Pergamon

Tetrahedron 56 (2000) 2513–2522

TETRAHEDRON

Synthesis of Alanine and Proline Amino Acids with Amino or Guanidinium Substitution on the Side Chain

Zhenyu Zhang,[a] Arthur Van Aerschot,[a] Chris Hendrix,[a] Roger Busson,[a] Frank David,[b] Pat Sandra[b] and Piet Herdewijn[a,*]

[a]*Laboratory of Medicinal Chemistry, Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium*

[b]*Research Institute for Chromatography, Kennedypark 20, B-8500 Kortrijk, Belgium*

Received 2 November 1999; revised 24 January 2000; accepted 10 February 2000

Abstract—Competitive binding of peptides containing basic amino acids to disrupt or prevent the Tat–TAR interaction could result in diminished transcription as well as translation and hence constitutes an alternative way of controlling HIV replication. Therefore, we synthesized guanidinium and amino containing amino acids, based on a proline or an alanine scaffold. The introduction of the guanidinium moiety was best accomplished using 1*H*-pyrazole-1-carboxamidine hydrochloride, with Pmc used for its protection. The absence of racemization, maintained throughout the whole synthesis, was confirmed by chiral purity determination. These building blocks were smoothly incorporated into oligopeptides, which proved their suitability for use in a combinatorial approach for selecting TAR binding ligands.

Introduction

In the past decade, remarkable clinical success in the field of target-directed AIDS treatment has been achieved with drugs against two important enzymes in the HIV-1 life cycle, the reverse transcriptase and the HIV protease.[1,2] However, the rapid development of viral resistance against existing drugs has stimulated the exploration of alternative targets.[3] One of these targets is the viral *trans*-activator protein, Tat. This regulatory protein is required early in the viral life cycle to increase the efficiency of transcriptional elongation by binding to a region near the start of transcription in the viral long terminal repeat (LTR) called the *trans*-activation response element.[4,5] The regulatory protein is one of the important viral proteins, which have scarcely been used as targets. A lower rate of resistance against TAR-binding compounds can be expected because the drugs would act at the level of gene expression and Tat–TAR interactions are highly conserved.[4] Moreover, mutations in the protein-binding site of the RNA will (most probably) result in replication of incompetent viruses.

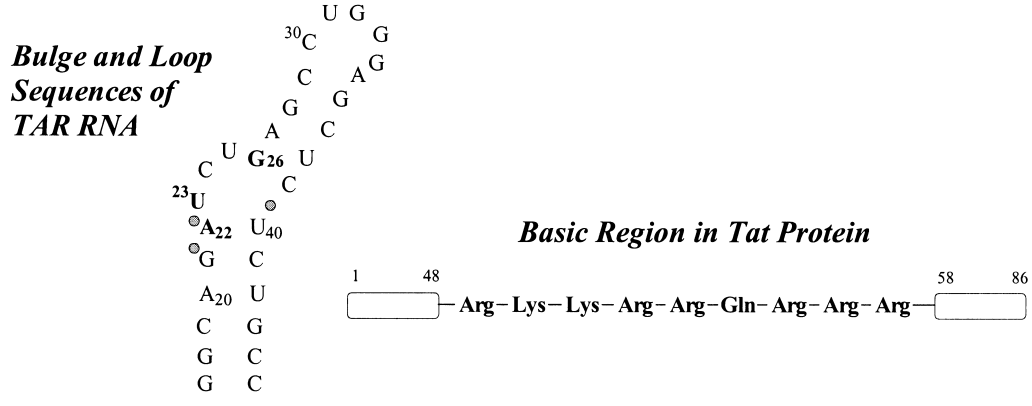


Figure 1. The basic region of Tat protein induces a local conformational rearrangement, which enhances the accessibility of unfunctional groups on TAR RNA.

Keywords: guanidinium substitution; Tat–TAR interaction; HIV replication.

* Corresponding author. Tel.: +32-16-33-73-87; fax: +32-16-33-73-40; e-mail: piet.herdewijn@rega.kuleuven.ac.be

0040–4020/00/$ - see front matter
PII: S0040-4020(00)00123-X

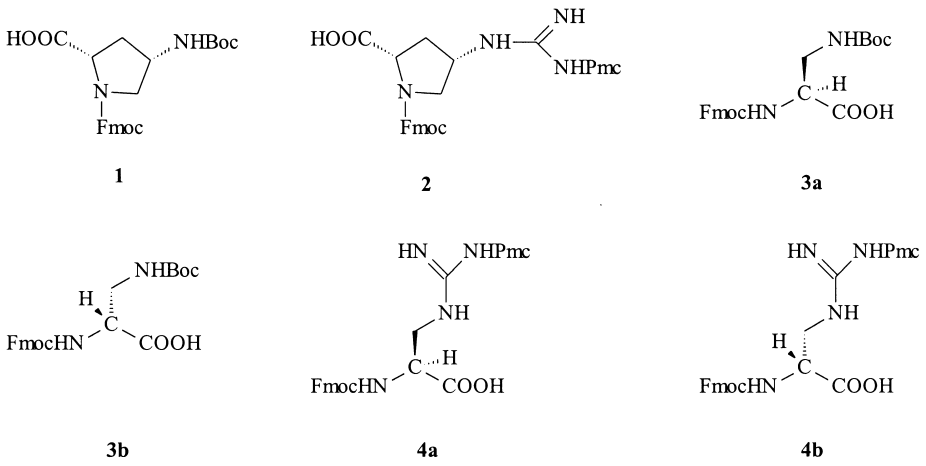


Figure 2. Orthogonal protected basic amino acids using proline **1**, **2** or alanine **3a**, **4a**, **3b**, **4b** as scaffold.

During past years, the interaction of Tat protein with TAR RNA has been studied in more detail. NMR studies showed that the accessibility of functional groups on TAR RNA to be recognized by Tat protein is enhanced by a local conformational rearrangement.[6–8] This folding process is induced by the basic region of Tat, which comprises a series of eight positively charged residues located within a stretch of nine amino acids[4] (Fig. 1). Following this observation, it has been suggested[8] that ligands carrying the basic guanidinium group may bind to TAR RNA and induce folding. As a result the ligand may block the regulatory function of the Tat–TAR complex.

Because of the complex interaction pattern involved in RNA structure organization and folding, de novo design of RNA-binding ligands is very difficult. Combinatorial chemistry might prove to be an alternative.[9–12] This approach allows the simultaneous synthesis of a large number of compounds that can be used for screening for TAR-binding properties.

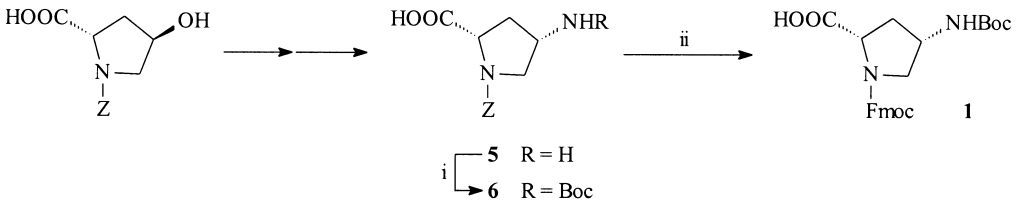
Since the library components are envisaged to compete with Tat for binding to TAR RNA, it seems reasonable to introduce functional groups interacting with TAR RNA via a similar mechanism. The basic region of Tat protein is extremely important for Tat–TAR RNA sequence specific interaction.[4,8] In this region eight positively charged amino acids are found, incorporating two lysine and six arginine residues. Therefore, a first library approach to select TAR binding compounds may be based on a selection of various positively charged and structurally more constrained residues that can be assembled in a peptide-like structure.

In a first step we plan to develop libraries of unnatural peptides containing one or more positively charged residues. Therefore we synthesized orthogonal protected amino acid analogues containing a basic functionality (amino or guanidine group) on the side chain. These amino acids are structurally based on a proline (**1**, **2**) or alanine (**3a**, **3b**, **4a**, **4b**) scaffold (Fig. 2).

