred at rt for 3 d. After addition of

Et_2O and filtration of the mixture on Celite, the solvents of the filtrate were evaporated, the residue dissolved in CH_2Cl_2 and washed with aqueous $NaHCO_3$ (5%, 3×). The combined aqueous phases were washed with CH_2Cl_2, the combined organic phases were dried ($MgSO_4$), and the solvent evaporated. Purification via CC (SiO_2, hexane/AcOEt 2:1) gave 5.31 g (76%) of the azirine **1f**. Colorless, viscous oil. IR ($CHCl_3$): 3000*m*, 2970*m*, 2940*m*, 2860*w*, 2840*w*, 1750*s*, 1685*s*, 1600*s*, 1500*m*, 1460*s*, 1425*s*, 1380*w*, 1365*m*, 1350*m*, 1320*m*, 1300*m*, 1275*m*, 1250*m*, 1155*s*, 1100*s*, 1070*m*, 1020*m*, 960*w*, 890*w*, 835*w*, 695*w*, 610*w*. ^1H-NMR ((D_6)DMSO, 85 °C): 7.45–7.33, 7.16–7.10, 6.52–6.51, 6.41–6.39 (4*m*, 8 arom. H); 3.78–3.70 (*m*, 2 H of $-CH_2NCH_2-$); 3.76 (*s*, 2 MeO); 3.52–3.42 (*m*, 2 H of $-CH_2NCH_2-$); 3.43 (*s*, MeN); 1.95–1.84 (*m*, 2 H of $-CH_2CCH_2-$); 1.74 (*s*, Me_2C); 1.42–1.34 (*m*, 2 H of $-CH_2CCH_2-$). ^{13}C-NMR ((D_6)DMSO, 85 °C): 165.7, 160.0, 152.9, 148.8, 142.1 (5*s*, C(2), 2 arom. *C*OMe, OCON, 2 arom. C); 128.7, 122.7, 116.8, 102.7, 98.1 (5*d*, 8 arom. CH); 80.1 (*s*, Me_2CO); 54.7 (*q*, 2 MeO); 42.5 (*t*, $-CH_2NCH_2-$); 35.1 (*q*, MeN); 34.2 (*t*, $-CH_2CCH_2-$); 28.2 (*q*, Me_2C); C(3) could not be detected. ESI-MS: 439 (28), 438 (100, $[M+1]^+$), 260 (34), 179 (25).

Reaction of azirine 1f with thiobenzoic acid. Synthesis of *N*-{1-[(3',5'-dimethoxy-1,1-dimethylbenzyloxy)carbonyl]-4-(*N*-methyl-*N*-phenylthiocarbamoyl)piperidin-4-yl}benzamide (8). To a solution of **1f** (428 mg, 0.978 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added a solution of thiobenzoic acid (152 mg, 1.10 mmol) in 2 mL of CH_2Cl_2. After stirring for 14 h at rt, the solvent was evaporated and the residue was purified by CC to give 453 mg (79%) of **8** as a yellow foam. IR ($CHCl_3$): 3450*w*, 3020*w*, 3000*m*, 2940*m*, 2860*w*, 2840*w*, 1690*s*, 1680*s*, 1630*w*, 1600*s*, 1510*m*, 1485*m*, 1460*m*, 1430*s*, 1365*m*, 1350*m*, 1320*m*, 1285*m*, 1255*m*, 1180*w*, 1160*s*, 1140*m*, 1110*m*, 1090*m*, 1070*m*, 1030*w*, 1000*w*, 975*w*, 960*w*, 925*w*, 885*w*, 835*w*. ^1H-NMR ((D_6)DMSO, 97 °C): 7.74–7.70, 7.54–7.37, 7.27–7.12 (3*m*, 10 arom. H, 1 NH); 6.49–6.48, 6.39–6.38 (2*m*, 3 arom. H); 3.87–3.79 (*m*, 2 H of $-CH_2NCH_2-$); 3.75 (*s*, 2 MeO); 3.61 (*s*, MeN); 3.18–3.07 (*m*, 2 H of $-CH_2NCH_2-$); 2.56–2.39 (*m*, $-CH_2CCH_2-$); 1.71 (*s*, Me_2C). ^{13}C-NMR ((D_6)DMSO, 97 °C): 207.8 (*s*, C=S); 165.3 (*s*, C=O); 160.0 (*s*, 2 arom. *C*OMe); 152.8 (*s*, OCON); 148.7, 147.9, 134.0 (3*s*, 3 arom. C); 130.5, 128.7, 127.3, 126.9, 126.6, 125.1 (6*d*, 10 arom. CH); 102.7, 98.2 (2*d*, 3 arom. CH); 80.0 (*s*, Me_2CO); 63.1 (*s*, C(4')); 54.7 (*q*, 2 MeO); 48.6 (*q*, MeN); 39.3 (*t*, $-CH_2NCH_2-$); 35.0 (*t*, $-CH_2CCH_2-$); 28.1 (*q*, Me_2C). ESI-MS: 598 (100, $[M+Na]^+$).

General procedure for the synthesis of dipeptide amides 10a–d (azirine coupling). To a solution of the corresponding azirine **1** in MeCN, THF or CH_2Cl_2 at 0 °C, Z- or Fmoc-protected D-Ala was added and the mixture stirred at rt for 16 h. After evapration of the solvent, the residue was purified by CC.

Z-D-Ala-Thp-N(Ph)Me (10a). The reaction of 400 mg (1.85 mmol) of azirine **1d** and 457 mg (2.05 mmol) of Z-D-Ala-OH in MeCN (4 mL) followed by CC (Et_2O/AcOEt 1:1) gave 746 mg (92%) of **10a**.

Colorless solid; mp 103–105 °C. IR ($CHCl_3$): 3520*m*, 3330*w*, 3070*w*, 3030*w*, 3000*m*, 2960*m*, 2930*w*, 2860*w*, 1690*s*, 1640*s*, 1595*m*, 1500*s*, 1470*w*, 1450*m*, 1430*w*, 1370*m*, 1350*m*, 1320*m*, 1290*m*, 1230*m*, 1170*w*, 1150*w*, 1110*m*, 1070*m*, 1030*w*, 1000*w*, 980*w*, 960*w*, 910*w*, 880*w*, 860*w*, 825*w*, 700*m*. ^{1}H-NMR ($CDCl_3$): 7.37–7.29, 7.14–7.12 (2*m*, 10 arom. H); 5.95 (*s*, 1 NH); 5.19 (*d*, *J* = 7.1, 1 NH); 5.10, 5.04 (2*d*, J_{AB} = 12.3, PhCH_2O); 3.74–3.62, 3.40–3.24 (2*m*, HC(2) of Ala, –CH_2OCH_2–); 3.21 (*s*, MeN); 2.33–2.25, 1.96–1.91 (2*m*, –CH_2CCH_2–); 1.26 (*d*, *J* = 7.0, Me of Ala). ^{13}C-NMR ($CDCl_3$): 171.3, 170.8 (2*s*, 2 C=O); 156.1 (*s*, OCON); 144.7, 135.9 (2*s*, 2 arom. C); 129.5, 128.5, 128.3, 128.0, 127.8, 127.2 (6*d*, 10 arom. CH); 67.2, 63.0, 62.9 (3*t*, Ph*C*H_2O, –CH_2OCH_2–); 58.1 (*s*, C(4')); 50.1 (*d*, HC(2) of Ala); 41.4 (*q*, MeN); 33.7, 33.1 (2*t*, –*C*H_2C*C*H_2–); 17.2 (*q*, Me of Ala). ESI-MS: 901 (47, [2*M*+Na]$^+$), 462 (100, [*M*+Na]$^+$). $[\alpha^{21}{}_{\mathrm{D}}]$ +20.6 (c 1.003).

