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Solid state ^{19}F NMR parameters of fluorine-labeled amino acids. Part II: Aliphatic substituents

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Abstract

A representative set of amino acids with aliphatic ^{19}F-labels has been characterized here, following up our previous compilation of NMR parameters for single ^{19}F-substituents on aromatic side chains. Their isotropic chemical shifts, chemical shift tensor parameters, intra-molecular ^{19}F dipole–dipole couplings and temperature-dependent T_1 and T_2 relaxation times were determined by solid state NMR on twelve polycrystalline amino acid samples, and the corresponding isotropic ^{19}F chemical shifts and scalar couplings were obtained in solution. Of particular interest are amino acids carrying a trifluoromethyl-group, because not only the ^{19}F chemical shift but also the intra-CF_3 homonuclear dipolar coupling can be used for structural studies of ^{19}F-labeled peptides and proteins. The CF_3-groups are further compared with CH_2F-, CD_2F-, and CD_3-groups, using both ^{19}F and ^{2}H NMR to describe their motional behavior and to examine the respective linebroadening effects of the protonated and deuterated neighbors. We have also characterized two unnatural amino acids in which a CF_3-label is rigidly connected to the backbone by a phenyl or bicyclopentyl moiety, and which are particularly well suited for structure analysis of membrane-bound polypeptides. The ^{19}F NMR parameters of the polycrystalline amino acids are compared with data from the correspondingly labeled side chains in synthetic peptides.

Keywords: Flourine; Solid state NMR; Amino acid; ^{19}F chemical shift anisotropy

1. Introduction

For analyzing a solid compound by NMR it is usually essential to know the fundamental parameters of the nucleus at the site of interest, such as its chemical shift anisotropy and dipolar couplings in the static limit. In the preceding contribution we had outlined the advantages of ^{19}F-labelling for studying peptides and proteins, and had compiled the NMR parameters for aromatic amino acids with a single ^{19}F-substituent on the ring [1]. These aromatic groups can be readily incorporated into proteins biosynthetically, but their use in structural investigations may be complicated by the flexibility of the side chain and the lack of an axially symmetric electronic environment. Certain aliphatic ^{19}F-labels, on the other hand, can overcome this limitation, especially when carrying a CF_3-group. The advantage of this reporter-group over a single ^{19}F-substituent lies in its fast rotational averaging, which creates an environment that is axially symmetric to a first approximation, in contrast to most other ^{19}F CSA tensors. Furthermore, the CF_3-group provides structural information not only via its ^{19}F chemical shift anisotropy, but also via its intra-CF_3 homonuclear dipolar coupling [2–7]. The present compilation of ^{19}F NMR parameters is thus focused on

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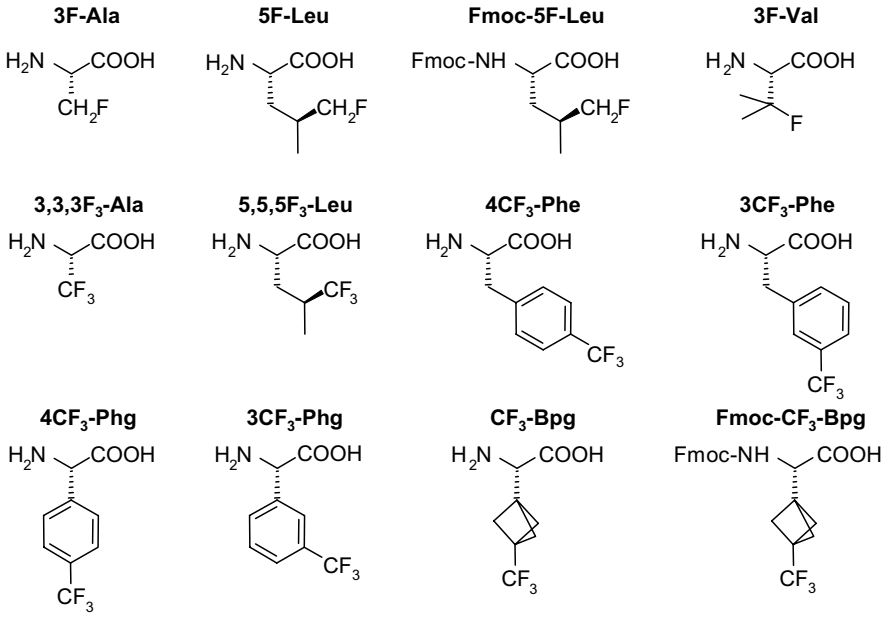

Fig. 1. Structures of the ^{19}F-labeled amino acids carrying an alipahtic ^{19}F-substituent: 3F-alanine (3F-Ala), 5F-leucine (5F-Leu), Fmoc-5F-leucine (Fmoc-5F-Leu), 3F-valine (3F-Val), 3,3,3F_3-alanine (3,3,3F_3-Ala), 5,5,5F_3-leucine (5,5,5F_3-Leu), 4CF_3-phenylalanine (4CF_3-Phe), 3CF_3-phenylalanine (3CF_3-Phe), 4CF_3-phenylglycine (4CF_3-Phg), 3CF_3-phenylglycine (3CF_3-Phg), 3-(trifluoromethyl)bicyclo-pent[1,1,1]-1-yl-glycine (CF_3-Bpg), Fmoc-3-(trifluoromethyl)bicyclo-pent[1,1,1]-1-yl-glycine (Fmoc-CF_3-Bpg). Shown are the L- or (2S,4S)-stereoisomers. Several deuterated leucine derivatives included in this study are not shown: 5,5,5D_3-leucine (5,5,5D_3-Leu) and 5F-5,5D_2-leucine (5F-5,5D_2-Leu).

aliphatic ^{19}F-segments, as illustrated in Fig. 1, with special attention to CF_3-labels in aliphatic and aromatic amino acids. The study includes two unnatural CF_3-labeled amino acids that have been specifically developed to describe the conformation, alignment and mobility of membrane-bound peptides, namely CF_3-labeled phenylglycine (Phg) and bicyclopentylglycine (Bpg) [4–8].

