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NMR-based molecular ruler for determining the depth of intercalants within the lipid bilayer Part I. Discovering the guidelines

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ABSTRACT

The development of "molecular rulers" would allow one to quantitatively locate intercalants within the liposomal bilayer. To this end, we have attempted to correlate the ^{13}C NMR chemical shift of a polarizable "reporter" carbon (e.g., carbonyl) of the intercalant—with the $E_T(30)$ polarity it experiences, and with its Angstrom distance from the interface. This requires families of molecules with the same two "reporter carbons" separated by a fixed distance, residing at various depths/polarities within the bilayer. The families studied included 4,4-dialkylcyclohexa-2,5-dienones **1**, benzenediacetic esters **15**, benzenedipropionic esters **17**, 4-alkoxybenzaldehydes **19** and methyl 4-alkoxybenzoates **22**. These compounds possessed the following characteristics: (1) a planar backbone; (2) polar/hydrophilic "head" groups; (3) modular hydrophobic tails; (4) large changes in the ^{13}C NMR chemical shift ($\Delta\delta$) of the reporter atoms with solvent polarity. These studies revealed a fifth requirement, namely: (5) the reporter carbons must not be strongly conjugated, lest it reflect the charge build-up at another site within the conjugated system.

1. Introduction

We have been exploring the active oxygen chemistry of substrates intercalated within the phospholipid bilayer of model liposomes (Frimer et al., 1983, 1996; Frimer, 1985; Strul et al., 1993, 1994; Weitman et al., 2001; Afri et al., 2002, 2004a,b; Bronshtein et al., 2004; Gamliel et al., 2008). Our studies indicate that there is a clear correlation between the location of a substrate within the bilayer and its biochemical activity. In order to determine the location of an intercalant within the lipid bilayer, we have used an NMR technique which is based on two observations (Maciel and Ruben, 1963; Maciel and Natterstad, 1965; Ueji and Makamaura, 1976; Menger et al., 1978, 1988; Menger, 1979; Janzen et al., 1989). Firstly, an excellent correlation exists between the ^{13}C NMR chemical shift (δ) of a polarizable carbon (e.g., the carbonyl or nitronyl carbon) and the Dimroth–Reichardt (Dimroth et al., 1963; Reichardt, 1965, 1988, 1994) $E_T(30)$ polarity parameter of the solvent in which the spectrum was obtained (Gamliel, 2005). Once this chemical shift–polarity correlation has been determined, one can work backwards, utilizing the observed chemical shift to determine the microenvironment polarity felt by the polar carbon in a particular situation.

The second observation is that the liposomal bilayer consists of a gradient of polarities ranging from that of water ($E_T(30)$ = 63.1 kcal/mol) at the lipid/water interface down to hexane ($E_T(30)$ = 31.0 kcal/mol) within the fatty acid chains. (Presumably, there is no change in polarity within the lipid slab.) The chemical shift–polarity correlation makes it possible to use chemical shifts as a polarity gauge thereby yielding a *qualitative* estimate of the substrate's distance from the interface. This then allows us to correlate a substrate's *average* location within the bilayer with its reactivity. We use the word "average" advisedly. The timescale of molecular motion is faster than that of the NMR experiment; hence, the values we obtain by the above method are actually average locations. The NMR chemical shift data will reflect polarity due to electrostatic fields from the time-averaged structure, as well as from water penetration and perhaps partial exit of the surfactants.

Given this caveat, we nevertheless believe that this ^{13}C NMR chemical shift/polarity correlation technique is a useful tool for approximating the location of substrates within lipid bilayers. Indeed, this technique has enabled us to determine the location of a variety of biologically active compounds including the natural antioxidants vitamin E and ubiquinol (Afri et al., 2004a), the photodynamic cancer therapy agent hypericin (Weitman et al., 2001) and various derivatives of dichlorodihydrofluorescein (DCFH) (Afri et al., 2004b) involved in oxyradical detection.

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Despite the above success, still lacking was a means for converting this qualitative data into *quantitative* values, namely, the vertical distance in Å from the water phase. To this end, we needed to determine whether any correlation – linear, stepwise or otherwise – existed between the polarity felt by a given carbon and its depth. In order to map the bilayer, we required molecules of differing lipophilicities, residing at various depths within the bilayer, with two or more "reporter carbons." This would allow us to link two or more vertical points within a bilayer by both distance (determined by *ab initio* or molecular mechanics calculations) and $E_T(30)$ polarity values. Such an assortment of molecules would eventually enable us to develop a "molecular ruler" correlating $E_T(30)$ polarity data with Angstrom distances from the water phase.

The literature does record other methods for measuring the depth of an organic molecule in non-homogeneous media, notably London's fluorescence-based Parallax method (Abrams and London, 1992; Chattopadhyay and London, 1987; Asuncion-Punzalan and London, 1995), and Walker and Steel's solvatochromic UV technique (Walker and Steel, 2003; Steel et al., 2002). The major drawback of these techniques is that the fluorescence or UV absorption arises from a reporter chromophore or molecule, leading to very low precision in locating the source of the spectrum. By contrast, the aforementioned NMR technique uses a single carbon as the reporter element.

Our efforts towards developing such a molecular ruler are described in this and the following companion paper (Cohen et al., 2008a) The former describes our efforts in developing reliable criteria for reporter molecules and selecting appropriate candidates, while the latter describes our initial results in developing a molecular ruler.

2. Materials and methods

2.1. General

Ab initio calculations were performed with Gaussian 03 performed at the B3LYP level using 6–31G* basis set. Gaussian, Inc., Pittsburgh PA, 2003. Molecular modeling calculations were carried out with PCMODEL version 7.50.00, Serena Software, Bloomington, Indiana, USA—which uses the MMX force field.

The NMR spectra were recorded on either 300 MHz (^{1}H) and 75 MHz (^{13}C) or 50.3 MHz (^{13}C). NMR spectra were generally taken at 25 ± 1 °C, with the exception of the dimyristoylphosphatidylcholine (DMPC) liposome solutions which were run on 150.9 MHz (^{13}C) at 45 °C, above the phase transition temperature (T_C) of DMPC (Zachariasse et al., 1981). Below this temperature, the mobility of the intercalated molecules is low and the resulting NMR absorptions are very broad. Raising the temperature sharpens the peaks but does not seem to affect the chemical shifts. The NMR spectra were generally recorded while locked on the deuterium signals of the respective solvent. The chemical shifts were measured relative to internal tetramethylsilane (TMS) or solvent (Gottlieb et al., 1997), except in the case of the aqueous liposome solutions in which we calibrated the spectrum according to the trimethylammonium peak at 54.6 ppm. KH_2PO_4 and KOH were used in the preparation of a 0.1 M phosphate buffer solution (pH 7.8 and containing 10^{-4} M EDTA). The general procedure for the preparation of DMPC liposomal suspensions for NMR studies has been previously described (Afri et al., 2002). The substrate concentration was generally 0.05 M and the intercalant:lipid molar ratio was 1:5, except for the case of compound **1**, where the substrate concentration was 0.009 M and the intercalant:lipid molar ratio was 1:4. The designation "t" refers to a second order triplet resulting from virtual coupling. In the assignments below, the carbons and attached hydrogens were numbered to allow convenient comparison of the spectral data of the various derivatives, and not necessarily according to the rules of nomenclature. The NMR numbering for compounds **1**, **2**, **6**, **9**, **11**, **15**, **17**, **19**, **22** and **23** is exemplified in Scheme 1.

2.2. General procedure for preparation of DMPC liposomal solutions

All glassware was first rinsed with conc. HCl to remove all traces of detergents and then with doubly distilled water. In a typical liposome preparation, the compounds to be intercalated (henceforth dubbed "intercalants") and DMPC in a molar ratio of 1:5 (except for the case of compound **1**, where the ratio was 1:4) were dissolved in chloroform in a vial. The solvent was then evaporated with a gentle steam of N_2 while rotating in the palm of the hand, leaving a uniformly thin layer of lipid on the walls of the vial. The vial was finally set in a cotton-packed RB flask and the solvent was removed by rotary evaporation, leaving a uniformly thin film layer of lipid on the walls of the vial. The vial was then charged with 0.1 M phosphate buffer with 10% D_2O (pH 7.8) yielding a cloudy solution in the exact desired molar concentration. The lipid solution was vortexed for 10 min to obtain multilamellar liposomes. The liposomal solution was then sonicated until a clear solution of essentially unilamellar liposome was obtained. Sonication was effected with a MSE Titanium Probe Ultrasonic Disintegrator model MK2 at 20 kHz output frequency. Previous cryo-TEM work (Afri et al., 2004a) confirms that these conditions produce primarily unilamellar liposomes.

To verify that the various intercalants lie within the lipid bilayer and not the aqueous phase, the liposomes were centrifuged down (25,000 × *g* for 15 min) to a lipid pellet. The supernatant liquid was decanted and replaced by buffer, and the pellet was redispersed by vortexing the sample. NMR spectra of the starting and final liposomal and supernatant solutions revealed that the substrate indeed resides within the lipid bilayer exclusively.

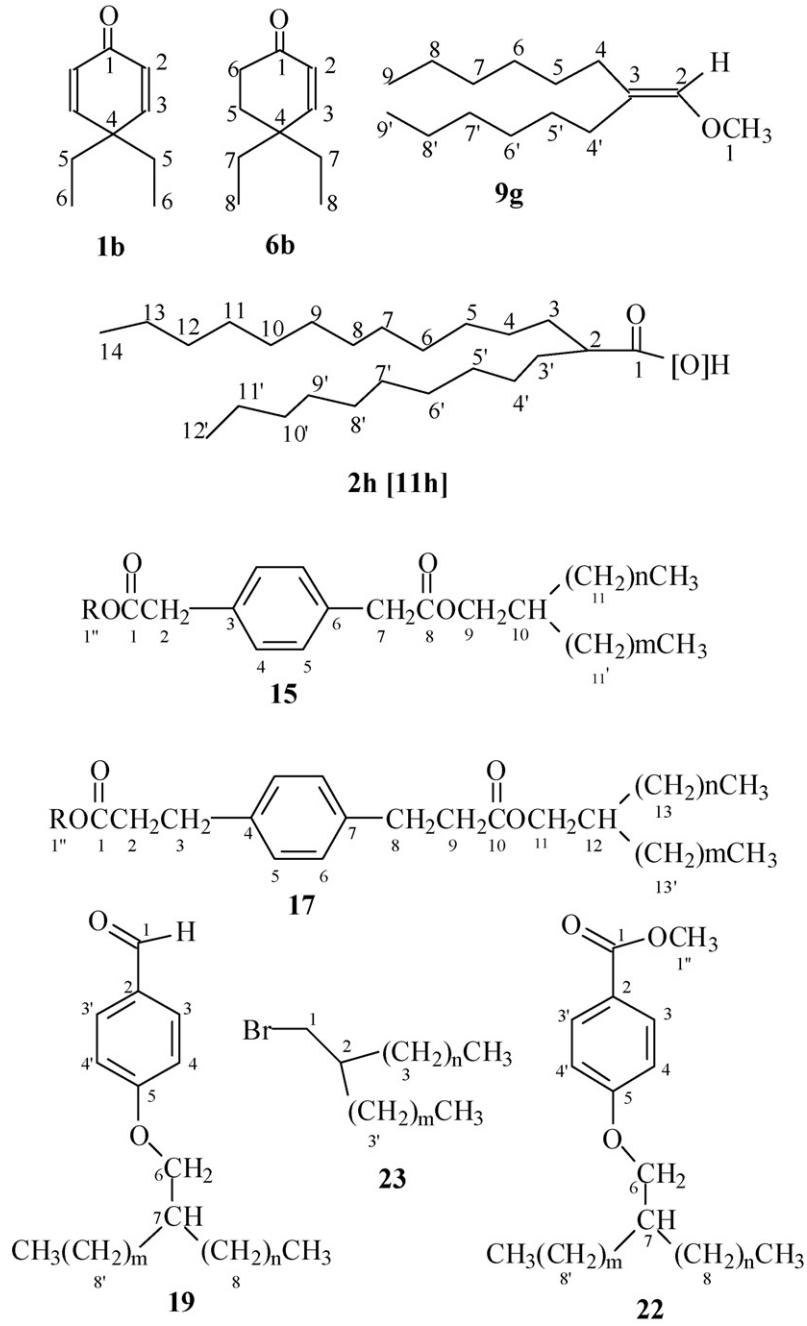
2.3. Substrates—preparation and spectral data

2.3.1. 4,4-Dialkylcyclohexa-2,5-dien-1-one (*1*)

The title dienones were prepared by a modification of the Zimmerman procedure (Zimmerman et al., 1971), as follows: 4,4-dialkylcyclohexenone (**6**, 0.013 mol), DDQ (5.9 g 0.026 mol), and purified dioxane (45 mL) were heated under nitrogen at 95–100 °C for 22 h. The cooled reaction solution was poured into a 1:1 mixture of ether/pentane (40 mL) and washed with 5% aqueous sodium hydroxide and water (40 mL). The organic phase was dried over $MgSO_4$ and rotary evaporated to dryness. The crude product was chromatographed on silica gel, eluting with petroleum ether/ethyl acetate (90:10), yielding the desired products as oils in 75–85% yield for **1a–d**, but substantially lower for **1e–h**. Dienones **1a** (Bordwell and Wellman, 1963; Zimmerman et al., 1967; Gramlich, 1979) **1c** (Dreiding, 1957) and **1d** (Bordwell and Wellman, 1963; Gramlich, 1979; Zimmerman and Schuster, 1962) are known.

