Nuclear Magnetic Resonance and LC/MS Characterization of Native and New Mass-labeled Fluorinated Telomer Alcohols, Acids and Unsaturated Acids

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Introduction

A variety of fluorinated compounds are used in a multitude of consumer products because of their ability to repel water and oil, resistance to heat, and chemical inertness. Recently, scientists and regulators have begun raising concerns about the potential health and environmental impact of perfluorinated compounds [1-7]. Exposure to perfluoroalkyl acids, such as Perfluorooctanoic acid (PFOA), has been identified[8] as a potential human health concern. A study has shown[9] that telomer alcohols such as 2-perfluorooctylethanol can be metabolized by living organisms or biodegrade under environmental conditions to sequentially give the saturated fluorinated telomer acid (2-perfluorooctylethanoic acid), then the unsaturated telomer acid (2H-Perfluorooct-2-enoic acid), and eventually PFOA.

Additional experimental work is necessary to determine the extent, if any, to which telomer product degradation may be a source of PFOA. The analysis for fluorinated compounds in environmental samples is performed, primarily, using LC/MS techniques. These analyses have been hindered by the lack of any commercially available mass-labeled fluorinated compounds for use as surrogates[10] and thus may be restricting the amount of research conducted in this area.

We have now synthesized the mass-labeled perfluoroalkyl telomer alcohols and the corresponding acids and unsaturated acids. We report in this study their ^{1}H-, ^{2}H-, ^{19}F- and ^{13}C-NMR characterizations along with GC/MS and LC/MS data and evaluation of their use as surrogate standards.

Methods and Materials

Chemicals. The fluorinated telomer compounds reported in this paper were synthesized and purified using conventional synthetic procedures. Abbreviations used for the fluorinated compounds are shown in Table 1.

Table 1. Abbreviations used for the fluorinated telomer compounds.

Name	Abbreviation for the native compound	Abbreviation for the mass labeled compound
2-Perfluorohexylethanol	FHET	MFHET
2-Perfluorooctylethanol	FOET	MFOET
2-Perfluorodecylethanol	FDET	MFDET
2-Perfluorohexylethanoic acid	FHEA	MFHEA
2-Perfluorooctylethanoic acid	FOEA	MFOEA
2-Perfluorodecylethanoic acid	FDEA	MFDEA
2H-Perfluorooct-2-enoic acid	FHUEA	MFHUEA
2H-Perfluorodec-2-enoic acid	FOUEA	MFOUEA
2H-Perfluorododec-2-enoic acid	FDUEA	MFDUEA

NMR Experiments. The NMR experiments were performed on a Bruker 400MHz instrument.

Gas Chromatography / Mass Spectrometry. The analysis was carried out using a Shimadzu GCMS-QP2010. Temperature program used on column DB-5 (30m x 0.25mm x 0.25um): 30°C, 10 minutes isothermal; 10°C/min. to 325°C and hold for 15 minutes; splitless injector port set at 120°C; detector set at 250°C. Scan from 50 to 1000amu.

Liquid Chromatography / Mass Spectrometry. LC/MS were determined using an Alltech 426 pump and Micromass Quattro micro detector. The LC traces were obtained by injection of 20uL into an Alltech Econosil C18 column (250cm, 4.6 mm ID, 5 um pore size) and using a methanol/water (75:25) solvent system. The MS was running in single reaction monitoring mode with a cone angle of 14 V. The full mass spectra were obtained by direct 10uL injection to the MS running in full scan mode from 50 m/z to 650 m/z with a cone voltage of 14 V.

Results and Discussion

NMR for the Fluorinated Telomer Compounds. The NMR data for the native and mass labeled fluorinated telomer compounds are reported in Tables 2-5. Assignments of the resonances pertain to the numbering schemes shown in figure 1. Tables 2 and 3 only report the NMR data for the perfluorohexyl derivatives as the ^{1}H, ^{2}H and ^{13}C-NMR data do not change when the perfluoroalkyl group changes from 6 to 8 or 10 carbons. Tables 4 and 5 include the ^{19}F-NMR data for the longer perfluoroalkyl compounds as these data are, of course, dependent on the number of fluorine atoms present.

The mass labeled fluorinated telomer alcohols are M+4 surrogate standards in which the labeling was achieved by introducing two ^{13}C for carbons A and B and replacing the two protons on carbon A with two deuteriums. The changes are evident when comparing NMR data between native and mass labeled compounds, the later containing the following: deuterium resonances in the ^{2}H-NMR, evidence of ^{13}C coupling in the ^{1}H- and ^{2}H-NMR, and strong ^{13}C resonances for the two carbons enriched with ^{13}C.

