

Origin of problems related to Staudinger reduction in carbopeptoid syntheses

Barbara Csordás¹ · Adrienn Nagy¹ · Veronika Harmat¹ · Virág Zsoldos-Mády² · Ibolya Leveles³ · István Pintér¹ · Viktor Farkas² · András Perczel^{1,2}

¹ Laboratory of Structural Chemistry and Biology, Institute of Chemistry, Eötvös Loránd University, Pázmány Péter s. 1/A, Budapest 1117, Hungary

² MTA-ELTE Protein Modelling Research Group, Pázmány Péter s. 1/A, Budapest 1117, Hungary

³ BME Department of Applied Biotechnology and Food Science, Szent Gellért tér 4, Budapest 1111, Hungary

Abstract We report the solid phase synthesis of –GG-X-GG– type α/β -carbopeptoids incorporating RibAFU(ip) (**1a**, *tX*) or XylAFU(ip) (**2a**, *cX*) sugar amino acids. 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., N₃-RibAFU(ip)-OH) via Staudinger reaction with *n*Bu₃P can be successfully applied. Both X-ray diffraction, ¹H- and ³¹P-NMR, and theoretical (QM) data support and explain why the application of Ph₃P as Staudinger reagent is “ineffective” in the case of a *cis* stereoisomer, if *cX* is attached to the preceding residue with a peptide (–CONH–) bond. The failure of the poly-peptide chain elongation with N₃-*cX* originates from the “coincidence” of a steric crowdedness and an electronic effect disabling the mandatory nucleophilic attack during the hydrolysis of a quasi penta-coordinated triphenylphosphinimine. 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

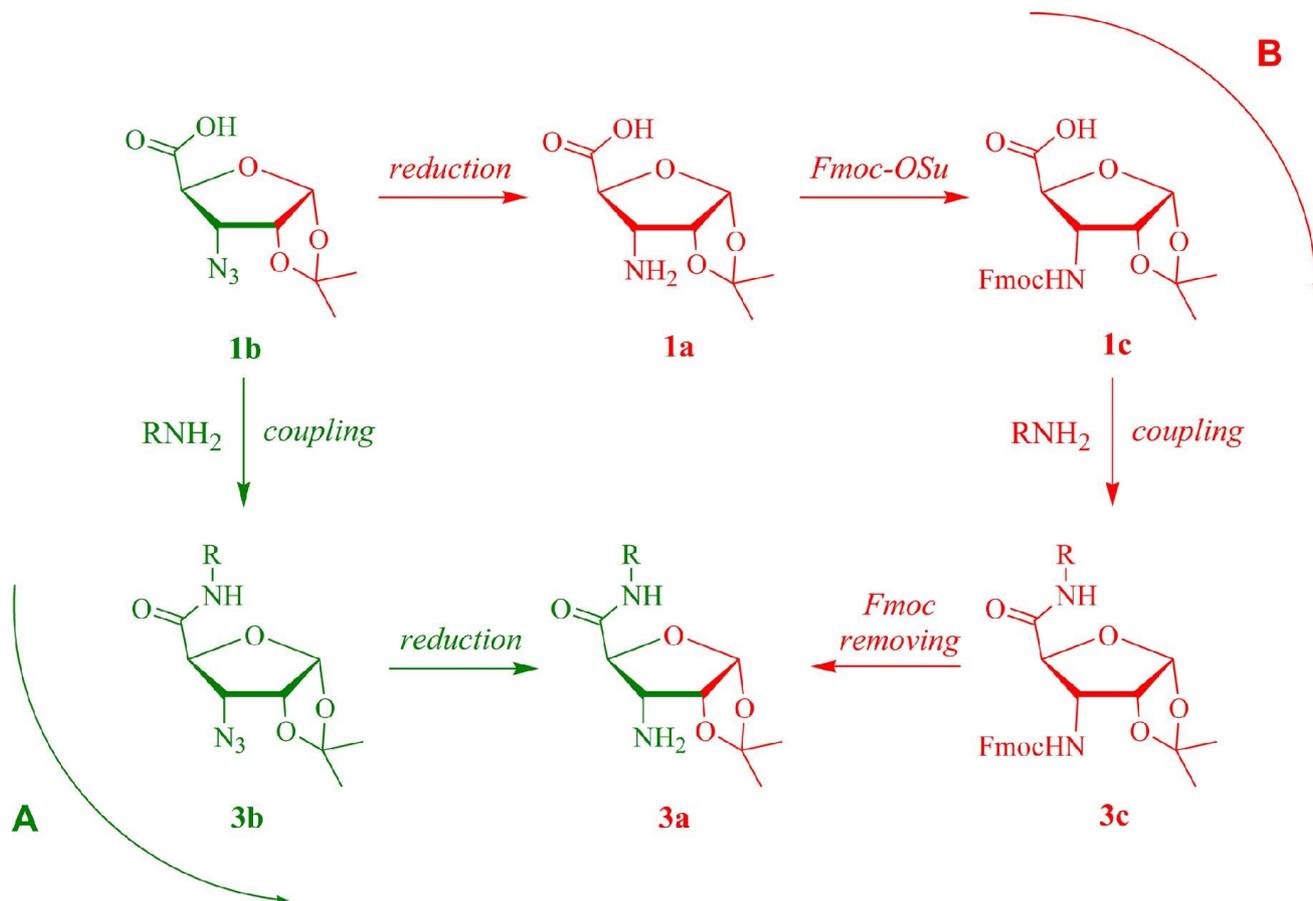
Oligopeptides containing β -amino acids have become increasingly popular in foldamer chemistry and drug research.

