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ORIGINAL ARTICLE

An efficient route towards a new branched tetrahydrofurane δ-sugar amino acid from a pyrolysis product of cellulose

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Abstract (1*R*,5*S*)-1-Hydroxy-3,6-dioxa-bicyclo[3.2.1] octan-2-one, is a bicyclic lactone obtained in gram-scale by catalytic pyrolysis of the renewable source cellulose. Now it has been used as a chiral building block in the preparation of the new δ-sugar amino acid, (3*R*,5*S*)-5-(aminoethyl)-3-hydroxytetrahydrofurane-3-carboxylic acid, by an efficient synthesis in five steps with a 67% overall yield. The structure of this tetrahydrofurane amino acid, isolated in protonated form, was assigned by extensive mono- and bidimensional ^{1}H- and ^{13}C-NMR analysis and mass spectrometry, including measurements by electrospray and matrix-assisted laser desorption ionization techniques, the latter one for high-resolution experiments. This amino acid is an isoster of dipeptide glycine-alanine (*H-Gly-Ala-OH*), with a potential use in the access of new peptidomimetics with conformationally restricted structures due to the presence of tetrahydrofurane ring. As a preliminary study in order to disclose this effect, density functional theory calculation performed in water using polar continuum model was applied to the new amino acid and *H-Gly-Ala-OH* dipeptide, so that to evaluate and compare the relative torsional angles for the energy-minimized structures.

Keywords Sugar amino acids · Chiral building block · Dipeptide isoster · DFT calculation · Hydroxylactone · Cellulose pyrolysis

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Introduction

Sugar amino acids (SAAs) are carbohydrate derivatives widely used as building blocks, allowing to mimic nature in organic synthesis. In recent years they have been involved in the development of glycomimetics and peptidomimetics (Schweizer 2002; Chakraborty et al. 2004), with application also in drug design (Gruner et al. 2002). The advantages are linked to their high density of functional groups present with a well-defined stereochemistry, making them peculiar chiral synthones able to introduce the suitable hydrophobic or hydrophilic substitution patterns. A relevant example is given by the potential of creating combinatorial amide libraries of compounds in order to emulate the diversity of biomolecules (Edwards et al. 2004). In addition, the incorporation at a specific location in a peptide sequence can induce a conformational restriction responsible for defined folding in peptides (Lohof et al. 2000).

The structures of sugar amino acids include variations on the relative position of amino and carboxylic groups (α, β,…) and on the oxygen heterocycle (with oxirane, oxetane, tetrahydrofurane and tetrahydropyrane as the major types) (Risseeuw et al. 2007). In particular, δ-SAAs are isosters of scissible dipeptide bonds (Trabocchi et al. 2008) and for this reason they have been thoroughly investigated in drug design owing to the expected greater stability towards enzymatic proteolysis with respect to natural peptides. Moreover, the δ-tetrahydrofurane series is characterized by a relative rigidity able to introduce structural constraints and making these SAAs attractive candidates as scaffolds in peptidomimetics, as well as by a propensity to induce conformational preferences in relatively short sequences (Smith et al. 1998; Simone et al. 2008). Most of these compounds consist of a linear chain of carbon atoms in accordance with their origin from simple carbohydrates (Risseeuw et al. 2007). Starting

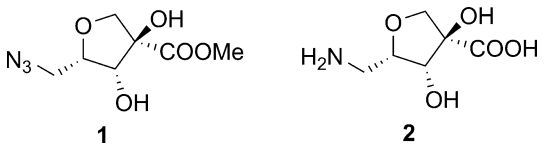


Fig. 1 Structure of the reported azido ester **1** as scaffold of the branched δ-tetrahydrofurane SAA **2**

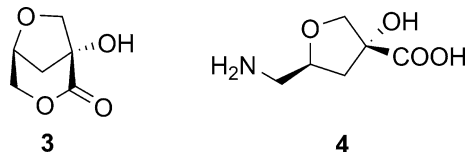


Fig. 2 Structures of the cellulose pyrolytic product **3**, used as the chiral building block for the synthesis of the new branched δ-tetrahydrofurane sugar amino acid **4**

from a protected ribonolactone affordable from D-ribose the methyl (3*R*,4*R*,5*S*)-5-azidomethyl 3,4-dihydroxy-tetrahydrofuran-3-carboxylate (**1**; Fig. 1) and the corresponding epimer at 5 position were recently synthesized as the first δ-SAA scaffolds containing a branched carbon chain. The sequence included a long series of steps among which the reduction of the lactone, a function required in order to insert the carbon branching by means of an aldol condensation with formaldehyde. The lactone was successively regenerated by oxidation followed by cyclisation of the ribonolactone derivative affording the tetrahydrofurane ring (Simone et al. 2008). Previously the same authors had reported that azido ester **1** was unable to give the methyl ester of amino acid **2** (Fig. 1) by palladium catalytic hydrogenation, due to the spontaneous cyclization to bicylic lactame (Simone et al. 2005).