Fmoc-D-Ala-Tht-N(Ph)Me (10b). The reaction of 500 mg (2.15 mmol) of azirine **1e** and 737 mg (2.34 mmol) of Z-D-Ala-OH in THF (5 mL) followed by CC (hexane/AcOEt 1:2) gave 1074 mg (92%) of **10b**. Colorless solid; mp 130–132 °C. IR ($CHCl_3$): 3420*m*, 3340*m*, 3000*m*, 2960*m*, 1690*s*, 1640*s*, 1595*m*, 1495*s*, 1465*w*, 1450*m*, 1430*w*, 1370*m*, 1350*w*, 1320*m*, 1280*m*, 1250*m*, 1170*w*, 1130*w*, 1105*w*, 1070*m*, 1040*w*, 975*w*. ^{1}H-NMR ($CDCl_3$): 7.76, 7.56 (2*d*, *J* = 7.4, 4 Fmoc-H); 7.39 (*d*, *J* = 7.4, 2 Fmoc-H); 7.33–7.25, 7.14–7.11 (2*m*, 7 arom. H); 5.95 (*s*, 1 NH); 5.22 (*d*, *J* = 7.6, 1 NH); 4.44–4.32 (*m*, Fmoc-CH_2); 4.20 (*t*, *J* = 6.6, Fmoc-C(9)H); 3.60 (*quint*, *J* = 7.2, HC(2) of Ala); 3.21 (*s*, MeN); 2.69–2.65, 2.52–2.24 (2*m*, 4 CH_2); 1.24 (*d*, *J* = 7.1, Me of Ala). ^{13}C-NMR ($CDCl_3$): 171.7, 170.8 (2*s*, 2 C=O); 156.2 (*s*, OCON); 144.9, 143.7, 143.5, 141.3 (4*s*, 5 arom. C); 129.5, 127.8, 127.2, 127.1, 124.8, 120.1 (6*d*, 13 arom. CH); 67.2 (*t*, Fmoc-CH_2); 59.7 (*s*, C(4')); 50.0 (*d*, HC(2) of Ala); 47.0 (*d*, Fmoc-C(9)H); 41.5 (*q*, MeN); 34.2, 33.7 (2*t*, –*C*H_2C*C*H_2–); 23.1, 22.9 (2*t*, –CH_2SCH_2–); 17.3 (*q*, Me of Ala). ESI-MS: 566 (100, [*M*+Na]$^+$). $[\alpha^{21}{}_{\mathrm{D}}]$ +22.2 (c 0.962).

Z-D-Ala-Pip(Ddz)-N(Ph)Me (10c). The reaction of 536 mg (1.23 mmol) of azirine **1f** and 295 mg (1.36 mmol) of Z-D-Ala-OH in CH_2Cl_2 (10 mL) led to analytically pure crystalline **10c** (771 mg, 95%). Colorless powder; mp 150–152 °C. IR (KBr): 3430*m*, 3060*w*, 3000*w*, 2980*m*, 2930*m*, 2880*w*, 2830*w*, 1715*s*, 1690*s*, 1670*s*, 1650*m*, 1640*w*, 1630*w*, 1610*s*, 1590*s*, 1545*m*, 1540*m*, 1525*m*, 1495*m*, 1470*m*, 1455*m*, 1415*m*, 1380*m*, 1360*m*, 1315*m*, 1300*m*, 1285*m*, 1275*m*, 1255*s*, 1205*m*, 1155*m*, 1140*m*, 1100*m*, 1070*m*, 1050*s*, 1035*m*, 1025*m*, 995*w*, 965*w*, 940*m*, 845*m*, 770*m*, 740*m*, 730*m*, 700*m*. ^{1}H-NMR ((D$_6$)DMSO): 7.86 (*s*, 1 NH); 7.35–7.27 (*m*, 10 arom. H); 7.15 (*d*, *J* = 7.3, 1 NH); 6.34–6.42, 6.38–6.36 (2*m*, 3 arom. H); 5.09, 5.01 (2*d*, J_{AB} = 12.6, PhCH_2O); 4.02–3.50 (*m*, HC(2) of Ala, 2 H of –CH_2NCH_2–); 3.72 (*s*, 2 MeO); 3.25–2.75 (*m*, 2 H of –CH_2NCH_2–); 3.18 (*s*, MeN); 2.11–1.58 (*m*, –CH_2CCH_2–); 1.67 (*s*, Me_2C); 1.20 (*d*, *J* = 7.1, Me of Ala). ^{13}C-NMR ((D$_6$)DMSO): 171.7, 171.2 (2*s*, 2 C=O); 160.2 (*s*, 2 arom. *C*OMe); 155.6, 152.9 (2s, 2 OCON); 149.1, 145.1, 137.0 (3*s*, 3 arom. C); 128.9, 128.2, 127.7, 127.5, 127.2, 126.7 (6*d*, 10 arom. CH); 102.6, 97.9 (2*d*, 2 arom. CH); 80.2 (*s*, Me_2*C*O); 65.2 (*t*, Ph*C*H_2O); 57.3

(*s*, C(4')); 55.0 (*q*, 2 MeO); 50.7 (*d*, HC(2) of Ala); 39.9 (*q*, MeN); 39.7, 39.2 (2*t*, –CH_2NCH_2–); 32.0, 31.4 (2*t*, –*C*H_2*C*H_2–); 28.9, 28.5 (2*q*, *Me*$_2$C); 18.3 (*q*, Me of Ala). ESI-MS: 683 (100, [*M*+Na]$^+$). Anal. Calcd for $C_{36}H_{44}N_4O_8$ (660.77): C 65.44, H 6.71, N 8.48. Found: C 65.33, H 6.71, N 8.31.

Fmoc-D-Ala-Pip(Ddz)-N(Ph)Me (10d). The reaction of 860 mg (1.97 mmol) of azirine **1f** and 617 mg (1.98 mmol) of Fmoc-D-Ala-OH in CH_2Cl_2 (10 mL) followed by CC (hexane/AcOEt 1:2) gave 1249 mg, 85%) of **10d**. Colorless powder; mp 170–171 °C. IR (KBr): 3440*m*, 3060*w*, 2980*m*, 2940*m*, 2880*w*, 2830*w*, 1710*s*, 1695*s*, 1685*s*, 1675*s*, 1615*s*, 1590*s*, 1540*m*, 1530*s*, 1500*m*, 1470*m*, 1450*m*, 1430*m*, 1415*s*, 1380*m*, 1365*w*, 1315*m*, 1300*m*, 1280*m*, 1235*s*, 1205*m*, 1195*m*, 1160*m*, 1130*m*, 1100*m*, 1070*m*, 1050*m*, 1030*m*, 1000*w*, 980*w*, 975*w*, 940*m*, 850*m*, 770*m*, 740*m*, 760*m*, 740*m*, 700*m*. ^{1}H-NMR ((D_6)DMSO): 7.85, 7.69 (2*d*, *J* = 7.4, 4 Fmoc-H); 7.44–7.14 (*m*, 9 arom. H, 1 NH); 6.86 (*d*, *J* = 7.9, 1 NH); 6.49–6.46, 6.40–6.36 (2*m*, 3 arom. H); 4.43–4.21 (*m*, Fmoc-CH_2, HC(2) of Ala); 4.04–4.34 (*m*, Fmoc-C(9)H); 3.75 (*s*, 2 MeO); 3.74–3.50 (*m*, 2 H of –CH_2NCH_2–); 3.20 (*s*, MeN); 3.25–3.07 (*m*, 2 H of –CH_2NCH_2–); 2.15–1.97 (*m*, –CH_2CCH_2–); 1.70 (*s*, Me_2C); 1.25 (*d*, *J* = 7.0, Me of Ala). ^{13}C-NMR ((D_6)DMSO): 172.1, 171.7 (2*s*, 2 C=O); 160.6 (*s*, 2 arom. *C*OMe); 156.1, 153.4 (2*s*, 2 OCON); 149.5, 145.5, 144.2, 141.1 (4*s*, 6 arom. C); 129.3, 128.0, 127.6, 127.4, 127.1, 125.7, 120.4, 103.0, 98.3 (9*d*, 16 arom. CH); 80.6 (*s*, Me_2*C*O); 66.0 (*t*, Fmoc-*C*H_2); 57.7 (*s*, C(4')); 55.4 (*q*, 2 MeO); 50.1, 47.0 (2*d*, HC(2) of Ala, Fmoc-C(9)H); 40.0 (*q*, MeN); 39.3, 38.5 (2*t*, –CH_2NCH_2–); 32.6, 31.9 (2*t*, –*C*H_2*C*H_2–); 29.5, 28.8 (2*q*, *Me*$_2$C); 18.8 (*q*, Me of Ala). ESI-MS: 771 (100, [*M*+Na]$^+$).

