Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 6, pp. 547-550, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00070-4

SYNTHESIS OF (-)-6-(3'-AMINOPROPYLOXY)METHYL-4-DEMETHOXY-6,7-DIDEOXY-DAUNOMYCINONE, A NEW DNA INTERCALATOR RELATED TO ANTHRACYCLINES

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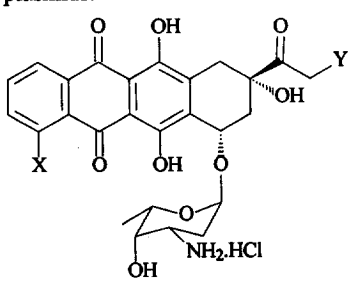
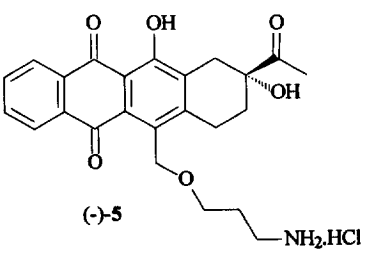
Abstract: *The hydrochloride of (-)-(8R)-8-acetyl-7,8,9,10-tetrahydro-6,8-dihydroxy-11-[(3'-aminopropyloxy)methyl]naphthacene-5,12-dione was derived from 1-(dimethoxymethyl)-2,3,5,6--tetramethylidene-7-oxabicyclo[2.2.2]heptane and shown to be a moderate intercalator of calf thymus DNA type XV.*

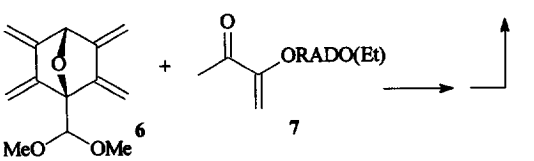
Adriamycin (**1**) and daunomycin (**2**) are the most valuable anticancer drugs in present clinical use.[1] Their effectiveness however, is restricted due to accute bone marrow toxicity and cardiotoxicity.[2] Since the cytotoxic mechanism by which these drugs act is still unclear,[3] the influence of structural alteration on activity remains an important issue.[4a] For instance, the 4-demethoxy derivatives **3** and **4** exhibit improved pharmaceutical profiles.[4b] We report here the synthesis of the anthracycline analogue (-)-**5** obtained in a highly enantioselective fashion. Compound (-)-**5** is the first member of a new class of anthracyclinones C-substituted at C(6) with a (3-aminopropyloxy)methyl group that may imitate the L-daunosaminyl unit in **1-4**.[5] Furthermore, the benzylic system at C(6) might compensate the lack of a benzylic nucleofugal group at C(7).[6] Preliminary assays have shown compound (-)-**5** to intercalate into calf thymus DNA and to inhibit topoisomerase I induced relaxation of circular plasmids.



1 X = OMe, Y = OH
2 X = OMe, Y = H
3 X = H, Y = OH
4 X = Y = H



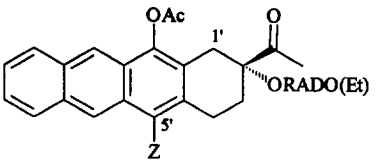
(-)-**5**



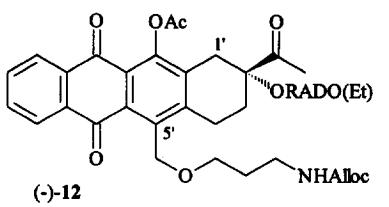
6 **7**

The pure (>95% d.e. 400 MHz ^{1}H-NMR) naphthacenecarbaldehyde derivative (-)-**8** was prepared *via* the BF_3·Et_2O-promoted Diels-Alder addition of tetraene **6** to 1-acetylvinyl (1'R,5'S,7'R)-3'-ethyl-2'-oxo-6',8'-