Biomass treatment includes the conversion to biofuels able to meet the energy demand under investigation as alternative fuel source with environmental respect, but also to produce chemicals, including building blocks to be employed in the synthesis of useful compounds. Cellulose, a polysaccharide found in plant materials, is the most abundant biosynthesized organic substance on earth, and therefore represents a key biomass component. The relevance and interest of this process is linked to the use of renewable source of energy, due the employment of pure cellulose and of cellulosic materials of recovery (i.e. wood). Cellulose can be converted into a large series of compounds by several processes and among them pyrolysis, which is a one-step thermal degradation under non-oxidative conditions leading to a liquid fraction (bio-oil). It contains low molecular weight compounds, including polyfunctional dehydrated C6 monomers named anhydro-monosaccharides, peculiar for retaining the structural record of the original stereochemistry in glucose units of cellulose and thus existing in pure enantiomeric form, which accounts for their employment as a chiral building block.

Hydroxylactone **3** (Fig. 2), namely (1*R*,5*S*)-1-hydroxy-3,6-dioxa-bicyclo[3.2.1]octan-2-one, is an anhydro monosaccharide first isolated in a 1 mg amount from Lewis acid-catalysed pyrolysis of cellulose (Furneaux et al. 1988). Recently, some of us reported an analytical study on the effect of different catalysts on the pyrolytic behaviour of cellulose. In particular, it was shown that the levels of production of **3** were significantly increased in the presence of commercial nanopowder metal oxides, with evidence that nano-sized feature of aluminium titanate (Al Ti) oxides resulted a determinant factor for its activity (Fabbri et al. 2007a). A preparative process was defined to give bio-oils enriched of hydroxylactone **3**. It proved that the behaviour of the Al Ti nanopowder catalyst, which could be recycled in its use quite effectively, resulted uniquely in promoting both the formation of the main anhydromonosaccharide product **3** in gram scale and in favoring its isolation from the pyrolytic liquid (Fabbri et al. 2007b). Later, the efficiency of the catalyst was verified also in the analytical pyrolysis of a series of carbohydrates, with levoglucosan and its 4-*O*-acetyl derivative producing **3** in yields comparable to those of starch and cellulose (Torri et al. 2009a). More recently, mesoporous materials doped with different metals were also investigated, with the best production of **3** was achieved with Sn-MCM-41 system (Torri et al. 2009b). These conditions open the route to its potential use as chiral building block, never scrutinized before probably due to the low production of **3**. Recent applications were done obtaining a suitable amide by the effective and eco-friendly microwave assisted methodology (Fabbri et al. 2007b), and later with the application as a monomer in the synthesis of polyesters by ring-opening polymerisation with L-lactide (Dobrzynski et al. 2009).

We report here on a simple and efficient route to the novel amino acid **4** (Fig. 2), showing the methyleneamino and the carboxylic acid components in 2,4-position of the tetrahydrofurane ring with a *cis* relative configuration. The synthesis employs **3** as chiral reagent, which enables to overcome the need of oxidation/reduction steps of the lactone moiety to insert the ramification and its ring closure into tetrahydrofurane. It is the first production of a δ-tetrahydrofurane SAA containing a branched carbon chain obtained in a free form, after the report of the first branched δ-SAA scaffold derived from D-ribose (Simone et al. 2008).

Experimental

Materials and methods

The reagents and solvents were used in chemical reactions without purification. High purity microgranular cellulose

