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A POTENTIALLY SIMPLE ^{13}C NMR METHOD TO ASSIGN REGIO- AND STEREOCHEMISTRY OF (2'-DEOXY-2'-FLUOROARABINOFURANOSYL) NUCLEOSIDES.

Muzammil M. Mansuri*, Bala Krishnan and John C. Martin
Bristol-Myers Squibb Company, 5 Research Parkway, Wallingford CT 06492-7660

Abstract The details of an NMR method of distinguishing between α- and ß- anomers of 2'-deoxy-2'-fluoro-arabinofuranosyl nucleosides are presented.

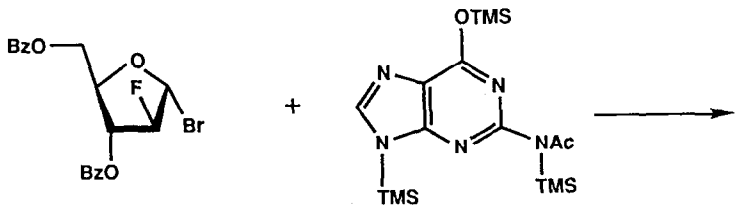
2'-Deoxy-2'-fluoroarabinofuranosyl nucleosides have attracted much attention as potential antiviral agents [1-3]. As only the ß-anomers of these analogs show biological activity against the herpes family of viruses, a simple method for distinguishing between the α- and ß-forms is necessary. We present here a simple method for assigning the α- and ß- anomers based on ^{13}C NMR coupling constants. This approach results from observations made during our synthesis of 9-(2'-deoxy-2'-fluoro-ß-D-arabinofuranosyl)guanine (FADG, **1**), an analogue of 2'-deoxyguanosine (**2**) [4].

The coupling of bromosugar **3** with the silylated base **4** yielded the regio and stereoisomers **5-8** which were separated and collected by semi-preparative HPLC IBM reverse phase column [5-7]. The separation gave pure samples of **5-7**; **8** was contaminated with some impurities. The use of ^{13}C NMR to assign N-7 and N-9 regioisomers, was suggested earlier by Fischer et al. [8]. The ^{13}C NMR spectra of **5-8** were therefore collected and compared with those of the N-9 (**10**) and N-7 (**11**) isomers of the condensation products of the 2-O-(acetoxymethyl)-1,3-di-O-benzyl-glycerol and N2-acetylguanine [9]. Comparison of the chemical shifts for carbons C-5 and C-8 of **10** and **11** with the chemical shifts for the corresponding carbons of **5-8** allowed us to assign **5** and **6** as the N-9 regioisomers, **7** and **8** were the N-7 regioisomers (Table 1).

The stereochemistry of **5-8** was initially assigned based on the long range coupling of 2-3 Hz between the purine H-8 proton and the 2'-fluoro substituent for the ß-anomers [4,11], in the α -forms the H-8 proton appeared as a singlet. Also, the H-1' - H-2' coupling constants were larger for the ß anomers [5,9,10]. Taken together, the H-8 - F and H-1' - H-2' coupling constants suggested that **5** was the N-9 α anomer, while **6** was the N-9 ß-anomer. Nuclear Overhauser experiments were used to confirm these assignments. Similar analysis identified **7** as the N-7 α-anomer, and **8** the N-7 ß-anomer.

TABLE 1. ^{13}C NMR Chemical shifts for C-5 and C-8 carbons of purine nucleosides

	5	6	7	8	10	11	
C-5	121.02	121.04	111.01	111.00	120.28	111.01	ppm
C-8	136.39	138.23	141.00	142.58	139.93	144.92	ppm



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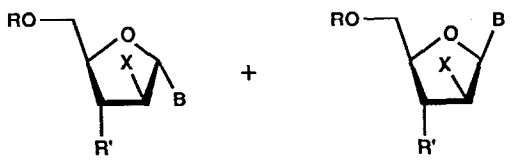


5 R = Bz, R'= OBz, X = F
B = N-9(2-acetylguanine)
7 R = Bz, R'= OBz, X = F
B = N-7(2-acetylguanine)
9 R = H, R' = OH, X = F
B = N-9-guanine

13 R = H, R' = OH, X = F
B = N-9(6-chloropurine)
15 R = Bz, R' = H, X = F
B = N-9(N-benzoyladenine)
17 R = R' = H, X = F
B = N-9(6-chloropurine)

