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Modified Neurotensin

Stereoselective Synthesis of β-(5-Arylthiazolyl) α-Amino Acids and Use in Neurotensin Analogues

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Abstract: A series of new unnatural amino acids bearing a β-arylthiazole side chain was synthesized by exploiting a diastereoselective alkylation starting from glycine *tert*-butyl ester Schiff base with hydroxypinanone as the chiral inducer. This strategy afforded β-arylthiazole alanines in good chemical yields and with 98 % *ee*. Due to their aromatic properties, these newly generated amino acids were used to prepare neurotensin (NT)[8–13] analogues by serving as replacements for the native Tyr11 residue. Incorporation of the (L)-(+)-(β-phenylthiazol-4-yl)alanine residue at NT[8–13] position 11 improved plasma stability and selectivity towards NTS1, while also preserving native receptor binding affinity and biological activity.

Introduction

The field of new synthetic peptides and peptidomimetics is experiencing renewed interest in recent years and several peptides are currently used for therapeutic applications in the treatment of cancer, diabetes, cardiovascular or renal insufficiency diseases.[1] The challenge is to synthesize more active and more stable analogues by introducing non-proteinogenic amino acids into the peptide sequence, thus allowing modulation of properties such as steric hindrance, polarity, conformational capabilities and proteolytic stability.[2] Therefore, the development of efficient sources for unnatural amino acids useful in high-throughput synthesis of customized peptides is of crucial interest. Among heteroaromatic amino acids, some containing a thiazole moiety are known to display antibacterial,[3] antitrypanosomal,[4] and antimalarial properties.[5] Many macrolactams containing thiazole-derived amino acids isolated from marine organisms have generated a great deal of synthetic interest because of their high cytotoxicity, as well as their metal binding and transport properties.[6] Synthetic thiazole and aryl-substituted thiazole amino acids have a broad spectrum of applications. Functionalized thiazole alanines have been used as organocatalysts for intramolecular Stetter reactions[7] and α-thiazole alanines are often found in synthetic peptides as bioisosteres of natural occurring amino acids; such peptides have had diverse applications such as in the preparation of integrin inhibitors,[8] glutamate carboxypeptidase II inhibitors,[9] or peptidase IV inhibitors.[10] Furthermore, the metal complexation properties of the thiazole rings were also exploited for the development of molecules with promising anti-cancer activity.[11] The synthesis of (L)-(2-arylthiazol-4-yl)alanines was described either by a stereoconservative procedure from hexafluoroacetone-protected (L)-aspartic and (L)-glutamic acids,[12] or from (*S*)-methyl 2-amino-5-bromo-4-oxopentanoate by a Hantzsch-type condensation.[13]

We recently reported an efficient stereoselective synthesis of silicon-containing and unsaturated amino acids exploiting hydroxypinanone-induced diastereoselective alkylation chemistry.[14] We report herein the first efficient stereoselective synthesis of (L)-(β-arylthiazol-4-yl)alanines. These new unnatural α-amino acids are very promising by virtue of their interesting properties resulting from their aromatic side chains. The diaromatic system can, indeed, play an important role in ligand–receptor interactions as a result of hydrophobic properties and π-stacking interactions. To demonstrate one valuable application of these new entities, they have been introduced into the structure of neurotensin (NT)[8–13] (H-Lys-Lys-Pro-Tyr-Ile-Leu-OH) via replacement of the Tyr11 residue. NT is a neuropeptide that elicits changes in blood pressure, variation in body temperature, and naloxone-insensitive analgesic responses.[15] Structure–activity studies have demonstrated that the minimal sequence required for full biological activity is the C-terminal hexapeptide NT[8–13].[16] Among the known NT receptors, it has been shown that NTS1 is overexpressed in various relevant tumors, including ductal breast cancer[17] and pancreatic tumors.[18] NTS2 recently emerged as an attractive target in pain treatment.[19] From a drug design perspective, it is therefore

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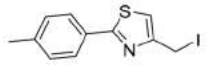
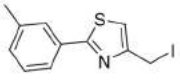
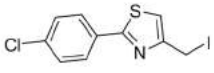
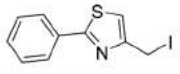
crucial to develop novel therapeutic agents that display selectivity for one receptor subtype. In this respect, position 11 of NT plays a key role in dictating receptor selectivity.[20] Based on the extended aromaticity of the β-arylthiazole moiety, relative to the Tyr11 aromatic side-chain, and comparable to a Trp side chain, we have introduced these new unnatural amino acids into NT[8–13] and evaluated their influence on binding, stability and activities of the resulting peptide analogues relative to the native peptide.

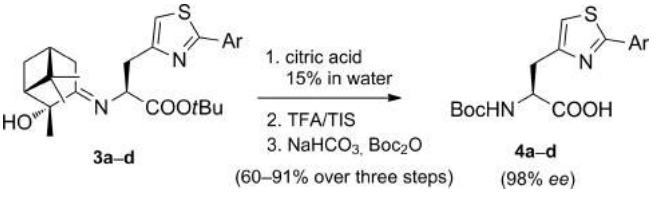
Results and Discussion

The synthesis of (L)-(2-arylthiazol-4-yl)alanines was accomplished using as the key reaction the diastereoselective alkylation of (1*R*,2*R*,5*R*)-2-hydroxy-3-pinanone Schiff base of glycine *tert*-butyl ester **2** (Scheme 1). The 2-aryl-4-(iodomethyl)thiazoles derivatives **1a**–**d** were obtained using Hantzsch condensation of various thiobenzamides with 1,3-dichloroacetone, followed by treatment with potassium iodide (Scheme 1).[21] The alkylation of **2** was performed by initial addition of LDA at –78 °C, followed by addition of 4-iodomethyl-2-arylthiazoles **1a**–**d**. After stirring for 12 h at –78 °C, and then for 2 h at –10 °C, corresponding alkylated Schiff bases **3a**–**d** and **3a′**–**d′** were each obtained as mixtures of two diastereomers in ratios up to 96:4 as determined by ^{1}H NMR of crude products and isolated in 60–82 % yield (Scheme 1, Table 1).

Table 1. , yield and chiral induction of the alkylation step.

Entry	Alkylating agent	Compounds	Ratio **3/3'**	Yield [%]
1		**3a**, **3a'**	95:5	69
2		**3b**, **3b'**	96:4	64
3		**3c**, **3c'**	93:7	82
4		**3d**, **3d'**	96:4	60

After separation of major diastereoisomer **3** by chromatography, the chiral auxiliary was removed by hydrolysis and free amino acids were obtained by treatment with trifluoroacetic acid (TFA) in the presence of triisopropylsilane. Finally, the amine functions were Boc-protected thus affording amino acids **4a**–**d** in 60–91 % yield over three steps and in > 98 % *ee* (as detected by chiral RP-HPLC) (Scheme 2). To evaluate *ee* values racemic amino acids were prepared by alkylation of diethyl acetamidomalonate with 2-aryl-4-(chloromethyl)thiazoles followed by treatment with Boc_2O.