*2.3.1.1. 4,4-Diethylcyclohexa-2,5-dien-1-one (**1b**).* ^{1}H NMR ($CDCl_3$) δ 6.69 (AA′XX′, 2H, H_3), 3.64 (d, J = 10.22 Hz, 2H, H_2), 1.69 (q, J = 7.5 Hz, 4H, H_5), 0.75 (t, J = 7.5 Hz, 6H, H_6); ^{13}C NMR ($CDCl_3$) δ 186.3 (C_1), 154.4 (C_3), 130.2 (C_2), 46.7 (C_4), 31.8 (C_5), 8.5 (C_6); MS (EI) m/z 150.1 (M, 50.8%); HRMS (EI) m/z calcd for $C_{10}H_{14}O$ (M^+) 150.1044, found 150.1014; IR (KBr) 1665.24 (CO stretch) cm^{-1}.

*2.3.1.2. Spiro[5.5]undec-1,4-dien-3-one (**1c**).* This compound is known in the literature (Dreiding, 1957), but some of the spectral data are lacking. ^{1}H NMR ($CDCl_3$) δ 7.08 (AA′XX′, 2H, H_3), 6.25 (d, J = 10.14 Hz, 2H, H_2), 1.61 (m, 10H, H_5–H_7); ^{13}C NMR ($CDCl_3$) δ 186.2 (C_1), 155.2 (C_3), 128.1 (C_2), 40.9 (C_4), 35.5 (C_5), 25.5 (C_6), 21.6 (C_7); MS (CI) m/z 163 (MH^+, 81%); HRMS (CI) m/z calcd for $C_{11}H_{15}O$



Scheme 1. The NMR numbering for compounds **1**, **2**, **6**, **9**, **11**, **15**, **17**, **19**, **22** and **23**.

(MH$^+$) 163.1214, found 163.1223; IR (KBr) 1663.46 (CO stretch) cm^{-1}.

*2.3.1.3. 4,4-Dibutylcyclohexa-2,5-dien-1-one (**1e**).* ^{1}H NMR ($CDCl_3$) δ 6.7 (AA′XX′, 2H, H_3), 6.34 (AA′XX′, 2H, H_2), 1.617 (m, 4H, H_5), 1.24 (m, 4H, H_7) 1.09 (m, 4H, H_6), δ 0.84 ("t", 6H, H_8); ^{13}C NMR ($CDCl_3$) δ 187.0 (C_1), 155.6 (C_3), 130.2 (C_2), δ 46.5 (C_4), 39.8 (C_5), 26.8 (C_6), 23.1 (C_7), 13.9 (C_8); HRMS (EI) *m*/*z* calcd $C_{14}H_{22}O$ (M$^+$) 206.1670, found 206.1667; IR (KBr) 1665.87 (CO stretch), 1624.50 (C=C) cm^{-1}.

*2.3.1.4. 4,4-Dipentylcyclohexa-2,5-dien-1-one (**1f**).* ^{1}H NMR ($CDCl_3$) δ 6.74 (AA′XX′, 2H, H_3), 6.34 (AA′XX′, 2H, H_2), 1.63 (m, 4H, H_5), 1.22 (m, 8H, H_7, H_8) 1.18 (m, 4H, H_6), 0.86 ("t", 6H, H_9); ^{13}C NMR ($CDCl_3$) δ 187.1(C_1), 155.6 (C_3), 130.3 (C_2), 46.7 (C_4), 40.1 (C_5), 32.3 (C_7), 22.5(C_6), 22.7 (C_8), 14.2 (C_9); HRMS (EI) *m*/*z* calcd for $C_{16}H_{26}O$ (M$^+$) 234.1983, found 234.1988; IR (KBr) 1665.37 (CO stretch) 1622.90 (C=C) cm^{-1}.

*2.3.1.5. 4,4-Dihexylcyclohexa-2,5-dien-1-one (**1g**).* ^{1}H NMR ($CDCl_3$) δ 6.69 (AA′XX′, 2H, H_3), 6.34 (AA′XX′, 2H, H_2), 1.63 (m, 4H, H_5), 1.21 (m, 12H, H_7, H_9) 1.18 (m, 4H, H_6), 0.86 ("t", 6H, H_{10}); ^{13}C NMR ($CDCl_3$) δ 187.1(C_1), 155.6 (C_3), 130.3 (C_2), 46.7 (C_4), 40.2 (C_5), 31.3 (C_8), 29.8 (C_7), 24.7 (C_6), 22.7 (C_9), 14.1 (C_{10}); HRMS (EI) *m*/*z* Calcd $C_{18}H_{30}O$ (M$^+$) 262.2296, found 262.2287; IR (KBr) 1665.77 (CO stretch) 1623.70 (C=C) cm^{-1}.

*2.3.1.6. 4-Dodecyl-4-decylcyclohexa-2,5-dien-1-one (**1h**).* ^{1}H NMR ($CDCl_3$) δ 6.72 (AA′XX′, 2H, H_3), 6.34 (AA′XX′, 2H, H_2), 1.63 (m, 4H,

H_5), 1.21 (m, 32H, H_7–H_{13}, $H_{7'}$–$H_{13'}$, H_{14} and H_{15}), 1.19 (m, 4H, H_6 and $H_{6'}$), 0.86 ("t", 6H, H_{16} and $H_{14'}$); ^{13}C NMR ($CDCl_3$) δ 186.7 (C_1), 155.4 (C_3), 130.1 (C_2), 46.5 (C_4), 40.0 (C_5 and $C_{5'}$), 31.9 (C_{14} and $C_{12'}$), 30.0, 29.62, 29.57 29.4 (C_7–C_{11}, $C_{7'}$–$C_{11'}$, C_{12} and C_{13}), 24.6 (C_6 and $C_{6'}$), 22.7 (C_{15} and $C_{13'}$), 14.1 (C_{16} and $C_{14'}$); HRMS (DCI, CH_4) m/z calcd for $C_{28}H_{50}O$ (M^+) 402.3861, found 402.3860; IR (KBr) 1660.48 (CO stretch), 1624.10 (C=C) cm^{-1}.

2.3.2. Dialkylacetaldehydes **2**

The title compounds were required as precursors for the synthesis of dialkylcylohexenones **6**. Symmetrical aldehydes **2a**–**d** (R=R′) are commercially available. Symmetrical aldehydes **2e**–**g** were prepared (Taber et al., 1989; Hu and Mattern, 2000) via the acid catalyzed hydrolysis of the below-synthesized enol ethers **9**, as follows: Aqueous HCl (5 mL of a 10% solution) was added to the enol ether **9** (0.05 mol) and refluxed in 20 mL THF. After 12 h, the reaction mixture was partitioned between ether and water. The combined organic phases were dried with $MgSO_4$ and concentrated *in vacuo* to give the aldehydes **2** in a 70% yield. Asymmetrical aldehyde **2h** was essentially prepared as described by Corey and Suggs (Corey and Suggs, 1975) beginning with the commercially available alcohol 2-decyltetradecan-1-ol **10**. The desired product was isolated by filtration of the organic extracts through a silica sinter and the solvent was removed by evaporation. The aldehydes underwent extremely facile autoxidation to the corresponding carboxylic acids (**11**) and had to be used soon after their preparation. In the HRMS the parent peak was essentially absent; hence, for analysis purposes we relied on the HRMS analysis of the derivative acids **11**. We note that the NMR spectra of the derived acids contain varying amounts of the corresponding aldehyde.

*2.3.2.1. 2-Butylhexanal (**2e**).* ^{1}H NMR ($CDCl_3$) δ 9.519 (d, J = 2.4 Hz, 1H, H_1), 2.184 (ttd, J = 9.5, 7 and 3 Hz, 1H, H_2), 1.623 (m, 2H, H_{3a} and $H_{3a'}$), 1.415 (m, 2H, H_{3b} and $H_{3b'}$) 1.256 (m, 8H, H_4, $H_{4'}$, H_5 and $H_{5'}$), 0.853 ("t", 6H, H_6 and $H_{6'}$); ^{13}C NMR ($CDCl_3$) δ 205.5 (C_1), 51.9 (C_2), 29.5, 28.6 (C_3, $C_{3'}$, C_4 and $C_{4'}$), 22.7 (C_5 and $C_{5'}$), 13.8 (C_6 and $C_{6'}$); HRMS m/z (EI) calcd for $C_{10}H_{20}O$ (M^+) 156.1514, found 156.1508 IR (KBr) 1737.72 cm^{-1} (CO stretch).

*2.3.2.2. 2-Pentylheptanal (**2f**).* ^{1}H NMR ($CDCl_3$) δ 9.53 (d, J = 3 Hz, 1H, H_1), 2.19 (ttd, J = 9.5, 7 and 3 Hz, 1H, H_2), 1.58 (m, 2H, H_{3a}, $H_{3a'}$), 1.42 (m, 2H, H_{3b}, $H_{3b'}$) 1.25 (m, 12H, H_4–H_6 and $H_{4'}$–$H_{6'}$), 0.85 ("t", 6H, H_7 and $H_{7'}$); ^{13}C NMR ($CDCl_3$) δ 205.8 (C_1), 52.1 (C_2), 32.0 (C_5 and $C_{5'}$), 29.0 and 26.9 (C_4, $C_{4'}$, C_5 and $C_{5'}$), 22.6 (C_6 and $C_{6'}$), 14.1 (C_7 and $C_{7'}$); HRMS (EI) m/z calcd for $C_{12}H_{23}O$ (M^+-1) 183.1749, found 183.1746. IR (KBr) 1727.41 cm^{-1} (CO stretch).

*2.3.2.3. 2-Hexyloctanal (**2g**).* ^{1}H NMR ($CDCl_3$) δ 9.54 (d, J = 3, 1H, H_1), 2.20 (ttd, J = 9.5, 7 and 3 Hz, 1H, H_2), 1.58 (m, 2H, H_{3a} and $H_{3a'}$), 1.43 (m, 2H, H_{3b} and $H_{3b'}$) 1.26 (m, 16H, H_4–H_7 and $H_{4'}$–$H_{7'}$), 0.86 ("t", 6H, H_8 and $H_{8'}$); ^{13}C NMR ($CDCl_3$) δ 205.8(C_1), 52.1 (C_2), 31.8 (C_6 and $C_{6'}$), 29.5, 29.1 (C_3–C_5 and $C_{3'}$–$C_{5'}$), 27.2 (C_6 and $C_{6'}$), 22.7 (C_7 and $C_{7'}$), 14.2 (C_8 and $C_{8'}$); MS (CI, ammonia) m/z 213 (MH^+); IR (KBr) 1732.69 cm^{-1} (CO stretch).

*2.3.2.4. 2-Decyltetradecanal (**2h**).* ^{1}H NMR ($CDCl_3$) δ 9.54 (d, J = 3 Hz, 1H, H_1), 2.20 (ttd, J = 9.5 Hz, 7 and 3 Hz, 1H, H_2), 1.59 (m, 2H, H_{3a} and $H_{3a'}$), 1.45 (m, 2H, H_{3b} and $H_{3b'}$) 1.25 (m, 36H, H_4–H_{11}, $H_{4'}$–$H_{11'}$, H_{12} and H_{13}), 0.86 ("t", 6H, H_{14} and $H_{12'}$); ^{13}C NMR ($CDCl_3$) δ 206.0 (C_1), 52.2 (C_2), δ 32.1 (C_{12} and $C_{10'}$), 29.9, 29.8, 29.7, 29.6, 29.5, 29.1 and 27.3 (C_3–C_9, $C_{3'}$–$C_{9'}$, C_{10} and C_{11}), 27.2 (C_6 and $C_{6'}$), 22.9 (C_{13} and $C_{11'}$), 14.3 (C_{14} and $C_{12'}$); IR (KBr) 1727.41 cm^{-1} (CO stretch).

2.3.3. 4,4-Dialkylcyclohex-2-en-1-ones **6**

Dialkylcylohexenones **6a**–**h** were synthesized as precursors for the preparation of 4,4-dialkylcyclohexa-2,5-dien-1-one (**1**), described in the next section. The dialkylcylohexenones were prepared from the abovementioned dialkylacetaldehydes **2** (0.1 mol scale) and methyl vinyl ketone (**3**), following the general procedure of Dauben and coworkers for the synthesis of **6a** (Dauben et al., 1968). In the case of enones **6a**–**d**, the crude product was chromatographed on silica gel, eluting with petroleum ether/ethyl acetate (90:10), giving the desired product in a 70–85% yield. Cyclohexenones **6a** (Zimmerman and Schuster, 1962; Chan and Epstein, 1973; Gramlich, 1979), **6b** (Chaki et al., 2002), **6c** (Snider et al., 1993) and **6d** (Zimmerman et al., 1971; Chan and Epstein, 1973; Frimer et al., 1989) are well known in the literature. In the case of the longer chain enones **6e**–**h**, the yields were substantially lower, presumably due to steric hindrance. The product was used in its crude state for the preparation of dienones **1**; nevertheless, their identity was confirmed by the following characteristic peaks: ^{1}H NMR ($CDCl_3$) δ 6.7 (d, J = 10 Hz, 1H, H_3), 5.9 (d, J = 10 Hz, 1H, H_2); ^{13}C NMR ($CDCl_3$) δ 200 (C_1), 159 (C_3), 128 (C_2). For convenience and clarity in the spectral data, the carbons were numbered as shown in Scheme 1.