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 studies 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; Pils 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), particularly, five- or six-membered cyclic amino sugar carboxylic acids, appeared as appropriate building blocks for carbopeptoid 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-cyclopentane carboxylic acid (*trans*-ACPC) and *trans*-2-amino-cyclohexane carboxylic acid (*trans*-ACHC) were synthesized and built into homo- and heterooligomers to test their self-assembling abilities (Pandey et al. 2011; Giri et al. 2012; Risseuw et al. 2013).

Amino furanuronic acids (AFUs, Fig. 1) and their homooligomers are part of several bioactive macromolecules (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 α -glucose. Furthermore, the relatively rigid conformation of the furanoid ring, in between the ϕ and ψ torsional angles, might give the right amount of internal flexibility $\theta \sim 60 \pm 30^\circ$ 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



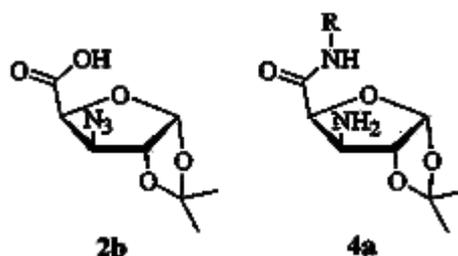
Scheme 1 Direct coupling of the “starting” azido compound (**1b**) followed by reduction provides in two steps (*A* route: **1b** → **3b** → **3a**) the very same product as the “conventional” Fmoc-strategy that provides in four consecutive steps (*B* route: **1b** → **1a** → **1c** → **3c** → **3a**)

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 derivatives for direct peptide couplings. The reduction of the N_3 functionality after peptide bond formation—once coupling 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-established conditions 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); however, 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 conformational propensities of oligopeptides (Shi et al. 2005, Beck et al. 2008), where X is either RibAFU(ip) (**1a**, tX) or XylAFU(ip) (**2a**, cX). Comparing synthetic routes, yields, etc., we could elucidate the stereochemical influence of the *trans(ribo)* and *cis(xylo)* configuration both on the reduction of the azido group and subsequent peptide bond formation. To our great surprise the phosphinimino group on the furanoid building block of *cis* (and only *cis*) conformation 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 Me₃P, the unexpected stability of the intermediate phosphinimino 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 molecular 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 F₂₅₄ (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

measured conditions of the Staudinger reaction (Staudinger and Meyer 1919; Staudinger and Hauser 1921; Leffler and Temple 1967; Gololobov et al. 1981; Gololobov 1992; Simone et al. 2005; Brase et al. 2005; Van Rompaey et al. 2005; Temelkoff et al. 2006): a method introduced for

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 quadrupole 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 ¹³C- and ³¹P-NMR spectra were measured with Bruker Avance 250 spectrometer in CDCl₃, D₂O, CD₃OD and DMSO-d₆ 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 diffractometer data were recorded with a PANalytical Xpert Pro MPD XRD X-ray diffractometer with X'celerator detector at the Budapest University of Technology and Economics.

Peptide synthesis

2-Chlorotriethyl 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 dissolved 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 continued 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 Ph₃P and Ph₃PO was removed by filtration. Evaporation of the filtrate furnished the crude product which was triturated 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*. ¹H-NMR (CDCl₃, 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 CH₃).

N-9-Fluorenylmethoxycarbonyl-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 ($c \approx 35$ mmol/l) and reduced with H₂ 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 %). *R_f*: 0.51 (chloroform–methanol 3:2). FTIR–ATR: ν_{max} : 3170 (ν NH₃), 2750–2460 and 2170–1900 diffuse (ν NH₃), 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*. ¹H NMR (D₂O, 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, CH₃), 1,39 (s, 3H, CH₃) 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 NaHCO₃ 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 \times). The combined organic phases were washed with NaHCO₃. The aqueous phase is adjusted to pH 1 with 2 N HCl and extracted with

for 2 h. The solid was filtered and the filtrate was concentrated in vacuo to give the crude product containing **2a** and Ph₃PO. The mixture was dissolved in dichloromethane, and ethyl acetate was added to precipitate a cream-colored solid.

EtOAc (3 \times). 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 %). *R_f*: 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 dissolved in a mixture of THF (3 mL) and methanol (1 mL). After stirring for 30 min under N₂, triphenylphosphine (0.088 g, 0.336 mmol) in THF (2 mL) and aqueous ammonium 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 Ph₃P and Ph₃PO. 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. *R_f*: 0.86 (chloroform–methanol 3:1). FTIR–ATR: 3375, 3353, 1652, 1545 cm⁻¹. ESI-MS: [M+H]⁺ 217.2 *m/z*. ¹H-NMR (CDCl₃, 250 MHz): δ ppm 6.47 (broad m, 1H, NH-CH₃), 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, $J_{\text{NH-CH}_3} = 5.0$ Hz, 3H, amide-CH₃), 1.47 and 1.27 (s, isopropylidene CH₃).

(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 Ph₃P and Ph₃PO. 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 %). *R_f*: 0.86 (chloroform–methanol 3:1). The spectral 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

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.

$^1\text{H-NMR}$ (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 crystals (**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} . $^1\text{H NMR}$ (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), $[\alpha]_{\text{D}}^{24}$ 1.50 and 1.03 (s, isopropylidene CH_3). $^{31}\text{P NMR}$ (CDCl_3 , 250 MHz): δ ppm 29.23 (N=PPh₃).

