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Interaction between concanavalin A and vesicles or monolayers formed by fluorocarbon amphiphiles with sugar headgroups

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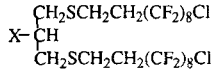
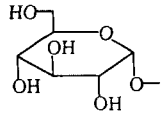
Fluorocarbon amphiphiles with α-glucosyl units as headgroups can form stable vesicles and monolayers. Their vesicle agglutination occurs in the presence of concanavalin A (Con A), which can be reversed by addition of glucose. Monolayers derived from these amphiphiles are also significantly affected by Con A in the subphase. These results can be explained in terms of recognition between Con A and sugar headgroups of membrane-forming constituents. The agglutination is dependent upon headgroup composition, but independent of surface charges of the membranes.

The glycocalix on the cell membrane surface determines surface recognition of cells. Many authors have reported that sugar-incorporated liposomes can functionally be membrane mimetic systems in the agglutination through interaction with lectins.[1–3] Furthermore, phospholipid monolayers represent powerful tools for probing structure-activity relationships of biomembranes.[4–6] We report here the synthesis of three fluorocarbon amphiphiles with sugar headgroups and the recognition interaction of their vesicles and monolayers with concanavalin A (Con A).

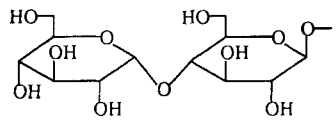
The fluorocarbon amphiphiles used are of the following structures:



X =

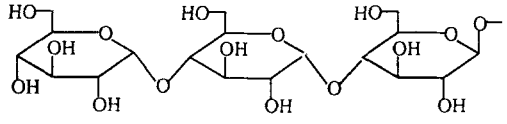


DFG



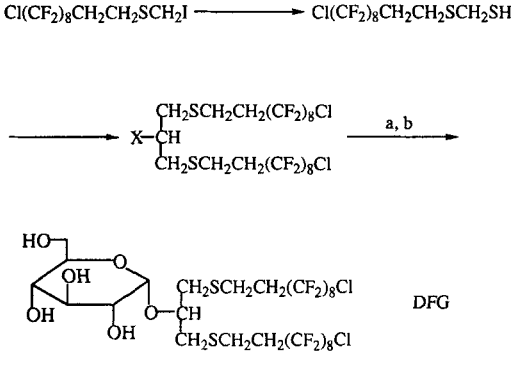
DFM

* To whom correspondence should be addressed.



DFMT

Their synthesis is shown in Scheme 1. The products were unambiguously characterized by their ^{19}F and ^{1}H NMR spectroscopic properties. The anomeric configurations of amphiphiles were determined by comparison of experimental coupling constants between anomeric protons and adjacent protons of peracetyl derivatives with values calculated based on Karplus' curve.[7] The coupling constants are 10.0 Hz for peracetyl DFM and 11.8 Hz for peracetyl DFMT, which corresponds to axial-axial orientations, i.e., β-anomer. However, the coupling constant for the anomeric proton of peracetyl DFG is 3.9 Hz, corresponding to the equitorial-axial orientation of an α-anomer.



