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Carbohydrate Research 258 (1994) 11–26

CARBOHYDRATE RESEARCH

Interunit, through-space $^{1}H-^{19}F$ and $^{13}C-^{19}F$ spin–spin couplings of 5-fluorinated arbekacins

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(Received June 29th, 1993; accepted November 23rd, 1993)

Abstract

5-Deoxy-5-fluoroarbekacin (**1**) and 5-deoxy-5,5-difluoroarbekacin (**2**) showed interunit, through-space $^{1}H-^{19}F$ spin–spin couplings at the signals for H-1′, 4″, 5″, 6″a and 6″b with F-5*eq*. These couplings were detected by a new NMR method, ^{1}H-detected 2D $^{1}H-^{19}F$ chemical shift correlation spectroscopy. These two compounds also displayed interunit, through-space $^{13}C-^{19}F$ couplings at the resonances for C-1′ and 5″ with F-5*eq*. These couplings were corroborated by 1D $^{13}C\{^{1}H\}\{^{19}F\}$ triple-resonance techniques. Triple-resonance ^{13}C-detected 2D $^{13}C-^{19}F$ COSY experiments were also carried out. The interunit conformational information obtained with the through-space $^{1}H-^{19}F$ and $^{13}C-^{19}F$ couplings was further supported by heteronuclear $^{1}H\{^{19}F\}$ NOE experiments.

1. Introduction

It is well known that values of $^{1}H-^{19}F$ spin–spin couplings ($J_{H,F}$) are much greater and much more dependent on the steric environment compared with the corresponding $J_{H,H}$ values. In spite of this feature, the practical use of long-range $^{5}J_{H,F}$ and $^{6}J_{H,F}$ values in conformational analysis seems small or negligible for fluoromonosaccharides [1]. Although $J_{C,F}$ values are easily measured in normal proton–broadband decoupling ^{13}C NMR spectra, $^{4}J_{C,F}$ values have only been obtained for a limited number of fluoromonosaccharides [1].

In 1961, Davis, et al. [2] reported a through-space $^{1}H-^{19}F$ spin–spin coupling for the first time to explain the long-range coupling between two of the phenyl protons and fluorine nuclei in 1,1-difluoro-1,2-dibromo-2-phenylethane. Since then many papers dealing with the phenomenon have appeared [3]. Through-space $^{13}C-^{19}F$ couplings have been known since Jerome and Servis [4] reported their

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SSDI 0008-6215(93)E0425-Z

observations in fluorophenanthrene derivatives in 1972. F.B. Mallory et al. [5,6] studied through-space $^1H-^{19}F$ (^5J and ^6J) couplings of fluoropolycyclic aromatic hydrocarbons, in which fluorine projected into a bay region. Recently Sardella, et al. [7,8] also reported $^1H-^{19}F$ (^5J and ^6J) and $^{13}C-^{19}F$ (^4J and ^5J) couplings of analogous aromatic compounds. Rae et al. [9] carried out theoretical calculations for determination of signs of through-space $^1H-^{19}F$ and $^{13}C-^{19}F$ coupling constants of fluoro aromatic compounds.

In the carbohydrate field, to the best of our knowledge, only a few examples of through-space $^1H-^{19}F$ and $^{13}C-^{19}F$ couplings have been reported. The examples of through-space $^1H-^{19}F$ couplings from our laboratories were as follows. The axial hydroxy proton at C-4′ and the axial F-2′ of 7-*O*-(3-*O*-benzoyl-2,6-dideoxy-2-fluoro-α-L-talopyranosyl)daunomycinone [10] showed a through-space coupling in $CDCl_3$ ($^5J_{H,F} \sim 10$ Hz). In this case, the hydroxy proton was considered to be fixed and pointing in the direction of the electron-rich F-2′ by a hydrogen bond. The resulting antiperiplanar conformation of the hydroxy proton and H-4′ was supported by the large vicinal coupling of $^3J_{H\text{-}4',OH}$ (10.5 Hz). It was also found that the hydroxy proton at C-3′ and F-3″ of 7-*O*-[2,6-dideoxy-2-fluoro-4-*O*-(3-fluorotetrahydropyran-2-yl)-α-L- talopyranosyl]daunomycinone [10] showed an interglycosidic, through-space $^1H-^{19}F$ coupling in $CDCl_3$ ($^7J_{H,F}$ 6 Hz). The intramolecular hydrogen bond, again, must play an important role in the through-space coupling. Rae, Contreras, and colleagues [11] recently reported that 2-fluoro-*N*-methylbenzamide showed long-range couplings between ^{19}F and nuclei in the amide moiety. These couplings were also explained by through-space mechanisms involving the hydrogen bond between the aromatic fluorine and the amide hydrogen.

In this paper we describe some interesting interunit, through-space $^1H-^{19}F$ (^5J, ^7J, and ^8J) and $^{13}C-^{19}F$ (^4J and ^6J) couplings of 5-fluorinated arbekacins [12]. We used $^1H\{^{19}F\}$ * double-resonance methods to determine $^1H-^{19}F$ couplings large enough to observe in one-dimensional (1D) ^1H NMR spectra. In order to detect smaller interunit $^1H-^{19}F$ couplings (< 1 Hz), we successfully introduced ^1H-detected two-dimensional (2D) $^1H-^{19}F$ chemical-shift correlation spectroscopy ($^1H-^{19}F$ COSY). Recently, Hughes et al. [13] reported detection of long-range $^1H-^{19}F$ couplings in fluorocorticosteroids using a ^{19}F-detected heteronuclear equivalent of the COSY pulse sequence. Interunit $^{13}C-^{19}F$ couplings were corroborated by $^{13}C\{^1H\}\{^{19}F\}$ triple-resonance techniques. We also carried out several conventional homo and heteronuclear ($^1H\{^{19}F\}$) nuclear Overhauser effect (NOE) experiments to evaluate the conformational information obtained with the through-space $^1H-^{19}F$ and $^{13}C-^{19}F$ couplings.

2. Results and discussion

Interunit $^1H-^{19}F$ and $^{13}C-^{19}F$ couplings of 5-deoxy-5-fluoroarbekacin (**1**) *[12] in D_2O.*—Proton chemical shifts, assigned by the COSY [14] spectrum, and coupling

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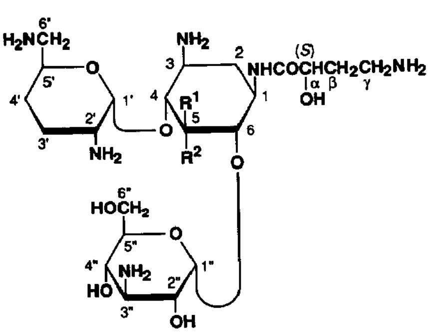
* { } Denotes irradiated spin.

