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SYNTHESIS OF PERFLUOROALKYL STEROIDS AS CO-EMULSIFYING AGENTS FOR 1-BROMOPERFLUOROOCTANE AND OTHER PERFLUOROCOMPOUNDS

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SUMMARY

Syntheses of steroids substituted with perfluoroalkyl groups at C-3, C-7, and C-20 positions on the steroid nucleus are described. Synthetic methods employed included coupling of perfluoroalkylcopper with allylic bromides and Grignard reactions. Free radical additions of perfluoroalkyl iodide to unsaturated steroids and reaction of perfluoroalkyl Grignard reagents with 6-ketosteroid were unsuccessful. Perfluoroalkyl-substituted steroids are desired for testing as co-emulsifying agents in perfluorooctyl bromide/water emulsions which are used as blood substitutes (*synthetic blood*). A rationale for the choice of perfluorooctyl bromide as the oxygen-carrying agent in the fluorocarbon-based blood substitute and on the use of perfluoroalkyl-substituted steroids as co-emulsifying agents is also reported.

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BACKGROUND INFORMATION

Two symposia proceedings in 1970 and 1975 [1,2] greatly stimulated the interest of organic fluorine chemists in the synthesis of perfluorocarbon compounds for use as the oxygen-carrying component in blood substitutes [3-8]. The physical properties of 1-bromoperfluorooctane and its safe use as an X-ray radiopaque [9-11] convinced our San Diego group that 1-bromoperfluorooctane would be the best perfluorocarbon for our investigations of synthetic blood. We concluded that our major research goal should be to develop a superior surfactant system for emulsifying perfluorocarbons in water.

There are significant advantages in the use of 1-bromoperfluorooctane as the oxygen-carrying component in synthetic blood. First, the work of Long shows that 1-bromoperfluorooctane has a relatively short half life for removal from tissues [11,12]. Second, the bromine atom confers an increased capability for dissolving oxygen compared to bromine-free perfluorocarbons [13]. This can be illustrated by comparing the solubility of oxygen in 1-bromoperfluorooctane [13] with solubility in the C_7-C_9 n-perfluoroalkanes [6], Table 1. The molecular weights and molecular size of 1-bromoperfluorooctane and perfluorononane are very nearly the same and should dissolve similar amounts of oxygen [6]; the difference between the two structures is substitution of a trifluoromethyl group by a sterically similar bromine atom. We believe the enhanced solubility of oxygen in 1-bromoperfluorooctane is due to a weak interaction between an electron in an antibonding π-molecular orbital of oxygen and the antibonding σ-molecular orbital of the C-Br bond .

Table 1

Solubility of Oxygen in Selected Perfluorocarbon Compounds at 25°C.

Perfluorocompound	Formula	Molecular Weight	Solubility of O_2, mL/100 mL solvent	Reference
n-perfluoroheptane	C_7F_{16}	388	54.8	6
n-perfluorooctane	C_8F_{18}	438	52.1	6
n-perfluorononane	C_9F_{20}	488	49.6	6
1-bromoperfluoro-octane	$C_8F_{17}Br$	499	52.7	13

Third, the bromine atom permits a superior method of analysis for residual 1-bromoperfluorooctane in tissues. This will be very important when seeking F.D.A. approval for use in humans. Bromine occurs naturally as 50.54% ^{79}Br and 49.46% ^{81}Br. When ^{81}Br is irradiated by neutrons, ^{82}Br is formed which has an extremely convenient half-life of 35.5 hours for emission of its unique gamma-ray spectrum. Animal organs and tissues contain a negligible amount of bromine. Neutron activation analysis is a powerful tool that can be used to analyze body organs for residual 1-bromoperfluorooctane. The knowledge that we can use neutron-activation analysis to prove elimination of 1-bromoperfluorooctane from animal tissue has made our choice of perfluorocarbon derivative much easier. A fourth and very important factor in our choice is that 1-bromoperfluorooctane can be synthesized commercially by two different processes. 1-Iodo-perfluorooctane is manufactured by telomerization of tetrafluoroethylene (E.I. duPont de Nemours and Co., Inc. in the U.S.A.); the iodo compound is readily converted to 1-bromoperfluorooctane. The 3M Company produces 1-bromoperfluorooctane as a mixture of ca. 83% 1-bromoperfluorooctane and ca. 17% 1-bromoperfluoro-6-methylheptane (perfluoro-iso-octyl-bromide) from intermediates obtained by electrochemical fluorination. In our hands this mixture of normal and iso-perfluorooctyl bromide is as effective in synthetic blood as pure 1-bromoperfluorooctane from the DuPont Company. A fifth reason for our choice is the unusually high rate of elimination of 1-bromoperfluorooctane from animals. Reiss [7] has pointed out in a graph of retention time versus the molecular weight of a fluorocarbon derivative that 1-bromoperfluoroctane is a deviant point. It has too low a retention time. Finally, a sixth and perhaps the most important advantage of 1-bromoperfluorooctane is that 1-bromoperfluorooctane forms more stable emulsions in water than perfluoroalkanes or perfluorocycloalkanes which do not contain the very polarizable bromine atom [14].

