Novel β-galactosidase-specific O^2-glycosylated diazeniumdiolate probes

Barry J. Bedell, D. Scott Bohle, Zhijie Chua, Alexander Czerniewski, Alan C. Evans, and Shadreck Mzengeza

Abstract: Three β-galactosidase-specific nitric-oxide-releasing diazeniumdiolate conjugated probes were prepared as a prelude to studies of new potential molecular MRI imaging agents. A glycosylated derivative, **2e**, designed to be trafficked across cell membranes, was also prepared. We report, in detail, the synthesis and characterization of these probes. In addition, the release of diazeniumdiolate from the probes by β-galactosidase-catalyzed hydrolysis was used to estimate their efficacy as serum-stable, specific NO donors.

Key words: nitric oxide, diazeniumdiolate, glycosylated, galactosidase.

Résumé : Comme prélude à des études de nouveaux produits pouvant éventuellement être utilisés comme agents moléculaires en imagerie de résonance magnétique (IRM), on a préparé trois nouvelles sondes spécifiques pour les β-galactosidase et conjuguées à un diazéniumdiolate qui libère de l'oxyde nitrique. On a aussi préparé un dérivé glycosylé, **2e**, qui devrait pouvoir pénétrer à l'intérieur des membranes des cellules. On rapporte, en détail, la synthèse et la caractérisation de ces sondes. De plus, on utilise le fait que l'hydrolyse catalysée par le β-galoactosidase libère le diazéniumdiolate des sondes pour évaluer leur efficacité comme donneurs spécifiques de NO, stables dans le sérum.

Mots-clés : oxyde nitrique, diazéniumdiolate, glycosylé, galactosidase.

Introduction

With the numerous biological roles of nitric oxide (NO), there is now great interest in developing NO-releasing compounds capable of providing required quantities of NO to specific biological tissues without disturbing other NO-sensitive physiology.[1–19] Because of their NO-releasing ability, diazeniumdiolates have become especially attractive for their potential use in novel drug therapies.[13,20–22] However, to design site-specific diazeniumdiolates, careful functionalization of these compounds is required. This strategy has been successfully achieved by derivatizing the terminal oxygen (O^2) of the diazeniumdiolate functionality.[6] To date, several O^2-protected diazeniumdiolates that only release NO in targeted biological tissues have been prepared, Scheme 1.[3,6,19,23–29] For example, Keefer and co-workers have reported O^2-vinyl diazeniumdiolates and their ability for liver-selectivity and activation by hepatic cytochrome P450.[6] They have also shown that O^2-acetoxymethyl diazeniumdiolates were esterase-sensitive and exhibited considerable anti-leukemia activity.[30] A number of peptide-diazeniumdiolate conjugates were recently prepared by Tang and Wang and found to be activated by α-chymotrypsin or prostate-specific antigens.[31]

Related efforts have led to the development of glycosylated derivatives of diazeniumdiolates, Scheme 2.[19,32–34] Such compounds constitute a novel series of tissue-specific diazeniumdiolates. For example, a library of pyranosyl derivates of diazeniumdiolates was recently prepared by Valdez et al.,[19] and these derivatives have the ability to pharmacologically target NO to pathogen-infected macrophages.

These recent examples of glycosylated diazeniumdiolate derivatives were not the first to be synthesized. Almost 25 years ago, Vasella and co-workers reported two sugar derivatives containing the diazeniumdiolate functionality and prepared them by a route involving oxidation of sugar oximes or sugar hydroxylamines[35] (see Scheme 3).

Analogous with the initial Valdez or Tang and Wang carbohydrate-substituted diazeniumdiolates, Vasella and co-workers' compounds are O^2-substituted diazeniumdiolates. Unlike the recent N-bound diazeniumdiolates, those two products are C-bound diazeniumdiolates. Jochims and co-workers have recently prepared a number of C-bound glycosylated diazeniumdiolates, Scheme 4, via nitrosation of

Received 17 February 2010. Accepted 2 June 2010. Published on the NRC Research Press Web site at canjchem.nrc.ca on 23 August 2010.

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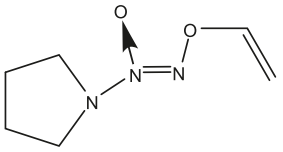
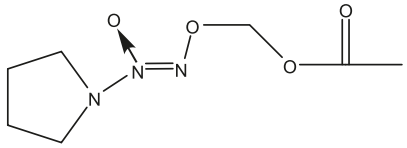
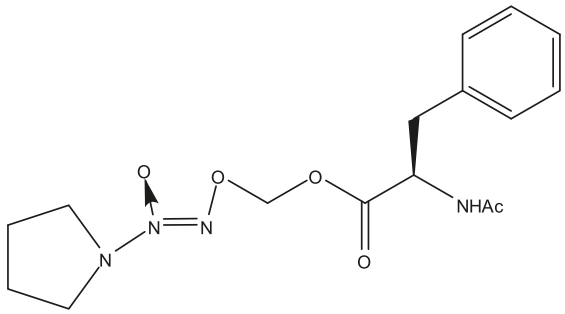
Scheme 1. Selected derivatives of pyrrolidine diazeniumdiolate prepared by Keefer and co-workers (see ref. 6).



Liver-selective PYRNONO vinyl derivative

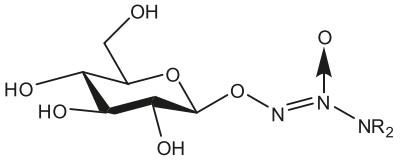


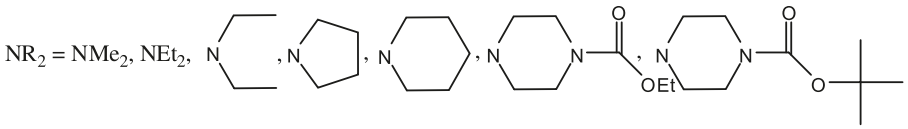
Esterase-active PYRNONO acetoxy derivative



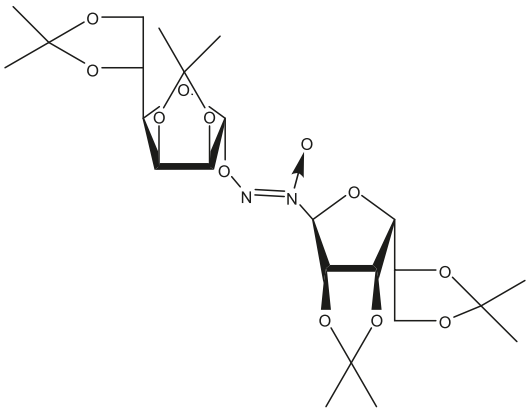
α-Chymotrypsin-active PYRNONO phenylalanine derivative

Scheme 2. Glycosylated derivatives of diazeniumdiolates prepared by Valdez et al. (see ref. 19).

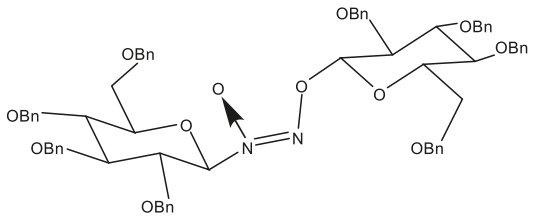




Scheme 3. Vasella and co-workers' sugar diazeniumdiolates prepared by oxidation of sugar oximes or sugar hydroxylamines.



Diisopropylidene-protected mannosyl dimer diazeniumdiolate



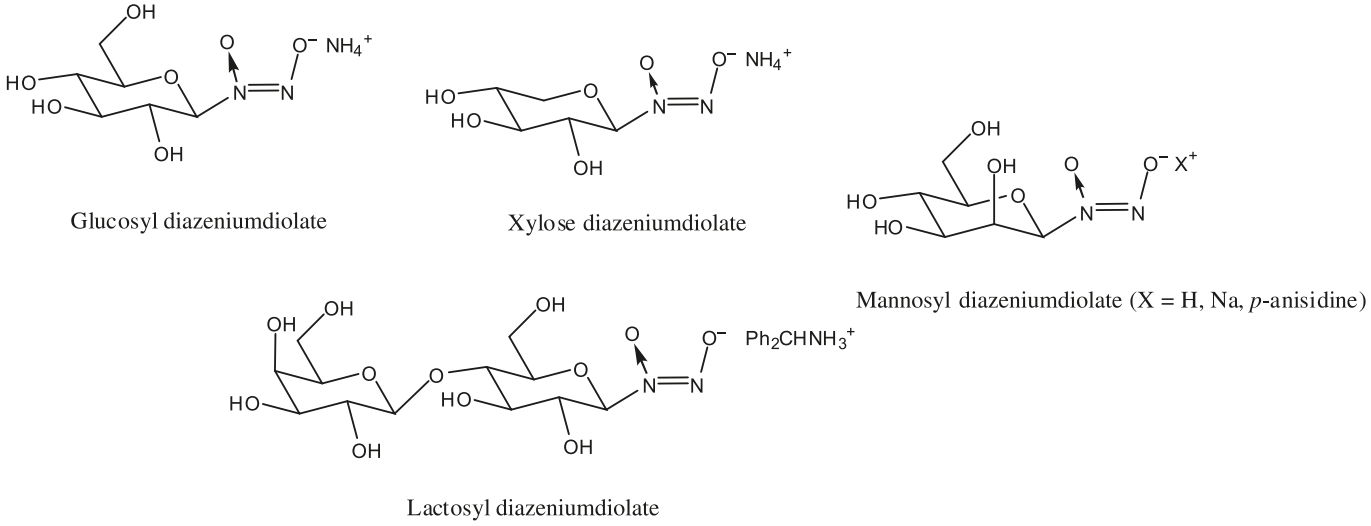
Benzyl-protected glucosyl dimer diazeniumdiolate

sugar oximes or sugar hydroxylamines.[36] The nitrosation of oximes or hydroxylamines are well-known routes for the preparation of C-bound diazeniumdiolates.[37–39]

As part of our effort to create new molecular magnetic resonance imaging (MRI) agents, we have designed and prepared a series of new glycosylated diazeniumdiolate compounds. We anticipate that these NO-releasing probes can non-invasively monitor β-galactosidase (β-gal) activity by MRI (e.g., perfusion MRI) based on the vasoactive properties of NO. As proof-of-principle, our monosaccharide-diazeniumdiolate derivatives are designed to target cells expressing a high level of β-gal, since the lacZ gene is commonly used as a reporter of gene expression. Specificity is proposed to arise from the coupling of a β-gal active component, namely a galactose molecule, with an amine-bound diazeniumdiolate salt via the glycosidic bond of the sugar.