*2.3.3.1. 4,4-Diethylcyclohex-2-en-1-one (**6b**).* This compound is known in the literature (Chaki et al., 2002), but some of the spectral data are lacking. ^{1}H NMR ($CDCl_3$) δ 6.72 (d, J = 10.28 Hz, 1H, H_3), 5.93 (d, J = 10.28 Hz, 1H, H_2), 2.44 (t, J = 7.12 Hz, 2H, H_6), 1.85 (t, J = 7.12 Hz, 2H, H_5), 1.51 (q, J = 7.52, 4H, H_7), 0.89 (t, J = 7.52 Hz, 6H, H_8); ^{13}C NMR ($CDCl_3$) δ 199.8 (C_1), 158.9 (C_3), 128.0 (C_2), 38.3 (C_4), 33.9 (C_6), 30.0 (C_5), 29.4 (C_7), 8.2 (C_8); MS (CI, CH_4) m/z 153 (CI, MH^+, 90%); HRMS (CI, CH_4) m/z calcd for $C_{10}H_{17}O$ (MH^+) 153.1279, found 153.1272.

2.3.4. Enol ethers **9e**–**g**

The title compounds were prepared as precursors to the corresponding aldehydes **6**, following procedures adapted from the literature (Taber et al., 1989; Hu and Mattern, 2000). *n*-Butyllithium (31 mL, 0.0502 mol, 1.6 M in hexanes) was added dropwise to methoxymethyltriphenylphosphonium chloride (18.1 g, 0.053 mol) in anhydrous ether (50 mL) at 0 °C. The ice bath was removed, and the deep red solution was stirred at room temperature for 4 h. The solution was again cooled to 0 °C and the commercially available dialkyl ketone **7** (0.0176 mol) in ether (20 mL) was added dropwise. The solution was allowed to stir at room temperature for 48 h. Following a water extraction, the precipitated phosphorous salts were filtered off, the organic filtrate was concentrated *in vacuo* and cooled to 10 °C, at which point more phosphorous salts precipitated. The supernatant liquid was chromatographed and eluted with hexane to give the desired enol ether in 35–40% yield. For convenience and clarity in the spectral data, the carbons were numbered as shown below.

*2.3.4.1. 2-Butyl-1-methoxy-1-hexene (**9e**).* ^{1}H NMR ($CDCl_3$) δ 5.75 (s, 1H, H_2), 3.51 (s, 3H, H_1), 2.05 (m, 2H, $H_{4'}$), 1.86 (m, 2H, H_4), 1.33 (m, 8H, H_5, $H_{5'}$, H_6 and $H_{6'}$), 0.91 ("t", 6H, H_7, $H_{7'}$); ^{13}C NMR ($CDCl_3$) δ 141.9 (C_2), 118.8 (C_3), 59.1 (C_1), 31.2, 30.5 and 30.0 (C_4, C_5 and $C_{5'}$), 26.5 ($C_{4'}$), 22.7 and 22.4 (C_6 and $C_{6'}$), 13.9 (C_7 and $C_{7'}$); IR (KBr) 1666 cm^{-1} (C=C stretch).

*2.3.4.2. 1-Methoxy-2-pentyl-1-heptene (**9f**).* ^{1}H NMR ($CDCl_3$) δ 5.73 (s, 1H, H_2), 3.50 (s, 3H, H_1), 2.03 (m, 2H, $H_{4'}$), 1.85 (m, 2H, H_4), 1.29 (m, 12H, H_5–H_7, and $H_{5'}$–$H_{7'}$), 0.89 ("t", 6H, H_8 and $H_{8'}$); ^{13}C NMR ($CDCl_3$) δ 142.0 (C_2), 119.1 (C_3), 59.3 (C_1), 32.0, 31.8, 31.6 (C_4, C_6 and $C_{6'}$), 28.1, 27.6 and 26.5 ($C_{4'}$, C_5 and $C_{5'}$), 22.7 (C_7 and $C_{7'}$), 13.9 (C_8 and $C_{8'}$); HRMS m/z (EI) calcd for $C_{13}H_{26}O$ (M^+) 198.1983, found 198.1979. IR (KBr) 1666.77 cm^{-1} (C=C stretch).

*2.3.4.3. 2-Hexyl-1-methoxy-1-octene (**9g**).* ^{1}H NMR ($CDCl_3$) δ 5.73 (s, 1H, H_2), 3.50 (s, 3H, H_1), 2.03 (m, 2H, $H_{4'}$), 1.85 (m, 2H, H_4), 1.28 (m, 16H, H_5–H_8 and $H_{5'}$–$H_{8'}$), δ 0.88 ("t", 6H, H_9 and $H_{9'}$); ^{13}C NMR ($CDCl_3$) δ 141.9 (C_2), 118.9 (C_3), 59.2 (C_1), 27.1–31.9 (C_4–C_7 and $C_{4'}$–$C_{7'}$), 22.8, 22.7 (C_8 and $C_{8'}$), 14.2, 14.1 (C_9 and $C_{9'}$); HRMS *m/z* (EI) calcd for $C_{15}H_{30}O$ (M^+) 226.2296, found 226.2296; IR (KBr) 1667 cm^{-1} (C=C stretch).

*2.3.4.4. 2-Pentylheptanoic acid (**11f**).* ^{1}H NMR ($CDCl_3$) δ 2.23 (m, 1H, H_2), 1.58 (m, 2H, H_{3a}, $H_{3a'}$), 1.42 (m, 2H, H_{3b}, $H_{3b'}$), 1.25 (m, 12H, H_4–H_6 and $H_{4'}$–$H_{6'}$), 0.85 ("t", 6H, H_7 and $H_{7'}$); ^{13}C NMR ($CDCl_3$) δ 182.3 (C_1), 45.7 (C_2), 32.0 (C_5 and $C_{5'}$), 29.0 and 26.9 (C_4, $C_{4'}$, C_5 and $C_{5'}$), 22.6 (C_6 and $C_{6'}$), 14.1 (C_7 and $C_{7'}$); HRMS (EI) *m/z* calcd for $C_{12}H_{25}O_2$ (MH^+) 201.1855, found 201.1851; IR (KBr) 1701.74 cm^{-1} (CO stretch).

*2.3.4.5. 2-Hexyloctanoic acid (**11g**).* ^{1}H NMR ($CDCl_3$) δ 2.32 (m, 1H, H_2), 1.58 (m, 2H, H_{3a} and $H_{3a'}$), 1.43 (m, 2H, H_{3b} and $H_{3b'}$), 1.26 (m, 16H, H_4–H_7 and H_4–$H_{7'}$), 0.86 ("t", 6H, H_8 and $H_{8'}$); ^{13}C NMR ($CDCl_3$) δ 182.5 (C_1), 45.5 (C_2), 31.8 (C_6 and $C_{6'}$), 29.5 and 29.1 (C_3–C_5 and $C_{3'}$–$C_{5'}$), 27.2 (C_6 and $C_{6'}$), 22.7 (C_7 and $C_{7'}$), 14.2 (C_8 and $C_{8'}$); HRMS (EI) *m/z* calcd for $C_{14}H_{28}O_2$ (M^+) 228.2089 found 228.2088; IR (KBr) 1706.93 cm^{-1}(CO stretch).

*2.3.4.6. 2-Decyltetradecanoic acid (**11h**).* ^{1}H NMR ($CDCl_3$) δ 2.35 (m, 1H, H_2), 1.59 (m, 2H, H_{3a} and $H_{3a'}$), 1.45 (m, 2H, H_{3b} and $H_{3b'}$), 1.25 (m, 36H, H_4–H_{11}, $H_{4'}$–$H_{11'}$, H_{12} and H_{13}), 0.86 ("t", 6H, H_{14}. and $H_{12'}$); ^{13}C NMR ($CDCl_3$) δ 181.7 (C_1), 45.6 (C_2), δ 32.1 (C_{12} and $C_{10'}$), 29.9, 29.8, 29.7, 29.6, 29.5, 29.1 and 27.3 (C_3–C_9, $C_{3'}$–$C_{9'}$, C_{10} and C_{11}), 27.2 (C_6 and $C_{6'}$), 22.9 (C_{13} and $C_{11'}$), 14.3 (C_{14} and $C_{12'}$); HRMS (EI) *m/z* calcd for $C_{24}H_{48}O_2$ (M^+) 368.3654, found 368.3670; IR (KBr) 1707.03 cm^{-1} (CO stretch).

*2.3.5. 1,4-Phenylenediacetate diesters (**15**) and 1,4-phenylenedipropionate diesters (**17**)*

In a dry three-necked 100 mL flask, equipped with a pressure-equalizing funnel, an N_2 inlet adapter, glass stopper and magnetic stirring, was placed 1,4-phenylenediacetic acid (1.5 g, 7.7 mmol) or 1,4-phenylenedipropionic acid (1.7 g, 7.7 mmol) in dried distilled CH_2Cl_2 (25 mL). The mixture was stirred until the diacid dissolved. Thionyl chloride (5.6 mL, 77 mmol) was then slowly added over the course of 10 min to the reaction solution from a pressure-equalizing funnel. During the addition, the glass stopper was briefly removed and pyridine (155 μL, 1.9 mmol) was added to the solution against the increased N_2 stream. The resulting clear solution was magnetically stirred at R.T. overnight. The solvent and excess thionyl chloride were then evaporated in the hood with a high stream of N_2 through the inlet adapter leaving a solid residue of diacid chloride.

The three-necked flask containing the diacid chloride (7.7 mmol) was again equipped with an N_2 inlet adapter and glass stoppers and charged with dry distilled CH_2Cl_2 (25 mL), the branched long chain alcohol (R′OH in Scheme 6; 4.7 mmol) and pyridine (155 μL, 1.9 mmol). The clear solution was allowed to continue stirring for a week at R.T. The short chain alcohol (methanol, ethanol or *i*-propanol; ROH in Scheme 6; 10 mL) was then added to the solution which was stirred for 30 min at R.T. The solvent was rotary evaporated to dryness. The residue was taken up in $CHCl_3$ and the organic phase was extracted twice with brine, dried with $MgSO_4$, and rotary evaporated to dryness. The crude material was purified by silica column chromatography (eluting with 15% ethyl acetate in hexane), which yielded the desired product after solvent removal. The R_f values given below are for TLC runs, eluting with the same solvent mixture, unless otherwise indicated. The NMR carbon numbering for diesters **15** and **17** is shown above (Scheme 1).

*2.3.5.1. 4-(2-Methylbutoxycarbonylmethyl)phenylacetic acid methyl ester (**15a**, n = 1, m = 0).* 25.8% yield; R_f = 0.23; ^{1}H NMR (acetone-d_6) δ 7.33 (s, 4H, H_4 and H_5), 4.00, 4.08, 1.81 (ABX system 1H each, J_{AB} = 10, J_{BX} = 7, J_{AX} = 6 Hz, $2H_9$, H_{10}), 3.71 (s, 4H, H_2 and H_7), 3.70 (s, 3H, H_1), 1.53 (dqd, *J* = 15, 7.5 and 6 Hz, 1H, H_{11}), 1.28 (dqd, *J* = 15, 7.5 and 6 Hz, 1H, H_{11}), 1.01 (d, *J* = 6.5 Hz, 3H, $H_{11'}$ or H_{12}), 0.99 (t, *J* = 7.5 Hz, 3H, H_{12} or $H_{11'}$); ^{13}C NMR (acetone-d_6) δ 172.2 (C_1), 171.8 (C_8), 134.3 (C_3 or C_6), 134.1 (C_6 or C_3), 130.19 (C_4 or C_5), 130.16 (C_5 or C_4), 69.6 (C_9), 52.0 ($C_{1''}$), 41.2 (C_2 or C_7), 40.9 (C_7 or C_2), 35.0 (C_{10}), 26.6, 16.6 and 11.5 ($C_{11'}$, C_{11} and C_{12}); MS (CI, CH_4) *m/z* 278 (M^+, 12.54%), 219 (M−$COOCH_3$, 22.4%), 163 (M−$COOCH_2CH(CH_3)CH_2CH_3$, 98.8%), 104 (M−$COOCH_3$,$COOCH_2CH(CH_3)CH_2CH_3$, 49.4%), 208 ($MH-CH_2(CH_3)CH_2CH_3$, 100%); HRMS (CI, CH_4): calcd ($C_{16}H_{22}O_4$, M^+) 278.1518, obsd 278.1503.

