Arabinose-derived bicyclic amino acids: synthesis, conformational analysis and construction of an $\alpha_v\beta_3$-selective RGD peptide

Francesco Peri,*[a] Roberta Bassetti,[a] Enrico Caneva,[b] Luca de Gioia,[a] Barbara La Ferla,[a] Marco Presta,[c] Elena Tanghetti[c] and Francesco Nicotra*[a]

[a] *Department of Biotechnology and Biosciences, University of Milano-Bicocca, I-20126 Milano, Italy. E-mail: francesco.peri@unimib.it, fancesco.nicotra@unimib.it; Fax: +39.02.64483565*
[b] *Department of Organic and Industrial Chemistry, Via Venezian 21, I-20133, Milano, Italy*
[c] *Unit of General Pathology and Immunology, Department of Biomedical Sciences and Biotechnology, School of Medicine, University of Brescia, I-25123, Brescia, Italy*

Received (in Cambridge, UK) 16th November 2001, Accepted 18th January 2002
First published as an Advance Article on the web 7th February 2002

The synthesis, NMR structure determination, and molecular modelling of the conformationally restricted diastereomeric sugar azido acids **1** and **2** are presented. The bicyclic structures of these compounds are obtained through a iodocyclization reaction on the *C*-allyl glycoside of the D-arabinofuranose. Cyclic tetrapeptide **11** containing the amino acid derived from **1** linked to the RGD sequence has been synthesized; this compound was found to be a selective antagonist of $\alpha_v\beta_3$ integrins expressed on GM 7373 cells.

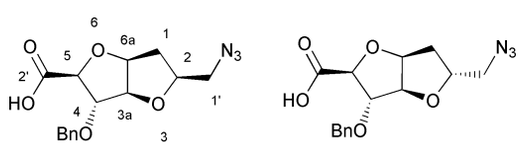
Introduction

An increasing interest has recently been devoted to sugar-derived amino acids (SAAs) that have been used as conformationally restricted non-peptide isosteres and have been introduced into the peptide backbone in order to achieve desirable secondary structures. In this context, the pyran ring of SAAs derived from α-D-glucopyranose was used as a rigid or semi-rigid template to induce linear, β-turn and γ-turn conformations in peptides.[1] The same sugar amino acids have been used to induce the bioactive conformation in small RGD (Alg-Gly-Aso) cyclic peptides, obtaining selective antagonists for the $\alpha_v\beta_3$ integrin.[2] In a slightly different conceptual approach, α-D-glucopyranose was employed as a peptidomimetic scaffold devoid of an amide backbone to accommodate the side-chain functionalities responsible for the biological activity of the peptide hormone somatostatin.[3] In this context, RGD mimics[4] and thrombin inhibitors[5] were prepared by assembling amino acid side chain functionalities on D-glucose. The same sugar was used to replace the ester-amide backbone of the cyclic depsipeptide hapalosin.[6] Recently, this approach has been extended to a model study in solution for the preparation of solid-phase libraries of compounds based on the α-D-glucose scaffold.[7] Finally, sugar-derived amino acid monomers were assembled to form oligomers capable of adopting defined secondary structures in solution. Oligomers of pyranose sugar amino acids[8] have been synthesized, and furanosidic sugar amino acids have been used as rigid platforms in the design and synthesis of carbopeptoids.[9] Homo-oligomers of tetrahydrofuran amino acids were shown to adopt a novel repeating β-turn-type secondary structure in tetramers[10] and a left-handed helical conformation in octamers[11] (the generic name of foldamers has been proposed for this type of structure).[12]

SAAs possess a cyclic pyranose or furanose core that reduces the overall conformational mobility of the molecule and allows it to maintain a defined mutual disposition of the amino and carboxylic groups. This is an important prerequisite to the induction of peptide sequences in which SAAs are inserted to fold into regular secondary structures. In this paper we present the design and the synthesis of SAAs with a bicyclic core: the conformational freedom of such molecules is further reduced by the presence of two fused five-membered rings. The use of bicyclic compounds derived from natural products as dipeptide analogues to stabilize the peptide chain in a reverse-turn conformation has been the object of extensive studies.[13] Among these, 5,5-[14] and 6,5-bicyclic[15] compounds have been reported as β-turn mimetics.

Results and discussion

We reported in a note[16] the synthesis and the structural characterization of the bicyclic sugar azido acids **1** and **2** (Fig. 1) and



1 **2**

Fig. 1 Structures of the azido acid **1** and **2** (epimers on C-2). Numbering shown is non-systematic.

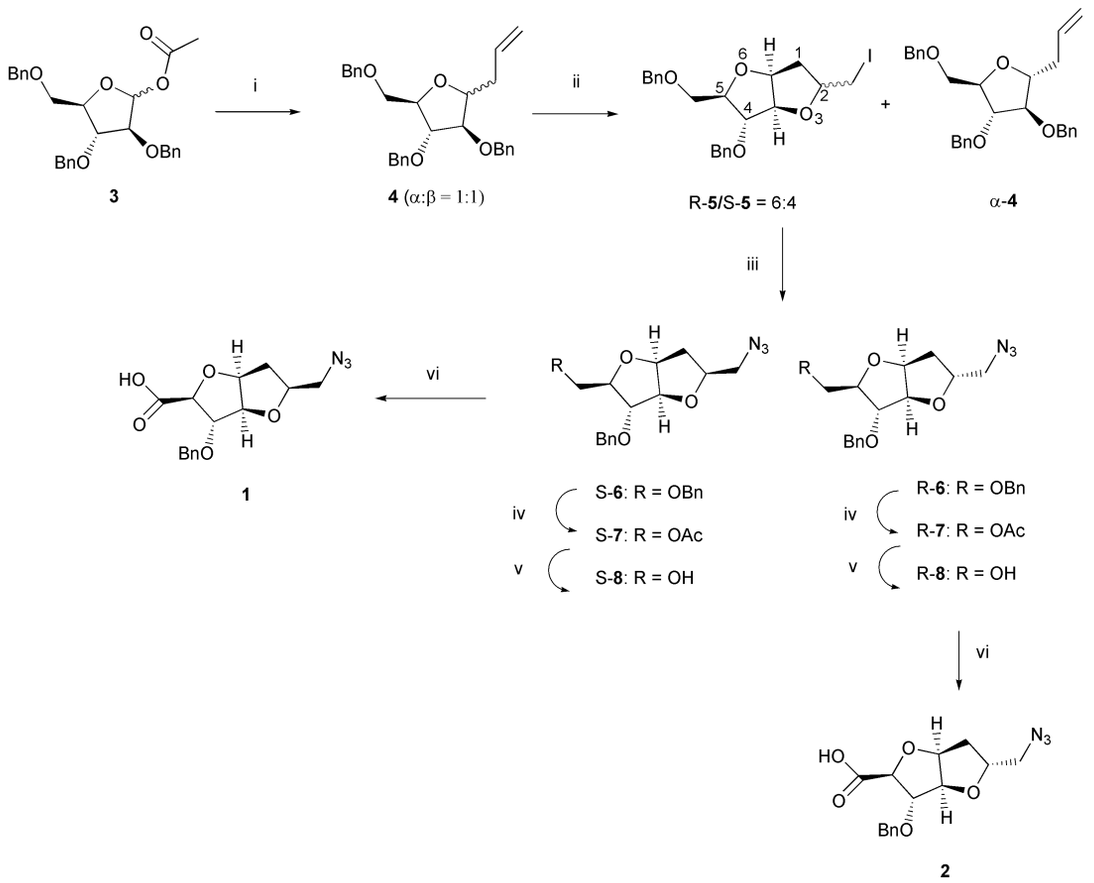
the incorporation of **1** into a cyclic RGD peptide. In this paper we report the synthetic procedure with full experimental details, the conformational analysis of azido acids **1** and **2**, and the biological activity of the peptide.

Compounds **1** and **2** can be directly condensed to *N*-protected amino acids in the solid phase by using a coupling method based on simultaneous *in situ* activation of the carboxylic group and reduction of the azide functionality.[17] The bicyclic structures of **1** and **2** were obtained according to the synthetic strategy shown in Scheme 1.

