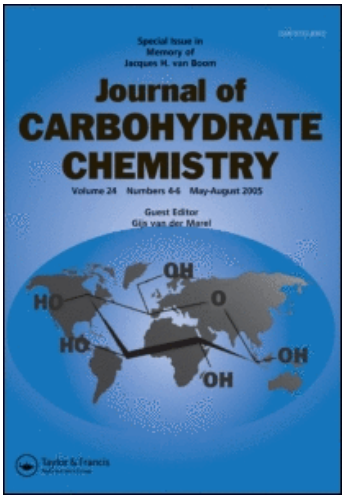

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Coloring Carbohydrates: Investigation of Azulene Derivatives as Blue Protecting Groups

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Coloring Carbohydrates: Investigation of Azulene Derivatives as Blue Protecting Groups

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Coloring carbohydrate derivatives by a chromophore tag greatly facilitates all purification steps during a synthetic sequence, according to a methodology called "chromophore-supported purification" (CSP). Herein an Fmoc-analogous blue protective group for CSP is introduced, based on guaiazulene. Following a mechanistic rational, the synthesis and introduction of this new protecting group is shown, together with its removal under variable conditions and its application for the synthesis of glycoclusters of a glycopeptide and glycopeptoid type.

Keywords Protecting groups; Guaiazulene; CSP; Glycopeptides; Glycopeptoids

INTRODUCTION

Recently, we introduced a concept for the parallelization and speed-up of organic synthesis by means of colored azulene derivatives, called "chromophore-supported purification," (CSP).[1] Due to the visibility of such colored tags, the purification of synthesis products by chromatography is facilitated and accelerated. Hence, in contrast to established methods such as solid or fluorous phase synthesis,[2] the principal idea of CSP is not to circumvent the time-consuming chromatography step, but to simplify this procedure. The CSP methodology is especially advantageous for chemical reactions that cannot be optimized to

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proceed without side-product formation and require chromatography in order to obtain pure products.

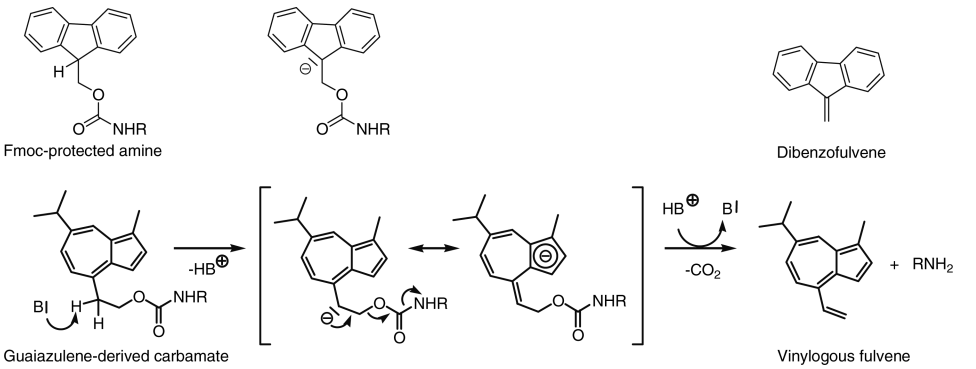
The synthesis of multivalent glycoconjugates, such as glycoclusters and glycodendrons,[3] which is important for many of our projects, is typically afflicted with the occurrence of structurally defective side products, requiring extensive and lengthy purification. To facilitate chromatographic purification of such mixtures, the CSP methodology is especially appealing. In line with our previous developments,[1] we now report an azulene-derived chromophore, which can be employed as a protecting group to allow CSP of branched glycopeptides. Moreover, we show the general compatibility of the CSP concept with chemical transformations, typically used to synthesize this type of biologically interesting glycoconjugates.

Azulenes are bicyclic hydrocarbons that hold intensive color without any additional chemical functionality. Especially, the lipophilic properties of azulenes are of advantage for CSP on polar stationary phases, such as silica gel, which is mainly used in preparative chromatography. In addition, the lipophilicity of the azulene moiety adds an appealing advantage to the compounds presented herein. We reasoned that azulene-modified ligands might show enhanced affinities for their receptors due to the addition of favorable hydrophobic interactions to the ligand-receptor complex.[4]

The azulene derivative guaiazulene (**1**) is inexpensive and of deep blue color. Its 4-methyl group can be easily deprotonated resulting in a resonance-stabilized anion. Based on this characteristic property of **1**, we reasoned that the development of an Fmoc-analogous protective group should be possible by introduction of a methylene oxycarbonyl moiety at the CH-acidic position of guaiazulene (Sch. 1). Deprotonation of a respective carbamate by a suitable base should furnish an intermediate carbanion, which would collapse in a β-elimination process, extruding carbon dioxide and leading to the vinylogous pentafulvene together with the deprotected amine.

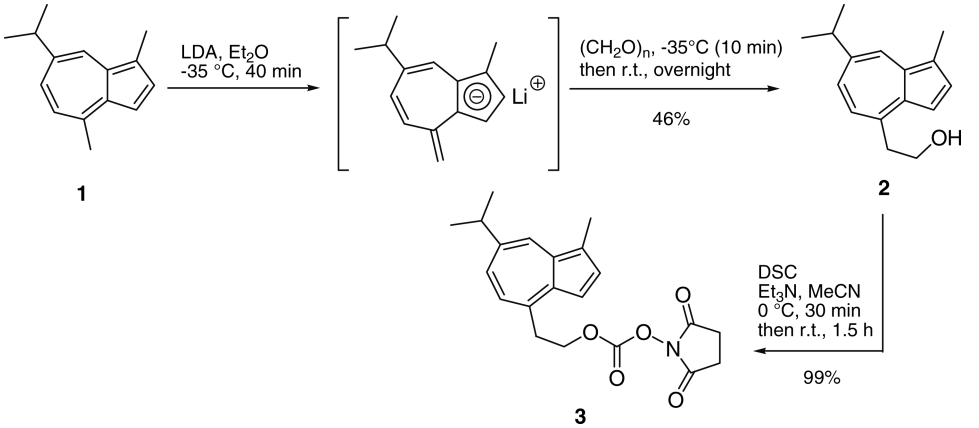
RESULTS AND DISCUSSION

Based on this rationale, we synthesized the alcohol **2** via deprotonation of **1** and treatment with paraformaldehyde in 46% yield based on converted azulene.[1] This alcohol had to be converted into an activated carbonic acid derivative, in order to allow the installation of the azulene oxycarbonyl residue as a protective group. Among a number of tested possibilities, the oxysuccinimidoyl moiety turned out to be best suited for activation. The preparation of the respective carbonate **3** using *N*, *N*′-disuccinimidylcarbonate (DSC) is simple and proceeds in almost quantitative yield (Sch. 2). Moreover, **3** is easily obtained as solid material, which is stable enough to allow storage for later use.

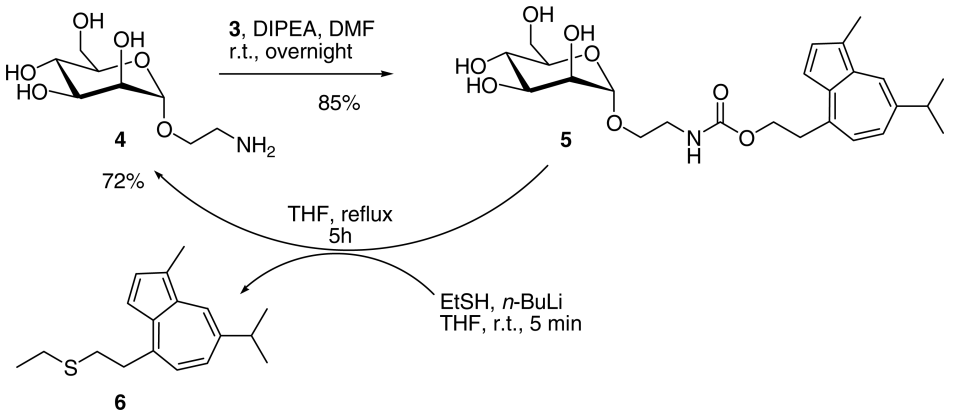


Scheme 1: In analogy to Fmoc-protected amines, guaiazulene-derived protection of amines should be possible leading to colored amino-functionalized derivatives. Deprotonation at the 4-methylene group of the guaiazulene subunit initiates β-elimination to furnish the deprotected amine and a dibenzofulvene-analogous guaiazulene-derived alkene.