In addition, we further explored the idea of tissue-specific glycosylated diazeniumdiolates by designing a diazeniumdio-

Scheme 4. Glucosyl, xylosyl, lactosyl, and mannosyl diazeniumdiolate derivatives prepared by Jochims and co-workers (see ref. 36).

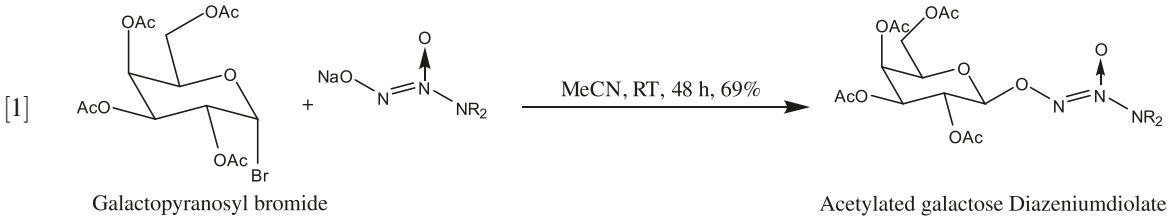


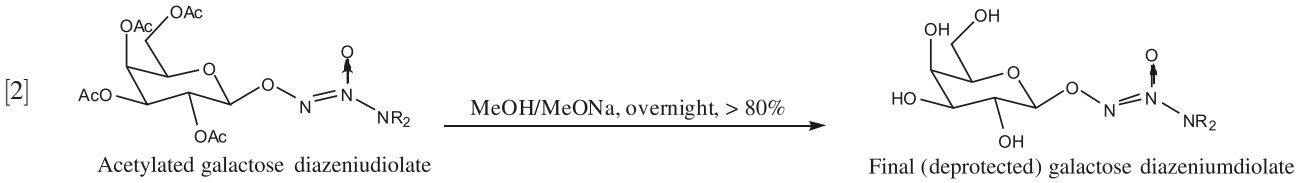
late glycoside derivative capable of crossing cell membranes. Our strategy was accomplished by appending the galactose-diazeniumdiolate derivative to another moiety that traffics the glycoside across plasma membranes (Scheme 5).[40–43] The ester conjugates of glucose, substituted at the pyranose C6-position, have high affinities for glucose transporters, such as GLUT-1.[7] Thus, a "bifunctional" NO-releasing probe was prepared with glucose coupled via its C-6 linkage, for trafficking via a GLUT-1 transporter, and galactose coupled via a glycosidic bond to a diazeniumdiolate core, for the probe's β-gal activation, Scheme 6.

We thus prepared "bifunctional" NO-releasing probes designed to be potentially activated by β-gal-expressing cells. Herein, the preparation and characterization of these new probes are described in detail.

Results

The synthetic scheme employed to design our probes is based on a halide-displacement reaction.[19,32,34,44] The diazeniumdiolate sodium salt was coupled with an acetylated pyranosyl bromide, eq. [1]. Base-catalyzed hydrolysis of the acetyl protecting groups then afforded the deprotected glycosylated diazeniumdiolate, eq. [2].



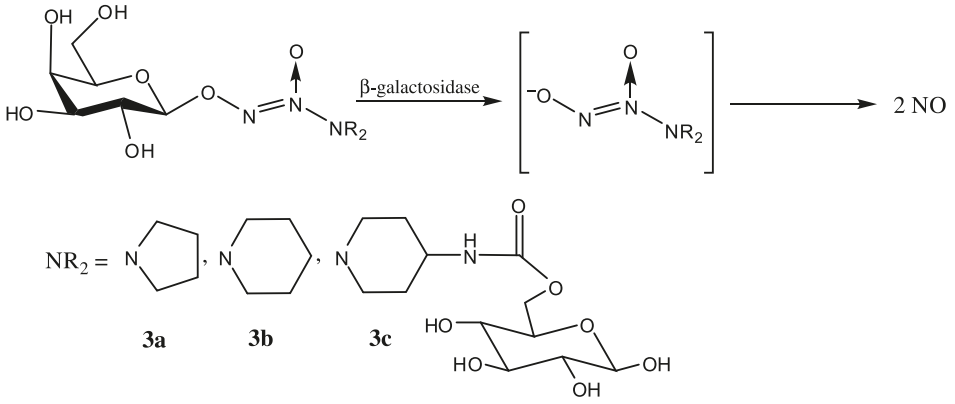


The presence of acetyl protecting groups on all of the pyranose C-2 to C-6 hydroxyl functionalities ensure that it is the diazeniumdiolate oxygen, and not the hydroxyl groups, which adds to the pyranose anomeric position. The acetyl protecting groups also aid in the purification and isolation of the glycosylated probe.

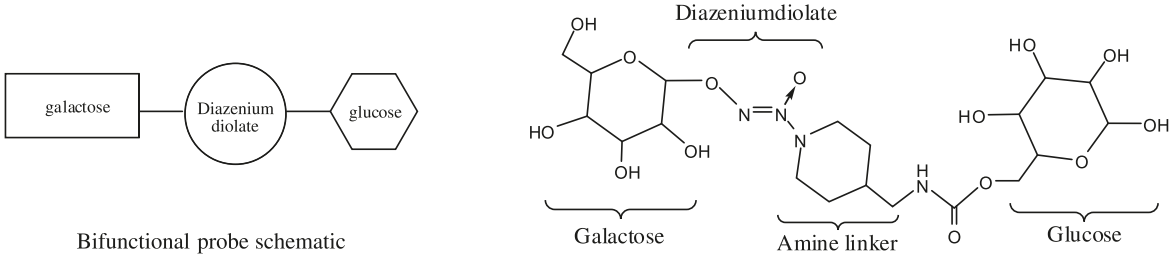
The "bifunctional" probes are comprised of three integral components as shown in Scheme 6. These three components include (*I*) a glucose moiety, coupled via its C-6 linkage and (*ii*) a galactose entity, coupled via a glycosidic bond to (*iii*) a piperidine-bound diazeniumdiolate core. Synthesis of the bifunctional probes involved the use of a *tert*-butyloxycarbonyl (Boc)-protected secondary amine, representing the precursor of the secondary amine linker.

The diazeniumdiolate **1c** was prepared from the Boc-protected secondary amine, eq. [3], with typical conditions for

Scheme 5. β-gal hydrolysis of constructed glycosylated diazeniumdiolate probes.

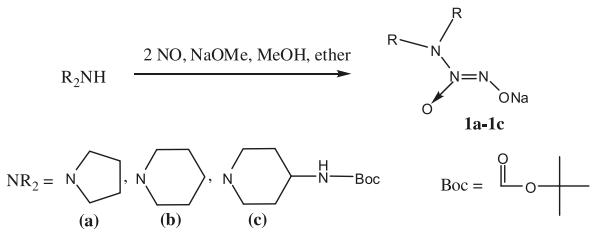


Scheme 6. Schematic (left) and detailed (right) structure of the proposed probes construction.

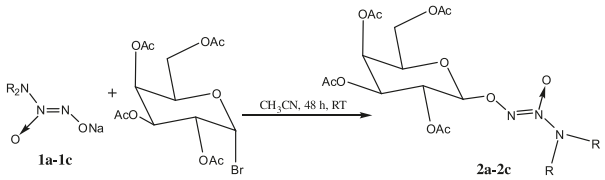


generating secondary-amine-bound diazeniumdiolates.[45,46] The diazeniumdiolates were then coupled with galactosyl bromide, eq. [4], to give the O^2-protected adducts **2a**–**2c** in good yields. The presence of a Boc protecting group in **1c** prevented any competitive coupling to galactosyl bromide by the protected primary amine.

