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Carbohydrate Research 341 (2006) 322–331
Carbohydrate RESEARCH

New regioselective derivatives of sucrose with amino acid and acrylic groups

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Received 28 February 2005; received in revised form 10 November 2005; accepted 24 November 2005
Available online 27 December 2005

Abstract—We report here a range of new sucrose derivatives obtained from '3-ketosucrose' in aqueous medium with few reaction steps. As an intermediate, 3-amino-3-deoxy-α-D-allopyranosyl β-D-fructofuranoside (**1**) was obtained via the classical route of reductive amination with much improved yield and high stereoselectivity. Building blocks for polymerization were synthesized by introduction of acrylic-type side chains, for example, with methacrylic anhydride. Corresponding polymers were synthesized. Aminoacyl and peptide conjugates were obtained through conventional peptide synthesis with activated and protected amino acids. Deprotection yielded new glycoderivatives having an unconventional substitution pattern, namely 3-(aminoacylamino) allosaccharides. Both mono- and di-peptide conjugates of allosucrose have been synthesized.

Keywords: Sucrose derivatives; Amino derivatives of sucrose; 3-Ketosucrose; Regioselective substitution; Polymer building blocks; Allosucrose aminoacyl conjugates

1. Introduction

Sucrose chemistry has elicited much interest since sucrose (α-D-glucopyranosyl β-D-fructofuranoside) is available on a large scale with high purity at low cost. However, the functionality of sucrose, with eight nearly equivalent hydroxyl groups, makes selective synthesis of derivatives laborious and difficult. Furthermore, the preferred route for potential technical applications are reactions in water, avoiding protecting groups, which require highly selective catalysis. Enzymes offer the advantage of high regio- and stereoselectivity. *Agrobacterium tumefaciens* elaborates a dehydrogenase, which oxidizes sucrose and other disaccharides at the 3-position yielding α-D-*ribo*-hex-3-ulopyranosyl-β-D-fructofuranoside ('3-ketosucrose').[1] We have already described the conditions of fermentation where sufficient enzymatic activity is produced, and optimized conditions for oxidation, in order to achieve good yields and low byproduct concentration levels for the 3-keto derivatives of sucrose, isomaltulose, leucrose, lactose, and a sugar alditol, α-D-glucopyranosyl-(1→6)-mannitol.[2] The biotransformation has been optimized and scaled up to give 70% yield of 3-ketosucrose, and even a 90% yield with 3-keto-isomaltulose.[3]

Subsequent chemical steps provide access to a range of products, including α-D-allopyranosyl β-D-fructofuranoside (allosucrose) and allose,[4] the corresponding cyanohydrin,[5] oximes, and 3-amino-3-deoxy-α-D-allopyranosyl β-D-fructofuranoside (**1**).[6] N-Acylation yielded the dodecanamide, which exhibits surfactant properties.[6] All of these routes proceed with high regioselectivity, and mostly also with high stereoselectivity and yield, in aqueous solution without protecting groups. Furthermore, Grignard reactions gave C–C

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doi:10.1016/j.carres.2005.11.033

linked derivatives with alkyl as well as allyl substituents.[6] The reductive amination could be improved to give substantially higher yields of 70% of **1**, using hydrazine, fresh Raney nickel catalyst, and providing control of the pH, as is described here.

Further reactions leading to polymer building-blocks of different structures, and the corresponding polymers, as well as amino acid conjugates, are described in this paper. Polymers having saccharide building blocks of different structural architecture, where the saccharide units are integrated into the polymer main chain,[7–9] or appended as side chains, bound via amino functional groups,[11,12] or by C–C-bonds[13] have found major interest.

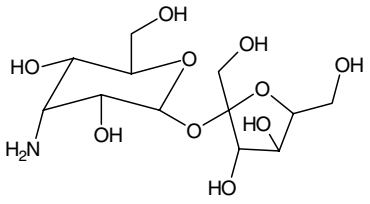
Glycoconjugates have become a field of great interest due to their manifold biological functions. A recent example is 1,5-anhydro-D-fructose, used as a synthon for various approaches.[14] Conjugates of carbohydrates and amino acids occur naturally in glycopeptides, glycoproteins, and in minor amounts in bacterial lipopolysaccharides. All of these substances are of major biological importance in various immunological processes.[15,16] Conjugates of carbohydrates also play a major role in a range of important antibiotics. Chemical synthesis of model compounds such as asparagine derivatives linked to oligosaccharides, generally require the use of protecting groups in the saccharide and the amino acid moiety in order to achieve selective coupling, and many blocking and deblocking steps are required for such syntheses.[17] Sucrose, despite its obvious advantages, has found much less attention as a building block, because of the difficulties of sucrose chemistry already mentioned. Here we present the synthesis of some aminoacyl derivatives of 3-amino-3-deoxy-α-D-allopyranosyl β-D-fructofuranoside, which requires protecting groups only for the amino acid moiety.

2. Results and discussion

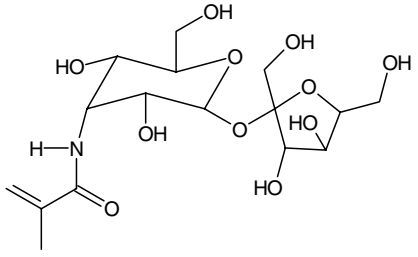
2.1. Polymer building blocks and polymers

Polyvinylsaccharides have interesting potential in such fields as cosmetics, pharmaceuticals, and as polymeric surfactants.[10–13] Most vinylsaccharides have been synthesized from reducing mono- and di-saccharides via reductive amination, a simple technique that proceeds in water with no need for protecting groups.[10–12] Under such conditions, sucrose derivatives of regioselective substitution pattern are accessible only via 3-ketosucrose and by subsequent reductive amination to 3-amino-3-deoxy-α-D-allopyranosyl β-D-fructofuranoside (**1**), followed by reaction with methacrylic anhydride to give the methacrylamide derivative: 3-deoxy-3-*N*-methacrylamido-α-D-allopyranosyl β-D-fructofuranoside (MA-sucrose) (**2**).[6] It seemed advisable to design an additional derivative with a spacer in order to distance the acrylic group from the rigid sucrose moiety, giving more flexibility and less steric hindrance for subsequent polymerization steps, to be described subsequently. Compound **1** is reacted in an equimolar amount of 2-isocyanato methyl methacrylate at 0–7 °C. The product was extracted with diethyl ether and freeze dried to yield over 90% of 3-deoxy-3-*N*′-methacryloyloxyethylureido-α-D-allopyranosyl βD-fructofuranoside (MAE-sucrose) (**3**), an acrylic acid derivative with a urea spacer.