General procedure for the selective methanolysis of dipeptide amides 10a–d. The corresponding dipeptide amide **10** was dissolved in MeOH at 0 °C, and HCl gas was bubbled through the solution until it reached ca. 60 °C. Then, the mixture was stirred for 2 h at rt, 1N aqueous HCl was added, and the mixture extracted with CH_2Cl_2 (3×). The combined organic layer was dried with $MgSO_4$ and the solvent evaporated in vacuo. The residue was purified by CC or crystallization.

Z-D-Ala-Thp-OMe (11a). The reaction of 1.020 g (2.32 mmol) of **10a** in MeOH (10 mL) followed by CC (hexane/AcOEt 1:5) gave 775 mg (92%) of **11a**. Colorless foam. IR ($CHCl_3$): 3520*m*, 3330*w*, 3020*w*, 3000*m*, 2960*m*, 2860*m*, 1740*s*, 1700*s*, 1500*s*, 1465*w*, 1455*m*, 1435*w*, 1380*w*, 1350*w*, 1320*m*, 1305*m*, 1290*m*, 1230*s*, 1155*w*, 1140*m*, 1115*m*, 1070*m*, 1030*w*, 1015*w*, 1000*w*, 910*w*, 880*w*, 840*w*, 700*m*. ^{1}H-NMR ($CDCl_3$): 7.35–7.27 (*m*, 5 arom. H); 6.79 (*s*, 1 NH); 5.36 (*d*, *J* = 7.3, 1 NH); 5.15, 5.10 (2*d*, J_{AB} = 12.1, PhC*H*$_2$O); 4.27 (*quint*, *J* = 7.1, HC(2) of Ala); 3.81–3.72 (*m*, 2 H of –CH_2OCH_2–); 3.70 (*s*, MeO); 3.62–3.48 (*m*, 2 H of –CH_2OCH_2–); 2.21–2.10, 1.96–1.89 (2*m*, –CH_2CCH_2–); 1.37 (*d*, *J* = 7.1, Me of Ala). ^{13}C-NMR ($CDCl_3$): 173.2, 172.0 (2*s*, 2 C=O); 156.4 (*s*, OCON); 136.0 (*s*, 1 arom. C); 128.6, 128.4, 128.1 (3*d*, 5 arom. CH); 67.3, 63.3 (2*t*, Ph*C*H_2O, –CH_2OCH_2–); 56.5 (*s*, C(4')); 52.6 (*s*, MeO); 50.2 (*d*, HC(2) of Ala); 32.6, 32.5 (2*t*, –*C*H_2*C*H_2–); 17.5 (*q*, Me of Ala). ESI-MS: 387 (100, [*M*+Na]$^+$). [$\alpha^{21}{}_D$] +22.6 (c

1.045).

Fmoc-D-Ala-Tht-OMe (11b). The reaction of 659 mg (1.21 mmol) of **10b** in MeOH (5 mL) followed by CC (hexane/AcOEt 1:2) gave 534 mg (94%) of **11b**. Colorless foam. IR ($CHCl_3$): 3430*m*, 3340*w*, 3060*w*, 3010*m*, 2950*m*, 1735*s*, 1695*s*, 1505*s*, 1465*w*, 1450*m*, 1435*m*, 1380*w*, 1350*w*, 1320*m*, 1280*m*, 1220*s*, 1110*w*, 1075*m*, 1060*m*, 1020*w*, 930*w*, 710*m*, 670*m*. ^{1}H-NMR ($CDCl_3$): 7.75, 7.57 (2*d*, *J* = 7.4, 4 Fmoc-H); 7.39, 7.29 (2*t*, *J* = 7.4, 4 Fmoc-H); 6.77 (*s*, 1 NH); 5.50 (*d*, *J* = 7.7, 1 NH); 4.40 (*d*, *J* = 6.7, Fmoc-CH_2); 4.35–4.27 (*m*, HC(2) of Ala); 4.20 (*t*, *J* = 6.8, Fmoc-C(9)H); 3.68 (*s*, MeO); 2.74–2.63, 2.55–2.45, 2.33–2.15 (3*m*, 4 CH_2); 1.37 (*d*, *J* = 6.9, Me of Ala). ^{13}C-NMR ($CDCl_3$): 173.4, 171.9 (2*s*, 2 C=O); 156.4 (*s*, OCON); 143.6, 141.3 (2*s*, 4 arom. C); 127.8, 127.1, 125.0, 120.1 (4*d*, 8 arom. CH); 67.3 (*t*, Fmoc-CH_2); 58.1 (*s*, C(4’)); 52.5 (*q*, MeO); 50.3 (*d*, HC(2) of Ala); 47.0 (*d*, Fmoc-C(9)H); 33.3 (*t*, $-\mathit{C}H_2C\mathit{C}H_2-$); 23.5, 23.4 (2*t*, $-CH_2SCH_2-$); 18.0 (*q*, Me of Ala). ESI-MS: 491 (100, $[M+\mathrm{Na}]^+$). $[\alpha^{21}{}_\mathrm{D}]$ +12.1 (c 0.962).

Z-D-Ala-Pip-OMe (11c). A solution of 1.742 g (2.64 mmol) of **10c** in MeOH (20 mL) was reacted with HCl gas. Then, the solvent was evaporated under vacuum, the residue was dissolved in CH_2Cl_2 and washed with 1N aqueous NaOH. The aqueous phase was extracted with CH_2Cl_2, the combined organic phase was dried ($MgSO_4$) and the solvent evaporated. CC (Et_2O/AcOEt 1:4) gave 650 mg (68%) of **11c**. Colorless foam. ^{1}H-NMR ($CDCl_3$): 7.35–7.25 (*m*, 5 arom. H, 1 NH); 5.70 (*d*-like, 1 NH); 5.11 (br. *s*, $PhC\mathit{H}_2O$); 4.35–4.25 (*m*, HC(2) of Ala); 3.75 (br. *s*, 1 NH); 3.67 (*s*, MeO); 2.99–2.70 (*m*, $-CH_2NCH_2-$); 2.10–2.00 (*m*, $-CH_2CCH_2-$); 1.38 (*d*, *J* = 7.0, Me of Ala). ^{13}C-NMR ($CDCl_3$): 173.5, 172.2 (2*s*, 2 C=O); 156.2 (*s*, OCON); 136.3 (*s*, 1 arom. C); 128.5, 128.2, 128.0 (3*d*, 5 arom. CH); 67.0 (*t*, $Ph\mathit{C}H_2O$); 57.2 (*s*, C(4’)); 52.5 (*q*, MeO); 47.1 (*d*, HC(2) of Ala); 41.5 (*t*, $-CH_2NCH_2-$); 32.0 (*t*, $-\mathit{C}H_2C\mathit{C}H_2-$); 18.2 (*q*, Me of Ala). CI-MS: 364 (100, $[M+1]^+$), 256 (32).