[3]

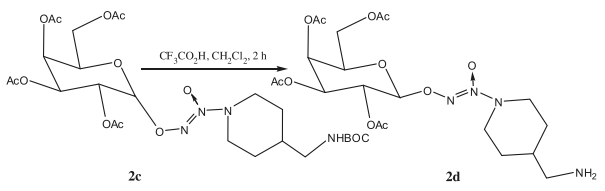


[4]

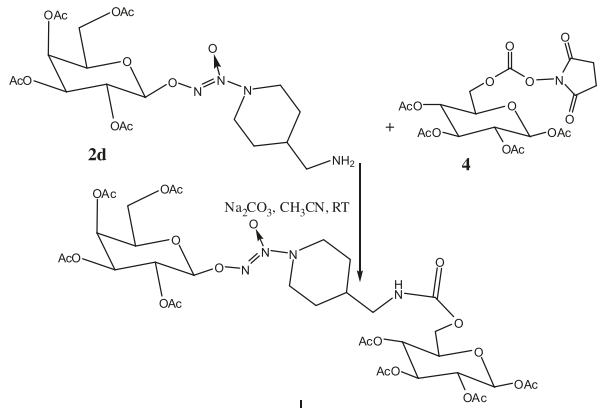


Removal of the Boc protecting group was then readily effected under acidic conditions,[47,48] eq. [5], and lead to the generation of the free primary amine in **2d**, which was then coupled with an activated glucose ester to give **2e**, eq. [6]. Removal of all protecting groups using NaOMe generated the final products **3a**–**3c**, eqs. [7] and [8].

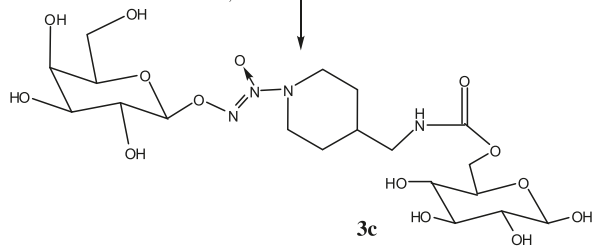
[5]



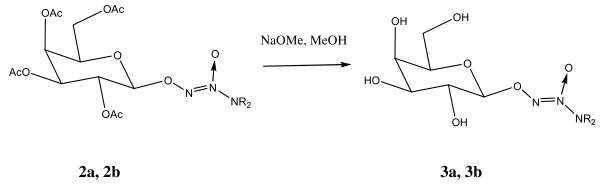
[6]



[7]

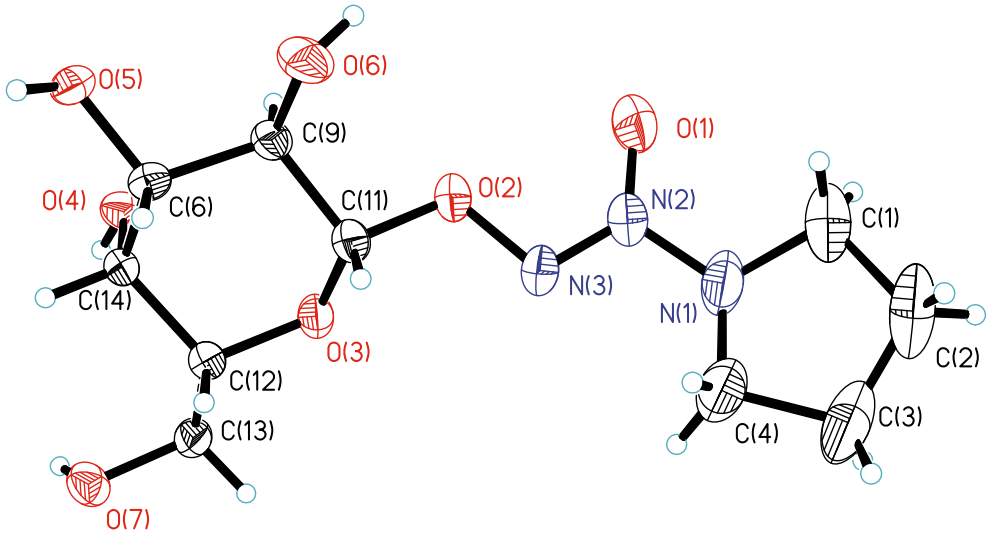


[8]



To verify the final product stereochemistry and linkage, a single-crystal X-ray diffraction structure for **3a** was determined, Fig. 1. Crystals for this adduct adopt the monoclinic

Fig. 1. X-ray crystal structure of **3a**. Important metric parameters (bond lengths, Å) and (angles, °): N(2)—N(1) 1.384(3), N(2)—N(3) 1.265(3), N(2)—O(1) 1.246(3), N(3)—O(2) 1.395(3), N(1)—C(4) 1.475(4), N(1)—C(1) 1.469(5), O(2)–N(3)–N(2) 106.7(2), O(1)–N(2)–N(3) 127.0(2), O(1)–N(2)–N(1) 117.3(2), N(3)–N(2)–N(1) 115.4(2). Hydrogen atoms are omitted for clarity.



non-centrosymmetric space group $P2_1$ with $Z = 2$. Complete details of the structure and refinement are provided in the Experimental and Supplementary data sections.

Enzymatic hydrolysis by β-galactosidase of compounds 3a–3c

Glycosylation of the diazeniumdiolate oxygen protects the diazeniumdiolate from its rapid reversion to two nitric oxides and the secondary amine, that is, the reverse of eq. [3] at neutral pH. The strong n–π* transition at ~260 nm in diazeniumdiolates allows for the facile monitoring of its hydrolytic breakdown. Since the half-life of the deprotected diazeniumdiolate under these conditions is less than 2 s, the loss of extinction at 256 nm corresponds to the limiting slow enzymatic cleavage of the C–O bond by galactosidase. As shown in the Supplementary data in that buffer and ion concentration, the half-life for **3a** at pH = 7.3, 37 °C, and [β-gal] = 8 nmol/L, is less than 120 s, which corresponds to at least a five orders of magnitude enhancement in the decomposition of these compounds over background degradation. Similar results are observed for **3b** and **3c** and for related glycosylated diazeniumdiolates.[32] Under physiological levels of cation concentration, these β-gal-promoted hydrolysis rates are likely to be faster and thus these half-lives and the resulting enhancement described earlier in the text is likely to be an underestimate of their activity in vivo.

Discussion

Diazeniumdiolate glycosylation not only confers probe specificity, but it is also a useful protecting-group strategy. In general, diazeniumdiolate anions are susceptible to acid-promoted thermal and photolytic decomposition with their O^2-alkyl and coordinated complexes being much more stable under all of these conditions.[28,49] For example, **3a** in phosphate buffer solution (PBS, 0.1 mol/L, pH 7.4) at 37 °C has a half-life of several days (>10 days), whereas the corresponding pyrrolidine diazeniumdiolate **1a**, under the same conditions, has a half-life of 1.8–2.8 s.[28] Compound **3a** can be stored as a solid at –20 °C with a minimum shelf-life of six months and can remain in PBS at room temperature for at least 10 days. Consequently, glycosylation of diazeniumdiolate ions not only offers a convenient route to deliver NO tissue-specifically, but it has the added advantage of significantly increasing the stability of the diazeniumdiolate in a physiological milieu.

Moreover, the probe's durability in physiological media may be enhanced by increasing the half-life of the diazeniumdiolate itself. This half-life depends on the nature of the substituent directly attached to the first nitrogen atom of the diazeniumdiolate functionality (i.e., –**N**(O)=NO⁻). For example, the piperidine diazeniumdiolate, **1b**, has a half-life of ~11 s in phosphate buffer solution (PBS, 0.1 mol/L, pH 7.4) at 37 °C, thus nearly an order of magnitude greater than the pyrrolidine substituent in **1a** with its half-life of (~1.8 s) under the same conditions. However, to ensure an instantaneous release of NO by the probes upon enzymatic hydrolysis, a diazeniumdiolate salt with a half-life that is not significantly longer either must be selected. Since the rate of β-galactosidase hydrolysis of the glycosylated diazeniumdiolate derivatives was monitored via the degradation of their corresponding diazeniumdiolate salts (generated upon enzyme hydrolysis) using UV spectroscopy, selecting a very slow NO-releasing diazeniumdiolate for the probe construction has the disadvantage of masking the kinetic parameters associated with enzyme hydrolysis. Furthermore, extensive structural modification of the diazeniumdiolate component in a given probe may lead to lowered rates of the probe's enzyme hydrolysis, and thus complicate the probe's NO release upon activation due to poorer enzyme recognition of the modified substrate. What was thus sought in the probe's design was a balance between the probe durability under physiological conditions and instantaneous NO release upon the probe's activation.

Overall, three glycosylated diazeniumdiolate probes (**3a**–**3c**) and their intermediates were generated. Their preparation employed a halide displacement reaction between an acetylated glycoside halide (gal–Br) and a diazeniumdiolate salt.

NMR spectroscopy confirmed that a single β-anomer was produced as has been found in related coupling products.[32] The coupling reactions usually took 2–3 days to proceed to completion. The slow pace of the reaction could be, at least partially, explained by the fact that the sodium diazeniumdiolate salts dissolve very slowly in MeCN. Yields for the coupling reactions averaged ~60% after chromatographic purification. A slight excess of diazeniumdiolate salt was used during these reactions to maximize the yields. An optimal ratio consisted of 5.8 equiv. diazeniumdiolate salt for every 5.5 equiv. of gal–Br. Exceeding this ratio did not significantly affect the reaction rate or yield. The use of rigorously dry conditions, that is doubly distilled acetonitrile stored in an inert-atmosphere box, led to reproducible and higher yields. The coupling reactions were undertaken in the glovebox as well. Despite these measures, TLC comparisons using solutions of degraded diazeniumdiolate salts in wet MeCN, along with ^{1}H NMR evaluations of the reaction mixtures, showed that the most important impurities that appeared during the reaction were mainly nitrosamines generated by diazeniumdiolate salt degradation. These degradation products made up roughly about 20%–30% (by weight) of the reaction material. Some unidentified sugar impurities during these coupling reactions were also detected by NMR, but they were not further examined. As described in the Supplementary data, chromatographic purification is critical to obtain pure final products.