Table 1
^1H NMR spectral data for **1** (DCl salt, pD 6.96) in D_2O at 500 MHz at 49°C

^1H	δ (ppm)		$J_{H,H}$ and $J_{H,F}$ (Hz)
1	~ 4.12	[a]	[b]
2*ax*	1.74	q	$J_{1,2ax} = J_{2ax,2eq} = J_{2ax,3} = 12.5$ [b]
2*eq*	2.12	dt	$J_{1,2eq} = J_{2eq,3} = 4$
3	3.27	ddd	$J_{3,4}$ 10 [b]
4	4.04	ddd	$J_{4,5}$ 9, $J_{4,F}$ 11
5	4.80	dt	$J_{5,6}$ 8.5, $J_{5,F}$ 50.5
6	~ 4.16	[a]	$J_{6,F}$ ~ 12
1′	5.55	d	$J_{1',2'}$ 3.5 [b]
2′	3.55	[a]	
3′*ax*	~ 2.05	[a]	
3′*eq*	~ 2.05	[a]	
4′*ax*	1.62	[a]	
4′*eq*	1.94	[a]	
5′	4.23	ddt	$J_{4'ax,5'}$ 11, $J_{4'eq,5'}$ 3, $J_{5',6'a}$ 7.5, $J_{5',6'b}$ 3
6′a	3.11	dd	$J_{6'a,6'b}$ 13.5
6′b	3.27	dd	
1″	5.13	d	$J_{1'',2''}$ 4
2″	3.77	dd	$J_{2'',3''}$ 11
3″	3.39	t	$J_{3'',4''}$ 10
4″	3.67	dt	$J_{4'',5''}$ 10, $J_{4'',F}$ 1
5″	3.93	ddd	$J_{5'',6''a}$ 4, $J_{5'',6''b} = 2.5$ [b]
6″a	3.79	dd	$J_{6''a,6''b}$ 12.5 [b]
6″b	3.83	dd	[b]
α	4.31	dd	J_{α,β_a} 9, J_{α,β_b} 4
β_a	1.99	ddt	J_{β_a,β_b} 14.5, $J_{\beta_a,\gamma_a} = J_{\beta_a,\gamma_b} = 7$
β_b	2.19	ddt	$J_{\beta_b,\gamma_a} = J_{\beta_b,\gamma_b} = 7$
$\gamma_{a,b}$	3.21	t	

[a] Not assignable in first order. [b] The presence of $J_{H,F}$ was detected in the ^1H-detected 2D $^1H-^{19}F$ COSY experiment.

constants ($J_{H,H}$ and $J_{H,F}$) of **1** are shown in Table 1. During the signal assignments, we found small splittings (J 1 Hz) of the triplet for H-4″ (Fig. 1a). At first, we could not imagine that F-5 caused the splittings because of the number of bonds between them (^8J). On the other hand, only geminal and vicinal couplings could be observed in the rather broadened 1D ^{19}F NMR spectrum of **1** (Table 4). The long-range $^1H-^{19}F$ coupling, however, was straightforwardly borne out by the $^1H\{^{19}F\}$ experiment. The double triplet for H-4″ turned into a sharp triplet by irradiating the signal for F-5 (Fig. 1b). Then we applied the HMBC pulse sequence, proposed by Bax and Summers [15] for determining long-range $^1H-^{13}C$ connectivity, in order to detect other smaller interunit $^1H-^{19}F$ couplings. The pulse sequence employed for this purpose is shown in Fig. 2. Because of the nonexistence of one-bond correlations, we removed the low-pass J-filter from the original pulse sequence and added a presaturation pulse to suppress the HOD signal. The ^1H-detected 2D $^1H-^{19}F$ COSY spectrum of **1** revealed that interunit polarization transfer occurred from F-5 not only to H-4″ but also to H-1′ (^5J), H-5″



	R¹	R²
1	F	H
2	F	F
3	H	F

(^7J), H-6"a(^8J), and H-6"b (^8J) (Fig. 3). These couplings, except for $J_{\text{H-4}'',\text{F-5}}$, were too small to observe in the normal 1D ^{1}H NMR spectrum of **1**. It was very convenient that we could observe ^{1}H–^{19}F couplings ranging from less than 1 Hz to 50.5 Hz in the 2D spectrum by setting the duration of Δ_2 at 0.1667 s (J 3 Hz).

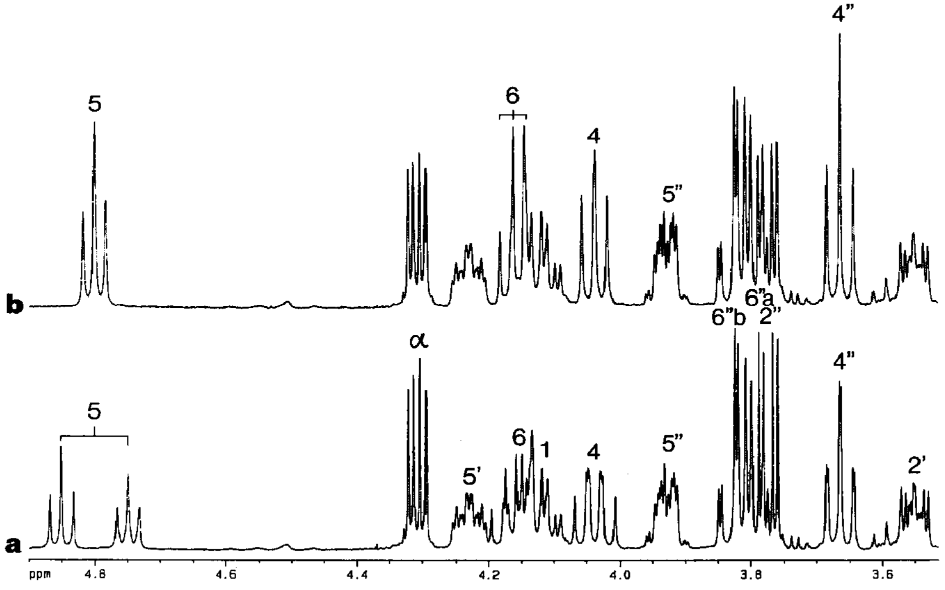


Fig. 1. ^{1}H{^{19}F} Experiment on **1** (DCl salt, pD 6.96) in D_2O, recorded at 500 MHz for ^{1}H, at 49°C with presaturation of the HOD signal. (a) Partial ^{1}H NMR spectrum and assignments. (b) ^{1}H{^{19}F} NMR spectrum, irradiating F-5.

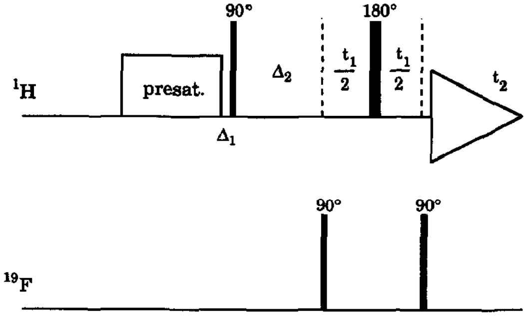


Fig. 2. The pulse sequence for ^{1}H-detected 2D ^{1}H–^{19}F COSY spectra with presaturation of the HOD signal, where Δ_1 is the delay for power switching (23 μs) and $\Delta2$ (1/2 J) is the delay for evolution of ^{1}H–^{19}F couplings.

