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SYNTHESIS OF THE RIBOSIDE ANALOG OF THE PYRIMIDINE ADDUCT FORMED IN THE REACTION BETWEEN THE CARCINOGEN N-HYDROXY-N-2 ACETYLAMINOFLUORENE AND DNA

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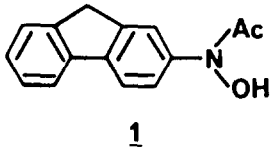
Summary : A general synthesis has been achieved to obtain carcinogen modified guanosines. The method was applied to the preparation of the N- 2,5-diamino--4-oxo-3H-pyrimidine-6-yl-N-(β-D-ribofuranosyl)-N'-(2-fluorenyl)urea 6.

Identification of the adducts formed in the reaction between chemical carcinogens and DNA is of major importance for the understanding of tumor formation. In the in vivo and in vitro reaction between the metabolites of the hepatocarcinogen N-hydroxy-2-acetylaminofluorene 1 and DNA, three major adducts were identified as 2, 3 and 4[1-2].



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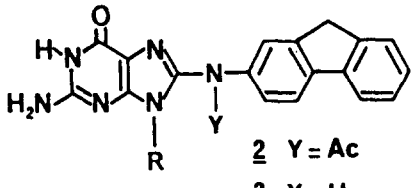
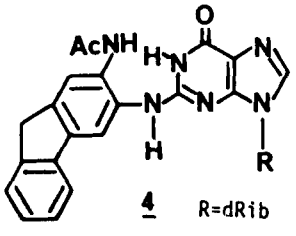