6 R = Bz, R' = OBz, X = F
B = N-9(2-acetylguanine)
8 R = Bz, R' = OBz, X =F
B = N-7(2-acetylguanine)
1 R = H, R' = OH, X = F
B = N-9-guanine
2 R = H, R' = OH, X = H
B = N-9-guanine

12 R = H, R'= OH, X = F
B = N-9-adenine
14 R = H, R' = OH, X = F
B = N-9(6-chloropurine)
16 R = Bz, R' = H, X = F
B = N-9(N-benzoyladenine)
18 R = R' =H, X = F
B = N-9(6-chloropurine)

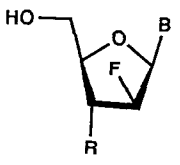
While examining the ^{13}C NMR spectra of these four compounds, **5-8**, we made the observation that the C-1' - F coupling constants were very different for the two sets of anomers. The α-anomers **5** and **7** had C-1' - F coupling constants of 36.2 Hz and 35.5 Hz, respectively, while the corresponding C-1' - F coupling constants for the ß-anomers **6** and **8** were 17.2 and 16.3 Hz. A number of other 2'-fluoro purine compounds were then examined (Table 2). The results suggest that in all the cases tested the α-anomers have significantly larger C-1' - F coupling constants ($J_{C-1'-F}$ = 35-36 Hz) than the corresponding ß-anomers ($J_{C-1'-F}$ = 16-17 Hz). We also examined a number of pyrimidine 2'-fluoroarabino nucleosides. The α- and ß- anomers of both 3', 5' dibenzoyl FEAU and 3', 5' dibenzoyl FIAU as well as the ß-anomers of the literature compounds 19 - 21, all have C-1'-F coupling contacts consistent with those seen with the purine analogues (Table 2). It appears that the two bond coupling is independent of the electronegativity of the base substituent attached to the anomeric carbon. The orientation of the substituent, however, has a large effect on the magnitude of the coupling constant. When the substituent attached to C-1' is gauche to the fluorine, as in the ß anomers, the coupling

constant is smaller than when the base substituent is trans to the fluorine as in the α anomers. Similar effects of orientation on the coupling constants are seen with fluoropyranosyl acetates and 2-deoxyfluoro sugars [12,13].

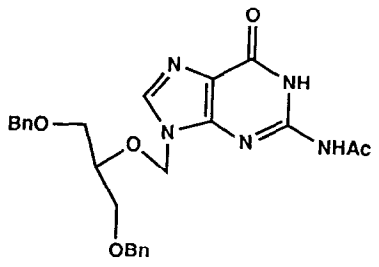
TABLE 2. C-1'-F coupling constants for α and ß anomers

Compound	α anomer J_{C-1-F}, Hz	ß anomer J_{C-1-F}, Hz	Ref
5	36.2	---	--
6	---	17.2	--
7	35.3	---	--
8	---	16.3	--
FADG, (1, 9)	36.2	16.9	4
FADA (12)	---	17.3	10
3',5'-dibenzoyl FEAU	35.2	17.45	11
3',5'-dibenzoyl FIAU	35.3	16.36	--
13, 14	36.1	16.9	14
15, 16	36.0	16.2	15
17, 18	36.1	16.2	15
3',5'-dibenzoyl FEAU	35.2	17.45	11
3',5'-dibenzoyl FIAU	35.3	16.36	--
19	---	16.5	16
20	---	16.5	16
21	---	16.8	16

We find that the C-1' - F coupling constants are diagnostic for the stereochemistry of the nucleoside analogue in 2'-deoxy-2'-fluoro-ß-D-arabinofuranosyl compounds. This appears to be true for pyrimidine and purine 2'-deoxy-2'-fluoro and 2',3'-dideoxy 2'-fluoro analogues.



19 R = H, B = cytosine
20 R = H, B = thymine
21 R = N_3, B = thymine



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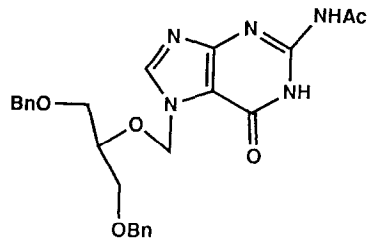

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