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The synthesis and antibacterial activity of doxycycline neoglycosides

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Abstract

A set of 37 doxycycline neoglycosides mediated via a C9 alkoxyamino-glycyl-based spacer reminiscent to that of tigecycline were prepared. The *in vitro* antibacterial assays against representative drug resistant Gram negative and Gram positive strains revealed a sugar-dependent activity profile and one doxycycline neoglycoside, the 2 -amino- -D-glucoside conjugate, to rival that of the parent pharmacophore. In contrast, the representative tetracycline-susceptible strains *E. coli* 25922 was found to be relatively responsive to a range of doxycycline neoglycosides. This study also extends the use of aminosugars in the context of neoglycosylation via a simple two step strategy anticipated to be broadly applicable for neoglycorandomization.

Tetracyclines are broad-spectrum antibiotics that have been in clinical use for over six decades and inhibit bacterial protein synthesis by binding bacterial 30S rRNA.^{1,2} Bacterial resistance to tetracyclines has spurred continual clinical development of analogues to circumvent primary resistance mechanisms,³ with tigecycline as the latest member approved for clinical use (Table 1). A semi-synthetic derivative of 9-amino minocycline, tigecycline is considered a new antibiotic class (the glycyclines) by virtue of its novel mode of 30S rRNA binding and extended spectrum of antibacterial activity.⁴⁻⁶ This unique activity derives from a key *tert*-butylation of the 9-glycylamino minocycline core and other short chain alkyl substitutions of the core architecture also provide antibacterial advantages.⁷⁻⁹ The success of glycyclines exemplifies the potential for antibiotic development through very subtle structural modifications of the privileged tetracycline pharmacophore.

In nature, such subtle modifications occur via a range of simple tailoring reactions including acylation,¹⁰ alkylation,¹¹ and glycosylation.¹² With respect to the latter, while naturally-occurring tetracycline glycosides have been reported including dactylocycline,¹³ TAN-1518,¹⁴ and SF2575,¹⁵ the systematic differential glycosylation of the tetracycline scaffold has not been pursued. Among emerging strategies to enable the rapid systematic differential glycosylation of complex natural products,^{12,16} neoglycosylation employs a mild

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Supporting Information Synthetic procedures of sugars, ¹H, ¹³C, and related spectra of the synthesized compounds, activity assessment results. This material is available free of charge via the internet at <http://pubs.acs.org>.

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chemo-selective reaction between free reducing sugars and alkoxyamine-bearing neoglycons.¹⁷⁻²⁰ As a result, neoglycosylation avoids the many protection/deprotections or anomeric activation manipulations typically required for glycoside synthesis by conventional methods.²¹ Inspired by both the impact of 9-glycylamino-tetracycline modification upon activity and the striking structural similarity between the tigecycline 9-glycylamino spacer and previously reported glycyl-based linkers for neoglycosylation,^{18,20b} herein we report the synthesis of a set of differentially glycosylated 9-(methoxyglycyl)amino-doxycyclines as a simple tetracycline model. This study also highlights the first general application of aminosugars in the neoglycosylation reaction. Activity assessment of the doxycycline neoglycosides revealed the antibacterial potency of one specific aminosugar derived doxycycline neoglycoside to rival that of the parent pharmacophore. In addition, tetracycline sensitive *E. coli* was found to be relatively responsive to a range of doxycycline neoglycosides with a bias toward C-2 -substituted glucosides as most advantageous in this regard. Given the range of divergent activities reported for doxycycline analogues (including anticancer,²² anti-inflammatory,²³ antiprotozoal,²⁴ antihelminthic,²⁵ multiple sclerosis,²⁶ or neurodegenerative disease²⁷), the diversification strategies and analogues highlighted herein may extend to other applications.

RESULTS AND DISCUSSION

The targeted doxycycline neoglycosides were specifically designed to incorporate a glycyl spacer reminiscent of tigecycline. The synthesis of the doxycycline neoglycon **4** (Scheme 1, Figure 1) was initiated by regio-selective nitration of doxycycline **1**.⁹ Subsequent reduction of 9-nitro-doxycycline afforded 9-amino-doxycycline (**2**, 99% in 2 steps from **1**) and acylation of **2** with succinimidyl ester **3**¹⁸ followed by borane-trimethylamine-mediated reduction gave neoglycon **4** (84% in 2 steps from **2**) to set the stage for neoglycosylation. Cumulatively, 0.56 gm of neoglycon **4** (4 steps, 84% overall yield) was produced to enable the synthesis of the diverse set of doxycycline neoglycosides as described below.

Small scale optimization of **4** neoglycosylation with D-glucose revealed DMF/2.5% TFA, 40 °C, 12 h as the best conditions to afford desired neoglycoside while minimizing degradative side reactions (10 mg scale, 46%). Using these optimized conditions, the neoglycosylation of **4** using a diverse range of monosaccharides (**5-40**) was subsequently pursued (Figure 1 and Table 2). Saccharides employed in this endeavor included a representative D-tetrose (D-erythrose for **Dx13**), D-pentoses (D-xylose for **Dx02**, D-ribose for **Dx07**, D-arabinose for **Dx12**, 2-azido-D-xylose for **Dx39**, 4-azido-L-ribose for **Dx41**), D- and L-hexoses (L-rhamnose for **Dx03**, D-fucose for **Dx06**, forosamine for **Dx14**, digitoxose for **Dx22**, 2-deoxy-D-glucose for **Dx38**, D/L-glucose for **Dx01/Dx11**, D/L-galactose for **Dx04/Dx19**, D-mannose for **Dx30**, deoxy-fluoro-D-glucoses for **Dx18/Dx29**, azido-deoxy-D-glucoses for **Dx16/Dx17**, 2-azido-D-mannose for **Dx31**, 2-azido-D-galactose for **Dx33**, 3-O-methy-D-glucose for **Dx10**, *N*-acetyl-D-glucosamine for **Dx05**, *N*-trifluoroacetyl-D-glucosamine for **Dx37**, *N*-allyloxycarbonyl-D-glucosamine for **Dx15**, streptozocin for **Dx21**, 6-*N*-decanoyl-D-glucosamine for **Dx25**, *N*-acetylmuramic acid for **Dx23**), acid-bearing sugars (D-glucuronic acid for **Dx09**) and a disaccharide (cellobiose for **Dx20**). The yield of neoglycosylation varied by sugar with an average isolated yield of 50% for most hexoses under standard conditions (DMF/2.5% TFA, 40 °C, 12 h). Using the same conditions, pentoses led to lower isolated yield (ranges from 11% to 41%) while decomposition was predominate in the tetrose-based reaction at longer reactions times. Milder conditions (3:1 DMF/AcOH, 40 °C, 6 h) enabled the desired tetrose neoglycoside in 44% isolated yield. Consistent with previous studies,¹⁷⁻²⁰ the α -anomer was the predominate product with notable exceptions including D-erythroside, D-arabinside, D-riboside, 2-deoxy-D-glucoside, and D-mannoside (Table 2, Figure 1).

The incompatibility of unprotected aminosugars with neoglycosylation has dramatically restricted their prior use in neoglycorandomization.¹⁸⁻²⁰ Specifically, while amine-bearing neoglycons can be compensated for via additional monosaccharide, an excess of aminosugar is believed to compete for the initial oxime-forming stage of the chemoselective neoglycosylation reaction. To address this limitation, the current study employed a small set of free azidosugars described in the previous paragraph (Figure 1, **Dx16**, **Dx17**, **Dx31**, **Dx33**, **Dx39**, **Dx41**) to enable the synthesis of the corresponding neoglycosides as requisite precursors of aminosugar-bearing analogues. For this work, 2-azido-D-glucose (**21**), 2-azido-D-mannose (**33**), and 2-azido-D-galactose (**34**) were synthesized from the corresponding D-glucosamine hydrochloride, D-mannosamine hydrochloride, and D-galactosamine hydrochloride, respectively via amino-azide interconversion using perfluorobutylsulfonyl azide as the diazo transfer agent.²⁹ In addition, two azido pentoses (**39** and **40**) were synthesized via selective protection of D-lyxose (See Figure S3, supporting information) and subsequent azide installation as an alternative to previously reported strategies.³¹ Neoglycosylation using this azidosugar set was accomplished with an overall average isolated yield of 36% under standard conditions. In all cases, post-neoglycosylation reduction (Pd/CaSO₄, H₂) furnished the desired aminosugar products (Figure 1, **Dx26**, **Dx27**, **Dx32**, **Dx34**, **Dx40**) with an overall average yield of 26% (two steps from neoglycon **4**). Intriguingly, while the doxycycline 2-azido-D-neoglucoside anomers (**Dx17**, 1:1 /) could not be resolved chromatographically, the corresponding (**Dx26a**) and (**Dx26b**) anomers of the 2-amino-D-neoglucoside product were obtained with an isolated yield of 20% and 22%, respectively, after preparative HPLC.

