Origin of problems related to Staudinger reduction in carbopeptoid syntheses

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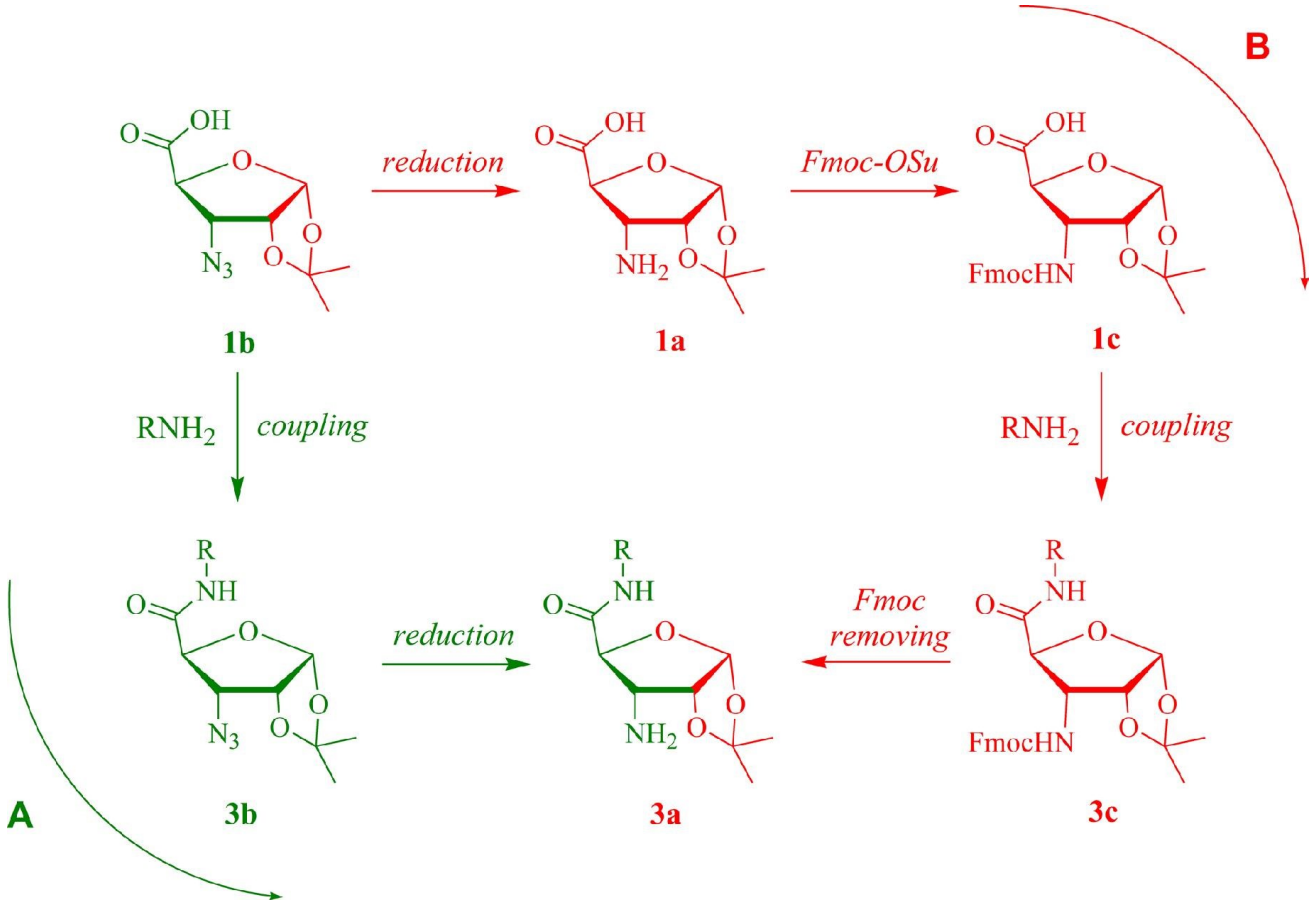
Abstract We report the solid phase synthesis of –GG-**X**-GG– type α/β-carbopeptoids incorporating RibAFU(ip) (**1a**, *t*X) or XylAFU(ip) (**2a**, *c*X) sugar amino acids. Though coupling efficacy is moderate, both the lengthier synthetic route using Fmoc deriva-tive (e.g., Fmoc-RibAFU(ip)-OH) and the azido deriva-tive (e.g., N_3-RibAFU(ip)-OH) via Staudinger reaction with nBu$_3$P can be successfully applied. Both X-ray dif-fraction, ^{1}H- and ^{31}P-NMR, and theoretical (QM) data support and explain why the application of Ph_3P as Staudinger reagent is "ineffective" in the case of a *cis* stereoisomer, if *c*X is attached to the preceding residue with a peptide (–CONH–) bond. The failure of the poly-peptide chain elongation with N_3-*c*X originates from the "coincidence" of a steric crowdedness and an electronic effect disabling the mandatory nucleophilic attack dur-ing the hydrolysis of a quasi penta-coordinated triph-enylphosphinimine. Nevertheless, the synthesis of the above α/β-chimera peptides as completed now by a new pathway via 1,2-*O*-isopropylidene-3-azido-3-deoxy-*ribo*-and -*xylo*-furanuronic acid (H-RibAFU(ip)-OH **1a** and H-XylAFU(ip)-OH **2a**) coupled with N-protected α-amino acids on solid phase could serve as useful examples and starting points of further synthetic efforts.

Introduction

Oligopeptides containing β-amino acids have become increasingly popular in foldamer chemistry and drug research. The enzyme resistance of α/β-peptides, coupled to their inherent feature of self-assembly (Beke et al. 2008) and a propensity for forming nanostructures, initiated stud-ies of particular importance, with different β-amino acids of modified side chains used as protein mimetics (Cheng et al. 2001; Hecht and Huc 2007; Horne and Gellman 2008; Pilsl and Reiser 2011; Guichard and Huc 2011; Martinek and Fülöp 2012; Cabrele et al. 2014; Mándity and Fülöp 2015). β-Amino acid building blocks of cyclic skeleton are of special interest, as the ring structure puts constraint on the central θ-backbone torsional angle adjacent to ϕ and ψ and thus stabilizes specific secondary structural elements (Beke et al. 2004). Recently, sugar amino acids (SAA), par-ticularly, five- or six-membered cyclic amino sugar carbox-ylic acids, appeared as appropriate building blocks for car-bopeptoid foldamers (Herradón and Seebach 1989; Kessler et al. 1995; Long et al. 1999; Simone et al. 2005; Sharma et al. 2008, 2011; Andreini et al. 2009). Numerous furanoid and pyranoid carbohydrate analogs of *trans*-2-amino-cyclo-pentane carboxylic acid (*trans*-ACPC) and *trans*-2-amino-cyclohexane carboxylic acid (*trans*-ACHC) were synthe-sized and built into homo- and heterooligomers to test their self-assembling abilities (Pandey et al. 2011; Giri et al. 2012; Risseeuw et al. 2013).

Amino furanuronic acids (AFUs, Fig. 1) and their homooligomers are part of several bioactive macromol-ecules (Gruner et al. 2001, 2002a; Chandrasekhar et al. 2009). Among them, H-RibAFU(ip)-OH, (**1a**) (Gruner et al. 2002b) and its *xylo* epimer H-XylAFU(ip)-OH (**2a**) (Chandrasekhar et al. 2004) as hydrophilic analogs of *cis*- and *trans*-ACPC monomers were incorporated into β-peptidic heterooligomers. Derivatives of the two epimers are preferred building blocks, as they can be synthesized from D-glucose. Furthermore, the relatively rigid conforma-tion of the furanoid ring, in between the ϕ and ψ torsional angles, might give the right amount of internal flexibility $\theta \sim 60 \pm 30°$ to the appropriately protected derivatives.

