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SYNTHESIS OF 4'-EPI-IODO-4'-DEOXY-DAUNORUBICIN, A POTENTIAL CANCER RADIOTHERAPEUTIC AGENT

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Abstract: We have prepared 4'-*epi*-iodo-4'-deoxy-daunorubicin (IDDNR)(**1**), a doxorubicin analog, via a 5-step synthesis involving a protected daunorubicin triflate derivative (**4**). This triflate derivative will allow the facile and regiospecific nucleophilic preparation of I-125 or Br-80m labelled analogs of IDDNR. Auger electron-emitting I-125- or Br-80m-labelled analogs of IDDNR may have potential as cancer radiotherapeutic agents.

Doxorubicin (DOX) is an anthracycline antibiotic that is one of the most effective and wide spectrum antineoplastic agents currently available.[1] The use of this compound has, however, been limited by incidence of cumulative dose-dependent cardiomyopathy and loss of efficacy due to multidrug resistance.[1] Progress toward improving DOX therapy has been made along the lines of developing new analogs (such as daunorubicin and epirubicin) developing new drug delivery systems and studies on mechanisms of resistance and cardiac toxicity to block these unwanted effects.[2] One approach that may improve the therapeutic index for DOX is to couple Auger electron-emitting radioisotopes to this compound. Auger electron-emitting radioisotopes, such as I-125 and Br-80m, are known to be highly cytotoxic when localized in cell nuclei due to highly localized energy deposition by low energy Auger and Coster-Kronig electrons.[3,4] Recently, 5-iodo-2'-deoxyuridine (IUdR), a thymidine analog which is incorporated into DNA, was tested in humans with liver metastases from colorectal cancers and found to be effectively taken up by hepatic cells.[5] This finding encourages further efforts to establish dosing protocols using this approach in cancer therapy.[5]

The mechanism involved in the antibiotic action of DOX is related to its ability to intercalate between adjacent DNA base pairs causing topoisomerase II inhibition, which leads to the production of hydroxyl free radicals that have both antitumour effects and toxicity toward healthy tissues.[1] The intercalation model proposed for DOX-DNA interaction envisions two adjacent DNA base pairs becoming 6.8 Å apart in order to accommodate the planar intercalating DOX molecule.[6] Since irreparable strand breaks form the basis for cytotoxicity, this tight geometry between DOX and DNA would most likely insure that the decay of I-125 or Br-80m bound to DOX would cause DNA strand breaks since 70% of strand breaks occur within 15 Å of I-125 decay[7] while the average range of Auger electrons emitted upon Br-80m decay is 7 Å.[8] Furthermore since very small amounts of Auger electron emitting radioisotopes need to be localized in cell nuclei to be cytotoxic, in the order of 10^{-15} M in the case of Br-80m[4], it may be possible that the enhanced cytotoxicity of Auger electron-emitting DOX analogs will allow significant dose reduction minimizing unwanted pathologies. In this approach, the anthracycline molecule serves as the carrier that transports the radioisotope to the sensitive DNA in actively cycling cells. Since Auger electron-emitting radioisotopes have very limited cytoxicity when localized outside

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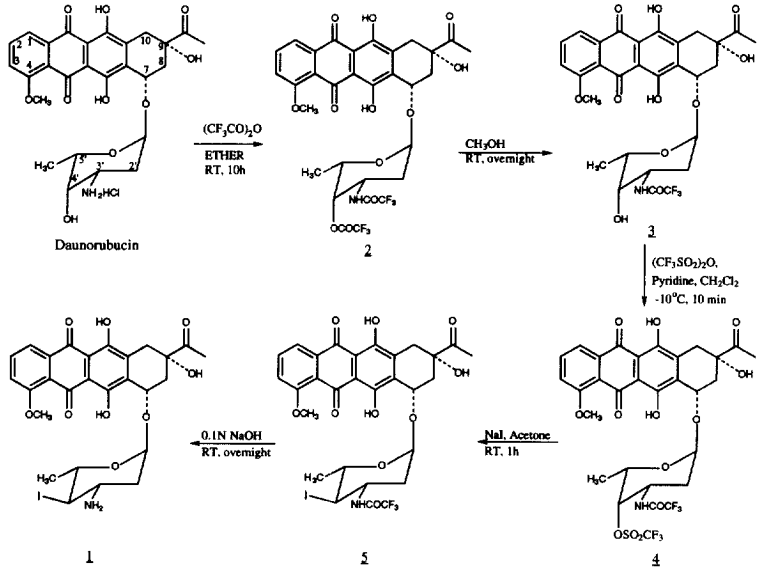
the cell nucleus[4], less actively dividing normal cells will essentially be spared.

