Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Research 343 (2008) 2057–2066
Carbohydrate RESEARCH

The Amadori rearrangement as key reaction for the synthesis of neoglycoconjugates

Tanja M. Wrodnigg,* Christiane Kartusch and Carina Illaszewicz

Glycogroup, Institut für Organische Chemie, Technische Universität Graz, Stremayrgasse 16, A-8010 Graz, Austria

Received 15 October 2007; received in revised form 20 February 2008; accepted 26 February 2008
Available online 2 March 2008

Presented at Eurocarb 14th Lübeck, Germany, September 2007

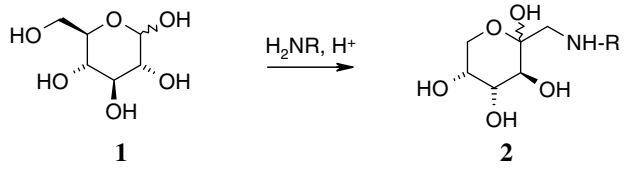
Abstract—The Amadori rearrangement was introduced as a key step for the conjugation of carbohydrate moieties with suitable amines such as aliphatic amines and amino acid derivatives. The rearrangement products were further transformed into the corresponding 1-*N*,2-*O* cyclic carbamates employing triphosgene to obtain anomerically stable glycoconjugates. The reaction conditions were probed on a model substrate, 3,5-di-*O*-benzyl-α,β-D-glucofuranose and further applied to D-*glycero*-D-*gulo*-heptose, which gave 'D-*gluco*-conjugates' in the α-anomeric form exclusively in high isolated yields.

Keywords: Amadori rearrangement; Carbohydrate-amino acid conjugate; Neoglycoconjugate; Protein modification

1. Introduction

The Amadori rearrangement[1] (Scheme 1), the reaction between aldoses such as D-glucose (**1**) and suitable amines, leads to 1-amino-1-deoxy ketoses (**2**) and is known as the first and initial step of the Maillard reaction cascade,[2] the non-enzymatic browning of food. Additionally, this reaction is a form of post-translational modification of biomolecules which has been linked to diseases such as diabetes, cataract, Alzheimer's, dialysis related amylosis, atherosclerosis and Parkinson's diseases as well as physiological aging.[3]

The preparative use of this rearrangement is rather limited because of many difficulties accompanying the reaction: several steps during the isomerisation are reversible and a range of side products can be formed. Additionally, it is difficult to isolate the rearrangement product from the obtained compound mixture and unreacted starting materials. Furthermore, the rearrangement product can occur as a mixture of furanoid and pyranoid α- and β-anomers. The product itself can enter the Maillard reaction cascade[2] pathway to give corresponding side and degradation products. Consequently, only a few preparatively useful examples for this reaction are known in the literature.[4] However, the Amadori rearrangement allows for simple introduction of an amine onto position C-1 of a carbohydrate moiety and does not require any protecting group manipulation.



1 **2**

Scheme 1. Amadori rearrangement.

With the acceptance and appreciation of the importance of the carbohydrate part of biomolecules, protein modification, such as the synthesis of glycoconjugates and neoglycoconjugates,[5] has been addressed extensively during recent years. Glycoconjugates—structures where carbohydrate units are covalently bound to biomolecules such as peptides, proteins or lipids—are ubiquitous in all living organisms and do participate in the cell–cell communication, recognition as well as signalling

* Corresponding author. Tel.: +43 316 873 8744; fax: +43 316 873 8740; e-mail: t.wrodnigg@tugraz.at

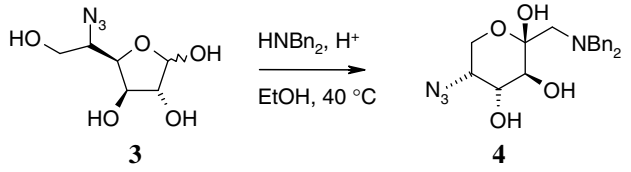
0008-6215/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2008.02.022

processes based on carbohydrate–carbohydrate or carbohydrate–protein interactions.[6] Furthermore, carbohydrate modification influences the physiological behaviour of therapeutic compounds such as their life time, absorption or penetration in physiological systems dramatically. For example, it was shown that modification of insulin by just one sugar residue improved its intestinal adsorption as well as resistance against enzymatic degradation in vivo.[7] Reliable methods for the synthesis of glycoconjugates and neoglycoconjugates allow for the preparation of sufficient quantities of pure materials compared to the typical mixtures obtained from tedious isolation techniques.[8] Different chemical[9] as well as enzymatic[10] synthetic approaches were introduced for the selective modification of biomolecules in the literature. To synthesise conjugates, which are not sensitive towards hydrolysis, such as the naturally occurring O- and N-glycosidic linkages, the synthesis of stable *C*-glycosyl compounds[11] became yet another important synthetic target, which are thought to have great therapeutic potential.[12]

Here we report the development of a method for the modification of amino acid derivatives using the Amadori rearrangement as a key reaction for the synthesis of *C*-glycosyl analogues of glycoconjugates. Probing of the reaction conditions as well as product characterisation was performed on a model substrate **5**, the devised methods were applied to heptose **16** for the synthesis of D-*gluco* configured carbohydrate-amino acid conjugates. Such structures can find use as building blocks for classical peptide synthesis. The method can also be applied for selective modification of lysine residues in peptides and smaller proteins.

2. Results and discussion

During our synthetic studies towards C-1 amino derivatives of the powerful glucosidase inhibitor 2,5-imino-2,5-dideoxy-D-mannitol,[13] we found that the Amadori rearrangement of 5-azido-5-deoxy-α,β-D-glucofuranose **3** gave excellent preparative isolated yields of the corresponding 1-amino-5-azido-1,5-dideoxy-β-D-fructose **4**[14] (Scheme 2). In this particular case, only the pyranoid form of compound **4** can be formed because of the azido substituent at position C-5. The relatively bulky dibenzylamine at position C-1 prefers the equatorial position in the β-form.

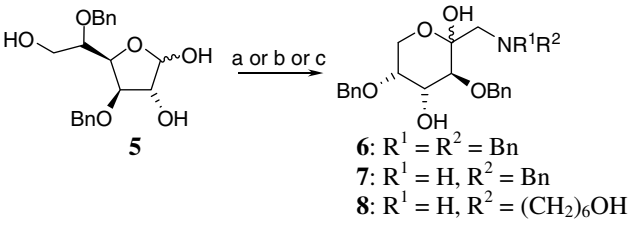


3 **4**

Scheme 2. Preparative application of the Amadori rearrangement.

Based on this findings 3,5-di-*O*-benzyl-α,β-D-glucofuranose (**5**)[15] was selected as a model compound for probing the reaction conditions of the rearrangement with free aliphatic amines, amine hydrochlorides as well as free and protected amino acid derivatives. The substitution pattern should allow easy rearrangement to the pyranoid 1-amino-1-deoxyketose, which is expected to occur in the β-anomeric form. Additionally, the two benzyl groups permit tracking of the reaction by UV and are rather unpolar groups, which should enable easier purification of the desired products from the reaction mixture by silica gel chromatography as well as better identification. For aliphatic amines such as dibenzylamine, benzylamine and 6-aminohexanol 1 equiv of acetic acid in ethanol was applied for the Amadori rearrangement. Contrasting with our observation to obtain only the respective β-anomers of the rearrangement products, we isolated α/β-mixtures with almost every amines employed such as with compounds **6** (1:3, 71%), **7** (2:7, 55%) and **8** (1:4, 52%), respectively (Scheme 3). In contrast to compound **4**, structures **6**, **7** as well as **8** have two rather large substituents, which might be responsible for the formation of both anomeric forms in the $^{5}C_{2}$ and $^{2}C_{5}$ conformation for the α- and β-anomer, respectively. In both forms two substituents are found in axial positions whereas the 1-amino substituent takes the sterically more convenient equatorial position.

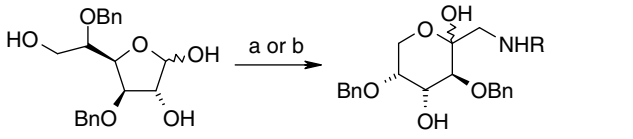
With amine hydrochlorides, addition of 1 equiv of Et_3N, which initiates the liberation of the free amine from its hydrochloride form, turned out beneficial in terms of rearrangement product formation. Furthermore, this prevents glycoside formation of the free aldose with the alcohol used. Under these conditions, the hydrochlorides of 6-amino hexanoic acid methyl ester and L-glycine benzyl ester were reacted successfully with model aldose **5**. Again, α/β-mixtures of compounds **9** (1:2) and **10** (1:3) were formed (Scheme 4). Isolated yields turned out to be low, the reaction had to be stopped with a notable amount of starting material remaining in order to avoid too much degradation. Additionally, the purification turned out to be very difficult, careful chromatography was necessary to obtain analytically pure samples for characterisation. Other reaction conditions, such as in the absence of



5

6: $R^1 = R^2 = Bn$
7: $R^1 = H$, $R^2 = Bn$
8: $R^1 = H$, $R^2 = (CH_2)_6OH$

Scheme 3. Reagents and conditions: (a) $HNBn_2$, AcOH, EtOH, 71%; (b) H_2NBn, AcOH, EtOH, 55%; (c) $H_2N(CH_2)_6OH$, AcOH, EtOH, 52%.