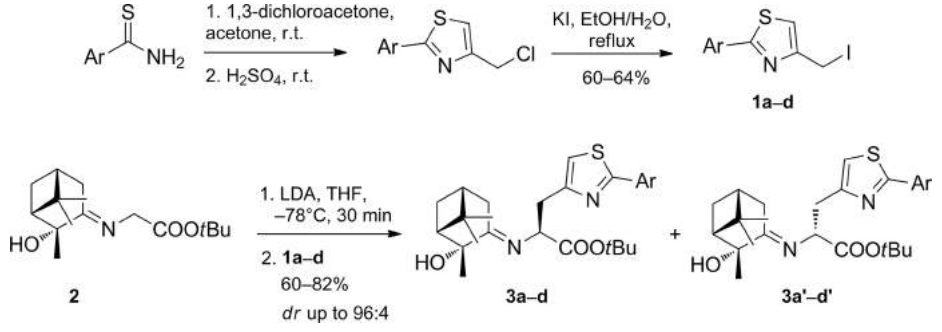
Scheme 2. Synthesis of Boc protected amino acids **4a**–**d**.

It is well-known that, depending on the chirality of the hydroxypinanone moiety, it is possible to control the stereochemistry of the final product. The formation of the (L)-enantiomer of **4d** was confirmed by measuring the optical rotation of the corresponding deprotected amino acid ***int*-(L)-4d** ($[\alpha]_D^{20}$ = –9.9 {*c* = 1.0, H_2O} see Supporting Information), and comparing with the literature value ($[\alpha]_D^{20}$ = –9.5 {*c* = 1.0, H_2O}).[12b] Thus, starting from (1*R*,2*R*,5*R*)-hydroxypinan-3-one, ***S*-4a**–**d** were obtained, in agreement with the ability of this chiral moiety to dictate generation of the absolute *S*-configuration.[22] The enantiomeric purities for **4a**–**d** were determined by chiral RP-HPLC analyses (≥ 98 % *ee*).

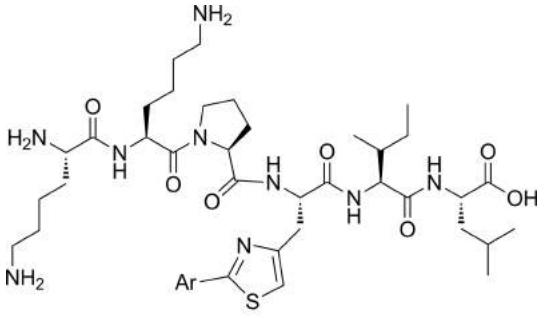
To validate the potential of these novel unnatural amino acids, they were introduced into the neurotensin (NT) peptide to explore structure-activity relationships and to improve metabolic peptide stabilities.[23] For this purpose, we synthesized four new NT analogues, replacing the Tyr11 residue with (L)-(+)-(β-arylthiazol-4-yl)alanines **4a**–**d** (Figure 1).

Hexapeptide NT[8–13] analogues were synthesized in solution using a [3 + 3] fragment condensation strategy. This allowed us to avoid racemisation during the coupling step owing to the presence of a owing to the presence of a proline residue.[24] All coupling reactions for the preparation of both tripeptides and hexapeptides were performed using BOP as the condensation agent and diisopropylamine as base, in DMF.

Boc-deprotection of the dipeptide Boc-Ile-Leu-OMe with TFA followed by condensation with **4a**–**d** afforded corresponding



Scheme 1. (+)-(1*R*,2*R*,5*R*)-2-hydroxy-3-pinanone-induced diastereoselective alkylation.



JMV 5618 compound **5**: Ar = *p*-tolyl
JMV 5619 compound **6**: Ar = *m*-methyl
JMV 5620 compound **7**: Ar = *p*-Cl-C_6H_4
JMV 5621 compound **8**: Ar = Ph

Figure 1. NT[8–13] analogues containing (L)-(+)-(2-arylthiazol-4-yl)alanines **4a–d**.

tripeptides Boc-**4a**–**d**-Ile-Leu-OMe in very good yields (>83 %). Further Boc-deprotection of these fragments was performed in the presence of triisopropylsilane as a scavenger to avoid *tert*-butylation of the 2-phenylthiazole moiety.[24] The condensation of *N*-deprotected tripeptides with the Boc-Lys(Z)-Lys(Z)-Pro-OH fragment afforded corresponding hexapeptides Boc-Lys(Z)-Lys(Z)-Pro-**4a**–**d**-Ile-Leu-OMe in acceptable yields (26–35 %) considering the steric hindrance of two tripeptides. Further saponification of the methyl ester in the presence of KOH, followed by a final one-pot Z- and Boc-deprotection in TFA/TFMSA/TIS, afforded the corresponding fully deprotected hexapeptides (**5**–**8**, Figure 1). Peptides **5**–**8** were then purified by preparative RP-HPLC.

We first evaluated the ability of these NT[8–13] derivatives **5**–**8** carrying β-arylthiazole alanine residues to inhibit the binding of ^{125}I-Tyr^3-NT to membranes prepared from cells stably expressing either hNTS1 or hNTS2 receptors. As shown in Table 2, insertion of the arylthiazole residues into position 11 decreased peptide binding affinities, relative to native NT[8-13], to both NTS1 and NTS2.

Table 2. Binding and stability of neurotensin analogues.

Compound	hNTS1	hNTS2	Selectivity	Plasma
	IC_{50} [nM]	IC_{50} [nM]	hNTS1 vs. hNTS2	stability (half-life min)
NT[8–13]	1.01 ± 0.09	5.52 ± 2.23	5.46	0.78 ± 0.10
5 (JMV5618)	1377 ± 312	86.9 ± 16.2	0.06	1.80 ± 0.14
6 (JMV5619)	1285 ± 217	80.0 ± 25.3	0.06	1.40 ± 0.11
7 (JMV5620)	55.1 ± 17.7	81.1 ± 13.6	1.47	1.25 ± 0.06
8 (JMV5621)	3.46 ± 1.34	139 ± 22.5	40.3	2.70 ± 0.51

Compounds **5** and **6**, incorporating either (L)-(+)-(β-*p*-tolylthiazol-4-yl)alanine or (L)-(+)-(β-*m*-tolylthiazol-4-yl)alanine residue, respectively at position 11, displayed improved selectivity to NTS2 with respect to **7** and **8**. This is in accordance with previous studies demonstrating that replacement of Tyr11 leads to improved NTS2 receptor selectivity.[20a] However, we found that binding interactions with compound **8**, in which the Tyr11 residue was substituted by a (L)-(+)-(β-phenylthiazol-4-yl)alanine residue, remained in the low nanomolar range with an IC_{50} of 3.46 ± 1.345 nM for hNTS1 but displayed a binding profile with hNTS2 (139 ± 22.5 nM) similar to those noted with hNTS2 and compounds **5**–**7**. These results suggest that the presence of rich electron-donating groups on the arylthiazoles in compounds **5** and **6** are deleterious to NTS1 interactions relative to cases involving a basic arylthiazole group (compound **8**). Furthermore, incorporation of (L)-(+)-(β-arylthiazol-4-yl)alanine residues at position 11 only slightly improved peptide stabilities and resistance to enzyme degradation (Table 2). This may be explained by the fact that these chemical modifications do not appear to prevent Lys8–Lys9 amino-terminal cleavage by the endopeptidase EC 3.4.24.15.[25]

Based on the higher binding affinity of compounds **7** and **8** for hNTS1, we next investigated the impact of (L)-(+)-(2-arylthiazol-4-yl)alanine incorporation on biological activity. A growing body of work has revealed that activation of NTS1 following intravenous administration of NT compounds results in a drop in blood pressure.[15,26] We thus injected compounds **7** and **8** to Sprague Dawley rats and monitored variations in arterial blood pressure via direct and continuous intra-carotid measurements (Figure 2).

