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Novel Anthracycline Oligosaccharides: Influence of Chemical Modifications of the Carbohydrate Moiety on Biological Activity

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Abstract—Several observations highlight the importance of the carbohydrate moiety for the biological activity of antitumoural anthracyclines. Here is reported the synthesis, cytotoxicity and topoisomerase II-mediated DNA cleavage intensity of the new oligosaccharide anthracyclines **1**–**4** modified in the sugar residue. Evaluation of cytotoxic potency on different cell lines, resulted in quite similar values among the different analogues. On the other hand, topoisomerase II-mediated DNA breaks level was different for the various compounds, and was not related to cytotoxicity, thus supporting previous observations reported for some monosaccharide anthracyclines modified in the carbohydrate portion.

Introduction

Anthracycline antibiotics are still among the most useful drugs in cancer therapy because of their clinical efficacy against a broad spectrum of solid tumours and leukemias. The importance of this class of antitumour agents has stimulated a number of studies aimed to achieve a deeper knowledge of the mechanism of action of these drugs and to identify the critical structural motives, which might improve their pharmacological properties and reduce their toxicity. DNA intercalation was proved to be a necessary but not sufficient condition for exhibiting antitumoural activity. Interference with the catalytic cycle of the enzyme topoisomerase II is considered as the main biological event responsible for the cytotoxic effect of anthracyclines.[1] Experimental data show that antracyclines stabilize a DNA–enzyme complex, wherein DNA strands are cut and covalently linked to the protein, hindering DNA strand religation by topoisomerase II and thus enhancing DNA breaks level.[2–5] From the model of DNA–drug complex, the non-intercalating portions, that is the α-hydroxy carbonyl side chain and the carbohydrate moiety, appear positioned in the DNA minor groove.[6,7] Moreover, experimental results demonstrate that chemical modifications in the carbohydrate moiety of the molecule, can determine changes in the drug preferred base sequence at the cleavage site.[8] From these observations it was supposed that, in the DNA–enzyme–drug complex, the anthracycline molecule is placed at the protein–DNA interface and that the non-intercalating chemical moieties could play an important role in the formation and stabilisation of the ternary complex.[9,10]

We started a research program aimed at the synthesis of new anthracyclines modified in the carbohydrate moiety in order to obtain derivatives with improved pharmacological profile. In particular, on the base of the currently accepted model of the ternary complex, an elongation of the sugar portion could increase molecular interactions between drug and DNA and/or topoisomerase II and provide more information on the structural requirements of this part of the molecule to obtain an improved biological activity.

We have already described new disaccharide anthracyclines belonging both to the daunomycin series and the doxorubicin series in which the amino sugar, different from the natural and synthetic disaccharide anthracyclines till then reported, was not directly linked to the aglycone moiety.[11] Evaluation of topoisomerase II-mediated DNA breaks and of antitumour activity

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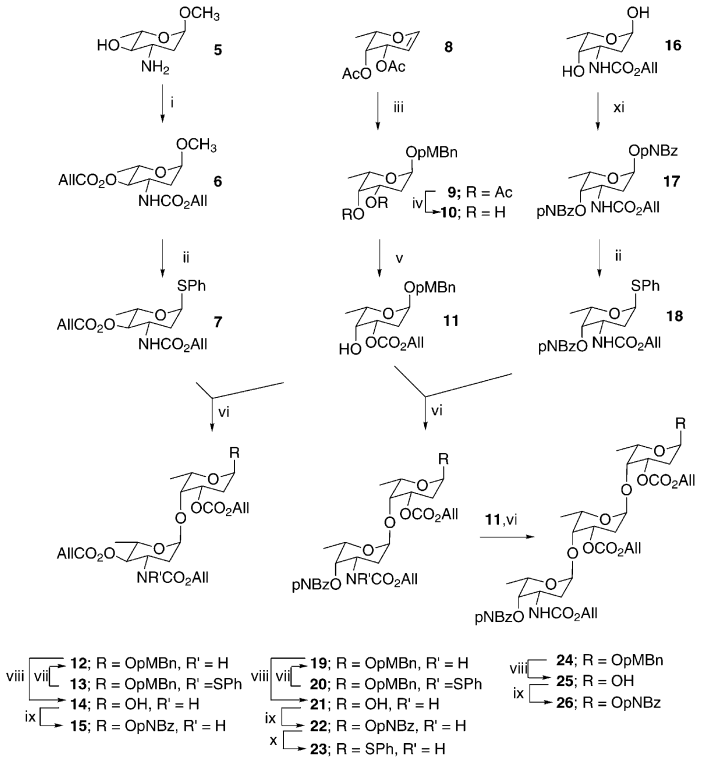


Scheme 1. (i) AllOCOCl, py, CH_2Cl_2, rt, 4 h; (ii) $PhSSiMe_3$, $CF_3SO_3SiMe_3$, CH_2Cl_2, rt, 2 h; (iii) (*p*-MeO) $C_6H_4CH_2OH$ (*p*-MBnOH), Ph_3PHBr, CH_2Cl_2, rt, 1 h; (iv) 1 N MeONa, MeOH, rt, 2 h; (v) (a) Bu_2SnO, toluene, reflux, 12 h; (b) AllOCOCl, Et_3N, CH_2Cl_2, −40 °C, 2 h; (vi) IDCP, $(CH_2Cl)_2$–Et_2O, M.S. 4 Å, 10 °C, 2 h; (vii) Ph_3P, 1 h; (viii) $Ce(NH_4)_2(NO_3)_6$, CH_3CN–H_2O, rt, 2 h; (ix) (*p*-NO_2)C_6H_4COCl (*p*-NBzCl), py, CH_2Cl_2, 0 °C, 1 h; (x) $PhSSiMe_3$, $CF_3SO_3SiMe_3$, CH_2Cl_2, 0 C, 1 h; (xi) *p*-NBzCl, py, CH_2Cl_2, rt, 1 h.

confirmed the critical role of the sugar moiety for the pharmacological properties of this new class of anthracyclines.[12] In particular, 1,4-α-linkage between the C-4 position of the first sugar and the anomeric carbon of the second carbohydrate unit of the disaccharide, turned out to be the optimal configuration for the biological activity. One of these new anthracyclines, MEN 10755, was identified as the best compound in this series. This new derivative showed remarkable efficacy in a number of xenografted (gynaecological, lung, prostatic) human tumour in nude mouse, both sensitive and resistant to doxorubucin.[13] Besides, in animal models MEN 10755 evidenced lower cardiotoxic effects with respect to doxorubicin.[14]

In order to establish more precise structure–activity relationships and to achieve a deeper knowledge of the influence of the carbohydrate portion on the biological activity for this new class of oligosaccharide anthracyclines, we designed novel oligosaccharide analogues containing chemical and configurational modifications in the carbohydrate moiety.

Results

Synthesis

Here we report the synthesis of new oligosaccharide anthracyclines **1**—**4**. They were respectively the 4″-epi-derivative of MEN 10755 (**1**), the trisaccharide (**2**), in which the aminosugar daunosamine is separated from the aglycone by two 2-deoxy-L-fucose units, the 3′-deoxy derivative of MEN 10755 (**3**), and the compound bearing a combination of the two modifications, that is epimerization in 4″ and removal of the hydroxy group in 3′ (**4**) (Fig. 1).

A convergent synthetic strategy was adopted for the synthesis of these compounds, implying the separate construction of the aglycone and of the oligosaccharide moiety and their successive coupling to give the desired glycoside.

Oligosaccharides were assembled starting from glycosyl donors activated at the anomeric position with thiophenyl group. Thioglycosides have been recently introduced as potential glycosyl donors as they have the advantage to be stable to normal protecting group manipulation encountered in oligosaccharide synthesis, although they may be selectively activated to become glycosyl donors.[15,16] Condensations between the thioglycosides and the glycosyl acceptors were performed in the presence of a preformed soluble complex of iodonium ion.[17] This protocol allowed to overcome the known low reactivity of the C-4 axial hydroxyl group of the glycosyl acceptor,[18] to obtain the glycosidic bond in the desired α configuration and to avoid the formation of transglycosylation products. All the glycosyl acceptors were protected at the anomeric position with *p*-methoxybenzyl group. This group, in contrast to the common anomeric protecting groups, which require acid or reductive conditions for their deprotection, was removable under neutral condition without affecting the glycosidic bond.[19] Allyloxycarbonyl group, used for

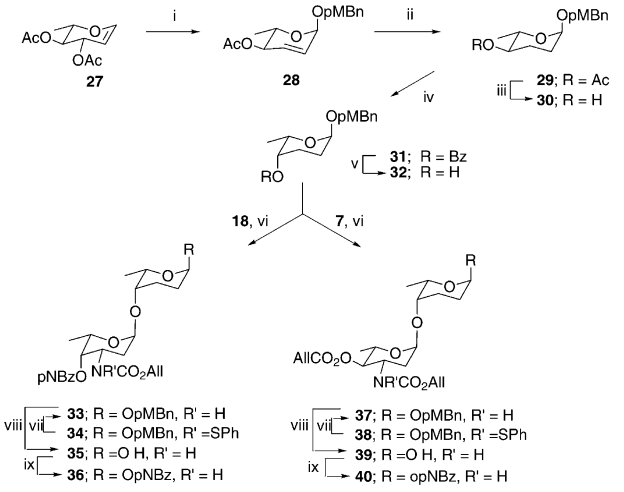
protecting the other hydroxyl or amino functions present in the carbohydrate molecule, was equally cleavable under neutral condition, allowing the base sensitive hydroxy function in the side chain of the anthracycline molecule, not to be affected in the final deprotection.[20] The trisaccharide was obtained through a stepwise synthetic sequence first obtaining the disaccharide moiety, then coupling it with another molecule of glycosyl acceptor, with both the glycosylations carried out in the presence of iodonium ion as promoter.

Starting materials for the disaccharide **12** were phenyl 3-*N*,4-*O*-di-allyloxycarbonyl-1-thio- acosaminide **7** and *p*-methoxybenzyl 3 - *O* - allyloxycarbonyl-2-deoxy-L-fucoside **11** (Scheme 1).

