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Synthesis and Cytotoxic Activity of a New Potent Daunomycinone Derivative

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Abstract—The preparation and cytotoxic activity of 4′-azido-3′-bromo-3′-deamino-4′-deoxydaunorubicin is described. The new compound was found to be less active in vitro than adriamycin against L1210 and the sensitive cell lines KB-3-1 and MES-SA, but retained interesting cytotoxicity against the adriamycin resistant subline KB-A1 and the multidrug resistant MES-SA/Dx5 subline.

The anthracycline glycosides, especially doxorubicin (adriamycin) and daunorubicin are potent chemotherapeutic agents, with clinical utility against a wide range of human malignancies.[1] However, their long-term effectiveness is often limited by a dose-related cumulative cardiotoxicity and the development of acquired drug resistance, mediated by overexpression of the ATP-dependent efflux proteins P-glycoprotein and multidrug resistance protein (MRP) in chemosensitive tumors of the MDR phenotype.[2,3] These two main problems have received much attention during the past two decades and consequently they have stimulated a massive research effort, directed toward understanding of their cytotoxic mechanism of action and the determination of the factors which favor the appearance of anthracycline-induced cardiotoxicity and cancer cell resistance. Despite this effort, the mechanism of action of anthracyclines, exemplified by doxorubicin, has not yet fully clarified and it is a subject of controversy, but it is widely admitted that it is multimodal in nature.[4] It is believed that the information provided by structure–activity studies would be useful for the design of new derivatives, endowed with an improved tolerance profile and the absence of cardiotoxicity, whereas retaining activity against MDR cells.

The modification of the sugar part of anthracyclines has been extensively used as an effective approach to overcome MDR[4–6] and substituents that increase the lipophilicity of this moiety could provide useful lead compounds.[7,8] Analogues having the amino group at C-3′ replaced with a halogen (Cl, Br, I) have already been found to be active against resistant tumor cells.[9,10] We have previously reported on the synthesis and biological evaluation of a daunorubicin analogue possessing a C-3′ chlorine atom instead of the 3′-amino group and an amino functionality at C-4′ in the place of the 4′-hydroxyl.[11] These alterations are advantageous concerning the activity against adriamycin-resistant cell lines.

Having in mind the above-mentioned considerations, we have synthesized the 4′-azido-3′-bromo-3′-deamino-4′-deoxydaunorubicin (**8**), using the 3-bromosugar **2** as starting material (Scheme 1), which has been prepared according to known procedures, starting from 3,4-di-*O*-acetyl-L-rhamnal (**1**).[12] Treatment of the anomeric mixture of **2** with monmorillonite in anhydrous benzene and methanol afforded both anomers of methyl-4-*O*-acetyl-3-bromo-2,3,6-trideoxy-L-*arabino*-hexopyranoside (**3**). Transesterification with sodium methoxide in methanol provided a 6:4 α/β anomeric mixture of the corresponding hexopyranosides **4**, which were efficiently separated by flash chromatography. The α-anomer was then treated with trifluoromethanesulfonic anhydride in anhydrous dichloromethane in the presence of pyridine to give the corresponding triflate. This compound was not isolated, but was subjected to reaction with sodium azide in anhydrous dimethylformamide to afford the azido analogue **5** in 65% yield,[13] together with 15% of a

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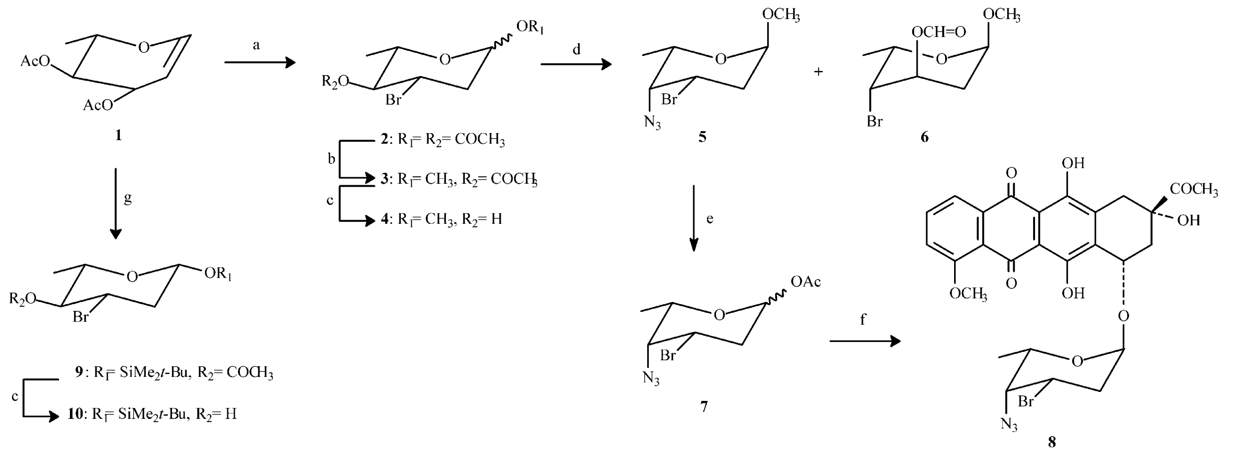
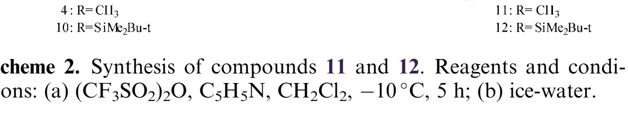


