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Naturalised Dyes: A Simple Straightforward Synthetic Route to a New Class of Dyes – Glycoazodyes (GADs)

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We report a process to naturalise synthetic azodyes through their linkage with lactose, or its monosaccharide components, either galactose or glucose, to enhance their solubility, as many other natural dyes. This modification allows the dyeing process of textile materials to take place in water without the addition of surfactants or other additives. The synthesis of the first generation of glycoazodyes (GADs) is carried out with the use of a diester linker to bond the azodye and the sugar. Preliminary tinctorial tests showed that this first generation of GADs is soluble in water and that these new dyes are multipurpose and are able to dye different fabrics well.

Introduction

Ten thousand different dyes are currently used in the textile industry, totalling 7×10^5 tons/year.[1] These dyes fall into a number of chemical families of which azodyes constitute the largest and most important commercial class. These molecules contain one, two or three azo groups bound to benzene, naphthalene or heteroaromatic rings (mono-, di- or triazodyes, respectively). Other important classes are anthraquinonic dyes and indigoids.[2] Dyes are generally applied in an aqueous solution and require auxiliary chemicals to improve the dyeing process, for example surfactants to increase solubility, mordants to enhance the fastness of the dye on the fibre and salts to adjust the pH. Effluent from textile industries therefore contains a broad spectrum of contaminants such as highly hydrophobic dyes, suspended solids, chlorinated organic solvents, surfactants, mordants, metals, etc.[3] Reactive dyes are another class of environmentally dangerous textile dyes; as the name suggests they are very reactive molecules that form covalent bond with fibres, but also react with water leading to inherent losses. In addition, these molecules can degrade to aromatic amines, which further damage the environment.[1] Selective treatment of effluent is not possible because the type and extent of contamination varies depending on the fabric dyed or the class of dye used. Because most pollutants present in the water have different physical and chemical properties all that is normally done is to destroy the chromophores by oxidative treatment of the contaminated waters (ozone, H_2O_2, UV), which removes only the visible pollution.[4–6] These treatments are efficient for the depletion of the colour, but the yield of these is variable and the final oxidation products[7] are often unknown. Most importantly the colourless oxidation products are possibly even more dangerous than the starting dyes.[8] For example, the major disadvantage of using ozone is that it could form toxic byproducts even from biodegradable substances.[6,9] A biological approach is also difficult because of the large variety of species present. Therefore, increasing attention has been devoted to natural dyes, with the aim of finding environmentally friendly materials. Natural dyes extracted from plants or animals do not cover all the market requirements, and their isolation from natural sources is difficult. Furthermore, the impact on the environment is far from negligible when high quantities are required. These factors, together with the cost of these processes, have slowed their development.

In order to devise a new strategy to solve these problems, we turned to naturalisation of synthetics dyes, through linkage with a sugar. The aims are: (1) preparation of multipurpose dyes able to dye different fabrics (synthetic, natural, artificial); (2) achievement of hydrophilicity of dyes through their glycoconjugation, so that dying processes with disperse dyes could be driven without surfactants, that are very difficult to treat; (3) attainment of easier dying processes avoiding high temperatures and high pressures; (4) increased affinity towards the textile, improving efficacy, reducing waste; (5) possibility of efficient and more selective waste treatment, with the use of, for instance, live microorganisms to attack the sugar moiety and consequently the

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covalently bonded chromophoric part, or the use of enzymes able to destroy dyes; (6) use of carbohydrates largely and cheaply available such as D-glucose, D-galactose and lactose; normally discarded in huge quantities in the environment with an impact that is not negligible.

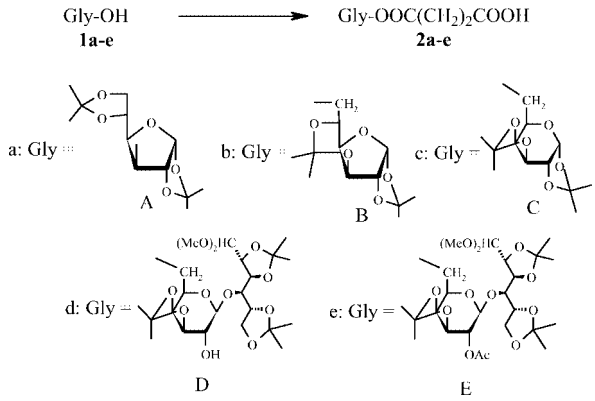
Glycosylation is a general topic in natural compounds, other glycoconjugates known are: glycolipids, glycopeptides, lipopolysaccharides, glycoproteins (also proteoglycans).[10] Carminic acid is a red glycosylated dye that occurs naturally in the cochineal insect and it is highly hydrophilic; the most important characteristic that we propose to transfer to synthetic dyes.

Conjugation of azodyes with carbohydrates has been, to the best of our knowledge, reported only in two cases. In the first report,[11] it was used for developing a methodology for the identification of the linking point of disaccharides, whereas in the second report,[12] glycoconjugation was proposed for the enhancement of the hydrophilicity of textile dyes, but the use of a low-yielding glycosylation reaction appears hardly applicable to industrial developments.

Results

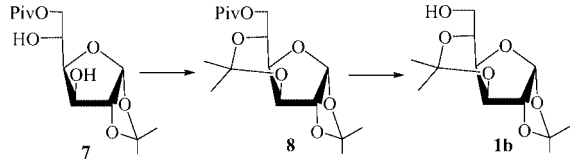
Here we present the synthesis of a new class of naturalised, multipurpose, glycoconjugated dyes,[13] which consist of a synthetic dye linked to a saccharide from natural and renewable sources, such as lactose, D-glucose or D-galactose, through a bifunctional linker. We decided to modify azodyes on their side chain in order to avoid changes in the chromophoric group so that their original colour was maintained. The choice of difunctional linkers to be used for the linkage of glycide and dye was devised on the basis of their easy availability and the simplicity of the practical procedure with the final aim of an industrial development of the new glycoazodyes (GADs). The succinyl bridge, previously proposed as a linker between glycosyl donors and acceptors in intramolecular aglycon delivery (IAD) glycosylations,[14] meets our requirements and has been selected for the synthesis of the first generation of GADs. Figure 1 shows the two complementary approaches: *path a* begins by attaching the linker to a protected glycide, whereas *path b* starts in reverse. The resulting structures are diesters, with the formation of esteric bonds between the sugar and the succinyl bridge and, on the other side, between the succinyl bridge and the hydroxy group on the side chain of the azodye.

A key point of this research is the influence of the structure of the glycide moiety on the tinctorial properties of the target GADs. For this purpose, five different protected glycides (**1a**–**e**, Scheme 1) have been selected in order to prepare a collection of final modified dyes **5**, characterised by a significant variation on the glycide structure. The planned structural differences are: (1) the succinyl–azodye linking position (3-*O*-D-glucose versus 6-*O*-D-glucose), (2) the glycide stereochemistry (6-*O*-D-glucose versus 6-*O*-D-galactose), (3) the glycide size (monosaccharides versus disaccharides).



Scheme 1. Monosuccinates of protected glycides.

Isopropylidene protection was chosen because of the ready availability of starting materials **1a**–**e** (see Experimental Section and Scheme 2 for **1b**) and the very mild deprotection conditions that should not affect the ester functions of the succinyl bridge. Monosaccharide semisuccinates **2a**–**c** (Scheme 1) were prepared in good yields (72–96% yield) by treatment of pertinent acetonides **1a**–**c** with succinic anhydride (1.2 equiv.) in toluene, in the presence of triethylamine (TEA) (1 equiv.) and 4-dimethylaminopyridine (DMAP) (0.1 equiv.). Fully protected lactose semisuccinate



Scheme 2. Preparation of 1,2:3,5-di-*O*-isopropylidene-D-glucofuranose.

