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Synthesis of Water-Soluble Large Naturalised Dyes Through Double Glycoconjugation

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Recently we started to develop a new class of dyes based upon the glycoconjugation of disperse dyes with mono- or disaccharides. We used the term "naturalised" to describe these dyes because we used natural lactose in the glycoconjugation process, and also because they are similar to real natural dyes, the hydrosolubility of which is based upon the saccharidic moiety attached to the chromophore, as in carminic acid or carthamin. Synthetic dyes submitted to the process of naturalisation consist of small chromophoric molecules, prevalently azoic, whereas larger dye molecules with a higher mass did not show appreciable solubility in water when a single molecule of lactose was added through a bivalent spacer, which is in contrast to the smaller dyes. To overcome this difficulty, herein we present the first approach to the insertion of two lactose units by a double glycoconjugation process. The procedure here presented allows the successful insertion of a spacer, the malonic acid, which can be linked with two lactose units so that even large dyes become soluble.

Introduction

Disperse dyes, still very important in the dyeing of many materials, consist mainly of azo and anthraquinonic chromophores and are essentially insoluble in water, in which they form a dispersion.[1] The process of dyeing synthetic fabrics, like polyester, acetate, acrylic and polyamide, becomes possible by adding liquors and surface agents. Consequently they are able to approach the hydrophobic fibres with which they have an affinity, and to dye them. But the use of these materials raises some concern because of the considerable quantities of additives that are necessary for dyeing in water. Moreover, the environmental impact both of the dyes and of the additives cannot be neglected. The textile industry consumes high percentages of the total surfactant production,[2] which causes considerable difficulties in purification plants as the elimination of surfactants is difficult. In fact, biological treatment, especially under aerobic aqueous conditions, is not easy in their presence. Under anaerobic conditions surfactants are not biodegradable and therefore they remain in solution affecting aquatic life. The synergistic effect with other toxic chemicals that may be present in waste waters increases their negative effects on the environment.[3] In this context, the surfactants present in the waste waters of textile industries must be reduced to acceptable levels before being discharged into the environment, and this is not an easy task because whatever technology is adopted,[2] for example, chemical or electrochemical oxidation, membrane technology, chemical precipitation, photocatalytic degradation and adsorption or electrosorption onto various adsorbents, the costs are prohibitive. Furthermore, the rinsing that is required to remove surfactants from clothes once dyed because of the danger to human health,[4,5] creates a significant quantity of waste water. We could take into consideration many other factors, but it can be concluded that the textile industry needs to make many changes, particularly with respect to the dyeing processes, materials and waste treatment.

In an attempt to reduce the environmental impact of the dyeing process and to improve the performances of the materials and procedures involved, we have recently developed a new class of dyes, which we have named "naturalised dyes"[6] that do not require chemical additives during the dyeing process, enable dyeing to be performed at lower temperatures and in a shorter time, and are multipurpose, with each of them able to dye different fibres, be they natural, artificial or synthetic.

We started with the glycoconjugation of synthetic dyes because saccharidic moieties are found in natural compounds. For instance, carthamin[7] (Figure 1) is a natural red double-glycoconjugated pigment derived from safflower (*Carthamus tinctorius*).

Safflower has been cultivated since ancient times and was used as a dye in ancient Egypt[8] and later in European countries, where it was used extensively for dyeing wool and in the carpet industry. Note that carthamin is highly hydro-

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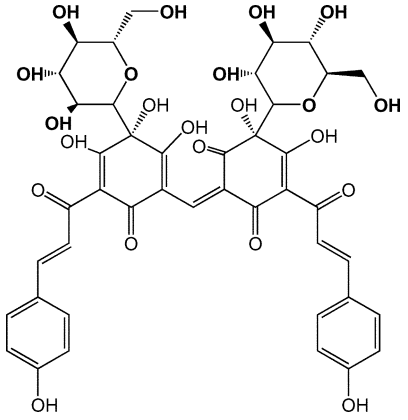


Figure 1. The structure of carthamin that includes twosaccharidic molecules.

philic and this is the most important characteristic that we propose to transfer to (or improve on in) commercial or synthetic dyes. Carthamin also attracted our attention because the chromophore is large and even though many polar groups, for example, hydroxys and carbonyls, are present, the two units of saccharide are covalently bonded to it to render it water-soluble. The results of a correlated, contemporary study of this compound have been reported in the literature.[9]

As described in our previous papers, we proceeded to glycoconjugate the azo dyes with lactose or with its monosaccharidic components, glucose and galactose, through ester[10,11] or ether[12,13] spacers bonded between the glycosidic moiety and the dye. The different glycol-azo dyes (GADs) synthesised are soluble in water and are multipurpose as they dye all the fabrics that we tested: wool, silk, nylon, polyester, acrylic and acetate. In the case of cotton, the results were contradictory. The dyeing process with these GADs was carried out in water without additives and under mild conditions of temperature and pressure.

The limitation of this procedure is that it is effective for small molecules, with molecular weights not larger than 250–300; difficulties arose with larger dyes. To overcome these difficulties, we decided to perform a more extensive glycoconjugation using other alternatives described in a European patent recently submitted. These approaches involve either the use of glutamic acid as the bridge with two acid units remaining free to bond with two lactose units or the preparation of a unique saccharidic moiety formed from two molecules of lactose covalently bonded.[14] Herein we present another approach that produces results in accord with our expectations. We proceeded therefore to prepare double-conjugated compounds using malonic acid derivatives as the spacer.

Results and Discussion

Coupling of the Starting Dyes D1–5

Five different commercial dyes (Figure 2) were selected for the double glycoconjugation procedure, after which they each displayed all the properties characteristic of naturalised dyes.

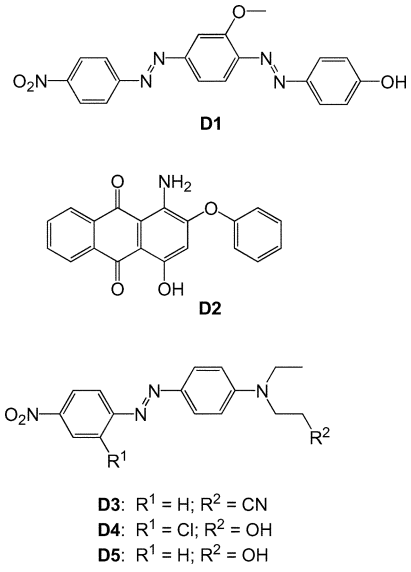
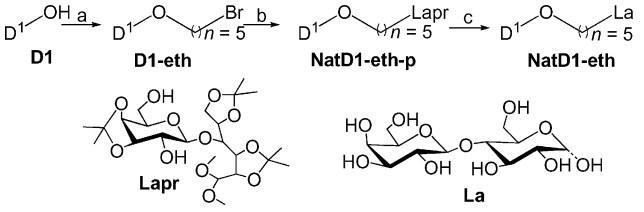


Figure 2. The commercial dyes used for the double glycoconjugation procedure.

The synthetic path depends on the nature and the position of the functional group in the starting molecules. Starting with **D1**, we prepared first the diethereal derivative according to a recently proposed procedure[12] and obtained the naturalised ethereal lactosidic derivative **NatD1-eth** (Scheme 1). This derivative was not soluble in water, insensitive to warming, stirring, powdering and microwaving. All attempts to solubilise it led to two-phase systems, with the water phase almost completely clear and uncoloured.

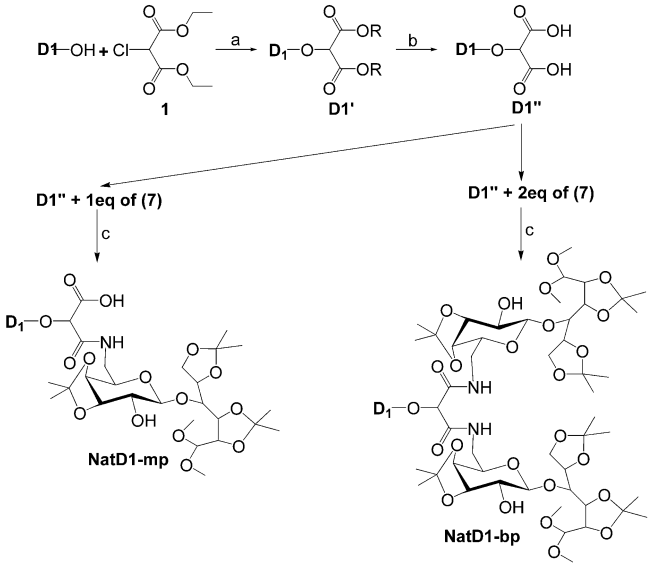


Scheme 1. Preparation of the diethereal glycoconjugated derivative of **D1**. Reagents and conditions: a) dibromopentane, KOH, 18-crown-6, room temp., 67%; b) derivative **7**, KOH, 18-crown-6. room temp., 54%; c) TFA, room temp., 3 h, 94%.

Owing to this failure, we tried a more extensive glycoconjugation of the dye **D1**, hoping that with a different lipophilic dye/hydrophilic lactose ratio the dye would become soluble in water and multipurpose as far as its tinctorial ability is concerned.

The required naturalised dye **NatD1-b** was obtained from the bis-azo dye **D1** by using diethyl chloromalonate as the spacer directly bonded to the aromatic hydroxy group of **D1**. The phenolic group of **D1** was treated with diethyl chloromalonate in THF and sodium hydride at room temperature to afford **D1′**, this step was followed by saponification with potassium hydroxide in a water/1,4-dioxane mix-

ture to provide the corresponding diacid **D1**′′. Next, the diacid **D1**′′ was coupled with the protected amino-lactose **7** (Scheme 2).



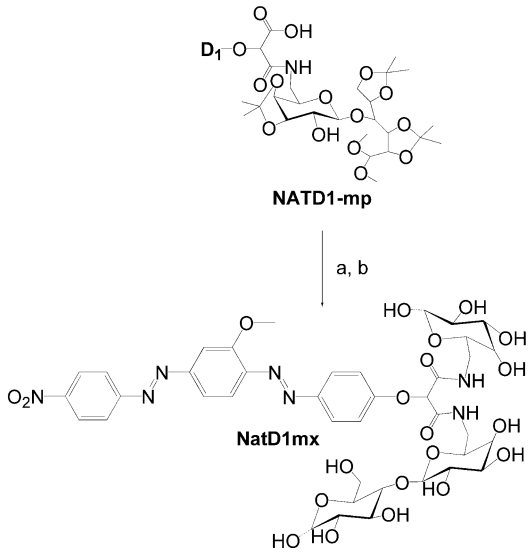
Scheme 2. Preparation of the mono- and double-conjugated naturalised dye **D1** depending on the stoichiometry of **7**. Reagents and conditions: a) NaH, THF, 0 °C to room temp. 20 h, 82%; b) KOH, dioxane/water (1:1), room temp., 2 h, 96%; c) NMM, DMTMM, THF, amino-lactose derivative **7**, room temp., 20 h, 77% for **NatD1-mp** and 92% for **NatD1-bp**.

During the coupling of the diacid **D1**′′ and the glycide **7**, the activated acid intermediate can be attacked by one or two molecules of amino-lactose derivative **7**. With 1 equiv. of **7**, the mono-glycoconjugated derivative **NatD1-mp** was formed, but with 2 equiv. of **7**, the product consists of a double-glycoconjugated derivative **NatD1-bp**.

The final mono-glycoconjugated naturalised dye **NatD1-m** was obtained after deprotection of the **NatD1-mp** in TFA at room temperature as an anomeric mixture (Scheme 3).

The same procedure was applied to **NatD1-bp** to give the double-glycoconjugated **NatD1-b**.

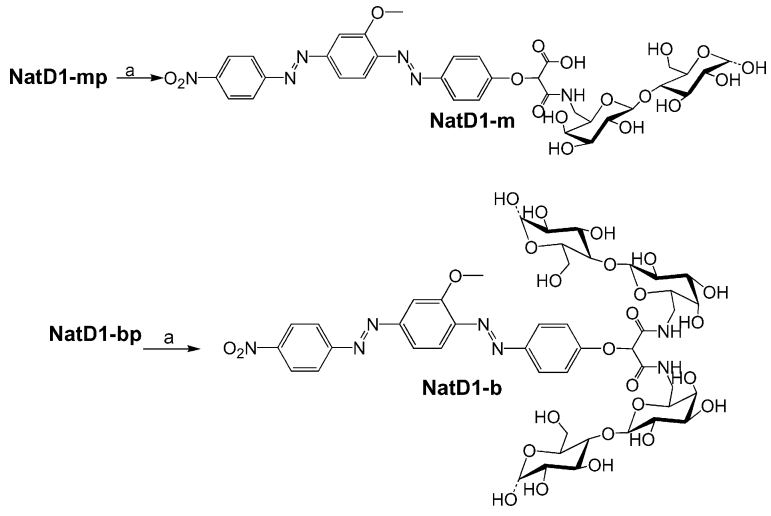
Finally, the mixed glycoconjugated product **NatD1mx** was prepared in which the protected amino-galactose **8**[14a] was attached to the other acid functionality of the malonic unit of **NatD1-mp** (Scheme 4).



Scheme 4. Preparation of mixed **NatD1mx** from **NatD1-mp**. Reagents and conditions: a) DMTMM, NMM, galactosamine **8**,[14a] THF, room temp., 20 h, 67%; b) TFA, room temp., 4 h, 95%.

With all these derivatives of the starting dye **D1**, we can compare their solubility in water and their glycidic content (Table 1).

We have to realise that products **NatD1-m** and **NatD1-eth** are insoluble in water; the solubility starts with product **NatD1mx** and is immediate with **NatD1-b**. Therefore we can conclude that for these derivatives to be soluble, a minimum percentage weight of 50% of the glycidic moiety is required, whereas at 40% they are completely insoluble.



Scheme 3. Deprotection of the compounds **NatD1-mp** and **NatD1-bp** to give the final products **NatD1-m** and **NatD1-b**. Reagents and conditions: a) TFA, room temp., 3 h, 98%.

Table 1. Formulae of the naturalised derivatives of the dye **D1** and the molecular weights of their lipophilic (chromophore and spacer) and glycidic moieties (saccharide) and their molecular weight ratios and solubility.

Naturalized dye	Chromophore (formula weight)	Spacer (formula weight)	Saccharide (formula weight)	MW [g/mol]	Ratio saccharide/ MW [%]	Solubility[a][g/L]
NatD1-m	$C_{19}H_{14}N_5O_4$ 376.36	$C_3H_4NO_3$ 101.067	$C_{12}H_{20}O_{10}$ 324.292	$C_{34}H_{38}N_6O_{17}$ 801.71	40.4	Insoluble
NatD1-eth	$C_{19}H_{14}N_5O_4$ 376.36	$C_5H_{10}O_2$ 102.135	$C_{12}H_{20}O_{10}$ 324.292	$C_{36}H_{44}N_5O_{16}$ 803.79	40.5	Insoluble
NatD1mx	$C_{19}H_{14}N_5O_4$ 376.36	$C_3H_4NO_3$ 101.067	$C_{18}H_{32}O_{15}$ 488.454	$C_{40}H_{49}N_7O_{21}$ 962.88	50.7	69.34
NatD1-b	$C_{19}H_{14}N_5O_4$ 376.36	$C_3H_4NO_3$ 101.067	$C_{24}H_{40}O_{20}$ 648.584	$C_{46}H_{58}N_6O_{27}$ 1124.01	57.7	71.47

[a] The solubility was measured at room temperature.

