Tetrahedron Letters,Vol.28,No.2,pp 147-150,1987
Printed in Great Britain

0040-4039/87 $3.00 + .00
Pergamon Journals Ltd.

SYNTHESIS AND 1H NMR SPECTRUM OF N6',N9-OCTAMETHYLENEPURINE CYCLOPHANE

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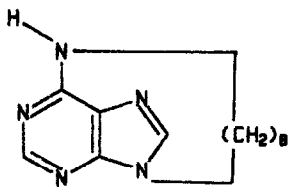
Summary: N6',N9-Octamethylenepurine cyclophane, 1 ,has been synthesized by intramolecular cyclization. Its pmr spectrum shows all 16 methylene protons to be resolved, their chemical shifts ranging from -0.6 to +4.8 ppm.

Much interest has been expressed in the chemical shift effects of the aromatic purine and pyrimidine heterobases present in ribo- and deoxyribo-oligonucleotides[1]. These highly anisotropic ring systems exert pronounced shift effects in both the flexible single stranded state[2] as well as in the helical double stranded state[3]. To this point, the spatial definition of the diamagnetic susceptibility anisotropy of the heterobases has been based upon theroretical calculations involving the use of ring currents[4], atomic susceptibility tensors[5], and values relative to those of benzene[6]. We report here the synthesis of a chiral cyclophane molecule, N6',N9-octamethylenepurine, 1 , to act as an nmr probe for the study of the diamagnetic anisotropy about the adenine system[7].

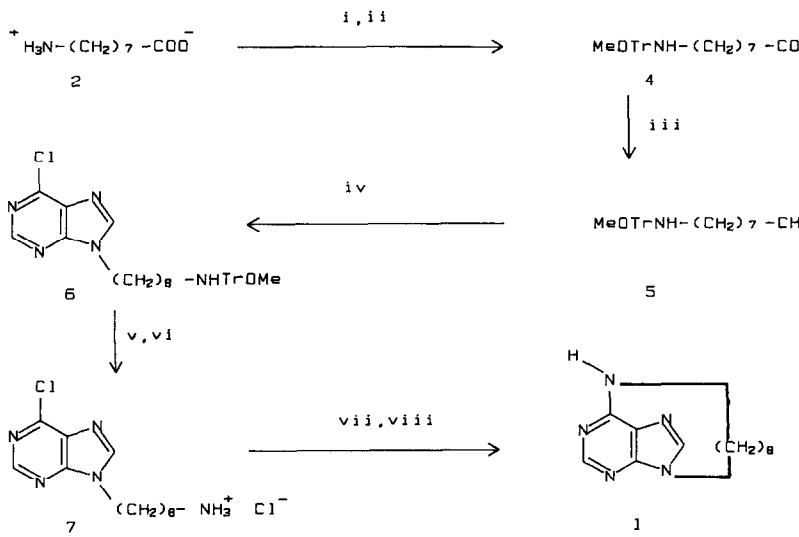


1

The choice of the octamethylene chain was based on the inspection of molecular models which indicated that this chain length was sufficiently constrained to permit minimal motion of the methylenes but not so taut as to introduce significant bending of the planar purine ring. The synthesis of 1 was accomplished as indicated in Scheme 1. 8-Aminocaprylic acid 2 was converted to ethyl-8-aminooctanoate[8] 3 followed by condensation with p-anisylchlorodiphenylmethane[9] (1eq Et_3N in $CHCl_3$). Reduction of 4 with lithium aluminum hydride in anhydrous diethyl ether produced the alcohol 5 . This protected amino-alcohol was combined with 6-chloropurine using the method of Mitsunobu[10] and gave the N-9 alkyl-6-chloropurine 6 in 65% yield. The monomethoxytrityl group was carefully hydrolyzed by treatment with trifluoroacetic acid (2eq) and methanol (4eq) in

methylene chloride for 0.5 hours at room temperature. The amine was isolated as the hydrochloride salt 7 by aqueous extraction and lyophilization. In view of the rapid displacement of the 6-chloro group in the parent 6-chloropurine by primary amines in aprotic solvents[11], compound 7 was converted to the free amine by dissolution in a minimum volume of acetonitrile containing diisopropylethylamine (10 eq) at -23°C. The solution was then diluted with more acetonitrile until the concentration of 7 was $1X10^{-3}$M. After 12 hours at reflux, thin layer chromatography showed the complete conversion of 8 into 1 with only minor amounts of other products. The cyclophane was quite stable and could be purified by standard column chromatography (silica gel; $CHCl_3$:MeOH; 99.5:0.5) and showed an M+ ion at m/z 245 and λmax(EtOH) 278nm(ε =10557).

Scheme 1



i) $SOCl_2$, EtOH; ii) MeOTrCl, Et_3N, $CHCl_3$; iii) $LiAlH_4$, Et_2O; iv) Ph_3P, 6-chloropurine, diethyl azodicarboxylate, THF, 48h; v) TFA, MeOH, CH_2Cl_2; vi) 0.1M HCl; vii) CH_3CN, -23°C, $(iPr)_2NEt$; viii) CH_3CN, reflux, 12h.

