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PREPARATION OF AN INTRAMOLECULAR ADENINE-THYMINE BASE PAIR

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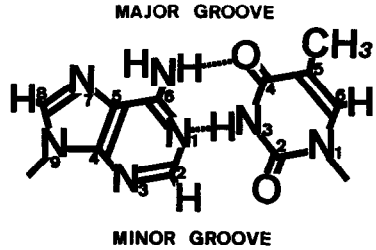
Summary: An intramolecular adenine-thymine derivative 5, prepared by connecting 9-(3-hydroxybenzyl)adenine and 1-(3-hydroxybenzyl)thymine with a dodecamethylene bridge via ether linkages, undergoes intramolecular adenine-thymine base pairing via hydrogen bonding in chloroform at -30 °C.

DNA binding proteins[2] and antibiotics[3] interact with peripheral nitrogen and oxygen atoms of complementary Watson-Crick base pairs of double helical DNA via hydrogen bonding. For example, netropsine and distamycine are known to bind in the minor groove of DNA with their amide groups hydrogen bonded to 3-N of adenine and 2-O of thymine (refer to structure 1).[3] For detailed physico-chemical characterization of such hydrogen bonds, use of standard base pairs is highly desirable. A problem, however, is that intermolecular association of lipophilic adenine (A) and thymine (T) derivatives in apolar organic media is weak ($K \approx 10^2$ M^{-1}),[4] so that an equimolar mixture of A and T usually contains monomers (A and T) and homodimers (AA and TT) in addition to desired hetero-dimer (AT); at higher concentrations, higher aggregates might also result.[5] Now, we wish to report on an intramolecular AT base pairing.

MAJOR GROOVE



MINOR GROOVE

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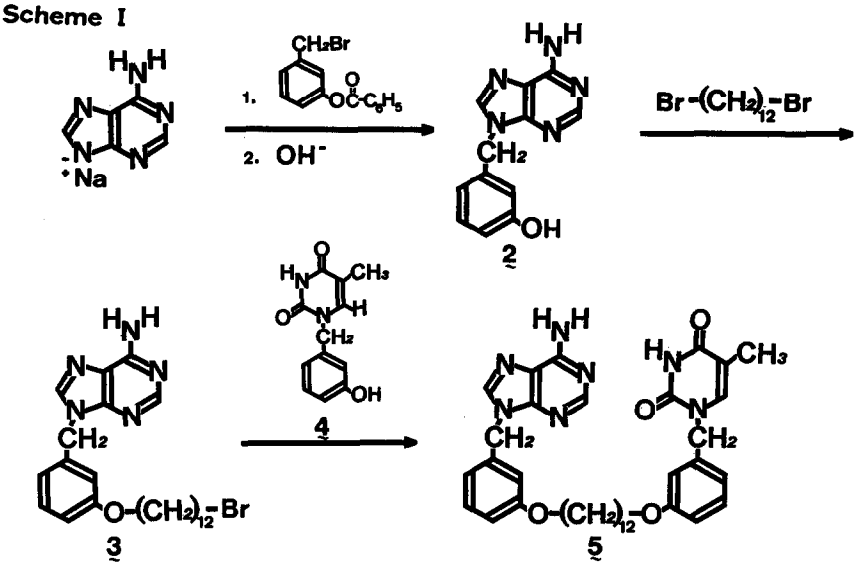
An intramolecular adenine-thymine derivative 5 was prepared, in which 9-(3-hydroxybenzyl)adenine and 1-(3-hydroxybenzyl)thymine moieties are connected with a dodecamethylene bridge via ether linkages. The synthetic route is shown in Scheme I. Thus, alkylation of sodium adenide[6] with 3-benzoyloxybenzyl bromide[7] and subsequent hydrolysis afforded 9-(3-hydroxybenzyl)adenine (2, 66 %). Reaction of 2 with an excess amount of 1,12-dibromododecane gave terminal bromide 3, which underwent further condensation with 1-(3-hydroxybenzyl)thymine (4)[8] to give desired compound

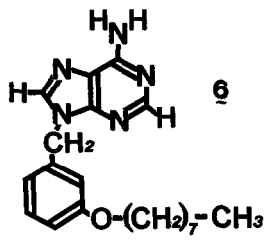
Scheme I



5 (90 %);[9] this compound was shown to be readily soluble in $CHCl_3$.

The adenine-thymine base pairing can be readily evaluated by ^1H NMR spectroscopy; upon formation of a hydrogen bonded adenine-thymine base pair, the thymine moiety undergoes a characteristic downfield shift of its imino proton resonance[10] and NOE correlations between this proton and neighboring protons in the adenine moiety are observable.[11] The ^1H NMR spectrum of compound 5 (1.0×10^{-1} or 1.0×10^{-2} M) in $CDCl_3$ at 25 °C showed the imino proton resonance at δ 9.4 or 8.5, as compared with δ 8.0 for reference thymine compound 6 under conditions where intermolecular hydrogen bonding is negligible. The NOE mesurements indicated that the imino proton is correlated with 2-H and 8-H of the adenine moiety (refer to structure 1 for numbering) in a similar manner as described below. The downfield shifts of the imino proton and the NOE results suggest that the adenine and thymine moieties are hydrogen bonded.[12] Vapor pressure osmometry showed that compound 5 in $CHCl_3$ at 32 °C is monomeric at least in the range $[5] \leq 5 \times 10^{-2}$ M, but some kind of aggregation takes place at higher concentrations.[13]