Carbon-13 chemical shifts, assigned by the ^{1}H-detected ^{1}H–^{13}C chemical shift correlation spectrum (HMQC [16]), along with coupling constants ($J_{C,F}$) of **1** are shown in Table 5. The interunit ^{13}C–^{19}F couplings were observed at the signals for C-1′ (^4J 8.0 Hz) and C-5″ (^6J 5.4 Hz) in the ^{13}C{^{1}H} spectrum. We utilized

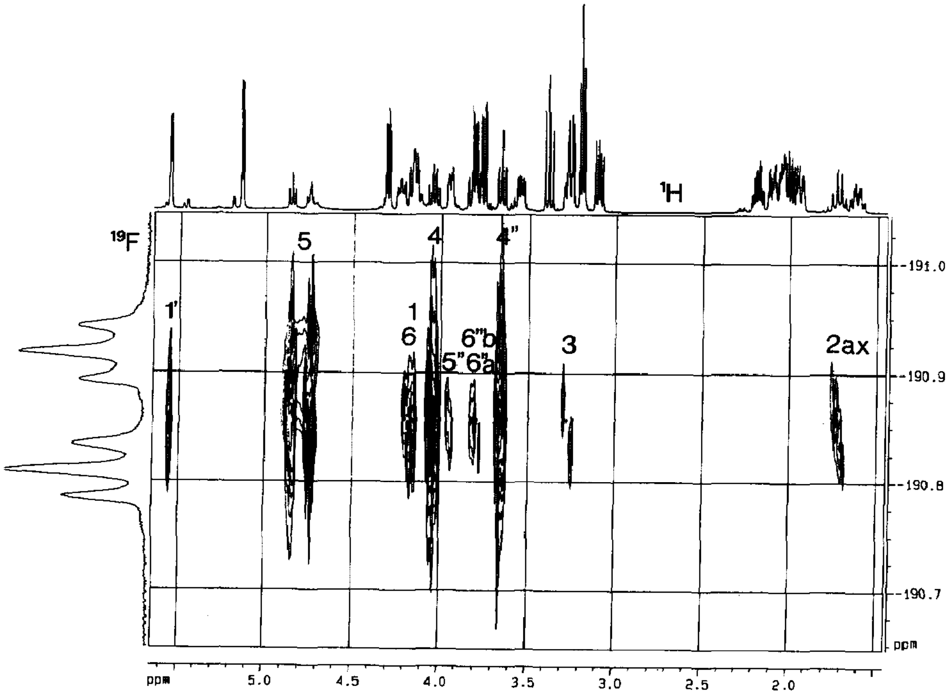


Fig. 3. ^{1}H-Detected 2D ^{1}H–^{19}F COSY spectrum of **1** (DCl salt, pD 6.96) in D_2O, recorded at 500 MHz for ^{1}H, with presaturation of the HOD signal (Δ_2 = 0.1667 s). The high-field triplet of H-5 is distorted by the presaturation.

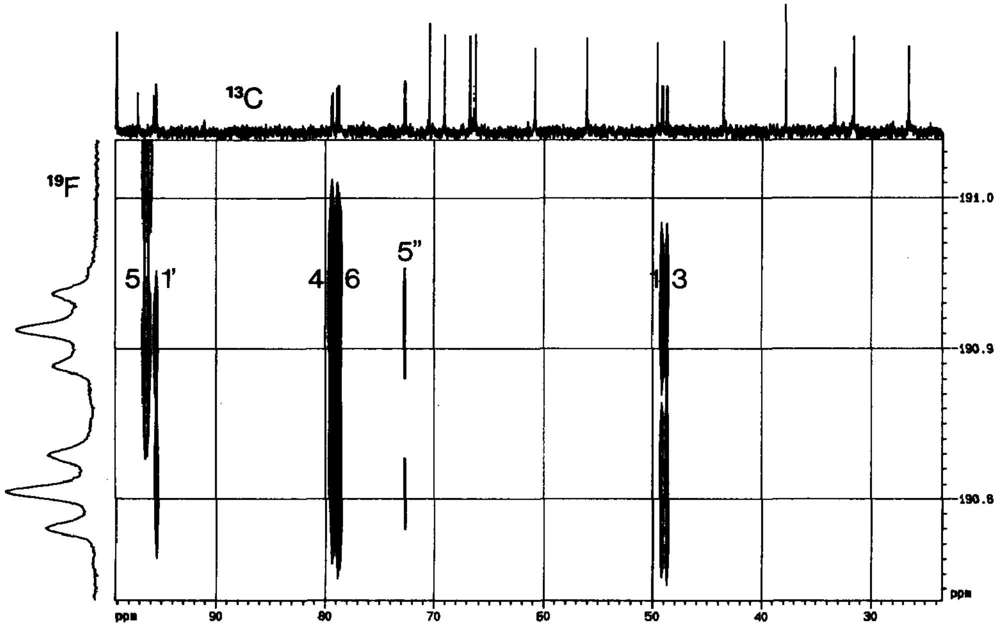


Fig. 4. ^{13}C-Detected 2D ^{13}C–^{19}F COSY spectrum of **1** (DCl salt, pD 6.96) in D_2O, recorded at 126 MHz for ^{13}C, with ^{1}H- and ^{19}F-decoupling. Two fixed delays were set at 0.025 and 0.0125 s.

^{13}C{^{1}H}{^{19}F} triple-resonance techniques to confirm these couplings. All of the doublets ($J_{C,F}$ 5.4–180.8 Hz) observed in the ^{13}C{^{1}H} spectrum successfully collapsed to singlets upon irradiation of the signal for F-5.

Since we had succeeded in detecting very small ^{1}H–^{19}F couplings of **1** by the ^{1}H-detected 2D ^{1}H–^{19}F COSY spectrum, we tried triple-resonance ^{13}C-detected 2D ^{13}C–^{19}F COSY experiments. The pulse sequence for COLOC [17] was modified by substituting ^{19}F-pulses for ^{1}H-pulses. Simultaneous ^{19}F- and ^{1}H-decouplings were made during acquisition. As shown in Fig. 4, we could detect all correlations for the ^{13}C doublets, but there were no other couplings observed.