The proton nmr spectrum of 1 , recorded at 500 MHz, was dramatic in comparison with that of benzene[10]paracyclophane[7] and is presented in Figure 1. Each pair of methylene protons is diastereotopic and the assignments noted in Figure 1 were initially based on an analysis of the corresponding homonuclear 2D-COSY experiment which enabled all 16 protons of the alkyl chain to be determined. Assignment of the relative stereochemistry[12], H_S or H_R, of each proton proved more difficult and relied on the determination of the coupling constants by spin simulation, application of the Karplus equation, and comparison with an energy minimized stereostructure of 1 (see Figure 2), obtained with the use of Chemgraph[13] and the molecular mechanics programme

MMP2[14]. The very high field signal at -0.64 ppm was assigned to C(5')-H_R from the observation, made during application of the MMP2 programme, that as the atoms N(6'), N(6')-H, and C(1') were forced to become progressively more coplanar with the purine ring system, the C(5')-H_R atom re-oriented itself and protruded directly into the imidazole ring.

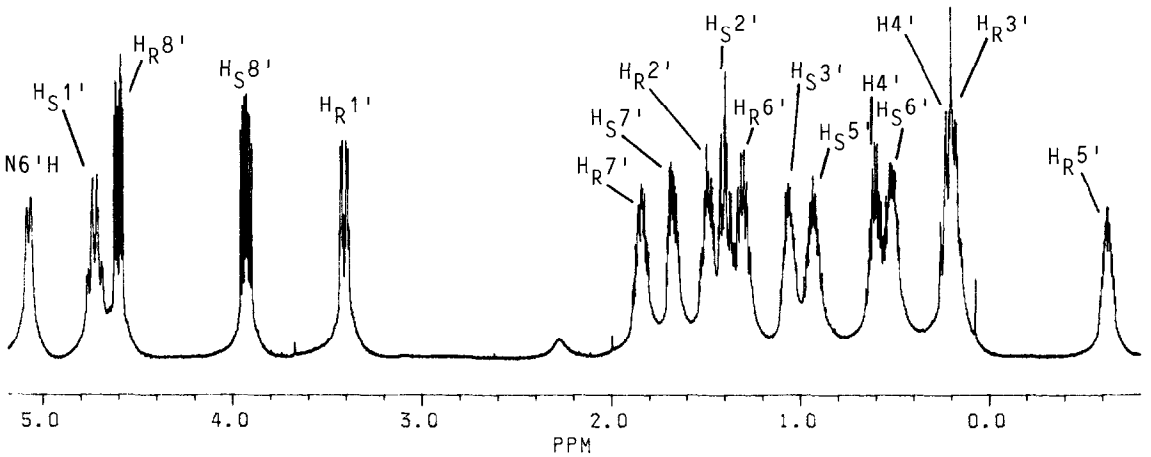


Figure 1. ^1H NMR spectrum of 1 at 500 MHz in deuteriochloroform, 30 °C.

The nmr spectrum of 1 showed that protons on carbons 3', 4', 5', and 6' were moved to a high field region between 0.0 and 0.75 ppm, while protons on carbons 2', 6', and 7' moved to lower field in comparison with the simple purine-amine precursor 7[15]. Some of the diastereotopic geminal protons showed substantial chemical shift differences, for example the protons on C(1') were separated by 1.3 ppm and those on C(5') differed by 1.6 ppm. These differences are anticipated on the basis of the expected highly anisotropic nature of the purine ring system and strongly suggest that compound 1 will be an excellent model to further refine this anisotropy. Currently work is in progress to fully define the conformational states of the alkyl chain of 1 using variable temperature nmr, solid state nmr, and X-ray crystallographic analysis.

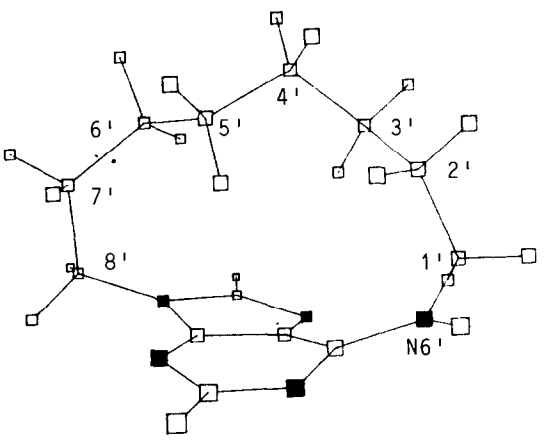


Figure 2. Stereostructure of 1. ■ = N

Acknowledgement: We thank the National Science and Engineering Research Council of Canada for financial assistance.

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11. 6-Chloropurine with n-butylamine in DMSO-d_6 shows a half-life at 25°C of approximately 6 minutes.
12. The methylene protons are labelled here as H_R or H_S according to whether it is a pro-R or pro-S proton.
13. Chemgraph, created by E. K. Davies, Chemical Crystallography Laboratory, Oxford University, developed and distributed by Chemical Design Ltd. Oxford.
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15. Selected spectroscopic properties: Compound 6 ^1H ($CDCl_3$) δ, 4.23 (C(1') CH_2), 2.08 (C(8') CH_2), 1.87 (C(2') CH_2), 1.43 (C(7') CH_2), 1.28 (C(3')-C(6') CH_2s). Compound 7 ^1H (D_2O) δ, 4.50 (C(1') CH_2), 3.13 (C(8') CH_2), 2.00 (C(2') CH_2), 1.76 (C(7') CH_2), 1.37 (C(3')-C(6') CH_2s).

(Received in USA 25 August 1986)