*2.3.5.2. 4-(2-Ethylbutoxycarbonylmethyl)phenylacetic acid methyl ester (**15b**, n = 1, m = 1).* 13.33% yield; R_f = 0.2; ^{1}H NMR ($CDCl_3$) δ 7.23 (s, 4H, H_4 and H_5), 4.00 (d, *J* = 6 Hz, 2H, H_9), 3.66 (s, 3H, $H_{1''}$), 3.59 (s, 2H, H_2 or H_7), 3.58 (s, 2H, H_7 or H_2), 1.49 (sept, *J* = 6 Hz, 1H, H_{10}), 1.31 (quint, *J* = 6.7 Hz, 4H, H_{11} and $H_{11'}$), 0.85 (t, *J* = 7.3 Hz, 6H, H_{12} and $H_{12'}$); ^{13}C NMR ($CDCl_3$) δ 171.9 (C_1), 171.6 (C_8), 133.1 (C_3 or C_6), 132.7 (C_6 or C_3), 129.5 (C_4 or C_5), 129.4 (C_5 or C_4), 66.9 (C_9), 52.0 ($C_{1''}$), 41.1 (C_2 or C_7), 40.8 (H_7 or C_2), 40.3 (C_{10}), 23.3 (C_{11} and $C_{11'}$), 11.0 (C_{12} and $C_{12'}$); MS (CI, CH_4) *m/z* 292 (M^+, 7.62%), 233 (M−$COOCH_3$, 26.81%), 163 (M−$COOCH_2CH(CH_2CH_3)_2$, 68.56%), 104 (M−$COOCH_3$–$COOCH_2CH(CH_2CH_3)_2$, 35.05%), 208 ($MH-CH_2CH(CH_2CH_3)_2$, 100%); HRMS (CI, CH_4): calcd ($C_{17}H_{24}O_4$, M^+) 292.1675, obsd 292.1660.

*2.3.5.3. 4-(2-Ethylhexyloxycarbonylmethyl)phenylacetic acid methyl ester (**15c**, n = 3, m = 1).* 13.29% yield. R_f = 0.2; ^{1}H NMR ($CDCl_3$) δ 7.24 (s, 4H, H_4 and H_5), 4.01 (d, *J* = 6 Hz, 2H, H_9), 3.68 (s, 3H, $H_{1''}$), 3.6 (s, 2H, H_2 or H_7), 3.59 (s, 2H, H_7 or H_2), 1.56 (m, 1H, H_{10}), 1.31 (m, 8H, H_{11}, H_{11}, H_{12} and H_{13}), 0.88 (t, *J* = 7.5 Hz, 3H, H_{14} or $H_{12'}$), 0.86 (t, *J* = 7.5 Hz, 3H, $H_{12'}$ or H_{14}); ^{13}C NMR ($CDCl_3$) δ 171.8 (C_1), 171.6 (C_8), 133.1 (C_3 or C_6), 133.7 (C_6 or C_3), 129.5 (C_4 or C_5), 129.4 (C_5 or C_4), 67.2 (C_9), 51.9 ($C_{1''}$), 41.1 (C_2 or C_7), 40.8 (C_7 or C_2), 38.7 (C_{10}), 30.4, 28.8, 23.8, 22.9, 14.0, 11.0 (C_{11}–C_{14} and $C_{11'}$–$C_{12'}$); MS (CI, CH_4) *m/z* 320.197 (M^+, 7.2%), 163 (M−$COOCH_2CH(CH_2CH_3)(CH_2)_3CH_3$, 46.55%), 104 (M−$COOCH_3$–$COOCH_2CH(CH_2CH_3)(CH_2)_3CH_3$, 42.22%), 207.94 ($MH^+-CH_2CH(CH_2CH_3)(CH_2)_3CH_3$, 100%); HRMS (CI, CH_4): calcd ($C_{19}H_{28}O_4$, M^+) 320.1987, obsd 320.1967.

*2.3.5.4. 4-(2-Butyloctoxycarbonylmethyl)phenylacetic acid methyl ester (**15d**, n = 5, m = 3).* 27.5% yield; R_f = 0.6; ^{1}H NMR ($CDCl_3$) δ 7.22 (s, 4H, H_4 and H_5), 3.98 (d, *J* = 6 Hz, 2H, H_9), 3.65 (s, 3H, $H_{1''}$), 3.583 (s, 2H, H_2 or H_7), 3.579 (s, 2H, H_7 or H_2), 1.6 (bs, 1H, H_{10}), 1.24 bs, 16H, H_{11}–H_{15} and $H_{11'}$–$H_{13'}$), 0.88 (m, 3H, $H_{14'}$ or H_{16}), 0.86 (t, *J* = 6 Hz, 3H, H_{16} or H_{16}); ^{13}C NMR ($CDCl_3$) δ 172.0 (C_1), 171.7 (C_8), 132.9 (C_3 or C_6), 132.8 (C_6 or C_3), 129.6 (C_4 or C_5), 129.5 (C_5 or C_4), 67.7 (C_9), 52.1 ($C_{1''}$), 41.2 (C_2 or C_7), 40.9 (C_7 or C_2), 37.3 (C_{10}), 31.9, 31.3, 31.0, 29.7, 28.9, 26.7, 23.0, 22.7, 14.2, 14.1 (C_{11}–C_{16} and $C_{11'}$–$C_{14'}$); MS (CI, CH_4), *m/z* 376.26 (M^+, 0.53%), 163 (M−$COOCH_2CH((CH_2)_3CH_3)(CH_2)_5CH_3$, 66.65%), 104 (M−$COOCH_3$–$COOCH_2CH((CH_2)_3CH_3)(CH_2)_5CH_3$, 50.86%), 207.97 (M−$CH_2CH((CH_2)_3CH_3)(CH_2)_5CH_3$, 100%); HRMS (CI, CH_4): calcd ($C_{23}H_{36}O_4$, M^+) 376.2614, obsd 376.2611.

*2.3.5.5. 4-(2-Hexyldecyloxycarbonylmethyl)phenylacetic acid methyl ester (**15e**, n = 7, m = 5).* 14.26% yield; R_f = 0.37; ^{1}H NMR ($CDCl_3$) δ 7.22 (s, 4H, H_4 and H_5), 3.98 (d, *J* = 6 Hz, 2H, H_9), 3.65 (s, 3H, $H_{1''}$), 3.581 (s, 2H, H_2 or H_7), 3.576 (s, 2H, H_7 or H_2), 1.6 (bs, 1H, H_{10}), 1.24 (bs, 24H, H_{11}–H_{17} and $H_{11'}$–$H_{15'}$), 0.88 ("t",

6H, $H_{16'}$ and H_{18}); ^{13}C NMR ($CDCl_3$) δ 171.8 (C_1), 171.5 (C_8), 133.1 (C_3 or C_6), 132.7 (C_6 or C_3), 129.5 (C_4 or C_5), 129.4 (C_5 or C_4), 67.6 (C_9), 51.9 ($C_{1''}$), 41.1 (C_2 or C_7), 40.7 (C_7 or C_2), 37.3 (C_{10}), 31.9, 31.8, 31.2, 29.9, 29.6, 29.5, 29.3, 26.75, 26.6, 22.7, 22., 14.1, 14.1 (C_{11}–C_{18} and $C_{11'}$–$C_{16'}$); MS (CI, CH_4) *m/z* 433.33 (MH^+, 13.57%), 163 (M–$COOCH_2CH((CH_2)_5CH_3)(CH_2)_7CH_3$, 12.4%), 104 (M–$COOCH_3$, $COOCH_2CH((CH_2)_5CH_3)(CH_2)_7CH_3$, 12.46%), 209 (MH–$CH_2CH((CH_2)_5CH_3)(CH_2)_7CH_3$, 100%), 865.57 ($MMH^+$, 70.58%); HRMS (CI, CH_4): calcd ($C_{27}H_{45}O_4$, MH^+) 433.3318, obsd 433.3311.

*2.3.5.6. 4-(2-Octyldodecyloxycarbonylmethyl)phenylacetic acid methyl ester (**15f**, n = 9, m = 7).* 23.32% yield; R_f = 0.4; ^1H NMR ($CDCl_3$) δ 7.23 (s, 4H, H_4 and H_5), 3.99 (d, J = 5.7 Hz, 2H, H_9), 3.66 (s, 3H, $H_{1''}$), 3.592 (s, 2H, H_2 or H_7), 3.586 (s, 2H, H_7 or H_2), 1.61 (bs, 1H, H_{10}), 1.26 (m, 32H, H_{11}–H_{19} and $H_{11'}$–$H_{17'}$), 0.89 (m, 6H, $H_{18'}$ and H_{20}); ^{13}C NMR ($CDCl_3$) δ 171.7 (C_1), 171.5 (C_8), 133.1 (C_3 or C_6), 132.7 (C_6 or C_3), 129.4 (C_4 or C_5), 129.4 (C_5 or C_4), 67.6 (C_9), 51.9 ($C_{1''}$), 41.1 (C_2 or C_7), 40.7 (C_7 or C_2), 37.3 (C_{10}), 31.9, 31.2, 29.9, 29.7, 29.59, 29.55, 29.4, 29.3, 26.7, 22.7, 14.1 (C_{11}–C_{20} and $C_{11'}$–$C_{18'}$); MS (CI, CH_4), *m/z* 489.3971 (MH^+, 0.75%), 163.01 (M–$COOCH_2CH((CH_2)_7CH_3)(CH_2)_9CH_3$, 37.38%), 104.02 (M–$COOCH_3$–$COOCH_2CH((CH_2)_7CH_3)(CH_2)_9CH_3$, 29.09%), 208.98 ($MH^+$–$CH_2CH((CH_2)_7CH_3)(CH_2)_9CH_3$, 100%); HRMS (CI, CH_4): calcd ($C_{31}H_{53}O_4$, MH^+) 489.3944, obsd 489.3971.

*2.3.5.7. 4-(2-Decyltetradecyloxycarbonylmethyl)phenylacetic acid methyl ester (**15g**, n = 11, m = 9).* 15.55% yield; R_f = 0.34; ^1H NMR ($CDCl_3$) δ 7.23 (s, 4H, H_4 and H_5), 3.98 (d, J = 6 Hz, 2H, H_9), 3.68 (s, 3H, $H_{1''}$), 3.604 (s, 2H, H_2 or H_7), 3.594 (s, 2H, H_7 or H_2), 1.6 (m, 1H, H_{10}), 1.26 (m, 32H, H_{11}–H_{21} and $H_{11'}$–$H_{19'}$), 0.89 ("t", 6H, $H_{20'}$ and H_{22}); ^{13}C NMR ($CDCl_3$) δ 172.0 (C_1), 171.8 (C_8), 133.2 (C_3 or C_6), 132.8 (C_6 or C_3), 129.6 (C_4 or C_5), 129.5 (C_5 or C_4), 67.8 (C_9), 52.1 ($C_{1''}$), 41.3 (C_2 or C_7), 40.9 (C_7 or C_2), 37.4 (C_{10}), 32.1, 31.3, 30.1, 29.8, 29.7, 29.5, 26.8, 22.8, 14.2 (C_{11}–C_{22} and $C_{11'}$–$C_{20'}$); MS (CI, CH_4), *m/z* 544.4505 (M^+, 2.48%), 163.07 (M–$COOCH_2CH((CH_2)_9CH_3)(CH_2)_{11}CH_3$, 25.31%), 104.03 (M–$COOCH_3$–$COOCH_2CH((CH_2)_9CH_3)(CH_2)_{11}CH_3$, 12.64%), 209.09 ($MH^+$–$CH_2CH((CH_2)_9CH_3)(CH_2)_{11}CH_3$, 100%), 208.09 (M–$CH_2CH(CH_2)_9CH_3)(CH_2)_{11}CH_3$), 81.24%); HRMS (CI, CH_4): calcd ($C_{35}H_{60}O_4$, M^+) 544.4491, obsd 544.4504.

*2.3.5.8. 4-(2-Ethylhexaloxycarbonylmethyl)phenylacetic acid ethyl ester (**15h**, n = 3, m = 1).* 17.46% yield; R_f = 0.58; ^1H NMR ($CDCl_3$) δ 7.24 (s, 4H, H_4 and H_5), 4.14 (q, J = 7.13 Hz, 2H, $H_{1''}$), 3.99 (d, J = 6 Hz, 2H, H_9), 4.00 (s, 2H, H_2 or H_7), 3.98 (s, 2H, H_7 or H_2), 1.62 (sept, J = 6 Hz, 1H, H_{10}), 1.28 (m, 11H, H_{11}–H_{13} and $H_{11'}$, $H_{2''}$), 0.87 (t, J = 7.5 Hz, 3H, $H_{12'}$ or H_{14}), 0.85 (t, J = 7.5 Hz, 3H, H_{14} or $H_{12'}$); ^{13}C NMR ($CDCl_3$) δ 171.9 (C_1), 171.7 (C_8), 133.1 (C_3 or C_6), 133.0 (C_6 or C_3), 129.6 (C_4 or C_5), 129.5 (C_5 or C_4), 67.4 (C_9), 61.0 ($C_{1''}$), 41.3 (C_2 or C_7), 41.2 (C_7 or C_2), 38.8 (C_{10}), 30.5, 28.3, 23.9, 23.1, 14.3, 14.2, 11.1 (C_{11}-C_{14}, $C_{11'}$–$C_{12'}$ and $C_{2''}$); MS (CI, CH_4), *m/z* 334.2161 (M^+, 11.65%), 216.193 (M–CO_2Et), 10.21%, 222.088 (MH–$CH_2CH(CH_2CH_3)(CH_2)_3CH_3$), 100%, 177.093 (MH–$CH_2CH(CH_2CH_3)(CH_2)_3CH_3$)–OEt, 51.76%, 149.069 (MH–$CH_2CH(CH_2CH_3)(CH_2)_3CH_3$)–COOEt, 50.29%), 104.058 (M–$COOCH_2CH(CH_2CH_3)(CH_2)_3CH_3$)–COOEt, 48.23%); HRMS (CI, CH_4): calcd ($C_{20}H_{30}O_4$, M^+) 334.2144, obsd 334.2161.