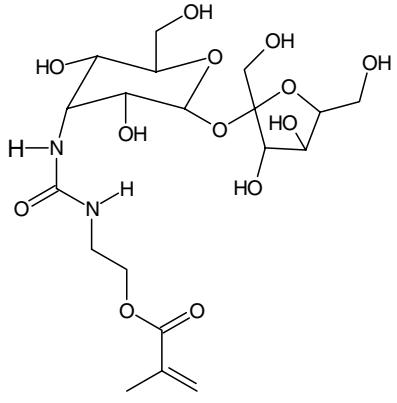
(**1**) 3-Amino-3-deoxy-α-D-allopyranosyl β-D-fructofuranoside



(**2**) 3-Deoxy-3-*N*-methacrylamido-α-D-allopyranosyl β-D-fructofuranoside (MA-sucrose)



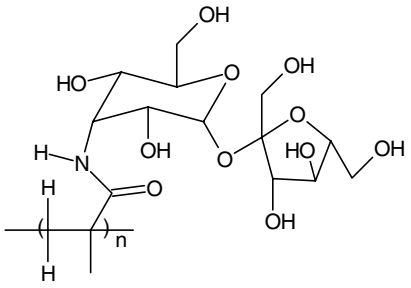
(**3**) 3-Deoxy-3-*N*′-methacryloyloxyethylureido-α-D-allopyranosyl β-D-fructofuranoside (MAE-sucrose)



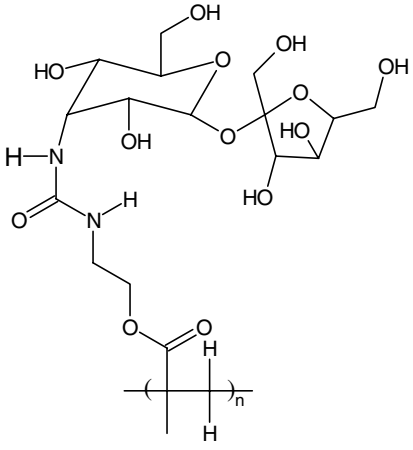
Polymerization to give homopolymers was performed in oxygen-free water under a nitrogen atmosphere with a

radical initiator ($Na_2S_2O_8$–$Na_2S_2O_5$, usually 1%). Reactions with MA-sucrose gave, at a monomer concentrations in the range of 0.244 mol/L, 40–60 °C after 6–48 h, dialysis and freeze drying, a 38–50% yield of polymer **4**. The molar mass was in the range of 61,000 (60 °C) to 350,000 (g/mol) (40 °C) (for details and further examples, see Experimental). ^{1}H NMR spectra show that the signals associated with the double bond of the methacrylic group at 5.78 and 5.50 ppm had disappeared. Polymerization of MAE-sucrose (**3**) gave, at 60 °C, under otherwise similar conditions, a 58–88% yield of polymer **5**, with a molar mass in the range of 490,000–1,900,000 (g/mol). Thus the introduction of a spacer between the sucrose moiety and the acrylic substituent had a dramatic effect on the molar mass, which was higher by an order of magnitude, and the yield, which was increased significantly. It may be assumed that steric hindrance restricts the growth rate of MA-sucrose, affecting both yield and chain length, as has been observed with similar systems.[10]

(**4**) Poly-[3-deoxy-3-*N*-methacrylamido-α-D-allopyranosyl β-D-fructofuranoside] (poly-MA-sucrose)



(**5**) Poly-[3-deoxy-3-*N*′-methacryloyloxyethylureido-α-D-allopyranosyl β-D-fructofuranoside] (poly-MAE-sucrose)



Copolymerization of MA-sucrose both with acrylamide and acrylonitrile was successfully performed, with significantly higher yields as compared to the homopolymerization.[18]

2.2. Amino acid conjugates of sucrose

Here we present the synthesis of some aminoacyl derivatives of 3-amino-3-deoxy-α-D-allopyranosyl β-D-fructofuranoside, which requires protecting groups only for the amino acid moiety. The resulting amide linkage is similar to the unconventional binding of saccharides and amino acids in bacterial lipopolysaccharides,[19,20] whereas glycoproteins have a N- or O-glycosylic linkage connecting the two moieties.

The sucrose–amino acid conjugates are new compounds, which might be useful in different aspects of biochemical or medical research. Coupling of amino acids to the 3-position in the sucrose molecule via 3-amino-allosucrose is rather unconventional. The synthesis does not require protecting groups in the sucrose derivative, although it is not trivial, because of its limited range of stability,[6] undergoing decomposition both at elevated pH and hydrolysis of the glycosidic bond at acidic pH.

In many glycoproteins, the carbohydrate residue is linked to the protein backbone through an aspartoyl–glycosylamine bond. Since aspartic acid often occurs in natural glycoconjugates, **1** was coupled with the protected amino acid benzyl-*N*-benzyloxycarbonyl-L-aspartate (Boc-Asp-OBzl) by treatment with *N*,*N*′-dicyclohexylcarbodiimide in pyridine–water according to the method of Kiyozumi et al.[21] After removing the side product (*N*,*N*′-dicyclohexylurea) and the residual amino acid, compound **6** was separated by column chromatography on silica gel (Fig. 1). Identification was performed by fast-atom bombardment MS and ^{13}C NMR spectroscopy. Selective deprotection of the C-terminus was possible by hydrogenolysis of **6** with Pd/charcoal catalyst. Complete deprotection of **7** was accomplished by cleavage of the benzyloxycarbonyl group with trifluoroacetic acid (TFA, 90%) to yield the amino acid conjugate 3-(4-L-aspartylamido)-3-deoxy-α-D-allopyranosyl β-D-fructofuranoside **8** (Fig. 1). Purification was in all cases possible by means of column chromatography. As well as the aspartic acid derivative, some other amino acids were used for the condensation reaction with **1**. Products of these reactions are shown in Figure 2.

By acidic hydrolysis it was possible to cleave the glycosidic linkage of **8**. Fructose and 3-(4-L-aspartylamido)-3-deoxy-α-D-allopyranose (**13**) were separated by ion-exchange chromatography. This result also shows that the corresponding monosaccharide derivatives are readily accessible by this route.