Conclusion

The three β-galactosidase-specific probes described here release NO upon β-gal hydrolysis. Glycosylation of the diazeniumdiolate core leads to both probe stability and specificity. These novel probes are currently being evaluated for their potential to detect β-gal in vivo using MRI.

Experimental

General methods and materials used in these preparations and for the characterization of new compounds are described in recent papers.[50] Where required, the probes were characterized by ^{1}H NMR in 0.01 mol/L NaOD in D_2O.

Sodium 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (1a)

This salt was prepared by the method of Saavedra[6] et al. in 61% yield and has the following characteristic data: UV (10 mmol/L NaOH, RT) λ_{max} (ε): 252 nm (7.4 mmol/L^{-1} cm^{-1}). $UV_{kinetics}$ (in 75% excess PBS (0.1 mol/L, pH 7.36)) half-lives: 3.20 s and 1.82 s at RT and 37 °C, respectively. FTIR ν (cm^{-1}): 3455 (br), 3378 (br), 2970 (m), 2880 (m), 1664 (w), 1608 (m), 1463 (w), 1393 (m), 1359 (m), 1266 (m), 1226 (s), 1184 (s), 984 (m), 972 (m), 905 (m) ^{1}H NMR (270 MHz, D_2O/NaOD) δ: 3.21 (m, 4H, J = 5.9 Hz), 1.90 (m, 4H, J = 5.3 Hz). ^{13}C NMR (67.5 MHz, D_2O/NaOD) δ: 51.38 (2C, C-1 and C-4), 22.40 (2C, C-2 and C-3). DSC onset temperature at 169.5 °C, decomposition temperature at 176.7 °C, ΔH = +151.1 kJ mol^{-1}. MS (MALDI-TOF) m/z: 153.1 [M]$^+$.

Sodium 1-(piperidin-1-yl)diazen-1-ium-1,2-diolate (1b)

A solution of piperidine (5.00 g, 58.0 mmols) in acetonitrile (20 mL), ether (20 mL), and 25% sodium methoxide in methanol (11.9 mL, 1 equiv.) was prepared. A white powder was ultimately obtained (8.3 g; 85% yield). UV (10 mmol/L NaOH, RT) λ_{max} (ε): 250 nm (8.8 mmol/L^{-1} cm^{-1}). $UV_{kinetics}$ (in 75% excess PBS (0.1 mol/L, pH 7.36)) half-lives: 26 s and 11 s at RT and 37 °C, respectively. FTIR ν (cm^{-1}): 3450 (br), 3364 (br) 2969 (m), 2964 (m), 2951 (m), 2942 (s), 2926 (m), 2233 (br, w), 2170 (w), 1676 (m), 1654 (m), 1538 (w), 1473 (m), 1453 (m), 1442 (m), 1435 (m), 1381 (m), 1356 (s), 1291 (m), 1270 (m), 1216 and 1183 (s), 1137 (m), 1096 (m), 1064 (m), 1031 (m), 944 (s), 913 (m), 884 (m), 863 (w), 828 (m), 726 (s). ^{1}H NMR (400 MHz, D_2O/NaOD) δ: 2.70 (t, 4H, J = 5.6 Hz), 1.38 (t, 4H, J = 5.6 Hz), 1.1**2** (t, 2H, J = 5.6 Hz). ^{13}C NMR (75 MHz, D_2O/NaOD) δ: 52.77 (C-1 and C-5), 24.34 (C-2 and C-4), 22.11 (C-3). DSC onset temperature at 195.9 °C, decomposition temperature at 197.4 °C, ΔH = +46.1 kJ mol^{-1}. MS (EI) m/z: 167.1 [M]$^+$, 114 [M – NO]$^+$, 84 [M – 2NO]$^+$, 56 [M – 2NO – 2CH$_2$]$^+$, 42 [M – 2NO – 3CH$_2$]$^+$. MS (MALDI-TOF) m/z: 168.5 [M + H]$^+$. The key characteristic data concur with those briefly reported elsewhere.[45]

Sodium 1-(4-Boc-4-aminomethylpiperidin-1-yl)diazen-1-ium-1,2-diolate (1c)

A solution of Boc-protected 4-aminomethylpiperidine (2.50 g, 11.7 mmols) in methanol (2.00 mL) and ether (40 mL) and 25% sodium methoxide in methanol (8.00 mL, 39.1 mmol) was prepared. A white powder was ultimately derived (2.97 g; 97% yield). Alternatively, the same reaction conditions were undertaken, but this time without ether and stirred at room temperature for 2–3 days. During this time period, the flask was periodically recharged with NO gas (up to 60 psi) until no significant gas consumption was noted over the course of several hours. No precipitate appeared during this time period, but upon pressure release and removal of solvent in vacuo, a yellowish oil was isolated. The product was obtained by performing a trituration on this yellow oil by dissolving it initially in hot ethanol and allowing the product to precipitate as a snow-white powder with the ensuing addition of excess cold hexane or ether. This powder was collected by filtration, rinsed with ether, and dried in a vacuum at room temperature (58% yield). UV (10 mmol/L NaOH, RT) λ_{max} (ε): 250 nm (7.8 mmol/L^{-1} cm^{-1}), 326 nm (2.88 mmol/L^{-1} cm^{-1}). $UV_{kinetics}$ (in 75% excess PBS (0.1 mol/L, pH 7.36)) half-lives: 33 s and 24 s at RT and 37 °C, respectively. FTIR ν (cm^{-1}): 3359 (s), 2978 (m), 2967 (m), 2951 (m), 2931 (m), 2858 (m), 2254 (m), 1686 (s), 1527 (s), 1473 (m), 1446 (m), 1386 (m), 1364 (s), 1268 (s), 1247 (s), 1177 (s), 1140 (m) 1088 (w), 1043 (w), 1014 (w), 996 (w), 964 (s), 955 (w), 916 (w), 880 (w), 868 (w), 779 (w), 623 (w), 583 (w). ^{1}H NMR (400 MHz, CD_3OD-NaOH (0.1 mol/L)) δ: 3.20 (d, 2H, J = 10.8 Hz, H-1 and H-5), 3.00 (m, 3H, H-1′, H-5′ and H-3), 1.81 (m, 2H, H-2 and H-4), 1.50 (m, 2H, partly overlapped by *t*-butyl peak, methylene Hs), 1.43 (s, 9H, *t*-butyl H), 1.40 (m, 2H, partly overlapped by *t*-butyl peak, H-2′ and H-4′). ^{13}C NMR (75 MHz, CD_3OD-NaOH (0.1 mol/L)) δ: 157.46 (Boc's C=O), 78.65 (Boc's tertiary carbon), 52.24 (piperidine's C-1 and C-5), 45.25 (4-amino methylene carbon), 35.77 (piperidine's C-3), 28.95 (piperidine's C-2 and C-4), 27.67 (Boc's methyl carbons). DSC onset temperature at 172.3 °C, decomposition temperature at 173.3 °C, ΔH = +50.4 kJ mol^{-1}. MS (ESI, + c) m/z: 318.9 [M + H]$^+$,

266.0 [M – Na – NO + H]$^+$, 237.1 [M – Na – 2NO + 2H]$^+$. MS (ESI, – c) *m/z*: 295.0 [M – Na]$^-$, 235.2 [M – Na – 2NO]$^-$. MS/MS (ESI) *m/z*: 318.9 [M + H]$^+$, 289 [M – NO + H]$^+$. MS (MALDI-TOF): *m/z*: 318.0 [M]$^+$. HR-MS (ESI) calcd. for $Na_2C_{11}H_{21}N_4O_4$ [M + H]$^+$: 319.1358. Found: 319.1351. Anal. calcd. for $Na_2C_{11}H_{20}N_4O_4$: C, 41.51; H, 6.33; N, 17.60. Found: C, 41.27; H, 6.57; N, 17.68.

General procedure for the synthesis of O^2-glycosylated diazeniumdiolates

To a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (5.5 equiv.) in anhydrous acetonitrile (20 mL), the desired solid diazeniumdiolate salt (5.8 equiv.) was added and stirred until all the galactopyranosyl bromide had reacted (2–3 days) as monitored by TLC (ethyl acetate:hexane, 2:3). Upon filtration, the solvent was removed under vacuum and the product was dissolved in ethyl acetate (25.0 mL). The organic layer was washed with distilled water (3 × 15 mL/wash), dried over Na_2SO_4 (2 g), filtered, and its solvent removed under vacuum. The isolated material was purified by column chromatography (silica gel, ethyl acetate:hexane, 1:3) to yield a clear colorless oil. To generate the deacetylated version of the O^2-glycosylated diazeniumdiolate, a few drops of sodium methoxide (25% in methanol) were added to a solution of the protected/acetylated glycosylated diazeniumdiolate in anhydrous methanol (20.0 mL), and the solution was stirred at RT overnight. TLC (methanol:ethyl acetate, 3:7) showed the presence of a new product. The deacetylated product is precipitated in (∼97%) by allowing its solution in ethanol, which was diluted with ether, to stand in an ice bath overnight. An alternative synthetic procedure to prepare the deacetylated product involved bypassing the column chromatography step during the synthesis of acetylated glycosylated diazeniumdiolate and carrying out the deprotection step right after the workup of the acetylated glycosylated diazeniumdiolate synthesis. A solid hygroscopic white product was generated (∼65% overall). Spectroscopic results were identical whichever methodology was employed.