The Dreiding model of **1** indicates that it is possible to arrange F-5 close to H-1′ and H-5″. Therefore, it was considered that the through-space mechanism must be playing an important role in the interunit couplings of F-5 with H-1′ and H-5″, and C-1′ and C-5″. As Sardella et al. [6] described for fluoropolycyclic aromatic hydrocarbons, these interunit couplings could be caused by overlapping of an F-5 lone pair and the C-1′–H-1′ as well as C-5″–H-5″ bond orbital. This hypothesis can explain why the coupling between F-5 and H-1′(^{5}J) (C-1′(^{4}J)) exists, while there are no couplings between F-5 and H-1″ (^{5}J) (C-1″(^{4}J)). The lack of coupling between F-5 and C-2 (^{4}J) also support the hypothesis. A small contribution of a through-bond mechanism for the coupling between F-5 and H-1′, however, could not be denied, because the 2D ^{1}H–^{19}F COSY spectra (Fig. 3) showed coupling between F-5 and H-2*ax* (^{5}J). The couplings of F-5 with H-4″, H-6″a, and H-6″b could be interpreted by a through-space and through-bond two-step mechanism.

Table 2
^{1}H NMR spectral data for **2** (DCl salt, pD 4.87) in D_2O at 500 MHz

^{1}H	δ (ppm)		$J_{H,H}$ and $J_{H,F}$ (Hz)
1	4.26	dt	$J_{1,2ax}$ 12, $J_{1,2eq}$ 4.5, $J_{1,6}$ 11[b]
2*ax*	~2.03		overlapped with H-3′*ax* [b,c]
2*eq*	2.37	dt	$J_{2eq,3}$ 4.5, $J_{2ax,2eq}$ 13
3	3.70		[b]
4	4.63	ddd	$J_{3,4}$ 10.5, $J_{4,F\text{-}5ax}$ 18.5, $J_{4,F\text{-}5eq}$ 1
6	4.44	ddd	$J_{6,F\text{-}5ax}$ 20, $J_{6,F\text{-}5eq}$ 3.5
1′	5.70	d	$J_{1',2'}$ 3.5 [c]
2′	3.63	ddd	$J_{2',3'ax}$ 12.5, $J_{2',3'eq}$ 5
3′*ax*	~2.07		overlapped with H-2*ax*
3′*eq*	~2.15	[a]	
4′*ax*	1.67	[a]	
4′*eq*	~1.98		overlapped with H-β_a
5′	4.20	[a]	
6′a	3.15	dd	$J_{5',6'a}$ 7, $J_{6'a,6'b}$ 13.5
6′b	3.32	dd	$J_{5',6'b}$ 3.5
1″	5.16	d	$J_{1'',2''}$ 3.5 [b]
2″	3.85	dd	$J_{2'',3''}$ 11
3″	3.44	t	$J_{3'',4''}$ 10
4″	3.70	t	$J_{4'',5''}$ 10 [c]
5″	3.93	ddd	$J_{5'',6''a}$ 5, $J_{5'',6''b}$ 2 [c]
6″a	3.81	dd	$J_{6''a,6''b}$ 12.5 [c]
6″b	3.86	dd	[c]
α	4.33	dd	J_{α,β_a} 9, J_{α,β_b} 4
β_a	~1.98		overlapped with H-4′*eq*
β_b	2.20	ddt	$J_{\beta_a,\beta_b} = 14$, $J_{\beta_b,\gamma_a} = J_{\beta_b,\gamma_b} = 7.5$
$\gamma_{a,b}$	3.20	t	$J_{\beta_a,\gamma} = 7.5$

[a] Not assignable in first order. [b,c] The presence of $J_{H,F\text{-}5ax}$ and $J_{H,F\text{-}5eq}$, respectively, was detected in the ^{1}H-detected 2D ^{1}H–^{19}F COSY experiment.

Interunit ^{1}H–^{19}F and ^{13}C–^{19}F couplings of 5-deoxy-5,5-difluoroarbekacin (**2**) *[12] in D_2O.*—Proton chemical shifts, assigned by the COSY spectrum, and coupling constants ($J_{H,H}$ and $J_{H,F}$) of **2** are shown in Table 2. There were no obvious interunit ^{1}H–^{19}F couplings appeared in the normal 1D ^{1}H NMR spectrum. Only vicinal ^{1}H–^{19}F couplings were observed at the signals for H-4 ($J_{H\text{-}4,F\text{-}5ax}$ 18.5, $J_{H\text{-}4,F\text{-}5eq}$ 1 Hz) and H-6 ($J_{H\text{-}6,F\text{-}5ax}$ 20, $J_{H\text{-}6,F\text{-}5eq}$ 3.5 Hz). On the other hand, the signal for F-5*eq* appeared as abroad doublet in the 1D ^{19}F NMR spectrum and gave no information regarding $J_{H,F\text{-}5eq}$ (Table 4). These couplings were confirmed by selective irradiation at the signals for F-5*eq* (Fig. 5b) and F-5*ax* (Fig. 5c). The ^{1}H-detected 2D ^{1}H–^{19}F COSY spectrum of **2** (Fig. 6) showed interunit couplings of F-5*eq* with H-1′, H-4″, H-5″, H-6″a, and H6″b as described for **1**. Therefore, **2** must have a conformation similar to that of **1**. The 2D spectrum also included correlations between F-5*ax* and H-1″ (^5J), F-5*ax* and H-2*ax* (^5J), and F-5*eq* and H-2*ax* (^5J). According to the Dreiding model of **2**, F-5*ax* is not in the vicinity of H-1″ in the conformation derived above. Therefore a through-bond mechanism probably contributes to $J_{H\text{-}1'',F\text{-}5ax}$.

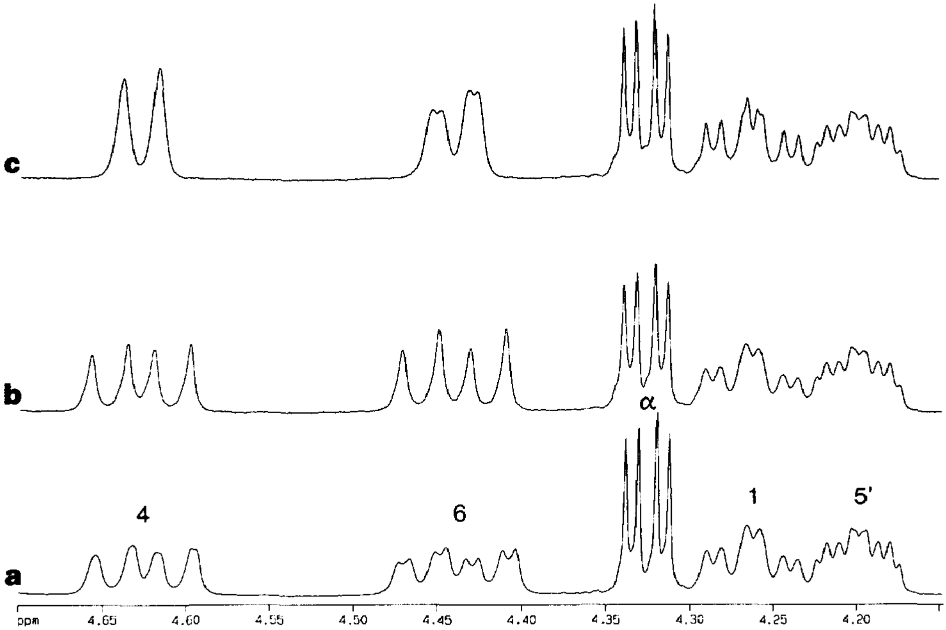


Fig. 5. $^{1}H\{^{19}F\}$ Experiments on **2** (DCl salt, pD 4.87) in D_2O, recorded at 500 MHz for ^{1}H, with presaturation of the HOD signal. (a) Partial ^{1}H NMR spectrum and assignments. (b) $^{1}H\{^{19}F\}$ NMR spectrum, irradiating F-5*eq*. (c) $^{1}H\{^{19}F\}$ NMR spectrum, irradiating F-5*ax*.