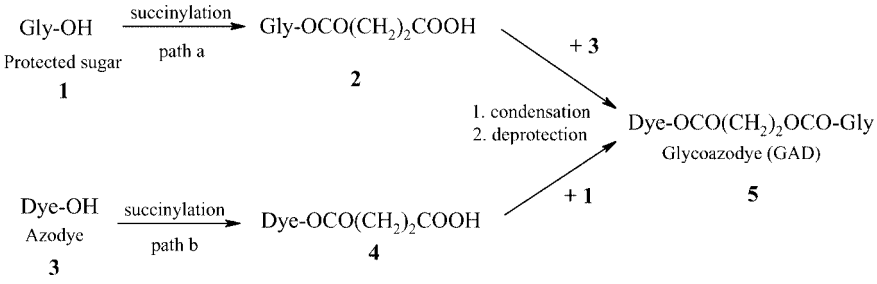
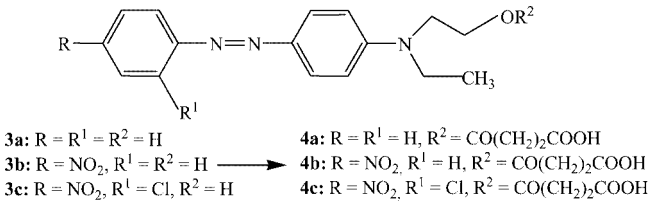


Figure 1. Schematic approaches to glycoazodyes (GADs).

2e was conveniently prepared from lactose in three steps: the first step was the acetonation of lactose,[15] subsequently the regioselective succinylation of the primary OH-6′ group of triacetonlactose dimethyl acetal (**1d**) (76% yield), and finally the routine acetylation of semisuccinate **2d** (Ac_2O/Py, 91% yield). Alternatively, **1e** (90% yield) was obtained from acetylation of the secondary 2′-OH group from **1d** through a two step reaction,[16] and afterwards **2e** was directly prepared by succinylation of **1e**.

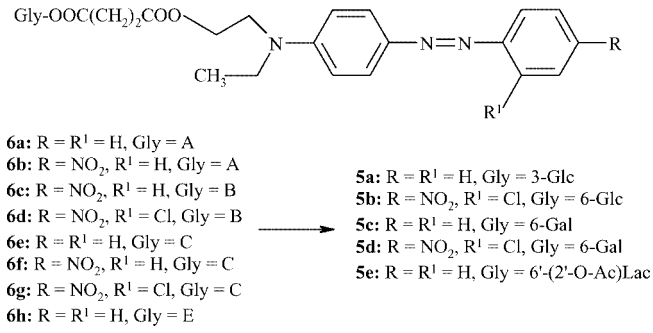
An alternative approach to the synthesis of GADs starts with the succinylation of an alcoholic azodye (Figure 1, path b) that was performed in high yields (90–98% yield) on commercially available red azodyes **3b** (Disperse Red 1) and **3c** (Disperse Red 13), and yellow azodye **3a**, prepared according to a literature method (Scheme 3).[17]



Scheme 3. Azodyes monosuccinates.

The linkage of semisuccinate sugars (**2**) with appropriate alcoholic dye **3** to give significant examples of protected GADs (**6a**,**b**,**d**,**e**,**g** and **h**) was performed in good to excellent yields by condensation with a slight excess of *N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride in THF in the presence of a catalytic amount of DMAP. The alternative route starting from semisuccinate dyes **4** with selected alcoholic protected sugars **1** gave comparable results both in terms of yields and practical procedure to give **6b**,**c** and **f**.

The final deprotection step was performed through treatment with 90% aqueous trifluoroacetic acid leading to the almost quantitative formation of dyes **5a**–**e** (Scheme 4). D-Glucose (**5a** and **5b**), 2′-*O*-acetyl-lactose (**5e**) and D-galactose (**5c** and **5d**) derivatives were isolated as mixtures of the two pyranose anomers; in the case of **5c** and **5d** further amounts of the α-furanose form were also found (about 10%). The identification of the isomeric forms of the deprotected GADs was made by comparison of their NMR spectroscopic data (Tables 1, 2, 3 and 4) with those reported in the literature for fully deprotected anomers.[18]



Scheme 4. Protected and deprotected GADs.

UV/Vis absorption spectra of the deprotected GADs presented slightly different absorptions when made in different solvents, such as methanol and acetone. In the experimental Section the UV/Vis absorption spectra of the industrial dye are also reported, to show the small hypsochromic shift that results from glycoconjugation.

We carried out preliminary tinctorial studies,[19] which showed that this first generation of GADs is soluble in water, as expected, and also that these dyes are multipurpose because they dye all of the fabrics we submitted to our attempts: wool, silk, nylon, polyester and polyurethane;

Table 2. ^{13}C NMR spectroscopic data (δ, ppm) in $CDCl_3$ of the glycide portion for protected 3-*O*-D-glucoyl (**2a**, **6a** and **6b**), 6-*O*-D-glucoyl (**2b**, **6c** and **6d**), and 6-*O*-D-galactoyl (**2c**, **6e**, **6f** and **6g**) derivatives.

Compound	C-1	C-2	C-3	C-4	C-5	C-6
2a	105.0	83.1	76.4	79.6	72.3	67.2
6a	104.8	83.1	76.3	79.5	72.3	67.6
6b	104.9	83.1	76.4	79.5	72.5	67.1
2b	106.2	83.7	74.8	79.2	69.9	64.5
6c	106.3	83.8	74.9	79.3	70.0	64.7
6d	106.2	83.7	74.8	79.2	69.9	64.6
2c	95.7	70.5[a]	70.1[a]	69.8[a]	65.4	63.2
6e	96.0	70.8[a]	70.4[a]	70.2[a]	65.7	63.5
6f	96.2	70.9[a]	70.6[a]	70.3[a]	65.4	63.7
6g	96.0	70.8[a]	70.4[a]	70.2[a]	65.7	63.6

[a] Assignments may have to be interchanged.

Table 1. ^1H NMR spectroscopic data (δ, ppm; *J*, Hz) in $CDCl_3$ of the glycide portion for protected 3-*O*-D-glucoyl (**2a**, **6a** and **6b**), 6-*O*-D-glucoyl (**2b**, **6c** and **6d**), and 6-*O*-D-galactoyl (**2c**, **6e**, **6f** and **6g**) derivatives.

Compound	1-H	2-H	3-H	4-H	5-H	6a-H	6b-H	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$
2a	5.87	4.50	5.26	4.21	4.10	4.10	4.10	3.7	0	2.2	1.7	n.d.	n.d.	n.d.
6a	5.86	4.50	5.25	4.19	4.19	4.10	4.10	3.7	0	2.7	n.d.	n.d.	n.d.	n.d.
6b	5.88	4.51	5.26	4.21	4.05	4.05	4.05	3.7	0	2.5	2.0	n.d.	n.d.	n.d.
2b	6.00	4.58	4.23	4.33	3.77	4.18	4.34	3.7	0	3.8	7.2	6.9	3.5	11.8
6c	6.00	4.58	4.23	4.36	3.79	4.17	4.34	3.7	0	3.8	7.0	7.0	3.4	11.8
6d	6.00	4.58	4.23	4.31	3.79	4.17	4.31	3.7	0	3.4	7.1	7.1	3.3	11.8
2c	5.54	4.34	4.63	4.30	4.01	4.18	4.21	5.0	2.5	7.9	1.8	7.4	4.8	10.2
6e	5.52	4.31	4.50	4.22	4.01	4.22	4.22	4.9	2.4	7.7	n.d.	n.d.	n.d.	n.d.
6f	5.53	4.33	4.62	4.23	4.03	4.27	4.31	5.0	2.5	7.9	1.9	7.1	5.1	10.9
6g	5.53	4.33	4.63	4.23	4.02	4.30	4.30	5.0	2.5	7.9	1.9	6.9	5.1	n.d.

Table 3. ^{13}C NMR spectroscopic data (δ, ppm) in Me_2SO of the glycide portion for deprotected 3-*O*-D-glucoyl (**5a**), 6-*O*-D-glucoyl (**5b**) and 6-*O*-D-galactoyl derivatives (**5c** and **5d**).

Compound	C-1	C-2	C-3	C-4	C-5	C-6
5b-α*p*	92.8	72.6	73.4	70.9	69.6	64.8
5a-α*p*	92.2	71.9	71.9	70.3	68.1	61.6
5b-β*p*	97.3	75.2	76.8	70.6	73.9	64.8
5a-β*p*	96.8	76.2	78.6	70.3	78.6	61.6
5c-β*f*	101.8	81.3	75.9	82.6	67.2	65.2
5d-β*f*	101.8	81.5	76.0	82.5	73.0	65.8
5c-α*p*	92.7	68.5	69.1	69.3	67.6	64.6
5d-α*p*	92.6	68.6	69.0	69.2	67.5	64.3
5c-β*p*	97.4	71.8	71.8	68.7	73.1	64.5
5d-β*p*	97.3	71.8	71.8	68.6	73.0	64.3

only cotton dyed poorly. The multipurpose nature of the industrial dyes is one of their most important properties because this would allow mixed fabrics, which are very common on the market, to be dyed. The results of one of these tests are reported in Figure 2. These tests were performed in water without the addition of surfactants, under mild conditions of temperature and pressure. To the best of our knowledge, disperse dyes, as our starting azodyes, do not effectively dye wool, and they dye polyester only under drastic conditions.