a) TMS Triflate/peracetyl glucose

b) MeO^-/MeOH

Scheme 1

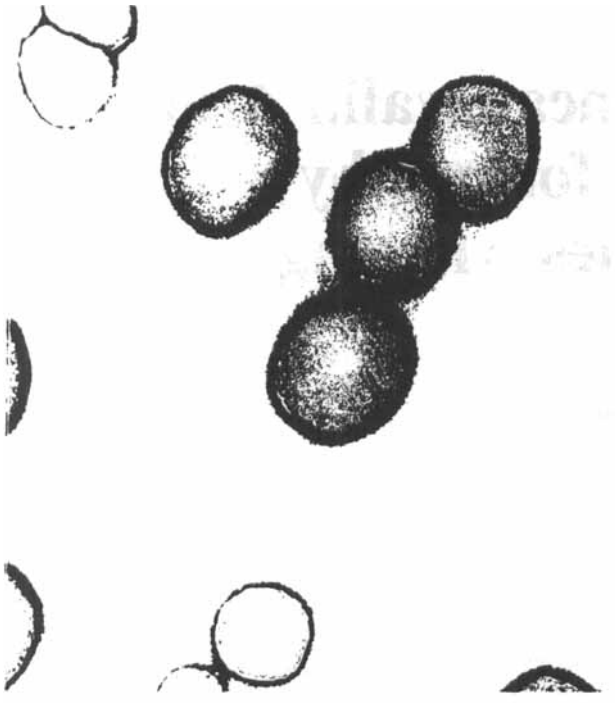


Figure 1 Electron micrography of DFM vesicle, magnification 60 000

Sonication of sugar-headed fluorocarbon amphiphiles dispersed in water generated optically transparent vesicle solutions. Electron micrographs confirmed the formation of unilamellar vesicles, whose diameters range from 3500 to 4000 Å, 3000 to 4000 Å and 1000 to 1500 Å for DFG, DFM, and DFMT, respectively. Figure 1 is the electron micrograph of DFM. All vesicles have a membrane thickness of about 30 Å, corresponding to the molecular length of an amphiphile bilayer. Vesicles of DFG, DFM, and DFMT, which are stabilized by the strong hydrophobic interaction between fluorocarbon chains, have excellent dispersion and transparency. Aqueous solutions of vesicles are found to be stable for weeks at room temperature.

A series of investigations have been reported on the agglutination caused by interactions between lectins and sugar-incorporated vesicles.[8,9] Agglutination occurs for vesicles upon addition of Con A, and is reversed by addition of glucose. As indicated in Fig 2, that Con A reacts with vesicles of DFG, DFM, and DFMT results in a fast increase in turbidity, accompanying precipitation for vesicle of DFG which causes a decrease in turbidity. Addition of glucose

