Synthesis of a metabolically stable modified long-chain fatty acid salt and its photolabile derivative

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Abstract An analogue of the long-chain fatty acid salt, sodium stearate, was synthesized in which the hydrogen atoms at carbons 2, 3, and 18 were replaced by fluorine. The key step in the synthesis was the addition of 3-iodo-2,2,3,3-tetrafluoropropanoic acid amide to 15,15,15-trifluoro-1-pentadecene. Radioactivity was introduced by catalytic reduction of 2,2,3,3,18,18,18-heptafluoro-4-octadecenoic acid amide with carrier-free tritium gas yielding a product with the specific radioactivity of 2.63 TBq/mmol. The resulting 2,2,3,3,18,18,18-heptafluoro-4-octadecenoic acid has a pK_a of about 0.5 and is completely dissociated under normal physiological conditions. The fluorinated fatty acid salt analogue is readily taken up into hepatocytes and proved to be metabolically inert. In an approach to the identification of proteins involved in long-chain fatty acid salt transport across membranes and intracellular compartments, the photolabile derivative 11,11-azo-2,2,3,3,18,18,18-heptafluoro[G-^{3}H]octadecanoic acid sodium salt was synthesized with a specific radioactivity of 2.63 TBq/mmol. Photolysis of the photolabile derivative, using a light source with a maximum emission at 350 nm, occurred with a half-life of 1.5 min. The generated carbene reacted with ^{14}C-labeled methanol and acetonitrile with covalent bond formation of 6–13%. Its efficacy for photoaffinity labeling was demonstrated by incorporation into serum albumin, the extracellular fatty acid salt-binding protein, as well as into the intracellular fatty acid salt-binding protein (FABP) of rat liver with the molecular weight of 14,000. —**Stoll, G. H., R. Voges, W. Gerok, and G. Kurz.** Synthesis of a metabolically stable modified long-chain fatty acid salt and its photolabile derivative. *J. Lipid Res.* 1991. **32:** 843–857.

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The metabolism of fatty acid salts requires efficient translocation of the salt across both the plasma membrane and the intracellular compartments. The molecular mechanisms of the different transport processes involved are highly controversial. On the one hand, transport across the plasma membrane has been suggested to occur only by simple diffusion (1–3), and on the other hand to be mediated by carrier systems (4, 5). The participation of albumin receptors that accelerate the dissociation of the fatty acid salt from the albumin-ligand complex prior to its translocation has been postulated (6) and questioned (7, 8).

Intracellular transport of fatty acid salts is assumed to be facilitated by relatively specific low molecular weight fatty acid salt-binding proteins (FABP), which are present in the cells of all organs involved in the uptake and utilization of long-chain fatty acid salts. However, their detailed physiological functions are still unclear (9, 10).

One of the main difficulties in the study of the different transport processes of free fatty acid salts is that translocation might be controlled by the rate of subsequent metabolic reactions. Furthermore, metabolism of salts of fatty acids impedes the study of outward fluxes and equilibrium exchange experiments using intact cells. With the exceptions of ω-oxidation (11), (ω-1)-oxidation (12), and, possibly, α-oxidation (13, 14), the initial step in metabolism of long-chain fatty acid salts is their activation to the corresponding CoA derivatives. The activated fatty acids are the starting metabolites for biosynthetic reactions such as desaturation, elongation, and acyl-transfer, as well as for catabolism by β-oxidation—reactions that occur in mitochondria and in peroxisomes (15). Assuming that (ω-1)-oxidation does not play an important role, prevention of *a*) α- and ω-oxidation of the free fatty acid salts, *b*) of β-oxidation of the activated fatty acids, and *c*) of the activation reaction should provide new possibilities for the study of fatty acid salt transport. Replacement of

Abbreviations: CMC, critical micellar concentration; DCI, direct chemical ionization; EI, electron impact ionization; FABP, fatty acid salt-binding protein; HPTLC, high performance thin-layer chromatography; LSC, liquid scintillation counting; SDS/PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; TLC, thin-layer chromatography.

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hydrogen by fluorine at the relevant positions of the fatty acid salt influences the reactivities in such a manner that metabolism should be prevented (16) and only transport processes should occur.

In order to study the mechanisms of transport of fatty acid salts across the plasma membrane and the intracellular compartments, we have devised the synthesis of α,β,ω-fluorinated, metabolically stable analogues of fatty acid salts. The identification of proteins that interact with free fatty acid salts under physiological conditions is of prime importance. To make this possible by photoaffinity labeling, we have also synthesized a photolabile derivative of the metabolically stable analogue of sodium stearate, and have established its suitability for the identification of fatty acid salt-binding proteins.

MATERIALS AND METHODS

Materials

Silica gel 60 (40–63 μm) and silica gel plates (silica gel 60, 10 × 20 cm) for TLC and for HPTLC were purchased from E. Merck (Darmstadt, Germany). Amberlite XAD-2 (analytical grade) was from Serva Feinbiochemica GmbH & Co. (Heidelberg, Germany). *o*-Nitrophenyl selenocyanate was obtained from Fluka Feinchemikalien GmbH (Neu-Ulm, Germany). Palladium on charcoal (5% Pd) was purchased from Aldrich GmbH & Co. KG (Steinheim, Germany). Sulfur tetrafluoride was from DuPont de Nemours & Co. (Wilmington, DE). Tetrafluoroethylene monomer, inhibited with D-pinen, was a gift from Hoechst AG (Frankfurt, Germany). Rat serum albumin (essentially fatty acid-free) was obtained from Sigma Chemie GmbH (Deisenhofen, Germany). Tritium gas (carrier-free, 96.2 GBq/ml) was purchased from MEDI PRO AG (Teufen, Switzerland). [^{14}C]Methanol (185 MBq/mmol) and [1-^{14}C]acetonitrile (18.5 MBq/mmol) were from ARC (St. Louis, MO). All other chemicals were of the highest quality available from commercial sources.

Animals

Male Wistar rats (Tierzuchtanstalt Jautz, Hannover, Germany) weighing 150–200 g were used. The animals had free access to food (standard rat diet Altromin 300 R, Altromin GmbH, Lage, Germany) and tap water.

Liver infusion experiments

The sodium salt of 2,2,3,3,18,18,18-heptafluorostearate, 0.1–1 nmol, containing 0.1–1 kBq of the tritium-labeled compound, was dissolved either in 500 μl of 0.15 M NaCl, pH 7.0, or rat serum. The solutions were injected into a peripheral mesenterial vein of rats anesthetized with pentobarbital (3 mg of sodium pentobarbital/100 g of body weight, i.p.). Considering a portal flow of about 10 ml/min (17), the compounds were infused over a 30-sec period so that their final concentration in blood did not exceed 5 μM. Bile was collected at different times beginning 10 min before start of injection. Radioactivity in bile was determined by liquid scintillation counting, and the nature of the secreted compounds was determined by HPTLC using the solvent systems described below (Syntheses). Radioactivity on thin-layer plates was detected with a radioscanner (TLC-Analyzer LB 2820, Berthold, Wildbad, Germany).

Isolation and incubation of hepatocytes

The standard isolation and incubation medium in all experiments with hepatocytes contained 118 mM NaCl, 4.74 mM KCl, 1.2 mM $MgCl_2$, 0.59 mM KH_2PO_4, 0.59 mM Na_2HPO_4, 24 mM $NaHCO_3$, 1.25 mM $CaCl_2$, and 5.5 mM D-glucose, and was saturated with carbogen (95% O_2/5% CO_2) and adjusted to pH 7.4. Isolated hepatocytes from livers of pentobarbital-anesthetized rats (3 mg of sodium pentobarbital/100 g of body weight, i.p.) were prepared by collagenase perfusion (18). The yield of hepatocytes was about 2 × 10^8 cells/liver. Cell viability was estimated by determining Trypan blue exclusion. Only cell suspensions with a viability of >90% were used.

Isolated hepatocytes (2 × 10^6 cells/ml) in standard isolation buffer were incubated with a 1–3 μM concentration of sodium salt of 2,2,3,3,18,18,18-heptafluorooctadecanoic acid, containing 0.1–1 MBq tritium-labeled compound. The cell suspension was kept under an atmosphere of carbogen and gently shaken for 2 h at 37°C. The cell suspension was then centrifuged, and the supernatant was evaporated to dryness in vacuo. The cell pellet and the residue after evaporation were extracted five times with 200 μl of methanol. The combined methanol extracts were evaporated and the residues were finally dissolved in 20 μl of methanol. These solutions were analyzed by HPTLC using the solvent systems described below (Syntheses).

Photolysis and photoaffinity labeling

Photolysis and photoaffinity labeling were carried out at 30°C in a Rayonet RPR 100 reactor (The Southern Ultraviolet Company, Hamden, CT) equipped with 16 RPR 3500 Å lamps (19, 20). Ultraviolet absorption spectra were measured with a Perkin-Elmer UV/VIS-Spectrometer Lambda 5 (Perkin-Elmer, Überlingen, Germany).

Photoaffinity labeling of rat serum albumin and of FABP from rat liver was performed in a solution of 15 nM (37 kBq) of 11,11-azo-2,2,3,3,18,18,18-heptafluoro[G-^{3}H]octadecanoic acid sodium salt and of 1 μM protein in 0.1 M potassium sodium phosphate buffer, adjusted to pH 7.2. Further experimental details of photoaffinity labeling of a mixture of proteins are given in the legend to Fig. 6. Irradiation was performed at 350 nm for dif-

ferent lengths of time. Incorporation of radioactivity into protein was determined after SDS/PAGE.

Polyacrylamide gel electrophoresis and detection of radioactivity

Discontinuous sodium dodecylsulfate/polyacrylamide gel electrophoresis using vertical slab gels (200 × 180 × 2.8 mm) and detection of radioactivity were performed exactly as described (21).

Analysis of organic compounds

Elemental analyses were carried out with a Perkin-Elmer 240 analyzer (Perkin-Elmer, Friedrichshafen, Germany). Melting points were determined with a Büchi hotstage apparatus (Büchi, Flawil, Switzerland) and are uncorrected. ^{1}H-NMR spectra were measured on a Bruker-250-MHz spectrometer (Bruker GmbH, Karlsruhe, Germany); ^{3}H-NMR spectra were obtained with a Bruker AM 360 pulse spectrometer (Bruker GmbH) operating at 384 MHz; values are in parts per million relative to tetramethylsilane as internal standard. ^{19}F-NMR spectra were measured on a Varian EM 390-A spectrometer (Varian GmbH, Darmstadt, Germany); values are in parts per million relative to fluorobenzol as internal standard. Mass spectra were recorded with a Finnigan 44S mass spectrometer connected with a data unit SS 2000 (Finnigan, Sunnyvale, CA). All synthesized compounds were ionized by EI (electron impact ionization) with an electron energy of 70 eV, and some synthesized substances were ionized by DCI (direct chemical ionization) with an electron energy of 170 eV using isobutane or ammonia as a reactant gas at a pressure of 30 Pa. In both cases positive ions were recorded (22). Radioactivity determination was carried out with a Packard model 3375 Tri-Carb liquid scintillation spectrometer (Canberra Packard International S.A., Zürich, Switzerland) using a Lumagel^R cocktail. All solvents were dried over molecular sieves type 3A or 4A and freshly distilled before use. The organic compounds were visualized after TLC by spraying the dried plates with 0.1 N $KMnO_4$ and subsequent heating at 80°C for 5 min.