The commercially available 2,3,5-tri-*O*-benzyl-D-arabinofuranose was converted into the anomeric acetate **3** (mixture of anomers), which was allylated by treatment with allyltrimethylsilane (ATMS)† in the presence of the Lewis acid

† *Abbreviations*: ATMS, allyltrimethylsilane; DIC, *N,N′*-diisopropylcarbodiimide; DIPEA, diisopropylamine; DMAP, 4-(dimethylamino)-pyridine; FCS, fetal calf serum; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, *N*-hydroxybenzotriazole; NIS, *N*-iodosuccinimide; Pmc, 2,2,5,7,8-pentamethylchroman-6-ylsulfonyl; SASRIN, Super Acid Sensitive ResIN; TFA, trifluoroacetic acid; TIS, triisopropylsilane; TMS tetramethylsilane.

 DOI: 10.1039/b110453e



Scheme 1 *Reagents and conditions (and yields)*: i) ATMS, $BF_3{\cdot}Et_2O$, dry CH_3CN, rt, (75%); ii) NIS, dry THF, rt (80% on the β-anomer); iii) Bu_4NN_3, toluene, 60 °C (60%, separation of diastereomers by column chromatography); iv) Ac_2O–TFA, rt (95%); v) MeONa, MeOH, rt (75%); vi) CrO_3–H_2SO_4, acetone–water, rt (72%).

promoter $BF_3{\cdot}Et_2O$. The reaction, effected at rt in dry acetonitrile, afforded **4**[18] as a 1 : 1 mixture of α and β diastereomers (75% yield). Iodocyclization (Scheme 1) was then carried out with NIS in dry THF at rt affording bicyclic iodo ether **5** as a mixture of diastereomers (80% yield based on the β-anomer), the major isomer having the 2*R* configuration. This reaction is the crucial step for the synthesis of the bicycle and its mechanism is based on the opening of the intermediate iodonium ion by attack of the γ-benzyloxy groups in the 5-*exo*-mode with formation of a cyclic iodo ether with concomitant debenzylation.[19] Only the β-anomer of **4** reacted and this allowed its easy separation from the α-anomer. Pure α-anomer of **4** was then isolated and characterized. The reaction of the iodo derivative **5** with tetrabutylammonium azide in toluene gave azido derivative **6** (60 °C, 60% yield). Compounds (*R*)-**6** and (*S*)-**6** were separated by flash chromatography and their relative configurations at C-2 assigned by NOESY analysis: an intense correlation peak was observed between H-2 and H-3a protons for the diastereomer (*S*)-**6**, that was absent for (*R*)-**6**. The subsequent reactions were carried out separately on the two diastereomers with similar yields. Compound **6** was regioselectively debenzylated at the primary hydroxy group by acetolysis (Ac_2O, CF_3COOH, rt, 95%), followed by saponification of the acetate on C-2′ (75%) and Jones oxidation (72%). Anomers **1** and **2** (*S* or *R* configuration at C-2), were obtained with similar yields over the last three synthetic steps.

In a first qualitative investigation of the conformation of **1** by ^{1}H-NMR spectroscopy in $CDCl_3$, intense NOEs were observed for H-6a/H-1_b, H-2/H-1_b, H-2/H-3a and H-5/H-6a couples (Fig. 2). These NOEs indicate that the hydrogen couples H-2/H-3a and H-5/H-6a are in close spatial proximity; this situation is compatible with a conformation of the bicycle in which the two tetrahydrofuran rings are disposed with both the oxygen atoms pointing upward. A quantitative conformational analysis of

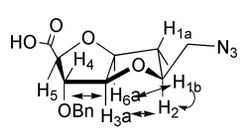


Fig. 2 Observed NOEs in **1**.

1 was then performed by executing five 2D NOESY experiments with different mixing-time-values,[20] between 0.6 and 1.4 seconds, to cover the full range of molecular relaxation times in the two-spin approximation. Since the presence of conformational equilibria may introduce errors in quantitative NMR-derived data, molecular dynamics simulations were performed for compound **1**. The structures obtained after the simulated annealing MD procedure were finally optimized by MM.[21] Final structures were clustered to obtain distinct conformer families and ranked by potential-energy values. Crucial structural properties of the low-energy conformers were compared with the analogous data inferred from NOE experiments. Some interproton distances relevant for the definition of the conformation of **1** derived from NOE and from MD-MM are compared in Table 1.

As exemplified from the obtained data, in compound **1** the distances calculated by MM/MD are in excellent agreement with those derived from NOE experiments. The only discrepancy between the two sets of measures are H-3a/H-4 and H-3a/H-2 distances that are underestimated by NMR calculations. This is due to the total superimposition between the H-3a and the benzyl methylene signals in the ^{1}H-NMR spectrum, leading to an increment of NOEs volume and a consequent interproton distance reduction. It is, however, noteworthy that all the high-energy conformers of **1** obtained by MM/MD are characterized by molecular geometries presenting interproton distances that differ consistently from those derived from NOE experiments.

Table 1 Reference geminal interproton distances in Å

Protons	MM/MD (**1**)	MM/MD (**2**)	NOE (**1**)
$1'_a$–$1'_b$*	1.76	1.76	1.80
5–6a	2.71	2.84	2.61
3a–5	2.86	3.12	2.70
3a–6a	2.46	2.46	2.21
2–3a	2.82	3.80	2.49
3a–4	2.93	2.98	2.47
6a–1_b	2.39	2.37	2.28
6a–1_a	2.77	2.77	2.68
2–1_b	2.33	3.05	2.30
$1'_a$–1_a			2.61
$1'_b$–1_b			2.65

The coherence between the NMR and MD/MM data in compound **1** indicates that the MM^+ forcefield is suitable to carry out a reliable conformational analysis for these structures: starting from this assumption the conformers corresponding to the energy minima for the azido acid **2** were obtained by MD/MM (Table 1). A conformer of **2** corresponding to an absolute energy minimum was unambiguously found, presenting a spatial disposition of the atoms constituting the bicycle different to that of **1**. In Fig. 3 the minimum-energy conformers found for compounds **1** and **2** are shown.

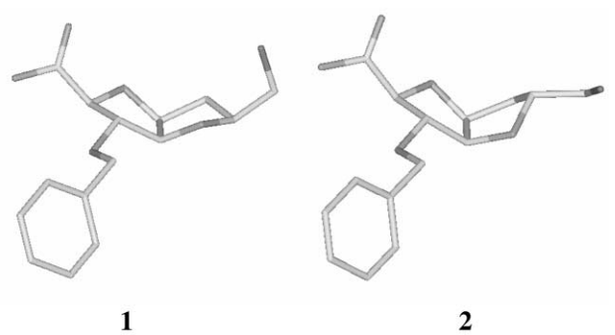


Fig. 3 Minimum-energy conformers of **1** and **2**.

We envisaged the use of conformationally constrained SAA **1** in the construction of a cyclic peptide containing the Arg-Gly-Asp sequence, with the aim of obtaining new inhibitors of the adhesive interaction between the integrins and their receptor.[22] Cyclic peptide **11** was synthesized on a solid phase (Scheme 2) using the Fmoc–tBu strategy.