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 dissolved 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 chromatography 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. $^1\text{H NMR}$ (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, isopropylidene 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 residue was dissolved in ether (1 mL) and dropped into hexane (tenfold volume) to precipitate the product, which was filtered 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} . $^1\text{H NMR}$ (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). $^{31}\text{P NMR}$: δ ppm 29.1 (N=PPh₃).

N-Methyl-1,2-O-isopropylidene-3-deoxy-3-triphenylphosphinimino- 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 precipitate 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 undissolved solid was filtered again. The filtrate was concentrated 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); $[\alpha]_{\text{D}}^{25}$ -75 (c 1, EtOH). RP-HPLC: 15.37 min (R-N = PPh₃). FTIR-ATR: ν_{max} : 3330, 2983, 1807, 1754, 1702, 1526 cm^{-1} . $^1\text{H NMR}$ (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). $^{13}\text{C NMR}$ (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). $^{31}\text{P NMR}$: δ ppm 40.55 (NH⁺=PPh₃). A sample of the crude **4g-HCl** was recrystallized from methanol to give large crystals appropriate 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 mixture 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 Ph₃PO. 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, conversion: 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. $^1\text{H-NMR}$ (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 (NCH₃), 1.36 and 1.17 (s, isopropylidene CH_3). $^{13}\text{C NMR}$ (CDCl_3 , 250 MHz): δ ppm

167.72 (C=O), 111.44 (C-NMe₂), 104.45 (C-1), 85.58 (C-2), 77.91 (C-4), 58.27 (C-3), 36.9, 35.6 (N-CH₃), 26.6, 25.9 (CH₃).

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 concentrated 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; *R_f*: 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; ¹H NMR (250 MHz, CDCl₃): (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, CH₃), 1.25 (s, 3H, CH₃); (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 CH₃) 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 (C³-NH₂), 1.36 and 1.17 (s, isopropylidene CH₃).

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 N₃-RibAFU(ip)-OH (**1b**) and N₃-XylAFU(ip)-OH (**2b**) was completed using partly the well-known general pathways starting from α -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 N₃ band at the IR spectrum (2113–2116 cm⁻¹).

Subsequent treatment of the sterically hindered **2d** with Ph₃P in THF/MeOH at room temperature provided Ph₃P=N-*c*XGG-Resin. Formation of it was shown by acidic cleavage of a portion from the resin giving Ph₃P=N-*c*XGG-OH: supported by the appearance of the singlet peak at 40.5 ppm (³¹P-NMR) and the lack of the N₃ IR band. To get the appropriate amine, **2h**, several attempts were carried out under usual conditions with NH₄OH. 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				
	<i>N</i> -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
<i>N</i> -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 mentionedotherwise ^e *n*Bu₃P/OH or piperidine^f Estimated based on back-calculations

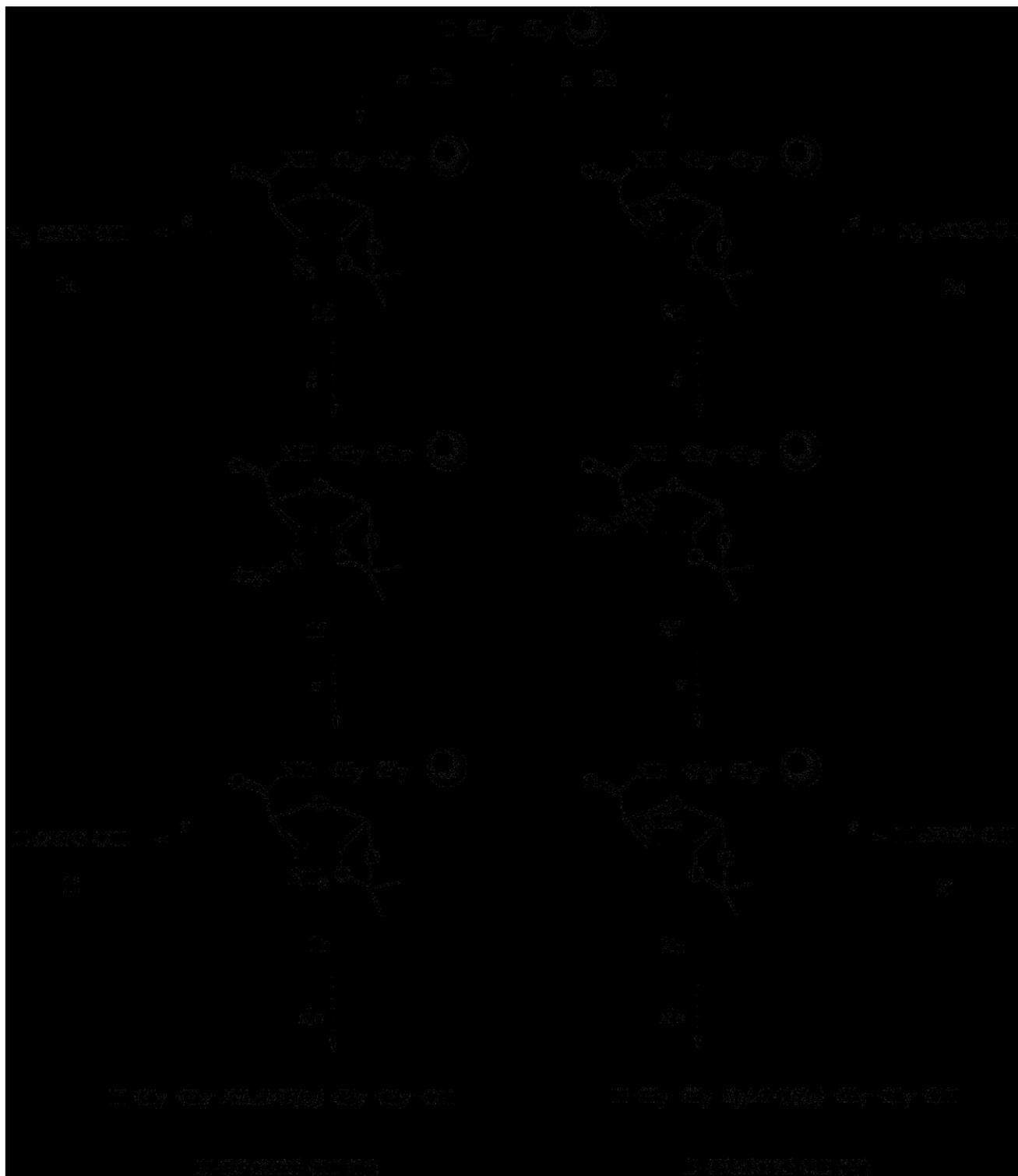
ethanolic KOH were used. The unexpectedly high stability of the intermediate triphenylphosphinimine impeded the formation of **2h** and, thus, the forthcoming peptide bond formation. Unlike Ph₃P, when the more reactive *n*Bu₃P was used (Scheme 3), reduction of N₃– was achieved. The application of the Me₃P (John and Wittmann 2015) was omitted as it is an expensive and hazardous reagent, and thus unfit for large scale production. Furthermore, **1d** gave Ph₃P=N-*t*XGG with Ph₃P 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 *n*Bu₃P in THF-MeOH 1:1 gave *n*Bu₃P=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 –NH₂ groups in the region of 1665–1663, 1596–1541, 1460–1445 cm^{–1} and, at the same