5

9: R = $(CH_2)_5COOMe$
10: R = CH_2COOBn

Scheme 4. Reagents and conditions: (a) $H_2N(CH_2)_5COOMe$, Et_3N, EtOH, 25%; (b) H_2NCH_2COOBn, Et_3N, EtOH, 15%.

catalyst, with AcOH, NaOAc or $NaHCO_3$, gave either no reaction at all or almost complete degradation of the starting materials.

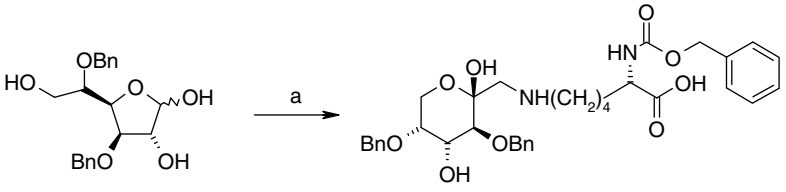
Partially protected Cbz-Lys-OH was reacted without any addition of acid, the carboxylic acid function itself providing the acidic catalyst for the isomerisation (Scheme 5). Interestingly, in this particular case only the β-anomer **11** of the rearrangement product was isolated, although the yield was very low due to solubility problems of the reactants and tedious purification by silica gel chromatography. From the TLC only one adequate rearrangement product was seen.

Next, the reaction conditions for the formation of a 1-*N*,2-*O*-cyclic carbamate with triphosgene[16] was investigated. Addition of 1,4-dioxane to the aqueous reaction system for better solubility, as well as a large excess of Na_2CO_3 to buffer, turned out to improve the conversion. Compounds **12**–**14** were isolated as α/β-anomeric mixtures ranging between 1:1 and 1:4 in yields of 80%, 30% and 27%, respectively. However, compound **15** was isolated as a pure β-anomer in 65% yield (Scheme 6).

The yields obtained for the Amadori rearrangement of the model compound **5** and in some cases for the cyclic carbamate formation turned out to be unexpected low. We think that this was caused by the nature of the very polar products obtained as well as degradation processes under the reaction conditions employed. Silica gel chromatography was performed as the purification protocol, but was clearly showing limits for such highly polar compounds. Despite preparative problems with the model compound **5** we decided to apply the reaction conditions to D-*glycero*-D-*gulo*-heptose **16** aiming at sugar amino acid conjugates featuring D-*gluco* configuration in the carbohydrate moiety.

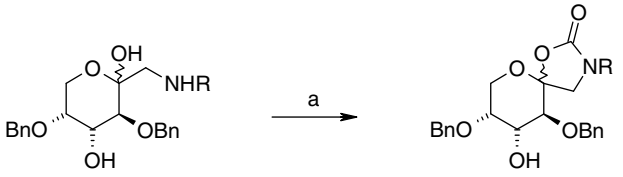
The reaction of this commercially available heptose gave good to excellent isolated yields in most cases of the pure α-anomer for the rearrangement products **17** (93%), **18** (95%), **19** (80%), **20** (82%) and **22** (73%) (Scheme 7). The all-equatorial arrangement of the hydroxyl groups in the product representing D-*gluco*-configuration was expected to be a strong driving force for the product formation in this particular case. Furthermore, in the α-anomeric form the $^5C_2$ pyranoid conformation allows the amino substituent to occupy the more comfortable equatorial position. However, employing L-glycine benzyl ester hydrochloride, only a moderate yield of 55% was obtained for the rearrangement product **21**.

Formation of the corresponding cyclic carbamates was achieved by employing the reaction conditions probed on the model compound leading to pure α-anomeric forms of the respective 1-amino-1-*N*,2-*O*-carbonyl-D-*gluco*-heptulose derivatives **23**–**27**. In case of



5 **11**

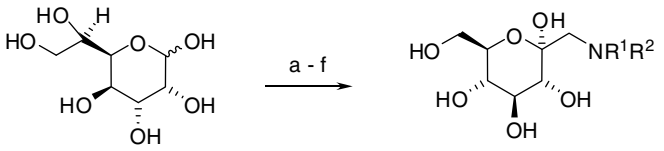
Scheme 5. Reagents and condition: (a) Cbz-Lys-OH, EtOH, 10%.



7: R = Bn
8: R = $(CH_2)_6OH$
9: R = $(CH_2)_5COOMe$
10: R = $(CH_2)_4CH(NHCbz)COOH$

12: R = Bn
13: R = $(CH_2)_6OH$
14: R = $(CH_2)_5COOMe$
15: R = $(CH_2)_4CH(NHCbz)COOH$

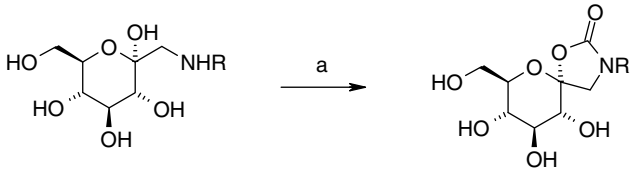
Scheme 6. Reagents and conditions: (a) 1.6 equiv $(CCl_3O)_2CO$, >6 equiv Na_2CO_3, H_2O, if necessary addition of 1,4-dioxane; yields for compounds **12**–**15**: **12**: 80%, **13**: 30%, **14**: 27%, **15**: 65%.



16

17: $R^1 = R^2 = Bn$
18: $R^1 = H$, $R^2 = Bn$
19: $R^1 = H$, $R^2 = (CH_2)_6OH$
20: $R^1 = H$, $R^2 = (CH_2)_5COOMe$
21: $R^1 = H$, $R^2 = CH_2COOBn$
22: $R^1 = H$, $R^2 = (CH_2)_4CH(NHCbz)COOH$

Scheme 7. Reagents and conditions: (a) $HNBn_2$, AcOH, EtOH, 93%; (b) H_2NBn, AcOH, EtOH, 95%; (c) $H_2N(CH_2)_6OH$, AcOH, EtOH, 80%; (d) $H_2N(CH_2)_5COOMe$, Et_3N, EtOH, 82 %; (e) H_2NCH_2COOBn, Et_3N, EtOH, 55%; (f) Cbz-Lys-OH, EtOH, 73%.



18: R = Bn
19: R = $(CH_2)_6OH$
20: R = $(CH_2)_5COOMe$
21: R = CH_2COOBn
22: R = $(CH_2)_4CH(NHCbz)COOH$

23: R = Bn
24: R = $(CH_2)_6OH$
25: R = $(CH_2)_5COOMe$
26: R = CH_2COOH
27: R = $(CH_2)_4CH(NHCbz)COOH$

Scheme 8. Reagents and conditions: (a) 1.6 equiv $(CCl_3O)_2CO$, >6 equiv Na_2CO_3, H_2O; yields for compounds **23**–**27**: **23**: 60%, **24**: 85%, **25**: 60%, **26**: 90%, **27**: 26%.

Amadori product **21**, under the fairly basic conditions employed for carbamate formation the benzyl ester was cleaved leading to free acid **26**. Yields in almost all examples were found in a satisfying range of 60–90%, however compound **27** was isolated in only 36% due to purification problems of this very polar compound (Scheme 8).

In conclusion, we have developed a method for the conjugation of carbohydrate moieties to amine functions of amino acid derivatives employing the Amadori rearrangement as the key step. The anomeric hydroxyl function obtained during the rearrangement can, if necessary, be stabilised by formation of a cyclic carbamate between the anomeric hydroxyl group and the amine at position C-1. By this procedure, *C*-glycosyl type glycoconjugates were obtained. In order to probe the reaction conditions for the rearrangement as well as cyclic carbamate formation partly protected 3,5-di-*O*-benzyl-D-glucofuranose **5** was chosen as a model substrate, however the yields obtained for the Amadori rearrangement products turned out to be only moderate to low. Additionally, α/β anomic mixtures in the $^5C_2$ and $^2C_5$ conformation, respectively, were obtained. Obviously the benzyl groups at positions 3 and 5 were not suitable for favouring only the β-form in its $^2C_5$ conformation as expected. Contrary to these findings, when D-*glycero*-D-*gulo*-heptose **16** was applied as a substrate, which allows for synthesis of a carbohydrate amino acid conjugates with D-*gluco* configuration in the sugar moiety, the method worked nicely. With this commercially available heptose, the rearrangement products were isolated in very good yields in pure α-form. Investigations exploiting various aldoses as starting materials to obtain different configurations of the glycose moieties bound to amino acids as well as fine tuning of the reaction conditions in order to carry out the modification method in physiological media are in progress.

3. Experimental

3.1. General methods

NMR spectra were recorded on a Varian INOVA 500 operating at 500.619 MHz (^1H), and at 125.894 MHz (^{13}C). $CDCl_3$ was employed for the protected compounds and methanol-d_4 or D_2O for free sugars. Chemical shifts are listed in δ employing residual, non-deuterated solvent as the internal standard. Optical rotations were measured on a Perkin Elmer 341 polarimeter at the wavelength of 589 nm and a path length of 10 cm at 20 °C. Analytical TLC was performed on

precoated aluminium plates Silica Gel 60 F254 (E. Merck 5554), detected with UV light (254 nm), 10% vanillin/sulfuric acid as well as ceric ammonium molybdate (100 g ammonium molybdate/8 g ceric sulfate in 1 L 10% H_2SO_4) and heated on a hotplate. Preparative TLC was performed on precoated glass plates Silica Gel 60 F254, 0.5 mm (E. Merck 5744). For column chromatography Silica Gel 60 (230–400 mesh, E. Merck 9385) was used.