In planning our research one of our initial uncertainties was whether the extensive solubility studies of oxygen in perfluoroorganic compounds could be applied to emulsions of perfluoroorganic compounds in water. In results published earlier in this Journal [15] we found that emulsions of perfluoroorganic compounds followed Henry's Law and that data of the solubility of oxygen in pure perfluoroorganic compounds could be used directly for emulsions of perfluoroorganic compounds. Our next step was a study of possible alternative surfactants for emulsify-

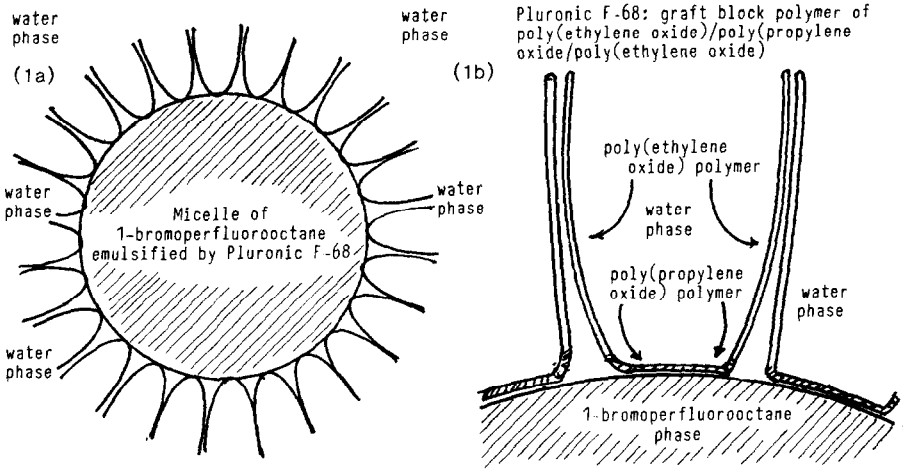

Fig. 1. Possible structure for 0.1 micron 1-bromoperfluorooctene droplet emulsified in water by Pluronic F-68: (a) Pluronic F-68 oriented with poly-(propylene oxide) polymer graft on surface of 1-bromoperfluorooctane droplet; poly(ethylene oxide) polymer grafts in water phase; (b) Expanded cross section.

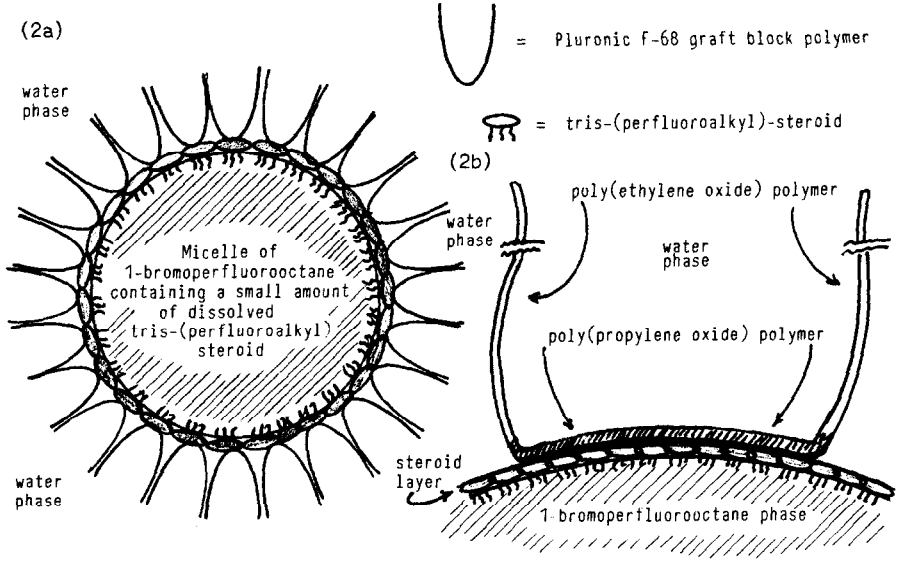

Fig. 2. Concept of a steroid substituted on the alpha side by perfluoroalkyl groups acting as a coemulsifying agent for 1-bromoperfluorooctane in water. Pluronic F-68 or other emulsifying agent interacting with the beta side of the steroid: (a) Steroid molecules drawn grossly oversize to illustrate concept; (b) Expanded cross section showing approximately 100 Å poly(propylene oxide) polymer chain interacting with steroid molecules at surface of droplet.

ing perfluoroalkanes and perfluorocycloalkanes. We reached the conclusion that it was unlikely that we could find a better surfactant than those that were in use in the late 1970's [16]. The successful use of commercially available Pluronic F-68 (Wyandotte trade mark) to give perfluorocarbon emulsions of limited stability prompted us to ask; "How?"

One of us (CMS) proposed a model for a micelle of Pluronic F-68 emulsion of 1-bromoperfluorooctane which has undergone evolutionary change to the current model shown in Fig. 1.* Regardless of the reality of the model, it seemed clear to us that the interactions between the lypophilic poly(propyleneoxide) portion of the Pluronic F-68 and 1-bromoperfluorooctane were not sufficiently strong to maintain a stable emulsion. We conceived the idea that an interface was required to give greater micelle stability and that an ideal interface would be a steroid substituted on the α-side by perfluoroalkyl groups as shown in Fig. 2. We decided that we should investigate the possible synthesis of perfluoroalkyl steroids as coemulsifiers in synthetic blood formulations.

A literature search showed that the only perfluoroalkyl steroids known were trifluoromethyl and perfluoroisopropyl steroids [17-19]. We concluded that synthesis of long-chained perfluoroalkyl compounds would allow us to explore some new chemistry and at the same time provide us with the desired co-emulsifying agents. This paper will describe our initial exploratory work to synthesize perfluoroalkyl-substituted steroids and the limited results achieved. Subsequent papers describe systematic syntheses of mono-, di-, and tri-α-substituted perfluoroalkenyloxy and perfluoroalkanoyloxy cholanoic acid derivatives.

SYNTHESIS OF PERFLUOROALKYL-SUBSTITUTED STEROIDS

Free Radical Addition of 1-Iodoperfluoroalkanes to Unsaturated Steroids

We attempted to add 1-iodoperfluorooctane and 1-iodoperfluorodecane to cholesteryl benzoate and cholesterol under free radical conditions. We recovered unreacted steroids. The C_8- and C_{10}-iodoperfluoroalkanes were converted to perfluorohexadecane and 1-hydroperfluorooctane, and to perfluoroeicosane and 1-hydroperfluorodecane, respectively.