The set of 37 doxycycline neoglycosides were tested for antibacterial activity using a panel of four bacterial strains comprised of a tetracycline susceptible Gram negative strain (*E. coli* 25922) and two drug-resistant clinical isolates (the Gram positive *S. aureus* R2507 and Gram negative *E. coli* 1-849) (Table 3 and Table S2, supporting information). While the mechanisms of drug resistance in these latter two strains have not been determined, both are known to display ~10-fold tetracycline/doxycycline resistance compared to their wild-type counterparts. This cumulative assessment enabled the following observations. First, consistent with previous studies,⁹ 9-amino-doxycycline (**2**) and doxycycline (**1**) were nearly equipotent with 2 MIC values of 1 µg/ml, 2 µg/ml, 4 µg/ml versus 1 values of 1 µg/ml, 8 µg/ml, 2 µg/ml against *E. coli* 25922, *E. coli* 1-849, and *S. aureus* R2507, respectively (Table S2, supporting information). In comparison, neoglycon **4** displayed a slight reduction in potency depending upon strain tested (ranging from 2-4 fold). While a general trend of further reduced potency upon neoglycosylation of **4** was observed, the 2-amino- -D-neoglucoside (**Dx26a**, MIC 4 µg/ml) afforded a slight improvement over **1** or neoglycon **4** against the tetracycline resistant strain *E. coli* 1-849 (Table S2, supporting information). In addition, the response of the tetracycline sensitive *E. coli* 25922 was more tolerant to sugar conjugate variation, with four additional neoglycosides (**Dx13**, **Dx14**, **Dx32**, **Dx37**) displaying similar activities to the neoglycon **4** (MIC 4 µg/ml). Finally, while neoglycosylation generally reduced potency in the context of the tetracycline resistant strain *S. aureus* R2507, the conjugation to 2-deoxy-2-substituted glucoside analogues were found to be the most active conjugates. The overall trend of reduced activity observed upon neoglycosylation may derive from disfavored interactions with key contributors to binding short chain alkylated glycylicylines – specifically, helices H34/H18 and/or C1054 of 30S rRNA^{5,6} – where contacts afforded by the aminosugar substitution in **Dx26a** may partially compensate for unfavored interactions.

The cytotoxicity of doxycycline neoglycon **4** and the nine most potent antibacterial neoglycosides was subsequently assessed using both the non-small cell cancer cell line A549 and a comparator normal lung fibroblast cell line IMR 90 (Table 3). This analysis revealed no statistically significant cytotoxicity at 10 µM - a dose well above the typical

serum concentration used to treat bacterial infections with existing clinical tetracyclines (0.2 to 5 $\mu\text{g/ml}$).³² This preliminary analysis suggests the general toxicity of the parent **1** and corresponding neoglycosides as potentially similar.

In summary, this study highlights the first systematic differential glycosylation of the privileged tetracycline scaffold. While neoglycosylation at C9 was predominately detrimental, one analogue, the 2-amino- β -D-glucoside conjugate **Dx26a**, afforded a slight antibacterial benefit over the parental doxycycline against the tetracycline resistant strain *E. coli* 1-849 and corresponding low general cytotoxicity. While the influence of sugar conjugation upon *in vivo* drug properties (ADMET) remains to be determined, this study highlights the amenability of this complex scaffold to neoglycosylation and opens the door to similar modifications at other key positions of the tetracycline architecture, particularly those known to be influenced via glycosylation. In addition, this study extends the use of aminosugars in the context of neoglycosylation via a simple two step strategy anticipated to be broadly applicable for neoglycorandomization.

EXPERIMENTAL SECTION

General Experimental Procedures

Reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were used without purification unless otherwise noted. Dichloromethane was freshly distilled from calcium hydride under nitrogen atmosphere. Pyridine and triethylamine were distilled and stored over 4 Å molecular sieves. Analytical TLC was performed using Sorbent Technologies silica gel glass TLC plates (EMD chemical Inc, PA, USA). Visualization was accomplished with UV light (254 nm) followed by staining with diluted sulfuric acid (5% in ethanol) solution and heating. Mass spectrometric data were obtained on a Waters (Milford, MA) LCT time-of-flight spectrometer for electrospray ionization (ESI). NMR spectra were obtained on either a Varian Unity Inova 400 or 500 MHz instrument (Palo Alto, CA) using 99.8% CDCl_3 with 0.05% v/v TMS or 99.8% CD_3OD from Cambridge Isotopes (Cambridge Isotope Laboratories, MA, USA). ^1H and ^{13}C chemical shifts were referenced to TMS for both CDCl_3 and CD_3OD . Multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Chemical shifts are reported in parts per million (ppm) and coupling constants *J* are given in Hz.

Chromatography Methods

Method A: Normal phase flash chromatography was performed using 40–63 μm particle-sized silica gel using ethyl acetate and hexane or methanol and methylene chloride as the mobile phase. **Method B:** Pilot purification was conducted by flash column chromatography using an Alltech C_{18} Extrac-Clean column (10000 mg, 75 mL, Alltech Associates, Deerfield, IL, USA) with gradient elution water/acetonitrile 100/0 to 50/50 containing 0.1% TFA. **Method C:** Semi-preparative reverse-phase HPLC was conducted using a Gemini C_{18} (5 μm , 250 \times 10 mm, Phenomenex, Torrance, California, USA) using a gradient of 5% B to 55% B over 27 min, 55% B to 100% B over 1 min, 100% B for 5 min, 100% B to 5% B over 1 min, 5% B for 4 min (A = dH_2O with 0.1% TFA; B = acetonitrile; flow rate = 5 mL min^{-1} ; λ_{254} nm). The desired fractions were collected and concentrated under reduced pressure, frozen at -80°C , and lyophilized. **Method D:** Analytical reverse-phase HPLC was conducted with a Luna C_{18} (4.6 mm \times 250 mm, Phenomenex, Torrance, California, USA) with a gradient of 5% B to 55% B over 20 min, 55% B to 100% B over 1 min, 100% B for 5 min, 100% B to 5% B over 1 min, 5% B for 3 min (A = dH_2O with 0.1% TFA; B = acetonitrile; flow rate = 1 mL min^{-1} ; λ_{254} nm). HPLC peak areas were integrated with Star Chromatography Workstation software (from Varian, Palo Alto, CA, USA) and the percent conversion calculated as a percent of the total peak area.

Cytotoxicity assays

A resazurin-based cytotoxicity assay, also known as AlamarBlue assay, was used to assess the cytotoxicity of agents against the human lung non-small cell carcinoma cell line A549 cell line and normal fibroblast IMR 90 cell lines where degree of cytotoxicity was based upon residual metabolic activity as assessed via reduction of resazurin (7-hydroxy-10-oxido-phenoxazin-10-ium-3-one) to its fluorescent product resorufin. A549 and IMR 90 cells, purchased from ATCC (Manassas, VA, USA), were grown in DMEM/F-12 Kaighn's modification and MEM/EBSS media, respectively (Thermo scientific HyClone, Logan, UT, USA), with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin, 2 mM L-glutamine. Cells were seeded at density 2×10^3 cells per well onto 96-well culture plates with a clear bottom (Corning, NY, USA), incubated 24 hours at 37 °C in a humidified atmosphere containing 5% CO₂ and were exposed to standard toxin (positive controls - 1.5 mM hydrogen peroxide, 10 µg/ml actinomycin D) and test compounds (selected doxycycline neoglycosides at final concentration of 10 µM) for 2 days. Resazurin (150 µM final concentration) was subsequently added to each well, plates were shaken briefly for 10 seconds and were incubated for another 3 h (A549 cells) and 5 h (IMR 90 cells) at 37 °C to allow viable cells to convert resazurin into resorufin. The fluorescence intensity for resorufin was detected on a scanning microplate spectrofluorometer FLUOstar Omega (BMG LABTECH GmbH, Ortenberg, Germany) using an excitation wavelength of 560 nm and an emission wavelength of 590 nm. The assay was repeated in 3 independent experimental replications. In each replication, the resorufin values of treated cells were normalized to, and expressed as percent of, the mean resorufin values of untreated, metabolically active cells (100%, all cells are viable).

Antibacterial assays

Antibacterial assays using the two community acquired clinically-resistant isolates *S. aureus* R2507 and *E. coli* 1-849 (JMI Laboratories, North Liberty Iowa), and the standard *E. coli* 25922 model (ATCC, Manassas, VA), were conducted as previously described.^{20g}

Stability of Doxycycline Neoglycoside Stock Solutions

A 3 mM DMSO solution of **Dx26a** was stored at -20 °C for 12 months. Subsequent purity assessment by HPLC revealed no change under these storage conditions over time.