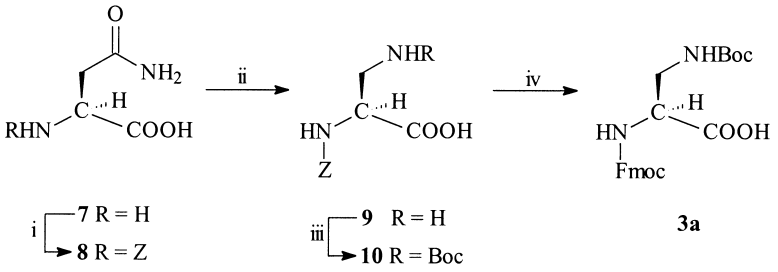
All building blocks were functionalized to be incorporated into peptides following the Fmoc-strategy. During the total synthesis the enantiomeric purity of these residues was properly retained as confirmed by chiral purity analysis. To evaluate the potential use of these modified α-amino acids as components in an oligopeptide library, the modified amino acids were incorporated in a peptide sequence. Following deprotection and cleavage from the solid support, the unnatural peptide was analyzed for its purity and correct structure, and the yield of incorporation was determined. This work lets us conclude that the newly described amino acids may be useful building blocks in a potential anti-Tat library.

Results and Discussion

The synthesis of 4-amino-*N*-benzyloxycarbonyl-L-*cis*-proline, (**5**) has been previously reported.[13] Since peptide assembly using the Fmoc-strategy was envisaged, the Boc group was used to protect the primary amino group of **5**. Therefore, **5** was reacted with ditertbutyl dicarbonate in the presence of 10% Na_2CO_3, affording **6** in 93% yield. Removal of the Z group by catalytic hydrogenation enabled the final protection of the α-amine with Fmoc-Cl under



Scheme 1. (i) 1.2 equiv. $(Boc)_2O$ in 10% Na_2CO_3 and dioxane; 93%, (ii) a. H_2, Pd/C in MeOH and H_2O, 45 psi; b. 1.2 equiv. Fmoc-Cl in 10% Na_2CO_3 and dioxane; 81%.

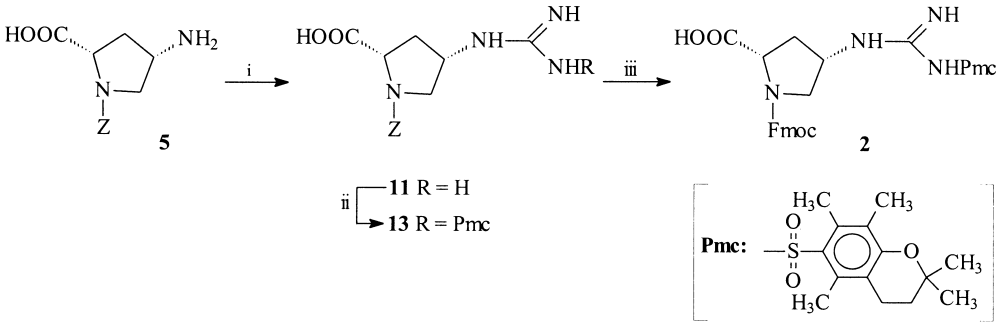


Scheme 2. (i) 1.2 equiv. Z-Cl in 10% Na_2CO_3 and dioxane; 84%, (ii) 1.5 equiv. [bis(trifluroacetoxy)iodo]-benzene in DMF/H_2O (1:1) and pyridine; 47%, (iii) 1.2 equiv. $(Boc)_2O$ in 10% Na_2CO_3 and dioxane; 91%, (iv) a. H_2, Pd/C in MeOH and H_2O, 45 psi; b. 1.2 equiv. Fmoc-Cl in 10% Na_2CO_3 and dioxane; 81%.

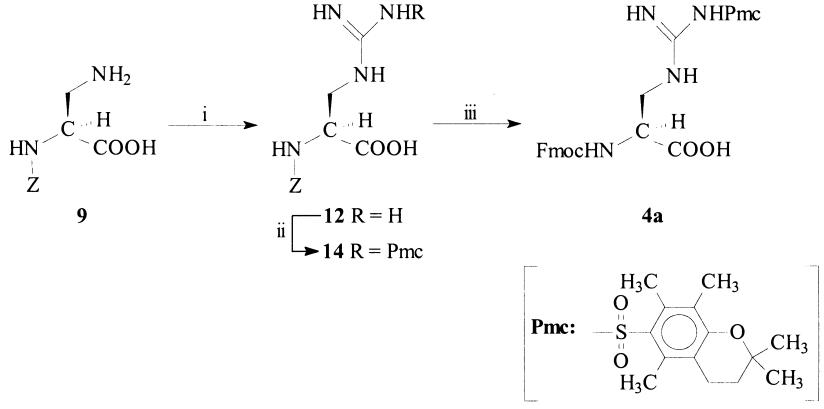
Schotten–Baumann conditions. The desired product **1** was obtained in 81% yield (Scheme 1).

For the synthesis of the amino analogue **3**, we first started from serine. However, mesylation of its β-alcohol group easily led to an elimination reaction. Therefore we investigated opening of serine-β-lactone with ammonia.[14] This led predominantly to formation of the amide of serine instead of the desired β-amino alanine derivative. Finally, we utilized a modified Hoffmann rearrangement as reported by Waki et al.,[15] starting from L-asparagine (**7**) (Scheme 2). Here also the α-amino function of asparagine was protected with a benzyloxycarbonyl group to give **8** in a yield of 84%. This derivative was converted into the desired diaminopropionic acid by addition of a solution of [bis(trifluroacetoxy)iodo]-benzene in a mixture of DMF-water (1:1) and pyridine and **9** was obtained in 47% yield as crystalline material from ether. The β-amino group was protected with a Boc group to yield **10**. The Z group was cleaved and replaced with Fmoc to afford **3a** in 80% yield.

Several approaches for the conversion of an amino group into a guanidine function have been reported in literature.[16] Guanylation via the formation of a carbodiimide intermediate, prepared in situ by reaction of di-Boc protected thiourea with Mukaiyama's reagent[16,17] or with $HgCl_2$,[18] has been widely used. More recently, triflated di-Boc guanidine was reported[19] to effectively carry out guanidine formation starting from an amine. However, both approaches suffer from an unpleasant and complicated manipulation. Moreover a di-Boc protected approach was not very attractive to us as it rendered the guanidine moiety less stable during subsequent reactions (often the guanidine group was degraded to yield the starting amino analogue).[20]



Scheme 3. (i) 1 equiv. 1*H*-pyrazole-1-carboxamidine hydrochloride in 1.0 M Na_2CO_3; 40%, (ii) 1.8 equiv. Pmc-Cl in 3.2 M NaOH and acetone at 0°C; 50%, (iii) a. H_2, Pd/C in MeOH and H_2O, 45 psi; b. 1.2 equiv. Fmoc-Cl in 10% Na_2CO_3 and dioxane; 81%.



Scheme 4. (i) 1 equiv. 1*H*-pyrazole-1-carboxamidine hydrochloride in 1.0 M Na_2CO_3; 40%, (ii) 1.8 equiv. Pmc-Cl in 3.2 M NaOH and acetone at 0°C; 52%, (iii) a. H_2, Pd/C in MeOH and H_2O, 45 psi; b. 1.2 equiv. Fmoc-Cl in 10% Na_2CO_3 and dioxane; 81%.

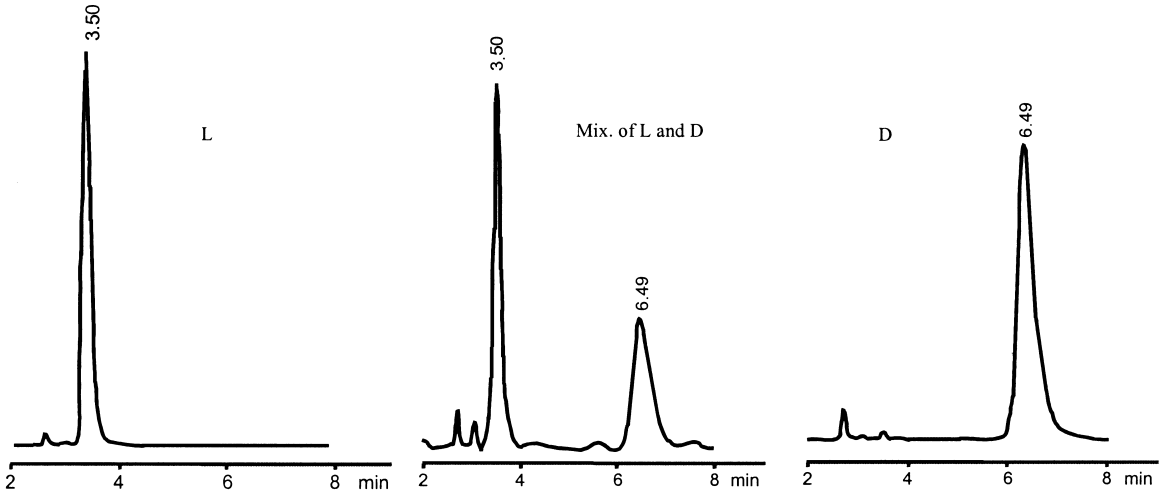


Figure 3. Analysis of the enantiomeric purity of **4a**(L) and **4b**(D) by using HPLC (Chirobiotic T-5 μm column).