was from Fluka. Mesophase of the 41 type doped with tin (Sn-MCM-41) was prepared as previously described (Torri et al. 2009b). All evaporations were carried out at room temperature at reduced pressure. The reaction yields were calculated on the products after chromatographic purification. Thin layer chromatography (TLC) was carried out on *Merck Kieselgel 60 PF*$_{254}$ and flash-chromatography (FC) on Merck silica gel 60 (15–25 μm), preparative thin layer chromatography was realized on 20 × 20 cm *Merck Kieselgel 60 F*$_{254}$ 0.5 mm plates. Infrared spectra were recorded by using a FT-IR Tensor 27 Bruker spectrometer (Attenuated Transmitter Reflection, ATR configuration) at 1 cm^{-1} resolution in the absorption region $\Delta\tilde{\nu}$ 4,000–1,000 cm^{-1}. A thin solid layer is obtained by evaporation of methanol solution of the sample. The instrument was purged with a constant dry air flux and clean ATR crystal as background was used. Spectra processing was made using Opus software package. Melting points were determined on Reichert Thermovapor microscope and the data are uncorrected. Polarimetric data were obtained with a Bellingham & Stanley Limited ADP 440 apparatus, reporting $[\alpha]_D$ in dm^{-1} deg ml g^{-1}. NMR spectra were recorded on a Bruker-Avance 400 spectrometer by using a 5 mm BBI probe with 90° proton pulse length of 8 μs at a transmission power of 0 db; ^1H at 400 MHz and ^{13}C at 100 MHz in $CDCl_3$ (by previous treatment on basic alumina to avoid acidic traces), δ values in ppm, in $CDCl_3$ relative to the solvent residual signals $\delta_H = 7.25$ and $\delta_C = 77.00$ ppm, or in D_2O relative to $\delta_H = 4.70$, *J* values in Hz. Structural assignments are from $^1H,^1H$-COSY, heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC) experiments. Electrospray ionization (ESI)-MS mass spectra were taken with a Bruker Esquire-LC spectrometer with an electrospray ion source used in positive or negative ion mode by direct infusion of a methanolic solution of the sample, under the following conditions: source temperature 300°C, drying gas N_2, 4 l/min, positive ion mode, ISV 4 kV, OV 38.3 V, scan range 100–1,000 *m/z*. Matrix assisted laser desorption ionization-time of flight (MALDI-TOF) measurements were performed on Bruker Daltonics Ultraflex MALDI-TOF-TOF mass spectrometer equipped with a reflectron unit. The acceleration voltage was set at 20 kV. For desorption of the components, a nitrogen laser beam ($\lambda = 337$ nm) was focused on the template. The laser power level was adjusted to obtain high signal-to-noise ratios, while ensuring minimal fragmentation of the parent ions. All measurements were carried out in the delayed extraction mode, allowing the determination of monoisotopic mass values (*m/z*; mass-to-charge ratio). After crystallization at ambient conditions, positive ion spectra were acquired in the reflectron mode, giving mainly singly protonated molecular ions ($[M + H]^+$). Samples were directly applied onto the stainless-steel spectrometer plate as 1 μl droplets, followed by the addition of 1 μl of DHB-matrix solution (0.5 M of 2,5-DHB in methanol). Every mass spectrum represents the average of about 100 single laser shoots. Calibration for high resolution experiments was performed on DHB peaks at *m/z* 177 ($[M + Na]^+$), 154 ($[M]^{+\cdot}$) and 137($[M + H–H_2O]^+$).

Quantum chemical calculations were performed on a Pentium IV/3.6 GHz personal computer using the Gaussian 03W revision E.01 package program set (Frisch et al. 2004). Restricted DFT was used, applied for geometry optimization in water using Polar Continuum Model (PCM) and invoking gradient geometry optimisation. The basis set of choice resulted 6–31 G (d) for all the atoms. The gradient-corrected DFT with the three-parameter hybrid functional (B3) (Becke 1993) for the exchange part and the Lee–Yang–Parr (LYP) correlation function (Lee et al. 1988) were utilized.

*Production of (1R,5S)-1-hydroxy-3,6-dioxa-bicyclo[3.2.1] octan-2-one (**3**) from cellulose* Cellulose (10 g) was mixed with 10 g of mesoporous phase Sn-MCM-41, synthesized as reported (Torri et al. 2009b). Four batches were pyrolysed at 500°C for 5 min under nitrogen. The bio-oil (around 50% yield, containing 12% of **3**) was obtained by trapping the evolved products with cold acetonitrile. Column chromatography on silica gel eluting with 9:1 cyclohexane/ethyl acetate and then pentane/dichloromethane gradient elution, following vacuum distillation and filtration over charcoal furnished **3** with 99% purity by GC analysis and 65% recovery from bio-oil, showing superimposable NMR and MS data to the ones previously reported (Fabbri et al. 2007b).

*(3R,5S)-Methyl 3-hydroxy-5-(hydroxymethyl)tetrahydrofuran-3-carboxylate (**5**)* To a solution of **3** (288 mg, 2.0 mmol.) in anhydrous methanol (5 ml) triethylamine (0.28 ml, 2.0 mmol) was added. The resulted mixture was stirred for 12 h at room temperature. After evaporation of the solvent in vacuo, the residue was subjected to flash chromatography on silica gel with dichloromethane/methanol gradient elution, to give the ester **5** as viscous clear oil (334 mg, 95%).

$[\alpha]_D^{20} = +10.2°$ (c 0.5 in MeOH). Optical activity, NMR and MS data are in accordance with those reported (Furneaux et al. 1988; Fabbri et al. 2007b).