Another convenient protecting group used is fluorenyl-9-methoxycarbonyl-group (Fmoc), which gives

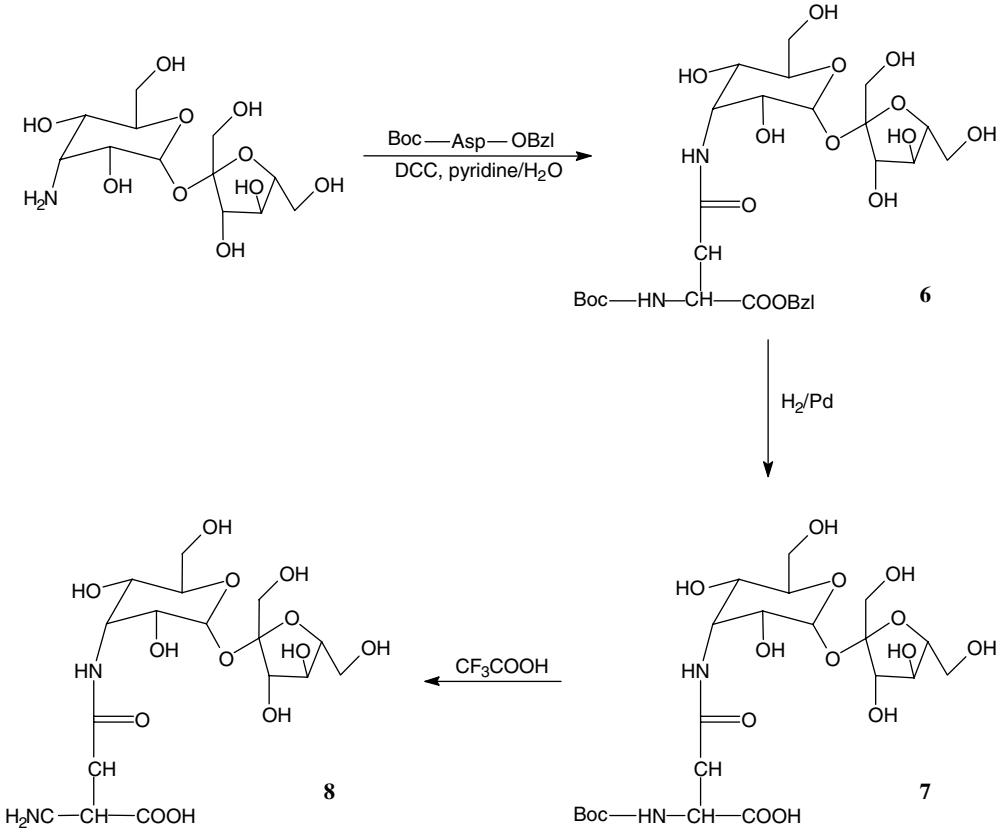


Figure 1. Synthesis of 3-(*N-tert*-butoxycarbonyl-L-aspartic acid-4-amido-1-benzyl ester)-3-deoxy-α-D-allopyranosyl β-D-fructofuranoside (**6**), 3-(*N-tert*-butoxycarbonyl-L-aspartic acid 4-amido)-3-deoxy-α-D-allopyranosyl β-D-fructofuranoside (**7**), and 3-(L-aspartic acid 4-amido)-3-deoxy-α-D-allopyranosyl β-D-fructofuranoside (**8**).

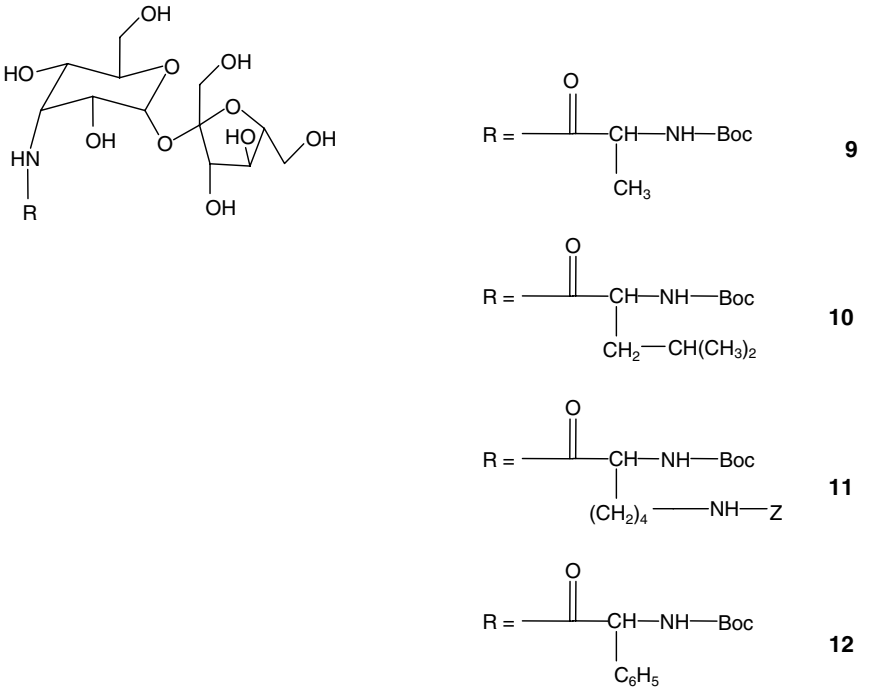


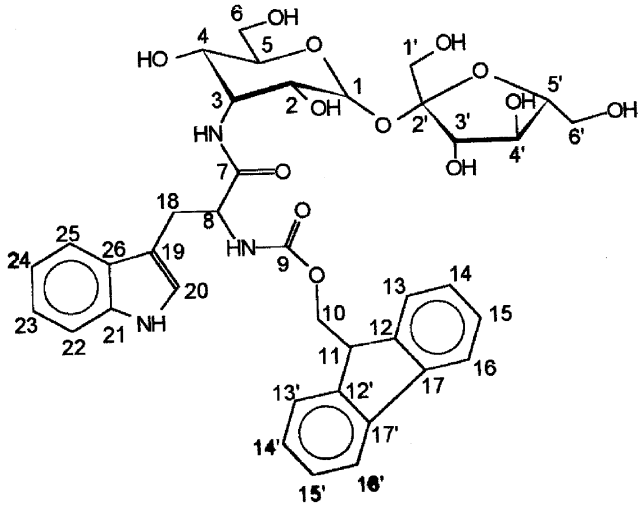
Figure 2. Synthesis of further aminoacyl derivatives of **1**: 3-(*N-tert*-butoxycarbonyl-L-alanylamido) (**9**), 3-(*N-tert*-butoxylcarbonyl-L-leucylamido) (**10**), 3-(N_α-*tert*-butoxylcarbonyl-N_ε-*Z*-L-lysyl amido) (**11**), and 3-(*N-tert*-butoxylcarbonyl-L-phenylalanylamido) (**12**).

quantitative yields. It is stable during the coupling procedure and is easily removed under mild conditions by, for instance, morpholine at a pH of ~8. Activation of the carboxyl group was performed with ethyl-2-ethoxy-1,2-dihydro-1-quinolinecarboxylate (EEDQ) in the following examples, with yields around 60%. The products were characterized as mentioned before, except that electrospray ionization was used for MS. Fmoc-tryptophan was thus coupled to **1**, using nearly equimolar quantities, in a solvent mixture of ethanol, 2-propanol, and acetone, with activation by EEDQ. Extraction, freeze drying, and purification with silica gel gave a 60% yield of the 3-Deoxy-3-(*N*-Fmoc-L-tryptophanylamido)-α-D-allopyranosyl β-D-fructofuranoside (**14**).