Syntheses

Preparative column chromatography was performed on 25 × 5 cm columns of silica gel 60 (40–63 μm) using the flash technique (23). Solvent systems for chromatographic separations were: solvent system 1, cyclohexane–ethyl acetate 15:1 (v/v); solvent system 2, cyclohexane–ethyl acetate 5:1 (v/v); solvent system 3, cyclohexane–ethyl acetate 4:1 (v/v); solvent system 4, cyclohexane–ethyl acetate 2:1 (v/v); solvent system 5, cylcohexane–ethyl acetate 1:1 (v/v); solvent system 6, cyclohexane–chloroform–ethyl acetate–acetone 8:2:2:1 (v/v/v/v); solvent system 7, cyclohexane–ethyl acetate–petrolether (60–70°C)–chloroform 4:2:2:1 (v/v/v/v); solvent system 8, ethyl acetate–petrolether (60–70°C) 2:1 (v/v); solvent system 9, chloroform–methanol 5:1 (v/v); solvent system 10, chloroform–methanol 3:1 (v/v); solvent system 11, chloroform–methanol 1:1 (v/v).

3-Iodo-2,2,3,3-tetrafluoropropanoic acid amide (V) (**Fig. 1**). The 3-iodo-2,2,3,3-tetrafluoropropanoic acid amide was synthesized principally according to Brown and Wetzel (24). A sample of 5.09 g (20 mmol) of 3-iodo-2,2,3,3-tetrafluoropropionitrile (IV), synthesized according to Krespan (25), and 815 μl (20 mmol) of dry methanol were placed in a 50-ml stainless steel autoclave. The autoclave was cooled in liquid nitrogen and evacuated to a pressure of 100 Pa. Six hundred μl (20 mmol) of anhydrous hydrogen chloride was condensed into the autoclave. The autoclave was closed under vacuum and allowed to warm slowly to room temperature and kept there for 24 h. The autoclave was opened cautiously, and the crude product was purified by flash chromatography using solvent system 4. The yield of pure product was 4.9 g (18 mmol, 90% yield). MP, 125°C; TLC: R_f = 0.07 (solvent system 2), 0.23 (solvent system 4); ^{1}H-NMR ($CDCl_3$): δ = 6.25 (d,b,CONH$_2$); ^{19}F-NMR ($CDCl_3$): δ = −53.5 (t,CF$_2$–3,J_{FF} = 7 Hz), −105.7 (t,CF$_2$–2); mass spectrum (EI): *m/z* = 208, M–(F + $CONH_2$); 177, M–CF_2CONH_2; 144 M–I; mass spectrum (DCI, isobutane): *m/z* = 272, $(M + H)^+$; 271, M^+; anal. calcd. for $C_3H_2F_4INO$ (270.95): C, 13.30, H, 0.74, N, 5.17; found: C, 13.50, H, 0.80, N, 5.05.

1-Bromo-15,15,15-trifluoropentadecane (IX) (Fig. 1). The 1-bromo-15,15,15-trifluoropentadecane was prepared principally according to Dmowski and Kolinski (26). The reaction was carried out in a 50-ml stainless steel autoclave operated at autogenous pressure. Ten g (30 mmol) of 15-bromopentadecanoic acid (VIII), synthesized according to Chuit and Hausser (27), was placed in the autoclave. The autoclave was cooled in liquid nitrogen and evacuated to a pressure of 100 Pa. Subsequently, 5.2 ml (93 mmol) of sulfur tetrafluoride was condensed into the autoclave. The charged autoclave was placed in a rocking muffle furnace and kept at 20°C for 19 h, and then heated to 120°C for 6 h. After cooling, the reaction gases were discharged and passed through three subsequent washers, charged with 20% aqueous KOH, in order to guarantee complete absorption. The remaining products were poured carefully onto ice in a polyethylene beaker, and the resulting mixture was slowly and cautiously neutralized with a saturated $NaHCO_3$ solution. The reaction product was extracted with three portions of 150 ml of chloroform. The combined chloroform extracts were washed with 0.1 N $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated. The crude product was purified by flash chromatography using cyclohexane as solvent yielding 8.3 g (24 mmol, 80% yield) of pure product. TLC: R_f = 0.30 (cyclohexane), 0.62 (solvent system 4); ^{1}H-NMR ($CDCl_3$): δ = 1.30 (m,CH$_2$-4/5/6/7/8/9/10/11/

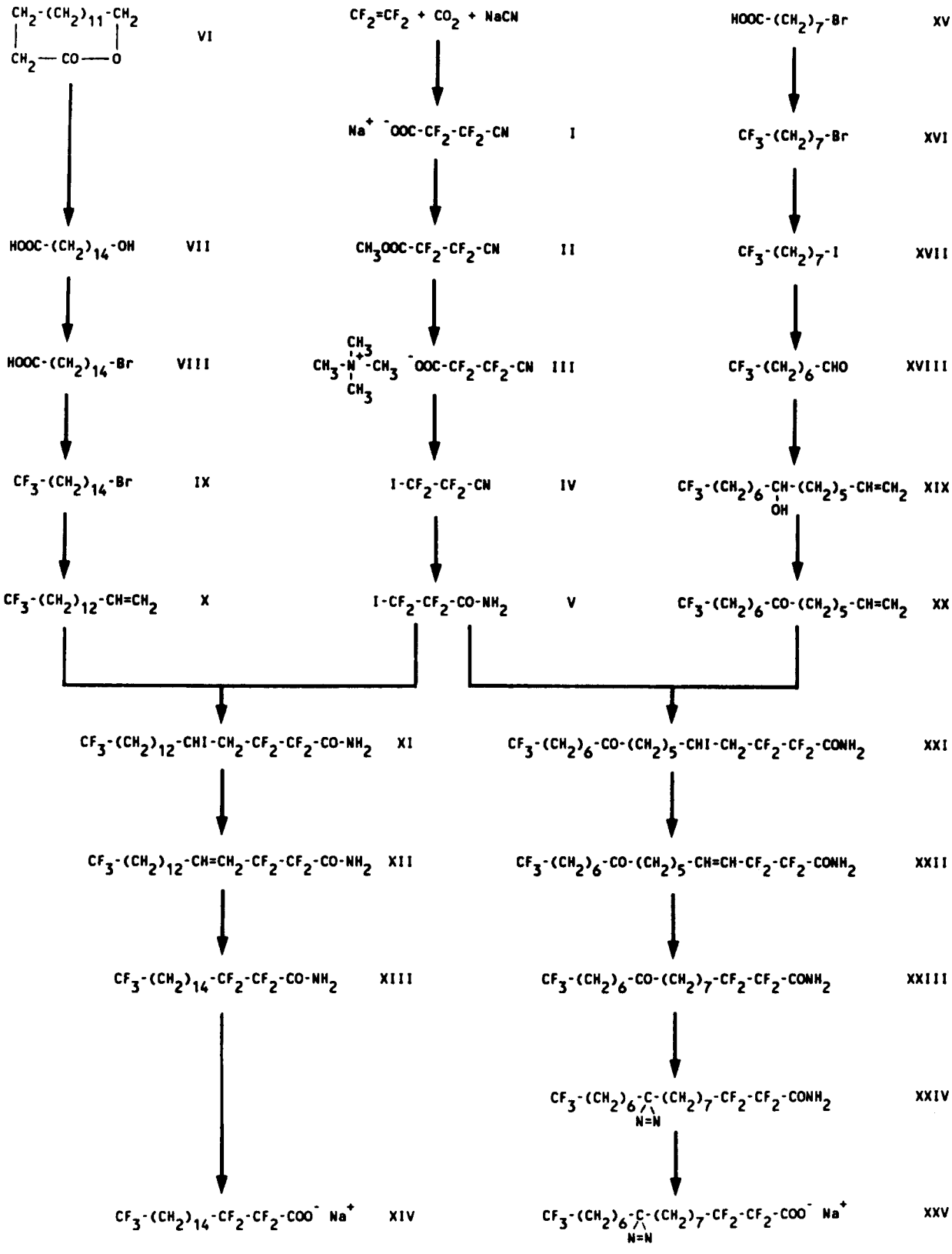

Fig. 1. Reaction scheme for the synthesis of α,β,ω-fluorinated fatty acid salts and their diazirine derivatives.

12), 1.40 (m,C$\underline{H}_2$-3), 1.52 (m,C$\underline{H}_2$-13), 1.85 (m,C$\underline{H}_2$-2), 2.05 (m,C$\underline{H}_2$-14), 3.4 (t,C$\underline{H}_2$-1); ^{19}F-NMR ($CDCl_3$): δ = −59.7 (s,C$\underline{F}_3$-15); mass spectrum (EI): *m/z* = 346, $(M+H)^+$; 265, M-Br; anal. calcd. for $C_{15}H_{28}BrF_3$ (345.29): C, 52.18, H, 8.17; found: C, 52.02, H, 8.12.