Compound **7** was obtained from methyl-L-acosaminide **5**,[21,22] after protection of the free hydroxyl and amino functions with allyloxycarbonyl group, and activation at the anomeric position by treatment with phenylthiotrimethylsilane and trimethylsilyl trifluoromethanesulfonate (trimethylsilyltriflate).[23] Addition of *p*-methoxybenzyl alcohol[24] to the easily available 3, 4-di-*O*-acetyl-L-fucal **8**,[25] de-*O*-acetylation and successive regioselective protection in C-3 position, through formation of a cyclic stannyl ether, of the resulting *p*-methoxybenzyl fucoside **10**[26,27] gave the glycosyl acceptor **11**. Condensation of this latter with a slight excess of glycosyl donor **7** in the presence of iodonium dicollidine perchlorate (IDCP),[28] resulted in the formation of the desired disaccharide **12**, exclusively in α configuration, in addition to the corresponding sulfenamide derivative **13** (30%).[29] This latter was easily converted into the useful disaccharide by adding triphenylphosphine to the reaction mixture. Quantitative removal of the anomeric *p*-methoxybenzyl group in **12** with ammonium cerium (IV) nitrate, followed by activation with 4-nitrobenzoyl chloride furnished the glycosyl donor **15** in 80% of yield.

The carbohydrate fragments forming the trisaccharide moiety were *p*-methoxybenzyl 2-deoxy fucoside **11** and thiophenyldaunosaminide **18** (Scheme 1). This latter was obtained from 3-*N*-allyloxycarbonyl-L-daunosamine **16**[30] after treatment with *p*-nitrobenzoyl chloride to give **17** and successive reaction with (phenylthio)-trimethylsilane and trimethylsilyl triflate. The trisaccharide **24** was easily accessible through a stepwise sequence first condensing the thiophenyldaunosaminide **18** with the *p*-methoxybenzyl 2-deoxy fucoside **11** in the presence of iodonium dicollidine perchlorate to obtain the disaccharide **19** with the desired α 1,4 linkage. Activation of the anomeric position of this disaccharide through direct substitution of the *p*-methoxybenzyl group with the thiophenyl group, failed. Therefore, this conversion was performed through deprotection with ammonium cerium(IV) nitrate, reaction with *p*-nitrobenzoyl chloride to give **22** and than with (phenylthio)trimethylsilane and trimethylsilyltriflate to obtain the glycosyl donor **23**. This latter was coupled, following the same procedure of the previous glycosylation, with another molecule of 2-deoxyfucoside **11** yielding to the trisaccharide **24**. The usual sequence of deprotection and activation at the anomeric position, gave the activated trisaccharide **26**.

The two 3′-deoxy disaccharides **33** and **37** were obtained condensing, respectively, the thiophenyl daunosaminide **18** and thiophenyl acosaminide **7**, with *p*-methoxybenzyl 2, 3, 6-trideoxy-L-*lyxo*-hexopyranoside **32** (Scheme 2).



Scheme 2. (i) *p*-MBnOH, 0.22 M $SnCl_4$, CH_2Cl_2, rt, 2 h; (ii) H_2, $[(Ph)_3P]_3RhCl$, benzene, rt, dark, 2 h; (iii) 1 M NaOMe, MeOH, rt, 2 h; (iv) DEAD, Ph_3P, $PhCO_2H$, CH_2Cl_2, rt, 2 h; (v) K_2CO_3, MeOH, rt, 2 h; (vi) IDCP, $(CH_2Cl)_2$–Et_2O, M.S. 4 Å, 5–10 °C, 3 h; (vii) Ph_3P, 1 h; (viii) $Ce(NH_4)_2(NO_3)_6$, CH_3CN–H_2O, rt, 2 h; (ix) *p*-NBzCl, py, CH_2Cl_2, 0 °C, 2 h.

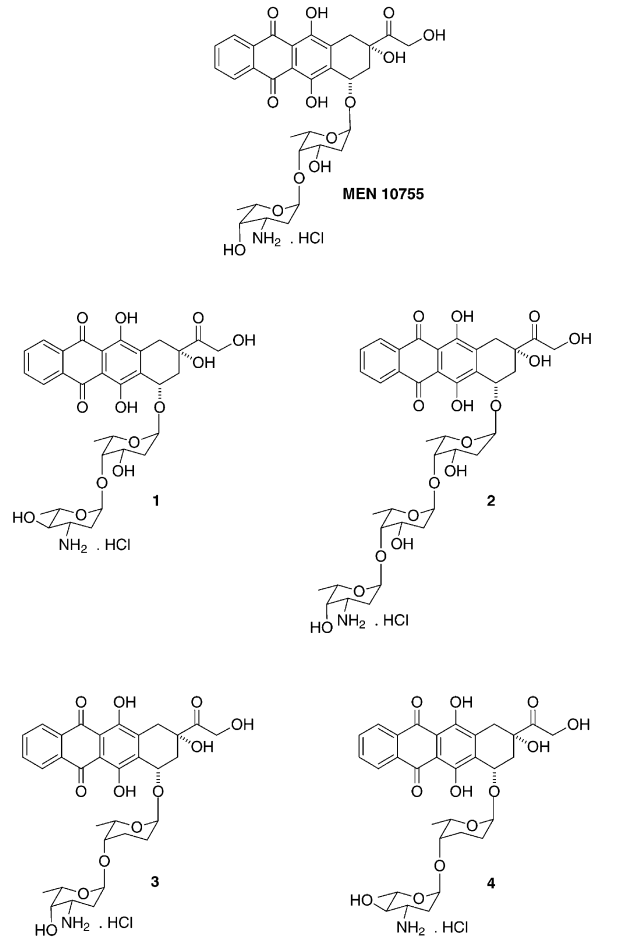
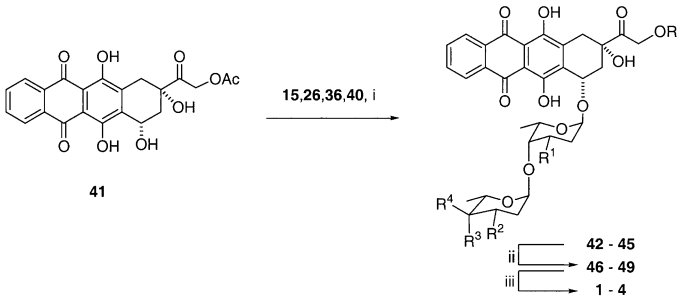


Figure 1. Molecular structures of MEN 10755 and oligosaccharide modified anthracyclines.



	R	R_1	R_2	R_3	R_4
42	-Ac	$-OCO_2All$	$-NHCO_2All$	-H	$-OCO_2All$
43	-Ac	$-OCO_2All$	$-OCO_2All$		-H
44	-Ac	-H	$-NHCO_2All$	-OpNBz	-H
45	-Ac	-H	$-NHCO_2All$	-H	$-OCO_2All$
46	-H	$-OCO_2All$	$-NHCO_2All$	-H	$-OCO_2All$
47	-H	$-OCO_2All$	$-OCO_2All$		-H
48	-H	-H	$-NHCO_2All$	-OH	-H
49	-H	-H	$-NHCO_2All$	-H	$-OCO_2All$

Scheme 3. (i) TMSOTf, CH_2Cl_2–Et_2O, M.S. 4 Å, −20 °C, 2 h; (ii) 0.5 M K_2CO_3, MeOH–CH_2Cl_2, −10 °C, 1 h; (iii) (a) [$(Ph_3P)_4$]Pd, Me_2NTMS, TMSOAc, CH_2Cl_2, rt, dark, 0.5 h; (b) 0.001 M aq HCl.

Table 1. Cytotoxic activity of the disaccharide anthracyclines on human carcinoma cell lines[a]

Cell line	MEN 10755	1	2	3	4
A2780	33±11	11±5	109±42	7±1	9±1
GCL-4	36±22	11±3	353±127	16±4	24±13
H460	76±12	17.5±18	>1000	7.8±5.2	29±19
PC-3	1250±778	890±610	>1000	350±212	215±65
CALU-3	239±9	58±13	>1000	123±40	13±2
DU-145	80±20	12±2	>1000	18±0.5	14±7
SW-684	23±1	41±13	>1000	106±23	19±2

[a]IC_{50} (nM) values were determined from dose–response curves from at least three indipendent experiments. Human tumor cell lines are A2780 (ovarian), GCL-4 (SCLC), H460 (lung), PC-3 (prostate), CALU-3 (lung), DU-145 (prostate), SW-684 (fibrosarcoma).

This latter was obtained in a multistep sequence from 3,4-di-*O*-acetyl-L-rhamnal **27**[25] through a $SnCl_4$ catalyzed addition of *p*-methoxybenzyl alcohol with allylic rearrangement,[31] reduction of the double bond in non-hydrogenolytic conditions to **29** and de-*O*-acetylation to **30**. Inversion of the configuration of the hydroxy group in C-4 position was performed following Mitsunobu procedure[32] to give **31**, later deprotected according to the standard procedure to **32**.

IDCP promoted coupling of **32** with the two glycosyl donors **18** and **7**, followed the same chemical and stereochemical course as above described, giving disaccharides **33** and **37** with an α interglycosidic linkage. Conversion of **33** and **37** to the glycosylating disaccharides **36** and **40** was performed according to the previously described sequence of deprotection with ammonium cerium(IV) nitrate and successive activation with *p*-nitrobenzoyl chloride at the anomeric position.

The fully protected glycosides **42–45** were obtained by coupling the 14-acetoxyidarubicinone **41** with the activated carbohydrate residues **15**, **26**, **36** and **40**, using trimethylsilyltriflate as promoter, according to a known procedure.[33] The new glycosides were produced in good yields and with the glycosidic bond in the desired α configuration. Mild deprotection of the ester groups with potassium carbonate afforded the partially deprotected glycosides **46–49**. Successive removal of the allyloxycarbonyl groups in neutral conditions with tetrakis(triphenylphosphine)palladium led respectively to the final glycosides **1–4** isolated as hydrochloride salt (Scheme 3).