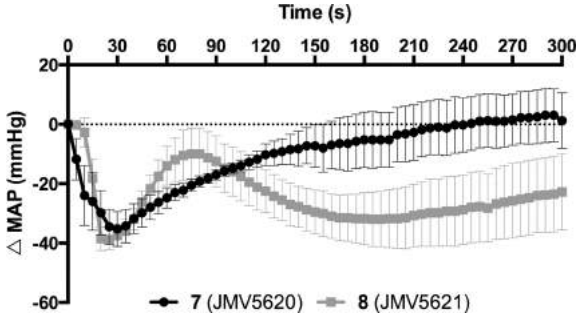


Figure 2. Effects of intravenous injection of compounds **7** (JMV5620) and **8** (JMV5621) on mean arterial pressure (ΔMAP) in anaesthetized rats. Tracings depicting the hypotensive effects after administration of each compound at 1 mg/kg. Data are expressed as means ± SEM obtained with 4 animals.

Intravenous bolus administration of 1 mg/kg of compounds **7** and **8** led to a short-lasting depressor phase of nearly 40 Torr followed by a pressor phase starting after 30 s. This return of arterial blood pressure to baseline values within less than 1 min is probably induced by the release of catecholamines from the adrenal medulla.[27] Finally, compound **8** produced a final sustained and pronounced hypotensive phase, as previously observed with the native NT peptide.[23a] However, in the same experimental paradigm, compound **7** failed to induce a sustained hypotensive effect at the employed dosage.

Conclusions

In conclusion, we have developed a synthesis of new β-arylthiazole-based α-amino acids using diastereoselective alkylation of (1*R*,2*R*,5*R*)-2-hydroxy-3-pinanone Schiff base with good yields and 98 % *ee*. Introduction of these new amino acids at position 11 of NT[8–13] was achieved yielding new NT analogs. Incorporation of (L)-(+)-(2-phenylthiazol-4-yl)alanine residue in position 11 of NT[8–13] resulted in NT analogs possessing good affinity for, and selectivity towards, NTS1, while also preserving the hy-

potensive properties of native NT. Modifications to the absolute configuration and aromatic portions of the arylthiazole-derived amino acids are currently under investigation.

Experimental Section

General: All reactions involving air-sensitive reagents were performed under argon. Thin layer chromatography was carried out using Merck Kieselgel 60 F254 sheets. Spots were visualized by treatment with iodine in the case of alkylated Schiff bases and by treatment with an ethanolic solution of ninhydrin 10 %, followed by heating in the case of amino acids and peptides. Purifications were performed with column chromatography using silica gel (Merck 60, 230–400 mesh) or with a Biotage Isolera 4 instrument using SNAP KP-SIL flash cartridges. Melting points were determined with a Buchi 510 melting point apparatus and are uncorrected. Infrared spectra were recorded using a Perkin–Elmer spectrum 100 FT-IR spectrometer. Proton nuclear magnetic resonance ^{1}H NMR and carbon nuclear magnetic resonance ^{13}C NMR spectra were recorded with a Bruker Avance DPX-300 spectrometer at 300 and 75 MHz respectively. All chemical shifts were recorded as values (ppm) relative to internal tetramethylsilane when $CDCl_3$ was used as solvent. Melting points were determined with a Büchi 510 melting point apparatus and are uncorrected. Low-resolution electrospray ionization (ESI) mass spectra were recorded using a micromass platform electrospray mass spectrometer. Spectra were recorded in positive mode (ESI^+). Optical rotations values were measured with a Perkin–Elmer 341 polarimeter (20 °C, sodium ray). High resolution mass spectra (HRMS) were recorded using a Synapt G2-S (Waters) mass detector operating at capillary tension of 1 kV and cone tension of 20 V. HPLC analyses were performed with a Beckman System Gold 126 instrument variable detector using column Chiracel OD-RH (0.46 cm × 15 cm), flow: 1 mL/min, H_2O (0.1 % TFA)/CH_3CN (0.1 % TFA).

General Procedure for the Preparation of 4-(Iodomethyl)-2-arylthiazole 1a–d: A mixture of thiobenzamide (2.05 g, 15 mmol) and 1,3-dichloroacetone (1.91 g, 15 mmol) dissolved in anhydrous acetone (20 mL) was stirred at room temperature for 24 h. The formed precipitate was filtered, dried and treated with concentrated sulfuric acid. After 3 h, the mixture was poured into ice and the formed precipitate was filtered, washed with water until neutral pH and dried. The resulting solid (2.1 g, 10 mmol) was dissolved in acetone (20 mL) and treated with a solution of NaI (1.8 g, 12 mmol) in acetone (20 mL). The reaction mixture was refluxed for 1 h and filtered. The filtrate was partially concentrated under reduced pressure, poured into ice and the formed precipitate of 4-(iodomethyl)-2-phenylthiazole **1d** was filtered and dried. The crude product was recrystallized from ethanol/H_2O. The 4-(iodomethyl)-2-aryl thiazoles **1a**–**c** were obtained by the same procedure, starting from 1,3-dichloroacetone and the corresponding 4-methylthiobenzamide, 3-methylthiobenzamide and 4-chlorothiobenzamide, respectively.

4-(Iodomethyl)-(2-*p*-tolyl)thiazole (1a): Compound **1a** (63 % yield) was obtained as a white solid, following the described general procedure, starting from 4-methylthiobenzamide (2.26 g, 15 mmol) and 1,3-dichloroacetone (1.91 g, 15 mmol), m.p. 128 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.39 (s, 3 H, CH_3), 4.57 (s, 2 H, CH_2I), 7.21 (s, 1 H, $H_{thiazole}$), 7.24 (d, J = 8.0 Hz, 2 H, H_{arom}), 7.83 (d, J = 8.0 Hz, 2 H, H_{arom}) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = –0.8, 21.9, 116.1 127.0, 130.1, 131.2, 141.0, 154.3, 169.1 ppm. FT-IR: $\tilde{\nu}$ = 3080, 1540, 1450, 1410, 1390, 1160, 990, 950, 820 cm^{-1}. HRMS calcd. for $[M + H]^+$ $C_{11}H_{11}NSI$: 315.9657, found 315.9660.

4-(Iodomethyl)-(2-*m*-tolyl)thiazole (1b): Compound **1b** (64 % yield) was obtained as a white solid following the described general procedure, starting from 3-methylthiobenzamide (2.26 g, 15 mmol) and 1,3-dichloroacetone (1.91 g, 15 mmol), m.p. 71 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.45 (s, 3 H, CH_3), 4.60 (s, 2 H, CH_2I), 7.29 (s, 2 H, $H_{thiazole\ and}$ H_{arom}), 7.33–7.35 (m, 1 H, H_{arom}), 7.74 (d, J = 8.0 Hz, 1 H, H_{arom}), 7.81 (s, 1 H, H_{arom}) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = –0.9, 21.8, 116.4, 124.3, 127.6, 129.3, 131.6, 133.7, 139.3, 154.4, 169.2 ppm. FT-IR: $\tilde{\nu}$ = 3080, 1590, 1460, 1435, 1415, 1162, 1144, 1002, 782, 761 cm^{-1}. HRMS calcd. for $[M + H]^+$ $C_{11}H_{11}NSI$: 315.9657, found 315.9656.