Carbon-13 chemical shifts, assigned by the HMQC spectrum, and coupling constants ($J_{C,F}$) of 2 are shown in Table 5. The interunit $^{13}C-^{19}F$ couplings were observed at the signals for C-1′ (^{4}J 9.4 Hz) and C-5″ (^{6}J 3.3 Hz) in the $^{13}C\{^{1}H\}$ spectrum (Fig. 7a). Selective ^{19}F-decoupling experiments ($^{13}C\{^{1}H\}\{^{19}F\}$) to determine the coupling fluorine were successfully achieved using continuous-wave irradiation. When F-5*eq* was irradiated, the doublets for C-1, C-3, C-1′, and C-5″ turned into singlets as mentioned for **1**, indicating that these ^{13}C nuclei couple with F-5*eq* (Fig. 7b). In contrast with the foregoing results, the signals for C-1, C-3, C-1′, and C-5″ remained doublets when F-5*ax* was irradiated (Fig. 7c). The triplets for C-4, C-5, and C-6 turned into doublets in both of the $^{13}C\{^{1}H\}\{^{19}F\}$ spectra. The contribution of a through-space mechanism must be large for $J_{C-1',F-5eq}$ and $J_{C-5'',F-5eq}$ as described for **1**.

In order to investigate interunit couplings of F-5*ax*, ^{1}H, ^{19}F, and ^{13}C NMR spectra of 5-deoxy-5-epifluoroarbekacin (**3**) [18] were recorded and assigned (Table 3, 4, and 5, respectively). The ^{1}H-detected $^{1}H-^{19}F$ COSY spectrum of **3** showed interunit couplings of F-5 with H-1′ and H-1″. No interunit $^{13}C-^{19}F$ couplings, however, were observed for **3**. Therefore, it was concluded that F-5*ax* of **3** is not in the vicinity of H-1′ and H-5″ as well as F-5*ax* of **2**.

NOE spectra of **1** *and* **2**.—In order to evaluate the conformational information obtained by interunit, through-space $^{1}H-^{19}F$ and $^{13}C-^{19}F$ spin–spin couplings,

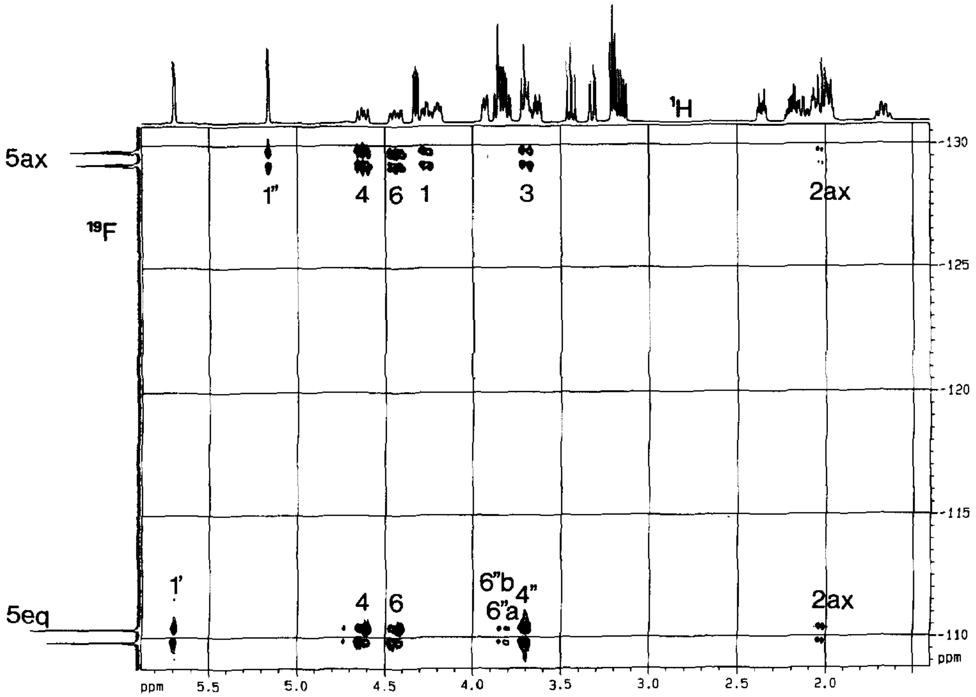


Fig. 6. ^{1}H-Detected 2D ^{1}H–^{19}F COSY spectrum of **2** (DCl salt, pD 4.87) in D_2O, recorded at 500 MHz for ^{1}H, with presaturation of the HOD signal ($\Delta_2 = 0.2$ s). The correlation between H-5″ and F-5*eq* appeared at lower contour slices.

NOE studies were carried out. The ROESY [19] spectrum of **1** showed interunit correlations regarding only H-4–H-1′ and H-6–H-1″. The Dreiding model indicates that these protons can exist near each other in the conformation derived above. The heteronuclear ^{1}H{^{19}F} NOE difference spectra supported the conformation more precisely. When F-5 of **1** was preirradiated, the spectrum afforded significant enhancements to the signals for H-1′ and H-5″, indicating that F-5 is spatially close to both of these protons (Fig. 8b). The small enhancement of the H-1″ signal could be explained by an indirect pathway via H-4. The same conclusion was drawn for **2** from the ^{1}H{^{19}F} NOE difference spectrum obtained by preirradiation at the signal for F-5*eq* (Fig. 9b). Only small enhancements on the signals for H-1′ and H-5″ were observed when F-5*ax* was preirradiated (Fig. 9c). These small enhancements could be caused indirectly via F-5*eq*.