The dipeptide Arg(Pmc)-Gly was assembled on SASRIN® resin with an average loading of about 0.5 mmol g^{-1}, then compound **1** was coupled in the presence of HBTU, HOBt and DIPEA. The solid-phase reduction of the azide proved to be problematic. Using PPh_3 in THF–water mixture,[23] the phosphazene intermediate obtained was difficult to transform quantitatively into the azide by treatment with acids or bases; $SnCl_2$ and PhSH in presence of Et_3N [24] led to cleavage of the peptide from resin; dithiothreitol (DTT) in presence of diazabicycloundecene (DBU) gave an extremely low reduction yield even after several reiterations of the reaction; finally, trimethylsilyl chloride in dry CH_2Cl_2 cleaved the peptide from the resin. We then explored the possibility of reducing the *N*-terminal azide and simultaneously couple the Fmoc-Asp(tBu), in a one-pot reaction, by using DIC, Bu_3P, and HOBt in dry DMF–toluene (2 : 1) at rt for 24 h.[25] This reaction was found to be very efficient and high yielding (about 95%, as assessed from the loading value calculated after Fmoc removal from the aspartic acid residue), in sharp contrast with the inefficiency of other well-established methods we employed to reduce solid-phase-bound azide. The reduction could be monitored conveniently by the disappearance of the N_3 stretch (2100 nm) in the IR spectrum obtained from a few resin beads. After *N*-terminal Fmoc deprotection, the tetrapeptide **9** was cleaved from the resin (1% TFA in CH_2Cl_2 with immediate neutralization of the effluents with pyridine) maintaining all protecting groups, purified by flash chromatography on silica gel, cyclized in a dilute solution of DMF (peptide concn. 0.5 mM) in the presence of HBTU, HOBt and DIPEA and finally totally deprotected with TFA–TIS–water (95 : 2.5 : 2.5), affording **11** in 35% yield over the last two steps. This compound was finally tested for its capacity to inhibit selectively the $\alpha_v\beta_3$-mediated endothelial cell adhesion. Fetal bovine aortic endothelial GM 7373 cells,[26] that express $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins,[27] were used for the inhibition tests. Experiments performed with specific, neutralizing anti-integrin antibodies had demonstrated that $\alpha_v\beta_3$ integrin mediates adhesion and spreading of GM 7373 cells on immobilized vitronectin (VN) whereas $\alpha_5\beta_1$ integrin is involved in the interaction of these cells with immobilized fibronectin (FN).[27] Control cRADfV peptide is instead ineffective. On this basis, we evaluated the capacity of **11** to affect the adhesion of GM 7373 cells to immobilized VN and FN. Non-tissue culture plastic 96-well plates were incubated with carbonate buffer containing 20 μg ml^{-1} VN (Fig. 4, open

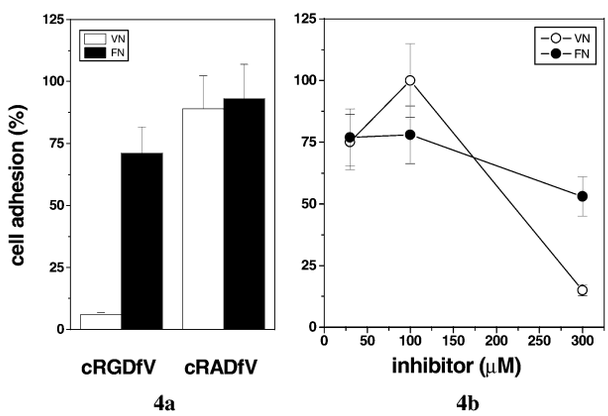
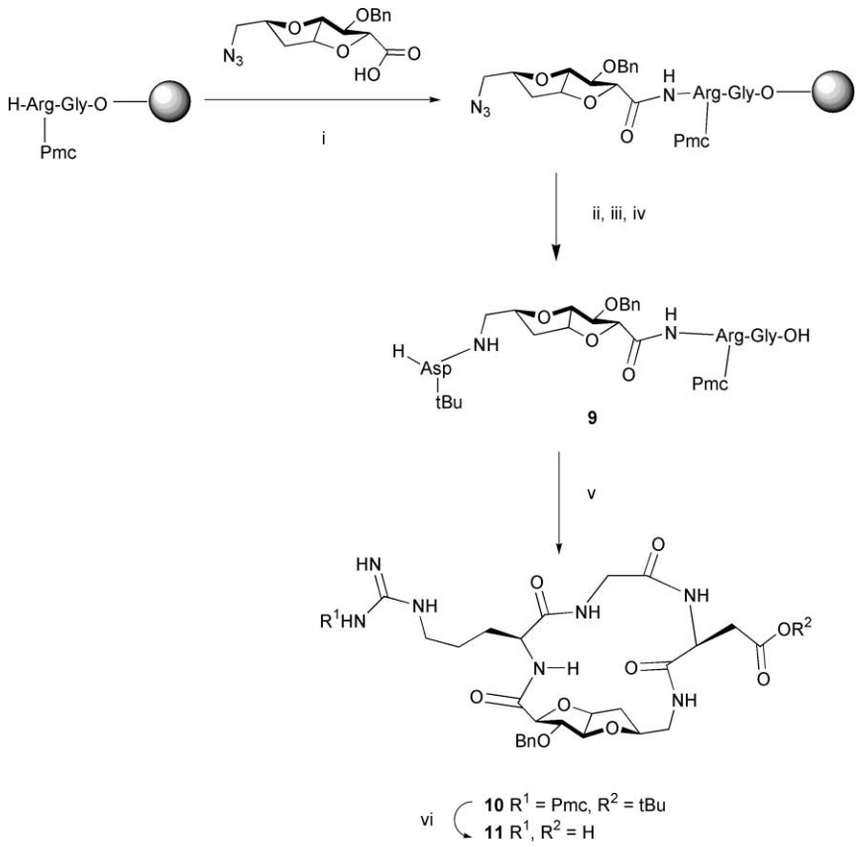


Fig. 4 Inhibition of integrin-dependent endothelial cell adhesion.

symbols) or FN (closed symbols). Then, GM 7373 cells were seeded onto coated plates in the absence or in the presence of 30 μM cRGDfV or cRADfV (Fig. 4a) or of increasing concentrations of **11** (Fig. 4b). Cells were allowed to adhere for 2 h at 37 °C. Then, adherent cells were fixed, stained, and plates were read with a microplate reader at 595 nm. Data are the mean ± S.D. of three determinations and are expressed as % of cell adhesion observed in the absence of antagonist. In agreement with its higher affinity for $\alpha_v\beta_3$ integrin, the cyclic cRGDfV peptide inhibits more efficiently GM 7373 cell adhesion to VN-coated plastic in respect to FN-coated substratum (Fig. 4a). Control cRADfV peptide is instead ineffective. As shown in Fig. 4b, **11** inhibits endothelial cell adhesion to VN, exerting only a limited effect on FN-mediated cell adhesion.

Conclusions

Sugar azido acids **1** and **2** were synthetically derived from the common precursor D-arabinofuranose. Through molecular dynamics and molecular modelling calculations a minimum-energy conformation was unambiguously found for **1**, that showed interproton distances very similar to those derived from NOE experiments in solution. The minimum-energy conformation of **2** was also calculated; both **1** and **2** present a very limited conformational mobility due to the bicyclic structure that makes the corresponding amino acids interesting building blocks for the construction of conformationally restricted peptides. The amino acid derived from **1** was inserted in the cyclic RGD peptide **11**. We demonstrated *in vitro* that, like cyclic cRGDfV peptide, **11** acts as selective antagonist of $\alpha_v\beta_3$ integrins expressed on GM 7373 cells.



Scheme 2 *Reagents and conditions (and yields)*: i) HBTU, HOBt, DIPEA, DMF, rt, 4 h; ii) Fmoc-Asp(tBu), DIC, Bu_3P, HOBt in dry DMF–toluene (2 : 1), rt (95%); iii) 20% piperidine in DMF; iv) 1% TFA in CH_2Cl_2, neutralization of the effluents with pyridine; v) cyclization: 0.5 mM peptide in DMF, HBTU, HOBt, DIPEA, 72 h, rt (47%); vi) 95% TFA, 2.5% TIS, 2.5% water, 10 h, rt (75%).

Experimental

General procedures

All solvents were dried over molecular sieves (Fluka), for at least 24 h prior to use. Thin-layer chromatography (TLC) was performed on Silica Gel 60 F_{254} plates (Merck) with detection with UV light when possible, or charring with a solution containing conc. H_2SO_4–MeOH–H_2O in the proportions 5 : 45 : 45. Flash column chromatography was performed on silica gel 230–400 mesh (Merck). N^{α}-Fmoc-protected amino acids were purchased from Calbiochem-Novabiochem AG (Laufelfingen, CH) with the following protecting groups for their side chains: pentamethylchroman-6-ylsulfonyl (Pmc) for arginine; *tert*-butyl (tBu) for aspartic acid. SASRIN® resin was purchased from Bachem (CH). Solid-phase peptide synthesis was carried out manually in plastic syringe with septum and Teflon stopcock on the bottom. Usual work-up refers to dilution with an organic solvent, washing with water to neutrality (pH test paper), drying with Na_2SO_4, filtration, and evaporation under reduced pressure. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer, using TMS as internal standard. All chemical shifts (δ) are quoted in ppm and coupling constants (J) in Hz. The numbering used for the difuran NMR assignments is as shown in structure **5** Scheme 1.