4-(Iodomethyl)-2-(*p*-chlorophenyl)-thiazole (1c): Compound **1c** (63 % yield) was obtained as a white solid, following the described general procedure, starting from 4-chlorothiobenzamide (2.57 g, 15 mmol) and 1,3-dichloroacetone (1.91 g, 15 mmol), m.p. 123 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.56 (s, 2 H, CH_2I), 7.25 (s, 1 H, $H_{thiazole}$), 7.40 (d, J = 8.0 Hz, 2 H, H_{arom}), 7.88 (d, J = 8.0 Hz, 2 H, H_{arom}) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = –1.9, 116.1, 127.6, 129.0, 131.6, 136.1, 154.1, 166.9 ppm. FT-IR: $\tilde{\nu}$ = 3080, 1370, 990, 920, 840, 755, 704 cm^{-1}. HRMS calcd. for $[M + H]^+$ $C_{10}H_8NSCII$: 335.9111, found 335.9112.

4-(Iodomethyl)-2-phenylthiazole (1d): Compound **1d** (60 % yield) was obtained as a white solid using thiobenzamide (2.05 g, 15 mmol) and 1,3-dichloroacetone (1.91 g, 15 mmol), m.p. 104 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.58 (s, 2 H, CH_2I), 7.25 (s, 1 H, $H_{thiazole}$), 7.43–7.45 (m, 3 H, H_{arom}), 7.93–7.96 (m, 2 H, H_{arom}) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = –1.3, 116.2, 126.8, 129.1, 130.4, 133.5, 154.2, 168.6 ppm. FT-IR: $\tilde{\nu}$ = 3080, 1509, 1497, 1460, 1144, 1003 cm^{-1}. HRMS calcd. for $[M + H]^+$ $C_{10}H_9NSI$: 301.9500, found 301.9499.

Diasteroselective Synthesis of Compounds 3a–d: To a solution of diisopropylamine (0.62 mL, 4.40 mmol) in dry THF (8.5 mL) was added slowly *n*-butyllithium 2.5 M in *n*-hexane (1.69 mL, 4.22 mmol) at –10 °C. After 30 min, the mixture was cooled to –78 °C and Schiff base **2** (494 mg, 1.76 mmol) dissolved in THF (1.5 mL) was added. After 30 min, one of the corresponding 4-(iodomethyl)-2-arylthiazoles **1a**–**d** (3.17 mmol, 1.8 equiv.) dissolved in THF (1.5–2 mL) was added slowly. The mixture was stirred at –78 °C for 12 h, and then at –10 °C for 2 h. The reaction mixture was quenched with a saturated solution of ammonium chloride (10 mL). THF was removed under reduced pressure and the aqueous phase was extracted three times with ethyl acetate (3 × 10 mL). The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated in vacuo. The diastereomeric ratio was determined by ^{1}H NMR of the crude product. Separation of diastereoisomers **3a–d** was performed by flash chromatography on silica gel using (cyclohexane/ethyl acetate, 9:1, then 8:2, and 7:3 with 1 % Et_3N) as eluent.

Compound 3a: Compound **3a** (304 mg, 57 %, *dr* 95:5) was obtained as a yellow oil following the general alkylation procedure of Schiff base **2** (317 mg, 1.13 mmol) and 4-(iodomethyl)-2-(*p*-tolyl)thiazole (**1a**) (640 mg, 2.03 mmol). R_f = 0.34 (cyclohexane/ethyl acetate, 7:3). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.27 (s, 3 H, $CH_{3\ bridge}$), 1.14 (s, 3 H, $CH_{3\ bridge}$), 1.26 (s, 3 H, C(OH)CH_3), 1.44 (s, 9 H, O*t*Bu), 1.86–1.93 (m, 2 H, CH_2CHC(OH)CH_3), 2.18–2.37 (m, 5H (m, 2 H, CHC$(CH_3)_2$ overlapped with s, 3 H, Ar-CH_3)), 2.50–2.56 (m, 2 H, CH_2CN), 3.19–3.27 (m, 1 H, CH_2CH_α), 3.43–3.49 (m, 1 H, CH_2CH_α), 4.75–4.79 (m, 1 H, CH_2CH_α), 6.86 (s, 1 H, $CH_{thiazole}$), 7.22 (d, J = 7.81 Hz, 2 H, H_{arom}), 7.74 (d, J = 7.98 Hz, 2 H, H_{arom}) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 21.4, 22.3, 27.2, 28.1, 28.4, 33.3, 34.8, 38.1, 38.3, 49.9, 62.1, 81.3, 115.0, 126.3, 129.5, 131.2, 140.1, 154.4, 167.9, 170.1, 178.8 ppm.

Compound 3b: Compound **3b** (304 mg, 57 %, *dr* 96:4) was obtained as a yellow oil following the general alkylation procedure of

Schiff base **2** (317 mg, 1.13 mmol) and 4-(iodomethyl)-2-(*m*-tolyl)thiazole **1b** (640 mg, 2.03 mmol). R_f = 0.34 (cyclohexane/ethyl acetate, 7:3). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.20 (s, 3 H, $CH_{3\ bridge}$), 1.06 (s, 3 H, $CH_{3\ bridge}$), 1.18 (s, 3 H, C(OH)CH_3), 1.44 (s, 9 H, O*t*Bu), 1.86–1.93 (m, 2 H, CH_2CHC(OH)CH_3), 2.21–2.38 (m, 5H (m, 2 H, CHC$(CH_3)_2$ overlapped with s, 3 H, Ar-CH_3)), 2.45–2.55 (m, 2 H, CH_2CN), 3.11–3.19 (m, 1 H, Het-CH_2CH_α), 3.35–3.41 (m, 1 H, Het-CH_2CH_α), 4.65–4.69 (m, 1 H, Het-CH_2CH_α), 6.80 (s, 1 H, $CH_{thiazole}$), 7.09–7.21 (m, 2 H, H_{arom}), 7.54–7.59 (m, 2 H, H_{arom}) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 21.1, 22.0, 26.9, 27.0, 27.8, 28.1, 33.0, 34.5, 37.8, 38.0, 49.6, 61.8, 81.1, 115.1, 123.2, 126.6, 128.5, 130.4, 133.4, 138.3, 154.2, 167.7, 169.8, 178.6 ppm.

Compound 3c: Compound **3c** (364 mg, 44 %, *dr* 93:7) was obtained as colorless oil following the general alkylation procedure of Schiff base **2** (494 mg, 1.76 mmol) and 4-(iodomethyl)-2-(*p*-chlorophenyl)thiazole (**1c**) (1.06 g, 3.16 mmol). R_f = 0.34 (cyclohexane/ethyl acetate, 7:3). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.21 (s, 3 H, $CH_{3\ bridge}$), 1.10 (s, 3 H, $CH_{3\ bridge}$), 1.19 (s, 3 H, C(OH)CH_3), 1.39 (s, 9 H, O*t*Bu), 1.81–1.87 (m, 2 H, CH_2CHC(OH)CH_3), 2.20–2.36 (m, 2 H, CHC$(CH_3)_2$), 2.48–2.62 (m, 2 H, CH_2CN), 3.19–3.24 (m, 1 H, CH_2CH_α), 3.37–3.38 (m, 1 H, CH_2CH_α), 4.68–4.72 (m, 1 H, CH_2CH_α), 6.87 (s, 1 H, $CH_{thiazole}$), 7.32 (d, J = 8.6 Hz, 2 H, H_{arom}), 7.75 (d, J = 8.6 Hz, 2 H, H_{arom}) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 22.2, 27.1, 28.0, 28.3, 33.3, 34.7, 38.0, 38.2, 49.9, 62.0, 81.5, 115.7, 127.5, 129.1, 132.2, 135.8, 154.8, 166.3, 169.9, 179.1 ppm.

