Enantioselective Synthesis of *cis* and *trans* 4-Aminopipecolic Acids as γ-Amino Acids for the Construction of Cyclic RGD-containing Peptidomimetics Antagonists of $\alpha_V\beta_3$ Integrin

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Abstract: A stereodivergent strategy to obtain enantiopure *cis* and *trans* 4-aminopipecolic acids (4-APAs) in a suitably protected form for peptide synthesis has been devised starting from a common, known precursor in turn easily prepared from commercial (*R*)-4-cyano-3-hydroxybutyric acid ethyl ester. The two isomers were efficiently obtained in 40% and 23% overall yields, respectively, in seven and ten steps. To demonstrate their usefulness in peptidomimetic synthesis, both 4-APA isomers were incorporated as γ-amino acid in a cyclic RGD-containing sequence, although for the *trans* 4-APA isomer a further amino acid in the sequence (L-Phe) was needed to allow ring closure. The two cyclopeptides were tested as $\alpha_V\beta_3$ integrin antagonists in comparison with cilengitide.

Introduction

Cis and *trans* 4-aminopipecolic acids **1** and **2** (Figure 1, a) are endocyclic-N^{α}/exocyclic-N^{γ}-constrained, naturally occurring[1] basic amino acids with great potential in medicinal chemistry not only as α-amino acids with restricted φ, χ1, χ2, and/or χ3 torsion angles, but also as rigid γ-amino acids. Surprisingly, only few examples of peptidomimetics incorporating 4-aminopipecolic acids as either α- or γ-amino acids exist, or synthetic bioactive compounds embedding them.[2] Also, a very limited number of stereoselective synthesis of compounds **1** and **2** have been reported, often leading to mixtures of isomers or compounds with unsuitable protection for peptide synthesis.[2b, 3] The most efficient strategies for the synthesis of both *cis* and *trans* 4-APAs make use of commercially available, but very expensive, *N*-Boc protected *cis* 4-hydroxypipecolic acid.[2b,3a] Since we had reported, a few years ago, on efficient chemical and chemo-enzymatic syntheses of *cis* 4-hydroxy pipecolic acids,[4] we decided to exploit our previous knowledge to perform a stereodivergent, enantioselective synthesis of 4-aminopipecolic acids and to demonstrate their usefulness in the preparation of peptidomimetics. In particular, we wanted to use them as conformationally constrained γ-amino acids for the preparation of cyclic RGD-containing peptidomimetics as antagonists of $\alpha_V\beta_3$ integrin receptor. This integrin is a heterodimeric transmembrane receptor for extracellular matrix (ECM) proteins which promote adhesion, migration, and proliferation of cells.[5]

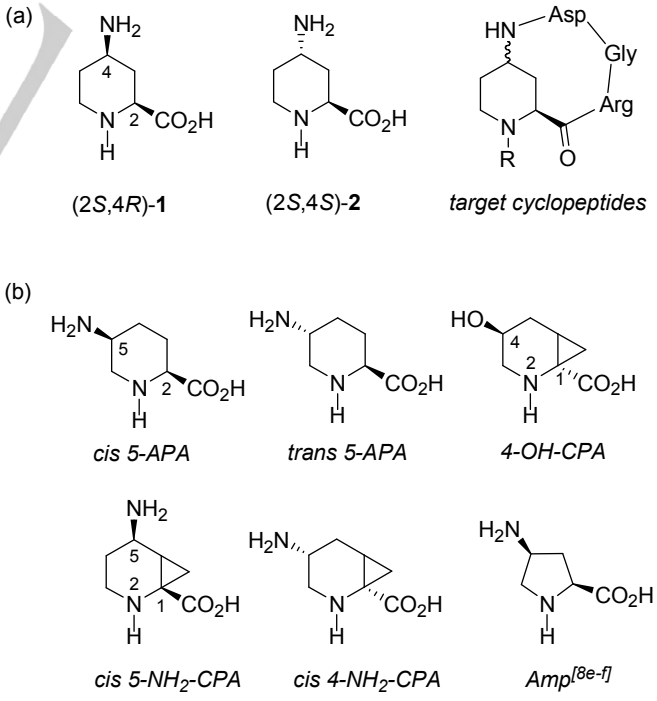
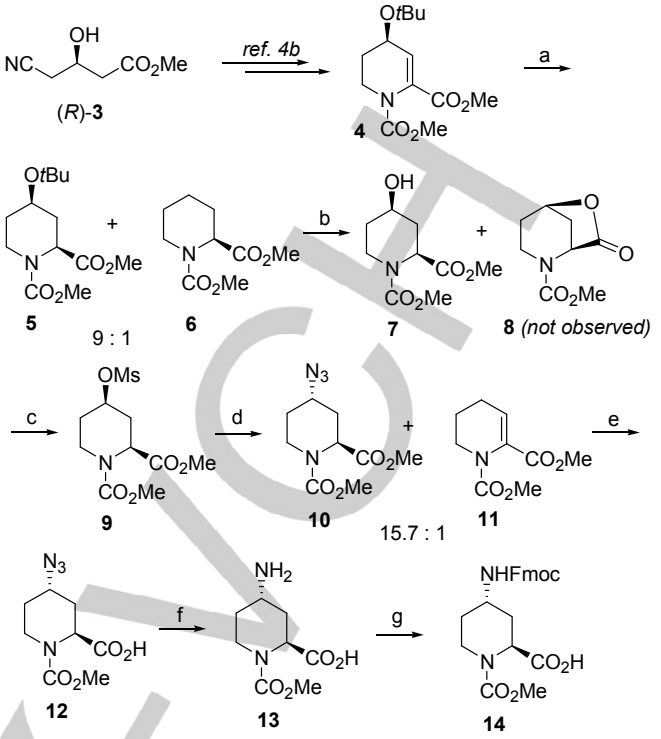


Figure 1. (a) *Cis* and *trans* 4-aminopipecolic acids **1** and **2**, and the target $\alpha_V\beta_3$ integrin ligands. (b) Hydroxy- and aminopipecolic acid derivatives, and 4-aminoproline.

The $\alpha_V\beta_3$ integrin receptor, which recognizes the RGD sequence of vitronectin,[6] has a critical role in tumor-induced angiogenesis and metastasis formation.[7] Therefore, RGD-containing peptides and peptidomimetics[8] as well as RGD mimetics[5a] are currently evaluated as antagonists to suppress the events mediated by this integrin and as possible shuttles for the targeted delivery of drugs and diagnostics.[7b, 9] In line with this, we have recently reported that pipecolic acid derivatives such 4- and 5-aminocyclopropane pipecolic acids,[10] 4-hydroxycyclopropane pipecolic acids,[11] and 5-aminopipecolic acids[12] are all suitable, rigid amino acids on which to build the RGD sequence to obtain highly active $\alpha_V\beta_3$ integrin receptor antagonists (Figure 1, b). These aminopipecolic acid derivatives are homologous of 4-aminoproline (Figure 1), a derivative of 4-hydroxyproline which has been extensively exploited in the last decade for the generation of $\alpha_V\beta_3$ integrin ligands and drug conjugates.[8h,i],[9f-h] As a completion of our studies on pipecolic acid derivatives,[4, 10-12,13] in this paper we wish to report on our efforts to prepare orthogonally protected *cis* and *trans* 4-aminopipecolic acids suitable for peptide synthesis and their use in the construction of RGD peptidomimetics as integrin antagonists.

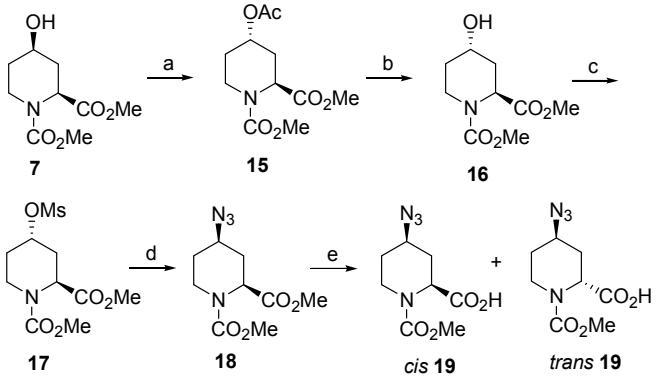
Results and Discussion

Having identified in *cis* 4-hydroxypipecolic acid the key intermediate for the synthesis of both *cis* and *trans* 4-aminopipecolic acids, we decided to prepare its *N*-CO_2Me protected form **7** (Scheme 1) from commercially available (*R*)-**3** as we have previously reported.[4b] However, some unexpected complications arose when repeating that sequence on a larger scale (15 mmol) and further experimentation was thus required to have a reliable and robust methodology in hand. So, while the transformation of (*R*)-**3** into α,β-unsaturated ester **4** proceeded smoothly as reported,[4b] the hydrogenation of the latter under the original (wet 10% Pd/C in EtOAc) conditions caused the almost total loss of the *t*-butoxy group to form **6**. This was reasonably due to the proneness of **4** to form an allylic cationic species, fully conjugated with the nitrogen atom, upon protonation of the *t*-butoxy oxygen atom under acid/aqueous conditions. Only by using dry 10% Pd/C in anhydrous THF, i.e. having care to remove water and any acid source from the reaction medium, the reaction provided diastereopure *cis* **5** quantitatively, with just a low content of the unwanted byproduct. Deprotection of the 4-OH group was first attempted with *p*TsOH in acetonitrile, but this caused epimerization at C2 and partial formation of lactone **8**. In the next attempt with TFA in dichloromethane at room temperature, quantitative formation of lactone **8** was observed.[14] However, we were glad to find that by carrying out this reaction at 0 °C, only desired alcohol **7** was obtained in 69% yield. Simple functional group manipulation then allowed us to convert this *cis* 4-hydroxypipecolic acid derivative into target *N*-Fmoc-protected *trans* 4-aminopipecolic acid **14**. The only problem we encountered was the partial elimination from mesylate **9** to form, after double bond migration, α,β-unsaturated ester **11** when we carried out the nucleophilic displacement by NaN_3 in DMF at 100 °C.

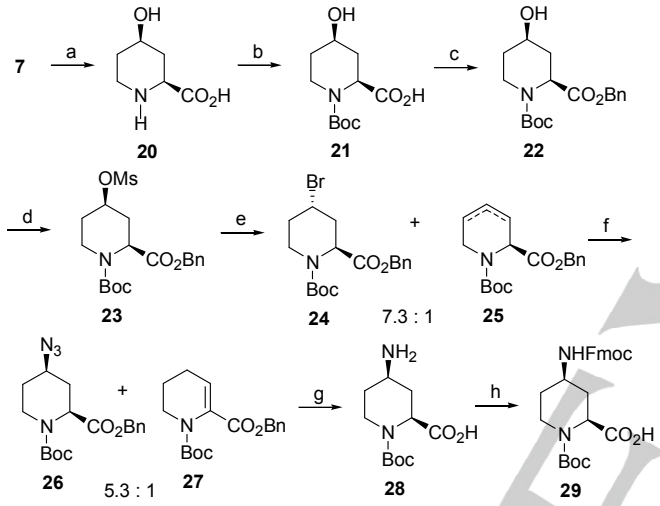


Scheme 1. Synthesis of Fmoc-protected *trans* 4-APA **14**. (a) H_2, 10% Pd/C, THF, 24 h (100%); (b) TFA, DCM, 0 °C, 4 h (69%); (c) MsCl, Et_3N, DCM, -30 to 25 °C, 2 h (92%); (d) NaN_3, DMF, 100 °C, 3 h (80%); (e) 1N NaOH, MeOH, 2 h (100%); (f) H_2, 10% Pd/C, MeOH, 24 h (100%); (g) FmocOSu, 10% aq. Na_2CO_3, THF, 0 to 25 °C, 19 h (79%).

