Synthesis, Antimicrobial Activity and *In Vivo* Fluorine NMR of a Hexafluorinated Derivative of Tilmicosin†

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A new fluorinated analog of tilmicosin was synthesized by the reductive amination of desmycosin with 3,5-bis(trifluoromethyl)piperidine. Despite an apparently small change in structure, the fluorinated analog had much less *in vitro* antimicrobial activity than tilmicosin and it failed to protect 3-day old chicks against a *Pasteurella multocida* challenge at 64 mg/kg sc. In a preliminary *in vivo* fluorine NMR experiment in a female Sprague-Dawley rat, a ^{19}F NMR signal was detected in the liver one hour after ip administration of the fluorinated compound. Therefore, although this fluorinated derivative had less antimicrobial activity than tilmicosin, it may nevertheless provide a suitable model of tilmicosin for pharmacokinetic studies using *in vivo* fluorine NMR.

The use of fluorine NMR (^{19}F NMR) as a tool to investigate the disposition of drugs *in vivo* is a potentially important method for the non-invasive study of pharmacokinetics. The low level of background fluorine found in the body allows the distribution of a fluorinated compound to be followed over time by repeated assays on the same animal, which need not be sacrificed to obtain the data. For this purpose, it is necessary to synthesize fluorinated analogs whose biological activity and pharmacokinetics accurately model the parent drug.

High tissue concentrations are desirable for obtaining a strong *in vivo* ^{19}F NMR signal. Macrolide antibiotics are known to achieve very high tissue concentrations within a short time after administration.[1] A convenient procedure to measure macrolide concentrations in tissues and organs and to follow their uptake, distribution, and elimination would provide a very valuable research tool. Such studies would complement existing methods and allow a more thorough understanding of macrolide penetration and accumulation throughout the body.

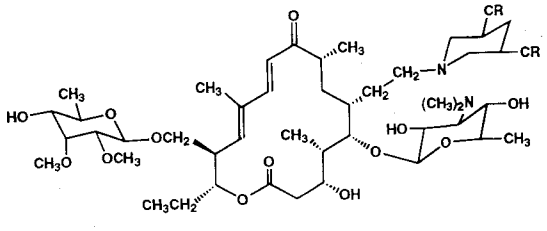
Tilmicosin (**1**) is a new macrolide antibiotic approved for treating respiratory disease in cattle.[2] In order to explore this biological application of spectoscopy, a fluorinated analog of tilmicosin was synthesized as a model compound for studying *in vivo* pharmacokinetics by ^{19}F NMR.††

20-Deoxo-20-(3,5-bis(trifluoromethyl)piperidinyl)-desmycosin (**2**) was selected as a convenient target for synthesis. The presence of six fluorine atoms per molecule would provide the advantage of a stronger ^{19}F NMR signal. In order to synthesize **2** from desmycosin (**4**), an efficient synthesis of 3,5-bis(trifluoromethyl)piperidine (**3**) was required.

Results

Attempts to prepare **3** from 2-hydrazino-3,5-bis(trifluoromethyl)pyridine (**5**) by first removing the hydrazine moiety by known oxidative methods using silver acetate or yellow mercuric oxide were unsuccessful in forming the intermediate 3,5-bis(trifluoromethyl)pyridine (**6**).[3,4] The literature reported that 2-chloropyridine derivatives could be dehalogenated over 5% palladium on carbon,

Fig. 1. The structures of tilmicosin (**1**; R=H_3) and its hexafluoro-analog (**2**; R=F_3).



† This publication is dedicated to Professor SATOSHI ŌMURA in honor of his 60th birthday.

†† Some of this material was presented at the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy, Orlando, Florida, October 1994, abstract number F170.

and pyridine derivatives could be hydrogenated to piperidine derivatives over platinum oxide.[5,6] We found that commercially available 2-chloro-3,5-bis(trifluoromethyl)pyridine (**7**) could be dehalogenated and hydrogenated to produce the desired product **3** in one step using a 1:10 mixed catalyst of 5% palladium on carbon and platinum oxide, respectively, in ethanol. This ethanolic solution of **3** was then mixed with desmycosin and treated with sodium cyanoborohydride to give the desired reductive amination product **2**.

The proton NMR spectrum of **2** was identical to that of tilmicosin except that the doublets at 0.80 and 1.00 ppm for the methyl groups of the dimethylpiperidinyl moiety were missing, and the multiplet at 0.50 ppm, which corresponds to one of the methylene protons at C-4 of the piperidinyl ring, had been shifted downfield.[7]

Fig. 2. Synthesis of 3,5-bis(trifluoromethyl)piperidine.