IR spectra were recorded on a Biorad FT-IR spectrometer FTS-40A coupled to an IR microscope Biorad UMA-40A. $[\alpha]_D$-Values were measured at 20 °C on a Perkin-Elmer 241 digital polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. Mass spectra were recorded on a MALDI 2 Kompakt Kratos instrument, using gentisic acid (2,5-dihydroxybenzoic acid, DHB) or α-cyano-4-hydroxycinnamic acid (CHCA) as matrices. Elemental analyses were performed with a Perkin-Elmer Series II Analyzer 2400.

Colorimetric assays for solid-phase peptide synthesis. TNBS (2,4,6-trinitrobenzenesulfonic acid) test:[28] two standard solutions are prepared as follows. Solution A: a 10% solution of diisopropylethylamine (DIPEA) in dry DMF; solution B: a 1% solution of TNBS in dry DMF. A small portion of the resin, previously washed with DMF, is suspended in a mixture of equal amounts of the two solutions. After a few minutes the presence of solid-phase-bound amino groups is detected by the appearance of an intense red color on the beads.

Purification of peptides was performed by semi-preparative reversed-phase HPLC on a Waters HPLC system (515 pumps) equipped with a Merck LiChrosper 100 RP_{18} column (250 × 10 mm; 10 μm), and elution with a flow rate of 7 ml min with linear gradients of eluent A (0.1% TFA in water) and eluent B (0.1% TFA in acetonitrile). Analytical HPLC purity checks were performed using a Merck LiChrosper 100 RP_{18} column (250 × 4 mm; 5 μm), elution at a flow rate of 1 ml min^{-1}, using gradients of A and B eluents. Detection at 214 and 280 nm with an dual-wavelength absorbance detector Waters 2487. Petroleum ether refers to the fraction with distillation range 40–60 °C.

1-(2′,3′,5′-Tri-*O*-benzyl-D-arabinofuranosyl)prop-2-ene (4)

To a stirred solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzyl-D-arabinofuranose (10.0 g, 21.6 mmol) in dry CH_3CN (75 ml) were added allyltrimethylsilane (6.9 ml, 43.2 mmol) and $BF_3{\cdot}Et_2O$ (2.7 ml, 21.6 mmol) at rt under argon atmosphere. After 1 h 30 min, solid Na_2CO_3 (5 g) was added to the solution to neutralize the acid, the solvent was partly evaporated, and the residue was diluted with CH_2Cl_2 (70 ml). The organic layer was submitted to usual work-up and purified by column flash-chromatography (9.5 : 0.5, petroleum ether–EtOAc) to afford **4** (7.2 g, 75%) as a colorless oil (mixture of α and β anomers; α : β = 1 : 1, as determined from the integration ratio of the ^{1}H-NMR signals). Anomers were not separable by chromatography. MS(MALDI-TOF): *m/z* 468.7 [M + H + Na]$^+$, 484.5

$[M + H + K]^+$; elemental analysis Calc. (%) for $C_{29}H_{32}O_4$: C, 78.35; H, 7.26. Found: C, 77.95; H, 6.48.

(2*R*, 4a*S*, 5*R*, 6*R*, 6a*R*) and (2*S*, 4a*S*, 5*R*, 6*R*, 6a*R*)-2-Iodomethyl-6-benzyloxy-5-(benzyloxymethyl)-hexahydrofuro[3,2-*b*]-furan (5)

To a solution of **4** (mixture of anomers, 0.436 g, 0.98 mmol) in dry THF (25 ml) was added NIS (0.663 g, 2.95 mmol) at rt under argon atmosphere. The reaction mixture was stirred at this temperature for 6 days. After this time, TLC analysis of the crude product (eluent 9.5 : 0.5, petroleum ether–AcOEt) showed that a part of **4** did not react (α-anomer) and that two distereomeric iodo derivatives were formed (two very close TLC spots with $R_f = 0.3$). The crude reaction product was then diluted with CH_2Cl_2 (25 ml), and the organic layer was subjected to usual work-up. The oily residue was purified by column chromatography (9.5 : 0.5, petroleum ether–AcOEt) to give **5** (mixture of diastereomers) as a pale yellow oil (0.185 g, 80% calculated on the β-anomer of **4**). The α-anomer of **4** was recovered unchanged.

(2*R*,4a*S*,5*R*,6*R*,6a*R*)- and (2*S*,4a*S*,5*R*,6*R*,6a*R*)-2-Azidomethyl-6-benzyloxy-5-(benzyloxymethyl)hexahydrofuro[3,2-*b*]furan [(*R*)-6)] and [(*S*)-6]

To a solution of **5** (mixture of diastereomers, 1.5 g, 3.15 mmol) was added tetrabutylammonium azide‡ (1.8 g, 6.3 mmol) under argon atmosphere and the mixture was stirred at 70 °C for 24 h. The solvent was then evaporated *in vacuo*, the residue was diluted with AcOEt, and after usual work-up and column chromatography (8 : 2, petroleum ether–AcOEt) the two diastereomers (*R*)-**6** and (*S*)-**6** were separated and isolated as colorless oils [500 mg of (*R*)-**6**, 40%; 250 mg of (*S*)-**6**, 20%].

(*R*)-6. δ_H (400 MHz; $CDCl_3$; 35 °C) 7.4–7.2 (10 H, m, H_{arom}), 4.79 (1 H, br t, $J_{6A,1}$ 5.2, H-6a), 4.71 (1 H, m, H-3a), 4.7–4.5 (4 H, $2AB_q$, CH_2Ph), 4.29 (1 H, m, H-2), 4.03 (1 H, ddd, *J* 6.2, *J* 6.2, *J* 3.8, H-5), 3.86 (1 H, dd, *J* 6.2, *J* 1.2, H-4), 3.61 (1 H, dd, *J* 10.5, *J* 3.8, H-2′a), 3.59 (1 H, dd, $J_{2'B,2'A}$ 10.5, $J_{2'B,5}$ 6.2, H-2′b), 3.52 (1 H, dd, $J_{1'A,1'B}$ 13.0, $J_{1'A,2}$ 3.5, H-1′a), 3.24 (1 H, dd, $J_{1'B,1'A}$ 13.0, $J_{1'B,2}$ 3.5, H-1′b), 2.19 (1 H, dd, $J_{1A,1B}$ 13.4, $J_{1A,6A}$ 5.2, H-1a), 1.78 (1 H, ddd, $J_{1B,1A}$ 13.4, $J_{1B,2}$ 10.2, $J_{1B,6A}$ 6.3, H-1b); δ_C (300 MHz, $CDCl_3$) 88.72, 85.60, 83.53, 83.35, 77.70, 73.41, 72.10, 70.18, 53.60, 35.82; $[\alpha]_D^{20}$ +21.4 (*c* 1, $CHCl_3$); MS (MALDI-TOF): *m*/*z* 419.2 $[M + H + Na]^+$, 435.0 $[M + H + K]^+$; $C_{22}H_{25}N_3O_4$ requires C, 66.82; H, 6.37; N, 10.63. Found: C, 67.53; H, 7.01; N, 11.21%.