The mass labeled fluorinated telomer acids and unsaturated acids are M+2 surrogate standards in which the labeling was achieved by introducing two ^{13}C for carbons A and B. Again, the changes are evident when comparing NMR data between native and mass labeled compounds, the later containing the following: carbon coupling in the ^{1}H-NMR, and strong ^{13}C resonances for the two carbons enriched with ^{13}C.

Isotopic purity of these compounds can be estimated using NMR spectroscopy. As an example with MFHET, there is a residual proton signal on carbon A (3.85 ppm, see figure 2) which is due to the fact that deuterium reagents are typically no more than 98% deuterated. In this case, the integration of the 3.85 ppm signal for MFHET corresponds to the presence of 2% proton. The protons on carbon B also show a small signal with no carbon coupling (2.29 ppm, see figure 2) due to the fact that ^{13}C reagents are never 100% ^{13}C. In this case, the integration estimates that the ^{13}C labeling was about the expected 99.1%. Very similar results were obtained with the longer perfluoroalkyl ethanol analogues. Statistical calculation using this data gives the abundance of the M+4 ion at 94.3% and the M+3 ion at 5.6%. A similar analysis can be done with the ^{1}H-NMR of the mass-labeled saturated and unsaturated acid, in which it is shown that the ^{13}C labeling is approximately 99.1%.

Figure 1: Structure, numbering scheme and abbreviation for the fluorinated telomer acids

Fluorinated telomer alcohols

10	9	8	7	6	5	4	3	2	1	B	A	C	
CF3-	CF2-	CF2-	CF2-	CF2-	CF2-	CF2-	CF2-	CF2-	CF2-	CH2-	CH2-	OH	FDET (10:2 FTOH)
		CF3-	CF2-	CF2-	CF2-	CF2-	CF2-	CF2-	CF2-	CH2-	CH2-	OH	FOET (8:2 FTOH)
				CF3-	CF2-	CF2-	CF2-	CF2-	CF2-	CH2-	CH2-	OH	FHET (6:2 FTOH)

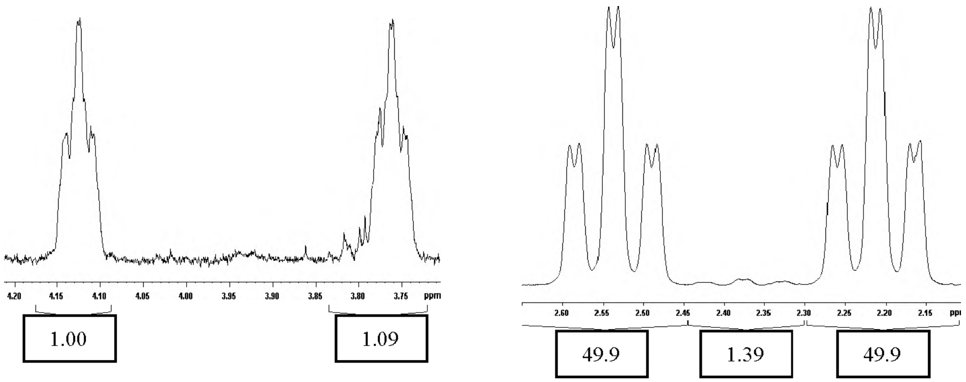
Fluorinated telomer acids

10	9	8	7	6	5	4	3	2	1	B	A	
CF3-	CF2-	CF2-	CF2-	CF2-	CF2-	CF2-	CF2-	CF2-	CF2-	CH2-	CO2H	FDEA
		CF3-	CF2-	CF2-	CF2-	CF2-	CF2-	CF2-	CF2-	CH2-	CO2H	FOEA
				CF3-	CF2-	CF2-	CF2-	CF2-	CF2-	CH2-	CO2H	FHEA

Fluorinated unsaturated telomer acids

10	9	8	7	6	5	4	3	2	1	B	A	
CF3-	CF2-	CF2-	CF2-	CF2-	CF2-	CF2-	CF2-	CF2-	CF=	CH-	CO2H	FDUEA
		CF3-	CF2-	CF2-	CF2-	CF2-	CF2-	CF2-	CF=	CH-	CO2H	FOUEA
				CF3-	CF2-	CF2-	CF2-	CF2-	CF=	CH-	CO2H	FHUEA

Figure 2: ^{1}H-NMR of MFHET (values in the boxes are the relative integration of the signals)



GC/MS of 2-Perfluoroalkylethanols. GC/MS analysis was done for the fluorinated telomer alcohols. The GC/MS of the three native alcohols (FHET/FOET/FDET) each gave reasonably shaped peaks with different retention times (see figure 3). The mass-labeled M+4 analogues also gave single peaks with similar retention times.