Scheme 1. Synthesis of compound **8**. Reagents and conditions: (a) (1) HBr/AcOH, rt, 2.5 h; (2) Ac_2O, C_5H_5N, rt, 24 h; (b) monmorillonite, MeOH, benzene, reflux, 6 h; (c) MeONa/MeOH, 0 °C, 14 h; (d) (1) $(CF_3SO_2)_2O$, C_5H_5N, CH_2Cl_2, −10 °C, 10 h; (2) NaN_3, DMF, 60 °C, 2 h; (e) AcOH/Ac_2O/H_2SO_4, rt, 2 h; (f) (1) HBr/AcOH, 0 °C, 10 min; (2) daunomycinone, $HgBr_2$, HgO, CH_2Cl_2, rt, 2 h; (g) (1) HBr/AcOH, rt, 2.5 h; (2) TBDMSCl, imidazole, CH_2Cl_2, rt, 40 h.

more polar by-product, which was unambiguously identified as **6** on the basis of 1-D and 2-D NMR data.[14] We hypothesized that the formation of this compound proceeds through a neighbouring-group mechanism, involving the nucleophilic attack of the carbonyl oxygen of DMF at C-3 of a bromonium ion intermediate, followed by hydrolysis of the iminium cation $ROC(H)={}^{+}N(CH_3)_2$. Analogous observations, concerning the reaction of carbohydrates with methanesulfonyl chloride or triflic anhydride in DMF have also been reported previously.[15,16]

Compound **5** was easily converted by acetolysis to a mixture of both anomers of the 1-*O*-acetyl derivative **7**. This mixture was treated with dry hydrogen bromide in benzene to provide the corresponding 1-bromide, which was used immediately and in 2-fold excess for coupling with daunomycinone. Coupling was carried out in dichloromethane solution under modified Koenings-Knorr conditions (yellow mercury II oxide and mercury II bromide with 4Å molecular sieves). The reaction after flash chromatography gave the α-anomer **8** in a rather good yield (70%).[17]

On the other hand, similar treatment of the β-anomer of **4** with trifluoromethanesulfonic anhydride in anhydrous dichloromethane in the presence of pyridine afforded exclusively the 4-bromo analogue **11** (Scheme 2)[18] and no trace of the corresponding triflate was detected. In our effort to investigate this unexpected reaction we have prepared the TBDMS analogue **10** as well,[19] starting from **1**, through the corresponding 1-TBDMS ester **9** (Scheme 1). Treatment of **10** with trifluoromethanesulfonic anhydride afforded again the corresponding 4-bromo analogue **12**[20] (Scheme 2). Based on these results we can assume that the formation of compounds **11** and **12** most likely proceed through a bromonium ion intermediate, which is probably easily accessible to nucleophilic attack in the case of the β-anomer.



Scheme 2. Synthesis of compounds **11** and **12**. Reagents and conditions: (a) $(CF_3SO_2)_2O$, C_5H_5N, CH_2Cl_2, −10 °C, 5 h; (b) ice-water.

The in vitro cytotoxic activity of compound **8** was evaluated using the murine leukemia L1210 cell line obtained from the American Type Culture Collection (Rockville Pike, MD, USA), the human epidermoid carcinoma cell line KB-3-1 and its 340-fold resistant to adriamycin (ADR) subline KB-A1, kindly provided by Dr. M. Gottesman (Bethesda, MD, USA), the human uterine sarcoma cell line MES-SA and its multidrug-resistant subline MES-SA/Dx5, obtained from the European Collection of Cell Cultures (Salisbury, ECACC). The cytotoxicity was measured by a modification of the Microculture Tetrazolium Assay, as previously described.[21,22] Results are expressed as IC_{50}, which is defined as the drug concentration inhibiting the proliferation by 50% with respect to untreated cells. The resistance of the MDR cell lines was expressed as the resistance factor (RF), which is the ratio between the IC_{50} on the resistant cells and the IC_{50} on the corresponding sensitive cells. The preliminary in vitro biological results are reported in Table 1.