Synthesis of the Amino Lactose 7 and Amino Galactose 8 Protected Derivatives

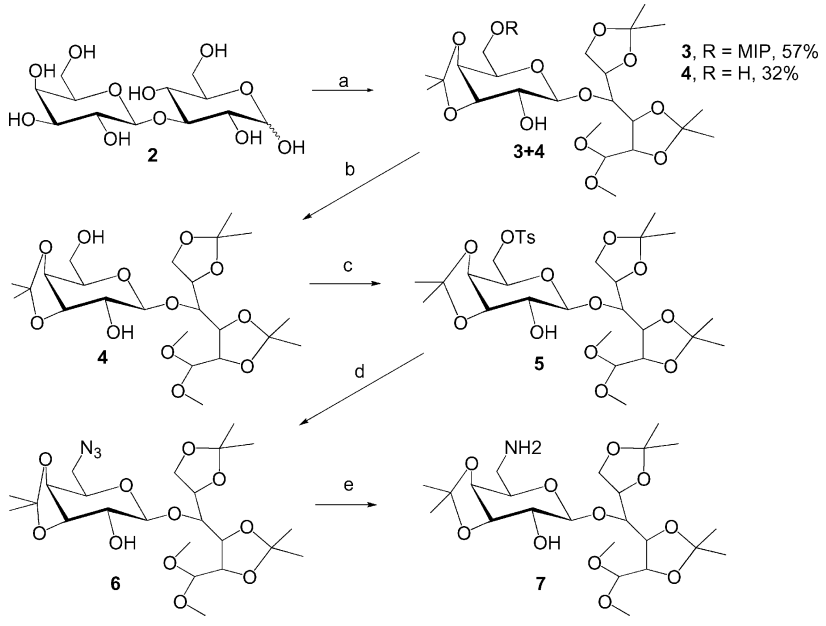
The protected amino-lactose **7** was obtained in four facile steps and 55% overall isolated yield from α,D-lactose **2** following the procedure reported in the literature[15a,15b] (Scheme 5). First, we treated α,D-lactose **2** with dimethoxypropane according to the reported procedure[16] to obtain a mixture of the protected lactoses **3** (R = H) and **4** (R = MIP). We then selectively deprotected the MIP group of **3** in the mixture by using H_2O/MeOH (1:10) at 80 °C, which fully transformed **3** into the protected product **4** in which the OH in the 6′-position was free. Subsequently, regioselective tosylation of the primary 6′-OH group in **4** with *p*-toluenesulfonyl chloride in the presence of pyridine/acetonitrile (2:1) gave the 6′-tosyl derivative **5**. In turn, derivative **5** was treated with an excess of sodium azide in DMSO at 100 °C to provide the 6′-azide-protected lactose **6**, which was transformed into **7** by hydrogenation on 10% Pd/C in methanol (Scheme 5).

The protected amino-galactose **8** was prepared according to the literature[15a] starting from commercial 1,2:3,4-bis-*O*-(1-methylethylidene)-α-D-galactopyranose.

Naturalisation of a Representative Anthraquinonic Dye

Anthraquinonic **D2** is a well-known textile disperse dye that is insoluble in water. Thus, dyeing processes involving **D2** are carried out in the presence of surface agents that bring the dye into contact with the surface of the liquid phase, from where it is distributed onto the fabric, often a synthetic material. Of course, the quantity of surface agents is considerable, in large excess with respect to the dye itself, and the treatment of waste water raises difficult problems. Moreover, the use of surface agents makes necessary many washes, therefore increasing water consumption. Because of all these problems, we wondered whether naturalisation of the **D2** dye was possible.

Because the presence of the amino group on the anthraquinonic ring could interfere with the following chemistry,



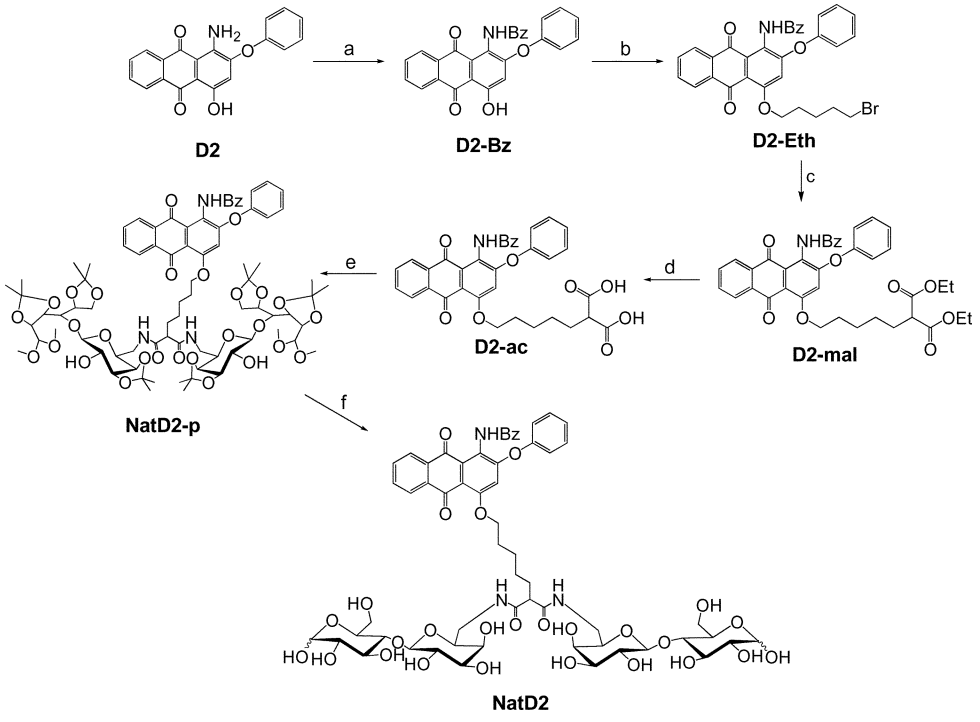
Scheme 5. Conversion of lactose **2** into the 6′-amino-protected lactose **7**. Reagents and conditions: a) DMP, TsOH, 80 °C, 5 h; b) H_2O/MeOH (1:10), 1.5 h, 80 °C, 95%; c) TsCl, pyridine/acetonitrile (2:1), room temp., 24 h, 82%; d) NaN_3, DMSO, 90 °C, 20 h, 81%; e) H_2, 10% Pd/C, MeOH, 96%.

we decided first to introduce a benzoyl group onto the NH_2 group (Scheme 6). Without benzoylation of the amino group, **D2** did not react to give **D2-Eth**, even under harsher reaction conditions and longer reaction times. After preparing **D2-Bz**, we tried to attach the malonate diethyl ester directly onto the hydroxy group on the anthraquinone by using 2-bromo- or 2-chloro derivatives of the malonic diester under basic or extremely basic conditions, but all our attempts were unsuccessful. Thus, we attempted a longer strategy that involved the preparation of the ethereal derivative **D2-Eth** using 1,5-dibromopentane and K_2CO_3 in acetone at reflux. **D2-Eth** was easily obtained and from this we arrived at the **D2-mal** adduct by using THF as the solvent, NaH as the base and diethyl malonate as the reagent. We proceeded then to saponify the diethyl ester of the malonate derivative and finally we isolated the diacid derivative after treatment with mineral acid. **D2-ac** was then treated with amino-lactose **7** in the presence of either coupling reagent to arrive at the protected naturalised **NatD2-p** product. The final step was the deprotection of this last compound using TFA, as already described, and finally we isolated **NatD2** in good yield. This product was readily soluble in water, even though we have introduced two spacers one of which is and lipophilic. However, the influence of the two lactose units in the naturalised **NatD2** raises the molecular weight of the hydrophilic moieties to 58.5% (see Table 1), a crucial value in terms of water solubility, as stressed above. Here, at variance with the azo dyes so far considered, we note that **NatD2** displays a different colour with respect to the initial **D2**, as two auxochromes of the anthraquinone derivative chromophore, the NH_2 and OH groups, have been modified.

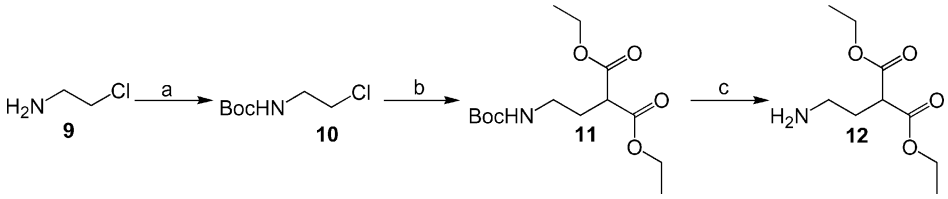
Naturalisation of Azo Dyes

The first problem concerning the solubilisation in water of heavy disperse dyes through a double glycoconjugation can be considered satisfied, but still the other decisive question about the ability to dye different fabrics with these double-lactose-conjugated derivatives remains unanswered. Therefore, we decided to compare these derivatives with those obtained through a single glycoconjugation, through which a multipurpose dye has been obtained and tested.

We started with the naturalisation of the dye family bearing a nitrile group (**D3**), which will be glycoconjugated for the first time. For this purpose, we synthesised the diethyl 2-(2-aminoethyl)malonate (**12**) in order to use its amino group in the coupling step with an eventual carboxylic acid deriving from the hydrolysis of the nitrile group of the dye **D3**. The malonate derivative **12** was prepared as shown in Scheme 7 from 2-chloroethanamine (**9**). First, we protected the amino group of **9** following the Boc strategy to yield **10** quantitatively. The *N*-Boc-2-chloroethanamine (**10**) was condensed with diethyl malonate diester in THF in the presence of the sodium hydride as base at reflux to give **11** in a high yield. The derivative **11** was eventually deprotected in 90% TFA to provide **12** in three steps from **9**.



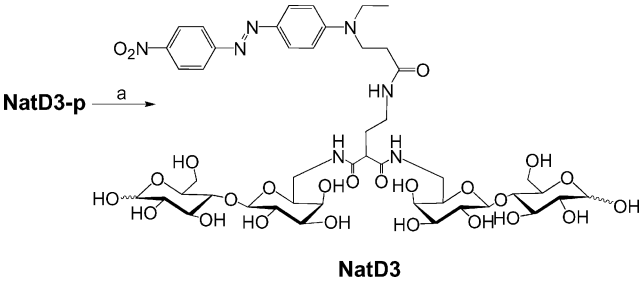
Scheme 6. Naturalisation of the anthraquinonic **D2**. Reagents and conditions: a) benzoyl chloride, pyridine, 0 °C to room temp., 88%; b) 1,5-dibromopentane, K_2CO_3, acetone, reflux, 20 h, 74%; c) diethyl malonate, NaH, THF, reflux, 20 h, 88%; d) KOH, dioxane/water, room temp., 97%; e) DMTMM, NMM, lactose derivative **7**, THF, room temp., 20 h, 97%.; f) TFA, room temp., 4 h, 98%.



Scheme 7. Reagents and conditions: a) $(Boc)_2O$, $NaHCO_3$, 1,4-dioxane/H_2O, 0 °C to room temp. 18 h, 97%; b) diethyl malonate diester, NaH, THF, reflux, 18 h, 84%; (c) TFA, –15 °C, 3 h, then NH_4OH, 91%.

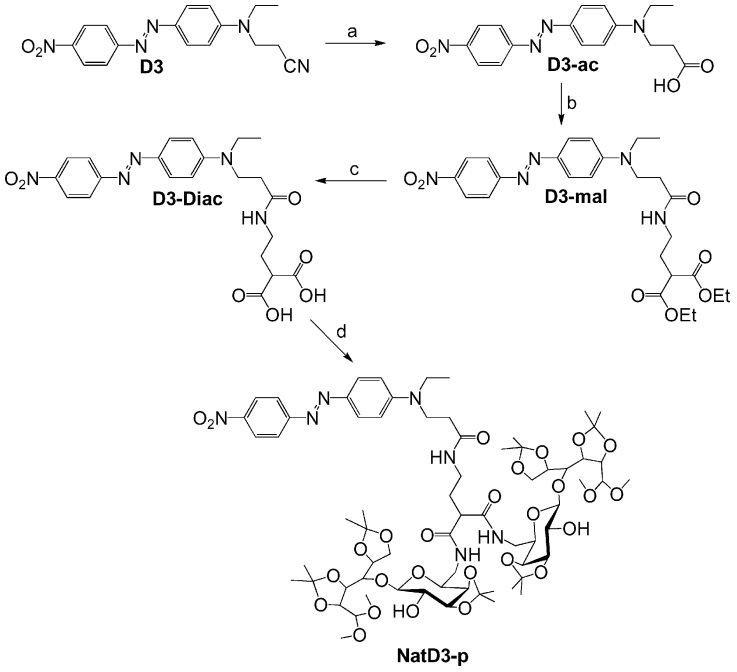
The first step of the derivatisation of **D3** was the attack on the nitrile group with 37% HCl at 90 °C to give the corresponding carboxylic acid **D3-ac** in quantitative yield (Scheme 8). **D3-mal** was then obtained from a coupling between the acid **D3-ac** and the malonate derivative **11** in THF as solvent under basic conditions at room temperature using either of two different coupling agents (see Table 2). The diethyl ester malonate derivative **D3-mal** was then submitted to saponification to produce the diacid derivative **D3-diac**. This was finally coupled with the 6′-amino-lactose **7** to produce **NatD3-p** using the coupling reagent 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM; see below for a discussion on the use of coupling reagents). **NatD3-p** is the expected naturalised derivative of the starting dye, still protected.

The deprotection step, illustrated in Scheme 9, carried out in the presence of TFA as usual, gave **NatD3** as the product.

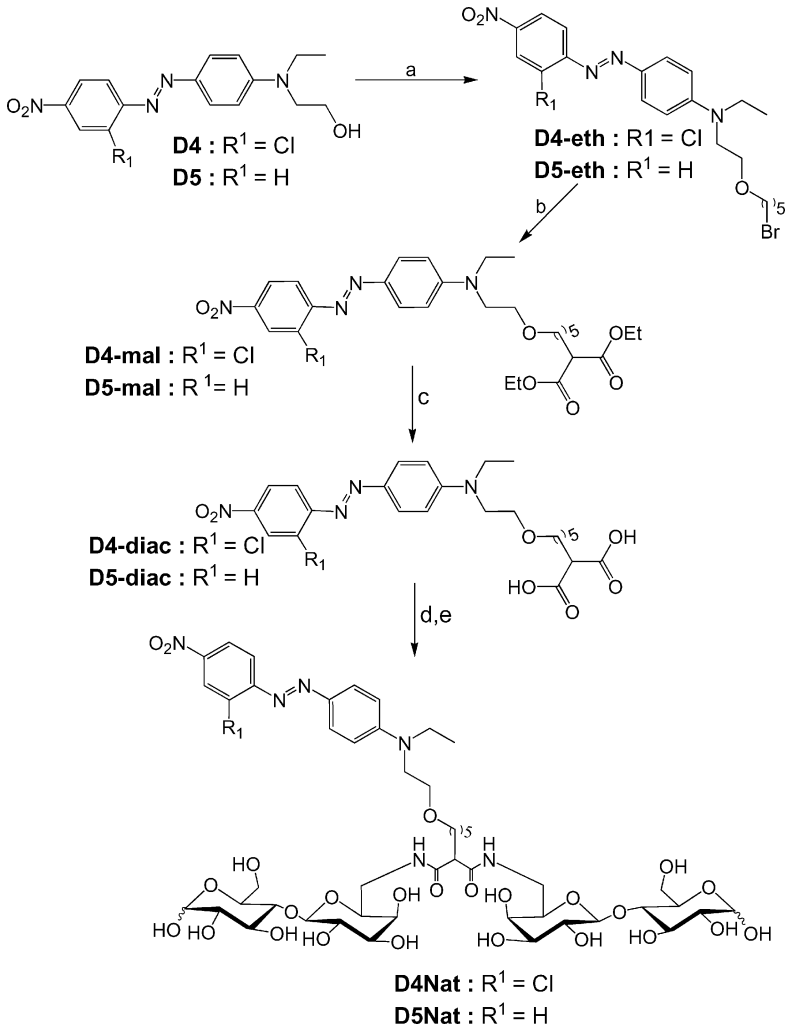


Scheme 9. Synthesis of **NatD3** from **NatD3-p**. Reagents and conditions: a) TFA, room temp., 4 h, 99%.

As far as the other two azoic dyes are concerned, we naturalised the dyes **D4** and **D5** using ethereal linkages, produced by the reaction of the hydroxy group of the dyes with 1,5-dibromopentane, as for the **D2** dye, which gave **D4-eth** and **D5-eth**, respectively. Nucleophilic substitution of the



Scheme 8. Synthesis of protected naturalised compound **NatD3-p** starting from the nitrile derivative **D3**. Reagents and conditions: a) 37% HCl, acetic acid, 90 °C, 3 h, 98%; b) derivative **11**, ethyl chloroformate, NEt_3, 20 h, 89%; c) KOH, dioxane/water, room temp., 98%; d) DMTMM, NMM, lactose derivative **7**, THF, room temp., 20 h, 92%.



Scheme 10. Naturalisation process for the dyes **D4** and **D5**. Reagents and conditions: a) 1,5-dibromopentane, KOH, 18-crown-6, THF, room temp., 20 h, 78%; b) diethyl malonate, NaH, THF, reflux, 20 h, 89%; c) KOH, dioxane/water, room temp., 93%; d) DMTMM, NMM, lactose derivative **7**, THF, room temp., 97%; e) TFA, room temp., 4 h, 98%.

remaining bromine atom was carried out by using NaH as base to transform the diethyl malonate diester into its corresponding anion so that the adducts **D4-mal** and **D5-mal** could be obtained. Saponification of the diesters was performed by using KOH and THF/water as the solvent, and successive acidification with HCl led to **D4-diac** and **D5-diac**. The final naturalised dyes **NatD4** and **NatD5**, respectively, were obtained according to the procedure described above, via **NatD4-p** and **NatD5-p** (Scheme 10).