Cytotoxic activity

Cytotoxicity of the anthracycline analogues **1–4** was evaluated on a panel of different human tumour cell lines and compared with the cytotoxic effect of MEN 10755 (Table 1). The disaccharide analogues showed a cytotoxic potency comparable to that of the reference compound, except in the PC-3 tumour line which was resistant to all the drugs. Obtained results pointed out that neither epimerization in C-4″ (**1**) nor removal of the OH group in C-3′ (**3** and **4**) notably affected the cellular response of the tested compounds. Introduction of a further carbohydrate residue in the trisaccharide derivative **2**, almost abolishes cytotoxic activity.

Topoisomerase II-mediated DNA cleavage

The DNA cleavage activity of the new compounds **1–4** in the presence of the enzyme topoisomerase II α was investigated in order to determine the influence of the chemical modifications at the 3′ and 4″ positions of these anthracycline analogues on the DNA cleavage

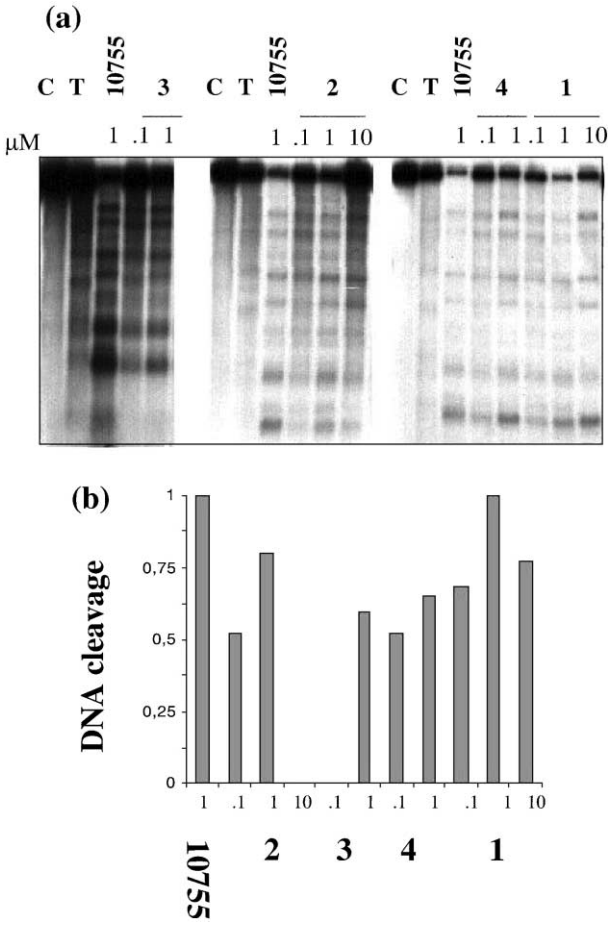


Figure 2. (a) Stimulation of topoisomerase II mediated DNA cleavage by the anthracyclines analogues **1**–**4** and the reference compound MEN 10755. Lane C, control DNA; lane T, topoisomerase II alone. (b) Quantification of topoisomerase II dependent DNA cleavage stimulated by anthracycline analogues in SV40 DNA. DNA cleavage levels were determined at different sites with an analysis performed on a Macintosh computer using the public domain NIH Image program (developed at the US National Institute of Health and available on the internet at http://RSB.info.nih.gov/nih-image). To normalize among different experiments, the stimulation factor of MEN 10755 at 1 M, which was included in all the gels, was used as an internal standard.

intensity. The 4″-epimer of MEN 10755, **1**, and the trisaccharide derivative **2**, showed an enzyme-mediated DNA cleavage level similar to MEN 10755. The two 3′-deoxy derivatives **3** and **4** were less active in mediating topoisomerase II α DNA breaks than the parent drug (Fig. 2).

Conclusions

With the exception of the trisaccharide analogue **2**, for which other biological events, such as cellular uptake, must be involved in order to explain its lack of cytotoxicity, the disaccharide derivatives **1**, **3**, **4** showed a cytotoxic potency similar to that of the reference compound. On the other hand, ability to poison topoisomerase II was different for these compounds. Epimerization of the C-4″ position (**1**) or introduction of a further carbohydrate residue (**2**), did not affect the capacity to induce DNA breaks, while removal of the hydroxyl group in C-3′ position of compounds **3** and **4**, seemed to influence the stability of the ternary complex, as intensity of topoisomerase II mediated DNA cleavage appeared to be slightly reduced.

These results seem to indicate that no direct correlation exist between the extent of enzyme-induced DNA cleavage and the cytotoxicity in this class of oligosaccharide anthracyclines. The same observations were reported for anthracycline monosaccharides modified in C-3′ position. It was found that these derivatives had an altered site specificity with respect to the parent compounds and that quantitative relations between levels of DNA cleavage and cytotoxicity were different for compounds that had different sequence specificity.[8,10] Thus, in addition to the level and persistence, the site selectivity of drug-stimulated DNA breaks may also contribute to the cytotoxic activities of anthracyclines. Hence, the need of further experiments to determine the sequence specificity of the oligosaccharide anthracyclines here reported.

Experimental

General remarks

NMR experiments were recorded on a Bruker Avance 400 MHz spectrometer equipped with a 5 mm inverse probe, and processed using Xwin-NMR version 2.1. The ESI-MS experiments were performed on a model QUATTRO triple Quadrupole mass spectrometer (Micromass, UK) equipped with an ESI interface. Solvents and reagents were dried before use according to standard procedures.[34] All reactions requiring anhydrous conditions were performed under a nitrogen atmosphere and starting materials were dried by coevaporations with anhydrous toluene. Molecular sieves 4 Å were activated by flaming under reduced pressure. 60 PF254 silica gel-coated aluminum sheets (Merck) were used for TLC analysis. Column chromatography was performed on silica gel 60, 230–400 mesh (Merck).

Methyl 3-*N*,4-*O*-di-allyloxycarbonyl-2, 3, 6-trideoxy-α-L-*arabino*-hexopyranoside (6). To a mixture of methyl 3-amino-2, 3, 6-trideoxy-α-L-*arabino*-hexopyranoside **5** (3 g, 19 mmol) in anhydrous dichloromethane (140 mL), anhydrous pyridine (2.44 mL, 30.4 mmol) and allylchloroformate (3.22 mL, 30.4 mmol) were added at −10 °C under nitrogen atmosphere. After 4 h at room temperature, water (50 mL) was added at 0 °C and after stirring 10 min, the organic layer was washed with saturated aq $NaHCO_3$ (100 mL), water (100 mL×2) and dried with Na_2SO_4. Evaporation of the solvent gave a residue, chromatographed on silica gel using petroleum ether/ethyl acetate (7:3) to afford 4.3 g (70%) of **6**. ^{1}H NMR (CDCl3) δ 6.0–5.8 (2H, m, 2×CH_2=C*H*), 5.4–5.2 (4H, m, 2×CH_2=CH), 4.8 (1H, br s, NH), 4.75 (1H, d, H-1), 4.65–4.55 (4H, m, 2×OCH_2–CH), 4.0–3.75 (1H, m, H-3), 3.7–3.5 (1H, m, H-5), 3.3 (3H, s, OCH_3), 3.15–3.0 (1H, m, H-4), 2.15–2.0 (1H, dd, H-2eq), 1.75–1.55 (1H, td, H-2ax), 1.3 (3H, d, H-6).

Phenyl 3-*N*,4-*O*-di-allyloxycarbonyl-1-thio-2, 3, 6-trideoxy-α-L-*arabino*-hexopyranoside (7). To a solution of methyl 3-*N*,4-*O*-di-allyloxycarbonyl-acosaminide **6** (5.7

g, 17.3 mmol) in anhydrous dichloromethane (60 mL), phenylthiotrimethyl silane (4.8 mL, 26 mmol), and, dropwise, trimethylsilyl triflate (5.0 mL, 26 mmol) were added at 0 °C. After stirring at room temperature for 2 h, the mixture was diluted with dichloromethane (60 mL), washed with saturated aq $NaHCO_3$ (60 mL), water (2×30 mL), dried with Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on silica gel using petroleum ether/ethyl acetate (8:2) to give 7 (6.6 g, 94%). ^{1}H NMR ($CDCl_3$) δ 7.5–7.4 (2H, m, Ph), 7.3–7.2 (2H, m, Ph), 6.0–5.8 (2H, m, 2×CH_2=C*H*), 5.5 (1H, d, H-1), 5.4–5.2 (4H, m, 2×CH_2=CH), 4.9 (1H, br s, NH), 4.7–4.5 (4H, m, 2×OCH_2–CH), 4.5–4.3 (2H, m, H-4, H-5), 4.2–4.1 (1H, m, H-3), 2.5 (1H, dd, H-2eq), 2.1 (1H, td, H-2ax), 1.2 (3H, d, H-6).

***p*-Methoxybenzyl 3,4-di-*O*-acetyl-2, 6-dideoxy-α-L-*lyxo*-hexopyranoside (9).** To a solution of crude 3,4-di-*O*-acetyl-L-fucal **8** (4.7 g, 21.9 mmol) and *p*-methoxybenzyl alcohol (3.9 g, 28.0 mmol) in anhydrous dichloromethane (70 mL), triphenylphosphine hydrobromide (0.35 g, 1 mmol) was added at 0 °C under nitrogen atmosphere. After 1 h at room temperature, the reaction mixture was diluted with dichloromethane (230 mL), the organic phase was washed with saturated aq $NaHCO_3$ (30 mL), water (2×30 mL) and dried over Na_2SO_4. Evaporation of the solvent furnished a residue (9 g) used without purification in the successive reaction. ^{1}H NMR ($CDCl_3$) δ 7.3–7.2 (2H, m, Ph), 6.9–6.8 (2H, m, Ph), 5.35–5.25 (1H, m, H-3), 5.15 (1H, s, H-4), 5.0 (1H, s, H-1), 4.7–4.6 (1H, d, OCH_2-Ph), 4.55–4.45 (1H, d, OCH_2–Ph), 4.1 (1H, q, H-5), 2.15 (3H, s, $OCOCH_3$), 2.1–2.0 (1H, td, H-2ax), 1.95 (3H, s, $OCOCH_3$), 1.95–1.85 (1H, dd, H-2eq), 1.1 (3H, d, H-6).

