Amino Acids
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ORIGINAL ARTICLE

C-3 epimers of sugar amino acids as foldameric building blocks: improved synthesis, useful derivatives, coupling strategies

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Abstract To obtain key sugar derivatives for making homooligomeric foldamers or α/β-chimera peptides, economic and multigram scale synthetic methods were to be developed. Though described in the literature, the cost-effective making of both 3-amino-3-deoxy-ribofuranuronic acid (H–*t***X–**OH) and its C-3 epimeric stereoisomer, the 3-amino-3-deoxy-xylofuranuronic acid (H–*c***X–**OH) from D-glucose is described here. The present synthetic route elaborated is (1) appropriate for large-scale synthesis; (2) reagent costs reduced (e.g. by a factor of 400); (3) yields optimized are ~80% or higher for all six consecutive steps concluding –*t***X**– or –*c***X**– and (4) reaction times shortened. Thus, a new synthetic route step-by-step optimized for yield, cost, time and purification is given both for D-xylo and D-ribo-amino-furanuronic acids using sustainable chemistry (e.g. less chromatography with organic solvents; using continuous-flow reactor). Our study encompasses necessary building blocks (e.g. –**X**–OMe, –**X–**O^{i}Pr, –**X**–NHMe, Fmoc–**X–**OH) and key coupling reactions making –Aaa–*t***X**–Aaa– or –Aaa–*t***X**–*t***X**–Aaa– type "inserts". Completed for both stereoisomers of **X**, including the newly synthesized Fmoc–*c***X–**OH, producing longer oligomers for drug design and discovery is more of a reality than a wish.

Keywords Sugar amino acids · Azido sugars · Nucleophilic substitution · Foldamers

Abbreviations

H-RibAFU(ip)-OH or *t*X	1,2-*O*-Isopropylidene-3-amino-3-deoxy-α-D-ribofuranuronic acid
H-XylAFU(ip)-OH or *c*X	1,2-*O*-Isopropylidene-3-amino-3-deoxy-α-D-xylofuranuronic acid
N_3-RibAFU(ip)-OH	1,2-*O*-Isopropylidene-3-azido-3-deoxy-α-D-ribofuranuronic acid
N_3-XylAFU(ip)-OH	1,2-*O*-Isopropylidene-3-azido-3-deoxy-α-D-xylofuranuronic acid
H-XylAFU(ip)-NHMe	*N*-Methyl-1,2-*O*-isopropylidene-3-amino-3-deoxy-α-D-xylofuranuronamide
H-RibAFU(ip)-NHMe	*N*-Methyl-1,2-*O*-isopropylidene-3-amino-3-deoxy-α-D-ribofuranuronamide
Ac-RibAFU(ip)-NHMe	*N*-Methyl-1,2-*O*-isopropylidene-3-acetamido-3-deoxy-α-D-ribofuranuronamide
Ac-XylAFU(ip)-NHMe	*N*-Methyl-1,2-*O*-isopropylidene-3-acetamido-3-deoxy-α-D-xylofuranuronamide

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Introduction

Chimeric synthetic polypeptides composed of α/β-peptide units are copolymers of emerging significance (Cheng et al. 2001; Horne and Gellman 2008; Pilsl and Reiser 2011; Guichard and Huc 2011; Chandrasekhar et al. 2010; Kiss and Fülöp 2014). Especially, those composed of functionalized cyclobutane (Declerck and Aitken 2011; Gorrea et al. 2012; Pohl et al. 2013; Altmayer-Henzien et al. 2015), cyclopentane (Martinek and Fülöp 2012; Rjabovs and Turks 2013) and cyclohexane (Martinek and Fülöp 2012) derivatives have biological importance and perspectives (Cabrele et al. 2014; Mándity and Fülöp 2015). To a great extent, the introduced non-natural amino acid derivatives (e.g. ACPC, ACHC) contain the same number of torsional angles (φ and ψ) as α-peptides do, two per residue; however, their backbone conformational behaviour is primarily determined by local configurational properties. Reduced mobility, if the conformation is carefully designed could increase binding ability of the polypeptide to other macromolecules, receptors and thus enhance bioactivity.

Cis and *trans* stereoisomers of both 2-aminocyclopentanecarboxylic acid (ACPC) and 2-aminocyclohexanecarboxylic acid (ACHC) derivatives are now extensively used to design chimeras in conjunction with acyclic γ- and/or δ-amino acid residues (Cabrele et al. 2014; Giuliano et al. 2013). However, as both ACPC and ACHC derivatives are hydrophobic, their hydrophilic related compounds would be of great relevance, not yet fully explored (Hetényi et al. 2009). Thus, their sugar analogues, called sugar amino acids (β-SAAs) are generally soluble in water. Also they would have similar conformational properties so they present considerable synthetic challenges.

Among β-furanoid sugar amino acids, 1,2-*O*-isopropylidene-3-amino-3-deoxy-α-D-ribofuranuronic acid (H-RibAFU(ip)-OH, –*t***X**–, **1**) and its C-3 epimer, H-XylAFU(ip)-OH, (–*c***X**–, **2**) have attracted special attention (Fig. 1). The small-scale synthesis of the *N*-protected derivatives of **1** and **2** from diacetone-glucofuranose (**3**) was described in various quantities (Gruner et al. 2001, 2002a, b; Chandrasekhar et al. 2004). Conditions of these syntheses are different and the results are sometimes poor.

Therefore, the ultimate goal of the present work was to find an efficient and economical synthetic pathway of multigram scale and high-yield preparation of both C-3 epimers (**1** and **2**) (Scheme 1). Key of this synthetic route is the introduction of the $-NH_2$ group, in other words making the *N*-terminal of the amino acid at the C-3 carbon atom, which is often a reduction following the sulfonate → azide replacement.

The importance of these nucleophilic substitutions is underlined by the review of Hale et al. (2015). This synthetic problem is of a challenge and merits a careful evaluation of the conditions. Consequently, the purpose of trying various alternative routes using low-cost chemicals, "greener" chemistry, giving higher yields is well-founded. In addition, we have elaborated synthetic routes converting both azido uronic acids (**6** and **7**) into key derivatives essential either for solid phase and in solution state peptide chemistry (e.g. Boc-, Fmoc-) as well as preliminary coupling reactions were conducted making di- and triamide nanosystems.

Therefore our primary interest was to make both H–*t***X**–OH and H–*c***X**–OH β-sugar amino acids, as well as the following key derivatives needed for solid phase and/or solution state synthesis:

1. C-terminal temporarily protected residue (e.g. –*t***X**–OMe, –*t***X**–O^{i}Pr),
2. the "next residue" mimicking C-terminal amide bond (e.g. –*c***X**–NHMe and –*t***X**–NHMe),

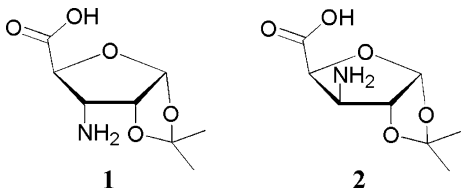
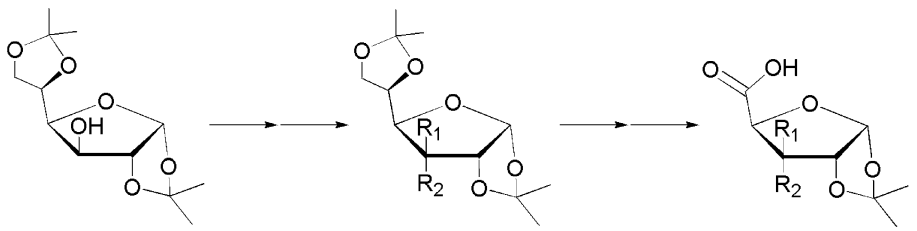
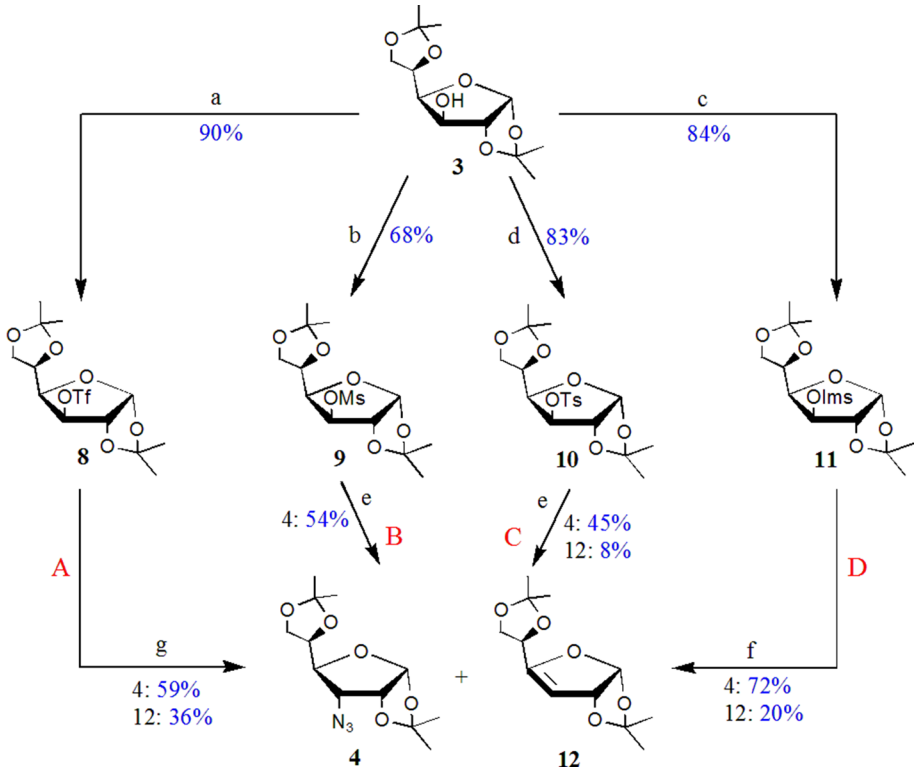


Fig. 1 The two central key products applied for further syntheses are H-RibAFU(ip)-OH (**1**, H-*t***X**-OH) and H-XylAFU(ip)-OH (**2**, H-*c***X**-OH)

Scheme 1 General synthetic pathway of 3-amino-3-deoxy-pentofuranuronic acids (**1** and **2**) from diacetone-glucofuranose (**3**)





Scheme 2 Synthesis of 1,2:5,6-di-*O*-isopropylidene-3-azido-3-deoxy-D-allofuranose. Reagents and conditions: *a* Tf_2O, Py, CH_2Cl_2; *b* MsCl, Py; *c* NaH, DMF, Im_2SO_2; *d* TsCl, Py; *e* NaN_3, DMSO, 140–145 °C; *f* NaN_3, DMSO, Bu_4NBr, 80 °C; *g* NaN_3, DMF, room temperature

3. *N*-terminal temporarily protected residue (e.g. Fmoc–*c***X**– and Fmoc–*t***X**–) were made.
4. Finally "insert", N_3–*t***X**–*t***X**–NHMe was synthesized to be ligated or elongated directly upon request.