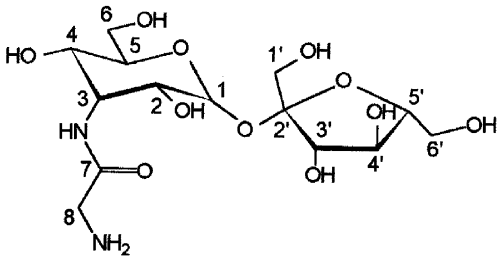
A dipeptide conjugate was synthesized with glycine, and subsequent phenylalanine coupling to the aminosucrose. Fmoc-glycine was coupled with **1** in a water–ethanol–2-propanol mixture with activation by EEDC. After purification with silica gel and freeze drying, a 64% yield of 3-deoxy-3-(*N*-Fmoc-L-glycylamido)-α-D-allopyranosyl β-D-fructofuranoside was obtained. Deprotection was straightforward with diethylamine, and extraction with ether to give 95% of 3-deoxy-3-(L-glycylamido)-α-D-allopyranosyl β-D-fructofuranoside (**15**). To this glycine derivative Fmoc-L-phenylalanine was coupled with activation by EEDC in ethanol. Purification by silica gel gave 50% of the dipeptide conjugate of allosucrose, 3-deoxy-3-(*N*-Fmoc-L-phenylalanyl-L-glycylamido)-α-D-allopyranosyl β-D-fructofuranoside (**16**).

The synthesis of new amino acid and dipeptide conjugates of sucrose via 3-amino-allosucrose thus with an unconventional regio-substitution pattern is demonstrated. The amino acids introduced may be neutral, or acidic, or basic, no protecting groups for the saccharide are required, and good yields can be obtained.

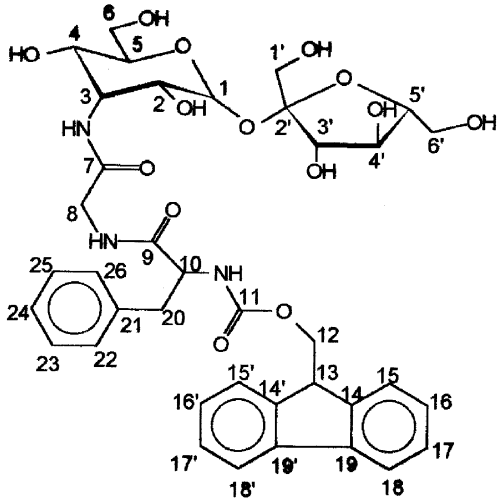
(**14**) 3-Deoxy-3-(*N*-Fmoc-L-tryptophanylamido)-α-D-allopyranosyl β-D-fructofuranoside



(**15**) 3-Deoxy-3-(L-glycylamido)-α-D-allopyranosyl β-D-fructofuranoside



(**16**) 3-Deoxy-3-(*N*-Fmoc-L-phenylalanyl-L-glycylamido)-α-D-allopyranosyl β-D-fructofuranoside



3. Experimental

3.1. General

TLC was conducted on aluminum sheets, precoated with 0.2 mm layers of Silica Gel 60F-254 (E. Merck, Darmstadt, Germany); the components were located either by exposure to UV light or by spraying with A (0.2% naphthoresorcin in ethanol–20% H_2SO_4 (1:1)), or B (MeOH–concd H_2SO_4 (1:1)) followed by heating at 120 °C. Column chromatography was performed on silica gel (230–400 mesh, E. Merck) with eluent C (EtOAc–EtOH–H_2O (5:3:1)) or D (MeOH–H_2O–NH_3 (25%) (5:1:0.5)). Other eluents are mentioned in individual sections. Preparation of **1** is described in Ref. 6.

Analysis of molar mass M_w and d.p. was accomplished by size-exclusion chromatography (SEC) with a multi-angle laser-light detector (MALLS) system. Two systems were applied for SEC: one from Shimadsu

with a differential refractometer detector RID-6A, a MALLS Dawn DSP detector (Laser photometer, Wyatt Technology Co. Santa Barbara, CA, USA) and a column of Polymer Standard Service (PSS Suprema 1000, 10 μm); second a system from Merck–Hitachi with a Waters Associates Differential Refractometer R 401, a column from Tosohaas Bioseparation Specialists (TSK-gel GMPWXL, 7.8 mm ID × 300 mm, 13 mm), together with the same MALLS system as already described. As solvent, twice distilled water with 0.05% NaN_3 (Aldrich) was used.

NMR spectra were recorded with a Bruker AM-400 instrument; ^{1}H NMR spectra at 400 MHz and ^{13}C NMR spectra at 100 MHz, if not stated otherwise. Several ^{13}C NMR spectra were recorded with a Bruker AM-300 instrument at 75.5 MHz, as noted. Mass spectra were mostly recorded with a Finnigan MAT 8430 instrument. Electrospray-MS (MS(ES)) was performed with a Fisons VG Trio 2000 instrument. Microanalyses were performed by Analytisches Labor des Institutes für Pharmazeutische Chemie, Technische Universität, Braunschweig.

L-Tryptophan, L-phenylalanine, Fmoc-Cl and EEDQ were purchased from Merck, Fmoc-L-glycine and Fmoc-L-phenylalanine from Novabiochem (all with purity >99%).

3.2. 3-Amino-3-deoxy-α-D-allopyranosyl β-D-fructofuranoside (1)

The preparation was modified to give improved yields. A sample (90.0 mL, 1.86 mol) of hydrazine hydrate was dissolved in water (1.5 L) and the pH adjusted to 6 with concd HCl. A solution (120.0 g, 0.35 mol) of 3-ketosucrose in 600 mL water was added over 5 h. The mixture was stirred at room temperature while the pH was held between 6.0 and 6.5 by adding hydrazine hydrate. After 19 h, 100 g Raney alloy was added and the suspension was added to a high-pressure apparatus. Hydrogenation was carried out at 50 °C and 80 bar H_2 pressure for 24 h. The alloy particles were filtered off and the filtrate was freeze dried. The crude material was passed through an ion-exchange column and the product isolated by chromatography (ion-exchanger Amberlite CG 120II in the NH_4^+-form, 0.5% aq NH_3 eluent) to yield 95.2 g (80%) of **1**. Analytical data were given in Ref. 6.