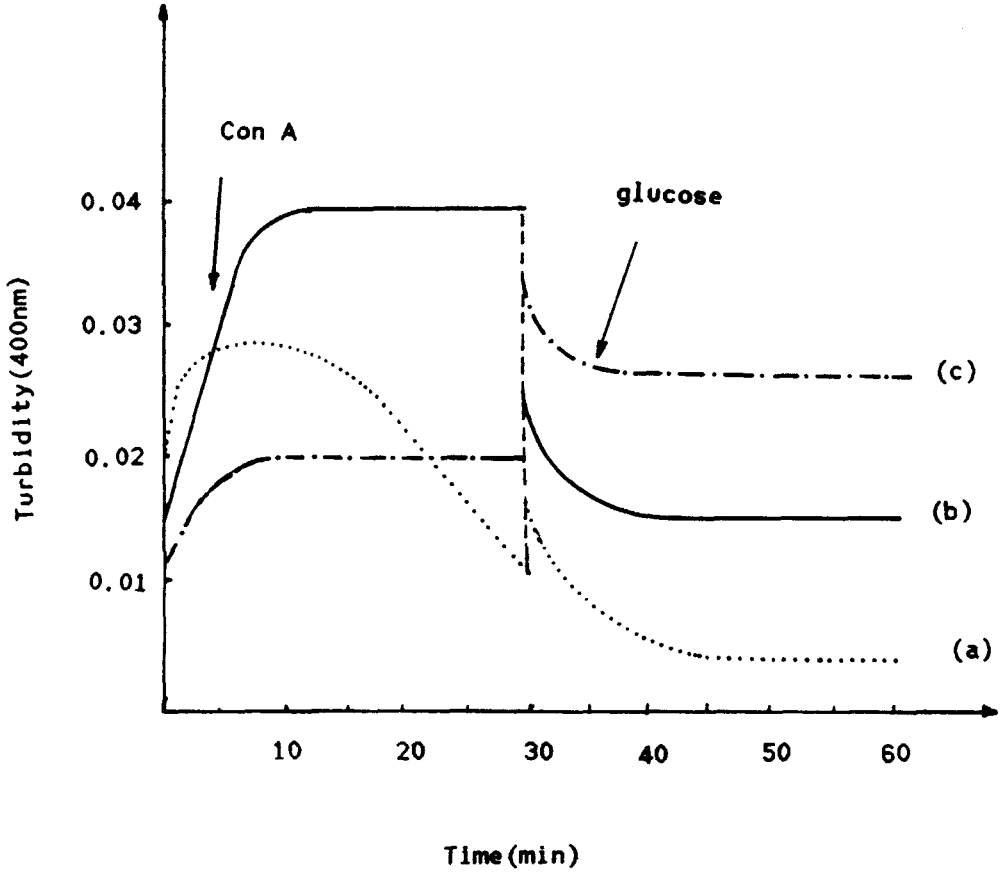


Figure 2 Interaction of Con A with vesicles of sugar-headed amphiphiles. Con A: 0.46 mg ml^{-1}. (a) DFG: 1.52×10^{-3} M, glucose: 0.027 M; (b) DFM: 1.33×10^{-3} M, glucose: 0.147 M; (c) DFMT: 1.18×10^{-3} M, glucose: 0.041 M

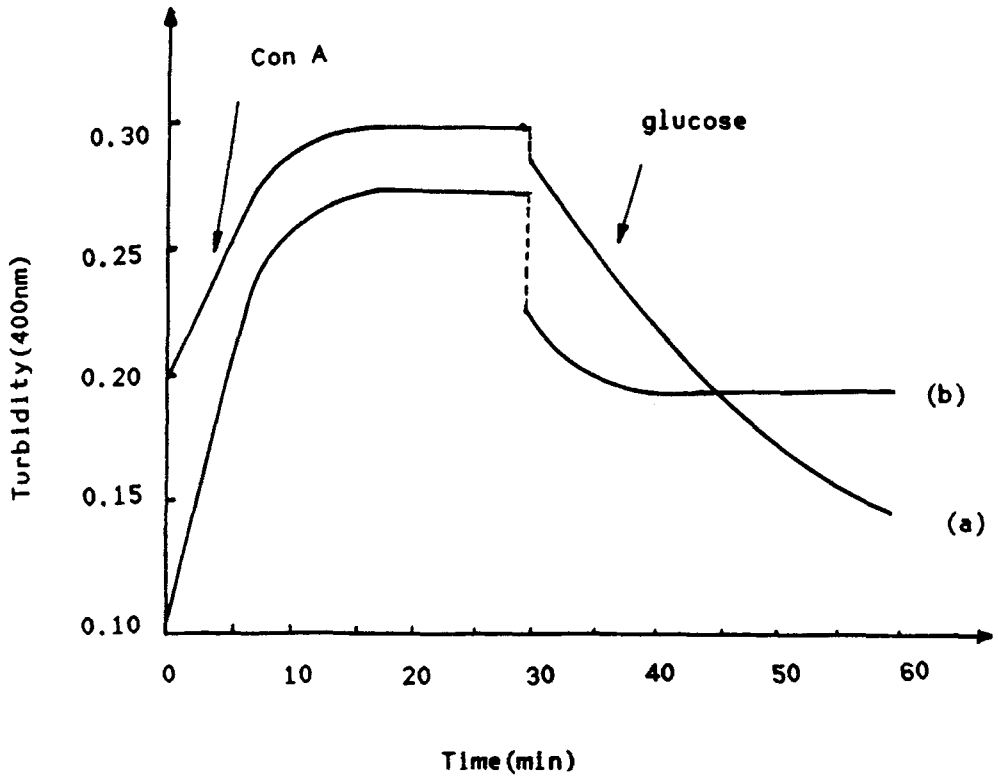
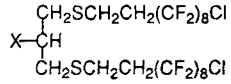


Figure 3 Interaction of Con A with covesicles. (a) DFMT+DDFSA (1.8×10^{-4} M, 4.6×10^{-3} M), Con A: 0.44 mg ml^{-1}, glucose: 0.18 M; (b) DFMT+DDFAB (3.0×10^{-4} M, 6.0×10^{-3} M), Con A: 0.46 mg ml^{-1}, glucose: 0.14 M

gradually abates the turbidity of vesicle solution. For vesicles of DFG, the precipitates caused by addition of Con A can redissolve to restore a transparent solution. As DFM and DFMT have larger hydrophilic groups, their vesicles are more stable and do not precipitate in cases where agglutination with Con A takes place. These results imply that the interaction between sugar-headed vesicles and Con A is reversible, which is characteristic of a recognition reaction. For the covesicles of the sugar-headed amphiphile DFMT with either cationic DDFAB or neutral DDFSA,[10] similar reversible agglutination behavior can be observed, as shown in Fig 3.