For the synthesis of the corresponding *cis* 4-APA (Scheme 2) from alcohol **7**, we first carried out a Mitsunobu reaction to invert the configuration at C4. However the reaction provided acetate **15** in low yield and in impure form. After deprotection and mesylation of the OH group, nucleophilic substitution by NaN_3 occurred smoothly to form **18.** No elimination products were observed as in this case the leaving group in **17** is equatorially oriented.[15] Unfortunately, when we tried to hydrolyze the ester under alkaline conditions, epimerization to form a mixture of *cis* and *trans* **19** was unavoidable, despite some precedents in literature on analogous systems in which epimerization was not reported.[3a] We therefore changed approach and a different way to obtain the free carboxylic acid was attempted. So, after exhaustive hydrolysis of **7**[4b] followed by *N*-Boc protection, compound **21** was protected as benzyl ester **22** (Scheme 3). The OH group was then converted into a leaving group and **23** finally treated with LiBr in dry DMF to perform the first inversion of configuration.[3a] In this case also, in contrast to what has been reported,[3a] we observed the formation of a 7.3 : 1 mixture of desired *trans* compound **24** and elimination products **25**. Treatment of this mixture with NaN_3 in DMF at 70 °C generated *cis* azide **26** together with α,β-unsaturated ester **27**, the latter deriving from both isomerization of **25** and elimination from azide **26** (in which the azide group is axially oriented).[15] Eventually, hydrogenation of the azide group with concurrent deprotection of the carboxylic group, followed by N-Fmoc protection, allowed us to obtain orthogonally protected *cis* 4-APA **29** which, however, could be only partially separated by chromatography on silica gel from the by-products formed in the previous steps of the synthesis.[16]

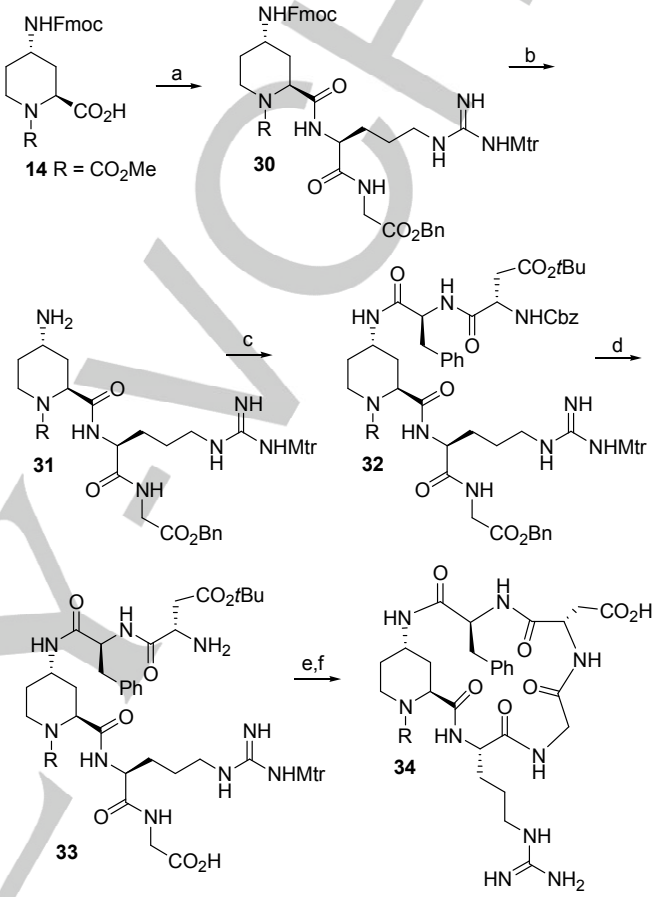


Scheme 2. Attempt at preparing *cis* 4-APA. (a) DIAD, Ph_3P, AcOH, THF, 0 °C, 4 h; (b) MeONa, MeOH, 0 °C, 6 h (33%, two steps); (c) MsCl, Et_3N, DCM, -30 to 25 °C (100 %); (d) NaN_3, DMF, 100 °C, 3 h (81%); (e) 1N NaOH, MeOH, 25 °C, 24 h.



Scheme 3. Synthesis of Fmoc-protected *cis* 4-APA **29**. (a) 2N HCl, reflux, 21 h (100%); (b) Boc_2O, Et_3N, MeOH, reflux, 21 h (100%); (c) BnBr, K_2CO_3, DMF, 0 to 25 °C, 24 h (89%); (d) MsCl, Et_3N, DCM, -30 to 25 °C (100%); (e) LiBr, DMF, 70 °C, 2 d (48%); (f) NaN_3, DMF, 70 °C, 22 h (98%); (g) H_2, 10% Pd/C, MeOH, 4.5 h (100%); (h) FmocOSu, 10% aq. Na_2CO_3, THF, 0 to 25 °C, 19 h (78%).

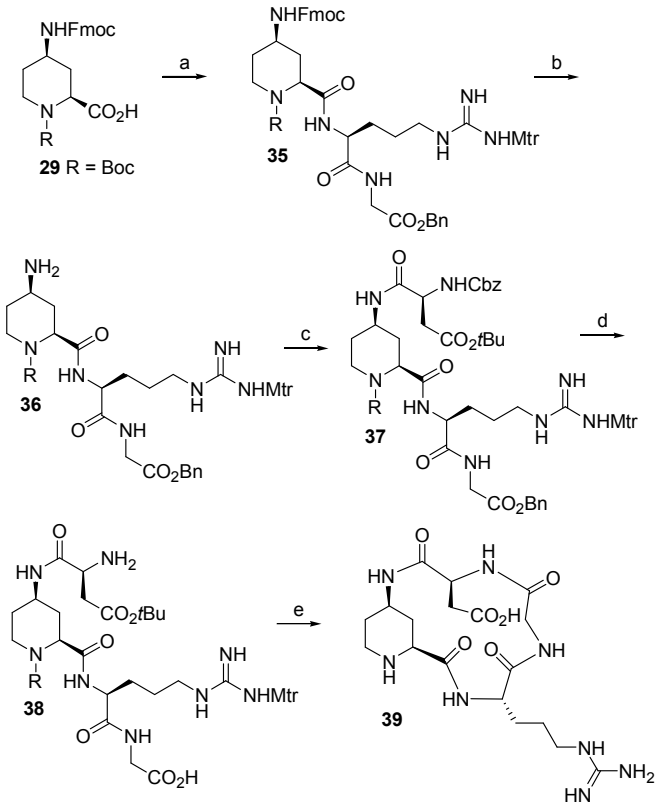
Both *trans* and *cis* 4-APA **14** and **29**, respectively, were then incorporated into cyclopeptides bearing the RGD (Arg-Gly-Asp) sequence recognized by the $\alpha v\beta_3$ integrin (Schemes 4 and 5). Due to the *trans* stereochemistry of 4-APA **14**, and the C-C bond at C2 being axially oriented,[15] we needed to embody a further amino acid into the sequence to allow the final ring closure. The results of a preliminary molecular modeling study (vide infra) resulted in L-Phe as the best amino acid to insert between 4-APA and the aspartate in order to match the binding conformation of cilengitide.[17] Thus, our *trans* 4-APA was coupled to the suitably protected dipeptide H-Arg(Mtr)-Gly-OBn to form **30** (86%), using DEPBT [3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one] as the coupling reagent in THF at 35 °C (Scheme 4). After deprotection of the amino group at C4, compound **31** was coupled to the Z-Asp(OtBu)-Phe-OH dipeptide under the above conditions to obtain **32** in excellent yield. This was subjected to hydrogenation to quantitatively form **33** which was in turn treated with DEPBT in a very dilute solution to attain cyclization. The reaction was stopped after 4 days and the cyclopeptide purified by semi-preparative HPLC providing compound **34** in pure form but in very low yield (11%). This is due to the many by-products formed during the last step. Indeed, despite lengthening one of the reacting arms with L-Phe, the *trans* nature of the 4-APA scaffold made cyclization very difficult.



Scheme 4. Synthesis of cyclopeptide **34**. (a) DEPBT, DIPEA, H-Arg(Mtr)-Gly-OBn, THF, 35 °C, 4 d (86%); (b) CH_2Cl_2/DEA 1:1, 3 h (100%); (c) DEPBT, DIPEA, Z-Asp(OtBu)-Phe-OH, THF, 35 °C, 4 d (98%); (d) H_2 (1 atm), 10% Pd/C, EtOH, 24 h (100%); (e) DEPBT, DIPEA, THF, 35 °C, 4 d (59%); (f) TFA/TIS/H_2O 95:2.5:2.5, 18 h (11%).

By an analogous synthetic approach, we converted *cis* 4-APA **29** into a cyclopeptide bearing the RGD sequence. Obviously, in this case we did not need a further amino acid into the sequence to facilitate cyclization. So (Scheme 5), after formation and deprotection of tripeptide **35** (which we could not purify properly) to generate **36**, the free amino group was reacted with the suitably protected aspartic acid to give **37** in 77% yield. After hydrogenation, cyclization and eventual exhaustive deprotection, cyclopeptide **39** was obtained in 24% after semi-preparative HPLC purification.

Cyclopeptides **34** and **39** were fully characterized by combining mono- and bi-dimensional homonuclear (^1H) experiments [proton, variable temperature (VT), gCOSY, and NOESY experiments in aqueous solution (D_2O/H_2O 1:9) and molecular modeling.



Scheme 5. Synthesis of cyclopeptide **39.** (a) DEPBT, DIPEA, H-Arg(Mtr)-Gly-OBn, THF, 35 °C, 4 d (93%); (b) CH_2Cl_2/DEA 1:1, 4 h (100%); (c) DEPBT, DIPEA, Z-Asp(OtBu)-OH, THF, 35 °C, 4 d (77%); (d) H_2 (1 atm), 10% Pd/C, EtOH, 24 h (100%); (e) DEPBT, DIPEA, THF, 35 °C, 4 d (50%); (f) TFA/TIS/H_2O 95:2.5:2.5, 18 h (24%).

For cyclopeptide **34**, ^{1}H NMR analysis revealed the presence of two sets of signals in a 1.4 : 1 ratio that are compatible with the existence of rotamers at the N-CO_2Me bond. As expected, our data seem to exclude the existence of a preferred conformation for compound **34**. The temperature coefficient values comprised between −5.4 and −8.0 ppb K^{-1} (Fig. S1, ESI) for the N–H protons of Phe, Asp, Arg and Gly indicate that none of these protons is locked in an intramolecular H-bonded state, and the analysis of the NOE contacts (Figure 2a) only showed the presence of medium–strong sequential CHα(i)/NH(i + 1) crosspeaks along the 4-APA-Arg-Gly-Asp-Phe sequence. The conformational analysis of compound **34** was also done *in silico* by temperature replica exchange molecular dynamics (T-REMD),[18] using a protocol that previously proved to be successful for similar questions.[11-12,19] Thus, 12 replica of 400 ns were performed with temperatures ranging from 300 to 860 K, without applying any restraint derived from the experimental NOEs. This resulted in three structures (**c0**, **c1** and **c2**) with significant populations (49%, 33%, and 13%, respectively) (Figure 3) in each of which atomic distances are such to justify only part of the observed NOEs.

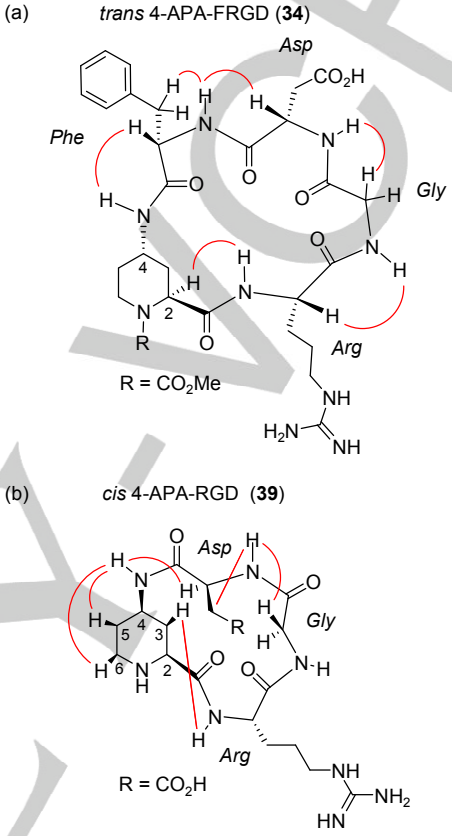


Figure 2. Selected experimental NOEs for compounds **34** and **39**.

For example, in **c0** the distance between 4-APA NH and Phe CHα (3.6 Å) and between Phe NH and Asp CHα (3.6 Å) are not consistent with the observed NOEs. In **c1** and **c2**, however, all distances are consistent with the observed NOEs with the exception of those between Phe NH and Asp CHα in **c1** (3.8 Å) and between 4-APA NH and Phe CHα in **c2** (3.5 Å). This could be explained by the existence of an equilibrium between these three conformations.