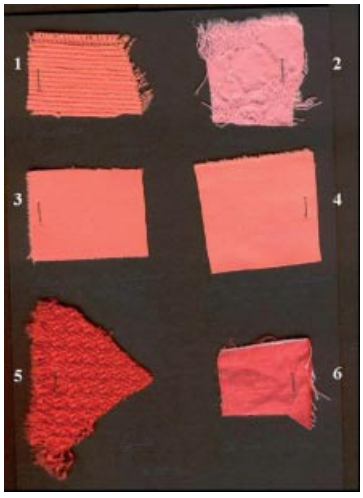


Figure 2. Tinctorial tests on **5d** performed on: 1) polyester, 2) cotton, 3) polyester \ polyurethane dyed as polyester, 4) polyester \ polyurethane dyed as wool, 5) wool, 6) cotton \ nylon.

Our tests have been carried out in standard conditions and they will be the subject of a dedicated report. Finally, fastness is very good, and in the case of polyester, excellent. These initial results have encouraged us to pursue the field of preparation of GADs, and to consider different types of linkers and classes of dyes, such as anthraquinonics.

Experimental Section

General: Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter at 20 ± 2 °C. ^1H NMR and ^{13}C NMR spectra were recorded with a Bruker AC 200, Varian Gemini instruments at 200 (^1H) and 50 (^{13}C) MHz or with a Varian INOVA600 spectrometer, in the appropriate solvent (internal standard Me_4Si). Assignments were made, when possible, with the aid of DEPT experiments, for comparison with values for known compounds and applying the known additivity rules,[20] and, in the case of anomeric mixtures, referring to the differences in the peak intensities. All reactions were followed by TLC on Kieselgel 60 F_{254} with detection by UV light and/or with ethanolic 10% phosphomolybdic or sulfuric acid, and heating. Kieselgel 60 (E. Merck, 70–230 and 230–400 mesh, respectively) was used for column and flash chromatography. Solvents were dried by distillation according to standard procedures[21] and stored over 4 Å molecular sieves that were activated for at least 24 h at 400 °C. Red azodyes **2b** (Disperse Red 1) and **2c** (Disperse Red 13) are commercially available (Sigma–Aldrich), yellow dye **2a** was prepared according to the literature.[17] 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**1a**) and 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**1c**) are commercially available (Fluka). Literature methods were used to prepare 2,3:5,6:3′,4′-tri-*O*-isopropylidenelactose dimethyl acetal (**1d**)[15] and its 2′-*O*-acetate **1e**.[16] The known 1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose[22] (**1b**) was prepared by the procedure reported below, starting from known 1,2-*O*-isopropylidene-6-*O*-pivaloyl-D-glucofuranose (**7**).[23]

1,2:3,5-Di-*O*-isopropylidene-α-D-glucofuranose (1b): To a solution of **7**[23] (14.0 g, 46.0 mmol) in DMP (350 mL) was added at room temperature TsOH (400 mg, 2.23 mmol). After 2 h stirring, the reaction mixture was neutralized with an excess of Et_3N (1.5 mL) and then coevaporated with toluene (5 × 50 mL) at reduced pressure. The resulting residue was diluted with H_2O (30 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The organic phase was dried with $MgSO_4$ and concentrated at reduced pressure. Purification of the residue by flash chromatography (hexane/EtOAc, 9:1) afforded **1,2:3,5-di-*O*-isopropylidene-6-*O*-pivaloyl-D-glucofuranose** (**8**) (14.5 g, 92%) as an oil. R_f = 0.54 (hexane/EtOAc, 7:3). $[\alpha]_D$ = +35.7 (*c* 1.0, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$): δ = 6.00 (d, $J_{1,2}$ = 3.7 Hz, 1 H, 1-H), 4.58 (d, 1 H, 2-H), 4.31 (dd, $J_{5,6b}$ = 3.6 Hz, $J_{6a,6b}$ =

Table 4. ^{13}C NMR spectroscopic data (δ, ppm) of the glycide portion for protected and deprotected lactose derivatives.

Compound	Solvent	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	C-1	C-2	C-3	C-4	C-5	C-6
2d	CD_3CN	104.1	74.6	80.0	77.7	71.6	64.1	106.4	77.8	77.9	74.3	78.6	66.1
2e	CD_3CN	100.8	73.8	76.7	75.9	71.4	63.9	106.2	77.8	78.1	74.6	78.7	65.5
6h	$CDCl_3$	100.1	72.5	77.1	73.4	70.6	63.4	104.9	75.1	77.9	73.8	78.1	64.6
β-lactose[a]	D_2O	103.7	72.0	73.5	69.5	76.2	62.0	96.6	74.8	75.3	79.2	75.6	61.1
β-**5e**	CD_3OD	104.1	71.9	74.0	68.9	75.2	62.0	96.7	75.5	75.1	80.5	74.8	60.7
α-lactose[a]	D_2O	103.6	72.0	73.5	69.5	76.2	62.0	92.7	72.2	72.4	79.3	71.0	61.0
α-**5e**	CD_3OD	103.8	71.5	74.0	68.2	75.1	62.0	92.4	72.3	73.5	81.1	70.3	60.3

[a] Taken from ref.[18]

11.5 Hz, 1 H, 6b-H), 4.30 (dd, $J_{3,4}$ = 4.0 Hz, $J_{4,5}$ = 7.1 Hz, 1 H, 4-H), 4.21 (d, 1 H, 3-H), 4.15 (dd, $J_{5,6a}$ = 7.7 Hz, 1 H, 6a-H), 3.78 (ddd, H, 5-H), 1.49, 1.36 [2s, each 3 H, C(CH_3)$_2$], 1.34 [s, 6 H, C(CH_3)$_2$], 1.21 [s, 9 H, (CH_3)$_3$C] ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 177.9 (*C*O), 112.0, 100.8 [2 × *C*(CH_3)$_2$], 106.3 (C-1), 83.7 (C-2), 79.3 (C-4), 74.8 (C-3), 70.2 (C-5), 63.9 (C-6), 38.6 [(CH_3)$_3$*C*], 27.0 [(*C*H$_3$)$_3$C], 26.9, 26.4, 23.8, 23.6 [2 × C(*C*H$_3$)$_2$] ppm. $C_{17}H_{28}O_7$ (344.41): calcd. C 59.29, H 8.19; found C 59.15, H 8.12.

To a solution of **8** (10.0 g, 31.9 mmol) in MeOH (200 mL) was added at room temperature a solution of 0.1 M NaOMe in MeOH (5 mL). After 24 h, the reaction mixture was neutralized with solid CO_2 and then concentrated to dryness. Purification of the residue by flash chromatography (hexane/EtOAc, 3:2) afforded **1b** (7.89 g, 95%) as a syrup. R_f = 0.42 (hexane/EtOAc, 3:2). [α]$_D$ = +37.9 (*c* 1.1, $CHCl_3$), ref.[22] [α]$_D$ = +39 (*c* 1.0, $CHCl_3$). The NMR spectroscopic data of **1b** completely agreed with those reported in literature.[20]

General Procedure for Succinylation of 1a–c: A mixture of the appropriate sugar (1.0 mmol), succinic anhydride (1.2 equiv.), 4-dimethylaminopyridine (0.1 equiv.) and Et_3N (1 equiv.) in toluene (4 mL) was stirred at reflux for 2.5 h and then concentrated at reduced pressure. The resulting residue was diluted with CH_2Cl_2 (5 mL), neutralized with AcOH, washed with H_2O (7 mL) and the aqueous phase extracted with CH_2Cl_2 (3 × 7 mL). The organic phase was dried with $MgSO_4$, concentrated and the resulting residue was purified by flash chromatography.