Compound 3d: Compound **3d** (315 mg, 44 %, *dr* 96:4) was obtained as a yellow oil following the general alkylation procedure of Schiff base **2** (466 mg, 1.56 mmol) and 4-(iodomethyl)-2-phenylthiazole **1d** (860 mg, 2.86 mmol). R_f = 0.3 (cyclohexane/ethyl acetate, 7:3). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.27 (s, 3 H, $CH_{3\ bridge}$), 1.14 (s, 3 H, $CH_{3\ bridge}$), 1.26 (s, 3 H, C(OH)CH_3), 1.44 (s, 9 H, O*t*Bu), 1.86–1.94 (m, 2 H, CH_2CHC(OH)CH_3), 2.18–2.37 (m, 2 H, CHC$(CH_3)_2$), 2.50–2.62 (m, 2 H, CH_2CN), 3.21–3.26 (m, 1 H, Het-CH_2CH_α), 3.44–3.50 (m, 1 H, Het-CH_2CH_α), 4.75–4.80 (m, 1 H, Het-CH_2CH_α), 6.91 (s, 1 H, $CH_{thiazole}$), 7.39–7.40 (m, 3 H, H_{arom}), 7.85–7.87 (m, 2 H, H_{arom}) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 22.2, 27.1, 28.0, 28.4, 33.3, 34.8, 37.8, 38.2, 49.9, 62.0, 81.3, 115.4, 126.3, 128.8, 129.8, 133.7, 154.5, 167.6, 170.1, 178.8 ppm.

Synthesis of Boc-(L)-(β-arylthiazole-4-yl)alanines 4a–d: To a solution of alkylated Schiff base **3a–d** (0.2 M) in THF, was added a solution of citric acid 15 % (10 mL for 1 mmol of **3a–d**). The mixture was stirred at room temperature for 3 d. After removing THF in vacuo, the aqueous layer was extracted with diethyl ether (ratio organic layer/aqueous layer = 1:1), in order to remove the hydroxypinanone. The pH was then adjusted to 8–9 by addition of K_2CO_3. The aqueous layer was extracted three times with dichloromethane (ratio organic layer/aqueous layer: 2:1). After concentration, the corresponding solution of (β-arylthiazol-4-yl)alanine *tert*-butyl ester was treated with a mixture of TFA/triisopropylsilane (10:1) (4 mL of TFA and 0.4 mL of triisopropylsilane for 1 mmol). The mixture was stirred at room temperature for 4 h. Then the solvent was evaporated and the acid excess was removed by coevaporation with cyclohexane. The crude product was treated with Boc_2O (1.2 equiv.), in THF/H_2O (1:1) at pH 8–9, adjusted with $NaHCO_3$. The reaction mixture was stirred at room temperature overnight. After completion of the reaction, THF was removed under reduced pressure and the pH was adjusted to 3 with citric acid. The aqueous layer was extracted three times with ethyl acetate (ratio organic layer/aqueous layer = 2:1). The organic layer was dried with anhydrous $MgSO_4$ and filtered. The solvent was removed to afford the corresponding Boc-(L)-(2-arylthiazole-4-yl)alanines **4a–d**.

Boc-(L)-(β-(*p*-tolyl)thiazole-4-yl)alanine (4a): Compound ***S*-4a** (198 mg, 91 % overall yield) were obtained as a yellow solid by treatment of mono-alkylated Schiff base **3a** (283 mg, 0.60 mmol), m.p. 97 °C. MS ESI$^+$ for $C_{18}H_{22}N_2O_4S$: [M + H]$^+$ = 363.3. $[\alpha]_D^{20}$ = +9 (c = 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.63 (s, 9 H, O*t*Bu), 2.58 (s, 3 H, CH_3), 3.44–3.58 (m, 2 H, CH_2), 4.73 (m, 1 H, CH_α), 6.19 (br. s, 1 H, N*H*), 7.28 (s, 1 H, $H_{thiazole}$), 7.43 (d, J = 7.8 Hz, 2 H, H_{arom}), 7.94 (d, J = 7.9 Hz, 2 H, H_{arom}), 10.39 (br. s, 1 H, OH) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 21.5, 28.3, 33.2, 53.2, 80.1, 115.3, 126.5, 129.7, 129.9, 141.2, 151.9, 155.6, 169.2, 173.7 ppm. HRMS calcd. for [M + H]$^+$ $C_{18}H_{23}N_2O_4S$: 363.1379, found 363.1380. The enantiomeric excess *ee* = 98.1 % was determined by RP-HPLC on chiral column OD-RH, ACN (0.1 % TFA)/H_2O (0.1 % TFA) 35:65, 1 mL min^{-1}, λ = 214 nm, 20 °C, t_R (*S*) = 18.97 min, t_R (*R*) = 20.67 min.

Boc-(L)-(β-(*m*-tolyl)thiazole-4-yl)alanine (4b): Compound ***S*-4b** (150 mg, 64 % overall yield) were obtained as a yellow solid by treatment of mono-alkylated Schiff base **3b** (300 mg, 0.65 mmol), m.p. 53–54 °C. MS ESI$^+$ for $C_{18}H_{22}N_2O_4S$: [M + H]$^+$ = 363.2. $[\alpha]_D^{20}$ = +13 (c = 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.37 (s, 9 H, O*t*Bu), 2.34 (s, 3 H, CH_3), 3.14–3.32 (m, 2 H, CH_2), 4.47 (m, 1 H, CH_α), 5.94 (d, J = 5.8 Hz, 1 H, N*H*), 7.03 (s, 1 H, $H_{thiazole}$), 7.19–7.26 (m, 2 H, H_{arom}), 7.57–7.60 (m, 2 H, H_{arom}), 10.42 (br. s, 1 H, OH) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 21.4, 28.3, 33.2, 53.2, 80.2, 115.7, 123.8, 127.1, 129.1, 131.7, 132.0, 139.1, 151.9, 155.7, 169.4, 173.6 ppm. HRMS calcd. for [M + H]$^+$ $C_{18}H_{23}N_2O_4S$: 363.1379, found 363.1375. The enantiomeric excess *ee* = 99.1 % was determined by RP-HPLC on chiral column OD-RH, ACN (0.1 % TFA)/H_2O (0.1 % TFA) 35:65, 1 mL min^{-1}, λ = 280 nm, 20 °C, t_R (*S*) = 19.25 min, t_R (*R*) = 21.53 min.