Results and discussion

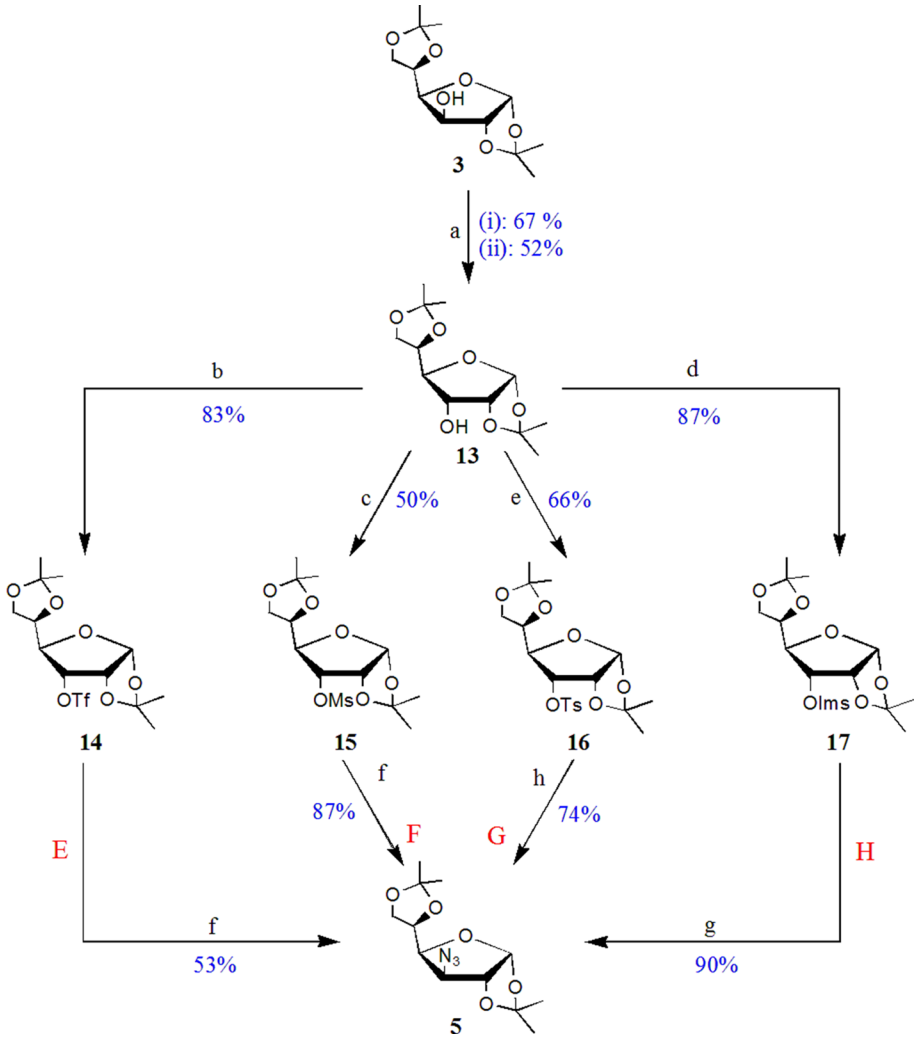
In order to achieve our goal to find more efficient and economical way for multigram scale synthesis of foldameric intermediate product **1** and **2** starting from D-glucose via diacetone-glucofuranose (**3**), the key step of the synthesis, the sulfonate ester → azide replacement had to be optimized. This transformation was performed earlier from various sulphonate esters under different conditions and in widely unlike scales. Consequently, one cannot find a comparative estimate of the published data which would establish the most reasonable and economic conditions for the synthesis of either the 3-azido-3-deoxy-allofuranose or the epimeric glucofuranose derivatives. In addition, although the synthesis of the *N*-protected sugar amino acid is described (Gruner et al. 2001, 2002a, b), the important intermediate free sugar amino acid **1** was not yet isolated and characterized.

Hence, we accomplished alternative routes with different sulfonate esters and under various reaction conditions, then we evaluated the results to choose the most advantageous protocol for producing both the 3-azido-3-deoxy-allo compound **4** (**A**, **B**, **C**, **D**) and the 3-azido-3-deoxy-gluco epimer **5** (**E**, **F**, **G**, **H**) (Schemes 2, 3) in multigram scale. Application of known synthetic methods was combined with reagent modification and yield optimization. By introducing mild reaction conditions, excluding column chromatography when possible resulted in ways to use less organic solvent and thus making our chemistry less polluting.

(A) Synthesis of N_3-RibAFU(ip)-OH (6) and N_3-XylAFU(ip)-OH (7)

Making 1,2:5,6-di-*O*-isopropylidene-3-azido-3-deoxy-α-D-allofuranose (**4**) is the key step for which the sulfonate ester (**8**, **9**, **10**, **11**) → azide replacement (Scheme 2) had to be achieved.

Following Gruner's suggestions (Gruner et al. 2002b) Route **A** via 3-*O*-trifluoromethanesulfonyl intermediate (**8**) was found to be effective (≈16 h, $T = 20$ °C) as trifluoromethanesulfonate is an excellent leaving group. However, this approach has drawbacks and disadvantages: (1) the reagent Tf_2O is expensive and environment unfriendly; (2) intermediate triflate (**8**) is unstable and thus, hard to store. Route **B** via 3-*O*-methanesulfonyl intermediate (**9**) was modified by carrying out in DMSO instead of DMF (Ferreira et al. 2010). Compared to Tf_2O, MsCl is a less expensive reagent; however, the reactivity of mesylate **9** was lower requiring higher temperature and longer time (≈181 h, $T = 140$ °C). In Route **C** via 3-*O*-toluene-sulfonyl



Scheme 3 Synthesis of 1,2:5,6-di-*O*-isopropylidene-3-azido-3-deoxy-D-glucofuranose. Reagents and conditions: *a* (i) PCC, DCM; (ii) $NaBH_4$, EtOH-H_2O or (i) TEMPO, BAIB, DCM; (ii) $NaBH_4$, EtOH-H_2O; *b* Tf_2O, Py, CH_2Cl_2; *c* MsCl, Py; *d* NaH, DMF, Im_2SO_2; *e* TsCl, Py; *f* NaN_3, DMSO, 120 °C; *g* NaN_3, toluene, Bu_4NBr, 80–90 °C; *h* NaN_3, DMSO, 135 °C

intermediate (**10**), Whistler's work was modified (Nayak and Whistler 1969), because we found that the reaction was faster in DMSO. Reaction time (≈54 h, $T = 140$ °C) was about one-seventh of the one published in DMF (360 h). In some cases, the influence of catalysts (Bu_4N-salts) or solvent (DMSO–water) was investigated, but their effect was found to be insignificant with respect to the overall yield. Route **D** via 3-*O*-(*N*-imidazole-1-sulfonyl) derivative (**11**), with modification of Vatéle's work (Vatéle and Hanessian 1996), was carried out in DMSO instead of toluene and resulted in better yield. The advantages of this route are evident: (1) the reagent *N,N'*-sulfuryl-diimidazole is easily prepared and far less expensive than Tf_2O; (2) the intermediate **11** is a crystalline and very stable product; (3) the formation of the azide **4** is fast (≈10 h, $T = 80$ °C) and the yield is excellent (72%). The latter yield found as an average after several repeats is higher than the one obtained in Gruner's experiment (**A**) and Vatéle's works (Vatéle and Hanessian 1996). The general problem of the above synthetic routes starting from sulfonate intermediates of *gluco* configuration (**8**, **9, 10, 11**) is that the 3-*O*-sulfonate group and the H-4 atom are in relative *trans* (antiperiplanar) position. Thus, under nucleophilic conditions *trans* elimination of the corresponding sulfonic acids cannot be prevented and the result is the formation of the unsaturated side product 1,2:5,6-di-*O*-isopropylidene-3-deoxy-α-D-erythro-hex-3-enofuranose (**12**). The ratio of side product **12** varied in total between 3 and 36% when syntheses were repeated.

The synthetic route yielding **5** (1,2:5,6-di-*O*-isopropylidene-3-azido-3-deoxy-α-D-glucofuranose) is longer as two additional steps are needed when compared with that making **4**: transformation of diacetone-glucofuranose (**3**) into diacetone-allofuranose (**13**) via the inversion of C-3 by oxido-reduction lengthens the protocol. Among the tested oxidation methods, either oxidation with PCC, or oxidation with TEMPO/BAIB was found to be the most effective. The sulfonate → azide replacement was conducted (Scheme 3) by using various 3-*O*-sulfonyl derivatives, like the triflate (**14**), the mesylate (**15**), the tosylate (**16**), and the imidazylate (**17**).

Table 1 Yields and costs of the synthesis of allofuranose (**4**) and glucofuranose (**5**)

Compound	Reaction route	Yield (%)		Price (USD)[a]	Reaction time (h)	
		3-*O*-SO_2R	3-N_3		3-*O*-SO_2R	3-N_3
1,2:5,6-Di-*O*-isopropylidene-3-azido-3-deoxy-α-D-allofuranose (**4**)	A[b]	90	59	1600.00	2	16
	B	68	54	14.50	48	181
	C	83	45	21.00	24	54
	D	84	72	4.00	1.5	10
1,2:5,6-Di-*O*-isopropylidene-3-azido-3-deoxy-α-D-glucofuranose (**5**)	E[c]	83	53	1600.00	2	20
	F	50	87	14.50	48	180
	G	66	74	21.00	24	52
	H	87	90	4.00	1.5	8

[a] Prices of chemicals used for 0.5 mol of starting material (**3** and **13**) (from the catalogue of Sigma-Aldrich 2016)

[b] Synthesis performed by modifying the reported protocol (Gruner et al. 2002b). This result served as primary standard for the comparison of other cases

[c] Synthesis performed by modifying the reported protocol (Austin et al. 1987). This result served as primary standard for the comparison of other cases

The reactions with **14**, **15** and **16** were carried out with modification of the earlier described methods (Ferreira et al. 2010; Austin et al. 1987), by using DMSO instead of DMF. Although the 3-*O*-imidazole-sulfonate ester **17** was known in the literature, its transformation into **5** has not been earlier executed. In Route **H** compound **17** gave the main product **5** with excellent yield, also in multigram scale.

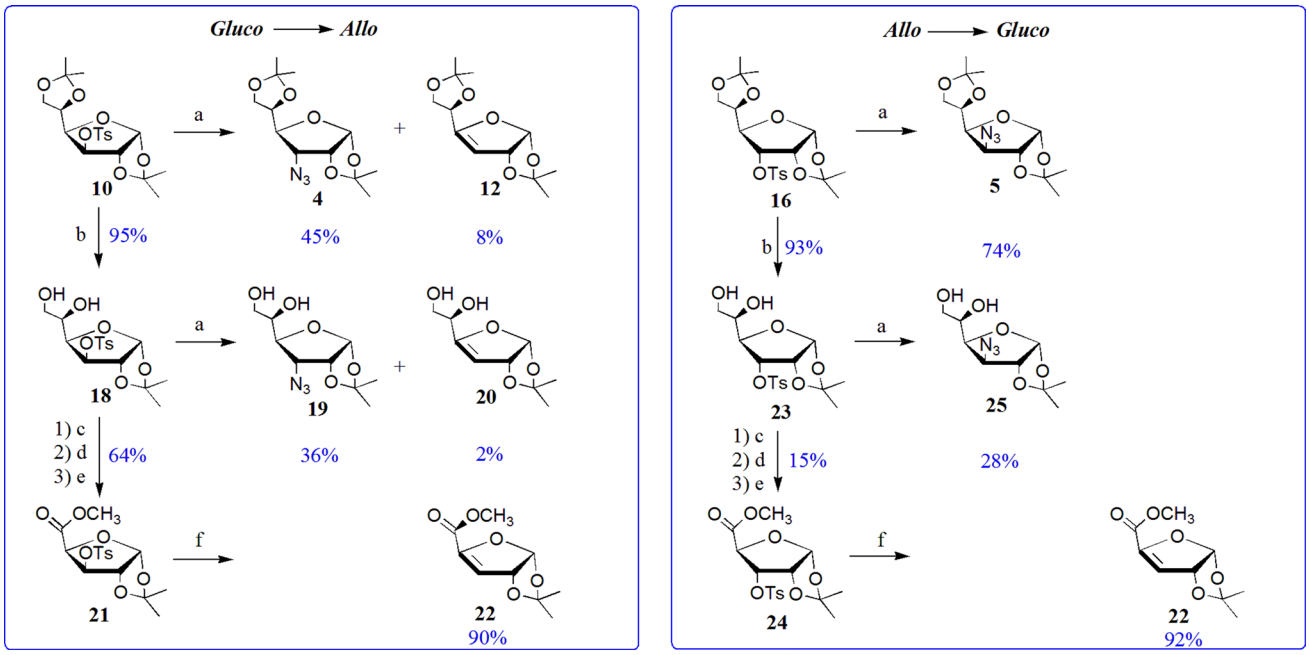
In summary, comparison of the yields of the *allo* compound **4** with those of the *gluco* compound **5** points out that data were higher for the latter almost in all cases. The main reason is that in the *gluco* sulfonate esters the configuration of the C-3 is opposite to that of *allo* derivatives. Hence, structural conditions for the elimination reaction producing unsaturated **12** are not given. In Table 1 the effectiveness of the various synthetic paths for the azides **4** and **5** are compared.