Coupling Reactions

The amidic bond between the dicarboxylic acid **D3-diac** and the amine group of derivative **7** was formed by the use of two coupling agents. Ethyl chloroformate is a common coupling reagent used for the formation of amidic compounds when they are derived from carboxylic acid and a small molecule like **12**, in this case affording the desired product in 91% yield after two cycles of activation with ethyl chloroformate.

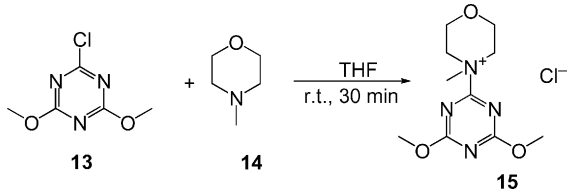
In contrast, the same coupling reaction with DMTMM gave 54% yield. This was at odds with the result of coupling of the same acid with a much larger amine, the 6′-amino-lactose **7**. The results of the two alternative approaches are reported in Table 2, which shows that the use of DMTMM[17] as the coupling reagent gives the best results, both in terms of yields and reaction times.

Table 2. Results of the condensation of the dicarboxylic acid **D3-ac** with the amine group of **7** using coupling agents.[a]

Entry	Coupling reagent	Base	Reaction time [h]	% Yield
1	DMTMM[17]	NMM	16	92
2	Ethyl chloroformate	Et_3N	30	66

[a] Reactions were carried out in THF as solvent at room temperature.

DMTMM (**15**) was prepared from 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT; **13**) and *N*-methylmorpholine (NMM; **14**) at room temperature (Scheme 11).



Scheme 11. Preparation of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) according to Kunishima et al.[17]

These triazinylammonium reagents react with the carboxylic acid to form the "triazine active ester"[18] in THF as solvent in 2 h and then this "super-active ester" couples easily with the amine group in high yield. In the other coupling process, the activation of the carboxylic acid using ethyl chloroformate led to the formation of the carboxylic anhydride, which has limited stability during the reaction, so that it was necessary to activate the acid twice to obtain the amidic compound in an acceptable yield. Similar results were obtained in the cases of **D4** and **D5**, for which the yields obtained with DMTMM as the coupling reagent were around 90%.

UV/Vis Spectra Studies

UV/Vis absorption spectra of the newly generated naturalised dyes are crucial because the modified dyes should display the same colour as the starting materials if the chromophore is not affected by the glycoconjugation process, as in dyes **D3**, **D4** and **D5**, whereas the phenolic hydroxy group was used to attach the spacer in dyes **D1** and **D2**. The UV/Vis spectra of the starting red dye **D4**, the protected dye **NatD4-p** and the deprotected dye **NatD4** in MeOH are shown in Figure 3.

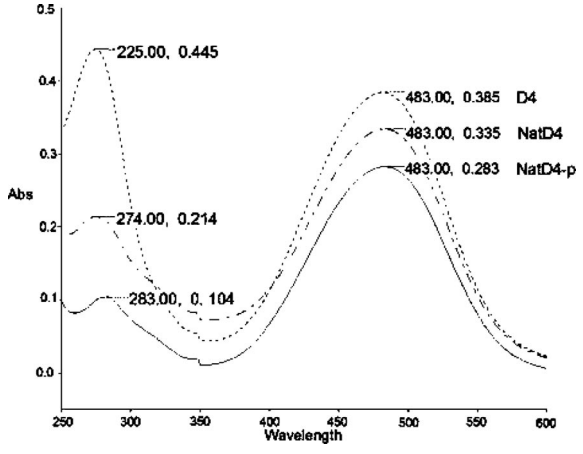


Figure 3. UV/Vis absorption spectra of the **D4**, **NatD4-p** and **Natd-4**.

The results reported in Table 3 show that λ_{max} does not exhibit any significant shift after the double glycoconjugation with the two glycide units. Also, molar extinction coefficient values can be considered almost constant, only very small differences being detected.

Table 3. UV/Vis absorption spectra of the dyes **D1**–**D5** and of their glycoconjugated derivatives. Entry **D2-x** formula is represented below.

Dye	λ_{max} (MeOH) [nm]	$\log(\varepsilon_{(max)}/M^{-1}cm^{-1})$
D1	415	4.3194
NatD1-p	416	4.3048
NatD1	416	4.3114
D2	502	4.5328
D2-x	503	4.6277
D2-bz	414	4.3364
NatD2-p	415	4.3344
NatD2	415	4.3374
D3	456	4.8383
NatD3-p	457	4.9304
NatD3	457	4.8324
D4	482	4.4356
NatD4-p	482	4.4344
NatD4	483	4.4329
D5	500	4.3006
NatD5-p	501	4.2863
NatD5	503	4.2948

Thus, derivatization of the alcoholic or carboxylic functions in dyes **D3**, **D4** and **D5** does not affect the UV/Vis spectrum as these groups are far from the chromophore. Very small changes are apparent in the spectra of compound **D1**, but on the other hand, even comparing the phenolic band with the corresponding band of anisole in practice shows no significant differences. The only electronic absorption spectrum that clearly changes is that of **D2-Bz**, in which the aromatic NH_2 group is changed and transformed into the amide derivative. Here, as expected, hypso- and hypochromic shifts are observed.

We also prepared compound **D2-x**, in which the phenolic hydroxy group was treated with ethyl bromoacetate without previous protection of the amino group. Compound **D2-x** in fact displays the same colour as the starting **D2** (Figure 4). But, as already stressed, without benzoylation of the amino group, the synthesis of **D2-eth** was hampered.

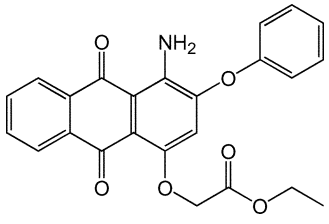


Figure 4. Formula of **D2-x**.

Tinctorial Tests

Tinctorial tests were carried out on the naturalised dyes **NatD1**, **NatD4** and **NatD5**. **NatD5** was compared with the same dye protected with monosuccinyllactose. The dyeing

Table 4. Dyeing conditions with naturalised dyes.

Fabric	Polyester	Cotton, step1	Cotton, step2	Acetate	Wool	Polyacrylic
Temperature [°C]	98	80	98	84	98	98
Dye concentration [%]	0.5	0.5	0.5	0.5	0.5	0.5
Time [min]	30	35	15	30	20	30
Acetic acid added [%]	1	–	–	1	1	2
Other	–	Na_2SO_4 (10 g/L)	Na_2CO_3 (0.5 g/L)	–	$(NH_4)_2SO_4$ (amount?)	–

conditions are reported in Table 4. Note that the dye percentages refer to the weight of the dyes in water, but as the naturalisation almost doubles the molecular weight of the dyes, a reliable comparison requires that the colour obtained with an approximately 1% solution of the naturalised dyes should be compared with a 0.5% solution of the corresponding starting dyes. The starting materials are insoluble in water and have a low affinity towards the fabrics. Therefore, during the dyeing process with the starting dyes, surface-active agents were added to disperse them in water and also many mordents to increase their affinity towards the fabrics. On the other hand, the original dyes become soluble in cold water after naturalisation through glycoconjugation with lactose. The solubilities of these dyes are reported in Table 5.

Table 5. Solubilities of the naturalised dyes.

Naturalised dyes	Solubility [g/L][a]
NatD1	71.86
NatD2	67.22
NatD3	83.47
NatD4	84.11
NatD5	82.65

[a] The solubility was measured at room temperature.

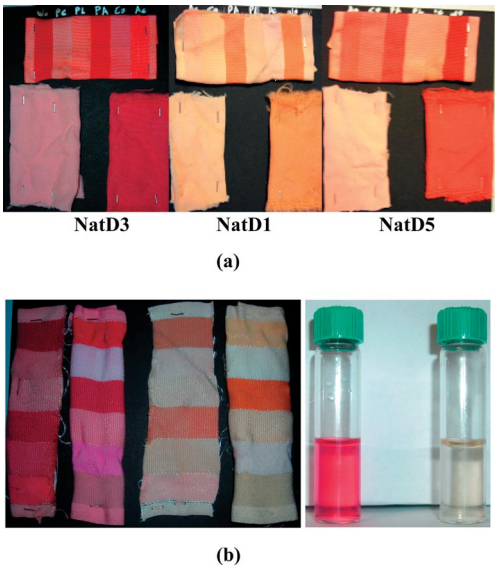


Figure 5. a) Tinctorial tests on **NatD1**, **NatD3** and **NatD5** carried out on wool (Wo, left), polyacrylic (PC), polyester (PL), Nylon (NY), cotton (Co) and polyacetate (Ac, right). b) **NatD5**, disperse red 1, **NatD1**, disperse orange 29 dyes used in the dyeing of fabric in water at 95 °C for 30 min in 0.5 (Nat dyes) or 0.25% (disperse dyes) solutions. The stripes are polyacetate, cotton, nylon, polyester, polyacrylic and wool starting from the top. On the left, the resulting water solution of 15 mL of water on 0.5 g of fabric dyed with disperse red 1 (left) and **NatD5** (right).

Thus, these naturalised dyes are multipurpose, that is, our second goal has been achieved even with two units of lactose bonded, as shown in Figure 5. The multicomponent fabric in fact shows that naturalised dyes are effective towards all the materials, wool, acetate, acrylic, nylon, polyester and even cotton (Figure 5, a), and this is at variance with dyes naturalised with a single lactose, with which cotton exhibited contradictory results. Moreover, dyeing is effective at temperatures below 100 °C in a short time (30 min as a maximum) and without the addition of dangerous surface agents or mordents and even cotton dyed better than with single lactose glycoconjugation.

In Figure 5 (b) two multicomponent fabrics dyed with **NatD1** and **NatD5** are compared with the same fabric dyed with the corresponding diperse dyes in water. The dyeing was carried out in the four cases by using a 0.5% solution of naturalised dyes and 0.25% of the disperse dye solutions. This difference is necessary because half of the molecular weight of the naturalised dyes is due to the glycidic moieties. The dyeing procedure involved keeping the fabric in the different dyes for 30 min at 95 °C. Before discussing the results let us consider that the disperse dyes are not soluble in water, except when the temperature of the water is raised. At that point equilibrium is established as the dye in solution wets the fabric, and this process produces the results shown in Figure 5 (b). Of course, a similar approach is of no industrial use, but here it allows an effective comparison. Looking at Figure 5 (b), and considering that the sequence of the strips from top to bottom is polyacetate, cotton, nylon, polyester, acrylic and wool, in both cases the disperse dyes, those in positions 2 and 4, appear to dye the polyacetate, mainly the nylon, and the polyester, but the dyeing failed in the case of cotton, acrylic and wool, bearing in mind that wool itself is a pale-brown colour. We can conclude that the naturalised dyes are multipurpose, but this is not the case for the disperse dyes. We then considered the quality of the dyeing. We weighed 0.5 g of each, **NatD5** and disperse red, and then added 15 mL of water and we kept the solutions at 95 °C for 30 min. The results are shown in the right-hand side of Figure 5 (b), the vial with the red solution is from the disperse dye, the almost transparent one from **NatD5**. Thus, we can say again that the dyeing of the naturalised materials is appropriate and the fastness is very good, whereas the disperse dye is lacquered onto the surface, mainly on the nylon strip.

Colour Fastness

Table 6 shows the wash and scraping, and light fastness data on wool of the naturalised dyes according to international fastness methods, ISO 105-C06 and ISO 105-X12, respectively. The fastness measurements definitively show that the dyes are not lacquered onto the surface of the material, but penetrate it and are efficiently bonded.

Table 6. Fastness tests for wool dyed with NatDs **1**, **2**, **3** and **5**.

Dyes	Wash fastness	Light fastness
NatD1	5	5
NatD2	4	4/5
NatD3	5	4/5
NatD5	5	5

Conclusions

From the results reported above, we can conclude that the double derivatisation of large-molecule dyes with lactose actually results in good solubility in water. This new property of the novel materials allows milder and simpler dyeing processes, avoiding the use of surface agents, mordants and other polluting additives, because the dyeing is efficient once the starting dyes have been glycoconjugated. And with two units of lactose instead of one, the dyeing process is even improved in some cases. Research towards other forms of glycoconjugation is underway in our laboratory, with encouraging results.

Experimental Section

General Methods: TLC was carried out on silica gel pre-coated plates (Merck; 60 Å F254) and spots located with (a) UV light (254 and 366 nm), (b) ninhydrin (solution in acetone), (c) fluorescamine, (d) I_2 or (e) a basic solution of permanganate [$KMnO_4$ (3 g), K_2CO_3 (20 g) and NaOH (0.25 g) in water (300 mL)]. Flash column chromatography (FCC) was carried out on Merck silica gel 60 (230–400 mesh) according to Still et al.[19] ^1H and ^{13}C NMR spectra were recorded at 200 MHz with Varian spectrometers in deuteriated solvents and are reported in parts per million (ppm) with the solvent resonance used as the internal reference. Mass spectra were recorded with a Thermo Fisher LCQfleet ion-trap instrument. (the spectra reported were also using the ESI +*c* technique). Elemental analysis was carried out with a Perkin–Elmer 240 C Elemental Analyzer. UV/Vis spectra were recorded with a Cary-4000 Varian spectrophotometer. The commercial dyes **D1** [disperse orange 29, 4-({2-methoxy-4-[(4-nitrophenyl)azo]phenyl}azo)phenol], **D2** (disperse red 60, 1-amino-4-hydroxy-2-phenoxyanthraquinone), **D3** [disperse orange 25, 3-(ethyl{4-[(4-nitrophenyl)azo]phenyl}amino)-propiononitrile], **D4** [disperse red 13, 2-(ethyl{4-[(2-chloro-4-nitrophenyl)azo]phenyl}amino)ethanol] and **D5** (disperse red 1, 2-(ethyl-{4-[(4-nitrophenyl)azo]phenyl}amino)ethanol] were purified by FCC with an appropriate eluent. Compounds **1**, **8**, **18**, **19**, *N*-methylmorpholine (NMM), 3,5-dimethoxy-1-chlorotriazine (CDMT), ethyl chloroformate and diethyl malonate are commercially available (Sigma–Aldrich) and used as such. Literature methods were used to prepare 2,3:5,6-bis-*O*-(1-methylethylidene)-4-*O*-[3,4-*O*-(1-methylethylidene)-β-D-galactopyranosyl] dimethyl acetal (**4**),[16] its 6-*O*-[(4-methylphenyl)sulfonyl **5**[17] and 6-amino-6-deoxy-1,2:3,4-di-*O*-isopropylidene-D-galactopyranoside (**8**).[15a]

Dyeing and Fastness Conditions: The dyeing process was designed to be as close as possible to the classic traditional method of dyeing described by Cardon.[20,21] This process was adapted to our dyeing methodology with a process bath of eight dyeing beakers (250 mL each) driven by a microprocessor time–temperature controller. The dyeing bath was prepared by stirring dye material (1%) in deionised water under magnetic stirring for 10 min. Later, the bath was cooled to room temperature (20 °C) to avoid felting, especially of the wool.[21] Then the fabrics and the dyeing bath were put together in the dyeing beaker (bath ratio, 1 g of fabric/50 mL of dye solution). The beakers were placed in the machine and the dyeing was carried out under mechanical stirring. The fastness to washing and scraping, and to light, was tested according to the well-known standard procedure.