*(3R,5S)-Methyl 3-hydroxy-5-(tosyloxymethyl)tetrahydrofuran-3-carboxylate (**6**)* To a solution of **5** (334 mg, 1.90 mmol) in 3 ml of anhydrous pyridine at 0°C was added slowly a solution of tosyl chloride (400 mg, 2.1 mmol) in anhydrous pyridine (2 ml). The solution was stirred at room temperature for 18 h and then poured in a beaker containing ice, the mixture was partitioned between

water and dichloromethane, the organic phases were separated (×3), combined and washed in sequence with 0.5 M aq. HCl, saturated solution of $NaHCO_3$ and water, dried on anhydrous Na_2SO_4 and concentrated in vacuo. The residue was subjected to flash-chromatography on silica gel with hexane/EtOAc gradient elution, to give tosylate **6** as a viscous yellowish oil (552 mg, 88%).

$[\alpha]_D^{20} = +19.7$ (c 0.04, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 7.80 (2H, d, $J = 8.4$ Hz, H-2′ and H-6′), 7.33 (2H, d, $J = 8.4$ Hz, H-3′ and H-5′), 4.39 (1H, m, H-5), 4.15 (1H, dd, $J = 10.6$, 3.8 Hz, H-6), 4.10 (1H, dd, $J = 10.6$, 4.8 Hz, H-6), 4.09 (1H, d, $J = 9.9$ Hz, H-2), 3.81 (3H, s, OMe), 3.77 (1H, d, $J = 9.9$ Hz, H-2), 3.38 (1H, br. s, OH), 2.44 (3H, s, Me-4′), 2.23 (1H, dd, $J = 12.8$, 9.9 Hz, H-4), 2.09 (1 H, dd, $J = 12.8$, 6.0 Hz, H-4). ^{13}C NMR ($CDCl_3$): δ 174.3 (COO), 146.7 (C-1′), 144.9 (C-4′), 129.8 (C-3′and C-5′), 127.9 (C-2′ and C-4′), 81.0 (C-3), 78.2 (C-5), 76.6 (C-2), 70.1 (C-6), 53.4 (OMe), 41.5 (C-4), 21.6 (q, Me-4′). Significant HMBC correlations: δ 7.80 ppm (H-2′ and H-6′) with C-1′; 7.33 (H-3′ and H-5′) with Me-4′; 4.15 (H-6) with C-4, 4.09 (H-2) with COO, 3.81 (OMe) with C-3 and COO, 3.77 (H-2) with COO, 2.09 (H-4) with C-3 and C-5. ESIMS (positive mode): *m/z* 353 ($[M + Na]^+$), 331 ($[M + H]^+$); ESI–MS/MS (331): 159($[M + H - TsO]^+$).

*(3R,5S)-Methyl 5-(azidomethyl)-3-hydroxytetrahydrofuran-3-carboxylate (**7**)* A mixture of tosylate **6** (552 mg, 1.67 mmol), sodium azide (163 mg, 2.5 mmol) and Aliquat® 336 (101 mg, 0.25 mmol) in anhydrous acetonitrile (20 ml) was refluxed for 17 h. After cooling and filtration, the solvent was removed in vacuo and the residue was subjected to flash-chromatography on silica gel with hexane/EtOAc gradient elution, to give the azide **7** as viscous clear oil (292 mg, 87%).

$[\alpha]_D^{20} = +90.3$ (c 0.06, MeOH). ^{1}H NMR ($CDCl_3$): δ 4.40 (1H, m, H-5), 4.23 (1H, d, $J = 9.5$ Hz, H-2), 3.85 (3H, s, OMe), 3.83 (1H, d, $J = 9.5$ Hz, H-2), 3.54 (1H, dd, $J = 12.8$, 3.6 Hz, H-6), 3.40 (1H, br. s, OH), 3.31 (1H, dd, $J = 12.8$, 5.6 Hz, H-6), 2.28 (1H, dd, $J = 12.8$, 9.9 Hz, H-4), 2.08 (1H, dd, $J = 12.8$, 5.4 Hz, H-4).^{13}C NMR ($CDCl_3$): δ 174.4 (COO), 77.8 (C-5), 81.6 (C-3), 78.2 (C-2), 53.3 (OMe), 53.2 (C-6), 53.3 (OMe), 42.3 (C-4). Significant HMBC correlations: δ 3.85 ppm (H-2) with C-4; 4.23 (H-2) with C-4, C-5 and C-4 COO, 3.85 (OMe) with COO, 4.40 (H-5) with C-2, C-6 and C-4, 3.54 (H-6) with C-4 and C-5; 3.31 (H-6) with C-2 and C-4; 2.28 (H-4) with C-6, C-2 and COO; 2.08 (H-4) with C-2. Significant HMBC correlations: δ 4.23 ppm (H-2) with C-5 and COO; 3.83 (H-2) with C-3, C-5 and COO; 3.54 (H-6) with C-4, C-5; 2.28 (H-4) with C-2, C-6,COO; 2.08 (H-4) with C-3. ESIMS (positive mode): *m/z* 224 ($[M + Na]^+$).