Z-D-Ala-Pip(Fmoc)-OMe (11d). A solution of 1.846 g (2.79 mmol) of **10c** in MeOH (20 mL) was reacted with HCl gas. Then, the solvent was evaporated under vacuum, the residue was dissolved in CH_2Cl_2 (20 mL), cooled to 0 °C, and 1.56 mL (11.2 mmol) Et_3N and 1.446 g (5.59 mmol) of Fmoc-chloride were added. After stirring for 14 h at rt, the mixture was washed with 1N HCl. The aqueous phase was extracted with CH_2Cl_2, the combined organic phase was dried ($MgSO_4$) and the solvent evaporated. CC (hexane/AcOEt 1:2) gave 1.236 g (76%) of **11d**. Colorless foam. IR ($CHCl_3$): 3430*w*, 3070*w*, 3000*w*, 2960*w*, 2870*w*, 1740*m*, 1695*s*, 1500*m*, 1480*m*, 1450*m*, 1435*m*, 1350*w*, 1335*w*, 1280*m*, 1270*m*, 1240*m*, 1205*m*, 1155*m*, 1135*w*, 1095*w*, 1065*m*, 1050*m*, 1040*w*, 1030*w*, 925*w*. ^{1}H-NMR ($CDCl_3$): 7.67, 7.47 (2*d*, *J* = 7.4, 4 Fmoc-H); 7.31 (*t*, *J* = 7.4, 2 Fmoc-H); 7.25–7.17 (*m*, 7 arom. H); 6.69 (*s*, 1 NH); 5.24 (*d*, *J* = 7.1, 1 NH); 5.05, 4.09 (2*d*, J_{AB} = 12.2, $PhC\mathit{H}_2O$); 4.40–4.32, 4.17–4.12, 3.83–3.57 (3*m*, Fmoc-CH_2, Fmoc-C(9)H, HC(2) of Ala, 2 H of $-CH_2NCH_2-$); 3.60 (*s*, MeO); 2.99–2.89 (*m*, 2 H of $-CH_2NCH_2-$); 1.86–1.79 (*m*, $-CH_2CCH_2-$); 1.27 (*d*, *J* = 7.0, Me of Ala). ^{13}C-NMR ($CDCl_3$): 172.9, 172.0

(2*s*, 2 C=O); 156.3, 154.8 (2*s*, 2 OCON); 143.9, 141.3, 135.9 (3*s*, 5 arom. C); 128.5, 128.3, 128.0, 127.6, 127.0, 124.8, 119.9 (7*d*, 13 arom. CH); 67.2 (*t*, Ph*C*H_2O, Fmoc-CH_2); 57.2 (*s*, C(4')); 52.5 (*q*, MeO); 50.3, 47.3 (2*d*, HC(2) of Ala, Fmoc-C(9)H); 39.5 (*t*, –CH_2NCH_2–); 31.7 (*t*, –*C*H_2C*C*H_2–); 17.3 (*q*, Me of Ala). ESI-MS: 608 (100, [*M*+Na]$^+$). $[\alpha^{21}{}_{D}]$ +10.4 (c 1.024).

The same product was obtained from **11c** (578 mg, 1.59 mmol) in CH_2Cl_2 (10 mL) at 0 °C by treatment with 270 μL (1.94 mmol) Et_3N and Fmoc-chloride (450 mg, 1.74 mmol). After stirring for 14 h at rt and purification by CC (hexane/AcOEt 1:2), 811 mg (87%) of **11d** were isolated.

Fmoc-D-Ala-Pip(Z)-OMe (11f). A solution of 1.371 g (1.83 mmol) of **10d** in MeOH (15 mL) was reacted with HCl gas. Then, the solvent was evaporated under vacuum, the residue was dissolved in CH_2Cl_2 (15 mL), cooled to 0 °C, and 1.10 mL (7.9 mmol) Et_3N and 624 mg (3.66 mmol) of Z-chloride were added. After stirring for 14 h at rt, the mixture was washed with 1N HCl. The aqueous phase was extracted with CH_2Cl_2, the combined organic phase was dried ($MgSO_4$) and the solvent evaporated. CC (hexane/AcOEt 1:2) gave 882 g (76%) of **11f**. Colorless foam. IR ($CHCl_3$): 3430*w*, 3330*w*, 3060*w*, 3000*m*, 2960*w*, 2870*w*, 1740*m*, 1690*s*, 1510*m*, 1500*m*, 1480*w*, 1450*m*, 1430*m*, 1385*w*, 1365*w*, 1355*w*, 1320*m*, 1280*m*, 1265*m*, 1230*m*, 1150*w*, 1135*w*, 1075*m*, 1020*w*, 1000*w*, 975*w*, 925*w*. ^{1}H-NMR ($CDCl_3$): 7.68, 7.47 (2*d*, *J* = 7.4, 4 Fmoc-H); 7.33–7.17 (*m*, 9 arom. H); 6.68 (*s*, 1 NH); 5.30 (*d*, *J* = 7.5, 1 NH); 5.03 (*s*, PhC*H*$_2$O); 4.32 (*d*, *J* = 6.8, Fmoc-CH_2); 4.19–4.08 (*m*, HC(2) of Ala); 4.11 (*t*, *J* = 6.8, Fmoc-C(9)H); 3.83–3.70 (*m*, 2 H of –CH_2NCH_2–); 3.60 (*s*, MeO); 3.13–3.01 (*m*, 2 H of –CH_2NCH_2–); 1.98–1.89 (*m*, –CH_2CCH_2–); 1.27 (*d*, *J* = 7.0, Me of Ala). ^{13}C-NMR ($CDCl_3$): 173.0, 172.1 (2*s*, 2 C=O); 156.2, 155.0 (2*s*, 2 OCON); 143.5, 141.2, 136.5 (3*s*, 5 arom. C); 128.4, 128.0, 127.8, 127.7, 127.0, 124.8, 120.0 (7*d*, 13 arom. CH); 67.2 (*t*, Ph*C*H_2O, Fmoc-*C*H_2); 57.3 (*s*, C(4')); 52.5 (*q*, MeO); 50.2, 47.0 (2*d*, HC(2) of Ala, Fmoc-C(9)H); 39.5 (*t*, –CH_2NCH_2–); 31.9, 31.7 (2*t*, –*C*H_2C*C*H_2–); 17.8 (*q*, Me of Ala). ESI-MS: 608 (100, [*M*+Na]$^+$). Anal. Calcd for $C_{33}H_{35}N_3O_7$ (585.66): C 67.68, H 6.02, N 7.17. Found: C 67.19, H 6.09, N 7.07. $[\alpha^{21}{}_{D}]$ +12.1 (c 1.061).