The azulene carbonate **3** was then tested as reagent for the amino group protection of the known 2-aminoethyl mannoside **4**.[5] Indeed, this reaction led to the *N*-protected mannoside **5** in high yield under the catalysis of DIPEA (Sch. 3). Various conditions were tried to remove the dye from the carbohydrate under mildly basic conditions. While for Fmoc deprotection mild bases such as morpholine are sufficient, the deprotection of this new urethane protecting group required stronger catalysts. In fact, lithium thioethanolate allowed deprotection via the devised β-elimination mechanism and led to the recovery of the free amine **4** in good yield after refluxing in THF (Sch. 3). Thioethanolate is nucleophilic enough to quench the vinylogous fulvene intermediate as the sulfide byproduct **6** that could be isolated and fully characterized. The formation



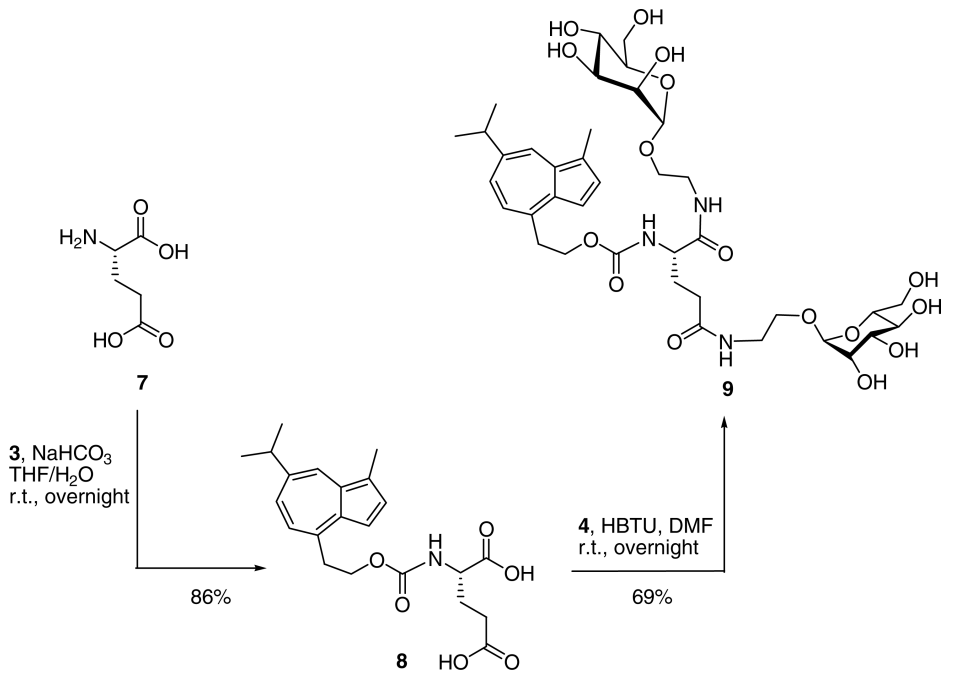
Scheme 2: Synthesis of the activated carbonic acid ester **3** is achieved from guaiazulene in two steps.



Scheme 3: The deep blue guaiazulene-derived carbonate **3** is suited for the protection of the amine **4** leading to mannoside **5**. The Fmoc-analogous ***N***-protective group can be removed with thioethanoate.

of this azulene sulfide proves the β-elimination mechanism, as direct thiolysis of the urethane **5** would give the alcohol **2** instead of sulfide **6**.

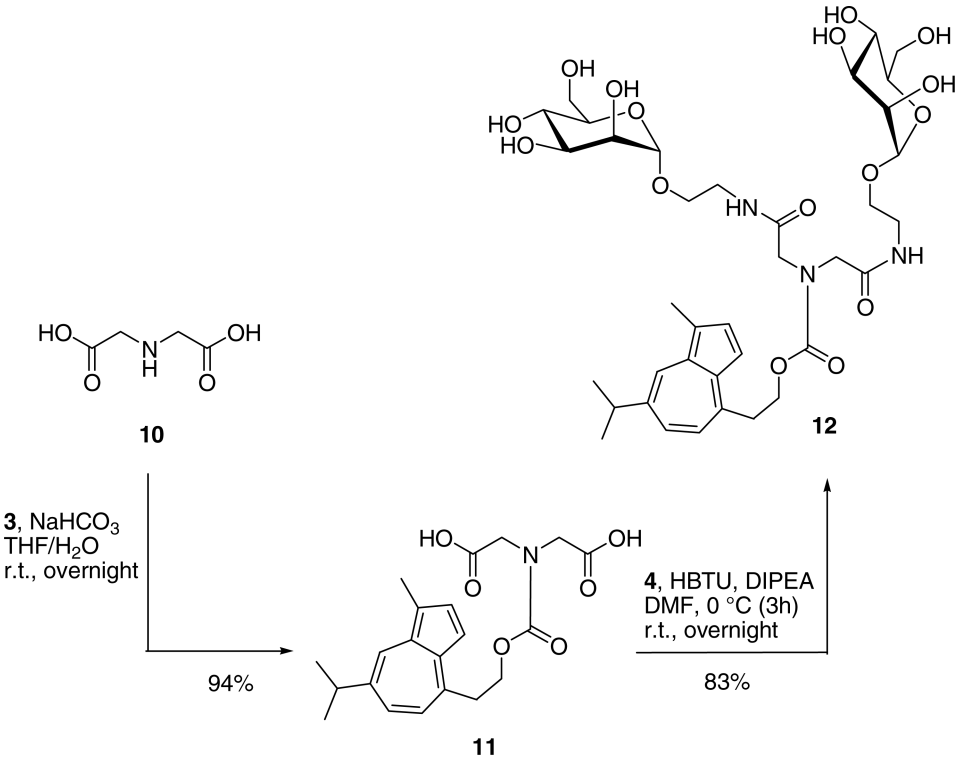
Next, we aimed at the utilization of **3** in the synthesis of a number of glycopeptide derivatives, which are of interest in our ongoing projects on the investigation of mannose-specific lectin binding.[6] Derivatization of L-glutamic



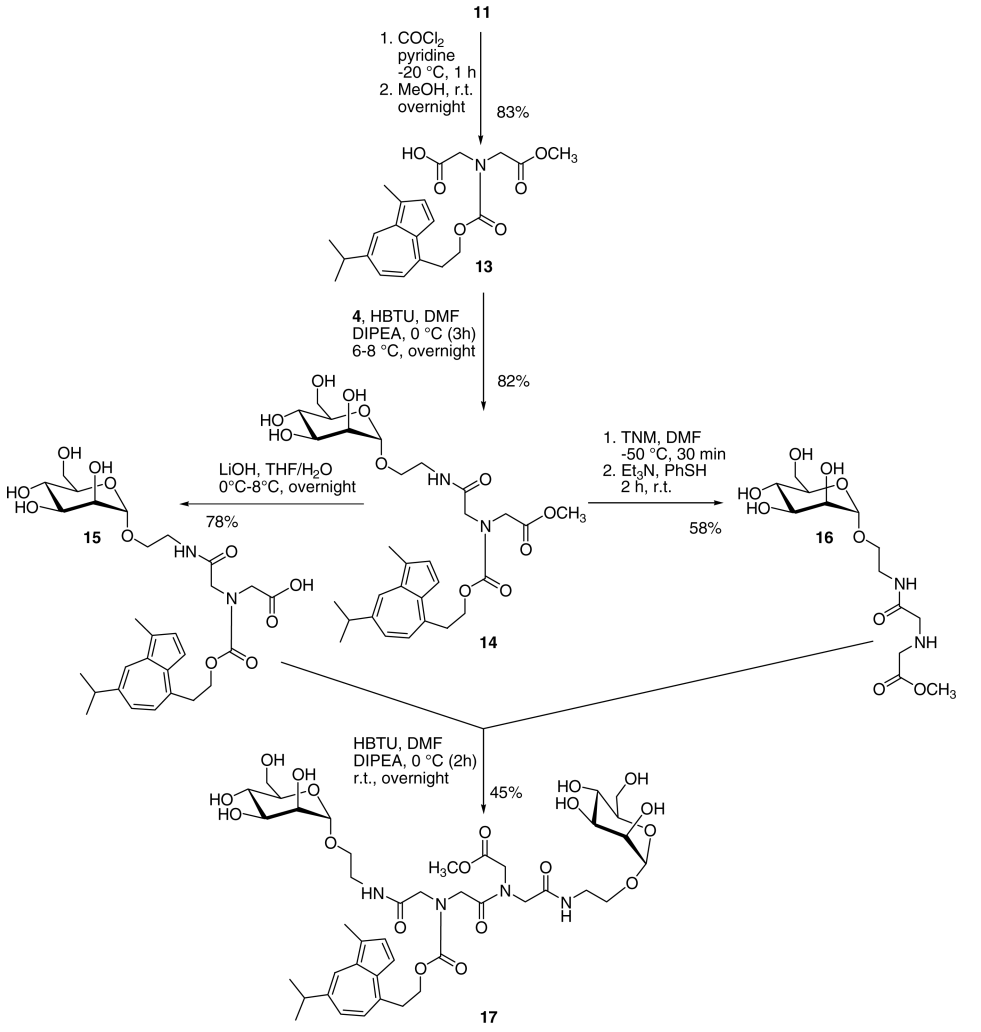
Scheme 4: The synthesis of the glyco-amino acid **9** is facilitated by the blue azulene chromophore, which is easily introduced using carbonate **3**.

acid (**7**) using the CSP reagent **3** gave the *N*-protected amino acid derivative **8** under Schotten-Baumann conditions. This diacid was submitted to a peptide coupling reaction with the unprotected mannoside **4** to yield the divalent glyco-amino acid[7] **9** (Sch. 4). In accordance with the principles of the CSP methodology, all the necessary purification steps in this synthetic sequence were efficiently simplified by the deep color of the respective derivatives.

This approach was extended to imidodiacetic acid (**10**) for the preparation of glycopeptoid clusters. Iminodiacetic acid is an appealing starting material in this project, as it is symmetric, in contrast to proteinogenic amino diacids, such as aspartic acid and glutamic acid. Accordingly, if only one of both carboxylic acid functions is functionalized (*vide infra*), no regioisomers can be formed in this transformation. In a first approach, protection of the secondary amine **10** using the carbonate **3**, again under Schotten-Baumann conditions, led to the dyed dicarboxylic acid **11** (Sch. 5). This, in turn, could be transformed to the divalent glycocluster **12** following a classical peptide coupling procedure employing the unprotected mannoside **4**. Glycopeptide **11** has prospects to be useful for the investigation of type 1 fimbriae-mediated bacterial adhesion,



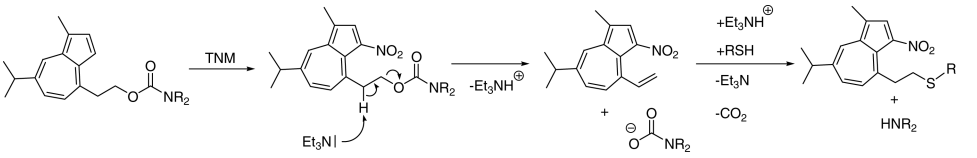
Scheme 5: Coloring imidodiacetic acid (**10**) to **11** allows straightforward synthesis of the divalent glycoconjugate **12**.