The reaction could be successfully carried out using 1*H*-pyrazole-1-carboxamidine hydrochloride as transferable amidine moiety.[21] After 3 h reaction of **5** with the pyrazole reagent in 1.0 M Na_2CO_3 at room temperature, **11** was obtained in 40% yield (Scheme 3). In a similar way, **9** was converted to **12** (Scheme 4).

For the protection of guanidine moieties, three types of protecting groups are available: a nitro group, a urethane group or an arylsulfonyl group. The nitro-protected arginine is prone to numerous side reactions during synthesis, as well as acylation and cleavage in solid phase synthesis,[22] whereas urethane-protected arginine suffers from side chain acylation and subsequent ornithine formation under piperidine treatment in Fmoc-strategy-based solid phase synthesis.[23] Because of these limitations, arylsulfonyl groups have been used widely since they can suppress side reactions to a minimum. However, arginines protected with these groups, such as the 4-methoxy-2,3,6-trimethylbenzenesulfonyl group (Mtr), have shown a slow coupling and cleavage rate in solid phase synthesis.[24] More recently the pentamethylchroman sulfonyl group (Pmc) was developed.[25] It inherits the advantage of the aryl sulfonyl group with respect to the suppression of side reactions and demonstrates facile cleavage under mild acidic conditions. Therefore, we introduced the Pmc group to the guanidine moieties of **11** and **12** under strongly alkaline conditions (in 3.2 M NaOH) to afford **13** and **14**, respectively, in 50% yield (Schemes 3 and 4). Following removal of the Z group, the α-amine was protected with Fmoc to yield **2** and **4a**, respectively, in 80%.

In order to evaluate whether introduction of the Pmc group under strongly basic conditions does not induce racemization, the chiral purity of the final compounds was verified. Hence, both L and D enantiomers of the alanine derivative

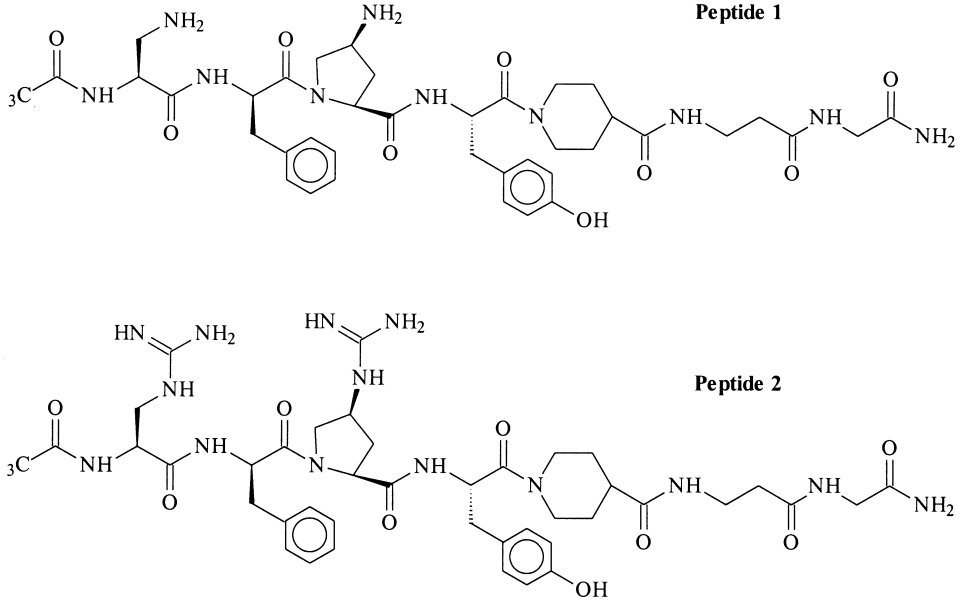


Figure 4. Structure of synthetic peptide consisting of amino–alanine, amino–proline, guanidino–alanine, guanidino–proline, phenylalanine, tyrosine, isonipecotic acid and β-alanine monomers.

Table 1. Coupling yields of the amino acids in solid phase peptide synthesis

Peptide 1	Gly	β-Ala	Inp	L-Tyr	L-Pro-NH_2 (**1**)	D-Phe	L-Ala-NH_2 (**3a**)
Coupling yield (%)	90	97.2	103.8	102	95.3	105	97
Peptide 2	Gly	β-Ala	Inp	L-Tyr	L-Pro-Gua (**2**)	D-Phe	L-Ala-Gua (**4a**)
Coupling yield (%)	90	97.2	103.8	102	77[a]/103	105	77[a]/93

[a] First figure is the yield from a single coupling step using HOAt/DIC activation, second figure represents the yield after a second coupling cycle.

(**4a**, **4b**) were prepared and subjected to chiral purity determination by liquid chromatography. In both cases, the spectra showed a single peak, while two well-separated peaks for the mixture of both isomers were obtained (Fig. 3). It is therefore clear that the enantiomeric purity of those compounds was sufficiently retained during the Pmc protection step as well as throughout the whole synthetic process.

Since the newly synthesized amino acids are to be used for the assembly of peptide libraries, their incorporation and deprotection efficiencies need to be controlled. Therefore, **1**, **2**, **3a** and **4a** were incorporated into two test peptides (Fig. 4), along with other amino acids, intended for use in our library. Assembly on solid support afforded good yield using HOAt/DIC activation.

Yield was determined using UV absorption of the piperidine-dibenzofulvene adduct. For most of the amino acids used, one coupling step was enough to obtain a yield of ca. 90%. The arginine analogues required a second coupling cycle to attain a yield of ca. 95% (Table 1). The yield over 100% is most probably due to the errors in the weight of small amounts of loaded beads. A capping and Fmoc deprotection reaction completed each coupling cycle.

Upon 90 min of treatment with the TFA-based cleavage cocktail containing thioanisole as scavenger, the protecting groups (Pmc, Boc, and *t*Bu) were removed and the peptides were released from the resin. The HPLC profile of the crude reaction mixture indicated that one major compound was formed (Fig. 5) (the peak which comes out with 76% B corresponds to thioanisole as verified by mass

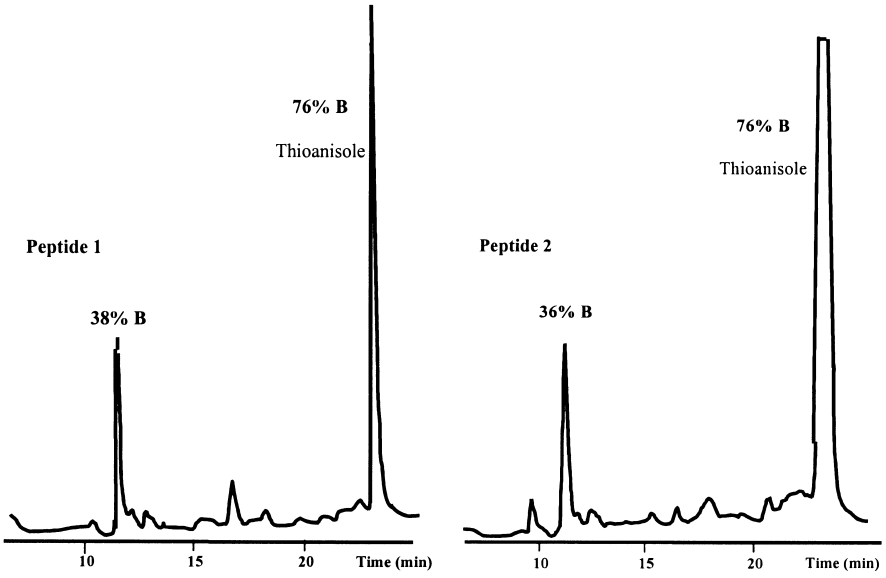


Figure 5. HPLC profiles of the peptides released from resin using a linear gradient of A: 5% CH_3CN in H_2O+0.1% TFA; and B: 80% CH_3CN in H_2O+0.1% TFA; flow rate: 1 ml/min.