O^2-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl) 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (2a)

To a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (400 mg, 0.976 mmol, 5.5 equiv.) in anhydrous acetonitrile (20.0 mL), **1a** (158 mg, 1.03 mmol, 5.8 equiv.) was added and stirred until all the galactopyranosyl bromide had reacted (2–3 days, as determined by TLC on silica ethyl acetate:hexane, 2:3, R_f = 0.24). A clear colorless oil (280 mg, 0.607 mmol, 62% yield) was obtained after purification by column chromatography (silica gel, ethyl acetate:-hexane, 1:3). UV (MeOH, RT) λ_{max} (ε): 251 nm (2.05 mmol/L^{-1} cm^{-1}). FTIR ν (cm^{-1}): 3475 (mbr), 2981 (m), 2886 (w), 1751 (s), 1652 (w), 1489 (w), 1436 (w), 1373 (s), 1233 (sbr), 1156 (w), 1127 (w), 1079 (s), 1059 (s), 989 (w), 956 (w), 917 (w), 902 (w), 883 (w), 851 (w), 823 (w), 777 (w), 713 (w), 676 (w), 655 (w), 627 (w), 601 (w), 557 (w), 525 (w), 493 (w), 461 (w), 432 (w), 415 (w). ^{1}H NMR (400 MHz, CD_3OD) δ: 5.40 (m, 3H, overlapped, H-1, H-2, and H-4), 5.22 (dd, 1H, *J* = 9.8, 3.4 Hz, H-3), 4.15 (m, 3H, overlapped, H-5, H-6, and H-7), 3.57 (t, 4H, *J* = 6.6 Hz, H-1′ and H-4′), 2.13 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 1.96 (s, 3H, overlapped, CH_3), 1.97 (m, 4H, overlapped, H-2′ and H-3′). ^{13}C NMR (75 MHz, CD_3OD) δ: 170.53 (C=O), 170.39 (C=O), 170.23 (C=O), 169.83 (C=O), 100.76 (C-1), 71.34 (C-5), 71.27 (C-3), 67.43 (C-2), 67.25 (C-4), 61.36 (C-6), 50.64 (C-1′ and C-4′), 22.76 (C-2′ and C-3′), 19.52, 19.51, 19.44, 19.40 (acetyl CH_3). MS (ESI) *m/z*: 484.1 [M + Na]$^+$. MS/MS (ESI) *m/z*: 484.0 [M + Na]$^+$, 371.0 [M + Na – pyrrolidine (NC_4H_8) – acetyl (C(=O)CH_3)]$^+$, 311.0 [M + Na – pyrrolidine (NC_4H_8) – 2NO – acetyl (C(=O)CH_3)]$^+$. MS/MS (ESI) *m/z*: 479.0 [M + NH_4]$^+$, 331.1 [M – pyrrolidine diazeniumdiolate (C_4H_8N–NONO)]$^+$. HR-MS (ESI) calcd. for $C_{18}H_{31}N_4O_{11}$ [M + NH_4]$^+$: 479.1989. Found: 479.1979. HR-MS (ESI) calcd. for $NaC_{18}H_{27}N_3O_{11}$ [M + Na]$^+$: 484.1543. Found: 484.1535. DSC onset temperature at 154.2 °C, decomposition temperature at 175.5 °C, Δ*H* = +53.0 kJ mol^{-1}. Anal. calcd. for $C_{18}H_{27}N_3O_{11}$: C, 46.85; H, 5.90; N, 9.11. Found: 47.05; H, 5.76; N, 9.05.

O^2-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl) 1-(piperidin-1-yl)diazen-1-ium-1,2-diolate (2b)

To a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (400 mg, 0.976 mmol, 5.5 equiv.) in anhydrous acetonitrile (20 mL), PipNONO **1b** (180 mg, 1.08 mmol, 5.8 equiv.) was added and stirred until all the galactopyranosyl bromide had reacted (2–3 days, TLC monitored, ethyl acetate:hexane, 2:3, R_f = 0.27). A clear colorless oil (282 mg, 0.593 mmol, 61% yield) was obtained after purification by column chromatography (silica gel, ethyl acetate:hexane, 1:3). UV (MeOH, RT) λ_{max} (ε) 227 nm (5.40 mmol/L^{-1} cm^{-1}). FTIR ν (cm^{-1}): 3480 (wbr), 2943 (m), 2860 (m), 1754 (s), 1508 (w), 1476 (w), 1447 (w), 1371 (m), 1228 (s), 1170 (w), 1129 (w), 1083 (m), 1062 (m), 1000 (w), 954 (w), 913 (w), 898 (w), 864 (w), 851 (w), 821 (w), 797 (w), 767 (w), 738 (w), 708 (w), 684 (w), 651 (w), 602 (w), 565 (w), 528 (w). ^{1}H NMR (400 MHz, CD_3OD) δ: 5.40 (m, 3H, overlapped, H-1, H-2, and H-4), 5.23 (dt, 1H, *J* = 7.2, 2.6 Hz, H-3), 4.18 (m, overlapped, 3H, H-5, H-6, and H-7), 3.43 (m, 4H, H-1′ and H-5′), 2.15 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 1.95 (s, 3H, CH_3), 1.74 (quint, 4H, *J* = 5.6, H-2′ and H-4′), 1.55 (quint, 2H, *J* = 5.6, H-3′). ^{13}C NMR (75 MHz, CD_3OD) δ: 170.82 (C=O), 170.71 (C=O), 170.19 (C=O), 169.78 (C=O), 100.81 (C-1), 71.43 (C-5), 71.21 (C-3), 67.35 (C-2), 67.25 (C-4), 61.30 (C-6), 51.92 (C-1′ and C-5′), 24.38 (C-3′), 23.25 (C-2′ and C-4′), 19.45, 19.39, 19.31 (acetyl CH_3). MS (ESI) *m/z*: 498.0 [M + Na]$^+$. MS/MS (ESI) *m/z*: 498.0 [M + Na]$^+$, 310.9 [M + Na – piperidine diazeniumdiolate (C_5H_{10}N–N(O)=NO) – acetyl (C(=O)CH_3)]$^+$, 294.9 [M + Na – piperidine diazeniumdiolate (C_5H_{10}N–N(O)=NO) – *O*-acetyl (O–C(=O)CH_3)]$^+$. HR-MS (ESI) calcd. for $NaC_{19}H_{29}N_3O_{11}$ [M + Na]$^+$: 498.1700. Found: 498.1688. HR-MS (ESI) calcd. for $C_{19}H_{33}N_4O_{11}$ [M + NH_4]$^+$: 493.2146. Found: 493.2138. DSC onset temperature at 213.8 °C, decomposition temperature at 221.0 °C, Δ*H* = + 103.9 kJ mol^{-1}. Anal. calcd. for $C_{19}H_{29}N_3O_{11}$: C, 48.00; H, 6.15; N, 8.84. Found: C, 48.24; H, 6.31; N, 8.40.

O^2-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl) 1-(4-Boc-4-aminomethylpiperi-din-1-yl)diazen-1-ium-1,2-diolate (2c)

To a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (400 mg, 0.976 mmol, 5.5 equiv.) in anhydrous

acetonitrile (20 mL), **1c** (330 mg, 1.04 mmol, 5.8 equiv.) was added and stirred until all the galactopyranosyl bromide had reacted (2–3 days, TLC monitored, ethyl acetate:hexane, 2:3, R_f = 0.13). A clear colorless oil (389 mg, 0.644 mmol, 66% yield) was isolated after purification by column chromatography (silica gel, ethyl acetate:hexane, 1:3). UV (MeOH, RT) λ_{max} (ε): 229 nm (5.10 mmol/L^{-1} cm^{-1}). FTIR ν (cm^{-1}): 3411 (wbr), 2980 (w), 2930 (w), 2850 (w), 1752 (s), 1513 (w), 1458 (w), 1438 (w), 1370 (m), 1224 (sbr), 1171 (w), 1137 (w), 1081 (m), 1063 (m), 1010 (w), 950 (w), 914 (w), 902 (w), 868 (w), 840 (w), 780 (w), 766 (w), 750 (w), 721 (w), 603 (w), 496 (w), 410 (w). ^{1}H NMR (400 MHz, CD_3OD) δ: 5.40 (m, 3H, overlapped, H-1, H-2, and H-4), 5.23 (dt, 1H, J = 7.2, 2.6 Hz, H-3), 4.18 (m, 3H, overlapped, H-5, H-6, and H-7), 4.01 (m, 2H, H-1″ and H-5″), 2.95 (m, 3H, overlapped, H-1′, H-3′, and H-5′), 2.15 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 1.96 (s, 3H, CH_3), 1.82 (m, 2H, H-2′ and H-4′), 1.60 (m, 2H, methylene Hs), 1.43 (s, 9H, *t*-butyl H), 1.38 (m, 2H, partly overlapped by *t*-butyl peak, H-2″ and H-4″). ^{13}C NMR (75 MHz, CD_3OD) δ: 170.81 (C=O), 170.70 (C=O), 170.18 (C=O), 169.78 (C=O), 157.46 (Boc's C=O), 100.81 (C-1), 78.79 (Boc's tertiary carbon), 71.42 (C-5), 71.20 (C-3), 67.32 (C-2), 67.22 (C-4), 61.26 (C-6), 50.91, 50.74 (C1′ and C-5′), 45.03 (4-aminomethyl carbon), 35.84 (C-3′), 28.11, 28.05 (C-2′ and C-4′), 27.58 (Boc CH_3), 19.38, 19.30 (acetyl CH_3). MS (ESI) *m/z*: 627.2 $[M + Na]^+$, 1230.7 $[2M + Na]^+$. MS/MS (ESI) *m/z*: 627.3 $[M + Na]^+$, 583.0 $[M + Na - CO_2]^+$, 567.5, 522.9, 441.5, 365.7, 217.9. HR-MS (ESI) calcd. for $NaC_{25}H_{40}N_4O_{13}$ $[M + Na]^+$: 627.2490. Found: 627.2474. DSC onset temperature at 192.6 °C, decomposition temperature at 220.2 °C, ΔH = +62.0 kJ mol^{-1}. Anal. calcd. for $C_{25}H_{40}N_4O_{13}$: C, 49.66; H, 6.67; N, 9.27. Found: C, 49.98; H, 6.54; N, 9.06.