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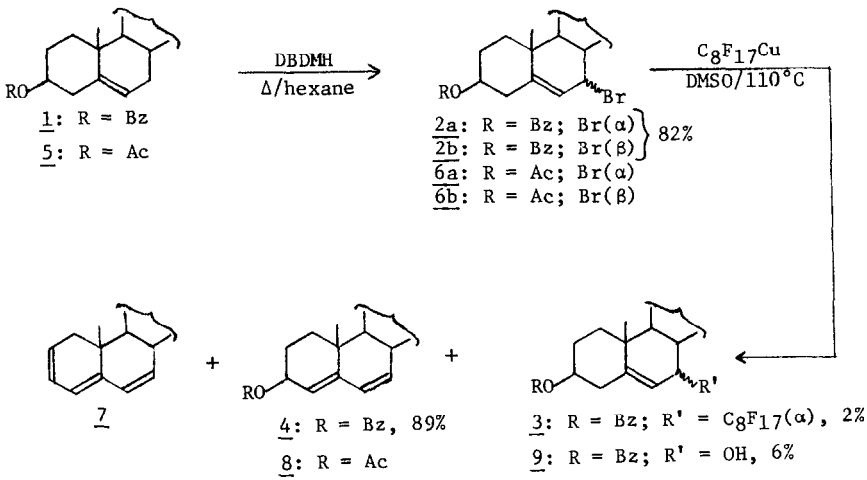
* We gratefully acknowledge the excellent criticism of our initial model made by a refree of this paper and have modified our initial model accordingly.

$$\text{Cholesteryl Benzoate (recovered)} + \begin{matrix} C_8F_{17}I \\ C_{10}F_{21}I \end{matrix} \xrightarrow[\text{conditions}]{\text{free radical}} \begin{matrix} C_{16}F_{34} \text{ or } C_{20}F_{42} \end{matrix} + \begin{matrix} C_8F_{17}\text{-H} \text{ or } C_{10}F_{21}\text{-H} \end{matrix}$$

Reactions of 1-iodoperfluorooctane and 1-iodoperfluorodecane under free-radical conditions with dienes, 7-dehydrocholesterol and ergosterol, resulted in formation of mixtures of addition and substitution products; coupling products were absent. Separation of products by chromatography gave fractions which contained fluorine as shown by C-F absorptions in the infrared and ^{19}F NMR spectra. Filtered light $\lambda > 460$ μm was used to prevent the known rearrangement of the light sensitive ring system [20]. The failure to obtain a significant amount of a major perfluoroalkyl-substituted steroid led us to abandon this line of research. We chose not to investigate further the potentially interesting mixture of products formed in these reactions as inconsistent with our goals.

Perfluoroalkylcopper Reactions with Steroidal Bromides

The use of perfluoroalkylcopper compounds to couple with aromatic iodides and allylic bromides as reported by McLoughlin and Thrower [21], Coe and Milner [22,23], and others [24] appeared attractive as a route to perfluoroalkyl steroids. Cholesteryl benzoate (1) was allylically brominated with 1,3-dibromo-5,5-dimethylhydantoin (DBDMH) to give a 82% yield of a 2.3:1.0 mixture of α- and β-7-bromocholesteryl benzoate (2a + 2b) [25] (Scheme 1). When N-bromosuccinimide in carbon tetrachloride or bromine in carbon disulfide [26] were substituted for DBDMH, lower yields of 2a + 2b were obtained. Reaction of the mixture of 2.3:1.0 α:β-7-bromocholesteryl benzoate (2a + 2b) with perfluorooctylcopper in dimethylsulfoxide (DMSO) at 110°C gave 2% 7α-perfluorooctylcholesteryl benzoate (3), 6% 7-hydroxycholesteryl benzoate (9), and 89% cholest-4,6-diene-3β-yl benzoate (4). At lower temperatures no perfluorooctyl compound was isolated: compound 4 was isolated in > 90% yield. The reaction of a mixture of α- and β-7-bromocholesteryl acetate (6a + 6b) with perfluorooctylcopper gave mostly cholest-2,4,6-triene (7) and a small amount of cholest-4,6-diene-3β-yl acetate (8) (Scheme 1).

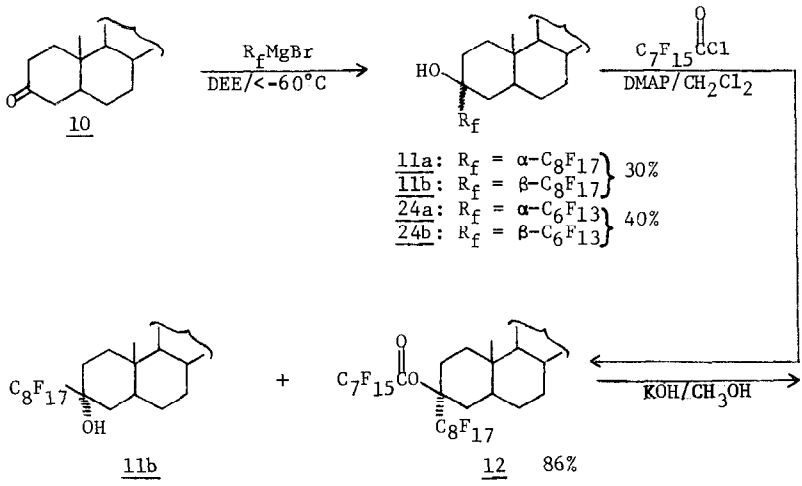


Scheme 1

We assigned the α-stereochemistry to the perfluorooctyl group in 3 from the chemical shifts and multiplicities of the C-6 and C-7 protons in the ^{1}H NMR spectra [27]. We interpreted the ^{1}H NMR data to indicate a dihedral angle of 40° between protons at C-6 and C-7 with the conformation of the C-7 proton to be quasiequatorial and the perfluorogroup quasiaxial.