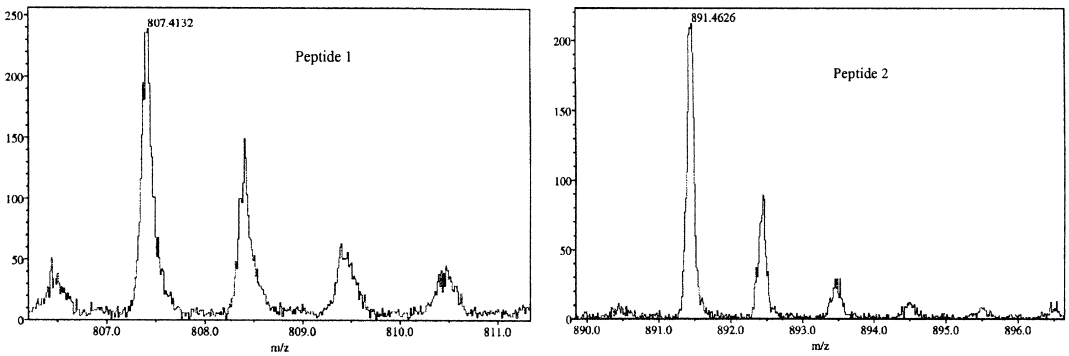


Figure 6. Mass spectra analysis of the major peaks isolated from HPLC purification proved the correct constitution of the peptides.

measurement). Subsequent analysis by mass spectrometry proved this major compound corresponded to the desired peptide sequence (Fig. 6), indicating successful incorporation and deprotection of the new amino and guanidinium containing amino acids.

Conclusion

A set of new chirally pure, orthogonally protected, basic amino acid analogues was synthesized. Their successful incorporation in a peptide was demonstrated. These new building blocks will be used in a potential anti-Tat library.

Experimental

The ^{1}H and ^{13}C NMR spectra were recorded with a Varian Gemini 200 spectrometer in the solvent indicated. For ^{1}H spectra tetramethylsilane (TMS) (for DMSO-d_6 the solvent signal at 2.5 ppm) was used as reference. For the ^{13}C spectra the center peak of the solvent multiplet, set at 77.00 ppm for $CDCl_3$ and at 39.60 ppm for DMSO-d_6, was used. In the case of N^{α}-protected proline derivatives, some peaks are double due to the presence of amide rotamers as a result of restricted rotation. Exact mass measurements by liquid secondary ion mass spectrometry (LSIMS) were obtained using a Kratos Concept 1*H* mass spectrometer, except for peptide 1 and peptide 2, which were analyzed on a PE SCIEX QSTAR™ Hybrid Quadrupole TOF System. Optical Rotation was measured with a Thorn NPL Automatic Polarimeter Type 243 at a temperature of 25°C in MeOH-dichloromethane (90/10) and MeOH for **3a**/**3b** and **4a**/**4b**, respectively. Infrared resonance was measured with a Spectrum™ RX I FT-IR System in reflectance mode. Melting point was measured with a Büchi SMP-20. Precoated Machery-Nagel Alugram® SilG/UV 254 plates were used for TLC and spots were examined with UV light, sulfuric acid/anisaldehyde spray, ninhydrine spray or Sakaguchi reagent. Column chromatography was performed on Acros silica gel (0.06–200 nm). Anhydrous solvents were obtained as follows: Pyridine and *N*,*N*-diisopropylethylamine (DIEA) were refluxed overnight over potassium hydroxide and distilled. *N*,*N*-dimethylformamide (DMF) was stored on activated molecular sieves for 3 days and was tested for the absence of dimethylamine by the bromophenol test prior to use. CH_3CN for HPLC was purchased from Rathburn (grade S) and water for HPLC purification was distilled twice. Tentagel-NH_2 was obtained from RAPP-Polymere (Tubingen, Germany). Dichloromethane (DCM), *N*,*N*-dimethylformamide, acetic anhydride (Ac_2O) and pyridine were obtained from BDH (Poole, England). 1-Hydroxy-7-azabenzotriazole (HOAt), Fmoc-β-alanine (β-Ala), Fmoc-glycine (Gly), Fmoc-D-phenylalanine (D-Phe), Fmoc-*O*-*t*-butyl-L-tyrosine (L-Tyr) were purchased from Advanced ChemTech (Louisville, Kentucky). Asparagine (L or D), *di*-*tert*-butyldicarbonate [$(Boc)_2O$], [bis(trifluoro-acetoxy)iodo]benzene, benzyl chloroformate, 9-fluorenylmethyloxycarbonyl chloride, 1*H*-pyrazole-1-carboxamidine, piperidine, trifluoroacetic acid (TFA), diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole (HOBt) were supplied by ACROS (Geel, Belgium). The *N*-Fmoc-isonipecotic acid was synthesized from isonipecotic acid, which was purchased from ACROS (Geel, Belgium).

4-*N*-*tert*-Butyloxycarbonyl-*N*-benzyloxycarbonyl-L-*cis*-proline (6). To a solution of 5.02 g (19 mmol) of **5** (13) in 29 ml of 10% Na_2CO_3 were added 19 ml of 1,4-dioxane and 4.97 g (23 mmol) of *di*-*tert*-butyldicarbonate at 0°C. The reaction mixture was stirred overnight at room temperature and was poured into 120 ml of H_2O after which the mixture was washed three times with diethyl ether. The aqueous layer was acidified with 2N HCl and the white suspension was extracted three times with ethyl acetate. The combined ethyl acetate layer was dried on anhydrous $MgSO_4$, filtered and evaporated to dryness. The crude product was crystallized from diethyl ether to afford compound **6** (6.5 g, 17.8 mmol, 93%). Mp 136°C; ν_{max} 3328, 2976 (NH, OH), 1694 (C═O), 1530, 1365, 12453, 738, 697 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H, Boc-CH_3), 1.90–2.65 (m, 2H, 3-H), 3.29–3.90 (m, 2H, 5-H), 4.10–4.60 (m, 2H, 2-H, 4-H), 5.00–5.29 (m, 2H, Z-CH_2), 7.29, 7.34 (two s, 5H, Z-aromatic) ppm. ^{13}C NMR ($CDCl_3$) δ 175.00 (COOH), 155.45 (2×OCON), 136.39 (Z-ar-Ci), 128.59, 128.14, 127.74 (Z-ar-Co,Cm,Cp), 79.82 (Boc-C), 68.10, 67.22 (Z-CH_2), 58.60 (C-2), 53.26 (C-5), 49.86 (C-4), 36.90, 34.38 (C-3), 28.25 (Boc-CH_3) ppm. Exact mass (LSIMS, diethanolamine) calcd for $C_{18}H_{23}N_2O_6$ $[M-H]^-$ 363.15560; found 363.15506; elem. anal. for $C_{18}H_{24}N_2O_6$ calcd C 59.33, H 6.64, N, 7.69; found C 59.22, H 6.73, N 7.50.