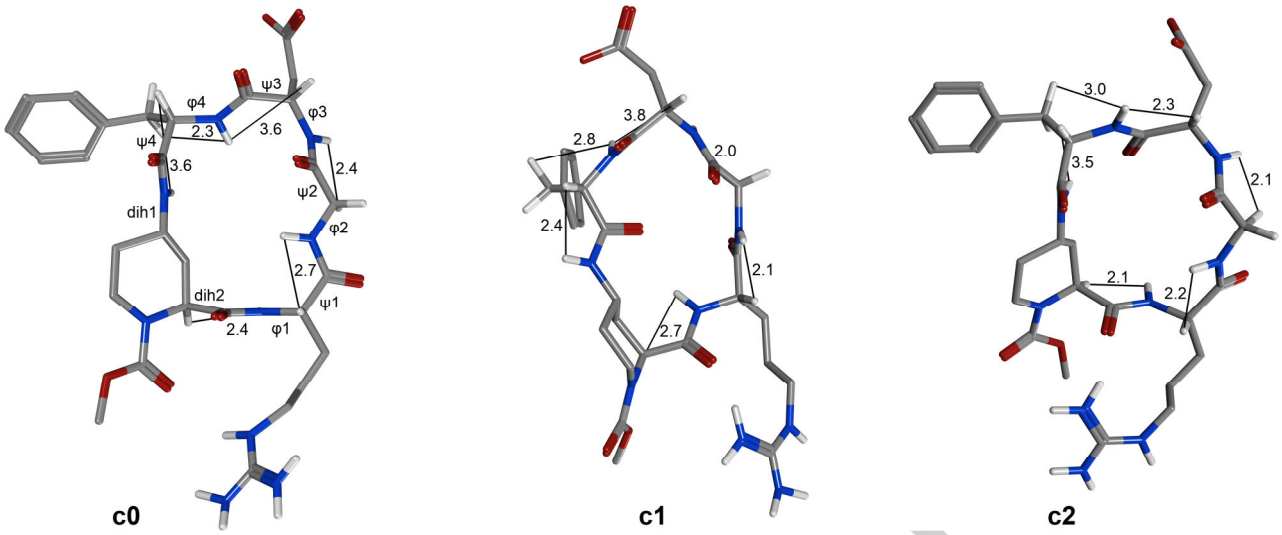


Figure 3. Representative conformations of the main clusters of compound **34**. Distances that are relevant to NOE experiments are reported in Å. Values of selected dihedrals are shown in Table 1.

In terms of backbone atom RMSD (**Table 1**), **c0**-**c2** structures match quite well that of cilengitide as measured in the X-ray structure of the $\alpha_V\beta_3$–cilengitide complex.[20] As suggested by our preliminary calculations, the benzyl group of the L-Phe of **34** overlaps quite well with that of D-Phe in cilengitide, although in **c0** and **c2** geometries only (Figure S2, ESI). Compared to cilengitide, relevant differences are observed in the orientation of some of the C=O and NH groups of the RGD moiety, as can also be evinced by the analysis of the corresponding φ and ψ dihedrals (Table 1, Figures 3 and S2, ESI). In particular, the c0 geometry shows quite divergent ψ1 and φ2 dihedrals, involving Arg and Gly, as well as ψ3, involving Asp. The other dihedrals, however, are of the same sign as observed in cilengitide. Concerning **c1**, a better match is observed for all RGD dihedrals, except ψ3 which shows the opposite sign as in cilengitide. Interestingly, the least populated **c2** shows a good match for all RGD dihedrals. Because of its importance for the biological activity, we also compared the distance between the Cβ atoms of Asp and Arg. As shown in Table 1, this distance is comparable to that measured for bound cilengitide,[20] even if, for this parameter also, the best match is obtained for c2.

A different behavior was instead observed for cyclopeptide **39**. The NOE contacts (Figure 2b) and the temperature coefficient values (Figure S1, ESI), especially those of Asp NH (-4 ppb K^{-1}) and 4-APA NH (-3.4 ppb K^{-1}), suggest the existence of a preferred and more rigid conformation. REMD simulations confirmed experimental data, where the clustering of the 300 K trajectory provided a highly populated principal cluster (**c0**, 87%; Figure 4). Additionally, all distances measured on the representative conformation of **c0** are consistent with the NOEs found. For example, the NOE crosspeaks between 4-APA NH and axial 6-H (d = 1.9 Å), and between Arg NH and equatorial 3-H (2.8 Å), are consistent with an optimal orientation of the 4-APA NH and Arg CO bond to form a γ-turn. The RMSD between the backbone atoms of **39 c0** and cilengitide is as low as 1.0 Å (Table 1) and all selected dihedrals match well those of the bounded cilengitide.

Table 1. Selected geometrical parameters (distances in Å, dihedrals in deg.) of compound **34** (clusters **c0**, **c1** and **c2**; Figure 3) and **39** (**c0**; Figure 4). The same parameters are measured in the X-ray of $\alpha_V\beta_3$–Cilengitide complex for comparison.

	34			**39**	Cilengitide
	c0	**c1**	**c2**	**c0**	
Dist. $C\beta_{Arg}$-$C\beta_{Asp}$	9.7	9.5	9.4	8.1	8.9
RMSD[a]	1.3	1.1	1.2	1.0	—
dih1[b]	85.9	152.9	72.1	−149.6	—
dih2[b]	159.8	−167.2	149.2	141.7	—
φ1	−119.9	−151.1	−126.3	−63.9	−114.5
ψ1	48.5	93.1	76.8	115.4	130.5
φ2	−170.5	142.9	148.4	87.0	84.0
ψ2	−136.1	−145.3	−124.5	−77.2	−136.2
φ3	−45.5	−62.4	−71.2	−141.0	−87.1
ψ3	−54.1	−55.7	135.1	97.3	61.4
φ4	−103.4	−87.0	58.4	—	172.1
ψ4	−70.4	158.9	−80.0	—	−122.7

[a] [a]Root mean squared displacement (Å) of backbone atoms of selected geometries compared to cilengitide. [b] The C5-C4-N-C=O(Phe) (dih1) and N1-C2-C(=O)-N(Arg) (dih2) dihedrals were considered.

Moreover, the $C\beta_{Arg}$-$C\beta_{Asp}$ distance (8.1 Å) results close to that observed for cilengitide (8.9 Å) and shorter than in **34** c0-c2 (9.4 – 9.7 Å).

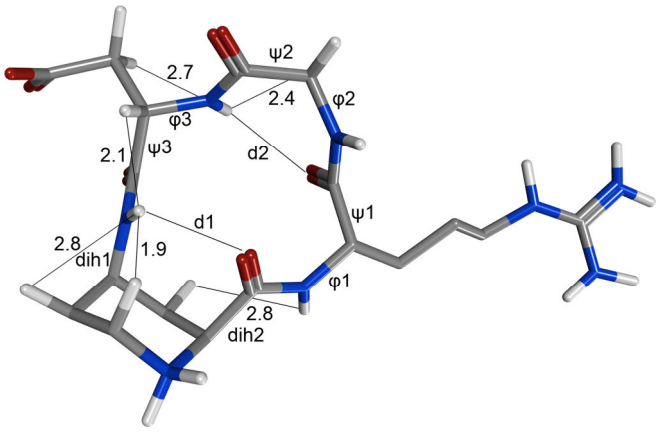


Figure 4. Representative conformation of the main cluster of compound **39**. Distances that are relevant to NOE experiments are reported in Å. Values of selected dihedrals are shown in Table 1.

The greater rigidity of cyclopeptide **39** compared to **34** can be explained by the shorter amino acid sequence (three instead of four amino acids) grafted onto the *cis* 4-APA scaffold with the two C2-CO and C4-N bonds bearing the RGD sequence being axially oriented. Additionally, **39** is likewise stabilized by two γ-turns involving Asp NH and Arg C=O (d1 = 2.6 Å) and 4-APA NH and 4-APA C=O (d2 = 2.5 Å).

Taken together, these data are in accordance with the inhibition activity of **34** (34.73 ± 10.62 μM) and **39** (1.15 ± 0.93 μM) relative to that of cilengitide (0.166 ± 0.08 μM) measured on WM266-4 metastatic human melanoma cell line overexpressing high levels of $\alpha_V\beta_3$ integrin (Figures S3 and S4, Supporting Information).[21, 22] Cyclopeptide **34** is about 200 times less active than cilengitide, this difference in potency likely being due to the energy required to switch from the preferred conformation in solution to the one that actually bind the receptor. It should be reminded that the same simulation protocol was also applied to cilengitide itself and that a very good match between the preferred conformation in solution and the bounded crystal structure was obtained.[17] In case of cyclopeptide **39**, its greater rigidity and better superposition with cilengitide accounts for it being only seven time less active than the latter.

Conclusion

In conclusion, a stereodivergent strategy to obtain enantiopure *cis* and *trans* 4-aminopipecolic acids (4-APAs) in a suitably protected form for peptide synthesis has been devised starting from a common known precursor (**4**), in turn easily prepared commercial (*R*)-4-cyano-3-hydroxybutyric acid ethyl ester. The two isomers were efficiently obtained in 40% and 23% overall yields, respectively, in seven and 10 steps. Both enantiomers of *cis* and *trans* 4-aminopipecolic acid can in principle be prepared given the commercial availability of both enantiomers of the starting material. To demonstrate their usefulness in peptidomimetics, both isomers were incorporated as γ-amino acid in a cyclic RGD-containing sequence, although for the *trans* 4-APA isomer a further amino acid in the sequence (L-Phe) was needed to allow ring closure. The two cyclopeptides were tested as $\alpha_V\beta_3$ integrin antagonist in comparison with cilengitide, which resulted only seven times more potent than cyclopeptide **39** deriving from *cis* 4-APA isomer and 200 times more potent than cyclopeptide **34** deriving from *trans* 4-APA isomer. The difference in potency between the two cyclopeptides can be explained by the higher molecular flexibility of cyclopeptide **34** compared to **39** and the better match of the latter with cilengitide in terms of backbone atoms and dihedrals. Further biological studies on compound **39** are being carried out and will be reported in due course.

Experimental Section

Calculations

Parameterization of trans- and cis-4-APA. Charge parameterization of *trans* 4-APA (as N1-methyl carbamate) and *cis*-4-APA (unprotected and protonated at N1) was performed using the R.E.D. software.[23] Both amino acids were capped by an acetyl and a NHMe at the 4-amino and at the C2 carboxy groups, respectively. A conformational search was then performed using the low mode molecular dynamics the MMFF94x force field, and the Born solvation model implemented in MOE,[24] and keeping the other parameters to default settings. Two low-energy conformations (the first and the third conformation for *trans*-4-APA, having opposite configuration of the tertiary amide group and different orientation of the acetylamido group at C4, and the first and fourth conformation for *cis*-4-APA, differing in dih1 and dih2 dihedrals, according to Figure Q) were used for charge parameterization. For each conformation, two different orientations were used to derive RESP charges. Quantum mechanical calculations were performed with Gaussian09[25] at the HF/6-31G* level, as requested by the force field.

REMD simulations. The starting conformations of compounds **34** and **39** were generated by a conformational search performed with MOE.[24] REMD simulations [were performed using the ff96 force field[26] coupled with the GB-OBC(II) solvent model[27] according to a protocol previously applied to similar synthetic peptides.[11, 12, 19a] The protocol was applied as described previously.[12] To evaluate convergence, the 300 K trajectories were subjected to a cluster analysis every 100 ns. Convergence was considered achieved after 300 ns of simulation, when the population of the three principal clusters in consecutive 100 ns batches differed by no more than 10%. REMD calculations were performed with *pmemd.cuda*,[28] while *cpptraj.cuda* executable was used for trajectory analyses.[29] Ten clusters were requested for clustering analysis, using the average-linkage algorithm, the pairwise mass-weighted RMSD on backbone heavy atoms as a metric and sampling one frame per picosecond.

Biological Tests

Expression of integrin receptors. Tumor cells were detached by gentle treatment with Accutase, washed, and incubated for 1 h at 4 °C in the presence of monoclonal antibody against different integrin receptors (1 μg each/50 μL PBS). We used anti-integrin $\alpha_V\beta_3$-FITC conjugated (11-0519-42 Thermofisher) and anti-$\alpha_V\beta_5$ monoclonal antibody (sc13588 Santa Cruz) followed by incubation with 1 μl/50 μl PBS of goat anti-mouse IgG FITC-conjugated (22549913 Immunotools) secondary antibody. Cells were analyzed at flow cytometer system (FACS Canto II Becton&Dickinson).

Inhibition of melanoma cell adhesion to RGD substrata. Highly expressing $\alpha_V\beta_3$ WM266.4 human melanoma cells were used for inhibition of adhesion experiments. 96 wells plates were coated overnight, at 4°C, with vitronectin (5 μg/mL) (140-09 Peprotech) or osteopontin (2 μg/mL 120-35 Peprotech). Plates were, then, washed with Phosphate Buffered Saline (PBS) solution and incubated at 37 °C for 1 h with PBS containing 1% Bovine Serum Albumin (BSA, A7906 Sigma). WM266.4

cells were centrifugated (RT, at 800*g*) in PBS, to remove serum, counted and suspended in serum-free medium at 7.0×10^5 cells/mL. Melanoma cell suspensions were pre-incubated with different amounts of the tested compounds (final concentration ranged from 30 μM to 1 nM) at 37 °C for 30 min to allow the ligand-receptor equilibrium to be reached. Next, cells were plated on RGD-containing substrata ($6\text{-}7\times10^4$ cells/well in 200μl volume) and incubated at 37 °C for 1 h. The assays were conducted in the presence of 2 mmol/L $MnCl_2$.[9f] At the end of the incubation, plates were washed with PBS to remove the non-adherent cells, and 200 μL of 0.5% crystal violet solution in 20% methanol were added. After 2 h of static incubation at 4 °C, plates were examined at 540 nm in a counter ELX800 (Bio TEK Instruments). Experiments were done in triplicate and repeated at least three times. The values are expressed as % inhibition ± SEM of cell adhesion relative to cells exposed to vehicle alone (PBS). IC_{50} values of the tested compounds were calculated using the online tool Quest Graph™ IC50 Calculator (AAT Bioquest, Inc., Sunnyvale, CA, USA) [IC50 Calculator AAT Bioquest. Available online: https://www.aatbio.com/tools/ic50-calculator (accessed on 5 December 2019)].