time, by the absence of the –N₃ band. In accordance, no signal was found in ³¹P-NMR, revealing the 100 % conversion to **1h** and **2h** (no traces of phosphinimines were pre-sent 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 xylofuranuronic one (*cis* **2b**) favored the *trans* diastereomer (76 vs. 54 % or 86 vs. 56 %). Consequently, the final difference 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 triphenylphosphinimine group in Ph₃P=N-*c*XGG-Resin as well as in the tripeptide Ph₃P=N-*c*XGG-OH is unprecedented. Generally, hydrolysis of any sugar phosphinimine will spontaneously take place, resulting in the corresponding 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 N₃-*t*X-OH with the “milder” Ph₃P or the more reactive *n*Bu₃P 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 –N₃ → –NH₂ reduction: even with the more reactive *n*Bu₃P 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* $n\text{Bu}_3\text{P}$, 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)

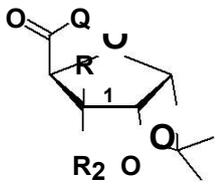
and 31 % for the *trans* derivatives. Below, we present a rational explanation of these differences by means of diffraction, spectroscopic and computational data.

As the least efficient step of the solid-phase synthesis is the otherwise attractive Staudinger reaction ($-\text{N}_3$ to $-\text{NH}_2$); both **1b** (N₃-RibAFU(ip)-OH) and **2b**



Scheme 4 Staudinger reaction of N₃-RibAFU(ip)-OH and N₃-XylAFU(ip)-OH as well as their derivatives, conditions: *i* Ph₃P, pyridine; *ii* aq. NH₄OH

Table 2 Reaction of 1,2-*O*-isopropylidene-3-azido-3-deoxy- α -*D*-furanuronic compounds with Ph₃P 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) 1g (Q: OH, R ¹ : H, R ² : N=PPh ₃)	82 % (93 %) No data
2b (Q: OH, R ¹ : N ₃ , R ² : H)	2a (Q: OH, R ¹ : NH ₂ , R ² : H) 2g (Q: OH, R ¹ : N=PPh ₃ , R ² : H)	81 % (94 %) 49 % (25 %)
3b (Q: NHMe, R ¹ : H, R ² : N ₃)	3a (Q: NHMe, R ¹ : H, R ² : NH ₂) 3g (Q: NHMe, R ¹ : H, R ² : N=PPh ₃)	69–82 % (77–92 %) No data
4b (Q: NHMe, R ¹ : N ₃ , R ² : H)	4a (Q: NHMe, R ¹ : NH ₂ , R ² : H) 4g (Q: NHMe, R ¹ : N=PPh ₃ , R ² : H)	0 % 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) 5g (Q: NMe ₂ , R ¹ : N=PPh ₃ , R ² : H)	45 % (50 %) 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

(N₃-XylAFU(ip)-OH) as well as their selected derivatives, N₃-RibAFU(ip)-NHMe (**3b**) and the corresponding *xyl*o epimer N₃-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 NHMe₂.) All azido monomers were reacted with triphenylphosphine in solution under the usual conditions (Scheme 4), the intermediate phosphinimines were treated in situ with bases, typically with NH₄OH (Scheme 4) leading to the appropriate product (Table 2).

Interestingly, both C-3-epimeric (free) acids, **1b** and **2b**, when reacted in solution with Ph₃P, followed by in situ phosphinimine hydrolysis (NH₄OH/H₂O, 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 ¹H-NMR spectra 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=PPh₃ intermediate was not isolated, while for **2g**, the *cis* or *xyl*o 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=PPh₃ 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, ¹H-NMR and ESI-MS data. In contrast to the above, under similar reaction conditions, **4b** afforded nothing of 3-amino-3-deoxy product **4a** (0 %) and only traces of the intermediate triphenylphosphinimine **4g** were detected. In a separate experiment under modified conditions, **4b** was transformed into the phosphinimine intermediate **4g**, but still in poor yield. Though



Scheme 5 Reaction conditions: *a* ClC(OOiBu)H, MeNH₂; *b* Ph₃P, pyridine; *c* aq.NH₄OH; *d* Ph₃P, Et₂O; *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 ³¹P-NMR spectra exhibiting characteristic signal of -N=PPh₃ at δ 29.10 ppm and that of -NH⁺=PPh₃ 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*Bu₃P reagent instead of Ph₃P.