Syntheses of the homo- or heterooligomers of furanoid sugar amino acids (AFUs) were performed, hitherto, with conventional methods of peptide synthesis starting from

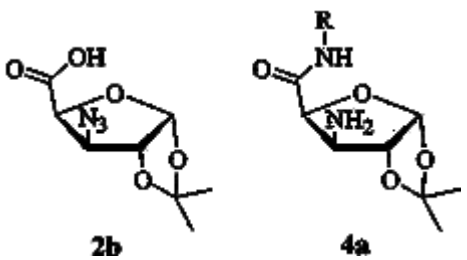


Scheme 1 Direct coupling of the "starting" azido compound (**1b**) followed by reduction provides in two steps (*A* route: **1b** → **3b** → **3a**) the very same product as the "conventional" Fmoc-strategy that provides in four consecutive steps (*B* route: **1b** → **1a** → **1c** → **3c** → **3a**)

various Fmoc- or Boc-protected AFU derivatives obtained from the corresponding azido-deoxy-furanuronic acids with catalytic hydrogenation and simultaneous Fmoc protection without isolation of the free β-amino acids (Gruner et al. 2002b; Chandrasekhar et al. 2004). Since the N_3 group could be considered as a "masked" NH_2 functionality, 3-azido-3-deoxy-furanoid intermediates should also be used, in principle, as temporary *N*-protected deriva-tives for direct peptide couplings. The reduction of the N_3 functionality after peptide bond formation—once cou-pling is completed—would result in the shortening of the synthetic pathway (the two-step route of **1b** → **3b** → **3a**) omitting the Fmoc protection/deprotection steps for each of the monomeric unit (Scheme 1) during the lengthier **1b** → **1a** → **1c** → **3c** → **3a** route.

Similar results might be expected from the *xylo* epimer **2b** on analogous pathways of giving product **4a** (Scheme 2).

This shortening of the reaction pathway, however, gives rise to difficulties during the catalytic hydrogenation of one N_3 group in the oligopeptides, particularly, during peptide



Scheme 2 The "starting" xylo epimer azido-compound (**2b**) and the related product (**4a**)

bond formation in the solid phase. Generally, problems arising from heterogenic phase hydrogenation can be overcome by reducing the azido group of a pentofuranuronic building block with tertiary phosphines under well-estab-azidosugars as early as 1964 (Messmer et al. 1964). The applicability of the Staudinger reaction in peptide chemistry was confirmed in the early 2000s (Saxon et al. 2000; Nilsson et al. 2000), and its applicability to solid phase synthesis was shown in the same year (Malkinson et al. 2000; Lundquist and Pelletier 2001). Staudinger ligation became a very useful technique in the field of bioconjugation methods (Schilling et al. 2011). An alternative could have been a 1,4-dithiothreitol (DTT)-mediated reduction on solid phase (Meldal et al. 1997; Tornøe et al. 2000); how-ever, both reaction conditions (higher *T*, e.g., 50 °C) and the requested reaction time make the latter reaction less favored.

Here, we present studies comparing the efficiency of A and B routes (Scheme 1) in making new –GG-**X**-GG– type pentapeptides, models used for studying the conforma-tional propensities of oligopeptides (Shi et al. 2005, Beck et al. 2008), where X is either RibAFU(ip) (**1a**, *t*X) or XylAFU(ip) (**2a**, *c*X). Comparing synthetic routes, yields, etc., we could elucidate the stereochemical influence of the *trans*(*ribo*) and *cis*(*xylo*) configuration both on the reduc-tion of the azido group and subsequent peptide bond for-mation. To our great surprise the phosphinimino group on the furanoid building block of *cis* (and only *cis*) conforma-tion proved unexpectedly stable. Although recently John and Wittmann (2015) published the reduction of a 3-azido-3-deoxy-ribofuranoid building block in a tetrapeptide with Me_3P, the unexpected stability of the intermediate phos-phinimino group demands explanation. We present a model based on X-ray diffraction, spectroscopic and theoretical (QM) data and describe multiple routes that allow efficient synthesis of α/β-heterooligopeptides in solid phase.

Experimental

Reagents and instrumentations

Reagents, starting materials and solvents were obtained from Alfa Aesar, Sigma-Aldrich, Merck, Reanal or VWR. Fmoc–Gly–Gly–OH was purchased from Reanal Private Ltd. For moisture-sensitive reactions, the solvents were distilled with standard procedures or dried by molecu-lar sieves in vacuo. Hydrogenation reactions were carried out in an H-Cube® and H-Cube Mini™ continuous-flow reactors (ThalesNano Inc, Budapest, Hungary). Column chromatography was performed on Kieselgel 60 silica gel (0.040–0.063 mm; Merck) with eluents indicated in the experiments. Reactions were monitored by thin-layer chromatography on Kieselgel 60 F_{254} (E. Merck). The plates were developed by UV detection (254 nm) and charring with aqueous sulfuric acid solution. Melting points were defined with a Boetius microscope apparatus. Optical rotations were measured by Carl Zeiss Jena Polamat A polarimeter. The products were analyzed by reverse-phase HPLC on a Phenomenex Jupiter C-18 column using water/acetonitrile mixtures of 0.1 % TFA in water (*A*) and 0.08 % TFA, 95 % acetonitrile in water (B) and UV detection at 220 and 280 nm. Some products were lyophilized and examined with Bruker Esquire 3000+ tandem quadru-pole mass spectrometer equipped with an electrospray ion source. The elemental analysis was performed on VARIO EL (Elementar Analysensysteme GmbH, Hanau, Germany) instrument. FTIR spectra were recorded with Bruker IFS 28 spectrometer by the ATR technique. 1D ^{13}C- and ^{31}P-NMR spectra were measured with Bruker Avance 250 spec-trometer in $CDCl_3$, D_2O, CD_3OD and DMSO-d_6 at room temperature. 2D NMR spectra were recorded with Bruker Avance-III 700 spectrometer at 288 K. Deuterated solvents were purchased from Sigma-Aldrich. The one-crystal dif-fraction data were recorded with a PANanalyticalXpert Pro MPD XRD X-ray diffractometer with X'celerator detector at the Budapest University of Technology and Economics.

Peptide synthesis

2-Chlorotrityl chloride resin was swollen in DCM. It was washed three times with DCM (10 mL/g resin) at room temperature. The Fmoc–Gly–Gly–OH (3 equiv. to the nominal capacity of the resin ~1.0 mmol/g) was dis-solved in DMF/THF 1:1 and DIEA (1 equiv.) was added to the solution. The reaction mixture was dropped slowly into the resin syrup after DIEA (2 equiv.) was added again. The reaction was allowed for 4 h or overnight. The resin was washed with 3× DCM, 3× *i*PrOH and 1× diethyl ether, and dried in vacuo. The capacity of the resin was determined by spectrometric measurement of the amount of Fmoc chromophore (Fmoc-piperidine adduct) released upon treatment of the resin with 20 % piperidine/DMF (Chan and White 2000).

The Fmoc group was removed by 2 % piperidine and 2 % DBU in DMF. The successful cleavage was analyzed by Kaiser test.

The synthesis of the model pentapeptides was contin-ued by Fmoc chemistry using DIC/HOBt or HATU/DIEA methods with repeated coupling if necessary. THF was used as cosolvent in the case of **1b** and **2b**.

After coupling the two azido derivatives (**1b** and **2b**), the efficiency was monitored by elemental analysis (N content).

The bench-scale test cleavage and the final cleavage from resin were carried out with DCM:MeOH:AcOH 8:1:1 mixture (5–10 mL/g resin) for 2 h. Resin was washed with 3× THF, 2× DMF, 2× *i*PrOH, 2× DMF, 2× *i*PrOH, 2× MeOH and 1× diethyl ether. The solvent was removed in

vacuo. Depending on the peptides, the crude product was precipitated with DCM/diethyl ether, EtOAc/hexane mixtures or lyophilized with dissolving water.

*1,2-O-Isopropylidene-3-amino-3-deoxy-α-d-ribofuranuronic acid (**1a**)*

1,2-*O*-Isopropylidene-3-azido-3-deoxy-α-d-ribofuranuronic acid (**1b**, 0.50 g, 2.18 mmol) was dissolved in dry pyridine (230.3 mL) and triphenylphosphine (1.262 g, 4.81 mmol) was added. The reaction mixture was allowed to stand for 2 h at room temperature. Ammonium hydroxide solution (25 %, 8.8 mL) was dropped to the mixture and it was allowed to stand for 3.5 h. The solution was filtered and concentrated in vacuo, the residue was treated with aqueous ammonia (12 %) and the solid containing Ph_3P and Ph_3PO was removed by filtration. Evaporation of the filtrate furnished the crude product which was tritu-rated with petroleum ether to give **1a** as pale yellow solid (0.412 g, conversion: 82 %, yield: 93 %). IR: 3170, 2750– 2460, 2170–1900, 1605 cm^{-1}. ESI-MS: [M+H] 204.1 *m/z*. ^{1}H-NMR ($CDCl_3$, 250 MHz): δ ppm 6.49 (broad m, N–H), 5.82 (d, $J_{1,2}$ = 3.4 Hz, 1H, H-1), 4.69 (t, $J_{2,3}$ = 4.5 Hz, 1H, H-2), 4.45 (d, $J_{3,4}$ = 9.5 Hz, 1H, H-4), 3.64 (dd, $J_{2,3}$ = 4.5 Hz, $J_{3,4}$ = 9.5 Hz, 1H, H-3), 1.54 and 1.34 (s, isopropylidene CH_3).