X = Br^+ $(CH_3)_3N^+$-CH_2CO_2- DDFAB

$HO_2CCH_2CH_2CO_2$- DDFSA

However, the results of vesicle formation by the non-sugar-headed fluorocarbon amphiphile DDFAB or diestearoylglycerophosphatidylcholine (DSPC) are rather different. Figure 4 indicates that the turbidity of these systems increases with addition of Con A, which, however, does not diminish by addition of glucose. Apparently, all vesicles containing sugar-headed amphiphiles, in spite of the type of surface charges, can be reversibly agglutinated by Con A. This implies that the agglutination is only dependent upon the match of the special sugar moiety for Con A. Con A causes vesicles of DDFSA, DDFAB and DSPC to experience an irreversible increase in turbidity, where lectin inducing fusion of vesicles or liposomes is a possible mechanism.[11,12]

All three amphiphiles can form monolayers at the gas/water interface. Their surface pressure-area isotherms are shown in Fig 5. It has been reported[13] that the sugar ring of 1,2-di-O-tetradecyl-3-O-(β-D-glucopyranosyl)-sn-glycerol is essentially fully extended away from its bilayer surface, while that of the corresponding α-anomer is almost parallel to the bilayer surface. Although the headgroups of amphiphiles hold a size order of DFMT > DFM > DFG, the α-linkage of DFG causes the sugar ring to orient parallel to the membrane surface, hence DFG requires a larger limited area (65.7 Å^2) in monolayer. The extension of the sugar rings of DFM and DFMT, which have β-linkage, into the water phase requires smaller limited area. The cross-sectioned area of a perfluorocarbon chain is 28 Å^2. DFM and DFMT have limited areas of 56.7 and 55.5 Å^2, respectively, in their monolayers, corresponding to the area that