Scheme 6: CSP-facilitated synthesis of the divalent glycopeptoid **17** through a convergent sequence based on the imidodiacetic acid monoester **14**.

where both multivalency and hydrophobicity have been shown to be of relevance.[8]

Next, the imidodiacetic acid derivative **11** was elaborated into an even more privileged molecule (Sch. 6). First, it was converted into the monomethyl ester **13** via intermediate formation of its cyclic anhydride, followed by base-catalyzed ring opening with methanol in situ (Sch. 6).[9] Employing DCC in THF in this step gave **13** in 49% only, whereas cyclization with phosgene (solution in toluene) in pyridine led to the product in over 80% yield. Under these reaction conditions, the azulene scaffolds in **11** and **13** are stable, although, in general, azulenes readily undergo electrophilic substitution reactions with phosgene already at low temperatures.[10] However, in this case



Scheme 7: Mild cleavage of the CSP protecting group is possible after nitration of the 3-position of the azulene moiety.

phosgene preferentially reacts with the carboxylic acid function of **11**, leaving the protecting group intact. This finding underlines the suitability of the azulene derivatives as chromophore for CSP, exemplifying that the stability of azulenes is no limiting factor in this methodology.

Carrying on with the blue monomethyl ester **13**, a first mannosyl residue was introduced by a typical peptide coupling reaction, leading to **14**. Eventually, this methyl ester **14** could be hydrolyzed with lithium hydroxide, affording the corresponding acid **15**. To obtain the complementary building block **16**, removal of the CSP protecting group from **14** had to be achieved. We reasoned that **14** is not stable under the reaction conditions, which were necessary for the deprotection of **5** (Sch. 3). Oligomerization by means of amide formation with the deprotected amino groups had to be prevented.

Hence, we needed to develop a modified deprotection procedure, in which the CH-acidity of the urethane protecting group is increased by derivatization in order to allow mild cleavage with weak bases. To modify **14** accordingly, the azulene scaffold was nitrated under conditions compatible with the rest of the molecule. Tetranitromethane (TNM) allowed selective nitration of the 3-position of the azulene moiety. As the electron-withdrawing nitro group is considerably increasing, the CH-acidity of the 4-methylene group adjacent to the aromatic system deprotection should now be possible under mild conditions. Indeed, treatment of **14** with TNM, followed by addition of triethylamine and thiophenol, led to the unprotected mannoside **16** in almost 60% yield (Sch. 6). A mechanistic rationale for this interesting procedure is presented in Scheme 7. Accordingly, the mild cleavage of the CSP protective group proceeds via a β-elimination with triethylamine after an initial nitration step. In order to quench the resulting vinylogous fulvene in form of a sulfide, thiophenol has to be added.

Finally, the carboxylic acid **15** and the secondary amine **16** could be linked in a peptide coupling reaction. This last step affords the divalent glycopeptoid **17** (Sch. 6), which is currently employed and further elaborated in our laboratory.

CONCLUSION

In summary, we have developed an Fmoc-analogous protecting group derived from the chromophore guaiazulene, which adds a deep blue color to all the

protected molecules. Herein, the activated guaiazulene derivative **3** was used to protect amino groups, but likely it can be used as a protective group for hydroxyl functions as well. Based on careful mechanistic reasoning, various deprotection conditions were elaborated to remove the color tag via β-elimination under anhydrous conditions. It was shown that a very selective reaction sequence starting with an electrophilic substitution of the azulene moiety allowed cleavage under mildly basic conditions, which might even be appropriate for compounds sensitive to racemization. This finding could be of special value if a particular orthogonality is required in a complex protecting group strategy.

The new protecting group can be applied in the so-called CSP methodology, which facilitates all purification steps of a synthetic sequence. It was shown that the CSP idea is of broad applicability. One protective group per molecule was sufficient to dye all compounds prepared in this work efficiently, although the guaiazulene portion was often only a small fraction of the molecular weight of the synthesized glycoclusters.

The blue protecting group was validated in the synthesis of a number of divalent glycoclusters and glycopeptoids. Currently, these molecules are under investigation as ligands of mannose-specific lectins, including the evaluation of the effect that the azulene moiety has on the respective binding affinity.

EXPERIMENTAL

General Methods

Thin layer chromatography was performed on silica gel plates (GF 254, Merck). Detection was effected by UV irradiation and subsequent charring with 10% sulfuric acid in ethanol followed by heat treatment. However, due to the inherent features of the CSP methodology no special detection procedure was required in most cases. Column chromatography was performed on silica gel 60 (230–400 mesh, particle size 0.040–0.063 mm, Merck). Optical rotations were measured on a Perkin-Elmer 241 polarimeter (Na-D-line: 589 nm, length of cell 1 dm). Optical rotations of azulene-protected compounds could not be recorded, as their intensive color inhibits detection. Melting points were measured with a Zeiss instrument and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX-500 (125.77 MHz for ^{13}C) and AV-600 (75.47 MHz for ^{13}C) spectrometers. Chemical shifts are reported relative to internal tetramethylsilane (δ 0.00 ppm). Assignment of NMR signals is based on 2D-NMR experiments (COSY, HSQC, HMBC). The azulene ring system is numbered according to IUPAC convention and abbreviated as "az" where appropriate. Assignments marked with an asterisk (*) are interchangeable. High-resolution mass analysis (HRES MS) was performed with an MAT 8230 (Finnegan) instrument. MALDI-TOF MS spectra were recorded with the

spectrometer Biflex III (Bruker-Daltonics). Air- and/or moisture-sensitive reactions were carried out under an atmosphere of argon. Anhydrous diethyl ether and THF were dried over sodium-potassium alloy. Anhydrous methanol was dried over magnesia. All the other anhydrous solvents were obtained commercially. All commercial reagents were used without purification unless otherwise noted.

*2-(7-Isopropyl-1-methylazulen-4-yl)ethyl N-succinimidyl carbonate (**3**)*

The alcohol **2** (2.28 g, 10.0 mmol) and disuccinimidyl carbonate (DSC, 3.84 g, 15.0 mmol) were dissolved in anhydrous acetonitrile (50 mL) and triethyl amine (4.18 mL, 30.0 mmol) was added under argon at 0°C. The solution was stirred for 30 min at this temperature and another 1.5 h at rt. The solution was concentrated to 10 mL at the rotary evaporator and diluted with ethyl acetate. The organic phase was washed three times with brine and dried over Na_2SO_4. After filtration, the solvent was removed at the rotary evaporator and the product was crystallized from diethyl ether and dried in vacuo to yield the title compound (3.64 g, 9.86 mmol, 99%) as a blue solid. Mp: 121°C; IR (film): $\nu = 2961$ ($CH_{aliph.}$) 1784, 1735 (C=O) cm^{-1}; ^1H NMR (300.13 MHz, $CDCl_3$): $\delta = 8.21$ (d, 1H, $^4J_{6,8} = 2.0$ Hz, H-8), 7.68 (dq, 1H, $^3J_{2,3} = 3.8$ Hz, $^4J_{2,Me} = 0.6$ Hz, H-2), 7.45 (dd, 1H, $^3J_{5,6} = 10.6$ Hz, H-6), 7.28 (d, 1H, H-3), 7.00 (d, 1H, H-5), 4.72–4.67 (m, 2H, $C\underline{H}_2O(CO)$), 3.61–3.56 (m, 2H, $C\underline{H}_2CH_2O(CO)$), 3.09 (sept, 1H, $^3J_{isopropyl} = 6.9$ Hz, az-$C\underline{H}(CH_3)_2$), 2.78 (s, 4H, $(CO)C\underline{H}_2C\underline{H}_2(CO)N$), 2.67 (d, 3H, az-$C\underline{H}_3$), 1.36 (d, 6H, az-$CH(C\underline{H}_3)_2$) ppm; ^{13}C NMR (75.47 MHz, $CDCl_3$): $\delta = 168.6$ (2 CON), 151.5 (OCOO), 141.3, 140.8 (C-4, C-7), 137.2 (C-2), 137.1, 136.6 (C-3a, C-8a), 135.2 (C-6), 133.8 (C-8), 125.8 (C-1), 124.7 (C-5), 112.2 (C-3), 71.1 ($CH_2\underline{C}H_2O$), 38.3 (az-$\underline{C}H(CH_3)_2$), 36.8 ($\underline{C}H_2CH_2O$), 25.4 ($(CO)\underline{C}H_2\underline{C}H_2(CO)N$), 24.7 (az-$CH(\underline{C}H_3)_2$), 12.9 (az-$CH_3$) ppm; MALDI-TOF MS (no matrix): $m/z = 396.8$ $[M+H]^+$ (calc.: 369.16 for $C_{21}H_{23}NO_5$); HRES MS: $m/z = 369.15550$ (calc.: $m/z = 369.15762$ for $C_{21}H_{23}NO_5$).