3.2. General method A (Amadori rearrangement with free amines)

The respective aldose was dissolved in abs EtOH, approx. 1.2 equiv of the free amine and 1.2 equiv of AcOH were added and the reaction was stirred at 40 °C until TLC showed satisfying conversion of the starting material. The reaction mixture was concentrated under diminished pressure and the obtained residue separated by flash chromatography with the respective solvent system indicated in Section 3.

3.3. General method B (Amadori rearrangement with amine hydrochlorides)

The respective amine hydrochloride was dissolved in abs EtOH, 1 equiv of Et_3N was added and the mixture stirred for 20 min at rt. To this mixture the respective aldose was added and the reaction stirred at 40 °C until TLC showed satisfying conversion of the starting material. The reaction mixture was concentrated under diminished pressure and the product was purified by flash chromatography with the respective solvent system indicated in Section 3.

3.4. General method C (Amadori rearrangement with amino acid derivatives having a free carboxylic acid residue)

The respective aldose was reacted in EtOH with the amino acid derivative at 40–60 °C until TLC showed satisfying conversion of the starting material. The reaction mixture was concentrated under diminished pressure and the obtained compound mixture purified by flash chromatography with the respective solvent system indicated in Section 3.

3.5. General method D (cyclic carbamate formation with triphosgene)

The respective Amadori rearrangement product was dissolved in water, for better solubility 1,4-dioxane can be added and a large excess of Na_2CO_3, typically >6 equiv, was added at 0 °C. After 15 min triphosgene was added and the reaction mixture stirred for 20 min at 0 °C followed by stirring at room temperature (rt) until TLC showed conversion of the starting material. The reaction was concentrated under diminished pressure and the obtained compound mixture separated by flash chromatography with the respective solvent mixture indicated in Section 3.

3.6. 1-(*N*,*N*-Dibenzyl)amino-3,5-di-*O*-benzyl-1-deoxy-α,β-D-fructopyranose (6)

Aldose **5** (1.0 g, 2.8 mmol) was reacted as described in general method A with dibenzylamine (0.6 mL, 3.3 mmol, 1.2 equiv) and AcOH (0.2 mL, 3.3 mmol, 1.2 equiv) in EtOH (10 mL). After storage of the reaction mixture in the fridge for 20 h compound **6** (1.0 g, 1.8 mmol, 71%) was isolated by filtration as an α/β-mixture in a ratio of 1:3. ^{1}H NMR ($CDCl_3$): δ 4.80 (d, 1H, J 12.2 Hz, OCH_2Phβ), 4.72 (d, 1H, J 11.2 Hz, OCH_2Phβ), 4.66 (d, 1H, J 12.2 Hz, OCH_2Phα), 4.62 (d, 1H, J 12.2 Hz, OCH_2Phα), 4.56 (d, 1H, J 11.7 Hz, OCH_2Phβ), 4.52 (d, 1H, J 11.7 Hz, OCH_2Phα), 4.47 (d, 1H, J 11.2 Hz, OCH_2Phβ), 4.31 (d, 2H, J 11.7 Hz, OCH_2Phα), 4.20 (m, 1H, H-5β), 4.13–4.10 (m, 6H, H-6eβ, H-3α, H-5α, H-6aα, NCH_2Phα), 4.06 (m, 2H, H-6aβ, NCH_2Phα), 3.98 (d, 2H, J 12.2 Hz, NCH_2Phβ), 3.64 (dd, 1H, $J_{6a,6e}$ 11.2 Hz, $J_{6e,5}$ 4.9 Hz, H-6eα), 3.51–3.47 (m, 3H, $J_{4,5}$ 4.4 Hz, J 13.7 Hz, H-4β, 2 × NCH_2Phβ), 3.35 (d, 1H, J 13.7 Hz, NCH_2Phα), 3.26 (m, 1H, H-4α), 3.05 (dd, 1H, $J_{1,1'}$ 13.7 Hz, H-1β), 2.82 (dd, 1H, $J_{1,1'}$ 13.7 Hz, H-1α), 2.63 (dd, 1H, H-1′β), 2.46 (dd, 1H, H-1′α). ^{13}C NMR: δ 98.0 (C-2β), 95.5 (C-2α), 79.4, 78.7 (2C, C-4α and β), 77.6, 77.4 (2C, C-3α and β), 74.6, 72.9, 71.7, 71.0 (4C, 2×OCH$_2$Ph, α and β), 71.41 (2C, C-5α and β), 59.6 (C-6β), 57.8 (C-6α), 59.3 (2C, NCH$_2$Ph); 57.3, 57.0 (2C, C-1α and β). Anal. Calcd for $C_{34}H_{37}NO_5$: C, 75.67; H, 6.91. Found: C, 75.62; H, 6.97.

3.7. 1-(*N*-Benzyl)amino-3,5-di-*O*-benzyl-1-deoxy-α,β-D-fructopyranose (7)

General method A was applied to aldose **5** (1.1 g, 3.0 mmol) using EtOH (15 mL), benzylamine (0.4 mL, 3.6 mmol, 1.2 equiv) and AcOH (0.2 mL, 3.7 mmol, 1.2 equiv). Silica gel chromatography with 1:2 cyclohexane–EtOAc containing 1% of pyridine gave compound **7** (751.0 mg, 1.7 mmol, 55%) as a yellow oil. Compound **7** was difficult to purify and gave very complex NMR spectra and was unambiguously characterised by conversion into compound **12**.

3.8. 1-(*N*-6-Hydroxyhexyl)-amino-3,5-di-*O*-benzyl-1-deoxy-α,β-D-fructopyranose (8)

General method A was applied to aldose **5** (1.2 g, 3.4 mmol), 6-aminohexanol (0.5 g, 4.1 mmol, 1.2 equiv) and AcOH (0.23 mL, 4.1 mmol, 1.2 equiv) in EtOH

(15 mL). Silica gel chromatography (7:1 EtOAc–MeOH containing 1% of concd NH_3) gave compound **8** (0.8 g, 1.7 mmol, 52%) as a slightly yellow oil in an α/β ratio of 1:4 determined by NMR. The β-anomer of compound **8** was isolated after extensive silica gel chromatography using 1:15 EtOAc–MeOH containing 0.5% of concd NH_3. β-Anomer: $[\alpha]_D$ −41.2 (*c* 2.4, MeOH); ^{1}H NMR (methanol-d_4): δ 4.97 (d, 1H, *J* 11.7 Hz, OCH_2Ph), 4.69 (d, 1H, *J* 12.7 Hz, OCH_2Ph), 4.67 (d, 1H, *J* 11.2 Hz, OCH_2Ph), 4.66 (d, 1H, *J* 12.2 Hz, OCH_2Ph), 4.57 (m, 1H, H-5), 4.07 (dd, 1H, $J_{3,4}$ 9.8 Hz, $J_{4,5}$ 3.4 Hz, H-4), 3.83 (d, 1H, $J_{1,1'}$ 12.7 Hz, H-1), 3.79–3.76 (m, 2H, $J_{6a,6e}$ 11.7 Hz, H-3, H-6a), 3.72 (m, 1H, H-6e), 3.56–3.51 (m, 3H, H-1, 2 × H-12), 3.32 (m, 1H, H-1′), 2.51 (t, 2H, 2 × H-7), 1.51 (m, 2H, H-9), 1.41 (m, 2H, 2 × H-8), 1.36–1.29 (m, 4H, 2 × H-11, 2 × H-10). ^{13}C NMR: δ 97.8 (C-2), 78.2, 78.0 (2C, C-3, C-4), 75.0 (O*C*H$_2$Ph), 71.5 (C-5), 71.1 (O*C*H$_2$Ph), 61.7 (2C, C-6, C-12), 59.9 (C-1), 49.7 (C-7), 32.4 (C-11), 29.5 (C-8), 27.0, 25.7 (2C, C-9, C-10). Anal. Calcd for $C_{26}H_{37}NO_6$: C, 67.95; H, 8.11. Found: C, 67.91; H, 8.17.

3.9. 1-(*N*-Methoxycarbonylpentyl)amino-3,5-di-*O*-benzyl-1-deoxy-α,β-D-fructopyranose (9)

Following General method B, 6-amino hexanoic acid methyl ester hydrochloride (1.3 g, 7.1 mmol, 1,5 equiv) was reacted in EtOH (10 mL) and Et_3N (0.98 mL, 7.1 mmol, 1.5 equiv) with aldose **5** (1.7 g, 4.7 mmol). Silica gel chromatography (5:1 EtOAc–MeOH containing 1% of concd NH_3) gave compound **9** (0.6 g, 1.2 mmol, 25%) as a slightly yellow oil in an α/β ratio of 1:2 determined by NMR. The β-anomer was isolated after extensive silica gel chromatography using 15:1 EtOAc–MeOH containing 0.5% of concd NH_3. β-Anomer: $[\alpha]_D$ −32.7 (*c* 3.2, MeOH); ^{1}H NMR (methanol-d_4): δ 4.97 (d, 1H, *J* 11.7 Hz, OCH_2Ph), 4.70–4.64 (m, 3H, 3 × OCH_2Ph), 4.57 (m, 1H, H-5), 4.09–4.06 (m, 1H, $J_{3,4}$ 9.3 Hz, $J_{4,5}$ 4.4 Hz, H-4), 3.80–3.74 (m, 3H, H-3, H-6a, H-1), 3.76 (m, 1H, H-6e), 3.66 (m, 4H, OCH_3, H-1′), 3.32 (m, 2H, H-7), 2.50 (t, 2H, H-11), 1.39 (d, 2H, H-8), 1.28–1.23 (m, 4H, H-9, H-10). ^{13}C NMR: δ 97.8 (C-2), 78.2, 78.0 (2C, C-3, C-4), 75.0, 71.1 (2C, 2 × O*C*H$_2$Ph), 71.5 (C-5), 60.4 (C-6), 59.9 (C-1), 50.9 (O*C*H$_3$), 49.4 (C-7), 33.6 (C-11), 29.1, 26.6, 24.7 (3C, C-8, C-9, C-10). Anal. Calcd for $C_{27}H_{37}NO_7$: C, 66.51; H, 7.65. Found: C, 66.46; H, 7.71.