*Chiral shift-reagent study with **7*** To compound **7** (2.0 mg, 0.01 mmol) in 0.5 ml of $CDCl_3$ a 0.11 M solution of $Eu(tfc)_3$ in $CDCl_3$ was added by 1–30 µl portions, observing proton shifts without splitting of the signals. After the addition of 0.30 mol equiv of shift-reagent $\Delta\delta$ for the signals of the protons were: 2.79 (OH), 0.79 (H-5), 0.75 and 0.73 (2H-4), 0.67 and 0.65 (2H-2), 0.59 and 0.37 (2H-6), 0.42 ppm (OMe).

*(3R,5S)-5-(Azidomethyl)-3-hydroxytetrahydrofuran-3-carboxylic acid (**8**)* To a solution of **7** (146 mg, 0.725 mmol) in THF (5 ml) was added NaOH 0.5 M (1.5 ml, 0.363 mmol) and stirred for 3 h. The mixture was passed through a column of Amberlyst 15 (H-form), washing with acetonitrile and water. The eluate, having a pH value of 4, was evaporated in vacuo to give the acid **8** (135 mg, 99%), pure enough to be used in the next step.

White powder. $[\alpha]_D^{20} = +87.4$ (c 0.2, EtOH). ^{1}H NMR ($CDCl_3$): δ 4.45 (1H, m, H-5), 4.30 (1H, d, $J = 9.6$ Hz, H-2), 3.90 (1H, d, $J = 9.6$ Hz, H-2), 3.85 (1H, s, HO-3), 3.55 (1H, dd, $J = 12.8$, 3.5 Hz, H-6), 3.36 (1H, dd, $J = 12.8$, 5.6 Hz, H-6), 2.37 (1H, dd, $J = 12.9$, 9.8 Hz, H-4), 2.16 (1H, dd, $J = 12.9$, 5.8 Hz, H-4).^{13}C NMR ($CDCl_3$): δ 177.5 (COOH), 83.3 (C-3), 78.8 (C-2), 78.3 (C-5), 53.1 (C-6), 42.2 (C-4). Significant HMBC correlations: δ 4.30 ppm (H-2) with C-4, C-5 and COO; δ_H 3.90 (H-2) with C-4, C-5 and COO; 3.55 and 3.36 (2H-6) with C-4 and C-5; 2.37 (H-4) with C-2, C-6 and COO. ESIMS (negative mode): *m/z* 186 ($[M–H]^-$).

*(1R,5S)-1-hydroxy-6-oxa-3-azabicyclo[3.2.1]octan-2-one(**9**)* A solution of **7** (73 mg, 0.36 mmol) in 5 ml of ethanol was hydrogenated for 3 h at room temperature and atmospheric pressure in presence of 10% Pd/C. After filtration, the solvent was evaporated in vacuo to obtain compound **9** (38 mg, 73%).

^{1}H NMR ($CDCl_3$): δ 5.50 (1H, br. s, NH), 4.56 (1H, br. s, H-5), 4.00 (1H, d, $J = 8.0$ Hz, H-2), 3.73 (1H, d, $J = 8.0$ Hz, H-2), 3.46 (1H, d, $J = 12.6$ Hz, H-6), 3.36 (1H, d, $J = 12.6$, H-6), 2.50 (1H, dd, $J = 11.9$, 5.7 Hz, H-4), 2.06 (1H, br. d, $J = 11.8$ Hz, H-4).^{13}C NMR ($CDCl_3$): δ 175.0 (CONH), 85.0 (C-3), 74.5 (C-5), 73.3 (C-2), 45.2 (C-6), 54.0 (C-4). Significant HMBC correlations: δ 4.00 and 3.73 ppm (2H-2) with C-5 and C=O; 2.06 (H-4) with C=O. ESIMS (positive mode): *m/z* 144 ($[M + H]^+$), 166 ($[M + Na]^+$).

((2S,4R)-4carboxy-4-hydroxytetrahydrofuran-2-yl) methanaminium salt A solution of **8** (135 mg, 0.72 mmol) in 10 ml of ethanol was hydrogenated for 3 h at room temperature and atmospheric pressure in presence of 10% Pd/C. After filtration, the solvent was evaporated in vacuo to obtain **4**, as protonated form due to the acid condition of the starting reagent (110 mg, 95%).