(4S,4aR,5S,5aR,6R,12aS)-4-(dimethylamino)-9-amino-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (2)

To a solution of doxycycline hyclate (2.00 g, 3.9 mmol) in concentrated H₂SO₄ solution was added KNO₃ (0.46 g, 4.6 mmol) and the reaction was stirred under argon and monitored by HPLC (method D) for consumption of starting material. After 3 h, the reaction solution was added to ether at 0 °C in a dropwise fashion to afford a heavy yellow precipitate that was subsequently collected by vacuum filtration and washed with cold ether (20 mL, x3). The yellow precipitate was dissolved in 40 mL degassed MeOH and to this 20 mg Pd/CaSO₄ was added. The flask was sealed, degassed and exposed to a H₂ balloon with stirring for 12 hours. The reaction was subsequently filtered through celite, the collected residue washed with MeOH (10 mL, x3) and the combined filtrate concentrated to 10 mL. The concentrated MeOH solution was subsequently added to the solution of cold EtOAc/hexane (1/1) in a dropwise fashion to afford a heavy precipitate that was subsequently collected via filtration, washed with cold hexane and dried under vacuum. Compound **2** (2.16 g, 3.9 mmol) was obtained in a yield of 99% after two steps as a yellow powder: ¹H NMR (CD₃OD, 500 MHz) 8.50 (s, 1 H), 4.15 (s, 1 H), 3.3 (m, 1 H), 3.23 (m, 6 H), 3.0 - 3.1 (m, 3 H), 3.0 (m, 3H), 2.59 (dd, *J* = 15.7, 13.5 Hz, 2 H), 2.4 (m, 2 H), 1.7 (m, 1 H); ¹³C NMR (CD₃OD, 100 MHz) 192.8, 172.4, 159.7, 154.6, 151.3, 142.7, 137.5, 130.6, 124.8, 116.6, 107.9, 95.8,

94.6, 73.8, 53.6 (2 carbons), 45.9, 41.6, 38.9, 31.2, 16.4, 15.8; HRESIMS m/z 460.17299 [$M + H$]⁺ (Calcd for C₂₂H₂₆N₃O₈ 460.1714).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxyglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (4)

To a solution of **2** (0.56 g, 1.2 mmol) in 4 mL DMF was added 0.5 mL pyridine and succinimidyl ester **3** (0.48 g, 2.4 mmol)¹⁸ and the reaction was stirred at room temperature for 24 h. The reaction was quenched by removing the solvent in *vacuo* and then the residue was dissolved with 3 mL MeOH to which was added 40 mL EtOAc followed by 20 mL hexane to afford a heavy yellow precipitate. The precipitate was filtered, washed with cold hexane (10 mL, x3) and dried under vacuum. The yellow solid was dissolved with water and the pH of the mixture was adjusted to 7 by adding 5% NH₄OH in a dropwise fashion. The solvent was removed under vacuum and the residue lyophilized overnight to give a yellow solid. The corresponding solid was dissolved in 20 mL EtOH to which was added BH₃•Me₃N (0.88 g, 12 mmol) and 2 mL 50% HCl in ethanol. The reaction was stirred under room temperature for 6 h (until the reaction was complete based upon HPLC - method D), solvent removed under vacuum, and the residue dissolved in 3 mL MeOH. Addition of 40 mL EtOAc followed by 10 mL hexane afforded a heavy precipitate which was collected via filtration and purified by flash reverse-phase (method B). Purified doxycycline neoglycon **4** (0.56 g, 1.0 mmol) was obtained with a yield of 84% after two steps from **2** as a yellow powder: ¹H NMR (CD₃OD, 500 MHz) 8.49 (d, *J* = 8.5 Hz, 1 H), 7.01 (d, *J* = 8.8 Hz, 1 H), 4.43 (s, 1 H), 3.67 (s, 5 H), 3.6 (m, 1 H), 2.98 (s, 6 H), 2.9 (m, 1 H), 2.8 (m, 1 H), 2.6 (m, 1 H), 1.59 (d, *J* = 8.1 Hz, 3 H); ¹³C NMR (CD₃OD, 125 MHz) 172.9, 168.3, 160.4, 160.1, 151.6, 142.8, 126.7, 125.2, 117.4, 115.5, 115.2, 107.4, 95.0, 73.5, 68.8, 65.9, 63.9, 61.2 (2 carbons), 54.1, 46.7, 44.2, 41.8, 38.6, 15.0; HRESIMS m/z 547.20165 [$M + H$]⁺ (Calcd for C₂₅H₃₁N₄O₁₀ 547.2035).

General procedure of neoglycosylation

Neoglycon **4** (10–40 mg) and reducing sugar (1.2 - 2.0 eq) were dissolved in 1 mL anhydrous DMF and to this was added 25 μL TFA. The reaction was stirred at 40 °C for 24 h and monitored by HPLC (method D). Upon completion, solvent was removed under vacuum and the residue was dissolved in 300 μL MeOH containing 0.01% NH₄OH. The solution was centrifuged and the collected supernatant was purified via semi-preparative HPLC (method C). The final purified doxycycline neoglycosides were obtained in a yield ranging from 20%-69% from doxycycline neoglycon **4**.

General hydrogenation procedure for azidosugar appended doxycycline neoglycosides

Doxycycline neoglycoside (**Dx16**, **17**, **31**, **33**, **39**) was dissolved into 10 mL degassed MeOH to which was added 1 mg Pd/CaSO₄. The vial was sealed, degassed and exposed to a H₂ balloon for 12 h with stirring. The reaction was subsequently filtered over celite, washed with 10 mL MeOH and the filtrate dried under vacuum. The residue was dissolved in 300 μL MeOH containing 0.01% NH₄OH, centrifuged and the collected supernatant was purified via semi-preparative HPLC (method C). The final purified aminosugar-appended doxycycline neoglycosides were obtained with an isolated yield ranging from 20%-31% after two steps from the doxycycline neoglycon **4**.

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*- β -D-glucosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx01)

yellow powder; $^1\text{H NMR}$ (CD_3OD , 500 MHz) 8.31 (d, $J = 8.3$ Hz, 1 H), 6.99 (d, $J = 8.1$ Hz, 1 H), 4.26 (d, $J = 8.7$ Hz, 1 H), 3.6 – 3.9 (m, 10 H), 3.74 (s, 3 H), 2.89 (s, 6 H), 2.80 (dd, $J = 12.1$, 7.4 Hz, 1 H), 2.7 (m, 1H), 2.58 (dd, $J = 12.4$, 8.3 Hz, 1 H), 1.56 (d, $J = 6.8$ Hz, 3 H); HRESI m/z 709.25484 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{31}\text{H}_{41}\text{N}_4\text{O}_{15}$ 709.2563).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*- β -D-xylosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx02)

yellow powder; $^1\text{H NMR}$ (CD_3OD , 500 MHz) 8.31 (d, $J = 8.5$ Hz, 1 H), 6.98 (d, $J = 8.3$ Hz, 1 H), 4.41 (s, 2 H), 4.19 (d, $J = 8.8$ Hz, 1 H), 3.92 (dd, $J = 11.2$, 5.4 Hz, 1 H), 3.3 – 3.8 (m, 3 H), 3.73 (s, 3 H), 3.1 - 3.2 (m, 3 H), 3.01 (s, 6 H), 2.94 (dd, $J = 12.0$, 9.8 Hz, 1 H), 2.8 (m, 1 H), 2.59 (dd, $J = 12.2$, 8.3 Hz, 1 H), 1.56 (d, $J = 6.8$ Hz, 3 H); HRESI m/z 679.24703 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{30}\text{H}_{39}\text{N}_4\text{O}_{14}$ 679.2457) .

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*- β -L-rhamnosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx03)

yellow powder; $^1\text{H NMR}$ (CD_3OD , 500 MHz) 8.41 (d, $J = 8.3$ Hz, 1 H), 6.97 (d, $J = 9.0$ Hz, 1 H), 4.44 (d, $J = 1.0$ Hz, 1 H), 4.3 (m, 1H), 4.20 (t, $J = 3.1$ Hz, 1 H), 4.06 (d, $J = 2.7$ Hz, 1 H), 3.9 – 4.0 (m, 1 H), 3.73 (s, 3 H), 3.72 (s, 2 H), 3.5 (m, 1 H), 3.5 (m, 1 H), 2.8 (m, 7 H), 2.5 (m, 2 H), 1.55 (d, $J = 6.8$ Hz, 3 H), 1.32 (d, $J = 5.9$ Hz, 3 H); HRESI m/z 693.26213 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{31}\text{H}_{41}\text{N}_4\text{O}_{14}$ 693.2614).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*- β -D-galactosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx04)

yellow powder; $^1\text{H NMR}$ (CD_3OD , 500 MHz) 8.32 (d, $J = 8.4$ Hz, 1 H), 6.99 (d, $J = 8.5$ Hz, 1 H), 4.36 (s, 1 H), 4.26 (d, $J = 9.0$ Hz, 1 H), 3.8 - 4.0 (m, 2 H), 3.6 (s, 3 H), 3.4 - 3.8 (m, 7 H), 3.93 (s, 6 H), 2.8 (m, 2 H), 2.58 (dd, $J = 12.0$, 8.2 Hz, 1 H), 1.56 (d, $J = 6.7$ Hz, 3 H); HRESI m/z 709.25597 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{31}\text{H}_{41}\text{N}_4\text{O}_{15}$ 709.2563).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-2'-deoxy-2'-*N*-acetyl-amino- β -D-glucosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx05)