15,15,15-Trifluoro-1-pentadecene (X) (Fig. 1). The 15,15,15-trifluoro-1-pentadecene was synthesized principally according to Sharpless and Young (28) and Grieco, Gilman, and Nishizawa (29). A vigorously stirred suspension of 2.33 g (10 mmol) of *o*-nitrophenyl selenocyanate in 33 ml of dry ethanol was cooled to 0°C and slowly treated with 0.46 g (12 mmol) of sodium borohydride under an atmosphere of dry nitrogen. A sample of 3.2 g (10 mmol) of 1-bromo-15,15,15-trifluoropentadecane (IX) was added, and the resulting mixture was stirred at room temperature for 13 h. The ethanol was removed, and the residue was redissolved in 50 ml of tetrahydrofuran. After cooling to 0°C, 13 ml of 30% aqueous hydrogen peroxide was added dropwise with vigorous stirring. The reaction mixture was allowed to warm up to ambient temperature and stirred for an additional 24 h. The reaction mixture was diluted with 100 ml of water, and the resulting mixture was extracted with three portions of 200 ml chloroform. The extracts were combined and dried over Na_2SO_4. After removal of solvent, the crude product was purified by flash chromatography using cyclohexane as solvent. The yield of pure product was 2.2 g (8.5 mmol, 85% yield). TLC: R_f = 0.39 (cyclohexane), 0.67 (solvent system 4); ^{1}H-NMR ($CDCl_3$): δ = 1.30 (m,C$\underline{H}_2$-4/5/6/7/8/9/10/11/12), 1.52 (m,C$\underline{H}_2$-13), 2.05 (m,C$\underline{H}_2$-3/14), 4.85 (d,b,J = 8 Hz,C$\underline{H}$-1,*trans*), 4.95 (d,b,J = 15 Hz,C$\underline{H}$-1,*cis*), 5.8 (m,C$\underline{H}$-2); ^{19}F-NMR ($CDCl_3$): δ = −60 (s,C$\underline{F}_3$-15); mass spectrum (EI): *m/z* = 265, $(M+H)^+$; 264, M^+; anal. calcd. for $C_{15}H_{27}F_3$ (264.38): C, 68.15, H, 10.29; found: C, 68.34, H, 10.40.

2,2,3,3,18,18,18-Heptafluoro-5-iodooctadecanoic acid amide (XI) (Fig. 1). The 2,2,3,3,18,18,18-heptafluoro-5-iodooctadecanoic acid amide was synthesized principally according to Chen, He, and Yang (30). A mixture of 2.3 g (8.7 mmol) of 15,15,15-trifluoro-1-pentadecene (X), 1.2 g (4.3 mmol) of 3-iodo-2,2,3,3-tetrafluoropropanoic acid amide, 1 ml of dry N,N-dimethylformamide, and 0.02 g (0.36 mmol) of freshly etched iron powder was stirred at 80°C for 2 h. After cooling to room temperature, the reaction mixture was taken up in solvent system 2, and the product was isolated by flash chromatography using solvent system 2. The yield of pure product was 1.7 g (3.2 mmol, 75% yield). MP, 65°C; TLC: R_f = 0.14 (solvent system 2), 0.37 (solvent system 4); ^{1}H-NMR ($CDCl_3$): δ = 1.30 (m,C$\underline{H}_2$-7/8/9/10/11/12/13/14/15), 1.52 (m,C$\underline{H}_2$-16), 1.75 (m,C$\underline{H}_2$-6), 2.05 (m,C$\underline{H}_2$-17), 2.90 (m,C$\underline{H}_2$-4), 4.35 (m,C$\underline{H}$-5), 6.25 (d,b,CONH$\underline{H}_2$); ^{19}F-NMR ($CDCl_3$): δ = −62.4 (s,C$\underline{F}_3$-18), −107.3 (s,C$\underline{F}_2$-3), −108.1 (s,C$\underline{F}_2$-3), −115 (s,C$\underline{F}_2$-2); mass spectrum (EI): *m/z* = 408, M-I; 388, M-(I+HF); mass spectrum (DCI, ammonia): *m/z* = 553, $(M+NH_4)^+$; 441, M-$CF_2\dot{C}ONH_2$; anal. calcd. for $C_{18}H_{29}F_7NO$ (535.33): C, 40.39, H, 5.46, N, 2.62; found: C, 40.65, H, 5.45, N, 2.56.

2,2,3,3,18,18,18-Heptafluoro-4-octadecenoic acid amide (XII) (Fig. 1). The 2,2,3,3,18,18,18-heptafluoro-4-octadecenoic acid amide was synthesized principally according to Tarrant, Lovelace, and Lilyquist (31). A mixture of 1 g (1.9 mmol) of 2,2,3,3,18,18,18-heptafluoro-5-iodooctadecanoic acid amide (XI) and 1 ml of tri-n-butylamine was stirred at 170°C for 45 min. After cooling to room temperature, the reaction mixture was taken up in solvent system 4, and the product was isolated by flash chromatography using solvent system 4. The yield of pure product was 0.62 g (1.5 mmol, 80% yield). MP, 60°C; TLC: R_f = 0.35 (solvent system 4), 0.05 (solvent system 2); ^{1}H-NMR ($CDCl_3$): δ = 1.30 (m,C$\underline{H}_2$-7/8/9/10/11/12/13/14/15), 1.52 (m,C$\underline{H}_2$-16), 2.05 (m,C$\underline{H}_2$-17), 2.19 (m,C$\underline{H}_2$-6), 5.65 (m,b,C$\underline{H}$-5), 6.35 (m,b,CONH$\underline{H}_2$,C$\underline{H}$-4); ^{19}F-NMR ($CDCl_3$): δ = −58.8 (s,C$\underline{F}_3$-18; t,J_{HF} = 10.7 Hz), −100 (s,C$\underline{F}_2$-3,*trans*; d,J_{HF} = 15.6 Hz), −104.6 (s,C$\underline{F}_2$-3,*cis*; d,J_{HF} = 11 Hz), −113.6 (s,C$\underline{F}_2$-2,*trans*), −113.9 (s,C$\underline{F}_2$-2,*cis*); mass spectrum (EI): *m/z* = 407, M^+; 387, M-HF; 367, M-2HF; 347, M-3HF; anal. calcd. for $C_{18}H_{28}F_7NO$ (407.42): C, 53.07, H, 6.93, N, 3.43; found: C, 53.33, H, 6.97, N, 3.51.

2,2,3,3,18,18,18-Heptafluorooctadecanoic acid amide (XIII) (Fig. 1). The 2,2,3,3,18,18,18-heptafluorooctadecanoic acid amide was synthesized principally according to Coe and Milner (32). A mixture of 1 g (2.5 mmol) of 2,2,3,3,18,18,18-heptafluoro-4-octadecenoic acid amide (XII), 1 g of palladium on charcoal (5% Pd) catalyst, and 150 ml of absolute ethanol was shaken in an atmosphere of hydrogen at room temperature for 3 h. When hydrogen uptake was completed (about 100 ml), the palladium catalyst was filtered off, and washed with a few portions of ether. The ethanol and ether solutions were combined and evaporated under reduced pressure. The crude product was purified by flash chromatography using solvent system 3. The yield of pure product was 1 g (2.45 mmol, 98% yield). MP, 105°C; TLC: R_f = 0.37 (solvent system 4), 0.10 (solvent system 3); ^{1}H-NMR ($CDCl_3$): δ = 1.30 (m,C$\underline{H}_2$-6/7/8/9/10/11/12/13/14/15), 1.52 (m,C$\underline{H}_2$-5/16), 2.05 (m,C$\underline{H}_2$-4/17), 6.00 (d,b,CONH$\underline{H}_2$); ^{19}F-NMR ($CDCl_3$): δ = −64.7 (s,C$\underline{F}_3$-18; t,J_{HF} = 11.2 Hz), −114.2 (s,CF_2-3; t,J_{HF} = 18.6 Hz), −120.1 (s,C$\underline{F}_2$-2); mass spectrum (EI): *m/z* = 409, M^+; 389, M-HF; 369, M-2HF; 349, M-3HF; anal. calcd. for $C_{18}H_{30}F_7NO$ (409.43): C, 52.81, H, 7.39, N, 3.42; found: C, 53.21, H, 7.45, N, 3.41.

2,2,3,3,18,18,18-Heptafluorooctadecanoic acid sodium salt (XIV) (Fig. 1). A mixture of 0.5 g (12.5 mmol) of NaOH, 0.55 g (1.3 mmol) of 2,2,3,3,18,18,18-heptafluorooctadecanoic acid amide (XIII), 10 ml of dioxane, 10 ml of water, and 5 ml of ethanol was stirred overnight at room temperature. The sodium salt of the fatty acid was separated

by adsorption chromatography on Amberlite XAD-2 yielding 0.57 g (1.3 mmol, 98% yield) of pure product. TLC: R_f = 0.08 (solvent system 9), 0.70 (solvent system 11); anal. calcd. for $C_{18}H_{28}F_7NaO_2$ (432.40): C,50.00, H, 6.53; found: C, 50.31, H, 6.78.

2,2,3,3,18,18,18-Heptafluorooctadecanoic acid. In a small flask 2 g (4.6 mmol) of the sodium salt of 2,2,3,3,18,18,18-heptafluorooctadecanoic acid (XIV) was added to 4 ml of concentrated sulfuric acid. The liberated free acid was isolated by high vacuum distillation as a viscous oil, which solidified in the receiver. The solid product was recrystallized from CCl_4 forming shiny colorless crystals. The yield of pure product was 1.5 g (3.7 mmol, 80% yield). MP, 58°C; ^{1}H-NMR ($CDCl_3$): δ = 1.35 (m,C$\underline{H}_2$-6/7/8/9/10/11/12/13/14/15), 1.55 (m,C$\underline{H}_2$-5/16), 2.05 (m,C$\underline{H}_2$-4/17), 10.8 (s,b,COO$\underline{H}$); ^{19}F-NMR ($CDCl_3$): δ = −62.8 (s,C$\underline{F}_3$-18; t,J_{HF} = 10.9 Hz), −113.8 (s,C$\underline{F}_2$-3; t,J_{HF} = 19 Hz), −125.6 (s,C$\underline{F}_2$-2); mass spectrum (EI): *m/z* = 350, M-3HF; 346, M-(CO_2+HF); mass spectrum (DCI, isobutane): *m/z* = 411, $(M+H)^+$; 372, M-(HF+F); anal. calcd. for $C_{18}H_{29}F_7O_2$ (410.42): C, 52.68, H, 7.12; found: C, 52.55, H, 7.08.

1-Bromo-8,8,8-trifluorooctane (XVI) (Fig. 1). The 1-bromo-8,8,8-trifluorooctane was synthesized analogous to 1-bromo-15,15,15-trifluoropentadecane (IX). Ten g (45 mmol) of 8-bromooctanoic acid (XV) was treated with 7.6 ml (135 mmol) of sulfur tetrafluoride. The crude product was purified by flash chromatography using solvent system 1. The yield of pure product was 9.7 g (39 mmol, 87% yield). TLC: R_f = 0.06 (cyclohexane), 0.64 (solvent system 4); ^{1}H-NMR ($CDCl_3$): δ = 1.30 (m,C$\underline{H}_2$-4/5), 1.40 (m,C$\underline{H}_2$-3), 1.52 (m,C$\underline{H}_2$-6), 1.85 (m,C$\underline{H}_2$-2), 2.05 (m,C$\underline{H}_2$-7), 3.41 (t,C$\underline{H}_2$-1); ^{19}F-NMR ($CDCl_3$): δ = −59.7 (s,C$\underline{F}_3$-8; t,J_{HF} = 10.6 Hz); mass spectrum (EI): *m/z* = 248, $(M+H)^+$; 168, M-Br; anal. calcd. for $C_8H_{14}BrF_3$ (247.10): C, 38.89, H, 5.71; found: C, 39.20, H, 5.87.