***p*-Methoxybenzyl 2,6-dideoxy-α-L-*lyxo*-hexopyranoside (10).** To a solution of the crude *p*-methoxybenzyl fucoside **9** in methanol (70 mL), 1 N methanolic MeONa (4 mL) was added at 0 °C under nitrogen atmosphere. After 2 h at room temperature, the solution was neutralized with 0.75% HCl in ethanol. Removal of the solvent gave a residue, purified by flash chromatography using petroleum ether/ethyl acetate (1:1), and after the elution of *p*-methoxybenzyl alcohol, petroleum ether/acetone (1:2) to give 3.5 g of **10** (60%). ^{1}H NMR ($CDCl_3$) δ 7.3–7.2 (2H, m, Ph), 6.9–6.8 (2H, m, Ph), 4.95 (1H, s, H-1), 4.6–4.5 (1H, d, OCH_2–Ph), 4.4–4.3 (1H, d, OCH_2–Ph), 4.05–3.95 (1H, m, H-3), 3.95 (1H, q, H-5), 3.75 (3H, s, OCH_3), 3.6 (1H, s, H-4), 1.95–1.85 (1H, dd, H-2eq), 1.8–1.7 (1H, td, H-2ax), 1.35 (3H, d, H-6).

***p*-Methoxybenzyl 3-*O*-allyloxycarbonyl-2,6-dideoxy-α-L-*lyxo*-hexopyranoside (11).** A mixture of *p*-methoxybenzylfucoside **10** (1 g, 3.73 mmol) and dibutyltin oxide (1.1 g, 4.46 mmol) in anhydrous toluene (100 mL) was refluxed for 12 h with continuous azeotropic removal of water till concentration to half the volume. After cooling at −78 °C, anhydrous triethylamine (0.623 mL, 4.48 mmol) and allylchloroformate (0.474 mL, 4.48 mmol), were added. After 2 h at −45 °C, a precooled solution of oxalic acid (0.402 g, 4.46 mmol) in anhydrous acetonitrile (20 mL) was added at −78 °C. The reaction mixture was stirred for about 1 h and allowed to warm to room temperature. After filtration of the precipitate oxalates, the filtrate was concentrated in vacuo and chromatographed on silica gel using petroleum ether/diethyl ether (9:1) to afford **11** (0.64 g, 62% considering the recovered starting material). ^{1}H NMR ($CDCl_3$) δ 7.3–7.2 (2H, m, Ph), 6.9–6.8 (2H, m, Ph), 6.0–5.8 (1H, m, CH_2=C*H*), 5.4–5.2 (2H, m, CH_2=CH), 5.1–5.0 (1H, m, H-3), 5.0 (1H, d, H-1), 4.7–4.55 (3H, m, OCH_2–CH, OCH_2–Ph), 4.4 (1H, d, OCH_2–Ph), 4.0 (1H, q, H-5), 3.8 (1H, d, H-4), 3.7 (3H, s, OCH_3), 2.1 (1H, td, H-2ax), 2.0 (1H, dd, H-2eq), 1.3 (3H, d, H-6).

3-*N*-allyloxycarbonyl-1,4-di-*O*-*p*-nitrobenzoyl-2, 3, 6-trideoxy-α-L-*lyxo*-hexopyranose (17). To a solution of 2.7 g (11.7 mmol) of 3-*N*-allyloxycarbonyldaunosamine **16** in anhydrous pyridine (50 mL), *p*-nitrobenzoylchloride (6.5 g, 35 mmol), was added at 0 °C under nitrogen atmosphere. After 1 h at room temperature, the mixture was diluted with dichloromethane (70 mL) and stirred for 5 min with ice-cold water (100 mL). The organic layer was washed with ice-cold 2 M H_2SO_4 (3×50 mL), saturated aq $NaHCO_3$ (3×50 mL), water (50 mL), dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by crystallization (acetone/chloroform/hexane = 1:1:1) to afford 2.2 g of α anomer of **17**. The mother liquor were chromatographed using petroleum ether/ethyl acetate (7:3) to give 2.7 g of the anomeric mixture with a 79% total yield. ^{1}H NMR ($CDCl_3$) α anomer δ 8.45–8.15 (8H, m, Ph), 6.6 (1H, s, H-1), 6.0–5.8 (1H, m, CH_2=C*H*), 5.55 (1H, s, H-4), 5.35–5.15 (2H, m, CH_2=CH), 4.8 (1H, d, NH), 4.6–4.5 (3H, m, OCH_2–CH, H-3), 4.35 (1H, q, H-5), 2.25–2.15 (2H, m, H-2), 1.2 (3H, d, H-6).

Phenyl 3-*N*-allyloxycarbonyl-4-*O*-*p*-nitrobenzoyl-1-thio-2, 3, 6-trideoxy-α-L-*lyxo*-hexopyranoside (18). To a solution of 3-*N*-allyloxycarbonyl-1, 4-di-*O*-*p*-nitrobenzoyl-L-daunosamine **17** (1.14 g, 2.15 mmol) in anhydrous dichloromethane (11.4 mL) phenylthiotrimethyl silane (0.59 mL, 3.22 mmol), and, dropwise, trimethylsilyl triflate (0.62 mL, 3.22 mmol) were added at 0 °C and under nitrogen atmosphere. After stirring at room temperature for 2 h, the mixture was diluted with dichloromethane (10 mL), washed with saturated aq $NaHCO_3$ (10 mL), water (2×10 mL), dried with Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on silica gel using petroleum ether: ethyl acetate (8:2) to give **18** (0.96 g, 95%). ^{1}H NMR ($CDCl_3$) δ 8.4–8.1 (5H, m, Ph), 7.5–7.2 (4H, m, Ph), 6.0–5.8 (1H, m, CH_2=C*H*), 5.75 (1H, d, H-1), 5.5 (1H, s, H-4), 5.35–5.15 (2H, m, CH_2=CH), 4.8–4.65 (2H, m, H-5, NH), 4.6–4.5 (2H, m, OCH_2–CH), 4.4 (1H, m, H-3), 2.35 (1H, td, H-2ax), 2.2 (1H, dd, H-2eq), 1.2 (3H, d, H-6).

General procedure A for the synthesis of oligosaccharides 12, 19, 24, 33, 37

To a solution of the glycosyl acceptor (1 mmol) and the glycosyl donor (1.4 mmol) in anhydrous diethyl ether (16 mL) and 1,2 dichloroethane (4 mL), activated molecular sieves 4 Å (2.2 g) were added. The mixture was stirred for 20 min at room temperature and then, at 10 °C, iodonium dicollidine perchlorate (IDCP) (1.95

mmol) was added. After 1 h at this temperature, TLC showed the formation of the disaccharide with the desired α glycosidic bond, and of the *N*-sulphenamide derivative of the same disaccharide (ratio 6:4). After adding triphenylphosphine (1.9 mmol), the reaction mixture was stirred till complete conversion of the sulphenamide derivative to the desired disaccharide (1 h). The mixture was diluted with diethyl ether (20 mL) and filtered, washed with 10% aq $Na_2S_2O_3$ (20 mL), saturated aq $NaHCO_3$ (20 mL), water (2×20 mL) and dried over Na_2SO_4. Evaporation of the solvent and flash chromatography of the residue on silica gel using petroleum ether/ethyl acetate (8:2), gave the desired disaccharides.

General procedure B for the synthesis of the activated oligosaccharides 15, 22, 26, 36, 40

To a solution of the protected disaccharides **12**, **19**, **24**, **33** and **37** (1 mmol) in acetonitrile/water (9:1, 10 mL), ammonium cerium (IV) nitrate (2 mmol) was added. After 2 h at room temperature, the reaction mixture was diluted with ethyl acetate (10 mL). The separated aqueous layer was extracted with ethyl acetate (2×10 mL) and the combined organic extracts were treated with 10% aq $Na_2S_2O_5$ (20 mL). The obtained precipitate was filtered and the organic phase washed with saturated aq $NaHCO_3$ (20 mL), water (2×20 mL) and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure gave a residue (quantitative reaction) used in the next reaction without purification. To a solution of crude deprotected disaccharide **14**, **21**, **25**, **35** and **39** (1 mmol), in anhydrous pyridine (6 mL), *p*-nitrobenzoylchloride (3 mmol) was added at 0 °C. After stirring for 2 h, at the same temperature, water (3 mL) was added to the reaction mixture and after 10 min, it was diluted with dichloromethane (10 mL). The organic layer was washed with cold 2 M H_2SO_4 (4×10 mL) and ice, cold saturated aq $NaHCO_3$ (4×10 mL), water (2×10 mL), and dried over Na_2SO_4. Concentration in vacuo and flash chromatography on silica gel using petroleum ether/ethyl acetate (8:2) gave the activated disaccharides.

p-Methoxybenzyl 4-*O*-(3-*N*,4-*O*-di-allyloxycarbonyl-2,3,6-trideoxy-L-*arabino*-hexopyranosyl)-3-*O*-allyloxycarbonyl-2,6-dideoxy-L-*lyxo*hexopyranoside (12)

The thiophenyl acosaminide **7** and the *p*-methoxybenzylfucoside **11** were reacted according to the general procedure A to give **12** (74%). ^{1}H NMR ($CDCl_3$) δ 7.2–6.9 (4H, m, Ph), 6.0–5.8 (3H, m, 3×CH_2=C*H*), 5.4–5.0 (8H, m, 3×CH_2=CH, NH, H-1), 4.85 (1H, s, H-1′), 4.7–4.1 (11H, m, 3×OCH_2–CH, OCH_2–Ph, H-3, H-4, H-5′), 3.9 (1H, q, H-5), 3.85 (1H, s, H-4), 3.8 (3H, s, OCH_3), 2.4 (1H, dd, H-2′eq), 2.2 (1H, td, H-2′ax), 1.9 (1H, dd, H-2eq), 1.8 (1H, td, H-2ax), 1.3–1.1 (6H, m, H-6, H6′). ESMS *m*/*z* calcd for $C_{32}H_{43}NO_{13}$ (M + H)+ 650.7, found 650.5.

4-*O*-(3-*N*,4-*O*-di-allyloxycarbonyl-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)-3-*O*-allyloxycarbonyl-2, 6-dideoxy-1-*O*-*p*-nitrobenzoyl-α-L-*lyxo*-hexopyranosyde (15).

