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LIPID ANALOGS OF THE NODULATION FACTORS USING THE UGI/PASSERINI MULTICOMPONENT REACTIONS: PRELIMINARY STUDIES ON THE CARBOHYDRATE MONOMER

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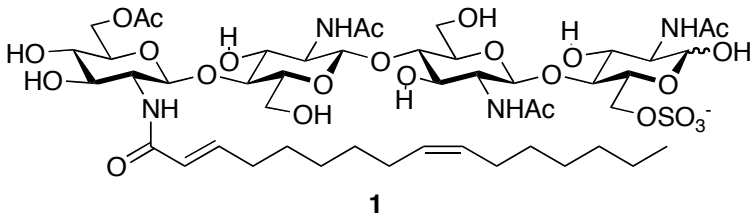
Abstract – Treament of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-isocyano-β-D-gluco-pyranose and the corresponding β-allyl glycoside with octanal and acetic acid provide the Passerini products in excellent yields whereas the corresponding Ugi transformation in the presence of *i*-propylamine proceeded with much lower efficiency. This work represents a preliminary investigation on a project directed towards the modification of the lipid moiety of the nodulation factors.

Carbohydrates and glycoconjugates have proven to be very important molecules in biology because they are involved in numerous cellular communication events.[1] The synthesis of carbohydrate derivatives has become a fundamental research area, in which a rapid access to molecular diversity is one of the promising strategies for the discovery of bioactive compounds.[1,2] Within this context, isocyanide-based multicomponent reactions such as the Passerini[3] or Ugi[4] reactions are attractive solutions since they require the synthesis of relatively simple precursors that can rapidly lead to a highly diverse range of derivatives which can be screened for biological activity.[5]

We decided to focus on the reaction of the peracetylated derivatives of 2-isocyanoglucose with either an α-acetyl or a β-allyl group on the anomeric position. This work represents a preliminary investigation on a project directed towards the modification of the lipid moiety of the nodulation factors (Nod Factors) such as the following lipo-chitooligosaccharide **1**. The structure of **1** is typical of a class of glycolipids secreted by rhizobia.[6] They are essential signaling molecules initiating symbiosis with legumes, resulting in the formation of rhizobia-infected nodules on plant roots in which the atmospheric nitrogen is fixed.[7] Structure-activity relationships have shown that a structural modification of the lipid unit induces a

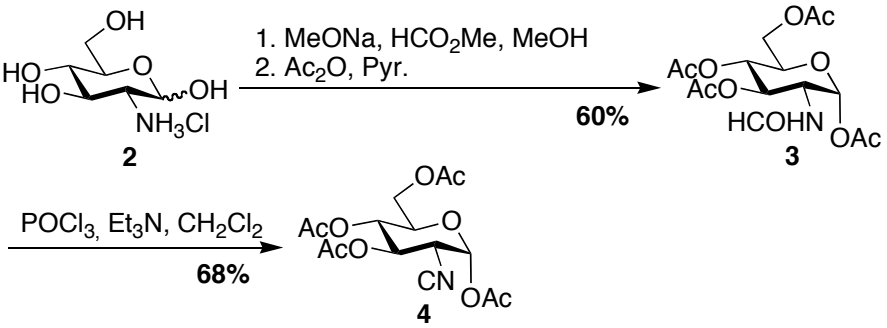
variation in the biological activity in alfalfa (*Medicago sativa*) roots.[8] The chemistry described here is somewhat complementary to Ziegler's studies,[9] which involved 1-isocyanoglucose, and 2-isocyanoglucose with an anomeric β-acetyl group.[10]



1

Figure

Glucosamine hydrochloride **2** was reacted with methyl formate in the presence of sodium methoxide, leading to the quantitative formation of *N*-formyl glucosamine[11] which appears, on the NMR timescale, as a mixture of anomers and rotamers around the amide bond (approx. 9/1 *trans* vs. *cis* amide). This intermediate was transformed, with acetic anhydride and pyridine, into the corresponding peracetate **3**, which was isolated in the form of its α anomer[12] in a 60% overall yield. Dehydration with phosphorus oxychloride afforded the corresponding isocyanide **4**[12] in 68% yield.