*2-[2-(7-Isopropyl-1-methyl-azulen-4-yl)ethoxycarbonylamido]ethyl α-D-mannopyranoside (**5**)*

Mannoside **4** (159 mg, 712 μmol) and carbonate **3** (290 mg, 784 μmol) were dissolved in anhydrous DMF (4 mL) and diisopropylethyl amine (DIPEA, 146 μL, 855 μmol) was added at rt under argon. The solution was stirred overnight and the solvent was removed at the rotary evaporator. Column chromatography on silica gel (ethyl acetate/methanol = 9:1) afforded the product as a blue resin (289 mg, 605 μmol, 85%). ^1H NMR (300.13 MHz, CD_3OD): $\delta =$ 8.22 (d, 1H, $^4J_{6,8} = 2.0$ Hz, H-8_{az}), 7.65 (d, 1H, $^3J_{2,3} = 3.8$ Hz, H-2_{az}), 7.49 (dd, 1H, $^3J_{5,6} = 10.8$ Hz, H-6_{az}), 7.36 (d, 1H, H-3_{az}), 7.07 (d, 1H, H-5_{az}), 4.80 (d, 1H, $^3J_{1,2} = 1.6$ Hz, H-1_{man}), 4.45 (t, 2H, $^3J_{a,b} = 7.2$ Hz, $NH(CO)OC\underline{H}_2CH_2$), 3.88 (dd, 1H, $^3J_{5,6} = 2.4$ Hz, $^3J_{6,6'} = 11.8$ Hz, H-6_{man}), 3.86 (dd, 1H, $^3J_{2,3} = 3.2$ Hz,

H-2$_{man}$), 3.80–3.72 (m, 1H, man-OCHH), 3.75 (dd, 1H, $^3J_{3,4} = 9.2$ Hz, H-3$_{man}$), 3.75 (dd, 1H, $^3J_{5,6'} = 5.7$ Hz, H-6$'_{man}$), 3.65 (dd ≈ t, 1H, $^3J_{4,5} = 9.1$ Hz, H-4$_{man}$), 3.62–3.45 (m, 2H, man-OCHH, H-5$_{man}$), 3.47 (t, 2H, NH(CO)OCH$_2$CH$_2$), 3.37–3.27 (m, man-OCH$_2$CH$_2$ + MeOH), 3.11 (sept ≈ quin, 1H, $^3J_{isopropyl} = 6.9$ Hz, az-CH(CH$_3$)$_2$), 2.66 (d, 3H, az-CH$_3$), 1.39 (d, 6H, az-CH(CH$_3$)$_2$) ppm; ^{13}C NMR (75.47 MHz, CD$_3$OD): δ = 159.0 (OCON), 144.9 (C-4), 141.3 (C-7), 139.0 (C-3a*), 137.9 (C-2), 137.7 (C-8a*), 136.0 (C-6), 133.9 (C-8), 126.4 (C-1), 125.8, (C-5), 113.5 (C-3), 101.6 (C-1$_{man}$), 74.7 (C-5$_{man}$), 72.5 (C-3$_{man}$), 72.1 (C-2$_{man}$), 68.5 (C-4$_{man}$), 67.5, 66.2 (NH(CO)OCH$_2$CH$_2$, man-OCH$_2$CH$_2$), 62.9 (C-6$_{man}$), 41.6 (man-OCH$_2$CH$_2$), 39.4 (CH(CH$_3$)$_2$), 38.7 (NH(CO)OCH$_2$CH$_2$), 25.1 (CH(CH$_3$)$_2$), 12.9 (az-CH$_3$) ppm; MALDI-TOF MS (no matrix): m/z = 477.1 [M]$^+$; 500.1 [M+Na]$^+$ (477.24 calc. for $C_{25}H_{35}NO_8$).

*Deprotection of mannoside **5**: Synthesis of **4** and ethyl-[2-(7-isopropyl-1-methyl-azulen-4-yl)ethyl]sulfide **6***

Ethanethiol (40.9 μL, 553 μmol) was dissolved in anhydrous THF (1 mL) under argon and a solution of *n*-butyl lithium (277 μL, 442 μmol) in *n*-hexane was added slowly. This mixture was stirred for 5 min at rt and then a solution of protected mannoside **5** (53.0 mg, 111 μmol) in anhydrous THF (1 mL) was added and the reaction mixture was refluxed for 5 hours. It was diluted with water and the mixture washed three times with diethyl ether. The aqueous phase was concentrated at the rotary evaporator and purification of the residue by column chromatography on silica gel (methanol → methanol/water, 1:1) gave the deprotected amine **4** (18 mg, 80 μmol, 72%) as a colorless solid. Its spectroscopic data were in accordance with the literature.[5] Concentration of the combined organic phases gave the guaiazulene sulfide side product **6** (23 mg, 85 μmol, 76%) as a blue oil after column chromatography on silica gel (toluene/cyclohexane, 1:1). ^{1}H NMR (300.13 MHz, DMSO-D$_6$): δ = 8.18 (d, 1H, $^4J_{6,8} = 2.2$ Hz, H-8), 7.65 (d, 1H, $^3J_{2,3} = 3.8$ Hz, H-2), 7.52 (dd, 1H, $^3J_{5,6} = 10.8$ Hz, H-6), 7.27 (d, 1H, H-3), 7.10 (d, 1H, H-5), 3.35 (m$_c$, 2H, CH$_2$CH$_2$S), 3.10 (sept ≈ quint, 1H, $^3J_{isopropyl} = 6.9$ Hz, az-CH(CH$_3$)$_2$), 2.90 (m$_c$, 2H, CH$_2$CH$_2$S), 2.64−2.57 (m, 5H, CH$_3$CH$_2$S, az-CH$_3$), 1.31 (d, 6H, az-CH(CH$_3$)$_2$), 1.19 (t, 3H, $^3J_{CH3,CH2} = 7.4$ Hz, CH$_3$CH$_2$S) ppm; ^{13}C NMR (75.47 MHz, CDCl$_3$): δ = 146.6, 139.9 (C-4, C-7), 136.7 (C-2, C-3a*), 135.8 (C-8a*), 135.2 (C-6), 133.3 (C-8), 124.9 (C-1), 124.6 (C-5), 112.1 (C-3), 37.8 (CH$_2$CH$_2$S), 37.4 (az-CH(CH$_3$)$_2$), 32,0, 25.1 (CH$_2$SCH$_2$), 24.6 (az-CH(CH$_3$)$_2$), 14.7 (SCH$_2$CH$_3$), 12.8 (az-CH$_3$) ppm; HRES MS: m/z = 272.15980 (calc.: m/z = 272.15988 for $C_{18}H_{24}S$).

*N-[2-(7-Isopropyl-1-methylazulen-4-yl)-ethoxycarbonyl]-L-glutaminic acid (**8**)*

Carbonate **3** (100 mg, 271 μmol) and L-glutamic acid **7** (40.1 mg, 271 mmol) were dissolved in a mixture of THF and water (7 mL, 1:1). Sodium hydrogen carbonate (74.8 mg, 893 mmol) was added and the solution was stirred

overnight at rt. The mixture was diluted with brine and water (1:1) and the aqueous phase was overlaid with ethyl acetate and then acidified by addition of hydrochloric acid (2 M). The phases were separated and the organic phase was washed repeatedly with brine until the aqueous phase remained neutral. The organic phase was then dried over Na_2SO_4. After filtration the solvent was removed in vacuo to afford the product as a blue oil (93.1 g, 232 μmol, 86%). ^{1}H NMR (300.13 MHz, CD_3OD): $\delta = 8.22$ (d, 1H, $^4J_{6,8} = 1.8$ Hz, H-8), 7.66 (d, 1H, $^3J_{2,3} = 3.8$ Hz, H-2), 7.50 (dd, 1H, $^3J_{5,6} = 10.7$ Hz, H-6), 7.36 (d, 1H, H-3), 7.09 (d, 1H, H-5), 4.48 (m_c, 2H, az-$CH_2C\underline{H}_2O$), 4.23 (dd, 1H, $^3J_{a,b} = 4.8$ Hz, $^3J_{a,c} = 9.4$ Hz, $NC\underline{H}CO_2H$), 3.50 (m_c, 2H, az-$C\underline{H}_2CH_2O$), 3.12 (sept, 1H, $^3J_{isopropyl} = 6.9$ Hz, az-$C\underline{H}(CH_3)_2$), 2.67 (s, 3H, az-CH_3), 2.42 (m_c, 2H, $NCHCH_2C\underline{H}_2CO_2H$), 2.28–2.14 (m, 1H, $NCHC\underline{H}HCH_2CO_2H$), 1.98–1.85 (m, 1H, $NCHCH\underline{H}CH_2CO_2H$, 1.40 (d, 6H, az-$CH(C\underline{H}_3)_2$) ppm; ^{13}C NMR (75.47 MHz, CD_3OD): $\delta =$ 176.4, 175.4 (2 COOH), 158.7 (O(CO)N), 144.9 (C-4), 141.3 (C-7), 139.0 (C-3a*), 137.9 (C-2), 137.7 (C-8a*), 136.0 (C-6), 134.0 (C-8), 126.4 (C-1), 125.9 (C-5), 113.5 (C-3), 66.4 ($CH_2\underline{C}H_2O$), 54.5 ($N\underline{C}H-CO_2H$), 39.4 (az-$\underline{C}H(CH_3)_2$), 38.6 ($\underline{C}H_2CH_2O$), 31.2 ($NCHCH_2\underline{C}H_2CO_2H$), 27.9 ($NCH\underline{C}H_2CH_2CO_2H$), 25.1 (az-$CH(\underline{C}H_3)_2$), 12.9 (az-$CH_3$) ppm; MALDI-TOF MS (no matrix): $m/z = 401.6$ $[M]^+$ (401.18 calc. for $C_{22}H_{27}NO_6$).