All of the interunit ^{1}H–^{19}F and ^{13}C–^{19}F spin–spin couplings and NOEs of **2** are displayed in Fig. 10. It is noteworthy that the interunit spin–spin couplings include the information for protons spatially far from F-5*eq*, which could not be obtained by NOE experiment. Therefore, it was proved that detection of such interunit correlations by utilizing ^{1}H-detected 2D ^{1}H–^{19}F COSY spectra and ^{13}C/^{1}H/^{19}F triple-resonance techniques, such as ^{13}C-detected 2D ^{13}C–^{19}F COSY spectra and

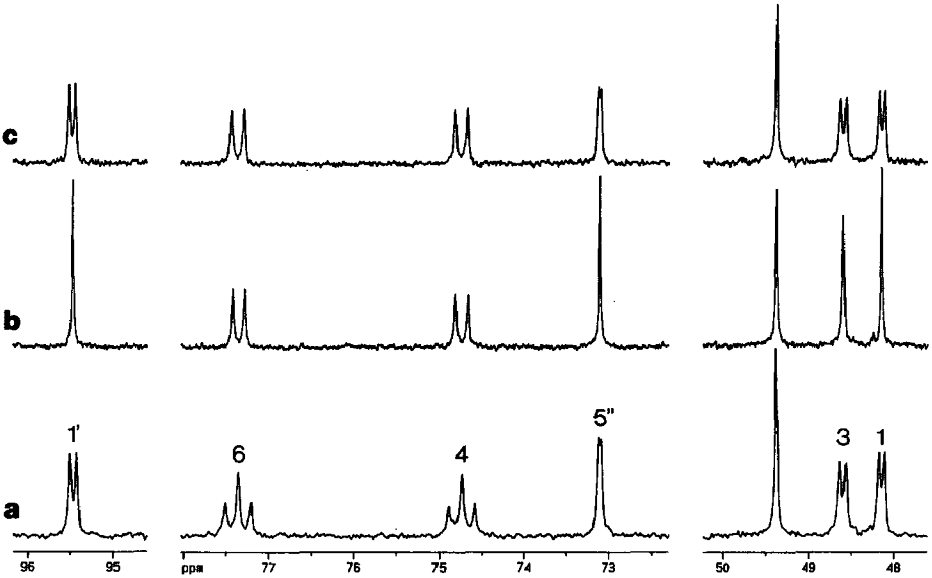


Fig. 7. $^{13}C\{^1H\}\{^{19}F\}$ Triple-resonance experiments on **2** (DCl salt, pD 4.87) in D_2O, recorded at 126 MHz for ^{13}C, with ^1H-broadband decoupling. Expanded regions in (a) $^{13}C\{^1H\}$ NMR spectrum, (b) $^{13}C\{^1H\}\{^{19}F\}$ NMR spectrum, irradiating F-5*eq*, and (c) $^{13}C\{^1H\}\{^{19}F\}$ NMR spectrum, irradiating F-5*ax*.

1D $^{13}C\{^1H\}\{^{19}F\}$ spectra, offer the possibility for additional conformational information to the results obtained by conventional NOE experiments.

3. Experimental

Sample preparation.—To a solution of **1** (52.4 mg, carbonate) in D_2O (0.4 mL, MSD Isotopes) was added 20% DCl in D_2O (Merck) until the solution showed pD ~ 1. After bubbling Ar through for a few minutes to eliminate CO_2 in the solution, NaOH in D_2O and diluted DCl in D_2O (if necessary) were used to adjust the pD values to give ~ 0.6 mL of solution. The pD values were measured on a Toa ion meter IM-40S with a Toa pH combination electrode GS-5018S (3 × 180 mm). Argon was bubbled through again before measurement of spectra. Samples of **2** (51.1 mg) and **3** (25.8 mg) were prepared in a similar manner.

NMR spectra.—All spectra were recorded on a Bruker AMX 500 spectrometer equipped with a 5-mm $^{13}C/^1H/^{19}F$ triple-resonance probe operating at 500.13 MHz for ^1H, 470.54 MHz for ^{19}F, and 125.77 MHz for ^{13}C. The sample temperature was maintained at 30°C, unless otherwise stated. Proton chemical shifts were reported in ppm relative to internal tetramethylsilane (Me_4Si). Fluorine-19 chemical shifts were reported in ppm relative to external 3% $CFCl_3$ in CCl_4. Carbon-13 chemical shifts were reported in ppm relative to Me_4Si using 1,4-dioxane (δ 67.4)

Table 3
^{1}H NMR spectral data for **3** (DCl salt, pD 4.20) in D_2O at 500 MHz

^{1}H	δ (ppm)		$J_{H,H}$ and $J_{H,F}$ (Hz)
1	4.38	dt	$J_{1,2ax}$ 12.5, $J_{1,2eq}$ 4, $J_{1,6} = 11$ [b]
2*ax*	1.94	q	$J_{2ax,2eq} = J_{2ax,3} = 12.5$ [b]
2*eq*	2.40	dt	$J_{2eq,3}$ 4
3	3.87		overlapped with H-2″ [b]
4	4.33		overlapped with H-1 and α, $J_{4,F}$ 28
5	5.62	br d	$J_{5,F}$ 52
6	4.19	br dd	$J_{6,F}$ 28
1′	5.53	d	$J_{1',2'}$ 3.5 [b]
2′	3.65		overlapped with H-4″
3′*ax*	~ 2.11	[a]	
3′*eq*	~ 2.15	[a]	
4′*ax*	2.66	dq	$J_{3'eq,4ax} = 4$, $J_{3'ax,4'ax} = J_{4'ax,4'eq} = J_{4'ax,5'} = 12.5$
4′*eq*	~ 1.98		overlapped with H-2*ax* and β_a
5′	4.17		overlapped with H-6
6′a	3.14	dd	$J_{5',6'a}$ 8, $J_{6'a,6'b}$ 14
6′b	3.33	dd	$J_{5',6'b}$ 3
1″	5.23	d	$J_{1'',2''}$ 3.5 [b]
2″	3.87		overlapped with H-3
3″	3.45	t	$J_{2'',3''} = J_{3'',4''} = 10.5$
4″	3.65	t	$J_{4'',5''}$ 10.5
5″	~ 3.96	[a]	
6″a	3.76	dd	$J_{5'',6''a}$ 7, $J_{6''a,6''b}$ 12
6″b	~ 3.98	[a]	
α	~ 4.35		overlapped with H-1 and 4
β_a	~ 2.02		overlapped with H-4′*eq*
β_b	2.22	[a]	
$\gamma_{a,b}$	3.22	t	$J_{\beta_a,\gamma} = J_{\beta_b,\gamma} = 7$

[a] Not assignable in first order. [b] The presence of $J_{H,F}$ was detected in the ^{1}H-detected 2D ^{1}H–^{19}F COSY experiment.

Table 4
^{19}F NMR spectral data for **1** (DCl salt, pD 6.96), **2** (DCl salt, pD 4.87), and **3** (DCl salt, pD 4.20) in D_2O at 471 MHz

	1	**2**	**3**
(ppm)			
F-5*ax*		− 129.54 (dt)	− 216.99 (dt)
F-5*eq*	− 190.86 (dt)	− 110.13 (d)	
(Hz)			
$J_{H\text{-}4,F\text{-}5ax}$		18.5	28
$J_{H\text{-}4,F\text{-}5eq}$	11	1 [a]	
$J_{H\text{-}5,F\text{-}5ax}$			52
$J_{H\text{-}5,F\text{-}5eq}$	50.5		
$J_{H\text{-}6,F\text{-}5ax}$		20	28
$J_{H\text{-}6,F\text{-}5eq}$	12	3.5 [a]	
$J_{H\text{-}4'',F\text{-}5eq}$	1 [a]		
$J_{F\text{-}5ax,F\text{-}5eq}$		248	

[a] Observable only in ^{1}H NMR spectra.