Boc-(L)-(β-(*p*-chlorophenyl)thiazole-4-yl)alanine (4c): Compound ***S*-4c** (238 mg, 82 % overall yield) were obtained as a yellow solid by treatment of mono-alkylated Schiff base **3c** (371 mg, 0.76 mmol), m.p. 143 °C. MS ESI$^+$ for $C_{17}H_{19}ClN_2O_4S$: [M + H]$^+$ = 383.2. $[\alpha]_D^{20}$ = +14 (c = 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.60 (s, 9 H, O*t*Bu), 3.46–3.51 (m, 2 H, CH_2), 4.77–4.78 (m, 1 H, CH_α), 6.04 (d, J = 5.6 Hz, 1 H, N*H*), 7.43 (s, 1 H, $H_{thiazole}$), 7.54 (d, J = 8.3 Hz, 2 H, H_{arom}), 7.95 (d, J = 8.5 Hz, 2 H, H $_{arom}$), 9.89 (br. s, 1 H, OH) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 28.3, 33.1, 53.1, 80.3, 116.1, 127.7, 129.3, 131.1, 136.5, 152.4, 155.7, 167.4, 174.5 ppm. HRMS calcd. for [M + H]$^+$ $C_{17}H_{20}N_2O_4SCl$: 383.0832, found 383.0834. The enantiomeric excess *ee* = 98 % was determined by RP-HPLC on chiral column OD-RH, ACN (0.1 % TFA)/H_2O (0.1 % TFA) 35:65, 1 mL min^{-1}, λ = 214 nm, 20 °C, t_R (*S*) = 19.75 min, t_R (*R*) = 22.25 min.

Boc-(L)-(β-phenylthiazole-4-yl)alanine (4d): Compound ***S*-4d** (142 mg, 60 % overall yield) were obtained as a yellow solid by treatment of mono-alkylated Schiff base **3d** (310 mg, 0.68 mmol), m.p. 57 °C. MS ESI$^+$ for $C_{17}H_{20}N_2O_4S$: [M + H]$^+$ = 349.2. $[\alpha]_D^{20}$ = +17 (c = 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.57 (s, 9 H, O*t*Bu), 3.33–3.54 (m, 2 H, CH_2), 4.64–4.65 (m, 1 H, CH_α), 6.11 (d, J = 2.1 Hz, 1 H, N*H*), 7.38 (s, 1 H, $CH_{thiazole}$), 7.58–7.59 (m, 3 H, H_{arom}), 7.98–7.99 (m, 2 H, H_{arom}), 9.06 (br. s, 1 H, OH) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 28.3, 33.2, 53.2, 80.3, 115.9, 126.5, 129.3, 130.9, 132.0, 133.0, 152.0, 155.7, 169.2, 173.2 ppm. HRMS calcd. for [M + H]$^+$ $C_{17}H_{21}N_2O_4S$: 349.1222, found 349.1225. The enantiomeric excess *ee* = 97 % was determined by RP-HPLC on chiral column (OD-RH, ACN (0.1 % TFA)/H_2O (0.1 % TFA) 30:70, 1 mL min^{-1}, λ = 214 nm, 20 °C, t_R (*S*) = 27.65 min, t_R (*R*) = 30.38 min.

General Procedure for Coupling N-Protected Amino Acid with *C*-Protected Amino Acid: To a solution of a C-protected amino acid (1 equiv.) in DMF (10 mL/mmol) were added BOP (1 equiv.), the N-protected amino acid (1 equiv.) and then diisopropylethylamine (2.5 equiv.). The reaction mixture was stirred at room temperature for 1.5 h. After removing DMF, the crude product was diluted in

ethyl acetate (20 mL/mmol). The organic layer was washed with a solution of potassium hydrogen sulfate 1 M (3 × 20 mL), a saturated solution of sodium hydrogen carbonate (3 × 20 mL) and then a saturated solution of sodium chloride (3 × 20 mL). The organic layer was then dried with anhydrous $MgSO_4$, filtered and concentrated.

General Procedure for Deprotecting Boc Group: A solution of a N-protected peptide in TFA (4 mL/mmol) was stirred at room temperature for 1 h. In the case of peptides containing amino acids **4a**–**d**, which are sensitive to Friedel and Crafts alkylation, 0.1 mol-% of triisopropylsilane was added to TFA. Excess TFA was then removed in vacuo using cyclohexane as co-solvent.

Boc-Lys(Z)-Pro-OMe: The dipeptide was obtained as described in protocol 2.3.1 starting from H-Pro-OMe and Boc-Lys(Z)-OH. The dipeptide was purified by flash chromatography on silica gel (cyclohexane/ethyl acetate, 4:6) and isolated with 61 % yield. Analytical RP-HPLC: t_R = 2.3 min. R_f = 0.35 (cyclohexane/ethyl acetate, 4:6). ESI-MS: $[M + H]^+$ = 492.2.

Boc-Lys(Z)-Lys(Z)-Pro-OMe: Boc-Lys(Z)-Pro-OMe (2.65 g, 5.4 mmol) was *N*-deprotected according to procedure 2.3.2. with 20 mL of TFA. The obtained TFA•H-Lys(Z)-Pro-OMe was coupled in DMF (50 mL) with Boc-Lys(Z)-OH•DCHA (3.02 g, 1 equiv.) according to protocol 3.2.1 with BOP (2.4 g, 1 equiv.) and diisopropylamine (2.35 mL, 2.5 equiv.). The tripeptide was purified by silica flash chromatography (cyclohexane/ethyl acetate, 1:9 v/v) and isolated with 62 % yield. Analytical RP-HPLC: t_R = 2.6 min. ESI-MS: $[M + H]^+$ = 754.4.

Boc-Lys(Z)-Lys(Z)-Pro-OH: Boc-Lys(Z)-Lys(Z)-Pro-OMe (4.25 g, 5.86 mmol) was dissolved in methanol (55 mL) and was treated with a solution of KOH (4 N) (23.45 mmol, 5.86 mL). The reaction mixture was stirred overnight at room temperature. After the evaporation of methanol, the pH of the aqueous solution was adjusted to 2–3 with citric acid. The product was extracted three times with ethyl acetate (ratio organic layer: aqueous layer = 2:1). The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated, affording the tripeptide in quantitative yield. Analytical RP-HPLC: t_R = 2.3 min. ESI-MS: $[M + H]^+$ = 740.4.

Boc-Ile-Leu-OMe: H-Leu-OMe (3 g, 7.62 mmol) was coupled in DMF (80 mL) with Boc-Ile-OH (1.76 g, 1 equiv.) according to protocol 2.3.1 with BOP (3.37 g, 1 equiv.) and diisopropylamine (3.31 mL, 2.5 equiv.). The dipeptide was purified by silica gel chromatography (cyclohexane/ethyl acetate, 7:3) and isolated with 93 % yield. R_f = 0.5 (cyclohexane/ethyl acetate, 7:3). ESI-MS: $[M + H]^+$ = 435.0.

General Procedure for Synthesis of Boc-(L)-4a–d-Ile-Leu-OMe: A solution of Boc-Ile-Leu-OMe was stirred in TFA (4 mL/mmol) and 0.1 mol-% of triisopropylsilane at room temperature for 1 h. Excess TFA was then removed in vacuo using cyclohexane as a co-solvent. To a solution of the corresponding salt TFA•H-Ile-Leu-OMe in DMF (10 mL/mmol) were added successively, BOP (1 equiv.), then N-protected amino acid **(L)-4a**–**d** (1 equiv.) and diisopropylethylamine (2.5 equiv.). The reaction mixture was stirred at room temperature for 1.5 h. After removing DMF, the crude product was diluted in ethyl acetate (20 mL/mmol). The organic layer was washed with a solution of potassium hydrogen sulfate 1*M* (3 × 20 mL), a saturated solution of sodium hydrogen carbonate (3 × 20 mL) and then a saturated solution of sodium chloride (3 × 20 mL). The organic layer was then dried with anhydrous $MgSO_4$, filtered and concentrated. The tripeptides were isolated in 82–97 % yield.