Chemistry

Anhydrous solvents were prepared accordingly to the standard techniques. Commercially available reagents were used without further purification. Melting points were recorded on a Büchi B-540 apparatus and are uncorrected. Chromatographic separations were performed under pressure on silica gel (Merck 70-230 mesh) by using flash column techniques; R_f values refer to TLC carried out on 0.25 mm silica gel plates (F254) with the same eluent as indicated for column chromatography. ^{1}H NMR (400, 500 MHz) and ^{13}C NMR (100.4 MHz) spectra were recorded either on a Bruker Avance II 500 MHz Ultrashield or on Varian Inova and Mercury (400 MHz) spectrometers in the specified deuterated solvent at 25 °C. Solvent reference lines were set at 7.26 and 77.00 ($CDCl_3$), 3.31 and 49.00 (CD_3OD) in ^{1}H and ^{13}C NMR spectra, respectively. Mass spectra were carried out by direct inlet of a 10 ppm solution in CH_3OH on a LCQ FleetTM Ion Trap LC/MS system (Thermo Fisher Scientific) with electrospray ionization (ESI) interface in the positive ion mode. HRMS analyses were performed under conditions of ESI-MS through direct infusion of a 1 uM solution in MeOH in a TripleTOF® 5600+ mass spectrometer (Sciex, Framingham, MA, U.S.A.), equipped with a DuoSpray® interface operating with an ESI probe. Optical rotations were determined with a JASCO DIP-370 instrument. HPLC analyses were performed on a Dionex Acclaim 120, C18, 5 μm, 4.6-250 mm reverse-phase analytical column at a flow rate of 1 mL min^{-1} and using water–acetonitrile gradient eluent buffered with 0.1% TFA. Purifications of a small sample of **29** and of cyclopeptides **34** and **39** were carried out by semi-preparative HPLC using a Dionex Ulltimate 3000 system equipped with a Alltech Alltima C18, 10 μm, 250 mm × 10 mm, reverse-phase column, using a water–acetonitrile gradient eluent buffered with 0.1% TFA and at a flow rate of 5 mL min^{-1}. Signals were monitored at 223 nm with a UV-detector. Compound **4** was synthesized as reported.[4b]

Dimethyl (2*S*,4R)-4-*tert*-Butoxypiperidine-1,2-dicarboxylate (5): To a stirred suspension of $NaHCO_3$ (242 mg) in anhydrous THF (30.3 mL) was added 10% Pd/C (198 mg) under nitrogen atmosphere. The mixture was flushed with H_2 and then stirring was continued for 30 min. The solution of **4** (315 mg) in anhydrous THF (2.8 mL) was added via microsyringe and the suspension was stirred at room temperature overnight under static H_2 pressure. After filtration through a Celite layer and evaporation of the solvent, compound **5** (319 mg, 100%) was obtained as a 10:1 mixture (colourless oil) with the elimination product **6** and it was directly used in the next step. Analytical and spectroscopic data are identical to those reported in literature.[4b]

Dimethyl (2*S*,4*R*)-4-Hydroxypiperidine-1,2-dicarboxylate (7): Compound **5** (319 mg) was dissolved in anhydrous CH_2Cl_2 (6.9 mL) under nitrogen atmosphere and cooled to 0 °C in an ice bath. TFA (1.22 mL, 15% vol/vol) was added dropwise and the solution was stirred for 4 h at 0 °C under nitrogen pressure. Solution was then poured into water (15 mL) and neutralized by slow addition of saturated aqueous sodium bicarbonate and then of solid sodium bicarbonate. The resulting mixture was extracted with CH_2Cl_2 (4 × 15 mL) and the combined organic layers were dried over Na_2SO4, filtered and evaporated under reduced pressure. The crude residue was purified by flash chromatography (*n*-hexane-EtOAc, 1 : 2; R_f 0.30) to afford pure **7** (175 mg, 69%) as a colourless oil. Analytical and spectroscopic data are identical to those reported in literature.[4b]

(1*S*,5*R*)-7-Oxo-6-oxa-2-aza-bicyclo[3.2.1]octane-2-carboxylic acid methyl ester (8): Compound **5** (354 mg) was dissolved in anhydrous CH_2Cl_2 (7.6 mL) under nitrogen atmosphere. TFA (1.34 mL, 15% vol/vol) was added dropwise and the solution was stirred for 22 h at room temperature, under nitrogen pressure. Solution was then poured into water (15 mL) and neutralized by slow addition of saturated aqueous sodium bicarbonate before and directly of sodium bicarbonate after. The resulting mixture was extracted with CH_2Cl_2 (4 × 15 mL) and the combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude residue (which was a 1.6:1 mixture with the alcohol **7**) was purified by flash chromatography (*n*-hexane-EtOAc, 1 : 2; R_f 0.45) to afford pure **8** (87 mg, 38%) as a colourless oil. $[\alpha]_D^{22}$ –15.5 (c 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.97 (t, *J* = 4.8 Hz, 1 H), 4.91 – 4.64 (m, 1 H), 4.20 – 3.97 (m, 1 H), 3.72 (s, 3 H), 3.31 – 3.15 (m, 1 H), 2.36 – 2.25 (m, 1 H), 2.12 – 2.00 (m, 1 H), 1.95 (d, *J* = 12.0 Hz, 1 H), 1.93 – 1.84 (m, 1 H). ^{13}C NMR (100.4 MHz, $CDCl_3$) δ (ppm): 172.9, 155.0, 76.8, 53.5, 53.1, 38.5, 36.6, 28.5. MS (ESI) m/z (%): 208 ($[M + Na]^+$, 100), 393 ($[2M + Na]^+$, 29). HRMS (ESI/TOF) m/z: $[M + H]^+$ Calcd. for $C_8H_{11}NO_4$: 186.0761. Found: 186.0781.[4b]

Dimethyl (–)-(2*S*,4*S*)-4-Methanesulfonyloxypiperidine-1,2-dicarboxylate (9): Methanesulfonyl chloride (192 μL, 2.48 mmol) was added dropwise to a solution of **7** (415 mg, 1.91 mmol) and trimethylamine (346 μL, 2.48 mmol) in anhydrous CH_2Cl_2 (20.1 mL), cooled to -30 °C and under nitrogen atmosphere. After 5 min, the cooling bath was removed and the reaction mixture was stirred for 2 h at room temperature. Water (12 mL) was added, followed by a dropwise addition of a 0.5 N solution of HCl (6.7 mL) and, after 10 min, the product was extracted with CH_2Cl_2 (3 × 17 mL). The combined organic layers were purified by flash chromatography (*n*-hexane-EtOAc, 4 : 5; R_f 0.20) to afford compound **9** (520 mg, 92%) as a colourless oil. $[\alpha]_D^{18}$ –11.4 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) (1.4 : 1 mixture of rotamers) δ (ppm): 5.05 (br s, 1 H), 4.94 (d, *J* = 6.7 Hz, 1 H, major rotamer), 4.79 (d, *J* = 6.6 Hz, 1 H, minor rotamer), 4.10 (dd, *J* = 13.4, 4.1 Hz, 1 H, minor rotamer), 3.96 (dd, *J* = 13.7, 4.1 Hz, 1 H, major rotamer), 3.75 (br s, 3 H), 3.74 (br s, 3 H, major rotamer), 3.69 (br s, 3 H, minor rotamer), 3.44 (t, *J* = 13.1 Hz, 1 H, major rotamer), 3.35 (t, *J* = 13.2 Hz, 1 H, minor rotamer), 2.97 (s, 3 H), 2.73 (br s, 1 H, minor rotamer), 2.69 (br s, 1 H, major rotamer), 2.01 (ddd, *J* = 14.8, 7.1, 2.1 Hz, 2 H), 1.85 – 1.68 (m, 1 H). ^{13}C NMR (100.4 MHz, $CDCl_3$) (mixture of rotamers) δ (ppm): 171.4, 156.8 and 156.2, 74.4, 53.1 and 52.5, 50.8 and 50.4, 38.5, 35.8 and 35.4, 31.8 and 31.6, 29.8 and 29.6. MS (ESI) m/z (%): 318 ($[M + Na]^+$, 100), 334 ($[M + K]^+$, 38), 613 ($[2M + Na]^+$, 48). HRMS (ESI/TOF) m/z: $[M + H]^+$ Calcd. for $C_{10}H_{17}NO_7S$: 296.0799. Found: 296.0793.

Dimethyl (–)-(2*S*,4*S*)-4-Azidopiperidine-1,2-dicarboxylate (10): Sodium azide (790 mg, 12.15 mmol) was added under nitrogen atmosphere to a solution of **9** (480 mg, 1.62 mmol) in anhydrous DMF (14.7 mL). The mixture was heated to 100 °C (external) for 3 h and then diluted with water (150 mL). The product was extracted with Et_2O (3 × 150 mL) and the combined organic extracts were dried over Na_2SO_4. After filtration and evaporation of the solvent, the crude residue was purified by flash chromatography (*n*-hexane-EtOAc, 3 : 1; R_f 0.24), and compound **10** (312 mg, 80%) was obtained as a pale yellow oil and as a 94:6 mixture with the elimination product **11**. $[\alpha]_D^{20}$ –6.2 (c 1.05, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) (1.4 : 1 mixture of rotamers) δ (ppm): 5.07 (br

d, *J* = 5.2 Hz, 1 H, major rotamer), 4.91 (br d, *J* = 5.2 Hz, 1 H, minor rotamer), 4.24 (br d, *J* = 13.1 Hz, 1 H, minor rotamer), 4.09 (br d, *J* = 13.8 Hz, 1 H, major rotamer), 3.74 (br s, 3 H), 3.72 (br s, 3 H, major rotamer), 3.69 (br s, 3 H, minor rotamer), 3.35 (tt, *J* = 12.0, 4.0 Hz, 1 H), 3.07 (t, *J* = 13.4 Hz, 1 H, major rotamer), 2.97 (t, *J* = 12.8 Hz, 1 H, minor rotamer), 2.47 (br t, *J* = 11.1 Hz, 1 H), 1.95 (br t, *J* = 14.8 Hz, 1 H), 1.73 – 1.56 (m, 1 H), 1.46 (qd, *J* = 12.7, 4.3 Hz, 1 H). ^{13}C NMR (100.4 MHz, $CDCl_3$) (mixture of rotamers) δ (ppm): 171.1 and 171.0, 156.5 and 155.9, 55.2, 53.9 and 53.7, 53.1 and 52.6, 40.2, 32.0 and 31.9, 30.4 and 30.3. MS (ESI) m/z (%): 265 ([M + Na]$^+$, 87).

(–)-(2*S*,4*S*)-4-Azidopiperidine-1,2-dicarboxylic acid 1-methyl ester (12): Aqueous 1 N NaOH (1.48 mL) was added to a solution of **10** (240 mg, 0.99 mmol) in methanol (2.9 mL) and the resulting mixture was vigorously stirred for 2 h at room temperature. The methanol was removed under reduced pressure and the residue was diluted in water (23 mL). The resulting solution was acidified to pH 2 by the addition of aqueous 1 N HCl and the product was extracted with CH_2Cl_2 (3 × 23 mL). The aqueous layer was then further acidified to pH 1 and the product was extracted again with CH_2Cl_2 (3 × 23 mL). The combined organic extracts were dried over Na_2SO_4 and, after filtration and evaporation of the solvent, compound **12** (226 mg, quantitative) was obtained as a white solid. $[\alpha]_D^{20}$ –5.5 (c 0.96, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) (1.4 : 1 mixture of rotamers) δ (ppm): 10.8 (br s, 1 H), 5.11 (br d, *J* = 5.5 Hz, 1 H, major rotamer), 4.96 (br d, *J* = 5.1 Hz, 1 H, minor rotamer), 4.24 (br d, *J* = 13.0 Hz, 1 H, minor rotamer), 4.11 (br d, *J* = 13.8 Hz, 1 H, major rotamer), 3.74 (br s, 3 H, major rotamer), 3.71 (br s, 3 H, minor rotamer), 3.42 (tt, *J* = 11.9, 3.8 Hz, 1 H), 3.17 – 2.95 (m, 1 H), 2.51 (br t, *J* = 14.2 Hz, 1 H), 1.98 (br t, *J* = 14.9 Hz, 1 H), 1.69 (td, *J* = 12.8, 6.3 Hz, 1 H), 1.48 (qd, *J* = 12.6, 4.6 Hz, 1 H). ^{13}C NMR (100.4 MHz, $CDCl_3$) (mixture of rotamers) δ (ppm): 175.9 and 175.8, 156.9 and 156.1, 55.1, 53.7 and 53.6, 53.4 and 53.3, 40.3, 31.8 and 31.7, 30.3 and 30.2. MS (ESI) m/z (%) (negative mode): 227 ([M - 1]$^-$, 81), 455 ([2M - 1]$^-$, 100).