(*S*)-6. δ_H (400 MHz; $CDCl_3$; 35 °C) 7.4–7.2 (10 H, m, Ar*H*), 4.72 (1 H, br t, $J_{6A,1}$ 4.4, H-6a), 4.65–4.45 (5 H, m, 2 × CH_2Ph and H-3a), 4.24 (1 H, m, H-2), 4.03 (1 H, ddd, $J_{5,4}$ 6.0, $J_{5,6}$ 3.7, H-5), 3.94 (1 H, br d, $J_{4,5}$ 6.0, H-4), 3.65 (1 H, dd, $J_{2'A,2'B}$ 10.3, $J_{2'A,5}$ 3.0, H-2′a), 3.58 (1 H, dd, $J_{2'B,2'A}$ 10.3, $J_{2'B,5}$ 5.9, H-2′b), 3.48 (1 H, dd, $J_{1'A,1'B}$ 12.5, $J_{1'A,2}$ 8.1, H-1′a), 3.16 (1 H, dd, $J_{1'B,1'A}$ 12.5, $J_{1'B,2}$ 4.0, H-1′b), 2.20 (1 H, ddd, $J_{1A,1B}$ 14.1, $J_{1A,2}$ 8.4, $J_{1A,6A}$ 5.6, H-1a), 1.91 (1 H, m, H-1b); δ_C (300 MHz, $CDCl_3$) 90.00, 85.40, 85.03, 83.63, 80.23, 73.64, 72.57, 70.00, 55.28, 35.80; $[\alpha]_D^{20}$ +29.3 (*c* 1, $CHCl_3$); MS (MALDI-TOF): *m*/*z* 396.4 $[M + H]^+$, 419.6 $[M + H + Na]^+$, 435.3 $[M + H + K]^+$; $C_{22}H_{25}N_3O_4$ requires C, 66.82; H, 6.37; N, 10.63. Found: C, 66.51; H, 6.81; N, 11.01%.

‡ Tetrabutylammonium azide was prepared from sodium azide according to the following procedure: to a stirred solution azide (228 mg, 3.5 mmol) in water (2 ml) was added Bu_4NOH (9.2 ml of a 20% aqueous solution) and the solution was stirred at rt for 30 min. After this time, the aqueous solution was extracted with CH_2Cl_2 (10 ml × 3), the organic layer was dried (Na_2SO_4), and the solvent removed *in vacuo* by adding toluene, until a solid powder is obtained. **CAUTION:** Bu_4NN_3 became explosive when solution was evaporated to dryness and the solid residue was heated.

(2*R*,4a*S*,5*R*,6*R*,6a*S*)- and (2*S*,4a*S*,5*R*,6*R*,6a*R*)-2-Azidomethyl-6-benzyloxy-5-(acetoxymethyl)hexahydrofuro[3,2-*b*]furan [(*R*)-7] and [(*S*)-7]

A solution of (*R*)-**6** or (*S*)-**6** (480 mg, 1.2 mmol) in 4 : 1 Ac_2O–TFA (10 ml) was stirred at room temperature for 45 min. After this time, the reaction was quenched by addition of a mixture of ice and water and the product was extracted with AcOEt. After usual work-up and column chromatography (8 : 2, petroleum ether–AcOEt) pure compound (*R*)-**7** or (*S*)-**7** was obtained as colorless oil (395 mg, 95% for both compounds).

(*R*)-7. δ_H (400 MHz; $CDCl_3$; 35 °C) 7.4–7.2 (5 H, m, H_{arom}), 4.79 (1 H, br t, $J_{6A,1}$ 4.3, H-6a), 4.65 (1 H, m, H-3a), 4.60 (2 H, AB_q, CH_2Ph), 4.29 (1 H, m, H-2), 4.25 (1 H, dd, $J_{2'A,2'B}$ 11.9, $J_{2'A,5}$ 3.8, H-2′a), 4.12 (1 H, dd, $J_{2'B,2'A}$ 11.9, $J_{2'B,5}$ 6.4, H-2′b), 4.02 (1 H, m, H-5), 3.80 (1 H, br d, $J_{4,3A}$ 5.7, H-4), 3.52 (1 H, dd, $J_{1'A,1'B}$ 13.1, $J_{1'A,2}$ 3.4, H-1′a), 3.22 (1 H, dd, $J_{1'B,1'A}$ 13.1, $J_{1'B,2}$ 5.1, H-1′b), 2.17 (1 H, m, H-1a), 2.11 (1 H, s, CH_3), 1.78 (1 H, m, H-1b); δ_C (300 MHz, $CDCl_3$) 170.8, 88.38, 85.26, 83.64, 82.08, 77.81, 72.22, 64.18, 53.55, 35.68, 20.78; $[\alpha]_D^{20}$ +28.0 (*c* 1, $CHCl_3$); MS (MALDI-TOF): *m*/*z* 371.3 $[M + H + Na]^+$, 387.6 $[M + H + K^+]^+$; $C_{17}H_{21}N_3O_5$ requires C, 58.78; H, 6.09; N, 12.10. Found: C, 59.11; H, 5.91; N, 11.87%.

(*S*)-7. δ_H (400 MHz; $CDCl_3$; 35 °C) 7.4–7.2 (5 H, m, H_{arom}), 4.73 (1 H, br t, $J_{6A,1}$ 4.9, H-6a), 4.60–4.50 (3 H, m, H-3a, CH_2Ph), 4.25 (3 H, m, H-2, H-2′a, H-2′b), 4.04 (1 H, m, H-5), 3.91 (1 H, br d, *J* 5.6, H-4), 3.48 (1 H, dd, $J_{1'A,1'B}$ 12.8, $J_{1'A,2}$ 8.0, H-1′a), 3.23 (1 H, dd, $J_{1'B,1'A}$ 12.8, $J_{1'B,2}$ 4.0, H-1′b), 2.22 (1 H m, H-1a), 2.09 (1 H, s, CH_3), 1.91 (1 H, m, H-1b); δ_C (300 MHz, $CDCl_3$) 185.5, 89.67, 85.00, 83.90, 83.57, 80.72, 72.69, 64.15, 55.13, 35.73, 20.84; $[\alpha]_D^{20}$ +48.9 (*c* 1, $CHCl_3$); MS (MALDI-TOF): *m*/*z* 371.6 $[M + H + Na]^+$, 387.8 $[M + H + K]^+$; $C_{17}H_{21}N_3O_5$ requires C, 58.78; H, 6.09; N, 12.10. Found: C, 58.51; H, 6.31; N, 11.77%.

(2*R*,4a*S*,5*R*,6*R*,6a*S*)- and (2*S*,4a*S*,5*R*,6*R*,6a*R*)-2-Azidomethyl-6-benzyloxy-5-(hydroxymethyl)hexahydrofuro[3,2-*b*]furan [(*R*)-8] and [(*S*)-8]

To a solution of (*R*)-**7** or (*S*)-**7** (1 g, 2.88 mmol) in dry MeOH (50 ml) was added sodium metal in catalytic amount under argon atmosphere. The solution was stirred at room temperature for 30 min. After this time Amberlite IRA-120 (H^+) resin was added and the mixture was vigorously stirred for 10 min. Then resin was removed by filtration and the solvent was evaporated. After usual work-up and chromatography (1 : 1, petroleum ether–AcOEt), pure compound (*R*)-**8** or (*S*)-**8** was recovered as colorless oil (660 mg, 75% for both compounds).

(*R*)-8. δ_H (400 MHz; $CDCl_3$; 35 °C) 7.4–7.2 (5H, m, H_{arom}), 4.78 (1 H, br t, $J_{6A,1}$ 4.3, H-6a), 4.65 (1 H, dd, $J_{3A,4}$ 4.3, H-3a), 4.62 (2 H, AB_q, CH_2Ph), 4.29 (1 H, m, H-2), 3.90 (2 H, m, H-4, H-5), 3.81 (1 H, dd, $J_{2'A,2'B}$ 11.4, $J_{2'A,5}$ 2.3, H-2′a), 3.63 (1 H, dd, $J_{2'B,2'A}$ 11.4, $J_{2'B,5}$ 3.4, H-2′b), 3.51 (1 H, dd, $J_{1'A,1'B}$ 13.2, $J_{1'A,2}$ 3.7, H-1′a), 3.22 (1 H, dd, $J_{1'B,1'A}$ 13.2, $J_{1'B,2}$ 5.0, H-1′b), 2.14 (1 H, dd, $J_{1A,1B}$ 13.6, $J_{1A,2}$ 5.5, H-1a), 1.94 (1 H, m, H-1b); δ_C (300 MHz, $CDCl_3$) 88.84, 84.62, 84.58, 83.35, 77.80, 72.27, 62.67, 53.61, 35.76; $[\alpha]_D^{20}$ +21.9 (*c* 1, $CHCl_3$); MS (MALDI-TOF): *m*/*z* 328.2 $[M + Na]^+$, 344.1 $[M + K]^+$; $C_{15}H_{19}N_3O_4$ requires C, 59.01; H, 6.27; N, 13.76. Found: C, 57.11; H, 6.79; N, 13.23%.