3-*O*-(3-Carboxypropanoyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (2a): The succinylation of **1a** (3.05 g, 11.7 mmol) afforded **2a** (3.74 g, 88.6%) as a clear syrup after flash chromatographic purification (hexane/EtOAc + 0.1% AcOH, 55:45). R_f = 0.35 (hexane/EtOAc + 1% AcOH, 1:1). [α]$_D$ = –27.2 (*c* 1.1, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): see Table 1 and δ = 2.69 [m, 4 H, CO(CH_2)$_2$CO], 1.52, 1.41, 1.32, 1.31 [4s, each 3 H, 2 × C(CH_3)$_2$] ppm. ^{13}C NMR (50 MHz, $CDCl_3$): see Table 2 and δ = 177.6 (*C*OOH), 170.6 (*C*OO), 112.3, 109.4 [2 × *C*(CH_3)$_2$], 28.8 [CO(*C*H$_2$)$_2$CO], 26.8, 26.7, 26.1, 25.1 [2 × C(*C*H$_3$)$_2$] ppm. $C_{16}H_{24}O_9$ (360.36): calcd. C 53.33, H 6.71; found C 53.52, H 6.78.

6-*O*-(3-Carboxypropanoyl)-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose (2b): The succinylation of **1b** (5.78 g, 22.17 mmol) afforded **2b** (5.75 g, 72%) as a solid after flash chromatographic purification (EtOAc/*i*PrOH, 9:1). R_f = 0.35 (hexane/EtOAc + 0.1% AcOH, 2:3). M.p. 94–96 °C (chrom). [α]$_D$ = +30.2 (*c* 1.1, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): see Table 1 and δ = 10.61 (br. s, 1 H, OH), 2.68 [br. s, 4 H, CO(CH_2)$_2$CO], 1.49, 1.37, 1.35, 1.33 [4s, each 3 H, 2 × C(CH_3)$_2$] ppm. ^{13}C NMR (50 MHz, $CDCl_3$): see Table 2 and δ = 177.7 (*C*OOH), 171.8 (*C*OO), 112.1, 100.9 [2 × *C*(CH_3)$_2$], 28.7, 28.6 [CO(*C*H$_2$)$_2$CO], 26.9, 26.3, 23.7, 23.7 [2 × C(*C*H$_3$)$_2$] ppm. $C_{16}H_{24}O_9$ (360.36): calcd. C 53.33, H 6.71; found C 53.12, H 6.76.

6-*O*-(3-Carboxypropanoyl)-1,2:3,4-di-*O*-isopropylidene-β-D-galactopyranose (2c): The succinylation of **1c** (5.00 g, 19.2 mmol) afforded **2c** (6.64 g, 96%) as a solid after crystallisation (EtOAc/hexane). R_f = 0.42 (EtOAc). [α]$_D$ = –38.7 (*c* 1.1, $CHCl_3$). M.p. 105–107 °C (from EtOAc/hexane). ^{1}H NMR (200 MHz, $CDCl_3$): see Table 1 and δ = 10.32 (br. s, 1 H, OH), 2.67 [br. s, 4 H, CO(CH_2)$_2$CO], 1.51, 1.45, 1.34, 1.33 [4s, each 3 H, 2 × C(CH_3)$_2$] ppm. ^{13}C NMR (50 MHz, $CDCl_3$): see Table 2 and δ = 176.6 (*C*OOH), 171.7 (*C*OO), 109.1, 108.3 [2 × *C*(CH_3)$_2$], 28.3 [CO(*C*H$_2$)$_2$CO], 25.4, 25.3, 24.4, 23.9 [2× C(*C*H$_3$)$_2$] ppm. $C_{16}H_{24}O_9$ (360.36): calcd. C 53.33, H 6.71; found C 53.16, H 6.72.

4-*O*-[6-*O*-(3-Carboxypropanoyl)-3,4-*O*-isopropylidene-β-D-galactopyranosyl]-2,3:5,6-di-*O*-isopropylidene-*aldehydo*-D-glucose Dimethyl Acetal (2d): The succinylation of **1d**[15] (3.53 g, 6.94 mmol) according to the general procedure, was, in this case, prolonged for 24 h, and afforded, after flash chromatographic purification (hexane/EtOAc, 3:7; followed by hexane/EtOAc + 0.1% AcOH, 3:7) pure **2d** (3.20 g, 76% yield) as a syrup. R_f = 0.12 (hexane/EtOAc + 1% AcOH, 1:1). [α]$_D$ = +31.1 (*c* 1.0, $CHCl_3$). ^{1}H NMR (200 MHz, CD_3CN): δ = 4.41 (d, $J_{1',2'}$ = 8.1 Hz, 1 H, 1′-H), 4.30 (m, 2 H, 6′a-H, 6′b-H), 4.22 (dd, $J_{1,2}$ = 6.7 Hz, $J_{2,3}$ = 4.7 Hz, 1 H, 2-H), 4.20 (d, 1 H, 1-H), 4.15–3.93 (m, 7 H, 3′-H, 4′-H, 5′-H, 4-H, 5-H, 6a-H, 6b-H), 3.86 (dd, $J_{3,4}$ = 1.5 Hz, 1 H, 3-H), 3.39, 3.38 (2s, each 3 H, 2 × OCH_3), 3.34 (dd, $J_{2',3'}$ = 7.2 Hz, 1 H, 2′-H), 2.58 [m, 4 H, CO(CH_2)$_2$CO], 1.44, 1.39, 1.34, 1.32 [4s, each 3 H, 2 × C(CH_3)$_2$], 1.30 [s, 6 H, C(CH_3)$_2$] ppm. ^{13}C NMR (50 MHz, CD_3CN): see Table 4 and δ = 174.2, 173.1 (2 × C=O), 110.8, 110.5, 109.1 [3 × *C*(CH_3)$_2$], 56.4, 54.3 (2 × OCH_3), 29.5, 29.1 [CO(*C*H$_2$)$_2$CO], 28.4, 27.6, 26.9, 26.6, 26.5, 25.2 [3 × C(*C*H$_3$)$_2$] ppm. $C_{27}H_{44}O_{15}$ (608.64): calcd. C 53.28, H 7.29; found C 53.20, H 7.18.

4-*O*-[2-*O*-Acetyl-6-*O*-(3-carboxypropanoyl)-3,4-*O*-isopropylidene-β-D-galactopyranosyl]-2,3:5,6-di-*O*-isopropylidene-*aldehydo*-D-glucose Dimethyl Acetal (2e): A solution of **2d** (2.52 g, 4.13 mmol), Ac_2O (10 mL) in pyridine (20 mL) was stirred at room temperature for 18 h and then repeatedly coevaporated with toluene at reduced pressure. Purification of the residue by flash column chromatography (hexane/EtOAc + 0.1% AcOH, 1:1) afforded pure **2e** (2.47 mg, 91%) as a syrup. R_f = 0.24 (hexane/EtOAc + 0.1% AcOH, 2:3). [α]$_D$ = +22.4 (*c* 1.0, $CHCl_3$). ^{1}H NMR (200 MHz, CD_3CN): δ = 4.86 (dd, $J_{1',2'}$ = 8.3 Hz, $J_{2',3'}$ = 6.6 Hz, 1 H, 2′-H), 4.55 (d, 1 H, 1′-H), 4.35–4.12 (m, 7 H, 1-H, 2-H, 5-H, 3′-H, 4′-H, 6′a-H, 6′b-H), 4.02 (dd, $J_{2,3}$ = 6.4 Hz, $J_{3,4}$ = 1.5 Hz, 1 H, 3-H), 3.98 (m, 1 H, 4-H), 3.91–3.72 (m, 3 H, 6a-H, 6b-H, 5′-H), 3.38, 3.37 (2s, each 3 H, 2 × OCH_3), 2.58 [m, 4 H, CO(CH_2)$_2$CO], 2.06 (s, 3 H, CH_3CO), 1.45, 1.42, 1.29, 1.28, 1.27, 1.26 [6s, each 3 H, 3 × C(CH_3)$_2$] ppm. ^{13}C NMR (50 MHz, CD_3CN): see Table 4 and δ = 174.0, 173.1, 170.5 (3 × C=O), 111.2, 110.9, 108.9 [3 × *C*(CH_3)$_2$], 56.2, 54.2 (2 × O*C*H$_3$), 29.6, 29.1 [CO(*C*H$_2$)$_2$CO], 28.0, 27.7, 26.8, 26.5, 26.4, 24.8 [3 × C(*C*H$_3$)$_2$], 21.1 (*C*H$_3$CO) ppm. $C_{29}H_{46}O_{16}$ (650.68): calcd. C 53.53, H 7.13; found C 53.34, H 7.08.

Alternatively, **2e** (6.18 g, 90%) was prepared by succinylation of **1e**[16] (6.20 g, 11.2 mmol) according to the general procedure described above.