1-Iodo-8,8,8-trifluorooctane (XVII) (Fig. 1). The 1-iodo-8,8,8-trifluorooctane was synthesized principally according to Johnson and Pelter (33). A sample of 8.6 g (35 mmol) of 1-bromo-8,8,8-trifluorooctane (XVI) was added to a stirred refluxing mixture of 12 g (40 mmol) of NaI and 40 ml of acetone. Refluxing was maintained for an additional 18 h. The reaction mixture was poured onto ice and extracted with three portions of 150 ml of ether. The combined ether extracts were dried over $MgSO_4$ and evaporated in vacuo. The crude product was purified by flash chromatography using solvent system 1. The yield of pure product was 9.4 g (32 mmol, 90% yield). TLC: R_f = 0.07 (cyclohexane), 0.62 (solvent system 4); ^{1}H-NMR ($CDCl_3$): δ = 1.30 (m,C$\underline{H}_2$-4/5), 1.40 (m,C$\underline{H}_2$-3), 1.52 (m,C$\underline{H}_2$-6), 1.85 (m,C$\underline{H}_2$-2), 2.05 (m,C$\underline{H}_2$-7), 3.20 (t,C$\underline{H}_2$-1); ^{19}F-NMR ($CDCl_3$): −59.6 (s,C$\underline{F}_3$-8; t,J_{HF} = 10.6 Hz); mass spectrum (EI): *m/z* = 294, M^+; 167, M-I; 155, M-(C+I); anal. calcd. for $C_8H_{14}F_3I$ (294.10): C, 32.67, H, 4.80; found: C, 32.48, H, 4.65.

8,8,8-Trifluorooctane-1-al (XVIII) (Fig. 1). The 8,8,8-trifluorooctane-1-al was synthesized principally according to Johnson and Pelter (33). To a stirred mixture of 140 ml of dimethyl sulfoxide and 19 g (0.226 mol) of sodium hydrogen carbonate was added 9.7 g (33 mmol) of 1-iodo-8,8,8-trifluorooctane (XVII) at 150°C under an atmosphere of dry nitrogen. After 4 min, the mixture was rapidly cooled and poured into water. The aqueous solution was extracted four times with 50 ml of ether. The combined ether extracts were washed with brine, dried over $MgSO_4$, and after removal of solvent, the crude product was purified by flash chromatography using solvent system 1. The yield of pure product was 4.3 g (24 mmol, 72% yield). TLC: R_f = 0.17 (solvent system 1), 0.42 (solvent system 4); ^{1}H-NMR ($CDCl_3$): δ = 1.30 (m,C$\underline{H}_2$-4/5), 1.52 (m,C$\underline{H}_2$-6), 1.65 (m,C$\underline{H}_2$-3), 2.05 (m,C$\underline{H}_2$-7), 2.42 (t,C$\underline{H}_2$-2), 9.80 (s,C$\underline{H}$O-1); ^{19}F-NMR ($CDCl_3$): δ = −59.6 (s,C$\underline{F}_3$-8; t,J_{HF} = 10.6 Hz); mass spectrum (EI): *m/z* = 164, M-H_2O; 154, M-CO; 138, M-C_2H_4O; mass spectrum (DCI, isobutane): *m/z* = 365, $(M+M+H)^+$; 239, $(M+C(C\underline{H}_3)_3)^+$; 183, $(M+H)^+$; anal. calcd. for $C_8H_{13}F_3O$ (182.19): C, 52.74, H, 7.19; found: C, 53.12, H, 7.43.

8-Hydroxy-15,15,15-trifluoro-1-pentadecene (XIX) (Fig. 1). The 8-hydroxy-15,15,15-trifluoro-1-pentadecene was synthesized principally according to Marshall (34). A sample of 0.23 g (10 mmol) of magnesium turnings was suspended in 1 ml of dry ether, and the suspension was allowed to react with a few drops of a solution of 1.65 g (9 mmol) of 7-bromo-1-heptene in 1.5 ml of dry ether. The bromo compound was previously prepared according to Casteignau and Villessot (35). When the violent reaction had started, the main part of the 7-bromo-1-heptene solution was slowly added. After refluxing for 30 min, the mixture was cooled in ice, and a solution of 1.7 g (9 mmol) of 8,8,8-trifluorooctane-1-al (XVIII) in 2.5 ml of dry ether was added slowly, so that the temperature did not exceed 0°C. To complete the reaction, the mixture was stirred at room temperature for 1 h and then decomposed with 1 N aqueous hydrochloric acid. The ether phase was separated, and the aqueous phase was extracted with three portions of 150 ml of chloroform. The combined organic phases were dried over Na_2SO_4. After removal of solvent, the crude product was purified by flash chromatography using solvent system 2. The yield of pure product was 1.9 g (6.8 mmol, 75% yield). TLC: R_f = 0.23 (solvent system 2), 0.44 (solvent system 4); ^{1}H-NMR ($CDCl_3$): δ = 1.30 (m,C$\underline{H}_2$-4/5/6/10/11/12), 1.41 (m,C$\underline{H}_2$-7/9), 1.52 (m,C$\underline{H}_2$-13), 2.05 (m,C$\underline{H}_2$-3/14), 3.60 (m,C$\underline{H}$-8), 4.85 (d,b,J = 8 Hz,C$\underline{H}$-1,*trans*), 4.95 (d,b,J = 15 Hz,C$\underline{H}$-1,*cis*), 5.8 (m,C$\underline{H}$-2); ^{19}F-NMR ($CDCl_3$): δ = −60.2 (s,C$\underline{F}_3$-15; t,J_{HF} = 10.5 Hz); mass spectrum (EI): *m/z* = 262, M-H_2O; 234, M-(H_2O+C_2H_4); 220, M-(H_2O+C_3H_6); anal. calcd. for $C_{15}H_{27}F_3O$ (280.38): C, 64.26, H, 9.71; found: C, 64.60, H, 9.98.

8-Oxo-15,15,15-trifluoro-1-pentadecene (XX) (Fig. 1). The 8-oxo-15,15,15-trifluoro-1-pentadecene was synthesized principally according to Bowers et al. (36). A cold solution of 267 g (2.7 mol) of chromic acid in 230 ml of concentrated sulfuric acid and 400 ml of water was adjusted to a final volume of 1 l. 8-Hydroxy-15,15,15-trifluoro-1- pentadecene [4.38 g (15 mmol)] (XIX) was dissolved in 42 ml of dry acetone, and the chromic acid solution was added dropwise until a persistent orange-brown coloration indicated that oxidation was complete (about 3.5 ml). After dilution with 200 ml of water, the organic products were extracted three times with 150 ml of chloroform, and the combined chloroform extracts were washed with brine and dried over Na_2SO_4. After removal of solvent, the crude product was purified by flash chromatography using solvent system 1. The yield of pure product was 4 g (14.4 mmol, 96% yield). TLC: R_f = 0.30 (solvent system 1), 0.56 (solvent system 4); ^{1}H-NMR ($CDCl_3$): δ = 1.30 (m,C$\underline{H}_2$-4/5/11/12), 1.52 (m,C$\underline{H}_2$-6/10/13), 2.05 (m,C$\underline{H}_2$-3/14), 2.40 (t,C$\underline{H}_2$-7/9), 4.85 (d,b,J = 8 Hz,C$\underline{H}$-1,*trans*), 4.95 (d,b,J = 15 Hz,C$\underline{H}$-1,*cis*), 5.80 (m,C$\underline{H}$-2); ^{19}F-NMR ($CDCl_3$): δ = -60 (s,C$\underline{F}_3$-15; t,J_{HF} = 10.6 Hz); mass spectrum (EI): *m/z* = 209, M-CF_3; 181, M-C_7H_{12}; mass spectrum (DCI, isobutane): 279, $(M+H)^+$; anal. calcd. for $C_{15}H_{25}F_3O$ (278.36): C, 64.72, H, 9.05; found: C, 64.93, H, 9.31.

2,2,3,3,18,18,18-Heptafluoro-5-iodo-11-oxooctadecanoic acid amide (XXI) (Fig. 1). The 2,2,3,3,18,18,18-heptafluoro-5-iodo-11-oxooctadecanoic acid amide was synthesized analogous to 2,2,3,3,18,18,18-heptafluoro-5-iodooctadecanoic acid amide (XI). The crude product was purified by flash chromatography using solvent system 6. The yield of pure product was 90%. TLC: R_f = 0.22 (solvent system 6), 0.51 (solvent system 5); ^{1}H-NMR ($CDCl_3$): δ = 1.30 (m,C$\underline{H}_2$-7/8/14/15), 1.52 (m,C$\underline{H}_2$-9/13/16), 1.75 (m,C$\underline{H}_2$-6), 2.05 (m,C$\underline{H}_2$-17), 2.40 (t,C$\underline{H}_2$-10/12), 2.90 (m,C$\underline{H}_2$-4), 4.35 (m,C$\underline{H}_2$-4), 6.25 (d,b,CON$\underline{H}_2$); ^{19}F-NMR ($CDCl_3$): δ = -59.6 (s,C$\underline{F}_3$-18; t,J_{HF} = 10.6 Hz), -107.4 (s,C$\underline{F}_2$-3), -108.1 (s,C$\underline{F}_2$-3), -114.8 (s,C$\underline{F}_2$-2); mass spectrum (EI): *m/z* = 549, M^+; 530, M-F; 402, M-(I + HF); anal. calcd. for $C_{18}H_{27}F_7INO_2$ (549.31): C, 39.36, H, 4.95, N, 2.55; found: C, 39.62, H, 5.21, N, 2.60.