*N-9-Fluorenylmethyloxycarbonyl-1,2-O-isopropylidene-3-amino-3-deoxy-α-d-ribofuranuronic acid (**1c**)*

1,2-*O*-Isopropylidene-3-azido-3-deoxy-α-d-ribofuranuronic acid (**1b** 0.255 g; 1.11 mmol) was dissolved in methanol ($c \cong$ 35 mmol/l) and reduced with H_2 on 10 % Pd/C by H-Cube® reactor (parameters: 60 °C; 10 bar; 0.2 mL/min). After the reaction, the solution was concentrated in vacuo to give the product. The product is a pale yellow solid (0.20 g; 88 %). *Rf*: 0.51 (chloroform–methanol 3:2). FTIR–ATR: ν_{max}: 3170 (ν NH_3^+), 2750–2460 and 2170–1900 diffuse (ν NH_3^+), 1605 (ν COO–) cm^{-1}. ESI-MS: 204,1 $[M+H]^+$, fragment 146,2 $[M+H-58]^+$; 202,0 $[M-H]^-$; calculated: 203,19 *m/z*. ^{1}H NMR (D_2O, 250 MHz): δ 6,035 (d, 1H, H-1, $J_{1,2}$ = 3.7 Hz); 4,97 (t, 1H, H-2, $J_{2,3}$ = 4.6 Hz); 4,43 (d, 1H, H-4, $J_{3,4}$ = 10.1 Hz); 3,68 (dd, 1H, H-3, $J_{2,3}$ = 4.6 Hz, $J_{3,4}$ = 10.1 Hz), 1,58 (s, 3H, CH_3), 1,39 (s, 3H, CH_3) ppm.

The stirred solution of 1,2-*O*-isopropylidene-3-amino-3-deoxy-α-d-ribofuranuronic acid (0.20 g; 0.99 mmol) in methanol–water 2:1 (6.6 mL) is adjusted to pH 8 with saturated $NaHCO_3$ solution. The solution of Fmoc-OSu (0.37 g; 1.1 mmol; 1.1 equiv.) in THF (6.8 mL) was added to this solution and stirred for 48 h at room temperature. Solvents were removed in vacuo. The residue was suspended in water and extracted with EtOAc (3×). The combined organic phases were washed with $NaHCO_3$. The aqueous phase is adjusted to pH 1 with 2 N HCl and extracted with EtOAc (3×). The organic phase is washed with saturated NaCl solution, dried (MgSO4) and concentrated in vacuo. The product is a white solid (0.21 g; yield: 45 %). *Rf*: 0.50 (ethyl acetate–methanol 4:1); ESI-MS: 426.2 $[M+H]^+$; calculated: 425.19 *m/z*.

*N-Methyl-1,2-O-isopropylidene-3-amino-3-deoxy-α-d-ribofuranuronamide (**3a**)*

(A) *N*-Methyl-1,2-*O*-isopropylidene-3-azido-3-deoxy-α-d-ribofuranuronamide (**3b**, 0.054 g, 0.223 mmol) was dis-solved in a mixture of THF (3 mL) and methanol (1 mL). After stirring for 30 min under N_2, triphenylphosphine (0.088 g, 0.336 mmol) in THF (2 mL) and aqueous ammo-nium hydroxide (2 mL) were added to the reaction mixture. After stirring for 1.5 days, diluted aqueous ammonia (10 %) was added again to precipitate a mixture of Ph_3P and Ph_3PO. After filtration, the crude product was separated and recrystallized from ethyl acetate to give pure **3a** (0.037 g, conversion: 69 %, yield: 77 %); m.p.: 170–173 °C. *Rf*: 0.86 (chloroform–methanol 3:1). FTIR–ATR: 3375, 3353, 1652, 1545 cm^{-1}. ESI-MS: [M+H] 217.2 *m/z*. ^{1}H-NMR ($CDCl_3$, 250 MHz): δ ppm 6.47 (broad m, 1H, NH-CH_3), 5.77 (d, $J_{1,2}$ = 3.4 Hz, 1H, H-1), 4.57 (t, $J_{2,3}$ = 4.4 Hz, 1H, H-2), 4.06 (d, $J_{3,4}$ = 9.5 Hz, 1H, H-4), 3.12 (dd, $J_{2,3}$ = 4.4 Hz, $J_{3,4}$ = 9.5 Hz, 1H, H-3), 2.77 (d, J_{NH-CH3} = 5.0 Hz, 3H, amide-CH_3), 1.47 and 1.27 (s, isopropylidene CH_3).

(B) *N*-Methyl-1,2-*O*-isopropylidene-3-azido-3-deoxy-α-d-ribofuranuronamide (**3b**) (0.161 g, 0.665 mmol) was dissolved in dry pyridine (6 mL) and triphenylphosphine (0.338 g, 1.29 mmol) was added. The reaction mixture was made to stand for 2 h at room temperature. Then, aqueous ammonia (2.3 mL) was added and the mixture was left to stand for 2.5 h. The precipitate was filtered and the filtrate was concentrated in vacuo. Aqueous ammonia (10 %) was added again to precipitate a mixture of Ph_3P and Ph_3PO. After filtration and evaporation of the filtrate, the crude product was recrystallized from ethyl acetate to give pure **3a** (0.132 g, conversion: 82 %, yield: 92 %). *Rf*: 0.86 (chloroform–methanol 3:1). The spec-tral and chromatographic data are identical with those of product A).

*1,2-O-Isopropylidene-3-amino-3-deoxy-α-d-xylofuranuronic acid (**2a**)*

1,2-*O*-Isopropylidene-3-azido-3-deoxy-α-d-xylofuranuronic acid (**2b**, 0.937 g, 4.0 mmol) was dissolved in dry pyridine (35 mL) and triphenylphosphine (1.7 g, 6.4 mmol) was added. The mixture was stirred for 2–3 h at room temperature until TLC (hexane:EtOAc 1:4) indicated no starting compound and a solid precipitated. Aqueous ammonia (25 %, 13.2 mL) was added and stirring was continued for 2 h. The solid was filtered and the filtrate was concentrated in vacuo to give the crude product containing **2a** and Ph_3PO. The mixture was dissolved in dichloromethane, and ethyl acetate was added to precipitate a cream-colored solid. After filtration, the solid was triturated with methanol to afford pure **2a** as a white powder (0.763 g, conversion: 81 %, yield: 94 %), m.p.: >235°C. FTIR–ATR: 2977, 2465, 1637, 1560 cm^{-1}. ESI-MS: [M+H] 204.2 *m/z*. RP-HPLC: 18.7 min.

^{1}H-NMR (D_2O, 250 MHz): δ ppm 6.10–6.08 (H-1), 4.76–4.74 (H-4), 3.97–3.95 (H-2), 3.31–3.29 (H-3), 1.50 and 1.30 (s, isopropylidene CH_3).

*1,2-O-Isopropylidene-3-deoxy-3-triphenylphosphinimino-α-d-xylofuranuronic acid (**2g**)*

1,2-*O*-Isopropylidene-3-azido-3-deoxy-α-d-xylofuranuronic acid (**2b**, 0.13 g, 0.56 mmol) was dissolved in dry THF (3 mL), and triphenylphosphine (0.163 g, 0.62 mmol) was added. The reaction mixture was stirred for 2 h at room temperature until TLC (EtOAc:MeOH 4:1) indicated no starting compound. The solution was concentrated in vacuo to give a brown solid which was dissolved in THF again and dropped into hexane (20 fold volume). Filtration gave jonquil crys-tals (**2g**) (0.064 g, conversion: 49 %, yield: 25 %), m.p.: 132–136°C; R_f = 0.24 (MeOH:EtOAc 1:2). RP-HPLC: 14.58 min. ESI-MS: [M+H]: 464.3 *m/z*. FTIR–ATR: 2977, 2465, 1637, 1560 cm^{-1}. ^{1}H NMR ($CDCl_3$, 250 MHz): δ ppm 7.76–7.39 (aryl-H), 6.06 (H-1), 4.57 (H-4), 4.06 (H-2), 3.74–3.73 (H-3), $[\alpha]^{24}_{D}$ -5 1.50 and 1.03 (s, isopropylidene CH_3). ^{31}P NMR ($CDCl_3$, 250 MHz): δ ppm 29.23 (N=PPh_3).