Compound **12** was deprotected and activated as described in the general procedure B to give **15** (80%). ^{1}H NMR ($CDCl_3$) δ 8.4–8.1 (4H, m, Ph), 6.5 (1H, d, H-1), 6.0–5.8 (3H, m, 3×CH_2=C*H*), 5.4–5.1 (8H, m, 3×CH_2=CH, NH, H-3′), 4.9–4.5 (9H, m, 3×OCH_2–CH, H-1′, H-3, H-4′), 4.4–4.1 (2H, m, H-5, H-5′), 3.95 (1H, s, H-4), 2.5–1.7 (4H, m, H-2, H-2′), 1.3–1.1 (6H, m, H-6, H-6′). ESMS *m*/*z* calcd for $C_{31}H_{38}N_2O_{15}$ (M + H)+ 679.6, found 679.8.

p-Methoxybenzyl 4-*O*-(3-*N*-allyloxycarbonyl-2,3,6-trideoxy-4-*O*-*p*-nitrobenzoyl-α-L-*lyxo*-hexopyranosyl)-3-*O*-allyloxycarbonyl-2,6-dideoxy-α-L-*lyxo*-hexopyranoside (19).

Compounds **11** and **18** were reacted according to the general procedure A to afford **19** in 75% yield. ^{1}H NMR ($CDCl_3$) δ 8.4–8.2 (4H, m, Ph), 7.3–7.2 (2H, m, Ph), 7.0–6.8 (2H, m, Ph), 6.0–5.8 (2H, m, 2×CH_2=C*H*), 5.5–5.0 (8H, m, 2×CH_2=CH, H-1, H-1′, H-3, H-4′), 4.7–4.4 (9H, m, 2×OCH_2–CH, OCH_2–Ph, NH, H-3′, H-5′), 4.0 (1H, q, H-5), 3.9 (1H, s, H-4), 3.8 (3H, s, OCH_3), 2.3–1.8 (4H, m, H-2, H-2′), 1.3 (3H, d, H-6), 1.1 (3H, d, H-6′). ESMS *m*/*z* calcd for $C_{35}H_{42}N_2O_{14}$ (M + H)+ 715.7, found 715.9.

4-*O*-(3-*N*-allyloxycarbonyl-2,3,6-trideoxy-4-*O*-*p*-nitrobenzoyl-α-L-*lyxo*-hexopyranosyl)-3-*O*-allyloxycarbonyl-2,6-dideoxy-1-*O*-*p*-nitrobenzoyl-α-L-*lyxo*-hexopyranoside (22).

Following the general procedure B, **19** gave the activated disaccharide **22** in 83% yield. ^{1}H NMR ($CDCl_3$) δ 8.4–8.1 (8H, m, Ph), 6.5 (1H, s, H-1), 6.0–5.8 (2H, m, 2×CH_2=C*H*), 5.5 (1H, s, H-4′), 5.4–5.2 (5H, m, 2×CH_2=CH, H-3), 5.1 (1H, s, H-1′), 4.7–4.4 (7H, m, 2×OCH_2–CH, NH, H-3′, H-5′), 4.2 (1H, q, H-5), 4.0 (1H, s, H-4), 2.5 (1H, td, H-2eq), 2.2–2.1 (2H, m, H-2′), 2.0 (1H, td, H-2ax), 1.3 (3H, d, H-6), 1.1 (3H, d, H-6′). ESMS *m*/*z* calcd for $C_{34}H_{37}N_3O_{16}$ (M + H)+ 744.7, found 744.9.

Phenyl 4-*O*-(3-*N*-allyloxycarbonyl-2,3,6-trideoxy-4-*O*-*p*-nitrobenzoyl-α-L-*lyxo*-hexopyranosyl)-2,6-dideoxy-3-*O*-allyloxycarbonyl-1-thio-α-L-*lyxo*-hexopyranoside (23).

To a solution of the disaccharide **22** (0.5 g, 0.67 mmol) in anhydrous dichloromethane (25 mL), phenylthiotrimethyl silane (0.31 mL, 1.68 mmol), and, dropwise, trimethylsilyl triflate (0.26 mL, 1.34 mmol) were added at 0 °C. After stirring at 0 °C for 1 h, the mixture was diluted with dichloromethane (10 mL), washed with saturated aq $NaHCO_3$ (10 mL), water (2×10 mL), dried with Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on silica gel using petroleum ether/ethyl acetate (8:2) to give **23** (0.25 mg, 54%). ^{1}H NMR ($CDCl_3$) δ 8.4–8.2 (4H, m, Ph), 7.6–7.2 (5H, m, Ph), 6.0–5.8 (2H, m, 2×CH_2=C*H*), 5.7 (1H, d, H-1), 5.5 (1H, s, H-4′), 5.4–5.0 (6H, m, 2×CH_2=CH, H-1′, H-3), 4.7—4.4 (8H, m, 2×OCH_2–CH, NH, H-3′, H-5, H-5′), 4.0 (1H, s, H-4), 2.5 (1H, td, H-2eq), 2.2–1.8 (3H, m, H-2′, H-2ax), 1.3 (3H, d, H-6), 1.1 (3H, d, H-6′).

p-Methoxybenzyl 4-*O*-4-*O*-(3-*N*-allyloxycarbonyl-4-*O*-*p*-nitrobenzoyl-2, 3, 6-trideoxy-α-L-*lyxo*-hexopyranosyl)-3-*O*-allyloxycarbonyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl-3-*O*-allyloxycarbonyl-2,6-dideoxy-α-L-*lyxo*-hexopyranoside (24).

To a solution of the disaccharide **23** (220

mg, 0.32 mmol) and the *p*-methoxybenzyl fucoside **11** (113 mg, 0.32 mmol) in anhydrous diethyl ether (6 mL) and 1,2 dichloroethane (1.5 mL), activated molecular sieves 4 Å (750 mg) were added. The mixture was stirred for 20 min at room temperature and then, at 10 °C, iodonium dicollidine perchlorate (220 mg, 0.48 mmol) was added. After 1.5 h at this temperature, the mixture was diluted with diethyl ether (10 mL) and filtered, washed with 2% aq $Na_2S_2O_3$ (10 mL), water (2×10 mL) and dried over Na_2SO_4. Evaporation of the solvent and flash chromatography of the residue on silica gel using petroleum ether/ethyl acetate (7.5:1.5), afforded the desired trisaccharide in 41% yield. ^{1}H NMR ($CDCl_3$) δ 8.4–8.2 (4H, m, Ph), 7.3 (2H, m, Ph), 6.9 (2H, m, Ph), 6.0–5.8 (3H, m, 3×CH_2=C*H*), 5.5 (1H, s, H-4″), 5.4–5.0 (11H, m, 3×CH_2=CH, H-1, H-1′, H-1″, H-3, H-3′), 4.7–4.4 (11H, m, 3×OCH_2–CH, OCH_2–Ph, NH, H-3″, H-5″), 4.3 (1H, q, H-5), 4.0–3.8 (3H, m, H-4, H-4′, H-5), 3.8 (3H, s, OCH_3), 2.4–1.8 (6H, m, H-2, H-2′, H-2″), 1.4–1.1 (9H, m, 3 × H-6, H-6′, H-6″). ESMS *m*/*z* calcd for $C_{45}H_{56}N_2O_{19}$ (M + H)+ 929.9, found 929.7.

4-*O*-[4-*O*-(3-*N*-allyloxycarbonyl-2,3,6-trideoxy-4-*O*-*p*-nitrobenzoyl-α-L-*lyxo*-hexopyranosyl)-3-*O*-allyloxycarbonyl - 2,6 - dideoxy - α - L - *lyxo* - hexopyranosyl] - 3 - *O*-allyloxycarbonyl - 2,6 - dideoxy - 1 - *O* - *p* - nitrobenzoyl-α-L-*lyxo*-hexopyranoside (26). The trisaccharide **24** was treated according to the general procedure B to give **26** (60%). ^{1}H NMR ($CDCl_3$) δ 8.4–8.2 (8H, m, Ph), 6.0–5.8 (4H, m, 3×CH_2 = C*H*, H-1), 5.5 (1H, s, H-4″), 5.4–5.3 (6H, m, 3×CH_2=CH), 5.2 (1H, m, H-3), 5.1 (1H, s, H-1), 5.0 (1H, s, H-1′), 4.8 (1H, dt, H-3), 4.7–4.5 (8H, m, 3×OCH_2–CH, NH, H-5″), 4.4 (1H, m, H-3″), 4.3 (1H, q, H-5″), 4.0 (1H, s, H-4′), 3.9 (1H, s, H-4), 3.8 (1H, q, H-5), 2.4–1.8 (6H, m, H-2, H-2′, H-2″), 1.3 (3H, d, H-6), 1.2 (3H, d, H-6′), 1.1 (3H, d, H-6″). ESMS *m*/*z* calcd for $C_{44}H_{51}N_3O_{21}$ (M + H)+ 958.9, found 958.7.

***p*-Methoxybenzyl 4-*O*-acetyl-2,3,6-trideoxy-α-L-*arabino*-hex-2-enopyranoside (28).** To a solution of **27** (2 g, 9.3 mmol) and *p*-methoxybenzyl alcohol (1.9 g, 14 mmol) in anhydrous dichloromethane (60 mL), 0.22 M $SnCl_4$ in dichloromethane (4.0 mL, 0.88 mmol) was added at 0 °C. After 2 h at room temperature the reaction mixture was diluted with dichloromethane (20 mL). The organic phase was washed with saturated aq $NaHCO_3$ (30 mL) and water (2×20 mL), then dried over Na_2SO_4. Evaporation of the solvent in vacuo afforded a residue chromatographed on silica gel using petroleum ether/diethyl ether (8:2) to afford **28** (1.9 g, 70% yield). ^{1}H NMR ($CDCl_3$) δ 7.3–6.9 (4H, m, Ph), 6.2 (1H, q, H-3), 5.8 (1H, dd, H-2), 4.8 (1H, s, H-1), 4.6–4.4 (2H, m, OCH_2–Ph), 4.2 (1H, m, H-5), 3.7 (3H, s, OCH_3), 3.6 (1H, m, H-4), 2.1 (3H, s, COCH3), 1.2 (3H, d, H-6).