2. Materials and methods

2.1. Amino acids

All amino acids and their abbreviations are summarized in Fig. 1 (for stereoisomers see Table 1). The polycrystalline ^{19}F-labeled amino acids were used as supplied by the following commercial sources: 3F-Ala was purchased from Bachem (Bubendorf, Switzerland), 3,3,3F_3-Ala from Chempur (Karlsruhe, Germany), 3F-Val and 5,5,5F_3-Leu from Lancaster (now Alfa Aesar, Karlsruhe, Germany), 4CF_3-Phe and 4CF_3-Phg from ABCR (Karlsruhe, Germany). The leucine derivatives 5F-(2S,4S)-leucine (5F-Leu(2S,4S)), 5F-(2S,4R)-leucine (5F-Leu(2S,4R)), Fmoc-5F-(2S,4S)-leucine (Fmoc-5F-Leu) were synthesized by D. Young and J.-D. Charrier (University of Sussex, UK) as previously described [9,10], as was 5F-5,5D_2-(2S,4S)-leucine (5F-5,5D_2-Leu), while 5,5,5D_3-L-leucine (5,5,5D_3-Leu) was supplied from Cambridge Isotopes (Andover, MA, USA). 3-(Trifluoromethyl)bicyclo-pent[1,1,1]-1-yl-glycine (CF_3-Bpg) was synthesized as described [7,11], and 3CF_3-Phe was a gift from T. Asakura (Tokyo University, Japan).

2.2. NMR measurements

The static solid state NMR spectra and relaxation data were measured at 470.3 MHz ^{19}F resonance frequency and 76.8 MHz ^{2}H frequency on a Varian Unity Inova (Varian Inc., Palo Alto, CA, USA) and a Bruker Avance spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany). For solid state ^{19}F- and ^{2}H NMR, a quadruple-tuned (^{1}H/^{19}F/X/Y) 5 mm MAS probe, a double-tuned (^{1}H/^{19}F) 4 mm MAS probe, and a double-tuned (^{1}H/^{19}F) static flat-coil probe from Doty Scientific (Columbia, SC, USA) were used. Proton-decoupling up to 40 kHz was achieved with this hardware. The 90°-pulse length was between 2 and 3 μs for ^{19}F and 4.8 μs for ^{2}H observation. Static ^{1}H-decoupled ^{19}F NMR spectra were acquired using a Hahn echo or solid echo with a delay time of 25 μs, or following a single-pulse excitation, depending on the presence of strong homonuclear ^{19}F–^{19}F dipolar couplings (see discussion below). Most T_1 values were determined by inversion recovery (only in the case of very long T_1 by saturation recovery), and the T_2 data was measured from echo experiments. A temperature range from −60 to +60 °C was covered with an accuracy of ±2 °C. Unless stated otherwise, magic angle spinning experiments were acquired at 564.7 MHz ^{19}F resonance frequency on a Bruker Avance 600 MHz spectrometer, using a 4 mm ^{1}H/^{19}F/X MAS probe at 12.5 kHz spinning speed (Bruker Biospin GmbH, Rheinstetten, Germany). Liquid state ^{19}F NMR spectra were acquired at 470.3 MHz on a Varian Unity Inova widebore spectrometer using a 5 mm liquid state probe, or at 376 MHz on a Bruker standard bore DMX

spectrometer. All ^{19}F NMR spectra are referenced with $CFCl_3$ set to 0 ppm (using in the solid state NMR experiments as secondary standard 100 mM NaF at 25 °C, set to −119.5 ppm) [12,13].

2.3. NMR pulse sequences for CF_3-groups

CF_3-labels provide the opportunity to observe and analyze two different kinds of interactions as a source of structural information, namely the ^{19}F chemical shift anisotropy and the homonuclear intra-CF_3-group dipolar couplings. However, the presence of both interactions at comparable strengths makes it difficult to acquire undistorted spectra, which are needed to deconvolute the two contributions. That is because static solid state NMR spectra are usually acquired with echo experiments to overcome the problems associated with receiver dead-time and ringing. The difficulty with CF_3-groups is that different echoes are required to refocus either the CSA (Hahn echo: 90°-τ-180°-τ-acquisition) or the homonuclear dipolar coupling (solid echo: 90°-τ-90°-τ-acquisition). Each echo sequence is optimized to observe one specific interaction but will lead to strong distortions of the respective other interaction. To assess the feasibility of acquiring undistorted static spectra of CF_3-labeled amino acids, the lineshapes of 4CF_3-Phg were compared using different pulse sequences. Fig. 2 shows the ^{19}F NMR spectra obtained with a single-pulse experiment (Fig. 2a), a Hahn echo experiment (Fig. 2b) and a solid echo experiment (Fig. 2c). In addition, the solid echo sequence with EXORCYCLE phase cycling, as proposed by Antonijevic and Wimperis [14] to refocus both CSA and dipolar coupling simultaneously, was tested (Fig. 2d).