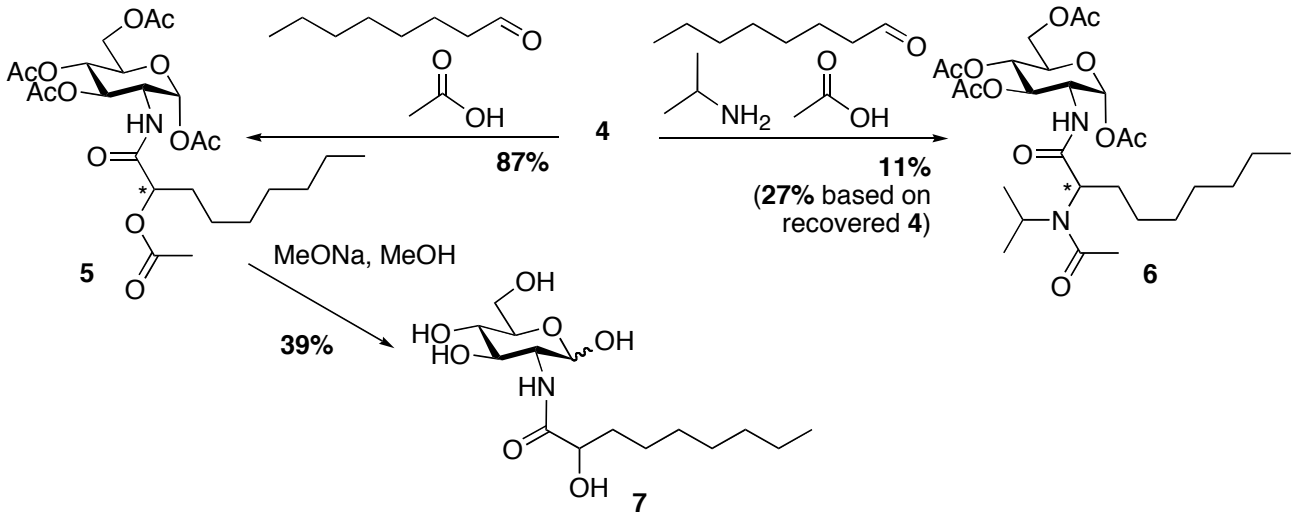


Scheme 1

The reaction of isocyanide **4** under Passerini conditions with ocatanal and acetic acid appeared to be sluggish and required 2 equiv. of both the aldehyde and the acid to reach completion. After 24 h, the expected products **5** were isolated in an 87% overall yield, as an equimolar mixture of the two possible diastereomers. It should be noted that all of these experiments were run under an inert atmosphere, since in the presence of air, a noticeable amount of aldehyde was oxidized to the corresponding acid which could take part in the reaction leading to an undesired side-product. The Passerini products **5** were deacetylated in moderate yield (39%) to the α-hydroxy amide **7** (α:β ratio of 3:1). Hydroxylated lipids have been found as *N*-substituents of D-glucosamine in the structurally diverse Nod factors.[13]

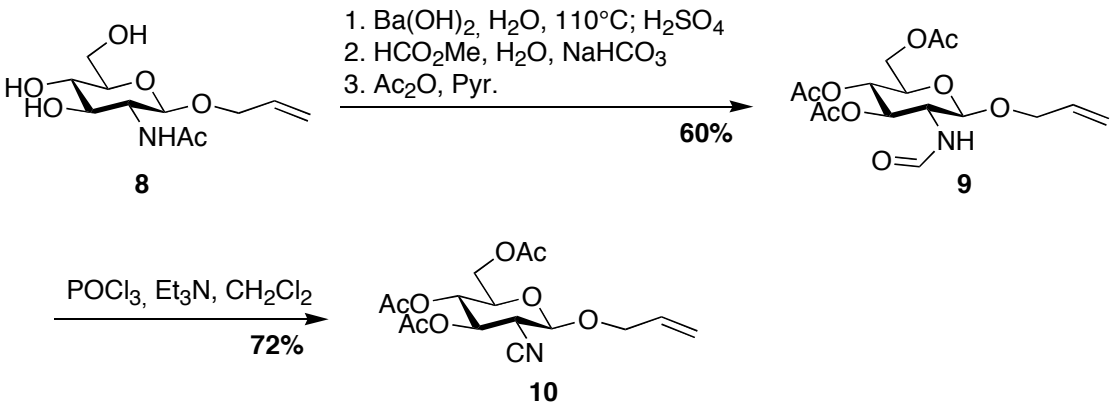
The poor reactivity of isocyanide **4** appeared more obvious in the Ugi reaction, where conversion was very slow and only an 11% yield of the expected products **6** was achieved. Once again, no

stereoselectivity was observed and an equimolar mixture of both diastereomers was isolated. Addition of zinc chloride seemed to accelerate the reaction, but led to the concomitant formation of byproducts including the Passerini products, as observed by TLC analysis.



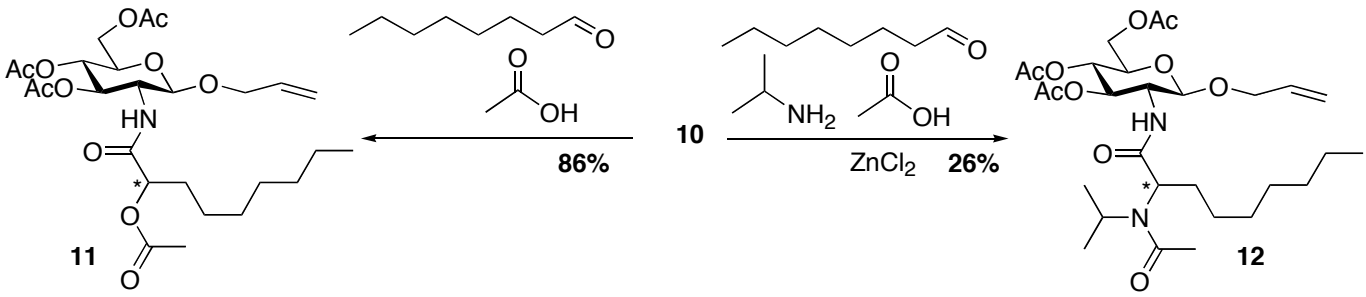
Scheme 2

In order to test whether the poor reactivity of the isocyanide was due to the presence of the anomeric α acetate and to better mimic the structure of the Nod Factors, we decided to perform these reactions on an allyl β-glycoside. The β-allyl glycoside of *N*-acetyl glucosamine **8**, available in one step by anomeric *O*-alkylation of *N*-acetyl-D-glucosamine,[14] was first deacetylated (90%) by treatment in refluxing aqueous barium hydroxide, and the resulting amine was isolated in the form of its sulfuric acid salt. Formylation in methanol in the presence of sodium methoxide failed due to the poor solubility of the amine, but could be achieved in 80% yield in water in the presence of sodium hydrogencarbonate. Peracetylation (Ac_2O, pyridine, 83%) provided the *N*-formyl derivative **9** which was dehydrated ($POCl_3$, Et_3N) to the corresponding isocyanide **10** in 72% yield.