***p*-Methoxybenzyl 4-*O*-acetyl-2, 3, 6-trideoxy-α-L-*arabino*-hexopyranoside (29).** To a solution of compound **28** (500 mg, 1.7 mmol) in anhydrous benzene (25 mL) chlorotris(triphenylphosphine)rhodium(I) (78.6 mg, 0.085 mmol) was slowly added. After 2 h in the dark, under hydrogen atmosphere (1 atm), the solvent was evaporated to dryness. The residue was purified by flash chromatography on silica gel using dichloromethane to obtain **29** (450 mg, 90%). ^{1}H NMR ($CDCl_3$) δ 7.3–6.9 (4H, m, Ph), 4.8 (1H, s, H-1), 4.6–4.4 (2H, m, OCH_2–Ph), 4.5 (1H, m, H-4), 3.9–3.8 (1H, m, H-5), 3.7 (3H, s, OCH_3), 2.1 (3H, s, $COCH_3$), 1.9–1.7 (4H, m, H-2, H-3), 1.2 (3H, d, H-6).

***p*-Methoxybenzyl 4-*O*-benzoyl-2,3,6-trideoxy-α-L-*lyxo*-hexopyranoside (31).** Removal of the acetyl group was performed according to the known procedures adding, at 0 °C, 1 N methanolic NaOMe (400 mL) to a solution of compound **29** (90 mg, 0.3 mmol) in methanol (700 mL). After 2 h at room temperature, the mixture was neutralized with 0.75% HCl in ethanol. After concentration of the solvent the residue was chromatographed on silica gel, using petroleum ether/ethyl acetate (1:1) to give the deprotected *p*-methoxybenzyl 2,3,6-trideoxy-α-L-*lyxo* hexopyranoside **30** (60%). 252 mg (1 mmol) of this compound, triphenylphosphine (393 mg, 1.5 mmol) and benzoic acid (183 mg, 1.5 mmol) were dissolved in anhydrous tetrahydrofuran (10 mL). A solution of diethyl azodicarboxylate (0.236 mL, 1.5 mmol) in tetrahydrofuran (2 mL) was added dropwise while stirring at room temperature. After 2 h the reaction was stopped. Prolonged reaction times did not increase the yield of the reaction. After removal of the solvent the residue was redissolved in dichloromethane (20 mL), washed with saturated aq $NaHCO_3$ (10 mL), water (10 mL), dried over Na_2SO_4 and evaporated in vacuo. Flash chromatography of the residue on silica gel using dichloromethane, gave **31** (178 mg, 50% yield, 80% considering the recovered starting material). ^{1}H NMR ($CDCl_3$) δ 7.7–7.4 (5H, m, Ph), 7.3–6.9 (4H, m, Ph), 5.1 (1H, s, H-1), 5.0 (1H, s, H-4), 4.7–4.5 (4H, m, OCH_2–Ph), 4.2 (1H, q, H-5), 3.8 (3H, s, OCH_3), 2.3–1.9 (4H, m, H-2, H-3), 1.3 (3H, d, H-6).

***p*-Methoxybenzyl 2,3,6-trideoxy-α-L-*lyxo*-hexopyranoside (32).** K_2CO_3 (15 mg, 0.11 mmol) was added at room temperature to a solution of *p*-methoxybenzyl 4-*O*-benzoyl-2,3,6-trideoxy fucose **31** (392 mg, 1.1 mmol) in methanol (50 mL). After 2 h the solvent was evaporated to dryness and the residue flash chromatographed on silica gel using dichloromethane/methanol (98:2) to give **32** (260 mg, 94%). ^{1}H NMR ($CDCl_3$) δ 7.3–6.9 (4H, m, Ph), 4.8 (1H, s, H-1), 4.6–4.4 (2H, m, OCH_2–Ph), 4.0 (1H, q, H-5), 3.8 (3H, s, OCH_3), 3.5. (1H, d, H-4), 2.2–1.5 (4H, m, H-2, H-3), 1.3 (3H, d, H-6).

***p*-Methoxybenzyl 4-*O*-(3-*N*-allyloxycarbonyl-4-*O*-*p*-nitrobenzoyl-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)-2,3,6 - trideoxy - α - L - *lyxo* - hexopyranoside (33).** The *p*-methoxybenzyl 2,3,6-trideoxy fucoside **32** was reacted with the thiophenyl daunosaminide **18**, following the general procedure A to give **33** (84%). ^{1}H NMR ($CDCl_3$) δ 8.4–8.2 (4H, m, Ph), 7.3–6.9 (4H, m, Ph), 6.0–5.8 (1H, m, CH_2=CH), 5.5 (1H, s, H-5′), 5.3–5.1 (2H, m, CH_2=C*H*), 5.1 (1H, s, H-1′), 4.9 (1H, s, H-1), 4.7–4.4 (6H, m, OCH_2–CH, OCH_2–Ph, NH, H-3′), 4.3 (1H, q, H-5), 3.9 (1H, m, H-5′), 3.8 (3H, s, OCH_3), 3.5. (1H, s, H-4), 2.2–1.5 (6H, m, H-2, H-2′, H-3), 1.3 (3H, d, H-6), 1.2 (3H, d, H-6′). ESMS *m*/*z* calcd for $C_{30}H_{36}N_2O_{11}$ (M + H)+ 601.2, found 601.4.

4-*O*-(3-*N*-allyloxycarbonyl-4-*O*-*p*-nitrobenzoyl-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)-2,3,6-trideoxy-1-*O*-*p*-nitrobenzoyl - α - L - *lyxo* - hexopyranoside (36). The disaccharide **33** was treated according to the general procedure B to give **36** (80%). ^{1}H NMR ($CDCl_3$) δ 8.4–8.2 (8H, m, Ph), 6.5 (1H, br s, H-1), 6.0–5.8 (1H, m, CH_2=C*H*), 5.5 (1H, s, H-4′), 5.4–5.2 (2H, m, CH_2=CH), 5.1 (1H, br s, H-1′), 4.7–4.4 (4H, m, OCH_2–CH, H-3′, NH), 4.3 (1H, q, H-5′), 4.2 (1H, q, H-5), 3.7 (1H, s, H-4), 2.4–1.8 (6H, m, H-2, H-2′, H-3), 1.3 (3H, d, H-6), 1.2 (3H, d, H-6′). ESMS *m*/*z* calcd for $C_{30}H_{33}N_3O_{13}$ $(M+H)^+$ 644.2, found 644.5.

***p*-Methoxybenzyl 4-*O*-(3-*N*, 4-*O*-di-allyloxycarbonyl-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)-2,3,6-trideoxy-α-L-*lyxo*-hexopyranoside (37).** The *p*-methoxybenzyl 2,3,6-trideoxy fucoside **32** was reacted with the thiophenyl acosaminide **7**, following the general procedure A to give **37** (75%). ^{1}H NMR ($CDCl_3$) δ 7.3–6.8 (4H, m, Ph), 6.0–5.8 (2H, m, 2×CH_2=C*H*), 5.4–5.2 (4H, m, 2×CH_2=CH), 5.0 (1H, d, NH), 4.9 (1H, s, H-1′), 4.8 (1H, dt, H-3), 4.7–4.5 (5H, m, 2×OCH_2–CH, H-1), 4.5–4.3 (2H, m, OCH_2–Ph), 4.0 (1H, q, H-5), 3.9 (1H, m, H-3′), 3.8 (3H, s, OCH_3), 3.7. (1H, br s, H-4), 3.5 (1H, m, H-5′), 2.2–1.5 (6H, m, H-2, H-2′, H-3), 1.3 (3H, d, H-6), 1.2 (3H, d, H-6′). ESMS *m*/*z* calcd for $C_{26}H_{35}NO_{10}$ (M + H)+ 522.2, found 522.3.

4-*O*-(3-*N*,4-*O*-di-allyloxycarbonyl-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)-2,3,6-trideoxy-1-*O*-*p*-nitrobenzoyl-α-L-*lyxo*-hexopyranoside (40). Application of the general procedure B to the disaccharide **37** gave **40** in 80% yield. ^{1}H NMR ($CDCl_3$) δ 8.4–8.2 (4H, m, Ph), 6.5 (1H, bs, H-1), 6.0–5.8 (2H, m, CH_2=C*H*), 5.4–5.2 (4H, m, CH_2=CH), 5.0 (1H, d, NH), 4.9–4.8 (2H, m, H-3′, H-1′), 4.7–4.5 (5H, m, OCH_2–CH, H-4′), 4.3 (1H, m, H-5′), 4.1 (1H, q, H-5), 4.0 (1H, s, H-4), 2.2–1.5 (6H, m, H-2, H-2′, H-3), 1.3 (3H, d, H-6), 1.2 (3H, d, H-6′). ESMS *m*/*z* calcd for $C_{26}H_{32}N_2O_{12}$ (M + H)+ 565.2, found 565.4.

General procedure C for the synthesis of fully protected glycosides 42–45

To a mixture of 14-acetoxy-4-demethoxyadriamycinone (1 mmol) **41**, disaccharide (1 mmol) and activated molecular sieves (5 g) in anhydrous dichloromethane/diethyl ether (3:1, 110 mL), trimethylsylil triflate (1.5 mmol) was added dropwise at −30 °C. After 1.5 h, 2% aq $NaHCO_3$ (60 mL), at 0 °C, and then dichloromethane (125 mL) were added to the reaction mixture. The organic layer was washed with water (100 mL), dried over Na_2SO_4 and evaporated in vacuo. The crude residue was flash chromatographed on silica gel using dichloromethane/acetone (19:1) to afford the protected glycosides.