An iodinated DOX analog, 4'-iodo-4'-deoxy-doxorubicin, was recently synthesized, evaluated and found to have better therapeutic index than DOX itself[10] while the *epi*-analog of DOX, epirubicin, was found to be less cardiotoxic than DOX itself.[11] Based on this structure-activity relationship, it is not unreasonable to expect that 4'-*epi*-iodo-4'-deoxy-daunorubucin (IDDNR) will maintain antibiotic activity but more important to our approach, retain its DNA intercalating property. Our goal in this research is to develop procedures for the routine regioselective synthesis of [I-125]- and [Br-80m]-labelled IDDNR analogs with the label at the 4'-*epi*-position. Here we report the synthesis of IDDNR (**1**) using a triflate derivative (**4**) as starting material (Scheme 1).



SCHEME 1

Methods and Materials: The HPLC analyses were carried out using a HPLC system consisting of a Gilson pump and UV detector (254 nm) and Rainin Dynamax CN (5 μ), 250 mm x 4.6 mm column. The mobile phase used was 0.05 M aqueous phosphate buffer (pH adjusted to 3.0) + acetonitrile (60:40). Rainin Dynamax software was used for HPLC data acquisition and processing. All the ^{1}H NMR measurements were made on either Brucker AM-400 WB or Brucker AM-500 instrument in $CDCl_3$ solvent with TMS as internal standard. Fast Atom Bombardment mass spectra were recorded using Katos Ltd. MS-50TC instrument. Daunomycin hydrochloride was obtained from Sigma Chemical Co (St. Louis, MO) while the other reagents were obtained fron Aldrich Chemical Co. (Milwaukee, WI).

Chemistry: 4'-*epi*-iodo-4'-deoxy-daunorubicin (IDDNR) (**1**) was prepared starting from commercial daunorubicin HCl (DNR) via a 5-step synthesis (Scheme 1). The first step in Scheme 1 is the high yield conversion (95%) of DNR to its *N*, 4'-*O*-bis-trifluoroacetyl-DNR (**2**) using excess trifluoracetic anhydride.

Selective O-hydrolysis of *N,O*-bis-trifluoroacetyl-DNR with methanol provided *N*-trifluoro-acetyl-DNR (**3**). Treatment of *N*-trifluoroacetyl-DNR with triflic anhydride in the presence of pyridine in dichloromethane gave the corresponding triflate (**4**) in 75% yield. Nucleophilic conversion of the triflate to (**1**) was achieved in ca. 50% yield by treatment with NaI in acetone followed by base hydrolysis of the protective *N*-trifluoroacetyl moeity. Analysis by ^{1}H NMR confirmed the intermediates and product to be the desired compounds.[12] Synthesis of 4'-*epi*-bromo-4'-deoxy-daunorubicin is currently underway using a similar scheme.