3.10. 1-(*N*-Benzyloxycarbonylmethyl)amino-3,5-di-*O*-benzyl-1-deoxy-α,β-D-fructopyranose (10)

General method B was applied to aldose **5** (1.2 g, 3.4 mmol) employing L-glycine benzyl ester hydrochloride (1.0 g, 5.1 mmol, 1.5 equiv), Et_3N (0.7 mL, 5.1 mmol, 1.5 equiv) in EtOH (10 mL). Silica gel chromatography (1:3 cyclohexane–EtOAc containing 1% of concd NH_3) gave compound **10** (252.5 mg, 0.5 mmol, 15%) as a slightly yellow oil in an α/β ratio of 1:3 determined by NMR. The β-anomer was isolated after extensive silica gel chromatography using 2:1 cyclohexane–EtOAc containing 0.5% of concd NH_3. β-Anomer: $[\alpha]_D$ −15.9 (*c* 1.1, MeOH); ^{1}H NMR (methanol-d_4): δ 5.12 (dd, 2H, *J* 12.2 Hz, COOCH_2Ph), 4.93 (d, 1H, *J* 11.2 Hz, OCH_2Ph), 4.68–4.62 (m, 3H, *J* 11.7 Hz, *J* 11.1 Hz, 3 × OCH_2Ph), 4.54 (m, 1H, H-5), 4.06 (dd, 1H, $J_{3,4}$ 9.8 Hz, $J_{4,5}$ 3.4 Hz, H-4), 3.78 (d, 1H, H-6a), 3.70 (m, 2H, $J_{6a,6e}$ 12.2 Hz, H-6e, H-3), 3.37–3.34 (m, 2H, H-7), 2.70 (2 × d, 2H, $J_{1,1'}$ 13.2 Hz, H-1, H-1′). ^{13}C NMR: δ 98.2 (C-2), 78.1, 77.8 (2C, C-3, C-4), 75.1, 71.4, (2C, O*C*H$_2$Ph), 71.0 (C-5), 66.2 (COO*C*H$_2$Ph), 59.7 (C-6), 54.1 (C-1), 50.5 (C-7). Anal. Calcd for $C_{29}H_{33}NO_7$: C, 68.62; H, 6.55. Found: C, 68.58; H, 6.60.

3.11. 1-(*N*-(5*S*-Benzyloxycarbonyl)amino-6-carboxypentyl)amino-3,5-di-*O*-benzyl-1-deoxy-β-D-fructopyranose (11)

General method C was applied to compound **5** (1.5 g, 4.3 mmol) and Cbz-Lys-OH (1.4 g, 5.1 mmol, 1.2 equiv) in EtOH (15 mL). Crystallisation from MeOH gave compound **11** (152.0 mg, 0.2 mmol, 10%) of the pure β-anomer. $[\alpha]_D$ −16.8 (*c* 1.1, DMF); ^{1}H NMR (DMSO-d_6): δ 4.98 (m, 2H, OCH_2Ph), 4.89 (d, 1H, *J* 11.2 Hz, OCH_2Ph), 4.64 (dd, 2H, *J* 12.7 Hz, *J* 13.2 Hz, OCH_2Ph), 4.57 (d, 1H, *J* 9.7 Hz, OCH_2Ph), 4.32 (m, 1H, H-5), 3.96 (dd, 1H, $J_{3,4}$ 9.8 Hz, H-4), 3.78 (d, 1H, H-11), 3.75 (d, 1H, $J_{6a,6e}$ 12.7 Hz, H-6a), 3.69 (d, 1H, H-6e), 3.54 (d, 1H, H-3), 2.98 (d, 1H, H-1), 2.82 (d, 1H, $J_{1,1'}$ 12.7 Hz, H-1′), 2.56 (m, 2H, H-7), 1.60 (m, 2H, H-10), 1.48 (m, 2H, H-8), 1.24–1.08 (m, 2H, H-9). ^{13}C NMR: δ 96.4 (C-2), 79.9, 78.5, 74.6 (3C, C-3, C-4, C-5), 71.7, 70.4, 65.9 (3C, 2 × *C*H$_2$Ph, C-14), 61.2 (C-6), 55.4 (C-1), 54.2 (C-11), 49.0 (C-7), 30.7 (C-10), 25.4 (C-8), 21.5 (C-9). Anal. Calcd for $C_{34}H_{42}N_2O_9$: C, 65.58; H, 6.80. Found: C, 65.52; H, 6.85.

3.12. 1-(*N*-Benzyl)amino-3,5-di-*O*-benzyl-1-*N*,2-*O*-carbonyl-1-deoxy-α,β-D-fructopyranose (12)

General method D was applied to compound **7** (0.3 g, 0.6 mmol) in water and 1,4-dioxane (25 mL, 1/1 v/v), Na_2CO_3 (390 mg, 3.7 mmol, 6.1 equiv) and triphosgene (0.3 g, 1.0 mmol, 1.5 equiv). The reaction mixture was concentrated under diminished pressure, diluted with CH_2Cl_2, washed with 6% HCl and satd $NaHCO_3$, dried over Na_2SO_4 and concentrated again. Purification using silica gel chromatography (1:1 cyclohexane–EtOAc containing 1% of pyridine) gave compound **12** (231.0 mg,

0.5 mmol, 80%) in an α/β ratio of 1:1 as a slightly yellow oil. Extensive purification gave analytical samples of the two anomers. β-Anomer: $[\alpha]_D$ −42.6 (*c* 1.0, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 4.89 (d, 1H, *J* 11.7 Hz, OC*H*$_2$Ph), 4.66 (d, 1H, *J* 11.7 Hz, OC*H*$_2$Ph), 4.52 (d, 1H, *J* 11.7 Hz, OC*H*$_2$Ph), 4.45 (d, 1H, *J* 11.7 Hz, OC*H*$_2$Ph), 4.41 (d, 1H, $J_{1,1'}$ 15.1 Hz, H-1), 4.22 (d, 1H, H-1′), 4.11 (ddd, 1H, H-5), 3.89 (dd, 2H, $J_{6a,6e}$ 13.2 Hz, H-6a, H-6e), 3.73 (m, 1H, $J_{4,5}$ 3.4 Hz, H-4), 3.52 (d, 1H, $J_{3,4}$ 9.3 Hz, H-3), 4.91 (d, 1H, $J_{10,10'}$ 9.8 Hz, H-7), 3.07 (d, 1H, H-7′). ^{13}C NMR: δ 155.2 (N*C*OO), 101.2 (C-2), 76.5 (C-3), 75.5 (C-4), 73.6, 70.7 (2C, O*C*H$_2$Ph), 70.1 (C-5), 60.7 (C-6), 51.0 (C-7), 46.7 (C-1). Anal. Calcd for $C_{28}H_{29}NO_6$: C, 70.72; H, 6.15. Found: C, 70.67; H, 6.19. α-Anomer: $[\alpha]_D$ −5.8 (*c* 1.3, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 4.56 (d, 1H, *J* 11.7 Hz, OC*H*$_2$Ph), 4.55 (d, 1H, *J* 12.2 Hz, OC*H*$_2$Ph), 4.52 (d, 1H, *J* 12.2 Hz, OC*H*$_2$Ph), 4.42 (d, 1H, *J* 11.7 Hz, OC*H*$_2$Ph), 4.32 (dd, 2H, $J_{1,1'}$ 15.6 Hz, H-1), 4.01 (dd, 1H, $J_{6a,6e}$ 12.2 Hz, $J_{5,6a}$ 8.8 Hz, H-6a), 3.94 (m, 1H, H-4), 3.74 (m, 1H, H-5), 3.66 (d, 1H, $J_{3,4}$ 4.9 Hz, H-3), 3.52 (dd, 1H, $J_{5,6e}$ 3.9 Hz, H-6e), 3.23 (d, 1H, $J_{10,10'}$ 10.3 Hz, H-7), 3.09 (d, 1H, H-7). ^{13}C NMR: δ 154.7 (N*C*OO), 99.9 (C-2), 76.6 (C-3), 72.9 (C-4), 70.4, 70.2 (2C, O*C*H$_2$Ph), 66.0 (C-5) 60.2 (C-6), 52.5 (C-7), 44.8 (C-1).