In conclusion, Routes **B** and **C** are less expensive ways than Route **A**, however, due to the low yield and the long reaction time, they are not good alternatives. Following Route **D** via 3-*O*Ims derivative (**11**) both costs (<1/400th) and reaction time (10 h ≈ 2/3) can be lowered significantly; moreover, yield turned out to be excellent (72%) and thus this is the method of choice, appropriate for large-scale synthesis (Table 1). Similarly, Route **H** via 3-*O*Ims derivative (**17**) was found to be the most suitable one for large-scale synthesis of **5**, as both cost (1/400th) and reaction time (8 h ≈ 2/5) are lower than that of Route **E** of the described alternative (Austin et al. 1987). In addition, yield was almost excellent (90%) (Table 1). In addition, Route **H** avoids the use of the environment unfriendly Tf_2O and introduced an easily isolable and stable intermediate **17**.

Substituent effect of C-4 on the 3-O-sulfonate → azide replacement reaction

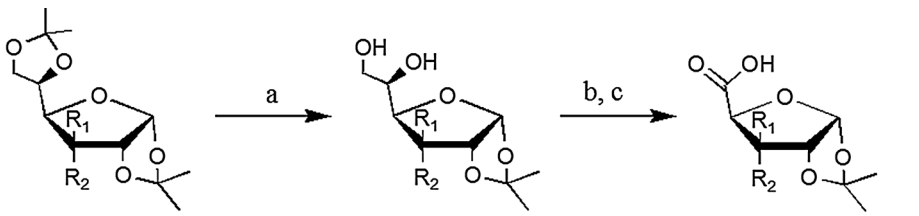
In all cases of sulfonate → azide replacement the yield of the 3-azido-3-deoxy-allofuranose product (**4**) was influenced by the *trans* elimination of the different sulfonic acid derivatives under basic conditions: furnishing the undesired 3,4-unsaturated derivative **12** (Watterson et al. 1999). The chance of the *trans* elimination is controlled by the partial positive charge of H-4, determined by electronic and/or steric effect of the substituent at C-4 due to its proximity to the reaction centre. To elucidate this influence we compared the reaction of six derivatives having three different substituents at C-4. During the removal of the 5,6-*O*-isopropylidene protecting group of 3-*O*-(*N*-imidazole-1-sulfonyl) derivative (**11**) unexpected synthetic challenges occurred (Szaniszló et al., to be published), the reaction with the 3-*O*-toluene-sulfonyl intermediate (**10**) is discussed here.

The –OTs → $–N_3$ replacement reactions of *gluco* derivative 1,2:5,6-di-*O*-isopropylidene-3-*O*-sulfonate (**10**, Scheme 4) and the derived partially unprotected 1,2-*O*-isopropylidene-3-*O*-sulfonate (**18**) yield the target 3-azido-3-deoxy compounds (**4** and **19**) and the 3,4-unsaturated side products (**12** and **20**) similarly. Thus, in both cases the steric effect determines the product primarily. In contrast to this, starting from **21** the exclusive formation of the corresponding unsaturated side product **22** (90%) is explained by the strong electron withdrawing effect of $–COOCH_3$ group, remarkably increasing the partially positive charge of H-4. In the parallel reactions of the corresponding *allo* derivatives (**16**, **23** and **24**) the relative *cis* configuration



Scheme 4 Competing substitution and elimination reaction for 3-*O*-tosylates. Reagents and conditions: *a* NaN_3, DMSO, 135–145 °C; *b* AcOH–H_2O 3:1; *c* $NaIO_4$, MeOH; *d* $KMnO_4$, AcOH–H_2O 1:1; *e* CH_2N_2/Et_2O, DCM; *f* NaN_3, DMSO, 80 °C

Scheme 5 Synthesis of N_3-RibAFU(ip)-OH and N_3-XylAFU(ip)-OH. Reagents and conditions: *a* AcOH–H_2O 3:1; *b* $NaIO_4$, MeOH; *c* $KMnO_4$, AcOH–H_2O 1:1

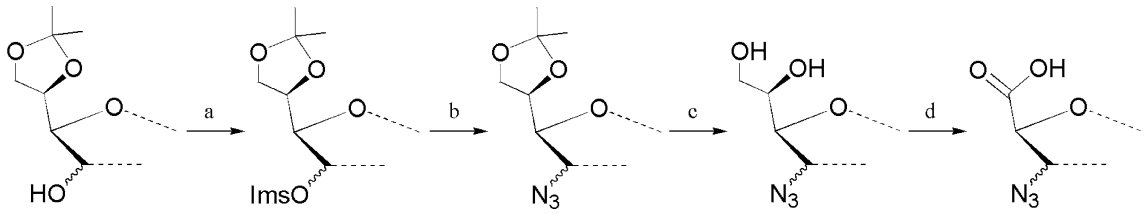


of substituents at C-3 and C-4 gives no a priori possibility of the *trans* elimination. The expectation fulfilled both for **16** and **23,** where no 3,4-unsaturated side products were detected. At the same time, ester **24** afforded exclusively the 3,4-unsaturated compound **22** (92%) also isolated from the similar reaction of **21**. The reason for this feature is the electronic effect of $–COOCH_3$ group is sufficiently strong to induce the *cis* elimination of toluenesulfonic acid, giving **22** as the main product. Hence, 3-*O*-sulfonate → azide replacement for both *gluco* and *allo* series is advantageously executed at the beginning of the reaction pathway, preferably on 1,2:5,6-di-*O*-isopropylidene compounds.

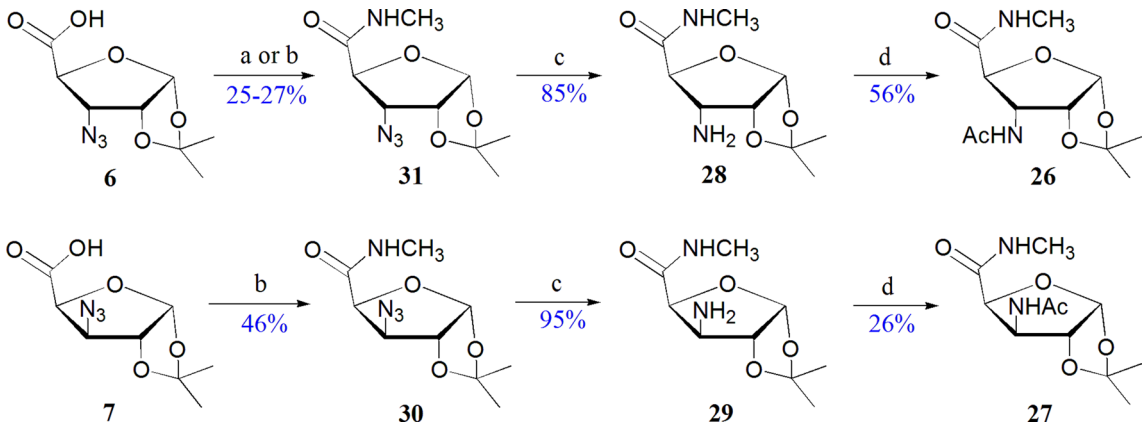
The formation of the unsaturated by-product **12** brings with the technical difficulty of its separation from the main product **4**. The column chromatography used by Vatéle and Hanessian (1996) was an appropriate choice for small quantities such as 300 mg, but not when synthesis is made on a larger scale. Therefore, we have reconsidered the purification process and have developed the following alternative working up, clearly more economic and environmentally friendly. The rough product was treated with $KMnO_4$ to oxidize the unsaturated by-product **12** into a vicinal diol and the water-soluble derivative easily extracted into the water phase. This improvement omitted the use of any chromatography (see "Methods").

Transformation of gluco and allo 3-azido-3-deoxy intermediates (4 and 5) into N_3-RibAFU(ip)-OH and N_3-XylAFU(ip)-OH

Selective removal of 5,6-*O*-isopropylidene protecting group of both *trans* and *cis* intermediates (**4** and **5**) with the conventional procedure (Zsoldos-Mády and Zbiral 1986) resulted in the 5,6-dihydroxy derivatives (**19** and **25**). After oxidative cleavage of diol compounds with sodium metaperiodate, the oxidation of the aldehydes was in situ performed into the carboxylic acids **6** and **7** (Scheme 5).



Scheme 6 The ultimate simplified reaction route to make azido-furanuronic acids as foldameric building blocks. Used reagents: *a* Im_2SO_2; *b* NaN_3; *c* AcOH; *d* $NaIO_4$ and $KMnO_4$



Scheme 7 Synthesis of AFU diamides for both C-3-epimers. Reagents and conditions: *a* (i) oxalyl chloride, DMF, THF, (ii) 2 M $MeNH_2$/THF; *b* (i) $ClCOO^tBu$, Et_3N, DMF, (ii) 2 M $MeNH_2$/THF; *c* H-Cube® H_2, CH_3OH, 10% Pd/C; *d* Ac_2O–pyridine

In conclusion, in line with Scheme 6 we propose the following reaction route, all steps now optimized using reagents of low costs, with unavoidable side products repressed and/or eliminated.

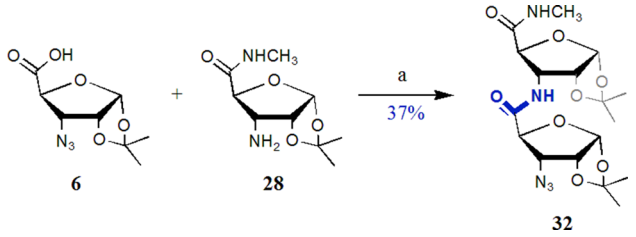
(B) Preparation of β-amino acid model compounds as LEGO-type bricks for foldamer research field

The 3-azido group of compounds **6** and **7** was reduced on two alternative ways: (a) hydrogenation in H-Cube® with Pd/C; (b) transformation via iminophosphorane ($Ph_3P{=}N–$) under the conditions of Staudinger reaction and subsequent hydrolysis (Csordás et al. 2016). Advantages of the use of H-Cube® continuous-flow reactor instead of the traditional catalytic hydrogenation are the higher yield and the easier separation of the product.

As mentioned earlier H-AFU-OH derivatives might be significant in peptide–foldamer sciences. The appropriate reaction conditions of peptide bond formation with H-AFU-OH are depending both on structure and substituents of the β-SAAs. Both *N*-acylation and N(Me)-amide formation reactions were tested on our AFUs (**1** and **2**) giving either Ac-RibAFU(ip)-NHMe (**26**) or the *xylo* epimer Ac-XylAFU(ip)-NHMe (**27**), the simplest and smallest models of homo- and heterooligomers of peptides (Perczel et al. 1991; James et al. 2009; Knapp et al. 2014). Both 3-azido-3-deoxy-furanuronic acids (**6** and **7**) are adequate intermediates for the synthesis of **26** and **27**, respectively, standing for β-amino acids with amide bonds at both ends (Scheme 7).

N-Methyl-carboxamide moiety was formed from both **6** and **7** with conventional amidation methods. Reduction of the azido group was carried out in an H-Cube® reactor with Pd/C, resulting in amino compound **28** and **29** in excellent yield (85–95%).

Interesting to note that by using the Staudinger reaction severe synthetic difficulties were observed, namely with the *xylo*(*cis*) epimer (**30**) the failure of the hydrolysis of the unexpectedly stable phosphinimine was observed. The rational explanation of the unexpected stability was found in steric repulsions and electronic interactions caused by the relative *cis* position of the vicinal triphenylphosphinimino group at C-3 and the substituents of C-4. These structural conditions were established by 3D-NMR and X-ray diffraction studies and corroborated by DFT calculations (Csordás et al. 2016).



Scheme 8 Coupling of sterically hindered trans-β-furanoid SAA dipeptide, namely N_3-RibAFU(ip)-RibAFU(ip)-NHMe. Reagents and conditions: *a* ClCOO^{t}Bu, Et_3N, DMF, −15 °C

*Coupling trans AFU derivatives making new β-peptide derivative (**32**)*

Foldamer synthesis with sterically hindered amines is a challenge and thus, preliminary reaction in optimizing conditions for making short sequences from β-amino acids is a requirement: dimeric, tetrameric and hexameric units form some of them were already studied (Chandrasekhar et al. 2004, 2005, 2008; Jagannadh et al. 2006; Pandey et al. 2011; Giri et al. 2012; Threlfall 2008). Here, we present the synthesis of *N*-terminal "masked", C-terminal protected -**X**-**X**- type β-dipeptide, by coupling *N*-methylated 3-amino-3-deoxy-ribofuranuronamide (**28**) with the related 3-azido-3-deoxy-ribofuranuronic acid (**6**, Scheme 8). The use of compound **6** as a reaction partner is based on the scope of the azido group being indeed a robust and uniform "mask" of the -NH_2 functionality, hence, it might play the role of both Fmoc-NH and Boc-NH functions widely used in peptide synthesis.