*2.3.5.9. 4-(2-Ethylhexaloxycarbonylmethyl)phenylacetic acid isopropyl ester (**15i**, n = 3, m = 1).* 3.42% yield; R_f = 0.38; ^1H NMR ($CDCl_3$) δ 7.22 (s, 4H, H_4 and H_5), 4.99 (sept, J = 6.2 Hz, 1H, $H_{1''}$), 3.99 (d, J = 6 Hz, 2H, H_9), 3.58 (s, 2H, H_2 or H_7), 3.54 (s, 2H, H_7 or H_2), 1.54 (sept, J = 6 Hz, 1H, H_{10}), 1.25 (m, 14H, H_{11}–H_{13} and $H_{11'}$, $H_{2''}$), 0.84 (m, 6H, H_{14} and $H_{12'}$); ^{13}C NMR ($CDCl_3$) δ 171.7 (C_1), 171.0 (C_8), 133.1 (C_3 or C_6), 132.9 (C_6 or C_3), 129.4 (C_4 or C_5), 129.4 (C_5 or C_4), 68.2 ($C_{1''}$), 67.2 (C_9), 41.3 (C_2 or C_7), 41.2 (C_7 or C_2), 38.7 (C_{10}), 30.4, 28.9, 23.8, 23.0, 14.1, 11.0 (C_{11}–C_{14} and $C_{11'}$–$C_{12'}$), 21.8 (2 $C_{2''}$); MS (CI, CH_4), *m/z* 349.2401 (MH^+, 23.28%), 348.232 (M^+, 17.41%), 307.185 (MH_2^+–$CH(CH_3)_2$, 36.35%), 237.108 (MH^+–$CH_2CH(CH_2CH_3)(CH_2)_3CH_3$, 80.59%), 195.065 ($MH_3^+$–$CH_2CH(CH_2CH_3)(CH_2)_3CH_3$–$CH(CH_3)_2$, 100%), 150.069 ($MH_2^+$–$CH_2CH(CH_2CH_3)(CH_2)_3CH_3$–$COOCH(CH_3)_2$, 49.11%), 105.067 ($MH^+$–$COOCH_2CH(CH_2CH_3)(CH_2)_3CH_3$–$COOCH(CH_3)_2$,42.44%); HRMS (CI, CH_4): calcd ($C_{21}H_{33}O_4$, MH^+) 349.2379, obsd 349.2401.

*2.3.5.10. 3-{4-[2-(2-Methylbutoxycarbonyl)ethyl]phenyl}propionic acid methyl ester (**17a**, n = 1, m = 0).* 10.65% yield; R_f = 0.28; ^1H NMR (acetone-d_6) δ 7.15 (s, 4H, H_5 and H_6), 3.89, 3.97, 1.66 (ABX system 1H each, J_{AB} = 10.7, J_{BX} = 7, J_{AX} = 6 Hz, $2H_{11}$, H_{12}), 3.66 (s, 3H, $H_{1''}$), 2.92 (t, J = 7.8 Hz, 2H, H_3 or C_8), 2.91 (t, J = 7.8 Hz, 2H, H_8 or C_3), 2.61 (t, J = 7.8 Hz, 2H, H_2 or C_9), 2.6 (t, J = 7.8 Hz, 2H, H_9 or C_2), 1.41 and 1.18 (AB system, 1H each, $2H_{13}$), 0.88 (t, J = 7.5 Hz, 3H, H_{14}), 0.87 (d, J = 6.5 Hz, 3H, $H_{13'}$); ^{13}C NMR (acetone-d_6) δ 173.3 (C_1), 173.0 (C_{10}), 139.5 (C_4 and C_7), 129.5 (C_5 and C_6), 69.2 (C_{11}), 51.6 ($C_{1''}$), 36.3 (C_2 or C_9), 36.1 (C_9 or C_2), 35.0 (C_{12}), 31.2 (C_3 or C_8), 31.1 (C_8 or C_3), 26.6, 16.6, 11.5 ($C_{13'}$ and C_{13}–C_{14}); MS (CI, CH_4), *m/z* 306.1853 (M^+, 91.43%), 307.1898 (MH^+, 76.6%), 236.107 (MH–$CH_2CH(CH_3)CH_2CH_3$, 35.03%), 219.112 (M–CH_3–$CH_2CH(CH_3)CH_2CH_3$, 49.15%), 176.092 (MH^+–CH_3–$COOCH_2CH(CH_3)CH_2CH_3$, 100%), 162.072 (M–CH_3–$CH_2COOCH_2CH(CH_3)CH_2CH_3$, 21.06%), 117.069 (M–$COOCH_3$–$CH_2COOCH_2CH(CH_3)CH_2CH_3$, 39.13%); HRMS (CI, CH_4): calcd ($C_{18}H_{27}O_4$, MH^+) 307.1909, obsd 307.1898.

*2.3.5.11. 3-{4-[2-(2-Ethylbutoxycarbonyl)ethyl]phenyl}propionic acid methyl ester (**17b**, n = 1, m = 1).* 15.24% yield; R_f = 0.4; ^1H NMR ($CDCl_3$) δ 7.12 (s, 4H, H_5 and H_6), 3.99 (d, J = 6 Hz, 2H, H_{11}), 3.66 (s, 3H, $H_{1''}$), 2.92 (t, J = 8 Hz, 2H, H_3 or C_8), 2.91 (t, J = 8 Hz, 2H, H_8 or C_3), 2.61 (t, J = 8 Hz, 2H, H_2 or C_9), 2.6 (t, J = 8 Hz, 2H, H_9 or C_2), 1.48 (quint, t, J = 7.5 and 6 Hz, 1H, H_{12}), 1.31 (quint, J = 7.5 Hz, 4H, $H_{13'}$ and H_{13}), 0.86 (t, J = 7.5 Hz, 6H, $H_{14'}$ and H_{14}); ^{13}C NMR ($CDCl_3$) δ 173.5 (C_1), 173.2 (C_{10}), 138.6 (C_4 or C_7), 138.5 (C_7 or C_4), 128.5 (C_5 or C_6), 128.5 (C_6 or C_5), 66.6 (C_{11}), 51.7 ($C_{1''}$), 40.3 (C_{12}), 36.0 (C_2 or C_9), 35.8 (C_9 or C_2), 30.7 (C_3 or C_8), 30.6 (C_8 or C_3), 23.3, 11.1 (C_{13}, $C_{13'}$, C_{14} and $C_{14'}$); MS (CI, CH_4), *m/z* 320.1994 (M^+, 33.49%), 321.207 (MH^+, 11.47%), 236.104 (MH^+–$CH_2CH(CH_2CH_3)_2$, 43.23%), 219.102 (M–CH_3–$CH_2CH(CH_2CH_3)_2$, 24.53%), 176.085 (M–CH_3–$COOCH_2CH(CH_2CH_3)_2$, 100%), 162.068 (M–CH_3–$CH_2COOCH_2CH(CH_2CH_3)_2$, 19.5%), 117.065 ($MH^+$–$COOCH_3$–$CH_2COOCH_2CH(CH_2CH_3)_2$, 34.86%); HRMS (CI, CH_4): calcd ($C_{19}H_{29}O_4$, MH^+) 320.2066, obsd 321.2068.

*2.3.5.12. 3-{4-[2-(2-Ethylhexyloxycarbonyl)ethyl]phenyl}propionic acid methyl ester (**17c**, n = 3, m = 1).* 5.77% yield; R_f = 0.57 (30% ethyl acetate in hexane); ^1H NMR ($CDCl_3$) δ 7.11 (4H, s, H_5 and H_6), 3.99, 3.97, 1.54 (ABX system 1H each, J_{AB} = 12, J_{BX} = 6, J_{AX} = 5.4 Hz, $2H_{11}$ and H_{12}), 3.66 (s, 3H, $H_{1''}$), 2.92 (t, 2H, J = 8.2 Hz, H_3 or C_8), 2.91 (t, 2H, J = 8.2 Hz, H_8 or C_3), 2.61 (t, J = 8.2 Hz, 2H, H_2 or C_9), 2.6 (t, J = 8.2 Hz, 2H, H_9 or C_2), 1.31 (m, 8H, $H_{13'}$ and H_{13}–H_{15}), 0.89 (t, J = 6 Hz, 3H, $H_{14'}$ or H_{16}), 0.86 (t, J = 7.5 Hz, 3H, H_{16} or $H_{14'}$); ^{13}C NMR ($CDCl_3$) δ 173.4 (C_1), 173.2 (C_{10}), 138.6 (C_4 or C_7), 138.5 (C_7 or C_4), 128.5 (C_5 or C_6), 128.5 (C_6 or C_5), 67.0 (C_{11}), 51.7 ($C_{1''}$), 38.8 (C_{12}), 36.0 (C_2 or C_9), 35.8 (C_9 or C_2), 30.7 (C_3 or C_8), 30.6 (C_8 or C_3), 30.5, 29.0, 23.8, 23.1, 14.1, 11.1 (C_{13}–C_{16} and $C_{13'}$–$C_{14'}$); MS (CI, CH_4), *m/z* 348.231 (M^+, 15.95%), 349.242 (MH^+, 5.73%), 236.091 (MH–$CH_2CH(CH_2)_3CH_3(CH_2CH_3)$, 58.63%), 219.127 (MH–$CH_3$–$CH_2CH(CH_2)_3CH_3(CH_2CH_3)$, 13.23%), 176.076 (M–$CH_3$–$COOCH_2CH(CH_2)_3CH_3(CH_2CH_3)$, 100%), 162.078 (M–$CH_3$,$CH_2COOCH_2CH(CH_2CH_3)_2$, 18.47%), 117.07 ($MH^+$–$COOCH_3$–

$CH_2COOCH_2CH(CH_2)_3CH_3(CH_2CH_3)$, 25.66%); HRMS (CI, CH_4): calcd ($C_{21}H_{32}O_4$, M^+) 348.23, obsd 348.231.

*2.3.5.13. 3-{4-[2-(2-Butyloctoxycarbonyl)ethyl]phenyl}propionic acid methyl ester (**17d**, n = 5, m = 3).* 10.35% yield; R_f = 0.5 (30% ethyl acetate in hexane); ^{1}H NMR ($CDCl_3$) δ 7.12 (s, 4H, H_5 and H_6), 3.97 (d, J = 6 Hz, 2H, H_{11}), 3.66 (s, 3H, $H_{1''}$), 2.91 (t, J = 8 Hz, 4H, H_3 and H_8), 2.61 (t, J = 7.1 Hz, 4H, H_2 and H_9), 1.6 (m, 1H, H_{12}), 1.26 (m, 16H, $H_{13'}$–$H_{15'}$ and H_{13}–H_{17}), 0.88 ("t", 6H, $H_{16'}$ and H_{18}); ^{13}C NMR ($CDCl_3$) δ 173.4 (C_1), 173.1 (C_{10}), 138.6 (C_4 or C_7), 138.4 (C_7 or C_4), 128.4 (C_5 and C_6), 67.3 (C_{11}), 51.6 ($C_{1''}$), 37.3 (C_{12}), 36.0 (C_2 or C_9), 35.8 (C_9 or C_2), 31.9 (C_3 or C_8), 31.3 (C_8 or C_3), 31.0, 30.6, 30.6, 29.7, 29.0, 26.7, 23.0, 22.7, 14.2, 14.1 (C_{13}–C_{18} and $C_{13'}$–$C_{16'}$); *m/z* (CI, CH_4), 404.294 (M^+, 7.72%), 236.099 ($MH-CH_2CH(CH_2)_5CH_3(CH_2)_3CH_3$), 78.13%), 190.115 ($MH_2-OCH_3-OCH_2CH(CH_2)_5CH_3(CH_2)_3CH_3$, 27.06), 176.088 ($M-CH_3-COOCH_2CH(CH_2)_5CH_3(CH_2)_3CH_3$, 100%), 162.022 ($M-CH_3-CH_2COOCH_2CH(CH_2)_5CH_3(CH_2)_3CH_3$, 17.53%), 117.05($MH-COOCH_3-CH_2COO$ $CH_2CH(CH_2)_5CH_3(CH_2)_3CH_3$, 20.96%); HRMS (CI, CH_4): calcd ($C_{25}H_{40}O_4$, M^+) 404.2927, obsd 404.2936.