Scheme 3

The reaction of **10** under Passerini conditions afforded the expected products **11** as an equimolar mixture of diastereomers and did not show any appreciable increase in reactivity as compared to isocyanide **4**. This was confirmed by the slow reaction of **10** under Ugi's conditions. After one week of reaction in the presence of zinc chloride, which in this case led to a clean reaction, 26% of the expected mixture of diastereomers **12** was isolated.



Scheme 4

With both isocyanides **4** and **10** in this study, the Ugi reaction proceeded significantly slower with a low yield than did the corresponding Passerini reaction, which provided the expected condensation products in excellent yields. In the present form it is clear that future synthetic work on more complex carbohydrates should be focused on the Passerini solution to provide structurally diverse α-hydroxy amide products for biological testing. Although not relevant to our study, the chirality of isocyanides **4** and **10** had no influence on the stereochemical outcome of both reactions, as stated many times in the past with chiral isocyanides.[5]

EXPERIMENTAL

All air sensitive reactions were carried out under argon atmosphere. Solvents were purified by distillation just prior to use or dried by standard methods. Dichloromethane was distilled from phosphorous pentoxide. Thin layer chromatography was performed on Merck $60F_{254}$ sheets with detection by UV and by charring with 5% ethanolic H_2SO_4. Silica gel SDS 60 ACC 35-70 μm was used for flash column chromatography. NMR spectra were taken on Brücker AC 200 and AC 250 spectrometers. For the allyl group, the following lettering was used for the protons: -CHdHd'CHa=CHbHc, with Ha and Hb in a *Z* relationship. Optical rotations were measured at 589 nm on a Perkin Elmer 341 polarimeter. Melting points (uncorrected) were measured on a Büchi 510 apparatus. Infrared spectra were taken on a Brücker IFS-66 Fourier transform spectrometer (KBr). Mass spectra were taken on a ThermoFinnigan Mat 95, with Electrospray ionization in the positive mode of detection (ESI^+). Elemental analyses were performed at the CNRS, Gif sur Yvette, France.

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-formamido-α–D-glucopyranose (3)[12]

Glucosamine hydrochloride (1.00 g) was added to 10.0 mL of a sodium methoxide solution (0.48 mol/L, MeOH), followed by 1.00 mL of methyl formate (16.6 mmol). After being stirred at rt for 2 h, the reaction mixture was diluted with 2 mL of MeOH, warmed at 40°C for 4 h, and stirred overnight at rt. The residue was concentrated and purified by flash chromatography over silica gel (EtOAc / MeOH / water 4:1:1) giving 961 mg (99%) of a white solid of 2-deoxy-2-formamido-D-glucopyranose[11]: mp 133°C; IR (cm^{-1}) : 3305 / 2993 / 2940 / 2890 / 1632 / 1564 / 1396 / 1322; ^{1}H NMR (250 MHz, D_2O) δ (ppm) : (two sets of signals due to the presence of the α / β anomers) 8.21 / 8.15 (2 s, 0.8 H, C*H*O^{trans}), 8.01 / 7.99 (2 s, 0.2 H, C*H*O^{cis}), 5.25 (d, *H*-1α^{cis}, $J_{1\alpha,2}$ 3.4 Hz), 5.21 (d, *H*-1α^{trans}, $J_{1\alpha,2}$ 3.4 Hz), 4.75 (d, *H*-1β^{trans}, $J_{1\beta,2}$ 8.3 Hz), 4.57 (d, *H*-1β^{cis}, $J_{1\beta,2}$ 8.3 Hz), 4.00 – 3.33 (m, 6 H, *H*-2, *H*-3, *H*-4, *H*-5, 2 *H*-6); ^{13}C NMR (62.5 MHz, D_2O) δ (ppm) : 167 (*C*HO^{cis}), 166 (*C*HO^{trans}), 97 (*C*-1β$^{trans\ and\ cis}$), 93 (*C*-1α$^{trans\ and\ cis}$), 78 / 76 / 72 (*C*-3^{cis}, *C*-4^{cis}, *C*-5^{cis}), 74 / 73 / 72 (*C*-3^{trans}, *C*-4^{trans}, *C*-5^{trans}), 63 (*C*-6^{cis}), 62 (*C*-6^{trans}), 58 (*C*-2^{cis}), 55 (*C*-2^{trans}); MS (ESI$^+$) m/z: 230.1 [M + Na]$^+$; HRMS (ESI$^+$) calcd. for $C_7H_{13}NO_6Na$ ([M+Na]$^+$): 230.0641; found m/z 230.0644.