Grignard Reaction of Perfluoroalkylmagnesium bromide with 3-keto, 6-keto, and 20-keto Steroid

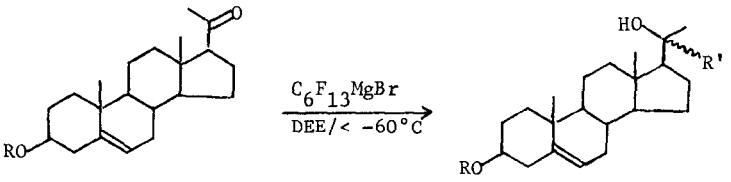
Perfluorooctylmagnesium bromide in diethyl ether (DEE) was prepared as described by Tamborski et al. [28,29] and reacted with 5α-cholestan-3-one (10) at -60°C or below [29,30] to give an epimeric mixture of 3α-perfluorooctyl-5α-cholestan-3β-ol (11a) and 3β-perfluorooctyl-5α-cholestan-3α-ol (11b) (Scheme 2). The epimeric alcohols 11a and 11b were separated by selectively esterifying epimer 11a with perfluorooctanoyl chloride and separating ester 12 from alcohol 11b. We chose to form perfluorooctyl ester because this gave us a steroid containing two perfluorinated chains for use in later solubility studies. Hydrolysis of ester 12 with alcoholic potassium hydroxide gave pure 11a. The ratio of 11a to 11b was ca. 85:15. Similarly, reaction of perfluorohexylmagnesium bromide with 10 in DEE at temperatures below -60°C gave an epimeric mixture of 24a and 24b in 39.5% yield (Scheme 2).



Scheme 2

Attempts to extend the Grignard coupling to 6-keto steroids failed. We prepared 6-keto-5α-cholestan-3β-ol (13) [31] and protected the hydroxyl group by conversion to 3-tetrahydropyranyloxy-6-keto-5α-cholestan (14). When 14 was reacted with perfluorooctylmagnesium bromide in DEE at < -60°C and work-up completed, no perfluorocarbon-substituted steroid could be detected; more than 90% of steroid 14 was recovered.

Next we studied the reaction of Grignard reagents with pregn-5-ene-3β-ol-20-one (15) (Scheme 3). Steroid 15 was reacted first with dihydropyran to protect the 3β-OH and then with perfluorohexylmagnesium bromide to give 20-perfluorohexyl-3β-tetrahydropyranyloxy-pregn-5-ene-20-ol (17). The tetrahydropyranyl group of 17 was removed by acid hydrolysis to give 20-perfluorohexyl-pregn-5-ene-3β,20-diol (18) in an overall yield of 70.0%.



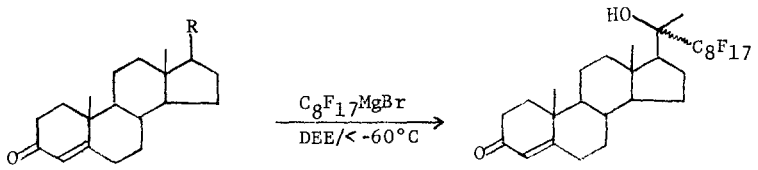
15: R = H
16: R = THP

17: R = THP; R' = C_6F_{13}
18: R = H; R' = C_6F_{13}, 70%
19: R = THP; R' = $C_8F_{17}CH_2CH_2$
20: R = H; R' = $C_8F_{17}CH_2CH_2$, 61%

Scheme 3

The availability of 2-perfluoroalkyl-1-iodoethane compounds and the probability that 2-perfluoroalkylethylmagnesium iodides would be less sterically hindered than perfluoroalkylmagnesium iodides, prompted us to investigate $R_fCH_2CH_2I$ compounds. 1H,1H,2H,2H-Perfluorodecyl iodide was reacted with activated magnesium [36] and iodine in DEE to give the corresponding Grignard reagent. Reaction of 1H,1H,2H,2H-perfluorodecylmagnesium iodide with 3β-tetrahydropyranyloxy-pregn-5-ene-20-one (16) in DEE and subsequent hydrolysis gave 20-(1H,1H,2H,2H-perfluorodecyl)-pregn-5-ene-3β,20-diol (20) in 61.2% yield.

At this point in our research we had achieved α-substitution of a perfluoroalkyl group at the 3-position of the ring and perfluoroalkylation of the 20-position of the side chain. Our next goal was to attach two perfluoroalkyl chains to the steroid. We chose to react pregn-4-ene-3,20-dione (21) with perfluorooctylmagnesium bromide in DEE (Scheme 4). Contrary to our expectations, preferential mono-perfluoroalkylation of 20-carbonyl occurred to give 20-perfluorooctyl-pregn-4-ene-3-one-20-ol (22). Perfluoroalkylation at 3-position was not detected. The low reactivity of 3-position in 21 has been attributed to the presence of a conjugated double bond at C-4. The low reactivity of α,β-unsaturated-3-keto compounds was further demonstrated by the reaction of perfluorooctylmagnesium bromide with cholest-4-ene-3-one (23) in DEE. Unreacted steroid 23 was recovered in amounts exceeding 90%.