*2.3.5.14. 3-{4-[2-(2-Hexyldecyloxycarbonyl)ethyl]phenyl}propionic acid methyl ester (**17e**, n = 7, m = 5).* 2.37% yield; R_f = 0.26; ^{1}H NMR ($CDCl_3$) δ 7.12 (s, 4H, H_5 and H_6), 3.98 (d, J = 6 Hz, 2H, H_{11}), 3.66 (s, 3H, $H_{1''}$), 2.92 (t, J = 8.2 Hz, 2H, H_3 or C_8), 2.91 (t, J = 8.2 Hz, 2H, H_8 or C_3), 2.61 (t, J = 7.2 Hz, 2H, H_9 or C_2), 2.6 (t, J = 7.2 Hz, 2H, H_2 or C_9), 1.6 (bs, 1H, H_{12}), 1.26 (m, 24H, $H_{13'}$–$H_{17'}$ and H_{13}–H_{19}), 0.883 (t, J = 7 Hz, 6H, $H_{18'}$), 0.88 (t, J = 7 Hz, 6H, H_{20}); ^{13}C NMR ($CDCl_3$) δ 173.5 (C_1), 173.2 (C_{10}), 138.6 (C_4 or C_7), 138.5 (C_7 or C_4), 128.5 (C_5 or C_6), 128.5 (C_6 or C_5), 67.4 (C_{11}), 51.7 ($C_{1''}$), 37.4 (C_{12}), 36.0 (C_2 or C_9), 35.8 (C_9 or C_2), 32.0 (C_3 or C_8), 31.9 (C_8 or C_3), 31.3, 31.3, 30.7, 30.6, 30.1, 29.7, 29.7, 29.4, 26.79, 26.76, 22.8, 22.8, 14.2 (C_{13}–C_{20} and $C_{13'}$–$C_{18'}$); MS (CI, CH_4), *m/z* 460.357 (M^+, 5.44%), 237.100 ($MH_2^+-CH_2CH(CH_2)_7CH_3(CH_2)_5CH_3$), 23.08%), 236.099 ($MH-CH_2CH(CH_2)_7CH_3(CH_2)_5CH_3$), 100%), 176.087 ($MH-CH_3COOH-CH_2CH(CH_2)_7CH_3(CH_2)_5CH_3$, 97.46%), 162.022 ($M-CH_2COOCH_3-CH_2CH(CH_2)_7CH_3(CH_2)_5CH_3$, 16.1%), 117.05 ($MH-COOCH_3-CH_2COO$ $CH_2CH(CH_2)_7CH_3(CH_2)_5CH_3$, 13.13%); HRMS (CI, CH_4): calcd ($C_{29}H_{48}O_4$, M^+) 460.355, obsd 460.357.

*2.3.5.15. 3-{4-[2-(2-Octyldodecyloxycarbonyl)ethyl]phenyl}propionic acid methyl ester (**17f**, n = 9, m = 7).* 0.29% yield; R_f = 0.75 (30% ethyl acetate in hexane); ^{1}H NMR ($CDCl_3$) δ 7.12 (s, 4H, H_5 and H_6), 3.97 (d, J = 6 Hz, 2H, H_{11}), 3.67 (s, 3H, $H_{1''}$), 2.91 (t, J = 7.8 Hz, 4H, H_3 and H_8), 2.61 (t, J = 7.8 Hz, 4H, H_2 and H_9), 1.6 (bs, 1H, H_{12}), 1.26 (m, 32H, $H_{13'}$–$H_{19'}$ and H_{13}–H_{21}), 0.88 ("t", 6H, $H_{20'}$ and H_{22}); ^{13}C NMR ($CDCl_3$) δ 173.5 (C_1), 173.3 (C_{10}), 138.7 (C_4 or C_7), 138.5 (C_7 or C_4), 128.5 (C_5 and C_6), 67.5 (C_{11}), 51.7 ($C_{1''}$), 37.4 (C_{12}), 36.1 (C_2 or C_9), 35.8 (C_9 or C_2), 32.05 (C_3 or C_8), 32.05 (C_8 or C_3), 31.4, 30.7, 30.7, 30.1, 29.8, 29.7, 29.7, 29.49, 29.46, 26.8, 22.8, 14.3 (C_{13}–C_{22} and $C_{13'}$–$C_{20'}$); MS (CI, CH_4), *m/z* 516.4175 (M^+, 2.59%), 236.10 ($MH-CH_2CH(CH_2)_9CH_3(CH_2)_7CH_3$), 98.68%), 176.08 ($M-COOCH_3-CH_2CH(CH_2)_9CH_3(CH_2)_7CH_3$, 91.75%), 117.07 ($MH^+-COOCH_3-CH_2COO-CH_2CH(CH_2)_9CH_3(CH_2)_7CH_3$, 30.54%); HRMS (CI, CH_4): calcd ($C_{33}H_{57}O_4$, MH^+) 517.4257, obsd 517.4232.

*2.3.5.16. 3-{4-[2-(2-Decyltetradecyloxycarbonyl)ethyl]phenyl}propionic acid methyl ester (**17g**, n = 11, m = 9).* 1.97% yield; R_f = 0.65 (30% ethyl acetate in hexane); ^{1}H NMR ($CDCl_3$) δ 7.12 (s, 4H, H_5 and H_6), 3.97 (d, J = 5.7 Hz, 2H, H_{11}), 3.67 (s, 3H, $H_{1''}$), 2.92 (t, J = 7.8 Hz, 4H, H_3 and H_8), 2.61 (t, J = 7.8 Hz, 4H, H_2 and H_9), 1.6 (bs, 1H, H_{12}), 1.26 (m, 40H, $H_{13'}$–$H_{21'}$ and H_{13}–H_{23}), 0.88'("t", 6H, $H_{22'}$ and H_{24}); ^{13}C NMR ($CDCl_3$) δ 173.5 (C_1), 173.3 (C_{10}), 138.7 (C_4 or C_7), 138.5 (C_7 or C_4), 128.5 (C_5 and C_6), 67.5 (C_{11}), 51.7 ($C_{1''}$), 37.4 (C_{12}), 36.1 (C_2 or C_9), 35.8 (C_9 or C_2), 32.1 (C_3 and C_8), 31.4, 30.7, 30.7, 30.1, 29.80, 29.75, 29.5, 26.8, 22.8, 14.3 (C_{13}–C_{24} and $C_{13'}$–$C_{22'}$); MS (CI, CH_4), *m/z* 572.476 (M^+, 4.94%), 573.488 (MH^+, 3.54%), 236.1 ($MH-CH_2CH(CH_2)_{11}CH_3(CH_2)_9CH_3$), 100%), 237.12($MH_2^+-CH_2CH(CH_2)_{11}CH_3(CH_2)_9CH_3$), 36.96%), 176.09 ($M-COOCH_3-CH_2CH(CH_2)_{11}CH_3(CH_2)_9CH_3$, 53.9%); HRMS (CI, CH_4): calcd ($C_{37}H_{65}O_4$, M^+) 573.4883, obsd 573.4885.

*2.3.6. Branched 4-alkoxybenzaldehydes (**19h–k**) and branched methyl 4-alkoxybenzoates (**21g–k**)*

The preparation of the title compounds was based on the literature procedure of Hong-bin et al. (2001) with major modification of the workup. A 100 mL round bottom flask was charged with 4-hydroxybenzaldehyde (**18**) or 4-hydroxymethylbenzoate (**21**) (24.6 mmol, 3 equiv.), K_2CO_3 (2 equiv.), and the required alkyl bromide (**23** in Schemes 8 and 9; synthesized as described below), dissolved in 25 mL of acetone (AR). The reaction flask was then fitted with a reflux condenser and the clear solution was refluxed for 24 h. The solution was evaporated to dryness, and the crude products were suspended in *n*-hexane. The unreacted starting materials (aldehyde **18** or ester **21**) were gravitationally filtered from the solution. The filtered solid was again suspended in *n*-hexane and the solution filtered. The combined filtrates were rotary evaporated and the crude product residue was silica column chromatographed. The column was eluted first with 670 mL of *n*-hexane per gram of crude product to remove excess alkyl bromide, followed by 50% ethyl acetate in hexane which yields the desired 4-alkoxybenzaldehyde (**19**) or 4-alkoxymethylbenzoate (**22**). The R_f values given below are for TLC runs eluting with 50% ethyl acetate in hexane, unless otherwise indicated. The products were identified by their spectral data.

*2.3.6.1. 4-(2-Butyloctoxy)benzaldehyde (**19h**).* 9.5% yield; R_f = 0.65; ^{1}H NMR ($CDCl_3$) δ 9.87 (s, 1H, H_1), 7.83, 7.00 (AA′XX′ system, 2H each, H_3, $H_{3'}$, H_4 and $H_{4'}$), 3.92 (d, J = 6 Hz, 2H, H_6), 1.81 (m, 1H, H_7), 1.3 (m, 16H, $H_{8'}$–$H_{10'}$ and H_8–H_{12}), 0.91 (t, J = 6.6 Hz, 3H, $H_{11'}$ or H_{13}), 0.89 (t, J = 6.6 Hz, 3H, H_{13} or $H_{11'}$); ^{13}C NMR ($CDCl_3$) δ 190.8 (C_1), 164.6 (C_5), 132.0 (C_3 and $C_{3'}$), 129.8 (C_2), 114.9 (C_4 and $C_{4'}$), 71.4 (C_6), 37.9 (C_7), 31.9 31.4, 31.1, 29.7, 29.1, 26.9, 23.1, 22.7, 14.2, 14.1 (C_8–C_{13}, $C_{8'}$–$C_{11'}$); MS (CI, CH_4), *m/z* 290.2237 (M^+, 38.79%), 291.233 (MH^+, 100%), 123.05 ($MH-CH_2{=}C(CH_2)_5CH_3(CH_2)_3CH_3$, 99.24%); HRMS (CI, CH_4): calcd ($C_{19}H_{31}O_2$, MH^+) 291.232, obsd 291.233.

*2.3.6.2. 4-(2-Hexyldecyloxy)benzaldehyde (**19i**).* 11.11% yield; R_f = 0.85; ^{1}H NMR ($CDCl_3$) δ 9.88 (s, 1H, H_1), 7.82, 6.99 (AA′XX′ system, 2H each, H_3, $H_{3'}$, H_4 and $H_{4'}$), 3.91 (d, J = 6 Hz, 2H, H_6), 1.8 (sept, J = 6 Hz, 1H, H_7), 1.3 (m, 24H, $H_{8'}$–$H_{12'}$ and H_8–H_{14}), 0.88 (m, 6H, $H_{13'}$ and H_{15}); ^{13}C NMR ($CDCl_3$) δ 191.0 (C_1), 164.7 (C_5), 132.1 (C_3 and $C_{3'}$), 129.9 (C_2), 114.9 (C_4 and $C_{4'}$), 71.5 (C_6), 38.0 (C_7), 32.0, 31.4, 30.1, 29.8, 29.7, 29.5, 27.0, 22.8, 14.2 (C_8–C_{15}, $C_{8'}$–$C_{13'}$); MS (CI, CH_4), *m/z* 346.2923 (M^+, 23.77%), 347.295 (MH^+, 59.76%), 224.24 ($MH^+-(CH_2)_7CH_3$, 10.08%), 123.04 ($MH-CH_2{=}C(CH_2)_7CH_3(CH_2)_5CH_3$, 100%); HRMS (CI, CH_4): calcd ($C_{23}H_{39}O_2$, MH^+) 347.295, obsd 347.295.

*2.3.6.3. 4-(2-Octyldodecyloxy)benzaldehyde (**19j**).* 11.48% yield; R_f = 0.74; ^{1}H NMR ($CDCl_3$) δ 9.88 (s, 1H, H_1), 7.83, 6.998 (AA′XX′ system, 2H each, H_3, $H_{3'}$, H_4 and $H_{4'}$), 3.92 (d, J = 6 Hz, 2H, H_6), 1.82 (m, 1H, H_7), 1.27 (m, 32H, $H_{8'}$–$H_{14'}$ and H_8–H_{16}), 0.89 ("t", 6H, $H_{15'}$ and H_{17}); ^{13}C NMR ($CDCl_3$) δ 190.8 (C_1), 164.6 (C_5), 132.0 (C_3 and $C_{3'}$), 129.8 (C_2), 114.9 (C_4 and $C_{4'}$), 71.4 (C_6), 37.9 (C_7), 32.0, 31.4, 30.2, 30.1, 29.8, 29.73, 29.69, 29.5, 27.0, 26.9, 22.8, 14.2 (C_8–C_{17} and $C_{8'}$–$C_{15'}$); MS (CI, CH_4), *m/z* 402.39 (M^+, 34.21%), 403.35 (MH^+, 99.76%), 123.06 ($MH-CH_2{=}C(CH_2)_9CH_3(CH_2)_7CH_3$, 76.04%); HRMS (CI, CH_4): calcd ($C_{27}H_{47}O_2$, MH^+) 403.3576, obsd 403.3558.