2,2,3,3,18,18,18-Heptafluoro-11-oxo-4-octadecenoic acid amide (XXII) (Fig. 1). The 2,2,3,3,18,18,18-heptafluoro-11-oxo-4-octadecenoic acid amide was synthesized principally according to Tarrant et al. (31). A mixture of 2 g (3.6 mmol) of 2,2,3,3,18,18,18-heptafluoro-5-iodo-11-oxooctadecanoic acid amide (XXI), 10 ml of triethylamine, and 10 ml of dry toluene was stirred at reflux temperature for 24 h. One hundred and fifty ml of dry ether was added to the cooled mixture, and the precipitate was filtered off. The filtrate was evaporated under reduced pressure, and the crude product was purified by flash chromatography using solvent system 7. The yield of pure product was 1.4 g (3.3 mmol, 90% yield). TLC: R_f = 0.15 (solvent system 7), 0.46 (solvent system 4); ^{1}H-NMR ($CDCl_3$): δ = 1.30 (m,C$\underline{H}_2$-7/8/14/15), 1.52 (m,C$\underline{H}_2$-9/13/16), 2.05 (m,C$\underline{H}_2$-17), 2.19 (m,C$\underline{H}_2$-6), 2.40 (t,C$\underline{H}_2$-10/12), 5.65 (m,b,C$\underline{H}$-5), 6.35 (m,b,CON$\underline{H}_2$,C$\underline{H}$-4); ^{19}F-NMR ($CDCl_3$): δ = -58.7 (s,C$\underline{F}_3$-18; t,J_{HF} = 10.7 Hz), -100 (s,C$\underline{F}_2$-3,*trans*; d,J_{HF} = 15.6 Hz), -104.6 (s,C$\underline{F}_2$-3,*cis*; d,J_{HF} = 11 Hz), -113.6 (s,C$\underline{F}_2$-2,*trans*), -113.9 (s,C$\underline{F}_2$-2,*cis*); mass spectrum (EI): *m/z* = 421, M^+; 381, M-2HF; 361, M-3HF; mass spectrum (DCI, ammonia): 439, $(M+NH_4)^+$; 422, $(M+H)^+$; anal. calcd. for $C_{18}H_{26}F_7NO_2$ (421.40): C, 51.33, H, 6.22, N, 3.33; found: C, 51.07, H, 6.13, N, 3.22.

2,2,3,3,18,18,18-Heptafluoro-11-oxooctadecanoic acid amide (XXIII) (Fig. 1). The 2,2,3,3,18,18,18-heptafluoro-11-oxooctadecanoic acid amide was synthesized analogous to 2,2,3,3,18,18,18-heptafluorooctadecanoic acid amide (XIII). A sample of 2.1 g (5 mmol) of 2,2,3,3,18,18,18,-heptafluoro-11-oxo-4-octadecenoic acid amide (XXII) yielded, after flash chromatography using solvent system 8, 2.1 g (5 mmol, 95% yield) of pure product. MP, 58°C; TLC: R_f = 0.28 (solvent system 8), 0.16 (solvent system 4); ^{1}H-NMR ($CDCl_3$): δ = 1.30 (m,C$\underline{H}_2$-6/7/8/14/15), 1.52 (m,C$\underline{H}_2$-5/9/13/16), 2.05 (m,C$\underline{H}_2$-4/17), 2.40 (t,C$\underline{H}_2$-10/12), 6.56 (d,b,CON$\underline{H}_2$); ^{19}F-NMR ($CDCl_3$): δ = -64.7 (s,C$\underline{F}_3$-18; t,J_{HF} = 11.2 Hz), -114.2 (s,C$\underline{F}_2$-3; t,J_{HF} = 18.6 Hz), -120.1 (s,C$\underline{F}_2$-2); mass spectrum (EI): *m/z* = 424, $(M+H)^+$; 404, M-F; anal. calcd. for $C_{18}H_{28}F_7NO_2$ (423.42): C, 51.06, H, 6.67, N, 3.31; found: C, 51.55, H, 6.70, N, 3.40.

11,11-Azo-2,2,3,3,18,18,18-heptafluorooctadecanoic acid amide (XXIV) (Fig. 1). The 11,11-azo-2,2,3,3,18,18,18-heptafluorooctadecanoic acid amide was synthesized principally according to Kramer and Kurz (19). A sample of 1.2 g (2.8 mmol) of 2,2,3,3,18,18,18-heptafluoro-11-oxooctadecanoic acid amide (XXIII) was dissolved in 100 ml of dry methanol, and dry ammonia was bubbled through the solution at 0°C for 3 h. Under vigorous stirring a solution of 1.5 g (13 mmol) of hydroxylamine-O-sulfonic acid in 30 ml of dry methanol was added at 0°C during 20 min. All subsequent operations were performed in dim light. The mixture was allowed to warm up to room temperature, stirred for an additional 12 h, and filtered. After addition of 1 ml of triethylamine, the filtrate was evaporated to dryness. The residue was dissolved in 50 ml of dry methanol, and after addition of 3 ml of triethylamine, a solution of 1.5 g (6 mmol) of iodine in 10 ml of dry methanol was added at room temperature in the dark within 20 min. An orange-red coloration indicated the end of the reaction. The mixture was evaporated under reduced pressure, and the crude product was purified by flash chromatography using solvent system 2. The yield of pure product was 0.37 g (0.85 mmol, 30% yield). TLC: R_f = 0.33 (solvent system 4), 0.59 (solvent system 5); ^{1}H-NMR ($CDCl_3$): δ = 1.35 (m,C$\underline{H}_2$-6/7/8/9/10/12/13/14/15), 1.55 (m,C$\underline{H}_2$-5/16), 2.05 (m,C$\underline{H}_2$-4/17), 6.56 (d,b,CON$\underline{H}_2$); ^{19}F - NMR ($CDCl_3$):

δ = −63.8 (s,C$\underline{F}_3$-18; t,J_{HF} = 11.2 Hz), −114.2 (s,CF_2-3; t,J_{HF} = 18.6 Hz), −120.1 (s,C$\underline{F}_2$-2); UV (methanol): λ_{max} = 349 nm (ϵ = 81 $M^{-1}cm^{-1}$); mass spectrum (EI): *m/z* = 407, M-N_2; 367, M-(N_2 + 2HF); mass spectrum (DCI, ammonia): *m/z* = 453, $(M + NH_4)^+$; 425, M-(N_2-NH_4); anal. calcd. for $C_{18}H_{28}F_7N_3O$ (435.43): C, 49.65, H, 6.48, N, 9.65; found: C, 49.91, H, 6.78, N, 9.45.

11,11-Azo-2,2,3,3,18,18,18-heptafluorooctadecanoic acid sodium salt (XXV) (Fig. 1). The 11,11-azo-2,2,3,3,18,18,18-heptafluorooctadecanoic acid sodium salt was synthesized analogous to 2,2,3,3,18,18,18-heptafluorooctanoic acid sodium salt (XIV). The crude product was purified by adsorption chromatography on Amberlite XAD-2. A sample of 1 g (2.3 mmol) of 11,11-azo-2,2,3,3,18,18,18-heptafluorooctadecanoic acid amide (XXIV) yielded 1 g (2.2 mol, 95% yield) of pure product. TLC: R_f = 0.11 (solvent system 9), 0.60 (solvent system 11); anal. calcd. for $C_{18}H_{26}F_7NaN_2O_2$ (458.40): C, 47.16, H, 5.70, N, 6.11; found: C, 47.43, H, 5.84 N, 6.21.

Syntheses of radioactively labeled fatty acid derivatives

2,2,3,3,18,18,18-Heptafluoro[G-^{3}H]octadecanoic acid amide (XIII) (Fig. 1). The tritiation of 2,2,3,3,18,18,18-heptafluoro-4-octadecenoic acid amide (XII) was performed in a high-vacuum glass apparatus. A 2-ml break-seal ampoule with 185 GBq of carrier-free tritium gas was connected to the vacuum line, the apparatus was evacuated to 0.01 Pa, and the break seal was opened with a small bar magnet. The tritium gas was transferred by means of a Toepler pump into a 2-ml reaction flask containing 9 mg (21 μmol) of 2,2,3,3,18,18,18-heptafluoro-4-octadecenoic acid amide (XII), 10 mg of 5% palladium on charcoal, and 500 μl of dry ethyl acetate. The reaction mixture was stirred at room temperature until the tritium uptake was completed (2 h). Residual tritium gas was pumped out and, after combustion over CuO at 450°C, converted to radioactive waste. The solvent was evaporated. Labile tritium was removed by repeated addition of methanol (3 parts per 1 ml) and subsequently freeze dried. The catalyst was filtered off and washed four times with ethyl acetate. The combined filtrates were evaporated; the residue was shown by TLC/radio-TLC (TLC-Analyzer LB 2820, Berthold, Wildbad, Germany) in system 2 to be chemically and radiochemically pure. It was finally dissolved in 50 ml of dry methanol in order to reduce radiolytic decomposition. The specific activity of the compound (6.4 GBq/mg, 2.63 TBq/mmol) was determined by LSC (Lumagel^R, Tri-Carb spectrometers, Canberra Packard International, Model 3375). The chemical yield was quantitative; the radiochemical yield was 31%, as calculated from the specific activity of the compound. ^{1}H-NMR ($CDCl_3$): δ = 1.30 (m,C$\underline{H}_2$-6/7/8/9/10/11/12/13/14/15), 1.52 (m,C$\underline{H}_2$-5/16), 2.05 (m,C$\underline{H}_2$-4/17), 6.00 (d,b,CON$\underline{H}_2$); ^{3}H-NMR ($CDCl_3$): δ = 1.28 (m,CH$\underline{T}$-6/7/8/9/10/11/12/13/14/15), 93.5%, 1.56 (m, CH$\underline{T}$-5/16), 6.5%. The solution was kept in the dark at −80°C in glass ampoules sealed under high vacuum.

2,2,3,3,18,18,18-Heptafluoro[G-^{3}H]octadecanoic acid sodium salt (XIV) (Fig. 1). A sample of 18.4 GBq (7 μmol) 2,2,3,3,18,18,18-heptafluoro[G-^{3}H]octadecanoic acid amide was dissolved in a solution of 1 ml dioxane and 1 ml of 10 mM NaOH. The resulting solution was stirred under an atmosphere of dry nitrogen at room temperature overnight. After removal of solvents by a stream of dry nitrogen, the residue was taken up in 500 μl of methanol, and the product was isolated by HPTLC using solvent system 10. The radiochemical yield of pure product was 18.3 GBq (99%). The compound showed a specific activity of 2.63 TBq/mmol. In order to reduce radiolytic decomposition, it was dissolved in methanol (18.5–37 kBq/μl) and stored as mentioned above.

2,2,3,3,18,18,18-Heptafluoro-11-oxo[G-^{3}H]octadecanoic acid amide (XXIII) (Fig. 1). The 2,2,3,3,18,18,18-heptafluoro-11-oxo[G-^{3}H]octadecanoic acid amide was synthesized and stored absolutely analogous to 2,2,3,3,18,18,18-heptafluoro[G-^{3}H]octadecanoic acid amide. Twenty μmol of 2,2,3,3,18,18,18-heptafluoro-11-oxo-4-octadecenoic acid amide yielded 52.5 GBq (100%) product having a specific activity of 2.63 TBq/mmol.