*N-Methyl-1,2-O-isopropylidene-3-amino-3-deoxy-α-d-xylofuranuronamide (**4a**)*

N-Methyl-1,2-*O*-isopropylidene-3-azido-3-deoxy-α-d-xylofuranuronamide (**4b**, 0.05 g, 0.2 mmol) was dis-solved in pyridine (1 mL); then tributylphosphine (53.2 µL, 0.21 mmol) was added with syringe to the solution. The reaction mixture was stirred for 1.5 h. Dilution with water and addition of aqueous ammonia (25 %) resulted in a solid. After filtration and evaporation of the filtrate, the treatment of the residue was repeated to give a syrupy crude product which was purified by column flash chromatogra-phy with an eluent mixture EtOAc:MeOH 9:1. The fraction of high polarity (R_f ~ 0.1) afforded pure **4a** as white solid (0.015 g, conversion: 30 %, yield: 35 %), m.p.: >300°C. FTIR–ATR: 3403, 1683, 1412 cm^{-1}. ESI-MS: [M+H] 217.2 *m/z*. RP-HPLC 19.1 min. ^{1}H NMR (D_2O, 250 MHz): δ ppm 6.03–6.02 (H-1), 4.04–4.01 (H-4), 3.65–3.63 (H-2), 2.68–2.66 (H-3), 1.95 (NH-Me), 1.44 and 1.26 (s, isopro-pylidene CH_3).

*N-Methyl-1,2-O-isopropylidene-3-deoxy-3-triphenylphosphinimino-α-d-xylofuranuronamide (**4g**)*

N-Methyl-1,2-*O*-isopropylidene-3-azido-3-deoxy-α-d-xylofuranuronamide (**4b**, 0.3 g, 1.23 mmol) was dissolved in diethyl ether (3 mL); then triphenylphosphine (0.38 g, 1.44 mmol, 1.2 equiv.) was added. After stirring for 2 h, the reaction mixture was concentrated in vacuo and the resi-due was dissolved in ether (1 mL) and dropped into hexane (tenfold volume) to precipitate the product, which was fil-tered to give crystals of pure **4g** (0.045 g, conversion: 15 %, yield: 8 %), m.p.: 93–95°C; R_f = 0.22 (EtOAc:MeOH 9:1). ESI-MS: [M+H] 477.3 *m/z*. RP-HPLC: 18.81. FTIR– ATR: 3345, 2985, 1669, 1543 cm^{-1}. ^{1}H NMR (d_4-MeOH, 250 MHz): δ ppm 5.99–5.97 (H-1), 4.72–4.71 (H-4), 3.98– 3.97 (H-2), 3.94–3.93 (H-3), 2.77 (NH-Me), 1.41 and 1.24 (s, isopropylidene CH_3). ^{31}P NMR: δ ppm 29.1 (N=PPh_3).

*N-Methyl-1,2-O-isopropylidene-3-deoxy-3-triphenylphosphiniminio-α-d-xylofuranuronamide chloride (**4g·HCl**)*

To the solution of *N*-methyl-1,2-*O*-isopropylidene-3-azido-3-deoxy-α-d-xylofuranuronamide (**4b**, 1.0 g, 3.8 mmol) in methanol (40 mL) triphenylphosphine (1.59 g, 6.0 mmol) was added. Methyl *tert*-butyl ether was dropped to the reaction mixture until dissolution of triphenylphosphine. The mixture was stirred for 2–3 h at room temperature and then water was dropped into the solution until a precipi-tate appeared; then stirring was continued for 2–3 h. The mixture was filtered and the filtrate was concentrated in vacuo. Methanol was added to the residue and the undis-solved solid was filtered again. The filtrate was concen-trated and traces of water were removed by evaporation of propanol-2. The residue was dissolved in methyl *tert*-butyl ether (50 mL); the solution was acidified to pH 2–3 with 6 N methanolic hydrochloric acid and stirred overnight. The separated cream-colored solid was filtered to give crude **4g·HCl** (0.7 g, conversion: 70 %, yield: 36 %), m.p.: >235°C; R_f = 0.21 (EtOAc:MeOH 4:1); $[\alpha]_D^{25}$ −75 (c 1, EtOH). RP-HPLC: 15.37 min (R-N = PPh_3). FTIR–ATR: ν_{max}: 3330, 2983, 1807, 1754, 1702, 1526 cm^{-1}. ^{1}H NMR (d_4-MeOH, 250 MHz): δ ppm 6.12–6.11 (H-1), 4.64–4.62 (H-4), 4.53–4.51 (H-2), 3.64–3.58 (H-3), 2.65 (NH-Me), 1.30 and 1.20 (s, isopropylidene CH_3). ^{13}C NMR (d_4-MeOH, 250 MHz): δ 169.9 (C-5), 114.07 (C-7), 106.36 (C-1), 86.3–86.2 (C-2), 81.14–81.05 (C-4), 61.31 (C-3), 26.87 (C-6), 26.43–26.24 (C-8 and C-9). ^{31}P NMR: δ ppm 40.55 (NH^+=PPh_3). A sample of the crude **4g·HCl** was recrystallized from methanol to give large crystals appro-priate for X-ray diffraction analysis.

*N,N-Dimethyl-1,2-O-isopropylidene-3-amino-3-deoxy-α-d-xylofuranuronamide (**5a**)*

To the solution of *N*,*N*-dimethyl-1,2-*O*-isopropylidene-3-azido-3-deoxy-α-d-xylofuranuronamide (**5b**, 0.29 g, 1.13 mmol) in dry pyridine (3 mL) triphenylphosphine (0.326 g, 1.24 mmol) was added and the reaction mix-ture was standing for 2 h at room temperature. Water was added dropwise until a precipitate appeared, then aqueous ammonia (25 %) was added to the mixture and was left to stand overnight. The solid was filtered and the filtrate was concentrated in vacuo to give a drab solid containing **5a** and Ph_3PO. After repeated treatment of this mixture with aqueous ammonia, finally, the components were separated by flash column chromatography (ethyl acetate:methanol 9:1). The combined fractions containing the product were concentrated to give **5a** as a brown oil (0.13 g, conver-sion: 45 % yield: 50 %); R_f: 0.28 (EtOAc:MeOH 2:1);

24.7° (c 0.85, $CHCl_3$). FTIR–ATR: 3296, 2986, 1644 cm^{-1}. ESI-MS: [M+H] 231.2 *m/z*. RP-HPLC: 2.86, 7.58, 18.9. ^{1}H-NMR (250 MHz, $CDCl_3$): δ ppm 5.89–5.87 (H-1), 4.72–4.71 (H-4), 4.3–4.29 (H-2), 3.56–3.55 (H-3), 2.97–2.83 (NMe2), 1.89 (NCH_3), 1.36 and 1.17 (s, iso-propylidene CH_3). ^{13}C NMR ($CDCl_3$, 250 MHz): δ ppm

167.72 (C=O), 111.44 (*C*-NMe_2), 104.45 (C-1), 85.58 (C-2), 77.91 (C-4), 58.27 (C-3), 36.9, 35.6 (N-*C*H_3), 26.6, 25.9 (*C*H_3).

*N,N-Dimethyl-1,2-O-isopropylidene-3-deoxy-3-triphenylphosphinimino-α-d-xylofuranuronamide (**5g**) and N,N-dimethyl-1,2-O-isopropylidene-3-amino-3-deoxy-α-d-xylofuranuronamide (**5a**)*

To the solution of *N*,*N*-dimethyl-1,2-*O*-isopropylidene-3-azido-3-deoxy-α-d-xylofuranuronamide (**5b**, 0.30 g, 1.16 mmol) in diethyl ether (3 mL), triphenylphosphine (0.335 g, 1.27 mmol) was added. After standing for 2 h at room temperature, the reaction mixture was concen-trated in vacuo. The residue was dissolved in ether (1 mL) and dropped into hexane (tenfold volume); it was then allowed to stand overnight at 0 °C when crystals of **5g** separated (0.029 g, conversion: 10 %); m.p.: 60–64 °C; *Rf*: 0.2 (EtOAc:MeOH 4:1). FTIR–ATR: 3227, 2931, 1643, 1436 cm^{-1}. ESI-MS: [M+H] 491.4 *m*/*z*. RP-HPLC: amine 14.47, two phosphinimine conformers 18.89 and 28.31; ^{1}H NMR (250 MHz, $CDCl_3$): (a) iminophosphorane major conformer δ ppm 7.63–7.50 (aryl-H), 5.96–5.94 (H-1), 4.78–4.77 (H-4), 4.36–4.34 (H-2), 4.98–4.96 (H-3), 3.04 and 2.90 (2 NMe), 1.44 (s, 3H, CH_3), 1.25 (s, 3H, CH_3); (b) iminophosphorane minor conformer δ ppm 7.47–7.37 (aryl-H), 5.90–5.89 (H-1), 4.95–4.94 (H-4), 4.51–4.49 (H-2), 4.89–4.87 (H-3), 2.99 and 2.87 (2 NMe), 1.44 and 1.22 (2 CH_3) ppm; (c) amino compound δ ppm 6.24–6.22 (H-1), 4.89–4.86 (H-4), 4.29–4.28 (H-2), 3.64–3.63 (H-3), 2.72 and 2.53 (2 NMe), 2.16 (C^3-NH_2), 1.36 and 1.17 (s, isopropylidene CH_3).