Coupling was carried out in solution under the usual conditions of peptide bond formation and the expected β-dipeptide (**32**) was isolated and purified. Though the yield is poor and needs to be improved and optimized, it is important that –*t***X**-*t***X**– can be achieved, thus direct coupling is possible.

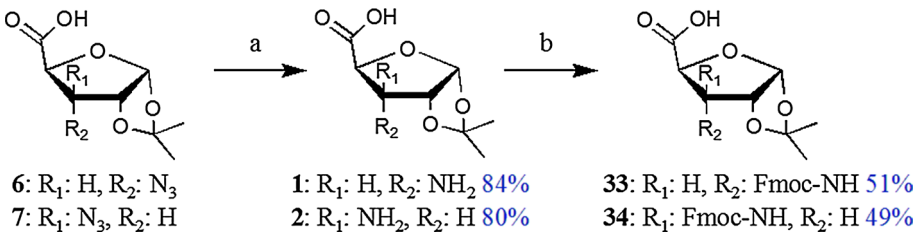
Synthesis of N-protected building block for SPPS

In modern peptide chemistry, the development of synthetic routes for the access of new *N*-protected monomeric amino acids as building block is essential. Thus, we have synthesized Fmoc-protected derivatives of –**X**– by using a continuous-flow hydrogenation reactor and subsequently Fmoc-OSu reagent (Scheme 9). At present Boc-protection is unsuitable as both Boc- and 1,2-*O*-isopropylidene group is acid labile.

The yields of hydrogenation are good (>80%), by using a continuous-flow reactor, a method fast, economic, useful and environmentally friendly. The subsequent reaction with Fmoc-OSu was found to perform poorer: the *N*-protected Fmoc-products were of moderate yield (51–49%). Nevertheless, in this way both Fmoc-RibAFU(ip)-OH (**33**) and its stereoisomer Fmoc-XylAFU(ip)-OH (**34**) are made, the former one synthesized for the first time. Thus, both Fmoc-key intermediates of AFU are now available for SPPS.

Conclusion

As we established, the success of the 6(7) stepped synthesis of β-sugar amino acids, as 3-amino-3-deoxy-pentofuranuronic acids (**1** and **2**) depends strongly on the efficacy of the transformation of the key step of 3-*O*-sulfonyl ester into the 3-azido-3-deoxy derivative. From D-glucose, the most abundant carbohydrate source, both C-3 epimers of the azido-furanuronic acid of both D-ribo and D-xylo configuration were obtained within 6(7) consecutive reaction steps. Yields are now optimized, only scalable and robust reactions are used. With detailed experimental studies on various fully protected 3-*O*-sulfonyl-furanoses we have established optimum conditions forming key intermediates and derivatives. As described recently (Pandey et al. 2011; Giri et al. 2012), newly synthesized sugar amino acid derivatives including the herein reported Fmoc–**X**–, key reactions and intermediates of the sterically hindered secondary amines were probed to make successfully –Aaa–*t***X**–Aaa– and –Aaa–*t***X**–*t***X**–Aaa– type "inserts" for α/β-chimera peptides. Thus, we have shown that both C-3 epimers of 3-azido-3-deoxy-pentofuranuronic acid, N_3-RibAFU(ip)-OH (**6**) and N_3-XylAFU(ip)-OH (**7**) are suitable building blocks to be inserted into β/β-oligopeptides. Making N_3-RibAFU(ip)-RibAFU(ip)-NHMe (**32**) is still a challenge due to steric hindrance, a step to be improved (Fig. 2).



Scheme 9 Synthesis of Fmoc-protected β-amino acid derivatives (Fmoc-RibAFU(ip)-OH and Fmoc-XylAFU(ip)-OH) for solid phase peptide synthesis. Reagents and conditions: *a* H-Cube® H_2, CH_3OH, 10% Pd/C, *b* MeOH-H_2O 2:1, $NaHCO_3$, Fmoc-OSu/THF

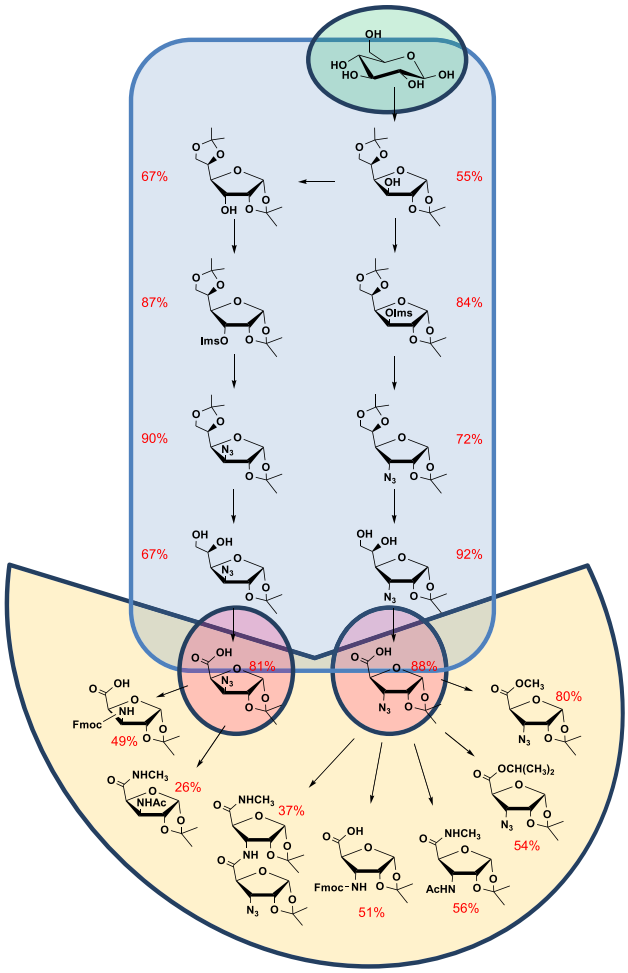


Fig. 2 Selected key Lego modules of water-soluble β-sugar amino acids, **6** and **7** made resourcefully from D-Glc

These ones and similar compounds are expected to be important intermediates both for academic research as well as for manufacturing biocompatible nanomaterials. The herein presented synthetic route was selected in line with sustainable or green(er) chemistry (e.g. most chromatography with organic solvents eliminated, continuous-flow reactor used for hydrogenation, highly toxic reagents replaced), by using the more economic azide intermediates when possible and thus, shortened by two reaction steps. All at a lower cost, derivatives now available on multigram scale, products obtained at a shorter time. Encompassing most necessary building blocks also tested in key coupling reactions for both stereoisomers of **X** gives "pros and cons" of the route to be selected for further optimization of coupling strategies and conditions in making longer oligomers perhaps ligated into polypeptides, chimera and foldamers.

Experimental section

Methods

Reagents, starting materials and solvents were obtained from Sigma-Aldrich, Merck, Reanal and VWR. Moisture-sensitive solvents either were distilled as standard procedure or dried on molecular sieve. Column chromatography was performed on Kieselgel 60 silica gel (0.040–0.063 mm; Merck). 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. MS spectra were performed with Bruker Esquire 3000+ tandem quadrupole mass spectrometer equipped with an electrospray ion source. FTIR spectra were recorded on a Bruker IFS 28 spectrometer by ATR technique. ^{1}H-NMR spectra were measured with Bruker Avance 250 spectrometer in $CDCl_3$, D_2O, DMSO-d_6 at room temperature. Deuterated solvents were purchased from Sigma-Aldrich.

*1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose (**3**)*

From α-D-glucose (100.0 g; 0.55 mol) with Schmidt's method (Schmidt 1963). The product **3** formed white crystals (79.8 g; 55%). M. p.: 112–113 °C; lit. m.p. (Schmidt 1963): 110 °C; $R_f = 0.74$ (hexane–EtOAc 1:2).

*1,2:5,6-Di-O-isopropylidene-α-D-allofuranose (**13**)*

(I) From **3** (5.0 g; 0.02 mol) with Austin's method (Austin et al. 1987), the product **13** formed white crystals (3.3 g; 67%). M.p.: 75–77 °C; lit. m.p. (Austin et al. 1987): 77–78 °C; $R_f = 0.57$ (hexane–EtOAc 1:1).
(II) 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**3**) (5.0 g; 0.02 mol) was dissolved in DCM (20 ml) then TEMPO (0.75 g; 4.7 mmol; 0.25 eqv.) and BAIB (2.5 eqv.) were added in several portions. The reaction mixture was stirred for 2 days at room temperature. The solution was diluted with DCM (100 ml) and washed with saturated $Na_2S_2O_3$ solution (2 × 50 ml). The aqueous phase was extracted with DCM (5 × 50 ml). The organic phase was washed with saturated $NaHCO_3$ solution (2 × 50 ml) and brine (2 × 50 ml). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo. The crude ketone was transformed without any isolation and purification. The reduction was carried out as in the case of method (A). The crude material was purified with column chromatography (hexane–EtOAc 1:1) to give the pure product **13** (2.6 g; 52%).

Synthesis of 3-*O*-sulfonate derivatives

*1,2:5,6-Di-O-isopropylidene-3-O-toluenesulfonyl-α-D-glucofuranose (**10**)*

(I) 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**3**) (3.9 g; 15.0 mmol) was dissolved in dry pyridine (15 ml) and *p*-toluenesulfonyl chloride (5.7; 29.9 mmol) was added to the solution with stirring at room temperature. The suspension was stirred for 1 day and allowed to stand for overnight at 0 °C. The reaction mixture was poured into ice-water, the precipitate was filtered, washed with cold water. The cream-coloured crude material was recrystallized from ethanol (95%) to give white crystalline product **10** (5.1 g; 83%). M.p.: 125–127 °C; lit. m.p. (Whistler and Doner 1972): 122–123 °C; $R_f = 0.80$ (hexane–EtOAc 2:1).
(II) From **3** (19.5 g; 75.0 mmol) the yield was 81% (25.1 g).

*1,2:5,6-Di-O-isopropylidene-3-O-toluenesulfonyl-α-D-allofuranose (**16**)*

(I) From 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (**13**) (10.0 g; 38.0 mmol) the reaction was carried out with the same method as the formation of **10**. After recrystallization of the crude product from ethanol (90%) the product **16** was obtained as white crystals (6.3 g; 63%). M.p.: 118–120 °C; lit. m.p. (Theoneste et al. 2009): 116–118 °C; $R_f = 0.64$ (hexane–EtOAc 1:1).
(II) From **13** (19.5 g; 75.0 mmol) the yield was 66% (20.5 g).

*1,2:5,6-Di-O-isopropylidene-3-O-methanesulfonyl-α-D-glucofuranose (**9**)*

1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**3**) (2.0 g; 7.7 mmol) was dissolved in dry pyridine (6 ml), cooled at 0 °C and methanesulfonyl chloride (0.8 ml) was dropped to the solution. The reaction mixture was allowed to stand for 2 days at 0 °C. After the solution was poured into ice-water, the solid crude product was filtered, washed with cold water and recrystallized from methanol (50%) to give white crystalline product **9** (1.8 g; 68%). M.p.: 83–84 °C; lit. m.p. (Helferich et al. 1939): 83–84 °C; $R_f = 0.44$ (hexane–EtOAc 2:1).

*1,2:5,6-Di-O-isopropylidene-3-O-methanesulfonyl-α-D-allofuranose (**15**)*

From 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (**13**) (0.50 g; 1.9 mmol) the reaction was carried out with the same method as the formation of **9**. Recrystallization of the crude product from ethanol gave white crystalline product **15** (0.32 g; 50%). M.p.: 133–136 °C; lit. m.p. (Reckendorf and Meyer 1968): 130–133 °C; $R_f = 0.44$ (hexane–EtOAc 1:1).