General Procedure for Succinylation of 3a–c: A mixture of the appropriate dye (1 mmol), succinic anhydride (1.2 equiv.), 4-dimethylaminopyridine (0.1 equiv.) and Et_3N (1 equiv.) in CH_2Cl_2 (10 mL) was stirred at room temperature. After 20 h, H_2O (10 mL) was added to the reaction mixture, which was then neutralized with AcOH and extracted with CH_2Cl_2 (2 × 10 mL). The organic phase was dried with Na_2SO_4, concentrated and the resulting residue was purified by flash chromatography eluting with the appropriate eluting mixture.

4-(2-{ethyl[4-(phenyldiazenyl)phenyl]amino}ethoxy)-4-oxobutanoic Acid (4a): The succinylation of **3a** (0.99 g, 3.67 mmol) afforded pure **4a** (1.34 g, 98%) as an orange solid after flash chromatographic purification (EtOAc, then EtOAc + 0.1% AcOH). R_f = 0.28 ($CHCl_3$/EtOAc/MeOH, 8:1.7:0.3). M.p. 108–110 °C (chrom). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.84 (m, 4 H, 2′-H, 6′-H, 8′-H, 12′-H), 7.65 (br. s, 1 H, COOH), 7.46 (m, 3 H, 3′-H, 4′-H, 5′-H), 6.77 (m, 2 H, 9′-H, 11′-H), 4.30 (t, *J* = 6.3 Hz, 2 H, CH_2O), 3.64 (t, *J* = 6.3 Hz, 2 H, CH_2CH_2N), 3.48 (q, *J* = 7.1 Hz, 2 H, CH_3CH_2N), 2.64 [m, 4 H, CO(CH_2)$_2$CO], 1.21 (t, *J* = 7.1 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 177.8 (*C*OOH),

172.1 (*C*OO), 153.1 (C-1′), 149.9 (C-10′), 143.6 (C-7′), 129.4, 128.9, 125.2, 122.2, 111.2 (Ar-CH), 61.8 (CH_2O), 48.6, 45.5 (CH_2N), 28.7 [$CO(CH_2)_2CO$], 12.2 (CH_3) ppm. $C_{20}H_{23}N_3O_4$ (369.42): calcd. C 65.03, H 6.28, N 11.37; found C 64.98, H 6.15, N 11.25.

4-[2-ethyl-({4-[(4-nitrophenyl)diazenyl]phenyl}amino)ethoxy]-4-oxobutanoic Acid (4b): The succinylation of **3b** (5.32 g, 16.9 mmol) afforded pure **4b** (6.87 g, 98%) as a red solid after flash chromatographic purification (EtOAc, then EtOAc + 0.1% AcOH). R_f = 0.53 (EtOAc + 1% ACOH). M.p. 175–177 °C (chrom). ^{1}H NMR (200 MHz, CD_3CN/D_2O): δ = 8.30, 7.88 (AA′XX′ system, 4 H, 2′-H, 2′-H, 5′-H, 6′-H), 7.84, 6.86 (AA′XX′ system, 4 H, 8′-H, 9′-H, 11′-H, 12′-H), 4.26 (t, J = 5.8 Hz, 2 H, CH_2O), 3.67 (t, J = 5.8 Hz, 2 H, CH_2CH_2N), 3.49 (q, J = 7.1 Hz, 2 H, CH_3CH_2N), 2.51 [m, 4 H, $CO(CH_2)_2CO$], 1.16 (t, J = 7.1 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, CD_3CN/D_2O): δ = 177.8 (*C*OOH), 174.5 (*C*OO), 157.6 (C-1′), 152.6 (C-10′), 148.2 (C-4′), 144.2 (C-7′), 127.0, 125.7, 123.3, 112.5 (Ar-CH), 62.0 (CH_2O), 49.5, 46.3 (CH_2N), 32.5, 31.4 [$CO(CH_2)_2CO$], 12.4 (CH_3) ppm. $C_{20}H_{22}N_4O_6$ (414.42): calcd. C 57.97, H 5.35, N 13.52; found C 57.91, H 5.33, N 13.48.

4-[2-ethyl-({4-[(2-chloro-4-nitrophenyl)diazenyl]phenyl}amino)-ethoxy]-4-oxobutanoic Acid (4c): The succinylation of **3c** (1.51 g, 4.3 mmol) afforded pure **4c** (1.74 g, 90%) as a red solid after flash chromatographic purification (EtOAc, then EtOAc + 0.1% AcOH), R_f = 0.54 (EtOAc + 1% AcOH). M.p. 98–100 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.39 (d, $J_{3',5'}$ = 2.4 Hz, 1 H, 3′-H), 8.14 (dd, $J_{5',6'}$ = 8.9 Hz, 1 H, 5′-H), 7.94, 6.81 (AA′XX′ system, 4 H, 8′-H, 9′-H, 11′-H, 12-H), 7.78 (d, 1 H, 6′-H), 4.33 (t, J = 6.3 Hz, 2 H, CH_2O), 3.70 (t, J = 6.3 Hz, 2 H, CH_2CH_2N), 3.54 (q, J = 7.0 Hz, 2 H, CH_3CH_2N), 2.66 [m, 4 H, $CO(CH_2)_2CO$], 1.26 (t, J = 7.0 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 177.2 (*C*OOH), 172.1 (*C*OO), 153.0 (C-1′), 151.6 (C-10′), 147.2 (C-4′), 144.4 (C-7′), 134.8 (C-2′), 126.9, 126.9, 126.0, 122.6, 118.0, 111.5, 111.5 (Ar-CH), 61.6 (CH_2O), 48.7, 45.8 (CH_2N), 28.7, 28.6 [$CO(CH_2)_2CO$], 12.2 (CH_3) ppm. $C_{20}H_{21}ClN_4O_6$ (448.87): calcd. C 53.52, H 4.72, N 12.48; found C 53.49, H 4.70, N 12.43.

General Procedure for the Preparation of Protected GADs 6a–h: A mixture of the appropriate monosuccinate (1 mmol), the appropriate alcohol (1.2 equiv.), *N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (1.2 equiv.), 4-dimethylaminopyridine (0.28 equiv.) in THF (10 mL) was stirred at room temperature for 20 h and then concentrated at reduced pressure. The resulting residue was diluted with EtOAc (15 mL) and washed with saturated aqueous $NaHCO_3$ (15 mL) and brine (15 mL). The organic phase was dried with Na_2SO_4, concentrated and the resulting residue was purified by flash chromatography eluting with the appropriate eluting mixture.

Protected GAD 6a: The condensation of **2a** (1.00 g, 2.79 mmol) and **3a** (899 mg, 3.35 mmol) afforded pure **6a** (1.54 g, 90%) as a yellow–orange solid foam after flash chromatographic purification (petroleum ether/EtOAc, 3:1). R_f = 0.42 (petroleum ether/EtOAc, 3:1). ^{1}H NMR (200 MHz, $CDCl_3$): see Table 1 and δ = 7.85 (m, 4 H, 2′-H, 6′-H, 8′-H, 12-H), 7.43 (m, 3 H, 3′-H, 4′-H, 5′-H), 6.76 (m, 2 H, 9′-H, 11′-H), 4.31 (t, J = 6.3 Hz, 2 H, CH_2O), 3.61 (t, J = 7.1 Hz, 2 H, CH_2CH_2N), 3.43 (q, J = 7.1 Hz, 2 H, CH_3CH_2N), 2.62 [m, 4 H, $CO(CH_2)_2CO$], 1.51, 1.39, 1.30, 1.29 [4 s, each 3 H, $2\times C(CH_3)_2$], 1.19 (t, J = 7.1 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): see Table 2 and δ = 171.8, 170.7 ($2\times$C=O), 152.9 (C-1′), 149.8 (C-10′), 143.5 (C-7′), 129.3, 128.7, 124.9, 122.0, 111.1 (Ar-CH), 112.1, 109.1 [$2\times C(CH_3)_2$], 61.7 (CH_2O), 48.5, 45.4 (CH_2N), 28.8, 28.7 [$CO(CH_2)_2CO$], 26.7, 26.6, 26.1, 26.0 [$2\times C(CH_3)_2$], 12.2 (CH_3) ppm. $C_{32}H_{41}N_3O_9$ (611.68): calcd. C 62.83, H 6.76, N 6.87; found C 63.01, H 6.92, N 6.99.