yellow powder; $^1\text{H NMR}$ (CD_3OD , 500 MHz) 8.49 (d, $J = 8.3$ Hz, 1 H), 6.98 (d, $J = 8.3$ Hz, 1 H), 4.42 (d, $J = 10.0$ Hz, 1 H), 4.32 (s, 1 H), 3.9 (m, 2 H), 3.7 (m, 3 H), 3.63 (s, 3 H), 3.6 (m, 6 H), 3.50 (t, $J = 8.8$ Hz, 1 H), 2.92 (s, 6 H), 2.7 - 2.8 (m, 2 H), 2.58 (dd, $J = 12.1$, 8.2 Hz, 1 H), 2.08 (s, 3 H), 1.55 (d, $J = 6.8$ Hz, 3 H). HRESI m/z 750.28313 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{33}\text{H}_{44}\text{N}_5\text{O}_{15}$ 750.2828).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*- β -D-fucosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx06)

yellow powder; $^1\text{H NMR}$ (CD_3OD , 500 MHz) 8.30 (d, $J = 8.4$ Hz, 1 H), 6.96 (d, $J = 8.4$ Hz, 1 H), 4.22 (d, $J = 8.6$ Hz, 1 H), 3.8 - 3.9 (m, 3 H), 3.72 (s, 3 H), 3.5 - 3.7 (m, 5 H), 2.80 (br. s, 6 H), 2.6 (m, 3 H), 1.55 (d, $J = 6.6$ Hz, 3 H), 1.28 (d, $J = 6.4$ Hz, 3 H); HRESI m/z 693.26212 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{31}\text{H}_{41}\text{N}_4\text{O}_{14}$ 693.2641).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-*D*-riboseglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx07)

yellow powder; ¹H NMR (CD₃OD, 500 MHz) 8.35 (d, *J* = 8.3 Hz, 1 H), 6.99 (d, *J* = 8.1 Hz, 1 H), 4.59 (m, 1 H), 4.54 (d, *J* = 8.8 Hz, 1 H), 4.19 (m, 1 H), 4.15 (m, 2 H), 4.08 (t, *J* = 5.5 Hz, 1 H), 3.8 - 3.9 (m, 1 H), 3.75 (s, 3 H), 3.6 - 3.7 (m, 2 H), 3.59 (dd, *J* = 9.2, 2.8 Hz, 1 H), 3.47 (s, 1 H), 2.8 (s, 6 H), 2.6 (m, 3 H), 1.58 (d, *J* = 7.1 Hz, 3 H); HRESI m/z 679.24632 [M + H]⁺ (Calcd for C₃₀H₃₉N₄O₁₄ 679.2457).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-β-*D*-glucuronosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx09)

yellow powder; ¹H NMR (CD₃OD, 500 MHz) 8.24 (d, *J* = 8.4 Hz, 1 H), 6.97 (d, *J* = 8.3 Hz, 1 H), 4.7 (m, 1 H), 4.56 (d, *J* = 6.1 Hz, 1 H), 4.51 (m, 1 H), 4.1 (m, 1 H), 4.0 (m, 1 H), 3.68 (s, 3 H), 3.6 - 3.7 (m, 3 H), 3.5 (m, 1 H), 3.4 (m, 1 H), 3.2 (m, 1 H), 2.7 (s, 6 H), 2.6 (m, 2 H), 2.58 (dd, *J* = 11.6, 7.4 Hz, 1 H), 1.56 (d, *J* = 7.3 Hz, 3 H). HRESI m/z 737.24934 [M + H]⁺ (Calcd for C₃₂H₄₁N₄O₁₆ 737.2512).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-3'-*O*-methyl-β-*D*-glucosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx10)

yellow powder; ¹H NMR (CD₃OD, 500 MHz) 8.31 (d, *J* = 8.5 Hz, 1 H), 6.98 (d, *J* = 8.3 Hz, 1 H), 4.27 (d, *J* = 9.3 Hz, 1 H), 3.8 (m, 2 H), 3.78 (s, 2 H), 3.73 (s, 3 H), 3.67 (s, 3 H), 3.6 - 3.7 (m, 2 H), 3.5 (m, 1 H), 3.4 (m, 1 H), 3.3 (m, 1 H), 3.1 (m, 1 H), 2.84 (s, 6 H), 2.8 (m, 1 H), 2.7 (m, 1 H), 2.58 (dd, *J* = 12.1, 7.9 Hz, 1 H), 1.56 (d, *J* = 7.1 Hz, 3 H); HRESI m/z 723.27208 [M + H]⁺ (Calcd for C₃₂H₄₃N₄O₁₅ 723.2719).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-β-*L*-glucosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx11)

yellow powder; ¹H NMR (CD₃OD, 500 MHz) 8.31 (d, *J* = 8.6 Hz, 1 H), 6.98 (d, *J* = 8.6 Hz, 1 H), 4.26 (d, *J* = 8.2 Hz, 1 H), 4.2 (m, 1H), 3.8 (m, 1 H), 3.86 (s, 2 H), 3.8 (m, 1 H), 3.75 (s, 3 H), 3.72 (m, 1 H), 3.67 (m, 1 H), 3.60 (t, *J* = 8.2 Hz, 1 H), 3.3 - 3.5 (m, 2 H), 2.87 (s, 6 H), 2.79 (dd, *J* = 12.8, 6.7 Hz, 1 H), 2.6 (m, 1 H), 2.57 (dd, *J* = 12.4, 8.1 Hz, 1 H), 1.56 (d, *J* = 6.6 Hz, 3 H); HRESI m/z 709.25706 [M + H]⁺ (Calcd for C₃₁H₄₁N₄O₁₅ 709.2563).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-*D*-arabinosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx12)

yellow powder; ¹H NMR (CD₃OD, 500 MHz) 8.32 (d, *J* = 8.3 Hz, 1 H), 6.99 (d, *J* = 8.1 Hz, 1 H), 4.3 (m, 1 H), 4.18 (d, *J* = 9.0 Hz, 1 H), 3.7 - 4.0 (m, 3 H), 3.72 (s, 6 H), 3.5 - 3.6 (m, 2 H), 2.93 (s, 6 H), 2.8 (m, 2 H), 2.58 (dd, *J* = 12.2, 8.1 Hz, 1 H), 1.56 (d, *J* = 6.8 Hz, 3 H); HRESI m/z 679.24591 [M + H]⁺ (Calcd for C₃₀H₃₉N₄O₁₄ 679.2457).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-*D*-erythrosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx13)

yellow powder; ¹H NMR (CD₃OD, 500 MHz) 8.38 (d, *J* = 8.5 Hz, 1 H), 6.98 (d, *J* = 8.1 Hz, 1 H), 4.67 (m, 1 H), 4.57 (s, 1 H), 4.22 (d, *J* = 4.5 Hz, 1 H), 4.0 (m, 1 H), 3.73 (s, 3 H), 3.6 - 3.7 (m, 2 H), 3.44 (m, 2 H), 3.2 (m, 1 H), 2.77 (s, 6 H), 2.6 (m, 1 H), 2.5 (m, 2 H), 1.56 (d, *J* = 6.8 Hz, 3 H); HRESI m/z 649.23770 [M + H]⁺ (Calcd for C₂₉H₃₇N₄O₁₃ 649.2352).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-2',3',4',6'-tetra-deoxy-4-*N*'-dimethylamino-β-*D*-glucosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx14)

yellow powder; ¹H NMR (CD₃OD, 500 MHz) 8.41 (d, *J* = 8.5 Hz, 1 H), 6.99 (d, *J* = 8.3 Hz, 1 H), 4.50 (d, *J* = 9.9 Hz, 1 H), 4.40 (s, 2 H), 3.8 - 3.9 (m, 2 H), 3.70 (s, 3 H), 3.6 - 3.7 (m, 1 H), 3.63 (s, 1 H), 3.57 (dd, *J* = 11.4, 8.7 Hz, 1 H), 2.94 (s, 6 H), 2.89 (s, 6 H), 2.7 - 2.8 (m, 2 H), 2.59 (dd, *J* = 12.3, 8.4 Hz, 1 H), 2.3 (m, 1 H), 2.1 (m, 1 H), 1.9 (m, 2 H), 1.56 (d, *J* = 6.8 Hz, 3 H), 1.38 (d, *J* = 5.9 Hz, 3 H); HRESI *m/z* 688.31860 [M + H]⁺ (Calcd for C₃₃H₄₆N₅O₁₁ 688.3188).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-2'-deoxy-2'-*N*'-allyloxycarbonylamino-*D*-glucosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx15)

yellow powder; ¹H NMR (CD₃OD, 500 MHz) 8.48 (d, *J* = 8.3 Hz, 0.5 H), 8.47 (d, *J* = 8.3, 0.5 H), 7.02 (d, *J* = 7.8 Hz, 0.5 H), 7.00 (d, *J* = 6.4 Hz, 0.5 H), 6.93 (m, 1 H), 5.33 (dd, *J* = 11.0, 0.9 Hz, 1 H), 5.14 (d, *J* = 10.6 Hz, 1 H), 4.7 (m, 1 H), 4.69 (d, *J* = 4.5 Hz, 0.5 H), 5.0 (m, 1 H), 4.46 (d, *J* = 9.4 Hz, 0.5 H), 4.2 (m, 1 H), 3.9 (m, 1 H), 3.7 - 3.8 (m, 2 H), 3.70 (s, 2 H), 3.67 (s, 3 H), 3.5 (m, 2 H), 2.89 (s, 6 H), 2.7 (m, 2 H), 2.60 (d, *J* = 12.1, 7.9 Hz, 1 H), 1.59 (d, *J* = 6.6 Hz, 3 H). HRESI *m/z* 792.29846 [M + H]⁺ (Calcd for C₃₅H₄₆N₅O₁₆ 792.2934).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-6'-deoxy-6'-azido-β-*D*-glucosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx16)