1-Bromopentoxy-4-({2′-methoxy-4′-[(4′′-nitrophenyl)azo]phenyl}-phazo)enol (D1-eth). General Procedure A: A mixture of the **D1** (1.0 g, 2.65 mmol), KOH (0.715 g, 7.96 mmol) and 18-crown-6 ether (0.01 equiv.) in THF (10 mL) was stirred at room temperature for 1 h. Then 1,5-dibromopentane (1.09 mL, 5.31 mmol) was added and the mixture was stirred under the same conditions for several hours. TLC analysis (EtOAc/petroleum ether, 1:10) revealed the complete disappearance of the starting material (R_f = 0.13) and the formation of a major faster-moving product (R_f = 0.44). The crude solution was neutralised with saturated NH_4Cl (15 mL) and extracted with DCM (3 × 20 mL). The organic phase was dried with Na_2SO_4, concentrated under reduced pressure and the resulting residue was purified by flash chromatography (EtOAc/petroleum ether, 1:10) to yield **D1-eth** (1.23 g, 67%). ^1H NMR (200 MHz, $CDCl_3$): δ = 8.44–8.36 (m, 2 H, Ar-H), 8.18–7.91 (m, 4 H, Ar-H), 7.88–7.76 (m, 3 H, Ar-H), 7.02 (m 2 H, Ar-H), 3.63–3.40 (m, 7 H, PhOCH_3, OCH_2, CH_2Br), 1.77 (m, 2 H, CH_2CH$_2$Br), 1.48 [m, 4 H, (CH_2)$_2$] ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 159.61 (Ar-C), 156.85 (Ar-C), 155.22 (Ar-C), 153.86 (Ar-C), 148.48 (Ar-C), 148.07 (Ar-C), 143.98 (Ar-C), 125.06 (Ar-C), 124.44 (Ar-C), 123.26 (Ar-C), 118.32 (Ar-C), 117.33 (Ar-C), 114.76 (Ar-C), 109.98 (Ar-C), 67.34 (OCH_2), 56.18 (PhOCH_3), 33.98 (CH_2Br), 31.34 (CH_2CH_2Br), 27.88 (OCH$_2$$CH_2$), 26.90 (CH$_2CH_2$$CH_2$) ppm. MS (ESI): *m*/*z* = 526.10 [M + 1]$^+$. $C_{24}H_{24}BrN_5O_4$ (526.38): calcd. C 54.76, H 4.60; found C 54.71, H 4.55.

Synthesis of NatD1-eth-p: The product **NatD1-eth-p** was prepared according to the general procedure **A** using the following quantities: **D1-eth** (1.00 g, 1.90 mmol), KOH (0.33 g, 5.71 mmol), lactose derivative **4** (0.97 g, 1.9 mmol) in dry THF (25 mL). After work-up the crude product was purified by FCC (EtOAc/petroleum ether, 2:1, R_f = 0.53) to afford **NatD1-eth-p** (1.48 g, 54%) as an orange solid. ^1H NMR (200 MHz, $CDCl_3$): δ = 8.44 (m, 2 H, Ar-H), 8.16–7.91 (m, 4 H, Ar-H), 7.88–7.76 (m, 3 H, Ar-H), 7.01 (m, 2 H, Ar-H), 4.61 (t, *J* = 7.4 Hz, 1 H), 4.53 (m, 1 H), 4.36 (m, 1 H), 4.25 (m, 1 H), 4.15–3.90 (m, 9 H), 3.74–3.49 (m, 8 H), 3.44–3.43 (2 s, 6 H, 2 OCH$_3$), 1.91 (m, 2 H, OCH$_2$CH_2), 1.65 (m, 4 H, CH_2CH$_2$$CH_2$), 1.55–1.30 [6 s, 18 H, 3 C(CH_3)$_2$] ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 159.75 (Ar-C), 157.02 (Ar-C), 155.67 (Ar-C), 153.97 (Ar-C), 148.80 (Ar-C), 147.57 (Ar-C), 144.71 (Ar-C), 125.37 (Ar-C), 124.76 (Ar-C), 123.55 (Ar-C), 118.83 (Ar-C), 117.68 (Ar-C), 115.81 (Ar-C), 114.78 (Ar-C), 110.08, 109.87, 108.53 [C(CH$_3$)$_2$], 72.17–68.28 (2 OCH_2), 56.45 [C(OCH_3)$_2$], 53.88 (PhOCH_3), 29.60, 28.95, 28.91, 28.08, 27.28, 26.55, 26.27, 25.50, 22.51 ppm; see Table 7 for the glycidic moiety. UV/Vis: λ_{max} = 416 nm; ε = 20133 M^{-1}cm^{-1}. MS (ESI): *m*/*z* = 954.43 [M + 1]$^+$. $C_{47}H_{63}N_5O_{16}$ (954.04): calcd. C 59.17, H 6.66; found C 59.12, H 6.61.

Synthesis of NatD1-eth. General Procedure B: A solution of **NatD1-eth-p** (1.00 g, 1.05 mmol) in 90% aqueous TFA (15 mL) was stirred

Table 7. ^{13}C NMR spectroscopic data for the glycide portion of the protected lactose derivatives.

Compound	Solvent	δ [ppm]											
		C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	C-1	C-2	C-3	C-4	C-5	C-6
6	$CDCl_3$	103.54	79.66	79.06	71.51	72.17	40.14	107.9	75.54	77.42	74.04	76.22	67.67
7	$CDCl_3$	103.33	79.49	78.01	73.48	70.97	42.22	106.51	73.94	75.65	74.48	75.19	64.60
NatD1-ethp	$CDCl_3$	102.92	81.33	79.67	72.57	71.41	62.39	106.78	74.27	78.15	73.81	75.95	65.38
NatD1-bP	$CDCl_3$	103.60	79.08	78.08	72.66	70.72	40.38	106.11	74.21	76.61	71.39	76.14	64.72
NatD1-mP	$CDCl_3$	103.74	79.68	78.40	74.27	71.94	40.89	106.61	74.44	77.88	73.57	77.66	64.57
NatD2-p	$CDCl_3$	103.34	81.33	79.57	73.35	72.04	40.46	106.07	75.11	78.99	73.79	77.59	64.80
NatD3-p	$CDCl_3$	103.43	79.02	77.18	73.97	72.45	40.63	106.24	74.15	76.87	73.82	77.82	64.60
NatD4-p	Me_2SO	103.49	79.06	77.97	74.09	72.61	40.49	106.03	74.22	77.07	73.21	76.81	64.63
NatD5-p	Me_2SO	103.12	79.64	78.04	73.83	71.22	40.06	106.78	75.10	76.54	72.25	76.42	64.22

Table 8. ^{13}C NMR spectroscopic data (δ, ppm) for the glycide portion of deprotected lactose derivatives.

Compound	Solvent	δ [ppm]											
		C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	C-1	C-2	C-3	C-4	C-5	C-6
β-Lactose[a]	D_2O	103.74	72.00	73.51	69.54	76.28	62.06	96.62	74.83	75.32	79.28	75.66	61.1
NatD1-eth-β	acetone	102.31	72.62	73.39	68.42	74.72	67.51	95.12	74.21	75.12	80.08	75.33	60.82
NatD1-m-β	acetone	102.88	72.73	73.69	68.41	74.84	40.39	95.75	74.55	75.07	80.18	75.22	60.64
NatD1-b-β	acetone	102.27	72.73	73.69	68.41	74.84	40.39	95.85	74.55	75.17	80.48	75.51	60.63
NatD2-β	D_2O	102.64	72.04	73.33	68.89	74.56	40.67	95.51	73.35	74.92	80.62	75.52	60.90
NatD3-β	D_2O	103.25	72.64	73.38	68.67	74.47	40.37	95.73	73.15	74.60	80.51	75.52	60.34
NatD4-β	D_2O	102.74	72.29	73.58	68.36	74.75	40.34	95.86	74.52	75.22	80.86	75.36	60.43
NatD5-β	D_2O	102.74	72.80	73.45	68.33	74.38	40.44	95.35	74.16	75.38	80.86	75.71	60.25
α-Lactose[a]	D_2O	103.62	72.04	73.5	69.5	76.2	62.0	92.74	72.23	72.4	79.34	71.03	61.17
NatD1-eth-α	acetone	102.31	72.49	73.06	68.26	74.45	67.51	95.06	72.76	72.16	81.22	71.39	60.79
NatD1-m-α	acetone	102.68	72.53	73.84	68.35	74.62	40.10	95.72	72.36	72.27	80.79	71.82	60.57
NatD1-b-α	acetone	102.84	72.44	73.84	68.35	74.62	40.10	95.67	72.53	72.27	80.71	71.82	60.55
NatD2-α	D_2O	102.48	72.00	72.29	68.79	73.53	40.47	95.47	72.08	71.98	81.44	71.66	60.43

[a] Taken from ref.[22]

at room temperature for 3 h. TLC (EtOAc/petroleum ether, 2:1) indicated that the starting material (R_f = 0.44) had completely reacted with the formation of the deprotected compound **NatD1-eth** (R_f = 0.1). The violet solution was repeatedly co-evaporated with toluene (5 × 25 mL) at reduced pressure to give the final product **NatD1-eth** (0.78 g, 94%) as a red powder consisting of a mixture of α- and β-pyranosic anomers in a ratio of 50:50, calculated on the basis of the relative C-1 signal intensities. ^1H NMR(200 MHz, acetone): δ = 8.44 (m, 2 H, Ar-H, α- and β-pyranose), 8.23–7.89 (m, 4 H, Ar-H, α and β-pyranose), 7.79–7.64 (m, 3 H, Ar-H, α and β-pyranose), 7.11 (m, 2 H, Ar-H, α- and β-pyranose), 5.11–4.51 (m, 2 H, α-β pyranose), 4.32–4.19 (m, 4 H), 3.87–3.79 (m, 6 H), 3.71–3.41 (m, 9 H), 1.87 (m, 2 H, OCH_2CH_2, α- and β-pyranose), 1.64 (m, 4 H, $CH_2CH_2CH_2$, α- and β-pyranose) ppm.^{13}C NMR (50 MHz, $CDCl_3$): δ = 159.85 (Ar-C), 157.08 (Ar-C), 155.48 (Ar-C), 154.17 (Ar-C), 148.76 (Ar-C), 148.23 (Ar-C), 144.20 (Ar-C), 125.33 (Ar-C), 124.51 (Ar-C), 123.43 (Ar-C), 118.60 (Ar-C), 117.62 (Ar-C), 115.17 (Ar-C), 114.28 (Ar-C), 109.50 (Ar-C), 70.99–68.55 (2 OCH_2), 56.58 ($PhOCH_3$), 30.30, 29.44, 28.27 ppm; see Table 8 for the glycidic moiety. UV/Vis: λ_{max} = 416 nm; ε = 20157 $M^{-1}cm^{-1}$. MS (ESI): *m/z* = 788.33 $[M + 1]^+$. $C_{36}H_{45}N_5O_{15}$ (787.78): calcd. C 54.89, H 5.76; found C 54.77, H 5.69.

Diethyl 2-[4-({2′-methoxy-4′-[(4′′-nitrophenyl)azo]phenyl}azo)phenoxy]malonate (D1′): A mixture of 60% NaH (0.11 g, 2.65 mmol) in THF (2 mL) was added slowly at 0 °C to a solution of **D1** (1.0 g, 2.65 mmol) in THF (10 mL) and the resulting mixture was stirred at room temperature for 30 min. Diethyl chloromalonate (**1**; 1.30 mL, 2.65 mmol) was added and the mixture was stirred at room temperature for several hours. TLC analysis (EtOAc/petroleum ether, 3:10) revealed the disappearance of the starting material (R_f = 0.21) and the formation of a major product (R_f = 0.59). The crude solution was diluted with DCM (15 mL) and washed with water (3 × 20 mL). The organic phase was dried with Na_2SO_4, concentrated under reduced pressure and the resulting residue was purified by flash chromatography (EtOAc/petroleum ether, 3:10, R_f = 0.59) to yield **D1′** (1.15 g, 82%) as an orange solid. ^1H NMR (200 MHz, $CDCl_3$): δ = 8.41–8.33 (m, 2 H, Ar-H), 8.09–7.94 (m, 4 H, Ar-H), 7.81–7.67 (m, 3 H, Ar-H), 7.11 (m, 2 H, Ar-H), 5.31 (s, 1 H, C*H*), 4.35 (q, *J* = 7.00 Hz, 4 H, $2CH_2CH_3$), 4.12 (s, 3 H, $PhOCH_3$), 1.32 (t, *J* = 7.00 Hz, 6 H, 2 CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 164.88 (*C*O), 159.22 (Ar-C), 157.13 (Ar-C), 155.50 (Ar-C), 154.13 (Ar-C), 148.77 (Ar-C), 148.65 (Ar-C), 144.35 (Ar-C), 125.18 (Ar-C), 124.69 (Ar-C), 123.52 (Ar-C), 118.50 (Ar-C), 117.65 (Ar-C), 115.65 (Ar-C), 114.96 (Ar-C), 105.54 (*C*H), 62.76 (CH_2CH_3), 56.59 ($PhOCH_3$), 14.22 (2 CH_3) ppm. MS (ESI): *m/z* = 536.17 $[M + 1]^+$. $C_{26}H_{25}N_5O_8$ (535.51): calcd. C 58.31, H 4.71; found C 58.24, H 4.65.

Synthesis of D1′′. General Procedure C: A 1 N solution of KOH (10 mL) was added to a solution of **D1′** (1.00 g, 1.87 mmol) in dioxane (10 mL) and the resulting mixture was stirred at room temperature for 2 h. TLC (ethyl acetate) indicated the disappearance of the starting product (R_f = 0.81) and the formation of one major spot (R_f = 0.1). The solution was diluted with water (25 mL) and extracted with chloroform (3 × 20 mL). The aqueous solution was acidified with an aqueous solution of 1 N HCl to pH = 2 and extracted with chloroform (30 mL). The organic layer was dried with Na_2SO_4 and filtered. The solvent was removed under reduced pressure to afford **D1′′** (0.87 g, 96%) as a black solid. ^1H NMR (200 MHz, [D_6]acetone): δ = 8.51–8.43 (m, 2 H, Ar-H), 8.22–7.97 (m, 4 H, Ar-H), 7.82–7.74 (m, 3 H, Ar-H), 7.19–7.11 (m, 2 H, Ar-H), 4.13 (s, 1 H, C*H*), 3.74 (s, 3 H, $PhOCH_3$) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 163.57 (*C*O), 159.69 (Ar-C), 157.19 (Ar-C), 155.01 (Ar-C), 154.96 (Ar-C), 147.87 (Ar-C), 147.83 (Ar-C), 144.40 (Ar-C), 124.91 (Ar-C), 124.81 (Ar-C), 123.56 (Ar-C), 117.44 (Ar-

C), 117.39 (Ar-C), 115.64 (Ar-C), 114.56 (Ar-C), 105.02 (*C*H), 56.02 (PhO*C*H$_3$) ppm. MS (ESI): *m*/*z* = 480.21 [M + 1]$^+$. $C_{22}H_{17}ClN_5O_8$ (514.86): calcd. C 55.12, H 3.57; found C 55.01, H 3.44.

Synthesis of NatD1-mp. General Procedure D: NMM (0.23 mL, 2.08 mmol) was added to a solution of **D1′′** (1.00 g, 2.08 mmol) in THF (15 mL) and the mixture was stirred at room temperature for 5 min. The resulting solution was cooled to 0 °C, DMTMM (0.58 g, 2.08 mmol) was added and the mixture was stirred at room temperature for 20 h. After this time TLC (EtOAc/petroleum ether, 5:1) indicated the formation of the activated intermediate (R_f = 0.64) and the disappearance of the starting acid (R_f = 0.1). Protected amino-lactose **7** (1.06 g, 2.08 mmol) was added and the reaction mixture was stirred at room temperature for 16 h. TLC (EtOAc/petroleum ether, 5:1) showed the formation of one major spot at (R_f = 0.38). The reaction mixture was evaporated to dryness under reduced pressure and the residue was dissolved in chloroform (20 mL) and washed with a solution of 5% HCl (20 mL) and water (3 × 20 mL). The organic solution was dried with Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue (obtained as a red solid) was purified by flash chromatography (EtOAc/petroleum ether, 5:2, R_f = 0.38) to afford **NatD1-mp** (1.85 g, 77%) as an orange solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.43 (m, 2 H, Ar-H), 8.12–7.89 (m, 4 H, Ar-H), 7.79–7.63 (m, 3 H, Ar-H), 7.03 (m, 2 H, Ar-H), 4.72 (d, J_{HH} = 7.1 Hz, 1 H), 4.53 (dd, J_{HH} = 6.3, J_{HH} = 7.0 Hz, 1 H), 4.36 (d, 1 H), 4.25 (m, 2 H), 4.15–3.97 (m, 6 H), 3.74–3.49 (m, 7 H), 3.44–3.43 (2 s, 6 H, 2 OCH_3), 1.48–1.29 [various overlapping signals, 18 H, 3 C(CH_3)$_2$] ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 165.78 (*C*O), 158.49 (Ar-C), 155.66 (Ar-C), 154.61 (Ar-C), 154.31 (Ar-C), 148.70 (Ar-C), 148.43 (Ar-C), 144.53 (Ar-C), 125.57 (Ar-C), 124.10 (Ar-C), 123.61 (Ar-C), 118.86 (Ar-C), 117.46 (Ar-C), 115.27 (Ar-C), 110.52 (Ar-C), 110.37, 109.55, 108.08 [*C*(CH$_3$)$_2$], 105.47 (*C*H), 56.32 [C(O*C*H$_3$)$_2$], 54.45 (PhO*C*H$_3$), 28.80, 27.67, 26.36, 25.96, 25.40, 24.79 [C(*C*H$_3$)$_2$] ppm; see Table 7 for the glycidic moiety. UV/Vis: λ_{max} = 416 nm; ε = 20209 M^{-1}cm^{-1}. MS (ESI): *m*/*z* = 969.44 [M + 1]$^+$. $C_{45}H_{56}N_6O_{18}$ (968.97): calcd. C 55.78, H 5.83; found C 55.67, H 5.71.