Synthesis of dipeptide Z-D-Ala-Pip(Boc)-OMe (11e). To a solution of **11d** (1.183 g, 1.97 mmol) in CH_2Cl_2 (5 mL) was added piperidine (0.5 mL). The mixture was stirred at rt for 1 h, the solvent evaporated, the residue dissolved in CH_2Cl_2, and 860 mg (3.94 mmol) Boc-anhydride and 550 μL (3.94 mmol) Et_3N were added. After stirring for 14 h at rt, the solvent was evaporated and the residue purified by CC (hexane/AcOEt 1:2) yielding 643 mg (70%) of **11e**. Colorless foam. IR ($CHCl_3$): 3430*m*, 3340*w*, 3060*w*, 3000*m*, 2980*m*, 1730*s*, 1680*s*, 1510*m*, 1500*s*, 1450*m*, 1430*m*, 1390*m*, 1370*m*, 1320*m*, 1280*s*, 1240*s*, 1170*m*, 1150*m*, 1095*w*, 1065*m*, 1030*w*, 1010*w*, 975*w*, 930*w*, 860*w*. ^{1}H-NMR ($CDCl_3$): 7.34–7.29 (*m*, 5 arom. H); 6.86 (*s*, 1 NH); 5.46 (*d*, *J* = 7.5, 1 NH); 5.12, 5.07 (2*d*, J_{AB} = 12.2, PhC*H*$_2$O); 4.29–4.20, 3.85–3.71 (2*m*, HC(2) of Ala, 2 H of –CH_2NCH_2–); 3.67 (*s*, MeO); 3.04–2.91 (*m*, 2 H of –CH_2NCH_2–);

2.02–1.85 (*m*, –CH_2CCH_2–); 1.46 (*s*, Me_3C); 1.36 (*d*, *J* = 7.0, Me of Ala). ^{13}C-NMR ($CDCl_3$): 173.2, 172.1 (2*s*, 2 C=O); 156.2, 154.3 (2*s*, 2 OCON); 135.9 (*s*, 1 arom. C); 128.5, 128.2, 127.9 (3*d*, 5 arom. CH); 79.7 (*s*, Me_3*C*); 67.1 (*t*, Ph*C*H_2O); 57.3 (*s*, C(4’)); 52.4 (*q*, MeO); 50.2 (*d*, HC(2) of Ala); 39.1 (*t*, –CH_2NCH_2–); 31.9, 31.6 (2*t*, –*C*H_2C*C*H_2–); 28.3 (*q*, *Me*$_3$C); 17.6 (*q*, Me of Ala). ESI-MS: 486 (100, [*M*+Na]$^+$). Anal. Calcd for $C_{23}H_{33}N_3O_7$ (463.53): C 59.60, H 7.18, N 9.07. Found: C 59.33, H 7.29, N 8.76. [$\alpha^{21}{}_{D}$] +11.5 (c 0.989).

Selective deprotection of the terminal amino group of dipeptide esters 11a,b.

H-D-Ala-Thp-OMe (12a). To a solution of dipeptide **11a** (167 mg, 0.458 mmol) in MeOH (3 mL) was added 16.7 mg of Pd/C (10%). This mixture was stirred under an H_2-atmosphere at rt until disappearance of **11a** (TLC). After filtration via Celite, the solvent of the filtrate was evaporated and the residue dried in HV: 85 mg (81%) of **12a**. White powder. ^{1}H-NMR (CF_3CO_2D): 4.52 (*q*, *J* = 7.0, HC(2) of Ala); 4.39–4.31, 4.18–3.99 (2*m*, –CH_2OCH_2–); 3.45 (*s*, MeO); 2.62–2.53, 2.06–1.98 (2*m*, –CH_2CCH_2–); 1.70 (*d*, *J* = 7.0, Me of Ala). ^{13}C-NMR (CF_3CO_2D): 172.8, 171.7 (2*s*, 2 C=O); 62.1 (*t*, –CH_2OCH_2–); 55.6 (*s*, C(4’)); 54.0 (*s*, MeO); 50.3 (*d*, HC(2) of Ala); 34.6, 33.5 (2*t*, –*C*H_2C*C*H_2–); 18.2 (*q*, Me of Ala). CI-MS: 461 (35, [2*M*+1]$^+$), 231 (100, [*M*+1]$^+$).

H-D-Ala-Tht-OMe (12b). The dipeptide **11b** (120 mg, 0.256 mmol) was dissolved in piperidine (3 mL) at rt. After 10 min, the precipitate was filtered and washed with AcOEt to give 48 mg (76%) of **12b**. White powder. ^{1}H-NMR (CF_3CO_2D): 4.50 (*q*, *J* = 7.0, HC(2) of Ala); 3.41 (*s*, MeO); 2.63–2.55, 2.23–2.18, 2.02–1.95, 1.87–1.79 (4*m*, 4 CH_2); 1.68 (*d*, *J* = 7.0, Me of Ala). ^{13}C-NMR (CF_3CO_2D): 174.8, 174.3 (2*s*, 2 C=O); 60.0 (*s*, C(4’)); 52.6 (*s*, MeO); 48.4 (*d*, HC(2) of Ala); 37.8, 36.6 (2*t*, –*C*H_2C*C*H_2–); 24.4, 23.4 (2*t*, –CH_2SCH_2–); 20.8 (*q*, Me of Ala). CI-MS: 461 (35, [2*M*+1]$^+$), 231 (100, [*M*+1]$^+$).

Synthesis of tripeptides 14 via coupling of dipeptides with aspartic acid derivatives.

Z-Asp(OBn)-D-Ala-Thp-OMe (14a). To a solution of **11a** (764 mg, 2.10 mmol) in MeOH (10 mL)/DMF (15 mL) was added Pd/C (10%, 38 mg) and the mixture was treated with H_2 until the disappearance of **11a** (TLC). The catalyst was removed by filtration via Celite and MeOH was evaporated. To the remaining solution were added Z-Asp(OBn)-OH (**13a**, 900 mg, 2.52 mmol), PyBOP (1.312 g, 2.52 mmol), and Et_3N (700 μL, 3 mmol), and the mixture was stirred for 16 h at rt. Evaporation of the solvent, CC (Et_2O/AcOEt 1:2), and crystallization from hexane/AcOEt/MeOH yielded 956 mg (80%) **14a**. Colorless crystals; mp 127–128 °C. IR ($CHCl_3$): 3420*m*, 3070*w*, 3030*m*, 3000*m*, 2960*m*, 2860*w*, 1760*s*, 1715*s*, 1695*s*, 1680*s*, 1515*s*, 1505*s*, 1455*m*, 1445*m*, 1430*w*, 1405*w*, 1390*m*, 1355*m*, 1290*m*, 1260*m*, 1190*m*, 1160*m*, 1145*m*, 1105*m*, 1075*w*, 1050*m*, 1030*w*, 1000*w*, 980*w*, 970*w*, 960*w*, 920*w*, 845*w*. ^{1}H-NMR ($CDCl_3$): 7.28–7.21 (*m*, 10 arom. H); 6.92–6.88 (*m*, 2 NH); 5.93 (*d*, *J* = 8.4, 1 NH); 5.07–4.97

(*m*, 2 PhCH_2O); 4.55–4.48 (*m*, HC(2) of Asp); 4.40 (*quint*, J = 7.3, HC(2) of Ala); 3.70–3.63, 3.56–3.51 (2*m*, –CH_2OCH_2–); 3.61 (*s*, MeO); 3.05–2.70 (*m*, CH_2 of Asp); 2.11–1.85 (*m*, –CH_2CCH_2–); 1.27 (*d*, J = 7.0, Me of Ala). ^{13}C-NMR ($CDCl_3$): 173.3, 171.5, 170.7 (3*s*, 3 C=O); 156.1 (*s*, OCON); 135.6, 135.0 (2*s*, 2 arom. C); 128.6, 128.4, 128.1, 128.0 (4*d*, 10 arom. CH); 67.5, 66.9 (2*t*, 2 PhCH_2O); 63.3, 63.2 (2*t*, –CH_2OCH_2–); 56.6 (*s*, C(4')); 52.4 (*s*, MeO); 51.4, 49.0 (2*d*, HC(2) of Ala, HC(2) of Asp); 35.9 (*t*, CH_2 of Asp); 32.6, 32.2 (2*t*, –CH_2*C*CH_2–); 16.8 (*q*, Me of Ala). ESI-MS: 592 (100, [*M*+Na]$^+$). [$\alpha^{21}{}_D$] +8.7 (c 0.946).