O^2-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl) 1-(4-aminomethylpiperidin-1-yl)diazen-1-ium-1,2-diolate (2d)

A solution of compound **2c** (389 mg, 0.644 mmol, 1 equiv.) in distilled dichloromethane (DCM, 20.0 mL), and trifluoroacetic acid (TFA, 10.0 mL) was prepared initially at around 0 °C and then stirred under N_2 at RT for 2–3 h. NMR studies in deuterated DCM/TFA solutions demonstrated the complete absence of the Boc methyl peak after this time period with near 100% conversion. Prolonged exposure of this product under such acidic conditions, however, led to deterioration of the product. The acid was subsequently removed from solution via a vacuum line with successive dilutions using DCM (100 mL). The residue was dissolved with aqueous $NaCO_3$ (1.0 mol/L solution) and extracted thrice with DCM (30 mL/extraction). The combined extracts were dried over Na_2SO_4 (2 g), filtered, and the solvent removed using a vacuum line to yield a sticky yellow oil (310 mg). The product turned out to be reactive with silica gel, and hence was not further purified by column chromatography. The yield for the ensuing intermediate of this product (see synthesis of protected (**2e**)) was 79% assuming the 310 mg isolated in this synthesis is essentially compound **2d**. On the other hand, during another trial of these syntheses, compound **2e** was isolated with an 81% yield relative to **2c**. This result suggests that the yield of compound **2d** obtained between these two synthetic steps is very high. UV (MeOH, RT) λ_{max} (ε): 230 nm (3.86 mmol/L^{-1} cm^{-1}). FTIR ν (cm^{-1}): 3431 (mbr), 2960 (w), 2926 (w), 2860 (w), 1752 (s), 1681 (s), 1508 (w), 1432 (w), 1384 (m), 1373 (m), 1232 (sbr), 1182 (m), 1134 (m), 1080 (m), 1062 (s), 992 (w), 955 (w), 915 (w), 837 (m), 801 (m), 780 (w), 723 (m), 601 (w), 519 (w), 495 (w), 462 (w), 409 (w). ^{1}H NMR (400 MHz, CD_3OD) δ: 5.40 (m, 3H, overlapped, H-1, H-2, and H-4), 5.23 (dt, 1H, J = 7.2, 2.6 Hz, H-3), 4.12 (m, 3H, overlapped, H-5, H-6, and H-7), 4.06 (m, 2H, H-1″ and H-5″), 3.00 (dd, 2H, J = 22.4, 10 Hz, H-1′ and H-5′), 2.88 (t, 1H, J = 6.8 Hz, H-3′), 2.15 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 1.95 (s, 3H, CH_3), 1.90 (m, 2H, H-2′ and H-4′), 1.81 (m, 2H, methylene Hs), 1.49 (m, 2H, H-2″ and H-4″). ^{13}C NMR (75 MHz, CD_3OD) δ: 170.80 (C=O), 170.63 (C=O), 170.14 (C=O), 169.83 (C=O), 100.78 (C-1), 71.47 (C-5), 71.13 (C-3), 67.29 (C-2), 67.19 (C-4), 61.21 (C-6), 53.63 (DCM solvent), 50.36, 50.17 (C1′ and C-5′), 43.86 (4-aminomethyl carbon), 33.61 (C-3′), 27.58, 27.48 (C-2′ and C-4′), 19.37, 19.28 (acetyl CH_3). MS (ESI) *m/z*: 527.1 $[M + Na]^+$, 505.0 $[M + H]^+$, 1008.8 $[2M + H]^+$, 1030.7 $[2M + Na]^+$. MS/MS (ESI) *m/z*: 505.0 $[M + H]^+$, 390.8 [M – 4-aminomethylpiperidine (NC_5H_9–CH_2NH_2)]$^+$. MS/MS (ESI) *m/z*: 527.0 $[M + Na]^+$, 399.9 [M + Na – 4-aminomethylpiperidine (NC_5H_9–CH_2NH_2) – nitrogen]$^+$, 371.0, 353.0, 341.1, 310.9, 295.0. HR-MS (ESI) calcd. for $C_{20}H_{33}N_4O_{11}$ $[M + H]^+$: 505.2146. Found: 505.2134. DSC onset temperature at 150.4 °C, decomposition temperature at 174.5 °C, ΔH = +52.5 kJ mol^{-1}.

O^2-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl) 1-(4-[1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranosyl]-4-aminomethylpiperidin-1-yl)diazen-1-ium-1,2-diolate (2e)

To a solution of **2d** (310 mg, 0.615 mmol, 1 equiv.) in anhydrous acetonitrile (20 mL), Na_2CO_3 (80.0 mg, 0.755 mmol, 1.2 equiv.) was added followed by a solution of GluDSC (345 mg, 0.705 mmol, 1.15 equiv.) in anhydrous acetonitrile (10 mL). The solution was stirred under N_2 at RT overnight. The solution was passed through a fine frit filter, and the solvent was removed in vacuo. The residue was dissolved in dichloromethane (DCM, 10 mL). This organic layer was washed thrice with distilled water (15 mL/wash), dried over Na_2SO_4 (2 g), filtered, and its solvent removed in vacuo. TLC demonstrated the appearance of a single new spot (ethyl acetate:hexane, 7:3, with R_f = 0.34). Interestingly, over time, two new spots appeared on the TLC plate above the initial new spot (R_f = 0.66, 0.76). NMR and MS studies however show that the TLC spot with R_f = 0.34 corresponds to the desired compound. The isolated material was purified by column chromatography (silica gel, ethyl acetate:hexane, 7:3) to yield a clear, colorless oil (427 mg, 0.486 mmol, 79%). UV (MeOH, RT) λ_{max} (ε): 229 nm (4.33 mmol/L^{-1} cm^{-1}). FTIR ν (cm^{-1}): 3391 (wbr), 2942 (w), 2872 (w), 1756 (s), 1652 (w), 1520 (w), 1436 (w), 1371 (m), 1221 (sbr), 1153 (w), 1079 (m), 1039 (m), 952 (w), 912 (w), 902 (w), 847 (w), 775 (w), 743 (w), 702 (w), 647 (w), 601 (w), 558 (w), 496 (w), 410 (w). ^{1}H NMR (400 MHz, CD_3OD) δ: 5.82 (d, 1H, J = 8.4 Hz, glucopyranosyl H-1), 5.40 (m, 3H, overlapped, H-1, H-2, and H-4), 5.33 (t, 1H, J = 9.6 Hz, glucopyranosyl H-3), 5.23 (dt 1H, J = 6.8, 2.4 Hz, galactopyranosyl H-3), 5.06 (t, 1H, J = 9.8 Hz, dd overlapping, 1H, J = 29.2, 9.6 Hz, glucopyranosyl H-2

and H-4), 4.24 (m, 1H, galactopyranosyl H-7) 4.14 (m, 4H, overlap of glucopyranosyl H-5 and H-6 as well as galactopyranosyl H-5 and H-6), 4.03 (m, 3H, overlap of glucopyranosyl H-7 and piperidinyl H-1″ and H-5″), 2.98 (m, 2H, overlap of piperidinyl H-1′, H-5′, and H-3′), 2.16 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.02 (s, 6H, CH_3), 2.01 (s, 3H, CH_3), 1.97 (s, 3H, CH_3), 1.96 (s, 3H, CH_3), 1.85 (m, 2H, piperidinyl H-2′ and H-4′), 1.58 (m, 2H, piperidinyl methylene Hs), 1.36 (m, 2H, piperidinyl H-2″ and H-4″). ^{13}C NMR (75 MHz, CD_3OD) δ: 170.87, 170.75, 170.39, 170.22, 170.02, 169.83, 169.26 (acetyl C=O), 157.46 (amide's C=O), 100.82 (C-1), 91.72 (C-1″), 73.05 (C-3″), 72.92 (C-5″), 71.46 (C-5), 71.21 (C-3), 70.50 (C-2″), 68.10 (C-4″), 67.37 (C-2), 67.25 (C-4), 61.93 (C-6″), 61.34 (C-6), 50.96, 50.76 (C1′ and C-5′), 45.63, 45.50 (4-aminomethyl carbon), 35.60 (C-3′), 28.02, 27.97 (C-2′ and C-4′), 19.47, 19.36, 19.32 (acetyl CH_3). MS (ESI) *m/z*: 901.1 $[M + Na]^+$. MS/MS (ESI) *m/z*: 901.0 $[M + Na]^+$, 841.0 [M + Na – *O*-acteyl (O–C(=O)CH_3) – H]$^+$, 715.1, 540.0 [M + Na – acetylated pyranose ($C_{14}H_{19}O_9$) – 2NO]$^+$, 509.1, 449.0. HR-MS (ESI) calcd. for $NaC_{35}H_{50}N_4O_{22}$ $[M + Na]^+$: 901.2814. Found: 901.2788. DSC onset temperature at 164.9 °C, decomposition temperature at 188.7 °C, ΔH = +58.5 kJ mol^{-1}. Anal. calcd. for $C_{35}H_{50}N_4O_{22}$: C, 47.84; H, 5.73; N, 6.38. Found: C, 47.94; H, 6.00; N, 6.19.