3.13. 1-(*N*-6-Hydroxyhexyl)amino-3,5-di-*O*-benzyl-1-*N*,2-*O*-carbonyl-1-deoxy-α,β-D-fructopyranose (13)

General method D was applied to compound **8** (0.6 g, 1.4 mmol) in water and 1,4-dioxane (24 mL, 2/1 v/v), Na_2CO_3 (1.5 g, 13.8 mmol, 10 equiv) and triphosgene (0.7 g, 2.2 mmol, 1.5 equiv). Silica gel chromatography (1:1 cyclohexane–EtOAc containing 0.5% of concd NH_3) gave compound **13** (204.0 mg, 0.4 mmol, 30%) in an α/β ratio of 1:4, purification gave analytical samples of the two anomers. α-Anomer (48 mg, 0.1 mmol): $[\alpha]_D$−8.5 (*c* 2.2, MeOH); ^1H NMR (methanol-d_4): δ 4.88 (m, 1H, OC*H*$_2$Ph), 4.74 (d, 1H, *J* 11.2 Hz, OC*H*$_2$Ph), 4.64 (m, 1H, OC*H*$_2$Ph), 4.60 (d, 1H, *J* 11.2 Hz, OC*H*$_2$Ph), 4.04 (dd, 1H, $J_{6a,6e}$ 12.2 Hz, $J_{5,6a}$ 5.9 Hz, H-6a), 3.95 (m, 1H, $J_{4,5}$ 2.5 Hz, H-4), 3.81 (d, 1H, $J_{3,4}$ 6.8 Hz, H-3), 3.76 (m, 1H, H-5), 3.63 (d, 1H, $J_{1,1'}$ 10.3 Hz, H-1′), 3.60 (dd, 1H, $J_{5,6e}$ 2.4 Hz, H-6e), 3.50 (t, 2H, H-12), 3.36 (d, 1H, H-1), 3.20 (m, 2H, H-7), 1.46 (m, 4H, H-8, H-11), 1.32, 1.23 (2 × m, 4H, H-9, H-10). ^{13}C NMR: δ 157.0 (NCOO), 102.6 (C-2), 78.7 (C-3), 74.5 (O*C*H$_2$Ph), 73.4 (C-5), 71.2 (O*C*H$_2$Ph), 68.2 (C-4), 61.7 (C-12), 60.3 (C-6), 48.7 (C-1), 43.3 (C-7), 32.3 (C-11), 26.9, 26.1, 25.3 (3C, C-9, C-10, C-11). Anal. Calcd for $C_{27}H_{35}NO_7$: C, 66.79; H, 7.27. Found: C, 66.73; H, 7.32. β-Anomer (70.7 mg, 0.15 mmol): $[\alpha]_D$ −80.9 (*c* 2.3, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 5.04 (d, 1H, *J* 11.7 Hz, OC*H*$_2$Ph), 4.75 (d, 1H, *J* 11.7 Hz, OC*H*$_2$Ph), 4.66 (d, 1H, *J* 12.2 Hz, OC*H*$_2$Ph), 4.56 (d, 1H, *J* 11.7 Hz, OC*H*$_2$Ph), 4.16 (m, 1H, H-4), 3.93 (dd, 2H, $J_{6a,6e}$ 13.2 Hz, H-6a, H-6e), 3.81 (m, 1H, H-5), 3.67 (d, 1H, $J_{3,4}$ 9.8 Hz, H-3), 3.56 (t, 2H, H-12), 3.41 (d, 1H, $J_{1,1'}$ 9.3 Hz, H-1), 3.22 (d, 1H, H-1′), 3.19 (t, 2H, H-7), 1.65 (m, 4H, H-8, H-11), 1.31, 1.19 (m, 4H, H-9, H-10). ^{13}C NMR: δ 156.4 (NCOO), 102.4 (C-2), 78.2 (C-3), 76.9 (C-4), 75.0, 72.1 (2C, O*C*H$_2$Ph), 71.3 (C-5), 62.7, 62.1 (2C, C-6, C-12), 52.6 (C-1), 43.6 (C-7), 32.6 (C-11), 27.3, 26.2, 25.3 (3C, C-9, C-10, C-11).

3.14. 1-(*N*-Methoxycarbonylpentyl)-amino-3,5-di-*O*-benzyl-1-*N*,2-*O*-carbonyl-1-deoxy-α,β-D-fructopyranose (14)

General method D was applied to compound **9** (0.42 g, 0.9 mmol) in water and 1,4-dioxane (15 mL, 2/1 v/v), Na_2CO_3 (0.9 g, 8.8 mmol, 10 equiv) and triphosgene (0.42 g, 1.4 mmol, 1.5 equiv). Silica gel chromatography (1:1 cyclohexane–EtOAc containing 0.5% of concd NH_3) gave compound **14** (0.12 g, 0.25 mmol, 27%) in an α/β ratio of 1:1. Purification gave analytical samples of the two anomers. α-Anomer: $[\alpha]_D$ −17.1 (*c* 3.2, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 4.61 (d, 1H, *J* 11.2 Hz, OC*H*$_2$Ph), 4.54 (m, 2H, 2×OC*H*$_2$Ph), 4.64 (d, 1H, *J* 11.7 Hz, OC*H*$_2$Ph), 4.06–3.99 (m, 2H, H-4, H-6e), 3.79 (m, 1H, H-5), 3.68 (br d, 1H, $J_{3,4}$ 4.39 Hz, H-3), 3.58 (s, 3H, OC*H*$_3$), 3.55 (dd, 1H, $J_{6a,6e}$ 11.7 Hz, $J_{5,6a}$ 4.4 Hz, H-6a), 3.3 (d, 1H, $J_{1,1'}$ 10.2 Hz, H-1); 3.16 (d, 1H, H-1′), 3.10 (m, 2H, H-7), 2.20 (t, 2H, H-11), 1.53, 1.37, 1.18 (m, 6H, H-8, H-9, H-10). ^{13}C NMR: δ 174.2 (COOMe), 155.8 (NCOO), 100.8 (C-2), 77.8 (C-3), 74.0, 71.6 (2C, O*C*H$_2$Ph), 71.2 (C-5), 67.0 (C-4), 59.4 (C-6), 52.1 (C-1), 51.8 (O*C*H$_3$), 43.7 (C-7), 34.0 (C-11), 27.1, 26.1, 24.7 (3C, C-8, C-9, C-10). Anal. Calcd for $C_{28}H_{35}NO_8$: C, 65.48; H, 6.87. Found: C, 65.42; H, 6.91. β-Anomer: $[\alpha]_D$ −63.4 (*c* 2.7, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 5.03 (d, 1H, *J* 11.7 Hz, OC*H*$_2$Ph), 4.75 (d, 1H, *J* 11.7 Hz, OC*H*$_2$Ph), 4.65 (d, 1H, *J* 11.7 Hz, OC*H*$_2$Ph), 4.55 (d, 1H, *J* 11.7 Hz, OC*H*$_2$Ph), 4.15 (m, 1H, $J_{4,5}$ 9.3 Hz, H-4), 3.93 (dd, 2H, $J_{6a,6e}$ 13.2 Hz, H-6a H-6e), 3.80 (m, 1H, H-5), 3.65 (d, 1H, $J_{3,4}$ 10.3 Hz, H-3), 3.64 (s, 3H, OC*H*$_3$), 3.40 (d, 1H, $J_{1,1'}$ 9.3 Hz, H-1), 3.21 (d, 1H, H-1′), 3.17 (t, 2H, H-7), 2.22 (t, 2H, H-11), 1.57, 1.42, 1.24 (3 × m, 6H, H-8, H-9, H-10). ^{13}C NMR: δ 174.2 (COOMe), 156.3 (NCOO), 102.3 (C-2), 78.2 (C-3), 76.8 (C-5), 75.0, 72.0 (2C, O*C*H$_2$Ph), 68.3 (C-4), 62.1 (C-6), 52.6 (C-1), 51.8 (O*C*H$_3$), 43.6 (C-7), 34.0 (C-11), 27.2, 26.2, 24.7 (3C, C-8, C-9, C-10).

3.15. 1-(*N*-(5*S*-Benzyloxycarbonyl)amino-6-carboxypentyl)-amino-3,5-di-*O*-benzyl-1-*N*,2-*O*-carbonyl-1-deoxy-β-D-fructopyranose (15)

General method D was applied to compound **11** (0.1 g, 0.2 mmol) in water and 1,4-dioxane (10 mL, 1/1 v/v),

Na_2CO_3 (0.4 g, 3.6 mmol, 16 equiv) and triphosgene (0.11 g, 0.4 mmol, 1.7 equiv). Compound **15** (95.3 mg, 0.2 mmol, 65%) was obtained in the pure β form by silica gel chromatography (3:1 EtOAc–MeOH containing 0.2% of concd NH_3). Compound **15** was not soluble in any common solvent, no optical rotation could be measured. ^{1}H NMR (DMSO-d_6): δ 5.00 (m, 2H, OCH_2Ph), 4.93 (d, 1H, J 11.2 Hz, OCH_2Ph), 4.66 (m, 2H, J 9.3 Hz, J 9.8 Hz, OCH_2Ph), 4.57 (m, 1H, J 10.2 Hz, OCH_2Ph), 3.90–3.82 (m, 3H, H-1, H-4, H-6a), 3.97 (dd, 2H, $J_{1,1'}$ 13.7 Hz, $J_{6a,6e}$ 10.3 Hz, H-1′, H-6e), 3.49 (m, 1H, H-11), 3.38 (m, 1H, H-5), 3.22 (d, 1H, $J_{3,4}$ 9.8 Hz, H-3), 3.00 (m, 2H, H-7), 1.65, 1.52 (m, 6H, H-8, H-9, H-10). ^{13}C NMR: δ 163.0 (COOH), 156.8, 156.1 (2C, NCOO, NH*C*OOBn), 102.8 (C-2), 78.1, 77.8 (2C, C-3, C-5), 74.9 (O*C*H$_2$Ph), 71.8 (C-4), 71.0 (O*C*H$_2$Ph), 66.0 (C-14), 63.4 (C-6), 54.4 (C-1), 52.7 (C-11), 51.8 (C-7), 31.8 (C-10), 24.5 (C-8), 22.9 (C-9). Anal. Calcd for $C_{35}H_{40}N_2O_{10}$: C, 64.80; H, 6.22. Found: C, 64.75; H, 6.28.