11,11-Azo-2,2,3,3,18,18,18-heptafluoro[G-^{3}H]octadecanoic acid amide (XXIV) (Fig. 1). A methanolic solution of 9.2 GBq (3.5 μmol) 2,2,3,3,18,18,18-heptafluoro-11-oxo[G-^{3}H]octadecanoic acid amide was desiccated. The dry residue was dissolved in 5 ml of methanol, and dry ammonia was bubbled through the solution at 0°C for 3 h. Under stirring, a solution of 10 mg (90 μmol) of hydroxylamine-O-sulfonic acid in 500 μl of dry methanol was added at 0°C within 20 min. The mixture was allowed to warm up to room temperature and stirred for an additional 16 h. The precipitate was removed; the filtrate was treated with 10 μl of triethylamine. The solution was evaporated to dryness, and the residue was dissolved in 2 ml of dry methanol. After addition of further 25 μl of triethylamine, an iodine crystal was added at 0°C to the solution. The reaction mixture was stirred at 0°C for 6 h and then concentrated in the dark with dry nitrogen to a final volume of 500 μl. The residual solution was subjected to a preparative HPTLC separation using a plate of 20 × 10 cm. The chromatogram was developed using solvent system 4. The radiochemical yield of pure product was 5.4 GBq (58%). The specific activity was unchanged (2.63 TBq/mmol); the radioactive product was stored as described.

11,11-Azo-2,2,3,3,18,18,18-heptafluoro[G-^{3}H]octadecanoic acid sodium salt (XXV) (Fig. 1). The 11,11-azo-2,2,3,3,18,18,18-heptafluoro[G-^{3}H]octadecanoic acid sodium salt was synthesized and stored analogous to 2,2,3,3,18,18,18-heptafluoro[G-^{3}H]octadecanoic acid amide yielded 2.58 GBq (98%) product, having a specific activity of 2.63 TBq/mmol.

RESULTS AND DISCUSSION

Syntheses

In order to study exclusively transport and translocation processes with fatty acid salts, metabolic reactions, both catabolic and anabolic, must be prevented as far as possible. Catabolic reactions occur at α-, ω-, and (ω-1)-positions of the fatty acid salts without previous activation, whereas β-oxidation as well as all anabolic reactions require formation of CoA derivatives. All degradation reactions of importance should be prevented by the introduction of fluorine in α-, β-, and ω-position of the fatty acid salts. Furthermore, fluorination of the α- and β-positions of fatty acids increases the acidity to such a degree that any activation reaction should be impeded.

Because transport and activation of fatty acid salts depend on their chain length (37), a synthesis strategy for α,β,ω-fluorinated fatty acid salts had to be conceived, which allowed a wide variation of chain length. In order to identify the polypeptides interacting with fatty acid salts by photoaffinity labeling, the introduction of the photoactivatable diazirine group had also to be designed for variable positions.

Because the successive introduction of fluorine in α- and β-positions by direct fluorination of the respective oxo groups proved not to be feasible, the synthesis of derivatives of the long-chain fatty acid stearic acid, 2,2,3,3,18,18,18-heptafluorooctadecanoic acid (XIV) and 11,11-azo-2,2,3,3,18,18,18-heptafluorooctadecanoic acid (XXV), was worked out according to the general scheme shown in Fig. 1.

The principle of the conceived synthesis is based on a radical addition of 3-iodinated perfluorinated propanoic acid amide (V) to ω-fluorinated 1-alkenes (X, XX). The addition reaction may also be carried out with the corresponding nitrile (IV) or with esters, but resulted in markedly lower yields of addition products. The radical addition reaction allows the synthesis of α,β,ω-fluorinated alkane carboxylic acids with high yields and a great variability of chain length. Moreover, it is easily feasible to introduce a further functional group into the α,β,ω-fluorinated fatty acid. Thus, in order to obtain a photolabile derivative, the oxo precursor group of the photoactivatable diazirine group can be introduced in a chosen position by use of appropriate oxo-1-alkenes. The oxo group does not interfere with the radical addition reaction. The radical addition reaction with 3-iodo-2,2,3,3-tetrafluoropropanoic acid amide (V) results in the formation of 5-iodo-derivatives (XI, XXI), which allow the generation of a reactive double bond adjacent to the tetrafluoroethylene group. This reactive double bond facilitates a complete hydrogenation under mild conditions, essential for the introduction of tritium with carrier-free tritium gas.

The key intermediate 3-iodo-2,2,3,3-tetrafluoropropanoic acid amide (V) was obtained from 3-iodo-2,2,3,3-tetrafluoropropionitrile (IV). This compound (IV) was synthesized according to Krespan (25) starting from gaseous tetrafluoroethylene via the intermediate compounds 3-cyano-2,2,3,3-tetrafluoropropionate (I), methyl 3-cyano-2,2,3,3-tetrafluoropropionate (II), and tetramethylammonium 3-cyano-2,2,3,3-tetrafluoropropionate (III). The nitrile reacted with equimolar amounts of methanol and anhydrous hydrogen chloride in an evacuated autoclave with good yields to 3-iodo-2,2,3,3-tetrafluoropropanoic acid amide (V), an excellent building block for the synthesis of α,β-tetrafluorinated alkane carboxylic acids.

The other building block, the ω-trifluoro-1-alkene, was obtained by fluorination of a carboxylic group of an appropriate ω-bromoalkane carboxylic acid with sulfur tetrafluoride, because direct fluorination of alkene carboxylic acids with sulfur tetrafluoride resulted in very low yields. Subsequent to the introduction of the three fluorine atoms, the required ω-trifluoro-1-alkene was generated by HBr elimination via the intermediary formation of the corresponding *o*-nitrophenyl selenoxide (28, 29).

In order to synthesize the α,β,ω-fluorinated analogue of stearate, 15-pentadecanolide (VI) was saponified with barium hydroxide in methanol. The resulting 15-hydroxypentadecanoic acid (VII) was brominated with an HBr–H_2O–CH_3COOH mixture to 15-bromopentadecanoic acid (VIII), which was fluorinated with sulfur tetrafluoride to 1-bromo-15,15,15-trifluoropentadecane (IX). Further conversion of 1-bromo-15,15,15-trifluoropentadecane yielded 15,15,15-trifluoro-1-pentadecene (X), which was subsequently coupled with the central building block 3-iodo-2,2,3,3-tetrafluoropropanoic acid amide (V). In the presence of catalytic amounts of various metals, fluoroalkyl iodides are added to carbon–carbon multiple bonds presumably by a radical chain mechanism initiated by a single electron transfer (SET) (30). Accordingly, 15,15,15-trifluoro-1-pentadecene (X) was converted to 2,2,3,3,18,18,18-heptafluoro-5-iodooctadecanoic acid amide (XI). After HI elimination, 2,2,3,3,18,18,18-heptafluoro-4-octadecenoic acid amide (XII) was hydrogenated in the presence of a palladium catalyzer under atmospheric pressure at room temperature. The resulting 2,2,3,3,18,18,18-heptafluorooctadecanoic acid amide (XIII) was hydrolyzed to the sodium salt of 2,2,3,3,18,18,18-heptafluorooctadecanoic acid (XIV).

For the preparation of a photoactivatable analogue of this fatty acid sodium salt, only the synthesis of the olefinic building block must be varied (Fig. 1). In the ω-fluorinated 1-alkene, the required oxo group was introduced by a Grignard reaction of 8,8,8-trifluorooctane-1-al (XVIII) with 7-bromo-1-heptene resulting in the forma-

tion of 8-hydroxy-15,15,15-trifluoro-1-pentadecene (XIX). 8,8,8-Trifluorooctane-1-al (XVIII) was obtained in good yields starting from 8-bromooctanoic acid (XV). The acid (XV) was transformed to 1-bromo-8,8,8-trifluorooctane (XVI) by fluorination with sulfur tetrafluoride and after Br/I exchange with NaI, 1-iodo-8,8,8-trifluorooctane (XVII) was oxidized to 8,8,8-trifluorooctane-1-al (XVIII). This three step synthesis of 8,8,8-trifluorooctane-1-al resulted in higher yields than an alternative synthesis by reduction of 8,8,8-trifluorooctanoic acid, which was obtained by fluorination of octanedioic acid with sulfur tetrafluoride. 8-Hydroxy-15,15,15-trifluoro-1-pentadecene (XIX) was oxidized with chromic acid to 8-oxo-15,15,15-trifluoro-1-pentadecene (XX), which was coupled with the key intermediate 3-iodo-2,2,3,3,-tetrafluoropropanoic acid amide (V) to 2,2,3,3,18,18,18-heptafluoro-5-iodo-11-oxooctadecanoic acid amide (XXI). Elimination of hydrogen iodide resulted exclusively in the formation of 2,2,3,3,18,18,18-heptafluoro-11-oxo-4-octadecenoic acid amide (XXII). Hydrogenation then led to 2,2,3,3,18,18,18-heptafluoro-11-oxooctadecanoic acid amide (XXIII). The oxo compound was converted to the photoactivatable diazirine derivative (19, 38) 11,11-azo-2,2,3,3,18,18,18-heptafluorooctadecanoic acid amide (XXIV). Mild hydrolysis finally led to the requested sodium salt of 11,11-azo-2,2,3,3,18,18,18-heptafluorooctadecanoic acid (XXV).

Syntheses of tritium-labeled derivatives

Double bonds adjacent to the tetrafluoroethylene group exhibit relatively good reactivity in catalytic hydrogenation. Accordingly, the catalytic reduction of the 4,5-unsaturated precursors 2,2,3,3,18,18,18-heptafluoro-4-octadecenoic acid amide (XII) and 2,2,3,3,18,18,18-heptafluoro-11-oxo-4-octadecenoic acid amide (XXII) with carrier-free tritium gas, even under reduced pressure (40–50 kPa), proved to be nonproblematic. In order to achieve a complete saturation of the double bond for both compounds, a 3 molar excess of tritium gas was used. Comparison of the specific activities observed (2.63 TBq/mmol for both tritiated derivatives of XIII and XXIII) with the theoretical value (2.15 TBq/mmol), revealed that in both cases, due to simultaneous H/T-exchange processes, more than the two tritium atoms theoretically expected were incorporated (2.4 ^{3}H-atoms/molecule). Evaluation of the ^{3}H-NMR spectrum of labeled 2,2,3,3,18,18,18-heptafluoro[G-^{3}H]octadecanoic acid amide (XIII) showed that only 6% of the tritium was actually attached to carbon 5. The other 94% was distributed along the carbon chain. This indicates that under the reaction conditions used, a double bond shift had occurred prior to the addition of tritium, an effect similar to that observed for the catalytic tritiation of elaidic acid to [G-^{3}H]stearic acid (39). Surprisingly, no tritium at all could be detected at position 4.