Discussion: Various approaches to deliver cytotoxic Auger electron-emitting radioisotopes to cell nuclei have included the use of several I-125-labelled compounds: acridine,[13] iododeoxyuridine,[14] iodoestradiol,[15] and more recently, rhodamine.[16] Br-80m (t1/2=4.4h) labelled estrogen receptor (ER) ligands, bromovinylestradiol and bromo-bis(4-hydroxyphenyl)phenylethylene, have also been proposed as radiotherapeutic agents since estrogen ligands when complexed with ER are tightly associated with nuclear DNA and chromatin.[9] The present report is the first to propose a common chemotherapeutic compound for the delivery of Auger electron-emitting radioisotopes in the treatment of cancer. I-125-labeled IDDNR can be easily prepared from commercially available I-125 and the triflate derivative (**4**) prepared here. Also Br-80m-labeled 4'-*epi*-bromo-4'-deoxy-daunorubicin (BDDNR) can be prepared from the same starting material and nuclear reactor or cyclotron-produced Br-80m.[17] Either compound can be tested to determine the feasibility of using this family of DNA-intercalating antibiotics labelled with Auger electron-emitting radioisotopes as cancer radiotherapeutic agents.

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12. **(1):** Yield 70%. ^{1}H NMR δ ($CDCl_3$) 1.55 (3H, d, $C_{5'}$-CH_3, J = 6.0 Hz), 1.95-2.38 (4H, m, $H_{2'a}$, $H_{2'e}$, H_{8A} & H_{8B}), 2.42 (s, $COCH_3$), 2.90 (1H, d, H_{10A}, $J_{H10A-H10B}$ = 19.4 Hz), 3.13(1H, d, H_{10B},$J_{H10B-H10A}$ = 17.9 Hz), 3.64 (2H, bs, NH_2), 3.92 (1H, dd, $H_{3'}$, $J_{H3'-H4'}$ = 11.1 Hz & $J_{H3'-H5'}$ = 11.1 Hz), 4.09 (3H, s, OCH_3), 4.20-4.32 (1H, m, $H_{4'}$), 4.34-4.45 (1H, m, $H_{5'}$), 5.27 (1H, bs, H_7), 5.61 (1H, bs, $H_{1'}$), 7.40 (1H, d, H_3, J_{H3-H2} = 7.5 Hz), 7.79 (1H, dd, H_2, J_{H2-H1} = 8.2 Hz & J_{H2-H3} = 8.2 Hz), 8.05 (1H, d, H_1, J_{H1-H2} = 7.4 Hz), 13.28 (1H, s, ArOH), and 13.97 (1H, s, ArOH).

(2): TLC (silica gel; $CHCl_3$-acetone, 6:1). Yield >95%. ^{1}H NMR δ ($CDCl_3$) 1.23 (3H, d, $C_{5'}$-CH_3, J = 6.5 Hz), 1.90-2.05 (2H, m, $H_{2'a}$ & $H_{2'e}$), 2.20 (1H, dd, H_{8A}, $J_{H8A-H8B}$ = 14.7 Hz & J_{H8A-H7} = 4.1 Hz), 2.31 (1H, bd, H_{8B}, $J_{H8B-H8A}$ = 14.0 Hz), 2.43 (3H, s, $COCH_3$), 2.97 (1H, d, H_{10A}, $J_{H10A-H10B}$ = 18.8 Hz), 3.26 (1H, bd, H_{10B}, $J_{H10B-H10A}$ = 18.9 Hz), 3.96-4.20 (1H, m, $H_{4'}$), 4.10 (3H, s, OCH_3), 4.32-4.50 (1H, m, $H_{5'}$), 5.30 (1H, bs, H_7), 5.41 (1H, bs, $H_{3'}$), 5.62 (1H, bs, $H_{1'}$), 5.99 (1H, d, NH, $J_{NH-H4'}$ = 7.1 Hz), 7.41 (1H, d, H_3, J_{H3-H2} = 8.6 Hz), 7.80 (1H, dd, H_2, J_{H2-H1} = 7.9 Hz & J_{H2-H3} = 7.9 Hz), 8.06 (1H, d, J_{H1-H2} = 7.6 Hz), 13.28 (1H, s, ArOH), and 13.97 (1H, s, ArOH).