Pyridine (4.80 mL, 60 mmol) and acetic anhydride (2.80 mL, 30 mmol) were added to 930 mg (4.49 mmol) of the preceding *N*-formyl derivative, and the reaction mixture was stirred at rt for 2 h. After concentration, the oily residue was taken in CH_2Cl_2 and washed with aqueous HCl (0.1 N). Drying (Na_2SO_4) and concentration of the organic layer gave 1.43 g of an orange foam which was recristallized from Et_2O yielding 1.02 g (61%) of **3** as a white solid. **3**: $[\alpha_D]^{20}_{589}$ +88 (c 0.675, $CHCl_3$); mp 131°C; IR (cm^{-1}) : 3402 / 2969 / 1746 / 1697 / 1508 / 1380 / 1225; ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm) : 8.11 (s, 0.8 H, C*H*O^{trans}), 7.99 (d, 0.2 H, C*H*O^{cis}, *J* 11,2 Hz), 6.18 (d, 1 H, *H*-1α, $J_{1\alpha,2}$ 3.4 Hz), 5.87 (d, 0.8 H, N*H*^{trans}, $J_{NH,2}$ 8.8 Hz), 5.86 (m, 0.2 H, N*H*^{cis}), 5.27 / 5.18 (m, 2 H, *H*-3 et *H*-4), 4.54 (ddd, 0.8 H, *H*-2^{trans}, $J_{2,3}$ 9.8 Hz), 3.76 (m, 0.2 H, *H*-2^{cis}), 4.24 / 4.03 (2 dd, 2 H, *H*-6a,b, $J_{a,b}$ 12.7 Hz, $J_{6a,5}$ 3.9 Hz *and* $J_{6b,5}$ 2.4 Hz), 4.00 (m, 1 H, *H*-5), 2.16 / 2.06 / 2.03 / 2.02 (4 s, 12 H, C*H*$_3$CO); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ (ppm) : (two sets of signals due to the presence of two slow equilibrating *trans* / *cis* rotamers of the formamide) 172 / 171 / 169 / 168 (4 CH_3*C*O), 161 (*C*HO), 90 (*C*-1α), 70 / 69 / 67 (*C*-3, *C*-4, *C*-5), 61 (2 *C*-6), 50 (*C*-2), 21 / 20 (4 *C*H_3CO); MS (ESI$^+$) m/z: 398.1 [M + Na]$^+$; HRMS (ESI$^+$) calcd. for $C_{15}H_{21}NO_{10}Na$ ([M+Na]$^+$): 398.1063; found m/z 398.1060.

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-isocyano-α-D-glucopyranose (4)[12]

Triethylamine (6.5 mL, 46 mmol) was added under an argon atmosphere to a solution of **3** (500 mg, 1.33 mmol) in anhydrous CH_2Cl_2 (20 mL), followed by dropwise addtion of $POCl_3$ (1.0 mL, 10.7 mmol) at 0°C. The reaction mixture was stirred for 2 h at rt under slightly positive argon pressure, and after dilution with CH_2Cl_2, washed with a saturated $NaHCO_3$ solution, dried (Na_2SO_4) and concentrated. The residue

was purified by flash chromatography over silica gel (cyclohexane / EtOAc 2:1), and crystallized from CH_2Cl_2 / hexane yielding 323 mg (68%) of a white solid. **4**: $[\alpha_D]^{20}_{589}$ +149 (c 0.375, $CHCl_3$); mp 113°C; IR (cm-1) : 2977 / 2156 / 1768 / 1390 / 1221; ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm) : 6.39 (d, 1 H, *H*-1α, $J_{1\alpha,2}$ 3.4 Hz), 5.53 (dd, 1 H, *H*-3, $J_{3,2}$ 10.7 Hz *and* $J_{3,4}$ 9.7 Hz), 5.01 (dd, 1 H, *H*-4, $J_{4,5} \sim J_{4,3}$), 4.28 (dd, 1 H, *H*-6a, $J_{a,b}$ 12.4 Hz *and* $J_{6a,5}$ 3.6 Hz), 4.12 – 4.00 (m, 2 H, *H*–5 and *H*-6b), 3.97 (dd, 1 H, *H*-2), 2.20 / 2.08 / 2.03 / 2.00 (4 s, 12 H, C*H*$_3$CO); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ (ppm) : 171 / 170 / 169 / 168 (4 CH_3*C*O), 162 (*C*N), 88 (*C*-1α), 70 / 69 / 67 (*C*-3, *C*-4, *C*-5), 61 (*C*-6), 55 (*C*-2), 21 / 20 (4 *C*H_3CO); MS (ESI$^+$) m/z: 380,1 [M + Na]$^+$; HRMS (ESI$^+$) calcd. for $C_{15}H_{19}NO_9Na$ ([M+Na]$^+$): 380,0958; found m/z 380,0954.