*1,2:5,6-Di-O-isopropylidene-3-O-trifluoromethanesulfonyl-α-D-glucofuranose (**8**)*

1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**3**) (3.0 g; 0.01 mol) was dissolved in dichloromethane (30 ml) containing pyridine (2.8 ml). The reaction mixture was cooled at −10 °C and under N_2-atmosphere trifluoromethanesulfonic anhydride (2.9 ml) was dropped to the solution. The reaction mixture was stirred for 2 h. After the solution was poured into ice-water, the aqueous phase was extracted with dichloromethane. The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo. The white crystalline product **8** crystallized from *n*-hexane (4.1 g; 90%). M.p.: 76–77 °C; lit. m.p. (Hall and Miller 1976): 70 °C; $R_f = 0.61$ (hexane–EtOAc 1:2).

*1,2:5,6-Di-O-isopropylidene-3-O-trifluoromethanesulfonyl-α-D-allofuranose (**14**)*

From 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (**13**) (0.50 g; 2.0 mmol) the reaction was carried out with the same method as the formation of **8**. The product **14** was pale yellow syrup (0.62 g; 83%). $R_f = 0.38$ (hexane–EtOAc 4:1).

*1,2:5,6-Di-O-isopropylidene-3-O-(N-imidazole-1-sulfonyl)-α-D-glucofuranose (**11**)*

(I) 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**3**) (1.0 g; 4.0 mmol) was dissolved in DMF (20 ml), cooled at 0 °C and under N_2-atmosphere NaH (0.14 g; 6.0 mmol) was added to the solution. The suspension was stirred for 30 min and cooled to −35 to −40 °C. After *N*′,*N*-sulfonyldiimidazole (1.2 g; 6.0 mmol) in DMF (12 ml) was dropped to the reaction mixture and stirred for 30 min again at −40 °C. MeOH was added (0.8 ml) to the solution and stirred for 30 min at −40 °C. The solution was poured into ice-water (200 ml). The precipitate was filtered, washed with cold water to get the white crystalline product **11** (1.2 g; 80%). M.p.: 97–98 °C; lit. m.p. (Vatéle and Hanessian 1996): 98–99 °C; $R_f = 0.70$ (EtOAc–hexane 1:1).
(II) From **3** (25.0 g; 96.0 mmol) the yield was 84% (31.5 g).

*1,2:5,6-di-O-isopropylidene-3-O-(N-imidazole-1-sulfonyl)-α-D-allofuranose (**17**)*

(I) From 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (**13**) (1.0 g; 4.0 mmol) the reaction was carried out with the same method as the formation of **11**, but after the solution was poured into ice-water, the aqueous phase was

extracted with diethyl ether (4 × 60 ml). The combined organic extracts were washed with water (4 × 30 ml) to pH 7. The organic phase was dried ($MgSO_4$), filtered and concentrated in vacuo to give colourless oil (1.4 g; 87%). $R_f = 0.40$ (EtOAc–hexane 1:1); ESI-MS: 391.2 $[M+H]^+$, calculated: 390.4 *m/z*.
(II) From **13** (25.0 g; 96.0 mmol) the yield was 80% (30.0 g).

Sulfonate ester → azide S_N-reaction

*1,2:5,6-Di-O-isopropylidene-3-azido-3-deoxy-α-D-allofuranose (**4**)*

(**A**) 1,2:5,6-Di-*O*-isopropylidene-3-*O*-trifluoromethanesulfonyl-α-D-glucofuranose (**8**) (0.56 g; 1.4 mmol) was dissolved in DMF (25.5 ml). Sodium azide (0.19 g; 2.9 mmol; 2 eqv.) and catalytic amount of tetra-*N*-butylammonium hydrogensulfate (0.002 g; 0.006 mmol) were added to the solution and stirred at 50 °C for 3 h and at room temperature for 13 h. The reaction was followed by TLC (hexane–EtOAc 3.5:1). The reaction mixture was concentrated in vacuo, the residue was dissolved in EtOAc (20 ml) and extracted with water (2 × 5 ml). The aqueous phase was extracted with EtOAc (3 × 3 ml). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated in vacuo to afford the crude product, which was the mixture of **4** and **12**. The crude product was purified by flash chromatography (hexane–EtOAc 3.5:1, 3.2:1, 2.5:1). The product **4** was colourless oil (0.18 g; 59%). $R_f = 0.53$ (hexane–EtOAc 3:1); FTIR-ATR: ν_{max}: 2106 (ν N_3) cm^{-1}. The by-product **12** formed white crystals (0.09 g; 36%). M.p.: 49–50 °C; lit. m.p. (Whistler and Doner 1972): 51 °C; $R_f = 0.75$ (hexane–EtOAc 3:1); FTIR-ATR: ν_{max}: 1672 (ν C=C) cm^{-1}.
(**B**) 1,2:5,6-Di-*O*-isopropylidene-3-*O*-methanesulfonyl-α-D-glucofuranose (**9**) (0.34 g; 1.0 mmol) was dissolved in DMSO (8 ml). Sodium azide (0.59 g; 9.0 mmol; 9 eqv.) was added to the solution and stirred at 140–145 °C for 181 h. The processing of the reaction mixture was analogous to the above. The crude product was homogeneous containing only the main product **4**, so further purification was not necessary. The product **4** was colourless oil (0.12 g; 54%). Spectral and chromatographic data of the product is in agreement with the data of the product by (**A**).
(**C**): (I) 1,2:5,6-Di-*O*-isopropylidene-3-*O*-toluenesulfonyl-α-D-glucofuranose (**10**) (1.2 g; 3.0 mmol) was dissolved in DMSO (33 ml). Sodium azide (1.2 g; 18.0 mmol; 6 eqv.) was added to the solution and stirred at 140–145 °C for 54 h. The reaction was followed by TLC (hexane–EtOAc 3.5:1). The reaction mixture was poured into ice-water (250 ml), extracted with petroleum ether (6 × 15 ml) and with ethyl acetate (2 × 15 ml). The combined organic extracts were washed with brine (1 × 20 ml), dried (Na_2SO_4), filtered and concentrated in vacuo to afford the crude product, which was the mixture of **4** and the by-pro duct **12**. The crude product was purified by column chromatography (hexane–EtOAc 4:1, 3:1). The product **4** was co lourless oil (0.26 g; 41%). The by-product **12** formed white crystals (0.02 g; 3%). Spectral and chromatographic data of the products are in agreement with the data of the products by (**A**).
(**C**): (II) From **10** (25.1 g; 60.6 mmol) the yield was 45% (7.8 g) for **4** and 8% (1.2 g) for **12**.
(**C**): (III) From **10** (0.41 g; 1.0 mmol) the reaction occurred analogously to method (**C**): (I), with sodium azide (4 eqv.) and catalytic amount of tetra-*N*-butylammonium hydrogensulfate (0.006 eqv.) was added to the solution. The reaction mixture was stirred at 140–145 °C for 33 h. The product **4** was colourless oil (0.09 g; 44%), the by-product **12** formed white crystals (0.01 g; 7%). Spectral and chromatographic data of the products are in agreement with the data of the products by (**A**).
(**C**): (IV) From **10** (24.1 g; 58.1 mmol) the reaction occurred analogously to method (**C**): (I), with tetra-*N*-butylammonium chloride (0.006 eqv.) catalyst. The reaction mixture was stirred at 130–135 °C for 73 h. The crude product was purified by column chromatography (hexane–acetone 14:1). The product **4** was colourless oil (5.4 g; 33%), the by-product **12** formed white crystals (1.5 g; 11%). Spectral and chromatographic data of the products are in agreement with the data of the products by (**A**).
(**C**): (V) From **10** (25.0 g; 60.3 mmol) the reaction occurred analogously to method (**C**): (I), in DMSO–H_2O 96:4. The crude product was purified by flash chromatography (hexane–acetone 14:1). The yield for **4** was 37% (6.3 g) and for **12** was 7% (1.1 g). Spectral and chromatographic data of the products are in agreement with the data of the products by (**A**).
(**D**): (I) 1,2:5,6-Di-*O*-isopropylidene-3-*O*-(*N*-imidazole-1-sulfonyl)-α-D-glucofuranose (**11**) (1.1 g; 2.8 mmol) was dissolved in toluene (33 ml). Tetra-*N*-butylammonium bromide (2.7 g; 8.4 mmol; 3 eqv.) and NaN_3 (0.55 g; 8.5 mmol; 3 eqv) were added to the solution and stirred for 30 min at room temperature. After the reaction mixture was refluxed for 10 h at 80 °C. The reaction was followed by TLC (EtOAc–hexane 1:1). After the reaction was complete, $KMnO_4$ solution (0.45 g; 1 eqv.) was added to the reaction mixture and stirred for 5 h. The organic phase, which contained only the main product **4** was separated and washed with water (2 × 10 ml). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo to afford the product **4** as colourless oil (0.42 g; 52%). Spectral and chromatographic data of the product are in agreement with the data of the product by (**A**).
(**D**): (II) From **11** (31.5 g; 80.8 mmol) the yield was 62% (14.3 g).

(**D**): (III) From **11** (0.3 g; 0.77 mmol) the reaction was analogous with method (**D**): (I), but the solvent was DMSO instead of toluene and the crude product, which was the mixture **4** and **12** was purified by column chromatography (hexane–acetone 14:1). The yield was 72% (0.16 g) for **4** and 20% (0.04 g) for **12**.

*1,2:5,6-Di-O-isopropylidene-3-azido-3-deoxy-α-D-glucofuranose (**5**)*

(**E**) From 1,2:5,6-di-*O*-isopropylidene-3-*O*-trifluoromethane sulfonyl-α-D-allofuranose (**14**) (0.56 g; 1.4 mmol) was dissolved in DMSO (25.5 ml). Sodium azide (0.48 g; 7.3 mmol; 5 eqv.) and catalytic amount of tetra-*N*-butylammonium hydrogensulfate (0.002 g; 0.006 mmol) were added to the solution and refluxed at 120 °C for 20 h. The reaction was followed by TLC (hexane–EtOAc 3.5:1), cooled at room temperature and streamed to ice-water (100 ml). The solution was extracted with petroleum ether (8 × 20 ml) and the combined organic extracts were washed with brine (3 × 20 ml), dried ($MgSO_4$), filtered and concentrated in vacuo to afford a golden yellow oil. The crude product was purified by column chromatography (hexane–EtOAc 3.5:1) to give a light yellow oil (0.16 g; 53%). R_f = 0.83 (EtOAc–hexane 1:1); FTIR-ATR: ν_{max}: 2111 (ν N_3) cm^{-1}.
(**F**) From 1,2:5,6-di-*O*-isopropylidene-3-*O*-methanesulfonyl-α-D-allofuranose (**15**) the reaction was analogous to the reaction of **14**. The reaction mixture was stirred at 120 °C for 180 h. The yield was 87% for **5**. Spectral and chromatographic data of the product are in agreement with the data of the product by (**E**).
(**G**) 1,2:5,6-Di-*O*-isopropylidene-3-*O*-toluenesulfonyl-α-D-allofuranose (**16**) the reaction was analogous to the reaction of **14**. The reaction mixture was stirred at 135 °C for 52 h. The yield was 74% for **5**. Spectral and chromatographic data of the product are in agreement with the data of the product by (**E**).
(**H**): (I) 1,2:5,6-Di-*O*-isopropylidene-3-*O*-(*N*-imidazole-1-sulfonyl)-α-D-allofuranose (**17**) (1.4 g; 3.5 mmol) was dissolved in toluene (40 ml). Tetra-*N*-butylammonium bromide (3.3 g; 0.01 mol; 3 eqv) and NaN_3 (0.67 g; 0.01 mol; 3 eqv) were added to the solution and stirred for 30 min at room temperature. After the reaction mixture was refluxed for 8 h between 80 and 90 °C. The reaction was followed by TLC (EtOAc–hexane 1:1). The precipitated NaBr was filtered. The organic phase was washed with water (5 × 10 ml), with saturated $NaHCO_3$ solution (2 × 10 ml) and brine (1 × 20 ml), dried ($MgSO_4$), filtered and concentrated in vacuo to afford the product **5** as colourless oil (0.89 g; 90%).
(**H**): (II) From **17** (30.0 g; 76.9 mmol) the yield was 88% (21.9 g). Spectral and chromatographic data of the product are in agreement with the data of the product by (**E**).