yellow powder; ¹H NMR (CD₃OD, 400 MHz) 8.36 (d, *J* = 8.5 Hz, 1 H), 7.01 (d, *J* = 8.3 Hz, 1 H), 4.45 (s, 2 H), 4.36 (d, *J* = 8.5 Hz, 1 H), 3.90 (s, 1 H), 3.8 (m, 1 H), 3.77 (s, 3 H), 3.7 - 3.8 (m, 2 H), 3.7 (m, 1 H), 3.6 (m, 2 H), 3.4 - 3.5 (m, 2 H), 2.98 (s, 6 H), 2.87 (t, *J* = 9.3 Hz, 1 H), 2.8 (m, 1 H), 2.61 (dd, *J* = 12.2, 8.3 Hz, 1 H), 1.59 (d, *J* = 6.8 Hz, 3 H); HRESI *m/z* 734.26611 [M + H]⁺ (Calcd for C₃₁H₄₀N₇O₁₄ 734.2628).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-2'-deoxy-2'-azido-*D*-glucosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx17)

yellow powder; ¹H NMR (CD₃OD, 500 MHz) 8.46 (d, *J* = 8.5 Hz, 0.5 H), 8.45 (d, *J* = 8.5 Hz, 0.5 H), 6.98 (d, *J* = 8.2 Hz, 0.5 H), 6.97 (d, *J* = 8.0 Hz, 0.5 H), 4.80 (s, 0.5 H), 4.38 (s, 1 H), 4.27 (d, *J* = 9.5 Hz, 0.5 H), 3.8 - 4.0 (m, 2 H), 3.76 (s, 2 H), 3.7 (m, 1 H), 3.64 (s, 3 H), 3.57 (dd, *J* = 11.5, 8.3 Hz, 1 H), 3.49 (t, *J* = 9.4 Hz, 1 H), 3.4 (m, 1 H), 3.2 (m, 1 H), 2.94 (s, 6 H), 2.8 (m, 2 H), 2.58 (dd, *J* = 12.2, 8.3 Hz, 1 H), 1.56 (d, *J* = 6.8 Hz, 3 H); HRESI *m/z* 734.26129 [M + H]⁺ (Calcd for C₃₁H₄₀N₇O₁₄ 734.2628).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-3'-deoxy-3'-fluoro-β-*D*-glucosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx18)

yellow powder; ¹H NMR (CD₃OD, 500 MHz) ppm 8.32 (d, *J* = 8.3 Hz, 1 H), 6.99 (d, *J* = 7.8 Hz, 1 H), 4.37 (t, *J* = 8.7 Hz, 1 H), 4.29 (d, *J* = 9.3 Hz, 1 H), 3.9 (m, 2 H), 3.61 (s, 2 H), 3.77 (s, 1 H), 3.74 (s, 3 H), 3.5 - 3.7 (m, 3 H), 3.5 (m, 1 H), 3.44 (dt, *J* = 3.4, 1.6 Hz, 1 H), 3.2 (m, 1 H), 2.90 (s, 6 H), 2.8 (m, 2 H), 2.59 (dd, *J* = 12.5, 8.3 Hz, 1 H), 1.56 (d, *J* = 6.9 Hz, 3 H); HRESI *m/z* 711.25146 [M + H]⁺ (Calcd for C₃₁H₄₀FN₄O₁₄ 711.2520).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*- β -L-galactosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx19)

yellow powder; $^1\text{H NMR}$ (CD_3OD , 500 MHz) 8.33 (d, $J = 8.3$ Hz, 1 H), 6.98 (d, $J = 8.3$ Hz, 1 H), 4.39 (s, 1 H), 4.26 (d, $J = 9.3$ Hz, 1 H), 3.9 (m, 1 H), 3.87 (d, $J = 3.2$ Hz, 1 H), 3.8 (m, 1 H), 3.76 (s, 2 H), 3.73 (s, 3 H), 3.7 (m, 1 H), 3.6 (m, 1 H), 3.60 (dd, $J = 9.4, 4.5$ Hz, 1 H), 3.6 (m, 2 H), 2.95 (s, 6 H), 2.8 (m, 2 H), 2.58 (dd, $J = 12.0, 8.3$ Hz, 1 H), 1.56 (d, $J = 6.8$ Hz, 3 H); HRESI m/z 709.25671 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{31}\text{H}_{41}\text{N}_4\text{O}_{15}$ 709.2563).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*- β -D-cellobiosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx20)

yellow powder; $^1\text{H NMR}$ (CD_3OD , 500 MHz) 8.32 (d, $J = 8.3$ Hz, 1 H), 6.99 (d, $J = 8.3$ Hz, 1 H), 4.42 (d, $J = 8.0$ Hz, 1 H), 4.41 (s, 1 H), 4.30 (d, $J = 9.2$ Hz, 1 H), 3.9 (m, 2 H), 3.89 (s, 2 H), 3.8 (m, 1 H), 3.75 (s, 3 H), 3.66 (dd, $J = 12.0, 5.9$ Hz, 1 H), 3.6 (m, 3 H), 3.4 (m, 3 H), 3.3 (m, 1 H), 3.22 (t, $J = 8.6$ Hz, 1 H), 3.2 (m, 1 H), 2.95 (s, 6 H), 2.8 (m, 1 H), 2.7 (m, 1 H), 2.59 (dd, $J = 12.2, 8.3$ Hz, 1 H), 1.55 (d, $J = 6.8$ Hz, 3 H); HRESI m/z 871.31004 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{37}\text{H}_{51}\text{N}_4\text{O}_{20}$ 871.3091).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-2'-deoxy-2'-([methyl(nitroso)amino]carbonyl)amino-D-glucosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx21)

yellow powder; $^1\text{H NMR}$ (CD_3OD , 500 MHz) 8.51 (d, $J = 8.4$ Hz, 0.5 H), 8.50 (d, $J = 8.5$ Hz, 0.5 H), 6.98 (d, $J = 8.8$ Hz, 1 H), 4.79 (s, 0.5 H), 4.65 (d, $J = 10.0$ Hz, 0.5 H), 4.4 (s, 2 H), 4.07 (dd, $J = 11.3, 9.1$ Hz, 0.5 H), 3.9 – 4.0 (m, 2 H), 3.75 (t, $J = 5.7$ Hz, 0.5 H), 3.70 (s, 1 H), 3.7 (m, 2 H), 3.63 (s, 3 H), 3.5 – 3.6 (m, 2 H), 3.4 (m, 1 H), 3.39 (d, $J = 8.5$ Hz, 1 H), 3.17 (s, 3 H), 3.06 (s, 3 H), 3.03 (s, 3 H), 2.8 (m, 2 H), 2.6 (m, 1 H), 1.57 (d, $J = 6.8$ Hz, 1.5 H), 1.54 (d, $J = 6.6, 1.5$ Hz); HRESI m/z 794.28488 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{33}\text{H}_{44}\text{N}_7\text{O}_{16}$ 794.2839).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*- β -D-digitoxosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx22)

yellow powder; $^1\text{H NMR}$ (CD_3OD , 500 MHz) 8.42 (d, $J = 8.3$ Hz, 1 H), 6.98 (d, $J = 8.5$ Hz, 1 H), 4.37 (s, 2 H), 4.10 (dd, $J = 2.7$ Hz, 2.2 Hz, 1 H), 4.00 (dd, $J = 6.1, 2.2$ Hz, 1 H), 3.7 – 3.8 (m, 4 H), 3.69 (s, 3 H), 3.5 – 3.7 (m, 3 H), 3.4 (m, 1 H), 3.2 (m, 1 H), 2.94 (s, 6 H), 2.7 – 2.8 (m, 2 H), 2.6 (m, 1 H), 1.56 (d, $J = 6.8$ Hz, 3 H); HRESI m/z 677.26693 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{31}\text{H}_{41}\text{N}_4\text{O}_{13}$ m/z 677.2665).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-2'-deoxy-2'-acetyl-amino-3'-O-(1''-carboxyethyl)-D-glucosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx23)