O^2-β-D-galactopyranosyl 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (3a)

A few drops of NaOMe (25% in methanol) were added to a solution of **2a** (163 mg, 0.353 mmol) in anhydrous methanol (20.0 mL), and the mixture was stirred at RT overnight. TLC (methanol:ethyl acetate, 3:7, R_f = 0.32) showed presence of a new product, which was isolated as a white powder (77.7 mg, 0.265 mmol, 75%) after purification by column chromatography (silica gel, methanol:ethyl acetate, 1:4). The product was also obtained in better yield (97%) by allowing it to precipitate in solution. NMR studies confirmed that the material obtained in this manner is identical to the product obtained after chromatography. Alternatively, bypassing the column chromatography step during the synthesis of **2a** and carrying out the deprotection step right after the **2a** workup generated an overall yield of 67%. Spectroscopic results were identical whichever methodology was employed. UV (Tris Buffer (0.1 mol/L, pH 7.4) + $(NH_4)SO_4$ (1.7 mol/L) + $MgCl_2$ (10 mmol/L), RT) λ_{max} (ε): 256 nm (6.86 mmol/L^{-1} cm^{-1}). FTIR ν (cm^{-1}): 3528 (m), 3434 (br), 3210(sbr), 2979 (m), 2968 (m), 2916 (m), 2883 (m), 2866 (m), 1752 (w), 1642 (m), 1472 (s), 1384 (s), 1347 (m), 1285 (m), 1249 (m), 1211 (w), 1196 (w), 1150 (s), 1124 (w), 1102 (s), 1086 (s), 1067 (s), 1044 (s), 1003 (s), 985 (m), 976 (w), 929 (w), 907 (w), 887 (w), 849 (w), 761 (w), 673 (w), 654 (w), 620 (w), 562 (w), 502 (w), 488 (w), 449 (w). ^{1}H NMR (400 MHz, CD_3OD) δ: 4.91 (d, 1H, *J* = 8.0 Hz, H-1), 3.86 (d, 1H, *J* = 3.2 Hz, H-4), 3.77 (d, 1H, *J* = 9.6 Hz, H-2), 3.73 (m, 2H, H-6 and H-7), 3.62 (t, 1H, *J* = 6.0 Hz, H-5), 3.56 (quintet, 4H, *J* = 6 Hz, H-1′ and H-4′), 3.53 (m, 1H, overlapped, H-6), 1.97 (m, 4H, H-2′ and H-3′). ^{13}C NMR (75 MHz, CD_3OD) δ: 104.21 (C-1), 76.14 (C-3), 73.64 (C-5), 69.29 (C-2), 68.85 (C-4), 61.12 (C-6), 50.70 (pyrrolidine's C-1′ and C-4′), 22.64 (pyrrolidine's C-2 and C-3′). MS (ESI) *m/z*: 316.1 $[M + Na]^+$, 609.0 $[2M + Na]^+$. MS/MS (ESI) *m/z*: 316.0 $[M + Na]^+$, 246.9 [M + Na – pyrrolidine (C_4H_8)]$^+$, 203.1, 185.9 [M + Na – pyrrolidine diazeniumdiolate (C_4H_8N–N(O)=NO)]$^+$, 157.9, 155.8. HR-MS (ESI) calcd. for $NaC_{10}H_{19}N_3O_7$ $[M + Na]^+$: 316.1121. Found: 316.1112. DSC onset temperature at 145.5 °C, decomposition temperature at 171.0 °C, ΔH = +37.05 kJ mol^{-1}. Anal. calcd. for $C_{10}H_{19}N_3O_7$: C, 40.95; H, 6.53; N, 14.33. Found: C, 40.45; H, 6.86; N, 14.04.

O^2-β-D-galactopyranosyl 1-(piperidin-1-yl)diazen-1-ium-1,2-diolate (3b)

A few drops of sodium methoxide (25% in methanol) were added to a solution of **2c** (190 mg, 0.400 mmol) in anhydrous methanol (20 mL) and stirred at RT overnight. TLC (methanol:ethyl acetate, 3:7, R_f = 0.36) showed the presence of a new product, which was isolated as a white powder (88.4 g, 0.288 mmol, 72%) after purification by column chromatography (silica gel, methanol:ethyl acetate, 1:4). The product was also obtained in better yield (94%) by allowing it to precipitate in solution. NMR studies confirmed that the material obtained in this manner is identical to the product obtained after chromatography. Alternatively, bypassing the column chromatography step during the synthesis of **2c** and carrying out the deprotection step right after the **2c** workup generated an overall yield of 65%. Spectroscopic results were identical whichever methodology was employed. UV (Tris Buffer (0.1 mol/L, pH 7.4) + $(NH_4)SO_4$ (1.7 mol/L) + $MgCl_2$ (10 mmol/L), RT) λ_{max} (ε): 224 nm (9.44 mmol/L^{-1} cm^{-1}). FTIR ν (cm^{-1}): 3418 (sbr), 3015 (w), 2965 (m), 2928 (m), 2886 (m), 2857 (m), 2705 (m), 2530 (m), 2408 (m), 1645 (m), 1483 (s), 1456 (w), 1384 (s), 1348 (w), 1297 (w), 1230 (m), 1151 (m), 1104 (s), 1085 (s), 1048 (s), 1004 (s), 962 (w), 923 (w), 898 (w), 875 (w), 864 (w), 854 (w), 823 (w), 758 (w), 694 (w), 660 (w), 614 (w), 558 (w), 489 (w). ^{1}H NMR (400 MHz, CD_3OD) δ: 4.96 (d, 1H, *J* = 8.0 Hz, H-1), 3.86 (d, 1H, *J* = 2.8 Hz, H-4), 3.80 (d, 1H, *J* = 9.6 Hz, H-2), 3.74 (m, 2H, H-6 and H-7), 3.63 (t, 1H, *J* = 6.0 Hz, H-5), 3.56 (dd, 1H, *J* = 9.6, 3.6 Hz, H-3), 3.41 (m, 4H, H-1′ and H-5′), 1.73 (quintet, 4H, *J* = 5.6 Hz, H-2′ and H-4′), 1.53 (quintet, 2H, *J* = 5.6 Hz, H-3′). ^{13}C NMR (75 MHz, CD_3OD) δ: 104.39 (C-1), 76.25 (C-3), 73.65 (C-5), 69.26 (C-2), 68.85 (C-4), 61.13 (C-6), 52.10 (C-1′ and C-5′), 24.51 (C-3′), 23.27 (C-2′ and C-4′). MS (ESI) *m/z*: 330.1 $[M + Na]^+$, 637.0 $[2M + Na]^+$, 944 $[3M + Na]^+$. MS/MS (ESI) *m/z*: 330.0 $[M + Na]^+$, 216.0 [M + Na - piperidine nitrosamine (i.e., - $C_5H_{10}N$–N=O)]$^+$, 203.1, 186.0 [M + Na – piperidine diazeniumdiolate ($C_5H_{10}N$–N(O)=NO)]$^+$, 158.1, 141.0, 128.0 [piperidine diazeniumdiolate – oxygen ($C_5H_{10}N$–N(O)=N)], 125.0, 113.9 [piperidine nitrosamine ($C_5H_{10}N$–N=O)]$^+$. HR-MS (ESI) calcd. for $NaC_{11}H_{21}N_3O_7$ $[M + Na]^+$: 330.1277. Found: 330.1267. DSC onset temperature at 153.9 °C, decomposition temperature at 164.1 °C, ΔH = +18.3 kJ mol^{-1}.