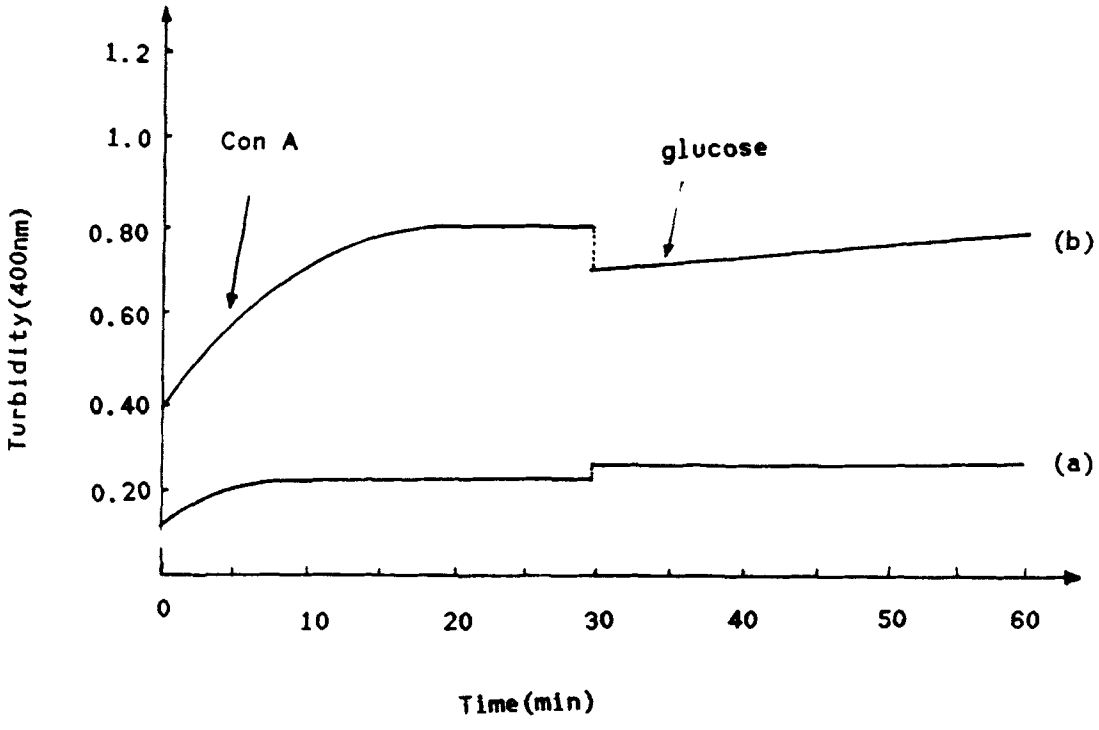


Figure 4 Interaction of Con A with non-sugar headed vesicles. (a) DDFAB: 3.56×10^{-3} M, Con A: 0.44 mg ml^{-1}, glucose: 0.11 M; (b) DSPC: 5.37×10^{-3} M, Con A: 0.42 mg ml^{-1}, glucose: 0.20 M

double fluorocarbon chains require. DFG consists of only one glucosyl unit, therefore, its headgroup has weaker hydrophilicity. When it spreads at the air/water interface, it readily forms monolayer domains, which causes a larger free space. As a result, it directly forms liquid condensed monolayers. In contrast, the stronger hydrophilicity of the headgroups of DFM and DFMT results in the formation of liquid expanded monolayers before a liquid condensed monolayer and a solid monolayer appear (Fig 5). Concanavalin A exhibits significant effects on the isotherms of these three amphiphiles. In the presence of Con A, liquid expanded membranes appear much earlier, and no solid membranes are observed, consequently the strength of the membranes obviously decreases.

Mixed membranes are prepared by solubilizing sugar-headed amphiphiles into a membrane of DDFAB, which is more similar to cell membranes. For mixed systems, typical gas, liquid expanded, liquid condensed, and solid membranes can be observed as the surface pressure consecutively increases. In the presence of Con A in the subphase, their isotherms only show liquid monolayers and a gas or solid monolayer does not form, as shown in Fig 6.

The interaction between Con A and sugar headgroups can be studied at the two-dimensional level by measuring their surface pressure-area isotherms. Generally, Con A exists as a tetramer where each subunit carries one binding site.[14] The combination of Con A with sugar headgroups of amphiphiles largely increases the volume of the hydrophilic part. Under higher surface pressures, the headgroups tightly arrange in the membrane, however, the hydrophobic chains are still loosely packed. Consequently, the solid monolayer does not come up and the stability of membranes lowers (Fig 7).

Our results give a clue to the possible means of forming Con A crystal in two dimensions through monolayer techniques.[15,16] Because the strong hydrophobic interaction between fluorocarbon chains can significantly strengthen the membrane stability, further work in this field should be intriguing.

MATERIALS AND METHODS

The ^1H and ^{19}F NMR spectra were recorded on a Bruker AM-400 and a FX-90Q spectrometer, respectively. MS spectra were recorded on a Finnigan Mass Spectrometer. Turbidity measurements were conducted on a Perkin-Elmer 241MC Spectrophotometer. The thermometers were not calibrated.

The synthesis of peracetylglucose, peracetylmaltose, and peracetylmaltotriose was achieved by following the reported procedures.[17]