3.16. 1-(*N*,*N*-Dibenzyl)amino-1-deoxy-α-D-*gluco*-hept-2-ulopyranose (17)

Heptose **16** (115.1 mg, 0.6 mmol) was reacted as described in general method A with dibenzylamine (0.13 mL, 0.7 mmol, 1.2 equiv) and AcOH (40.0 μL, 0.7 mmol, 1.2 equiv) in EtOH (8 mL). Silica gel chromatography (1:2 cyclohexane–EtOAc) gave compound **17** (200 mg, 0.5 mmol, 93%) as a white precipitate. $[\alpha]_D$ +45.4 (*c* 1.2, MeOH); ^{1}H NMR ($CDCl_3$): δ ^{1}H NMR ($CDCl_3$): δ 3.97 (d, 2H, J 13.2 Hz, NCH_2Ph), 3.75–3.67 (m, 3H, H-4, H-7, H-6), 3.62 (d, 1H, $J_{6,7'}$ 7.7 Hz, H-7′), 3.50 (d, 2H, J 13.2 Hz, NCH_2Ph), 3.47 (dd, 1H, $J_{5,4}$ 8.3 Hz, $J_{5,6}$ 9.3 Hz, H-5), 3.12 (d, 1H, $J_{3,2}$ 9.3 Hz, H-3), 0.02 (d, 1H, $J_{1,1'}$ 13.2 Hz, H-1), 2.67 (d, 1H, H-1′). ^{13}C NMR: δ 97.0 (C-2), 75.1 (C-4), 73.3 (C-3), 72.1 (C-6), 70.3 (C-5), 62.2 (C-7), 59.0 (2C, N*C*H$_2$Ph), 57.2 (C-1). Anal. Calcd for $C_{21}H_{27}NO_6$: C, 64.77; H, 6.99. Found: C, 64.73; H, 7.04.

3.17. 1-(*N*-Benzyl)amino-1-deoxy-α-D-*gluco*-hept-2-ulopyranose (18)

Heptose **16** (251.6 mg, 1.2 mmol) was reacted as described in general method A with benzylamine (0.17 mL, 1.6 mmol, 1.3 equiv) and AcOH (100.0 μL, 1.6 mmol, 1.3 equiv) in EtOH (10 mL). Silica gel chromatography (5:1 chloroform–MeOH containing 1% of concd NH_3) gave compound **18** (341.0 mg, 1.1 mmol, 95%) as a slightly yellow oil. $[\alpha]_D$ +37.3 (*c* 0.5, MeOH); ^{1}H NMR (methanol-d_4): δ 4.16 (d, 1H, J 12.5 Hz, NCH_2Ph), 4.13 (d, 1H, NCH_2Ph), 3.83 (d, 1H, $J_{7,7'}$ 11.2 Hz, H-7), 3.76–3.73 (m, 1H, H-6), 3.69–3.65 (m, 2H, $J_{3,4}$ 9.3 Hz, $J_{4,5}$ 7.3 Hz, H-4, H-7′), 3.31–3.28 (m, 2H, $J_{5,6}$ 9.8 Hz, H-3, H-5), 3.10 (d, 1H, $J_{1,1'}$ 12.2 Hz, H-1), 3.05 (d, 1H, H-1). ^{13}C NMR: δ 96.6 (C-2), 74.4 (C-4), 73.3 (C-3), 73.0 (C-5), 70.4 (C-6), 61.6 (C-7), 53.9 (C-1), 53.1 (NH*C*H$_2$Ph). Anal. Calcd for $C_{14}H_{21}NO_6$: C, 56.18; H, 7.07. Found: C, 56.15; H, 7.10.

3.18. 1-(*N*-6-Hydroxyhexyl)amino-1-deoxy-α-D-*gluco*-hept-2-ulopyranose (19)

General method A was applied to aldose **16** (210.4 mg, 1.0 mmol) in EtOH (10 mL), 6-aminohexanol (155.0 mg, 1.3 mmol, 1.3 equiv) and AcOH (80.0 μL, 1.3 mmol, 1.3 equiv). Silica gel chromatography (6:1 chloroform–MeOH containing 1% of concd NH_3) gave compound **19** (245.4 mg, 0.8 mmol, 80%) as a slightly orange oil. $[\alpha]_D$ +38.3 (*c* 3.7, MeOH); ^{1}H NMR (methanol-d_4): δ 3.82 (dd, 1H, $J_{7,7'}$ 11.7 Hz, $J_{6,7}$ 1.5 Hz, H-7), 3.75–3.71 (m, 1H, H-6), 3.70–3.66 (m, 2H, $J_{6,7'}$ 5.4 Hz, $J_{3,4}$ 9.3 Hz, H-4, H-7′), 3.55 (t, 2H, H-13), 3.32–3.28 (m 2H, $J_{5,4=5,6}$ 9.3 Hz, H-3, H-5), 3.03–3.00 (n.d., 2H, H-1, H-1′), 2.89–2.79 (m, 2H, H-8), 1.65–1.63 (m, 2H, H-9), 1.56–1.53 (m, 2H, H-12), 1.40–1.39 (m, 4H, H-10, H-11). ^{13}C NMR: δ 95.9 (C-2), 74.2 (C-4), 73.4 (C-3), 73.2 (C-6), 70.4 (C-5), 61.6 (C-13), 61.5 (C-7), 54.1 (C-1), 49.1 (C-8), 32.3 (C-12), 27.4 (C-9), 26.6, 25.5 (2C, C-10, C-11). Anal. Calcd for $C_{13}H_{27}NO_7$: C, 50.47; H, 8.80. Found: C, 50.44; H, 8.84.

3.19. 1-(*N*-Methoxycarbonylpentyl)amino-1-deoxy-α-D-*gluco*-hept-2-ulopyranose (20)

Following general method B, 6-amino hexanoic acid methyl ester hydrochloride (290.0 mg, 1.6 mmol, 1.6 equiv) in EtOH (8 mL) and Et_3N (225.0 μL, 1.6 mmol, 1.6 equiv) was applied to aldose **16** (207.2 mg, 1.0 mmol). Silica gel chromatography (4:1 chloroform–MeOH containing 1% of concd NH_3) gave compound **20** (272 mg, 0.8 mmol, 82%) as a yellow oil. $[\alpha]_D$ +31.1 (*c* 4.0, MeOH); ^{1}H NMR (methanol-d_4): δ 3.45 (dd, 1H, $J_{6,7}$ 1.0 Hz, $J_{7,7'}$ 11.2 Hz, H-7), 3.77–3.74 (m, 1H, H-6), 3.70–3.66 (m, 2H, $J_{6,7'}$ 4.9 Hz, H-4, H-7′), 3.65 (s, 3H, OCH_3), 3.33 (d, 1H, $J_{3,4}$ 9.3 Hz, H-3), 3.30 (dd, 1H, $J_{5,4}$ 9.8 Hz, $J_{5,6}$ 7.3 Hz, H-5), 3.22 (d, 1H, $J_{1,1'}$ 12.7 Hz, H-1), 3.17 (d, 1H, H-1′), 3.02 (br t, 2H, H-8), 2.36 (t, 2H, H-12), 1.73 (q, 2H, H-11), 1.66 (q, 2H, H-9), 1.41 (q, 2H, H-10). ^{13}C NMR: δ 174.5 (C-13), 95.1 (C-2), 74.0 (C-4), 73.5 (C-6), 73.4 (C-3), 70.3 (C-5), 61.3 (C-7), 53.3 (C-1), 50.9 (OCH_3), 48.3 (C-8), 33.2 (C-12), 26.0 (C-10), 25.5 (C-11), 24.2 (C-9). Anal. Calcd for $C_{14}H_{27}NO_8$: C, 49.84; H, 8.07. Found: C, 49.79; H, 8.09.

3.20. 1-(*N*-Benzyloxycarbonylmethyl)amino-1-deoxy-α-D-*gluco*-hept-2-ulopyranose (21)

General method B was applied to L-glycine benzyl ester hydrochloride (440 mg, 2.2 mmol, 1.5 equiv) in EtOH

(8 mL), Et_3N (215.0 μL, 1.5 mmol, 1.0 equiv) and heptose **16** (300.0 mg, 1.4 mmol). Silica gel chromatography (6:1 chloroform–MeOH containing 1% of concd NH_3) gave compound **21** (275 mg, 0.8 mmol, 55%) as a yellow oil. $[\alpha]_D$ +35.8 (*c* 2.5, MeOH); ^1H NMR (methanol-d_4): δ 5.19 (br s, 2H, OCH_2Ph), 3.79 (br d, 1H, $J_{7,7'}$ 10.7 Hz, H-7), 3.74–3.71 (m, 1H, H-6), 3.69–3.61 (m, 4H, H-4, H-7′, H-8), 3.32–3.29 (m, 2H, H-3, H-5), 2.94 (bs, 2H, H-1, H-1′). ^{13}C NMR: δ 171.4 (C-9), 96.6 (C-2), 74.4 (C-4), 73.1 (C-3), 73.0 (C-6), 70.5 (C-5), 66.3 (C-10), 61.6 (C-7), 54.2 (C-1), 49.9 (C-8). Anal. Calcd for $C_{16}H_{23}NO_8$: C, 53.78; H, 6.49. Found: C, 53.75; H, 6.52.