White crystalline solid, m.p. 192–194°C. $[\alpha]_D^{20} = +24.3$ (c 0.15, H_2O). IR *v*max: 2,910 (v br) 1,716 (vs), 1,591 (s),

1,502, 1,394, 1,225 (s), 1,134, 1,024 (vs), 812 and 710 cm^{-1}. ^1H NMR (D_2O): δ 4.40 (1H, m, H-5), 4.11 (1H, d, $J = 9.8$ Hz, H-2), 3.80 (1H, d, $J = 9.8$ Hz, H-2), 3.17 (1H, dd, $J = 13.0$, 3.1 Hz, H-6), 3.07 (1H, dd, $J = 13.0$, 8.7 Hz, H-6), 2.20 (2H, m, H-4).^{13}C NMR (D_2O): δ 176.8 (COOH), 81.4 (C-3), 76.8 (C-2), 75.1 (C-5), 41.5 (C-4), 41.3 (C-6). Significant HMBC correlations: δ 4.40 ppm (H-5) with C-2; 4.11 and 3.80 (2H-2) with C-3, C-4, C-5 and COO; 3.17 and 3.06 (2H-6) with C-4, C-5; 2.20 (2H-4) with C-3, C-6 and COO. ESIMS (positive mode): *m/z* 162 ($[M + H]^+$); ESI–MS/MS (162): 145 ($[M + H - NH_3]^+$), 144 ($[M + H - H_2O]^+$), 117 ($[M + H - CO_2]^+$), 116 ($[M + H - HCOOH]^+$), 99 ($[C_5H_7O_2]^+$). HR-MALDI-TOF/TOF MS (positive mode): *m/z* 162.0751 ± 0.005, calculated for $C_6H_{12}NO_4$ ($[M + H]^+$) 162.0766.

Results and discussion

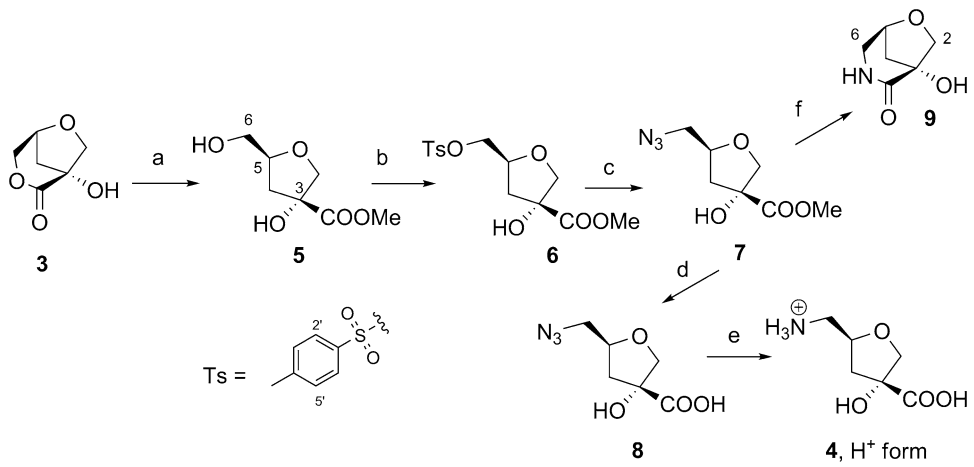
Synthesis of sugar amino acid 4

Hydroxylactone **3**, here obtained in higher yields by a modified procedure involving Sn-MCM-41 system ("Experimental"), was converted into the new amino acid **4** according to the sequence illustrated in Scheme 1. All the products were purified by silica gel chromatography and structurally characterized ("Experimental").

In details, the lactone group of **3** could be easily opened to the methyl ester derivative **5** by triethylamine treatment in methanol at room temperature for 12 h. The product was obtained in 95% yield and characterized by ^1H- and ^{13}C-NMR technique, observing superimposable data with the ones already reported (Furneaux et al. 1988; Fabbri et al. 2007b). The value of optical activity ("Experimental") was also in agreement with literature, supporting the (3*R*, 5*S*) stereochemistry. By conversion of the primary hydroxyl group through the reaction with 1.2 molar equivalents of *p*-toluenesulfonyl chloride in pyridine by stirring at room temperature overnight, tosylate **6** was obtained in 88% yield. The sterically HO-3 did not require time demanding protection/deprotection steps, but it is noteworthy that an excess of tosyl chloride was able to produce also the ditosylate derivative by conversion of the tertiary hydroxyl function.

The azide intermediate **7** was achieved by nucleophilic substitution of tosylate **6** with sodium azide in a heterogeneous system containing methyltrioctylammonium chloride (Aliquat® 336). Although the use of this catalyst is generally reported in water (Balo et al. 1998), in our case acetonitrile resulted a better solvent giving 85% yield, whereas in water we observed partial hydrolysis of the tosylate group. Otherwise the standard method involving the use of sodium azide in dimethylformamide (DMF) as solvent, applied to tosylate **6** furnished a very low yield of azide **7**, due to the difficult isolation of the water-soluble product. The conversion of azido ester **7** to amino acid **4** involved a first hydrolysis by aqueous sodium hydroxide in tetrahydrofurane (THF) at room temperature for 3 h providing pure azido acid **8** in quantitative amount, which was later subjected to Pd/C catalysed hydrogenation in ethanol for 3 h with a 95% yield. Otherwise, the direct reduction to amine of the azido function in compound **7** did not work, but intramolecular cyclization to lactame **9** occurred, in line with the compound **1** giving a corresponding lactame (Simone et al. 2005), whose structure was firmly established by X-ray crystallographic analysis (Punzo et al. 2004).