It is seen that application of the Hahn echo sequence to polycrystalline 4CF_3-Phg resulted in a distorted spectrum. The use of a solid echo did not show dispersive distortions, but yielded a comparatively featureless lineshape, in which especially the low-field edge is not sufficiently resolved. EXORCYCLE phase cycling did not improve the solid echo spectrum. Despite a dead time of approximately 5 μs, it thus turned out that single-pulse excitation yielded the best lineshape, which is free from distortions and contains all expected features of the CF_3-triplet in a powder spectrum. Note that an important prerequisite to obtain such a clean spectrum was the availability of very short 90°-pulses of ∼2 μs. All compounds containing a CF_3-group were therefore characterized using single-pulse excitation. Naturally, single-pulse excitation also performed well in the experiments where substantial line narrowing was achieved using magic angle spinning.

2.4. Data analysis

To characterize the chemical shift anisotropy, we used the parameters δ_{11}, δ_{22}, δ_{33}, Δ and η, as defined in the preceding Part I [1]. The isotropic chemical shift δ_{iso} and chemical shift anisotropy parameters of the monofluorine-substituted amino acids were determined from side-

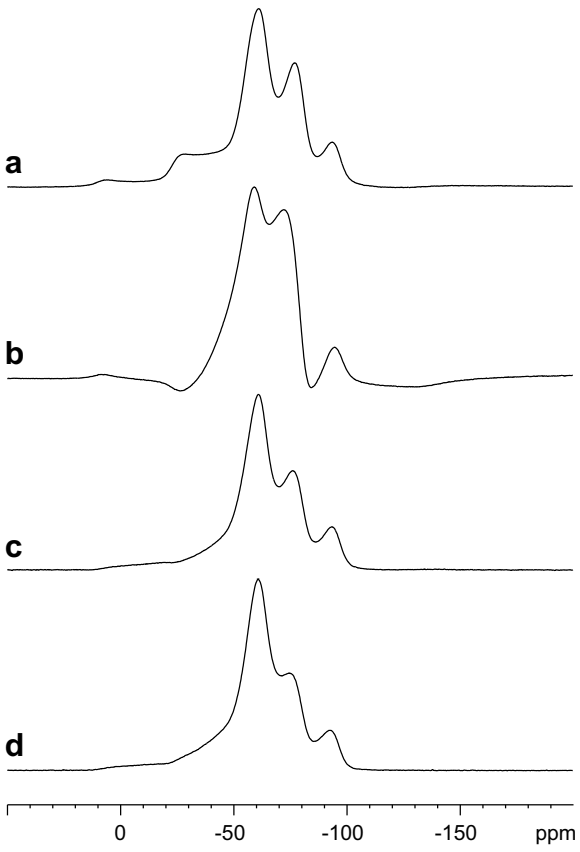

Fig. 2. Different pulse sequences are compared to acquire static ^{19}F-solid state NMR spectra of CF_3-groups that possess both chemical shift anisotropy and homonuclear ^{19}F dipolar couplings: single-pulse excitation (a), Hahn echo (b), solid echo (c), and solid echo with EXORCYCLE (d).

band intensities of MAS spectra as described, using the Herzfeld–Berger algorithm [15].

In the case of CF_3-labeled amino acids, the intra-CF_3-group dipolar couplings were determined by simulating the static spectra using the SIMPSON package [16]. An FID of 256 points with 4 μs dwell time following excitation by a single 2 μs pulse and a 5.5 μs delay accounting for the experimental dead time was generated, and powder averaging was performed using 678 crystallite orientations. The calculated FID was zero-filled to 8192 points and Fourier-transformed using exponential line broadening of 2.7 kHz. Fitting was done by visual inspection, since iterative fitting did not converge due to the low spectral intensity at the low field-edge. Since this edge does not enter the error function sufficiently, it is not capable of forcing the fit to reproduce this important feature of the lineshape adequately. As illustrated in Figs. 3a and c, the simulated lineshapes reproduce the experimental spectra of 4CF_3-Phg and CF_3-Bpg very well.

The chemical shift anisotropies of the CF_3-labeled amino acids were determined from fitting the MAS spectra. The intensities of the center band plus two sidebands on each side were extracted and fitted by SIMPSON simulations using the minuit minimizing functions [17]. FIDs of 32 points were calculated that are spaced by a dwell time

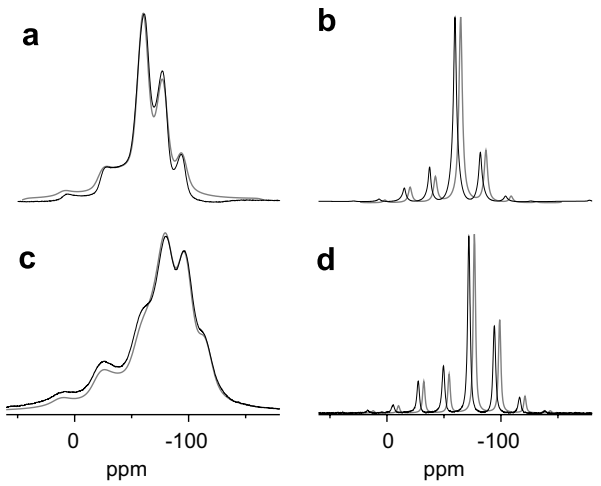

Fig. 3. Static and MAS spectra, as shown for $4CF_3$-Phg (a,b) and CF_3-Bpg (c,d), used to extract the homonuclear dipolar ^{19}F couplings and ^{19}F chemical shift anisotropies. The static lineshapes (a,c) were fitted to extract the dipolar coupling (fitted spectra drawn as gray lines), and the sideband intensities of the MAS spectra (b,d) were analyzed by fitting the experimental spectra (fitted lines drawn in gray and shifted to the right for clarity).