yellow powder; $^1\text{H NMR}$ (CD_3OD , 500 MHz) 8.50 (d, $J = 8.4$ Hz, 0.5 H), 8.49 (d, $J = 8.5$ Hz, 0.5 H), 6.98 (d, $J = 7.6$ Hz, 0.5 H), 6.97 (d, $J = 7.5$ Hz, 0.5 H), 4.74 (d, $J = 3.2$ Hz, 0.5 H), 4.7 (m, 1 H), 4.64 (dd, $J = 13.1, 6.3$ Hz, 1 H), 4.59 (d, $J = 6.9$ Hz, 1 H), 4.56 (d, $J = 9.7$ Hz, 0.5 H), 4.43 (d, $J = 9.7$ Hz, 1 H), 4.41 (s, 2 H), 3.6 – 4.0 (m, 3 H), 3.74 (s, 1 H), 3.63 (s, 3 H), 3.5 – 3.6 (m, 2 H), 2.95 (s, 6 H), 2.83 (t, $J = 11.1$ Hz, 1 H), 2.8 (m, 1 H), 2.59 (dd, $J = 11.9, 8.3$ Hz, 1 H), 2.08 (s, 1.5 H), 2.07 (s, 1.5 H), 1.55 (d, $J = 6.9$ Hz, 1.5 H), 1.49 (d, $J = 7.0$ Hz, 1.5 H), 1.41 (d, $J = 6.9$ Hz, 1.5 H), 1.38 (d, $J = 6.9$ Hz, 1.5 H); HRESI m/z 822.30525 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{36}\text{H}_{48}\text{N}_5\text{O}_{17}$ 822.3040).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-6'-deoxy-6'-*N*'-decanoylamino- β -D-glucosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx25)

yellow powder; $^1\text{H NMR}$ (CD_3OD , 500 MHz) 8.32 (d, $J = 8.3$ Hz, 1 H), 6.98 (d, $J = 8.3$ Hz, 1 H), 4.45 (d, $J = 7.8$ Hz, 1 H), 4.26 (d, $J = 8.8$ Hz, 1 H), 3.8 (m, 2 H), 3.74 (s, 3 H), 3.6 (m, 3 H), 3.3 - 3.5 (m, 2 H), 3.1 - 3.2 (m, 2 H), 2.90 (s, 6 H), 2.8 (m, 1 H), 2.7 (m, 1 H), 2.59 (dd, $J = 11.7, 8.3$ Hz, 1 H), 2.21 (q, $J = 7.1$ Hz, 2 H), 1.6 (m, 2 H), 1.56 (d, $J = 6.8$ Hz, 3 H), 1.3 (m, 12 H), 0.85 (t, $J = 6.8$ Hz, 3 H); HRESI m/z 862.41238 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{41}\text{H}_{60}\text{N}_5\text{O}_{15}$ 862.4080).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-2'-deoxy-2'-amino- α -D-glucosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx26a)

yellow powder; $^1\text{H NMR}$ (CD_3OD , 500 MHz) 8.28 (d, $J = 8.4$ Hz, 1 H), 6.96 (d, $J = 8.6$ Hz, 1 H), 5.30 (d, $J = 3.5$ Hz, 1 H), 4.1 (m, 1 H), 3.95 (s, 3 H), 3.7 - 3.8 (m, 3 H), 3.74 (dd, $J = 11.7, 5.4$ Hz, 1 H), 3.6 - 3.7 (m, 3 H), 3.4 (m, 1 H), 3.2 (m, 1 H), 3.04 (dd, $J = 10.6, 4.0$ Hz, 1 H), 2.86 (s, 3 H), 2.85 (s, 3 H), 2.7 (m, 1 H), 2.6 (m, 1 H), 2.56 (dd, $J = 12.0, 7.7$ Hz, 1 H), 1.55 (d, $J = 6.8$ Hz, 3 H); HRESI m/z 708.27445 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{31}\text{H}_{42}\text{N}_5\text{O}_{14}$ 708.2723).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-2'-deoxy-2'-amino- β -D-glucosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx26b)

yellow powder; $^1\text{H NMR}$ (CD_3OD , 500 MHz) 8.21 (d, $J = 8.3$ Hz, 1 H), 7.00 (d, $J = 8.3$ Hz, 1 H), 4.55 (d, $J = 10.0$ Hz, 1 H), 4.3 (s, 1 H), 3.94 (s, 2 H), 3.91 (dd $J = 11.0, 1.5$ Hz, 1 H), 3.8 (m, 1 H), 3.7 (m, 2 H), 3.64 (s, 3 H), 3.56 (dd, $J = 9.8, 8.4$ Hz, 1 H), 3.44 (dt, $J = 3.5, 1.6$ Hz, 1 H), 3.2 (m, 1 H), 3.07 (t, $J = 10.0$ Hz, 1 H), 2.90 (s, 6 H), 2.8 (m, 2 H), 2.59 (dd, $J = 12.1, 8.2$ Hz, 1 H), 1.57 (d, $J = 6.8$ Hz, 3 H); HRESI m/z 708.27352 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{31}\text{H}_{42}\text{N}_5\text{O}_{14}$ 708.2723).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-6'-deoxy-6'-amino- β -D-glucosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx27)

yellow powder; $^1\text{H NMR}$ (CD_3OD , 400 MHz) 8.30 (d, $J = 9.0$ Hz, 1 H), 7.00 (d, $J = 8.4$ Hz, 1 H), 4.37 (d, $J = 8.8$ Hz, 1 H), 4.21 (dd, $J = 5.8, 3.3$ Hz, 1 H), 3.7 - 3.8 (m, 2 H), 3.77 (s, 2 H), 3.7 (m, 1 H), 3.68 (s, 1 H), 3.64 (s, 3 H), 3.3 - 3.4 (m, 3 H), 3.2 (m, 1 H), 2.8 (s, 6 H), 2.6 (m, 3 H), 1.41 (d, $J = 7.5$ Hz, 3 H); HRESI m/z 708.27284 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{31}\text{H}_{42}\text{N}_5\text{O}_{14}$ 708.2723).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-2'-fluoro- β -D-glucosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx29)

yellow powder; $^1\text{H NMR}$ (CD_3OD , 500 MHz) 8.43 (d, $J = 8.3$ Hz, 1 H), 6.98 (d, $J = 8.5$ Hz, 1 H), 4.48 (dd, $J = 9.0, 2.0$ Hz, 1 H), 4.40 (d, $J = 9.0$ Hz, 1 H), 4.28 (t, $J = 8.9$ Hz, 1 H), 4.2 (m, 1 H), 3.8 - 3.9 (m, 3 H), 3.75 (s, 3 H), 3.7 (m, 1 H), 3.6 (m, 1 H), 3.3 (m, 1 H), 3.2 (m, 1 H), 2.87 (s, 6 H), 2.78 (dd, $J = 12.8, 6.7$ Hz, 1 H), 2.7 (m, 1 H), 2.57 (dd, $J = 12.5, 8.1$ Hz, 1 H), 1.55 (d, $J = 7.1$ Hz, 3 H); HRESI m/z 711.25609 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{31}\text{H}_{40}\text{FN}_4\text{O}_{14}$ 711.2520).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-D-mannosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx30)

yellow powder; ^1H NMR (CD_3OD , 500 MHz) ppm 8.39 (d, J = 8.3 Hz, 1 H), 6.99 (d, J = 7.1 Hz, 1 H), 4.47 (d, J = 1.0 Hz, 1 H), 4.34 (d, J = 2.3 Hz, 1 H), 4.2 (m, 2 H), 4.21 (t, J = 3.4 Hz, 1 H), 4.1 (m, 1 H), 4.0 (m, 1 H), 3.90 (dd, J = 9.2, 2.4 Hz, 1 H), 3.75 (s, 2 H), 3.74 (s, 1 H), 3.73 (s, 1 H), 3.5 – 3.7 (m, 2 H), 2.89 (s, 3 H), 2.88 (s, 3 H), 2.7 (m, 2 H), 2.6 (m, 1 H), 1.55 (d, J = 6.9 Hz, 3 H); HRESI m/z 709.25859 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{31}\text{H}_{41}\text{N}_4\text{O}_{15}$ 709.2563).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-2'-deoxy-2'-azido-D-mannosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx31)

yellow powder; ^1H NMR (CD_3OD , 500 MHz) ppm 8.40 (d, J = 8.4 Hz, 0.5 H), 8.36 (d, J = 8.4 Hz, 0.5 H), 6.99 (d, J = 8.3 Hz, 1 H), 4.77 (d, J = 0.5 Hz, 0.5 H), 4.69 (s, 0.5 H), 4.54 (s, 0.5 H), 4.44 (d, J = 2.9 Hz, 0.5 H), 4.2 - 4.3 (m, 3 H), 4.16 (t, J = 3.2 Hz, 1 H), 4.0 - 4.1 (m, 1 H), 3.8 (m, 2 H), 3.75 (s, 3 H), 3.5 - 3.7 (m, 2 H), 2.91 (s, 6 H), 2.8 (m, 1 H), 2.6 (m, 2 H), 1.56 (d, J = 6.8 Hz, 3 H); HRESI m/z 734.26404 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{31}\text{H}_{40}\text{N}_7\text{O}_{14}$ 734.2628).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-2'-deoxy-2'-amino-D-mannosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx32)