Amino acid was isolated in protonated form, due to the acidic conditions present in the workup of the azido carboxylate when it was subjected to a filtration on a cationic resin before reduction by Pd/C catalysed hydrogenation.



Scheme 1 Arbitrary numbering is for convenience. Reaction reagents and conditions: **a** MeOH/Et_3N (2.5 eq), r.t., 12 h, 95%; **b** TsCl (1.2 eq)/Py, r.t., overnight, 88%; **c** NaN_3 (1.5 eq)/Aliquat®336 (0.1 eq), CH_3CN, reflux 17 h, 85%; **d** 0.5 M aq.NaOH (1 eq), THF, r.t., 3 h, H^+-form Amberlyst 15, 99%; **e** H_2, $p = 1$ atm, Pd/C 10%; EtOH, 3 h, 95%; **f** H_2, $p = 1$ atm, Pd/C 10%; EtOH, 1 h, 73%

Electrospray (ESI)-mass spectra showed the signal at *m/z* 162 corresponding to the molecular ion of the protonated form, and fragmentation experiments on this selected mass value gave signals for the loss of ammonia, water, carbon dioxide and formic acid. High-resolution measurement by matrix-assisted laser desorption ionization (MALDI)-MS confirmed the molecular composition $C_6H_{12}NO_4$. ^{1}H-and ^{13}CNMR spectra recorded in D_2O allowed to fully assign the structure, supported by one bond and long range heterocorrelation (HSQC and HMBC, respectively). By infrared spectrum in attenuated transmitter reflection (ATR) configuration on a solid sample a strong peak at 1,716 cm^{-1} attributable to stretching of the CO group in the carboxylic unit was evident, as well as a very broad and intense signal centered at 2,910 cm^{-1} due to CH and OH stretching. The OH groups are probably involved in inter- and intra-molecular hydrogen bonds as suggested by the asymmetric broadening of the band and the decreased in wavenumber value if compared to the free group (Perrin and Nielson 1997). Otherwise, in the structure of amino acid **4**, or in its protonated form, the number of hydrogen bond donor/acceptor atoms is also higher than in hydroxylactone **3**, whose OH group was involved in intramolecular hydrogen bonding with the vicinal carbonyl group, as confirmed by FT-IR spectroscopic analysis in solid state and in solution (Dobrzynski et al. 2009).

The enantiomeric purity of **4** is expected by the use of hydroxylactone **3** as building block existing in pure enantiomeric form due to its natural origin from cellulose, combined to a sequence of reactions not affecting the configuration of the asymmetric centres. It is in line with the optical activity shown by the final aminoacid **4**, as well as for each intermediate of the synthesis. A further support came from chiral shift reagent study, where ^{1}HNMR spectrum of azido ester **7** was investigated in the presence of europium trifluorohydroxymethylene-D-camphorato, $Eu(tfc)_3$. The choice of the product **7** is imposed by its solubility in $CDCl_3$ and above all by the presence of a well-detectable singlet NMR signal (the COOMe group) to be monitored by addition of increasing amount of Eu-complex. Otherwise $Eu(tfc)_3$ was selected as the chiral shift reagent for its ability to complex with tertiary α-hydroxyl methyl esters (Fraser 1983). No splitting was observed for the COOMe singlet, neither for each other signal; these results speaking for the lack of diastereomeric complexes and confirming the enantiomeric purity of the compound, but shifts were observed for all the protons, in downfield as typical when induced by europium complexes. The strongest one was for OH proton, giving indication of the kind of chelation with this functional group, whereas a pronounced shift was obtained also for H-5 located in the same side of the plane with OH, thus confirming their relative *syn* position.