Table 5
^{13}C NMR spectral data for **1** (DCl salt, pD 6.96), **2** (DCl salt, pD 4.87), and **3** (DCl salt, pD 4.20) in D_2O at 126 MHz

^{13}C	**1**		**2**			**3**	
	δ (ppm)	$J_{C,F}$ (Hz)	δ (ppm)	$J_{C,F_{ax}}$ (Hz)	$J_{C,F_{eq}}$ (Hz)	δ (ppm)	$J_{C,F}$ (Hz)
1	49.22	11.0	48.18		8.2	47.02	4.8
2	33.44		30.23			30.56	
3	48.75	11.1	48.64		8.9	48.29	5.1
4	79.41	16.0	74.78	19.4	19.4	72.79	17.8
5	96.47	180.8	120.58	252.1	252.1	87.90	182.4
6	78.88	18.2	77.40	19.4	19.4	78.79	17.1
1′	95.51	8.0	95.50		9.4	91.06	
2′	49.70		49.41			49.08	
3′	21.65		21.08			21.67	
4′	26.64		26.31			26.43	
5′	66.39		66.92			66.75	
6′	43.62		43.45			43.55	
1″	99.10		99.99			100.42	
2″	69.09		68.71			68.77	
3″	56.15		56.04			56.11	
4″	66.77		66.41			67.07	
5″	72.74	5.4	73.16		3.3	73.41	
6″	60.87		60.85			61.53	
C=O	176.41		176.61			176.68	
α	70.50		70.47			70.55	
β	31.71		31.67			31.70	
γ	37.88		37.88			37.89	

as the internal reference. Spectral simulations and determinations of the sign of couplings were not carried out.

^{1}H NMR spectra were acquired in 8 scans over a 5050-Hz spectral width in 16K data points (acquisition time 1.622 s) with presaturation (3 s) of the HOD signal. The pulse width was 12.0 μs (49° flip angle). The free induction decays (FIDs) were Fourier transformed without any resolution enhancement.

COSY spectra were recorded in the absolute value mode using the pulse sequence: presaturation $-90°-t_1-57°-$FID. A 2.0-s presaturation pulse was used. The 90° pulse width was 22.0 μs. Spectra were obtained in 8 scans for each of the 256 FIDs which contained 2K data points in F_2 over a 5050 Hz spectral width. Zero-filling in the F_1 dimension produced a 1024×1024 data matrix. During 2D Fourier transformation, a sine-bell window function was applied to both dimensions. The transformed data were subsequently symmetrized.

^{19}F NMR spectra were acquired at first in 48 scans over a 125-kHz spectral width in 32K data points including the signal of $CFCl_3$. The pulse width was 18.0 μs (35° flip angle). A 2.0-s relaxation delay was used. Then chemical shifts and coupling constants were measured from spectra obtained with narrowed spectral width (5500 Hz for **1** and **3**, and 15600 Hz for **2**). The FIDs were Fourier transformed without any resolution enhancement.

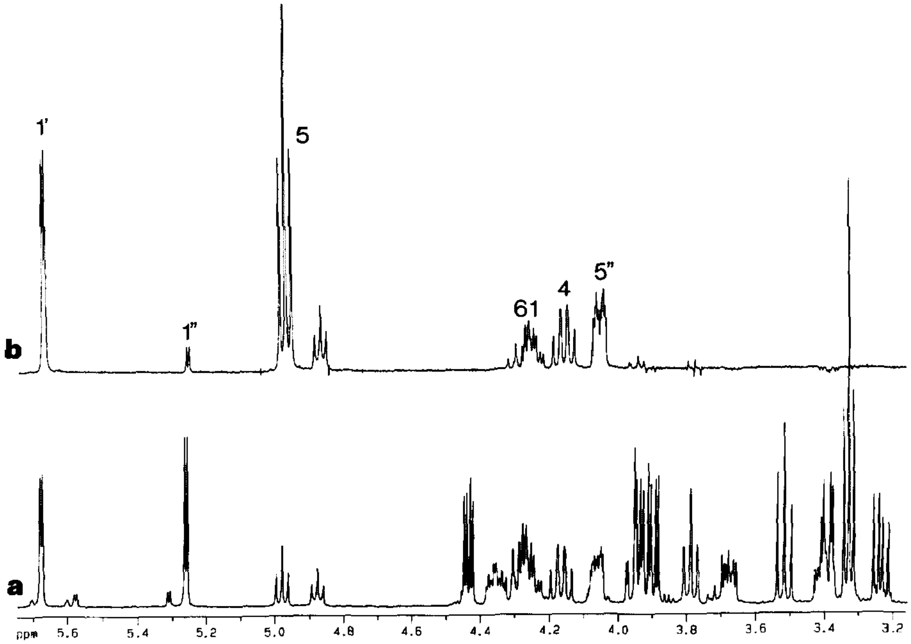


Fig. 8. $^1H\{^{19}F\}$ NOE experiment on **1** (DCl salt, pD 6.96) in D_2O, recorded at 500 MHz for ^1H, at 49°C with presaturation of the HOD signal. (a) Partial ^1H NMR spectrum, (b) $^1H\{^{19}F\}$ NOE difference spectrum, irradiating F-5. The intensity of the high-field triplet for H-5 was reduced by the presaturation.

$^1H\{^{19}F\}$ NMR spectra were taken with the parameters used for ^1H spectra except for ^{19}F decoupling using continuous-wave irradiation at the center of the ^{19}F signals during acquisition.

^1H-Detected 2D $^1H-^{19}F$ COSY spectra were obtained employing the pulse sequence in Fig. 2. A 2.0-s presaturation pulse was used to suppress the HOD signal. The 90° pulse width for ^1H and ^{19}F were 23.6 and 46.0 μs, respectively. The phase cycling used was the same as that of HMBC. Spectra were acquired in 80 scans for each of the 128 FIDs (256 FIDs for **2**) which contained 2K data points in F_2 over a 5050-Hz spectral width. During 2D Fourier transformation, a sine-bell squared window function was applied to both dimensions to produce a 256 × 1024 data matrix.

$^{13}C\{^1H\}$ NMR spectra were measured in 800 scans or more over a 33.3-kHz spectral width in 32K data points (acquisition time 0.492 s). The pulse width was 7.0 μs (60° flip angle). A 2.0-s relaxation delay was used. ^1H Broadband decoupling was carried out using WALTZ 16. The FIDs were processed using exponential multiplication (line broadening 2.0 Hz) for resolution enhancement.

$^{13}C\{^1H\}\{^{19}F\}$ NMR spectra were obtained with the parameters used for $^{13}C\{^1H\}$ spectra except for simultaneous ^{19}F-decoupling using continuous-wave irradiation at the center of the ^{19}F signals during acquisition.