Computation methods

Calculations were carried out using the Gaussian09 software (Gaussian 09, Frisch et al. 2009). The lowest energy conformers were obtained by completing the appropriate scans around the psi torsional for compounds **2g**, **4g** and **5g** at B3LYP/6-31+G(d) level of theory. The final geometry optimization and frequency calculations were done at the B3LYP/6-311++G(d,p) level, both in vacuo and in water (IEFPCM solvent model). Natural bond orbital (NBO) analysis was performed using the NBO 5.9 program (Glendening et al. 2001), using the in-house developed vis-ualization module.

Results and discussion

The syntheses of precursors N_3-RibAFU(ip)-OH (**1b**) and N_3-XylAFU(ip)-OH (**2b**) was completed using partly the well-known general pathways starting from d-glucose (Gruner et al. 2002b; Chandrasekhar et al. 2004). The 2-chlorotrityl chloride (2-Cl-Trt) resin was used as it offers the advantage of easy ligation of the first amino acid with ester bond and then after the final coupling the easy cleavage of the polypeptide under mild acidic conditions (Barlos et al. 1991; Kocsis et al. 2006; García-Martín et al. 2007). Determination of theoretical capacity and coupling efficiency of the resin was completed by elementary analysis (N %) and measurement of Fmoc capacity (Table 1). The first two glycine moieties were linked to the resin in the usual manner with Fmoc-GG-OH, followed by the subsequent removal of Fmoc protection. Ligation of both **1b** and **2b** was performed to the free N-terminus of the second Gly, either with the DIC/HOBt or HATU/DIEA coupling method in DMF:THF(1:1). Successful formation of azide intermediates **1d** and **2d** was established with bench-scale test cleavages giving **1e** and **2e** α/β-tripeptides, the structure of which was corroborated by the N_3 band at the IR spectrum (2113–2116 cm^{-1}).

Subsequent treatment of the sterically hindered **2d** with Ph_3P in THF/MeOH at room temperature provided Ph_3P=N-*c*XGG-*Resin*. Formation of it was shown by acidic cleavage of a portion from the resin giving Ph_3P=N-*c*XGG-OH: supported by the appearance of the singlet peak at 40.5 ppm (^{31}P-NMR) and the lack of the N_3 IR band. To get the appropriate amine, **2h**, several attempts were car-ried out under usual conditions with NH_4OH. However, hydrolysis was unsuccessful even if stronger bases, like tri-ethylamine (TEA), *N*,*N*-diisopropylethylamine (DIEA) and

Table 1 Selected analytical measures of the solid phase synthesis of pentapeptides: on-resin coupling and efficiency

Cl-Trt resin	Coupling with:	Fmoc-GG-OH				
	Resin capacity (mmol/g)[a]	0.59				
H-GG-Trt resin	Coupling with:	**1b**		**2b**		**1c**
	N-content (%)	3.15[b]	3.59[c]	2.27[b]	2.30[c]	2.13[c]
	Residual capacity (mmol/g)	0.45	0.51	0.32	0.33	0.51
	Efficiency of coupling (%)[d]	76	86	54	56	86
N-term deprotection	Efficiency of $-NH_2$ formation (%)[e,f]	31	37	27	18	>90
H-XGG-Trt resin	Coupling with:	Fmoc-GG-OH				
	Residual capacity (mmol/g)[a]	0.067	0.091	0.042	0.028	0.25
	Apparent efficiency of coupling (%)[d]	15	18	13	9	49
Fmoc-GGXGG-Trt resin	Overall efficiency (%)	11	15	7	5	42

[a] Fmoc capacity

[b] DIC/HOBt coupling [c] HATU/DIEA coupling

[d] 2 equiv. reagent used, unless mentioned otherwise [e] nBu$_3$P/OH or piperidine

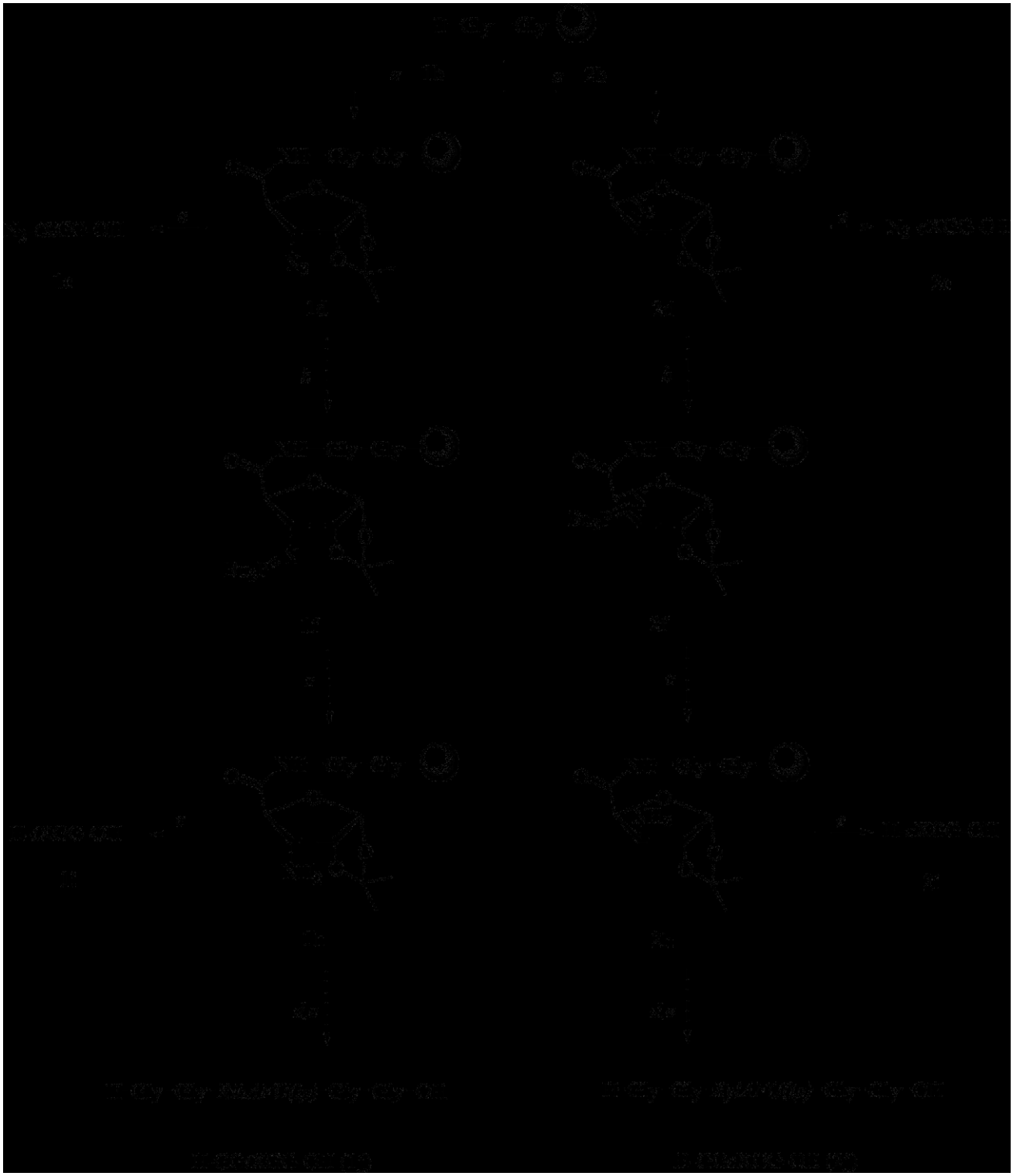
[f] Estimated based on back-calculations

ethanolic KOH were used. The unexpectedly high stability of the intermediate triphenylphosphinimine impeded the formation of **2h** and, thus, the forthcoming peptide bond formation. Unlike Ph$_3$P, when the more reactive nBu$_3$P was used (Scheme 3), reduction of N_3– was achieved. The application of the Me$_3$P (John and Wittmann 2015) was omitted as it is an expensive and hazardous reagent, and thus unfit for large scale production. Furthermore, **1d** gave Ph$_3$P=N-*t*XGG with Ph $_3$P in THF/MeOH at room temperature, hydrolyzed to **1h**, leading to the conclusion that in case of the *trans* isomer the nucleophilic attack on the P-atom is easy to achieve (see below).

