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DESIGN AND SYNTHESIS OF DNA INTERCALATING CROWN ETHER MOLECULES

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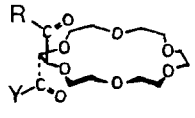
Abstract: We describe the synthesis of a series of novel mono- and bis-intercalant crown ethers **1** and **2**, having either a methidium or a psoralen function covalently attached to the macrocyclic ring with well-defined stereo chemistry. Their binding efficiency to DNA duplex has been evaluated.

The molecular recognition of DNA by small molecules such as drugs and its application in the field of chemotherapy[1] is one of many particularly important subjects of bioorganic chemistry[2]. In this respect, Dervan and collaborators[3] have recently prepared a bis-methidium spermine (an ethidium bromide analogue) as well as some sequence specific DNA cleaving molecules. With a similar approach, the group of Cantor[4], has made some interesting mono- and bis-psoralen intercalating molecules that can be photocrosslinked to DNA duplex. In this paper we would like to present the synthesis of a series of analogous mono- and bis-intercalating molecules **1** and **2** that are covalently attached to a crown ether.

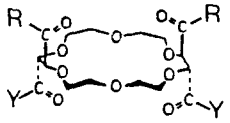
This approach offers the advantage that being now on a semi-rigid crown ether backbone with well-disposed orientation, the intercalating functions would be expected to show more specific and stronger binding properties with DNA receptor molecules. Such an assumption is indeed justified because it has recently been shown by NMR study with bis-acridine derivatives[5] that increasing the rigidity of the linking chain between the two intercalating functions enhances both their stability and the binding efficiency to a model DNA duplex.

The crown ether intercalants **1** and **2** were synthesized from condensation of either the 2-aminoethyl-*p*-carboxamide-methidium chloride[3] or 4'-aminomethyl-4,5'8-trimethylpsoralen (AMT)[6] with the mono- or the di-anhydride of optically active 18-crown-6 which incorporate one or two *R*,*R*-tartaric acid units[7,8].

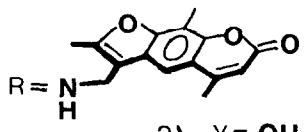
Mono-psoralen crown ether **1a** was prepared in 92% yield by the reaction of AMT with the 18-crown-6 mono anhydride[9] in CH_2Cl_2 at room temperature for 1 hr [white cryst., mp. 155-57°C; IR ($CHCl_3$) cm^{-1} 3250, 2995, 2900, 1730, 1700, 1680, 1600, 1520, 1110; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.93 (t, 1H, J = 4.2 Hz), 7.80 (s, 1H), 6.8 (vbr s, 1H, exchan-



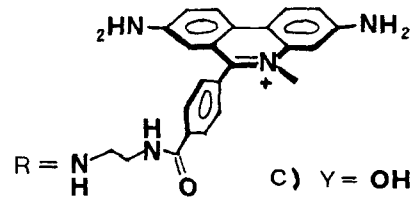
1



2



a) Y = OH
b) Y = OCH_3



c) Y = OH

geable with D_2O), 6.22 (d, 1H, J = 1.5 Hz), 4.62 (dd, 1H, J_1 = 14.7 Hz, J_2 = 6.7 Hz), 4.48 (dd, 1H, J_1 = 14.7 Hz, J_2 = 5.5 Hz), 4.35 (d, 1H, J = 2.3 Hz), 4.25 (d, 1H, J = 2.3 Hz), 3.75-3.45 (m, 14H), 3.3-3.2 (m, 4H), 2.95 (t, 2H), 2.56 (s, 3H), 2.55 (s, 3H), 2.52 (d, 3H, J = 1.1 Hz); MS m/e 592 ($M+H^+$), 591 (M^+)]. Compound **1a** on methylation with a large excess of ethereal CH_2N_2 furnished the corresponding methyl ester **1b** as a white solid, [mp. 65°-67°C, MS m/e 606 ($M+H^+$)]. This esterification never went to completion despite repeated treatment; the reason for this is not quite clear.

The reaction of 18-crown-6 di-anhydride[10] with 2 equiv. of AMT in the presence of 3 equiv. of triethylamine in $CH_2C\ell_2$ furnished the syn-bis-psoralen crown ether derivative **2a** in 72% yield as a white solid [m.p. 208-15°C (dec.); IR ($CHC\ell_3$/MeOH 10:1) cm^{-1} 3600-3150, 2870, 2810, 1730, 1700, 1670, 1620, 1598, 1110; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.74 (m, 1H), 8.15 (s, 1H), 8.04 (br s, 2H), 7.95 (m, 1H), 7.84 (s, 1H), 6.25 (s, 2H), 4.72 (dd, J_1 = 12 Hz, J_2 = 9 Hz, 1H), 4.61 (dd, J_1 = 6 Hz, J_2 = 12 Hz, 1H), 4.45 (dd, J_1 = 6 Hz, J_2 = 11 Hz, 1H), 4.25 (dd, J_1 = 6 Hz, J_2 = 12 Hz, 1H), 4.22 (s, 1H), 4.11 (s, 1H), 3.98 (s, 1H), 3.88 (s, 1H), 4.3-2.85 (br m, 16H), 2.50-2.70 (finely splitted s, 18H); MS m/e 460 ($M+2H^+$)/2]. Compound **2a** on repeated treatment with excess CH_2N_2 yielded the corresponding dimethyl ester **2b** in 40% yield as a white crystalline solid, [mp. 145°-50°C, MS m/e 828 ($M - CH_3COOH - COOCH_3$)].