3.21. 1-(*N*-(5*S*-Benzyloxycarbonyl)amino-6-carboxypentyl)amino-1-deoxy-α-D-*gluco*-hept-2-ulopyranose (22)

General method C was applied to heptose **16** (233.0 mg, 1.1 mmol) and Cbz-Lys-OH (500.0 mg, 1.7 mmol, 1.6 equiv) in EtOH (6 mL). Silica gel chromatography (3:1 chloroform–MeOH containing 1% of concd NH_3) gave compound **22** (393.1 mg, 0.8 mmol, 73%) as a yellow oil. $[\alpha]_D$ +41.5 (*c* 1.3, water); ^1H NMR (D_2O): δ 4.8 (d, 1H *J* 12.2 Hz, H-15), 4.74 (d, 1H, H-15), 3.70–3.68 (m, 2H, H-7, H-12), 3.63 (m, 1H, $J_{7,7'}$ 11.8 Hz, H-7′), 3.57–3.50 (m, 1H, H-6), 3.51 (dd, 1H, $J_{4,5}$ 8.3 Hz, $J_{3,4}$ 9.3 Hz, H-4), 3.21 (dd, 1H, $J_{5,6}$ 8.8 Hz, H-5), 3.18 (d, 1H, H-3), 2.97–2.92 (2 × br d, 2H, $J_{1,1'}$ 12.7 Hz, H-1, H-1′), 2.71–2.63 (m, 2H, H-8), 1.53–1.50 (m, 1H, H-11), 1.43–1.32 (m, 3H, 2 × H-9, H-11), 1.10–1.07 (m, 2H, H-10). ^{13}C NMR: δ 179.6 (C-13), 157.7 (C-14), 95.1 (C-2), 73.3 (C-4), 72.8 (C-3), 72.6 (C-6), 69.4 (C-5), 66.8 (C-15), 60.7 (C-7), 56.2 (C-12), 49.0 (C-1), 39.3 (C-8), 31.4 (C-11), 26.5 (C-9), 22.2 (C-10). Anal. Calcd for $C_{21}H_{32}N_2O_{10}$: C, 53.38; H, 6.83. Found: C, 53.34; H, 6.86.

3.22. 1-(*N*-Benzyl)amino-1-*N*,2-*O*-carbonyl-1-deoxy-α-D-*gluco*-hept-2-ulopyranose (23)

General method D was applied to compound **18** (313.6 mg, 1.1 mmol), Na_2CO_3 (690 mg, 6.5 mmol, 6.0 equiv) and triphosgene (320 mg, 1.1 mmol, 1.0 equiv) in water (8 mL). Silica gel chromatography (6:1 chloroform–MeOH containing 1% of concd NH_3) gave compound **23** (205.2 mg, 0.6 mmol, 60%) as a slightly yellow precipitate. $[\alpha]_D$ +56.3 (*c* 4.2, MeOH); ^1H NMR (methanol-d_4): δ 4.49 (d, 1H, *J* 15.6 Hz, NCH_2Ph), 4.41 (d, 1H, NCH_2Ph), 3.81–3.67 (m, 5H, H-1, H-4, H-6, H-7, H-7′), 3.44 (dd, 1H, $J_{5,3=5,4}$ 9.3 Hz, H-5), 3.34–3.31 (m, 2H, H-1′, H-3). ^{13}C NMR: δ 157.4 (C-8), 103.1 (C-2), 75.8 (C-4), 74.1 (C-6), 72.7 (C-3), 69.5 (C-5), 60.8 (C-7), 51.9 (C-1), 47.3 (C-9). Anal. Calcd for $C_{15}H_{19}NO_7$: C, 55.38; H, 5.89. Found: C, 55.36; H, 5.91.

3.23. 1-(*N*-6-Hydroxyhexyl)amino-1-*N*,2-*O*-carbonyl-1-deoxy-α-D-*gluco*-hept-2-ulopyranose (24)

General method D was applied to compound **19** (148.5 mg, 0.5 mmol), Na_2CO_3 (560 mg, 5.3 mmol, 6.1 equiv) and triphosgene (260 mg, 0.9 mmol, 1.8 equiv) in water (5 mL). Silica gel chromatography (6:1 chloroform–MeOH containing 1% of concd NH_3) gave compound **24** (136 mg, 0.4 mmol, 85%) as a slightly yellow precipitate. $[\alpha]_D$ +51.5 (*c* 4.4, MeOH); ^1H NMR (methanol-d_4): δ 3.82–3.79 (m, 2H, $J_{7,7'}$ 9.7 Hz, H-1, H-7), 3.74–3.72 (m, 2H, H-6, H-7′), 3.65 (dd, 1H, $J_{3,4}$ 9.3 Hz, $J_{4,5}$ 9.8 Hz, H-4), 3.54 (t, 2H, H-13), 3.45 (d, 1H, H-3), 3.43 (dd, $J_{5,6}$ 8.8 Hz, H-5), 3.39 (d, 1H, $J_{1,1'}$ 9.8 Hz, H-1′), 3.30–3.24 (m, 2H, H-8), 1.63–1.50 (m, 4H, H-11, H-12), 1.48–1.23 (m, 4H, H-9, H-10). ^{13}C NMR: δ 157.3 (NCOO), 102.9 (C-2), 75.7 (C-6), 74.1 (C-4), 72.7 (C-3), 69.6 (C-5), 61.7 (C-13), 60.9 (C-7), 52.1 (C-1), 43.3 (C-8), 32.3 (C-12), 26.9, 26.1, 25.3 (3C, C-9, C-10, C-11). Anal. Calcd for $C_{14}H_{25}NO_8$: C, 50.14; H, 7.51. Found: C, 50.09; H, 7.55.

3.24. 1-(*N*-Methoxycarbonylentyl)-amino-1-*N*,2-*O*-carbonyl-1-deoxy-α-D-gluco-hept-2-ulopyranose (25)

General method D was applied to compound **20** (198.2 mg, 0.6 mmol), Na_2CO_3 (690.0 mg, 6.5 mmol, 6.1 equiv) and triphosgene (320 mg, 1.1 mmol, 1.8 equiv) in water and 1,4-dioxane (8 mL, 1/1 v/v). Silica gel chromatography (6:1 chloroform–MeOH containing 1% of concd NH_3) gave compound **25** (129.0 mg, 0.4 mmol, 60%) as a yellow oil. $[\alpha]_D$ +55.5 (*c* 3.4, MeOH); ^1H NMR (methanol-d_4): δ 3.81–3.78 (m, 2H, $J_{7,7'}$ 10.3 Hz, H-1, H-7), 3.75–3.70 (m, 2H, H-6, H-7′), 3.65 (m, 4H, $J_{4,5}$ 10.8 Hz, H-4, OCH_3), 3.44–3.40 (m, 2H, $J_{1,1'}$ 9.8 Hz, $J_{5,6}$ 9.8 Hz, H-1′, H-5), 3.35 (d, 1H, $J_{3,4}$ 9.8 Hz, H-3), 3.30–3.24 (m, 2H, H-8), 2.30 (t, 2H, H-12), 1.64 (q, 2H, H-11), 1.58 (q, 2H, H-10), 1.34 (q, 2H, H-9). ^{13}C NMR: δ 174.8 (C-13), 157,3 (NCOO), 102.9 (C-2), 75.7 (C-6), 74.1 (C-4), 72.7 (C-3), 69.6 (C-5), 60.9 (C-7), 52.0 (C-1), 51.0 (OCH_3), 43.1 (C-8), 33.5 (C-12), 26.6 (C-10), 25.7 (C-9), 24.4 (C-11). Anal. Calcd for $C_{15}H_{25}NO_9$: C, 49.58; H, 6.93. Found: C, 49.55; H, 6.95.

3.25. 1-(*N*-Methoxycarbonylpentyl)amino-1-*N*,2-*O*-carbonyl-1-deoxy-α-D-gluco-hept-2-ulopyranose (26)

General method D was applied to compound **21** (226.5 mg, 0.6 mmol), Na_2CO_3 (660.0 mg, 6.2 mmol, 6.1 equiv) and triphosgene (300.0 mg, 1.0 mmol, 1.6 equiv) in water (5 mL). Silica gel chromatography (1:2 chloroform–MeOH containing 1% of concd NH_3) gave compound **26** (160.0 mg, 0.6 mmol, 90%) as a yellow oil. $[\alpha]_D$ +49.8 (*c* 1.7, water); ^1H NMR (D_2O): δ 3.93 (d, 1H, $J_{1,1'}$ 10.7 Hz, H-1), 3.90–3.79 (m, 4H, H-6, H-7,

H-8), 3.75 (dd, 1H, $J_{6,7'}$ 5.4 Hz, $J_{7,7'}$ 12.2 Hz, H-7′), 3.73 (dd, 1H, H-4), 3.61 (d, 1H, $J_{3,4}$ 9.8 Hz, H-3), 3.56 (d, 1H, H-1′), 3.52 (dd, 1H, $J_{4,5}$ 9.3 Hz, $J_{5,6}$ 9.8 Hz, H-5). ^{13}C NMR: δ 175.3 (COOH), 158.2 (NCOO), 103.3 (C-2), 75.1 (C-6), 73.6 (C-4), 71.9 (C-3), 69.1 (C-5), 60.7 (C-7), 53.4 (C-1), 47.1 (C-8). Anal. Calcd for $C_{10}H_{15}NO_9$: C, 40.96; H, 5.16. Found: C, 40.92; H, 5.19.