Synthesis of NatD1-bp: The product **NatD1-bp** was prepared according to the general procedure D using the following quantities: **D1′′**(0.80 g, 1.67 mmol), NMM (0.55 mL, 5.01 mmol), DMTMM (1.38 g, 5.01 mmol), amino-lactose derivative **7** (1.70 g, 3.34 mmol) in THF (20 mL). The crude material was purified by FCC (EtOAc/petroleum ether, 10:3, R_f = 0.39) to afford **NatD1-bp** (2.24 g, 92%) as an orange solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.38–8.27 (m, 2 H, Ar-H), 8.12–7.89 (m, 4 H, Ar-H), 7.75–7.64 (m, 3 H, Ar-H), 7.13 (m, 2 H, Ar-H), 4.61–4.59 (m, 2 H), 4.53 (m, 2 H), 4.36 (m, 2 H), 4.25 (m, 3 H), 4.15–3.90 (m, 12 H), 3.74–3.49 (m, 11 H), 3.44–3.43 (2 s, 12 H, 4 OCH_3), 1.60–1.20 (various overlapping signals, 36 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): 167.88 (2 *C*O), 159.86 (Ar-C), 157.08 (Ar-C), 155.47 (Ar-C), 154.10 (Ar-C), 148.75 (Ar-C), 148.35 (Ar-C), 144.28 (Ar-C), 125.31 (Ar-C), 124.68 (Ar-C), 123.51 (Ar-C), 118.49 (Ar-C), 117.62 (Ar-C), 115.07 (Ar-C), 110.25 (Ar-C), 110.14, 108.28, 106.20 [*C*(CH$_3$)$_2$], 105.53 (*C*H), 56.58 [C(O*C*H$_3$)$_2$], 54.89 (PhOCH$_3$), 28.27, 27.32, 26.62, 26.44, 25.87, 24.61 [C(*C*H$_3$)$_2$] ppm; see Table 7 for the glycidic moiety. UV/Vis: λ_{max} = 416 nm; ε = 20174 M^{-1}cm^{-1}. MS (ESI): *m*/*z* = 1458.77 [M + 1]$^+$. $C_{68}H_{95}N_7O_{28}$ (1458.53): calcd. C 56.00, H 6.57; found C 55.97, H 6.54.

Synthesis of 2-[4-({2′-Methoxy-4′-[(4′′-nitrophenyl)azo]phenyl}azo)-phphenoxy]malonic 6′-Amino-lactose Monoamide (NatD1-m): The product **NatD1-m** was prepared according to the general procedure **B** using the following quantities: **NatD1-mp** (1.8 g, 1.86 mmol) in TFA (15 mL) to afford **NatD1-m** (1.44 g, 98%) as an orange powder as a mixture of α- and β-pyranosic anomers in a ratio of 49:51 calculated on the basis of the relative C-1 signal intensities.^{1}H NMR (200 MHz, Acetone). δ = 8.43 (m, 2 H, Ar-H, both anomers), 8.28–7.88 (m, 4 H, Ar-H, both anomers), 7.77–7.61 (m, 3 H, Ar-H, both anomers), 7.19 (m, 2 H, Ar-H, both anomers), 5.10, 4.55 (m, 2 H, both anomers), 4.31–4.16 (m, 4 H, both anomers), 3.88–3.79 (m, 6 H, both anomers), 3.77–3.52 (m, 4 H, PhOCH_3, C*H*, both anomers), 3.44–3.41 (m, 2 H, both anomers) ppm. ^{13}C NMR (50 MHz, acetone): δ = 164.88 (*C*O), 159.22 (Ar-C), 157.13 (Ar-C), 155.50 (Ar-C), 154.13 (Ar-C), 148.77 (Ar-C), 147.65 (Ar-C), 144.35 (Ar-C), 125.18 (Ar-C), 124.69 (Ar-C), 123.51 (Ar-C), 118.50 (Ar-C), 117.65 (Ar-C), 115.64 (Ar-C), 114.96 (Ar-C), 105.55 (*C*H), 56.58 (PhOCH$_3$) ppm; see Table 8 for the glycidic moiety. UV/Vis: λ_{max} = 416 nm; ε = 20111 M^{-1}cm^{-1}. MS (ESI): *m*/*z* = 803.71 [M + 1]$^+$. $C_{34}H_{38}N_6O_{17}$ (802.70): calcd. C 50.87, H 4.77; found C 50.74, H 4.71.

Synthesis of 2-[4-({2′-Methoxy-4′-[(4′′-nitrophenyl)azo]phenyl}azo)-phphenoxy]malonic Di-6′-aminolactose Diamide (NatD1-b): The product **NatD1-b** was prepared according to the general procedure **B** using the following quantities: **NatD1-bp** (1.50 g, 1.03 mmol) in TFA (15 mL) to afford **NatD1-b**(1.13 g, 98%) as an orange powder as a mixture of α- and β-pyranosic anomers in a ratio of 40:60 calculated on the basis of the relative C-1 signal intensities. ^{1}H NMR (200 MHz, D_2O): δ = 8.35 (m, 2 H, Ar-H, both anomers), 8.18–7.89 (m, 4 H, Ar-H, both anomers), 7.81–7.64 (m, 3 H, Ar-H, both anomers), 7.74 (m, 2 H, Ar-H, both anomers), 5.08, 4.57 (3 m, 4 H, both anomers), 4.24–4.17 (m, 8 H, both anomers), 3.91–3.81 (m, 12 H, both anomers), 3.75–3.49 (m, 4 H, PhOCH_3, C*H*, both anomers), 3.43–3.40 (m, 4 H, both anomers) ppm. ^{13}C NMR (50 MHz, D_2O): δ = 164.77 (2 *C*O, both anomers), 159.31 (Ar-C, both anomers), 157.22 (Ar-C, both anomers), 155.59 (Ar-C, both anomers), 154.15 (Ar-C, both anomers), 148.88 (Ar-C, both anomers), 148.65 (Ar-C, both anomers), 144.43 (Ar-C, both anomers), 125.19 (Ar-C, both anomers), 124.65 (Ar-C, both anomers), 123.91 (Ar-C, both anomers), 118.68 (Ar-C, both anomers), 117.60 (Ar-C, both anomers), 115.55 (Ar-C, both anomers), 114.66 (Ar-C, both anomers), 105.64 (*C*H, both anomers), 56.58 (PhO*C*H$_3$, both anomers) ppm; see Table 8 for the glycidic moiety. UV/Vis: λ_{max} = 416 nm; ε = 20183 M^{-1}cm^{-1}. MS (ESI): *m*/*z* = 1126.01 [M + 1]$^+$. $C_{46}H_{59}N_7O_{26}$ (1126.00): calcd. C 49.07, H 5.28; found C 48.97, H 5.19.

Synthesis of NatD1mp: The product **NatD1mp** was prepared according to the general procedure D using the following quantities: **NatD1-mp** (0.50 g, 0.51 mmol), NMM (0.08 mL. 0.77 mmol), DMTMM (0.23 g, 0.77 mmol) and galactose derivative **8** (0.26 g, 0.51 mmol) in THF (20 mL). The crude adduct was purified by FCC (EtOAc/petroleum ether, 2:1, R_f = 0.49) to afford **NatD1mp** (0.47 g, 67%) as an orange solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.43 (m, 2 H, Ar-H), 8.19–7.99 (m, 4 H, Ar-H), 7.73–7.62 (m, 3 H, Ar-H), 7.22 (m, 2 H, Ar-H), 5.52 (d, J_{HH} = 5.00 Hz, 1 H), 4.68–4.52 (m, 3 H), 4.38–4.36 (m, 2 H), 4.27–4.25 (m, 3 H), 4.17–3.91 (m, 7 H), 3.76–3.55 (m, 9 H), 3.41–3.40 (2 s, 6 H, 2 OCH_3), 1.51–1.21 [various singlets, 30 H, 5 C(CH_3)$_2$] ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 169.05 (2 *C*O), 159.77 (Ar-C), 157.54 (Ar-C), 155.11 (Ar-C), 154.50 (Ar-C), 148.85 (Ar-C), 148.14 (Ar-C), 144.31 (Ar-C), 125.25 (Ar-C), 124.68 (Ar-C), 123.55 (Ar-C), 118.33 (Ar-C), 117.41 (Ar-C), 115.17 (Ar-C), 110.47, 110.24, 109.28, 108.84, 108.57 [5 *C*(CH$_3$)$_2$], 105.12 (C-1), 104.97 (*C*H), 100.11 (C-1′), 96.61 (C-1′′), 79.08, 77.27, 77.08 (C-3, C-5, C-3′), 75.61 (C-2), 76.45, 73.54, 73.21 (C-4, C-4′), 72.39 (C-2′), 70.88, 70.42 (C-2′′, C-4′′), 70.11 (C-3′′, C-5′), 67.77 (C-5′′), 64.72 (C-6), 40.77, 40.38 (C-6′,

C-6′′), 55.14 (PhOCH_3), 54.67 (2 OCH_3), 28.54, 28.27, 27.68, 27.32, 26.62, 26.44, 25.87, 25.35, 24.61, 24.51 [C(CH_3)$_2$] ppm. UV/Vis: λ_{max} = 416 nm; ε = 20189 $M^{-1}cm^{-1}$. MS (ESI): *m*/*z* = 1210.53 [M + 1]$^+$. $C_{57}H_{75}N_7O_{22}$ (1210.25): calcd. C 56.57, H 6.25; found C 56.51, H 6.18.

Synthesis of 2-[4-({2′-Methoxy-4′-[(4′′-nitrophenyl)azo]phenyl}azo)-phphenoxy]malonic 6′-Amino-lactose 6′-Aminogalactose Diamide (NatD1mx): The product **NatD1mx** was prepared according to the general procedure B using the following quantities: **NatD1mp** (0.3 g, 0.24 mmol) in TFA (7 mL) to afford **NatD1mx** (0.23 g, 95%) as an orange powder as a complex mixture of anomeric forms of either the lactose or galactose moiety. Selected ^{1}H NMR (200 MHz, D_2O): δ = 8.39 (m, 2 H, Ar-H), 8.21–7.89 (m, 4 H, Ar-H), 7.71–7.52 (m, 3 H, Ar-H), 7.19 (m, 2 H, Ar-H), 5.11–4.68 (m, 4 H), 4.31–4.21 (m, 6 H), 4.09–3.61 (m, 8 H), 3.76–3.40 (m, 7 H, both anomers) ppm. ^{13}C NMR (50 MHz, D_2O): δ = 163.55–163.11 (2 *C*O, both anomers), 159.23 (Ar-C, both anomers), 157.05 (Ar-C, both anomers), 155.12 (Ar-C, both anomers), 154.54 (Ar-C, both anomers), 148.44 (Ar-C, both anomers), 147.17 (Ar-C, both anomers), 144.11 (Ar-C, both anomers), 125.10 (Ar-C, both anomers), 124.35 (Ar-C, both anomers), 123.74 (Ar-C, both anomers), 117.57 (Ar-C, both anomers), 117.78 (Ar-C, both anomers), 114.00 (Ar-C, both anomers), 114.41 (Ar-C, both anomers), 104.42 (*C*H, both anomers), 102.50, 102.25 (C-1′, α- and β-pyranose), 101.10 (C-1′′, β-furanose), 97.44 (C-1′′, β-pyranose), 96.74 (C-1, β-pyranose), 92.88 (C-1′′, α-pyranose), 92.10 (C-1, α-pyranose), 82.75 (C-4′′, β-furanose), 81.54 (C-2′′, β-furanose), 80.88 (C-4, α- and β-pyranose), 55.77 (PhOCH_3, both anomers) ppm. UV/Vis: λ_{max} = 416 nm; ε = 20287 $M^{-1}cm^{-1}$. MS (ESI): *m*/*z* = 964.88 [M + 1]$^+$. $C_{40}H_{49}N_7O_{21}$ (963.86): calcd. C 49.84, H 5.12; found C 49.77, H 5.18.

Synthesis of 2,3:5,6-Bis-*O*-(1-methylethylidene)-4-*O*-[3,4-*O*-(1-methphylethylidene)]-6-*O*-[(azido)-β-D-galactopyranosyl]-aldehydo-D-glucose Dimethyl Acetal (6): The derivative **6** was prepared from the derivative **5** (1 mol) following the procedure reported in the literature[15b] using NaN_3 (1.5 mol) in DMSO (20 mL). The crude product was purified by FCC (AcOH/petroleum ether, 2:1, R_f = 0.54) to afford **6** in 81% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.60 (d, *J* = 7.9 Hz, 1 H), 4.53 (dd, *J* = 6.2, *J* = 7.8 Hz, 1 H), 4.28 (m, 1 H), 4.19 (m, 1 H), 4.17–3.96 (m, 6 H), 3.77–3.37 (m, 10 H), 1.40, 1.28, 1.20 (3 s, 18 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 109.78, 109.15, 108.94 [*C*(CH_3)$_2$], 56.32 [C(O*C*H$_3$)$_2$], 28.77, 27.33, 26.24, 26.34, 25.24, 24.57 [C(*C*H$_3$)$_2$] ppm; see Table 7 for the glycidic moiety. MS (ESI): *m*/*z* = 534.59 [M + 1]$^+$. $C_{23}H_{39}N_3O_{11}$: calcd. C 51.77, H 7.37; found C 51.69, H 7.31.

Synthesis of 2,3:5,6-Bis-*O*-(1-methylethylidene)-4-*O*-[3,4-*O*-(1-methphylethylidene)]-6-*O*-[(amino)-β-D-galactopyranosyl]-aldehydo-D-glucose Dimethyl Acetal (7): The derivative **7** was prepared from the derivative **6** following the procedure reported in the literature[15b] using 10% Pd in MeOH (10 mL) The crude reaction product was purified by precipitation from diethyl ether to afford **7** in 96% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.55 (d, *J* = 8.0 Hz, 1 H), 4.50 (dd, *J* = 6.2, *J* = 7.9 Hz, 1 H), 4.29 (m, 1 H), 4.19 (m, 1 H), 4.17–3.95 (m, 6 H), 3.77–3.38 (m, 10 H), 1.41–1.20 (3 s, 18 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 110.10, 109.59, 108.16 [*C*(CH_3)$_2$], 57.37 [C(O*C*H$_3$)$_2$], 28.16, 27.10, 26.43, 26.30, 25.86, 24.26 [Cph(*C*H$_3$)$_2$] ppm; see Table 7 for the glycidic moiety. MS (ESI): *m*/*z* = 508.39 [M + 1]$^+$. $C_{23}H_{41}NO_{11}$ (507.58): calcd. C 54.45, H 8.15; found C 54.39, H 8.04.

***N*-(4-Hydroxy-9,10-dioxo-2-phenoxyanthracen-1-yl)benzamide (D2-Bz):** A mixture of **D2** (1.0 g, 3.02 mmol) in pyridine (10 mL) was added with benzoyl chloride (0.35 mL, 3.02 mmol) at 0 °C and the mixture was stirred at room temperature for several hours. TLC analysis (EtOAc/petroleum ether, 3:2) revealed the complete disappearance of the starting material (R_f = 0.23) and the formation of a major faster-moving product (R_f = 0.54). The pyridine was removed under reduced pressure and the crude product was washed with a 10% HCl solution (20 mL) and extracted with DCM (3 × 20 mL). The organic phase was dried with Na_2SO_4, concentrated under reduced pressure and the resulting residue was purified by flash chromatography (EtOAc/petroleum ether, 3:2) to yield **D2-Bz** (1.15 g, 88%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.35–8.22 (m, 2 H, Ar-H), 7.8–7.7 (m, 2 H, Ar-H), 7.5–7.0 (m, 8 H, Ar-H), 6.78–6.75 (m, 2 H, Ar-H), 6.34 (m, 1 H, Ar-H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 186.23–185.37 (2 *C*O), 166.47 (NH*C*O), 160.85, 154.08, 134.28, 134.24, 133.72, 132.93, 132.20, 130.14, 129.50, 129.30, 128.69, 128.41, 127.98, 127.26, 126.58, 125.66, 121.07, 110.43, 110.29 (Ar-C) ppm. MS (ESI): *m*/*z* = 436.21 [M + 1]$^+$. $C_{27}H_{17}NO_5$ (435.44): calcd. C 74.48, H 3.94; found C 74.38, H 3.87.