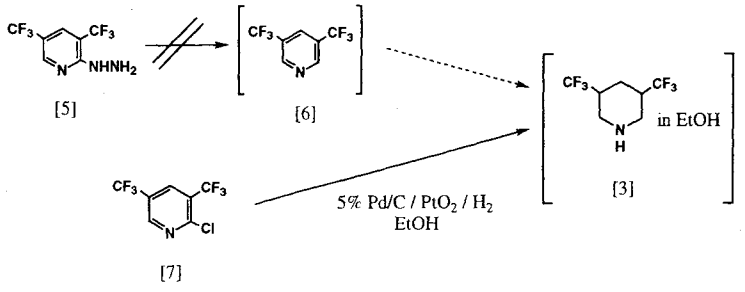


Fig. 3. Synthesis of hexafluoro-analog of trilmicosin.

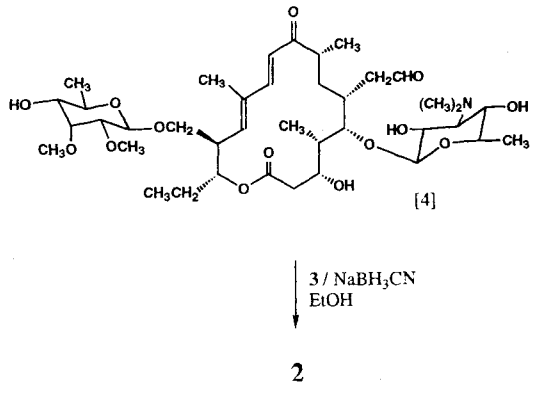


Fig. 4. The ^1H NMR (300 MHz) of tilmicosin (**1**) in $CDCl_3$ referenced to TMS.

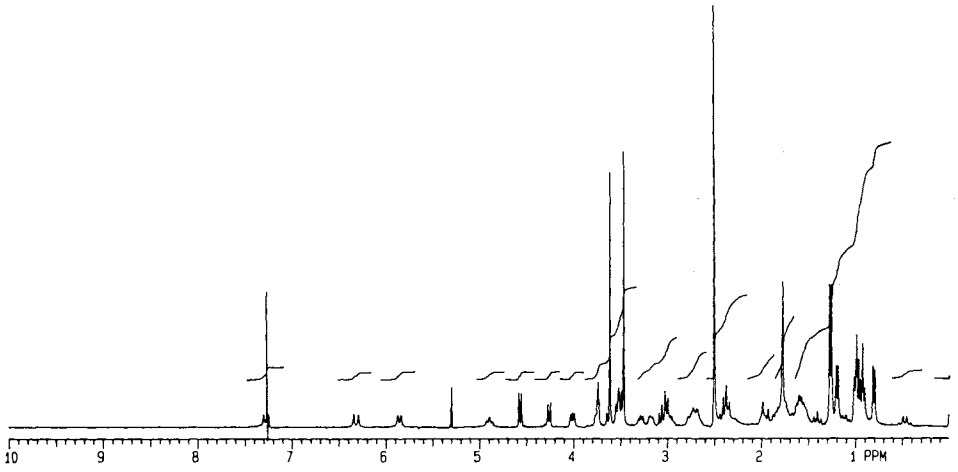


Fig. 5. The ^1H NMR (300 MHz) of hexafluoro-analog (**2**) of tilmicosin in $CDCl_3$ referenced to TMS.

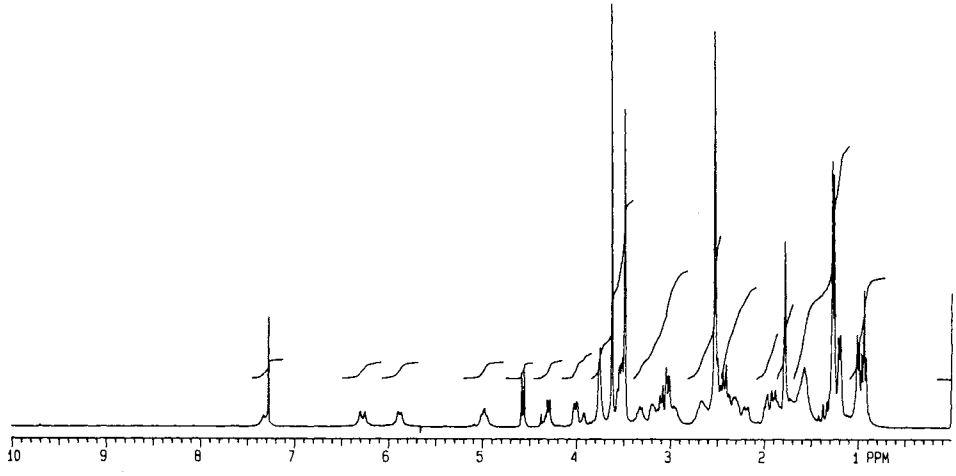


Fig. 6. The ^{19}F NMR (282 MHz) of hexafluoro-analog (**2**) of tilmicosin in water-ethanol (20:1 v/v).