4-*N*-*tert*-Butyloxycarbonyl-*N*-9-fluorenylmethyloxycarbonyl-L-*cis*-proline (1). Nitrogen was bubbled for 10 min through a solution of 5.00 g (13.7 mmol) of **6** in 45 ml of methanol and 30 ml of H_2O. To this solution was added 1 g of palladium on charcoal (10%). The suspension was hydrogenated overnight at 45 psi. After filtration, the obtained solution was evaporated to dryness. The residue was dissolved in 30 ml of 10% Na_2CO_3 in H_2O. To the solution was added 18 ml of 1,4-dioxane and 3.87 g (15 mmol) of 9-fluorenylmethyloxycarbonyl chloride at 0°C. The reaction mixture was stirred overnight and then poured into 100 ml of H_2O and the solution was extracted three times with diethyl ether. The aqueous layer was acidified with a 2N HCl in water and the white suspension was extracted three times with ethyl acetate. The combined organic layer was dried on anhydrous $MgSO_4$, filtered and evaporated to afford compound **1** (5.02 g, 11.1 mmol, 81%). Mp 104°C; ν_{max} 3331, 2974 (NH, OH), 1702 (C═O), 1522, 1364, 1249, 738 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H, Boc-CH_3), 1.90–2.65 (m, 2H, 3-H), 3.30–3.90 (m, 2H, 5-H), 4.08–4.61 (m, 5H, 2-H, 4-H, Fmoc-CH_2, Fmoc-9′-H), 7.20–7.80 (m, 8H, Fmoc-aromatic) ppm. ^{13}C NMR ($CDCl_3$) δ 174.97 (COOH), 157.02, 156.33, 155.45, 154.48 (OCON), 143.74 (Fmoc-10′), 141.28 (Fmoc-11′), 127.77 (Fmoc-3′), 127.10 (Fmoc-2′), 125.13 (Fmoc-1′), 119.97 (Fmoc-4′), 79.85 (Boc-C), 68.32, 67.83 (Fmoc-CH_2), 58.45 (C-2), 53.60, 53.26 (C-5), 49.95 (C-4), 46.95 (Fmoc-9′), 37.18, 34.69 (C-3), 28.25 (Boc-CH_3) ppm. Exact mass (LSIMS, diethanolamine) calcd for $C_{25}H_{27}N_2O_6$ $[M-H]^-$ 451.18690; found 451.18742; elem. anal. for $C_{25}H_{28}N_2O_6 \cdot C_4H_8O_2$ (1,4-dioxane) calcd C 64.43, H 6.71, N 5.18; found C 64.75, H 6.85, N 4.90.

N^{α}-Benzyloxycarbonyl-L-asparagine (8). To a solution of 6.05 g (40 mmol) of L-asparagine **7** in 100 ml of 10% Na_2CO_3 were added 56 ml of 1,4-dioxane and 6.9 ml (48 mmol) of benzyl chloroformate at 0°C. The reaction mixture was stirred overnight at room temperature and was poured into 300 ml of H_2O. The mixture was washed three times with diethyl ether. The aqueous layer was acidified with 2N HCl in water and a white precipitate fell out. The precipitate was filtered off and washed extensively with diethyl ether to afford compound **8** (9 g, 33.8 mmol, 84%). Mp 164°C; ν_{max} 3402, 3333 (NH), 1695 (C=O), 1638, 1534, 735, 694 cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.36–2.68 (dAB, 2H, J=6.0 and 15.4 Hz, β-H), 4.30–4.50 (m, 1H, α-H), 5.04 (s, 2H, Z-CH_2), 6.97 (s, 1H, NH), 7.35–7.60 (m, 7H, aromatic and 2×NH's) ppm. ^{13}C NMR (DMSO-d_6) δ 173.45 (COOH), 171.59 (C-γ), 156.15 (OCONH), 137.21 (Z-ar-Ci), 128.62, 128.07, 127.95 (Z-ar-Co,Cm,Cp), 65.64 (Z-CH_2), 50.80 (C-α), 36.87 (C-β) ppm. Exact mass (LSIMS, thioglycerol:NaOAc) calcd for $C_{12}H_{14}N_2O_5Na$ $[M+Na]^+$ 289.08006; found 289.07812; elem. anal. for $C_{12}H_{14}N_2O_5$ 0.4 H_2O calcd C 52.71, H 5.46, N 10.24; found C 52.84, H 5.09, N 10.05.

N^{α}-Benzyloxycarbonyl-L-2,3-diaminopropionic acid (9). To a stirred solution of 25.8 g (60 mmol) of [bis(trifluroacetoxy)iodo]-benzene in 320 ml (1/1, v/v) of dimethylformamide/water was added 10.64 g (40 mmol) of **8** at room temperature. After 15 min, 6.4 ml (80 mmol) of pyridine was added and stirring was continued for 4.5 h. The solvent was evaporated in vacuo and the residue was dissolved in 400 ml of water. The solution was washed extensively with diethyl ether and concentrated in vacuo. The residue was precipitated from ethanol/ether and purified by column chromatography with methanol in DCM to afford compound **9** (4.5 g, 18.9 mmol, 47%). Mp 229°C; ν_{max} 3296, 2909 (NH), 1690 (C=O), 1589 (COO^-), 1537 (NH), 738, 694 cm^{-1}. ^1H NMR (D_2O) δ 2.98–3.42 (dAB, 2H, J=5.0 and 13.6 Hz, β-H), 4.25–4.40 (m, 1H, α-H), 4.89 (s, 2H, Z-CH_2), 7.18 (s, 5H, Z-aromatic) ppm. ^{13}C NMR (D_2O) δ 171.81 (COOH), 157.82 (OCONH), 135.78 (Z-ar-Ci), 128.65, 128.38, 127.68 (Z-ar-Co,Cm,Cp), 67.31 (Z-CH_2), 51.26 (C-α), 39.51 (C-β) ppm. Exact mass (LSIMS, thioglycerol) calcd for $C_{11}H_{15}N_2O_4$ $[M+H]^+$ 239.10317; found 239.10742; elem. anal. for $C_{11}H_{14}N_2O_4$·0.1 CF_3COOH calcd C 53.89, H 5.69, N 11.22; found C 53.67, H 5.63, N 10.99 (CF_3COOH is used to keep the free amino function in a protonated form).

N^{α}-Benzyloxycarbonyl-N^{β}-*tert*-butyloxycarbonyl-L-α-2,3-diaminopropionic acid (10). To a solution of 4.5 g (19 mmol) of **9** in 47 ml of 10% Na_2CO_3 were added 28 ml of 1,4-dioxane and 6.18 g (28.4 mmol) of *di-tert*-butyl dicarbonate at 0°C. The reaction mixture was stirred overnight at room temperature and was poured into 100 ml of H_2O after which the mixture was washed three times with diethyl ether. The aqueous layer was acidified with 2N HCl in water and the white suspension was extracted three times with ethyl acetate. The combined ethyl acetate layer was dried on anhydrous $MgSO_4$, filtered and evaporated to dryness. The crude product was crystallized from diethyl ether to afford compound **10** (5.8 g, 17.2 mmol, 91%). Mp 144°C; ν_{max} 3332, 2976 (NH, OH), 1693 (C=O), 1514, 1365, 1250 738, 697 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.41 (s, 9H, Boc-CH_3), 3.25–3.75 (br, 2H, β-H), 4.25–4.50 (br, 1H, α-H), 5.11 (s, 2H, Z-CH_2), 7.32 (s, 5H, Z-aromatic) ppm. ^{13}C NMR ($CDCl_3$) δ 173.57(COOH), 157.09(OCONH), 136.06(Z-ar-Ci), 128.53, 128.14(Z-ar-Co,Cm,Cp), 82.03, 80.39 (Boc-C), 67.14 (Z-CH_2), 55.08, 54.29 (C-α), 42.97, 42.06 (C-β), 28.07 (Boc-CH_3) ppm. Exact mass (LSIMS, glycerol) calcd for $C_{16}H_{21}N_2O_6$ $[M-H]^-$ 337.13995; found 337.13980; elem. anal. for $C_{16}H_{22}N_2O_6$·0.5 H_2O calcd C 55.32, H 6.67, N 8.06; found C 55.59, H 6.49, N 7.94.