Table 2: ^{1}H- and ^{19}F-NMR data for the perfluorohexyl telomer compounds

	FHET		**FHEA**		**FHUEA**	
	ppm	**Coupling constant (Hz)**	**ppm**	**Coupling constant (Hz)**	**ppm**	**Coupling constant (Hz)**
	1H-NMR in d4-methanol					
A	3.85	t, $J_3(HC_A\text{- }C_BH)= 6.6$	No protons attached to Ca		No protons attached to Ca	
B	2.38	tt, $J_3(HC_B\text{- }C_AH)= 6.6$ $J_3(HC_B\text{- }C_1F)= 19.2$	3.33	dt, $J_3(HC_B\text{- }C_1F)= 18.4$	6.27	t, $J_3(HC_B\text{- }C_1F)= 18.4$
	19F-NMR in d4-methanol					
1	-112.0	m	-111.5	m	-113.7	m
2,3,4,5	-120.5, -121.4, -122.3 -124.7		-121.2, -122.2, -122.4, -125.7		-120.0, -124.1, -124.3, -127.5	
6	-79.5	$J_3(FC_6\text{- }C_5F)= 10.2$ $J_5(FC_6C_5C_4F)= 2$	-80.7	$J_3(FC_6\text{- }C_5F)= 9.4$	-82.6	$J_3(FC_6\text{- }C_5F)= 9.4$

Table 3: ^{1}H-, ^{2}H-, ^{13}C- and ^{19}F-NMR data for the mass-labeled perfluorohexyl telomer compounds.

	MFHET		MFHEA		MFHUEA	
	ppm	**Coupling constant (Hz)**	**ppm**	**Coupling constant (Hz)**	**ppm**	**Coupling constant (Hz)**
	1H-NMR in d4-methanol					
A	3.85[a]	dt, $J_1(HC_A)= 130$ $J_3(HC_A\text{- }C_BH)= 6.6$	No protons attached to Ca		No protons attached to Ca	
B	2.37	ddt, $J_3(HC_B\text{- }C_1F)= 19.2$ $J_1(HC_B)= 130$ $J_2(HC_BC_A)= 4.8$	3.33	dtt, $J_3(HC_B\text{- }C_1F)= 18.4$ $J_1(HC_B)= 132$ $J_2(HC_BC_A)= 7.2$	6.27	ddd, $J_3(HC_B\text{- }C_1F)= 31.6$ $J_1(HC_B)= 169$ $J_2(HC_BC_A)= 1$
	2H-NMR in d4-methanol					
B	3.97	d, $J_1(DC_B)= 22$	No deuteriums present		No deuteriums present	
	13C-NMR in d4-methanol					
A	54.8	dp, $J_1(C_B\,C_A)= 37$ $J_1(DC_B)= 22$	167.1	d, $J_1(C_B\,C_A)= 57$	167.1	d, $J_1(C_B\,C_A)= 74$
B	34.0	dt, $J_1(C_B\,C_A)= 37$ $J_2(C_B\text{- }C_1F)= 21$	37.3	dt, $J_1(C_B\,C_A)= 57$ $J_2(C_B\text{- }C_1F)= 22.2$	109.4	d, $J_1(C_B\,C_A)= 74$
	19F-NMR in d4-methanol					
Fluorine NMR signals and couplings are identical to the ones shown for the native analogues shown in Table 2.						

[a] This signal is reduced to 2% of its expected integration due to deuterium (98%) labeling

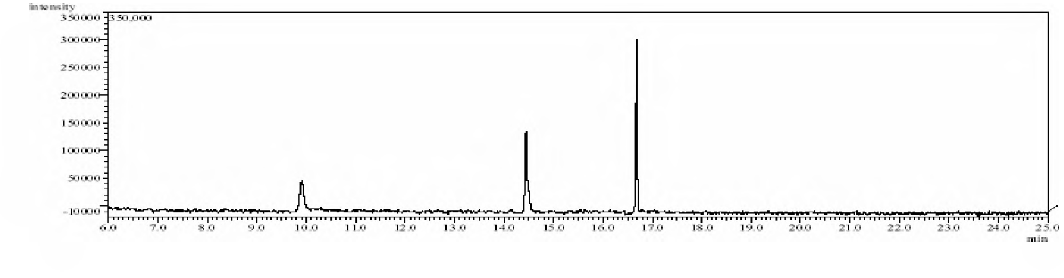
Table 4: ^{19}F-NMR data for the perfluorooctyl telomer compounds