*1,2-O-Isopropylidene-3-azido-3-deoxy-α-D-allofuranose (**19**)*

1,2-*O*-Isopropylidene-3-*O*-toluenesulfonyl-α-D-glucofuranose (**18**) (0.50 g; 1.3 mmol) was dissolved in DMSO (1.8 ml). Sodium azide (0.35 g; 5.2 mmol; 4 eqv.) in DMSO (9.4 ml) was dropped to the solution and stirred at 135 °C for 29 h. The reaction mixture was poured into ice-water (15.0 ml), extracted with ethyl acetate (7 × 3 ml) and the combined organic extracts were washed brine (1 × 5 ml), dried (Na_2SO_4), filtered and concentrated in vacuo to afford the crude product, which was the mixture of the main product **19** and the elimination by-product **20**. The crude product was purified by column chromatography (EtOAc–hexane 2:1). The product **19** formed white crystals (0.11 g; 36%). M.p.: 76–78 °C; lit. m.p. (Gomtsyan et al. 1992): 75–76 °C; R_f = 0.54 (EtOAc); FTIR-ATR: ν_{max}: 3500 and 3358 (ν OH), 2100 (ν N_3) cm^{-1}. The by-product **20** is yellow oil (0.006 g; 2%).

*1,2-O-Isopropylidene-3-azido-3-deoxy-α-D-glucofuranose (**25**)*

1,2-*O*-Isopropylidene-3-*O*-toluenesulfonyl-α-D-allofuranose (**23**) (0.50 g; 1.3 mmol) was dissolved in DMSO (1.8 ml). Sodium azide (0.52 g; 7.9 mmol; 6 eqv.) in DMSO (9.4 ml) was dropped to the solution and stirred at 135 °C for 28 h. The reaction mixture was poured into ice-water (15.0 ml), extracted with ethyl acetate (6 × 3 ml) and the combined organic extracts were washed with brine (1 × 5 ml), dried (Na_2SO_4), filtered and concentrated in vacuo to afford the product **25** as white crystals (0.09 g; 28%). M.p.: 82–85 °C; lit. m.p. (Kovár and Jarý 1969): 83–84 °C; R_f = 0.35 (hexane–EtOAc 1:1); FTIR-ATR: ν_{max}: 3404 (ν OH), 2102 (ν N_3) cm^{-1}. ^{1}H NMR (CD_3OD, 250 MHz) δ = 5.80 (d, J = 3.62 Hz, 1H), 4.66 (d, J = 3.63 Hz, 1H), 4.14 (d, J = 3.06 Hz, 1H), 3.72 (m, 2H), 3.54 (m, 1H), 3.28 (d, J = 1.52 Hz, 1H), 1.43 and 1.28 (s, 6H) ppm.

*Methyl 3-deoxy-l,2-O-isopropylidene-D-glycero-pent-3-enofuranuronate (**22**)*

(I) 1,2-*O*-Isopropylidene-3-*O*-toluenesulfonyl-α-D-xylofuranuronic acid methyl ester (**21**) (0.11 g; 0.28 mmol) was dissolved in DMSO (0.4 ml). Sodium azide (0.07 g; 1.1 mmol; 4 eqv.) in DMSO (2.1 ml) was dropped to the solution and stirred at 80 °C for 5 h. The reaction mixture was poured into ice-water (10 ml) and extracted with ethyl acetate (4 × 3 ml) and the combined organic extracts were washed with brine (1 × 5 ml), dried (Na_2SO_4), filtered and concentrated in vacuo to afford the product **22** as yellow oil (0.09 g; 90%). R_f = 0.70 (EtOAc–hexane 1:2); FTIR-ATR: ν_{max}: 1735 (ν C=O); 1627 (ν C=C) cm^{-1}. ^{1}H NMR ($CDCl_3$, 250 MHz) δ = 6.11 (d, J = 5.35 Hz, 1H), 6.03 (d,

$J = 1.93$ Hz, 1H), 5.31 (dd, $J = 2.34$ Hz, $J = 4.96$ Hz, 1H), 3.78 (s, 3H), 1.39 (s, 6H) ppm.
(II) From 1,2-*O*-isopropylidene-3-*O*-toluenesulfonyl-α-D-ribofuranuronic acid methyl ester (**24**) (0.10 g; 0.26 mmol) the reaction was carried out as in the case of method (I). The product **22** was yellow oil (0.05 g; 92%). Spectral and chromatographic data of the product are in agreement with the data of the product by (I).

Removal of OH-5 and OH-6 isopropylidene protecting group

*1,2-O-Isopropylidene-3-azido-3-deoxy-α-D-allofuranose (**19**)*

(I) 1,2:5,6-Di-*O*-isopropylidene-3-azido-3-deoxy-α-D-allofuranose (**4**) (2.7 g; 9.6 mmol) was dissolved in acetic acid (44 ml, 75%). The solution was stirred at room temperature for 2 days and concentrated in vacuo. After evaporation of the organic solvents, crude product was purified with column chromatography (EtOAc–hexane 2:1) to afford white crystals (2.2 g; 92%). Spectral and chromatographic data of the product are in agreement with the data of the product above.
(II) From **4** (14.0 g; 49.1 mmol) the yield was 90% (10.8 g).

*1,2-O-Isopropylidene-3-azido-3-deoxy-α-D-glucofuranose (**25**)*

(I) From 1,2:5,6-di-*O*-isopropylidene-3-azido-3-deoxy-α-D-glucofuranose (**5**) (10.0 g; 35.0 mmol) the reaction was carried out with the same method as the formation of **19**. After crystallization of the crude product from ethyl acetate–*n*-hexane 1:1 the pure product **25** was got in the form of white crystals (5.4 g; 67%). Spectral and chromatographic data of the product are in agreement with the data of the product above.
(II) From **5** (21.0 g; 73.7 mmol) the yield was 65% (11.7 g).

*1,2-O-Isopropylidene-3-O-toluenesulfonyl-α-D-glucofuranose (**18**)*

From 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluenesulfonyl-α-D-glucofuranose (**10**) (6.0 g; 14.5 mmol) the reaction was carried out with the same method as the formation of **19**. After purification of the crude product with column chromatography (EtOAc-hexane 2:1) the pure product **18** was obtained as colourless oil (5.2 g; 95%); $R_f = 0.62$ (EtOAc–hexane 2:1). FTIR-ATR: ν_{max}: 3520 (ν OH) cm^{-1}; ESI-MS: 392.3 $[M+NH_4]^+$, calculated: 374.41 *m/z*. ^{1}H NMR (DMSO-d_6, 250 MHz) $\delta = 7.83$ (d, $J = 8.31$ Hz, 2H), 7.47 (d, $J = 8.10$ Hz, 2H), 5.93 (d, $J = 3.83$ Hz, 1H), 4.68 (dd, $J = 3.32$ Hz, $J = 7.61$ Hz, 2H), 4.62 (d, $J = 6.18$ Hz, 1H), 4.54 (t, $J = 5.44$ Hz, 1H), 4.02 (dd, $J = 2.71$ Hz, $J = 9.01$ Hz, 1H), 3.48 (m, 1H), 2.42 (s, 3H), 1.37 (s, 3H), 1.23 (s, 3H) ppm.

*1,2-O-Isopropylidene-3-O-toluenesulfonyl-α-D-allofuranose (**23**)*

From 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluenesulfonyl-α-D-allofuranose (**16**) (2.0 g; 4.8 mmol) the reaction was carried out with the same method as the formation of **19**. After purification of the crude product with column chromatography (EtOAc–hexane 2:1) the pure product **23** was pale yellow oil (1.7 g; 93%). $R_f = 0.57$ (EtOAc–hexane 2:1); FTIR-ATR: ν_{max}: 3534 (ν OH) cm^{-1}; ESI-MS: 392.3 $[M+NH_4^+]^+$, calculated: 374.4 *m/z*. ^{1}H NMR (DMSO-d_6, 250 MHz) $\delta = 7.83$ (d, $J = 8.22$ Hz, 2H), 7.47 (d, $J = 8.18$ Hz, 2H), 5.93 (d, $J = 3.68$ Hz, 1H), 4.67 (dd, $J = 3.17$ Hz, $J = 10.09$ Hz, 2H), 4.59 (d, $J = 6.01$ Hz, 1H), 4.52 (t, $J = 5.16$ Hz, 1H), 4.03 (dd, $J = 2.53$ Hz, $J = 8.98$ Hz, 1H), 3.48 (m, 1H), 2.42 (s, 3H), 1.37 (s, 3H), 1.23 (s, 3H) ppm.

Formation of COOH functional group

*1,2-O-Isopropylidene-3-azido-3-deoxy-α-D-ribofuranuronic acid (**6**)*

(I) 1,2-*O*-Isopropylidene-3-azido-3-deoxy-α-D-allofuranose (**19**) (0.89 g; 3.7 mmol) was dissolved in MeOH–water 6:4 (10.5 ml) and cooled at 0 °C. Sodium periodate (0.88 g; 4.1 mmol) was dissolved in water (6.3 ml) and added to the solution of the starting material. The reaction mixture was stirred for a day at room temperature. The inorganic salts were precipitated by addition of methanol (15 ml), were filtered off and washed several times with MeOH. The solvent was removed and the crude product was reacted without purification. The aldehyde derivative was dissolved in acetic acid (18 ml, 50%). Potassium permanganate (0.83 g; 5.2 mmol) was dissolved in water (16 ml) and added to the solution. The reaction mixture was stirred for 12 h at room temperature, then was allowed to stand at 0 °C for 2 days. The reaction mixture was filtered, excess $KMnO_4$ was removed with sodium sulphite. The solution was adjusted to pH 2–3 with 2 M HCl and extracted with ethyl acetate (6 × 10 ml). The aqueous phase was saturated with sodium chloride and extracted with ethyl acetate (1 × 10 ml). The combined organic extracts were extracted with saturated sodium chloride solution (2 × 15 ml). The organic phase was dried ($MgSO_4$) and concentrated in vacuo to afford the crude product. Crystallization of the crude product from ethyl acetate–*n*-hexane afforded white crystals (0.74 g; 88% for 2 steps). M.p.: 110–112 °C; lit.

m.p. (Kulinkovich and Timoshchuk 1983): 107–108 °C; R_f = 0.44 (chloroform–methanol 3:2). FTIR-ATR: $\nu_{max:}$ 3500–2400 (ν COOH), 2107 (ν N_3), 1736 (ν C=O) cm^{-1}; ESI-MS: 228.2 $[M-H]^-$, calculated: 229.2 *m/z*; ^{1}H NMR ($CDCl_3$, 250 MHz) δ = 7.92 (br s, 1H), 5.87 (d, J = 3.1 Hz, 1H), 4.72 (br m, J = 4.2 Hz, 1H), 4.55 (d, J = 9.5 Hz, 1H), 3.68 (dd, J = 4.2 Hz, J = 9.5 Hz, 1H), 1.53 (s, 3H), 1.32 (s, 3H) ppm.
(II) From **19** (10.0 g; 40.8 mmol) the yield was 80% (7.5 g) for the 2 steps.

*1,2-O-Isopropylidene-3-azido-3-deoxy-α-D-xylofuranuronic acid (**7**)*

(I) From 1,2-*O*-isopropylidene-3-azido-3-deoxy–α-D-glucofuranose (**25**) (5.0 g; 0.02 mol) the reaction was carried out with the same method as the formation of **6**. In this case the reaction mixture was extracted with chloroform (1×) and dichloromethane (5×). The product **7** was pale yellow oil (3.8 g; 82% for 2 steps). R_f = 0.11 (hexane–EtOAc 1:4); FTIR-ATR: ν_{max}: 3462 (ν OH), 2108 (ν N_3), 1735 (ν C=O) cm^{-1}; ESI-MS: 228.2 $[M-H]^-$, calculated: 229.2 *m/z*.
(II) From **25** (10.0 g; 40.8 mmol) the yield was 78% (7.3 g) for the 2 steps.

*1,2-O-Isopropylidene-3-O-toluenesulfonyl-α-D-xylofuranuronic acid (**35**)*

From 1,2-*O*-isopropylidene-3-*O*-toluenesulfonyl-α-D-glucofuranose (**18**) (1.0 g; 2.7 mmol) the reaction was carried out with the same method as the formation of **6**. The product **35** was colourless oil (0.26 g; 27% for 2 steps). R_f = 0.70 (toluene–EtOAc–EtOH–AcOH 4:2:2:1). FTIR-ATR: ν_{max}: 3500–2400 (ν COOH), 1756 (ν C=O); ESI-MS: 376.3 $[M+NH_4]^+$, calculated: 358.37 *m/z*; ^{1}H NMR ($CDCl_3$, 250 MHz) δ = 7.75 (d, J = 8.36 Hz, 2H), 7.36 (d, J = 8.08 Hz, 2H), 6.12 (d, J = 3.42 Hz, 1H), 5.02 (d, J = 3.35 Hz, 1H), 4.93 (d, J = 3.43 Hz, 1H), 4.82 (d, J = 3.36 Hz, 1H), 2.45 (s, 3H), 1.49 (s, 3H), 1.34 (s, 3H) ppm.