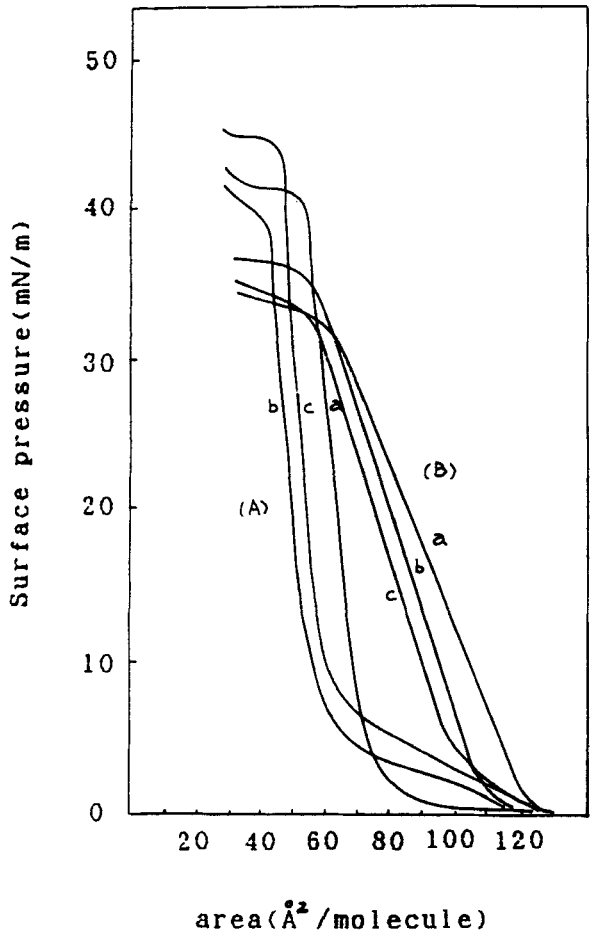


Figure 5 Surface pressure-area isotherms of (a) DFG, (b) DFM, (c) DFMT at 18°C; subphase; (A) water, (B) Con A in water, 2.8 mg l^{-1}

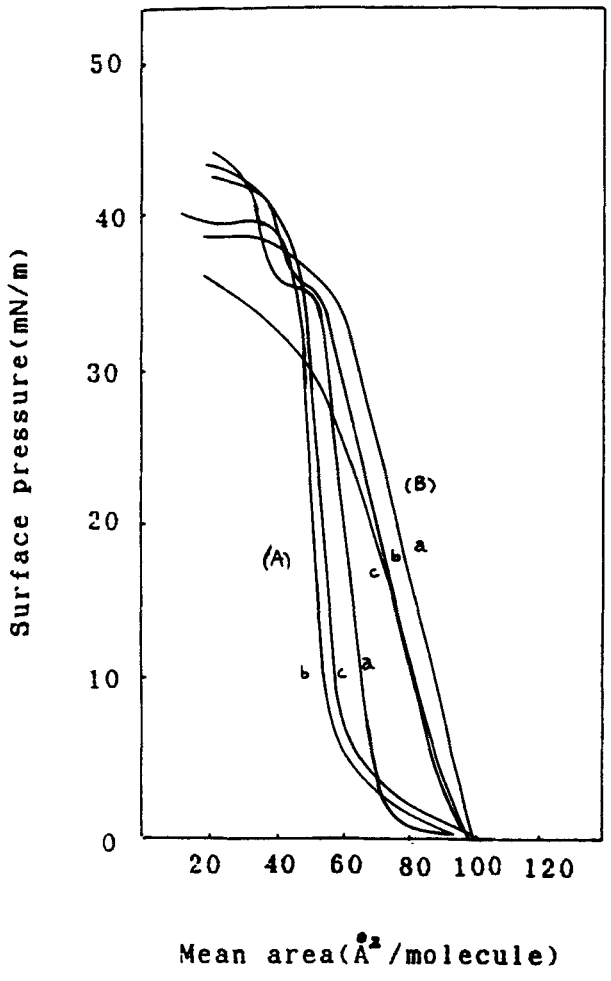


Figure 6 Isotherms of mixed monolayers of (a) DFG:DDFAB (1:0.37), (b) DFM:DDFAB (1:0.38) and (c) DFMT:DDFAB (1:0.39) at 18°C; subphase (A) water, (B) Con A in water, 2.8 mg l^{-1}

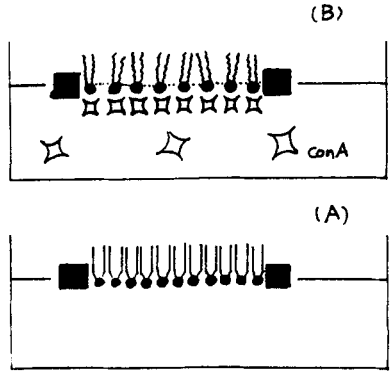


Figure 7 Schematic illustration of the effect of Con A in subphase on the monolayers of sugar-headed amphiphiles. Subphase (A) water, (B) Con A in water