***N*-[4-(5′-Bromopentoxy)-9,10-dioxo-2-phenoxyanthracen-1-yl]benzphamide (D2-Eth):** K_2CO_3 (1.25 g, 9.19 mmol) was added to a solution of disperse dye **D2-Bz** (1.0 g, 2.29 mmol) in acetone (25 mL) and the resulting mixture was stirred at room temperature for 1.5 h. Then 1,5-dibromopentane (0.94 mL, 6.88 mmol) and a catalytic quantity of 18-crown-6 were added and the resulting solution was heated at reflux for 20 h. At the end of the reaction, the resulting solution was filtered, diluted with water (40 mL) and the aqueous solution was extracted with chloroform (4 × 30 mL). Then the organic phase was dried with Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue (obtained as a red solid) was purified by flash chromatography (ethyl acetate/petroleum ether, 1:2, R_f = 0.59) to afford **D2-Eth** (0.99 g, 74%) as a yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.35–8.22 (m, 2 H, Ar-H), 7.8–7.7 (m, 2 H, Ar-H), 7.5–7.0 (m, 8 H, Ar-H), 6.78–6.75 (m, 2 H, Ar-H), 6.34 (m, 1 H, Ar-H), 3.45 (m, 4 H, O*C*H$_2$, *C*H$_2$Br), 1.94–1.85 (m, 2 H, *C*H$_2$CH$_2$Br), 1.84, 1.42 (2 × *C*H$_2$) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 187.14–181.88 (2 × *C*O), 164.06 (NH*C*O), 162.27, 153.00, 136.85, 134.65, 134.07, 132.19, 130.43, 129.62, 127.57, 127.52, 127.23, 126.38, 126.23, 120.75, 111.47, 108.764, 108.68 (Ar-C), 68.24 (O*C*H$_2$), 33.86 (*C*H$_2$Br), 32.69 (*C*H$_2$CH$_2$Br), 27.07 (OCH$_2$*C*H$_2$), 26.10 (CH$_2$*C*H$_2$CH$_2$) ppm. MS (ESI): *m*/*z* = 584.13 [M + 1]$^+$. $C_{32}H_{26}BrNO_5$ (584.47): calcd. C 65.76, H 4.48; found C 65.69, H 4.47

Diethyl 2-[5-(4-Benzoylamino-9,10-dioxo-3-phenoxyanthracen-1-ylphoxy)pentyl]malonate (D2-mal). General Procedure E: A mixture of 60% NaH (0.07 g, 1.71 mmol) in THF (2 mL) was added slowly at 0 °C to a solution of **D2-Eth** (1.00 g, 1.71 mmol) in THF (10 mL) and the resulting mixture was stirred at room temperature for 30 min. Diethyl malonate (0.28 mL, 1.71 mmol) was added and the mixture was stirred at reflux for 20 h. TLC analysis (EtOAc/petroleum ether, 2:5) revealed the disappearance of the starting material (R_f = 0.31) and the formation of a major product (R_f = 0.63). The crude solution was diluted with chloroform (15 mL) and washed with water (3 × 20 mL). The organic phase was dried with Na_2SO_4, concentrated under reduced pressure and the resulting residue was purified by flash chromatography (EtOAc/petroleum ether, 2:5, R_f = 0.63) to yield **D2-Mal** (1.13 g, 88%) as a red crystal. ^{1}H NMR (200 MHz, $CDCl_3$): this spectrum is identical to that of **D2-eth**, but has some more signals due to the presence of the malonic moiety: δ = 4.11 (q, *J* = 7.20 Hz, 4 H, 2*C*H$_2$CH$_3$), 3.75 (m, 1 H, C*H*), 3.31 (m, 2 H, OC*H*$_2$), 1.97–1.86 (m, 4 H, OCH$_2$C*H*$_2$, C*H*$_2$CH), 1.41–1.34 (m, 4 H, 2 × C*H*$_2$), 1.24 (t, *J* = 7.20 Hz, 6 H, 2 C*H*$_3$) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 187.15, 181.85 (2 *C*O), 170.55, 169.24, 164.06 (NH*C*O), 162.35, 153.00, 136.98, 134.60, 134.11, 132.01,

132.20, 130.39, 129.54, 129.22, 128.29, 127.59, 127.48, 127.23, 126.36, 126.17, 120.82, 111.45, 108.65, 108.38 (Ar-C), 68.00 (OCH_2), 61.29 (CH_2CH_3), 50.23 (*C*H), 28.83 (OCH_2CH_2), 27.63 (CH_2CH), 27.37 (CH_2CH_2CH), 27.16 ($CH_2CH_2CH_2CH_2$) ppm. MS (ESI): *m*/*z* = 664.33 $[M + 1]^+$. $C_{39}H_{37}NO_9$ (663.72): calcd. C 70.58, H 5.62; found C 70.44, H 5.57.

2-[5-(4-Benzoylamino-9,10-dioxo-3-phenoxyanthracen-1-yloxy)penphtyl]malonic Acid (D2-ac): The product **D2-ac** was prepared according to the general procedure C using the following quantities: **D2-mal** (1.00 g, 1.51 mmol), dioxane (10 mL), 1 N KOH (10 mL) to obtain **D2-ac** (0.86 g, 97%) as a yellow solid. R_f = (EtOAc) = 0.1. ^1H NMR (200 MHz, $CDCl_3$): δ = 8.38–8.29 (m, 2 H, Ar-H), 8.11–7.87 (m, 2 H, Ar-H), 7.64–7.29 (m, 8 H, Ar-H), 6.87 (m, 2 H, Ar-H), 6.25 (m, 1 H, Ar-H), 4.15 (m, 1 H, C*H*), 3.34 (m, 2 H, OCH_2), 1.96–1.84 (m, 4 H, OCH_2CH_2, CH_2CH), 1.42–1.35 (m, 4 H, $2\times CH_2$) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 187.49, 181.97 ($2\times CO$), 169.84, 169.41, 163.86 (3 *C*O), 162.51, 153.25, 137.44, 134.85, 134.39, 134.16, 132.19, 132.56, 130.56, 129.73, 129.32, 128.25, 127.42, 127.34, 127.25, 126.22, 126.04, 120.89, 111.58, 107.94, 107.88 (Ar-C), 71.22, 67.28 (OCH_2), 51.25 (*C*H), 27.95 (OCH_2CH_2), 27.66 (CH_2CH), 27.23 (CH_2CH_2CH), 27.08 ($CH_2CH_2CH_2CH_2$) ppm. MS (ESI): *m*/*z* = 608.61 $[M + 1]^+$. $C_{35}H_{29}NO_9$ (607.62): calcd. C 69.19, H 4.81; found C 69.08, H 4.75.

Synthesis of NatD2-p: The product **NatD2-p** was prepared according to the general procedure D using the following quantities: **D2-ac** (0.70 g, 1.16 mmol), NMM (0.38 mL, 3.34 mmol), DMTMM (0.94 g, 3.34 mmol) and protected amino-lactose **7** (1.18 g, 2.32 mmol) in THF (25 mL). The product was purified by FCC (EtOAc/petroleum ether, 2:5, R_f = 0.37) to afford **NatD2-p** (1.80 g, 97%) as a yellow solid ^1H NMR (200 MHz, $CDCl_3$): δ = 8.30–8.19 (m, 2 H, Ar-H), 7.81–7.72 (m, 2 H, Ar-H), 7.43–7.15 (m, 8 H, Ar-H), 6.84 (m, 2 H, Ar-H), 6.31 (m, 1 H, Ar-H), 4.69 (m, 2 H), 4.52 (m, 2 H), 4.38 (m, 2 H), 4.35–4.22 (m, 2 H) 4.17–3.95 (m, 13 H), 3.75–3.48 (m, 10 H), 3.43–3.42 (2 s, 12 H, 2 OCH_3), 1.88–1.65 (m, 4 H, OCH_2CH_2, CH_2CH), 1.49, 1.44, 1.42, 1.39 [4 s, 24 H, 4 $C(CH_3)_2$], 1.36–1.27 [m, 16 H, 2 CH_2, $C(CH_3)_2$] ppm. ^{13}C NMR (50 MHz, $CDCl_3$): 187.14, 181.98 (2 *C*O), 170.97, 170.77, 164.08 (3 *C*O), 162.29, 152.94, 136.68, 134.67, 134.42, 134.10, 132.19, 130.42, 129.64, 129.18, 128.33, 127.59, 127.16, 127.41, 125.81, 120.79, 111.44, 110.18, 108.66 (Ar-C), 108.42, 107.88, 107.21 [$C(CH_3)_2$], 68.80 (OCH_2), 54.92 [$C(OCH_3)_2$], 50.33 (*C*H), 33.92, 31.07, 29.05, 28.28, 27.69, 27.40, 27.10, 26.42, 24.84, 24.11 [$C(CH_3)_2$, CH_2] ppm; see Table 7 for the glycidic moiety. UV/Vis: λ_{max} = 415 nm; ε = 21597 $M^{-1}cm^{-1}$. MS (ESI): *m*/*z* = 1586.88 $[M + 1]^+$. $C_{81}H_{107}N_3O_{29}$ (1586.74): calcd. C 61.31, H 6.80; found C 61.25, H 6.71.

Synthesis of NatD2: The product **NatD2** was prepared according to the general procedure **B** using the following quantities: **NatD2-p** (1.0 g, 0.63 mmol) in TFA (15 mL) to afford **NatD2** (0.78 g, 98%) as a yellow powder as a mixture of α- and β-pyranosic anomers in the ratio of 50:50 calculated on the basis of the relative C-1 signal intensities. Selected ^1H NMR (200 MHz, D_2O): δ = 8.35–8.27 (m, 2 H, Ar-H, both anomers), 7.81–7.69 (m, 2 H, Ar-H, both anomers), 7.41–7.11 (m, 8 H, Ar-H, both anomers), 6.88 (m, 2 H, Ar-H, both anomers), 6.29 (m, 1 H, both anomers), 5.11–4.56 (m, 4 H, both anomers), 4.33–4.23 (m, 9 H, both anomers), 3.99–3.79 (m, 12 H, both anomers), 3.75–3.40 (m, 6 H, both anomers), 1.87–1.79 (m, 4 H, OCH_2CH_2, CH_2CH, both anomers), 1.48–1.36 (m, 4 H, 2 CH_2, both anomers) ppm. ^{13}C NMR(50 MHz, D_2O): δ = 187.49–181.97 (2 *C*O, both anomers), 169.84–169.41, 163.86 (*C*O), 162.51, 153.25, 137.44, 134.39, 134.16, 132.19, 130.56, 129.73, 129.32, 128.25, 127.42, 127.34, 127.25, 126.22, 126.04, 120.89, 111.58, 107.94, 107.88 (Ar-C), 67.28 (OCH_2), 51.25 (*C*H), 30.95 (OCH_2CH_2), 29.66 (CH_2CH), 28.23 (CH_2CH_2CH), 27.08 ($CH_2CH_2CH_2CH_2$) ppm; see Table 8 for the glycidic moiety. UV/Vis: λ_{max} = 415 nm; ε = 21747 $M^{-1}cm^{-1}$. MS (ESI): *m*/*z* = 1254.43 $[M + 1]^+$. $C_{59}H_{71}N_3O_{27}$ (1254.21): calcd. C 56.50, H 5.71; found C 56.42, H 5.65.

***tert*-Butyl 2-Chloroethylcarbamate (10):** A solution of $NaHCO_3$ (8.77 g, 104.34 mol) in water (15 mL) was added dropwise to a mixture of the 2-chloroethylamine hydrochloride (**9**) (2.49 g, 11.39 mmol), di-*tert*-butyl dicarbonate (9.68 g, 20.86 mmol) and 1,4-dioxane (10 mL) at 0 °C and the mixture was stirred at 0 °C for a further 18 h. After this time, the mixture was diluted with water (25 mL) and then extracted with DCM (3×25 mL). The combined organic phases were dried, filtered and concentrated to leave a crude brown oil. This oil was purified by column chromatography (EtOAc/petroleum ether, 3:1, R_f = 0.68) to give **10** (1.98 g; 97%) as a colourless oil. ^1H NMR (200 MHz, $CDCl_3$): δ = 3.56 (t, *J* = 4.8 Hz, 2 H, CH_2Cl), 3.42 (t, *J* = 4.8 Hz, 2 H, CH_2CH_2Cl), 1.39 (s, 9 H, 3 CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 155.52 (*C*O), 79.75 [$C(CH_3)_3$], 44.22 (CH_2Cl), 42.61 (CH_2 CH_2Cl), 28.46 [$(CH_3)_3$] ppm. MS (ESI): *m*/*z* = 180.24 $[M + 1]^+$. $C_7H_{14}ClNO_2$ (179.65): calcd. C 46.80, H 7.86; found C 46.78, H 7.82.

Diethyl 2-[2-(*tert*-Butoxycarbonylamino)ethyl]malonate (11): The product **11** was prepared according to the general procedure A using the following quantities: diethyl malonate (1.30 mL, 8.38 mmol), sodium hydride 60% (0.34 g, 8.38 mmol) and **10** (1.5 g, 8.39 mmol) in dry THF (25 mL). The crude was purified by FCC (EtOAc/petroleum ether, 3:1, R_f = 0.35) to afford **11** (1.95 g, 84%) as a yellow oil. ^1H NMR (200 MHz, $CDCl_3$): δ = 4.12 (q, *J* = 7.2 Hz, 4 H, 2 CH_2CH_3), 3.14 (m, 3 H, C*H*CO, CONHCH_2), 2.30 (t, *J* = 7.4 Hz, 2 H, CH_2CH), 1.39 (s, 9 H, 3 CH_3), 1.19 (t, *J* = 7.2 Hz, 6 H, 2 CH_2CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 168.36 (2 *C*O), 149.44 (*C*ONH), 82.73 [$C(CH_3)_3$], 61.46 (2 CH_2CH_3), 50.09 (*C*HCO), 44.81 (CH_2CH_2CH), 27.84 [$(CH_3)_3$], 21.51 (CH_2CH), 14.04 (2 CH_2CH_3) ppm. MS (ESI): *m*/*z* = 304.35 $[M + 1]^+$. $C_{14}H_{25}NO_6$ (303.35): calcd. C 55.43, H 8.31; found C 55.38, H 8.19.

Diethyl 2-(2-Aminoethyl)malonate (12): Trifluoroacetic acid (90%) was cooled to –15 °C and then **11** (1.00 g, 3.30 mmol-%) was added neat. The solution was then stirred at –15 °C for 3 h. After this time, the mixture was transferred slowly using a cannula into a saturated aqueous solution of NH_4OH cooled to –10 °C and then extracted with DCM. The combined organic phases were dried, filtered and concentrated under reduced pressure. The residue was purified by precipitation in water to afford **12** (0.66, 91%). ^1H NMR (200 MHz, $CDCl_3$): δ = 4.01 (q, *J* = 7.2 Hz, 4 H, 2 CH_2CH_3), 3.21 (m, 3 H, C*H*, CONHCH_2), 2.39 (t, *J* = 7.3 Hz, 2 H, CH_2CH), 1.19 (t, *J* = 7.2 Hz, 6 H, 2 CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 168.48 (2 *C*O), 61.49 (2 CH_2CH_3), 50.15 (*C*HCO), 44.80 (CH_2CH_2CH), 21.60 (CH_2CH), 14.04 (2 CH_2CH_3) ppm. MS (ESI): *m*/*z* = 204.41 $[M + 1]^+$. $C_9H_{17}NO_4$ (203.24): calcd. C 53.19, H 8.43; found C 53.07, H 8.32.

