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# Synthesis and Preliminary Evaluation of a New Class of Fluorinated Amphiphiles Designed for In-Plane Immobilisation of Biological Macromolecules

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**Abstract:** A series of new fluorinated amphiphilic structures has been synthesized. The molecules were designed to prevent the insertion of hydrophobic compounds in between the fatty chains of the amphiphile. Derivatives of vitamin A analogues were prepared in order to perform two-dimensional crystallisation experiments with retinoic acid receptors.


Molecular recognition is a key principle in the organisation of large protein architectures. It is of the outermost interest to have equally complex and interesting combinations of proteins outside biosystems[1]. Protein two-dimensional (2D) arrays represent one way for dealing with technologies developed in life processes and succeeding in practical applications. From "simple" anchoring of proteins on solid supports for purification purposes[2-6] or biosensor manufacturing[7-9], to highly ordered 2D crystals required for structural analysis[10-12], the common first step is the coverage of a surface by a protein. In particular, when 2D crystals of a water soluble protein are sought, a technique has been developed using ligand-functionalised lipid monolayers[13-17]. A water soluble ligand of the protein of interest is chemically anchored to a lipid moiety. The resulting amphiphilic molecule when deposited at the air-water interface spreads into a monolayer, displaying all the ligands into the aqueous medium. When introducing the protein into the subphase, molecular recognition between the macromolecule and the immobilised ligand leads to in-plane concentration of the polypeptide. According to the experimental conditions depending on intrinsic properties of the protein, the concentration process will or will not end in the formation of 2D crystals. Anyway, successful experiments absolutely require the 2D mobility of each lipid molecule within the monolayer. This requirement is satisfactorily met only when crystallisation experiments are performed with a fluid phase lipid layer[16].

In the course of our work, we are interested in 2D structural analysis of retinoid receptors. These nuclear proteins are involved in a broad range of biological processes such as embryogenesis, cellular differentiation and vertebrate homeostasis and are under the control of natural and synthetic analogues of vitamin A[18-22]. An interesting point emerges when dealing with a protein the ligand of which is hydrophobic. In such a case, the anchoring of the ligand onto a lipid moiety and its spreading at the air-water interface result in the insertion of the pharmacophore in between the lipid fatty chains. This definitely prevents the molecular

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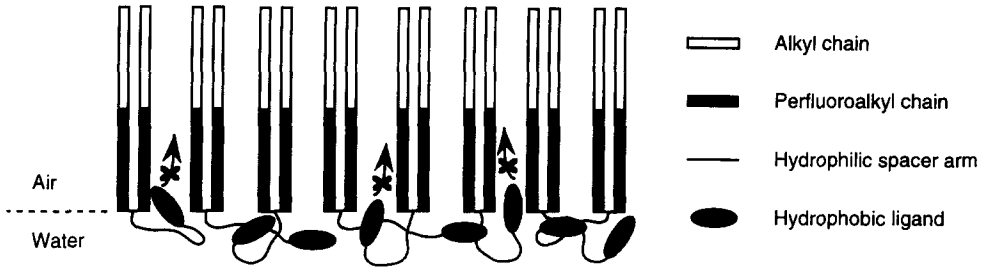
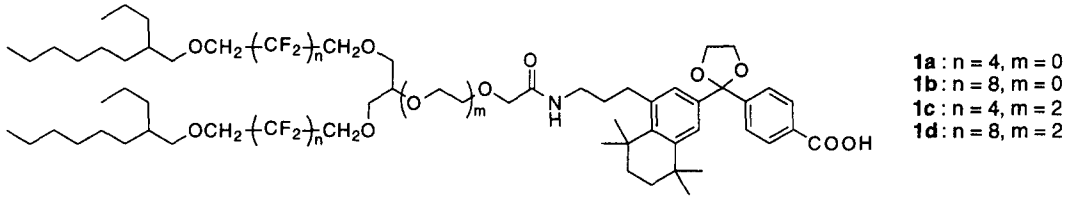
recognition process with the protein, *i.e.* concentration and finally 2D crystallisation of the macromolecule. In order to overcome that difficulty, we designed lipids which, when derivatised with a hydrophobic ligand, are able to express the pharmacophore towards an aqueous phase. The concept is based on the properties of perfluorinated compounds that do not mix neither with hydrocarbons nor with hydrophilic compounds[23-26] (Fig. 1).




- Figure 1 -

Provided the hydrophilic spacer arm between the lipid moiety and the ligand is shorter than the perfluorinated chain, insertion of the ligand in between the chains is not thermodynamically favoured. The pharmacophore is then confined at the frontier between the aqueous phase and the "fluorinated phase", thus remaining accessible to its receptor in solution.