*2.3.6.4. 4-(2-Decyltetradecyloxy)benzaldehyde (**19k**).* 19.73% yield; R_f = 0.78; ^{1}H NMR ($CDCl_3$) δ 9.88 (s, 1H, H_1), 7.83, 6.99 (AA′XX′ system, 2H each, H_3, $H_{3'}$, H_4 and $H_{4'}$), 3.91 (d, J = 6 Hz, 2H, H_6), 1.8 (m, 1H, H_7), 1.26 (m, 40H, $H_{8'}$–$H_{16'}$ and H_8–H_{18}), 0.88 ("t", 6H, $H_{17'}$ and H_{19}); ^{13}C NMR ($CDCl_3$) δ 191.0 (C_1), 164.7 (C_5), 132.1 (C_3 and $C_{3'}$), 129.8 (C_2), 114.9 (C_4 and $C_{4'}$), 71.4 (C_6), 38.0 (C_7), 32.1, 31.4, 30.1, 29.8, 29.5, 27.0, 22.8, 14.3 (C_8–C_{19}, $C_{8'}$–$C_{17'}$); MS (CI, CH_4), m/z 458.4299 (M^+, 72.66%), 459.419 (MH^+, 100%), 123.053 (MH−CH_2=C(CH_2)$_{11}$$CH_3$($CH_2$)$_9$$CH_3$, 64.65%; HRMS (CI, CH_4): calcd ($C_{31}H_{55}O_2$, MH^+) 459.42, obsd 459.419.

*2.3.6.5. Methyl-4-(2-ethylhexyloxy)benzoate (**22g**).* 7.1% yield; R_f = 0.7. The NMR spectral data of the title compound appear in the literature (Zhi-Kuan et al., 1999; Seto et al., 1994). MS (CI, CH_4), m/z 264.1724 (M^+, 38.65), 265.1764 (MH^+, 11.83%), 233.098 (M−OCH_3), 152.054 (M−CH_2=C(CH_2)$_3$$CH_3$$CH_2$$CH_3$,100%); HRMS (CI, CH_4): calcd ($C_{16}H_{24}O_3$, M^+) 264.1724, obsd 264.1724.

*2.3.6.6. Methyl-4-(2-butyloctoxy)benzoate (**22h**).* 2.35% yield; R_f = 0.75; ^{1}H NMR ($CDCl_3$) δ 7.97 and 6.90 (AA′XX′ system, 2H each, H_3, $H_{3'}$, H_4 and $H_{4'}$), 3.87 (d, J = 6 Hz, 2H, H_6), 3.87 (s, 3H, $H_{1''}$), 1.79 (m, 1H, H_7), 1.36 (m, 16H, $H_{8'}$–$H_{10'}$ and H_8–H_{12}), 0.9 (t, J = 6.6 Hz, 3H, $H_{11'}$ or H_{13}), 0.88 (t, J = 6.6 Hz, 3H, H_{13} or $H_{11'}$); ^{13}C NMR ($CDCl_3$) δ 167.0 (C_1), 163.3 (C_5), 131.6 (C_3 and $C_{3'}$), 122.4 (C_2), 114.2 (C_4 and $C_{4'}$), 71.2 (C_6), 51.9 ($C_{1''}$), 38.0 (C_7), 32.0 31.4, 31.1, 29.8, 29.2, 26.9, 23.2, 22.8, 14.2, 14.8 (C_8–C_{13} and $C_{8'}$–$C_{11'}$); MS (CI, CH_4), m/z 320.2362 (M^+, 36.78), 321.2486 (MH^+, 15.99%), 152.067 (M−CH_2=C(CH_2)$_5$$CH_3$($CH_2$)$_3$$CH_3$, 100%), 121.023 (M−$OCH_3$$CH_2$,$CH_2$=C($CH_2$)$_5$$CH_3$($CH_2$)$_3$$CH_3$, 60.79%); HRMS (CI, CH_4): Calcd ($C_{20}H_{32}O_3$, M^+) 320.2351, obsd 320.2362.

*2.3.6.7. Methyl-4-(2-Hexyldecyloxy)benzoate (**22i**) (Moeller and Wallat, 1986).* 12.3% yield; R_f = 0.66; ^{1}H NMR ($CDCl_3$) δ 7.98 and 6.91 (AA′XX′ system, 2H each, H_3, $H_{3'}$, H_4 and $H_{4'}$), 3.92 (s, 3H, $H_{1''}$), 3.87 (d, J = 5.4 Hz, 2H, H_6), 1.79 (m, 1H, H_7), 1.38 (m, 24H, $H_{8'}$–$H_{12'}$ and H_8–H_{14}), 0.88 (m, 6H, $H_{13'}$, H_{15}); ^{13}C NMR ($CDCl_3$) δ 167.1 (C_1), 163.4 (C_5), 131.7 (C_3 and $C_{3'}$), 122.4 (C_2), 114.3 (C_4 and $C_{4'}$), 71.3 (C_6), 52.0 ($C_{1''}$), 38.0 (C_7), 32.0, 32.0, 31.5, 30.1, 29.8, 29.7, 29.55, 27.0, 22.8, 14.2 (C_8–C_{15} and $C_{8'}$–$C_{13'}$); MS (CI, CH_4), m/z 376.304 (M^+, 44.22%), 377.304 (MH^+, 100%), 153.025 (MH−CH_2=C(CH_2)$_7$$CH_3$($CH_2$)$_5$$CH_3$, 43.27%), 121.03 (MH−$OCH_3$,$CH_2$=C($CH_2$)$_7$$CH_3$($CH_2$)$_5$$CH_3$, 24.66%); HRMS (CI, CH_4): calcd ($C_{24}H_{41}O_3$, MH^+) 377.3056, obsd 377.304.

*2.3.6.8. Methyl-4-(2-octyldodecyloxy)benzoate (**22j**).* 9.64% yield; R_f = 0.62; ^{1}H NMR ($CDCl_3$) δ 7.97, 6.90 (AA′XX′ system, 2H each, H_3, $H_{3'}$, H_4 and $H_{4'}$), 3.88 (s, 3H, $H_{1''}$), 3.87 (d, J = 4.6 Hz, 2H, H_6), 1.78 (m, 1H, H_7), 1.26 (m, 32H, $H_{8'}$–$H_{14'}$ and H_8-H_{16}), 0.88 ("t", 6H, $H_{15'}$ and H_{17}); ^{13}C NMR ($CDCl_3$) δ 167.1 (C_1), 163.4 (C_5), 131.7 (C_3 and $C_{3'}$), 122.4 (C_2), 114.2 (C_4 and $C_{4'}$), 71.2 (C_6), 52.0 ($C_{1''}$), 38.0 (C_7), 32.1, 31.4, 30.2, 30.1, 29.8, 29.7, 29.5, 29.5, 27.0, 22.8, 14.3 (C_8–C_{17} and $C_{8'}$–$C_{15'}$); MS (CI, CH_4), m/z 432.41 (M^+, 62.42%), 433.3686 (MH^+, 93.99%), 153.064 (MH−CH_2=C(CH_2)$_9$$CH_3$($CH_2$)$_7$$CH_3$ 100%); HRMS (CI, CH_4): calcd ($C_{28}H_{49}O_3$, MH^+) 433.3682, obsd 433.3626.

*2.3.6.9. Methyl-4-(2-decyltetradecyloxy) benzoate (**22k**).* 1.52% yield; R_f = 0.44 (80% hexane in ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 7.98, 6.9 (AA′XX′ system, 2H each, H_3, $H_{3'}$, H_4 and $H_{4'}$), 3.88 (d, J = 5.5 Hz, 2H, H_6), 3.87 (s, 3H, $H_{1''}$), 1.79 (m, 1H, H_7), 1.26 (m, 40H, $H_{8'}$–$H_{16'}$ and H_8–H_{18}), 0.88 ("t", 6H, $H_{17'}$ and H_{19}); ^{13}C NMR ($CDCl_3$) δ 167.0 (C_1), 163.3 (C_5), 131.6 (C_3 and $C_{3'}$), 122.4 (C_2), 114.2 (C_4 and $C_{4'}$), 71.2 (C_6), 51.9 ($C_{1''}$), 38.0 (C_7), 32.1, 31.4, 30.1, 29.82, 29.8, 29.75, 29.5, 29.49, 27.0, 22.8, 14.2 (C_8–C_{19} and $C_{8'}$–$C_{17'}$); MS (CI, CH_4), m/z 488.4215 (M^+, 31.35%), 489.429 (MH^+, 28.15%), 153.047(MH−CH_2=C(CH_2)$_{11}$$CH_3$($CH_2$)$_9$$CH_3$, 100%); HRMS (CI, CH_4): calcd ($C_{32}H_{57}O_3$, MH^+) 489.4308, obsd 489.4294.

*2.3.7. Unbranched 4-alkoxybenzaldehydes (**19a**–**f**) and branched 4-(2-ethylhexyloxy)benzaldehyde (**19g**)*

The unbranched 4-alkoxybenzaldehydes **19a**–**f** are commercially available, though we found it convenient to prepare **19b**–**f** (65–85% yield) and 4-(2-ethylhexyloxy)benzaldehyde **19g** via the classic Williamson procedure (Williamson, 1852), with major modifications in the workup. A 100mL round flask was charged with 4-hydroxybenzaldehyde (0.15 g, 1.23 mmol, 1 equiv.) and 0.25 M NaOH solution (9.84 mL, 1 equiv.) was stirred under air for 15 min at R.T. yielding a clear deep red solution. The water was removed by rotary evaporation and the remaining crude solid was dissolved in DMF (10 mL). The desired alkyl bromide (**23**, 1 equiv.) was added and the solution was then stirred for 3 days at R.T. During this period, the color of the solution gradually changed from deep red to brown-yellow. The DMF was removed by rotary evaporation and the oily liquid was dissolved in $CHCl_3$ and extracted with an equal volume of H_2O. The organic phase was dried over $MgSO_4$ and evaporated to dryness, and the crude material was purified on a silica column (eluting with 50% ethyl acetate in *n*-hexane), yielding the desired product after solvent removal.

*2.3.7.1. 4-(2-Ethylhexyloxy)benzaldehyde (**19g**) (Woo et al., 1997).* The title compound was obtained in a 27% yield. R_f (50% ethyl acetate in hexane) = 0.8; ^{1}H NMR ($CDCl_3$) δ 9.87 (1H, s, H_1), 7.82, 6.99 (AA′XX′ system, 2H each, H_3, $H_{3'}$, H_4 and $H_{4'}$), 3.92 (d, J = 6 Hz, 2H, H_6), 1.78 (m, 1H, H_7), 1.3 (m, 8H, $H_{8'}$ and H_8–H_{10}), 0.93 (t, J = 7.4 Hz, 3H, $H_{9'}$ or H_{11}), 0.9 (t J = 7.4 Hz, 3H, H_{11} or $H_{9'}$); ^{13}C NMR ($CDCl_3$) δ 191.0 (C_1), 164.6 (C_5), 132.1 (C_3 and $C_{3'}$), 129.8 (C_2), 114.9 (C_4 and $C_{4'}$), 71.0 (C_6), 37.4 (C_7), 30.6, 29.2, 23.9, 23.1, 14.2, 11.2 (C_8–C_{11} and $C_{8'}$–$C_{9'}$); MS (CI, CH_4), m/z 234.1612 (M^+, 43.02%), 235.1773 (MH^+, 41.38%), 123.04 (MH−CH_2=C(CH_2)$_3$$CH_3$$CH_2$$CH_3$, 94.13%); HRMS (CI, CH_4): calcd ($C_{15}H_{23}O_2$, MH^+) 235.1698, obsd 235.1676.

*2.3.8. Unbranched methyl 4-alkoxybenzoates (**22a**–**f**)*

The unbranched esters, methyl 4-alkoxybenzoates **22a**–**f**, are commercially available; nevertheless, we found it convenient to prepare **22b**–**f** according to literature procedure (Fieser, 1957) in 20–45% yields.

*2.3.9. Preparation of the branched alkyl bromides (**23h**–**k**)*

The branched alkyl bromides **23h**–**k**, required for the synthesis of the branched derivatives of 4-alkoxybenzaldehydes **19** and methyl 4-alkoxybenzoates **22**, were prepared by bromination of the corresponding alcohols following the procedure of Balachander and Sukenik (1990). The unbranched bromides and branched bromides **23g** and **23i** (City Chemical) are commercially available, but the latter was conveniently synthesized by this procedure as well. Bromides **23h** (Gaertner, 1965), **j** (Ellinger et al., 2007) and **k** (Pisula et al., 2004) have been previously reported, though the spectral data are incomplete for the first and last and, therefore, reported below.

*2.3.9.1. 2-Butyloctyl bromide (**23h**).* 70.2% yield; ^{1}H NMR ($CDCl_3$) δ 3.45 (d, J = 4.8 Hz, 2H, H_1), 1.6 (m, 1H, H_2), 1.3 (m, 16H, H_3–H_7 and $H_{3'}$–$H_{5'}$), 0.92 (m, 6H, H_8 and $H_{6'}$); ^{13}C NMR ($CDCl_3$) δ 39.7 (C_1 and C_2), 32.7, 32.4, 32.0, 29.6, 28.9, 26.7, 23.0, 22.8, 14.2, 14.16 (C_3–C_8, $C_{3'}$–$C_{6'}$).