Boc-(L)-4a-Ile-Leu-OMe: 143 mg of Boc-Ile-Leu-OMe (0.4 mmol) and 145 mg of **(L)-3a** (0.4 mmol) were used to afford 198 mg of Boc-**(L)-4a**-Ile-Leu-OMe as a colorless oil in 82 % yield; R_f = 0.55 (cyclohexane/ethyl acetate, 8:2); t_R = 2.8 min. ESI-MS: $[M + H]^+$ = 603.8.

Boc-(L)-4b-Ile-Leu-OMe: 143 mg of Boc-Ile-Leu-OMe (0.4 mmol) and 145 mg of **(L)-3b** (0.4 mmol) were used to afford 200 mg of Boc-**(L)-4b**-Ile-Leu-OMe as a colorless oil in 83 % yield; R_f = 0.55 (cyclohexane/ethyl acetate, 8:2); t_R = 2.8 min. ESI-MS: $[M + H]^+$ = 603.8.

Boc-(L)-4c-Ile-Leu-OMe: 143 mg of Boc-Ile-Leu-OMe (0.4 mmol) and 153 mg of **(L)-3c** (0.4 mmol) were used to afford 219 mg of Boc-**(L)-4c**-Ile-Leu-OMe as a colorless oil in 88 % yield; R_f = 0.55 (cyclohexane/ethyl acetate, 8:2); t_R = 2.8 min. ESI-MS: $[M + H]^+$ = 624.3.

Boc-(L)-4d-Ile-Leu-OMe: 143 mg of Boc-Ile-Leu-OMe (0.4 mmol) and 139 mg of **(L)-3d** (0.4 mmol) were used to afford 229 mg of Boc-**(L)-3c**-Ile-Leu-OMe as a colorless oil in 97 % yield; R_f = 0.55 (cyclohexane/ethyl acetate, 8:2). t_R = 2.7 min. ESI-MS: $[M + H]^+$ = 589.8.

General Procedure for Synthesis of Boc-Lys(Z)-Lys(Z)-Pro-(L)-4a–d-Ile-Leu-OMe: A solution of Boc-**(L)-4a–d**-Ile-Leu-OMe was stirred in TFA (4 mL/mmol) and 0.1 mol-% of triisopropylsilane at room temperature for 1 h. Excess TFA was then removed in vacuo using cyclohexane as a co-solvent. To a solution of the corresponding salt TFA•H-**(L)-4a–d**-Ile-Leu-OMe in DMF (2 mL for 0.5 mmol) was added BOP (1.5 equiv.), then Boc-Lys(Z)-Lys(Z)-Pro-OH (1 equiv.) and diisopropylamine (2.5 equiv.). The mixture was stirred overnight at room temperature, DMF was removed and the crude product was diluted in ethyl acetate (20 mL/mmol). The organic layer was successively washed with a solution of potassium hydrogen sulfate 1M (3 × 20 mL), a saturated solution of sodium hydrogen carbonate (3 × 20 mL) and then a saturated solution of sodium chloride (3 × 20 mL). The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel, using a gradient separation procedure with a mixture of chloroform/methanol (99:1, then 95:5).

Boc-Lys(Z)-Lys(Z)-Pro-(L)-4a-Ile-Leu-OMe: 118 mg of Boc-Lys(Z)-Lys(Z)-Pro•OH (0.16 mmol) and 98 mg of TFA•H-**(L)-4a**-Ile-Leu-OMe (0.16 mmol) were used to afford 102 mg of Boc-Lys(Z)-Lys(Z)-Pro-**(L)-4a**-Ile-Leu-OMe as a colorless oil. 35 % yield; t_R = 3 min. ESI-MS: $[M + H]^+$ = 1224.7; $[M + Na]^+$ = 1246.8.

Boc-Lys(Z)-Lys(Z)-Pro-(L)-4b-Ile-Leu-OMe: 244 mg of Boc-Lys(Z)-Lys(Z)-Pro•OH (0.33 mmol) and 203 mg of TFA•H-**(L)-4b**-Ile-Leu-OMe (0.33 mmol) were used to afford 143 mg of Boc-Lys(Z)-Lys(Z)-Pro-**(L)-4b**-Ile-Leu-OMe as a colorless oil. 30 % yield; t_R = 3 min. ESI-MS: $[M + H]^+$ = 1224.8.

Boc-Lys(Z)-Lys(Z)-Pro-(L)-4c-Ile-Leu-OMe: 240 mg of Boc-Lys(Z)-Lys(Z)-Pro•OH (0.35 mmol) and 223 mg of TFA•H-**(L)-4c**-Ile-Leu-OMe (0.35 mmol) were used to afford 130 mg of Boc-Lys(Z)-Lys(Z)-Pro-**(L)-4c**-Ile-Leu-OMe as a colorless oil. 30 % yield; t_R = 3 min. ESI-MS: $[M + H]^+$ = 1244.7.

Boc-Lys(Z)-Lys(Z)-Pro-(L)-4d-Ile-Leu-OMe: 288 mg of Boc-Lys(Z)-Lys(Z)-Pro•OH (0.4 mmol) and 246 mg of TFA•H-**(L)-4d**-Ile-Leu-OMe (0.4 mmol) were used to afford 122 mg of Boc-Lys(Z)-Lys(Z)-Pro-**(L)-4d**-Ile-Leu-OMe as a colorless oil. 26 % yield t_R = 2.9 min. ESI-MS: $[M + H]^+$ = 1210.8; $[M + Na]^+$ = 1232.7.

Boc-Lys(Z)-Lys(Z)-Pro-(L)-4a–d-Ile-Leu-OH: Boc-Lys(Z)-Lys(Z)-Pro-**(L)-4a–d**-Ile-Leu-OMe was dissolved in methanol (2 mL/0.1 mmol) and was treated with a solution of KOH (4N) (5 equiv.). The reaction mixture was stirred overnight at room temperature. After the evaporation of methanol, the aqueous solution was treated with citric acid to pH = 2–3. The product was extracted three times with ethyl acetate (ratio organic layer: aqueous layer = 2:1). The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated.

Boc-Lys(Z)-Lys(Z)-Pro-(L)-4a-Ile-Leu-OH: 69 mg of Boc-Lys(Z)-Lys(Z)-Pro-**(L)-4a**-Ile-Leu-OH (0.056 mmol) were used to afford 55 mg of Boc-Lys(Z)-Lys(Z)-Pro-**(L)-4a**-Ile-Leu-OH as a colorless oil in 81 % yield; t_R = 2.8 min. ESI-MS: $[M + H]^+$ = 1210.7; $[M + Na]^+$ = 1232.7.

Boc-Lys(Z)-Lys(Z)-Pro-(L)-4b-Ile-Leu-OH: 122 mg of Boc-Lys(Z)-Lys(Z)-Pro-**(L)-4b**-Ile-Leu-OMe (0.1 mmol) were used to afford 121 mg of Boc-Lys(Z)-Lys(Z)-Pro-**(L)-4b**-Ile-Leu-OMe 99 % yield; t_R = 2.8 min. ESI-MS: $[M + H]^+$ = 1210.7.

Boc-Lys(Z)-Lys(Z)-Pro-(L)-4c-Ile-Leu-OH: 130 mg of Boc-Lys(Z)-Lys(Z)-Pro-**(L)-4c**-Ile-Leu-OMe (0.1 mmol) were used to afford 127 mg of Boc-Lys(Z)-Lys(Z)-Pro-**(L)-4c**-Ile-Leu-OH as a colorless oil in 99 % yield; t_R = 2.8 min. ESI-MS: $[M + H]^+$ = 1230.7; $[M + Na]^+$ = 1252.6.