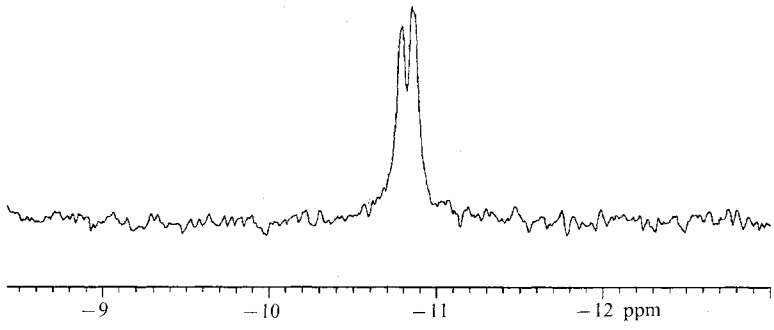


Table 1. *In vitro* MIC values (in μg/ml) for tilmicosin (**1**) and hexafluorotilmicosin (**2**).

Organism	Strain	(1)	(2)	Organism	Strain	(1)	(2)
Staphylococcus aureus	X1.1	<0.25	<0.25	*Pasteurella haemolytica*	128K	nd	8
	V41	<0.25	<0.25		129R	2	2
	X400	<0.25	<0.25		133A	nd	8
	F15R	nd	16	*P. multocida*	60A	6.25	32
	S13E	<0.25	<0.25		77G	nd	32
S. epidermidis	270	128	128		108E	2	64
	222	<0.25	<0.25		116E	nd	16
	F5C	nd	1		129M	bd	8
	92E	nd	0.5		1411	2	16
	F5R	<0.12	1	*Escherichia coli*	EC14	64	128
Streptococcus pyogenes	C203	1	16		TEM	4	128
S. pneumoniae	PARK	1	8		28C	>64	>256
Enterococcus faecium	X66	1	4	*Klebsiella pneumoniae*	X26	8	128
	2041	2	8		X68	128	128
Haemophilus influenzae	C.L.	2	32	*Salmonella* sp.	X514	32	128
	76	2	16		1335	64	128
Pasteurella haemolytica	115B	8	32		33C	64	>256
	119E	nd	16		66B	>64	>256

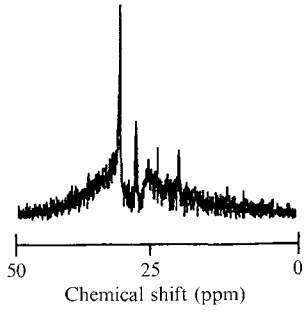
The mass spectrum and elemental analysis were both correct for **2**. The fluorine NMR showed a doublet at −10.8 ppm with a splitting of 17.5 Hz.

The fluorinated derivative **2** exhibited good activity against strains of staphylococci, but was less active than tilmicosin against streptococci and Gram-negative cocci. It was relatively inactive against *Enterobacteriaceae* and *Pseudomonas* species.

To explore how well **2** modeled the *in vivo* efficacy of tilmicosin, a disease challenge model with *P. multocida* strain 60A in 3-day old chicks was conducted. However, **2** failed to show any protection from the infection at 64 mg/kg sc while tilmicosin afforded protection at 32 mg/kg sc. This result was in accord with the *in vitro* MIC values which showed that **2** was only 1/5 as active as tilmicosin against *P. multocida* strain 60A. An *in vivo* challenge study against a more sensitive organism such as *S. aureus* needs to be conducted to more accurately assess the relative *in vivo* efficacy of **2** and tilmicosin.

Despite the lack of *in vivo* activity against the *Pasteurella* challenge, an initial experiment was performed to assess the feasibility of *in vivo* ^{19}F NMR spectroscopy. **2** was administered ip at 50 mg/kg in a 2% EtOH-saline buffer solution with a trace amount of tartaric acid to an anesthetized female Sprague-Dawley rat (approximately 250 mg body weight). The fluorine spectrum was acquired using a 2.7 cm diameter transmit/receive single turn surface coil. The pulse delay was set to 3 seconds and 256 transients were acquired. Exponential line broadening of 50 Hz was applied to the time domain data. Accumulation was recorded in the liver one hour post administration. Although the lineshape of the fluorine resonance was broadened and the resolution was reduced by paramagnetic susceptibility effects due to the naturally high levels of iron in the liver, a strong signal was received. No signals were detected in the *in vivo* ^{19}F NMR scan before the administration of **2**. Shift differences were noted between the *in vivo* ^{19}F NMR and the analytical ^{19}F NMR due to the use of different data acquisition parameters on different field

Fig. 7. The *in vivo* F NMR spectrum of **2** in the female Sprague-Dawley rat liver 1 hour post ip administration.



strength instruments.