Sugar amino acid **4** as H-Gly-Ala-OH isoster

It has been reported that δ-amino acids are able to affect the structure of the peptides where they are involved (Edwards et al. 2006). Amino acid **4** is comparable to *H-Gly-Ala-OH* dipeptide, with the peculiarity of assuming a restricted conformation due to the presence of the tetrahydrofurane ring, formally derived by closure between oxygen atom of peptide bond and methyl group of alanine residue. In particular 2,5-*cis*-tetrahydrofurane amino acid provide building blocks for peptides, whose β-turn-type structure is due to hydrogen bonds, as predicted by calculation in the gas phase (Jockusch et al. 2006). By using density functional theory (DFT) method with polarizable continuum model, stable conformers can be calculated in water to simulate solvation effect (Iwaoka et al. 2002). Under these conditions, the comparison of energy minimized structures has been here achieved for *H-Gly-Ala-OH* dipeptide and compound **4** (Fig. 3). Fleet and co-workers, for example have shown that quite short oligomers of tetrahydrofurane dipeptide isosteres derived from carbohydrates adopt regular conformations in solution due to stabilization by hydrogen bonds (Smith and Fleet 1999).

In order to disclose the effect given by **4** as a replacement for the *Gly-Ala* residue in a peptide sequence, the values of the its dihedral angles corresponding to the rotations about OC–N bond (ω_i angle) and about N–CCO bond in the following aminoacid residue (Φ_{i+1} angle) as reported in Fig. 3, have been taken into account. In detail, the torsional angles were evaluated for the energy-minimized structures of compound **4** and *H-Gly-Ala-OH*

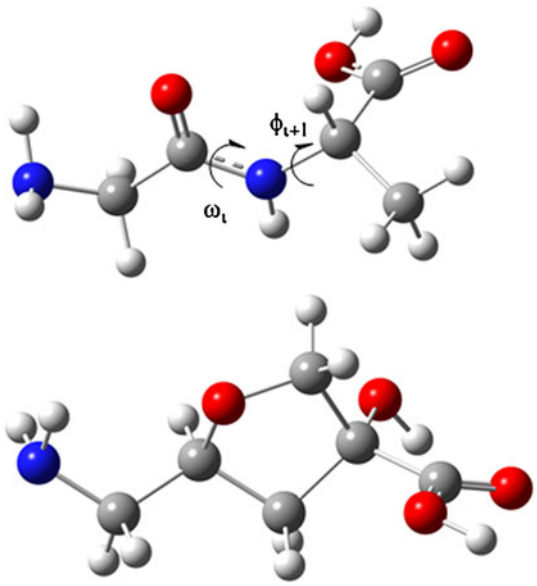
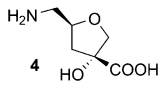
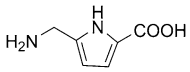


Fig. 3 Energy minimized structures by DFT-B3LYP 6–31 G (d) calculation in water of Gly-Ala dipeptide, with indication of the torsional angles (*top*), and of amino acid **4** (*bottom*)

Table 1 Torsional angles for the energy-minimized structures of sugar aminoacid **4**, *H-Gly-Ala-OH* and 5-(aminomethyl)pyrrole-2-carboxylic acid (**10**) by DFT-B3LYP 6–31 G (d) calculation in water

Compound	ω_i	Φ_{i+1}
H-Gly-Ala-OH	+173	−98
 **4**	−157	+145
 **10**	+178	+179

dipeptide by DFT calculation in water. A reversed trend was obtained (Table 1), so as to induce a probable change in conformation of the peptide with **4** replacing the *Gly-Ala* unit. Pyrrole amino acid **10** was also designed and reported as a constrained surrogate of *Gly-ΔAla* in peptidomimetic studies (Chakraborty et al. 2002). From a similar computation on this δ-amino acid we have obtained the dihedral angles reported in Table 1, showing an additional different combination of values, probably due to the presence of a planar structure.

Conclusion

The chiral building block hydroxylactone **3**, available in gram scale from cellulose pyrolysis in the presence of mesoporous material MCM-41 doped with tin, has been used for the synthesis of the new sugar amino acid **4**, obtained by an efficient route in five steps with a 67% overall yield. Standard functional group interconversions were applied, but each step was optimized for our substrates, as in the production of azido ester **7** where the use of acetonitrile in the place of water makes workup easier and improved the recovery of the product. Amino acid **4** is the first carbon-branched δ-tetrahydrofurane sugar amino acid obtained in free form (and isolated as protonated form), after the previous report of the scaffold described as azido methylester **1**. The structure of **4** in the protonated form was fully characterized by extensive NMR analysis, including experiments with chiral shifts' reagent in order to confirm its enantiomeric purity, and mass spectrometric measurements both by ESI-MS and high resolution MALDI-TOF/TOF experiments.

The amino acid reported here is expected to be a suitable monomer in the synthesis of peptides, as well as a building block towards new conformationally constrained peptidomimetics. It represents a *Gly-Ala* dipeptide isoster, with the peculiarity of assuming a restricted conformation. In order to evaluate the peculiarity of compound **4**, DFT calculation was performed and the values of torsional angles were compared to the ones computed for *H-Gly-Ala-OH* dipeptide and the known pyrrole amino acid **10**. These studies are under investigation.

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