O^2-β-D-galactopyranosyl 1-(4-β-D-glucopyranosyl-4-aminomethylpiperidin-1-yl)diazen-1-ium-1,2-diolate (3c)

To a solution of **2e** (427 mg, 0.486 mmol) in anhydrous methanol (20 mL), a few drops of sodium methoxide (25% by weight in methanol) were added, and the solution was stirred at RT overnight. TLC (ethyl acetate:hexane, 7:3) showed absence of starting material. The solvent volume

was reduced to 5 mL under vacuum and upon addition of ether (20 mL), the product precipitated out of solution as an expected white solid. Because of its hygroscopic nature, it was collected by filtration under N_2 and dried under reduced pressure overnight (250 mg, 95%). While the product could not be purified by column chromatography, it was nevertheless quite pure by NMR standards. UV (Tris Buffer (0.1 mol/L, pH 7.4) + $(NH_4)SO_4$ (1.7 mol/L) + $MgCl_2$ (10 mmol/L), RT) λ_{max} (ε): 229 nm (7.44 mmol/L^{-1} cm^{-1}). FTIR ν (cm^{-1}): 3407 (sbr), 2926 (w), 1699 (w), 1652 (w), 1579 (w), 1451 (w), 1384 (s), 1270 (w), 1222 (w), 1151 (w), 1080 (m), 1020 (w), 957 (w), 888 (w), 836 (m), 763 (w), 685 (w), 610 (w), 553 (w), 481 (w), 469 (w). ^{1}H NMR (400 MHz, CD_3OD) δ: 4.97 (d, 1H, J = 7.6 Hz, galactopyranosyl H-1), 4.44 (m, 1H, glucopyranosyl H-4), 4.32 (m, 1H, glucopyranosyl H-1), 4.22 (m, H, glucopyranosyl H-2), 4.00 (m, 2H, piperidinyl H-1″ and H-5″), 3.87 (m, 1H, galactopyranosyl H-3), 3.75 (m, 1H, partly overlapped, galactopyranosyl H-2), 3.61 (2 dds, 4H, partly overlapped, galactopyranosyl H-4, H-5, H-6 and H-7), 3.48 (m, 1H, glucopyranosyl H-3) 3.12 (m, 1H, glucopyranosyl H-5), 3.03 (m, 2H, piperidinyl methylene Hs), 2.93 (t, 2H, J = 10.8 piperidinyl H-1′ and H-5′), 2.45 (m, 2H, glucopyranosyl H-6 and H-7), 1.86 (m, 2H, piperidinyl H-2′ and H-4′), 1.63 (m, 1H, piperidinyl H-3′), 1.37 (m, 2H, piperidinyl H-2″ and H-4″). ^{13}C NMR (75 MHz, CD_3OD) δ: 157.46 (amide's C=O), 100.86 (C-1), 91.76 (C-1″), 73.08 (C-3″), 72.93 (C-5″), 71.48 (C-5), 71.21 (C-3), 70.49 (C-2″), 68.13 (C-4″), 67.38 (C-2), 67.23 (C-4), 61.92 (C-6″), 61.33 (C-6), 50.93, 50.73 (C1′ and C-5′), 45.65, 45.48 (4-aminomethyl carbon), 35.58 (C-3′), 28.06, 27.87 (C-2′ and C-4′). MS (ESI) m/z: 565.2 [M + Na]$^+$. MS/MS (ESI) m/z: 565.1 [M + Na]$^+$, 372.1 [M + Na – pyranose ($C_6H_{11}O_5$) – NO]$^+$, 341.1 [M + Na – pyranose ($C_6H_{11}O_5$) – 2NO – H]$^+$. HR-MS (ESI) calcd. for $NaC_{19}H_{34}N_4O_{14}$ [M + Na]$^+$: 565.1969. Found: 565.1966. DSC onset temperature at 146.5 °C, decomposition temperature at 176.7 °C, ΔH = +102 kJ mol^{-1}.

1,2,3,4-Tetra-*O*-acetyl-β-D-glucopyranosyl succinimidyl carbonate (GluDSC) (4)

To a solution of 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose (200 mg, 0.575 mmol, 1 equiv.) in anhydrous acetonitrile (25 mL), *N*,*N*′-disuccinimidyl carbonate (DSC, 221 mg, 0.863 mmol, 1.5 equiv.) and triethylamine (TEA, 240 μL, 1.73 mmol, 3 equiv.) were added, and the mixture was stirred until all the glucopyranose had reacted (overnight, TLC monitored, ethyl acetate:hexane, 3:1). The solvent was removed under vacuum and the residue was dissolved with aqueous $NaHCO_3$ (1.0 mol/L solution) and extracted with ethyl acetate (3 × 30 mL). The combined extracts were washed with brine (20 mL) and dried over Na_2SO_4 (2.0 g), filtered, and the solvent was removed under vacuum. The isolated material turned out as a yellow sticky paste (265 mg, 0.542 mmol, 94%). The product turned out to be reactive with silica gel and hence was not further purified by column chromatography. NMR studies have demonstrated nonetheless rather high purity (yield by NMR: 96%). UV (DCM, RT) λ_{max} (ε): 229 nm (0.509 mmol/L^{-1} cm^{-1}). FTIR ν (cm^{-1}): 3487 (wbr), 2950 (w), 1817 (m), 1791 (m), 1743 (s), 1636 (w), 1432 (w), 1370 (m), 1222 (sbr), 1166 (w), 1078 (m), 1040 (m), 915 (w), 899 (w), 853 (w), 838 (w), 817 (w), 794 (w), 779 (w), 762 (w), 645 (w), 602 (w), 555 (w), 492 (w). ^{1}H NMR (300 MHz, CD_2Cl_2) δ: 5.77 (d, 1H, J = 10.8 Hz, H-1), 5.30 (t, 1H, J = 12.4 Hz, H-3), 5.11 (dd, 1H, J = 9.3, 11.7 Hz, H-2), 5.08 (dd, 1H, J = 9.3, 13.8 Hz, H-4), 4.43 (dd, 1H, J = 16.0, 3.6 Hz, H-6), 4.35 (dd, 1H, J = 16.0, 7.0 Hz, H-7), 3.99 (ddd, 1H, J = 3.6 Hz, H-5), 2.83 (s, 4H, succinimide Hs), 2.13 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.01 (s, 3H, CH_3). ^{13}C NMR (75 MHz, CD_3OD) δ: 170.06, 169.74, 169.34, 169.11 (acetyl C=O), 168.82 (succinimide C=O), 151.45 (activated ester C=O), 91.61 (C-1), 72.48 (C-5), 72.14 (C-2), 70.13 (C-4), 68.58 (C-3), 68.17 (C-6), 25.83, 25.71 (succinimide CH_2), 20.81, 20.61, 20.58 (acetyl CH_3). MS (ESI) m/z: 512.1 [M + Na]$^+$. MS/MS (ESI) m/z: 512.0 [M + Na]$^+$, 452.0 [M + Na – *O*-acetyl (O–C(=O)CH_3) – H]$^+$. HR-MS (ESI) calcd. for $NaC_{19}H_{23}N_1O_{14}$ [M + Na]$^+$: 512.1016. Found: 512.1007.

X-ray diffraction crystal structure of 3a

Crystals of **3a** suitable for single-crystal X-ray diffraction were grown from water/methanol and crystallized in the monoclinic noncentrosymmetric space group $P2_1$ Z = 2, with unit cell dimensions of a = 4.8350(18), b = 9.435(3), and c = 15.552(6) Å; β = 92.423(5)°, α = γ = 90°; V = 708.8(4) Å^3; The structure was solved by direct methods, and the refined chirality was based upon that of the starting carbohydrate. Other key crystallographic data include ρ_c = 1.459 Mg m^{-3}; crystal size = 1.0 × 0.05 × 0.01 mm^3. Data: 2882 independent data with $I > 2\sigma(I)$, S_{gof} = 1.039; R_1 = 4.02%, wR_2 = 10.52%. For further details, see the Supplementary data section.

Investigation of the stability of compounds 3a–3c

The solid deprotected glycosylated diazeniumdiolates are quite hygroscopic and have a tendency to turn brown over the course of a few hours if exposed to warm moist air. However, NMR examination of these brown materials reveals that there is only slight degradation (<5%) of the products. Under N_2, the same materials do not change in consistency over the course of two weeks at room temperature. Again, NMR examination showed negligible degradation of these materials when stored at room temperature under N_2. The optimal conditions for these compounds are as dried solids at –20 °C under N_2 in the dark. Under these conditions, the compounds have been stored for up to six months without significant degradation. Probe stability in aqueous and alcoholic media was also determined at room temperature over 2–3 weeks using NMR (D_2O, CH_3OD) and by UV (H_2O, Tris buffer (0.1 mol/L, pH 7.4), CH_3OH). Degradation over this time period was in general minor (<10%) as revealed by either spectroscopic technique. The acetylated intermediate sugar diazeniumdiolates also presented similar stability. These oils were generally clear and colorless after purification (except for compound **3**) and slowly yellowed over 2–3 weeks when exposed to air at room temperature. NMR examination of these yellow oils show some signs of degradation (<2%). The optimal storage conditions for these oils were the same as for the deprotected sugars. However, both acetylated and deprotected sugar diazeniumdiolates degraded considerably (~40%) when exposed continually to UV light for prolonged periods of time (4–5 h).

Supplementary data

Supplementary data for this article (general methods, instrumentation, preparation, and diffraction data) are available on the journal Web site (canjchem.nrc.ca). CCDC 740859 contains the X-ray data in CIF format for this manuscript. These data can be obtained, free of charge, via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax +44 1223 336033; or deposit@ccdc.cam.ac.uk).

Acknowledgements

The authors thank the National Institutes of Health (NIH) (grant No. 5R33CA109965), Quebec Transgenic Research Network (QTRN), and Canada Research Chair (CRC) for financial support of this research, and the Canadian Institutes of Health Research (CIHR) for Trainee fellowship to AC.

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