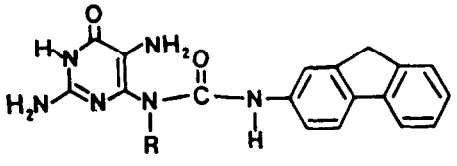
R=dRib

2 Y = Ac

3 Y = H



4 R=dRib

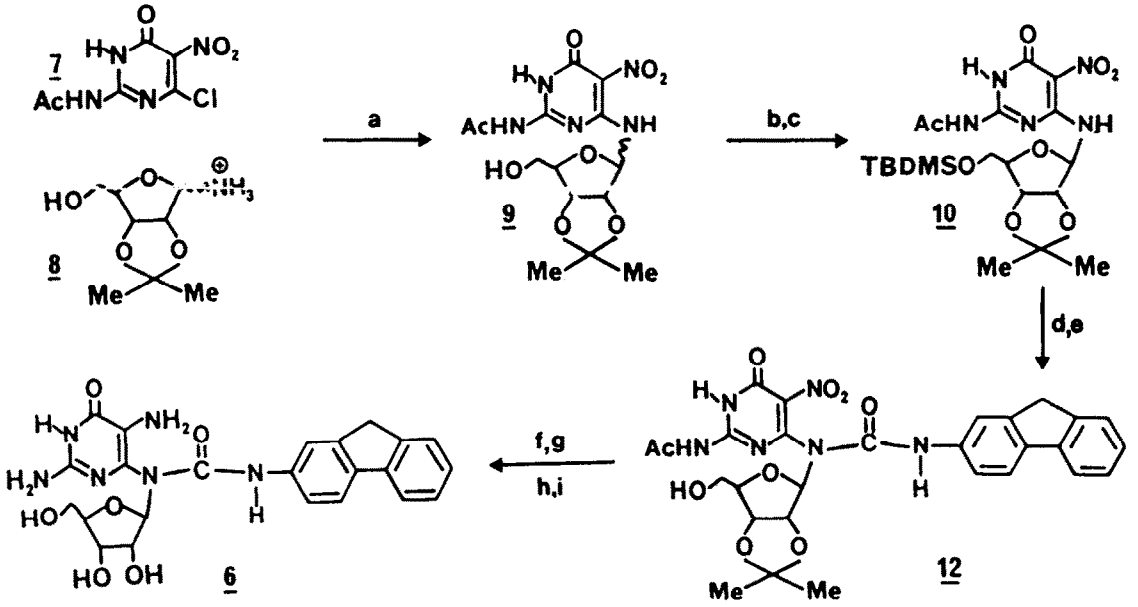


5 R=dRib

6 R=Rib

The formation of these products which result from reaction of the carcinogen with the C_8 and the N_2 positions of the guanine ring appears to be general for the mutagenic aryl amine metabolites[2]. More recently however it has been found in rat liver DNA exposed to 1 a new minor adduct identified as the urea 5[3,a,b,c,d]. This compound corresponds to the opening of the imidazole ring in 3 at the N_7-C_8 bond. A ring opened guanosine derivative has also been found for 2-naphthylamine[4]. The product corresponds to the cleavage of the imidazole between the N_9 and the C_8 atoms. Formation of such minor ring opened adducts might represent a more general phenomenon[5]. As a consequence it seemed desirable to develop a general synthetic route to such ring opened guanosine derivatives, which could be applied to a number of carcinogen amines : we report here our preliminary results relative to the preparation of the fluorenyl adduct 6 in the ribose series.

The synthesis involves building up of the pyrimidine ring 7 with subsequent fixation of the sugar moiety leading to the key intermediate 10 on which the aromatic amine is added as its isocyanate in the last steps of the scheme :



(a) Et_3N, DMF, rt, 18h. (b) crystallisation of the β-anomer. (c) TBDMSCl, pyridine, rt, 3h. (d) ArNCO, pyridine, rt, 3 days. (e) aq. HCl, pH 4, rt, 3H. (f) H_2/NiR, EtOH, rt, 2.5h. (g) NH_4OH, MeOH, 65°C, 14h. (h) DOWEX $50H^+$, MeOH, 40°C, 1h. (i) NH_3, MeOH.

Substitution of chloride at the C_6 position of the pyrimidine 7 (prepared in four steps[6] by conventional routes) by the isopropylidene ribofuranosylamine 8 (used as the p-toluene sulfonate salt[7]) gave a mixture of α/β anomers (45/55) from which the β isomer 9 was isolated by cristallisation (F 202-203°C)

and characterised[8] by ^1H NMR spectroscopy[9,a,b]. Protection of the 5'OH function by the t-butyldimethylsilyl group (TBDMS) afforded 10 (yield 90%) on which the fluorenyl moiety could be introduced by reaction with the corresponding fluorenyl isocycanate used in slight excess[10] (52% yield). The adduct 11 thus obtained was treated in acidic conditions to eliminate the 5'-silyl protection to yield 12 (90% yield) (F 251-255°C). For this reaction carefully controlled condition were used, which left the acetonide unaffected, i.e., vigourous stirring of 11 in a biphasic system constitued of HCl-H_2O, pH 4 and $CHCl_3$. Reduction of the nitro function leading to 13 (F 124-127°C) was achieved by catalytic hydrogenation over Raney nickel (53% yield). The 2-acetamido group was removed by ammonia and 14 was isolated (50% yield) (F 162-164°C). The transformation of 14 to the desired nucleoside 6 (F 132-134°C) was accomplished by unblocking the 2', 3'-OH functions through a Dowex treatment in methanol[11] (90% yield). All spectral determinations correspond to the desired N- 2,5-diamino-4-oxo-3H-pyrimidine-6-yl N-(β-D-ribofuranosyl)-N'-(2-fluorenyl)urea 6 : Noe measurements clearly confirm that the nucleoside possesses the β configuration[8].

The analytical data for 6 (Rf value, UV and CD spectra) are quite identical to those reported by M. Leng and coll.[3b] for one of the ring-opened aminofluorenyl-guanosine derivatives[13], which unambiguously confirms the structure assigned by the authors[3e,d].

Extensions of the synthesis to deoxyribose compounds are now under investigation.

Acknowledgement : We thank Dr. M. Leng for fruitful discussion. Financial support from ADRC is gratefully acknowledged.

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8. Significant spectral data are recorded below : (Fl = fluorenyl, Pyr : pyrimidine).