Table 1. Inhibition of proliferation of compound **8**

Cell line	IC_{50} (μM)[a] and resistance factor[b]		
	8	ADR	DNR
L1210	0.50 (±0.09)	0.025 (±0.007)	0.04 (±0.008)
KB-3-1	0.80 (±0.04)	0.021 (±0.008)	0.007 (±0.001)
KB-A1	0.90 (±0.05)	7.01 (±0.5)	2.07 (±0.09)
MES-SA	7.00 (±0.3)	0.25 (±0.05)	0.07 (±0.008)
MES-SA/Dx5	2.00 (±0.2)	12.00 (±0.8)	3.00 (±0.12)
RF (KB)	1.1	333.8	295.7
RF (MES)	0.28	48.0	42.8

[a] Values are means of three experiments, standard deviation is given in parentheses.

[b] IC_{50} resistant cells/IC_{50} sensitive cells.

Compound **8** was less potent than ADR and DNR when tested on the sensitive L-1210 cells. Similarly, this analogue exhibited a decreased cytotoxic activity in respect to ADR or DNR against KB-3-1 and MES-SA cell lines, but when tested on KB-A1 and MES-SA/Dx5 cell lines, which are resistant against ADR or DNR, it showed a clear advantage (R_f values 1.1 and 0.28, respectively). Additionally, compound **8** was found to be 2–3-fold more active than the 3′-chloro analogue, which was previously reported.[11] These data suggest that the lipophilicity and the volume of the sugar substituents may significantly affect the cytotoxicity. Consequently, further work is needed to define the structure- activity relationships of the compounds in this series.

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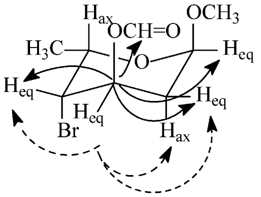
13. Data for compound **5**. Syrup, yield: 65%, $[\alpha]_D$−68° (*c* 1.0, CDCl$_3$). ^{1}H NMR (CDCl$_3$) δ ^{1}H NMR (CDCl$_3$) δ 4.65 (1H, d, J=3.2 Hz, H-1), 4.59 (1H, ddd, J=12.4/4.8/2.2 Hz, H-3), 4.02 (1H, qd, J=6.5/1.4 Hz, H-5), 3.64 (1H, brs, H-4), 3.28 (3H, s, CH$_3$O-1), 2.34 (1H, ddd, J=12.4/3.2 Hz, H-2ax), 2.19 (1H, dd, J=12.4/4.8 Hz, H-2eq), 1.28 (3H, d, J=6.5 Hz, H-6). ^{13}C NMR (CDCl$_3$) δ 100.4 (C-1), 69.2 (C-4), 68.6 (C-5), 56.9 (CH$_3$O-1), 49.6 (C-3), 37.9 (C-2), 18.9 (C-6). MS (CI-NH$_3$): *m*/*z* 267 ($[M+NH_4^+]^+$) 269 ($[M+2+NH_4^+]^+$).
14. Data for compound **6**. Syrup, yield: 15%, $[\alpha]_D$ −111° (*c* 0.3 CDCl$_3$). ^{1}H NMR (CDCl$_3$) δ 8.06 (1H, s, OCH=O), 5.31 (1H, ~brs, H-3), 4.76 (1H, d, J=3.1 Hz, H-1), 4.16 (1H, qd, J=6.4/1.6 Hz, H-5), 3.97 (1H, brs, H-4), 3.41 (3H, s, CH$_3$O-1), 2.47 (1H, brd, J=12.8 Hz, H-2ax), 1.87 (1H, d, J=12.8 Hz, H-2eq), 1.22 (3H, d, J=6.4 Hz, H-6). ^{13}C NMR (CDCl$_3$) δ 159.5 (OCH=O), 98.1 (C-1), 70.8 (C-3), 60.5 (C-5), 55.7 (CH$_3$O-1), 52.0 (C-4), 27.9 (C-2), 20.2 (C-6). MS (CI–NH$_3$): *m*/*z* 270 ($[M+NH_4^+]^+$) 272 ($[M+2+NH_4^+]^+$).

HMBC and COSY (dotted arrows) correlations at C-3 of **6**.