To elucidate the role of the carboxamido group, the related *N,N*-dimethylamide derivative **5b**, N₃-XylAFU(ip)-NMe₂, was synthesized from **2b** under similar conditions. Compound **5b** was treated with Ph₃P in pyridine and subsequently hydrolyzed by aq. NH₄OH to give the expected product **5a**, H-XylAFU(ip)-NMe₂. 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=PPh₃ group at C-3 atom of the ring (Fig. 2a).

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

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

	Conformers	ψ (°)	$d_{O\dots N}$ (Å)	$d_{O\dots P}$ (Å)	$G_{298.15\text{ K}}$ (kcal/mol)	Boltzmann distribution at $T = 298\text{ K}$ (%)	
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	<i>111.2</i>	<i>3.3</i>	<i>4.3</i>	<i>0.00</i>	<i>92.0</i>
	In water	Conf1	-35.3	4.1	5.6	1.66	5.6
		Conf2	<i>34.6</i>	<i>4.1</i>	<i>5.5</i>	<i>0.00</i>	<i>94.0</i>
		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	<i>121.5</i>	<i>3.4</i>	<i>3.8</i>	<i>0.00</i>	<i>100.0</i>
	In water	Conf1	-76.0	3.3	4.7	1.16	12.4
		Conf2	<i>115.7</i>	<i>3.5</i>	<i>4.0</i>	<i>0.00</i>	<i>87.6</i>
Q: NMe ₂ (5g)	In vacuo	Conf1	-81.9	3.3	4.4	3.08	0.3
		Conf2a	<i>147.9</i>	<i>3.2</i>	<i>3.4</i>	<i>0.20</i>	<i>41.5</i>
		Conf2b	<i>123.6</i>	<i>3.4</i>	<i>3.8</i>	<i>0.00</i>	<i>58.2</i>
	In water	Conf1	-77.0	3.3	4.7	<i>0.00</i>	<i>97.7</i>
		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

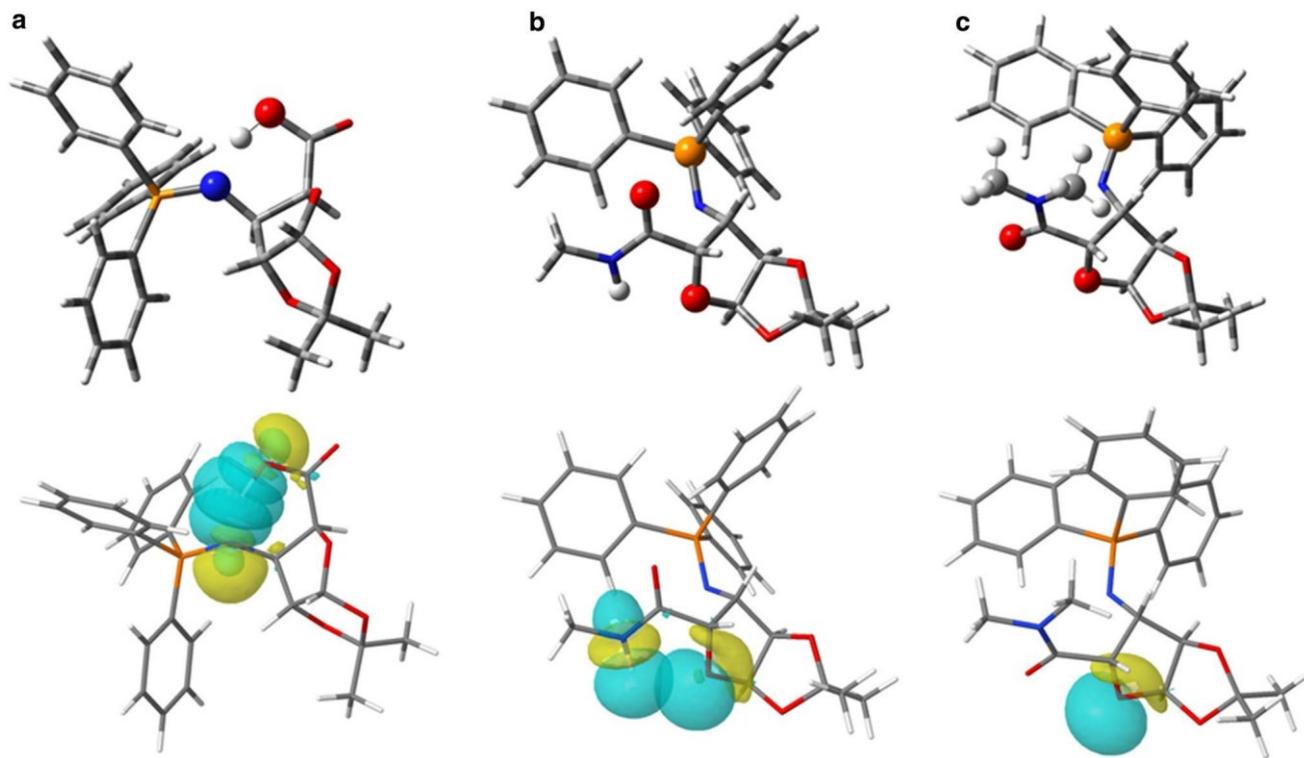
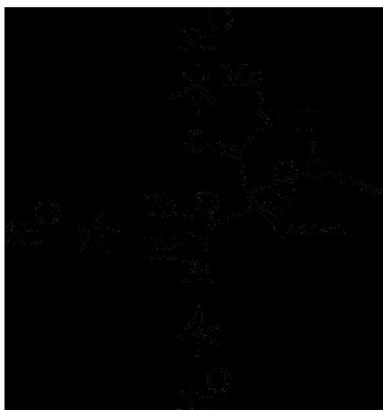