3-(*N*-Ethyl-*N*-{4′-[(4′′-nitrophenyl)azo]phenyl}amino)propanoic Acid (D3-ac): A 37% solution of HCl (l0 mL) was added to a solution of **D3** (3 g, 9.23 mmol) in AcOH (15 mL) and the resulting mixture was stirred at 90 °C for 3 h. The acid was removed under reduced pressure and the residue was dissolved in DCM (30 mL). The organic solution was washed with an aqueous solution of 3 N NaOH (3×25 mL) and the aqueous solution was acidified with a 1 N HCl solution to pH = 2 and extracted with DCM (3×30 mL). The organic solution was dried with Na_2SO_4 and filtered. The solvent was removed under reduced pressure to afford **D3-ac** (3.1 g, 98%) as a red solid. ^1H NMR (200 MHz, [D_6]acetone): δ = 8.38,

6.90 (AA′XX′ system, 4 H, Ar-H), 7.91, 7.84 (AA′XX′ system, 4 H, Ar-H), 3.85 (t, J = 6.8 Hz, 2 H, NCH_2CH$_2$), 3.65 (q, J = 7.1 Hz, 2 H, NCH_2CH$_3$), 2.72 (t, J = 6.8 Hz, 2 H, CH_2CO), 1.24 (t, J = 7.1 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, [D$_6$]acetone): δ = 169.69 (CO), 152.25 (Ar-C), 150.90 (Ar-C), 146.70 (Ar-C), 143.66 (Ar-C), 126.87–126.24 (Ar-C), 124.90 (Ar-C), 122.15 (Ar-C), 113.47–111.32 (Ar-C), 47.19 (NCH$_2$CH$_2$), 46.56 (NCH$_2$CH$_3$), 34.51 (NCH$_2$CH$_2$), 12.36 (CH$_3$) ppm. MS (ESI): m/z = 343.34 [M + 1]$^+$. $C_{17}H_{18}N_4O_4$ (342.35): calcd. C 59.64, H 5.30; found C 59.61, H 5.27.

3-Ethyl-(4-azo-4′-nitrophenylphenylamino)propanamide Diethyl Malonate (D3-mal): NEt$_3$ (0.62 mL, 4.38 mmol) and ethyl chloroformate (0.42 mL, 4.38 mmol) were added to a solution of **D3-ac** (1.5 g, 2.92 mmol) in dry THF (25 mL), and the resulting mixture was stirred at 0 °C for 30 min. The derivative **11** (0.59 g, 2.92 mmol) was added and the resulting solution was stirred at room temperature for 20 h. TLC analysis (EtOAc/petroleum ether, 1:2) revealed the formation of a major product (R_f = 0.67). The crude solution was diluted with chloroform (15 mL) and washed with water (3 × 20 mL). The organic phase was dried with Na_2SO_4, concentrated under reduced pressure and the resulting residue was purified by flash chromatography (EtOAc/petroleum ether, 1:2, R_f = 0.67) to yield **D3-mal** (1.74 g, 89%) as an orange solid. ^{1}H NMR (200 MHz, CDCl$_3$): δ = 8.36–7.95 (AA′XX′ system, 4 H, Ar-H), 7.95–6.82 (AA′XX′ system, 4 H, Ar-H), 4.22 (q, J = 7.3 Hz, 4 H, 2 CH_2CH$_3$), 3.88 (t, J = 6.9 Hz, 2 H, NCH_2CH$_2$), 3.64–3.49 (m, 5 H, NCH_2CH$_3$, NHCH_2, CHCO), 2.61 (t, J = 6.9 Hz, 2 H, NCH$_2$CH_2), 2.31 (m, 2 H, CH_2CHCO), 1.26 (m, 9 H, 3 CH$_3$) ppm. ^{13}C NMR (50 MHz, CDCl$_3$): δ = 170.67 (CO), 156.52 (Ar-C), 150.98 (Ar-C), 147.20 (Ar-C), 143.54 (Ar-C), 126.66–126.24 (Ar-C), 124.31 (Ar-C), 122.39 (Ar-C), 113.31–111.96 (Ar-C, 61.92 (2 CH$_2$CH$_3$), 55.86 (CH), 46.14 (NCH$_2$CH$_2$), 45.50 (NCH$_2$CH$_3$), 34.65 (CH$_2$CH$_2$CH), 31.31 (NCH$_2$CH$_2$), 29.82 (CH$_2$CH), 14.68 (2 CH$_2$CH$_3$), 12.55 (CH$_3$) ppm. MS (ESI): m/z = 528.42 [M + 1]$^+$. $C_{26}H_{33}N_5O_7$: calcd. C 59.19, H 6.30; found C 59.07, H 6.21.

3-Ethyl-(4-azo-4′-nitrophenylphenylamino)propanamide Malonic Acid (D3-Diac): The product **D3-Mal-diac** was prepared according to the general procedure **C** using the following quantities: **D3-mal** (1.70 g, 3.22 mmol) in dioxane (10 mL), a solution of 1 N KOH (10 mL) to afford **D3-diac** (1.50 g, 98%) as a red crystal. ^{1}H NMR (200 MHz, CDCl$_3$): δ = 8.37, 7.93 (AA′XX′ system, 4 H, Ar-H), 7.87, 6.75 (AA′XX′ system, 4 H, Ar-H), 3.88 (t, J = 6.8 Hz, 2 H, NCH_2CH$_2$), 3.63–3.49 (m, 5 H, NCH_2CH$_3$, NHCH_2, CHCO), 2.62 (t, J = 6.8 Hz, 2 H, NCH$_2$CH_2), 2.31 (m, 2 H, CH_2CHCO),1.24 (m, J = 7.0 Hz, 3 H, CH$_3$) ppm. ^{13}C NMR (50 MHz, CDCl$_3$): δ = 170.47 (CO), 156.52 (Ar-C), 150.98 (Ar-C), 147.21 (Ar-C), 143.67 (Ar-C), 126.66–126.26 (Ar-C), 124.61 (Ar-C), 122.38 (Ar-C), 113.33–111.96 (Ar-C), 55.86 (CH), 46.14 (NCH$_2$CH$_2$), 45.50 (NCH$_2$CH$_3$), 34.66 (CH$_2$CH$_2$CH), 31.24 (NCH$_2$CH$_2$), 29.82 (CH$_2$CH), 12.67 (CH$_3$) ppm. MS (ESI): m/z = 472.23 [M + 1]$^+$. $C_{22}H_{25}N_5O_7$ (471.47): calcd. C 56.05, H 5.34; found C 56.01, H 5.22.

Synthesis of NatD3-p: The product **NatD3-p** was prepared according to the general procedure D using the following quantities: **D3-diac** (1.90 g, 4.03 mmol), *N*-methylmorpholine (0.9 mL, 8.06 mmol), DMTMM (2.23 g, 8.06 mmol) and amino-lactose **7** (47.11 g, 8.06 mmol) in THF (15 mL) to afford **NatD3-p** (5.32 g, 92%) as a red crystal (R_f = 0.35, EtOAc/petroleum ether, 5:2). ^{1}H NMR (200 MHz, CDCl$_3$): δ = 8.33–7.87 (AA′XX′ system, 4 H, Ar-H), 7.87–6.75 (AA′XX′ system, 4 H, Ar-H), 4.67 (m, 2 H), 4.53 (m, 2 H), 4.33 (m, 2 H), 4.30–4.17 (m, 2 H), 3.82–3.43 (m, 15 H), 3.55–3.39 (m, 12 H), 3.37 (s, 12 H, 4 OCH_3), 2.64 (t, J = 6.9 Hz, 2 H, NHCH$_2$CH_2), 2.29 (m, 2 H, CH_2CHCO), 1.48–1.24 [m, 39 H, C(CH_3)$_3$, CH_3] ppm. ^{13}C NMR (50 MHz, CDCl$_3$): δ = 172.66 (2 CO), 170.76 (CO), 156.50 (Ar-C), 150.07 (Ar-C), 147.26 (Ar-C), 143.52 (Ar-C), 126.27 (Ar-C), 124.57 (Ar-C), 122.48 (Ar-C), 111.43 (Ar-C), 110.42, 110.24, 108.40 [3 C(CH$_3$)$_3$], 56.84 [2 O(CH$_3$)$_2$], 56.49 (CH), 46.79 (NCH$_2$CH$_2$), 45.84 (NCH$_2$CH$_3$), 34.34 (CH$_2$CH$_2$CH), 31.03 (NCH$_2$CH$_2$), 29.82 (CH$_2$CH), 28.26, 27.32, 26.55, 26.41, 25.83, 24.23 [C(CH$_3$)$_2$], 12.62 (CH$_3$) ppm; see Table 7 for the glycidic moiety and. UV/Vis: λ_{max} = 457 nm; ε = 85192 M^{-1}cm^{-1}. MS (ESI): m/z = 1450.71 [M + 1]$^+$. $C_{68}H_{103}N_7O_{27}$ (1450.59): calcd. C 56.30, H 7.16; found C 56.25, H 7.04.

Synthesis of NatD3: The product **NatD3** was prepared according to the general procedure B using the following quantities: **NatD3-p** (1.3 g, 1.62 mmol) and TFA (10 mL) to give **NatD3** (1.58 g, 99%) as an orange powder as a mixture of α- and β-pyranosic anomers in a ratio of 40:60 calculated on the basis of the relative C-1 signal intensities. Selected ^{1}H NMR (200 MHz, D$_2$O): δ = 8.37–7.91 (AA′XX′ system, 4 H, Ar-H, both anomers), 7.90–6.77 (AA′XX′ system, 4 H, Ar-H, both anomers), 5.07–4.59 (m, 4 H, both anomers), 4.24–4.17 (m, 8 H, both anomers), 3.89–3.76 (m, 14 H, both anomers), 3.62 (m, 9 H, both anomers), 2.63 (t, J = 7.00 Hz, 2 H, NHCH$_2$CH_2, both anomers), 2.29 (m, 2 H, CH_2CHCO, both anomers), 1.22 (m, J = 7.2 Hz, 3 H, CH$_3$, both anomers) ppm. ^{13}C NMR (50 MHz, D$_2$O$_3$): δ = 171.45–170.65 (CO), 155.55 (Ar-C), 150.98 (Ar-C), 147.20 (Ar-C), 143.54 (Ar-C), 126.66–126.24 (Ar-C), 124.61 (Ar-C), 122.39 (Ar-C), 113.31–111.96 (Ar-C), 55.86 (CH), 47.07, 43.14 (NCH$_2$CH$_2$), 45.02 (NCH$_2$CH$_3$), 34.66 (CH$_2$CH$_2$CH), 31.62 (NCH$_2$CH$_2$), 29.82 (CH$_2$CH), 12.56 (CH$_3$) ppm; see Table 8 for the glycidic moiety. UV/Vis: λ_{max} = 457 nm; ε = 85585 M^{-1}cm^{-1}. MS (ESI): m/z = 1118.14 [M + 1]$^+$. $C_{46}H_{67}N_7O_{25}$ (1118.07): calcd. C 49.42, H 6.04; found C 49.33, H 6.01.

5-Bromopentyl 2-(*N*-Ethyl-*N*-{4′-[(2′′-chloro-4′′-nitrophenyl)-phazo]phenyl}amino)ethyl Ether (D4-Eth): The product **D4-Eth** was prepared according to the general procedure **A** using the following quantities: **D4** (1.00 g, 3.72 mmol), KOH (1.05 g, 18.60 mmol), 18-crown-6 ether (0.01 equiv.) and dibromopentane (1.02 mL, 7.44 mmol) in THF (25 mL). The crude product was purified by FCC (EtOAc/petroleum ether, 3:1, R_f = 0.51) to afford **D4-Eth** (1.44 g, 78%). ^{1}H NMR (200 MHz, CDCl$_3$): δ = 8.33–7.95 (AA′XX′ system, 4 H, Ar-H), 7.91–6.77 (AA′XX′ system, 3 H, Ar-H), 3.64–3.36 (m, 10 H, 2 CH$_2$O, CH$_2$CH$_2$N, CH$_3$CH$_2$N, CH$_2$Br), 1.84 (m, 2 H, CH$_2$), 1.54 [m, 4 H, (CH$_2$)$_2$], 1.25 (t, J = 7.0 Hz, 3 H, CH$_3$) ppm. ^{13}C NMR (50 MHz, CDCl$_3$): δ = 153.00 (Ar-C), 151.73 (Ar-C), 146.87 (Ar-C), 144.19 (Ar-C), 133.70 (Ar-C), 126.87 (Ar-C), 125.85 (Ar-C), 122.49 (Ar-C), 117.92 (Ar-C), 111.61 (Ar-C), 71.40 (OCH$_2$), 68.28 (CH$_2$O), 52.07 (NCH$_2$CH$_2$), 46.33 (NCH$_2$CH$_3$), 32.46 (CH$_2$Br), 29.50 (CH$_2$CH$_2$Br), 28.79 (OCH$_2$CH$_2$), 25.94 [OCH$_2$CH$_2$CH$_2$], 12.4 (CH$_3$) ppm. MS (ESI): m/z = 497.13 [M + 1]$^+$. $C_{21}H_{26}BrClN_4O_3$ calcd. C 50.67, H 5.26; found C 50.44, H 5.17.

Diethyl 2-{5′-[2′′-(*N*-Ethyl-*N*-{4′′′-[(2′′′′-chloro-4′′′′-nitrophenyl)-phazo]phenyl}amino)ethoxy]pentyl}malonate (D4-mal): The product **D4-mal** was prepared according the general procedure E using the following quantities: **D4-Eth** (1.00 g, 2.01 mmol), diethyl malonate (0.3 mL, 2.01 mmol) and 60% NaH (0.08 g, 2.01 mmol) in THF (15 mL). The product was purified by FCC (EtOAc/petroleum ether, 3:1, R_f = 0.34) to obtain **D4-mal** (0.90 g, 87%). ^{1}H NMR (200 MHz, CDCl$_3$): δ = 8.32–7.96 (AA′XX′ system, 4 H, Ar-H), 7.92–6.78 (AA′XX′ system, 3 H, Ar-H), 4.10 (q, J = 7.0 Hz, 4 H, 2 CH$_2$CH$_3$), 3.61–3.45 (m, 6 H, NCH$_2$CH$_3$, NCH$_2$CH$_3$, NCH$_2$CH$_2$), 3.34 (m, 3 H, OCH$_2$, CH), 1.94 (m, 2 H, CH$_2$CHCO),

1.52 (m, 2 H, OCH_2CH_2), 1.35 (m, 4 H, CH_2CH_2CH, $CH_2CH_2CH_2$), 1.25 (m, 9 H, $3CH_3$) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 171.61 (2 CO), 153.02 (Ar-C), 151.67 (Ar-C), 146.97 (Ar-C), 144.29 (Ar-C), 133.76 (Ar-C), 126.86 (Ar-C), 125.90 (Ar-C), 122.50 (Ar-C), 117.92 (Ar-C), 111.59 (Ar-C), 71.49 (OCH_2), 68.29 (CH_2O), 61.05 (2 CH_2CH_3), 57.33 (CH_2CH_2O), 50.65 (*C*H), 46.32 (NCH_2CH_3), 32.46 (OCH_2CH_2), 29.62 (CH_2CH), 26.56 (CH_2CH_2CH), 24.04 ($CH_2CH_2CH_2$), 14.29 (2 CH_3), 12.40 (CH_3) ppm. MS (ESI): *m*/*z* = 577.12 $[M + 1]^+$. $C_{28}H_{37}ClN_4O_7$ (577.08): calcd. C 58.28, H 6.46; found C 58.11, H 6.41.

2-{5′-[2′′-(*N*-Ethyl-*N*-{4′′′-[(2′′′′-chloro-4′′′′-nitrophenyl)azo]-phenyl}phamino)ethoxy]pentyl}malonic Acid (D4-diac): The product **D4-diac** was prepared according to the general procedure C using the following quantities: **D4-mal** (0.90 g, 1.56 mmol), dioxane (10 mL) and 1 N KOH (10 mL) to obtain **D4-diac** (0.76 g, 93%) as a red solid. R_f (EtOAc) = 0.1.^1H NMR (200 MHz, $CDCl_3$): δ = 8.33–7.95 (AA′XX′ system, 4 H, Ar-H), 7.91–6.77 (AA′XX′ system, 3 H, Ar-H), 3.71 (m, 6 H, NCH_2CH_3, NCH_2CH_3, NCH_2CH_2), 3.41 (m, 3 H, OCH_2, C*H*), 1.97 (m, 2 H, CH_2CHCO), 1.55 (m, 2 H, OCH_2CH_2), 1.39 (m. 4 H, CH_2CH_2CH, $CH_2CH_2CH_2$), 1.24 (t, *J* = 6.9 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 169.87 (2 CO), 152.90 (Ar-C), 152.44 (Ar-C), 147.09 (Ar-C), 143.87 (Ar-C), 133.02 (Ar-C), 126.75 (Ar-C), 125.60 (Ar-C), 122.87–117.94 (Ar-C), 111.71 (Ar-C), 70.88 (OCH_2), 68.36 (CH_2O), 51.24 (NCH_2CH_2), 50.33 (*C*H), 45.87 (NCH_2CH_3), 30.24 (OCH_2CH_2), 27.94 (CH_2CH), 27.11 (CH_2CH_2CH), 25.90 ($CH_2CH_2CH_2$), 11.77 (CH_3) ppm. MS (ESI): *m*/*z* = 521.27 $[M + 1]^+$. $C_{24}H_{29}ClN_4O_7$ (520.97): calcd. C 55.33, H 5.61; found C 55.21, H 5.54.