21: R = C(=O)CH_3
23: R = C_8H_{17}

22 11%

Scheme 4

We are convinced that perfluoroalkyl Grignard reaction is a convenient method for preparing 3- and 20-perfluoroalkylated steroids. However, low temperature requirements limit the synthetic utility of this reaction, as reflected by the inability of perfluoroalkyl Grignards to react with 6-keto and α,β-unsaturated-3-keto steroids. These known experimental limitations caused us to shift our direction of research to other approaches. The success obtained with other methods will be reported in the subsequent papers in this series. Investigation of the surfactant properties of the perfluoroalkyl-substituted steroids are in progress; results obtained will be reported in a later paper.

EXPERIMENTAL

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. The infrared spectra (IR) were obtained on a Perkin Elmer Model 337 Grating Spectrophotometer. Proton-nuclear-magnetic resonance spectra (^1H NMR) were recorded on a Varian EM-390, 90 MHz, NMR Spectrometer, whereas fluorine-magnetic-resonance spectra (^{19}F NMR) were recorded on a JEOL-PS-100, high resolution NMR Spectrometer. Chemical shifts are reported relative to tetramethylsilane and trichlorofluoromethane, respectively. Elemental analyses were carried out by Galbraith Laboratories Inc. Solvents were purified as follows: hexane and dimethyl sulfoxide were dried and distilled over calcium hydride; diethyl ether (DEE) and tetrahydrofuran (THF) were distilled over sodium/benzophenone. Perfluorooctyl and perfluorohexyl iodides were separated from a mixture of telomeric iodides of tetrafluoroethylene (E.I. duPont de Numours and Co., Inc.) by vacuum distillation. The term 'brine' corresponds to a saturated sodium chloride solution in water.

Allylic bromination of cholesteryl benzoate

The procedure of P. N. Confalone et al. [25] was used to prepare 7-bromocholesteryl benzoate. From 5.75 gm (11.7 mmol) of cholesteryl benzoate (1) 5.44 gm (82.0%) of an epimeric mixture of ca. 68% 7-*alpha* and 32% 7-*beta*-bromocholesteryl benzoate was obtained. Recrystallization from benzene/acetone provided 2a + 2b as white needles: mp. 125-132°C (lit. mp. of 7β-bromocholesteryl benzoate [32]: 114-115°C; lit. mp. of 7α-bromocholesteryl benzoate [33]: 144.5-145.5°C); ^{1}H NMR ($CDCl_3$) δ7.96 (m, 2H, Ar$\underline{H}$), δ7.33 (m, 3H, Ar$\underline{H}$), δ5.67 (d, $J_{6,7\beta}$ = 5.25 Hz, 0.68H, C_6-$\underline{H}$) δ5.32 (d, $J_{6,7\alpha}$ = 2.0 Hz, 0.32H, C_6-$\underline{H}$), δ4.85 (m, 1H, C_3-$\underline{H}$), δ4.56 (dd, $J_{7\beta,6}$ = 5.25 Hz, $J_{7\beta,8}$ = 1.5 Hz, 0.68H, $C_{7\beta}$-$\underline{H}$), δ4.08 (complex d, $J_{7\alpha,8}$ = 7.5 Hz, 0.32H, $C_{7\alpha}$-$\underline{H}$) and δ2.49 (d, 2H, C_4-$\underline{H}_2$). Irradiation of the signal at δ4.56 caused the doublet at δ5.67 to collapse to a singlet, whereas irradiation of the signal at δ4.08 caused the signal at δ5.32 to collapse to a singlet. 7-Bromocholesteryl benzoate was unstable at room temperature and had to be stored at a low temperature (-20°C).

Preparation of perfluoroalkylcopper complex in DMSO

Perfluorooctylcopper complex in DMSO was prepared by following the procedure outlined by Coe and Milner [22,23]. A 100-mL, 3-necked, round-bottomed flask fitted with a magnetic stirring bar, rubber septums, and a nitrogen inlet/outlet was first degassed and then charged with 1.22 gm (19.0 mmol) of copper bronze and 25 mL of DMSO. Perfluorooctyl iodide (2.80 mL, 5.60 gm, 10.3 mmol) was added and the mixture heated at 110°C for 2 hours. After cooling to room temperature, 20 mL of DMSO was added and the complex stored under nitrogen until used.

Reaction of perfluorooctylcopper with 7-bromocholesteryl benzoate

The complex obtained above was treated with a solution of 2.00 gm (3.51 mmol) of 2a + 2b in 10 mL of THF. The mixture was stirred at room temperature for 24 hours. After dilution with DEE, unreacted copper and copper salts were separated by filtration. The filtrate was poured into a flask containing 5 mL of 10% NH_4Cl solution. The organic layer was separated and the aqueous layer extracted with DEE (4 x 100 mL). The

combined organic extracts were washed with brine (2 x 100 mL), 10% $Na_2S_2O_3$ (2 x 100 mL), water (3 x 100 mL), and brine (1 x 100 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a white solid residue. Recrystallization of the residue from methanol/water gave 1.45 gm of 4 (84.3%) as a white crystalline solid: mp. 124-127°C (lit. mp. [34] 125.5-126.5°C). Concentration of the mother liquor provided 0.277 gm of a pale yellow solid. Chromatography of the solid over alumina using CCl_4, 60% $CCl_4/CHCl_3$, 50% $CCl_4/CHCl_3$, and $CHCl_3$ as eluents provided an additional 76 mg of 4 (4.42%), 62 mg (2.0%) of 3 and 105 mg (5.9%) of an epimeric mixture of 7-hydroxycholesteryl benzoate (9). Compound 3 has the following spectral properties: IR (thin film) 3015, 2950, 2850, 1720, 1180-1270, 1170, 1120 and 1080 cm^{-1}; ^1H NMR ($CDCl_3$) δ7.90 (m, 2H, aromatic), δ7.33 (m, 3H, aromatic), δ5.66 (d, $J_{6,7\beta}$ = 5.0 Hz, C_6-H), δ4.86 (m, 1H, C_3-H), δ3.10 (m, 1H, $C_{7\beta}$-H) and δ2.50 (d, 2H, C_4-H_2). When the signal at δ3.10 was irradiated the doublet at δ5.66 collapsed to a sharp singlet. ^{19}F NMR ($CDCl_3$) δ80.2 (CF_3CF_2-, 3F) δ120.0-121.8 (-CF_2CF_2-, 12F) and δ126.0 (CF_3CF_2-, 2F); mass spectrum; m/e: 909 (M^+) and 787 ($M^+ - C_6H_5CO_2H$).