Fig. 2 Graphical representation with selective NBOs of key interactions in water of the lowest energy conformers of the appropriate phosphinimine: **a** **2g_conf2** (lone pair (LP) of N and antibond (BD*))

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*-methylfuranuronamide **4g**, the $O=C-NHMe$ group (at C-4) forms a $N-H\cdots O$ -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



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

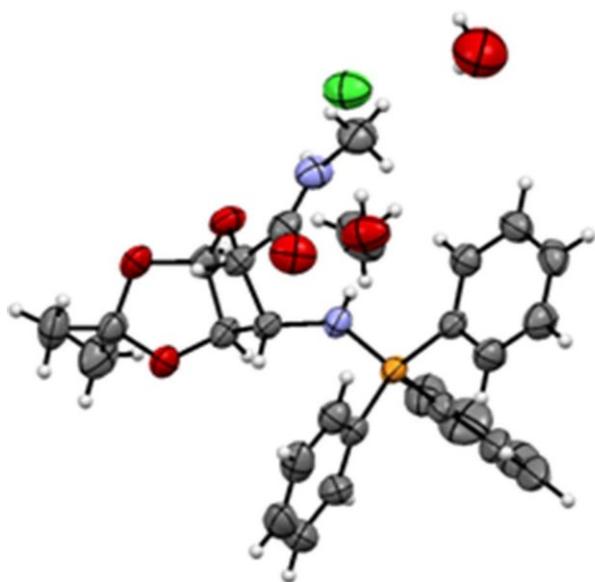
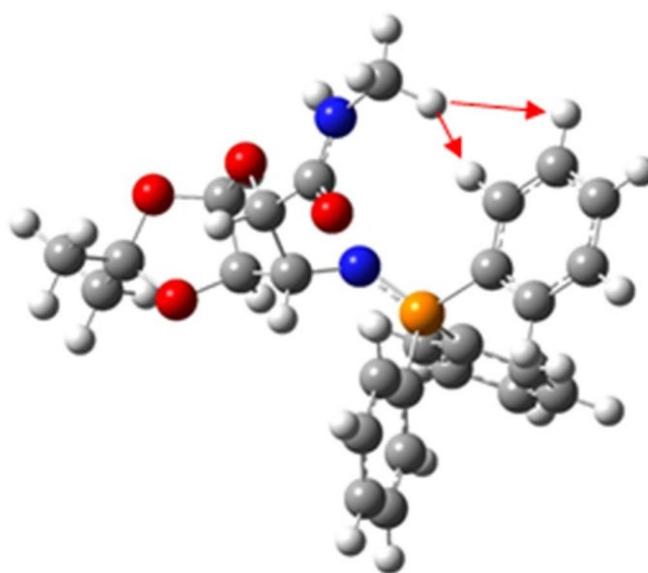


Fig. 3 Crystal structure of *N*-methyl-3-deoxy-3-triphenylphosphinimino-1,2-*O*-isopropylidene- α -D-xylofuranuronamide chloride (**4g·HCl**) by X-ray diffraction. Calculated structure of **4g_conf2** in

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**.

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 $N-H\cdots O$ -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)-NMe₂) 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



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 ($\psi^{X\text{-ray}} = 108.9^\circ$, $\psi^{ab\text{ initio}} = 115.7^\circ$), positioning the O-atom of C=O into the vicinity of the central P-atom ($d^{X\text{-ray}} = 3.9 \text{ \AA}$, $d^{ab\text{ initio}} = 4.0 \text{ \AA}$). This geometrical constellation makes any external nucleophilic attack disabling the hydrolysis impossible. Thus, the X-ray-determined crystalline conformer can be considered the frozen, stable and thus unreactive intermediate.

Based on homonuclear 2D ROESY NMR measurements (CDCl₃ at $T = 288 \text{ K}$), the very same conformer in protic solution could be confirmed for **4g** by the strong ROE cross-peaks of the H_#^δ and H_# 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 pentapeptides, both the RibAFU(ip) (**1a**, *tX*) and the XylAFU(ip) variant (**2a**, *cX*), can be synthesized on a solid state support. 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., N₃-RibAFU(ip)-OH) via Staudinger reaction with *n*Bu₃P can be successfully applied. X-ray diffraction, spectroscopic and theoretical (QM) data support and explain why the application of Ph₃P as Staudinger reagent is “ineffective” in the case of the *cis* stereoisomer (N₃-XylAFU(ip)-Gly-Gly-Resin) if *cX* is attached to the preceding residue with a peptide (–CONH–) bond. The failure of the polypeptide chain elongation with N₃-*cX* originates from the “coincidence” of a steric crowdedness and an electronic effect disabling the mandatory nucleophilic attack during the hydrolysis of the quasi pentacoordinated triphenylphosphinimine. 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 -*xyl*-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.