(*S*)-8. δ_H (400 MHz; $CDCl_3$; 35 °C) 7.4–7.2 (5 H, m, H_{arom}), 4.72 (1 H, bt, $J_{6A,1}$ 4.2, H-6a), 4.60 (2 H, AB_q, CH_2Bn), 4.48 (1 H, d, $J_{3A,4}$ 4.2, H-3a), 4.22 (1 H, m, H-2), 3.98 (2 H, m, H-4, H-5), 3.82 (1 H, dd, $J_{2'A,2'B}$ 11.8, $J_{2'A,5}$ 2.7, H-2′a), 3.68 (1 H, dd,

$J_{2'B,2'A}$ 11.8, $J_{2'B,5}$ 4.4, H-2′b), 3.46 (1 H, dd, $J_{1'A,1'B}$ 12.9, $J_{1'A,2}$ 6.7, H-1′a), 3.33 (1 H, dd, $J_{1'B,1'A}$ 12.9, $J_{1'B,2}$ 4.1, H-1′b), 2.25 (1 H, ddd, $J_{1A,1B}$ 14.2, *J* 8.2, *J* 6.0, H-1a), 1.94 (1 H, m, H-1b); δ_C (300 MHz, $CDCl_3$) 89.83, 86.67, 84.40, 83.73, 79.91, 72.63, 62.95, 54.97, 35.61; $[\alpha]_D^{20}$ +47.03 (c 1, $CHCl_3$); MS (MALDI-TOF): *m*/*z* 328.6 $[M + Na]^+$, 344.4 $[M + K]^+$. $C_{15}H_{19}N_3O_4$ requires: C, 59.01%; H, 6.27%; N, 13.76%. Found: C, 58.45%; H, 5.99%; N, 14.01%.

(2*R*,4a*S*,5*S*,6*S*,6a*R*)- and (2*S*,4a*S*,5*S*,6*S*,6a*R*)-2-Azidomethyl-6-benzyloxy-5-carboxyhexahydrofuro[3,2-*b*]furan (2) and (1)

To a solution of (*R*)-**8** or (*S*)-**8** (320 mg, 1.1 mmol) in acetone (20 ml) was added dropwise a solution of CrO_3 (550 mg, 5.5 mmol) in 3 M H_2SO_4 (13 ml) over a period of 10 min at 0 °C, the reaction mixture was then allowed to warm at room temperature and stirred for 3 h. The acetone was then evaporated *in vacuo* from the mixture and the product was extracted with AcOEt. The organic layer was washed twice with 0.1 M HCl, and after usual work-up and chromatography (9.5 : 0.45 : 0.05, $CHCl_3$–EtOH–AcOH), pure compound **1** or **2** was obtained as a colorless oil.

2. δ_H (400 MHz; $CDCl_3$; 35 °C) 7.4–7.2 (5 H, m, H_{arom}), 4.93 (1 H, m, H-6a), 4.69 (2 H, AB_q, C*H*$_2$Ph), 4.66 (1 H, m, H-5), 4.47 (1 H, br d, *J* 2.0, H-4), 4.39 (1 H, d, $J_{3A,4}$ 3.3, H-3a), 4.18 (2 H, m, H-2), 3.58 (1 H, dd, $J_{1'A,1'B}$ 12.8, $J_{1'A,2}$ 7.4, H-1′a), 3.33 (1 H, dd, $J_{1'B,1'A}$ 12.8, $J_{1'B,2}$ 3.7, H-1′b), 2.35 (1 H, m, H-1a), 2.12 (1 H, m, H-1b); δ_C (300 MHz, $CDCl_3$) 174.34, 87.39, 85.88, 85.14, 83.24, 79.42, 72.20, 54.41, 36.04; $[\alpha]_D^{20}$ −8.90 (*c* 1, $CHCl_3$); MS (MALDI-TOF): *m*/*z* 342.4 $[M + Na]^+$, 358.4 $[M + K]^+$; $C_{15}H_{17}N_3O_5$ requires C, 56.42; H, 5.37; N, 13.16. Found: C, 57.02; H, 5.67; N, 13.70%.

1. δ_H (400 MHz; $CDCl_3$; 35 °C) 7.4–7.2 (5 H, m, H_{arom}), 4.97 (1 H, m, H-6a), 4.67 (2 H, AB_q, C*H*$_2$Ph), 4.61 (1 H, m, H-5), 4.47 (1 H, br d, *J* 2.9, H-4), 4.42 (1 H, m, H-2), 4.39 (1 H, m, H-3a), 3.51 (1 H, dd, $J_{1'A,1'B}$ 13.2, $J_{1'A,2}$ 2.4, H-1′a), 3.33 (1 H, dd, $J_{1'B,1'A}$ 13.2, $J_{1'B,2}$ 3.9, H-1′b), 2.38 (1 H, m, H-1a), 1.96 (1 H, m, H-1b); δ_C (300 MHz, $CDCl_3$) 175.21, 87.95, 85.43, 84.78, 83.32, 79.91, 71.65, 53.81, 34.64; $[\alpha]_D^{20}$ −1.76 (*c* 1, $CHCl_3$); MS (MALDI-TOF): *m*/*z* 342.4 $[M + Na]^+$, 358.4 $[M + K]^+$; $C_{15}H_{17}N_3O_5$ requires C, 56.42; H, 5.37; N, 13.16. Found: C, 56.02; H, 5.91; N, 12.78%.

Solid-phase peptide synthesis

SASRIN® resin (0.5 g) was swollen in CH_2Cl_2 (4 ml) for 30 min, then treated with a solution of FmocGlyOH (420 mg, 0.7 mmol), DIC (216 µl, 0.7 mmol), and HOBt (189 mg, 0.7 mmol), and DMAP (11 mg, 0.05 mmol) in dry DMF (2.5 ml) for 12 h at rt. After this time, the resin was washed several times with DMF (5 ml) and CH_2Cl_2 (5 ml), and capped with Ac_2O–pyridine–DMF (2 : 4 : 94 by vol) in the presence of catalytic DMAP during 2 h. The resin was then rinsed with DMF and CH_2Cl_2 and treated with piperidine–DMF (1 : 4 by vol; 10, 10 and 5 min). Loading (0.47 mmol g^{-1}) was calculated by detecting UV absorbance of the effluents at 290 nm. The resin was then treated with a solution of FmocArg(Pmc)OH (311 mg, 0.47 mmol), HBTU (170 mg, 0.45 mmol), and DIPEA (160 µl, 0.94 mmol) in DMF (3 ml) for 2 h at, rt. The coupling was repeated until the TNBS test was negative. After washing and Fmoc deprotection, the resin was treated with azido acid **1** (220 mg, 0.7 mmol), HBTU (246 mg, 0.65 mmol), HOBt (95 mg, 0.7 mmol), and DIPEA (240 µl, 1.4 mmol) in DMF–CH_2Cl_2 (2 : 1 by vol; 3 ml) for 4 h at, rt. The resin was washed with DMF and CH_2Cl_2 and submitted to one-pot azide reduction and aspartic acid coupling reaction. To a solution of FmocAsp(tBu)OH (189 mg, 0.46 mmol) and HOBt (62 mg, 0.46 mmol) in dry DMF (3 ml) was added DIC (72 µl, 0.46 mmol) at 0 °C under argon atmosphere and the solution was stirred 10 min at this temperature. Then a solution of tributylphosphine in dry toluene (1.7 ml of a 0.6 mM solution) and the resin (0.5.g) were added and the suspension was allowed to warm to rt. and shaken under argon atmosphere for 24 h. The resin was rinsed with DMF and CH_2Cl_2 and, after *N*-terminal Fmoc removal, treated with 1% TFA in CH_2Cl_2 (3 × 10 min). Effluents containing peptide were neutralized at 0 °C with 2% pyridine in CH_2Cl_2. Solvents were evaporated *in vacuo*, the residue was diluted with CH_2Cl_2 (10 ml), and the organic layer was washed with water to partly remove pyridinium salts, dried (Na_2SO_4), and solvent was evaporated. TLC analysis of the crude revealed the presence of protected peptide (eluent $CHCl_3$–EtOH–AcOH 7 : 3 : 0.2, R_f = 3.5) which was purified by column chromatography ($CHCl_3$–EtOH–AcOH 7 : 3 : 0.2) and obtained as a colorless oil (120 mg, 0.13 mmol, 56% based on the loading of the resin). Peptide **9**: MS (MALDI-TOF): *m*/*z* 945.8 $[M + H]^+$, 968.2 $[M + Na]^+$, 983.7 $[M + K]^+$.