-dioxa-3'-azabicyclo[3.2.1]octane-7'-exo-carboxylate (**7**: 1-acetylvinyl-RADO(Et)[7]) following a procedure already described.[8] Reduction of (-)-**8** with $Na(CN)BH_3$ in chloroform-methanol acidified (pH=6) with AcOH (20 °C) gave the corresponding benzyl alcohol (-)-**9** (93%).[9] Its reaction with AcBr in chloroform (20 °C, 15 min.) afforded the bromide (-)-**10** (97%).[10] The treatment of (-)-**10** with the N-allyloxycarbamate of 3-amino-propanol and $AgOSO_2CF_3$ in anhydrous THF (4 Å molecular sieves, 20 °C, 5 min)[11] furnished the benzyl ether (-)-**11** (80%).[12] The oxidation of its anthracene moiety with 4 N aqueous Jones reagent (CrO_3/H_2SO_4) in acetone (0 °C , 30 min., then 20 °C, 1.5 h, work-up with isopropanol) led to the corresponding anthraquinone (-)-**12** (91%).[13]

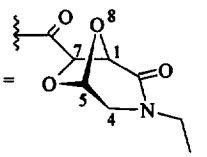


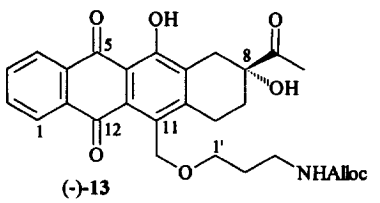
(-)-**8** : Z = CHO
(-)-**9** : Z = CH_2OH
(-)-**10**: Z = CH_2Br
(-)-**11**: Z = $CH_2O(CH_2)_3NHAlloc$



(-)-**12**

RADO(Et) =





(-)-**13**

⟶ (-)-**5**

Saponification of (-)-**12** with LiOH in aqueous THF (0 °C, 15 min)[14] gave (-)-**13**[15] which was contaminated by about 10 % of an unknown compound. Treatment of this crude product with $Pd(Ph_3P)_2(OAc)_2$ and Bu_3SnH in wet CH_2Cl_2[16] (20 °C, 20 min.) afforded the unprotected amine. After acidification with 0.1 M aqueous HCl (to pH=2.5-3.0) and purification by flash chromatography on silica gel (CH_2Cl_2:MeOH 4:1), the hydrochloride (-)-**5** was obtained pure in 45 % yield.[17] The structures of new compounds (-)-**5**, (-)-**9** - (-)-**13** were deduced from their spectral data, elemental analyses and mode of formation.

Binding affinity of (-)-**5** to calf thymus DNA Type XV (Sigma, purity checked by measuring the absorbance ratio at 260 nm and 280 nm (1.9); ε_{260}=12824 M^{-1} in base pair; dissolved in 10^{-5} molar TRIS-buffer, pH=7.4, 10^{-6} molar Na_2EDTA, 0.1 molar NaCl) was evaluated by absorbance titration[18] (Hewlett Packard 8450A diode array, 25 °C) and by fluorescence titration[19] (Perkin-Elmer LS-50B, 25 °C, 4 nm slit, λ_{ex}=406 nm, λ_{em}=561 nm). Aliquots of known concentration of (-)-**5** solution were added every 15 minutes to the DNA solution of known concentration. The baseline absorbance and fluorescence of DNA was substracted from all readings and adjustements were made to account for dilution (average of three readings). The data were analysed by Scatchard-plots[20] (see Figure). The dashed line shown in the Figure was calculated according to the neighbour exclusion model[21] and was obtained with n=7.0±0.1 as exclusion parameter in base pairs and with $K_i=(6.1\pm0.1)\times10^4$ M^{-1} as intrinsic binding constant. This suggests that (-)-**5** is a weaker intercalator than **1** ($K_i=(2\text{-}4)\times10^6$ M^{-1}) and **2** ($K_i=(4\text{-}6)\times10^6$ M^{-1})[22] but slightly better than daunomycinone ($K_i=(1\text{-}3)\times10^4$ M^{-1}).[22b]

Topoisomerase unwinding assay was also used to monitor the intercalation of (-)-**5** into DNA.[23] Reactions were carried out in 20 µl buffered solution containing 350 ng of pGEM plasmid and 5 units of topoisomerase I. Aliquots of (-)-**5** were added to realize solutions of 50, 100, 200 and 500 µM final