Determination of dissociation constant

Introduction of fluorine in the α-position leads to a strong increase in acid strength of alkane carboxylic acids (40, 41). The precise determination of the pK_a values of such moderately strong acids, having pK_a values less than 2, cannot be carried out by classical methods. Therefore, the dissociation constant of 2,2,3,3,18,18,18-heptafluorooctadecanoic acid was estimated in absolute chloroform by means of absorptiometry comparison with 4-aminoazobenzene and n-butylamine/2,4,6-trinitrophenol as indicators (42). **Fig. 2** demonstrates the equilibrium between 2,2,3,3,18,18,18-heptafluorooctadecanoic acid and 4-aminoazobenzene in chloroform and, as a means of comparison, equilibrium between the comparable strong acids difluoroacetic acid and trifluoroacetic acid. It is obvious that using chloroform as solvent, with 4-aminoazobenzene as base and indicator, the dissociation constant of 2,2,3,3,18,18,18-heptafluorooctadecanoic acid is only slightly lower than that of trifluoroacetic acid. In the same solvent, but with n-butylamine as base and 2,4,6-trinitrophenol as indicator, the dissociation constants of the two acids could not be distinguished. Thus, it is evident that the dissociation behavior of 2,2,3,3,18,18,18-heptafluorooctadecanoic acid is nearly identical to that of trifluoroacetic acid, whose pK_a value in water is about 0.5 (40, 41).

Determination of critical micellar concentration (CMC)

In order to estimate the CMC of the sodium salt of 2,2,3,3,18,18,18-heptafluorooctadecanoic acid, solutions of

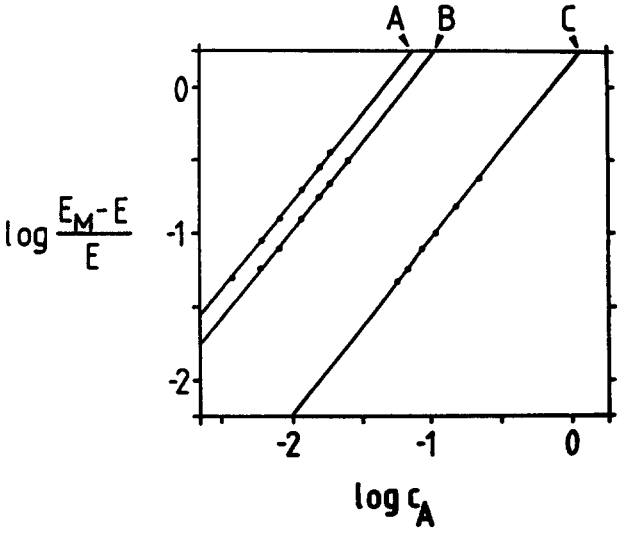

Fig. 2. Logarithmic graph of the decrease of the extinction (E_M = maximum extinction without acid) divided by the measured extinction of the indicator 4-aminoazobenzene (E) versus the concentration of free fatty acid (C_A); A, trifluoroacetic acid; B, 2,2,3,3,18,18,18-heptafluorooctadecanoic acid; C, difluoroacetic acid.

known concentrations, above CMC and containing the dye pinacyanol chloride, were titrated with an aqueous solution of the dye to the point of characteristic color change (43). With this method, a CMC of 5 μM at 25°C was found. This CMC is somewhat lower than that of the nonfluorinated sodium stearate. The observed difference must be attributed to the enhanced lipophilic effect of the ω-CF_3 group and to the α,β-tetrafluoroethylene group (44) as well as to an increased hydrophilic nature of the carboxylic group. The effects are responsible for the enhanced aggregation tendency of the α,β,ω-fluorinated stearate in aqueous solutions, a phenomenon which is also known from perfluorinated fatty acids (44).

Duration of photolysis

In order to minimize photolytical destruction of biological material, photoaffinity labeling should be carried out using light at wavelengths longer than 320 nm. Because the diazirine derivative of the fatty acid exhibits an absorption maximum exactly at 350 nm with a shoulder at about 365 nm (**Fig. 3**), all photoaffinity labeling studies were performed at 350 nm. To estimate the time necessary for photoaffinity labeling, time dependency of photolysis of 11,11-azo-2,2,3,3,18,18,18-heptafluorooctadecanoic acid amide in deuterated chloroform was followed spectrophotometrically (Fig. 3). Photolysis of the diazirine derivative

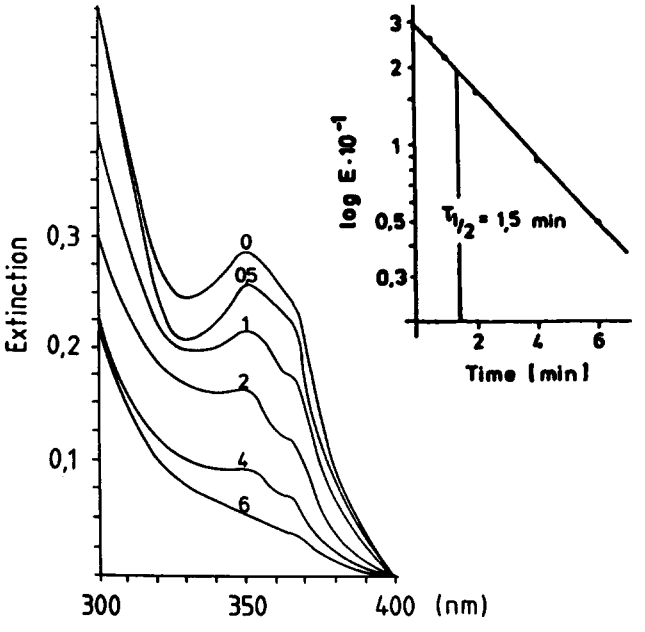

Fig. 3. Photolysis of 11,11-azo-2,2,3,3,18,18,18-heptafluorooctadecanoic acid amide monitored by ultraviolet spectroscopy. A 2.3-mM solution of 11,11-azo-2,2,3,3,18,18,18-heptafluorooctadecanoic acid amide in deuterated chloroform was photolyzed in a Rayonet RPR-100 photochemical reactor equipped with 16 RPR-3500 Å lamps for 0, 0.5, 1, 2, 4, and 6 min. The absorption of the diazirine group was recorded after different times of photolysis. The insert shows the determination of the half-life time of the diazirine group during photolysis.

followed, under the experimental conditions used, first order kinetics with a half-life time of 1.5 min (Fig. 3, insert).

Extent of photoaffinity labeling

In order to evaluate the suitability of diazirine derivatives for the identification of fatty acid salt-binding structures by photoaffinity labeling, we determined the extent of covalent bond formation on photolysis with solvents as well as with proteins known to interact specifically with fatty acid derivatives. For evaluation of the extent of covalent bond formation with solvents, photolysis was performed using [^{14}C]methanol and [1-^{14}C]acetonitrile. Subsequent to photolysis, which was performed in sealed ampoules, the radioactive solvent was removed by distillation. The residue was taken up in the same unlabeled solvent and again evaporated to dryness. This procedure had to be repeated until no traces of radioactivity were detectable in the distillate. The finally obtained residue was dissolved in a definite volume of solvent and examined qualitatively by radio-TLC as well as quantitatively for radioactivity. With methanol as solvent, 9–13% of the photolyzed diazirine derivative reacted, forming one main product, as demonstrated by TLC (**Fig. 4**, upper panel). Photolysis in acetonitrile resulted in 6–11% bond formation with the solvent. Two main products, whose identification was not intended, were found (Fig. 4, lower panel).

Photolysis of 1 μM rat serum albumin, which is involved in the transport of fatty acid salts in blood, in the presence of 15 nM (37 kBq) 11,11-azo-2,2,3,3,18,18,18-heptafluoro[G-^{3}H]octadecanoic acid sodium salt, revealed a clear incorporation of radioactivity into the protein. The amount of radioactivity covalently bound to albumin increased with irradiation time during the first 5 min (**Fig. 5**), as shown after SDS/PAGE (Fig. 5, insert). Without irradiation, albumin-bound radioactivity was negligible after SDS/PAGE, indicating that incorporation of radioactivity was a result of the irradiation. Under the experimental conditions used, rat serum albumin was found to be labeled in an extent of 1–3% (Fig. 5).

In order to estimate the relative specificity of the labeling process using the photolabile fatty acid derivative, albumin was subjected to photoaffinity labeling in the presence of other proteins. As revealed after SDS/PAGE of the protein mixture used (**Fig. 6**, upper panel) incorporation of radioactivity was only found in albumin (Fig. 6, lower panel), indicating that under the experimental conditions used only the fatty acid salt-binding protein albumin had been labeled.

Photolysis of 1 μM FABP from rat liver, isolated principally according to Dempsey et al. (45) and to Ockner and Manning (46), revealed in the presence of 15 nM (37 kBq) 11,11-azo-2,2,3,3,18,18,18-heptafluoro[G-^{3}H]octadecanoic acid sodium salt a clear labeling of the protein (**Fig. 7**). Under the ex-

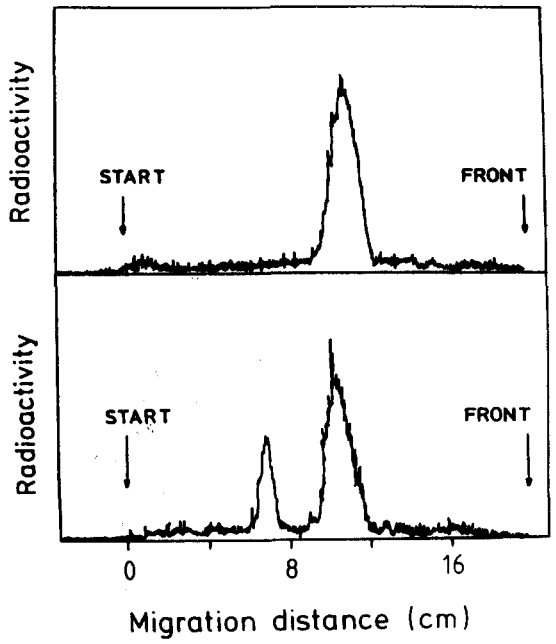

Fig. 4. TLC of the products obtained by photolysis of 11,11-azo-2,2,3,3,18,18,18-heptafluorooctadecanoic acid amide in radioactively labeled solvents. Samples (4.35 mg, 10 μM) of 11,11-azo-2,2,3,3,18,18,18-heptafluorooctadecanoic acid amide in 100 μl (5.5 MBq) of [1-^{14}C]acetonitrile or 100 μl of [^{14}C]methanol were photolyzed in sealed ampoules at 30°C in Rayonet RPR-100 photochemical reactor equipped with 16 RPR-3500 Å lamps for 10 min. After complete evaporation of the radioactive solvent, the residues were dissolved in definite volumes of the corresponding unlabeled solvents and subjected to thin-layer chromatography using solvent system 4. Upper panel, after photolysis in [^{14}C]methanol; lower panel, after photolysis in [1-^{14}C]acetonitrile.

perimental conditions used, FABP from rat liver was found to be labeled in an extent of 1–3% (Fig. 7). Labeling of both albumin, the extracellular fatty acid salt-binding protein, and of FABP, the protein involved in intracellular transport, demonstrates the suitability of the synthesized derivative for photoaffinity labeling.