*1,2-O-Isopropylidene-3-O-toluenesulfonyl-α-D-ribofuranuronic acid (**36**)*

From 1,2-*O*-isopropylidene-3-*O*-toluenesulfonyl-α-D-allofuranose (**23**) (1.0 g; 2.7 mmol) the reaction was carried out with the same method as the formation of **6**. The product **36** was pale yellow (0.16 g; 16% for 2 steps). R_f = 0.73 (toluene–EtOAc–EtOH–AcOH 4:2:2:1). FTIR-ATR: ν_{max}: 3500–2400 (ν COOH), 1747 (C=O) cm^{-1}; ESI-MS: 376.3 $[M+NH_4]^+$, calculated: 358.37 *m/z*; ^{1}H NMR (DMSO-d_6, 250 MHz) δ = 7.77 (d, J = 8.25 Hz, 2H), 7.48 (d, J = 8.16 Hz, 2H), 6.03 (d, J = 3.68 Hz, 1H), 4.87 (d, J = 3.27 Hz, 1H), 4.79 (d, J = 3.32 Hz, 1H), 4.69 (d, J = 3.72 Hz, 1H), 2.43 (s, 3H), 1.40 (s, 3H), 1.24 (s, 3H) ppm.

Formation of NH_2 from N_3-group

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

1,2-*O*-Isopropylidene-3-azido-3-deoxy-α-D-ribofuranuronic acid (**6**) (0.26 g; 1.1 mmol) was dissolved in methanol (31.8 ml, c = 35 mmol/l) and reduced with H_2 on 10% Pd/C by H-Cube (parameters: 60 °C, 10 bar, 0.3 ml/min). After the reaction, the solution was concentrated in vacuo to give the product **1** as pale yellow oil (0.19 g; 84%). R_f = 0.51 (chloroform–methanol 3:2). FTIR-ATR: $\nu_{max:}$ 3170 (ν NH_3^+), 2750–2460 and 2170–1900 diffuse (ν NH_3^+), 1605 (ν COO^-) cm^{-1}. ESI-MS: 204.1 $[M+H]^+$, calculated: 203.19 *m/z*. ^{1}H NMR (D_2O, 250 MHz) δ = 6.04 (d, J = 3.7 Hz, 1H), 4.97 (t, J = 4.60 Hz, 1H), 4.43 (d, J = 10.10 Hz, 1H), 3.68 (dd, J = 4.60 Hz, J = 10.10 Hz, 1H), 1.58 (s, 3H), 1.39 (s, 3H) ppm.

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

From 1,2-*O*-isopropylidene-3-azido-3-deoxy-α-D-xylofuranuronic acid (**7**) (0.20 g; 0.87 mmol) the reaction was carried out with the same method as the formation of **1**. The product was pale yellow oil (0.14 g; 80%). R_f = 0.50 (chloroform–methanol 3:2); FTIR-ATR: ν_{max}: 2977, 2465 (ν COOH), 1637 (ν COO^-), 1560, 1377 (ν_{as} COO^-, δ_s CH_3) cm^{-1}; ESI-MS: 204.2 $[M+H]^+$, calculated: 203.19 *m/z*. ^{1}H NMR (D_2O, 250 MHz) δ = 5.97 (d, J = 3.83 Hz, 1H), 4.79 (d, J = 3.85 Hz, 1H), 4.65 (overlapped by the signal of D_2O, 1H), 3.86 (d, J = 3.95 Hz, 1H), 1.39 and 1.21 (s, 6H) ppm.

Formation of ester derivatives

*1,2-O-Isopropylidene-3-O-toluenesulfonyl-α-D-xylofuranuronic acid methyl ester (**21**)*

1,2-*O*-Isopropylidene-3-*O*-toluenesulfonyl-α-D-xylofuranuronic acid (**35**) (0.20 g; 0.56 mmol) was dissolved in dichloromethane (10 ml) and cooled to 0 °C. The cold diazomethane/diethyl ether solution (15 ml) was dropped to the reaction mixture, while the yellow colour remained for 20 min. The rest of the diazomethane was removed with air and the solution was concentrated in vacuo to get the product **21** as pale yellow oil (0.20 g; 96%). R_f = 0.66 (EtOAc–hexane 1:1); FTIR-ATR:

ν_{max}: 1767 (ν C=O) cm^{-1}. ESI-MS: 390.3 $[M+NH_4]^+$, calculated: 372.40 *m/z*; ^1H NMR ($CDCl_3$, 250 MHz) δ = 7.77 (d, J = 8.32 Hz, 2H), 7.37 (d, J = 8.21 Hz, 2H), 6.07 (d, J = 3.50 Hz, 1H), 5.13 (d, J = 3.26 Hz, 1H), 4.84 (d, J = 3.31 Hz, 1H), 4.77 (d, J = 3.50 Hz, 1H), 3.58 (s, 3H), 2.46 (s, 3H), 1.48 (s, 3H), 1.32 (s, 3H) ppm.

*1,2-O-Isopropylidene-3-O-toluenesulfonyl-α-D-ribofuranuronic acid methyl ester (**24**)*

From 1,2-*O*-isopropylidene-3-*O*-toluenesulfonyl-α-D-ribofuranuronic acid (**36**) (0.12 g; 0.33 mmol) the reaction was carried out with the same method as the formation of **21**. The product **24** was pale yellow oil (0.12 g; 96%). R_f = 0.69 (EtOAc–hexane 1:1); FTIR-ATR: ν_{max}: 1766 (ν C=O) cm^{-1}. ESI-MS: 390.32 $[M+NH_4]^+$, calculated: 372.40 *m/z*; ^1H NMR ($CDCl_3$, 250 MHz) δ = 7.70 (d, J = 8.35 Hz, 2H), 7.30 (d, J = 8.09 Hz, 2H), 6.00 (d, J = 3.53 Hz, 1H), 5.06 (d, J = 3.32 Hz, 1H), 4.77 (d, J = 3.33 Hz, 1H), 4.71 (d, J = 3.54 Hz, 1H), 3.51 (s, 3H), 2.40 (s, 3H), 1.41 (s, 3H), 1.25 (s, 3H) ppm.

*1,2-O-Isopropylidene-3-azido-3-deoxy-α-D-ribofuranuronic acid methyl ester (**37**)*

From 1,2-*O*-isopropylidene-3-azido-3-deoxy-α-D-ribofuranuronic acid (**6**) (0.40 g; 1.7 mmol) the reaction was carried out with the same method as the formation of **21**. The crude product was purified by column chromatography (hexane–EtOAc 1:2). The product (**37**) was colourless yellow oil (0.34 g; 80%). R_f = 0.90 (hexane–EtOAc 1:2.5); FTIR-ATR: ν_{max}: 2115 (ν N_3), 1745 (ν C=O) cm^{-1}. ESI-MS: 261.2 $[M+NH_4]^+$, calculated: 243.2 *m/z*; ^1H NMR ($CDCl_3$, 250 MHz) δ = 5.86 (d, J = 3.43 Hz, 1H), 4.69 (t, J = 3.97 Hz, 1H), 4.50 (d, J = 9.58 Hz, 1H), 3.79 (s, 3H), 3.64 (dd, J = 4.50 Hz, J = 9.56 Hz, 1H), 1.52 (s, 3H), 1.31 (s, 3H) ppm.

*1,2-O-Isopropylidene-3-azido-3-deoxy-α-D-ribofuranuronic acid isopropyl ester (**38**)*

1,2-*O*-Isopropylidene-3-azido-3-deoxy-α-D-ribofuranuronic acid (**6**) (0.50 g; 2.2 mmol) was dissolved in CH_3CN (4.2 ml) and cooled at 0 °C. Oxalyl chloride (0.34 ml) and dry DMF (0.16 ml) were dropped to the solution and stirred for 5 h at room temperature. The solvent was removed, the residue was dissolved in $CHCl_3$ (1.7 ml) and cooled to 0 °C. 2-PrOH (6.0 ml) was dropped slowly to the solution and stirred for 45 min. The reaction mixture was poured onto iced water and extracted with DCM (5 × 8 ml). The organic phase was dried (Na_2SO_4) and concentrated. The crude product was isolated and purified by column chromatography (hexane–EtOAc 1:2) to give colourless oil (0.32 g; 54%). FTIR-ATR: ν_{max}: 2106 (ν N_3), 1738 (ν C=O), 1369 (ν_{as} C–CO–O) cm^{-1}. ^1H NMR ($CDCl_3$, 250 MHz): δ 5.84 (d, J = 3.49 Hz, 1H), 5.10 (sept, J = 6.31 Hz, J = 6.33 Hz, J = 6.33 Hz, 1H), 4.67 (m, 1H), 4.45 (d, J = 9.60 Hz, 1H), 3.63 (dd, J = 4.54 Hz, J = 9.56 Hz, 1H), 1.52 and 1.30 (s, 6H), 1.27 and 1.23 (d, 6H) ppm.

Synthesis of uronamide derivatives

*N-Methyl-1,2-O-isopropylidene-3-azido-3-deoxy-α-D-ribofuranuronamide (**31**)*

(I) 1,2-*O*-Isopropylidene-3-azido-3-deoxy-α-D-ribofuranuronic acid (**6**) (0.50 g; 2.2 mmol) was dissolved in dry THF (4.2 ml) and cooled to 0 °C. Oxalyl chloride (0.34 ml) and dry DMF (0.16 ml) were dropped to the solution and stirred for 5 h at room temperature. The solvent was removed, the residue was dissolved in DCM (1.7 ml) and cooled to 0 °C. Slowly 2 M $MeNH_2$/THF solution (6.0 ml) was dropped to the solution and it was stirred for 45 min. The reaction mixture was poured into ice-water and extracted with DCM (5 × 8 ml). The organic phase was dried (Na_2SO_4) and concentrated in vacuo. The crude product was isolated and purified by column chromatography (hexane–EtOAc 1:2) to give white crystals (0.14 g; 27%). M.p.: 170–173 °C; R_f = 0.34 (EtOAc–hexane 2:1); FTIR-ATR: ν_{max}: 3368 (ν NH), 2110 (ν N_3), 1652 (amide I, ν C=O and ν C–N), 1538 (amide II) cm^{-1}; ESI-MS: 243.2 $[M+H]^+$, calculated: 242.2 *m/z*; ^1H NMR ($CDCl_3$, 250 MHz) δ = 6.49 (broad m, 1H), 5.82 (d, J = 3.4 Hz, 1H), 4.69 (t, J = 4.5 Hz, 1H), 4.45 (d, J = 9.5 Hz, 1H), 3.64 (dd, J = 4.5 Hz, J = 9.5 Hz, 1H), 2.83 (d, J = 5.0 Hz, 3H), 1.54 (s, 3H), 1.34 (s, 3H) ppm.
(II) 1,2-*O*-Isopropylidene-3-azido-3-deoxy-α-D-ribofuranuronic acid (**6**) (0.50 g; 2.2 mmol) was dissolved in dry DMF (26.4 ml) and cooled to −15 °C. Triethylamine (0.35 ml) and isobutyl chloroformate (0.32 ml) were dropped to the solution and stirred. The temperature was kept between −10 and −15 °C (Solution I). Methylamine in THF (1.12 ml, 2 M) included TEA (0.35 ml) (Solution II) was cooled to 0 °C and added to the Solution I after 20 min. The reaction mixture was stirred for an hour between −10 and −15 °C and even an hour at 0 °C, then pH was checked (pH 7–8). The precipitate (TEA.HCl) was filtered, washed with cool DMF. The solvent was removed, the residue was dissolved in EtOAc (20 ml) and extracted with cool distilled water (2 × 10 ml), HCl solution (2 × 10 ml, 2 N), saturated solution of $NaHCO_3$ (2 × 10 ml) and brine (2 × 10 ml). The organic phase was dried ($MgSO_4$) and concentrated in vacuo. The crude pro duct was isolated and purified by column chromatography (hexane–EtOAc 1:2) to give white crystals (0.13 g; 25%). Spectral and chromatographic data of the product are in agreement with the data of the product by (II).