of the inverse of the spinning frequency, to sample the intensities at the sideband frequencies. Powder averaging was achieved by summing over 36 γ-angles and 144 random crystallite orientations. For CF_3-groups, a spin system of three coupled ^{19}F spins possessing the same chemical shift anisotropy and asymmetry was employed. Variations in the dipolar coupling and asymmetry parameter were found to only weakly change the calculated sideband intensities. Therefore, the dipolar coupling was fixed to the values obtained from the static ^{19}F NMR spectra, and the asymmetry parameter was set to zero assuming an essentially axially symmetric environment due to the averaging by fast methyl rotation. The fitted MAS sideband intensities obtained this way deviated from the experimental ones on average by 1–2%. The experimental spectra of $4CF_3$-Phg and CF_3-Bpg are compared in Figs. 3b and d with the calculated lineshapes, using the best fit dipolar coupling and chemical shift anisotropy.

3. Results and discussion

3.1. Liquid state NMR

As a first step in characterizing the isotropic chemical shift values (δ_{iso}), J-coupling constants, and the types of multiplets of the various ^{19}F-labeled amino acids, we examined their ^{19}F NMR spectra in aqueous solution. The results are summarized in Table 1. As expected from the large dispersion known for this nucleus, the isotropic chemical shifts cover a wide range from −60 to −230 ppm. Clear differences are noted between the mono-fluorinated analogues of alanine and leucine (shifts between −220 and −230 ppm), and the CF_3-substituted amino acids (shifts between −62 and −70 ppm). The ^{19}F-label in the tertiary carbon moiety of 3F-Val differs substantially from that in the methyl group of 3F-Ala or 5F-Leu. We also note that the chemical shifts of the CF_3-labeled amino acids fall into two groups, depending on whether the side chain is aromatic (shifts around −62 ppm) or aliphatic (shifts around −72 ppm).

3.2. ^{19}F CSA tensors of polycrystalline powders

The chemical shift data and dipolar couplings of the mono-fluorinated analogues and the CF_3-labeled compounds are compiled in Tables 2 and 3, respectively, as extracted from the corresponding static and MAS spectra (data not shown, but see representative Fig. 3, and Section 2). Isotropic chemical shifts δ_{iso} are found within 2 ppm of the values in aqueous solution, and may thus be considered as relatively robust towards changes in the external environment. The tensor anisotropies Δ cover a broad range from ∼10 ppm for 5F-Leu(2S,4R) up to ∼55 ppm for Fmoc-CF_3-Bpg. The smallest anisotropies of 10–20 ppm were observed for most of the 5F-Leu derivatives, while

Table 1
^{19}F NMR parameters of amino acids with aliphatic ^{19}F-labels, measured in aqueous solution at room temperature

Substance	Chirality	δ_{iso} (ppm)	^1J (Hz)	^2J (Hz)	Type of spectrum
3F-Ala	D/L	−229.20	47	29	Triplet of doublets
5F-Leu	(2S,4S)	−221.62	48	20	Triplet of doublets
5F-Leu	(2S,4R)	−220.83	46	21	Triplet of doublets
Fmoc-5F-Leu	(2S,4S)	−221.10	47	20	Triplet of doublets
5F-5,5FD_2-Leu	(2S,4S)	−222.94	6.8		Octet
3F-Val	D/L	−141.04			Not resolved[b]
3,3,3F_3-Ala	D/L	−70.61		10	Doublet
5,5,5$F_3$140-Leu	D/L	−73.43		19	Doublet of doublets
$4CF_3$-Phe	D/L	−62.35			Single line
$3CF_3$-Phe	D/L	−62.38			Single line
$4CF_3$-Phg	D/L	−62.68			Single line
$3CF_3$-Phg	D/L	−62.57			Single line
CF_3-Bpg	L	−77.24[a]			Single line

[a] Measured in D_2O, and referenced against C_6F_6 set to −164.9 ppm.
[b] Data acquired on a 400 MHz spectrometer did not provide the necessary resolution, all other data was obtained on a 500 MHz spectrometer.

Table 2
Solid state ^{19}F NMR chemical shift parameters of monofluorinated aliphatic amino acids, extracted from MAS spectra at 20 °C by Herzfeld–Berger analysis, and relaxation times T_1 and T_2 obtained from static ^{19}F NMR experiments at 20 °C

Substance	Chirality	δ_{11} (ppm)	δ_{22} (ppm)	δ_{33} (ppm)	δ_{iso} (ppm)	Δ (ppm)	η	T_1 (s)	T_2 (μs)
3F-Ala	D/L	−200	−235	−263	−232.7	33	0.84	19.5 ± 1.1 17.3 ± 1.6	14.7 ± 0.3
5F-Leu	(2S,4S)	−205	−218	−240	−221.4	−19	0.68	0.38 ± 0.03	54.0 ± 5.1
5F-Leu	(2S,4R)	−213	−224	−231	−222.4	10	0.72	0.52 ± 0.03	41.4 ± 3.2
Fmoc-5F-Leu	(2S,4S)	−175	−200	−240	−204.9	−35	0.71	3.3 ± 0.6	33.6 ± 1.4
5F-5,5D_2-Leu	(2S,4S)	−216.1	−226.5	−253.1	−231.9	−21.2	0.49	1.62 ± 0.03	36.2 ± 3.1
		−215.6	−235.0	−260.6	−237.1	−23.5	0.82		
3F-Val	D/L	−117.6	−131.6	−176.5	−141.9	−34.6	0.40	2.05 ± 0.04	36.0 ± 0.3