Synthesis of NatD4-p: The product **NatD4-p** was prepared according to the general procedure **D** using the following quantities: **D4-diac** (0.70 mL, 1.35 mmol), NMM (0.44 g, 4.04 mmol), DMTMM (1.12 g, 4.04 mmol) and protected amino-lactose **7** (1.37 g, 2.7 mmol) in THF (25 mL). The product was purified by FCC (EtOAc/petroleum ether, 5:1, R_f = 0.44) to afford **NatD4-p** (1.80, 97%) as an orange solid ^1H NMR (200 MHz, $CDCl_3$): δ = 8.39–7.93 (AA′XX′ system, 4 H, Ar-H), 7.93–6.78 (AA′XX′ system, 3 H, Ar-H), 4.65 (m, 2 H), 4.52 (m, 2 H), 4.33 (m, 2 H), 4.25 (m, 2 H), 4.14–3.85 (m, 12 H), 3.79–3.42 (m, 17 H), 3.40–3.39 (s, 12 H, 4 OCH_3), 1.98 (m, 2 H, CH_2CHCO), 1.57 (m, 2 H, OCH_2CH_2), 1.49–1.26 (various signals, 40 H) 1.22 (t, *J* = 7.1 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 173.09 (2 CO), 153.04 (Ar-C), 151.66 (Ar-C), 146.99 (Ar-C), 144.28 (Ar-C), 133.76 (Ar-C), 126.85 (Ar-C), 125.90 (Ar-C), 122.51–117.94 (Ar-C), 111.66 (Ar-C), 110.40, 110.19, 108.39 [$C(CH_3)_2$], 71.58, 68.28 (2 OCH_2), 53.16 (NCH_2CH_2), 50.72 (CH), 46.36 (NCH_2CH_3), 36.40 (OCH_2CH_2), 29.71 (CH_2CH), 29.42, 28.29, 27.41, 26.59, 26.41, 26.16, 25.82, 24.26 [$C(CH_3)_2$], 12.40 (CH_3) ppm; see Table 7 for the glycidic moiety. UV/Vis: λ_{max} = 483 nm; ε = 19333 $M^{-1}cm^{-1}$ ™S (ESI): *m*/*z* = 1499.77 $[M + 1]^+$. $C_{70}H_{107}ClN_6O_{27}$ (1500.09): calcd. C 56.05, H 7.19; found C 56.17, H 7.23.

Synthesis of NatD4: The product **NatD4** was prepared according to the general procedure **B** using the following quantities: **NatD4-p** (1.2 g, 0.55 mmol) and TFA (10 mL) to afford **NatD4** (1.0 g, 98%) as a red solid. ^1H NMR (200 MHz, D_2O): δ = 8.40–7.92 (AA′XX′ system, 4 H, Ar-H), 7.91–6.65 (AA′XX′ system, 3 H, Ar-H), 5.07–4.58 (m, 4 H), 4.24–4.17 (m, 8 H), 3.93–3.82 (m, 12 H), 3.66–3.56 (m, 6 H, NCH_2CH_3, NCH_2CH_3, NCH_2CH_2), 3.44–3.40 (m, 7 H), 1.96 (m, 2 H, CH_2CHCO), 1.54 (m, 2 H, OCH_2CH_2), 1.38 (m, 4 H, CH_2CH_2CH, $CH_2CH_2CH_2$), 1.26 (t, *J* = 6.8 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, D_2O): δ = 172.22, 169.86 (2 CO), 152.59 (Ar-C), 152.43 (Ar-C), 147.08 (Ar-C), 143.86 (Ar-C), 133.02 (Ar-C), 126.75 (Ar-C), 125.59 (Ar-C), 122.86–117.93 (Ar-C), 111.71 (Ar-C), 70.55, 67.92 (2 CH_2), 51.25 (NCH_2CH_2), 50.33 (*C*H), 45.88 (NCH_2CH_3), 30.25 (OCH_2CH_2), 28.33 (CH_2CH), 27.13 (CH_2CH_2CH), 25.90 (CH_2), 11.77 (CH_3) ppm; see Table 8 for the glycidic moiety. UV/Vis: λ_{max} = 483 nm; ε = 19718 $M^{-1}cm^{-1}$. MS (ESI): *m*/*z* = 1167.48 $[M + 1]^+$. $C_{48}H_{71}ClN_6O_{25}$ (1167.57): calcd. C 49.38, H 6.13; found C 49.37, H 6.07.

2-(*N*-Ethyl-*N*-{4′-[(4′′-nitrophenyl)azo]phenyl}amino)ethyl (5-Bromopentyl)] Ether (D5-Eth): The product **D5-Eth** was prepared according to the general procedure **A** using the following quantities: **D5** (1.0 g, 3.18 mmol), KOH (0.715g, 12.73 mmol), 18-crown-6 ether (0.01 equiv.) and dibromopentane (1.30 mL, 9.54 mmol) in THF (10 mL) to yield **D5-Eth** (1.15g, 78%) as a red solid. R_f (hexane/EtOAc, 5:1) = 0.79. ^1H NMR (200 MHz, $CDCl_3$): δ = 8.33–7.93 (AA′XX′ system, 4 H, Ar-H), 7.87–6.77 (AA′XX′ system, 4 H, Ar-H), 3.63–3.36 (m, 10 H, 2 CH_2O, CH_2CH_2N, CH_3CH_2N, CH_2Br), 1.84 (m, 2 H, CH_2), 1.54 [m, 4 H, $(CH_2)_2$], 1.25 (t, *J* = 7.0 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 156.62 (Ar-C), 151.47 (Ar-C), 147.12 (Ar-C), 143.41 (Ar-C), 126.29 (Ar-C), 124.37 (Ar-C), 122.46 (Ar-C), 111.40 (Ar-C), 71.25 (OCH_2), 68.46 (CH_2O), 50.51 (NCH_2CH_2), 46.14 (NCH_2CH_3), 33.88 (CH_2Br), 32.67 (CH_2CH_2Br), 29.00 (OCH_2CH_2), 25.04 ($OCH_2CH_2CH_2$), 12.4 (CH_3) ppm. MS (ESI): *m*/*z* = 463.21 $[M + 1]^+$. $C_{21}H_{27}BrN_4O_3$ (463.37): calcd. C 54.43, H 5.87; found C 54.33, H 5.76.

Diethyl 2-{5′-[2′′-(*N*-Ethyl-*N*-{4′′′-[(4′′′′-nitrophenyl)azo]phenyl}-amino)ethoxy]pentyl}malonate (D5-mal): The product **D5-Mal** was prepared according to the general procedure E using the following quantities: diethyl malonate (0.33 mL, 2.16 mmol), 60% sodium hydride (0.087g, 2.16 mmol) and **D5-Eth** (1.00g, 2.16 mmol) in dry THF (15 mL). The product was purified by FCC (EtOAc/petroleum ether, 1:5, R_f = 0.39) to afford **D5-mal** (1.042g, 89%) as a red crystal. ^1H NMR (200 MHz, $CDCl_3$): δ = 8.33–7.93 (AA′XX′ system, 4 H, Ar-H), 7.87–6.77 (AA′XX′ system, 4 H, Ar-H), 4.19 (q, *J* = 7.0 Hz, 4 H, 2 CH_2CH_3), 3.61–3.26 (m, 9 H, CH_2OCH_2, CH_2CH_2O, NCH_2CH_3, OCH_2, $CHCO$), 1.90 (m, 2 H, CH_2CHCO), 1.57 (m, 2 H, OCH_2CH_2), 1.35 (m, 4 H, CH_2CH_2CH, $CH_2CH_2CH_2$), 1.25 (m, 9 H, $3CH_3$) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 169.32 (2 CO), 156.57 (Ar-C), 151.50 (Ar-C), 147.13 (Ar-C), 143.34 (Ar-C), 126.42 (Ar-C), 124.60 (Ar-C), 122.42 (Ar-C), 111.44 (Ar-C), 71.42 (OCH_2), 68.34 (CH_2O), 61.39 (2 CH_2CH_3), 52.08 (*C*H), 50.53 (CH_2CH_2O), 46.20 (NCH_2CH_3), 29.52 (OCH_2CH_2), 28.80 (CH_2CH), 27.27 (CH_2CH_2CH), 25.96 ($CH_2CH_2CH_2$), 14.28 (2 CH_3), 12.42 (CH_3) ppm. MS (ESI): *m*/*z* = 543.42 $[M + 1]^+$. $C_{28}H_{38}N_4O_7$ (542.63): calcd. C 61.98, H 7.06; found C 61.89, H 7.01.

2-{5′-[2′′-(*N*-Ethyl-*N*-{4′′′-[(4′′′′-nitrophenyl)azo]phenyl}amino)-ethoxy]pentyl}malonic Acid (D5-diac): The product **D5-diac** was prepared according to the general procedure C using the following quantities: **D5-Mal** (1.00 g, 1.84 mmol), dioxane (10 mL) and 1 N KOH (10 mL) to obtain **D5-diac** (0.83g, 92%) as a red solid. R_f (EtOAc) = 0.1. ^1H NMR (200 MHz, $CDCl_3$): δ = 8.33–7.93 (AA′XX′ system, 4 H, Ar-H), 7.87–6.77 (AA′XX′ system, 4 H, Ar-H), 3.61–3.26 (m, 9 H, CH_2OCH_2, CH_2CH_2O, NCH_2CH_3, OCH_2, $CHCO$), 1.90 (m, 2 H, CH_2CHCO), 1.57 (m, 2 H, OCH_2CH_2), 1.35 (m, 4 H, CH_2CH_2CH, $CH_2CH_2CH_2$), 1.25 (t, *J* = 7.0 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 170.03 (2 CO), 156.13 (Ar-C), 152.20 (Ar-C), 147.11 (Ar-C), 142.99 (Ar-C), 126.59 (Ar-C), 124.58 (Ar-C), 122.19 (Ar-C), 111.95 (Ar-C), 70.91 (OCH_2), 68.34 (CH_2O), 51.32 (*C*H), 50.44 (NCH_2CH_2), 45.98 (NCH_2CH_3), 29.94 (OCH_2CH_2), 28.89 (CH_2CH), 27.13 ($CH_2CH_2CH_2$), 25.90

(CH_2CH_2CH), 11.87 (CH_3) ppm. MS (ESI): m/z = 487.31 [M + 1]$^+$. $C_{24}H_{30}N_4O_7$ (486.52): calcd. C 59.25, H 6.22; found C 59.23, H 6.19.

Synthesis of NatD5-bp: The product **NatD5-bp** was prepared according to the general procedure **D** using the following quantities: **D5-diac** (0.80 g, 2.54 mmol), NMM (0.84 g, 7.64 mmol), DMTMM (2.11 g, 7.64 mmol) and protected amino-lactose **7** (0.106 g, 4.44 mmol) in THF (20 mL). The product was purified by FCC (EtOAc/petroleum ether, 10:3, R_f = 0.39) to afford **NatD5-bp** (3.38g, 92%) as a red solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.34–7.92 (AA′XX′ system, 4 H, Ar-H), 7.91–6.87 (AA′XX′ system, 4 H, Ar-H), 4.66 (m, 2 H), 4.52 (m, 2 H), 4.33 (m, 2 H), 4.29–4.18 (m, 2 H), 4.14–3.95 (m, 12 H), 3.81–3.43 (m, 17 H), 3.42–3.38 (s, 12 H, 4 OCH_3), 1.98 (m, 2 H, CH_2CHCO), 1.55 (m, 2 H, OCH_2CH_2), 1.49–1.24 (various signals, 40 H), 1.21 (t, J = 7.0 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 172.94 (CO), 152.73 (Ar-C), 151.15 (Ar-C), 146.73 (Ar-C), 144.16 (Ar-C), 126.59 (Ar-C), 125.73 (Ar-C), 122.29 (Ar-C), 117.66–111.64 (Ar-C), 110.13, 109.92, 108.11 [$C(CH_3)_2$], 73.70, 67.81 (2 OCH_3), 55.82 [$C(OCH_3)_2$], 53.50 (CH), 50.49 (NCH_2CH_2), 46.18 (NCH_2CH_3), 36.00 (OCH_2CH_2), 29.29, 29.03, 27.9, 26.99, 26.16, 26.01, 25.76, 25.37, 23.79 [$C(CH_3)_2$, CH_3], 11.84 (CH_3) ppm; see Table 7 for the glycidic moiety. UV/Vis: λ_{max} = 501 nm; ε = 19333 $M^{-1}cm^{-1}$. MS (ESI): m/z = 1465.79 [M + 1]$^+$. $C_{70}H_{108}N_6O_{27}$: calcd. C 57.36, H 7.43; found C 57.18, H 7.35.

Synthesis of NatD5: The product **NatD5** was prepared according to the general procedure B using the following quantities: **NatD5-bp** (1.5 g, 1.00 mmol) in TFA (10 mL) to afford **NatD5** (1.10g, 97%) as a red solid as a mixture of α- and β-pyranosic anomers in a ratio of 50:50 calculated on the basis of the relative C-1 signal intensities. Selected ^{1}H NMR (200 MHz, D_2O): δ = 8.41–7.95 (AA′XX′ system, 4 H, Ar-H), 7.92–6.81 (AA′XX′ system, 4 H, Ar-H), 5.12–4.59 (m, 4 H), 4.25–4.19 (m, 8 H), 3.93–3.80 (m, 12 H), 3.62–3.27 (m, 9 H, CH_2OCH_2, CH_2CH_2O, NCH_2CH_3, OCH_2, $CHCO$), 3.42–3.39 (m, 4 H), 1.97 (m, 2 H, CH_2CHCO), 1.68 (m, 2 H, OCH_2CH_2), 1.46 (m, 4 H), 1.23 (t, J = 6.9 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, D_2O): δ = 171.85 (CO), 152.87 (Ar-C), 151.44 (Ar-C), 146.27 (Ar-C), 143.42 (Ar-C), 126.31 (Ar-C), 124.57 (Ar-C), 122.45 (Ar-C), 117.06–111.38 (Ar-C), 73.08–67.75 (2 CH_2), 55.02 (CH), 50.50 (NCH_2CH_2), 46.16 (NCH_2CH_3), 31.39 (OCH_2CH_2), 29.35 (CH_2CH), 26.31 ($CH_2CH_2CH_2$) 25.09 (CH_2CH_2CH), 12.43 (CH_3) ppm; see Table 8 for the glycidic moiety. UV/Vis: λ_{max} = 501 nm; ε = 19715 $M^{-1}cm^{-1}$. MS (ESI): m/z = 1133.47 [M + 1]$^+$. $C_{48}H_{72}ClN_6O_{25}$: calcd. C 50.88, H 6.40; found C 50.79, H 6.33.

Synthesis of D2-x: K_2CO_3 (1.25g, 9.06 mmol) was added to a solution of disperse dye **D2** (1.0 g, 3.02 mmol) in acetone (25 mL) and the resulting mixture was stirred at room temperature for 1.5 h. Ethyl bromoacetate (1.02 mL, 9.06 mmol) was added and the resulting solution was heated at reflux for 20 h. At the end of the reaction, the resulting solution was filtered, diluted with water (40 mL) and the aqueous solution was extracted with chloroform (4 × 30 mL). Then the organic phase was dried with Na_2SO_4 and filtered, the filtrate was concentrated under reduced pressure and the residue (obtained as a red solid) was purified by flash chromatography (ethyl acetate/petroleum ether, 3:2, R_f = 0.61) to afford **D2-x** (0.99 g, 79%) as a red solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.85–7.75 (m, 2 H, Ar-H), 7.5–7.0 (m, 5 H, Ar-H), 6.78–6.75 (m, 2 H, Ar-H), 6.30 (m, 1 H, Ar-H), 4.88 (s, 2 H), 4.09 (q, J = 7.1 Hz, 2 H, CH_2CH_3), 1.33 (t, J = 7.1 Hz, 3 H, CH_2CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 185.10,181.56 (2 CO), 154.14, 151.00, 139.94, 134.48, 133.79, 133.19, 132.97, 130.26, 126.74, 126.24, 125.46, 120.17, 117.69, 113.34, 112.52, 69.25 (OCH_2), 61.29 (CH_2CH_3), 14.34 (CH_3) ppm. MS (ESI): m/z = 418.32 [M + 1]$^+$. $C_{24}H_{19}NO_6$ (417.42): calcd. C 69.06, H 4.59; found C 68.97, H 4.49.

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