Preparation of perfluoroalkylmagnesium bromide

Perfluorooctylmagnesium bromide was prepared by metal-halogen exchange reaction. The procedure outlined by Tamborski et al. [28,29] was followed. A 3-necked, round-bottomed flask equipped with a magnetic stirring bar, rubber septums and a nitrogen inlet/outlet was charged with 5.6 mmol of perfluoroalkyl iodide and 61 mL of anhydrous DEE. The solution was cooled to -78°C in a dry ice/acetone bath and treated dropwise with 5.6 mmol of 3.0M phenylmagnesium bromide solution in DEE at such a rate that the temperature remained below -60°C. The resulting white slurry was stirred at -78°C for 1 hour. Perfluoroalkylmagnesium bromide thus prepared was used immediately for the subsequent perfluoroalkylation reaction.

3-Perfluorooctyl-5α-cholestan-3-ol (11a + 11b)

Perfluorooctylmagnesium bromide (8.97 mmol) was prepared by the above procedure and reacted with a solution of 3.10 gm (8.02 mmol) of 5α-cholestan-3-one (10) in 50 ml of DEE at -78°C. The rate of addition

was so maintained that at no time was the temperature allowed to exceed -60°C. The mixture was stirred at -78°C for 1 hour, -60°C for 3 hours and at -46°C for 1 hour. At this point the cooling bath was filled with dry ice and the mixture allowed to warm slowly to room temperature. The reaction mixture was diluted with DEE (50 mL) and slowly poured into a flask containing 50 gm of ice and 50 mL of 2% HCl. The organic layer was separated and the aqueous layer extracted with two 50-mL portions of DEE The combined organic layers were washed with water (3 x 150 mL), dried ($MgSO_4$), filtered and evaporated under reduced pressure. Crystallization of the semi-solid residue from 95% ethanol/water yielded 1.92 gm (29.7%) of a mixture of 11a and 11b as a white crystalline solid. An analytical sample was prepared by recrystallization from methanol/acetone: mp. 130-150°C; IR (KBr) 3600 (sharp), 3425, 2925, 2850, 1170-1250, 1150, and 1050 cm^{-1}; ^1H NMR ($CDCl_3$) δ0.90 (s, C_{19}-H_3) and δ0.66 (s, C_{18}-H_3); ^{19}F NMR ($CDCl_3$) δ80.2 (CF_3CF_2-, 3F), δ118.6 (SteroidCF_2-, 2F), δ121.4-121.8 (-CF_2CF_2-, 10F) and δ125.8 (CF_3CF_2, 2F).

Anal. Calcd. for $C_{35}H_{47}F_{17}O$: C, 52.1; H, 5.9; F, 40.0

Found: C, 52.3; H, 6.0; F, 40.0

3-Perfluorohexyl-5α-cholestan-3-ol (24a + 24b)

By following the above procedure 5.70 mmol of perfluorohexyl-magnesium bromide in DEE was reacted with 2.00 gm (5.17 mmol) of 5α-cholestan-3-one, to give after work-up a white semi-solid residue. Crystallization of the residue from methanol/DEE provided 1.43 gm (39.5%) of 24a + 24b as colorless needles: mp. 158.5-160°C; IR (KBr) 3600 (sharp), 3450 (br), 2950, 2860, 1160-1270, 1150 1060 and 1020 cm^{-1}; ^1H NMR ($CDCl_3$) δ0.93 (s, C_{19}-H_3) and δ0.70 (s, C_{18}-H_3); ^{19}F NMR ($CDCl_3$) δ81.0 (CF_3CF_2-, 3F), δ116.6-123.0 (-CF_2CF_2-, 8F) and δ126.6 (CF_3CF_2-, 2F)

Anal. Calcd. for $C_{33}H_{47}F_{13}O$: C, 56.1; H, 6.7; F, 34.9

Found: C, 56.1; H, 6.8; F, 34.9

Separation of 3α-perfluorooctyl-5α-cholestan-3β-ol (11a) from 3β-perfluoro-octyl-5α-cholestan-3α-ol (11b)

A 2-necked, round-bottomed flask equipped with a magnetic stirring bar, rubber septums and a nitrogen inlet/outlet was charged with 0.20 gm (0.25 mmol) of 11a + 11b, 45 mg (0.37 mmol) of 4-dimethylaminopyridine