Table 3
Solid state ^{19}F NMR parameters of CF_3-labeled amino acids in their polycrystalline forms and when incorporated in lyophilized peptides, extracted from MAS and static spectra at 20 °C as described in Section 2

Substance	δ_{11} (ppm)	δ_{22} (ppm)	δ_{33} (ppm)	δ_{iso} (ppm)	Δ (ppm)	η	D_{CF3}	T_1 (s)	T_2 (μs)
3,3,3F_3-Ala	−29	−96	−96	−73.8	44	0	19.5	0.32 ± 0.01	39.6 ± 2.8
5,5,5F_3-Leu	−17	−95	−95	−69.2	52	0	16.5	1.08 ± 0.03	24.0 ± 4.0
	−19	−97	−97	−71.3	52				
	−23	−101	−101	−74.9	52				
4CF_3-Phe	−26	−85	−85	−65.4	39	0	18.5	1.86 ± 0.07	23.5 ± 0.7
3CF_3-Phe	−48	−106	−106	−86.6	39	0	16.5	nd[a]	nd[a]
	−45	−104	−104	−84.4	39				
4CF_3-Phg	−22	−78	−78	−59.7	37	0	15.4	5.7 ± 0.4	31.4 ± 2.2
3CF_3-Phg	−25	−83	−83	−63.8	39	0	17.5	nd[a]	nd[a]
CF_3-Bpg	−21	−98	−98	−72.2	52	0	15.6	0.59 ± 0.01	51.6 ± 1.5
Fmoc-CF_3-Bpg	nd[a]	nd[a]	nd[a]	nd[a]	55[b]	0	15.6	0.42 ± 0.01	59.7 ± 1.5
4CF_3-Phg in GS [6]	−25	−76	−76	−58.5	34	0	15.6	nd[a]	nd[a]
CF_3-Bpg in PGLa [7]	−21	−100	−100	−73.4	53	0	15.7	nd[a]	nd[a]

[a] Not determined.
[b] Estimated from the static spectrum.

the other mono-fluorinated analogues possess anisotropies around 30 ppm. As seen with the isotropic chemical shifts, also the chemical shift anisotropies of CF_3-groups differ significantly between aromatic and aliphatic side chains. A CF_3-group on an aliphatic side chain has a large anisotropy of 45–55 ppm, whereas in aromatic amino acids it is only around 35–40 ppm. The asymmetry parameter shows a pronounced non-axiality for mono-fluorinated amino acids, with η in the range of 0.4–0.8. For the CF_3-labeled amino acids, the axial asymmetry could not be quantified by simulation from the MAS spectra due to its weak influence in the presence of the additional ^{19}F–^{19}F dipolar coupling. However, the static spectra (see Fig. 3a as an example) did not show any significant deviation from axial symmetry, thus justifying the assumption of η approaching zero.

3.3. Methyl rotation

An important aspect in the interpretation of the ^{19}F NMR data obtained from a fluorine-labeled methyl group (i.e. CF_3, CF_2H, CFH_2) is its motional behavior. Fast uniaxial rotation of the segment will lead to axially averaged CSA and dipolar tensors, and it will make them collinear with the orientation of the methyl group. Only in this case, when the principal tensor direction is independent of the local chemical environment, it is straightforward to determine the orientation of the labeled segment directly from the chemical shift data. The occurrence of fast methyl rotation can be readily detected in the case of a CF_3-group from the ^{19}F–^{19}F dipolar couplings. For all CF_3-labeled amino acids investigated here, a dipolar splitting of around 8 kHz (at the 90° edge of the convoluted powder lineshapes) was found. As this splitting is approximately half of the value expected for an immobile CF_3-group, it is clear that these CF_3-segments are averaged by fast uniaxial rotation on the timescale of the ^{19}F dipolar coupling (~10–100 μs) [18]. However, the ^{19}F NMR spectra of the single ^{19}F-substituent in the CFH_2- and CFD_2-segments of the leucine derivatives (see Fig. 1) were not that easy to interpret, since no previous reference compounds had been characterized to yield the static CSA values. We therefore investigated the segmental molecular averaging by solid state ^2H NMR, as illustrated in Fig. 4. Here, the large but unresolved deuterium quadrupole coupling of around 120 kHz indicates that the monofluoro-methyl group in polycrystalline 5F-5,5D_2-Leu is not engaged in uniaxial rotation faster than the 10 μs timescale (Fig. 4a). As a control, we also examined the analogous CD_3-group in 5,5,5D_3-Leu, which obviously undergoes fast rotational

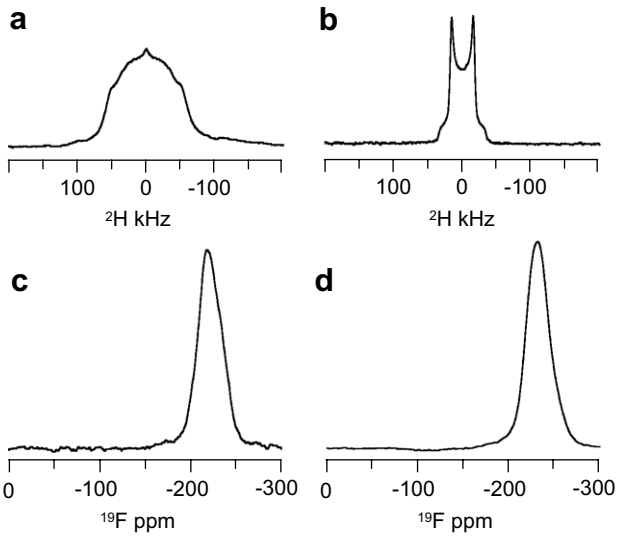

Fig. 4. ^{2}H NMR (a,b) and ^{19}F NMR (c,d) spectra of the leucine analogues 5F-5,5D$_2$-Leu (a,d) and 5,5,5D$_3$-Leu (b), and 5F-Leu (c). The asymmetric CD$_2$F-group does not rotate fast enough to average the quadrupolar interaction, resulting in a wide and featureless lineshape (a), while the CD$_3$-group shows a motionally averaged quadrupolar powder spectrum with a splitting of ~40 kHz. The ^{1}H-decoupled ^{19}F NMR spectrum of the CH$_2$F-group (c) has a slightly better linewidth than the deuterated CD$_2$F-group under ^{1}H-decoupling.