Acknowledgments The authors wish to thank László Kocsis and Gábor Szirbik from ThalesNano Inc. (Budapest, Hungary) for their help and advice in the hydrogenation reaction. The Biostruct

Laboratory at the Budapest University of Technology and Economics is acknowledged for collecting X-ray diffraction data. The authors thank Petra Rovó for her help in NMR measurements. This work was supported by grants from the Hungarian Scientific Research Fund (OTKA K72973, NK101072) and TÁMOP-4.2.1. B-09/1/KMR.

References

- Andreini M, Taillefumier C, Chretien F, Thery V, Chapleur Y (2009) Synthesis and solution conformation of homo- β -peptides consisting of N-mannofuranosyl-3-ulosonic acids. *J Org Chem* 74:7651–7659
- Barlos K, Chatzi O, Gatos D, Stavropoulos G (1991) 2-Chlorotriyl chloride resin—studies on anchoring of Fmoc-amino acids and peptide cleavage. *Int. J. Peptide Protein Res* 37:513–520
- Beck DAC, Alonso DOV, Inoyama D, Daggett V (2008) The intrinsic conformational propensities of the 20 naturally occurring amino acids and reflection of these propensities in proteins. *PNAS* 105:12259–12264
- Beke T, Csizmadia IG, Perczel A (2004) On the flexibility of β -peptides. *J Comput Chem* 25:285–307
- Beke T, Czajlik A, Bálint B, Perczel A (2008) A theoretical comparison of self-assembling α - and β -peptide nanostructures: toward design of β -barrel frameworks. *ACS Nano* 2:545–553
- Brase S, Gil C, Knepper K, Zimmermann V (2005) Organic azides: an exploding diversity of a unique class of compounds. *Angew Chem Int Ed* 44:5188–5240
- Cabrele C, Martinek TA, Reiser O, Berlicki Ł (2014) Peptides containing β -amino acid patterns: challenges and successes in medicinal chemistry. *J Med Chem* 57:9718–9739
- Chan WC, White PD (2000) Fmoc solid phase peptide synthesis—a practical approach. Oxford University Press, Oxford
- Chandrasekhar S, Reddy SM, Jagadeesh B, Prabhakar A, Ramana Rao MHV, Jagannadh B (2004) Formation of a stable 14-helix in short oligomers of furanoid *cis*- β -sugar-amino acid. *J Am Chem Soc* 126:13586–13587
- Chandrasekhar S, Rao CL, Seenaiiah M, Naresh P, Jagadeesh B, Manjeera D, Sarkar A, Bhadra MP (2009) Total synthesis of azumamide E and sugar amino acid-containing analogue. *J Org Chem* 74:401–404
- Cheng RP, Gellman SH, DeGrado WF (2001) Beta-peptides: from structure to function. *Chem Rev* 101:3210–3232
- Frisch MJ et al (2009) Gaussian 09, Revision B.01. Gaussian, Inc., Wallingford CT
- García-Martín F, Bayó-Puxan N, Cruz LJ, Bohling JC, Albericio F (2007) Chlorotriyl chloride (CTC) resin as a reusable carboxyl protecting group. *QSAR Comb Sci* 26:1027–1035
- Giri AG, Jogdand GF, Rajamohanam PR, Pandey SK, Ramana CV (2012) Synthesis and structural characterization of homochiral homo-oligomers of *cis*- γ -methoxy-substituted *cis*- and *trans*-furanoid- β -amino acids. *Eur. J. Org. Chem.* 2012:2656–2663
- Glendening ED, Badenhoop JK, Reed AE, Carpenter JE, Bohmann JA, Morales CM, Weinhold F (2001) NBO v. 5.9. 5.9 ed.