*N-[2-(7-Isopropyl-1-methylazulen-4-yl)-ethoxycarbonyl]-L-glutaminic acid-N′,N″-di[2-(α-D-mannopyranosyloxy)ethyl]-diamide (**9**)*

The glutamic acid derivative **8** (171 mg, 426 μmol) and the mannoside **4**[5] (209 mg, 937 μmol) were dissolved under argon in anhydrous DMF (5 mL) at rt. *O*-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU, 355 mg, 937 μmol) and DIPEA (159 μL, 937 μmol) were added and the solution was stirred overnight. The solvent was removed at the rotary evaporator and the residue purified by column chromatography on silica gel (ethyl acetate/methanol = 7:3) to afford the title glycocluster (238 mg, 293 μmol, 69%) as a blue resin. ^{1}H NMR (300.13 MHz, CD_3OD): $\delta = 8.23$ (d, 1H, $^4J_{6,8} =$ 1.8 Hz, H-8_{az}), 7.67 (d, 1H, $^3J_{2,3} = 4.0$ Hz, H-2_{az}), 7.51 (dd, 1H, $^3J_{5,6} = 10.8$ Hz, H-6_{az}), 7.37 (d, 1H, H-3_{az}), 7.11 (d, 1H, H-5_{az}), 4.81, 4.80 (each d, each 1H, $^3J_{1,2} = {}^3J_{1',2'} = 1.6$ Hz, 2 H-1_{man}), 4.54–4.43 (m, 2H, az-$CH_2C\underline{H}_2$), 4.10 (dd, 1H, O(CO)HNC$\underline{H}$), 3.89 (dd, 2H, $^2J_{6,6} = 11.8$ Hz, $^3J_{5,6} = 2.2$ Hz, 2 H-6_{man}), 3.86 (dd, 2H, $^3J_{2,3} = 3.3$ Hz, 2 H-2_{man}), 3.81–3.70 (m, 6H, 2 man-OC$\underline{H}HCH_2$, 2 H-3_{man}, 2 H-$6'_{man}$), 3.64, 3.63 (each dd ≈ t, each 1H, $^3J_{3,4} = 9.7$ and 9.6 Hz, $^3J_{4,5} = 9.8$ and 9.7 Hz, 2 H-4_{man}), 3.60–3.37 (m, 10H, 2 H-5_{man}, az-$C\underline{H}_2CH_2$, 2 man-OC$H\underline{H}CH_2$, 2 man-OCHHC$\underline{H}_2$), 3.12 (sept ≈ quint, 1H, $^3J_{isopropyl} = 6.9$ Hz, az-$C\underline{H}(CH_3)_2$), 2.67 (s, 3H, az-CH_3), 2.30 (m, 2H, O(CO)HNCHC$H_2C\underline{H}_2$(CO)N), 2.13–2.03 (m, 1H, O(CO)HNCHC$\underline{H}HCH_2$(CO)N), 1.96–1.85 (m, 1H, O(CO)HNCHCH$\underline{H}CH_2$(CO)N), 1.40 (d, 6H, az-$CH(C\underline{H}_3)_2$) ppm; ^{13}C NMR (75.47 MHz, CD_3OD): $\delta =$ 175.1, 174.5 (2 (CO)N), 158.5 (O(CO)N), 144.9

(C-4_{az}), 141.4 (C-7_{az}), 139.0 (C-$3a_{az}$*), 137.9 (C-2_{az}), 137.7 (C-$8a_{az}$*), 136.0 (C-6_{az}), 134.0 (C-8_{az}), 126.4 (C-1_{az}), 125.9 (C-5_{az}), 113.5 (C-3_{az}), 101.6, 101.6 (2 C-1_{man}), 74.8 (2 C-5_{man}), 72.5 (2 C-3_{man}), 72.0 (2 C-2_{man}), 68.7, 68.6 (2 C-4_{man}), 67.2, 67.0, 66.6 (az-$CH_2\underline{C}H_2O$, 2 man-$O\underline{C}H_2CH_2N$), 62.9 (2 C-6_{man}), 56.0 (NCH), 40.3, 40.3 (2 man-$OCH_2\underline{C}H_2N$), 39.4 (az-$\underline{C}H(CH_3)_2$), 38.5 (az-$\underline{C}H_2CH_2O$), 33.2, 29.5 ($CHN\underline{C}H_2\underline{C}H_2(CO)N$, 25.1 (az-$CH(\underline{C}H_3)_2$), 12.9 (az-$CH_3$) ppm; MALDI-TOF MS (no matrix): m/z = 811.1 $[M]^+$ (calc for $C_{38}H_{57}N_3O_{16}$: m/z = 811.37), m/z = 834.2 $[M+Na]^+$ (calc for $C_{38}H_{57}N_3O_{16}Na$: m/z = 834.36), m/z = 850.1 $[M+K]^+$ (calc for $C_{38}H_{57}N_3O_{16}K$: m/z = 850.34).

*N-[2-(7-Isopropyl-1-methyl-azulen-4-yl)]ethoxycarbonyl-imino diacetic acid (**11**)*

Carbonate **3** (369 mg, 1.00 mmol) and imino diacetic acid **10** (133 mg, 1.00 mmol) were dissolved in a mixture of THF and water (10 mL, 1:1). Sodium hydrogen carbonate (277 mg, 3.30 mmol) was added and the solution was stirred overnight at rt. The solution was diluted with brine and water (1:1) and the aqueous phase was overlaid with ethyl acetate and then acidified by addition of hydrochloric acid (2 M). The phases were separated and the organic phase was washed repeatedly with brine until the aqueous phase remained neutral. The organic phase was then dried over Na_2SO_4. After filtration, the solvent was removed in vacuo to afford the product (365 mg, 942 μmol, 94%) as blue solid. Mp: 126°C; IR (KBr): ν = 3600–2400 (bs, OH), 2958 ($CH_{aliph.}$), 1707 (COO) cm^{-1}; ^{1}H NMR (300.13 MHz, DMSO-D_6): δ = 8.19 (d, 1H, $^4J_{6,8}$ = 1.9 Hz, H-8), 7.66 (d, 1H, $^3J_{2,3}$ = 3.9 Hz, H-2), 7.50 (dd, 1H, $^3J_{5,6}$ = 10.7 Hz, H-6), 7.34 (d, 1H, H-3), 7.05 (d, 1H, H-5), 4.34 (t, 2H, $^3J_{a,b}$ = 6.7 Hz, az-$CH_2\underline{C}H_2$), 3.96, 3.89 (each s, each 2H, 2 $N\underline{C}H_2CO_2H$), 3.55–3.25 (m, 4H, az-$\underline{C}H_2CH_2$, 2 $COO\underline{H}$), 3.10 (sept, 1H, $^3J_{isopropyl}$ = 6.9 Hz, az-$C\underline{H}(CH_3)_2$), 2.61 (s, 3H, az-$C\underline{H}_3$), 1.32 (d, 6H, az-$CH(C\underline{H}_3)_2$) ppm; ^{13}C NMR (75.47 MHz, DMSO-D_6): δ = 170.9, 170.9 (2 CO_2H), 155.9 (OCON), 143.9 (C-4), 140.0 (C-7), 137.1 (C-3a*), 136.9 (C-2), 135.8 (C-8a*), 135.1 (C-6), 133.3 (C-8), 125.0 (C-1), 124.9 (C-5), 112.5 (C-3), 65.8 (az-$CH_2\underline{C}H_2$), 49.3, 49.0 (2 $N\underline{C}H_2CO_2H$), 37.4 (az-$\underline{C}H(CH_3)_2$), 36.9 (az-$\underline{C}H_2CH_2$), 24.6 ($CH(\underline{C}H_3)_2$), 12.8 (az-CH_3) ppm; MALDI-TOF MS (no matrix): m/z = 387.6 $[M]^+$ (calc for $C_{21}H_{25}NO_6$: m/z = 387.17); HRES MS: m/z = 387.16820 (calc for $C_{21}H_{25}NO_6$: m/z = 387.16818).

*N-[2-(7-Isopropyl-1-methyl-azulen-4-yl)-ethoxycarbonyl]imino diacetic acid N′,N″-di[2-(α-D-mannopyranosyloxy)-ethyl]diamide (**12**)*

The imino diacetic acid derivative **11** (200 mg, 516 μmol) and mannoside **4**[5] (254 mg, 1.14 mmol) were dissolved under argon in anhydrous DMF (7 mL). HBTU (431 mg, 1.14 mmol) and DIPEA (220 μL, 1.29 mmol) were added and the solution was stirred for 3 h at 0°C and then overnight at rt. The solvent was removed at the rotary evaporator and the residue purified by column