At lower temperatures, the imino proton resonance underwent further downfield shifts. In Figure 1 are shown the concentration dependencies of the imino proton resonances at -30 °C for compound 5 and equimolar mixtures of thymine and adenine references 6 and 7. In marked contrast to the intermolecular base pairing of 6 and 7, compound 5 shows only a very slight concentration dependence; this can be taken as evidence that



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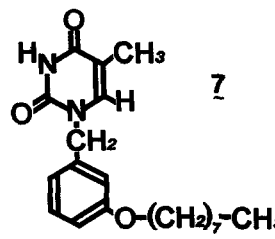

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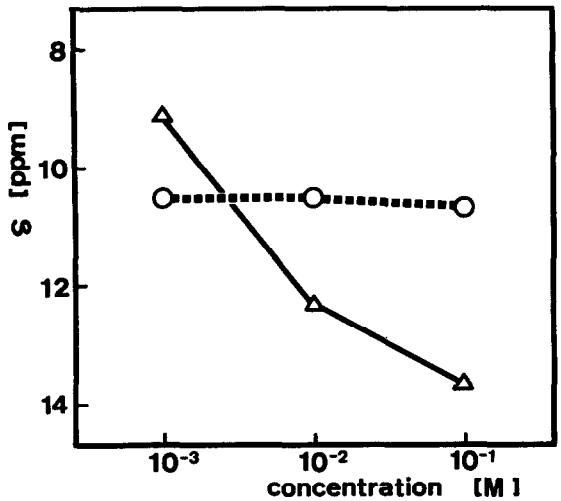
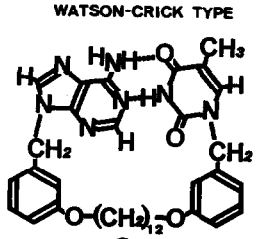


Figure 1. Concentration dependencies of the imino proton chemical shifts for 5 (O) and equimolar mixture of 6 and 7 (△) in $CDCl_3$ at -30 °C.

the hydrogen bonding is intramolecular in nature. A deeper insight into base pairing was provided by NOE measurements; irradiation of the imino proton at -10 °C resulted in NOE's at 2-H (0.64 %) and 8-H (1.91 %) of the adenine moiety, indicating that both the Watson-Crick (8) and Hoogsteen (9) base pairing,[14] leading respectively to NOE's at 2-H and 8-H, are involved. Examination of CPK molecular models indicates that such a base pairing is readily attainable, but the intramolecular bridging involved possibly causes a bond-length or bond-angle strain at the site of hydrogen bonds. This may be why compound 5 shows a considerably smaller downfield shift (ca. 3 ppm) as compared with the intermolecular association of 6 and 7 (ca. 6 ppm). Further work is now under way to shed more light on base pairs 8 and 9 and also on the molecular recognition thereof via hydrogen bonding interactions.

WATSON-CRICK TYPE



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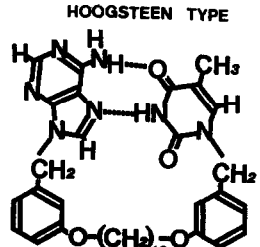
HOOGSTEEN TYPE



9

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References and Notes

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7. Obtained (84 %) by NBS-bromination of 3-benzoyloxytoluene in CCl_4.

8. Obtained (52 %) by direct alkylation of thymine with 3-benzoyloxybenzyl bromide in DMSO in the presence of K_2CO_3 followed by alkaline hydrolysis: cf. K. L. Carraway, P. C. Huang, and T. G. Scott, in "Synthetic Procedures in Nucleic Acid Chemistry", Vol. 1, W. W. Zorbach, Ed., Interscience Publishers,New York, N. Y., 1968.

9. Purified by means of preparative HPLC on a column of μ-porasil: ^1H NMR (10^{-1} M in $CDCl_3$ at 25 °C) δ 1.1-1.5 (m, 22 H, $OCH_2(CH_2)_{10}CH_2O$), 1.88 (S, 3 H, CH_3), 3.89-3.99 (two t, 4 H, OCH_2), 4.82 (s, 2 H, thymine-CH_2), 5.33 (s, 2 H, adenine-CH_2), 5.63 (s, 2 H, NH), 6.73-6.89 (m, 6H, aromatic-H), 6.97 (s, 1 H, 6-H on thymine ring), 7.29-7.50 (m, 2H, aromatic-H), 7.85 (s, 1 H, 2-H on adenine ring), 8.40 (s, 1 H, 8-H on adenine ring); EI-mass spectrum m/e 640 (M^+) (calcd for $C_{36}H_{45}O_4N_7$, M = 640).

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12. The chemical shifts of other protons, except the amino protons which undergo a slight downfield shift, are almost the same as those in references 6 and 7, indicaing that π-stacking interaction between two heterocycles in 5 is not important. For π-stacking interaction, see for example: (a) F. Seyama, K. Akahori, Y. Sakata, S. Misumi, M. Aida, and C. Nagata, J. Am. Chem. Soc., 110, 2192 (1988).

13. The apparent molecular weight at [5] = 1 x 10^{-1} M is ca. 830, which is 1.3 times as large as the true molecular weight (640).

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