A solution of peptide **9** (100 mg, 0.11 mmol), HBTU (58 mg, 0.15 mmol), HOBt (21 mg, 0.16 mmol) and DIPEA (36 µl, 0.21 mmol) in dry DMF (212 ml, concn. peptide = 0.5 mM) was stirred for 72 h at rt. Solvent was evaporated *in vacuo* and the crude residue was purified by RP-HPLC. After lyophilization peptide **10** was recovered as a colorless oil (48 mg, 47%). Peptide **10**: MS (MALDI-TOF): *m*/*z* 927.8 $[M + H]^+$, 950.2 $[M + Na]^+$, 965.7 $[M + K]^+$.

Peptide **10** (30 mg, 0.03 mmol) was dissolved in TFA–TIS–water (95 : 2.5 : 2.5 by vol; 1 ml) and the solution was stirred for 10 h at rt. Solvent was evaporated *in vacuo* and the crude reidue was purified by RP-HPLC. After lyophilization, peptide **11** was recovered as a white powder (13 mg, 75%). Peptide **11**: *m*/*z* 604.8 $[M]^+$, 627.2 $[M + Na]^+$, 643.7 $[M + K]^+$.

NMR Spectroscopy

^1H and ^{13}C NMR spectra were recorded with TMS as internal standard. The signals of the aromatic carbons in the ^{13}C NMR spectra are not reported. Diluted and degassed *d*-chloroform solutions of compounds (concentrations around 30–40 mM) were examined either on a Bruker AC-300 or an Avance-400 spectrometer. In order to select the best NMR method to analyze the conformation in solution of bicycle **1**, preliminary measurements were performed on a similar bicyclic compound. A first set of interproton distances was derived from NOE-difference-1D experiments,[29] repeated at different values of NOE generation time (distances were evaluated in the two-spin approximation[30] or, when possible, with the NOE ratio method).[31] A second set of measurement was obtained by a series (4 or 5) of 2D-NOESY experiments with different mixing-time-values,[32] between 0.4 and 1.3 seconds, to cover the full range of molecular relaxation times in the two-spin approximation. Examination of curves of cross-peaks volumes *vs.* NOESY mixing times allowed us to match maximum NOE value for each nucleus and to calculate distances with the well-known relationship between NOE enhancement and r^{-6}.[33] Finally, an ROESY experiment[34] was used, when necessary, to confirm uncertainties in NOE attributions. The geminal constant interproton distance (1.8 Å) between non-equivalent nuclei was taken as reference[31] in distance calculations either for 1D or for 2D experiments. Results derived from NOESY experiments were more regular, numerous and reliable in fitting with our MD simulation and so, representing the best reference, have been used to analyze conformations of **1** (five different experiments with mixing times between 0.6 and 1.4 s).

Molecular dynamics

The potential-energy surface has been modelled employing the MM+ forcefield,[21] according to the following strategy. Molecular geometries were optimized by MM and then subjected to up to 10 cycles of simulated annealing MD. In particular, the temperature was raised from 50 up to 700 K in 10 ps and

then lowered to 20 K in 20 ps. The temperature was raised to high values (700 K) to allow possible conformational transitions in the sterically constrained molecules **1** and **2**.

Cell cultures

Fetal bovine aortic endothelial GM 7373 cells were obtained from the NIGMS Human Genetic Mutant Cell Repository (Institute for Medical Research, Camden, NJ). They correspond to the BFA-1c multilayered transformed clone described by Grinspan *et al.*[26] GM 7373 cells were grown in Eagle's minimal essential medium (MEM) containing 10% fetal calf serum (FCS), vitamins, and essential and non-essential amino acids.

Cell-adhesion assay

100 µl aliquots of 100 mM $NaHCO_3$, pH 9.6 (carbonate buffer), containing 20 µg ml^{-1} of the adhesive molecule under test, were added to polystyrene non-tissue-culture microtiter plates. After 16 h of incubation at 4 °C the solution was removed and wells were washed three times with cold phosphate-buffered saline (PBS). For the cell-adhesion assay, confluent cultures of GM 7373 cells were trypsinized, washed, and resuspended with the appropriate medium. 50,000 GM 7373 cells were resuspended in 200 µl of medium/1% FCS and were immediately seeded onto coated wells. Routinely, cell adhesion was allowed to occur for 2 h at 37 °C. Then, wells were washed with 2 mM EDTA in PBS. Adherent cells were fixed in 3.7% paraformaldehyde–0.1 M sucrose in PBS, washed with PBS, and stained with Methylene Blue–Azur II (1 : 1 by vol). Plates were read with a microplate reader at 595 nm.

Acknowledgements

This work was supported in part by CNR (Progetto Finalizzato Biotecnologie), MURST (Cofin 2000), Associazione Italiana per la Ricerca sul Cancro, and Istituto Superiore di Sanità (AIDS Project) to M. P.

References

1 E. G. Von Roedern and H. Kessler, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 687; E. G. Von Roedern, E. Lohof, G. Hessler, M. Hoffmann and H. Kessler, *J. Am. Chem. Soc.*, 1996, **118**, 10165; B. Aguilera, G. Siegal, H. S. Overkleeft, N. J. Meeuwenoord, F. P. J. T. Rutjes, J. C. M. van Hest, H. E. Schoemaker, G. A. van der Marel, J. H. van Boom and M. Overhand, *Eur. J. Org. Chem.*, 2001, **8**, 1541.

2 (*a*) E. Lohof, E. Planker, C. Mang, F. Burkhart, M. A. Dechantsreiter, R. Haubner, H. J. Wester, M. Schwaiger, G. Holzemann, S. L. Goodman and H. Kessler, *Angew. Chem., Int. Ed.*, 2000, **39**, 2761; (*b*) R. Haubner, H. J. Wester, F. Burkhart, R. Senekowitsch-Schmidtke, W. Weber, S. L. Goodman, H. Kessler and M. Schwaiger, *J. Nucl. Med.*, 2001, **42**, 326; (*c*) C. Gibson, G. A. G. Sulyok, D. Hahn, S. L. Goodman, G. Holzemann and H. Kessler, *Angew. Chem., Int. Ed.*, 2001, **40**, 165; (*d*) N. Moitessier, S. Dufour, F. Chretien, J. P. Thiery, B. Maigret and Y. Chapleur, *Bioorg. Med. Chem.*, 2001, **9**, 511.

3 (*a*) R. Hirschmann, K. C. Nicolaou, S. Pietranico, J. Salvino, E. M. Leathy, P. A. Sprengler, G. Furst, A. B. Smith III, C. D. Strader, M. A. Cascieri and M. R. Candelore, *J. Am. Chem. Soc.*, 1992, **114**, 9217; (*b*) R. Hirschmann, W. Yao, M. A. Cascieri, C. D. Strader, L. Maechler, M. A. Cichy-Knight, J. Hines Jr., R. D. van Rijn, P. A. Sprengeler and A. B. Smith III, *J. Med. Chem.*, 1996, **39**, 2441; (*c*) R. Hirschmann, J. Hines Jr., M. A. Cichy-Knight, R. D. Van Rijn, P. A. Sprengeler, P. G. Spoors, W. C. Sheakespeare, S. Pietranico-Cole, J. Barbosa, J. Liu, W. Yao, S. Rohrer and A. B. Smith III, *J. Med. Chem.*, 1998, **41**, 1382.