3.3. 3-Deoxy-3-*N*-methacrylamido-α-D-allopyranosyl β-D-fructofuranoside (MA-sucrose) (2) (compare Ref. 6)

Methacrylic anhydride (1.1 mL, 6.4 mmol) was added dropwise to a cooled (−10 °C) suspension of 2.0 g (5.8 mmol) **1** in 10 mL MeOH. The mixture was stirred for 1 h at −10 °C, 3 h at −5 °C and 15 h at 4 °C. It was warmed to room temperature and poured into a 4:1 mixture of acetone–ether. The product **2** was filtered and washed with ice-cold ether to yield 1.6 g of **2** as white crystals (69%). ^{1}H NMR (400 MHz, D_2O) δ 5.78 (d, J 0.9 Hz, 1H, C═C─H_a), 5.51 (d, J 0.9 Hz, 1H, C═C─H_b), 5.45 (d, $J_{1',2'}$ 4.0 Hz, 1H, H-1′), 4.73 (t, $J_{2',1'}=J_{2',3'}$ 4.0 Hz, 1H, 2′-H), 4.27 (d, $J_{3,4}$ 8.7 Hz, 1H, 3-H), 4.06 (t, $J_{3,4}=J_{4,3}$ 8.6 Hz, 1H, 4-H), 4.01–3.84 (m, 8H, 5-H, 6-H_2, 3′-H, 4′-H, 5′-H, 6′-H_2), 3.64 (s, 2H, 1-H_2), 1.98 (s, 3H, CH_3). ^{13}C NMR (100 MHz, D_2O) δ 174.2 (NH*C*O), 140.8 (HC═*C*─CH_3), 122.0 (H*C*═C─CH_3), 104.6 (C-2), 92.4 (C-1′), 82.5 (C-5), 76.7 (C-3), 74.4 (C-4), 69.1 (C-5′), 65.9, 65.8 (C-2′, C-4′), 63.1 (C-1), 62.8 (C-6), 60.9 (C-6′), 53.5 (C-3′), 18.7 (CH_3).

3.4. 3-Deoxy-3-*N*′-methacryloyloxyethylureido-α-D-allopyranosyl β-D-fructofuranoside (3)

2-Isocyanato methyl methacrylate (0.83 mL, 5.9 mmol) was added dropwise to a cooled (0 °C) solution of 2 g (5.9 mmol) **1** in water. The mixture was stirred for 1 h at 0 °C and 16 h at 7 °C. After 1 h at room temperature, the mixture was extracted three times with 10 mL of ether. The product **3** was isolated by freeze drying, yield of 2.92 g (99%). IR (cm^{-1}): 3500–3020 (OH), 2934, 2894 (C–H), 1713 (C═O) (ester), 1642 (C═O) (urea), 1561 (N–H), 1176, 1121, 1052, 992 (C–OH); ^{1}H NMR (400 MHz, D_2O) δ 6.09 (d, J 0.9 Hz, 1H, C═C–H_a), 5.66 (d, J 0.9 Hz, 1H, C═C–H_b), 5.32 (d, $J_{1',2'}$ 3.5 Hz, 1H, H-1′), 4.72 (m, 1H, 2′-H under D_2O), 4.21 (d, $J_{3,4}$ 8.6 Hz, 1H, 3-H), 4.17 (t, J 4.9 Hz, 2H, NCH_2CH_2O), 4.05 (t, $J_{3,4}=J_{4,3}$ 8.6 Hz, 1H, 4-H), 3.87–3.48 (m, 10H, 1-H_2, 5-H, 6-H_2, 3′-H, 4′-H, 5′-H, 6′-H_2), 3.41 (t, J 4.9 Hz, 2H, NCH_2CH_2O), 1.87 (s, 3H, CH_3). ^{13}C NMR (100 MHz, D_2O) δ 172.3 (COO), 163.5 (NHCO), 138.4 (HC═*C*–CH_3), 129.5 (H*C*═C–CH_3), 106.5 (C-2), 94.7 (C-1′), 84.1 (C-5), 79.1 (C-3), 76.3 (C-4), 71.2 (C-5′), 68.0, 67.7 (C-2′, C-4′), 67.0 (NCH_2CH_2O), 64.6 (C-1), 64.1 (C-6), 62.7 (C-6′), 55.7 (C-3′), 41.4 (NCH_2CH_2O), 18.7 (CH_3). Anal. Calcd for $C_{19}H_{32}O_{13}$: C, 45.97; H, 6.45; N, 5.65. Found: C, 45.00; H, 6.78; N, 5.45.

3.5. Poly-(3-deoxy-3-*N*-methacrylamido-α-D-allopyranosyl β-D-fructofuranoside) (poly-MA-sucrose) (4)

Typical procedure for polymerization of **2** under nitrogen: A sample (0.5 g, 1.22 mmol) of **2** and 1 mol % $Na_2S_2O_8$ was dissolved in 0.5 mL water in a glass tube. Oxygen was removed by the freeze–thaw method and the mixture heated for 48 h to 60 °C. Then the tube was opened, cooled, and the solution was added to 10 mL water and dialyzed against water. The product **4** was isolated by freeze drying in a yield of 40%. Other reaction conditions assayed and results are collected in Table 1.

Table 1. Reaction conditions assayed for polymerization of **2**

Sample	Radical initiator (mol %)	Reaction temperature (°C)	Reaction time (h)	Yield [g (%)]	Molecular weight M_w (g/mol)
1	1[a]	60	48	0.20 (40)	61,400
2	1[b]	40	24	0.25 (50)	194,000
3	2[b]	40	48	0.23 (46)	349,000
4	2[b]	40	6	0.19 (38)	96,400

[a] $Na_2S_2O_8$.
[b] $Na_2S_2O_8/Na_2S_2O_5$.

$[\alpha]_D$ +20.3 (*c* 1.3, H_2O); IR (cm^{-1}): 3500–3020 (OH), 2927 (C–H), 1643 (C=O) (urea), 1526 (N–H), 1128, 1112, 1050, 990 (C–OH); ^1H NMR (400 MHz, D_2O) δ 5.43 (H-1′), 4.73 (2′-H), 4.60–3.30 (1-H, 3-H, 4-H, 5-H, 6-H, 3′-H, 4′-H, 5′-H, 6′-H), 2.70–1.60 (C*H*CH_3), 1.60–0.70 (CH_3). ^{13}C NMR (100 MHz, D_2O) δ 184.0 (NHCO), 106.6 (C-2), 92.5 (C-1′), 84.5 (C-5), 78.2 (C-3), 75.5 (C-4), 68.3 (C-5′), 64.7 (C-2′, C-4′), 62.7 (C-1), 62.8 (C-6), 58.1 (C-6′), 54.7 (*C*HCH$_3$), 48.3 (C-3′), 25–17 (*C*H_2CHCH$_3$, CH_2CH*C*H$_3$).