yellow powder; ^1H NMR (CD_3OD , 500 MHz) 8.28 (d, J = 8.8 Hz, 1 H), 6.99 (d, J = 7.6 Hz, 1 H), 4.96 (d, J = 0.5 Hz, 0.5 H), 4.76 (s, 1 H), 4.68 (s, 1 H), 4.65 (d, J = 3.5 Hz, 0.5 H), 4.5 (m, 1 H), 4.41 (s, 1 H), 4.18 (dd, J = 7.7, 4.5 Hz, 0.5 H), 3.8 – 4.1 (m, 3.5 H), 3.73 (s, 1.5 H), 3.72 (s, 1.5 H), 3.70 (s, 1 H), 3.6 (m, 1 H), 2.8 (m, 6 H), 2.7 (m, 2 H), 2.6 (m, 1 H), 1.56 (d, J = 6.8 Hz, 3 H); HRESI m/z 708.27411 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{31}\text{H}_{42}\text{N}_5\text{O}_{14}$ 708.2723).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-2'-deoxy-2'-azido- β -D-galactosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx33)

yellow powder; ^1H NMR (CD_3OD , 500 MHz) 8.47 (d, J = 8.5 Hz, 1 H), 6.99 (d, J = 8.5 Hz, 1 H), 4.39 (m, 1 H), 4.24 (d, J = 9.3 Hz, 1 H), 4.0 - 4.1 (m, 1 H), 3.94 (s, 1 H), 3.85 (d, J = 1.2 Hz, 1 H), 3.75 (s, 3 H), 3.7 (m, 3 H), 3.5 - 3.6 (m, 4 H), 2.94 (s, 6 H), 2.8 (m, 2 H), 2.59 (dd, J = 12.2, 8.3 Hz, 1 H), 1.58 (d, J = 7.1 Hz, 3 H); HRESI m/z 734.26589 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{31}\text{H}_{40}\text{N}_7\text{O}_{14}$ 734.2628).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-2'-deoxy-2'-amino- β -D-galactosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx34)

yellow powder; ^1H NMR (CD_3OD , 500 MHz) 8.20 (d, J = 8.3 Hz, 1 H), 6.97 (d, J = 8.3 Hz, 1 H), 4.57 (s, 1 H), 4.50 (d, J = 9.9 Hz, 1 H), 3.9 (m, 3 H), 3.7 - 3.8 (m, 4 H), 3.64 (s, 3 H), 3.4 (m, 1 H), 3.59 (t, J = 6.2 Hz, 1 H), 2.77 (s, 6 H), 2.5 (m, 3 H), 1.56 (d, J = 6.6 Hz, 3 H); HRESI m/z 708.27445 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_{31}\text{H}_{42}\text{N}_5\text{O}_{14}$ 708.2723).

(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-(dimethylamino)-9-(*N*-methoxy-*N*-2'-*N*-trifluoroacetyl-amino- β -D-glucosylglycyl)amino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide (Dx37)

yellow powder; ^1H NMR (CD_3OD , 500 MHz) 8.46 (d, J = 8.5 Hz, 1 H), 6.98 (d, J = 7.8 Hz, 1 H), 4.73 (d, J = 7.9 Hz, 1 H), 4.40 (d, J = 5.1 Hz, 1 H), 4.20 (dd, J = 12.0, 4.9 Hz, 1

H), 4.1 (m, 1 H), 4.0 (m, 3 H), 3.7 (m, 1 H), 3.64 (s, 3 H), 3.57 (m, 1 H), 2.95 (s, 6 H), 2.87 (m, 1 H), 2.78 (dd, $J = 12.5, 6.8$ Hz, 1 H), 2.59 (dd, $J = 12.2, 8.3$ Hz, 1 H), 1.55 (d, $J = 6.5$ Hz, 3 H); HRESI m/z 804.25463 $[M + H]^+$ (Calcd for $C_{33}H_{41}F_3N_5O_{15}$ 804.2546).

(4S,4aR,5S,5aR,6R,12aS)-4-(dimethylamino)-9-(*N*-methoxy-*N*-2'-deoxy-2'-D-glucosylglycyl)amino-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (Dx38)

yellow powder; 1H NMR (CD_3OD , 500 MHz) 8.43 (d, $J = 8.5$ Hz, 0.5 H), 8.42 (d, $J = 8.6$ Hz, 0.5 H), 7.02 (d, $J = 8.3$ Hz, 0.5 H), 7.00 (d, $J = 9.2$ Hz, 0.5 H), 5.20 (t, $J = 6.3$ Hz, 0.5 H), 4.71 (s, 1 H), 4.63 (t, $J = 4.8$ Hz, 0.5 H), 4.38 (s, 2 H), 4.39 (s, 1 H), 4.20 (m, 1 H), 3.74 (s, 1.5 H), 3.67 (s, 1.5 H), 3.6 (m, 3 H), 3.5 (m, 1 H), 2.96 (s, 6 H), 2.8 (m, 2 H), 2.61 (dd, $J = 4.9, 1.1$ Hz, 1 H), 2.4 (m, 1 H), 2.3 (m, 1 H), 1.59 (t, $J = 6.7$ Hz, 3 H); HRESI m/z 693.26248 $[M + H]^+$ (Calcd for $C_{31}H_{41}N_4O_{14}$ 693.2641).

(4S,4aR,5S,5aR,6R,12aS)-4-(dimethylamino)-9-(*N*-methoxy-*N*-2'-deoxy-2'-azido- β -D-xylosylglycyl)amino-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (Dx39)

yellow powder; 1H NMR (CD_3OD , 500 MHz) 8.47 (d, $J = 8.5$ Hz, 1 H), 6.98 (d, $J = 8.3$ Hz, 1 H), 4.38 (s, 1 H), 4.19 (d, $J = 9.0$ Hz, 1 H), 3.94 (t, $J = 5.4$ Hz, 1 H), 3.91 (s, 1 H), 3.88 (s, 1 H), 3.77 (s, 1 H), 3.74 (s, 3 H), 3.57 (dd, $J = 11.4, 8.6$ Hz, 1 H), 3.5 (m, 1 H), 3.4 (m, 1 H), 3.21 (d, $J = 10.9$ Hz, 1 H), 3.2 (m, 1 H), 2.94 (s, 6 H), 2.81 (d, $J = 11.4$ Hz, 1 H), 2.76 (t, $J = 5.8$ Hz, 1 H), 2.58 (dd, $J = 12.3, 8.3$ Hz, 1 H), 1.55 (d, $J = 6.9$ Hz, 3 H); HRESI m/z 704.25084 $[M + H]^+$ (Calcd for $C_{30}H_{38}N_7O_{13}$ 704.2528).

(4S,4aR,5S,5aR,6R,12aS)-4-(dimethylamino)-9-(*N*-methoxy-*N*-2'-deoxy-2'-amino- β -D-xylosylglycyl)amino-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (Dx40)

yellow powder; 1H NMR (CD_3OD , 500 MHz) ppm 8.16 (d, $J = 8.1$ Hz, 1 H), 6.96 (d, $J = 8.1$ Hz, 1 H), 4.57 (s, 1 H), 4.47 (d, $J = 10.0$ Hz, 1 H), 3.99 (dd, $J = 11.6, 5.0$ Hz, 1 H), 3.8 - 3.9 (m, 3 H), 3.7 (m, 1 H), 3.63 (s, 3 H), 3.4 - 3.6 (m, 2 H), 3.03 (t, $J = 9.9$ Hz, 1 H), 2.78 (s, 6 H), 2.6 (m, 3 H), 1.56 (d, $J = 6.6$ Hz, 3 H); HRESI m/z 678.25815 $[M + H]^+$ (Calcd for $C_{30}H_{40}N_5O_{13}$ 678.2617).

(4S,4aR,5S,5aR,6R,12aS)-4-(dimethylamino)-9-(*N*-methoxy-*N*-4'-deoxy-4'-azido- β -L-riboseglycyl)amino-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (Dx41)

yellow powder; 1H NMR (CD_3OD , 500 MHz) ppm 8.49 (d, $J = 8.3$ Hz, 1 H), 6.99 (d, $J = 8.5$ Hz, 1 H), 4.2 (m, 1 H), 4.22 (d, $J = 4.4$ Hz, 1 H), 4.01 (t, $J = 11.5$ Hz, 1 H), 3.95 (dd, $J = 10.6, 5.2$ Hz, 2 H), 3.90 (s, 1 H), 3.8 (m, 1 H), 3.78 (s, 3 H), 3.6 (m, 2 H), 3.5 (m, 1 H), 3.45 (t, $J = 4.2$ Hz, 1 H), 2.92 (s, 6 H), 2.8 (m, 1 H), 2.7 (m, 1 H), 2.57 (dd, $J = 12.2, 8.1$ Hz, 1 H), 1.57 (d, $J = 6.8$ Hz, 3 H); HRESI m/z 704.25589 $[M + H]^+$ (Calcd for $C_{30}H_{38}N_7O_{13}$ 704.2522).