4 K. C. Nicolaou, J. I. Trujillo and K. Chibale, *Tetrahedron*, 1997, **53**, 8751.

5 H. P. Wessel, D. Banner, K. Gubernator, K. Hilpert, K. Müller and T. Tschopp, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 751.

6 T. Q. Dinh, C. D. Smith, X. Du and R. W. Armstrong, *J. Med. Chem.*, 1998, **41**, 981.

7 R. Hirschmann, L. Ducry and A. B. Smith, *J. Org. Chem.*, 2000, **65**, 8307.

8 (*a*) E. F. Fuchs and J. Lehmann, *Chem. Ber.*, 1975, **108**, 2254; (*b*) E. F. Fuchs and J. Lehmann, *Carbohydr. Res.*, 1975, **45**, 135; (*c*) E. F. Fuchs and J. Lehmann, *Carbohydr. Res.*, 1976, **49**, 267; (*d*) K. C. Nicolaou, H. M. Florke, G. Egan, T. Barth and V. A. Estevez, *Tetrahedron Lett.*, 1995, **36**, 1775.

9 (*a*) T. K. Chakraborty, S. Ghosh, S. Jayaprakash, J. A. R. P. Sarna, V. Ravikanth, P. V. Diwan, R. Nagaraj and A. C. Kunwar, *J. Org. Chem.*, 2000, **65**, 7710; (*b*) T. K. Chakraborty, S. Ghosh, M. H. V. R. Rao, A. C. Kunwar, H. Cho and A. K. Ghosh, *Tetrahedron Lett.*, 2000, **41**, 10121; (*c*) T. K. Chakraborty, S. Jayaprakash, P. V. Diwan, R. Nagaraj, S. R. B. Jampani and A. C. Kunwar, *J. Am. Chem. Soc.*, 1998, **120**, 12962; (*d*) T. K. Chakraborty, S. Ghosh, S. Jayaprakash, J. A. R. P. Sharma, V. Ravikanth, P. V. Diwan, R. Nagaraj and A. C. Kunwar, *J. Org. Chem.*, 2000, **65**, 6441; (*e*) M. D. Smith, D. D. Long, D. G. Marquess, T. D. W. Claridge and G. W. J. Fleet, *Chem. Commun.*, 1998, 2039; (*f*) R. M. van Well, H. S. Overkleeft, M. Overhand, E. V. Carstenen, G. A. van der Marel and J. H. van Boom, *Tetrahedron Lett.*, 2000, **41**, 9331.

10 M. D. Smith, T. D. W. Claridge, G. E. Tranter, M. S. P. Sansom and G. W. J. Fleet, *Chem. Commun.*, 1998, 2041.

11 T. D. W. Claridge, D. D. Long, N. L. Hungerford, R. T. Aplin, M. D. Smith, D. G. Marquess and G. W. J. Fleet, *Tetrahedron Lett.*, 1999, **40**, 2199.

12 S. H. Gellmann, *Acc. Chem. Res.*, 1998, **31**, 173.

13 (*a*) J. P. Schneider and J. W. Kelly, *Chem. Rev.*, 1995, **95**, 2169; (*b*) S. Hannessian, G. McNaughton-Smith, H.-G. Lombart and W. D. Lubell, *Tetrahedron*, 1997, **53**, 12789; (*c*) G. Müller and H. Giera, *J. Comput.-Aided Mol. Des.*, 1998, **12**, 1.

14 (*a*) N. L. Subasinghe, R. J. Bontems, E. McIntee, R. K. Mishra and R. L. Johnson, *J. Med. Chem.*, 1993, **36**, 2356; (*b*) N. L. Subasinghe, E. M. Khalil and R. L. Johnson, *Tetrahedron Lett.*, 1997, **38**, 1317.

15 (*a*) U. Nagai and K. Sato, *Tetrahedron Lett.*, 1985, **26**, 647; (*b*) U. Nagai, K. Sato, R. Nakamura and R. Kato, *Tetrahedron*, 1993, **49**, 3577; (*c*) T.-A. Tran, R.-H. Mattern, Q. Zhu and M. Goodman, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 997; (*d*) J. H. Viles, S. U. Patel, J. B. O. Mitchell, C. M. Moody, D. E. Justice, J. Uppenbrink, P. M. Doyle, C. J. Harris, P. J. Saddler and J. M. Thornton, *J. Mol. Biol.*, 1998, **279**, 973; (*e*) J. Wagner, J. Kallen, C. Ehrhardt, J.-P. Evenou and D. Wagner, *J. Med. Chem.*, 1998, **41**, 3664.

16 F. Peri, L. Cipolla, B. La Ferla and F. Nicotra, *Chem. Commun.*, 2000, 2303.

17 J. P. Malkinson, R. A. Falconer and I. Toth, *J. Org. Chem.*, 2000, **65**, 5249.

18 A. Araki, Y. Younosuke, S. Kobayashi, Y. Naoki, K. Ishido, Y. Yoshiharu, J. Nagasawa and Y. Jun'ichi, *Carbohydr. Res.*, 1987, **171**, 125.

19 L. Cipolla, L. Lay and F. Nicotra, *J. Org. Chem.*, 1997, **62**, 6678.

20 B. H. Meier and R. R. Ernst, *J. Am. Chem. Soc.*, 1979, **101**, 6441.

21 N. L. Allinger, *J. Am. Chem. Soc.*, 1977, **99**, 8127.

22 (*a*) M. Aumalley, M. Gurrah, G. Müller, J. Calvete, R. Timpl and H. Kessler, *FEBS Lett.*, 1991, **291**, 50; (*b*) G. Müller, M. Gurrath, H. Kessler and R. Timpl, *Angew. Chem., Int. Ed. Engl.*, 1992, **31**, 326; (*c*) R. Haubner, R. Gratias, B. Diefenbach, S. L. Goodman, A. Jonczyk and H. Kessler, *J. Am. Chem. Soc.*, 1996, **118**, 7461; (*d*) A. Giannis and F. Rübsam, *Angew. Chem.*, 1997, **109**, 606; (*e*) A. C. Bach II, J. Espina, S. A. Jackson, P. F. W. Stouten, J. L. Duke, S. A. Mousa and W. F. De Grado, *J. Am. Chem. Soc.*, 1996, **118**, 293.

23 For reviews on phosphazene chemistry see: (*a*) A. W. Johnson, *Ylides and Imines of Phosphorus*, Wiley, New York, 1993; (*b*) P. Molina and M. J. Vilaplana, *Synthesis*, 1994, 1197.

24 K. Brickmann, Z. Q. Yuan, I. Sethson, P. Sommai and J. Kihlberg, *Chem. Eur. J.*, 1999, **5**, 2241.

25 Z. Tang and J. C. Pelletier, *Tetrahedron Lett.*, 1998, **39**, 4773.

26 J. B. Grinspan, N. M. Stephen and E. M. Levine, *J. Cell. Physiol.*, 1983, **114**, 328.

27 M. T. E. Rusnati, P. Dell'Era, A. Gualandris and M. Presta, *Mol. Biol. Cell*, 1997, **8**, 2449.

28 W. S. Hancock and J. E. Battersby, *Anal. Biochem.*, 1976, **71**, 261.

29 W. A. Gibbons, C. F. Beyer, J. Dadok, R. F. Sprecher and H. R. Wyssbrod, *Biochemistry*, 1975, **14**, 420.

30 J. H. Noggle and R. E. Schirmer, *The Nuclear Overhauser Effect, Chemical Applications*, Academic Press, London, 1971.

31 P. Mascagni and W. A. Gibbons, *J. Chem. Soc., Perkin Trans. 1*, 1985, 245.

32 B. H. Meier and R. R. Ernst, *J. Am. Chem. Soc.*, 1979, **101**, 6441.

33 R. A. Bell and J. K. Saunders, *Can. J. Chem.*, 1970, **48**, 1114.

34 A. Bax and D. G. Davis, *J. Magn. Reson.*, 1985, **63**, 207.