(DMAP), 2 drops of triethylamine and 10 mL of dichloromethane. Perfluorooctanoyl chloride (0.12 mL, 0.22 gm, 0.50 mmol) was added and the mixture stirred at room temperature for 5 hours. Solvent was evaporated and the residue partitioned between equal volumes of DEE and 0.5N HCl. The organic layer was separated and the aqueous layer extracted with DEE (2 x 10 mL). The combined ethereal layers were washed with 0.5N HCl (1 x 30 mL), H_2O (1 x 30 mL), 10% $NaHCO_3$ (1 x 30 mL), and brine (2 x 30 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a white solid residue. Presence of a weak, but sharp O-H stretching vibration at 3600 cm^{-1} in the infrared sepectrum indicated unreacted 11b. The residue was crystallized from methanol/DEE to give 0.26 gm (86%) of 12 as shiny white crystals: mp. 85-87°C; TLC ($CHCl_3$) R_F 0.76; IR (KBr) 2925, 2850, 1785, 1160-1270, 1080, and 1020 cm^{-1}; ^1H NMR ($CDCl_3$) δ2.34-2.59 (dd, 2H, C_4-H_2) and δ0.66 (s, C_{18}-H_3); ^{19}F NMR ($CDCl_3$) δ82.2 (CF_3CF_2-, 6F), δ118.0 (-CF_2C=O, 2F), δ120.0 (SteroidCF_2-, 2F), δ122.8-123.6 (-CF_2CF_2-, 18F), and δ127.2 (CF_3CF_2-, 4F).

Concentration of the mother liquor afforded a white solid consisting mainly of 11b. Recrystallization of crude 11b from methanol/DEE provided pure 11b as white needles: mp. 143-146°C; IR (KBr) 3600 (sharp) 2950, 2860, 1180-1250, 1150 and 1040 cm^{-1}. Based on the above data it was concluded that the original product mixture consisted of ca. 85% 11a and 15% 11b.

Hydrolysis of 12

A solution of 0.100 gm (.083 mmol) of 12 in 10 mL of 5% methanolic potassium hydroxide was refluxed on a steam bath for 2 hours and then allowed to stand at 30°C for 12 hours. The mixture was heated to reflux and slowly diluted with water till cloudiness persisted. DEE was added to clear up the cloudiness and the mixture allowed to stand on desk-top for 2 days. After cooling (ca. 5°C), the crystals were filtered, washed and dried (58 mg, 87%) under vacuum. Recrystallization from methanol/DEE provided pure 11a as white needles: mp.156-157.5°C; IR (KBr) 3400 (br), 2930, 2850, 1170-1250, 1150 and 1050 cm^{-1}.

3β-Tetrahydropyranyloxy-pregn-5-ene-20-one (16)

A solution of 5.00 gm (15.8 mmol) pregn-5-ene-3β-ol-20-one (15), 0.19 gm of p-toluenesulfonic acid monohydrate, 4.5 mL (50 mmol) of dihydropyran and 65 mL of anhydrous THF was heated under reflux for 1 hour and 20 minutes. The mixture was cooled to room temperature and 1.1 mL of tetramethylguanidine was added to neutralize the catalyst. The reaction mixture was diluted with water (200 mL), concentrated under reduced pressure and the resulting white solid extracted with DEE (3 x 50 mL). The organic layer was washed with brine (3 x 250 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a white solid residue having an odor of dihydropyran. Recrystallization of the crude product from methanol/water provided 5.37 gm (85.0%) of pure 16 as shiny white crystals: mp. 127-128.5°C (lit. mp. [35] 129-131°C); IR (KBr) 3010, 2970, 2850, 2825, 1710, 1180-1200, 1140, 1060 and 1030 cm^{-1}; ^1H NMR ($CDCl_3$) δ5.26 (d, J = 4.5 Hz, 1H, C_6-H), δ4.63 (s, 1H, C'_1-H), δ3.86 (m, 1H, C_3-H), δ3.50 (m, 2H, C'_5-H_2), δ2.07 (s, 3H, C_{21}-H_3) δ0.96 (s, C_{19}-H_3) and δ0.59 (s, C_{18}-H_3).

20-Perfluorohexyl-3β-tetrahydropyranyloxy-pregn-5-ene-20-ol (17)

Perfluorohexylmagnesium bromide (6.0 mmol), prepared by the procedure described earlier for perfluorooctylmagnesium bromide, was reacted with a solution of 2.0 gm of 16 in 49 mL of DEE. Usual precautions were taken to maintain the temperature below -60°C. The resulting mixture was stirred at -78°C for 1 hour, at -65°C for 1 hour, and between -45 to -55°C for another 4 hours. Work-up (as indicated for 11a + 11b) followed by the removal of solvent under reduced pressure gave a oily residue. Crystallization of the residue from methanol/DEE/water and collecting two crops for crystals provided 3.50 gm (89.8%) of pure 17: mp. 189-191°C; IR (KBr) 3400, 3010, 2930, 2845, 1170-1270, 1140, 1050 and 1020 cm^{-1}; ^1H NMR ($CDCl_3$) δ5.26 (d, J = 4.5 Hz, 1H, C_6-H), δ4.63 (s, 1H, C'_1-H), δ3.86 (m, 1H, C_3-H), δ3.46 (m, 2H, C'_5-H_2), δ1.53 (s, C_{21}-H_3), δ1.00 (s, C_{19}-H_3) and δ0.87 (s, 3H, C_{18}-H_3); ^{19}F NMR ($CDCl_3$) δ81.3 (CF_3CF_2-, 3F), δ117.0-123.8 (-CF_2CF_2-, 8F) and δ126.6 (CF_3CF_2-, 2F)

Anal. Calcd. for $C_{32}H_{41}F_{13}O_3$: C, 53.3; H, 5.7; F, 34.3

Found: C, 53.2; H, 5.6; F, 34.2

20-Perfluorohexyl-pregn-5-ene-3β,20-diol (18)