In conclusion, a fluorinated analog of the antibiotic tilmicosin has been synthesized and detected *in vivo* by ^{19}F NMR spectroscopy. This successful preliminary experiment suggests that ^{19}F NMR may be a useful tool for non-invasive pharmacokinetic studies. Future studies are needed to look for detectable signals from other tissues and organs, to follow the signals for a longer time course, to determine concentrations, and to determine if **2** is an accurate pharmacokinetic model for tilmicosin.

Experimental

General Procedures

^1H NMR spectra were measured in $CDCl_3$ solution on a Varian Gemini-300 spectrometer and are scaled from internal TMS. The analytical ^{19}F NMR spectrum was measured in water-ethanol (20:1) solution on a 7 Tesla GE/Tecmag spectrometer at a frequency of 282 MHz for fluorine. The ^{19}F NMR *in vivo* spectrum was measured at 4.7 Tesla on a Spectroscopy Imaging Systems Corporation (SISCO) imager/spectrometer with a custom built single loop surface coil tuned to 188 MHz for fluorine. IR spectra were obtained on a Nicolet 510P Optical Bench spectrometer. UV spectra were measured on a Shimadzu UV-2101 PC Spectrophotometer. Field desorption mass spectra (FD-MS) were obtained on a VG ZAB-3F mass spectrometer. Elemental analyses were performed on a Control Equipment Corporation 440 elemental analyzer for carbon, hydrogen and nitrogen. Elemental analyses for fluorine were measured using a Dr. Hans Hosli, Model A-1 furnace, a Brinkmann 665 Dosimat titrator and a Brinkmann PC/100 colorimeter.

The preparation of desmycosin was carried out at the Lilly fermentation facilities at Indianapolis, IN, U.S.A. 2-Chloro-3,5-bis(trifluoromethyl)pyridine and sodium cyanoborohydride were obtained from Aldrich Chemical Co., Milwaukee, WI, U.S.A.. 2-Hyrazino-3,5-bis(trifluoromethyl)pyridine was obtained from Ryan Scientific Co., Columbia, SC, U.S.A.

Antibiotic susceptibility data were obtained by broth or agar dilution procedures (Table 1). Determination of *in vivo* activity against an infection caused by *Pasteurella multocida* strain 60A was conducted with 3-day old chicks which were acclimated overnight with *ad libitum* access to feed and water. After two days of observation, the birds were inoculated intramuscularly in the thigh with 0.1 ml of 10^7 cfu/ml *P. multocida* strain 60A (cultured in Columbia broth at 37°C). The birds were subcutaneously administered compounds **1** and **2** (solubilized in DMSO) at 1 and 4 hours post-challenge. The birds were observed through Day 10 post treatment.

20-Deoxo-20-(3,5-bis(trifluoromethyl)piperidinyl)-desmycosin (2)

To a solution of **7** (5.0 g, 20.04 mmol) in EtOH (140 ml) was added 5% palladium on carbon (0.5 g) and platinum oxide (5.0 g), and the suspension was placed under hydrogen gas (60 psi) at 60°C for 6 hours. The catalysts were then removed by filtration, and desmycosin (11.04 g, 14.0 mmol) was added. After stirring the mixture at room temperature for 45 minutes, $NaBH_3CN$ (1.18 g, 19.0 mmol) was added and the solution was stirred an additional 5 hours at room temperature. The solvent was evaporated at room temperature under vacuum, and the residue was chromatographed on silica gel, eluting with MeOH, to give crude **2** (14.6 g) as an off-white solid. A pure sample of **2** was obtained by suspending the crude product (9.58 g) in pH 2 buffer (400 ml) and filtering. The filtrate was then basified with 5 N NaOH, saturated with NaCl, and extracted with EtOAc. The EtOAc was washed with brine, dried ($MgSO_4$) and evaporated at room temperature to give pure **2** (4.01 g); FD-MS *m/z*, 977 (M^+); UV λ_{max} nm (ε) 282 (21,807); IR ($CHCl_3$) cm^{-1}, 3029, 2975, 2937, 2881, 1730, 1592, 1456, 1378, 1314, 1289, 1254, 1166, 1129, 1081, 1058, 1008, 985, 962; ^1H NMR ($CDCl_3$), Fig. 5.

Anal Calcd for $C_{46}H_{74}F_6N_2O_{13}$:
C 56.55, H 7.63, N 2.87, F 11.67
Found: C 56.82, H 7.88, N 2.95, F 9.39

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