Regardless of the local *cis* or *trans* configuration of X, both **1d** and **2d** with nBu$_3$P in THF-MeOH 1:1 gave nBu$_3$P=N-intermediates which could be hydrolyzed with TEA and DIEA in the same solvent to give **1h** and **2h**, respectively. Bench-scale test cleavages gave intermediates H-*t*XGG-OH (**1i**) and H-*c*XGG-OH (**2i**) characterized by IR bands of –CONH– and $-NH_2$ groups in the region of 1665–1663, 1596–1541, 1460–1445 cm^{-1} and, at the same time, by the absence of the $-N_3$ band. In accordance, no signal was found in ^{31}P-NMR, revealing the 100 % conversion to **1h** and **2h** (no traces of phosphinimines were present after hydrolysis). Coupling of both **1h** and **2h** with Fmoc-GG-OH gave Fmoc-GG*t*XGG-*Resin* and Fmoc-GG*c*XGG-*Resin* intermediates (Table 1) converted directly into the final products. Identification of both pentapeptides, H-GG*t*XGG-OH (**1j**) and H-GG*c*XGG-OH (**2j**), was corroborated by ESI-MS ([M+H]$^+$ = 432.3) after cleavage from the resin under usual conditions. The very same pentapeptide incorporating *trans*X, H-GG*t*XGG-OH (**1j**) was synthesized using Fmoc-RibAFU(ip)-OH (**1c**) building block, along route **B** (Scheme 1) (for coupling efficacy see Table 1).

Data of these syntheses were compared and analyzed in two ways: (i) efficiency of route A and B giving the same ribofuranuronic product H-GG*t*XGG-OH (**1j**) and (ii) efficiency differences between the synthesis of ribofuranuronic H-GG*t*XGG-OH (**1j**) and xylofuranuronic H-GG*c*XGG-OH (**2j**) products, respectively. Comparison of the data of the two routes (Table 1) revealed a significant difference in the overall efficiency of both coupling methods in favor of route B: 42 vs. 15 % (or 11 %) for *t*X. Coupling of both ribofuranuronic building blocks **1b** and **1c** indicated the same efficiency: ~86 %. On the contrary, coupling of the ribofuranuronic building block (*trans* **1b**) vs the xylofura-nuronic one (*cis* **2b**) favored the *trans* diastereomer (76 vs. 54 % or 86 vs. 56 %). Consequently, the final differ-ence between route A and B obviously originates from the azide-phosphinimine-amine steps, or rather, from the difficulties of the hydrolysis of phosphinimines, contrary to the often used Fmoc deprotection. The stability of the triphenylphosphinimino group in Ph$_3$P=N-*c*XGG-*Resin* as well as in the tripeptide Ph$_3$P=N-*c*XGG-OH is unprec-edented. Generally, hydrolysis of any sugar phosphinimine will spontaneously take place, resulting in the correspond-ing aminosugar in alcoholic or aqueous solutions [e.g., β-D-glycopyranosyl-triphenylphosphinimine in water afforded β-D-glycopyranosyl amine with excellent yield (Kovács et al.1985)].

In conclusion, –GG-*t*X-GG– can be synthesized in solid phase support using N_3-*t*X-OH with the "milder" Ph $_3$P or the more reactive nBu$_3$P reagent via the Staudinger reaction, as well as by Fmoc-*t*X-OH (Table 1). Acylation with the *N*-protected sugar amino acids is of acceptable efficacy (76–86 %) for the *trans* and lower but still acceptable for the sterically more compact *cis* derivative (54–56 %). The subsequent acylation of these α/β-tripeptides with Fmoc-GG-OH, resulting in the appropriate α/β-pentapeptides is poorer (~50 %), as a primary amine needs to be coupled. However, the bottleneck of the total synthesis (Scheme 3) is clearly the $-N_3 \rightarrow -NH_2$ reduction: even with the more reactive nBu$_3$P efficacy can be as low as 18 % for the *cis*



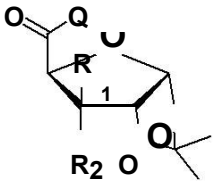
Scheme 3 Solid phase synthesis of α/β-pentapeptides. Reagents and conditions: *a* HOBt/DIC or HATU/DIEA; *b* nBu_3P, THF/MeOH, rt.; *c* TEA, DIEA, THF/MeOH, RT; *d* Fmoc-GG-OH with HOBt/DIC or HATU/DIEA; *e* DCM:MeOH:AcOH (8:1:1)

and 31 % for the *trans* derivatives. Below, we present a rational explanation of these differences by means of diffraction, spectroscopic and computational data.

As the least efficient step of the solid-phase syn-thesis is the otherwise attractive Staudinger reaction ($–N_3$ to $–NH_2$); both **1b** (N_3-RibAFU(ip)-OH) and **2b**

Scheme 4 Staudinger reaction of N_3-RibAFU(ip)-OH and N_3-XylAFU(ip)-OH as well as their derivatives, conditions: *i* Ph_3P, pyridine; *ii* aq. NH_4OH

Table 2 Reaction of 1,2-*O*-isopropylidene-3-azido-3-deoxy-α-D-furanuronic compounds with Ph_3P in solution



	Products[a]	Conversion (yield)[b]
1b (Q: OH, R^1: H, R^2: N_3)	**1a** (Q: OH, R^1: H, R^2: NH_2)	82 % (93 %)
	1g (Q: OH, R^1: H, R^2: N=PPh_3)	No data
2b (Q: OH, R^1: N_3, R^2: H)	**2a** (Q: OH, R^1: NH_2, R^2: H)	81 % (94 %)
	2g (Q: OH, R^1: N=PPh_3, R^2: H)	49 % (25 %)
3b (Q: NHMe, R^1: H, R^2: N_3)	**3a** (Q: NHMe, R^1: H, R^2: NH_2)	69–82 % (77–92 %)
	3g (Q: NHMe, R^1: H, R^2: N=PPh_3)	No data
4b (Q: NHMe, R^1: N_3, R^2: H)	**4a** (Q: NHMe, R^1: NH_2, R^2: H)	0 %
	4g (Q: NHMe, R^1: N=PPh_3, R^2: H)	15 % (8 %) [HCl salt 70 % (33 %)]
5b (Q: NMe_2, R^1: N_3, R^2: H)	**5a** (Q: NMe_2, R^1: NH_2, R^2: H)	45 % (50 %)
	5g (Q: NMe_2, R^1: N=PPh_3, R^2: H)	10 %

[a] Note that the general reaction route **b** → **a** via **g** follows the path according to Scheme 4

[b] Conversions and yields of amines and triphenylphosphinimines calculated from the starting azides

(N_3-XylAFU(ip)-OH) as well as their selected derivatives, N_3-RibAFU(ip)-NHMe (**3b**) and the corresponding *xylo* epimer N_3-XylAFU(ip)-NHMe (**4b**) were studied in solution. (The latter amide derivatives were synthesized with conventional amidation from **1b** and **2b,** and **4b** and **5b** derivatives from the reaction conducted with $NHMe_2$.) All azido monomers were reacted with triphenylphosphine in solution under the usual conditions (Scheme 4), the intermediate phosphimines were treated in situ with bases, typically with NH_4OH (Scheme 4) leading to the appropriate product (Table 2).

Interestingly, both C-3-epimeric (free) acids, **1b** and **2b,** when reacted in solution with Ph_3P, followed by in situ phosphinimine hydrolysis (NH_4OH/H_2O, pyridine at room temperature) afforded the expected furanuronic acids **1a** and **2a**, respectively (yields: 93–94 %). Both products were isolated as white solids identified by IR and ^{1}H-NMR spec-tra as well as with ESI-MS data.

Both of the above reactions, **1b** → **1a** and **2b** → **2a** proceed via the appropriate phosphinimino-intermediate, **1g** and **2g**. For the *trans*(*ribo*) derivative, **1g**, hydrolysis is spontaneous and quick and thus the –N=PPh_3 intermediate was not isolated, while for **2g**, the *cis* or *xylo* variant was obtained in stable crystalline form. The higher stability and thus a slower hydrolysis of the **2g** intermediate can be attributed to the intramolecular protonation of the N-atom of –N=PPh_3 by the adjacent and particularly close –COOH group at C-4 (see computations below). Note that in the case of **1g,** no such ion pair was formed in solution due to steric reasons, which resulted in its spontaneous hydrolysis and lower stability.