chromatography on silica gel (ethyl acetate/methanol = 9:1) to afford the title glycocluster (342 mg, 429 μmol, 83%) as blue amorphous solid. ^{1}H NMR (500.13 MHz, CD_3OD): δ = 8.23 (d, 1H, $^4J_{6,8}$ = 1.9 Hz, H-8$_{az}$), 7.67 (d, 1H, $^3J_{2,3}$ = 3.9 Hz, H-2$_{az}$), 7.52 (dd, 1H, $^3J_{5,6}$ = 10.7 Hz, H-6$_{az}$), 7.33 (d, 1H, H-3$_{az}$), 7.08 (d, 1H, H-5$_{az}$), 4.82, 4.78 (each d, each 1H, $^3J_{1,2}$ = 1.8 Hz, 2 H-1$_{man}$), 4.51 (t, 2H, $^3J_{a,b}$ = 6.7 Hz, az-$CH_2C\underline{H}_2$), 4.00 (m$_c$, 2H, N$C\underline{H}_2$(CO)N), 3.92 (s, 2H, N$C\underline{H}_2$(CO)N), 3.91–3.71 (m, 10H, 2 H-2$_{man}$, 2 H-6$_{man}$, 2 H-3$_{man}$, 2 manOCH$\underline{H}$CH_2, 2 H-6′$_{man}$), 3.65–3.40 (m, 12H, 2 H-4$_{man}$, 2 H-5$_{man}$, 2 manOC$\underline{H}$HCH_2, az-$CH_2C\underline{H}_2$, 2 manOC$\underline{H}_2CH_2$), 3.12 (sept, 1H, $^3J_{isopropyl}$ = 6.9 Hz, az-C$\underline{H}(CH_3)_2$), 2.67 (s, 3H, az-C$\underline{H}_3$), 1.40 (d, 6H, az-CH(C$\underline{H}_3)_2$) ppm; ^{13}C NMR (75.48 MHz, CD_3OD): δ = 172.1 (2 (CO)NH), 157.8 (OCON), 144.7 (C-4$_{az}$), 141.4 (C-7$_{az}$), 138.8 (C-3a$_{az}$*), 137.9 (C-2$_{az}$), 137.7 (C-8a$_{az}$*), 136.1 (C-6$_{az}$), 134.0 (C-8$_{az}$), 126.4 (C-1$_{az}$), 125.8 (C-5$_{az}$), 112.4 (C-3$_{az}$), 101.5 (2 C-1$_{man}$), 74.7 (2 C-5$_{man}$), 72.5 (2 C-3$_{man}$), 72.0 (2 C-2$_{man}$), 68.6, 68.5 (2 C-4$_{man}$), 68.0 (az-$CH_2C\underline{H}_2$), 67.0 (2 manO$C\underline{H}_2CH_2$), 62.8 (2 C-6$_{man}$), 53.9, 53.6 (2 N$C\underline{H}_2$(CO)N), 40.4, 40.3 (2 manOC$H_2C\underline{H}_2$), 39.4 (az-$C\underline{H}(CH_3)_2$), 38.2 (az-$C\underline{H}_2CH_2$), 25.1 (az-CH($C\underline{H}_3)_2$), 13.0 (az-CH_3) ppm; MALDI-TOF MS (no matrix): m/z = 820.2 [M+Na]$^+$ (calc for $C_{37}H_{55}N_3O_{16}Na$:m/z = 820.35), m/z = 837.1 [M+K]$^+$ (calc for $C_{37}H_{55}N_3O_{16}Na$: m/z = 836.32).

*N-[2-(7-Isopropyl-1-methyl-azulen-4-yl)]ethoxycarbonyl-iminodiacetic acid monomethylester (**13**)*

The imino diacetic acid derivative **11** (2.28 g, 5.89 mmol) was dissolved in anhydrous pyridine (25 mL) under argon and cooled to −20°C. A solution of phosgene in toluene (20%, 3.78 mL, 7.66 mmol) was added slowly via a syringe. Stirring was continued for 1 h at this temperature and then anhydrous methanol (23.9 μL, 5.89 mmol) was added. The reaction mixture was stirred overnight at rt, then the solvent was removed at the rotary evaporator, and the residue was dissolved in a sat. solution of sodium hydrogen carbonate. This aqueous solution was washed three times with ethyl acetate. The organic phase was dried over Na_2SO_4, the suspension was filtered, and the solvent was removed at the rotary evaporator to afford the product (1.97 g, 4.90 mmol, 83%) as a blue resin. IR (film): ν = 3063 (CH_{arom}), 2957, 2926, 2864 ($CH_{aliph.}$), 1751 (COOMe), 1708 (COOH, OCON) cm^{-1}.

NMR analysis showed two rotational isomers based on the configuration of the CN-bond of the urethane substructure; they were assigned as isomer and isomer': ^{1}H NMR (300.13 MHz, DMSO-D_6): δ = 8.19 (d, 2H, $^4J_{6,8}$ = $^4J_{6',8'}$ = 1.9 Hz, H-8, H-8′), 7.66 (d, 2H, $^3J_{2,3}$ = $^3J_{2'3'}$ = 3.8 Hz, H-2, H-2′), 7.51 (dd, 1H, $^3J_{5,6}$ = 10.7 Hz, H-6), 7.47 (dd, 1H, $^3J_{5'6'}$ = 10.7 Hz, H-6′), 7.34 (d, 1H, H-3), 7.32 (d, 1H, H-3′), 7.06 (d, 1H, H-5), 7.04 (d, 1H, H-5′), 4.33 (t, 4H, $^3J_{CH2,CH2}$ = 6.7 Hz, az-$CH_2C\underline{H}_2$, az-$CH_2C\underline{H}'_2$), 4.06−3.85 (m, 8H, N$C\underline{H}_2CO_2$Me, N$C\underline{H}_2CO_2$Me′, N$C\underline{H}_2CO_2$H, N$C\underline{H}_2CO_2$H′), 3.63, 3.57 (each s, each 3H, OMe, OMe′), 3.37 (t,

4H, az-C$\underline{H}_2$CH$_2$, az-C$\underline{H}_2$CH$_2'$), 3.17–3.03 (m, 2H, az-C$\underline{H}$(CH$_3$)$_2$, az-C$\underline{H}$(CH$_3$)$_2'$), 2.61 (s, 6H, az-C$\underline{H}_3$, az-C$\underline{H}_3'$), 1.32 (d, 12H, $^3J_{isopropyl} = {}^3J_{Isopropyl'} = 6.9$ Hz, az-CH(C$\underline{H}_3$)$_2$, az-CH(C$\underline{H}_3$)$_2'$) ppm; ^{13}C NMR (75.47 MHz, DMSO-D$_6$): $\delta = 171.1$ (2x), 170.0, 169.9 (CO_2Me, CO_2Me′, CO_2H, CO_2H′), 155.7, 155.4 (OCON, OCON′), 144.1, 143.9 (C-4, C-4′), 139.9, 139.9 (C-7, C-7′), 137.1, 137.1 (C-3a*, C-3a′*), 136.8 (C-2, C-2′), 135.8 (C-8a*, C-8a′*), 135.1, 135.0 (C-6, C-6′), 133.2 (C-8, C-8′), 125.0 (C-5), 124.9 (C-5′, C-1, C-1′), 112.5 (C-3, C-3′), 65.8 (az-CH$_2\underline{C}$H$_2$, az-CH$_2\underline{C}$H$_2'$), 51.8 (OMe, OMe′), 49.5, 49.4, 49.2, 48.7 (2 N$\underline{C}$H$_2$, 2 N$\underline{C}$H$_2'$), 37.4 (az-$\underline{C}$H(CH$_3$)$_2$, az-$\underline{C}$H(CH$_3$)$_2'$), 36.9, 36.7 (az-$\underline{C}$H$_2$CH$_2$, az-$\underline{C}$H$_2$CH$_2'$), 24.6 (az-CH($\underline{C}$H$_3$)$_2$, az-CH($\underline{C}$H$_3$)$_2'$), 12.8 (az-CH$_3$, az-CH$_3'$) ppm; MALDI-TOF MS (no matrix): $m/z = 401.0$ [M]$^+$ (401.18 calc. for $C_{22}H_{27}NO_6$); HRES MS: $m/z = 401.18370$ (calc. for $C_{22}H_{27}NO_6$: $m/z = 401.18384$).

*N-[2-(7-Isopropyl-1-methyl-azulen-4-yl)ethoxycarbonyl]-N-{[2-(α-D-mannopyranosyloxy)ethyl]carbamoylmethyl}glycin methylester (**14**)*

The monomethyl ester **13** (213 mg, 531 μmol) and mannoside **4**[5] (142 mg, 637 μmol) were dissolved under argon in anhydrous DMF (10 mL). HBTU (275 mg, 637 μmol) and DIPEA (108 μL, 637 μmol) were added. The reaction mixture was stirred for 3 h at 0°C and overnight at a temperature of 6°C to 8°C. Then the solution was diluted with ethyl acetate (40 mL) and washed with brine (40 mL). The aqueous phase was washed three times with ethyl acetate (10 mL) and all four ethyl acetate phases were combined. This washing procedure (addition of 40 mL brine and re-extraction with 3 × 10 mL ethyl acetate) was repeated once. The solvent was dried over Na_2SO_4; the suspension was filtered and then concentrated at the rotary evaporator. Column chromatography on silica gel (ethyl acetate/methanol = 4:1) afforded the product (263 mg, 434 μmol, 82%) as a blue amorphous solid.