3.6. Poly-(3-deoxy-3-*N*′-methacryloyloxyethylureido-α-D-allopyranosyl β-D-fructofuranoside) (poly-MAE-sucrose) (5)

Typical procedure for polymerization of **3** under nitrogen: A sample (0.5 g, 1 mmol) of **3** and 3 mol % $Na_2S_2O_8$ was dissolved in 0.5 mL water in a glass tube. Oxygen was removed by the freeze–thaw method and the mixture heated for 48 h to 60 °C. Then the tube was opened, cooled, and the solution was added to 10 mL water and dialyzed against water. The product **5** was isolated by freeze drying in a yield of 88%. Other reaction conditions assayed and results are collected in Table 2.

$[\alpha]_D$ +31.8 (*c* 1.5, H_2O); IR (cm^{-1}): 3500–3020 (OH), 2926 (C–H), 1720 (C=O) (ester), 1651 (C=O) (urea), 1520 (N–H), 1163, 1115, 1048, 971 (C–OH); ^1H NMR (400 MHz, D_2O) δ 5.40 (H-1′), 4.73 (2′-H), 4.60–3.30 (1-H, 3-H, 4-H, 5-H, 6-H, 3′-H, 4′-H, 5′-H, 6′-H, NC*H*$_2$CH$_2$O, NCH$_2$C*H*$_2$O), 2.70–1.60 (C*H*CH$_3$), 1.60–0.70 (CH_3). ^{13}C NMR (100 MHz, D_2O) δ 182.3 (COO), 163.4 (CONH), 106.6 (C-2), 94.7 (C-1′), 84.3 (C-5), 79.2 (C-3), 76.5 (C-4), 71.6 (C-5′), 68.2, 67.9 (C-2′, C-4′), 66.1 (NCH$_2$*C*H$_2$O), 64.7 (C-1), 64.3 (C-6), 62.9 (C-6′), 55.9 (C-3′), 47.4 (*C*HCH$_3$), 41.0 (N*C*H$_2$CH$_2$O), 21.3 (*C*H_2CHCH$_3$), 19.4 (CH_2CH*C*H$_3$).

3.7. 3-(*N*-*tert*-Butoxycarbonyl-L-aspartic acid-4-amido-1-benzyl ester)-3-deoxy-α-D-allopyranosyl β-D-fructofuranoside (6)

To a solution of **1** (0.59 mmol, 201 mg) in 4:1 pyridine–water (13 mL) *N*-*tert*-butoxycarbonyl-L-aspartic acid 1-benzyl ester (0.59 mmol, 193.5 mg) was added. The resulting solution was cooled to 0 °C. *N*,*N*′-Dicylclohexylcarbodiimide (1.0 mmol dissolved in 0.5 mL pyridine–water) was added dropwise with stirring. The mixture was warmed to room temperature and stirred for 17 h. Glacial acetic acid (one drop) was added and stirring was continued for 15 min. The mixture was filtered to remove the *N*,*N*′-dicyclohexylurea. Pyridine was coevaporated with several portions of toluene. The aqueous residue was extracted with Et_2O. The water phase was lyophilized and the crude product was applied for hydrogenation without further purification.

3.8. 3-(*N*-*tert*-Butoxycarbonyl-L-aspartic acid 4-amido)-3-deoxy-α-D-allopyranosyl β-D-fructofuranoside (7)

A suspension of **6** (0.31 mmol, 200 mg) in 50% aq MeOH (22 mL) was hydrogenated in the presence of 10% palladium–charcoal (50 mg) at atmospheric pressure for 4 h at room temperature. The catalyst was filtered off and washed with aq MeOH and the filtrates were evaporated. Compound **7** was purified by chromatography on silica gel. Elution with C gave the product (yield 44%). ^{13}C NMR (75 MHz, D_2O) δ 179.1 (COO), 175.1 (NHCOR), 158.2 (NHCOOR), 104.7 (C-2), 93.0 (C-1′), 82.6 (C-5), 82.0 (*C*(CH$_3$)$_3$), 77.3 (C-3), 75.0 (C-4), 69.5 (C-5′), 65.9, 65.8, (C-2′, C-4′), 63.2, 62.3 (C-1, C-6), 60.9 (C-6′), 54.1, 53.5 (C-3′, C-2$_{asp}$), 40.3 (C-3$_{asp}$), 28.5 (3 × CH_3). FABMS (glycerol) *m*/*z* 555 $[M-H]^-$, 393 $[M-\text{fructosyl}-H]^-$.

Table 2. Reaction conditions assayed for polymerization of **3**

Sample	Radical initiator (mol %)	Reaction temperature (°C)	Reaction time (h)	Yield [g (%)]	Molecular weight M_w (g/mol)
1	0.5	60	24	0.29 (58)	494,000
2	3.0	60	24	0.38 (76)	1,115,000
3	3.0	60	48	0.44 (88)	1,880,000
4	3.0	60	6	0.19 (38)	162,000

3.9. 3-(L-Aspartic acid 4-amido)-3-deoxy-α-D-allopyranosyl β-D-fructofuranoside (8)

Compound **7** (0.2 mmol, 112 mg) was dissolved in cooled 90% trifluoroacetic acid (0.8 mL) and kept for 15 min at 4 °C. Cold dried Et_2O (60 mL) was added and the precipitated product was filtered off and washed with Et_2O. Purification by chromatography on silica gel and elution with D gave the product (yield 77%). ^{13}C NMR (75 MHz, D_2O, acetone-d_6) δ 174.7 (COO), 174.1 (NHCOR), 104.8 (C-2), 93.2 (C-1′), 82.7 (C-5), 77.3 (C-3), 74.9 (C-4), 69.6, 66.2, 65.9 (C-2′, C-4′, C-5′), 63.2, 62.3 (C-1, C-6), 61.1 (C-6′), 53.5, 52.5 (C-3′, C-2_{asp}), 37.3 (C-3_{asp}). FABMS (glycerol) *m/z* 457 $[M+H]^+$, 295 $[M-\text{fructosyl}+H]^+$.

3.10. Synthesis of the aminoacyl derivatives 9, 10, 11, and 12

Compound **1** (0.59 mmol, 201 mg) was coupled with the protected amino acid as described for the preparation of **6**. After lyophilization the product was purified by column chromatography on silica gel. The following products were thus obtained.

3.11. 3-(*N*-*tert*-Butoxycarbonyl-L-alanylamido)-3-deoxy-α-D-allopyranosyl β-D-fructofuranoside (9)

Chromatography with eluent C gave the product (yield 40%). ^{13}C NMR (75 MHz, $(CD_3)_2SO$) δ 170.8 (NHCOR), 154.9 (NHCOOR), 104.2 (C-2), 91.6 (C-1′), 82.7 (C-5), 78.2 ($C(CH_3)_3$), 76.4 (C-3), 73.7 (C-4), 69.0, 65.5, 65.0 (C-2′, C-4′, C-5′), 61.8 (C-1, C-6), 60.1 (C-6′), 55.3 (C-3′), 49.5 (C-2_{ala}), 28.1 (C(CH_3)$_3$), 17.9 (C-3_{ala}).