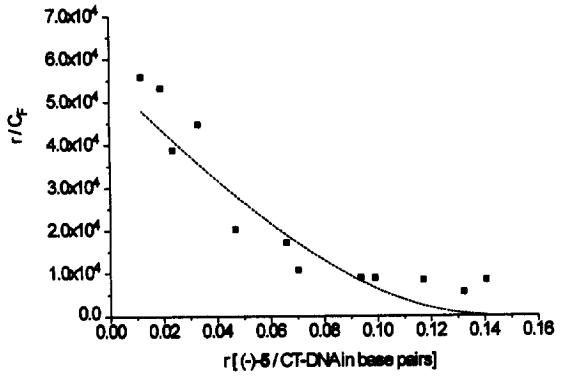


Figure : Scatchard-plot of equilibrium binding isotherm for (-)-**5** and calf thymus DNA interaction. Data are shown from fluorescence and absorbance titration, the dashed line was calculated for the neighbour exclusion model, assuming n = 7.0 ±0.1 and $K_i = (6.1 \pm 0.1)x10^4\ M^{-1}$.

concentration. Daunomycin was used as control. After 30 min incubation at 37 °C (without light), samples were extracted (phenol/chloroform) and subjected to electrophoresis (1% agarose, TRIS-borate, 10 V/cm). It was found that topoisomerase induced relaxation was inhibited at a concentration higher then 200 µM.

These preliminary results encourage us to prepare further derivatives of the new anthracycline analogue
(-)-**5**.

Acknowledgements: We thank *F. Hoffmann-La Roche & Co.*, AG (Basel), the *Fonds Herbette* (Lausanne) and the *Swiss National Science Foundation* for financial support. We are also grateful to Dr. Cécile Pasquier, Pierre-Yves Musard (University of Neuchâtel), Dr. Yegor Vassetzky (ISREC, Epalinges) and to Miss Isabelle Rochat for their advice and technical help.