diffusion in view of its ~40 kHz quadrupole splitting (Fig. 4b). We attribute the different mobilities of the mono- and trifluoro-substituted methyl groups to the broken symmetry of the CH$_2$F-rotor, which is distorted by the larger ^{19}F-substituent. A mono-fluorinated methyl group is therefore often disadvantageous for solid state ^{19}F NMR structure analysis using chemical shift anisotropies or dipolar couplings.

3.4. Influence of deuteration

Since proton-decoupling can be technically rather demanding in view of the close proximity of the ^{1}H-frequency to the ^{19}F-observation channel, the substitution of geminal protons by deuterons may seem a promising alternative to achieve line-narrowing in fluorine-labeled methyl groups [18]. However, the ^{19}F NMR spectra in Fig. 4 show that ^{1}H-decoupling with only 40 kHz produces equivalent line-narrowing in the ^{19}F NMR spectra of 5F-5,5H$_2$-Leu (Fig. 4c) than the effect of deuteration in 5F-5,5D$_2$-Leu (Fig. 4d). This observation is not surprising in view of the close packing of many other protons, besides the geminal ones, around the ^{19}F-label in a crystalline lattice. Furthermore, it turned out that neither ^{1}H-decoupling nor ^{2}H-decoupling alone had any significant impact on the linewidth in the ^{19}F-spectra of 5F-5,5D$_2$-Leu, whereas the 5F-5,5H$_2$-Leu spectra were significantly narrowed by ^{1}H-decoupling. Therefore, even with comparatively low decoupling field strengths, ^{1}H-decoupling of adjacent protons yields equal results as partial deuteration.

3.5. Orientational analysis of peptides

Based on two of the amino acids included in this study, a powerful labeling strategy to determine the conformation, alignment and mobility of polypeptides in membranes has been developed. Both 4CF$_3$-Phg and CF$_3$-Bpg possess a direct link between the CF$_3$-group and the peptide backbone, hence these ^{19}F-labels yield direct orientational constraints for the backbone that do not depend on any side chain torsion angle. Their intra-CF$_3$ homonuclear dipolar coupling has been successfully exploited in several recent ^{19}F NMR studies of membrane-bound peptides [4–8]. To judge whether the data obtained here from the polycrystalline amino acids can be transferred to such peptide samples, we have collected in Table 3 the parameters of 4CF$_3$-Phg incorporated in gramicidin S (positions Val2 and Val2′), and of CF$_3$-Bpg incorporated in PGLa (position Ile13) [6,7]. The respective CSA principal axis values of the free and incorporated amino acids are very close to one another (within 3 ppm), indicating a marginal influence of the external environment. Here, the differences appear to be less than for the aromatic amino acids described in Part I [1]. Also, the chemical shift anisotropy and dipolar coupling are only slightly reduced in the peptides compared to the solid amino acids, again less than for the aromatic substituents. For our CF$_3$-labels the data from the polycrystalline amino acids and peptides thus compare rather well. For structure analysis it is indeed even more advantageous to use the intra-CF$_3$ dipolar couplings than the chemical shifts, since the couplings are not affected by the external environment or the state of the sample at all [12,13]. We may thus conclude that the dipolar analysis of a CF$_3$-Phg or CF$_3$-Bpg with a simple 1-pulse experiment provides a robust route to obtain orientational information of membrane-bound peptides, even though it may have initially appeared difficult to find the appropriate pulse sequence or to deconvolute the dipolar coupling from the chemical shift interaction.

3.6. Relaxation

The longitudinal and transversal ^{19}F NMR relaxation times T_1 and T_2 were determined as a function of temperature in the range of −60 to +60 °C. As already noticed for the aromatic ring-substituted ^{19}F amino acids [1], also for the aliphatic compounds the T_2 relaxation times do not vary much with temperature, being in the range of 30–50 μs. In contrast to this rather uniform T_2-relaxation behavior, the T_1 relaxation times differ widely and exhibit more pronounced temperature dependencies (Fig. 5). Noteworthy are the T_1-minima observed for 3,3,3F$_3$-Ala, 5F-Leu(2S,4S), CF$_3$-Bpg and Fmoc-CF$_3$-Bpg at 20, 0, 30, and −20 °C, respectively. Other amino acids (e.g., 5F-5,5D$_2$-Leu, 5F-Leu(2S,4R)) exhibit particularly low T_1-values without any temperature dependence in the range covered, suggesting a broad T_1-minimum at ambient temperatures. More striking, however, is the fact that the