Fmoc-Asp(OtBu)-D-Ala-Tht-OMe (14b). To a solution of **11b** (262 mg, 0.559 mmol) in DMSO (3 mL) was added piperidine (49 mg, 0.575 mmol) at rt. After 40 min, the residual piperidine was removed by distillation. Then, 232 mg (0.564 mmol) Fmoc-Asp(O*t*Bu)-OH (**13b**) and 303 mg (0.583 mmol) PyBOP were added and the mixture stirred for 16 h at rt. The solvent was removed by distillation (high vacuum) and the residue purified by CC (hexane/AcOEt 1:3) to give 330 mg (92%) of **14b**. Colorless foam. IR ($CHCl_3$): 3420*w*, 3020*w*, 3000*m*, 2980*m*, 2950*w*, 1730*s*, 1715*s*, 1680*s*, 1505*s*, 1465*w*, 1450*m*, 1435*m*, 1420*w*, 1405*w*, 1395*w*, 1370*m*, 1315*m*, 1280*m*, 1260*m*, 1155*w*, 1105*w*, 1080*w*, 1065*m*, 1050*m*, 1020*w*, 930*w*, 920*w*, 900*w*, 880*w*, 855*w*, 840*w*, 820*w*. ^{1}H-NMR ($CDCl_3$): 7.76, 7.57 (2*d*, J = 7.4, 4 Fmoc-H); 7.40 (*t*, J = 7.4, 2 Fmoc-H); 7.34–7.30 (*m*, 2 Fmoc-H); 6.88 (*d*, J = 7.6, 1 NH); 6.75 (*s*, 1 NH); 5.88 (*d*-like, br, 1 NH); 4.51–4.43, 4.24–4.19 (2*m*, Fmoc-CH_2, Fmoc-C(9)H, HC(2) of Asp, HC(2) of Ala); 3.68 (*s*, MeO); 2.92–2.18 (*m*, CH_2 of Asp, 4 CH_2); 1.45 (*s*, Me_3C); 1.35 (*d*, J = 7.0, Me of Ala). ^{13}C-NMR ($CDCl_3$): 173.4, 171.2, 170.9, 170.6 (4*s*, 4 C=O); 156.1 (*s*, OCON); 143.5, 141.2 (2*s*, 4 arom. C); 127.7, 127.0, 124.8, 120.0 (4*d*, 8 arom. CH); 82.0 (*s*, Me_3*C*); 67.3 (*t*, Fmoc-CH_2); 58.1 (*s*, C(4')); 52.4 (*q*, MeO); 51.4, 48.9, 47.0 (3*d*, HC(2) of Ala, HC(2) of Asp, Fmoc-C(9)H); 37.1, 33.3, 33.2 (3*t*, CH_2 of Asp, –CH_2*C*CH_2–); 28.0 (*q*, *Me*$_3$C); 23.4 (*t*, –CH_2SCH_2–); 16.8 (*q*, Me of Ala). ESI-MS: 662 (100, [*M*+Na]$^+$), 440 (47, [*M*–Fmoc+Na]$^+$). [$\alpha^{21}{}_D$] +5.2 (c 1.085).

Z-Asp(OBn)-D-Ala-Pip(Boc)-OMe (14c). To a solution of **11e** (545 mg, 1.18 mmol) in MeOH (10 mL)/DMF (15 mL) was added Pd/C (10%, 47 mg) and the mixture was treated with H_2 until the disappearence of **11e** (TLC). The catalyst was removed by filtration via Celite and MeOH was evaporated. To the remaining solution were added Z-Asp(OBn)-OH (**13a**, 505 mg, 1.41 mmol), PyBOP (730 mg, 1.40 mmol), and Et_3N (200 μL, 1.4 mmol), and the mixture was stirred for 16 h at rt. Evaporation of the solvent and subsequent CC (Et_2O/AcOEt 1:1) yielded 657 mg (84%) **14c**. Colorless foam. IR ($CHCl_3$): 3420*m*, 3060*w*, 3020*m*, 3000*m*, 2970*m*, 1740*s*, 1715*s*, 1680*s*, 1515*m*, 1500*m*, 1495*m*, 1450*m*, 1430*m*, 1390*m*, 1365*m*, 1355*m*, 1280*m*, 1270*m*, 1240*m*, 1170*m*, 1150*m*, 1070*m*, 1050*w*, 975*w*, 920*w*, 855*w*, 845*w*. ^{1}H-NMR ($CDCl_3$): 7.35–7.26 (*m*, 10 arom. H); 6.92 (*s*, 1 NH); 6.87 (*d*, J = 7.3, 1 NH); 5.93 (*d*, J = 9.0, 1 NH); 5.15–5.02 (*m*, 2 PhCH_2O); 4.60–4.54 (*m*, HC(2) of Asp); 4.45 (*quint*, J = 7.3, HC(2) of Ala); 3.82–3.75 (*m*, 2 H of –CH_2NCH_2–); 3.67 (*s*, MeO); 3.16–3.03, 2.84–2.76 (2*m*, 2 H of –CH_2NCH_2–, CH_2

of Asp); 2.04–1.96 (*m*, –CH$_2$CCH$_2$–); 1.42 (*s*, Me$_3$C); 1.32 (*d*, *J* = 7.0, Me of Ala). ^{13}C-NMR (CDCl$_3$): 173.4, 171.7, 171.6, 170.8 (4*s*, 4 C=O); 156.2, 154.6 (2*s*, 2 OCON); 135.7, 135.1 (2*s*, 2 arom. C); 128.7, 128.5, 128.2, 127.9 (4*d*, 10 arom. CH); 79.8 (*s*, Me$_3$*C*); 67.6, 67.0 (2*t*, 2 Ph*C*H$_2$O); 57.6 (*s*, C(4')); 52.5 (*q*, MeO); 51.4, 49.1 (2*d*, HC(2) of Ala, HC(2) of Asp); 39.3, 35.9 (2*t*, –CH$_2$NCH$_2$–, CH$_2$ of Asp); 32.0, 31.6 (2*t*, –*C*H$_2$C*C*H$_2$–); 28.4 (*q*, *Me*$_3$C); 16.9 (*q*, Me of Ala). ESI-MS: 691 (100, [*M*+Na]$^+$). $[\alpha^{21}{}_{D}]$ +7.8 (c 0.992).