9 : ^{1}H NMR (DMSO d_6, 60MHz) : 9.55 (1H, br d, C_1,NH) ; 5.58-5.38 (1H, m, C_1,H) ; 2.21 (3H, s, CH_3-CO) ; 1.46 and 1.24 (6H, 2s, $2CH_3$).
MS m/e : M^+ 385 (13) ; 354 (14) ; 327 (10) ; 242 (30) ; 213 (100).

10 : ^{1}H NMR (DMSO d_6, 60MHz) : 0.92 (9H, s, $(CH_3)_3C$) ; 0.71 and 0.63 (6H, 2s, $(CH_3\text{-Si})_2$)
MS m/e : M^+ 499 (48) ; 484 (42) ; 469 (56) ; 385 (15) ; 328 (18) ; 242 (32).

11 : ^{1}H NMR (DMSO d_6, 60MHz) : 9.38 (1H, s, NH urea) ; 7.82-7.15 (7H, m, Fl1H) ; 5.96 (1H, d, C_1,H, J=2.9Hz).
MS m/e : M^+ 706 (12) ; 691 (17) ; 661 (41) ; 576 (26).

12 : ^{1}H NMR (DMSO d_6, 80MHz) : 3.68-3.38 (3H, m, C_5,H, C_5,OH). ^{13}C NMR (DMSO d_6, 20MHz) : 150.8 (1C, urea) ; 145-115 (12C, Fl) ; 36.8 (1C, CH_2Fl).
MS m/e : M^+ 592 (28) ; 534 (43) ; 328 (84) ; 207 (71).
IR (Nujol) : 1700cm^{-1}.

13 : ^{1}H NMR (DMSO d_6, 80MHz) : 6.75 (2H, s, C_5NH_2).
MS m/e : M^+ ($+NH_4^+$) 584 (4) ; M^+ 562 (19) ; 504 (37) ; 297 (32).

14 : ^{1}H NMR (DMSO d_6, 80MHz) : 9.72 (2H, s, C_2NH_2). ^{13}C NMR (DMSO d_6, 20MHz) : 157.6 (C_2).
MS m/e : M^+($+NH_4^+$) 558 (17) ; M^+ 520 (5) ; 505 (42).

6 : ^{1}H NMR (DMSO d_6, 200MHz) : 10.58 (2H, s, C_2NH_2) ; 8.77 (1H, s, NH urea) ; 8.49 (1H, s, N_3H) ; 7.76-7.51 (3H, m, Fl1H) ; 7.30-7.12 (4H, m, Fl1H) ; 6.41 (2H, s, C_5NH_2) ; 5.64 (1H, d, C_1,H, J=5.95Hz) ; 5.35 (1H, d, C_2,OH, J=6Hz) ; 5.07 (1H, d, C_3,OH, J=4.5Hz) ; 4.99 (1H, t, C_5,OH, J=4.5Hz) ; 4.35-4.32 (1H, m, C_2,H) ; 4.06-3.89 (1H, m, C_3,H) ; 3.86-3.80 (3H, m, C_4,H, CH_2Fl) ; 3.50-3.40 (2H, m, C_5,H_2). ^{13}C NMR (DMSO d_6, 20MHz) : Fl ring : 142 (C_{13}) ; 140.7 (C_{10}) ; 140.05 (C_{12}) ; 136.6 (C_{11}) ; 134.0 (C_2) ; 124.9 (C_6) ; 124 (C_7) ; 123.3 (C_8) ; 118.1 (C_4 or C_5) ; 117.2 (C_5 or C_4) ; 115 (C_3) ; 113.2 (C_1). Pyr. ring : 172.8 (C_4) ; 162.5 (C_6) ; 155,5 (C_2) ; 149.1 (C_5). Ribose : 89.8 ($C_{1'}$) ; 87.6 ($C_{4'}$) ; 82,8 ($C_{2'}$) ; 72.8 ($C_{3'}$) ; 58.7 ($C_{5'}$). NOE : $H_{2'}$ irradiation induces a 25% increase for the H_3, signal, and 0% for $H_{1'}$.
MS (silylated compound) m/e : 696 (71) ; 681 (35) ; 666 (31) ; 208 (70).

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12. All the yields are based on pure material isolated by chromatography.

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