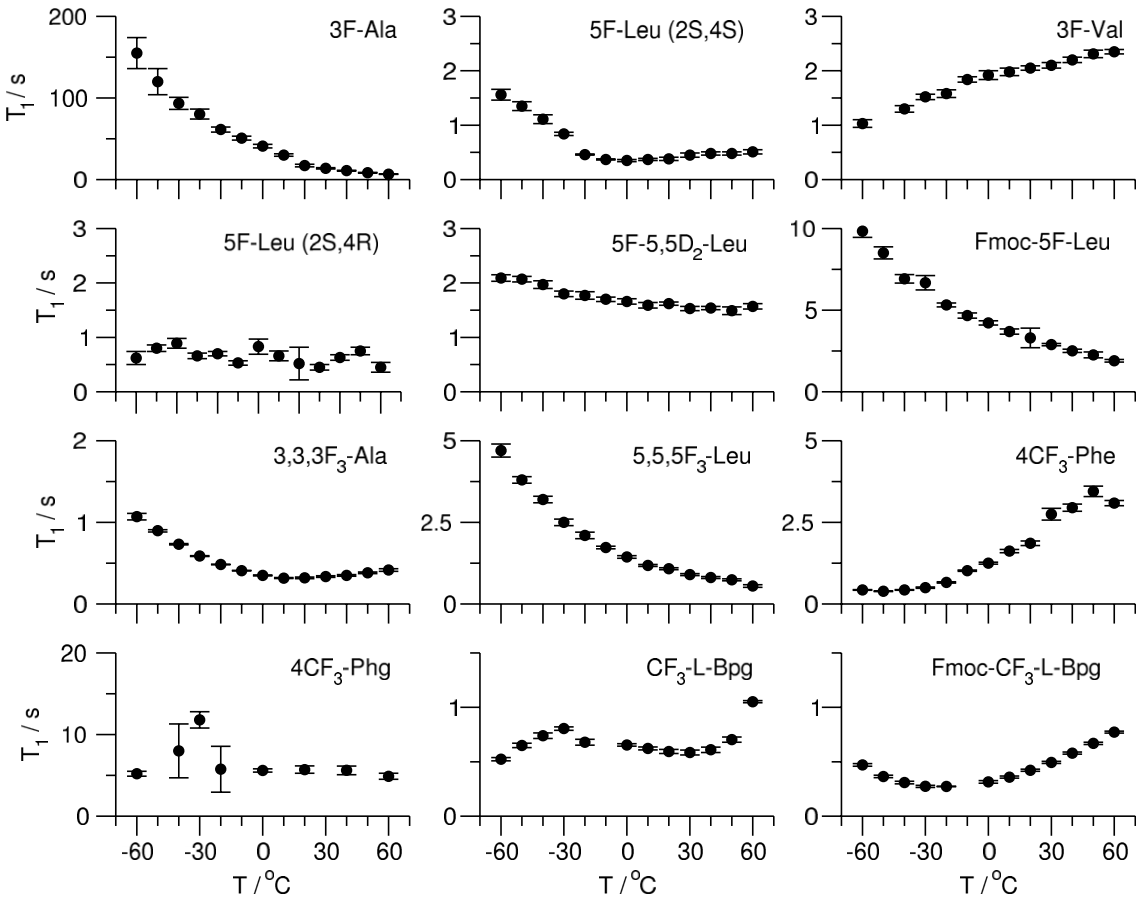

Fig. 5. Temperature dependence of T_1 relaxation times of the amino acids with ^{19}F substitutions in aliphatic positions. (Unless otherwise specified, the racemic mixtures were used for the relaxation measurements.)

T_1-values of the aliphatic fluorinated segments studied here are significantly shorter than those of the aromatic ring-substituted amino acids characterized in Part I [1]. While T_1 relaxation times of minutes were encountered for ^{19}F-substituents on aromatic rings, most T_1 values obtained for the fluorinated methyl groups are in the range of seconds or below (except for 3F-Ala below 20 °C). The T_1-minima observed here in a number of cases and the short T_1-times suggest the presence of molecular motions on or near the time scale of the inverse of the ^{19}F Larmor frequency (~10 ns) at ambient temperatures. Such motions seem to be absent when a ^{19}F-substituent is directly bound to an aromatic ring system.

4. Conclusions

To extend the compilation of ^{19}F NMR parameters for the most relevant amino acids, we have here examined aliphatic ^{19}F-labels, especially CF_3-groups, in aliphatic and aromatic side chains. Compared to the aromatic ring-substituted ^{19}F-labels characterized in Part I [1], the most pronounced differences were found in the relaxation behavior. Whereas the aromatic ^{19}F-substituents have long T_1 times in the range of several minutes, the labeled methyl groups never exceed a few seconds, which is advantageous for NMR acquisition. Furthermore, the isotropic chemical shifts measured in the liquid state were close to the values extracted from the solid state NMR spectra. They can be grouped into several subsets, namely as CF_3-groups (with shifts around −60 to −70 ppm), as single aromatic ^{19}F-substituents (around −110 ppm), and as mono-substituted aliphatic amino acids (shifts around −200 to −240 ppm). The chemical shift anisotropies of the fluorinated methyl-groups cover a wide range of ~10–55 ppm, and are generally smaller than the anisotropies found for aromatic ^{19}F-substituents. Large chemical shift tensor asymmetries were found for single ^{19}F-substituents on aromatic rings, as well as in mono-substituted methyl groups and methylene segments. In contrast, for CF_3-groups the effective CSA tensors are virtually axially symmetric, likely due to fast methyl rotation. Such motional averaging was hindered, on the other hand, in mono-fluorinated CFH_2-groups. Amongst all of the compounds studied here, the artificial amino acids 4CF_3-Phg and CF_3-Bpg have the most beneficial properties for solid state ^{19}F NMR orientational analysis of membrane-bound peptides, as they contain a CF_3-label that is directly connected to the backbone. The use of these labels opens up the opportunity to analyze the intra-group ^{19}F–^{19}F dipolar coupling in the CF_3-label as a structural reporter, using a 1-pulse experi-

ment, with exquisite sensitivity and no interference from any natural abundance background signals.