The same two-step reaction from **3b** lead to **3a** and the crystalline 3-amino-3-deoxy derivative was isolated in high yield (92 %), characterized by IR, ^{1}H-NMR and ESI-MS data. In contrast to the above, under similar reaction con-ditions, **4b** afforded nothing of 3-amino-3-deoxy product **4a** (0 %) and only traces of the intermediate triphenylphos-phinimine **4g** were detected. In a separate experiment under modified conditions, **4b** was transformed into the phos-phinimine intermediate **4g**, but still in poor yield. Though

Scheme 5 Reaction conditions: *a* ClC(OOiBu)H, $MeNH_2$; *b* Ph_3P, pyridine; *c* aq.NH_4OH; *d* Ph_3P, Et_2O; *e* HCl, MeOH

obtained, **4g** could be hydrolyzed into **4a** not even with stronger bases, such as trimethylamine (TEA), *N*,*N*-diisopropylethylamine (DIEA) and ethanolic KOH! The unexpectedly high stability of **4g** is further supported by the lack of characteristic phosphinimine reactions (e.g., with acetyl chloride, acetic anhydride or *p*-methoxybenzaldehyde) (Gololobov 1992). Subsequently, the stable crystalline tri-phenylphosphiniminium salt **4g·HCl** was isolated in good yield (70 %, Scheme 5). The presence of the phosphin-imine substituent was corroborated by ^{31}P-NMR spectra exhibiting characteristic signal of –N=PPh_3 at δ 29.10 ppm and that of –NH^+=PPh_3 at 40.55 ppm in **4g** and **4g·HCl**, respectively. Note that the target compound **4a** could only be obtained as a white solid from **4b** using *n*Bu_3P reagent instead of Ph_3P.

To elucidate the role of the carboxamido group, the related *N,N*-dimethylamide derivative **5b**, N_3-XylAFU(ip)-NMe_2, was synthesized from **2b** under similar conditions. Compound **5b** was treated with Ph_3P in pyridine and subsequently hydrolyzed by aq. NH_4OH to give the expected product **5a**, H-XylAFU(ip)-NMe_2. Though the yield of **5a** was moderate, the intermediate triphenylphosphinimine **5g** was not detected. This indicates that hydrolysis of **5g** takes place in essence, although the steric effect of the adjacent bulky dimethylcarboxamido group can still hinder an external nucleophile to reach the central P-atom. This gives some residual stability to **5g** as a triphenylphosphinimine, but this effect is indeed smaller compared to **2g** or **4g**.

To better understand the stability of iminophosphoranes at C-3 of furanoses, conformational search and subsequent DFT calculations were carried out in vacuo and in water on **2g**, **4g** and **5g**. The structural parameters were calculated of these three triphenylphosphinimine compounds to explain the differences in their reactivity. The results of ab initio calculations for the conformers with carboxylic and carboxamide groups at C-4 of the furanoid ring, i.e., those of **2g**, **4g** and **5g** are summarized in Table 3.

In case of the free acid **2g** in water, the **2g**_*conf2* is the most favored structure in which the OH of the 4-COOH group is directed toward the ylide *N*-atom of the N=PPh_3 group at C-3 atom of the ring (Fig. 2a).

This arrangement leads to the intramolecular protonation of the partially negative $N^{\delta-}$-atom resulting in explicit

Table 3 Ab initio determined relative energies of key phosphinimine intermediates and selected structural parameters

	Conformers		ψ(°)	$d_{O\ldots N}$ (Å)	$d_{O\ldots P}$ (Å)	$G_{298.15\ K}$ (kcal/mol)	Boltzmann distribution at T = 298 K (%)
Q: OH (**2g**)	In vacuo	Conf1	−47.0	4.1	5.7	4.13	0.1
		Conf2	46.6	4.1	5.4	1.45	7.9
		Conf3	*111.2*	*3.3*	*4.3*	*0.00*	*92.0*
	In water	Conf1	−35.3	4.1	5.6	1.66	5.6
		Conf2	*34.6*	*4.1*	*5.5*	*0.00*	*94.0*
		Conf3	108.3	3.3	4.4	3.31	0.3
Q:NHMe (**4g**)	In vacuo	Conf1	−83.7	3.2	4.3	5.84	0.0
		Conf2	*121.5*	*3.4*	*3.8*	*0.00*	*100.0*
	In water	Conf1	−76.0	3.3	4.7	1.16	12.4
		Conf2	*115.7*	*3.5*	*4.0*	*0.00*	*87.6*
Q: NMe_2 (**5g**)	In vacuo	Conf1	−81.9	3.3	4.4	3.08	0.3
		Conf2a	*147.9*	*3.2*	*3.4*	*0.20*	*41.5*
		Conf2b	*123.6*	*3.4*	*3.8*	*0.00*	*58.2*
	In water	Conf1	*−77.0*	*3.3*	*4.7*	*0.00*	*97.7*
		Conf2a	141.5	3.2	3.6	3.60	0.2
		Conf2b	108.6	3.6	4.2	2.26	2.1

Italics indicate the low-energy conformers

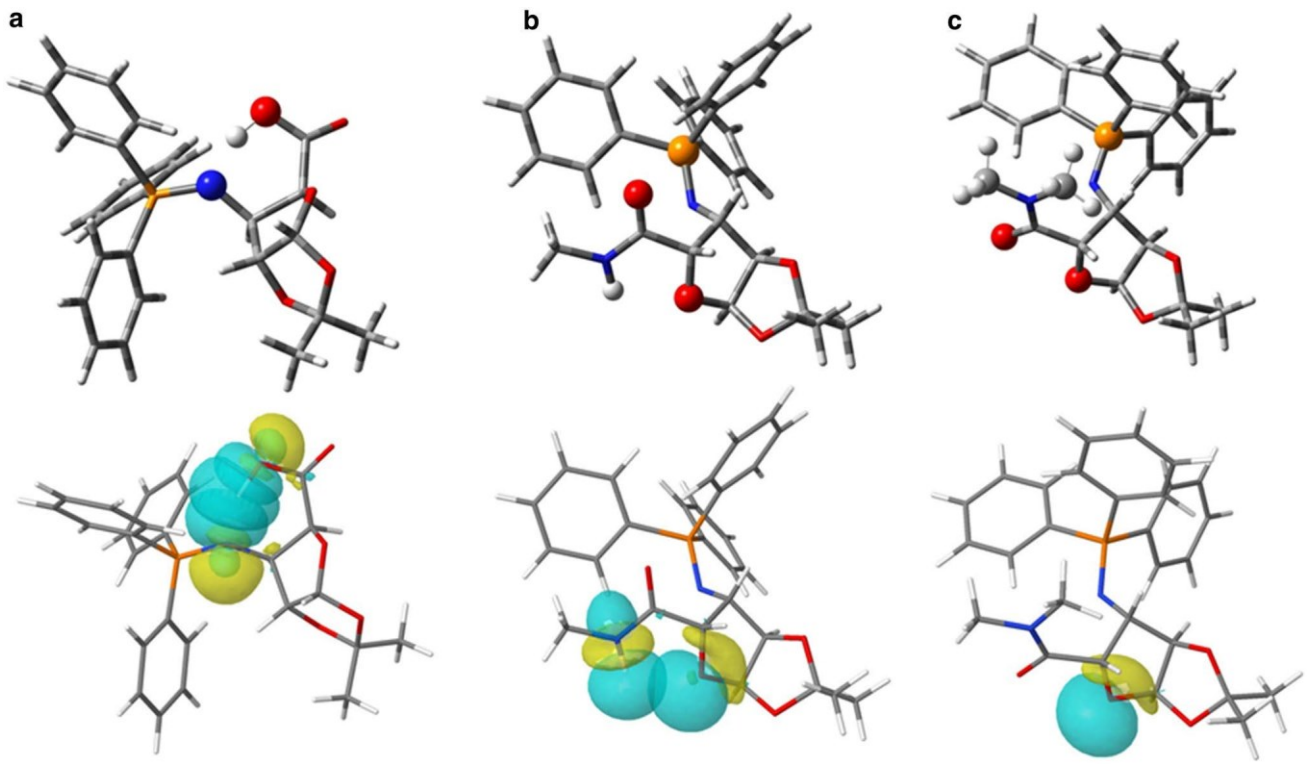
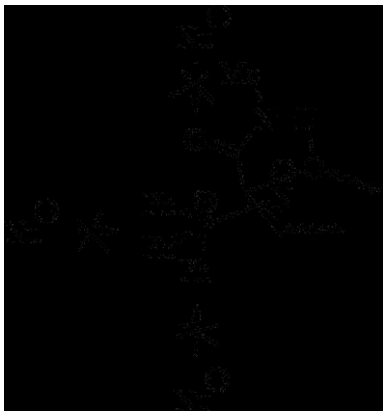


Fig. 2 Graphical representation with selective NBOs of key interactions in water of the lowest energy conformers of the appropriate phosphinimine: **a 2g**_conf2 (lone pair (LP) of N and antibond (BD*) of OH), **b 4g**_conf2 (lone pair (LP) of O(ring) and antibond (BD*) of NH) and **c 5g**_conf1 (lone pair (LP) of O(ring))

P^+-cation stabilized by the 4-COO^- anion (the calculated Mulliken charge on P is +0.16). The P^+-cation can be easily attacked by an external nucleophile to give the expected 3-amino-3-deoxy product (**2a**, H-XylAFU(ip)-OH) in a fast and quantitative manner.