3.12. 3-(*N*-*tert*-Butoxycarbonyl-L-leucylamido)-3-deoxy-α-D-allopyranosyl β-D-fructofuranoside (10)

Chromatography with eluent C gave the product (yield 30%). ^{13}C NMR (75 MHz, D_2O, acetone-d_6) δ 178.0 (NHCOR), 158.3 (NHCOOR), 105.0 (C-2), 93.0 (C-1′), 82.6 (C-5), 82.2 ($C(CH_3)_3$), 77.5 (C-3), 74.6 (C-4), 69.4, 66.1, 65.7 (C-2′, C-4′, C-5′), 62.9, 62.4 (C-1, C-6), 60.8 (C-6′), 55.3 (C-3′), 53.4 (C-2_{leu}), 40.8 (C-3_{leu}), 28.5 (C(CH_3)$_3$), 25.1 ($C(CH_3)_2$), 23.1 (C(CH_3)$_2$). FABMS (glycerol) *m/z* 555 $[M+H]^+$, 393 $[M-\text{fructosyl}+H]^+$.

3.13. 3-(N_α-*tert*-Butoxycarbonyl-N_ε-*Z*-L-lysylamido)-3-deoxy-α-D-allopyranosyl β-D-fructofuranoside (11)

^{13}C NMR (75 MHz, D_2O, acetone-d_6) δ 176.3 (NHCOR), 158.0, 157.9 (NHCOOR), 137.0 (quaternary aromatic carbon CH_2Ph), 128.9, 128.1, 128.0 (five tertiary aromatic carbons CH_2Ph), 104.5 (C-2), 92.5 (C-1′), 82.4 (C-5), 81.0 ($C(CH_3)_3$), 77.4 (C-3), 74.3 (C-4), 69.2, 65.7, 65.4 (C-2′, C-4′, C-5′), 66.5, 62.4 (C-1, C-6), 60.5 (C-6′), 56.0 (C-3′), 53.0 (C-2_{lys}), 40.6, 40.5 (C-3_{lys}, C-4_{lys}, C-5_{lys}, C-6_{lys}, CH_2Ph), 28.1 (C(CH_3)$_3$). FABMS (glycerol) *m/z* 726 $[M+Na]^+$.

3.14. 3-(*N*-*tert*-Butoxycarbonyl-L-phenylalanylamido)-3-deoxy-α-D-allogyranosyl β-D-fructofuranoside (12)

Chromatography with eluent C gave the product (yield 33%). ^{13}C NMR (75 MHz, D_2O, acetone-d_6) δ 176.3 (NHCOR), 158.0 (NHCOOR), 137.0 (quaternary aromatic carbon CH_2Ph), 128.9, 128.5, 128.1 (five tertiary aromatic carbons CH_2Ph), 104.5 (C-2), 92.5 (C-1′), 82.4 (C-5), 81.0 ($C(CH_3)_3$), 77.4 (C-3), 74.3 (C-4), 69.2, 65.7, 65.4 (C-2′, C-4′, C-5′), 66.5, 62.4 (C-1, C-6), 60.5 (C-6′), 56.0 (C-3′), 53.0 (C-2_{phe}), 40.5 (C-3_{phe}), 28.1 (C(CH_3)$_3$). FABMS (glycerol) *m/z* 611 $[M+Na]^+$, 589 $[M+H]^+$, 427 $[M-\text{fructosyl}+H]^+$.

3.15. 3-(L-Aspartic acid 4-amido)-3-deoxy-α-D-allopyranose (13)

A solution of **8** in 0.5 N HCI (5 mL) was heated at 60 °C for 10 min. After cooling to 20 °C, the solution was neutralized with NaOH. Compound **13** was separated from the reaction mixture by ion-exchange chromatography (separation conditions: column size 4 × 250 mm; packing: cation exchange resin, Ca^{2+}; eluent: H_2O).

3.16. Fmoc-L-tryptophan[22]

L-Tryptophan (100 mg, 0.49 mmol) in 50 mL of acetone/10% Na_2CO_3 solution was cooled with ice, and then fluorenyl-9-methoxycarbonyl chloride (Fmoc) (135 mg, 0.52 mmol), in 2 mL of acetone was added. The solution was stirred for 90 min at 0 °C and then 4 h at room temperature, and then poured into 100 mL of water and extracted three times with Et_2O. The pH of the solution was adjusted to 2 under cooling with ice, and the solution kept for 15 h at 5 °C. The white precipitate was then filtered off and washed three times with water to yield the product (190 mg, 91%). M_r 426.47 g/mol. ^1H and ^{13}C NMR spectra data are in accordance with the literature.[19] ES^+MS *m/z* 875 $[2M+Na]^+$, 465 $[M+K]^+$, 449 $[M+Na]^+$, 427 $[M+H]^+$.

3.17. 3-Deoxy-3-(*N*-Fmoc-L-tryptophanylamido)-α-D-allopyranosyl β-D-fructofuranoside (14)

Fmoc-L-tryptophan (1.37 g, 3.2 mmol) was dissolved in a mixture of 30 mL EtOH, 30 mL 2-propanol, and 10 mL acetone, and then a solution of **1** (1 g, 2.9 mmol) in water (40 mL) and ethanol (40 mL) was added, and then solution of EEDQ (0.87 g, 3.5 mmol) in a mixture of 2-propanol (20 mL), EtOH (10 mL), and 10 mL

acetone was added slowly. After 24 h of stirring, additional EEDQ (0.45 g, 1.8 mmol) in the same solvent mixture was added, and stirring was continued for 48 h, followed by the addition of water (100 mL). The alcohol was evaporated and the aqueous solution then extracted with 30 mL petroleum ether and three times with Et_2O (30 mL). The water solution was concentrated followed by freeze drying. The product was purified by chromatography on silica gel and elution consecutively with 100 mL petroleum ether, a 1:3 mixture of petroleum ether and Et_2O (100 mL), Et_2O (100 mL) and 2-propanol (200 mL), monitored by TLC (R_f in 2-propanol 0.67). The yield of the freeze-dried product was 60%. IR (cm^{-1}): 3350 (OH), 2926 (C–H), 1700 (C=O) (ester), 1671 (C=O) (amide), 1526 (N–H), 1478, 1450 (CH_2), 1249, 1105, 1050 (C–OH); ^1H NMR (300 MHz, D_2O, acetone-d_6) δ 7.74 (d, J 7.5 Hz, 2H, Aryl-H_{Fmoc}), 7.65 (d, J 7.6 Hz, 1H, $3'_{trp}$-H), 7.52 (d, J 7.40 Hz, 2H, Aryl-H_{Fmoc}), 7.31 (m, 3H, $2\times$Aryl-H_{Fmoc}, $6'_{trp}$-H), 7.22 (m, 2H, $2\times$Aryl-H_{Fmoc}), 7.04 (s, 1H, $1'_{trp}$-H), 6.99 (m, 2H, $4'_{trp}$-H, $5'_{trp}$-H), 5.38 (d, J 3.2 Hz, 1H, 1′-H), 4.57 (t, J 4.3 Hz, 1H, Aryl-C*H*–CH_2–O), 4.23 (d, $J_{3,4}$ 8.2 Hz, 1H, 3-H), 4.11 (m, 4H, Aryl-CH–C*H*$_2$–O, 2_{trp}-H, 4-H, 5H), 3.98 (m, 1H, 5′-H), 3.80 (m, 3H, 5-H, 2′-H, 5′-H), 3.70 (m, 6H, 6-H_2, 4′-H, 5′-H, 6′-H_2), 3.58 (m, 3H, 1-H_2, 3′-H), 3.29 (m, 2H, 3_{trp}-H). ES^+MS m/z 788 $[M+K]^+$, 772 $[M+Na]^+$, 750 $[M+H]^+$ 612, 588, 570, 181, 179.