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References

[1] U.H.N. Dürr, S.L. Grage, R. Witter, A.S. Ulrich, Solid-state ^{19}F NMR parameters of fluorine labeled amino acids. Part I: aromatic substituents, J. Magn. Reson. 191 (2008) 7–15.

[2] S.L. Grage, A.S. Ulrich, Orientation-dependent ^{19}F dipolar couplings within a trifluoromethyl group are revealed by static multipulse NMR in the solid state, J. Magn. Reson. 146 (2000) 81–88.

[3] S.L. Grage, D.R. Gauger, C. Selle, W. Pohle, W. Richter, A.S. Ulrich, The amphiphilic drug flufenamic acid can induce a hexagonal phase in DMPC: a solid state ^{31}P- and ^{19}F NMR study, Phys. Chem. Chem. Phys. 2 (2000) 4574–4579.

[4] R.W. Glaser, C. Sachse, U.H.N. Dürr, P. Wadhwani, A.S. Ulrich, Orientation of the antimicrobial peptide PGLa in lipid membranes determined from ^{19}F NMR dipolar couplings of 4-CF_3-phenylglycine labels, J. Magn. Reson. 168 (2004) 153–163.

[5] R.W. Glaser, C. Sachse, U.H.N. Dürr, P. Wadhwani, S. Afonin, E. Strandberg, A.S. Ulrich, Concentration-dependent realignment of the antimicrobial peptide PGLa in lipid membranes observed by solid-state ^{19}F NMR, Biophys. J. 88 (2005) 3392–3397.

[6] S.L. Grage, A.V. Suleymanova, S. Afonin, P. Wadwhani, A.S. Ulrich, Solid state NMR analysis of the dipolar couplings within and between distant CF_3-groups in a membrane-bound peptide, J. Magn. Reson. 183 (2006) 77–86.

[7] S. Afonin, P.K. Mikhailiuk, I.V. Komarov, A.S. Ulrich, Evaluating the use of CF_3-bicyclopentylglycine as a label for ^{19}F NMR structure analysis of membrane-bound peptides, J. Pept. Sci. 13 (2007) 614–623.

[8] E. Strandberg, P. Wadhwani, P. Tremouilhac, U.H.N. Dürr, A.S. Ulrich, Solid state NMR analysis of the PGLa peptide orientation in DMPC bilayers: structural fidelity of ^{2}H- versus high sensitivity of ^{19}F NMR, Biophys. J. 90 (2006) 1676–1686.

[9] D. Alexeev, P.N. Barlow, S.M. Bury, J.D. Charrier, A. Cooper, D. Hadfield, C. Jamieson, S.M. Kelly, R. Layfield, R.J. Mayer, H. McSparron, N.C. Price, R. Ramage, L. Sawyer, B.A. Starkmann, D. Uhrin, J. Wilken, D.W. Young, Synthesis, structural and biological studies of ubiquitin mutants containing (2S,4S)-5-fluoroleucine residues strategically placed in the hydrophobic core, ChemBioChem 4 (2003) 894–896.

[10] J.D. Charrier, D.S. Hadfield, P.B. Hitchcock, D.W. Young, Synthesis of (2S,4S)- and (2S,4R)-5-fluoroleucine and (2S,4S)- 5,5-H-2(2)-5-fluoroleucine, Org. Biomol. Chem. 2 (2004) 474–482.

[11] P.K. Mikhailiuk, S. Afonin, A.N. Chernega, E.B. Rusanov, M. Platonov, G. Dubinina, M. Berditsch, A.S. Ulrich, I.V. Komarov, Conformationally rigid trifluoromethyl-substituted α-amino acid designed for peptide structure analysis by solid state ^{19}F NMR, Angew. Chem., Int. Ed. 45 (2006) 5659–5661.

[12] R.W. Glaser, A.S. Ulrich, Susceptibility corrections in solid-state NMR experiments with oriented membrane samples. Part I: applications, J. Magn. Reson. 164 (2003) 104–114.

[13] R. Ulrich, R.W. Glaser, A.S. Ulrich, Susceptibility corrections in solid state NMR experiments with oriented membrane samples. Part II: Theory, J. Magn. Reson. 164 (2003) 115–127.

[14] S. Antonijevic, S. Wimperis, Refocussing of chemical and paramagnetic shift anisotropies in ^{2}H NMR using the quadrupolar-echo experiment, J. Magn. Reson. 164 (2003) 343–350.

[15] J. Herzfeld, A.E. Berger, Sideband intensities in NMR-spectra of samples spinning at the magic angle, J. Chem. Phys. 73 (1980) 6021–6030.

[16] M. Bak, J.T. Rasmussen, N.C. Nielsen, SIMPSON: a general simulation program for solid-state NMR spectroscopy, J. Magn. Reson. 147 (2000) 296–330.

[17] T. Vosegaard, A. Malmendal, N.C. Nielsen, The flexibility of SIMPSON and SIMMOL for numerical simulations in solid- and liquid-state NMR spectroscopy, Monatshefte für Chemie 133 (2002) 1555–1574.

[18] S.L. Grage, J.F. Wang, T.A. Cross, A.S. Ulrich, Solid-state ^{19}F NMR analysis of ^{19}F-labeled tryptophan in gramicidin A in oriented membranes, Biophys. J. 83 (2002) 3336–3350.