A typical procedure for synthesis of peracetyl DFM

0.886 g (1.9 mmol) of peracetyl maltose and 1.22 g (1.2 mmol) of 1,3-di(w-chloro-1'H,1'H,2'H,2'H-perfluoroundecanethio)-2-propanol[10] were dissolved in anhydrous 1,2-dichloroethane. In the presence of 4 Å molecular sieves, the mixture was stirred for 1 hour at room temperature. To the mixture 0.4 mL of trimethylsilyl triflate (TMStriflate) was added and the mixture was stirred at room temperature for 30 hours. The reaction was halted by addition of triethylamine and molecular sieves were removed by filtration. The combined filtrates were concentrated and the crude product was purified by column chromatography to afford 380 mg of pure product, yield 11.5%. m.p., 44–46 °C.

A typical procedure for the synthesis of DFM

To a suspension of 200 mg (0.12 mmol) of peracetyl DFM in 3 mL of anhydrous methanol, 1 mL of sodium methoxide solution (0.5 g of sodium dissolved into 100 mL of anhydrous methanol) was added. After stirring for 40 minutes at room temperature, 2 mL of water was then added to the mixture. The precipitate was filtered, and washed with anhydrous methanol.

After drying, a white solid of DFM was obtained, yield 93%. m.p. 112–114 °C. $[\alpha]_D = +43.8°$ (c, 0.0913 in methanol). ^1H NMR ($CDCl_3 + CD_3OD$): $\delta = 2.10$ (m, 4H, $2XCH_2CF_2$), 2.55 (m, 8H, $2XCH_2SCH_2$), 4.00 (d, J = 7.8 Hz, 1-H), 4.50 (d, J = 3.9 Hz, 1′-H). ^{19}F NMR: $\delta = 13.5$ (s, 4F, $2XCF_2Cl$), −32.25 (s, 4F, $2XCH_2CF_2$), −39.77 (t, 24F, $2X(CF_2)_6$). MS (m/z): 1372 (M^+).

DFG, yield 85.4%. m.p. 95–97 °C. $[\alpha]_D = +37.1°$ (c, 0.283 in methanol). ^1H NMR ($CDCl_3 + CD_3OD$): $\delta = 2.0$ (m, 4H, $2XCH_2CF_2$), 2.50 (m, 8H, $2XCH_2SCH_2$), 3.0 (t, J = 9.5 Hz, 1H), 3.05 (dd, J = 3.9 Hz, 1H), 3.20 (t, J = 9.5 Hz, 1H), 3.55 (m, 1H), 4.6 (d, J = 3.9 Hz, 1-H). IR: ν [cm^{-1}] = 3400. MS (m/z): 1210 (M^+). Calcd. for $C_{29}H_{24}O_6F_{32}S_2Cl_2$: C 28.74, H 1.98, F 50.21. Found: C 28.57, H 1.82, F 49.8.

DFMT, yield 78.4%. m.p. 141–143 °C. ^1H NMR ($CDCl_3 + CD_3$ $\delta = 2$, 10 (m, 4H, $2XCH_2CF_2$), 2.50 (m, 8H, $2XCH_2SCH_2$), 4.10 (d, J = 7.8 Hz, 1-H), 4.35 (d, J = 4.4 Hz, 1″-H), 4.60 (d, J = 3.9 Hz, 1′-H). IR: ν [cm^{-1}] = 3400. Ms (m/z): 1210 (M^+-2glu). Calcd. for $C_{41}H_{44}O_{16}F_{32}S_2Cl_2$: C 32.05, H 2.87, F 39.61. Found: C 31.58, H 2.34, F 39.35.

THE MEASUREMENTS OF ISOTHERMS

All the π-A isotherms were recorded on a home-made film balance at 18°C. Water of the subphase was double-distilled. The concentration of Con A in subphase was 2.8 mg/L.

ACKNOWLEDGMENT

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