*N-Methyl-1,2-O-isopropylidene-3-azido-3-deoxy-α-D-xylofuranuronamide (**30**)*

From **7** (0.5 g; 2.2 mmol) the reaction was carried out with the same method as the formation of **31** with method (II). The product **30** was pale yellow oil (0.24 g; 46%). $R_f = 0.4$ (EtOAc–hexane 1:1); FTIR-ATR: ν_{max}: 3349 (ν NH), 2106 (ν N_3), 1645 (amide I, ν C=O), 1535 (amide II) cm^{-1}. ESI-MS: 243.2 $[M+H]^+$, calculated: 242.2 *m/z*; ^1H NMR ($CDCl_3$, 250 MHz) $\delta = 6.45$ (broad m, 1H), 5.87 (d, $J = 3.32$ Hz, 1H), 4.70 (d, $J = 3.45$ Hz, 1H), 4.55 (m, 1H), 4.34 (d, $J = 3.43$, 1H), 2.83 (d, $J = 4.95$ Hz, 3H), 1.51 (s, 3H), 1.27 (s, 3H) ppm.

*N-Methyl-1,2-O-isopropylidene-3-amino-3-deoxy-α-D-ribofuranuronamide (**28**)*

(I) *N*-Methyl-1,2-*O*-isopropylidene-3-azido-3-deoxy-α-D-ribofuranuronamide (**31**) (0.24 g; 0.99 mmol) was dissolved in methanol (28.0 ml, $c = 35$ mmol/l) and reduced with H_2 on 10% Pd/C by H-Cube (parameters: 60 °C, 10 bar, 0.2 ml/min). After the reaction, the solution was concentrated in vacuo to give the product **28** as pale yellow oil (0.18 g; 85%). $R_f = 0.86$ (chloroform–methanol 3:1); FTIR-ATR: ν_{max}: 3375 and 3353 (ν NH_2 and NH), 1652 (amide I), 1545 (amide II) cm^{-1}; ESI-MS: 217.2 $[M+H]^+$, calculated: 216.2 *m/z*; ^1H NMR ($CDCl_3$, 250 MHz) $\delta = 6.44$ (broad m, 1H), 6.00 (d, $J = 3.4$ Hz, 1H), 4.80 (t, $J = 4.4$ Hz, 2H), 4.54 (d, $J = 9.5$ Hz, 1H), 2.81 (d, $J = 5.0$ Hz, 3H), 1.43 (s, 3H), 1.27 (s, 3H) ppm.
(II)*N*-Methyl-1,2-*O*-isopropylidene-3-azido-3-deoxy-α-D-ribofuranuronamide (**31**) (0.16 g; 0.67 mmol) was dissolved in dry pyridine (6 ml) and triphenylphosphine (0.34 g; 1.3 mmol) was added to the solution. The reaction mixture was allowed to stand for 2 h at room temperature. Ammonium hydroxide solution (2.3 ml, 25%) 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 triphenylphosphine and triphenylphosphine oxide was removed by filtration. Evaporation of the aqueous solution gave pale yellow oil as homogeneous product **28** (0.15 g; 92%). Spectral and chromatographic data of the product are in agreement with the data of the product from the H-Cube reactor.

*N-Methyl-1,2-O-isopropylidene-3-amino-3-deoxy-α-D-xylofuranuronamide (**29**)*

N-Methyl-1,2-*O*-isopropylidene-3-azido-3-deoxy-α-D-xylofuranuronamide (**30**) (0.31 g; 1.3 mmol) was dissolved in methanol (37 ml, 35 mmol/l). The starting material was reduced with H_2 on 10% Pd/C by H-Cube (parameters: 70 °C, 10 bar, 0.2 ml/min). The reaction time was 6 h to give a colourless oil (0.30 g; 95%). $R_f = 0.83$ (chloroform–methanol 3:1); FTIR-ATR: ν_{max}: 3340 (ν NH_2 and NH), 1655 (amide I), 1544 (amide II) cm^{-1}; ESI-MS: 217.2 $[M+H]^+$, calculated: 216.2 *m/z*; ^1H NMR ($CDCl_3$, 250 MHz) $\delta = 6.45$ (broad m, 1H), 6.00 (d, $J = 3.25$ Hz, 1H), 4.81 (dd, $J = 3.21$ Hz, 2H), 4.54 (d, $J = 3.17$ Hz, 1H), 2.81 (d, $J = 5.02$ Hz, 3H), 1.43 (s, 3H), 1.27 (s, 3H) ppm.

*N-Methyl-1,2-O-isopropylidene-3-acetamido-3-deoxy-α-D-ribofuranuronamide (**26**)*

N-Methyl-1,2-*O*-isopropylidene-3-amino-3-deoxy-α-D-ribofuranuronamide (**28**) (0.03 g; 0.14 mmol) was dissolved in cold mixture of pyridine (0.17 ml) and acetic anhydride (0.1 ml). The reaction mixture was allowed to stand at 0 °C for 2 days. The solution was dropped to ice-water (5 ml) and concentrated in vacuo. The crude product was crystallized from ethyl acetate–petroleum ether to give white crystals (0.02 g; 56%). M.p.: 208–210 °C; $R_f = 0.62$ (chloroform–methanol 3:2); FTIR-ATR: ν_{max}: 3391 and 3324 (ν NH), 1655 (amide I), 1546 (amide II) cm^{-1}; ESI-MS: 259.2 $[M+H]^+$, calculated: 258.2 *m/z*; ^1H NMR ($CDCl_3$, 700 MHz) $\delta = 6.42$ (broad m, 1H), 6.08 (d, $J = 7.2$ Hz, 1H), 5.79 (d, $J = 3.4$ Hz, 1H), 4.70 (t, $J = 4.2$ Hz, 1H), 4.23 (ddd, $J = 4.2$ Hz, $J = 10.2$ Hz, $J = 7.2$ Hz, 1H), 4.17 (d, $J = 10.2$ Hz, 1H), 2.78 (d, $J = 5.0$ Hz, 3H), 2.00 (s, 3H), 1.48 (s, 3H), 1.28 (s, 3H) ppm.

*N-Methyl-1,2-O-isopropylidene-3-acetamido-3-deoxy-α-D-xylofuranuronamide (**27**)*

From **29** (0.04 g; 0.18 mmol) the reaction was carried out with the same method as the formation of **26**. The product **27** was colourless yellow oil (0.015 g; 31%). $R_f = 0.70$ (chloroform–methanol 3:2); FTIR-ATR: ν_{max}: 3293 (ν NH), 1654 (amide I, ν C=O), 1544 (amide II) cm^{-1}; ESI-MS: 259.2 $[M+H]^+$, calculated: 258.2 *m/z*; ^1H NMR ($CDCl_3$, 250 MHz) $\delta = 6.83$ (m, 1H), 6.61 (m, 1H), 6.04 (m, 1H), 4.73 (m, 2H), 4.41 (m, 1H), 2.78 (d, 3H), 1.90 (s, 3H), 1.43 (s, 3H), 1.25 (s, 3H) ppm.

Synthesis of sugar amino acid dipeptide

*3-Azido-3-deoxy-1,2-O-isopropylidene-α-D-ribofuranuronil-N-methyl-1,2-O-isopropylidene-D-ribofuranuronamide (**32**)*

1,2-*O*-Isopropylidene-3-azido-3-deoxy-α-D-ribofuranuronic acid (**6**) (0.95 g; 0.42 mmol) was dissolved in dry DMF (2.5 ml) and cooled at −15 °C. Triethylamine (80 µl) and isobutyl chloroformate (75 µl) were dropped to the solution and stirred. The temperature was kept between −10

and −15 °C for 40 min (Solution I). *N*-methyl-1,2-*O*-isopropylidene-3-amino-3-deoxy-α-D-ribofuranuronamide (**28**) (0.09 g; 0.42 mmol) included TEA (80 µl) (Solution II) was cooled to 0 °C and added to the Solution I. The reaction mixture was stirred for an hour between −10 and −15 °C and even an hour at 0 °C, then pH was checked (pH 7–8). The precipitate (TEA.HCl) was filtered, washed with cool DMF. The solvent was removed. The crude product was isolated and purified by column chromatography (hexane–EtOAc 1:1) to give white crystals (0.07 g; 37%). M.p.: 171–174 °C; $R_f = 0.40$ (EtOAc); FTIR-ATR: ν_{max}: 3362 and 3229 (ν NH), 2110 (ν N_3), 1681 (ν C=O), 1652 (amide I), 1525 (amide II) cm^{-1}. ESI-MS: 428.3 $[M+H]^+$, calculated: 427.41 *m/z*. ^1H NMR ($CDCl_3$, 250 MHz) $\delta = 6.86$ (broad d, $J = 7.9$ Hz, 1H), 6.36 (broad m, 1H), 5.83 (d, $J = 3.5$, 1H), 5.81 (d, $J = 3.5$ Hz, 1H), 4.66 (t, $J = 4.4$ Hz, 1H), 4.64 (t, $J = 4.2$ Hz, 1H), 4.50 (d, $J = 9.5$ Hz, 1H), 4.23 (ddd, $J = 4.2$ Hz, $J = 9.9$ Hz, $J = 7.2$ Hz, 1H), 4.17 (t, $J = 9.9$ Hz, 1H), 3.63 (dd, $J = 4.4$ Hz, $J = 9.5$ Hz, 1H), 2.76 (d, $J = 5.0$ Hz, 3H), 1.51 (s, 3H), 1.48 (s, 3H), 1.31 (s, 3H), 1.28 (s, 3H) ppm.

Synthesis of *N*-protected derivatives

*1,2-O-Isopropylidene-N-(9-fluorenylmethoxy-carbonyl)-3-amino-3-deoxy-α-D-ribofuranuronic acid (**33**)*

The stirred solution of 1,2-*O*-isopropylidene-3-amino-3-deoxy-α-D-ribofuranuronic acid (**1**) (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 eqv.) 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 ($MgSO_4$) and concentrated in vacuo. The product formed colourless oil (0.21 g; 51%). $R_f = 0.50$ (ethyl acetate–methanol 4:1); FTIR-ATR: ν_{max}: 3500-2400 (ν COOH), 3349 (ν NH), 1719 (ν C=O), 1691 (ν aromatic), 1600 (amide I), 1523 (amide II) cm^{-1}; ESI-MS: 426.2 $[M+H]^+$, calculated: 425.19 *m/z*. ^1H NMR (DMSO-d_6, 250 MHz) $\delta = 7.74$ (m, 5H), 7.36 (m, 5H), 5.85 (d, $J = 3.29$ Hz, 1H), 4.61 (m, 1H), 4.28 (m, 3H), 4.06 (m, 1H), 1.46 (s, 3H), 1.26 (s, 3H) ppm.

*1,2-O-Isopropylidene-N-(9-fluorenylmethoxy-carbonyl)-3-amino-3-deoxy-α-D-xylofuranuronic acid (**34**)*

From 1,2-*O*-isopropylidene-3-amino-3-deoxy-α-D-xylofuranuronic acid (**2**) (0.13 g; 0.64 mmol) the reaction was carried out with the same method as the formation of (**33**). The product formed colourless oil (0.13 g; 49%). $R_f = 0.52$ (ethyl acetate–methanol 4:1); FTIR-ATR: ν_{max}: 3500–2400 (ν COOH), 1719 (broad, ν C=O, ν aromatic, amide I), 1519 (amide II) cm^{-1}; ESI-MS: 426.2 $[M+H]^+$, calculated: 425.19 *m/z*. ^1H NMR ($CDCl_3$, 250 MHz) $\delta = 7.55$ (m, 3H), 7.30 (overlapped by the signal of $CDCl_3$), 6.08 (d, $J = 3.07$ Hz, 1H), 4.97 (d, $J = 3.27$ Hz, 1H), 4.85 (d, $J = 3.26$ Hz, 1H), 4.46 (d, $J = 3.36$ Hz, 1H), 3.82 (m, 1H), 3.44 (m, 1H), 1.45 (s, 3H), 1.28 (s, 3H) ppm.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

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