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-(N-2-acetoxy-nonanoyl)-amino-α-D-glucopyranose (5)

Isocyanide **4** (50 mg, 140 μmol) was dissolved in CH_2Cl_2 (0.5 mL), and octanal (50 μL, 320 μmol) and acetic acid (20 μL, 340 μmol) were added under an argon atmosphere. After stirring for 24 h at rt, the reaction mixture was concentrated and purified by flash chromatography over silica gel (cyclohexane / EtOAc 2:1), yielding 66.5 mg (87%) of a slightly yellowish solid. **5**: mp 101°C; IR (cm^{-1}) : 3323 / 2928 / 2858 / 1751 / 1685 / 1529 / 1436 / 1371 / 1224 / 1043; ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm) : 6.20 / 6.15 (2 d, *H*-1α for both stereomers, $J_{1\alpha,2}$ 3.9 Hz), 6.19 (d, N*H*, $J_{NH,2}$ 12.7 Hz), 5.30 – 5.12 (m, 2 H, *H*-3 and *H*-4), 4.99 – 4.89 (m, 1 H, CH_2-C*H*-CO), 4.44 – 4.29 (m, 1 H, *H*-2), 4.23 (m, 1 H, *H*-6a), 4.06 – 3.92 (m, 2 H, *H*-5 and *H*-6b), 2.16 – 1.99 (10 s, 15 H, 5 C*H*$_3$CO for both diastereomers), 1.70 (m, 2 H, CO-CH-C*H*$_2$-CH$_2$), 1.30 – 1.10 (m, 10 H, 5 C*H*$_2$), 0.83 (t, 3 H, C*H*$_3$, *J* 6.0 Hz); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ (ppm) : 172 - 168 (12 signals, 6 *C*O for both diastereomers), 90 (2 signals, *C*-1α for both diastereomers), 74 (CH_2-*C*H-CO), 70 / 69 / 67 (6 signals, *C*-3, *C*-4, *C*-5 for both diastereomers), 61 (*C*-6), 51 (2 signals, *C*-2 for both diastereomers), 32 - 22 (5 *C*H_2), 21 (10 signals, 5 *C*H_3CO for both diastereomers), 14 (*C*H_3); MS (ESI$^+$) m/z: 545.2 [M + Na]$^+$; HRMS (ESI$^+$) calcd. for $C_{25}H_{39}NO_{12}Na$ ([M+Na]$^+$): 568.2370; found m/z 568.2371; Anal. Calcd. for $C_{25}H_{39}NO_{12}$: C, 55.04; H, 7.21; N, 2.57; O, 35.19. Found: C, 55.15; H, 7.47; N, 2.36; O, 35.25.

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-[N-2-(N-acetyl-N-isopropylamino)-nonanoyl]-amino-α-D-gluco-pyranose (6)

Isocyanide **4** (50 mg, 140 μmol) was dissolved in CH_2Cl_2 (0.5 mL), and octanal (25 μL, 160 μmol), an isopropylamine solution (140 μL, 10 % v/v, 163 μmol, CH_2Cl_2), and an acetic acid solution (100 μL, 10 % v/v, 160 μmol, CH_2Cl_2) were added. After stirring for 12 h at rt, the reaction mixture was concentrated and the resulting oily residue was purified by flash chromatography over silica gel (cyclohexane / EtOAc 2:1), yielding 9.0 mg (11%) of the expected product **6** and 29.5 mg of recovered starting material (59%). **6**: IR (cm^{-1}) : 3423 / 2930 / 2857 / 1750 / 1674 / 1622 / 1543 / 1433 / 1372 / 1223

/ 1020; ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm) : 8.39 / 8.13 (2 d, 1 H, N*H* for both diastereomers, $J_{NH,2}$ 9.3 Hz), 6.17 / 6.13 (2 d, *H*-1α for both diastereomers, $J_{1\alpha,2}$ 3.9 Hz), 5.40 – 4.88 (m, 2 H, *H*-3 and *H*-4), 4.39 (m, 1 H, *H*-2), 4.27 – 3.85 (m, 4 H, *H*-5, *H*-6a,b, $(CH_3)_2$-C*H*-N), 3.61 / 3.51 (2 t, 1 H, CO-C*H*-CH_2, *J* 7.3 Hz), 2.24 – 1.96 (10 s, 15 H, 5 *C*H_3CO for both diastereomers), 1.69 (m, 2 H, CO-CH-C*H*$_2$-CH_2), 1.27 – 1.19 (m, 10 H, 5 C*H*$_2$), 1.13 (m, 6 H, (C*H*$_3$)$_2$-CH-N), 0.84 (m, 3 H, C*H*$_3$); MS (ESI$^+$) m/z: 609.3 [M + Na]$^+$; HRMS (ESI$^+$) calcd. for $C_{28}H_{46}N_2O_{11}Na$ ([M+Na]$^+$): 609.2999; found m/z 609.3002.

2-Deoxy-2-N-(2-hydroxynonanoyl)-amino-D-glucopyranose (7)

A solution of sodium methoxide (0.1 M, 0.5 mL, 50 μmol, MeOH) was added to 25 mg of **5** (46 μmol) and the reaction mixture was stirred overnight at rt, then neutralized with Amberlite IR 120 H$^+$ resin, filtrated, concentrated and purified by silica gel chromatography (CH_2Cl_2 / MeOH 10:1), yielding product **7** as a white solid (6 mg, 39 %). **7**: ^{1}H NMR (250 MHz, D_2O) δ (ppm) :5.09 (2 d, 0.6 H, *H*-1α for both diastereomers, $J_{1\alpha,2}$ 2.9 Hz), 4.66 (2 d, 0.4 H, *H*-1β for both diastereomers, $J_{1\beta,2}$ 9.0 Hz), 4.02 (m, 1 H, CO-C*H*(OH)-CH_2-CH_2), 4.00 – 3.30 (m, 6 H, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6a,b), 1.74 / 1.61 (2 m, 2 H, CO-CH(OH)-C*H*$_2$-CH_2), 1.50 – 1.20 (m, 10 H, 5 C*H*$_2$), 0.89 (t, 3 H, C*H*$_3$, *J* 6.6 Hz); ^{13}C NMR (62.5 MHz, D_2O) δ (ppm) :177 (*C*O), 93 (*C*-1α), 73 - 72 (*C*-3, *C*-4, *C*-5, CH_2-*C*H(OH)-CO), 63 (*C*-6), 55 (*C*-2), 35 - 36 (*C*H$_2$-CH(OH)-CO), 33 - 24 (5 *C*H$_2$), 14 (*C*H$_3$); MS (ESI$^+$) m/z: 358.1 [M + Na]$^+$; HRMS (ESI$^+$) calcd. for $C_{15}H_{29}NO_7Na$ ([M+Na]$^+$): 358.1842; found m/z 358.18450.