(–)-(2*S*,4*S*)-4-(9*H*-Fluorenylmethoxycarbonylamino)-piperidine-1,2-dicarboxylic acid 1-methyl ester (14): To a solution of **12** (217 mg, 0.95 mmol) in anhydrous MeOH (18.1 mL), 10% Pd/C (32 mg, 0.03 mmol) was added under a nitrogen atmosphere. The resulting suspension was first flushed with hydrogen under vigorous stirring and then maintained under a hydrogen atmosphere (balloon) at room temperature. After 24 h, the mixture was filtered over a Celite pad, and the residual solution was evaporated under reduced pressure. The amino acid **13** (205 mg, quantitative) was obtained as a white solid and immediately used in the next step without further purifications. ^{1}H NMR (400 MHz, CD_3OD) (1.5 : 1 mixture of rotamers) δ (ppm): 4.76 (br d, *J* = 5.4 Hz, 1 H, minor rotamer), 4.68 (br d, *J* = 5.0 Hz, 1 H, major rotamer), 4.15 – 4.02 (m, 1 H), 3.70 (br s, 3 H, major rotamer), 3.67 (br s, 3 H, minor rotamer), 3.29 – 3.14 (m, 1 H), 3.09 (tt, *J* = 12.1, 3.6 Hz, 1 H), 2.67 – 2.52 (m, 1 H), 2.02 – 1.85 (m, 1 H), 1.65 – 1.51 (m, 1 H), 1.48 – 1.34 (m, 1 H). MS (ESI) m/z (%) (negative mode): 201 ([M - 1]$^-$, 100). A 10% aqueous solution of Na_2CO_3 (2.92 mL) was added to a suspension of amino acid **13** (192 mg, 0.95 mmol) in THF (2.3 mL) and the resulting mixture was cooled to 0 °C. After the addition of a solution of Fmoc–OSu (320 mg, 0.95 mmol) in THF (7.3 mL), the ice bath was removed and the mixture was vigorously stirred at room temperature for 19 h. The solvent was evaporated under reduced pressure and the residue was taken up in EtOAc (20 mL). The organic solution was washed once with water (15 mL) and once with satd NH_4Cl (10 mL). The aqueous phases were then reunited and extracted with EtOAc (5 × 20 mL). The aqueous layer was then acidified down to pH 2 by the addition of aqueous 0.5 N HCl and extracted again with EtOAc (10 × 20 mL). The combined organic extracts were dried over Na_2SO_4. After filtration and evaporation of the solvent, the crude residue was purified by flash chromatography, eluting first with DCM-MeOH, 20 : 1 and then with DCM-MeOH, 7.5 : 1 (R_f 0.27). Pure compound **14** (319 mg, 79%) was obtained as a clear syrup. $[\alpha]_D^{22}$ –8.6 (c 1.02, $CHCl_3$). ^{1}H NMR (400 MHz, CD_3OD) (1.3 : 1 mixture of rotamers) δ (ppm): 7.77 (d, *J* = 7.5 Hz, 2 H), 7.61 (d, *J* = 7.1 Hz, 2 H), 7.37 (t, *J* = 7.4 Hz, 2 H), 7.28 (t, *J* = 7.3 Hz, 2 H), 4.78 (br s, 1 H, minor rotamer), 4.72 (br s, 1 H, major rotamer), 4.32 (d, *J* = 6.5 Hz, 2 H), 4.18 (br d, *J* = 6.4 Hz, 1 H), 4.02 (br t, *J* = 14.0 Hz, 1 H), 3.67 (s, 3 H, minor rotamer), 3.65 (s, 3 H, major rotamer), 3.48 (br t, *J* = 11.2 Hz), 3.23 – 3.06 (m, 1 H), 2.53 – 2.39 (m, 1 H), 1.85 (br t, *J* = 12.8 Hz, 1 H), 1.50 (br s, 1 H), 1.39 – 1.23 (m, 1 H). ^{13}C NMR (100.4 MHz, CD_3OD) (mixture of rotamers) δ (ppm): 176.8 and 175.3, 158.7 and 158.5, 158.0, 145.3 (2 C), 142.6 (2 C), 128.7 (2 C), 128.1 (2 C), 126.1 (2 C), 120.9 (2 C), 67.7, 56.8 and 56.6, 53.4 and 53.3, 47.2, 41.9, 34.4 and 34.2, 32.5 and 32.4, 26.2. HRMS (ESI/TOF) m/z: [M + Na]$^+$ Calcd. for $C_{23}H_{24}N_2O_6Na$: 447.1527. Found: 447.1511.

Dimethyl (–)-(2*S*,4*S*)-4-Hydroxypiperidine-1,2-dicarboxylate (16): A solution of **7** (232 mg, 1.07 mmol) and Ph_3P (422 mg, 1.61 mmol) in anhydrous THF (8 mL) was added to a solution of diisopropyl azodicarboxylate (DIAD) (317 µL, 1.61 mmol) and acetic acid (92 µL, 1.61 mmol) in anhydrous THF (8 mL), cooled to 0 °C and under nitrogen atmosphere. After 1 h, a second portion of PPh_3 (84 mg, 0.32 mmol) and DIAD (31 µL, 0.16 mmol) was added and the mixture was stirred at 0 °C for an additional 3 h. The solvent was then removed under reduced pressure and the residue was taken up into *n*-hexane-Et_2O, 1 : 1 (20 mL). After filtration on a Celite pad and evaporation of the solvent, the crude residue was purified by flash chromatography (*n*-hexane-EtOAc, 1 : 2; R_f 0.35) to afford acetate **15** (in a 1 : 1.6 ratio with a residual impurity of reduced DIAD) as a colourless oil (193 mg). ^{1}H NMR (400 MHz, $CDCl_3$) (1.7 : 1 mixture of rotamers) δ (ppm): 5.11 – 5.00 (m, 1 H, minor rotamer), 5.01 – 4.86 (m, 1 H, major rotamer), 4.72 (tt, *J* = 11.5, 4.3 Hz, 1 H), 4.21 (br d, *J* = 12.9 Hz, 1 H, minor rotamer), 4.06 (br d, *J* = 12.2 Hz, 1 H, major rotamer), 3.75 (s, 3 H), 3.73 (br s, 3 H, major rotamer), 3.71 (br s, 3 H, minor rotamer), 3.22 – 3.00 (m, 1 H), 2.47 (br d, *J* = 12.1 Hz, 1 H), 2.06 – 1.95 (m, 4 H), 1.74 (td, *J* = 12.4, 6.3 Hz, 1 H), 1.54 – 1.40 (m, 1 H). A solution of **15** in anhydrous MeOH was cooled to 0 °C, under nitrogen atmosphere, and MeONa (84 mg, 1.55 mmol) was added. After 6 h, glacial acetic acid (825 µL) was added and the solvent was evaporated. The residue was diluted with water (40 mL), the product was extracted with EtOAc (4 × 50 mL) and the combined organic extracts were dried over Na_2SO_4. After filtration and evaporation of the solvent, the crude residue was purified by flash chromatography (*n*-hexane-EtOAc, 1 : 2; R_f 0.25) to afford **16** (76 mg, 33% over two steps) as a colourless oil. $[\alpha]_D^{21}$ –25.3 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) (1.4 : 1 mixture of rotamers) δ (ppm): 5.03 (br d, *J* = 4.7 Hz, 1 H, major rotamer), 4.88 (br d, *J* = 3.9 Hz, 1 H, minor rotamer), 4.18 (br d, *J* = 13.1 Hz, 1 H, minor rotamer), 4.05 (br d, *J* = 13.2 Hz, 1 H, major rotamer), 3.72 (s, 3 H), 3.71 (br s, 3 H, major rotamer), 3.68 (br s, 3 H, minor rotamer), 3.64 (tt, *J* = 11.2, 4.2 Hz, 1 H), 3.03 (dt, *J* = 27.3, 13.0 Hz, 1 H), 2.51 – 2.37 (m, 1 H), 2.21 – 1.96 (m, 1 H), 1.96 – 1.82 (m, 1 H), 1.60 (ddd, *J* = 12.8, 11.7, 6.3 Hz, 1 H), 1.40 (qd, *J* = 12.7, 4.4 Hz, 1 H). Spectroscopical data are identical to those reported in literature for its enantiomer.[4b]

Dimethyl (+)-(2*S*,4*R*)-4-Azidopiperidine-1,2-dicarboxylate (18): Prepared as reported for (–)-**9**, starting from **16** (76 mg, 0.35 mmol) and obtaining the intermediate O-mesylate **17** (99 mg, quantitative) after purification by flash chromatography (*n*-hexane-EtOAc, 1 : 1; R_f 0.28) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) (1.4 : 1 mixture of rotamers) δ (ppm): 5.12 (br s, 1 H, major rotamer), 4.97 (br s, 1 H, minor rotamer), 4.67 (tt, *J* = 11.5, 4.5 Hz, 1 H), 4.27 (br d, *J* = 13.7 Hz, 1 H, minor rotamer), 4.12 (br d, J = 13.5 Hz, 1 H, major rotamer), 3.78 (s, 3 H), 3.74 (br s, 3 H, major rotamer), 3.72 (br s, 3 H, minor rotamer), 3.18 – 3.05 (m, 1 H), 3.04 (s, 3 H), 2.66 (br s, 1 H, minor rotamer), 2.63 (br s, 1 H, major rotamer), 2.16 (br s, 1 H), 1.90 (ddd, J = 12.8, 11.9, 6.3 Hz, 1 H), 1.72 (ddd, J = 24.4, 12.6, 5.0 Hz, 1 H). The O-mesylate **17** (99 mg, 0.35 mmol) was treated with NaN_3 (170 mg, 2.62 mmol) as reported for compound (+)-**10** and pure (+)-**18** was obtained as a pale yellow oil (65 mg, 81%) after purification by flash chromatography (*n*-hexane-EtOAc, 1 : 1; R_f 0.42). $[\alpha]_D^{20}$ +39.1 (c 0.52, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) (1.4 : 1 mixture of rotamers) δ (ppm): 4.87 (br s, 1 H, major rotamer), 4.72 (br s, 1 H, minor rotamer), 4.02 (br d, *J* = 3.6 Hz, 1 H, minor rotamer), 3.98 (quint, *J* = 3.1 Hz, 1 H), 3.87 (br d, *J* = 11.9 Hz, 1 H, major rotamer), 3.78 (s, 3 H), 3.73 (br s, 3 H, major rotamer), 3.70 (br s, 3 H, minor rotamer), 3.39 – 3.17 (m, 1 H), 2.55 (br s, 1 H, minor rotamer), 2.52 (br s, 1 H, major rotamer), 1.98 (dd, *J* = 6.7, 2.9 Hz, 1 H, major rotamer), 1.98 (dd, *J* = 6.7, 2.9 Hz, 1 H, minor rotamer), 1.85 – 1.62 (m, 2

H). ^{13}C NMR (100.4 MHz, $CDCl_3$) (mixture of rotamers) δ (ppm): 171.4, 156.8, 54.4, 53.0, 52.4, 51.1 and 50.8, 36.2 and 36.0, 30.4 and 30.3, 28.7 and 28.5. MS (ESI) m/z (%): 243 ([M + H]$^+$, 19), 265 ([M + Na]$^+$, 100), 507 ([2M + Na]$^+$, 17). HRMS (ESI/TOF) m/z: [M + Na]$^+$ Calcd. for $C_9H_{14}N_4O_4Na$: 265.0907. Found: 265.0916.