Metabolic stability

For the investigation of transport of long-chain fatty acid salts in mammals, α,β,ω-fluorinated stearate and its photolabile derivative must be metabolically sufficiently stable under the conditions necessary for kinetic studies as well as for photoaffinity labeling experiments. The metabolic stability of the sodium salt of 2,2,3,3,18,18,18-heptafluoro[G-^{3}H]stearate was examined by infusion experiments using intact rat liver in situ, by perfusion of isolated rat liver, and by incubation of isolated rat liver hepatocytes. After a bolus injection of 0.1–1 MBq of the sodium salt of 2,2,3,3,18,18,18-heptafluoro[G-^{3}H]stearate into a mesenterial vein, more than 95% of the radioactivity was taken up into the liver. In the course of 2 h, only 3–5% of the radioactivity was found in bile and identified as completely unchanged 2,2,3,3,18,18,18-heptafluoro[G-^{3}H]stearate. Analysis of radioactivity remaining in liver, representing more than 90% of the tritium-labeled α,β,ω-fluorinated derivative, revealed the presence of only one compound, identical in its chromatographic behavior with the injected fatty acid salt. Practically the same results were obtained using isolated rat liver. Furthermore, after incubation of isolated rat liver hepatocytes

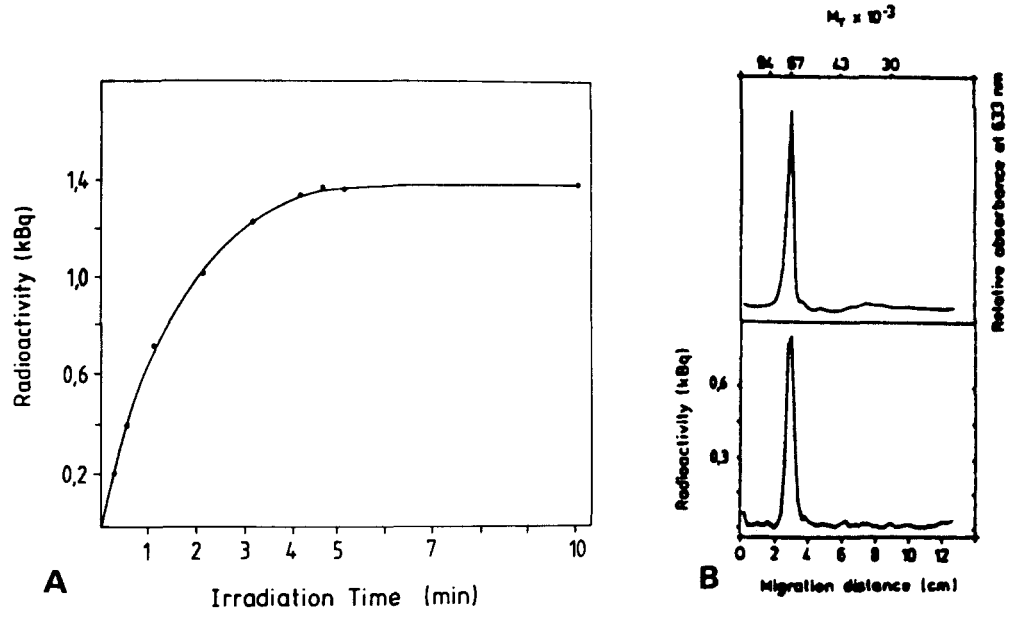

Fig. 5. Photoaffinity labeling of rat serum albumin by 11,11-azo-2,2,3,3,18,18,18-heptafluoro[G-^{3}H]octadecanoic acid sodium salt. A: Irradiation at 350 nm. B: Incorporation of radioactivity into albumin determined after SDS/PAGE by liquid scintillation counting.

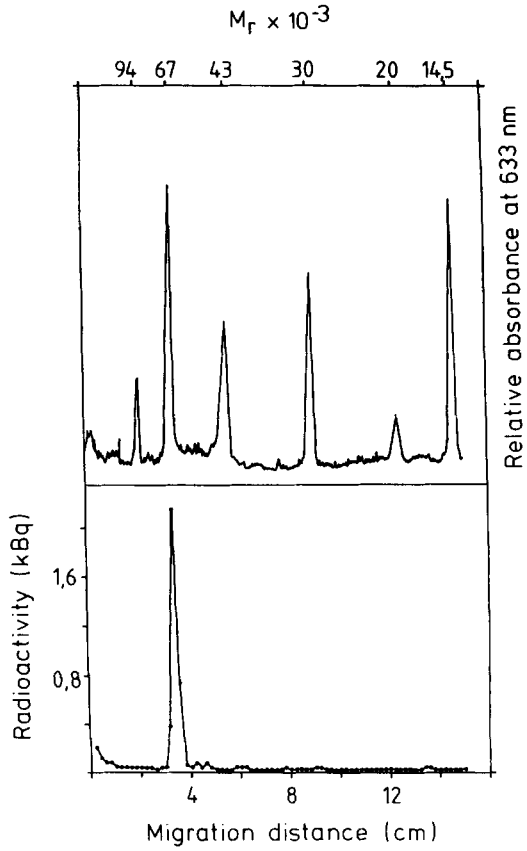

Fig. 6. Photoaffinity labeling of a mixture of purified proteins by 11,11-azo-2,2,3,3,18,18,18-heptafluoro[G-^{3}H]octadecanoic acid sodium salt. Phosphorylase b (64 μg), bovine serum albumin (83 μg), ovalbumin from chicken egg (147 μg), carbonic anhydrase from bovine erythrocytes (83 μg), trypsin inhibitor from soybean (80 μg), α-lactalbumin from bovine milk (121 μg), and 40 pmol (100 kBq) of 11,11-azo-2,2,3,3,18,18,18-heptafluoro[G-^{3}H]octadecanoic acid sodium salt dissolved in 400 μl of 0.1 M potassium sodium phosphate buffer, adjusted to pH 7.2, were irradiated at 350 nm for 8 min. Incorporation of radioactivity into proteins was determined after SDS/PAGE by liquid scintillation counting. Upper panel, distribution of proteins; lower panel, incorporation of radioactivity.

with 1–3 μM of 2,2,3,3,18,18,18-heptafluoro[G-^{3}H]stearate for 2 h, no biotransformation could be detected.

The final experimental conditions, particularly the low concentrations of the α,β,ω-fluorinated stearate, were chosen according to the requirements favorable for photoaffinity labeling experiments. Under these experimental conditions, no metabolite could be detected, despite the fact that no fluorine atoms were introduced in the (ω-1)-position. However, the possibility cannot be ruled out completely that with higher concentrations and longer times small amounts of the α,β,ω-fluorinated derivative may be biotransformed by nonspecific oxidation reactions.

Versatility for biological studies

The primary purpose of this study was to design metabolically stable derivatives of fatty acids that may be useful for photoaffinity labeling and to show their suitability for the identification of fatty acid salt-binding proteins in biological systems. The introduction of the small fluorine atoms in α-, β-, and ω-positions of long-chain fatty acid salts prevented, under the experimental conditions used, any further metabolic conversion without significantly changing the amphipathic structure of the long-chain fatty acid salts. It must be considered, however, that substitution of seven hydrogen atoms by fluorine in stearate could cause differences in some respects between the natural fatty acid salt and its heptafluoro derivatives. Results obtained with the heptafluoro derivatives should be complemented by competition experiments using the natural fatty acid salt as competing ligand, both in kinetic studies and in differential photoaffinity labeling, and caution is indicated in equating transport of the natural fatty acid salt and that of its heptafluoro derivatives.

The diazirine group was chosen as the photoactivatable group because it is less bulky than the azido group and susceptible to photolysis at longer wavelengths. Photolysis of diazirine derivatives occurs at 350 nm, a wavelength where biological systems are reasonably stable to irradiation. Furthermore, the half-life time of photolysis of aliphatic diazirines is shorter than that of azides, only requiring irradiation times for photoaffinity labeling in which an impairment of intact cells is of no importance. Thus, diazirine derivatives of fatty acids may favorably complement the potentialities opened by azido derivatives of fatty acids (47, 48).

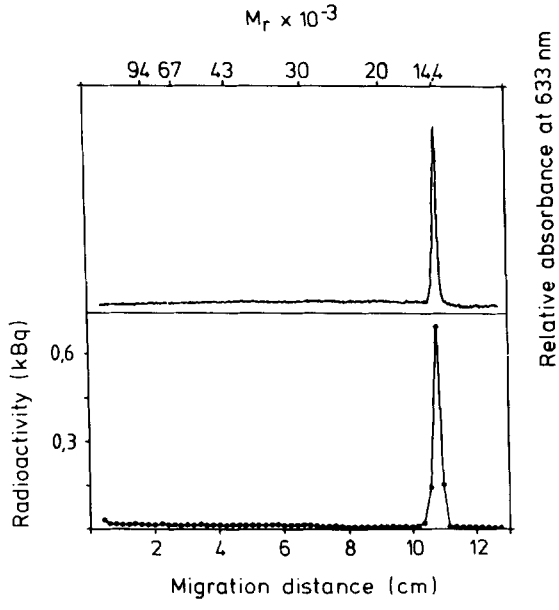

Fig. 7. Photoaffinity labeling of FABP from rat liver by 11,11-azo-2,2,3,3,18,18,18-heptafluoro[G-^{3}H]octadecanoic acid sodium salt. Irradiation at 350 nm was performed for 8 min. Incorporation of radioactivity into FABP was determined after SDS/PAGE by liquid scintillation counting.

The scheme of synthesis allows the introduction of the diazirine group at various positions of the α,β,ω-fluorinated fatty acid derivative. It must, however, be stated that a diazirine group adjacent to the tetrafluoroethylene or to the trifluoromethyl group is not favorable for photoaffinity labeling (49).

The metabolic stability of salts of α,β,ω-fluorinated fatty acids and the favorable photolytic properties of appropriate diazirine derivatives make them suitable for the study of biological transport processes as well as for the identification of transport systems involved in transport of salts of fatty acids.

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