Allyl 3,4,6-tri-O-acetyl-2-deoxy-2-formamido-β-D-glucopyranoside (9)

An aqueous solution (1 mL) of **8**[14] (25 mg, 96 μmol) was treated with barium dihydroxide (61 mg, 193 μmol). The mixture was refluxed for 3 h, then aqueous sulfuric acid (2 M) was added until pH 2. After filtration and concentration, the sulfate salt of allyl 2-amino-2-deoxy-β-D-glucopyranoside *was* obtained as a white solid (26 mg, 90%) which was used without further purification. ^{1}H NMR (200 MHz, D_2O) δ (ppm) : 5.97 (m, 1 H, *H*a), 5.36 (dddd, 1 H, *H*c, $J_{Hc,Ha}$ 17.2 Hz *and* $J_{Hc,Hb} \sim J_{Hc,Hd} \sim J_{Hc,Hd'}$ 1.6 Hz), 5.30 (dddd, 1 H, *H*b, $J_{Hb,Ha}$ 10.2 Hz and $J_{Hb,Hd} \sim J_{Hb,Hd'}$ 1.6 Hz), 4.78 (d, 1 H, *H*-1β, $J_{1\beta,2}$ 8.4 Hz), 4.40 (dddd, 1 H, *H*d, $J_{Hd,Hd'}$ 12.5 Hz and $J_{Hd,Ha}$ 5.6 Hz), 4.21 (dddd, 1 H, *H*d', $J_{Hd',Ha}$ 6.6 Hz), 3.92 (dd, 1 H, *H*-6a, $J_{6a,6b}$ 12.3 Hz and $J_{6a,5}$ 2.0 Hz), 3.73 (dd, 1 H, *H*-6b, $J_{6b,5}$ 5.1 Hz), 3.67 (dd, 1 H, *H*-3, $J_{3,4}$ 8.4 Hz *and* $J_{3,2}$ 10.6 Hz), 3.58 - 3.36 (m, 2 H, *H*-4 and *H*-5), 2.83 (dd, 1 H, *H*-2); ^{13}C NMR (62.5 MHz, D_2O) δ (ppm) : 135 (-OCH_2-*C*H=CH_2), 121 (-OCH_2-CH=*C*H$_2$), 101 (*C*-1β), 77 / 75 / 71 (*C*-3, *C*-4 and *C*-5), 72 (-O*C*H$_2$-CH=CH_2), 62 (*C*-6), 57 (*C*-2); MS (ESI$^+$) m/z: 220.2 [M + H]$^+$.

Sodium hydrogencarbonate (10 mg, 119 μmol) and methyl formate (20 μL, 324 μmol) were added to a solution of the preceding ammonium salt (20 mg, 66 μmol) in water (0.3 mL). After 24 and 36 h at rt

other portions of $NaHCO_3$ (2x10 mg, 2x119 μmol) and methyl formate (2x20 μL, 2x324 μmol) were added. The reaction mixture was concentrated after 48 h (total) and purified by flash chromatography over silica gel (EtOAc / MeOH / water 3:1:1), followed by passing through a column of Dowex H^+ (50Wx8), yielding after concentration 14 mg (80%) of allyl 2-deoxy-2-formamido-β-D-glucopyranoside as white solid: ^{1}H NMR (250 MHz, D_2O) δ (ppm) : 8.18 (s, 0.7 H, C*H*O^{trans}), 7.97 (s, 0.3 H, C*H*O^{cis}), 5.90 (m, 1 H, *H*a), 5.33 / 5.22 (2 m, 2 H, *H*b and *H*c), 4.60 (d, *H*-1β^{trans}, $J_{1\beta,2}$ 8.4 Hz), 4.56 (d, *H*-1β^{cis}), 4.33 / 4.15 (2 m, 2 H, Hd and Hd'), 3.90 (dd, 1 H, *H*-6a, $J_{6a,6b}$ 12.2 Hz and $J_{6a,5}$ 1.8 Hz), 3.80 – 3.65 (m, 1.7 H, *H*-6b and *H*-2^{trans}), 3.60 - 3.40 (m, 3 H, *H*-3, *H*-4 and *H*-5), 3.21 (dd, 0.3 H, *H*-2^{cis}, $J_{2,3}$ 8.5 Hz); ^{13}C NMR (62.5 MHz, D_2O) δ (ppm) : (two sets of signals due to the presence of two *trans* / *cis* rotamers of the formamide with slow equilibrium) 170 (*C*HO^{cis}), 166 (*C*HO^{trans}), 134 (-OCH$_2$-*C*H=CH$_2$), 120 (-OCH$_2$-CH=*C*H$_2$), 101 (*C*-1β), 77 (*C*-5), 75 / 72 (*C*-3, *C*-4), 73 (-O*C*H$_2$-CH=CH$_2$), 62 (*C*-6), 60 (*C*-2^{cis}), 56 (*C*-2^{trans}); MS (ESI$^+$) m/z: 517.2 [2M + Na]$^+$; 270.1 [M + Na]$^+$; HRMS (ESI$^+$) calcd. for $C_{10}H_{17}NO_6Na$ ([M+Na]$^+$): 270.0954; found m/z 270.0943.