N^{α}-(9-Fluorenylmethyloxycarbonyl)-N^{β}-*tert*-butyloxycarbonyl-L-α-2,3-diaminopropionic acid (3a). Nitrogen was bubbled for 10 min through a solution of 6.14 g (18.2 mmol) of **10** in 50 ml of methanol and 20 ml of H_2O. To this solution was added 1.2 g of palladium on charcoal (10%). The suspension was hydrogenated overnight at 45 psi. After filtration, the obtained solution was evaporated to dryness. The residue was dissolved in 45 ml of 10% Na_2CO_3 in H_2O and 25 ml of 1,4-dioxane and 5.17 g (20.0 mmol) of 9-fluorenylmethyloxycarbonyl chloride were added at 0°C. The reaction mixture was stirred overnight and then poured into 100 ml of H_2O and the solution was extracted three times with diethyl ether. The aqueous layer was acidified with a 2N HCl and the white suspension was extracted three times with ethyl acetate. The combined organic layer was dried on anhydrous $MgSO_4$, filtered and evaporated to afford compound **3a** (6.2 g, 14.6 mmol, 80%). Mp 129°C; ν_{max} 3334, 2977 (NH, OH), 1698 (C=O), 1518, 1366, 1251 738 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.41 (s, 9H, Boc-CH_3), 3.30–3.62 (br, 2H, β-H), 4.00–4.60 (br, 4H, α-H, Fmoc-CH2, Fmoc-9′-H), 7.18-7.80 (m, 8H, aromatic) ppm. ^{13}C NMR ($CDCl_3$) δ 173.85 (COOH), 157.18, 156.76 (2×OCONH), 143.86 (Fmoc-10′), 141.25 (Fmoc-11′), 127.68 (Fmoc-3′), 127.08 (Fmoc-2′), 125.16 (Fmoc-1′), 119.91 (Fmoc-4′), 80.33 (Boc-C), 67.22 (Fmoc-CH_2), 55.36 (C-α), 46.92 (Fmoc-9′), 42.03 (C-β), 28.10 (Boc-CH_3) ppm. Exact mass (LSIMS, glycerol) calcd for $C_{23}H_{25}N_2O_6$ $[M-H]^-$ 425.17125; found 425.16973; elem. anal. for $C_{23}H_{26}N_2O_6$·0.5 $C_4H_8O_2$ (1,4-dioxane) calcd C 63.82, H 6.43, N 5.95; found C 63.70, H 6.38, N 5.63; $[\alpha]_D^{25}=-6.20$ [c=0.72 in MeOH/DCM (90/10)]. Compound **3b** with $[\alpha]_D^{25}=+5.78$ [c=0.70 in MeOH/DCM (90/10)].was prepared from D-asparagine in exactly the same way as described for **3a**.

4-Guanidino-N^{α}-benzyloxycarbonyl-L-*cis*-proline (11). To a solution of 5.28 g (20 mmol) of **5** in 20 ml of 1.0 M Na_2CO_3 in H_2O was added 2.93 g (20 mmol) of 1*H*-pyrazole-1-carboxamidine hydrochloride. The reaction mixture was stirred overnight at room temperature and neutralized to pH 5–6 with 1N HCl. The solution was evaporated to dryness and the residue was adsorbed on silica gel and purified by column chromatography with methanol in DCM yielding 2.45 g (8.0 mmol, 40%) of compound **11** as a foam. All attempts for crystallization failed. ν_{max} 3356, 3196, 2920 (NH), 1682 (C=O), 1650, 1541, 770, 697 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.97–2.43 (m, 2H, 3-H), 3.20–3.67 (m, 2H, 5-H), 3.73–3.77 (m, 1H, 4-H), 3.98–4.08 (m, 1H, 2-H), 5.04 (s, 2H, Z-CH_2), 7.31, 7.35 (two s, 5H, Z-aromatic) ppm. ^{13}C NMR (DMSO-d_6) δ 175.21, 174.63 (COOH), 157.03 (Gua-C), 153.99 (OCON), 137.12 (Z-ar-Ci), 128.65, 127.74, 127.19 (Z-ar-Co,Cm,Cp), 68.18, 66.06

(Z-CH_2), 58.72, 58.51 (C-2), 51.64, 51.07 (C-5), 49.77, 49.10 (C-4), 36.20, 35.20 (C-3) ppm. Exact mass (LSIMS, thioglycerol) calcd for $C_{14}H_{19}N_4O_4$ $[M+H]^+$ 307.14062; found 307.13631.

4-(2,2,5,7,8-Pentamethylchroman-6-sulfonyl)-guanidino-N^{α}-benzyloxycarbonyl-L-*cis*-proline (13). To a solution of 1.4 g (4.6 mmol) of **11** in a mixture of 5.4 ml of 3.2 M NaOH and 15 ml of acetone were added 2.5 g (8.2 mmol) of 2,2,5,7,8-pentamethylchroman-6-sulfonyl chloride in 10 ml of acetone at 0°C. The mixture was stirred at 0°C for 2 h and another 2 h at room temperature. After acidification to pH 6–7 with 1N HCl, the acetone was removed under vacuo. The remaining solution was further acidified with 1N HCl to pH 3, diluted with water and extracted with ethyl acetate. The combined extract was dried on anhydrous $MgSO_4$, filtered and evaporated to dryness. The remaining oil was adsorbed on silica gel and purified by column chromatography with methanol in DCM yielding 1.3 g (2.27 mmol, 49%) of compound **13**. Mp 138°C; ν_{max} 3434, 3336 2977 (NH, OH), 1682 (C═O), 1620, 1547 (NH), 1297 (S═O), 1105, 742, 697 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.28 (s, 6H, 2×Pmc CH_3's), 1.62–2.53 (m, 15H, 2×CH_3's *o* to $-SO_{2-}$, 1×CH_3 *m* to $-SO_{2-}$, Pmc 3′-H, Pmc 4′-H, 3-H), 3.44-3.68 (m, 2H, 5-H), 4.32 (br, 2H, 4-H, 2-H), 4.95–5.15 (m, 2H, Z-CH_2), 6.20–7.00 (br, 3H, guanidino NH's), 7.25, 7.27 (m, 5H, Z-aromatic) ppm. ^{13}C NMR ($CDCl_3$) δ 175.88 (COOH), 155.48 (Gua-C), 154.72 (OCON), 153.88 (Pmc 9′), 136.03 (Z-ar-Ci), 135.61 (Pmc-6′), 135.03 (Pmc-5′), 132.87 (Pmc-7′) 128.53, 128.22, 127.93 (Z-ar-Co,Cm,Cp), 124.19 (Pmc-10′), 118.06 (Pmc-8′), 73.69 (Pmc-2′) 67.65 (Z-CH_2), 58.39, 57.67 (C-2), 52.50 (C-5), 50.20, 49.50 (C-4), 36.82, 35.23 (C-3), 32.68 (Pmc-4′), 26.71 (2×CH_3 on Pmc-C2′), 21.28 (Pmc-3′), 18.39, 17.33 (2×CH_3's *o* to $-SO_{2-}$), 11.99 (CH_3 *m* to $-SO_{2-}$) ppm. Exact mass (LSIMS, thioglycerol:TFA) calcd for $C_{28}H_{37}N_4O_7S_1$ $[M+H]^+$ 573.23828; found 573.2386; elem. anal. for $C_{28}H_{36}N_4O_7S_1 \cdot 1.5\ H_2O$ calcd C 56.08, H 6.55, N 9.34; found C 56.11, H 6.28, N 9.02.

4-(2,2,5,7,8-Pentamethylchroman-6-sulfonyl)-guanidino-N^{α}-9-fluorenylmethyloxycarbonyl-L-*cis*-proline (2). Nitrogen was bubbled for 10 min through a solution of 1.5 g (2.6 mmol) of **13** in 5 ml of methanol, 5 ml of dioxane and 1 ml of acetic acid. To this solution was added 0.3 g of palladium on charcoal (10%). The suspension was hydrogenated overnight at 45 psi. After filtration, the obtained solution was evaporated to dryness. The residue was dissolved in 20 ml of 10% aqueous Na_2CO_3. To the solution was added 12 ml of 1,4-dioxane and 0.8 g (3.1 mmol) of 9-fluorenylmethyloxycarbonyl chloride at 0°C. The reaction mixture was stirred overnight and was poured into 20 ml of H_2O and the solution was extracted three times with diethyl ether. The aqueous layer was acidified with a 2N HCl and the white suspension was extracted three times with ethyl acetate. The combined organic layer was dried on anhydrous $MgSO_4$, filtered and evaporated to dryness. The crude product was crystallized from diethyl ether to afford compound **2** (1.3 g, 2.0 mmol, 77%). Mp 199°C; ν_{max} 3448, 3345 2973 (NH, OH), 1702 (C═O), 1618, 1535 (NH), 1298 (S═O), 1108, 740 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.25, 1.28 (two s, 6H, 2×Pmc CH_3's), 1.68–2.62 (m, 15H, 2×CH_3's *o* to $-SO_{2-}$, 1×CH_3 *m* to $-SO_{2-}$, Pmc 3′-H, Pmc 4′-H, 3-H), 3.43-3.68 (m, 2H, 5-H), 4.08–4.44 (m, 5H, 4-H, 2-H, Fmoc CH, CH_2), 6.00–6.50 (m, 3H, guanidino NH's), 7.20–7.76 (m, 8H, aromatic's) ppm. ^{13}C NMR ($CDCl_3$) δ 175.29 (COOH), 155.67 (Gua-C), 155.23 (OCON), 153.82 (Pmc-9′), 143.82–118.01 (aromatic C's), 73.77 (Pmc-2′) 68.25 (Fmoc-CH_2), 58.36, 57.47 (C-2), 52.86 (C-5), 50.42, 49.55 (C-4), 47.04 (Fmoc-CH), 37.01, 35.08 (C-3), 32.73 (Pmc-4′), 26.79 (2×CH_3 on Pmc-2′), 21.36 (Pmc-3′), 18.52, 17.44 (2×CH_3's *o* to $-SO_{2-}$), 12.09 (CH_3 *m* to $-SO_{2-}$) ppm. Exact mass (LSIMS, thioglycerol: TFA) calcd for $C_{35}H_{41}N_4O_7S_1$ $[M+H]^+$ 661.26958; found 661.26696; elem. anal. for $C_{35}H_{40}N_4O_7S_1$ calcd C 63.62, H 6.10, N 8.48; found C 63.33, H 6.15, N 8.49.