	FOET		FOEA		FOUEA	
	ppm	**Coupling constant (Hz)**	**ppm**	**Coupling constant (Hz)**	**ppm**	**Coupling constant (Hz)**
	19F-NMR in d4-methanol					
1	-113.9	m	-111.5	m	-111.5	m
2,3,4,5,6,7	-122.1, -122.3 (2X), -123.1, -124.2, -126.5		-121.0, -121.2 (2X), -122.0, -122.3, -125.6		-118.1, -121.2, -121.3, -122.1, -122.2, -125.6	
8	-81.3	J_3(FC$_6$- C$_5$F)= 10.2	-80.7	J_3(FC$_6$- C$_5$F)= 10.2	-80.7	J_3(FC$_6$- C$_5$F)= 10.2

Table 5: ^{19}F-NMR data for the perfluorodecyl telomer compounds.

	FDET		FDEA		FDUEA	
	ppm	**Coupling constant (Hz)**	**ppm**	**Coupling constant (Hz)**	**ppm**	**Coupling constant (Hz)**
	19F-NMR in d4-methanol					
1	-112.1	m	-111.4	m	-113.7	m
2,3,4,5,6,7, 8,9	-120.2 (5X), -121.2, -122.0, -124.7		-121.0 (5X), -122.0, -122.3, -125.6		-119.9, -122.9 (4X), -123.9, -124.0, -127.4	
10	-79.8	J_3(FC$_6$- C$_5$F)= 10.2	-80.6	J_3(FC$_6$- C$_5$F)= 10.2	-82.5	J_3(FC$_{10}$- C$_9$F)= 10.2

Figure 3. GC/MS TIC Chromatogram of FHET/FOET/FDET

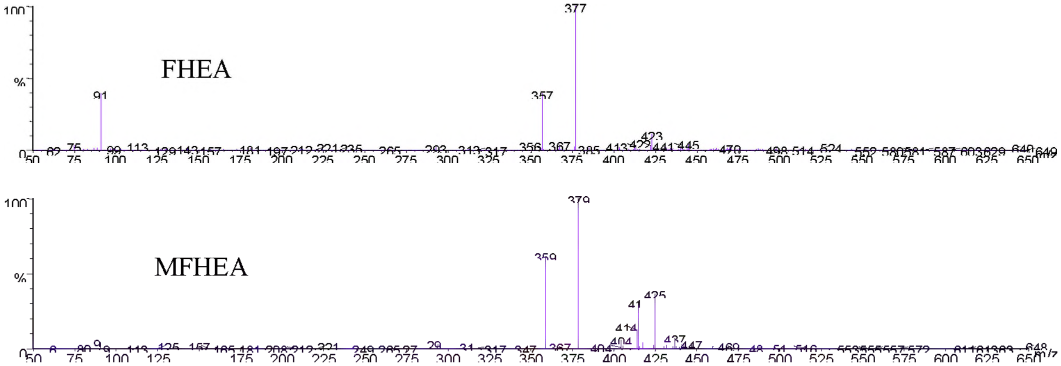


LC/MS of the Fluorinated Telomer Compounds. The full mass spectra for the 18 native and mass labeled fluorinated telomer compounds were obtained via direct injection to the mass spectrometer. All showed their parent molecular ion with the expected mass. The saturated acids also showed the distinctive loss of HF in the fragmentation pattern. The fragmentation pattern for the native compounds was similar to the mass labeled compounds except for the expected lower masses (as an example, see figure 4).

The LC trace of all six native and six mass labeled fluorinated telomer acids from the LC/MS analysis gave one reasonably shaped peak. The retention times (minutes) recorded for the compounds were the following: FHEA:FOEA:FDEA (4.9:5.1:6.1); FHUEA:FOUEA:FDUEA

(5.1:5.5:6.2). The retention times of the mass labeled compounds were identical to their native analogues.

Figure 4. Mass spectrum of FHEA and MFHEA



Conclusions

This work has provided NMR data for the pure native and mass labeled fluorinated telomer compounds. The mass labeled compounds should prove useful as surrogate standards for the mass spectral analysis and quantification of this group of fluorinated telomer compounds.

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