Pyridine (1 mL) and acetic anhydride (0.5 mL) were added to 9 mg of the preceding *N*-formyl derivative (37 μmol), and the reaction mixture was stirred at rt overnight, then concentrated and coevaporated several times with toluene, diluted in CH_2Cl_2, washed with aqueous HCl (1 M), dried and concentrated, yielding 12 mg of **9** (83%, white solid). **9**: ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm) : 8.17 (d, 0.6 H, C*H*O^{trans}, *J* 1.7 Hz), 8.02 (d, 0.4 H, C*H*O^{cis}, *J* 11.3 Hz), 6.00 (m, 1 H, N*H*), 5.80 (m, 1 H, *H*a), 5.40 - 5.00 (m, 4 H, *H*b, *H*c, *H*-3 and *H*-4), 4.74 (d, 0.6 H, *H*-1β^{trans}, $J_{1\beta,2}$ 8.3 Hz), 4.50 - 4.05 (m, 4.4 H, *H*d, *H*d', *H*-6a,b and *H*-1β^{cis}), 3.95 (m, 0,6 H, *H*-2^{trans}), 3.70 (m, 1 H, *H*-5), 3.46 (m, 0.4 H, *H*-2^{cis}), 2.10 – 2.03 (6 s, 9 H, C*H*$_3$CO); MS (ESI$^+$) m/z: 396.1 [M + Na]$^+$.

*Allyl 3,4,6-tri-O-acetyl-2-deoxy-2-isocyano-β-D-glucopyranoside (***10***)*

A solution of **9** (226 mg, 605 μmol) in anhydrous CH_2Cl_2 (15 mL) was treated with triethylamine (3.7 mL, 26.3 mmol) under an argon atmosphere, followed by dropwise addition of $POCl_3$ (700 μL, 7.5 mmol) at 0°C. After 2 h at rt under argon, the reaction mixture was diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, dried and concentrated. The residue was purified by flash chromatography over silica gel (cyclohexane / EtOAc 2:1), yielding 163 mg of a white solid (**10**, 72%). **10**: ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm) : 5.95 (dddd, 1 H, *H*a, $J_{Ha,Hc}$ 17.1 Hz, $J_{Ha,Hb}$ 10.0 Hz, $J_{Ha,Hd'}$ 6.6 Hz and $J_{Ha,Hd}$ 5.1 Hz), 5.40 (dddd, 1 H, *H*c, $J_{Hc,Hb}$ 1.8 Hz, $J_{Hc,Hd'}$ 1.4 Hz and $J_{Hc,Hd}$ 1.6 Hz), 5.30 (m, 2 H, *H*b and *H*-3), 4.96 (m, 1 H, *H*-4), 4.64 (d, 1 H, *H*-1β, $J_{1\beta,2}$ 8.0 Hz), 4.41 (m, 1 H, *H*d, $J_{Hd,Hd'}$ 12.8 Hz and $J_{Hd,Hb}$ 1.6 Hz), 4.28 (dd, 1 H, *H*-6b, $J_{6a,6b}$ 12.3 Hz and $J_{6b,5}$ 4.6 Hz), 4.19 (dddd, 1 H, *H*d', $J_{Hd',Hb}$ 1.4 Hz), 4.13 (dd, 1 H, *H*-6a, $J_{6a,5}$ 2.4 Hz), 3.74 (ddd, 1 H, *H*-5, $J_{5,4}$ 10.1 Hz), 3.66 (dd, 1 H, *H*-2, $J_{2,3}$ 10.5 Hz), 2.12 / 2.09 / 2.04 (3 s, 9 H,

C*H*$_3$CO); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ (ppm) : 171 / 170 / 169 (CH_3*C*O), 161 (*C*N), 133 (-OCH_2-*C*H=CH_2), 119 (-OCH_2-CH=*C*H_2), 99 (*C*-1β), 73 / 72 / 67 (*C*-3 *C*-4, *C*-5), 71 (-O*C*H_2-CH=CH_2), 61 (*C*-6), 57 (*C*-2), 21 / 20 (*C*H_3CO); MS (ESI$^+$) m/z: 378.1 $[M + Na]^+$; HRMS (ESI$^+$) calcd. for $C_{16}H_{21}NO_8Na$ ($[M+Na]^+$): 378.1165; found m/z 378.1165; Anal. Calcd. for $C_{16}H_{21}NO_8$: C, 55.04; H, 5.96; N, 3.94; O, 36.02. Found: C, 54.03; H, 6.09; N, 3.81; O, 35.86.