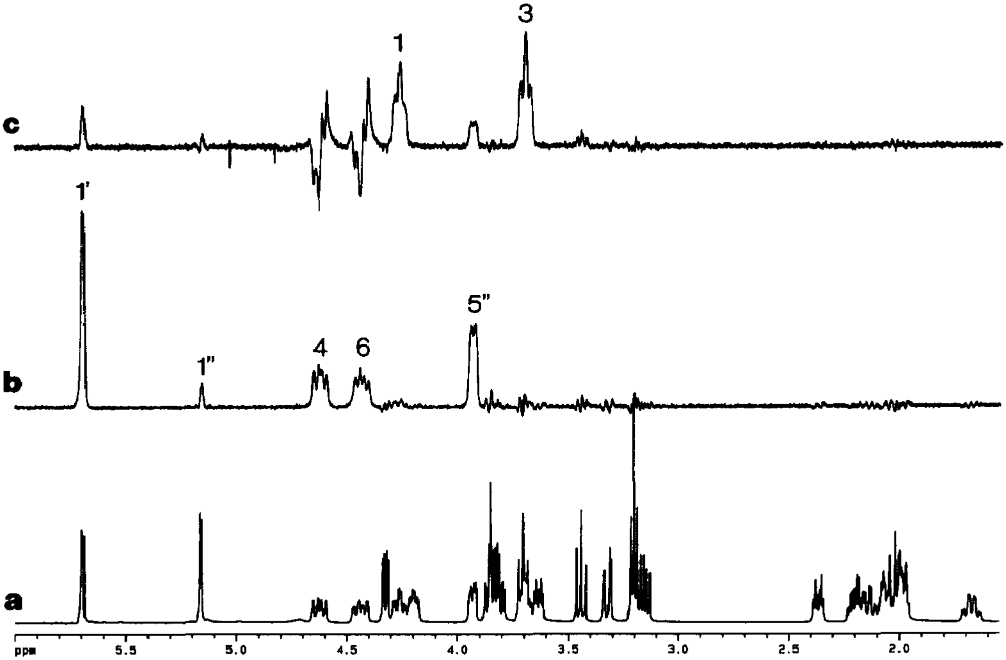


Fig. 9. ^{1}H{^{19}F} NOE experiments on **2** (DCl salt, pD 4.87) in D_2O, recorded at 500 MHz for ^{1}H, with presaturation of the HOD signal. (a) Partial ^{1}H NMR spectrum. (b) ^{1}H{^{19}F} NOE difference spectrum, irradiating F-5*eq*. (c) ^{1}H{^{19}F} NOE difference spectrum, irradiating F-5*ax*. Spectra b and c were obtained without spinning the sample.

^{13}C-Detected 2D ^{13}C–^{19}F COSY spectra were recorded using a modified version of the COLOC pulse sequence. ^{19}F-Pulses were substituted for ^{1}H-pulses. Simultaneous ^{1}H- and ^{19}F-decouplings were made using WALTZ 16 during

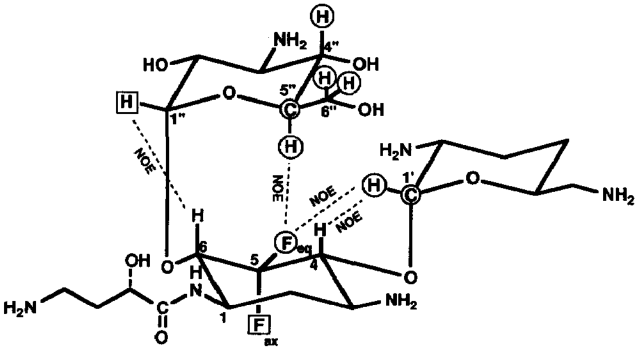


Fig. 10. Interunit, through-space ^{1}H–^{19}F and ^{13}C–^{19}F spin–spin couplings and NOEs of **2**: Ⓗ and $\boxed{H}$ denote protons coupling with F-5*eq* and F-5*ax*, respectively; Ⓒ denotes carbons coupling with F-5*eq*.

acquisition. The spectrum was taken in 340 scans for each of the 128 FIDs which contained 2K data points in F_2. During 2D Fourier transformation, a shifted since-bell squared window function was applied to both dimensions. Zero-filling and inversion in F_1 resulted in a 1024 × 1024 data matrix.

HMQC spectra were acquired using the standard pulse sequence incorporating presaturation of the HOD signal during relaxation delay (2.0 s) and inversion recovery (0.408 s) after a BIRD pulse. ^{13}C-Decoupling using GARP was also employed during acquisition. Spectra were taken in 8 scans for each of the 256 FIDs which contained 2K data points in F_2. Fixed delays used for the BIRD pulse, polarization transfer, and refocusing were set at 0.0035 s ($1/2\ J_{C,H}$). During 2D Fourier transformation, a shifted sine-bell squared window function was applied to both dimensions to produce a 256 × 1024 data matrix.

ROESY spectra were measured using the standard pulse sequence incorporating presaturation (2.0 s) of the HOD signal. The 90° pulse width was 22.0 s. A spin-locking field was applied for 0.24 s. Spectra were acquired in 128 scans for each of the 256 FIDs which contained 2K data points in F_2. During 2D Fourier transformation a shifted sine-bell squared window function was applied to both dimensions. Zero-filling in F_1 resulted in a 1024 × 1024 data matrix.

^{1}H{^{19}F} NOE difference spectra were acquired using a modified pulse sequence incorporating presaturation during the relaxation delay (10.0 s). The standard microprogram for NOE difference spectra was modified as follows. Each original transmitter phase was made double. Each original receiver phase was made double and every second phase was shifted by 180°. Irradiation was carried out alternately on resonance and off resonance. Thus we obtained a difference FID every two scans. On-resonance irradiation of F-5 for **1** was done at the 6 peaks of the double triplet for 0.117 s each, 30 times in total for 1 scan. On-resonance irradiation of F-5*eq* for **2** was done alternately at the 2 peaks of the broad doublet for 0.110 s each, 36 times in total for 1 scan. On-resonance irradiation of F-5*ax* for **2** was done in a similar manner as described for F-5 for **1**. Spectra were acquired in 3200 scans in 16K data points. The FIDs were Fourier transformed without resolution enhancement.

Acknowledgments

We are grateful to Dr. E. Umemura (Meiji Seika Kaisha, Ltd.) and Mr. T. Shitara (this institute) for their generosity in supplying the compounds used in this work. We express appreciation to Dr. Y. Kuroda, Dr. M.R. Wälchli, and Dr. M. Imachi (Bruker Japan Co., Ltd.) for helpful suggestions in designing the ^{13}C/^{1}H/^{19}F triple-resonance probe and in pursuing our experiments. Encouragement from Dr. T. Tsuchiya (this Institute) to carry out this work is gratefully acknowledged. We thank Ms. T. Yamaguchi and Mr. Y. Kobayashi for help in preparing the manuscript.

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