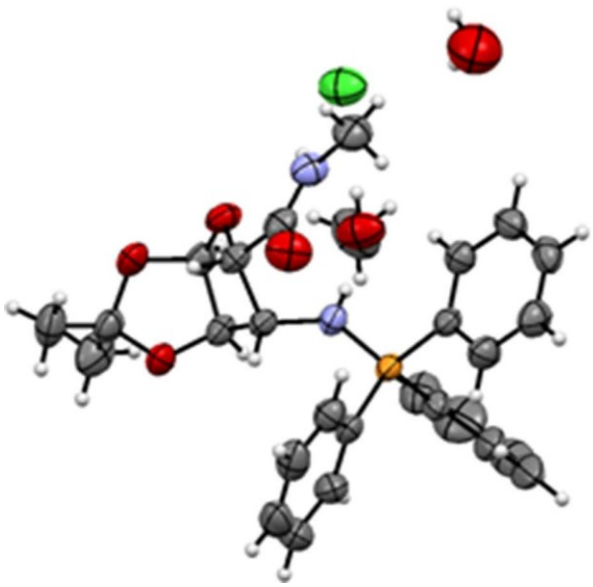
In contrast to this, as in the structure of the *N*-methyl-furanuronamide **4g,** the O=C-NHMe group (at C-4) forms a N–H..O-bridge with the oxygen atom of the furanoid ring stabilizing **4g**_*conf2*, in which there is no transferable proton for the ylide N-atom. In this conformer, the oxygen of the O=C group is oriented toward the partially positive $P^{\delta+}$-atom of the Ph_3P=N group at C-3 (Fig. 2b, the calculated Mulliken charge on P is +0.38). Due to the spatial arrangement of the three phenyl substituents around the P-atom, the equatorial N-atom of P=N along with the lone pairs of the carbonyl oxygen form a quasi penta-coordinated structure (Scheme 6) in which the unoccupied 3s* orbital of the central P-atom is in axial position shadowed by a lone pair of the carbonyl oxygen. Lower electron density on phosphorus atom enhances this interaction. This quasi penta-coordinate phosphorane system leaves no possibility for an external nucleophile to attack successfully on this central P-atom. Consequently, hydrolysis into 3-amino-3-deoxy product (**4a**, H-XylAFU(ip)-NHMe) does not occur due to the unexpected stability of the triphenylphos-phinimine compound **4g.**



Scheme 6 Tetra-coordinated P^+ in triphenylphosphinimine enables, while quasi penta-coordinated disables, hydrolysis via Nu attack

The reactivity of the triphenylphosphinimino compound **5g** has been found partly similar to that of the related **2g**. The difference is that in **5g** no transferable proton is given for producing an explicit P^+-cation in the Ph_3P=N group at C-3. Furthermore, there is no possibility in $-CONMe_2$ for an N–H..O-bridge to hinder the rotation of the carboxamide moiety. In addition, the electron density on P allows a nucleophilic attack on it (Fig. 2c, the calculated Mulliken charge on P is +0.29). Consequently, successful hydrolysis to 3-amino-3-deoxy compound **5a** (H-XylAFU(ip)-NMe_2) has occurred; however, due to the steric hindrance of the larger O=C-NMe_2 group the reaction rate is slower, compared to **2g**.

The above detailed theoretical considerations concerning **4g**, namely, the quasi penta-coordinated key structure, was also supported by the X-ray diffraction data (Fig. 3) of its protonated salt (**4g·HCl)**. Once again as in **4g**_*conf2*, the



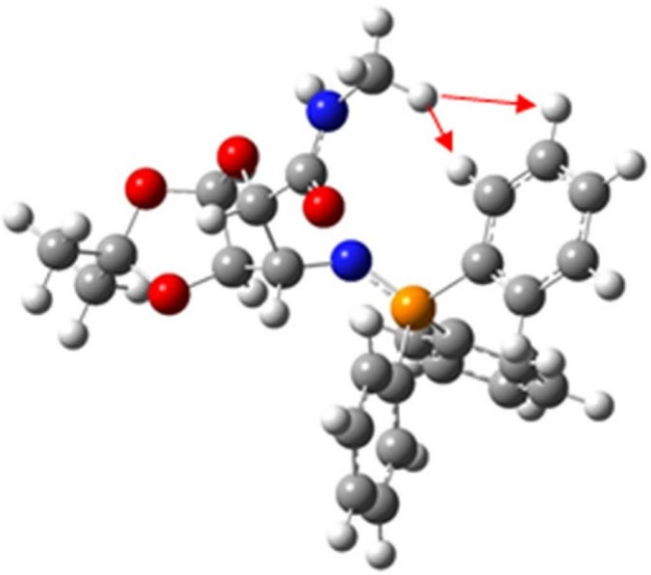


Fig. 3 Crystal structure of *N*-methyl-3-deoxy-3-triphenylphos-phiniminio-1,2-*O*-isopropylidene-α-D-xylofuranuronamide chloride (**4g·HCl**) by X-ray diffraction. Calculated structure of **4g**_conf2 in water (B3LYP/6-311++G(d,p), IEFPCM solvent model//B3LYP/6-31+G(d)). The *arrows* indicate the observed ROE cross-peaks

key role of N–H..O-bridge is evident, namely its anchoring the same conformation of the CONHMe moiety ($\psi^{\text{X-ray}}$ = 108.9°, $\psi^{\text{ab initio}}$ = 115.7°), positioning the O-atom of C=O into the vicinity of the central P-atom ($d^{\text{X-ray}}$ = 3.9 Å, $d^{\text{ab initio}}$ = 4.0 Å). This geometrical constellation makes any external nucleophilic attack disabling the hydrolysis impos-sible. Thus, the X-ray-determined crystalline conformer can be considered the frozen, stable and thus unreactive intermediate.

Based on homonuclear 2D ROESY NMR measurements ($CDCl_3$ at T = 288 K), the very same conformer in protic solution could be confirmed for **4g** by the strong ROE cross-peaks of the $H^{\delta}_{\#}$ and $H_{\varepsilon\#}$ protons of the phenyl rings with the methyl protons of the methylamide group (Fig. 3).

Conclusion

We have shown that the –GG-**X**-GG– type α/β pentapep-tides, both the RibAFU(ip) (**1a**, *t*X) and the XylAFU(ip) variant (**2a**, *c*X), can be synthesized on a solid state sup-port. Though coupling efficacy is moderate, both the lengthier synthetic route using Fmoc derivative (e.g., Fmoc-RibAFU(ip)-OH) and the azido derivative (e.g., N_3-RibAFU(ip)-OH) via Staudinger reaction with *n*Bu_3P can be successfully applied. X-ray diffraction, spectro-scopic and theoretical (QM) data support and explain why the application of Ph_3P as Staudinger reagent is "ineffec-tive" in the case of the *cis* stereoisomer (N_3-XylAFU(ip)-Gly-Gly-*Resin*) if *c*X is attached to the preceding residue with a peptide (–CONH–) bond. The failure of the polypep-tide chain elongation with N_3-*c*X originates from the "coin-cidence" of a steric crowdedness and an electronic effect disabling the mandatory nucleophilic attack during the hydrolysis of the quasi penta-coordinated triphenylphos-phinimine. This is in line with results of the reactions of the azido-furanuronic compounds with triphenylphosphine in solution. In spite of the herein revealed synthetic challenge of making α/β-chimera peptides on solid phase support, we have accomplished the synthesis of –GG-**X**-GG– type α/β-carbopeptides. On a new pathway 1,2-*O*-isopropylidene-3-azido-3-deoxy-*ribo*- and -*xylo*-furanuronic acids, namely that of H-RibAFU(ip)-OH (**1a**) and H-XylAFU(ip)-OH (**2a**) were coupled with *N*-protected α-amino acids on solid phase. The herein described synthetic routes are general by nature and could serve as useful examples for further synthetic works badly needed for making more effectively enzyme-resistant, inherently self-assembling and occasion-ally nanostructure-forming α/β-peptides for drug discovery.

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