(2*S*,4*R*)-4-Hydroxypiperidine-2-carboxylic acid Hydrochloride (1·HCl) (20): A vigorously stirred dispersion of **7** (83 mg, 0.41 mmol) in a 2 N HCl aqueous solution (23.4 mL) was refluxed for 21 h. The mixture was then cooled to room temperature, washed with Et2O (2 x 40 mL), and the aqueous layer concentrated under vacuum. The aminoacid **20** was obtained as a white solid (75 mg, quantitative) and immediately used in the next step without further purification. Analytical and spectroscopic data are identical to those reported in literature.[4c]

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-hydroxypiperidine-2-carboxylic acid (21): To a solution of compound **20** (75 mg, 0.41 mmol) in anhydrous methanol (9.1 mL), triethylamine (172 µL, 1.23 mmol) and Boc_2O (179 mg, 0.82 mmol) were added, under a nitrogen atmosphere, and the resulting reaction mixture was heated under reflux. After 21 h, the solvent was evaporated under reduced pressure and the residue was dissolved in H_2O (5 mL). $NaH_2PO_4{\cdot}2H_2O$ (7 mg) was added and the resulting solution was cooled to 0 °C, acidified down to pH 3 by addition of aqueous 0.5 N HCl and then stirred for 30 min at 0 °C. The product was then extracted with EtOAc (4 x 7 mL) and the combined organic extracts were dried over Na_2SO_4. After filtration and evaporation of the solvent, the crude *N*-Boc protected compound **21** (101 mg, quantitative) was obtained and used in the next step without further purification. The spectroscopical data are identical to those reported in the literature.[30]

2-Benzyl 1-*tert*-Butyl (–)-(2*S*,4*R*)-4-Hydroxypiperidine-1,2-dicarboxylate (22): To a solution of the *N*-Boc protected compound **21** (250 mg, 1.02 mmol) and K_2CO_3 (153 mg, 1.11 mmol) in anhydrous DMF (23 mL), cooled at 0 °C and under a nitrogen atmosphere, benzyl bromide (122 µL, 1.02 mmol) was added. The ice bath was removed and the resulting mixture was stirred at room temperature for 24 h. Water (115 mL) was added, the product was extracted with EtOAc (58 mL) and the organic extract dried over Na_2SO_4. After filtration and evaporation of the solvent, the crude product was purified by flash chromatography (*n*-hexane-EtOAc, 3 : 2; R_f 0.38) to afford pure **22** (305 mg, 89%) as a colourless oil. $[\alpha]_D^{20}$ –38.0 (c 1.04, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) (1.1 : 1 mixture of rotamers) δ (ppm): 7.39 – 7.27 (m, 5 H), 5.27 – 5.01 (m, 2 H), 4.87 (br s, 1 H, major rotamer), 4.67 (br s, 1 H, minor rotamer), 4.11 (br s, 1 H), 3.90 (br d, *J* = 11.3 Hz, 1 H, minor rotamer), 3.77 (br d, *J* = 10.1 Hz, 1 H, major rotamer), 3.47 – 3.24 (m, 1 H), 2.43 (br d, *J* = 13.2 Hz, 1 H), 1.90 (ddd, *J* = 14.4, 6.8, 2.3 Hz, 1 H), 1.80 – 1.49 (m, 3 H). ^{13}C NMR (100.4 MHz, $CDCl_3$) (mixture of rotamers) δ (ppm): 172.7 and 172.5, 155.8 and 155.3, 135.7, 128.5 (2 C), 128.2 (3 C), 80.1, 66.8, 63.3, 51.6 and 50.4, 36.1 and 35.0, 33.4, 31.1, 28.3 (3 C). MS (ESI) m/z (%): 358 ([M + Na]$^+$, 99), 693 ([2M + Na]$^+$, 100). HRMS (ESI/TOF) m/z: [M + Na]$^+$ Calcd. for $C_{18}H_{25}NO_5Na$: 358.1625. Found: 358.1623.

2-Benzyl 1-*tert*-Butyl (–)-(2*S*,4*R*)-4-Methanesulfonyloxypiperidine-1,2-dicarboxylate (23): Prepared as reported for (–)-**9**, starting from **22** (405 mg, 1.21 mmol) and obtaining pure O-mesylate **23** (419 mg, 84%) as a colourless oil, after purification of the crude by flash chromatography (*n*-hexane-EtOAc, 2 : 1; R_f 0.31). $[\alpha]_D^{19}$ –17.2 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) (1.2 : 1 mixture of rotamers) δ (ppm): 7.42 – 7.29 (m, 5 H), 5.29 – 5.10 (m, 2 H), 5.05 (br s, 1 H), 4.95 (br d, *J* = 6.8 Hz, 1 H, major rotamer), 4.74 (br d, *J* = 5.7 Hz, 1 H, minor rotamer), 4.03 (br d, *J* = 9.7 Hz, 1 H, minor rotamer), 3.91 (br d, *J* = 9.6 Hz, 1 H, major rotamer), 3.41 (br t, *J* = 13.2 Hz, 1 H, minor rotamer), 3.30 (br t, *J* = 11.9 Hz, 1 H, major rotamer), 2.81 (br s, 3 H, minor rotamer), 2.77 (br s, 3 H, major rotamer), 2.73 (br s, 1 H, minor rotamer), 2.69 (br s, 1 H, major rotamer), 2.11 – 1.96 (m, 2 H), 1.76 (br t, *J* = 11.8 Hz, 1 H), 1.45 (br s, 9 H, major rotamer), 1.39 (br s, 9 H, minor rotamer). ^{13}C NMR (100.4 MHz, $CDCl_3$) (mixture of rotamers) δ (ppm): 171.2 and 171.0, 155.6 and 155.1, 135.5, 128.6 (2 C), 128.3 (3 C), 80.6, 74.4 and 74.2, 67.2, 51.3 and 50.1, 38.3, 36.1 and 35.1, 31.6 and 31.4, 29.9 and 29.6, 28.3 (3 C). MS (ESI) m/z (%): 436 ([M + Na]$^+$, 39), 849 ([2M + Na]$^+$, 100). Elem. Anal. Calcd for $C_{19}H_{27}NO_7S$: C, 55.19; H 6.58; N 3.39. Found: C, 54.96; H, 6.81; N, 3.18.

2-Benzyl 1-(*tert*-Butyl) (2*S*,4*S*)-4-Bromopiperidine-1,2-dicarboxylate (24): To a solution of O-mesylate **23** (419 mg, 1.02 mmol) in anhydrous DMF (9.3 mL), LiBr (133 mg, 1.53 mmol) was added. The mixture was stirred for 2 days at 70 °C, then taken up into EtOAc (90 mL), washed with water (90 mL) and dried over Na_2SO_4. After filtration and evaporation of the solvent, the crude product was purified by flash chromatography (*n*-hexane-EtOAc, 7 : 1; R_f 0.45) and the pale yellow oil so obtained contained **24** (194 mg, 48%) as a 88:12 mixture with the elimination product **25**. $[\alpha]_D^{24}$ –38.4 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) (1.25 : 1 mixture of rotamers) δ (ppm): 7.40 – 7.27 (m, 5 H), 5.18 (br s, 2 H), 5.00 (br d, *J* = 3.5 Hz, 1 H, major rotamer), 4.77 (br d, *J* = 4.2 Hz, 1 H, minor rotamer), 4.13 – 3.99 (m, 1 H, minor rotamer), 3.99 – 3.82 (m, 2 H, major rotamer), 3.01 (br t, *J* = 12.3 Hz, 1 H, major rotamer), 2.92 (br t, *J* = 13.2 Hz, 1 H, minor rotamer), 2.78 (br t, *J* = 15.2 Hz, 1 H), 2.29 – 2.05 (m, 2 H), 1.89 (qd, *J* = 12.8, 4.9 Hz, 1 H), 1.44 (br s, 9 H, major rotamer), 1.35 (br s, 9 H, minor rotamer). ^{13}C NMR (100.4 MHz, $CDCl_3$) (mixture of rotamers) δ (ppm): 170.7 and 170.6, 155.3 and 154.9, 135.3, 128.6 (3 C), 128.2, 128.0, 80.7, 67.1, 56.1 and 55.2, 44.2 and 44.1, 42.6 and 41.9, 37.4, 36.4 and 36.3, 28.2 (3 C). MS (ESI) m/z (%): 420 ([M + Na]$^+$, 73) and 422 ([M + Na]$^+$, 66).

(2*S*,4*R*)-4-Azidopiperidine-1,2-dicarboxylic acid 2-benzyl ester 1-*tert*-butyl ester (26): Sodium azide (238 mg, 3.66 mmol) was added under nitrogen atmosphere to a solution of the bromide derivative **24** (194 mg, 0.49 mmol) in anhydrous DMF (4.9 mL). The mixture was heated to 70 °C (external) for 22 h and then diluted with water (50 mL). The product was extracted with Et_2O (3 × 50 mL) and the combined organic extracts were dried over Na_2SO_4. After filtration and evaporation of the solvent, the crude residue was then purified by flash chromatography (*n*-hexane-EtOAc, 7 : 1; R_f 0.27) and the pale yellow oil **26** (174 mg, 98%) was obtained as an 84:16 mixture with the elimination and consecutive isomerization product **27**. ^{1}H NMR (400 MHz, $CDCl_3$) (1.2 : 1 mixture of rotamers) δ (ppm): 7.44 – 7.27 (m, 5 H), 5.32 – 5.16 (m, 2 H), 4.86 (br s, 1 H, major rotamer), 4.67 (br s, 1 H, minor rotamer), 3.99 – 3.87 (m, 1 H), 3.36 – 3.10 (m, 1 H), 2.50 (br d, *J* = 12.9 Hz, 1 H), 1.93 (ddd, *J* = 14.4, 6.8, 3.0 Hz, 1 H), 1.85 – 1.61 (m, 2 H), 1.45 (br s, 9 H, major rotamer), 1.37 (br s, 9 H, minor rotamer). MS (ESI) m/z (%): 383 ([M + Na]$^+$, 100). HRMS (ESI/TOF) m/z: [M + H]$^+$ Calcd. for $C_{18}H_{24}N_4O_4$: 361.1870. Found: 361.1871.

(2*S*,4*R*)-4-Aminopiperidine-1,2-dicarboxylic acid 1-*tert*-butyl ester (28): To a solution of **26** (174 mg, 0.48 mmol) in anhydrous MeOH (12.6 mL), 10% Pd/C (16 mg, 15 µmol) was added under a nitrogen atmosphere. The resulting suspension was first flushed with hydrogen under vigorous stirring and then maintained under a hydrogen atmosphere (balloon) at room temperature. After 4.5 h, the mixture was filtered over a Celite pad, and the residual solution was evaporated under reduced pressure. The amino acid **28** (118 mg, quantitative) was obtained as a white solid and immediately used in the next step without further purification. ^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 4.48 (br s, 1 H), 3.90 – 3.79 (m, 1 H), 3.41 – 3.36 (m, 1 H), 3.33 – 3.31 (m, 1 H), 2.21 – 2.09 (m, 1 H), 2.08 – 1.96 (m, 1 H), 1.94 – 1.83 (m, 1 H), 1.83 – 1.71 (m, 1 H), 1.46 (s, 9 H). MS (ESI) m/z (%): 267 ([M + Na]$^+$, 100), 511 ([2M + Na]$^+$, 65).

(2*S*,4*R*)-4-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-piperidine-1,2-dicarboxylic acid 1-*tert*-butyl ester (29): A 10% aqueous solution of Na_2CO_3 (1.47 mL) was added to a suspension of amino acid **28** (118 mg, 0.48 mmol) in THF (1.5 mL) and the resulting mixture was cooled to 0 °C. After the addition of a solution of Fmoc–OSu (162 mg, 0.48 mmol) in THF (4.5 mL), the ice bath was removed and the mixture was vigorously stirred at room temperature for 19 h. The solvent was evaporated under reduced pressure and the residue was taken up in EtOAc (24 mL). The organic solution was washed once with water (24 mL) and once with satd

NH_4Cl (24 mL). The aqueous phases were then reunited and extracted with EtOAc (3 × 24 mL). The acqueos layer was then acidified to pH 3 by the addition of aqueous 1 N HCl and the product was extracted with EtOAc (8 × 24 mL). Finally, the aqueous layer was then further acidified to pH 2 and the product was extracted again with EtOAc (24 mL). The combined organic extracts were dried over Na_2SO_4. After filtration and evaporation of the solvent, the crude residue was chromatographed (MeOH-DCM, 1 : 20; R_f 0.22) to provide **29** (174 mg, 78%) as a colourless oil. A small sample of compound **29** was further purified by semi-preparative HPLC (water–acetonitrile eluent buffered with 0.1% TFA, acetonitrile from 50 to 90%; R_t = 10.48 min) for characterization. $[\alpha]_D^{20}$ –6.0 (c 1.0, CH_3OH). ^1H NMR (400 MHz, CD_3OD) δ (ppm): 7.79 (d, *J* = 7.5 Hz, 2 H), 7.69 – 7.59 (m, 2 H), 7.39 (t, *J* = 7.4 Hz, 2 H), 7.35 – 7.28 (m, 2 H), 4.52 (br s, 1 H), 4.39 – 4.16 (m, 3 H), 3.75 (br s, 2 H), 3.45 – 3.33 (m, 1 H), 2.59 – 2.49 (m, 1 H), 1.96 – 1.88 (m, 1 H), 1.86 – 1.78 (m, 1 H), 1.77 – 1.69 (m, 1 H), 1.46 (s, 9 H). ^{13}C NMR (100.4 MHz, CD_3OD) (mixture of rotamers) δ (ppm): 176.2 and 175.4, 158.1 and 157.4, 145.3 (2 C), 142.5 (2 C), 128.7 (2 C), 128.1 (2 C), 126.2 (2 C), 120.9 (2 C), 81.6 and 81.3, 67.8, 56.3 and 55.1, 45.5, 43.2 and 42.2, 37.8, 31.3, 29.7, 28.6 (3 C). MS (ESI) m/z (%): 489 ([M + Na]$^+$, 100). HRMS (ESI/TOF) m/z: [M + H]$^+$ Calcd. for $C_{26}H_{30}N_2O_6$: 467.2175. Found: 467.2177.