- Theoretical Chemistry Institute, University of Wisconsin, Madison, Wisconsin
- Gololobov YG (1992) Recent advances in the Staudinger reaction. *Tetrahedron* 48:1353–1406
- Gololobov YG, Zhmurova LN, Kasukhin LF (1981) Sixty years of Staudinger reaction. *Tetrahedron* 37:437–472
- Gruner SAW, Gy Kéri, Venetainer A, Kessler H (2001) Sugar amino acid containing somatostatin analogues that induce apoptosis in both drug-sensitive and multidrug-resistant tumor cells. *Org Lett* 3:3723–3725
- Gruner SAW, Locardi E, Lohof E, Kessler H (2002a) Carbohydrate-based mimetics in drug design: sugar amino acids and carbohydrate scaffolds. *Chem Rev* 102:491–514
- Gruner SAW, Truffault V, Voll G, Locardi E, Stöckle M, Kessler H (2002b) Design, synthesis, and NMR structure of linear and cyclic oligomers containing novel furanoid sugar amino acids. *Chem Eur J* 8:4365–4376
- Guichard G, Huc I (2011) Synthetic foldamers. *Chem Commun* 47:5933–5941
- Hecht S, Huc I (2007) Foldamers: structure, properties and applications. Wiley-VCH, Weinheim
- Herradón B, Seebach D (1989) Mono- and dialkylation of derivatives of (1*R*,2*S*)-2-hydroxycyclopentanecarboxylic acid and -cyclohexanecarboxylic acid via bicyclic dioxanones: selective generation of three contiguous stereogenic centers on a cyclohexane ring. *Helv Chim Acta* 72:690–714
- Horne WS, Gellman SH (2008) Foldamers with heterogeneous backbones. *Acc Chem Res* 41:1399–1408
- John F, Wittmann V (2015) Orthogonally protected furanoid sugar diamino acids for solid-phase synthesis of oligosaccharide mimetics. *J Org Chem* 80:7477–7485
- Kessler H, Diefenbach B, Finsinger D, Geyer A, Gurrath M, Goodman SL, Hölzemann G, Haubner R, Jonczyk A, Müller G, Graf von Roeder E, Wermuth J (1995) Design of superactive and selective integrin receptor antagonists containing the RGD sequence. *Lett Pept Sci* 2:155–160
- Kocsis L, Ruff F, Gy Orosz (2006) The effect of peptide length on the cleavage kinetics of 2-chlorotrityl resin-bound ethers. *J Pept Sci* 12:428–436
- Kovács J, Pintér I, Messmer A, Tóth G (1985) Unprotected sugar phosphinimines: a facile route to cyclic carbamates of aminosugars. *Carbohydr Res* 141:57–65
- Leffler JE, Temple RD (1967) Staudinger reaction between triarylphosphines and azides. A study of the mechanism. *J Am Chem Soc* 89:5235–5246
- Long DD, Hungerford NL, Smith MD, Brittain DEA, Marquess DG, Claridge TDW, Fleet GWJ (1999) From sequencamers to foldamers? Tetrameric furanose carbopeptoids from cis- and trans-5-aminomethyl-tetrahydrofuran-2-carboxylates. *Tetrahedron Lett* 40:2195–2198
- Lundquist JT, Pelletier JC (2001) Improved solid-phase peptide synthesis method utilizing α -azide-protected amino acids. *Org Lett* 3:781–783
- Malkinson JP, Falconer RA, Tóth I (2000) Synthesis of C-terminal glycopeptides from resin-bound glycosyl azides via a modified staudinger reaction. *J Org Chem* 65:5249–5252
- Mándity IM, Fülöp F (2015) An overview of peptide and peptoid foldamers in medicinal chemistry. *Exp Opin Drug Discov* 10:1163–1177
- Martinek TA, Fülöp F (2012) Peptidic foldamers: ramping up diversity. *Chem Soc Rev* 41:687–702
- Meldal M, Juliano MA, Jansson AM (1997) Azido acids in a novel method of solid-phase peptide synthesis. *Tetrahedron Lett* 38:2531–2534
- Messmer A, Pintér I, Szeg F (1964) Triphenylphosphine *N*-acetylglycosylimides and *N*, *N'*-bis(acetylglycosyl)carbodiimides. *Angew Chem Int Ed* 3:228
- Nilsson BL, Kiessling LL, Raines RT (2000) Staudinger ligation: a peptide from a thioester and azide. *Org Lett* 2:1939–1941
- Pandey SK, Jogdand GF, Oliveira JCA, Mata RA, Rajamohanan PR, Ramana CV (2011) Synthesis and structural characterization of homochiral homo-oligomers of parent *cis*- and *trans*-furanoid- β -amino acids. *Chem Eur J* 17:12946–12954
- Pils I, Reiser O (2011) α/β -Peptide foldamers: state of the art. *Amino Acids* 41:709–718
- Risseuw MDP, Overhand M, Fleet GWJ, Simone MI (2013) A compendium of cyclic sugar amino acids and their carbocyclic and heterocyclic nitrogen analogues. *Amino Acids* 45:613–689
- Saxon E, Armstrong JI, Bertozzi CR (2000) A “traceless” staudinger ligation for the chemoselective synthesis of amide bonds. *Org Lett* 2:2141–2143
- Schilling CI, Jung N, Biskup M, Schepers U, Bräse S (2011) Bioconjugation via azide–Staudinger ligation: an overview. *Chem Soc Rev* 40:4840–4871
- Sharma GVM, Nagendar P, Ramakrishna KVS, Chandramouli N, Choudhary M, Kunwar AC (2008) Three-residue turns in α/β -peptides and their application in the design of tertiary structures. *Chem Asian J* 3:969–983
- Sharma GVM, Reddy PS, Chatterjee D, Kunwar AC (2011) Synthesis and structural studies of homooligomers of geminally disubstituted β ,2-amino acids with carbohydrate side chain. *J Org Chem* 76:1562–1571
- Shi Z, Chen K, Liu Z, Ng A, Bracken WC, Kallenbach NR (2005) Polyproline II propensities from GGXGG peptides reveal an anticorrelation with β -sheet scales. *Proc Natl Acad Sci USA* 102:17964–17968
- Simone MI, Soengas R, Newton CR, Watkin DJ, Fleet GWJ (2005) Branched tetrahydrofuran α,α -disubstituted- δ -sugar amino acid scaffolds from branched sugar lactones: a new family of foldamers? *Tetrahedron Lett* 46:5761–5765
- Staudinger H, Hauser E (1921) Über neue organische phosphorverbindungen IV phosphinimine. *Helv. Chim. Acta* 4:861–886
- Staudinger H, Meyer J (1919) Über neue organische phosphorverbindungen III. Phosphinmethylderivate und phosphinimine. *Helv Chim Acta* 2:635–646
- Temelkoff DP, Zeller M, Norris P (2006) *N*-Glycoside neoglycotrimers from 2,3,4,6-tetra-*O*-acetyl- β -*D*-glucopyranosyl azide. *Carbohydr Res* 341:1081–1090
- Tornøe CW, Davis P, Porreca F, Meldal M (2000) α -Azido acids for direct use in solid-phase peptide synthesis. *J Pept Sci* 6:594–602
- Van Rompaey P, Jacobson KA, Gross AS, Gao ZG, Van Calenbergh S (2005) Exploring human adenosine A₃ receptor complementarity and activity for adenosine analogues modified in the ribose and purine moiety. *Biorg Med Chem* 13:973–983