Protected GAD 6b: The condensation of **4b** (157.7 mg, 0.381 mmol) and **1a** (118.9 mg, 0.457 mmol) afforded pure **6b** (150.1 mg, 60% yield) as a red syrup after flash chromatographic purification (hexane/EtOAc, 3:2). R_f = 0.42 (hexane/EtOAc, 2:3). ^{1}H NMR (200 MHz, $CDCl_3$): see Table 1 and δ = 8.32, 7.92 (AA′XX′ system, 4 H, 2′-H, 3′-H, 5′-H, 6′-H), 7.90, 6.80 (AA′XX′ system, 4 H, 8′-H, 9′-H, 11′-H, 12′-H), 4.33 (t, J = 6.3 Hz, 2 H, CH_2O), 3.70 (t, J = 6.3 Hz, 2 H, CH_2CH_2N), 3.54 (q, J = 7.1 Hz, 2 H, CH_3CH_2N), 2.67 [m, 4 H, $CO(CH_2)_2CO$], 1.51, 1.41, 1.32, 1.28 [4 s, each 3 H, $2\times C(CH_3)_2$], 1.26 (t, J = 7.1 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): see Table 2 and δ = 172.0, 170.9 ($2\times$C=O), 156.6 (C-1′), 151.1 (C-10′), 147.3 (C-4′), 143.7 (C-7′), 126.2, 124.6, 122.6, 111.3 (Ar-CH), 112.2, 109.3 [$2\times C(CH_3)_2$], 61.6 (CH_2O), 48.6, 45.6 (CH_2N), 28.7 [$CO(CH_2)_2CO$], 26.8, 26.6, 26.1, 25.2 [$2\times C(CH_3)_2$], 12.2 (CH_3) ppm. $C_{32}H_{40}N_4O_{11}$(656.70): calcd. C 58.53, H 6.14, N 8.53; found C 58.50, H 6.11, N 8.50.

6b was obtained also by the condensation of **3b** (1.00 g, 3.18 mmol) and **2a** (1.12 g, 3.12 mmol). Flash chromatographic purification (petroleum ether/EtOAc, 3:1) of the crude residue afforded pure **6b** (red syrup, 1.47 g, 72%) having NMR parameters identical to those of the sample prepared above.

Protected GAD 6c: The condensation of **4b** (1.32 g, 3.19 mmol) and **1b** (994.3 mg, 3.82 mmol) afforded pure **6c** (1.80 g, 86%) as a red syrup after flash chromatographic purification (hexane/EtOAc, 65:35). R_f = 0.52 (hexane/EtOAc, 2:3). ^{1}H NMR (200 MHz, $CDCl_3$): see Table 1 and δ = 8.31, 7.93 (AA′XX′ system, 4 H, 2′-H, 3′-H, 5′-H, 6′-H), 7.88, 6.79 (AA′XX′ system, 4 H, 8′-H, 9′-H, 11′-H, 12′-H), 4.30 (t, J = 6.3 Hz, 2 H, CH_2O), 3.68 (t, J = 6.3 Hz, 2 H, CH_2CH_2N), 3.53 (q, J = 7.1 Hz, 2 H, CH_3CH_2N), 2.66 [m, 4 H, $CO(CH_2)_2CO$], 1.49, 1.37, 1.36, 1.33 [4 s, each 3 H, $2\times C(CH_3)_2$], 1.26 (t, J = 7.1 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): see Table 2 and δ = 172.0, 171.9 ($2\times$C=O), 156.6 (C-1′), 151.1 (C-10′), 147.3 (C-4′), 143.3 (C-7′), 126.2, 124.6, 122.6, 111.3 (Ar-CH), 112.2, 101.0 [$2\times C(CH_3)_2$], 61.5 (CH_2O), 48.6, 45.6 (CH_2N), 28.8 [$CO(CH_2)_2CO$], 27.1, 26.4, 23.8, 23.7 [$2\times C(CH_3)_2$], 12.2 (CH_3) ppm. $C_{32}H_{40}N_4O_{11}$ (656.70): calcd. C 58.53, H 6.14, N 8.53; found C 58.75, H 6.05, N 8.33.

Protected GAD 6d: The condensation of **2b** (3.00 g, 8.32 mmol) and **3c** (3.48 g, 9.95 mmol) afforded pure **6d** (4.02 g, 70%) as a red syrup after flash chromatographic purification (hexane/EtOAc, 7:3). R_f = 0.54 (hexane/EtOAc, 3:2). ^{1}H NMR (200 MHz, $CDCl_3$): see Table 1 and δ = 8.39 (d, $J_{3',5'}$ = 2.4 Hz, 1 H, 3′-H), 8.16 (dd, $J_{5',6'}$ = 8.9 Hz, 1 H, 5′-H), 7.95, 6.80 (AA′XX′ system, 4 H, 8′-H, 9′-H, 11′-H, 12′-H), 7.78 (d, J = 8.9 Hz, 1 H, 6′-H), 4.29 (m, 2 H, CH_2O), 3.70 (t, J = 6.2 Hz, 2 H, CH_2CH_2N), 3.55 (q, J = 7.1 Hz, 2 H, CH_3CH_2N), 2.66 [m, 4 H, $CO(CH_2)_2CO$], 1.49, 1.36, 1.35, 1.33 [4 s, each 3 H, $2\times C(CH_3)_2$], 1.27 (t, J = 7.1 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): see Table 2 and δ = 171.9, 171.8 ($2\times$C=O), 151.5, 151.5 (C-1′, C-10′), 146.9 (C-4′), 144.2 (C-7′), 133.8 (C-2′), 126.8, 126.8, 125.8, 122.2, 117.8, 111.3, 111.3 (Ar-CH), 112.1, 100.9 [$2\times C(CH_3)_2$], 61.3 (CH_2O), 48.6, 45.6 (CH_2N), 28.7 [$CO(CH_2)_2CO$], 27.0, 26.3, 23.7, 23.6 [$2\times C(CH_3)_2$], 12.1 (CH_3) ppm. $C_{32}H_{39}ClN_4O_{11}$ (691.14): calcd. C 55.61, H 5.69, N 8.11; found C 55.58, H 5.76, N 8.04.

Protected GAD 6e: The condensation of **2c** (500 mg, 1.39 mmol) and **3a** (448 mg, 1.66 mmol) afforded pure **6e** (814 mg, 96%) as a yellow–orange solid foam after flash chromatographic purification (hexane/EtOAc, 3:2). R_f = 0.50 (petroleum ether/EtOAc/Et_3N, 3:2:0.1). M.p. 37–39 °C (chrom). ^{1}H NMR (200 MHz, $CDCl_3$): see Table 1 and δ = 7.85 (m, 4 H, 2′-H, 6′-H, 8′-H, 12′-H), 7.42 (m, 3

H, 3′-H, 4′-H, 5′-H), 6.77 (m, 2 H, 9′-H, 11′-H), 4.23 (m, 2 H, CH_2O), 3.59 (t, J = 6.2 Hz, 2 H, CH_2CH_2N), 3.43 (q, J = 6.9 Hz, 2 H, CH_3CH_2N), 2.63 [m, 4 H, $CO(CH_2)_2CO$], 1.50, 1.43, 1.30, 1.29 [4 s, each 3 H, $2\times C(CH_3)_2$], 1.18 (t, J = 6.9 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): see Table 2 and δ = 171.9 ($2\times C{=}O$), 152.9 (C-1′), 149.8 (C-10′), 143.4 (C-7′), 129.2, 128.7, 125.0, 122.0, 111.1 (Ar-CH), 109.3, 108.5 [$2\times C(CH_3)_2$], 61.4 (CH_2O), 48.4, 45.3 (CH_2N), 28.7 [$CO(CH_2)_2CO$], 25.8, 25.7, 24.7, 24.2 [$2\times C(CH_3)_2$], 12.0 (CH_3) ppm. $C_{32}H_{41}N_3O_9$ (611.70): calcd. C 62.83, H 6.76, N 6.87; found C 63.03, H 6.97, N 6.92.