A mixture of 1.10 gm (1.53 mmol) of 17, 0.30 gm of p-toluenesulfonic acid monohydrate and 15 mL of 95% ethyl alcohol in a 50-mL, round-bottomed flask was heated under reflux for 3.5 hours and at room temperature for 12 hours. The reaction mixture was diluted with DEE and poured into a beaker containing 20 mL of H_2O. The layers were separated and the aqueous layer extracted with DEE (2 x 40 mL). The combined ethereal layers were washed with brine (2 x 100 mL), dried ($MgSO_4$), and filtered. Removal of solvent under reduced pressure furnished 0.890 gm (91.7%) of 18 as a white powder. Recrystallization of crude 18 from methanol/water provided pure 18: mp. 184-85.5°C; IR (KBr) 3350, 3025, 2960, 2850, 1170-1270, 1150 and 1055 cm^{-1}; ^1H NMR ($CDCl_3$) δ5.13 (d, J = 5.0 Hz, 1H, C_6-H), δ3.40 (m, 1H, C_3-H), δ1.53 (s, C_{21}-H_3), δ1.00 (s, C_{19}-H_3) and δ0.86 (s, 3H, C_{18}-H_3); ^{19}F NMR ($CDCl_3$) δ81.2 (CF_3CF_2-, 3F), δ117.0-123.2 (-CF_2CF_2-, 8F) and δ126.6 (CF_3CF_2-, 2F).

20-(1H,1H,2H,2H-Perfluorodecyl)-pregn-5-ene-3β,20-diol (20)

A 3-necked, round-bottomed flask fitted with a magnetic stirring bar, a reflux condensor, an addition funnel and a nitrogen inlet/outlet was flame dried and charged with 0.610 gm (0.025 mmol) of activated magnesium [36], one crystal of iodine and 10 mL of DEE. 1H,1H,2H,2H-Perfluorodecyl iodide (14.3 gm, 25.0 mmol) dissolved in 11 mL of DEE was added to the refluxing DEE over 3.5 hours, after which enough DEE was added to bring the volume to 30 mL. The mixture was refluxed for 2.5 hours and treated with a solution of 5.65 gm (14.0 mmol) 16 in 15 mL of THF over 1 hour. The mixture was heated under reflux for 1 hour, cooled to room temperature and poured into a flask containing 200 mL of saturated NH_4Cl. The layers were separated and the aqueous layer extracted with 30-60° petroleum ether (2 x 50 mL). The organic extracts were combined, dried ($MgSO_4$), filtered and evaporated under reduced pressure to give a solid residue, which when recrystallized from isopropyl alcohol gave 9.62 gm (80.0%) of 19 as a white solid: mp. 159-174°C.

To 5.98 gm (7.05 mmol) of 19 in 50 mL of anhydrous ethyl alcohol was added 170 mg of p-toluenesulfonic acid monohydrate and the mixture heated under reflux for one hour. Solvent (ca. 20-25 mL) was evaporated and the mixture diluted with hot water till the cloud point was apparent.

Crystals that formed on cooling were separated, washed with cold aqueous ethyl alcohol and dried in a vacuum oven for 12 hours (4.85 gm, 90.0%). Recrystallization from 95% ethyl alcohol gave 20 as a white crystalline solid: mp. 168-169°C; IR (KBr) 3450 (s), 3020, 2850, 1120-1250, 1100 and 1020 cm^{-1}; ^1H NMR ($CDCl_3$) δ5.36 (d, 1H, J = 5.0 Hz, C_6-H), δ3.53 (m, 1H, C_3-H), δ1.29 (s, 3H, C_{21}-H_3), δ1.01 (s, C_{18}-H_3) and δ0.92 (s, C_{19}-H_3); ^{13}C NMR ($CDCl_3$) δ140.86 (C_5), δ121.53 (C_6), δ74.06 (C_{20}) and δ71.80 (C_3); High resolution mass spectrum: m/e 764.26 (m/e Calcd. for $C_{31}H_{37}F_{17}O_2$, 764.25).

20-Perfluorooctyl-pregn-4-ene-3-one-20-ol (22)

By following the direction outlined above, a solution of 3.00 gm (9.54 mmol) pregn-4-ene-3,20-dione (21) in 220 mL of DEE was reacted with 25.2 mmol of perfluorooctylmagnesium bromide in DEE at -78°C. Usual precautions were taken to maintain the temperature between -60°C and -70°C. After stirring the mixture at -78°C for 36 hours, at -60°C for 5 hours, and between -40°C and -50°C for 4 hours, the dry ice/acetone bath was removed and the mixture allowed to warm gradually to room temperature. Work-up as usual followed by the removal of solvent under reduced pressure provided a yellow semi-solid. Crystallization of the residue from methanol/water provided 1.05 gm of a white solid consisting mainly of 22 and a small amount of 21. The solid was dissolved in dichloromethane, impregnated on alumina and loaded onto a alumina column. The column was eluted with CH_2Cl_2, 90% CH_2Cl_2/methanol, 20% CH_2Cl_2/methanol, and 35% CH_2Cl_2/methanol. Appropriate fractions were combined and concentrated under reduced pressure to give 0.750 gm (10.7%) of 22. An analytical sample was prepared by recrystallization from methanol/water/DEE: mp. 190-203°C; IR (KBr) 3400, 3010, 2950, 1665 (C=O), 1620, 1170-1280, 1150 1110, and 950 cm^{-1}; ^1H NMR ($CDCl_3$) δ5.66 (s, 1H, C_4-H), δ1.60 (s, 3H, C_{21}-H_3), δ1.17 (s, 3H, C_{18}-H_3) and δ0.92 (s, 3H, C_{19}-H_3); ^{19}F NMR ($CDCl_3$) δ81.0 (CF_3CF_2-, 3F), δ116.2-121.8 (-CF_2CF_2-, 12F) and δ125.4 (CF_3CF_2-, 2F).

Anal. Calcd for $C_{29}H_{31}F_{17}O_2$: C, 47.4; H, 4.2; F, 44.0
Found: C, 47.0; H, 4.2; F, 43.2

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