7-[4-*O*-(2,3,6-trideoxy-3-*N*,4-*O*-di-allyloxycarbonyl-α-L-*arabino*-hexopyranosyl)-2,6-dideoxy-3-*O*-allyloxycarbonyl-α-L-*lyxo*-hexopyranosyl]-14-acetoxy-4-demethoxyadriamycinone (42). The aglycone **41** and the disaccharide **15** were reacted following the general procedure C to obtain the glycoside **42** (73%). ^{1}H NMR ($CDCl_3$) δ 13.6 (1H, s, OH-6), 13.3 (1H, s, OH-11), 8.4 (2H, m, H-1, H-4), 7.8 (2H, m, H-2, H-3), 6.0–5.8 (3H, m, 3×CH_2=C*H*), 5.6 (1H, s, H-1′), 5.0–5.4 (11H, m, 3×CH_2=CH, H-1″, H-3′, H-7, H-14), 4.9 (1H, d, H-4″), 4.7–4.5 (6H, m, 3×OCH_2–CH), 4.4 (1H, q, H-5″), 4.3 (1H, m, H-3″), 4.1 (1H, q, H-5′), 3.9 (1H, s, H-4′), 3.2 (2H, q, H-10), 2.5–1.7 (6H, m, H-2′, H-2″, H-8), 2.2 (3H, s, $COCH_3$), 1.4–1.2 (6H, m, H-6′, H-6″). ESMS *m*/*z* calcd for $C_{46}H_{51}NO_{20}$ (M + H)+ 938.9, found 938.7.

7-{3-*O*-allyloxycarbonyl-4-*O*-[3-*O*-allyloxycarbonyl-2,6-dideoxy-4-*O*-(3-*N*-allyloxycarbonyl-2,3,6-trideoxy-4-*O*-*p*-nitrobenzoyl-α-L-*lyxo*-hexopyranosyl)-α-L-*lyxo*-hexopyranosyl]-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl-14-acetoxy-4-demethoxyadriamycinone (43). The aglycone **41** and the trisaccharide **26** were coupled according to the general procedure C to afford the glycoside **43** (60%). ^{1}H NMR ($CDCl_3$) δ 13.6 (1H, s, OH-6), 13.2 (1H, s, OH-11), 8.4 (2H, m H-1, H-4), 8.3–8.2 (4H, m, Ph), 7.8 (2H, m, H-2, H-3), 6.0–5.7 (3H, m, 3×CH_2=C*H*), 5.55 (1H, br s, H-1′), 5.45 (1H, br s, H-4‴), 5.4–5.2 (9H, m, 3×CH_2=CH, H-7, H-14), 5.1 (1H, m, H-3″), 5.0 (1H, br s, H-1‴), 4.9 (1H, br s, H-1″), 4.8 (1H, m, H-3′), 4.7–4.5 (7H, m, 3×OCH_2–CH, NH), 4.35 (1H, q, H-5‴), 4.2 (1H, q, H-5″), 4.1 (1H, m, H-3‴), 4.05 (1H, q, H-5′), 3.95 (1H, br s, H-4″), 3.9 (1H, br s, H-4′), 3.15 (2H, q, H-10), 2.4–2.2 (2H, m, H-8), 2.2 (3H, s, $COCH_3$), 2.1–1.6 (6H, m, H-2′, H-2″, H-2‴), 1.3 (3H, d, H-6′) 1.2 (3H, d, H-6″), 1.1 (3H, d, H-6‴). ESMS *m*/*z* calcd for $C_{59}H_{64}N_2O_{26}$ $(M+H)^+$ 1218.2, found 1218.4.

7-[4-*O*-(2, 3, 6-Trideoxy-3-*N*-allyloxycarbonyl-4-*O*-*p*-nitrobenzoyl-α-L-*lyxo*-hexopyranosyl)-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]-14-acetoxy-4-demethoxyadriamycinone (44). The aglycone **41** and the disaccharide **36** were reacted following the general procedure C obtaining the glycoside **44** in 45% yield. ^{1}H NMR ($CDCl_3$) δ 13.6 (1H, s, OH-6), 13.3 (1H, s, OH-11), 8.4 (2H, m, H-1, H-4), 7.8 (2H, m, H-2, H-3), 6.0–5.8 (1H, m, CH_2=C*H*), 5.5 (1H, s, H-1′), 5.4–5.2 (3H, m, CH_2=CH, H-7), 4.5 (3H, m, H-14, NH), 4.9 (1H, s, H-1′), 4.8 (2H, d, OCH_2–CH), 4.6 (1H, d, OH-9), 4.2–3.9 (3H, m, H-3′, H-5′, H-5″), 3.7 (1H, d, H-4″), 3.6 (1H, s, H-4′), 3.4–3.0 (2H, q, H-10), 3.0 (1H, t, OH-14), 2.4–1.8 (11H, m, $COCH_3$, H-2′, H-2″, H-3′, H-8), 1.3–1.2 (6H, m, H-6′, H-6″). ESMS *m*/*z* calcd for $C_{45}H_{46}N_2O_{18}$ (M + H)+ 903.9, found 903.7.

7-[4-*O*-(3-*N*,4-*O*-di-allyloxycarbonyl-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]-14-acetoxy-4-demethoxyadriamycinone (45). The aglycone **41** and the disaccharide **40** were coupled according to the general procedure C. The obtained residue was purified by preparative HPLC (Merck column, LiChrosphere RP-18, 7 μm, 250×25 mm, CH_3CN + 0.1% TFA/H_2O + 0.1% TFA, 80:20, flow 8 mL/min) giving the glycoside **45** (55%). ^{1}H NMR ($CDCl_3$) δ 13.6 (1H, s, OH-6), 13.3 (1H, s, OH-11), 8.4 (2H, m, H-1, H-4), 7.8 (2H, m, H-2, H-3), 6.0–5.8 (2H, m, 2×CH_2=C*H*), 5.5 (1H, s, H-1′), 5.4–5.2 (5H, m, 2×CH_2=CH, H-1″), 4.9 (1H, s, H-4″), 4.8 (1H, d, NH), 4.7–4.5 (4H, m, 2×OCH_2–CH), 4.4 (2H, m, H-14), 4.2 (1H, m, H-3″), 4.1–3.9 (2H, m, H-5′, H-5″), 3.6 (1H, s, H-4′), 3.4–3.0 (2H, q, H-10), 2.4–2.0 (8H, m, H-2′, H-2″, H-3′, H-8), 2.1 (3H, s, $COCH_3$), 1.4–1.2 (6H, m, H-6′,

H-6″). ESMS *m/z* calcd for $C_{42}H_{47}NO_{17}$ $(M+H)^+$ 837.8, found 837.6.

General procedure D for the synthesis of partially protected glycosides 46–49

To a solution of fully protected glycoside (1 mmol) in methanol/dichloromethane (2:1, 106 mL), 0.5 M aq K_2CO_3 (3 mmol) was added at −10 °C. After stirring for 1 h at −10/−5 °C the reaction mixture was quenched with 0.1 M HCl. The aq layer was washed with chloroform until a colourless extract was obtained. The combined organic extracts were washed with water (2×50 mL), dried over Na_2SO_4 and concentrated in vacuo. Flash chromatography on silica gel using dichloromethane/ethanol (96:4) gave the partially deprotected glycoside.

7-[4-*O*-(2,3,6-trideoxy-3-*N*,4-*O*-di-allyloxycarbonyl-α-L-*arabino*-hexopyranosyl)-2,6-dideoxy-3-*O*-allyloxycarbonyl-α-L-*lyxo*-hexopyranosyl]-4-demethoxyadriamycinone (46). The fully protected glycoside **42** was partially deprotected according to the general procedure D to afford **46** (70%). ^{1}H NMR ($CDCl_3$) δ 13.6 (1H, s, OH-6), 13.3 (1H, s, OH-11), 8.4 (2H, m H-1, H-4), 7.9 (2H, m, H-2, H-3), 6.0–5.8 (3H, m, 3×CH_2=C*H*), 5.6 (1H, s, H-1′), 5.4–5.1 (8H, m, 3×CH_2=CH, H-7, OH-9), 4.9–4.5 (12H, m, 3×OCH_2–CH, NH, H-14, H-1″, H-3′, H-4″), 4.4–4.0 (3H, m, H-3″, H-5, H-5′), 3.9 (1H, s, H-4′), 3.2 (2H, q, H-10), 2.5–1.8 (6H, m, H-2′, H-2″, H-8), 1.4–1.2 (6H, m, H-6′, H-6″). ESMS *m/z* calcd for $C_{44}H_{49}NO_{19}(M+H)+$ 896.9, found 896.7.

7-{3-*O*-allyloxycarbonyl-4-*O*-[3-*O*-allyloxycarbonyl-2,6-dideoxy-4-*O*-(3-*N*-allyloxycarbonyl-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)-α-L-*lyxo*-hexopyranosyl]-2, 6-dideoxy-α-L-*lyxo*-hexopyranosyl}-4-demethoxyadriamycinone (47). Compound **43** was reacted following the general procedure D to obtain the glycoside **47** (73%). ^{1}H NMR ($CDCl_3$) δ 13.55 (1H, s, OH-6), 13.3 (1H, s, OH-11), 8.35 (2H, m H-1, H-4), 7.85 (2H, m, H-2, H-3), 6.0–5.8 (3H, m, 3×CH_2=C*H*), 5.57 (1H, s, H-1′), 5.4–5.2 (7H, m, 3×CH_2=CH, H-7), 5.15–5.0 (2H, m, OH-9, H-3″), 4.95 (1H, s, H-1‴), 4.87 (1H, s, H-1″), 4.85–4.7 (3H, m, H-3′, H-14), 4.65–4.5 (7H, m, 3×OCH_2–CH, NH), 4.4–4.3 (1H, m, H-5‴), 4.3–4.2 (1H, m, H-5″), 4.2–4.0 (2H, m, H-3‴, H-5′), 3.95 (1H, br s, H-4″), 3.9 (1H, s, H-4′), 3.6 (1H, s, H-4‴), 3.3 (1H, d, H-10), 3.05 (1H, d, H-10), 3.0 (1H, br s, OH-14), 2.4–1.6 (8H, m, H-2′, H-2″, H-2‴, H-8), 1.3 (3H, d, H-6′) 1.2 (3H, d, H-6″), 1.1 (3H, d, H-6‴). ESMS *m/z* calcd for $C_{50}H_{59}NO_{22}$ $(M+H)^+$ 1027.0, found 1027.1.