Protected GAD 6f: The condensation of **4b** (496 mg, 1.20 mmol) and **1c** (374 mg, 1.44 mmol) afforded pure **6f** (589 mg, 75%) as a red syrup after flash chromatographic purification (hexane/EtOAc, 3:2). R_f = 0.53 (hexane/EtOAc, 2:3). ^1H NMR (200 MHz, $CDCl_3$): see Table 1 and δ = 8.32, 7.92 (AA′XX′ system, 4 H, 2′-H, 3′-H, 5′-H, 6′-H), 7.90, 6.79 (AA′XX′ system, 4 H, 8′-H, 9′-H, 11′-H, 12′-H), 4.32 (t, J = 6.3 Hz, 2 H, CH_2O), 3.69 (t, J = 6.3 Hz, 2 H, CH_2CH_2N), 3.53 (q, J = 7.1 Hz, 2 H, CH_3CH_2N), 2.66 [m, 4 H, $CO(CH_2)_2CO$], 1.51, 1.45, 1.33, 1.32 [4 s, each 3 H, $2\times C(CH_3)_2$], 1.25 (t, J = 7.1 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): see Table 2 and δ = 172.1, 170.0 ($2\times C{=}O$), 156.6 (C-1′), 151.1 (C-10′), 147.3 (C-4′), 143.7 (C-7′), 126.2, 124.6, 122.6, 111.3 (Ar-CH), 109.6, 108.7 [$2\times C(CH_3)_2$], 61.5 (CH_2O), 48.7, 45.7 (CH_2N), 28.9 [$CO(CH_2)_2CO$], 26.0, 25.9, 24.9, 24.4 [$2\times C(CH_3)_2$], 12.2 (CH_3) ppm. $C_{32}H_{40}N_4O_{11}$ (656.70): calcd. C 58.53, H 6.14, N 8.53; found C 58.52, H 6.11, N 8.48.

Protected GAD 6g: The condensation of **2c** (1.04 g, 2.89 mmol) and **3c** (1.21 g, 3.47 mmol) afforded pure **6** (503 mg, 70%) as a red syrup after flash chromatographic purification (hexane/EtOAc, 7:3). R_f = 0.50 (hexane/EtOAc, 2:3). ^1H NMR (200 MHz, $CDCl_3$): see Table 1 and δ = 8.40 (d, $J_{3',5'}$ = 2.4 Hz, 1 H, 3′-H), 8.16 (dd, $J_{5',6'}$ = 8.9 Hz, 1 H, 5′-H), 7.95, 6.71 (AA′XX′ system, 4 H, 8′-H, 9′-H, 11′-H, 12′-H), 7.78 (d, J = 8.9 Hz, 1 H, 6′-H), 4.28 (m, 2 H, CH_2O), 3.70 (t, J = 6.3 Hz, 2 H, CH_2CH_2N), 3.55 (q, J = 7.1 Hz, 2 H, CH_3CH_2N), 2.66 [m, 4 H, $CO(CH_2)_2CO$], 1.51, 1.45, 1.33, 1.32 [4 s, each 3 H, $2\times C(CH_3)_2$], 1.27 (t, J = 7.1 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): see Table 2 and δ = 171.9 ($2\times C{=}O$), 152.7 (C-1′), 151.5 (C-10′), 146.9 (C-4′), 144.1 (C-7′), 133.7 (C-2′), 126.8, 126.8, 125.8, 122.4, 117.8, 111.3, 111.3 (Ar-CH), 109.4, 108.5 [$2\times C(CH_3)_2$], 61.2 (CH_2O), 48.6, 45.6 (CH_2N), 28.7 [$CO(CH_2)_2CO$], 25.8, 25.7, 24.7, 24.3 [$2\times C(CH_3)_2$], 12.1 (CH_3) ppm. $C_{32}H_{39}ClN_4O_{11}$ (691.14): calcd. C 55.61, H 5.69, N 8.11; found C 55.79, H 5.59, N 8.02.

Protected GAD 6h: The condensation of **2e** (350 mg, 0.54 mmol) and **3a** (175 mg, 0.66 mmol) afforded pure **6h** (270 mg, 55%) as a yellow solid foam after flash chromatographic purification (hexane/EtOAc, 1:1). R_f = 0.38 (petroleum ether/EtOAc, 1:1). ^1H NMR (200 MHz, $CDCl_3$): δ = 7.84 (m, 4 H, 2′-H, 6′-H, 8′-H, 12′-H), 7.43 (m, 3 H, 3′-H, 4′-H, 5′-H), 6.77 (m, 2 H, 9′-H, 11′-H), 5.00 (m, 1 H, 2′-H), 4.76 (d, $J_{1',2'}$ = 8.2 Hz, 1 H, 1′-H), 4.47–4.22 (m, 9 H, 2-H, 1-H, 6′b-H, 3′-H, 6′a-H, 4-H, 5-H, CH_2CO), 4.13–4.05 (m, 5 H, 6a-H, 6b-H, 3-H, 5′-H, 4′-H), 3.65 (t, J = 6.2 Hz, 2 H, CH_2CH_2N), 3.49 (q, J = 7.1 Hz, 2 H, CH_3CH_2N), 3.40, 3.39 (2s, 6 H, $2\times OCH_3$), 2.63 [s, 4 H, $(CH_2)_2CO$], 2.10 (s, 3 H, CH_3CO), 1.54, 1.46, 1.36, 1.35, 1.31, 1.30 [6 s, each 3 H, $C(CH_3)_2$], 1.23 (t, J = 7.1 Hz, 3 H, CH_3CH_2N) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): see Table 4 and δ = 174.0, 173.1, 170.5 ($3\times CO$), 153.0 (C-1′), 149.8 (C-10′), 143.6 (C-7′), 129.3, 128.8, 125.1, 122.1, 111.3 (Ar-CH), 110.8, 110.6, 107.8, [$3\times C(CH_3)_2$], 61.7 (CH_2O), 48.7, 45.4 (CH_2N), 28.8, 28.7 [$CO(CH_2)_2CO$], 27.6, 27.5, 26.3, 26.2, 26.1, 24.6 [$3\times C(CH_3)_2$], 20.9 (CH_3CO), 12.3 (CH_3) ppm. $C_{45}H_{62}N_3O_{16}$ (901.99): calcd. C 59.92, H 7.04, N 4.66; found C 60.21, H 7.22, N 4.80.

Deprotected GAD 5a: A solution of **6a** (1.54 g, 2.51 mmol) in 90% aq CF_3COOH (20 mL) was stirred at room temperature for 1.5 h and then repeatedly coevaporated with toluene at reduced pressure. The resulting residue was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The organic phase was dried with Na_2SO_4 and concentrated at reduced pressure to give **5a** (1.08 g, 81%) as a yellow powdery syrup. R_f = 0.25 (EtOAc/MeOH, 20:1) as a mixture of α-pyranose, β-pyranose and β-furanose anomers in the ratio of 35:55:10, calculated on the basis of the relative C-1 signal intensities (see Table 3). ^1H NMR (200 MHz, Me_2SO): δ = 7.77 (m, 4 H, 2′-H, 6′-H, 8′-H, 12′-H, both anomers), 7.47 (m, 3 H, 3′-H, 4′-H, 5′-H, both anomers), 6.85 (m, 2 H, 9′-H, 11′-H, both anomers), 6.53 (d, $J_{1,2}$ = 4.4 Hz, 1 H, 1-H, α-pyranose), 5.05 (m, 2 H, 3-H, both anomers), 4.72–4.50 (m, 2 H, both anomers), 4.20–4.18 (m, 2 H, both anomers), 3.64–3.21 (m, 6 H, CH_2O, CH_3CH_2N, CH_2CH_2N, both anomers), 2.53 [m, 4 H, $CO(CH_2)_2CO$, both anomers], 1.12 (m, 3 H, CH_3, both anomers) ppm. ^{13}C NMR (50 MHz, Me_2SO): see Table 3 for the glycide portion and for the succinyl and dye portions δ = 172.0, 171.6 ($2\times C{=}O$), 152.5 (C-1′), 150.3 (C-10′) 142.7 (C-7′), 129.6, 129.2, 125.0. 121.8, 111.4 (ArCH), 60.8 (CH_2O), 48.2 (CH_3CH_2N), 44.9 (CH_2CH_2N), 28.9 [$CO(CH_2)_2CO$], 12.0 (CH_3) ppm. $C_{26}H_{33}N_3O_9$ (531.56): calcd. C 58.75, H 6.26, N 7.91; found C 58.91, H 6.48, N 8.07.