3.26. 1-(*N*-5*S*-Benzyloxycarbonylamino-6-carboxypentyl)amino-1-*N*,2-*O*-carbonyl-1-deoxy-α-D-*gluco*-hept-2-ulopyranose (27)

General method D was applied to compound **22** (226.7 mg, 0.6 mmol), Na_2CO_3 (600.0 mg, 5.7 mmol, 6.3 equiv) and triphosgene (260.0 mg, 0.9 mmol, 1.6 equiv) in water (5 mL). Silica gel chromatography (5:1 chloroform–MeOH containing 1% of concd NH_3) gave compound **28** (101 mg, 0.2 mmol, 36%) as a yellow oil. $[\alpha]_D$ +54.2 (*c* 0.5, water); ^{1}H NMR (D_2O): δ 4.96 (d, 1H, *J* 12.2 Hz, H-15), 4.56 (d, 1H, H-15), 3.76 (m, 1H, H-12), 3.66–3.59 (m, 2H, H-1, H-7. H-7′), 3.59–3-57 (m, 1H, $J_{6,7'}$ 6.4 Hz, H-6), 3.55 (dd, 1H, $J_{3,4=4,5}$ 9.8 Hz, H-4), 3.36–3.32 (m, 2H, $J_{5,4}$ 6.8 Hz, H-3, H-5), 3.27 (d, 1H, $J_{1,1'}$ 10.3 Hz, H-1′), 3.09–2.97 (m, 2H, H-8), 1.63–1.54; 1.50–1.42 (2m, 2H, H-11), 1.40–1.30 (m, 2H, H-9), 1.16–1.06 (m, 2H, H-10. ^{13}C NMR: δ 179.8 (COOH), 157.9, 157.8 (2C, C-14, C-16), 103.1 (C-2), 74.9 (C-6), 73.4 (C-4), 71.9 (C-3), 68.9 (C-5), 67.0 (OCH_2Ph), 60.3 (C-7), 56.2 (C-12), 52.0 (C-1), 43.2 (C-8), 31.2 (C-11), 25.8 (C-9), 22.2 (C-10). Anal. Calcd for $C_{22}H_{30}N_2O_{11}$ C, 53.01; H, 6.07. Found: C, 52.98; H, 6.11.

Acknowledgement

Financial support by the Austrian Fonds zur Förderung der Wissenschaftlichen Forschung (FWF), Vienna, (Project P18998-N17) is gratefully acknowledged.

References

1. (a) Amadori, M. *Atti R. Accad. Lincei*, **1925**, *Rend* [6], 2, 337–342; (b) Hodge, J. E.; Fisher, B. E. *Meth. Carbohydr. Chem.* **1963**, *2*, 99–107; (c) Wrodnigg, T. M.; Eder, B. In *Glycoscience—Epimerization Isomerization Rearrangement Reactions of Carbohydrates*; Stütz, A. E., Ed.; Springer, 2001; pp 115–152.
2. (a) Davidek, T.; Blank, I. *Chimia* **2005**, *59*, 862; (b) Ames, J. M. *Biochem. Food Proteins* **1992**, 99–153.
3. (a) Suji, G.; Sivakami, S. *Biogerontology* **2004**, *5*, 365–373; (b) Singh, R.; Barden, A.; Mori, T.; Beilin, L. *Diabetologia* **2001**, *44*, 129–146.
4. (a) Hotchkiss, D. J.; Soengas, R.; Booth, K. V.; Weymouth-Wilson, A. C.; Eastwick-Field, V.; Fleet, G. W. J. *Tetrahedron Lett.* **2007**, *48*, 517–520; (b) Fernandez-Bolanos, J. G.; Ulgar, V.; Maya, I.; Fuentes, J.; Dianez, M. J.; Estrada, M. D.; Lopez-Castro, A.; Perez-Garrido, S. *Tetrahedron: Asymmetry* **2003**, *14*, 1009–1018; (c) Kadokawa, J.; Hino, D.; Karasu, M.; Tagaya, H.; Chiba, K. *Eur. Polym. J.* **2000**, *36*, 225–230; (d) Shu, L.; Shen, Y.-M.; Burke, C.; Goeddl, D.; Shi, Y. *J. Org. Chem.* **2003**, *68*, 4963–4965.
5. (a) Peri, F. *Mini-Rev. Med. Chem.* **2003**, *3*, 651–658; (b) Leeuwenburgh, M. A.; van der Marel, G. A.; Overkleeft, H. S. *Curr. Opin. Chem. Biol.* **2003**, *7*, 757–765; (c) Mellet, C. O.; Defaye, J.; Garcia Fernandez, J. M. *Biol. Zhurnal Armenii* **2001**, *53*, 70–81; (d) Roy, R. In *Carbohydrate Chemistry*; Boons, G.-J., Ed.; Blackie, 1998; pp 243–321; (e) Pachamuthu, K.; Schmidt, R. R. *Chem. Rev.* **2006**, *106*, 160–187.
6. (a) Varki, A. *Essentials of Glycobiology*; Cold Spring Harbor Laboratory Press: New York, 1999; (b) Varki, A. *Glycobiology* **1993**, *3*, 97–130; (c) Kamiya, Y.; Kato, K. *Trends Glycosci. Glycotechnol.* **2006**, *18*, 231–244.
7. Hashimoto, T.; Nomoto, M.; Komatsu, K.; Haga, M.; Hayashi, M. *Eur. J. Pharm. Biopharm.* **2000**, *50*, 197–204.
8. (a) Buskas, T.; Ingale, S.; Boons, G.-J. *Glycobiology* **2006**, *16*, 113R–136R; (b) Pratt, M. R.; Bertozzi, C. R. *Chem. Soc. Rev.* **2005**, *34*, 58–68; (c) Davis, B. G. *Chem. Rev.* **2002**, *102*, 579–601; (d) Guo, Z.; Shao, N. *Med. Res. Rev.* **2005**, *25*, 655–678.
9. (a) Brik, A.; Wong, C.-H. *Chem.-Eur. J.* **2007**, *13*, 5670–5675; (b) Hojo, H.; Nakahara, Y. *Biopolymers* **2007**, *88*, 308–324; (c) Macmillan, D. *Angew. Chem., Int. Ed.* **2006**, *45*, 7668–7672; (d) Mogemark, M.; Kihlberg, J. In *The Organic Chemistry of Sugars*; Levy, P. E., Fügedi, P., Eds.; CRC Press: Taylor and Francis, 2006; pp 755–801.
10. (a) Murata, T.; Usui, T. *Biosci. Biotechnol. Biochem.* **2006**, *70*, 1049–1059; (b) Seibel, J.; Joerdening, H.-J.; Buchholz, K. *Biocatal. Biotransform.* **2006**, *24*, 311–342; (c) Liu, L.; Bennett, C. S.; Wong, C.-H. *Chem. Commun.* **2006**, *1*, 21–33.
11. (a) Taylor, R. J. K.; McAllister, G. D.; Franck, R. W. *Carbohydr. Res.* **2006**, *341*, 1298–1311; (b) Dondoni, A.; Massi, A. *Acc. Chem. Res.* **2006**, *39*, 451–463; (c) Micova, J.; Steiner, B.; Koos, M.; Langer, V.; Gyepesova, D. *Carbohydr. Res.* **2004**, *339*, 2187–2195; (d) Postema, M. H. D.; Piper, J. L. *Org. Lett.* **2003**, *5*, 1721–1723; (e) Walter, A.; Westermann, B. *Synlett* **2000**, 1682–1684.
12. (a) Doores, K. J.; Gamblin, D. P.; Davis, B. G. *Chem.-Eur. J.* **2006**, *12*, 656–665; (b) Liakatos, A.; Kunz, H. *Curr. Opin. Mol. Ther.* **2007**, *9*, 35–44; (c) Freire, T.; Bay, S.; Vichier-Guerre, S.; Lo-Man, R.; Leclerc, C. *Mini-Rev. Med. Chem.* **2006**, *6*, 1357–1373; (d) Van Bambeke, F. *Curr. Opin. Invest. Drugs* **2006**, *7*, 740–749; (e) Becker, T.; Dziadek, S.; Wittrock, S.; Kunz, H. *Curr. Cancer Drug Targets* **2006**, *6*, 491–517; (f) Bryskier, A.; Veyssier, P. *Antimicrob. Agents Chemother.* **2005**, 880–905.
13. Wrodnigg, T. M. *Monatsh. Chem.* **2002**, *133*, 393–426.
14. (a) Wrodnigg, T. M.; Stütz, A. E.; Withers, S. G. *Tetrahedron Lett.* **1997**, *38*, 5463–5466; (b) Wrodnigg, T. M.; Gaderbauer, W.; Greimel, P.; Häusler, H.; Sprenger, F.; Stütz, A. E.; Virgona, C.; Withers, S. G. *J. Carbohydr. Chem.* **2000**, *19*, 975–990.
15. Estevez, J. C.; Fairbanks, A. J.; Fleet, G. W. J. *Tetrahedron* **1998**, *54*, 13591–13620.
16. (a) Lopez, O.; Maza, S. *Tetrahedron* **2005**, *61*, 9058–9069; (b) Bracher, F.; Litz, T. *J. Prakt. Chem.* **1995**, *337*, 516–518.