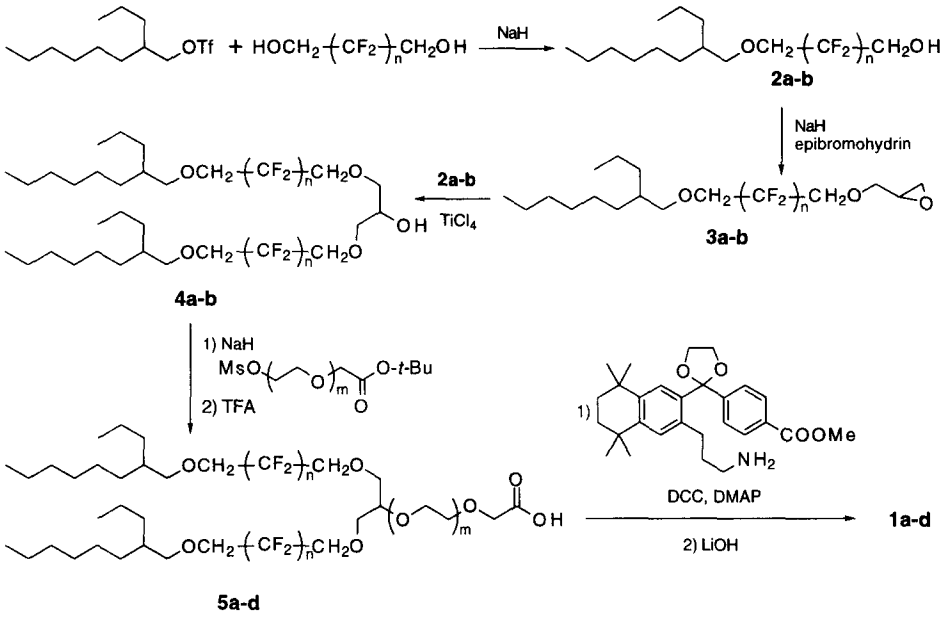
Therefore, in order to perform 2D crystallisation experiments with retinoid receptors, we synthesised different retinoid-lipid conjugates based on that concept (Fig. 2). To manage with monolayer stability and fluidity requirements, only the "lower" portion of the alkyl chains is perfluorinated and the "higher" hydrocarbon segment is branched.




- Figure 2 -

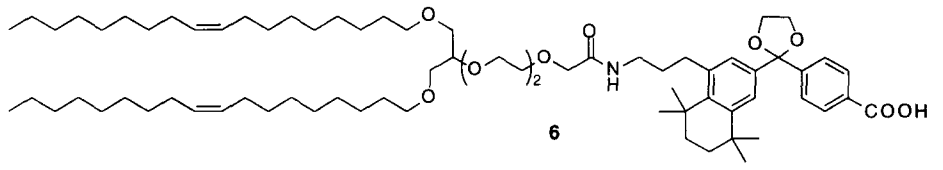
The synthesis of the lipid moiety was elaborated from perfluoroalkyl α,ω-dihydroxy compounds, by successive Williamson-type reactions (Fig. 3)†. That is the first reported synthesis of partially fluorinated amphiphiles where a long perfluoroalkyl spacer is introduced between the alkyl tail and the polar head of the molecule[27-34].

† The choice and synthesis of the ligand moiety will be discussed elsewhere




- Figure 3[35] -

Surface pressure *vs* area isotherms obtained for compounds **1a-d** confirm that these lipids always spread into fluid monolayers at the air-water interface, as was expected because of the presence of the branched alkyl chains[36]. Incubation experiments with a retinoid receptor (RAR-RXR heterodimer[37,38]) showed that the macromolecule efficiently and specifically binds and concentrates onto the lipid film[39]. This is definitely not the case when the non-fluorinated fluid lipid **6** is used (Fig. 4), thereby indicating that the ligand is buried in between the alkyl chains and is not accessible for the protein any more. These results unambiguously validate our hypothesis about hydrophobic ligand segregation into the lipid proximal aqueous phase. This type of fluorinated compounds constitutes an interesting new tool to investigate interactions between proteins and hydrophobic interfaces.



**6**

- Figure 4 -

Two-dimensional crystallisation experiments with different retinoid receptors are now in progress. It is to be noted that besides their use in the 2D crystallisation of soluble protein, the compounds described herein (especially **2**, **4** and **5** series, and derivatives) could be of special interest as detergents in membrane protein 3D

crystallisation experiments. Such structures have never been used yet and their potential remains to be discovered.

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