7-[4-*O*-(2,3,6-trideoxy-3-*N*-allyloxycarbonyl-α-L-*lyxo*-hexopyranosyl)-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]-4-demethoxyadriamycinone (48). The fully protected glycoside **44** was partially deprotected according to the general procedure D to afford **48** (70%). ^{1}H NMR ($CDCl_3$) δ 13.55 (1H, s, OH-6), 13.25 (1H, s, OH-11), 8.4 (2H, m, H-1, H-4), 7.8 (2H, m, H-2, H-3), 5.9 (1H, m, CH_2=C*H*), 5.45 (1H, s, H-1′), 5.4–5.2 (3H, m, CH_2=CH, H-7), 5.1 (3H, m, H-14, NH), 4.9 (1H, s, H-1′), 4.8 (2H, d, OCH_2–CH), 4.6 (1H, d, OH-9), 4.2–3.9 (3H, m, H-3′, H-5′, H-5″), 3.6 (2H, m, H-4′, H-4″), 3.3 (1H, d, H-10), 3.2–3.0 (2H, m, H-10, OH-14), 2.4–1.9 (8H, m, H-2′, H-2″, H-3′, H-8), 1.3–1.2 (6H, m, H-6′, H-6″). ESMS *m/z* calcd for $C_{36}H_{41}NO_{14}$ $(M+H)^+$ 712.7, found 712.9.

7-[4-*O*-(3-*N*,4-*O*-di-allyloxycarbonyl-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]-4-demethoxyadriamycinone (49). The glycoside **45** was reacted following the general procedure D to afford the glycoside **49** (71%). ^{1}H NMR ($CDCl_3$) 13.55 δ (1H, s, OH-6), 13.3 (1H, s, OH-11), 8.4 (2H, m, H-1, H-4), 7.9 (2H, m, H-2, H-3), 6.0–5.8 (2H, m, 2×CH_2=C*H*), 5.5–5.1 (6H, m, 2×CH_2=CH, H-1′, H-7), 5.0 (1H, s, H-1″), 4.9 (1H, s, H-4″), 4.8 (1H, m, NH), 4.75 (2H, d, H-14), 4.7–4.5 (4H, m, 2×OCH_2–CH), 4.4 (1H, q, H-5′), 4.2 (1H, m, H-3″), 4.0 (1H, m, H-5″), 3.6 (1H, s, H-4′), 3.3 (1H, d, H-10), 3.1–3.0 (2H, m, H-10, OH-14), 2.25–1.6 (8H, m, H-2′, H-2″, H-3′, H-8), 1.2 (6H, m, H-6′, H-6″). ESMS *m/z* calcd for $C_{50}H_{54}N_2O_{21}$ $(M+H)^+$ 852.3, found 852.6.

General procedure E for the synthesis of glycosides 1–4

To a solution of the partially deprotected glycoside (1 mmol) in dichloromethane (125 mL), *N*,*N*-dimethyltrimethylsilylamine (5 mmol), trimethylsilylacetate (5 mmol) and tetrakis-(triphenylphosphine)palladium (0.03 mmol) were added at room temperature. After stirring for 30 min in the dark, dichloromethane (500 mL) was added and the organic layer was washed with 5% aq NaCl (2×370 mL) and percolated through a column of Na_2SO_4 (125 g). The organic phase was concentrated to a small volume and evaporated several times with ethyl acetate, until complete removal of dichloromethane. After concentration of the solution to a small volume (125 mL), 0.001 M HCl (75 mL) was added and the separated organic layer was extracted with 0.001 M HCl (4×12 mL) until a colourless extract was obtained. The combined aqueous extracts were washed with ethyl acetate (3×250 mL), concentrated to a volume of about 55 mL (bath temperature <30 °C), and after adjusting the pH to a value of 4.0, added with acetonitrile (6 mL). This solution was filtered on a 10 cm reverse phase bad (Merck, LiChropep RP-18, 40–63 mm, substance/reverse phase 1/100, water (pH 4)/acetonitrile 2:1). After removal of the residual acetonitrile and adjusting the pH to 4.5, the eluate was lyophilized to give the final glycoside as hydrochloride.

7-[4-*O*-(2,3,6-trideoxy-3-amino-α-L-*arabino*-hexopyranosyl)-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl]-4-demethoxyadriamycinone (1). The partially protected glycoside **46** was treated as described in the general procedure E to give **1** (40%). ^{1}H NMR (DMSO) δ 8.4 (2H, m H-1, H-4), 8.1 (2H, m H-2, H-3), 5.6 (1H, d, OH-4″), 5.5 (1H, s, OH-9), 5.3 (1H, s, H-1′), 5.0 (1H, m, H-7), 4.9 (1H, s, H-1″), 4.8 (1H, t, OH-14), 4.7–4.5 (3H, m, OH-3′, H-14), 4.3–4.1 (2H, m, H-5″, H-5′), 3.8 (1H, m, H-3′), 3.6 (1H, s, H-4′), 3.3 (1H, m, H-3″), 3.1–3.0, (3H, m, H-4″, H-10), 2.2 (3H, m, H-2″eq, H-8), 1.9–1.6 (3H, m, H-2″ax, H-2′), 1.2 (3H, d, H-6′), 1.1 (3H, d, H-6″). ESMS *m/z* calcd for C32H37NO13 $(M+H)^+$ 644.6, found 644.7.

7-{4-*O*-[4-*O*-(2,3,6-trideoxy-3-amino-α-L-*lyxo*-hexopyranosyl)-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl]-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl}-4-demethoxyadriamycinone (2). Following the general procedure E the glycoside **47** gave **2** in 60% yield. ^{1}H NMR (DMSO) δ 13.6 (1H, s, OH-6), 13.2 (1H, s, OH-11), 8.35 (2H, m, H-1, H-4), 8.0 (2H, m, H-2, H-3), 5.50 (1H, s, OH-9), 5.30 (1H, d, OH-4‴), 5.25 (1H, br s, H-1′), 5.95 (1H, t, OH-14), 4.90–4.8 (3H, m, H-1″, H-1‴, H-7), 4.65 (1H, d, OH-3′), 4.60 (2H, d, H-14), 4.55 (1H, d, OH-3″), 4.40 (1H, q, H-5″), 4.30 (1H, q, H-5″), 4.15 (1H, q, H-5′), 3.90 (1H, m, H-3″), 3.75 (1H, m, H-3′), 3.50 (3H, m, H-4′, H-4″, H-4‴), 3.40 (1H, m, H-3‴), 3.00 (2H, s, H-10), 2.25–2.05 (2H, m, H-8), 1.95–1.50 (6H, m, H-2′, H-2″, H-2‴), 1.15 (3H, d, H-6′) 1.10 (3H, d, H-6″), 1.05 (3H, d, H-6‴). ESMS *m*/*z* calcd for $C_{38}H_{47}NO_{16}$ (M + H) + 774.8, found 774.6.

7-[4-*O*-(2,3,6-trideoxy-3-amino-α-L-*lyxo*-hexopyranosyl)-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]-4-demethoxyadriamycinone (3). According to the general procedure E, **48** afforded the final glycoside **3** (70%). ^{1}H NMR (DMSO) δ 8.4 (2H, m, H-1, H-4), 7.8 (2H, m, H-2, H-3), 5.5 (1H, s, OH-9), 5.4 (1H, d, OH-4″), 5.2 (1H, s, H-1′), 5.0 (1H, br s, H-7), 5.0 (1H, s, H-1″), 4.9 (1H, s, H-1″), 4.8 (1H, t, OH-14), 4.6 (2H, d, H-14), 4.2 (1H, q, H-5″), 3.8 (1H, m, H-5′), 3.6 (1H, s, H-3′), 3.5 (2H, d, H-4′, H-4″), 3.0 (2H, m, H-10), 2.2 (2H, m, H-8), 1.8–1.4 (6H, m, H-2′, H-2″, H-3′), 1.3–1.2 (6H, m, H-6′, H-6″). ESMS *m*/*z* calcd for $C_{32}H_{37}NO_{12}$ (M + H) + 628.6, found 628.7.

7-[4-*O*-(2,3,6-trideoxy-3-amino-α-L-*arabino*-hexopyranosyl)-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]-4-demethoxyadriamicinone (4). From the protected glycoside **49** following the general procedure E the glycoside **4** was obtained in 65% yield. ^{1}H NMR (DMSO) δ 8.4 (2H, m, H-1, H-4), 7.8 (2H, m, H-2, H-3), 5.9 (1H, d, OH-4″), 5.6 (1H, s, OH-9), 5.3 (1H, s, H-1′), 5.0 (1H, s, H-7), 5.0 (1H, s, H-1″), 4.9 (1H, s, H-1″), 4.8 (1H, t, OH-14), 4.5 (2H, d, H-14), 4.1 (1H, q, H-5′), 3.7 (1H, m, H-5″), 3.5 (1H, s, H-4′), 3.2 (1H, m, H-3″), 3.1–3.0 (2H, m, H-10), 2.2–2.0 (3H, m, H-2′ax, H-8), 1.8–1.5 (5H, m, H-2′eq, H-2″, H-3′), 1.3–1.2 (6H, m, H-6′, H-6″). ESMS *m*/*z* calcd for $C_{32}H_{37}NO_{12}$ (M + H) + 628.2, found 628.1.

Cytotoxicity assays

All cell lines were maintained in RPMI 1640 (Gibco/BRL, Gaithersburg, MD) supplemented with 10% fetal calf serum, 2 mM glutamine, 100 units penicillin and 100 μg streptomycin at 37 °C in a 5% CO_2, 95% air humidified incubator. After 24 h of incubation in the medium, cells were exposed to drugs. After 24 h, cells were washed with saline and incubated in drug-free medium for about three doubling times (72 h). Cellular viability was then measured by Sulforhodamine B (SRB) assay.[35] The IC_{50} (the concentration achieving 50% cellular mortality compared to untreated control) was evaluated by a curve in which the surviving percentage of cells was reported as a function of the drug concentration. The numbers in Table 1 are the means ± SE of three independent determinations.

Topoisomerase II mediated DNA cleavage

SV 40 DNA fragments were 5′ ^{32}P-labelled with T4 kinase and γ-^{32}P ATP. DNA cleavage reactions were performed at 37 °C for 20 min in 10 mM TRIS–HCl pH 6, 10 mM $MgCl_2$, 100 mM KCl, 1 mM ATP with or without drugs. Cleavage reactions were stopped with 1% SDS and 0.1 mg/ mL proteinase K and incubated at 42 °C for 45 min and then analyzed on 1% agarose gel. Autoradiograms of dried gels were carried out using Amersham Hyperfilms.

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