The mono-methidium derivative **1c** was obtained in 63% yield similarly from the 2-aminoethyl-p-carboxamide-methidium chloride[11] and 18-crown-6 mono-anhydride in DMSO for 2 hrs at room temperature [purple solid, m.p. 298-305°C (dec.); IR (KBr) cm^{-1} 3430, 3240, 3040, 2960, 2860, 1730, 1655, 1625, 1105; ^1H NMR (DMSO-d_6, 90 MHz) δ 9.14 (br m, 1H), 8.64 (d, J = 6.7 Hz, 2H), 8.16 (d, J = 7 Hz, 2H), 7.92 (br m, 1H), 7.81 (m, 2H), 7.57-7.30 (m, 4H), 6.5 (br s, 2H), 6.32 (s, 1H), 6.0 (vbr s, 2H), 3.98 (br s, 2H), 3.50-3.30 (m, 27H)]. The syn-dimethidium derivative **2c** was made similarly in 61%

yield [purple-red solid, mp. 360°C; IR (KBr) cm^{-1} 3380, 3230, 2940, 2880, 1725, 1655, 1625, 1545, 1115; ^1H NMR (DMSO-d_6, 90 MHz) δ 9.20-7.30 (m, 22H), 6.8 (br m, 1H), 6.50 (br m, 1H), 6.35 (br s, 2H), 4.2-3.7 (m, 28H), 3.20 (s, 6H), 3.40-3.0 (m, 8H)].

The assignment of the configuraiton of the syn and anti isomers of the above compounds were based on the polarity and mobility on t.l.c., spectral data and comparison with previous work.[10]

In the methidium series, preliminary bio-assays[12] to evaluate the selectivity to inhibit the restriction endonucleases aHa III and/or Bgl I on the pBR 327 plasmid were performed under standard conditions[3]. The results indicated that the binding behavior of the mono-crown ether **1c** was found to be comparable to carboxy-methidium (Dervan acid). However, there were little evidence that the syn-crown ether **2c** was able to compete for either of the restriction enzymes tested. Although examination of CPK models of **2c** and the DNA helix indicated that the methidium groups are capable of bis-intercalating, the molecule is probably too rigid and the intercalant functions still too close to the macrocyclic ring to properly bind to a super-coiled DNA plasmid.

The psoralen series **1a** and **2a** was also tested[12] for binding and photo-crosslinking to DNA using the previously described procedure[4]. The first results showed that the mono-compound **1a** is reactive but not the bis-syn- **2a**. One reason why the syn-compound does not intercalate into DNA is probably due to a favorable stacking between the psoralen rings. This phenomenon is in fact associated to a strong hypochromism observed in the UV spectrum and some characteristic resonance shifts in the NMR spectrum. Such hypothesis is very likely since the bis-anti-analog[13], where the two psoralen groups are now on either side of the crown ether ring, showed a good intercalating ability with DNA helices.

In summary, these observations point to the possibility of varying the length of the spacer arm between the macrocycle and the intercalant molecules in order to optimize their binding efficiency. Moreover, the pH and the nature of the solvent[14] should also have a profound effect on the mode of orientation of the macrocyclic ring and in turn on the binding properties of the intercalant residues it carries. Such variations merit further investigation and other studies are now under way on this basis.

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11. Made from acyl imidazolide ester of p-carboxy-methidium chloride (Dervan acid) and a large excess of ethylene diamine in DMSO for 1 hr at room temperature; purple solid, mp. 252-55°C, forms a 1:1 complex with DMSO, which is removed under vacuo at 100°C overnight [IR (KBr) cm^{-1} 3325, 3200, 3040, 2940, 1630, 1545, 1018, 998, 950; ^1H NMR (DMSO-d_6, 90 MHz) δ 9.42 (br s, 1H), 8.66 (d, J = 7.6 Hz, 2H), 8.40 (d, J = 7.5 Hz, 2H), 7.8-8.3 (m, 5H), 7.03 (br s, 2H), 6.57 (br s, 2H), 6.35 (s, 1H), 6.05 (br, s, 2H), 3.99 (s, 3H), 3.67 (m, 2H), 3.18 (m, 2H); MS m/e 391 ($M^+-CH_2NH_2$), 386 (M^+-Cl)].

12. In the laboratory of Professeur G. Boileau (Université de Montréal) for methidium derivatives and in the group of Professor C. Cantor (University of Columbia) for the psoralen compounds. For experimental procedure see: F.P. Gasparro, W.A. Saffran, C.R. Cantor, and R.L. Edelson, J. Investigative Dermato. **82**, 420 (1984).

13. Obtained as a mixture with syn isomer by the reaction between the dianhydride and AMT (2 equiv.) in CH_2Cl_2/R.T. in the absence of Et_3N, separated by chromatography over silica gel, overall yield 74%, ratio syn:anti = 2:1; anti isomer, white solid [mp. 215-20°C (dec.); IR ($CHCl_3$/MeOH 9:1) cm^{-1} 3250, 2940, 1730, 1705, 1670, 1640, 1660, 1110; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.79 (m, 2H), 7.74 (s, 2H), 6.20 (s, 2H), 4.59 (t, J = 5Hz, 4H, 4.37 (s, 2H), 4.30 (s, 2H), 3.80-3.15 (m, 16H), 2.63-2.41 (finely split m, 18H).

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