NMR analysis showed two rotational isomers based on the configuration of the CN-bond of the urethane substructure; they were assigned as isomer and isomer′: ^{1}H NMR (500.13 MHz, CD_3OD): $\delta = 8.23$ (d, 2H, $^4J_{6,8} = {}^4J_{6',8'} = 2.0$ Hz, H-8$_{az}$, H-8$_{az}'$), 7.67–7.65 (m, 2H, H-2$_{az}$, H-2$_{az}'$), 7.51 (dd, 1H, $^3J_{5,6} = 10.7$ Hz, H-6$_{az}$), 7.49 (dd, 1H, $^3J_{5',6'} = 10.7$ Hz, H-6$_{az}'$), 7.33 (d, 0.4H (slow relaxation), $^3J_{2,3} = 4.0$ Hz, H-3$_{az}$), 7.32 (d, 1H, $^3J_{2',3'} = 4.0$ Hz, H-3$_{az}'$), 7.06 (d, 1H, H-5$_{az}$), 7.05 (d, 1H, H-5$_{az}'$), 4.80 (d, 1H, $^3J_{1,2} = 1.8$ Hz, H-1$_{man}$), 4.74 (d, 1H, $^3J_{1',2'} = 1.8$ Hz, H-1′$_{man}$), 4.52 (t, 2H, $^3J_{a,b} = 6.7$ Hz, az-CH$_2$C$\underline{H}_2$), 4.51 (t, 2H, $^3J_{a',b'} = 6.7$ Hz, az-CH$_2$C$\underline{H}_2'$), 4.16–4.08 (m, 2H, NC$\underline{H}_2$), 4.00–3.94 (m, 4H, NC$\underline{H}_2$, NC$\underline{H}_2'$), 3.91 (s, 2H, NC$\underline{H}_2'$), 3.88–3.83 (m, 4H, H-2$_{man}$, H-2$_{man}'$, H-6$_{man}$, H-6$_{man}'$), 3.82–3.78 (m, 4H, (CO)OCH$_3$, man-OC$\underline{H}$H), 3.76–3.69 (m, 5H, H-3$_{man}$, H-3′$_{man}$, H-6′$_{man}$, H-6′$_{man}'$, man-OC$\underline{H}$H′), 3.66 (s, 3H, (CO)OCH$_3'$), 3.63 (dd ≈ t, 2H, $^3J_{3,4} = {}^3J_{3',4'} = {}^3J_{4,5} = {}^3J_{4',5'} = 9.6$ Hz, H-4$_{man}$, H-4$_{man}'$), 3.58–3.51 (m, 3H, H-5$_{man}$, H-5′$_{man}$, man-OCH$\underline{H}$), 3.50–3.38 (m, 8H) and 3.34–3.29 (m, 1H) (az-C$\underline{H}_2$CH$_2$, az-C$\underline{H}_2$CH$_2'$, man-OCH$_2$C$\underline{H}$H,

man-$OCH_2C\underline{H}H'$, man-$OCH_2CH\underline{H}$, man-$OCH_2CH\underline{H}'$, man-$OCH\underline{H}'$), 3.12 (sept, 2H, $^3J_{isopropyl} = {}^3J_{isopropyl'} = 6.9$ Hz, az-$C\underline{H}(CH_3)_2$, az-$C\underline{H}(CH_3)_2'$), 2.67 (s, 6H, az-$C\underline{H}_3$, az-$C\underline{H}_3'$), 1.40 (d, 6H, az-$CH(C\underline{H}_3)_2$), 1.40 (d, 6H, az-$CH(C\underline{H}_3)_2'$) ppm; ^{13}C NMR (125.76 MHz, CD_3OD): $\delta =$ 172.8, 172.4, 171.6 (2x), (CONH, CONH′, COOMe, COOMe′), 155.8 (2x) (OCON, OCON′), 144.8, 144.7 (C-4_{az}, C-$4_{az}'$), 141.4, 141.4 (C-7_{az}, C-$7_{az}'$), 139.0, 138.8 (C-$3a_{az}{}^*$, C-$3a_{az}{}'^*$), 137.9, 137.9 (C-2_{az}, C-$2_{az}'$), 137.7, 137.7 (C-$8a_{az}{}^*$, C-$8a_{az}{}'^*$), 136.1, 136.0 (C-6_{az}, C-$6_{az}'$), 134.0, 133.9 (C-8_{az}, C-$8_{az}'$), 126.4 (C-1_{az}), 125.8 (C-5_{az}), 125.6 (C-$1_{az}'$, C-$5_{az}'$), 113.4 (C-3_{az}, C-$3_{az}'$), 101.6 (C-1_{man}, C-$1_{man}'$), 74.7 (C-5_{man}, C-$5_{man}'$), 72.5 (C-3_{man}, C-$3_{man}'$), 72.0, 72.0 (C-2_{man}, C-$2_{man}'$), 68.7, 68.6 (C-4_{man}, C-$4_{man}'$), 68.0, 67.9 (az-$CH_2\underline{C}H_2$, az-$CH_2\underline{C}H_2'$), 67.0 (man$O\underline{C}H_2$, man$O\underline{C}H_2'$), 62.9 (C-6_{man}, C-$6_{man}'$), 53.1 (2 C, $N\underline{C}H_2$), 53.0, 52.9 (OMe, OMe′), 51.6, 51.0 (2 C, $N\underline{C}H_2'$), 40.3, 40.2 (man-$OCH_2\underline{C}H_2$, man$OCH_2\underline{C}H_2'$), 39.4 (az-$\underline{C}H(CH_3)_2$, az-$\underline{C}H(CH_3)_2'$), 38.2, 38.0 (az-$\underline{C}H_2CH_2$, az-$\underline{C}H_2CH_2'$), 25.1 (2x) (az-$CH(\underline{C}H_3)_2$, az-$CH(\underline{C}H_3)_2'$), 12.9 (az-CH_3, az-CH_3') ppm; MALDI-TOF MS (no matrix): $m/z = 606.2$ $[M]^+$ (606.28 calc. for $C_{30}H_{42}N_2O_{11}$).

*N-[2-(7-Isopropyl-1-methyl-azulen-4-yl)-ethoxycarbonyl]-N-{[2-(α-D-mannopyranosyloxy)ethyl]-carbamoylmethyl}glycin (**15**)*

The methyl ester **14** (122 mg, 201 μmol) was dissolved in a mixture of THF and water (1:1, 3.5 mL) and cooled to 0°C. Lithium hydroxide hydrate (11.0 mg, 261 μmol) was added and the solution was stirred for 5 h at this temperature and overnight at a temperature of 6°C to 8°C. Water (20 mL) was added and the solution was washed with ethyl acetate (20 mL). Ethyl acetate (20 mL) was added to the aqueous phase and the mixture was acidified with hydrochloric acid (2 M). The phases were separated and the aqueous phase was extracted three times with ethyl acetate (10 mL). The combined organic phases were washed with brine (20 mL), followed by re-extraction of the aqueous phase with ethyl acetate (3 × 10 mL). This washing procedure was repeated until the aqueous phase remained neutral. The combined organic phases were dried over Na_2SO_4 and after filtration the solvent was removed in vacuo to afford the product (93.0 mg, 157 μmol, 78%) as blue amorphous solid.

NMR analysis showed two rotational isomers based on the configuration of the CN-bond of the urethane substructure; they were assigned as isomer and isomer′: ^{1}H NMR (500.13 MHz, CD_3OD): $\delta = 8.23$ (d, 2H, $^4J_{6,8} = {}^4J_{6',8'} =$ 2.0 Hz, H-8_{az}, H-$8_{az}'$), 7.67 (d, 1H, $^3J_{2,3} = 3.7$ Hz, H-2_{az}), 7.67 (d, 1H, $^3J_{2',3'} =$ 3.7 Hz, H-$2'_{az}$), 7.52 (dd, 1H, $^3J_{5,6} = 10.7$ Hz, H-6_{az}), 7.52 (dd, 1H, $^3J_{5',6'} =$ 10.7 Hz, H-$6_{az}'$), 7.34 (d, 1H, H-3_{az}), 7.34 (d, 1H, H-$3_{az}'$), 7.09 (d, 1H, H-5_{az}), 7.08 (d, 1H, H-$5_{az}'$), 4.79 (d, 1H, $^3J_{1,2} = 1.8$ Hz, H-1_{man}), 4.73 (d, 1H, $^3J_{1',2'} =$ 1.8 Hz, H-$1_{man}'$), 4.53 (t, 2H, $^3J_{\alpha,b} = 6.7$ Hz, az-$CH_2C\underline{H}_2$), 4.52 (t, 2H, $^3J_{\alpha',b'} =$ 6.7 Hz, az-$CH_2C\underline{H}_2'$), 4.14–3.88 (m, 8H, $NC\underline{H}_2COO$, $NC\underline{H}_2COO'$, $NC\underline{H}_2CON$, $NC\underline{H}_2CON'$), 3.88–3.78 (m, 5H, H-2_{man}, H-$2_{man}'$, H-6_{man}, H-$6_{man}'$, man-$OC\underline{H}H$),