Deprotected GAD 5b: Compound **5b** was obtained, starting from **6d** (2.51 g, 3.63 mmol) in 94% yield through the same procedure described above for the preparation of **5a**: Product **5b** is a red amorphous, slightly hygroscopic solid. λ_{max} (methanol) = 492 nm; λ_{max} (acetone) = 500 nm; λ_{max} **3c** (acetone) = 512 nm. R_f = 0.11 (EtOAc) as a mixture of α and β-pyranose anomers in the ratio of about 1:1, calculated on the basis of the relative H-1 signal intensities. ^1H NMR (600 MHz, Me_2SO): δ = 8.41 (d, $J_{3',5'}$ = 2.1 Hz, 1 H, 3′-H, α- and β-pyranose), 8.22 (dd, $J_{5',6'}$ = 8.9 Hz, 5′-H, 1 H, α- and β-pyranose), 7.84 and 6.91 (AA′XX′ system, 4 H, 8′-H, 9′-H, 11′-H, 12′-H, α- and β-pyranose), 7.76 (d, J = 8.9 Hz, 1 H, 6′-H, α- and β-pyranose), 4.88 (d, $J_{1,2}$ = 3.2 Hz, 0.5 H, 1-H, α-pyranose), 4.28 (d, $J_{1,2}$ = 7.9 Hz, 0.5 H, 1-H, β-pyranose), 4.36 and 3.98 (2m, 4 H, 6a-H, 6b-H, CH_2O α- and β-pyranose), 3.78 (m, 2 H, CH_2CH_2N, α- and β-pyranose), 3.70 (m, 1 H, 5-H, α-pyranose), 3.53 (br. q, 2 H, CH_3CH_2N, α- and β-pyranose), 3.55 (m, 2 H, 2-H, 3-H, α-pyranose), 3.12–2.90 (m, 4 H, 4-H of α-pyranose and 2-H, 3-H, 4-H of β-pyranose), 2.88 (m, 1 H, 5-H, β-pyranose), 2.53 and 2.48 [2m, each 4 H, $CO(CH_2)_2CO$, α- and β-pyranose], 1.46 (m, 3 H, CH_3, α- and β-pyranose) ppm. ^{13}C NMR (50 MHz, Me_2SO): see Table 3 for the glycide portion and, for the succinyl and dye portions and δ = 172.8 ($2\times C{=}O$), 152.8 (C-1′), 152.6 (C-10′), 147.0 (C-4′), 143.9 (C-7′), 132.9 (C-2′), 127.2, 126.1, 123.3, 118.4, 112.2 (ArCH), 62.2 (CH_2O), 48.8 (CH_3CH_2N), 45.8 (CH_2CH_2N), 29.1 [$CO(CH_2)_2CO$], 12.5 (CH_3) ppm. $C_{26}H_{31}ClN_4O_{11}$ (611.01): calcd. C 51.11, H 5.11, N 9.17; found C 51.32, H 5.33, N 9.15.

Deprotected GAD 5c: Compound **5c** (1.10 g) was obtained, starting from **6e** (1.30 g, 2.13 mmol) in 97% yield through the same procedure described above for the preparation of **5a**. λ_{max} (methanol) = 409 nm, λ_{max} (acetone) = 413 nm, λ_{max} **3a** (acetone) = 417 nm. **5c** is a mixture of α-pyranose, β-pyranose and β-furanose anomers in the ratio of 35:55:10, calculated on the basis of the relative C-1 signal intensities (see Table 3). Selected ^1H NMR (200 MHz, Me_2SO) data (unresolved multiplets relative to all anomeric forms): δ = 7.77 (m, 4 H, 2′-H, 6′-H, 8′-H, 12′-H), 7.46 (m, 3 H, 3′-H, 4′-H, 5′-H), 6.84 (m, 2 H, 9′-H, 11′-H), 5.10–4.70 (m, 2 H), 4.21 (m, 2 H), 4.09 (m, 2 H), 3.70–3.30 (m, 7 H, CH_2O, CH_3CH_2N, CH_2CH_2N), 2.55 [m, 4 H, $CO(CH_2)_2CO$], 1.09 (m, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, Me_2SO): see Table 3 for the glycide portion

and for the succinyl and dye portions δ = 172.0 (2×C=O), 152.5 (C-1′), 150.3 (C-10′), 142.6 (C-7′), 129.6, 129.2, 125.0, 121.9, 111.4 (ArCH), 61.7 (CH_2O), 48.2 (CH_3CH_2N), 44.9 (CH_3CH_2N), 28.6 [$CO(CH_2)_2CO$], 12.0 (CH_3) ppm. $C_{26}H_{33}N_3O_9$ (531.57): calcd. C 58.75, H 6.26, N 7.90; found C 58.65, H 6.20, N 7.86.

Deprotected GAD 5d: Compound **5d** was obtained, starting from **6g** (1.09 g, 1.58 mmol) in 92% yield through the same procedure described above for the preparation of **5a**. Product **5d** is a red amorphous solid. λ_{max} (methanol) = 492 nm; λ_{max} (acetone) = 499 nm; λ_{max} **3c** (acetone) = 512 nm. R_f = 0.12 (EtOAc) as a mixture of α-pyranose, β-pyranose and β-furanose anomers in the ratio of 32:49:19, calculated on the basis of the relative C-1 signal intensities (see Table 3). ^{1}H NMR (200 MHz, Me_2SO): δ = 8.38 (m, 1 H, 3′-H), 8.20 (m, 1 H, 5′-H), 7.78 and 6.90 (2 m, 5 H, 6′-H, 8′-H, 9′-H, 11′-H, 12′-H), 5.10–4.60 (m, 1 H), 4.25 (m, 2 H), 4.10 (m, 1 H), 3.80–3.20 (m, 9 H, CH_2O, CH_3CH_2N, CH_2CH_2N), 2.56 [m, 4 H, $CO(CH_2)_2CO$], 1.16 (m, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, Me_2SO): see Table 3 for the glycide portion and for the succinyl and dye portions δ = 171.8 (2×C=O), 152.3 (C-1′), 152.0 (C-10′), 146.7 (C-4′), 143.4 (C-7′), 132.3 (C-2′), 126.6 and 111.8 (C-8′, C-9′, C-10′, C-11′), 125.6 (C-3′), 123.2 (C-5′), 118.0 (C-6′), 61.5 (CH_2O), 48.3 (CH_3CH_2N), 45.1 (CH_2CH_2N), 28.5 [$CO(CH_2)_2CO$], 12.0 (CH_3) ppm. $C_{26}H_{31}ClN_4O_{11}$ (611.01): calcd. C 51.11, H 5.11, N 9.17; found C 51.41, H 5.08, N 9.07.

Deprotected GAD 5e: Compound **5e** (818 mg) was obtained, starting from **6h** (1.20 g, 1.33 mmol) in 85% yield through the same procedure described above for the preparation of **5a**. Product **5e** is a yellow powdery syrup. R_f = 0.51 (EtOAc/MeOH, 4:1) as a mixture of α- and β-pyranose anomers in the ratio of 3:2, calculated on the basis of the relative H-1 signal intensities. ^{1}H NMR (200 MHz, CD_3OD): δ = 7.89 (m, 4 H, 2′-H, 6′-H, 8′-H, 12′-H), 7.52 (m, 3 H, 3′-H, 4′-H, 5′-H), 6.96 (m, 2 H, 9′-H, 11′-H), 5.10 (d, $J_{1,2}$ = 3.7 Hz, 1-H, α-pyranose); 4.52 (d, $J_{1,2}$ = 7.7 Hz, 1-H, β-pyranose); 4.38–4.23 (m, 4 H), 3.86–3.77 (m, 6 H), 3.68–3.40 (m, 6 H, CH_2O, CH_3CH_2N, CH_2CH_2N), 2.65 [m, 4 H, $CO(CH_2)_2CO$], 2.11 (s, 3 H, CH_3CO), 1.20 (t, 3 H, CH_3CH_2N) ppm. ^{13}C NMR (50 MHz, CD_3OD): see Table 4 for the glycide portion and for the succinyl and dye portions δ = 173.0, 172.8, 171.1 (3×C=O, α-pyranose), 172.5, 172.3, 171.2 (3×C=O, β-pyranose), 153.2 (C-1′, both anomers), 150.7 (C-10′, both anomers), 143.5 (C-7′, both anomers), 129.4, 129.0, 125.1, 122.0, 111.4 (Ar-CH, both anomers), 60.7 (CH_2O, β-pyranose), 60.3 (CH_2O, α-pyranose), 48.5 (CH_3CH_2N, both anomers), 45.2 (CH_2CH_2N, both anomers), 28.6 [$CO(CH_2)_2CO$, both anomers], 19.8 (CH_3CO, α-pyranose), 19.7 (CH_3CO, β-pyranose), 11.4 (CH_3, both anomers) ppm. $C_{34}H_{45}N_3O_{15}$ (735.73): calcd. C 55.50, H 6.16, N 5.71; found C 55.81, H 6.38, N 5.97.

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