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SYNTHESIS OF 1,14-DIAMINO-5,10-DIAZA-N^1,N^{14},5,10-TETRAKIS-/9-(β-D-RIBOFURANOSYL)PURIN-6-YL/-TETRADECANE - A NEW OLIGONUCLEOTIDE ANALOGUE WITH STACKED BASE RESIDUES

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Abstract: The title compound 1b was obtained by reaction of 6-chloro-9-(β-D-ribofuranosyl)purine (2) with N,N'-bis-(4-aminobutyl)-1,4-diaminobutane (3).

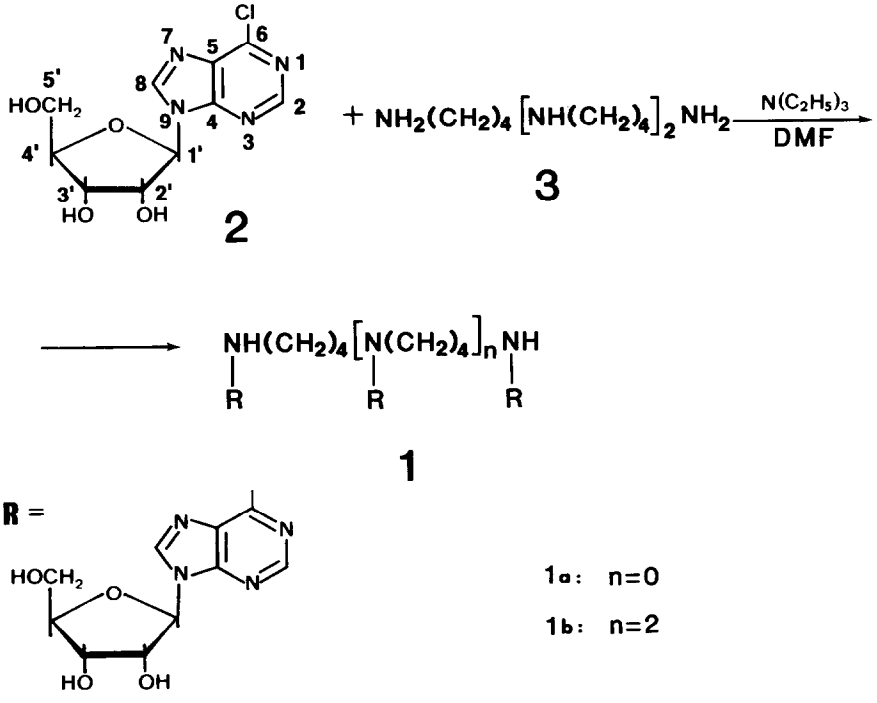
Bridged nucleosides, e.g. compound 1a, have been studied extensively[1-10] and their derivatives, suitably functionalized, have found use as valuable probes for enzymes such as ribosomal peptidyltransferase[3,7], snake venom phosphodiesterase[2] and codon-anticodon binding[8]. Similar dinucleosides linked by a single methylene group were obtained from hydrolysis of DNA and RNA treated with formaldehyde[11]. Spectral[6] and enzymic studies[2,3,7] have shown that bridged nucleosides containing 2 and 4 methylene groups as spacers can be regarded as analogues of dinucleoside phosphates. The availability of bridged nucleosides has been hitherto restricted to compounds having only two nucleoside residues in the molecule.

We have now prepared the first "bridged oligonucleoside" which contains four ribonucleoside moieties linked to a polyamine backbone - compound 1b. "Exhaustive nucleosidation" of polyamine 3 with chloro nucleoside 2 (5 molar equivalents) readily afforded product 1b in 70% yield (Scheme 1). Compound 1b has a plane of symmetry and comprises two different nucleoside residues - N^6-mono- and N^6-dialkylated adenosine. Thus, the FT-100 NMR spectrum of 1b exhibits a pattern of heterocyclic H_8 and H_2 protons as 2 + 1 singlets at δ 8.36, 8.32 and 8.19 whereas anomeric H_1' protons appear as two poorly resolved overlapped doublets centered at δ 5.86. After addition of D_2O, the pattern of the purine protons was reversed to give 1 + 2 singlets at δ 8.27 and 8.12 (poorly resolved). Integration showed the ratio of (H_8 + H_2):H_1 : C-methylene groups (δ 1.67) being 1.8 : 1 : 3.2 (calculated for structure 1b: 2 : 1 : 3).