Boc-Lys(Z)-Lys(Z)-Pro-(L)-4d-Ile-Leu-OH: 123 mg of Boc-Lys(Z)-Lys(Z)-Pro-**(L)-4d**-Ile-Leu-OMe (0.1 mmol) were used to afford 117 mg of Boc-Lys(Z)-Lys(Z)-Pro-**(L)-4d**-Ile-Leu-OH as a colorless oil. 98 % yield; t_R = 2.7 min. ESI-MS: $[M + H]^+$ = 1196.7.

Synthesis of H-Lys-Lys-Pro-(L)-4a–d-Ile-Leu-OH: A solution of Boc-Lys(Z)-Lys(Z)-Pro-**(L)-4a–d**-Ile-Leu-OH in a mixture of TFA/triisopropylsilane (2:1) was stirred at room temperature for 10 min. TFA (5 equiv.) was then added and the reaction was stirred for 30 min. After total conversion, TFA was removed by co-evaporation with cyclohexane and the crude product was purified by preparative HPLC, using a gradient separation procedure: 10 min from 0 to 25 % acetonitrile; then 30 min from 25–35 % acetonitrile, then 10 min from 35–100 % acetonitrile.

H-Lys-Lys-Pro-(L)-4a-Ile-Leu-OH (JMV5618): t_R = 1.7 min; $[M + Na]^+$ = 864.47. HRMS: calcd. for $C_{42}H_{68}N_9O_7S$: $[M + H]^+$ = 842.4962, found 842.4962.

H-Lys-Lys-Pro-(L)-4b-Ile-Leu-OH (JMV5619): t_R = 1.7 min; $[M + Na]^+$ = 864.48. HRMS: calcd. for $C_{42}H_{68}N_9O_7S$: $[M + H]^+$ = 842.4962, found 842.4959.

H-Lys-Lys-Pro-(L)-4c-Ile-Leu-OH (JMV5620): t_R = 1.7 min; $[M + Na]^+$ = 884.42. HRMS calcd. for $C_{41}H_{65}ClN_9O_7S$: $[M + H]^+$ = 862.4416, found 862.4417.

H-Lys-Lys-Pro-(L)-4d-Ile-Leu-OH (JMV5621): t_R = 1.6 min; $[M + Na]^+$ = 850.46. HRMS: calcd. for $C_{41}H_{66}N_9O_7S$: $[M + H]^+$ = 828.4806, found 828.4806.

Biological Results and Methods

Competitive Radioligand Binding Assay: CHO-K1 cells stably expressing hNTS1 or 1321N1 cells stably expressing hNTS2 were cultured respectively in Ham's F12 or DMEM. Culture media were supplemented with 10 % FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, 20 mM HEPES and 0.4 mg/mL G418 at 37 °C in a humidified chamber at 5 % CO_2. Competitive radioligand binding experiments were performed by incubating 50 µg of freshly prepared cells membranes, expressing either hNTS1 or hNTS2 with 0.1 nM (for hNTS1) or 0.4 nM (for hNTS2) ^{125}I-Tyr^3-NT (2200 Ci/mmol) and increasing concentrations of the analogues ranging from 10^{-11} to 10^{-5} M for 30 min at 25 °C in binding buffer (50 mM Tris-HCl, pH 7.5, 0.2 % BSA). After incubation, the binding reaction mixture was transferred in polyethylenimine-coated 96 well filter plates (Millipore, Billerica, MA). Reaction was terminated by filtration and plate was washed three times with 200 µL of ice-cold binding buffer. Glass filters were then counted in a γ-counter. Non-specific binding was measured in the presence of 10^{-5} M unlabeled NT[8–13] and represented less than 5 % of total binding. IC_{50} values were determined from the competition curves as the unlabeled ligand concentration inhibiting half of the [^{125}I][Tyr^3]Neurotensin-specific binding. Data were plotted using GraphPad Prism 6 using the One-site – Fit log (IC_{50}) and represent the mean ± SEM of at least three separate experiments.

Animal Procedures: All animal procedures were approved by the Ethical and Animal Care Committee of the Université de Sherbrooke and were in accordance with policies and directives of the Canadian Council on Animal Care. Adult male (250–300 g) Sprague-Dawley rats (Charles River Laboratories, St-Constant, Québec, Canada) were maintained on a 12 h light/dark cycle with free access to food and water. Rats were acclimatized for 4 d to the animal facility and for 2 d to manipulations and devices prior to behavioral studies.

Plasma Stability: Rat plasma was obtained from rat blood by keeping the translucent phase after centrifugation at 15,000 g over 5 min. Plasma stability assay was carried out by incubating each analog in rat plasma at final concentration of 0.156 mM for 0, 2, 5, 15, and 30 min at 37 °C before adding 50 µL of CH_3CN containing 0.1 mM Fmoc-Glycine as an internal standard. After centrifugation at 15,000 g for 30 min, supernatant was filtered through 0.22 µm filter and analyzed by UPLC/MS (Water H Class Acquity UPLC, mounted with Acquity UPLC BEH C18 column, 1.7 µm, 2.1 × 50 mm and paired to a SQ Detector 2). Quantification was done by determining the AUC (area under the curve) ratio of tested compound over AUC of Fmoc-Glycine. Remaining compound over time was plotted into GraphPad Prism 6 and the half-life of each compound was calculated using one-phase decay fit and represented the mean ± SEM of at least three separate experiments.

Blood Pressure Measurement: Male Sprague-Dawley rats were anesthetized with a mixture of ketamine/xylaxine (87 mg/kg: 13 mg/kg, i.m.) and placed in supine position on a heating pad. Mean, systolic and diastolic arterial blood pressure as well a heart rate were measured through a catheter (PE 50 filled with heparinized saline) inserted in the right carotid artery and connected to a Micro-Med transducer (model TDX-300, USA) linked to a blood pressure Micro-Med analyzer (model BPA-100c). Another catheter was inserted in the left jugular vein for bolus injections (1 mL/kg, 5–10 s) of vehicle (isotonic saline) or compounds **7** (JMV5620) and **8** (JMV5621) at 1 mg/kg. Rats were given vehicle first, then only one dose of one analogue before being euthanized. For relative potency evaluation, changes in blood pressure from baseline to post-injection in individual animals were determined. Data represents the mean ± SEM of at least four different experiments.

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Keywords: Medicinal chemistry · Receptors · Neurotensin analogues · Amino acids · Peptides · Alkylation

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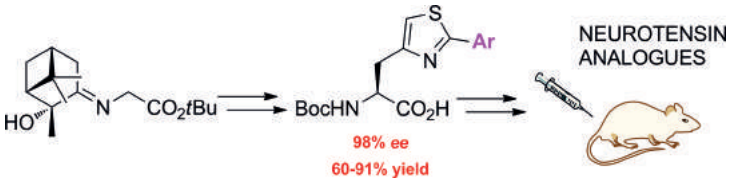
Modified Neurotensin



New β-arylthiazole alanines were synthesized in good chemical yields and with 98 % *ee* using a diastereoselective alkylation; these alanine derivatives were then used as Tyr11 replacements in the construction of neurotensin (NT)[8–13] analogues. The new NT analogues showed improved plasma stability and selectivity towards NTS1 thus preserving the hypotensive properties of the native peptide.

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