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9. Data of (-)-**9**: colourless crystals, m. p. 194-196 °C (EtOAc); $[\alpha]^{25}_D$ = -45 (c=1.0, CH_3CN);IR (KBr)ν: 3500, 1755, 1710, 1670 cm^{-1};^{1}H-RMN (250 MHz, $CDCl_3$) δ_H: 8.89 (s, 1H); 8.33 (s, 1H); 8.03 (m, 2H); 7.48 (m, 2H); 5.70 (d, ^{3}J=2, 1H); 5.38, 5.24 (2d, ^{2}J=12, 2H); 4.60 (s, 1H); 3.65-3.05 (m, 8H); 3.02 (d, ^{2}J=12, 1H); 2.50-2.40 (m, 1H); 2.58 (s, 3H); 2.28 (s, 3H); 2.12-1.98 (m, 1H); 0.98 (t, ^{3}J=7, 3H).
10. Data of (-)-**10**: yellow crystals, m. p. 173-174 °C (EtOAc); $[\alpha]^{25}_D$ = -65 (c=1.0, $CHCl_3$); ^{1}H-RMN (250 MHz, $CDCl_3$) δ_H: 8.64 (s, 1H); 8.33 (s, 1H); 8.05 (m, 2H); 7.51 (m, 2H); 5.78 (d, ^{3}J=2, 1H); 5.15, 5.08 (2d, ^{2}J=12, 2H); 4.64, 4.67 (2s, 2H); 3.47 (dd, ^{3}J=2, ^{2}J=12, 1H); 3.40-3.10 (m, 6H); 3.10 (d,^{2}J=12, 1H); 2.65-2.55 (m, 1H); 2.58 (s, 3H); 2.27 (s, 3H); 2.12-1.98 (m, 1H); 1.04 (t, ^{3}J=7, 3H).
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12. Data of (-)-**11**: colourless crystals, m. p. 175 °C (Et_2O); $[\alpha]^{25}_D$ = -23 (c=1.0, $CHCl_3$); ^{1}H-RMN (250MHz, $CDCl_3$) δ_H: 8.70 (s, 1H); 8.29 (s, 1H); 7.98 (m, 2H); 7.48 (m, 2H); 5.80 (d, ^{3}J=2, 1H); 5.82 (m, 2H); 5.30-5.02 (m, 2H); 5.11 (s, 2H); 4.70, 4.62 (2s, 2H); 4.45 (dm, 2H, ^{3}J=5.5); 3.73 (t, ^{3}J=6.0, 2H); 3.40 (dd, ^{3}J=2, ^{2}J=12, 1H); 3.45-3.2 (m, 8H); 3.10 (d, ^{2}J=12, 1H); 2.59 (s, 3H); 2.55-2.50 (m, 1H); 2.27 (s, 3H); 2.22-2.15 (m, 1H); 1.85 (m, 2H); 1.04 (t, ^{3}J=7, 3H).
13. Data of (-)-**12**: yellow solid; $[\alpha]^{25}_D$ = -13 (c=1.0, $CHCl_3$); IR (KBr) ν: 3450, 2900, 1750, 1720, 1670, 1320 cm^{-1}; ^{1}H-RMN (250 MHz, $CDCl_3$) δ_H: 8.13 (m, 2H); 7.73 (m, 2H); 5.90 (m, 1H); 5.82 (d, ^{3}J=2, 1H); 5.20 (m, 2H); 4.98 (br. s, 2H); 4.75, 4.70 (2s, 2H); 4.51 (d, ^{3}J=5.5, 2H); 3.75 (t, ^{3}J=5.5, 2H); 3.42 (dd, ^{3}J=2, ^{2}J=12, 1H); 3.40-3.00 (m, 8H); 3.15 (d, ^{2}J=12, 1H); 2.55 (s, 3H); 2.26 (s, 3H); 2.15-2.00 (m, 2H); 1.85 (m, 2H), 1.10 (t, ^{3}J=7, 3H).
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15. Data of (-)-**13**: yellow solid; $[\alpha]^{25}_D$ = -7 (c=0.5, $CHCl_3$); ^{1}H-RMN (250 MHz, $CDCl_3$) δ_H: 13.81 (s, 1H); 8.24 (m, 2H); 7.80 (m, 2H); 5.83 (m, 1H); 5.49 (br. t, 1H); 5.28-5.10 (m, 2H); 5.10, 4.83 (2d,^{2}J=10, 2H); 4.48 (dm, ^{3}J=5.5, 2H); 3.92 (s, 1H); 3.75 (m, 2H); 3.40-3.11 (m, 4H); 3.11, 2.98 (2d, ^{2}J=18, 2H); 2.40 (s, 3H); 2.12-1.90 (m, 2H); 1.84 (m, 2H).
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17. Data of (-)-**5**: yellow solid, m. p. of the N-p-nitrobenzoate : 86-88 °C; $[\alpha]^{25}_D$ = -52 (c=0.18, EtOH); UV (MeOH) $\lambda_{max}(\varepsilon)$: 265 (19600), 406 (3740); ^{1}H-RMN (250 MHz, CD_3OD) δ_H: 8.23 (m, 2H); 7.89 (m, 2H); 4.98 (2d, ^{2}J=10, 2H); 3.91 (t, ^{2}J=5, 2H); 3.30-2.90 (m, 6H); 2.41 (s, 3H); 2.15-1.85 (m, 4H); ^{13}C-RMN (100 MHz, CD_3OD) δ_C: 209.2, 190.06, 187.1 (3s); 162.5 (s); 148.7 (s); 136.5, 133.7, 132.1, 131.5, 130.8, 127.8 (6s); 136.0, 135.1, 128.2, 127.8 (4d, J=163); 76.9 (s); 69.9 (t, J=140); 67.0 (t, J=145); 40.0 (t, J=139); 33.2, 28.3 (2t, J=125); 30.6 (t, J=132); 24.7 (q, J=129); 24.2 (t, J=131).
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(*Received in Belgium* 21 *October* 1994)