(–)-(2*S*,4*S*)-4-(9-Fluorenylmethoxycarbonylamino)-1-(methoxycarbonyl)piperidine-2-Arg(Mtr)-Gly-OBn (30): DEPBT (359 mg, 1.20 mmol) and DIPEA (209 µL, 1.20 mmol) were added under a nitrogen atmosphere to a solution of **14** (256 mg, 0.60 mmol) in anhydrous THF (5.5 mL), cooled to 0 °C, and the resulting mixture was allowed to warm to room temperature. After 15 min, the reaction mixture was cooled again to 0 °C, and a solution of H-Arg(Mtr)-Gly-OBn (428 mg, 0.78 mmol) in anhydrous THF (5.5 mL) was added. The ice bath was removed and the mixture was stirred at 35 °C (external) for 24 h and then stirred at 25 °C for 3 days. The mixture was then diluted with EtOAc (225 mL), washed with satd NH_4Cl (2 × 60 mL), satd $NaHCO_3$ (2 × 60 mL), and finally with H_2O (2 × 60 mL). The organic extract was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude residue was purified by flash chromatography (MeOH-DCM, 1 : 20; R_f 0.41) and pure **30** (484 mg, 86 %) was obtained as a white gummy solid. m.p. = 147.4 – 151.3 °C. $[\alpha]_D^{24}$ –10.4 (c 1.04, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.73 (d, *J* = 7.4 Hz, 2 H), 7.66 (br s, 1 H), 7.51 (d, *J* = 7.3 Hz, 2 H), 7.37 (t, *J* = 7.5 Hz, 2 H), 7.32 – 7.16 (m, 7 H), 6.48 (s, 1 H), 6.31 (br s, 2 H), 5.13 – 4.95 (m, 3 H), 4.81 (br s, 1 H), 4.61 (br s, 1 H), 4.28 (br d, *J* = 5.1 Hz, 2 H), 4.15 – 3.94 (m, 3 H), 3.79 (s, 3 H), 3.68 (s, 3 H), 3.55 (br s, 1 H), 3.35 – 3.12 (m, 2 H), 2.65 (s, 3 H), 2.58 (s, 4 H), 2.08 (s, 3 H), 2.03 – 1.92 (m, 1 H), 1.92 – 1.48 (m, 7 H). ^{13}C NMR (100.4 MHz, $CDCl_3$) δ (ppm): 172.2, 169.8, 158.3, 156.5, 156.4, 155.7, 143.7, 141.2, 138.5, 136.4, 135.3, 133.5, 128.5, 128.2 (2 C), 128.2, 128.1 (2 C), 127.7 (2 C), 127.0 (2 C), 124.9, 124.7, 119.9 (2 C), 111.6, 67.0, 66.8, 66.7, 55.4, 54.9, 53.2, 47.1, 45.7, 41.3, 40.4, 33.4, 31.2, 29.7, 29.6, 25.5, 24.1, 18.3, 11.9. MS (ESI) m/z (%): 962 ([M + Na]$^+$, 100).

(2*S*,4*S*)-4-[L-Phe-Z-Asp(OtBu)]-1-(methoxycarbonyl)-4-APA-2-Arg(Mtr)-Gly-OBn (32): Compound **30** (244 mg, 0.26 mmol) was dissolved in a 1 : 1 CH_2Cl_2/diethylamine mixture (4.2 mL) under a nitrogen atmosphere. The resulting solution was stirred at room temperature for 4 h, meanwhile an additional 1 : 1 CH_2Cl_2/diethylamine mixture (1.5 mL) was added. The solution was concentrated under reduced pressure, the residue was taken up in CH_2Cl_2 (3.5 mL), filtered over a Celite pad and then concentrated again. The crude **31** was obtained as a pale yellow solid. ^1H NMR (400 MHz, $CDCl_3$) (2.1 : 1 mixture of rotamers) δ (ppm): 7.34 – 7.26 (m, 5 H), 6.50 (s, 1 H), 6.41 (br s, 1 H), 5.16 – 5.04 (m, 2 H), 4.97 (br s, 1 H, major rotamer), 4.86 (br s, 1 H, major rotamer), 4.58 (br s, 1 H), 4.16 – 4.03 (m, 3 H), 3.80 (s, 3 H), 3.67 (s, 3 H), 3.41 – 3.08 (m, 3 H), 3.01 (br s, 1 H), 2.65 (s, 3 H), 2.58 (s, 3 H), 2.58 (s, 3 H), 2.00 – 1.79 (m, 2 H), 1.70 (br s, 1 H), 1.56 (br s, 3 H), 1.30 – 1.22 (m, 3 H), 1.20 – 1.02 (m, 1 H). MS (ESI) m/z (%): 313 ([M – CH_2Ph]$^{2+}$, 100), 718 ([M + 1]$^+$, 97). DEPBT (200 mg, 0.67 mmol) and DIPEA (86 µL, 0.67 mmol) were added under nitrogen atmosphere to a solution of Z-Asp(OtBu)-L-Phe-OH (207 mg, 0.38 mmol) in anhydrous THF (4.5 mL), cooled at 0 °C, and the resulting mixture was allowed to warm to room temperature. After 15 min, this solution was slowly added to a solution of compound **31** in anhydrous THF (1.5 mL), precooled to 0 °C. The ice bath was removed and the resulting mixture was stirred at 35 °C. After 24 h, a second portion of Z-Asp(OtBu)-L-Phe-OH (61 mg, 0.13 mmol), DEPBT (77 mg, 0.26 mmol) and DIPEA (45 µL, 0.26 mmol) was added and the mixture was stirred at 35 °C for an additional 3 days. The mixture was then diluted with EtOAc (57 mL) and washed with satd NH_4Cl (2 × 16 mL), satd $NaHCO_3$ (2 × 16 mL) and finally H_2O (2 × 16 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude residue was purified by flash chromatography (MeOH/CH_2Cl_2 1:15, R_f 0.30) and pure **32** (284 mg, 98%) was obtained as a white solid. m.p. 103.4 – 109.1 °C. $[\alpha]_D^{26}$ –14.5 (c 0.99, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.73 (br m, 1 H), 7.45 – 7.05 (m, 15 H), 6.89 (br s, 1 H), 6.50 (s, 1 H), 6.37 (br s, 2 H), 5.88 (br s, 1 H), 5.10 (br s, 2 H), 5.09 – 4.91 (m, 2 H), 4.66 (br s, 1 H), 4.51 (br s, 1 H), 4.46 – 4.28 (m, 1 H), 4.16 – 3.91 (m, 4 H), 3.83 – 3.74 (m, 1 H), 3.79 (s, 3 H), 3.66 (s, 3 H), 3.43 – 3.09 (m, 3 H), 3.00 (br s, 2 H), 2.72 – 2.65 (m, 1 H), 2.67 (br s, 3 H), 2.60 (s, 3 H), 2.47 – 2.38 (m, 1 H), 2.10 (s, 3 H), 1.95 (br s, 1 H), 1.79 – 1.47 (m, 4 H), 1.38 (s, 9 H) 1.43 – 1.17 (m, 3 H). ^{13}C NMR (100.4 MHz, $CDCl_3$) (mixture of rotamers) δ (ppm): 172.1, 170.79 and 170.76, 170.64 and 170.57, 170.3, 169.8, 158.3, 157.1, 156.5, 156.1, 138.5, 136.4, 136.2, 135.8, 135.3, 135.2, 133.6, 129.2, 128.7, 128.53, 128.50, 128.4, 128.3, 128.2, 128.0, 127.0, 124.7, 111.7, 82.0, 67.3, 66.9, 55.4, 54.8, 54.4, 53.2, 52.6, 51.7, 44.3, 41.4 and 41.2, 40.3, 37.2, 32.4, 30.5, 29.7, 29.6, 29.3, 28.0 (3 C), 25.5, 24.1, 18.3, 11.9. MS/MS (ESI) [M + Na]$^+$ m/z (%): 1192 ([M + Na]$^+$, 4), 1136 (100).

(–)-Cyclo{Arg-Gly-Asp-L-Phe[1-(methoxycarbonyl)-4-APA]}·TFA (34): To a solution of **32** (284 mg, 0.25 mmol) in ethanol (14.3 mL), 10% Pd/C (157 mg, 0.15 mmol) was added under a nitrogen atmosphere. The resulting suspension was first flushed with hydrogen under vigorous stirring and then maintained under a hydrogen atmosphere (balloon) at room temperature. After 18 h, the mixture was filtered over a Celite pad, filtered again through a syringe filter (Nylon, 0.45 µm pores) and the residual solution was evaporated under reduced pressure. Compound **33** (213 mg, 90%) was obtained as a white solid and used in the next step without further purifications. MS (ESI) m/z (%): 968 ([M + Na]$^+$, 100). The crude **33** (213 mg, 0.23 mmol) was suspended in THF (66 mL) under a nitrogen atmosphere. The suspension was cooled to 0 °C, and DEPBT (202 mg, 0.68 mmol) and DIPEA (117 µL, 0.68 mmol) were added. The resulting mixture was stirred at 35 °C for 5 days and the diluted with EtOAc (22 mL), washed with satd NH_4Cl (2 × 15 mL), satd $NaHCO_3$ (2 × 15 mL), H_2O (2 × 15 mL) and finally dried over Na_2SO_4. After filtration and evaporation of the solvent, the residue was purified by flash chromatography (MeOH/CH_2Cl_2 1:20, R_f 0.10). Cromatography column was then washed with MeOH. The MeOH solution was concentrated, taken up in $CHCl_3$, washed with water (3 x 15 mL) and finally concentrated again. The cyclopeptide (116 mg, 59%) was obtained ad a white solid. MS (ESI) m/z (%): 950 ([M + Na]$^+$, 100). The crude cyclic tetrapeptide was dissolved in a 95:2,5:2,5 trifluoroacetic acid/triisopropylsilane/H_2O mixture (6,5 mL) and the resulting solution was stirred at room temperature for 24 h. The mixture was concentrated under reduced pressure, and the residue was taken up in H_2O (6 mL) and washed with Et_2O (4 × 6 mL). The aqueous layer was then concentrated under reduced pressure to afford the deprotected cyclic tetrapeptide as a trifluoroacetate salt. This crude was purified by semi-preparative HPLC (R_t = 10.33 min) and pure **34** (10.4 mg, 11%) was obtained as a white spongy solid by lyophilisation of the HPLC sample. The purity of the final compound was checked by analytical HPLC. $[\alpha]_D^{26}$ –36.0 (c 0.27, CH_3OH). ^1H NMR (500 MHz, D_2O/H_2O 1:9) (1.8 : 1 mixture of rotamers) δ (ppm): 8.61 (s, 1 H, NH Asp), 8.41-8.34 (m, 1 H, NH Arg), 8.13 (d, *J* = 6.0 Hz, 1 H, NH Phe), 8.02 – 7.97 (m, 1 H, NH Gly), 7.80 (d, *J* = 5.5 Hz, 1 H, NH 4-APA), 7.33 – 7.07 (m, 5 H, CH_{arom} Phe), 6.57 (br s, 5 H, NH_ε Arg), 4.85 (d, *J* = 13.9 Hz, 1 H, 2-H), 4.48 – 4.40 (m, 1 H, H_α Arg), 4.37 – 4.27 (m, 1 H, H_α Phe), 4.13 (dt, J = 9.0, 4.6 Hz, 1 H, H_α Asp), 4.07 – 3.91 (m, 2 H, H_α Gly + 6-H), 3.85 (br d, *J* = 17.4 Hz, 1 H, H_α Gly), 3.63 (s, 3 H, CO_2CH_3 major rotamer), 3.59 (s, 3 H, CO_2CH_3 minor rotamer), 3.28 – 3.19 (m, 1 H, 6-H), 3.17 – 3.03 (m, 4 H, 4-H +

2H$_\delta$ Arg + H$_\beta$ Phe), 2.93 (dd, *J* = 13.6, 9.9 Hz, 1 H, H$_\beta$ Phe), 2.53 (dd, *J* = 17.1, 9.1 Hz, 1 H, H$_\beta$ Asp), 2.45 (dd, *J* = 16.8, 4.8 Hz, 1 H, H$_\beta$ Asp), 2.25 – 2.11 (m, 2 H, H$_\gamma$ Arg), 2.10 – 2.00 (m, 1 H, 3-H), 1.88 – 1.77 (m, 2 H, 5-H + H$_\beta$ Arg), 1.74 (br d, *J* = 14.6 Hz, 1 H, H$_\beta$ Arg), 1.67 – 1.47 (m, 2 H, 3-H + 5-H). MS (ESI) m/z (%): 660 ([M + 1]$^+$, 100), 682 ([M + Na]$^+$, 18). HRMS (ESI/TOF) m/z: [M + H]$^+$ Calcd. for $C_{29}H_{41}N_9O_9$: 660.3100, Found: 660.3089.