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17. Data for compound **8**. Syrup, yield: 70%, ^{1}H NMR (CDCl$_3$) δ ^{1}H NMR (CDCl$_3$) δ 7.97 (1H, d, J=8.1 Hz, H-1), 7.75 (1H, t, J=8.1 Hz, H-2), 7.35 (1H, d, J=8.1 Hz, H-3), 5.38 (1H, d, J=2.7 Hz, H-1′), 5.18 (1H, brs, H-7), 4.39 (1H, ddd, J=13.2/4.8/2.2 Hz, H-3′), 4.28 (1H, s, OH-9), 4.23 (1H, qd, J=6.3/1.0 Hz, H-5′), 3.68 (1H, brs, H-4′), 3.16 (1H, dd, J=18.6/1.4 Hz, H-10eq), 2.83 (1H, d, J=18.6 Hz, H-10ax), 2.38 (3H, s, CH$_3$CO-9), 2.40–2.00 (4H, m, H-2′ax/H-2′eq/H-8ax/H-8eq), 1.33 (3H, d, J=6.3 Hz, H-6). ^{13}C NMR (CDCl$_3$) δ 211.3 (C-13), 187.1 (C-5), 186.8 (C-12), 161.1 (C-4), 156.3 (C-6), 155.7 (C-11), 135.8 (C-2), 119.8 (C-1), 118.5 (C-3), 101.2 (C-1′), 70.1 (C-4′), 67.7 (C-7), 66.6 (C-5′), 56.7 (CH$_3$O-4), 46.6 (C-3′), 35.1 (C-2′), 35.0 (C-8), 33.3 (C-10), 24.6 (C-14), 18.3 (C-6′). ES–MS: *m*/*z* 616 ($[M+H]^+$, 616 $[M+2+H]^+$.
18. Data for compound **11**. Syrup, yield: 76%, $[\alpha]_D$ −23° (*c* 0.9, CDCl$_3$). ^{1}H NMR (CDCl$_3$) δ 4.71 (1H, dd, J=9.8/2.1 Hz, H-1), 4.37 (1H, ~q, J=3.1/2.5/2.1 Hz, H-3), 4.02 (1H, dq, J=6.2/2.1 Hz, H-5), 3.87 (1C, m, H-4), 3.48 (3H, s, CH$_3$O-1), 2.29 (1H, brs, OH-3), 2.19 (1H, ddd, J=12.9/9.8/3.1 Hz, H-2ax), 1.78 (1H, ddd, J=12.9/2.5/2.1 Hz, H-2eq), 1.30 (3H, d, J=6.2 Hz, H-6). ^{13}C NMR (CDCl$_3$) δ: 99.8 (C-1), 70.6 (C-4), 67.6 (C-5), 57.0 (C-3), 56.7 (CH$_3$O-1), 33.8 (C-2), 20.2 (C-6).
19. Data for compound **10**. Syrup, yield: 72%, $[\alpha]_D$ −16° (*c* 0.4, CDCl$_3$). ^{1}H NMR (CDCl$_3$) δ 4.71 (1H, dd, J=10.1/3.0 Hz, H-1), 3.94 (1H, ddd, J=13.1/10.2/5.2 Hz, H-3), 3.32 (2H, m, Ç-4/H-5), 2.43 (1H, ddd, J=13.1/5.2/3.0 Hz, H-2eq), 2.11 (1H, ddd, J=13.1/10.1 Hz, H-2ax), 1.32 (3H, d, J=6.3 Hz, H-6), 0.86 (9H, s, (CH$_3$)$_3$C), 0.08 (3H, s, (CH$_3$)$_2$Si), 0.07 (3H, s, (CH$_3$)$_2$Si). ^{13}C NMR (CDCl$_3$) δ 96.2 (C-1), 73.7 (C-4), 70.8 (C-5), 48.9 (C-3), 43.2 (C-2), 26.6 ((CH$_3$)$_3$C), 25.9 ((CH$_3$)$_3$C), 18.1 ((CH$_3$)$_2$Si), 17.8 (C-6).
20. Data for compound **12**. Syrup, yield: 68%, $[\alpha]_D$ −12° (*c* 0.3, CDCl$_3$).^{1}H NMR (CDCl$_3$) δ 5.10 (1H, dd, J=10.1/2.0 Hz, H-1), 4.37 (1H, ~q, J=3.1/2.5/2.1 Hz, H-3), 4.01 (1H, dq, J=6.2/2.1 Hz, H-5), 3.82 (1C, m, H-4), 2.24 (1H, brs, OH-3), 2.15 (1H, ddd, J=13.2/10.1/3.1 Hz, H-2ax), 1.73 (1H, ddd, J=13.2/2.5/2.0 Hz, H-2eq), 1.26 (3H, d, J=6.2 Hz, H-6) 0.88 (9H, s, (CH$_3$)$_3$C), 0.11 (3H, s, (CH$_3$)$_2$Si), 0.09 (3H, s, (CH$_3$)$_2$Si). ^{13}C NMR (CDCl$_3$) δ 93.3 (C-1), 71.3 (C-3), 67.4 (C-5), 55.6 (C-4), 35.9 (C-2), 27.0 ((CH$_3$)$_3$C), 25.8 ((CH$_3$)$_3$C), 20.4 ((CH$_3$)$_2$Si), 18.2 (C-6). MS (CI–NH$_3$): *m*/*z* 342 ($[M+NH_4^+]^+$) 344 ($[M+2+NH_4^+]^+$).
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