3.18. 3-Deoxy-3-(L-glycylamido)-α-D-allopyranosyl β-D-fructofuranoside (15)

To a solution of **1** (1.5 g, 4.4 mmol) in water (70 mL) and EtOH was added a solution of Fmoc-glycine (1.45 g, 4.9 mmol) in a 3:2:1 mixture of EtOH–2-propanol–water and then a solution of EEDQ (1.31 g, 5.3 mmol) in 2-propanol (50 mL) was added slowly with stirring. After 20 h of reaction at room temperature water (200 mL) was added, the alcohol is removed in vacuo and the product isolated by freeze drying. For purification it was suspended in petroleum ether, ultrasonicated for 30 min, followed by filtration, and purification by chromatography on silica gel in 2-propanol. Freeze drying yielded 1.74 g (64%) of 3-deoxy-3-(Fmoc-L-glycylamino)-α-D-allopyranosyl β-D-fructofuranoside (R_f in 2-propanol 0.60), $M_r = 620.61$ g/mol. For deprotection 0.45 g (0.73 mmol) of the product was treated with Et_2NH (2 mL), stirred for 60 min at room temperature, and then water (150 mL) was added. The solution was then extracted three times with petroleum ether and two times with Et_2O. The aqueous solution was freeze dried and the product purified by chromatography on silica gel with 2-propanol. The yield was 0.275 g (0.7 mmol, 95%), $M_r = 398.37$ g/mol. ^1H NMR (400 MHz, D_2O) δ 5.32 (d, $J_{1',2'}$ 3.3 Hz, 1H, H-1′), 4.26 (d, $J_{3,4}$ 8.7 Hz, 1H, 3-H), 4.05 (t, $J_{3,4} = J_{4,3}$ 8.7 Hz, 1H, 4-H), 3.90 (m, 2H, 5-H, 5′-H), 3.83–3.62 (m, 10H, 1-H_2, 6-H_2, 2′H, 4′-H, 6′-H_2, NH_2CH_2–) 3.50 (t, J 5.5 Hz, 1H, 3′-H). ^{13}C NMR (100 MHz, D_2O) δ 176.0 (CONH), 104.9 (C-2), 92.2 (C-1′), 82.3 (C-5), 77.1 (C-3), 74.7 (C-4), 68.9 (C-5′), 65.9, 65.6 (C-2′, C-4′), 63.0 (C-6), 62.7 (C-1), 60.9 (C-6′), 57.4 (NH_2CH_2–), 53.4 (C-3′). ES^+MS m/z 399 $[M+H]^+$, 365, 237, 219, 181.

3.19. 3-Deoxy-3-(*N*-Fmoc-L-phenylalanyl-L-glycylamido)-α-D-allopyranosyl β-D-fructofuranoside (16)

Compound **15** (0.16 g, 0.4 mmol) and Fmoc-L-phenylalanine (0.155 g, 0.4 mmol) were dissolved in EtOH (50 mL) and then EEDQ (0.1 g, 0.4 mmol) in EtOH (20 mL) was added while stirring. The solution was stirred for a further 45 h at room temperature, and then water (250 mL) was added and the EtOH removed in vacuo. The product was purified by chromatography on silica gel with petroleum ether (100 mL), 100 mL of 1:3 EtOH–petroleum ether, and finally 2-propanol. The yield was 0.153 g (0.2 mmol, 50%), $M_r = 767.79$ g/mol. IR (cm^{-1}): 3350 (OH), 3065, 2929 (C–H), 1703 (C=O) (ester), 1603, (C=O) (amide), 1535 (N–H), 1498, 1477, 1451, 1413 (CH_2), 1258, 1106, 1050 (C–OH); ^1H NMR (300 MHz, pyridine-d_5) δ 7.82 (d, J 7.4 Hz, 2H, Aryl-H_{Fmoc}), 7.65 (t, J 7.5 Hz, 2H, Aryl-H_{Fmoc}), 7.41–7.29 (m, 9H, $5\times$Ph–H_{phe}, $4\times$Aryl-H_{Fmoc}), 5.38 (d, J 3.2 Hz, 1H, 1′-H), 4.61–4.18 (m, 10H, CH_2NH_2, Aryl-C*H*–CH_2–O, Aryl-CH–C*H*$_2$–O, 3-H, 5-H, 2′-H, 4′-H, 5′-H), 4.08 (d, $J_{3,4}$ 7.1 Hz, 1H, 3-H), 3.96 (m, 1H, 2_{phe}-H), 3.85 (m, 1H, 3′-H), 3.70 (m, 2H, 3_{phe}-H_2). ^{13}C NMR (75 MHz, pyridine-d_5) δ 172.6, 171.4 ($2\times$NHCOR), 157.2 (NHCOOR), 144.6, 141.6 (four aromatic quaternary carbons Fmoc), 138.5 (aromatic quaternary carbon phe), 130.0, 129.8, 129.7 (five tertiary aromatic carbons phe), 128.9, 128.0, 126.7, 121.7 (eight tertiary aromatic carbons Fmoc) 105.3 (C-2), 93.3 (C-1′), 84.4 (C-5), 79.9 (C-3), 75.4 (C-4), 71.3 (C-5′), 67.2 (Aryl-CH–*C*H_2–O), 67.0 (C-2_{phe}), 54.1 (C-3′), 46.8 ($NHCOCH_2$–), 38.9 (C-3_{phe}). ES^+MS m/z 790 $[M+Na]^+$, 768 $[M+H]^+$, 740, 696, 630, 612, 553, 522.

Acknowledgements

Suggestions by Dr. J. Seibel on the manuscript and support by the Federal Government/Fachagentur für Nachwachsende Rohstoffe e.V. (Grant No. 22001498) is gratefully acknowledged.

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