Fmoc-Asp(OtBu)-D-Ala-Pip(Z)-OMe (14d). To a solution of **11f** (250 mg, 0.472 mmol) in DMSO (4 mL) was added piperidine (45 μL, 0.456 mmol) at rt. After 40 min, the residual piperidine was removed by distillation. Then, 194 mg (0.472 mmol) Fmoc-Asp(O*t*Bu)-OH (**13b**) and 245 mg (0.471 mmol) PyBOP were added and the mixture stirred for 16 h at rt. The solvent was removed by distillation (high vacuum) and the residue purified by CC (hexane/AcOEt 1:3) to give 147 mg (44%) of **14d**. Colorless foam. IR (CHCl$_3$): 3450*w*, 3330*w*, 3060*w*, 3020*w*, 3000*m*, 2980*m*, 2950*w*, 1730*s*, 1710*s*, 1690*s*, 1515*s*, 1505*s*, 1450*m*, 1430*m*, 1395*w*, 1370*m*, 1320*m*, 1280*m*, 1255*m*, 1245*m*, 1150*m*, 1105*w*, 1060*m*, 1020*w*, 955*w*, 925*w*, 900*w*, 855*w*, 840*w*, 820*w*. ^{1}H-NMR (CDCl$_3$): 7.75, 7.56 (2*d*, *J* = 7.4, 4 Fmoc-H); 7.42–7.21 (*m*, 9 arom. H); 6.97 (*s*, 1 NH); 6.89 (*d*, *J* = 7.6, 1 NH); 5.81 (*d*-like, 1 NH); 5.10 (*s*, PhC*H*$_2$O); 4.50–4.41 (*m*, HC(2) of Asp, HC(2) of Ala, Fmoc-CH$_2$); 4.21 (*t*, *J* = 6.6, Fmoc-C(9)H); 3.89–3.73 (*m*, 2 H of –CH$_2$NCH$_2$–); 3.67 (*s*, MeO); 3.27–3.16 (*m*, 2 H of –CH$_2$NCH$_2$–); 2.98–2.87, 2.74–2.66 (2*m*, CH$_2$ of Asp); 2.07–1.98 (*m*, –CH$_2$CCH$_2$–); 1.41 (*s*, Me$_3$C); 1.35 (*d*, *J* = 7.1, Me of Ala). ^{13}C-NMR (CDCl$_3$): 173.1, 171.6, 170.7 (3*s*, 4 C=O); 156.1, 155.0 (2*s*, 2 OCON); 143.5, 143.4, 141.2, 136.6 (4*s*, 5 arom. C); 128.4, 127.9, 127.8, 127.5, 127.0, 124.8, 120.0 (7*d*, 13 arom. CH); 82.0 (*s*, Me$_3$*C*); 67.2, 67.1 (2*t*, Ph*C*H$_2$O, Fmoc-CH$_2$); 57.4 (*s*, C(4')); 52.4 (*q*, MeO); 51.4, 48.9, 47.0 (3*d*, HC(2) of Ala, HC(2) of Asp, Fmoc-C(9)H); 39.5, 37.0 (2*t*, –CH$_2$NCH$_2$–, CH$_2$ of Asp); 32.0, 31.6 (2*t*, –*C*H$_2$C*C*H$_2$–); 27.9 (*q*, *Me*$_3$C); 16.9 (*q*, Me of Ala). ESI-MS: 779 (100, [*M*+Na]$^+$). $[\alpha^{21}{}_{D}]$ +5.1 (c 1.097).

Preparation of the zwitterionic tripeptides 15a–d.

H-Asp-D-Ala-Thp-OMe (15a). To a solution of **14a** (125 mg, 0.219 mmol) in MeOH (10 mL) was added Pd/C (10%, 15 mg) and the mixture was treated with H$_2$ for 6 h. The catalyst was removed by filtration via Celite, MeOH was evaporated, and the residue dried (HV) to give 89 mg (98%) of **15a**. Colorless powder; mp 129–131 °C. IR (KBr): 3460*m*, 3300*m*, 3060*m*, 2960*m*, 1745*m*, 1730*m*, 1695*s*, 1680*s*, 1665*s*, 1645*m*, 1630*m*, 1580*m*, 1575*m*, 1565*s*, 1550*s*, 1540*s*, 1505*w*, 1495*w*, 1470*m*, 1450*m*, 1445*m*, 1430*m*, 1400*m*, 1390*m*, 1385*m*, 1345*w*, 1335*w*, 1310*w*, 1290*m*, 1260*m*, 1230*m*, 1150*m*, 1100*m*, 1070*m*, 1030*w*, 1015*w*, 1005*w*, 985*w*, 965*w*, 920*w*, 890*w*, 870*w*, 840*w*. ^{1}H-NMR (CD$_3$OD): 4.43–4.35 (*m*, HC(2) of Asp); 4.09–404 (*m*, HC(2) of Ala); 3.79–3.65 (*m*, –CH$_2$OCH$_2$–); 3.68 (*s*, MeO); 2.71–2.56 (*m*, CH$_2$ of Asp); 2.17–1.89 (*m*, –CH$_2$CCH$_2$–); 1.37 (*d*, *J* = 7.1, Me of Ala). ^{13}C-NMR (CD$_3$OD): 174.6, 173.6, 173.4, 168.6

(4*s*, 4 C=O); 62.2, 62.8 (2*t*, –CH$_2$OCH$_2$–); 56.4 (*s*, C(4')); 51.4 (*s*, MeO); 50.8, 48.9 (2*d*, HC(2) of Ala, HC(2) of Asp); 36.7 (*t*, CH$_2$ of Asp); 32.2, 31.6 (2*t*, –*C*H$_2$C*C*H$_2$–); 16.6 (*q*, Me of Ala). ESI-MS: 384 (27, [*M*+K]$^+$), 368 (23, [*M*+Na]$^+$), 346 (100, [*M*+1]$^+$). [α$^{21}_{\text{D}}$] +31.3 (c 1.020).

H-Asp-D-Ala-Tht-OMe (15b). To a solution of 50 mg (0.078 mmol) **14b** in CH$_2$Cl$_2$ (1 mL) was added piperidine (100 μL, 1.01 mmol) and the mixture stirred for 30 min at rt. Then, the solvent and piperidine were evaporated and the residue purified by CC (CH$_2$Cl$_2$/MeOH/NH$_3$(aq) 100:2.5:0.25) yielding 30 mg (92%) of *H-Asp(OtBu)-*D*-Ala-Tht-OMe* as a colorless foam. IR (CHCl$_3$): 3440*m*, 3020*w*, 2980*m*, 2950*w*, 1740*s*, 1730*s*, 1715*s*, 1695*s*, 1680*s*, 1660*s*, 1650*s*, 1510*s*, 1495*m*, 1480*m*, 1395*w*, 1370*m*, 1335*w*, 1330*w*, 1320*w*, 1280*s*, 1260*m*, 1150*s*, 1085*w*, 1060*w*, 1015*w*, 950*w*, 940*w*, 930*w*, 840*w*. ^{1}H-NMR (CDCl$_3$): 7.94 (*d*, *J* = 7.1, 1 NH); 7.21 (*s*, 1 NH); 4.45 (*quint*, *J* = 7.0, HC(2) of Ala); 3.64 (*s*, MeO); 3.64–3.57 (*m*, HC(2) of Asp); 2.79–2.64, 2.55–2.43, 2.30–2.05 (3*m*, CH$_2$ of Asp, 4 CH$_2$); 1.89 (*s*, NH$_2$); 1.39 (*s*, Me$_3$C); 1.35 (*d*, *J* = 7.0, Me of Ala). ^{13}C-NMR (CDCl$_3$): 173.3, 172.6, 170.7, 169.8 (4*s*, 4 C=O); 80.4 (*s*, Me$_3$*C*); 57.1 (*s*, C(4')); 52.4 (*q*, MeO); 50.9, 47.5 (2*d*, HC(2) of Ala, HC(2) of Asp); 40.2 (*t*, CH$_2$ of Asp); 32.2 (*t*, –*C*H$_2$C*C*H$_2$–); 27.1 (*q*, *Me*$_3$C); 22.5, 22.4 (2*t*, –CH$_2$SCH$_2$–); 15.9 (*q*, Me of Ala). ESI-MS: 440 (38, [*M*+Na]$^+$), 418 (100, [*M*+1]$^+$). [α$^{21}_{\text{D}}$] +21.1 (c 1.010). A solution of 85 mg (0.203 mmol) of the product in TFA (1 mL) was stirred for 2 h. After evaporation of TFA and drying of the residue (HV), ca. 100 mg of **15b**, still containing TFA, were obtained. ^{1}H-NMR (CD$_3$OD): 4.48–4.40, 4.23–4.17 (2 *m*, HC(2) of Asp, HC(2) of Ala); 3.67 (*s*, MeO); 3.05–2.75, 2.55–2.08 (2*m*, CH$_2$ of Asp, 4 CH$_2$); 1.37 (*d*, *J* = 7.1, Me of Ala). ^{13}C-NMR (CD$_3$OD): 175.4, 174.4, 172.9, 169.0 (4*s*, 4 C=O); 59.7 (*s*, C(4')); 51.4 (*q*, MeO); 49.3, 49.0 (2*d*, HC(2) of Ala, HC(2) of Asp); 36.0, 34.9, 34.2 (3*t*, CH$_2$ of Asp, –*C*H$_2$C*C*H$_2$–); 24.3, 24.2 (2*t*, –CH$_2$SCH$_2$–); 17.8 (*q*, Me of Ala).