(3): TLC (silica gel; $CHCl_3$-acetone, 4:1). ^{1}H NMR δ ($CDCl_3$) 1.60 (3H, d, $C_{5'}$-CH_3, J = 6.3 Hz), 1.82 (1H, ddd, $H_{2'a}$, $J_{H2'a-H1'}$ = 4.4, $J_{H2'a-H2'e}$ = 12.8 Hz & $J_{H2'a-H3'}$ = 12.8 Hz), 1.97 (1H, dd, $H_{2'e}$, $J_{H2'e-H2'a}$ = 13.3 Hz & $J_{H2'e-H3'}$ = 5.1 Hz), 2.16 (1H, dd, H_{8A}, $J_{H8A-H8B}$ = 14.5 & J_{H8A-H7} = 4.3 Hz), 2.32 (1H, bd, H_{8B}, $J_{H8B-H8A}$ = 14.0 Hz), 2.42 (3H, s, $COCH_3$), 2.97 (1H, d, H_{10A}, $J_{H10A-H10B}$ = 18.8 Hz), 3.28 (1H, bd, H_{10B}, $J_{H10B-H10A}$ = 18.8 Hz), 3.67 (1H, bs, $H_{3'}$), 4.08 (3H, s, OCH_3), 4.18-4.24 (1H, m, $H_{4'}$), 4.27 (1H, q, $H_{5'}$, J = 6.5 Hz), 5.29 (1H, bs, H_7), 5.52 (1H, d, $H_{1'}$, $J_{H1'-H2'a}$ = 4.0 Hz), 6.62 (1H, d, NH, $J_{NH-H4'}$ = 8.2 Hz), 7.40 (1H, d, H_3, J_{H3-H2} = 8.4 Hz), 7.79 (1H, dd, H_2, J_{H2-H1} = 8.4 Hz & J_{H2-H3} = 8.4 Hz), 8.06 (1H, d, H_1, J_{H1-H2} = 7.8 Hz), 13.29 (1H, s, ArOH), and 14.04 (1H, s, ArOH).

(4): TLC (silica gel; $CHCl_3$-acetone, 2:1). Yield 95%.

(5): TLC (silica gel, $CHCl_3$: acetone, 4:1). Yield >95%.^{1}H NMR δ ($CDCl_3$) 1.49 (3H, d, $C_{5'}$-CH_3, J = 5.1 Hz), 1.93 (1H, ddd, $H_{2'e}$, $J_{H2'a-H1'}$ = 3.7 Hz, $J_{H2'a-H2'e}$ = 12.8 Hz & $J_{H2'a-H3'}$ = 12.8 Hz), 2.06-2.14 (2H, m, $H_{2'a}$ & H_{8A}), 2.27 (1H, bd, H_{8B}, $J_{H8B-H8A}$ = 15.3 Hz), 2.38 (3H, s, $COCH_3$), 2.92 (1H, d, H_{10A}, $J_{H10A-H10B}$ = 18.4 Hz), 3.20 (1H, bd, H_{10B}, $J_{H10B-H10A}$ = 19.0 Hz), 3.85 (1H, dd, $H_{3'}$, $J_{H3'-H5'}$ = 10.8 Hz & $J_{H3'-H4'}$ = 10.8 Hz), 4.02 (3H, s, OCH_3), 4.08-4.18 (1H, m, $H_{3'}$), 4.28-4.36 (1H, m, $H_{5'}$), 5.22 (1H, bs, H_7), 5.52 (1H, d, $H_{1'}$, $J_{H1'-H2'a}$ = 2.8 Hz), 6.13 (1H, d, NH, $J_{NH-H3'}$ = 7.9 Hz), 7.33 (1H, d, H_3, J_{H3-H2} = 8.2 Hz), 7.73 (1H, dd, H_2, J_{H2-H1} = 7.9 Hz & J_{H2-H3} = 7.9 Hz), 7.99 (1H, d, H_1, J_{H1-H2} = 7.8 Hz), 13.31 (1H, s, ArOH), and 14.02 (1H, s, ArOH). MS: $(M + H)^+$ 734 m/e.

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