These results along with a negative ninhydrin test have shown that all amino and imino groups of polyamine 3 are substituted with nucleoside residues.

The UV and CD spectra of tetranucleoside 1b which, incidentally, are quite similar to dinucleoside[6] 1a are indicative of a base-base (stacking) interaction in the molecule. Thus, UV spectrum of 1b at pH 7 (0.01 M Na_2HPO_4) exhibited λ_{max} 271 nm and ε_{max} 50,800. As expected[12], the destacking of base residues occurred in ethanol and ε_{max} was increased to 65,800 (λ_{max} 274 nm). The CD spectra[13] showed a similar stacking - destacking pattern. A negative Cotton effect was observed at 282 nm (pH 7) with a molecular ellipticity of 59,100. The latter value decreased significantly in ethanol to 14,500 (CD_{max} 274 nm).

Scheme 1



The "tetrameric" structure of 1b is also supported by UV spectroscopy. Thus, ε_{max} value of an equimolar mixture of N^6-ethyl- and N^6, N^6- diethyladenosine[14] (λ_{max} 272 nm) multiplied by a factor of 2 (69,150) is in good accord with ε_{max} of unstacked tetranucleoside 1b (all spectra were measured in ethanol).

Compound 1b. A solution of chloro nucleoside[15] 2 (0.72 g, 2.5 mmol), polyamine 3 (0.12 g, 0.52 mmol) and triethylamine (0.42 mL, 3 mmol) in dimethylformamide (DMF, 20 mL) was stirred magnetically for 3 days at room temperature. The insoluble portion was filtered off and washed with methanol. The filtrate was evaporated and the residue was crystallized from 50% methanol to give compound 1b in two crops (0.36 g, 55%),mp. 150-156°C (transition point). Recrystallization from 50% ethanol (50mg/mL, 91% yield) gave 1b, mp. 153-156°C (transition point). Anal. Calcd. for $C_{52}H_{70}N_{20}O_{16}.2\ H_2O$: C 49.28, H 5.89, N 22.11. Found: C 49.54, H 6.00, N 21.86. Anhydrous 1b was obtained by drying in vacuo at 100°C immediately before analysis: Calcd. for $C_{52}H_{70}N_{20}O_{16}$: C 50.72, H 5.73, N 22.75. Found: C 50.86, H 5.92, N 22.49. Compound 1b was uniform on electrophoresis in 0.02M $Na_2B_4O_7$ (pH 9.2, 1h, 40 V/cm, Whatman No. 1 paper). The insoluble portion and mother liquors after the first crystallization were combined and they were passed through Dowex 50 WX 4, 200 - 400 mesh column (12.5 x 1.5 cm, H^+ form). The column was eluted with 50% methanol (300 mL) and 10% pyridine in 50% methanol (400 mL). The latter elution afforded after evaporation an additional 90 mg (14%) of compound 1b.

Tetranucleoside 1b was tested for cell culture antiviral activity with the following viruses: Influenza A, Parainfluenza 3, Coxsackie A-21, Equine Rhinovirus, Herpes Simplex-1, Herpes Simplex-2 and Vaccinia. With the exception of a small zone of antiviral activity for Coxsackie A-21 virus compound 1b was inactive and non-cytotoxic.

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14. It may be argued that N^6-butyladenosines are better models for comparison with tetranucleoside 1b. However, UV-spectra of N^6-butyladenosine virtually overlap with those of N^6-ethyladenosine[6].
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