H-Asp-D-Ala-Pip(Boc)-OMe (15c). To a solution of **14c** (252 mg, 0.377 mmol) in MeOH (10 mL) was added Pd/C (10%, 35 mg) and the mixture was treated with H$_2$ for 7 h. The catalyst was removed by filtration via Celite, MeOH was evaporated, and the residue dried (HV) to give 167 mg (100%) of **15c**. Colorless powder; mp 137–140 °C. IR (KBr): 3400*m*, 3250*m*, 3060*m*, 2985*m*, 1740*m*, 1730*m*, 1690*s*, 1680*s*, 1670*s*, 1635*m*, 1615*m*, 1570*m*, 1550*m*, 1540*m*, 1515*m*, 1505*m*, 1485*m*, 1470*m*, 1465*m*, 1455*m*, 1445*m*, 1430*s*, 1390*m*, 1370*m*, 1330*w*, 1280*m*, 1240*m*, 1170*m*, 1150*m*, 1090*w*, 1070*m*, 1010*w*, 975*w*, 920*w*, 850*w*, 810*w*. ^{1}H-NMR (CD$_3$OD): 4.40–4.33 (*m*, HC(2) of Asp); 4.10–4.04 (*m*, HC(2) of Ala), 3.83–3.74 (*m*, 2 H of –CH$_2$NCH$_2$–); 3.67 (*s*, MeO); 3.26–3.12 (*m*, 2 H of –CH$_2$NCH$_2$–); 2.74–2.54 (*m*, CH$_2$ of Asp); 2.12–1.89 (*m*, –CH$_2$CCH$_2$–); 1.46 (*s*, Me$_3$C); 1.37 (*d*, *J* = 7.1, Me of Ala). ^{13}C-NMR (CD$_3$OD): 176.0, 175.1, 170.4, 170.1 (4*s*, 4 C=O); 156.4 (*s*, OCON); 81.3 (*s*, Me$_3$*C*); 58.7 (*s*, C(4')); 53.0 (*q*, MeO); 52.3, 50.5 (2*d*, HC(2) of Ala, HC(2) of Asp); 41.2, 40.6 (2*t*, –CH$_2$NCH$_2$–); 38.2 (*t*, CH$_2$ of Asp); 33.1, 32.3 (2*t*, –*C*H$_2$C*C*H$_2$–); 28.7 (*q*, *Me*$_3$C); 18.0 (*q*, Me of Ala). ESI-MS: 467 (54, [*M*+Na]$^+$), 445 (100, [*M*+1]$^+$). [α$^{21}_{\text{D}}$] +18.8 (c 1.020).

H-Asp-D-Ala-Pip(Z)-OMe (15d). To a solution of 797 mg (1.05 mmol) **14d** in CH_2Cl_2 (5 mL) was added piperidine (5 mL) and the mixture stirred for 30 min at rt. Then, the solvent and piperidine were evaporated and the residue purified by CC (CH_2Cl_2/MeOH/NH_3(aq) 100:3:0.3) yielding 515 mg (91%) of *H-Asp(OtBu)*-D-*Ala-Pip(Z)-OMe* as a colorless foam. IR ($CHCl_3$): 3360*m*, 3060*w*, 3000*m*, 2980*m*, 2950*w*, 1740*m*, 1730*m*, 1710*m*, 1705*m*, 1690*s*, 1680*s*, 1520*m*, 1505*m*, 1470*w*, 1450*m*, 1435*m*, 1395*w*, 1370*m*, 1330*w*, 1280*m*, 1245*m*, 1150*m*, 1095*m*, 1070*m*, 1020*w*, 970*w*, 925*w*, 890*w*, 850*w*. ^{1}H-NMR ($CDCl_3$): 7.86 (*d*, *J* = 8.0, 1 NH); 7.38–7.28 (*m*, 5 arom. H); 7.23 (*s*, 1 NH); 5.11 (*s*, $PhCH_2O$); 4.55–4.44 (*m*, HC(2) of Ala); 3.90–3.78 (*m*, 2 H of $–CH_2NCH_2–$); 3.71 (*s*, MeO); 3.62–3.58 (*m*, HC(2) of Asp); 3.35–3.20 (*m*, 2 H of $–CH_2NCH_2–$); 2.87–2.65 (*m*, CH_2 of Asp); 2.08–2.01 (*m*, $–CH_2CCH_2–$); 1.86 (*s*, NH_2); 1.40 (*s*, Me_3C); 1.38 (*d*, *J* = 7.2, Me of Ala). ^{13}C-NMR ($CDCl_3$): 174.0, 173.3, 172.1, 171.3 (4*s*, 4 C=O); 155.1 (*s*, OCON); 136.7 (*s*, 1 arom. C); 128.5, 128.0, 127.9 (3*d*, 5 arom. CH); 81.6 (*s*, Me_3C); 67.2 (*t*, $PhCH_2O$); 57.4 (*s*, C(4')); 52.5 (*q*, MeO); 51.6, 48.5 (2*d*, HC(2) of Ala, HC(2) of Asp); 40.2, 39.7 (2*t*, $–CH_2NCH_2–$, CH_2 of Asp); 31.8 (*t*, $–CH_2CCH_2–$); 28.1 (*q*, Me_3C); 16.8 (*q*, Me of Ala). ESI-MS: 557 (22, $[M+Na]^+$), 535 (100, $[M+1]^+$). $[\alpha^{21}_D]$ +22.3 (c 0.990). A solution of 67 mg (0.125 mmol) of the product in TFA (1 mL) was stirred for 1.5 h, whereby the color of the solution turned to pale brown. After evaporation of TFA and drying of the residue (HV), it was dissolved in MeOH. Evaporation of the solvents and drying in HV yielded 82 mg of **15d**, which contained still TFA. ^{1}H-NMR (CD_3OD): 7.35–7.29 (*m*, 5 arom. H); 5.11 (*s*, $PhCH_2O$); 4.41–4.33, 4.21–4.16 (2*m*, HC(2) of Asp, HC(2) of Ala); 3.88–3.82 (*m*, 2 H of $–CH_2NCH_2–$); 3.66 (*s*, MeO); 3.31–3.20 (*m*, 2 H of $–CH_2NCH_2–$); 2.99–2.82 (*m*, CH_2 of Asp); 2.15–1.93 (*m*, $–CH_2CCH_2–$); 1.37 (*d*, *J* = 7.1, Me of Ala). ^{13}C-NMR (CD_3OD): 173.5, 173.4, 171.1, 167.4 (4*s*, 4 C=O); 155.3 (*s*, OCON); 136.5 (*s*, 1 arom. C); 128.1, 127.7, 127.4 (3*d*, 5 arom. CH); 66.9 (*t*, $PhCH_2O$); 57.2 (*s*, C(4')); 51.4 (*q*, MeO); 49.4, 49.1 (2*d*, HC(2) of Ala, HC(2) of Asp); 39.3, 39.2 (2*t*, $–CH_2NCH_2–$); 34.4 (*t*, CH_2 of Asp); 31.5, 30.7 (2*t*, $–CH_2CCH_2–$); 16.5 (*q*, Me of Ala). ESI-MS: 501 (13, $[M+Na]^+$), 479 (100, $[M+1]^+$).

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