(2*S*,4*R*)-4-(9-Fluorenylmethoxycarbonylamino)-1-(*tert*-butyloxycarbonyl)piperidine-2-Arg(Mtr)-Gly-OBn (35): DEPBT (225 mg, 0.75 mmol) and DIPEA (131 µL, 0.75 mmol) were added under a nitrogen atmosphere to a solution of **29** (174 mg, 0.37 mmol) in anhydrous THF (3.3 mL), cooled to 0 °C, and the resulting mixture was allowed to warm to room temperature. After 15 min, the reaction mixture was cooled again to 0 °C, and a solution of H-Arg(Mtr)-Gly-OBn (262 mg, 0.49 mmol) in anhydrous THF (3.3 mL) was added. The ice bath was removed and the mixture was stirred at 35 °C (external) for 3 days. The mixture was then diluted with EtOAc (135 mL), washed with satd NH_4Cl (2 × 35 mL), satd $NaHCO_3$ (2 × 35 mL), and finally with H_2O (2 × 35 mL). The organic extract was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude residue was chromatographed (MeOH/CH_2Cl_2 1:20, R_f 0.30) to give compound **35** (256 mg, 70%) as a white gummy solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.78 – 7.64 (m, 2 H), 7.64 – 7.43 (m, 2 H), 7.44 – 7.25 (m, 10 H), 6.51 (s, 1 H), 6.35 – 6.15 (m, 2 H), 5.15 – 5.00 (m, 2 H), 4.80 – 4.43 (m, 3 H), 4.35 – 4.20 (m, 1 H), 4.20 – 4.06 (m, 2 H), 4.01 – 3.84 (m, 2 H), 3.81 (s, 3 H), 3.47 – 3.03 (m, 3 H), 2.67 (s, 3 H), 2.60 (s, 3 H), 2.18 (d, *J* = 12.9 Hz, 1 H), 2.11 (s, 3 H), 2.02 – 1.83 (m, 2 H), 1.72 – 1.48 (m, 5 H), 1.51 – 1.29 (m, 9 H). MS/MS (ESI) [M + Na]$^+$ m/z (%): 1004 ([M + Na]$^+$, 6), 948 (18), 904 (100).

(–)-(2*S*,4*R*)-4-[Z-Asp(OtBu)]-1-(*tert*-butyloxycarbonyl)-4-APA-2-Arg(Mtr)-Gly-OBn (37): Compound **35** (256 mg, 0.26 mmol) was dissolved in a 1 : 1 CH_2Cl_2/diethylamine mixture (4.3 mL) under a nitrogen atmosphere. The resulting solution was stirred at room temperature for 4 h, meanwhile an additional 1 : 1 CH_2Cl_2/diethylamine mixture (2 mL) was added. The solution was concentrated under reduced pressure, the residue was taken up in CH_2Cl_2 (4 mL) and then concentrated again. The crude **36** was obtained as a pale yellow solid and used for the next step without further purifications. DEPBT (204 mg, 0.68 mmol) and DIPEA (119 µL, 0.68 mmol) were added under nitrogen atmosphere to a solution of Z-Asp(OtBu)-OH (116 mg, 0.34 mmol) in anhydrous THF (3 mL), cooled at 0 °C, and the resulting mixture was allowed to warm to room temperature. After 15 min, this solution was slowly added to a solution of compound **36** in anhydrous THF (1 mL), precooled to 0 °C. The ice bath was removed and the resulting mixture was stirred at 35 °C. After 24 h, a second portion of Z-Asp(OtBu)-OH (45 mg, 0.13 mmol), DEPBT (78 mg, 0.26 mmol) and DIPEA (46 µL, 0.26 mmol) was added and the mixture was stirred at 35 °C for an additional 3 days. The mixture was then diluted with EtOAc (40 mL) and washed with satd NH_4Cl (2 × 12 mL), satd $NaHCO_3$ (2 × 12 mL) and finally H_2O (2 × 12 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude residue was purified by flash chromatography (MeOH/CH_2Cl_2 1:20, R_f 0.12) and pure **37** (214 mg, 77%) was obtained as a white solid. m.p. = 56.4 – 63.0 °C. $[\alpha]_D^{20}$ –27.8 (c 0.97, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) (1 : 1 mixture of rotamers) δ (ppm): 7.45 – 7.25 (m, 10 H), 6.50 (s, 1 H), 6.42 – 6.16 (m, 2 H), 5.17 – 5.02 (m, 4 H), 4.81 – 4.61 (m, 1 H), 4.61 – 4.39 (m, 1 H), 4.28 – 4.21 (m, 1 H), 4.15 – 4.05 (m, 2 H), 3.98 – 3.89 (m, 1 H), 3.80 (s, 3 H), 3.37 – 3.10 (m, 2 H), 3.02 – 2.85 (m, 1 H), 2.80 – 2.65 (m, 1 H), 2.66 (s, 3 H), 2.59 (s, 3 H), 2.23 – 1.74 (m, 5 H), 2.11 (s, 3 H), 1.68 – 1.50 (m, 5 H), 1.43 (s, 9 H), 1.39 (s, 9 H, one rotamer), 1.37 (s, 9 H, one rotamer). ^{13}C NMR (100.4 MHz, $CDCl_3$) (mixture of rotamers) δ (ppm): 173.1, 172.1, 171.7, 170.9, 170.5, 169.7, 158.3, 156.5, 138.4, 136.4, 135.1, 133.3, 128.5, 128.4, 128.3, 128.2, 128.17, 128.16, 128.0, 127.7, 124.7, 124.6, 111.6, 81.8, 80.6, 66.9, 65.2, 63.8 and 63.7, 55.3, 52.5, 51.6, 50.8, 41.1, 40.4, 37.9, 37.2, 28.2, 27.9 and 27.8, 24.6, 24.1, 20.3, 18.3, 15.9, 11.9. MS (ESI) m/z (%): 1087 ([M + Na]$^+$, 100). HRMS (ESI/TOF) m/z: [M + Na]$^+$ Calcd. for $C_{52}H_{72}N_8O_{14}SNa$: 1087.4781. Found: 1087.4743.

(+)-Cyclo{Arg-Gly-Asp-4-APA}·TFA (39): To a solution of **37** (180 mg, 0.17 mmol) in ethanol (14.3 mL), 10% Pd/C (117 mg, 0.11 mmol) was added under a nitrogen atmosphere. The resulting suspension was first flushed with hydrogen under vigorous stirring and then maintained under a hydrogen atmosphere (balloon) at room temperature. After 21 h, the mixture was filtered over a Celite pad and the residual solution was evaporated under reduced pressure. Compound **38** (139 mg, 97%) was obtained as a white solid and used in the next step without further purifications. MS (ESI) m/z (%): 845 ([M - H_2O + Na]$^+$, 100). The crude **38** (139 mg, 0.17 mmol) was suspended in THF (50 mL) under a nitrogen atmosphere. The suspension was cooled to 0 °C, and DEPBT (153 mg, 0.51 mmol) and DIPEA (89 µL, 0.51 mmol) were added. The resulting mixture was stirred at 35 °C for 4 days and the diluted with EtOAc (25 mL) and washed with satd NH_4Cl (2 × 10 mL), satd $NaHCO_3$ (2 × 10 mL), H_2O (2 × 10 mL) and finally dried over Na_2SO_4. After filtration and evaporation of the solvent, the residue was purified by flash chromatography (MeOH/CH_2Cl_2 1:20, R_f 0.10). Cromatography column was then washed with MeOH. The MeOH solution was concentrated, taken up in $CHCl_3$ and washed with water (3 x 15 mL) and finally concentrated again. The cyclopeptide was obtained as a white solid (62 mg, 48%). MS (ESI) m/z (%): 845 ([M + Na]$^+$, 100). The crude cyclic tetrapeptide was dissolved in a 95:2,5:2,5 trifluoroacetic acid/triisopropylsilane/H_2O mixture (4,1 mL) and the resulting solution was stirred at room temperature for 23 h. The mixture was concentrated under reduced pressure, and the residue was taken up in H2O (5 mL) and washed with Et_2O (4 × 6 mL). The aqueous layer was then concentrated under reduced pressure to afford the deprotected cyclic tetrapeptide as a trifluoroacetate salt. This crude was purified by semi-preparative HPLC (R_f = 12.29 min) and pure **39** (10 mg, 12% over two steps) was obtained as a glassy solid by lyophilisation of the HPLC sample and the purity of the final compound was checked by analytical HPLC. $[\alpha]_D^{26}$ +20.3 (c 0.20, H_2O). ^{1}H NMR (500 MHz, D_2O/H_2O 1:9) δ (ppm): 9.02 (t, *J* = 6.5 Hz, 1 H, NH Gly), 8.97 (d, *J* = 5.0 Hz, 1 H, NH Arg), 8.19 (d, *J* = 9.5 Hz, 1 H, NH Asp), 7.26 (d, *J* = 7.5 Hz, 1 H, NH 4-APA), 7.11 (m, 1 H, 4-NH), 6.58 (m, 5 H, NH$_\varepsilon$ Arg), 4.66 (m, 2 H, H$_\alpha$ Asp + 2-H), 4.20 – 4.16 (m, 1 H, H$_\alpha$ Gly), 4.15 – 4.10 (m, 2 H, H$_\alpha$ Arg + 4-H), 3.83 (td, *J* = 13.5, 3.0 Hz, 1 H, 6 H), 3.48 – 3.42 (m, 1 H, H$_\alpha$ Gly), 3.23 – 3.17 (m, 1 H, 6-H'), 3.16 – 3.10 (m, 2 H, H$_\delta$ Arg), 2.85 – 2.73 (m, 2 H, H$_\beta$ Asp), 2.40 – 2.34 (m, 1 H, 3-H), 2.14 (d, *J* = 16.0 Hz, 1 H, 3-H'), 1.99 – 1.93 (m, 1 H, 5-H), 1.91 – 1.83 (m, 1 H, 5-H'), 1.74 – 1.67 (m, 2 H, H$_\beta$ Arg), 1.65 – 1.57 (m, 1 H, H$_\gamma$ Arg), 1.55 – 1.46 (m, 1 H, H$_\gamma$ Arg). ^{13}C NMR (100.4 MHz, $CDCl_3$) δ (ppm): 178.2, 177.6, 174.4, 174.3, 173.9, 159.6, 57.5, 52.3, 51.8, 51.7, 47.1, 43.3, 43.2, 39.6, 30.5, 29.0, 27.5, 27.0. MS ESI m/z (%): 455 ([M + 1]$^+$, 100). HRMS (ESI/TOF) m/z: [M + H]$^+$ Calcd. for $C_{18}H_{30}N_8O_6$: 455.2361. Found: 455.2363.

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Keywords: synthesis design • 4-aminopipecolic acids • peptidomimetics • integrins • replica exchange molecular dynamics

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[14] See Supporting Information for NMR spectra of lactone **8**

[15] In α-substituted N-acylpiperidines the carboxylic moiety at position 2 is axially oriented to remove the $A^{1,3}$-allylic strain with the N-protecting group. Thus the MsO group in **17** (Scheme 2) is equatorial and the azide group in **26** (Scheme 3) is axial. D. L. Comins, S. P. Joseph, in *Advances in Nitrogen Heterocycles*, Vol. 2 (Ed.: C. J. Moody), JAI Press, Greenwich (CT), **1996**, pp. 251–294.

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[21] With this cell line, the IC_{50} value obtained by us for cilengitide was much higher than that we have previously measured by using M21 melanoma cell line (3.8 ± 1.7 nM). See ref. [11]

[22] WM266.4 cells were analyzed for the expression of surface integrin receptor using cytofluorimetric assay. We found that human melanoma cells strongly express $\alpha_V\beta_3$ receptor while the expression of $\alpha_V\beta_5$ receptor was significantly lower (see Supporting Information)

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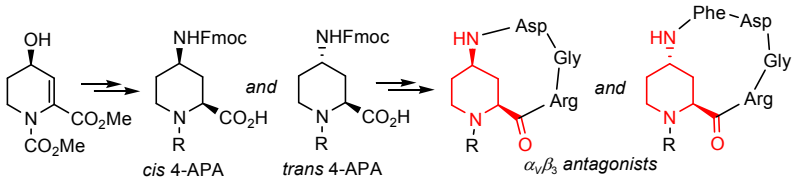

A stereodivergent preparation of *cis* and *trans* 4-aminopipecolic acids (4-APAs) was developed from a common precursor to obtain suitably protected, constrained γ-amino acids useful in peptidomimetic synthesis. Two antagonists of $\alpha_v\beta_3$ integrin were synthesized.