Allyl 3,4,6-tri-O-acetyl-2-deoxy-2-N-(2-acetoxynonanoyl)-amino-β-D-glucopyranoside (11)

Isocyanide **10** (40 mg, 113 μmol) was dissolved in CH_2Cl_2 (0.5 mL), and octanal (35 μL, 224 μmol) and acetic acid (13 μL, 225 μmol) were added. After 36 h of stirring at rt, the reaction mixture was concentrated and purified by flash chromatography over silica gel (cyclohexane / EtOAc 2:1). The coupling products **11** were isolated as a white solid (51.5 mg, 86 %). **11**: IR (cm^{-1}) : 3330 / 2928 / 2860 / 1750 / 1667 / 1537 / 1460 / 1376 / 1232 / 1048 / 972 / 903; ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm) : 6.12 / 6.07 (2 d, N*H* for both diastereomers, $J_{NH,2}$ 9.0 Hz and $J_{NH,2}$ 9.4 Hz), 5.82 (m, 1 H, *H*a), 5.35 - 4.96 (m, 5 H, *H*b, *H*c, *H*-3, *H*-4 and CH_2-C*H*-CO), 4.66 / 4.57 (2 d, 1 H, H_1β for both diastereomers, $J_{1\beta,2}$ 8.3 Hz), 4.39 – 3.86 (m, 5 H, *H*d, *H*d', *H*-2, *H*-6a and *H*-6b), 3.68 (m, 1 H, *H*-5), 2.15 – 1.99 (8 s, 12 H, C*H*$_3$CO for both diastereomers), 1.75 (m, 2 H, CO-CH-C*H*$_2$-CH_2), 1.32 – 1.15 (m, 10 H, C*H*$_2$), 0.85 (t, 3 H, C*H*$_3$, *J* 6.5 Hz); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ (ppm) : (two sets of signals due to the presence of two diastereomers) 171 - 169 (5 *C*O), 133 (-OCH_2-*C*H=CH_2), 118 (-OCH_2-CH=*C*H_2), 100 (*C*-1β), 74 (CH_2-*C*H-CO), 72 - 68 (*C*-3, *C*-4, *C*-5), 70 (-O*C*H_2-CH=CH_2), 62 (*C*-6), 54 (*C*-2), 32 - 22 (6 *C*H_2), 21 (4 *C*H_3CO), 14 (*C*H_3); MS (ESI$^+$) m/z: 566.3 $[M + Na]^+$; HRMS (ESI$^+$) calcd. for $C_{26}H_{41}NO_{11}Na$ ($[M+Na]^+$): 566.2580; found m/z 566.2577; Anal. Calcd. for $C_{26}H_{41}NO_{11}$: C, 57.45; H, 7.60; N, 2.58; O, 32.38. Found: C, 57.41; H, 7.69; N, 2.39; O, 32.41.

Allyl 3,4,6-tri-O-acetyl-2-deoxy-2-[N-(2-(N-acetyl-N-isopropylamino)-nonanoyl)-amino-β-D-glucopyraside (12)

Isocyanide **10** (23 mg, 65 μmol) was dissolved in CH_2Cl_2 (0.4 mL), and octanal (25 μL, 160 μmol), isopropylamine (15 μL, 175 μmol), and acetic acid (10 μL, 173 μmol) were added. After 1 h, a zinc chloride solution (140 μL, 1M, 140 μmol, THF) was added and the reaction mixture was stirred at rt for 1 week, concentrated and purified by flash chromatography over silica gel (cyclohexane / EtOAc 2:1). The expected coupling product was isolated as a slightly yellowish solid (10 mg, 26 %). **12**: ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm) : 5.87 (m, 1 H, *H*a), 5.49 – 5.00 (m, 4 H, *H*b, *H*c, *H*-3 and *H*-4), 4.98 and 4.92 (2 d, 1 H, *H*-1β for both diastereomers, $J_{1\beta,2}$ 8.3 Hz), 4.40 – 3.95 (m, 6 H, *H*d, *H*d', *H*-2, *H*-6a, *H*-6b and CO-C*H*-CH_2), 3.80 (m 1 H, $(CH_3)_2$-C*H*-N), 2.44 – 2.40 (2 s, N-CO-C*H*$_3$ for both diastereomers), 2.09 – 2.02 (6 s, C*H*$_3$CO for both diastereomers), 1.50 – 1.20 (m, 18 H, CO-CH-C*H*$_2$-CH_2, 5 C*H*$_2$ and

(CH_3)$_2$-CH-N), 0.87 (t, 3 H, , CH_3, *J* 6.8 Hz); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ (ppm) : (two sets of signals due to the presence of two diastereomers) 171 - 169 (5 *C*O), 133 (-OCH$_2$-*C*H=CH$_2$), 117 (-OCH$_2$-CH=*C*H$_2$), 100 (*C*-1β), 72 (CH$_2$-*C*H-CO), 71 - 69 (*C*-3, *C*-4, *C*-5), 70 (-O*C*H$_2$-CH=CH$_2$), 62 (*C*-6), 54 (*C*-2), 52 ((CH$_3$)$_2$-*C*H-N)), 32 - 23 (6 *C*H$_2$), 23 ((CH_3)$_2$-CH-N), 21 (4 *C*H$_3$CO), 14 (*C*H$_3$); MS (ESI$^+$) m/z: 607.3 [M + Na]$^+$; HRMS (ESI$^+$) calcd. for $C_{29}H_{48}N_2O_{10}Na$ ([M+Na]$^+$): 607.3207; found m/z 607.3213.

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