N^{α}-Benzyloxycarbonyl-β-guanidino-L-α-alanine (12). To a solution of 4.76 g (20 mmol) of **9** in 20 ml of 1.0 M Na_2CO_3 in H_2O was added 2.93 g (20 mmol) of 1*H*-pyrazole-1-carboxamidine hydrochloride. The reaction mixture was stirred overnight at room temperature and neutralized to pH 5–6 with 1N HCl. The solution was evaporated to dryness and the residue was adsorbed on silica gel and purified by column chromatography with methanol in DCM. The crude product was crystallized from methanol to yield 2.24 g (8.0 mmol, 40%) of compound **12**. Mp 179°C; ν_{max} 3414, 3315 2915 (NH), 1676 (C═O), 1654, 1527, 759, 699 cm^{-1}. ^1H NMR (DMSO-d_6) δ 3.45 (m, 2H, β-H), 4.03–4.13 (m, 1H, α-H), 5.03 (s, 2H, Z-CH_2), 7.34 (s, 5H, Z-aromatic) ppm. ^{13}C NMR (DMSO-d_6) δ 172.29 (COOH), 157.94 (Gua-C), 156.36 (OCONH), 137.18 (Z-ar-Ci), 128.62, 128.01, 127.89 (Z-ar-Co,Cm,Cp), 65.70 (Z-CH_2), 54.29 (C-α), 42.39 (C-β) ppm. Exact mass (LSIMS, thioglycerol:NaOAc) calcd for $C_{12}H_{16}N_4O_4Na$ $[M+Na]^+$ 303.10695; found 303.10774; elem. anal. for $C_{12}H_{16}N_4O_4$ calcd C 51.42, H 5.75, N 19.99; found C 51.27, H 5.56, N 19.36.

N^{α}-Benzyloxycarbonyl-β-(2,2,5,7,8-pentamethylchroman-6-sulfonyl)guanidino-L-α-alanine (14). To a solution of 1.4 g (5.0 mmol) of **12** in a mixture of 6 ml of 3.2 M NaOH and 16 ml of acetone were added 2.72 g (9 mmol) of 2,2,5,7,8-pentamethylchroman-6-sulfonyl chloride in 10 ml of acetone at 0°C. The mixture was stirred at 0°C for 2 h and another 2 h at room temperature. After acidification to pH 6–7 with 1N HCl, the acetone was removed under vacuo. The remaining solution was further acidified with 1N HCl to pH 3 and diluted with water, and was extracted with ethyl acetate. The combined extracts were dried on anhydrous $MgSO_4$, filtered and evaporated to dryness. The remaining oil was adsorbed on silica gel and purified by column chromatography with methanol in DCM yielding 1.4 g (2.6 mmol, 52%) of compound **14**. Mp 166°C; ν_{max} 3445, 3347 2974 (NH, OH), 1713 (C═O), 1622, 1547 (NH), 1298 (S═O), 1111, 743, 697 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.27 (s, 6H, 2×Pmc CH_3's) 1.74 (t, 2H, Pmc 3′-H), 2.05 (s, 3H, 1×CH_3 *m* to $-SO_{2-}$), 2.48–2.55 (m, 8H, 2×CH_3's *o* to $-SO_{2-}$, Pmc 4′-H), 3.58 (br, 2H, β-H), 4.32 (br, 1H, α-H), 5.02 (s, 2H, Z-CH_2), 6.57 (br, 3H, guanidino NH's), 7.25 (s, 6H, aromatic, α-NH), 9.71 (br, 1H, COOH) ppm. ^{13}C NMR ($CDCl_3$) δ 173.48 (COOH), 156.70 (Gua-C, OCON), 154.09 (Pmc-9′), 136.18 (Z-ar-Ci), 135.85 (Pmc-6′), 135.36 (Pmc-5′), 132.33 (Pmc-7′) 128.44, 127.86 (Z-ar-Co,Cm,Cp), 124.22 (Pmc-10′), 118.06 (Pmc-8′), 73.72 (Pmc-2′) 66.98 (Z-CH_2), 54.75

(C-α), 42.22 (C-β), 32.59 (Pmc-4′), 26.65 (2×CH_3 on Pmc C-2′), 21.22 (Pmc-3′), 18.33, 17.30, 11.96 (2×CH_3's *o* to $-SO_{2-}$, 1×CH_3 *m* to $-SO_{2-}$) ppm. Exact mass (LSIMS, thioglycerol:NaOAc) calcd for $C_{26}H_{34}N_4O_7SNa$ $[M+Na]^+$ 569.20460; found 569.20257; elem. anal. for $C_{26}H_{34}N_4O_7S$ 0.25 H_2O calcd C 56.66, H 6.31, N 10.17; found C 56.68, H 6.32, N 10.02.

***N*$^{\alpha}$-9-Fluorenylmethyloxycarbonyl-β-(2,2,5,7,8-pentamethylchroman-6-sulfonyl)-guanidino-L-α-alanine (4a).** Nitrogen was bubbled for 10 min through a solution of 4.8 g (8.7 mmol) of **14** in 15 ml of methanol, 15 ml of dioxane and 1ml of acetic acid. To this solution was added 1 g of palladium on charcoal (10%). The suspension was hydrogenated overnight at 45 psi. After filtration, the obtained solution was evaporated to dryness. The residue was dissolved in 50 ml of 10% aqueous Na_2CO_3. To the solution was added 30 ml of 1,4-dioxane and 2.5 g (9.6 mmol) of 9-fluorenylmethyloxycarbonyl chloride at 0°C. The reaction mixture was stirred overnight and was poured into 60 ml of H_2O and the solution was extracted three times with diethyl ether. The aqueous layer was acidified with a 2N HCl and the white suspension was extracted three times with ethyl acetate. The combined organic layer was dried on anhydrous $MgSO_4$, filtered and evaporated to dryness. The crude product was crystallized from diethyl ether to afford compound **4a** (4.5 g, 7.1 mmol, 81.5%). Mp 192°C; ν_{max} 3435, 3340 2967 (NH, OH), 1702 (C═O), 1619, 1542 (NH), 1296 (S═O), 1106, 735 cm^{-1}. ^1H NMR [$(CD_3)_2CO$] δ 1.25 (s, 6H, 2×Pmc CH_3's) 1.75(t, 2H, Pmc 3′-H), 2.00 (obscured by d_5-acetone, 3H, 1×CH_3 *m* to $-SO_{2-}$), 2.58–2.66 (m, 8H, 2×CH_3's *o* to $-SO_{2-}$, Pmc 4′-H), 3.61, 3.77 (br, 2H, β-H), 4.18–4.42 (m, 4H, α-H, Fmoc CH, CH_2), 6.40–7.00 (m, 3H, guanidino NH's), 7.28–7.88 (m, 8H, aromatics) ppm. ^{13}C NMR [$(CD_3)_2CO$] δ 172.19 (COOH), 157.60 (Gua-C), 157.20 (OCONH), 153.91 (Pmc-9′), 145.06–118.75 (aromatic C's) 74.25 (Pmc-2′) 67.47 (Fmoc-CH_2), 55.59 (C-α), 47.88 (Fmoc-CH), 42.73 (C-β), 33.32 (Pmc-4′), 26.92 (2×CH_3 on Pmc-2′), 21.89 (Pmc-3′), 18.83, 17.71, 12.21 (2×CH_3's *o* to $-SO_{2-}$, 1×CH_3 *m* to $-SO_{2-}$) ppm. Exact mass (LSIMS, thioglycerol:NaOAc) calcd for $C_{33}H_{38}N_4O_7SNa$ $[M+Na]^+$ 657.23590; found 657.23603; elem. anal. for $C_{33}H_{38}N_4O_7S$ calcd C 62.44, H 6.03, N 8.83; found C 62.18, H 6.31, N 8.67; $[\alpha]_D^{20}=-22.86$ (c=0.29 in MeOH). Compound **4b** with $[\alpha]_D^{20}=+23.70$ (c=0.31 in MeOH)was prepared from D-asparagine in exactly the same way as described for **4a**.