Supplementary Material

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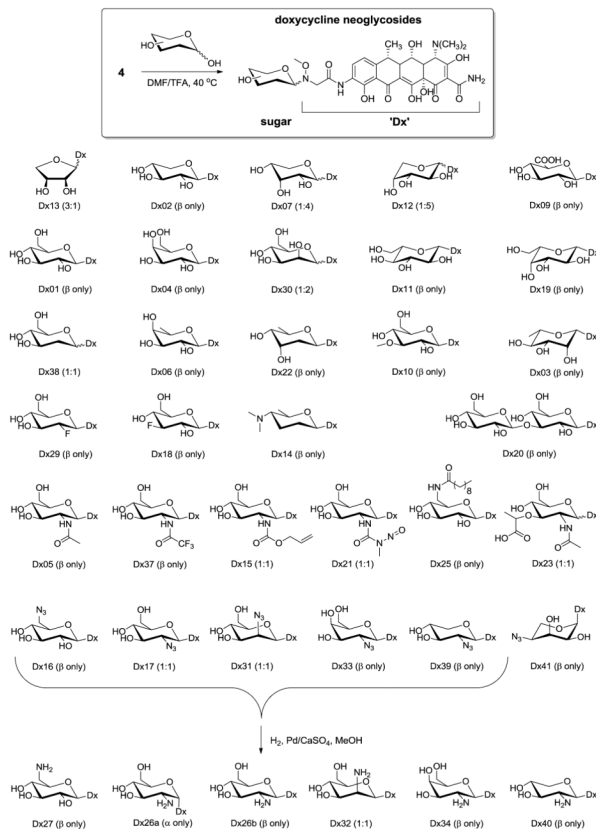
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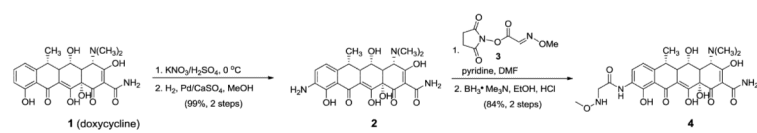
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**Figure 1.**

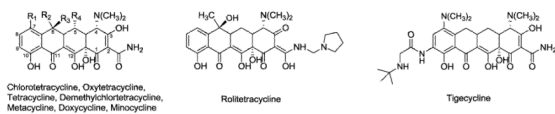
Synthesis of doxycycline neoglycosides with product anomeric ratios highlighted within parentheses (:). Neoglycosides are roughly organized by size (beginning with tetraoses in upper left) and increasing alteration of monosaccharide (vertical). The syntheses of target aminosugar-bearing neoglycosides are also highlighted (lower). Doxycycline neoglycoside is designated as 'Dx'.



Scheme 1.
Synthesis of the 9-amino doxycycline-based neoaglycon

Table 1

Representative structures of known tetracycline analogues.



Generic name	Chemical name	R ₄	R ₃	R ₂	R ₁	Trade Name	Yr of discovery	Source
Chlorotetracycline	7-Chlorotetracycline	H	OH	CH ₃	Cl	Aureomycin	1948	Natural occurring
Oxytetracycline	5-Hydroxytetracycline	OH	OH	CH ₃	H	Terramycin	1948	Natural occurring
Tetracycline	Tetracycline	H	OH	CH ₃	H	Sumycin	1953	Natural occurring
Demethylchlorotetracycline	6-Demethyl-7-chlorotetracycline	H	OH	H	Cl	Declomycin	1957	Natural occurring
Rolitetracycline	2- <i>N</i> -Pyrrolidinomethyltetracycline	H	OH	CH ₃	H	Colbiocin	1958	Semi-synthetic
Metacycline	6-Methylene-7-chlorotetracycline	OH	CH ₂	H	H	Randomycin	1965	Semi-synthetic
Doxycycline	6-Deoxy-5-hydroxytetracycline	OH	H	CH ₃	H	Vibramycin	1967	Semi-synthetic
Minocycline	7-Dimethylamino-6-demethyl-6-deoxytetracycline	H	H	H	N(CH ₃) ₂	Minocin	1972	Semi-synthetic
Tigecycline	9-(<i>t</i> -butylglycylamido)-minocycline	H	H	H	N(CH ₃) ₂	Tygacil	1993	Semi-synthetic

Table 2

Summary table of neoglycosides synthesized and tested.

Neoglycoside (see Figure 1)	Sugars utilized	Yield	/ ratio
Dx01	D-glucose (5) ^b	46%	only
Dx02	D-xylose (6) ^b	69%	only
Dx03	L-rhamnose (7) ^b	66%	only
Dx04	D-galactose (8) ^b	38%	only
Dx05	<i>N</i> -acetyl-D-glucosamine (9) ^b	30%	only
Dx06	D-fucose (10) ^b	25%	only
Dx07	D-ribose (11) ^b	14%	/ =1/4
Dx08	D-glucosamine hydrochloride (12) ^b	. <i>a</i>	. <i>a</i>
Dx09	D-glucuronic acid (13) ^b	45%	only
Dx10	3- <i>O</i> -methyl-D-glucose (14) ^b	51%	only
Dx11	L-glucose (15) ^b	34%	only
Dx12	D-arabinose (16) ^b	32%	/ =1/5
Dx13	D-erythrose (17) ^b	44%	/ =3/1
Dx14	forosamine (18) ^c	41%	only
Dx15	2-deoxy-2- <i>N</i> -alloc-D-glucosamine (19) ²⁸	47%	/ =1/1
Dx16	6-deoxy-6-azido-D-glucose (20) ^b	38%	only
Dx17	2-deoxy-2-azido-D-glucose (21) ^b	41%	/ =1/1
Dx18	3-deoxy-3-fluoro-D-glucose (22) ^b	48%	only
Dx19	L-galactose (23) ^b	48%	only
Dx20	D-cellobiose (24) ^b	23%	only
Dx21	streptozocin (25) ^b	11%	/ =1/1
Dx22	digitoxose (26) ^b	28%	only
Dx23	<i>N</i> -acetylmuramic acid (27) ^b	32%	/ =1/1
Dx24	3-deoxy-3- <i>N</i> -decanoyl-D-glucosamine (28) ^{20a}	. <i>a</i>	. <i>a</i>
Dx25	6-deoxy-6- <i>N</i> -decanoyl-D-glucosamine (29) ^{20a}	18%	only
Dx26a	2-deoxy-2-azido-D-glucose (21) ^b	20% 2 steps	only
Dx26b	2-deoxy-2-azido-D-glucose (21) ^b	22% 2 steps	only
Dx27	6-deoxy-6-azido-D-glucose (20) ^b	25% 2 steps	only
Dx28	2,3,4,6-tetraacetyl-D-glucose (30) ^c	. <i>a</i>	. <i>a</i>
Dx29	2-deoxy-2-fluoro-D-glucose (31) ^b	21%	only
Dx30	D-mannose (32) ^b	27%	/ =1/2
Dx31	2-deoxy-2-azido-D-mannose (33) ²⁹	32%	/ =1/1
Dx32	2-deoxy-2-azido-D-mannose (33) ²⁹	35% 2 steps	/ =1/1
Dx33	2-deoxy-2-azido-D-galactose (34) ²⁹	31%	only

Neoglycoside (see Figure 1)	Sugars utilized	Yield	/ ratio
Dx34	2-deoxy-2-azido-D-galactose (34) ²⁹	31% 2 steps	only
Dx35	2-deoxy-2- <i>N</i> -methyl-D-glucosamine (35) ³⁰	. <i>a</i>	. <i>a</i>
Dx36	2-deoxy-2- <i>N</i> -dimethyl-D-glucosamine (36) ³⁰	. <i>a</i>	. <i>a</i>
Dx37	2-deoxy-2- <i>N</i> -trifluoroacetyl-D-glucosamine (37) ³⁰	22%	only
Dx38	2-deoxy-D-glucose (38) ^{<i>b</i>}	37%	/ =1/1
Dx39	2-deoxy-2-azido-D-xylose (39) ^{<i>c</i>}	37%	only
Dx40	2-deoxy-2-azido-D-xylose (39) ^{<i>c</i>}	30% 2 steps	only
Dx41	4-deoxy-4-azido-L-ribose (40) ^{<i>c</i>}	33%	only

^{*a*} no reaction

^{*b*} commercially available

^{*c*} synthesized in this work

Table 3

Antibacterial activity and cytotoxicity of selected doxycycline neoglycosides

Neoglycoside (see Figure 1)	Sugar utilized	<i>E. coli</i> 25922 ($\mu\text{g/mL}$)	<i>E. coli</i> 1-849 ($\mu\text{g/mL}$)	<i>S. aureus</i> R2507 ($\mu\text{g/mL}$)	A549 viability (%)	IMR 90 viability (%)
4	none	4	8	4	95.9	99.2
Dx26a	2-deoxy-2-azido-D-glucose (21)	4	4	4	99.1	94.6
Dx15	2-deoxy-2- <i>N</i> -alloc-D-glucosamine (19)	8	16	8	98.8	97.6
Dx14	forosamine (18)	4	32	16	96.9	100.7
Dx37	2-deoxy-2- <i>N</i> -trifluoroacetyl-D-glucosamine (37)	4	32	16	98.7	109.1
Dx23	<i>N</i> -acetylmuramic acid (27)	8	32	16	97.7	104.4
<i>Dx32</i>	2-deoxy-2-azido-D-mannose (33)	4	32	32	97.1	98.9
Dx26b	2-deoxy-2-azido-D-glucose (21)	8	32	32	95.6	101.7
Dx12	D-arabinose (16)	8	64	32	97.2	101.7
Dx13	D-erythrose (17)	4	64	32	98.2	99.3