3.78–3.69 (m, 5H, H-3$_{man}$, H-3′$_{man}$, H-6′$_{man}$, H-6′$_{man}$′, man-OCHH′), 3.63 (dd ≈ t, 2H, $^3J_{3,4} = {}^3J_{3',4'} = {}^3J_{4,5} = {}^3J_{4',5'} = 9.6$ Hz, H-4$_{man}$, H-4$_{man}$′), 3.58–3.28 (m, 12H, H-5$_{man}$, H-5′$_{man}$, man-OCHH, man-OCHH′, az-CH$_2$CH$_2$, az-CH$_2$CH$_2$′, man-OCH$_2$CH$_2$, man-OCH$_2$CH$_2$′ + MeOH), 3.13, 3.13 (each sept, each 1H, $^3J_{isopropyl} = {}^3J_{isopropyl'} = 6.9$ Hz, az-CH(CH$_3$)$_2$, az-CH(CH$_3$)$_2$′), 2.67 (s, 6H, az-CH$_3$, az-CH$_3$′), 1.41 (d, 6H, az-CH(CH$_3$)$_2$), 1.41 (d, 6H, az-CH(CH$_3$)$_2$′) ppm; ^{13}C NMR (125.76 MHz, CD$_3$OD): δ = 174.0, 173.9, 172.0, 172.0 (CONH, CONH′, COOH, COOH′), 157.9 (OCON, OCON′), 144.7 (C-4$_{az}$, C-4$_{az}$′), 141.5 (C-7$_{az}$, C-7$_{az}$′), 138.9 (C-3a$_{az}$*), 138.0 (C-2$_{az}$), 138.0 (C-3a$_{az}$′*), 137.9 (C-2$_{az}$′), 137.8, 137.8 (C-8a$_{az}$*, C-8a$_{az}$′*), 136.1, 136.0 (C-6$_{az}$, C-6$_{az}$′), 134.0 (C-8$_{az}$, C-8$_{az}$′), 126.5 (C-1$_{az}$, C-1$_{az}$′), 125.9, 125.9 (C-5$_{az}$, C-5$_{az}$′), 113.4, 113.4 (C-3$_{az}$, C-3$_{az}$′), 101.7, 101.7 (C-1$_{man}$, C-1$_{man}$′), 74.7 (C-5$_{man}$, C-5$_{man}$′), 72.5, 72.5 (C-3$_{man}$, C-3$_{man}$′), 72.0, 72.0 (C-2$_{man}$, C-2$_{man}$′), 68.7, 68.6 (C-4$_{man}$, C-4$_{man}$′), 68.0, 68.0 (az-CH$_2$CH$_2$, az-CH$_2$CH$_2$′), 67.1, 67.0 (man-OCH$_2$, man-OCH$_2$′), 62.9, 62.9 (C-6$_{man}$, C-6$_{man}$′), 53.5, 53.4, 51.9, 51.5 (NCH$_2$COO, NCH$_2$COO′, NCH$_2$CON, NCH$_2$CON′), 40.4, 40.3 (man-OCH$_2$CH$_2$, man-OCH$_2$CH$_2$′), 39.4 (az-CH(CH$_3$)$_2$, az-CH(CH$_3$)$_2$′), 38.3, 38.2 (az-CH$_2$CH$_2$, az-CH$_2$CH$_2$′), 25.1, 25.1 (az-CH(CH$_3$)$_2$, az-CH(CH$_3$)$_2$′), 12.9, 12.9 (az-CH$_3$, az-CH$_3$′) ppm; MALDI-TOF MS (no matrix): m/z = 592.3 [M]$^+$ (592.26 calc. for $C_{29}H_{40}N_2O_{11}$).

*N-{[2-(α-D-Mannopyranosyloxy)ethyl]-carbamoylmethyl}glycin methylester (**16**)*

The protected glycopeptide **14** (60 mg, 99 μmol) was dissolved in anhydrous DMF (2 mL) under argon and cooled to −50°C. A solution of tetranitromethane (13.1 μL, 109 μmol) in anhydrous methanol (300 μL) was added and the reaction mixture was stirred for 30 min at this temperature. Thiophenol (152 μL, 1.48 mmol) and triethyl amine (54.6 μL, 396 μmol) were added and the solution was stirred for 2 h at rt. The solvent was removed at a rotary evaporator at a water bath temperature of 25°C. The residue was dissolved in water and washed twice with ethyl acetate. Then, the water was removed by lyophilization and the resulting residue was suspended in methanol and filtered, and the methanol was removed at the rotary evaporator. Column chromatography of the residue on silica gel (ethyl acetate/methanol/water, 10:10:1) afforded the product (20.1 mg, 57.0 μmol, 58%) as colorless resin. ^{1}H NMR (300.13 MHz, CD$_3$OD): 4.82 (d, 1H, $^3J_{1,2} = 1.7$ Hz, H-1), 3.88 (dd, 1H, $^2J_{6,6'} = 11.7$ Hz, $^3J_{5,6} = 2.3$ Hz, H-6), 3.85 (dd, 1H, $^3J_{2,3} = 3.5$ Hz, H-2), 3.85–3.79 (m, 1H, man-OCHHCH$_2$), 3.76 (s, 3H, OMe), 3.76–3.70 (m, 2H, H-3, H-6′), 3.64 (dd ≈ t, 1H, $^3J_{3,4} = 9.6$ Hz. $^3J_{4.5} = 9.9$ Hz, H-4), 3.64–3.45 (m, 5H, H-5, man-OCHHCH$_2$, man-OCH$_2$CHH, man-OCH$_2$CHH), 3.48 (s, 2H, NHCH$_2$COO*), 3.34 (s, 2H, NHCH$_2$CON*) ppm; ^{13}C NMR (75.47 MHz, CD$_3$OD): δ = 174.1, 174.1 (COO, CON), 101.6 (C-1), 74.8, 72.5, 72.0, 68.6 (C-2, C-3, C-4, C-5), 67.1 (C-6), 62.9 (man-OCH$_2$CH$_2$), 52.4 (NHCH$_2$COO*),

52.3 (OMe), 50.9 ($NH\underline{C}H_2CON^*$), 39.9 (man-$OCH_2\underline{C}H_2$) ppm; MALDI-TOF MS (DHB): m/z = 375.2 $[M+Na]^+$, 391.1 $[M+K]^+$ (352.15 calc. for $C_{13}H_{24}N_2O_9$).

*N-[2-(7-Isopropyl-1-methyl-azulen-4-yl)-ethoxycarbonyl]-N,N′-(di-{2-[α-D-mannopyranosyloxy]-ethyl}-carbamoylmethyl)glycylglycin methylester (**17**)*

The acid **15** (33 mg, 55 μmol) and the amide **16** (19 mg, 55 μmol) were dissolved under argon in anhydrous DMF (1 mL). HBTU (23 mg, 61 μmol) and DIPEA (14 μL, 83 μmol) were added and the solution was stirred for 2 h at 0°C and overnight at rt. The solvent was removed at the rotary evaporator and the product was purified by column chromatography on silica gel (ethyl acetate/methanol/water = 10:10:1). Product fractions were pooled, eluent was removed at the rotary evaporator, and the residue was dissolved in a small amount of water. Lyophilization of this solution afforded the product (23 mg, 25 μmol, 45%) as a blue lyophilizate. NMR analysis showed four rotational isomers based on two possible configurations of each of the CN-bonds of the urethane and the secondary amide substructure: ^{1}H NMR (500.13 MHz, CD_3OD): δ = 8.23–8.19 (m, 4H, 4 × H-8_{az}), 7.66–7.62 (m, 4H, 4 × H-2_{az}), 7.52–7.47 (m, 4H, 4 × H-6_{az}), 7.38–7.29 (m, 4H, 4 × H-3_{az}), 7.10–7.04 (m, 4H, 4 × H-5_{az}), 4.83–4.69 (m, 8H, 8 × H-1_{man}), 4.51–4.44 (m, 8H, 4 × az-$CH_2C\underline{H}_2$), 4.23–3.92 (m, 32H, 4 × $NC\underline{H}_2CO_2$, 12 × $NC\underline{H}_2(CO)N$), 3.86–3.65 (m, 116 H, 8 × H-2_{man}, 8 × H-3_{man}, 8 × H-4_{man}, 8 × H-5_{man}, 8 × H-6_{man}, 8 × H-$6'_{man}$, 4 × CO_2CH_3, 8 × man-$OC\underline{H}_2CH_2$, 8 × man-$OCH_2C\underline{H}_2$, 4 × az-$C\underline{H}_2CH_2$, 4 × az-$CH_2C\underline{H}_2$), 3.13 (m, 4H, 4 × az-$C\underline{H}(CH_3)_2$), 2.64–2.63 (m, 12H, 4 × az-$C\underline{H}_3$), 1.39–1.36 (m, 24H, 4 × az-$CH(C\underline{H}_3)_2$) ppm; ^{13}C NMR (125.76 MHz, CD_3OD): δ = 174.1, 173.0, 172.8, 172.6, 172.6, 172.0, 172.0, 171.9, 171.9, 171.9, 171.6, 171.6, 171.01, 171.9, 170.3, 170.2 (12 × CONH, 4 × $\underline{C}O_2CH_3$), 158.0, 158.0, 157.9, 157.9 (4 × OCON), 145.0, 144.8 (4 × C-4_{az}), 141.5 (4 × C-7_{az}), 139.2, 139.1, 138.9, 138.8 (4 × C-3a*), 138.0, 137.9 (4 × C-2_{az}), 137.8, 137.8, 137.7, 137.7 (4 × C-8a*), 136.2, 136.1 (4 × C-6_{az}), 134.1, 134.0, 134.0 (4 × C-8_{az}) 126.5 (4 × C-1_{az}), 125.9, 125.8, 125.8, 124.8 (4 × C-5_{az}), 113.7, 113.6, 113.5 (4 × C-3_{az}), 101.7, 101.6, 101.6 (8 × C-1_{man}), 74.9, 74.8, 74.8, 74.8 (8 × C-5_{man}), 72.5 (8 × C-3_{man}), 72.1, 72.0 (8 × C-2_{man}), 68.7, 68.6 (8 × C-4_{man}), 68.1, 68.0, 67.9 (4 × $\underline{C}H_2OCON$), 67.2, 67.1, 67.1, 67.0 (8 × man-$O\underline{C}H_2$), 63.0, 63.0 (8 × C-6_{man}), 53.8, 53.8, 53.5, 53.5, 53.3, 53.2, 53.1, 53.1, 52.8, 52.6, 52.5, 52.5, 52.2, 51.8, 51.5, 51.4, 51.0, 51.0, 51.0 (4 × $N\underline{C}H_2COOMe$, 12 × $N\underline{C}H_2CON$, 4 × $CO_2\underline{C}H_3$), 40.5, 40.4, 40.3, 40.3 (8 × man-$OCH_2\underline{C}H_2$), 39.4 (8 × az-$\underline{C}H(CH_3)_2$), 38.3, 38.1, 38.0 (4 × az-$\underline{C}H_2CH_2$), 25.1 (2×) (8 × az-$CH(\underline{C}H_3)_2$), 12.9 (4 × az-CH_3) ppm; MALDI-TOF MS (no matrix): m/z = 949.5 $[M+Na]^+$ (926.40 calc. for $C_{42}H_{62}N_4O_{19}$).

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