Determination of the enantiomeric purity of the alanine derivatives (4a, 4b). The enantiomeric purity of the newly synthesized amino acids was measured on a Hewlett Packard-1100 Liquid Chromatograph (Waldbronn, Germany). A mixture of the two isomers was injected onto a Chirobiotic T-5 μm column 250×4.6 mm (ASTEC, US). A complete separation was achieved with a mobile phase of 95% MeOH (0.01 M ammonium acetate) and 5% water, at a flow rate of 1.0 ml/min at 30°C. The peaks were detected by a diode array detector set at 220.4 nm and 254.4 nm. Subsequently the two isomers were injected one by one, with both compounds showing a single peak. The compound with the L configuration displayed a shorter retention time than the compound with the D configuration.

Solid phase synthesis of the heptapeptide

A. Solid phase peptide synthesis. After 15 min swelling of 39 mg of Rink amide MBHA resin (21.6 μmol) in CH_2Cl_2, 2 ml of 20% piperidine in DMF was added to the solid support. The beads were shaken for 15 min at room temperature and washed with 5×2 ml DMF, 5×2 ml CH_2Cl_2. A solution of Fmoc amino acid (86.4 μmol), HOAt (86.4 μmol), DIC (86.4 μmol), and DIEA (172.8 μmol) in 1 ml of DMF was added onto the beads without preactivation. The beads were shaken for 6 h, and afterwards washed with 5×1 ml DMF, 5×1 ml CH_2Cl_2. A solution of pyridine/acetic anhydride/*N*-methylimidazole (4:1:0.5) was added to the beads. After 10 min of shaking, the beads were washed with 5×1 ml of CH_2Cl_2, and 5×1 ml of DMF. The beads were treated with 1 ml of 20% piperidine for 15 min to remove the Fmoc protecting group, washed with 5×1 ml of DMF and 5×1 ml of CH_2Cl_2. After each complete coupling cycle, 5–6 mg of the beads were isolated, suspended in 25 ml of 20% piperidine in DMF over 10 min and subjected to a UV absorption measurement at 301 nm in order to determine the coupling yield. For all the amino acids, except arginine analogues for which a second coupling cycle was required, a single coupling cycle was enough to obtain a yield ca. 95%.

B. Removal of the protecting groups and cleavage of the heptapeptide from the solid support. After completion of the coupling of the last amino acid, the beads were treated with 1 ml of 20% piperidine in DMF over 15 min, washed with 5×1 ml of DMF, 5×1 ml of CH_2Cl_2, and then capped with 1 ml solution of pyridine/acetic anhydride/*N*-methylimidazole (4:1:0.5) over 10 min. After washing with 5×1 ml of CH_2Cl_2, and 5×1 ml of DMF, the resin was treated with 1 ml of a mixture of TFA/H_2O/thioanisole (10:0.5:0.5) for 90 min. The deprotection cocktail was removed and the beads were washed with 5×1 ml cold diisopropyl ether. The collected suspension was centrifuged for 10 min and the precipitated pellet was dried in vacuo, dissolved in H_2O, and purified on a Nova-Pak® C18 column (60 Å, 4 μm), 150/3.9 (Waters, US). The peptides were eluted off the column by using a linear gradient from 0 to 100% B in 30 min, with a flow rate of 1 ml/min [eluent A: 0.1% TFA in CH_3CN–H_2O (5/95); eluent B: 0.1% TFA in CH_3CN–H_2O (80/20)], and detected by UV at 220 nm. The major compound was isolated and analyzed by high resolution mass spectrometry (the peak which comes out with 76% B corresponds to thioanisole). Peptide 1: Exact mass (ESI) for $C_{39}H_{55}N_{10}O_9$ $[M+H]^+$ calcd 807.41532, found 807.4132. Peptide 2: Exact mass (ESI) for $C_{41}H_{59}N_{14}O_9$ $[M+H]^+$ calcd 891.45891, found 891.4626.

Acknowledgements

The work was supported by a grant from K. U. Leuven (GOA 97/11). We are grateful to Dr Volker Gnau (PE SCIEX) for recording the mass spectrum for peptide 1 and peptide 2. We thank Jaak Hoebus for optical rotations, Ivo Quintens for infrared resonance analyses, and Chantal Biernaux for editorial help.

References

1. Declercq, E. *Collect. Czech. Chem. Commun.* **1998**, *63*, 449–479.
2. Declercq, E. *Trends Drug Res. II* **1998**, 91–104.
3. Declercq, E. *Int. J. Antimicrob. Agents* **1997**, *9*, 21–36.
4. Kingsman, S. M.; Kingsman, A. J. *Eur. J. Biochem.* **1996**, *240*, 491–507.
5. Frankel, A. D. *Curr. Opin. Genet. Dev.* **1992**, *2*, 293–298.
6. Calnan, B. J.; Tidor, B.; Biancalana, S.; Hudson, D.; Frankel, A. D. *Science* **1991**, *252*, 1167–1171.
7. Puglisi, J. D.; Tan, R.; Canlan, B. J.; Frankel, A. D.; Williamson, J. R. *Science* **1992**, *257*, 76–80.
8. Aboul-ela, F.; Karn, J.; Varani, G. *J. Mol. Biol.* **1995**, *253*, 313–332.
9. Terrett, N. K.; Gardner, M.; Gordon, D. W.; Kobylecki, R. J.; Steele, J. *Tetrahedron* **1995**, *51*, 8135–8173.
10. Medynski, D. *Biotechnology* **1994**, *12*, 709–710.
11. Houghten, R. A.; Pinilla, C.; Blondelle, S. E.; Appel, J. R.; Dooley, C. T.; Cuervo, J. H. *Nature* **1991**, *354*, 84–86.
12. Youngquist, R. S.; Fuentes, G. R.; Lacey, M. P.; Keough, T. *J. Am. Chem. Soc.* **1995**, *117*, 3900–3906.
13. Löbberding, A., Mielke, B., Schwemler, C., Schwenner, E., Stropp, U., Springer, W., Kretschmer, A., Pötter, T. In *Nukleinsäuren-bindende Oligomere mit N-Verzweigung für Therapie und Diagnostik* (EP 0 646 595 A1).
14. Arnold, L. D.; Kalantar, T. H.; Vederas, J. C. *J. Am. Chem. Soc.* **1985**, *107*, 7105–7109.
15. Waki, M.; Kitajima, Y.; Izumiya, N. *Synthesis* **1981**, 266–268.
16. Yong, Y. F.; Kowalski, J. A.; Lipton, M. A. *J. Org. Chem.* **1997**, *62*, 1540–1542.
17. Shibanuma, T.; Shiono, M.; Mukaiyama, T. *Chem. Lett.* **1977**, 575–576.
18. Kim, K. S.; Qian, L. *Tetrahedron Lett.* **1993**, *34*, 7677–7680.
19. Feichtinger, K.; Zapf, C.; Sings, H. L.; Goodman, M. *J. Org. Chem.* **1998**, *63*, 3804–3805.
20. Uno, T.; Beausoleil, E.; Goldsmith, R. A.; Levin, B. H.; Zuckermann, R. N. *Tetrahedron Lett.* **1999**, *40*, 1475–1478.
21. Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R. *J. Org. Chem.* **1992**, *57*, 2497–2502.
22. Rzeszotarska, B.; Masiukiewicz, E. *Org. Prep. Proced. Int.* **1988**, *20*, 427–464.
23. Rink, H.; Sieber, P.; Raschdorf, F. *Tetrahedron Lett.* **1984**, *25*, 621–624.
24. Atherton, E.; Sheppard, R. C.; Wade, J. D. *J. Chem. Soc. Chem. Commun.* **1983**, 1060–1062.
25. Ramage, R.; Green, J.; Blake, A. J. *Tetrahedron* **1991**, *47*, 6353–6370.