*2.3.9.2. 2-Hexyldecyl bromide (**23i**).* 62.3% yield; ^{1}H NMR ($CDCl_3$) δ 3.44 (d, J = 6 Hz, 2H, H_1), 1.58 (m, 1H, H_2), 1.28 (m, 24H, H_3–H_9 and $H_{3'}$–$H_{7'}$), 0.89 (m, 6H, H_{10} and $H_{8'}$); ^{13}C NMR ($CDCl_3$) δ 39.7 (C2),

39.7 (C1), 32.7, 32.1, 32.0, 30.0, 29.7, 29.6, 29.5, 26.72, 26.7, 22.83, 22.81, 14.2 (C_3–C_{10}, $C_{3'}$–$C_{8'}$).

*2.3.9.3. 2-Decyltetradecyl bromide (**23k**).* 70% yield; ^{1}H NMR ($CDCl_3$) δ 3.44 (d, J = 4.8 Hz, 2H, H_1), 1.6 (m, 1H, H_2), 1.27 (m, 40H, H_3–H_{13} and $H_{3'}$–$H_{11'}$), 0.89 (m, 6H, H_{14} and $H_{12'}$); ^{13}C NMR ($CDCl_3$) δ 39.7 (C2), 39.6 (C1), 32.8, 32.82, 30.0, 29.83, 29.79, 29.6, 26.7, 22.9, 14.3 (C_3–C_{14}, $C_{3'}$–$C_{12'}$).

3. Results and discussion

3.1. Initial criteria for reporter molecules

A priori, four properties would seem to be necessary for an efficiently constructed "ruler": (1) the compounds should have a planar backbone—which simplifies distance and dihedral angle calculations. (2) The molecules should lie vertically within the lipid bilayer, i.e., perpendicularly to the water/lipid interface. Putting these first two requirements together suggested molecules with a hydrophilic/polar "head" group, a planar and symmetrical (or near-symmetrical) body, and symmetrical (or near-symmetrical) hydrophobic/lipophilic tails. (3) The hydrophobic tails should be modular, permitting the ready synthesis of a family of compounds with varying lipophilicity—and hence varying depths within the lipid bilayer. (4) Our experience (Frimer et al., 1983, 1996; Frimer, 1985; Strul et al., 1993,1994; Weitman et al., 2001; Afri et al., 2002, 2004a,b; Bronshtein et al., 2004) has further taught us that accurate ^{13}C chemical shift/E_T(30) correlations require systems in which the increase in the ^{13}C NMR chemical shift (henceforth, $\Delta\delta$) of the reporter carbons with solvent polarity is relatively large. In particular, we have found that results are reliable when the change in ^{13}C chemical shift is $\geq$1.8 ppm over ca. 20 kcal/mol E_T range (e.g., in going from methanol [E_T(30) = 55.4 kcal/mol] to benzene [E_T(30) = 34.3]. The $\Delta\delta$ is particularly large in conjugated systems capped at alternate ends by a donor and an acceptor moiety, where a large "push–pull" effect is present.

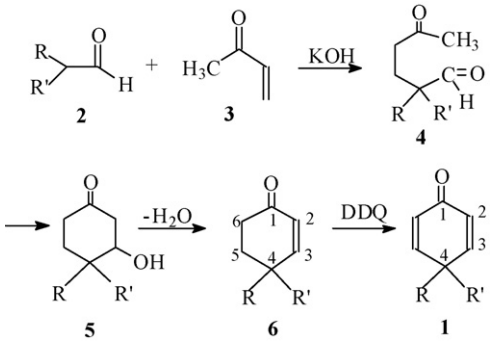
Based on the above criteria we began our search for appropriate compounds with the preparation of cyclohexadienones **1a**–**h**.

3.2. 4,4-Dialkylcyclohexa-2,5-dienones ***1a–h***

3.2.1. Synthesis

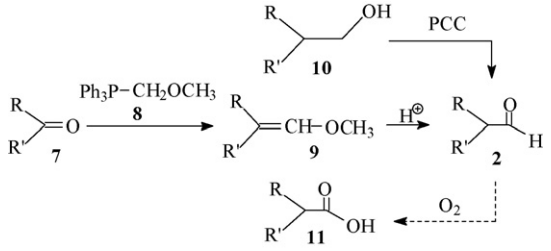
The title compounds were synthesized via a Robinson annulation approach, as outlined in Scheme 2 (Frimer et al., 1989).

In the first step, a Michael addition of the enolate of α-disubstituted aldehyde **2** to methyl vinyl ketone **3** yields dione **4**.



a:R=R'=CH_3; **b**: R=R'=CH_2CH_3; **c**: R=R'=-$(CH_2)_5$-;
d: R=R'=C_6H_5; **e**: R=R'=$(CH_2)_3CH_3$; **f**: R=R'=$(CH_2)_4CH_3$;
g: R=R'=$(CH_2)_5CH_3$; **h**:R=$(CH_2)_9CH_3$, R'=$(CH_2)_{11}CH_3$

Scheme 2. Synthetic route to cyclohexadienones **1**.



Scheme 3. Synthetic routes to α-disubstituted aldehydes **2**.

Aldol cyclization leads to β-hydroxyketone **5** which yields enone **6** upon dehydration. Oxidation of the latter with DDQ generates the desired cyclohexadienone **1**. The vinyl hydrogens of dienones **1** have an AA′XX′ splitting pattern which is characteristically different from that of the analogous hydrogens in enone system **6**.

Turning to the starting materials, the short-chain symmetrical (where R = R′) aldehydes **2a**–**d** are commercially available. The longer chain aldehydes **2e**–**g** could be synthesized via the hydrolysis of enol ethers **9** (Taber et al., 1989; Hu and Mattern, 2000) (Scheme 3). The latter were prepared in turn by treating commercially available symmetrical ketones **7** with the Wittig reagent methoxymethylenetriphenylphosphorane (**8**). The very long-chain asymmetrical aldehyde **6h** was finally prepared by the chromate oxidation of the corresponding alcohol **10** (Scheme 3) (Corey and Suggs, 1975).

Two comments are appropriate at this juncture. Firstly, this route to the aldehyde has been previously carried out in a one-pot method directly from the ketone, without the isolation of the intermediate enol ether **9** (Taber et al., 1989; Hu and Mattern, 2000). Nevertheless, it is preferable in our system to add this isolation step because it allows for removal of starting ketone **7**, whose polarity is very similar to that of the final aldehyde **2**. Secondly, we note that the aldehydes undergo very facile autoxidation to the corresponding carboxylic acids **11**; hence, the aldehydes were used immediately upon synthesis.

3.2.2. Locating cyclohexadienones ***1a–h*** within dimyristoylphosphatidylcholine (DMPC) liposomes

As noted above, it was our aim to determine the exact location of specific carbons in each compound in the liposomal bilayer. As we explained earlier, to do so requires developing a graph which correlates between the ^{13}C NMR chemical shift and solvent polarity for the various carbons of each compound. To this end, the ^{13}C NMR data were acquired for each of the compounds in four or five solvents of varying E_T(30) polarity, generally carbon tetrachloride (32.4), benzene (34.3), chloroform (39.1), acetone (42.2), acetonitrile (45.6) and methanol (55.4). In general, the correlations were excellent (correlation coefficient, $r^2 > 0.9$). From the accrued data, it becomes clear that there are certain carbons which are most sensitive to polarity change, i.e., had $\Delta\delta = \geq$1.8 ppm over an E_T range of ~20 kcal/mol. We then prepared ^{13}C NMR chemical shift/E_T(30) polarity correlation graphs (henceforth, simply dubbed "correlation graphs") for each of these sensitive "reporter" carbons.

The next step was to intercalate each of the various derivatives within the DMPC liposomal phospholipid bilayer and determine the chemical shifts of the "reporter" carbons. Based on the correlation graph, it was possible to calculate from the chemical shift values the corresponding microenvironment polarity (expressed as an E_T(30) value) presumably felt by a given reporter "carbon". The relative polarity values of these carbons give us a sense of the molecules location and orientation. (We note, parenthetically, that reporter moieties, such as carbonyls, might conceivably drag water

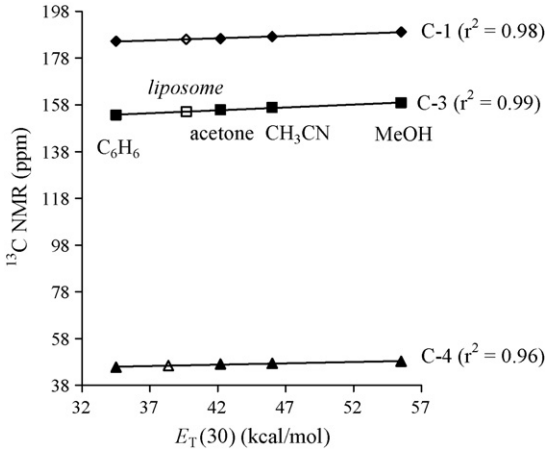
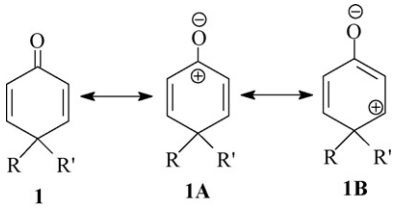


Fig. 1. Plots of the ^{13}C NMR chemical shift (ppm) for C-1, C-3 and C-4 of cyclohexadienone **1f** vs. $E_T(30)$ solvent polarity (kcal/mol). (The solid symbols represent chemical shift values in pure solvent, while hollow symbols are for those within liposomes.)



Scheme 4. Resonance structures of cyclohexadienones **1**.

with them into normally hydrophobic regions and, thus, give a false reading. We believe this to be highly unlikely, though. After all, the carbonyl is essentially isolated, surround on all sides by highly lipophilic groups which overwhelmingly determine the solvation and polarity properties of the microenvironment.)

When we followed this procedure for dienones **1**, we revealed that carbons C-1, C-3 and C-4 were the most sensitive to polarity change. Fig. 1 exemplifies this process for dienone **1f** in which the $\Delta\delta$ values in going from benzene to methanol were 3.91, 5.25 and 2.53 for carbons C-1, C-3 and C-4, respectively, with correlation factors (r^2) $\geq$0.96.

The sensitivity of carbons C-1, C-3 to solvent polarity is not at all surprising in light of the resonance structures of dienone system **1** (Scheme 4). We see that both C-1 and C-3 have large charge separation in structures **1A** and **1B**, respectively. The particularly large change in the chemical shift of C-3 ($\Delta\delta \approx 5$ ppm) suggests that charge separation becomes more facile as polarity increases, and

Table 1
Calculated polarity ($E_T(30)$ in kcal/mol) of carbons C-1, C-3 and C-4 of compounds **1a–h** intercalated within the liposomal bilayer

Compound	C-1	C-3	C-4
1a	58.80	57.70	49.18
1b	48.58	48.48	46.25
1c	46.45	45.61	43.64
1d	44.48	44.07	42.63
1e	42.03	41.62	40.14
1f	39.67	39.70	38.36
1g	38.14	38.49	36.38
1h	31.60	35.74	36.22

with it the prominence of resonance structure **1B**. The sizable $\Delta\delta$ of C-4 must result from an inductive effect.

Dienones **1a–h** were then intercalated into DMPC liposomes and the chemical shifts of carbons C-1, C-3 and C-4 were measured. The calculated polarity ($E_T(30)$ values) are listed in Table 1.

The following interim observations can be made about these results:

(1) The data of Table 1 confirm our assumption that long chain derivatives impart greater lipophilicity to molecules. Looking at the series **1a–h**, we see that the longer the chains are at C-4: the more lipophilic the molecule becomes; the deeper the molecule penetrates into the bilayer toward the lipid slab; and, as a result, the lower the $E_T(30)$ values of the reporter carbons become. Perhaps surprisingly in this regard, an *n*-butyl chain (**1e**) imparts greater lipophilicity than a benzene ring (**1d**).
(2) Nevertheless, Table 1 contains seemingly irresolvable contradictions. Thus, in derivative **1a**, C-1 and C-3 have essentially the same $E_T(30)$ polarity value, suggesting that this section of the molecule lies almost horizontally. However, these polarity values are almost 10 $E_T(30)$ units higher than C-4, suggesting that the molecules is nigh vertical.
(3) In derivatives **1b–g**, C-1 and C-3 have nearly the same polarity value again suggesting that the dienones lie almost parallel to the interface, horizontally in the bilayer—all this despite the polar carbonyl head group and very lipophilic tails. What is more, in the case of the most lipophilic dienone **1h**, the $E_T(30)$ value of carbonyl carbon C-1 is actually *lower* than both C-3 and the highly lipophilic C-4.

3.2.3. Implications to the construction of a molecular ruler

As noted above, the calculated E_T results left us quite confused. We had expected to see a correlation between the lipophilicity of the substituents on the various intercalants and their depth in the bilayer. This was in general observed, perhaps most clearly in the case of dienones **1**. Proceeding from **1a** (where the total number of carbons in R and R′ is 2) to **1h** (where this number has gone

Table 2
Calculated polarity of carbons C-I and C-II of methyl alkyl diesters **15a–g** intercalated within the liposomal bilayer

Compound	Carbons in R′	Carbonyl-I[a] (kcal/mol)	Carbonyl-II[b] (kcal/mol)	$\Delta E_T/\Delta$Å$_{I-II}$[c] (kcal/mol Å)	
15a	5	44.06	39.35	0.63	
15b	6	44.57	39.47	0.96	
15c	8	44.44	39.30	0.69	
15d	12	44.20	38.20	0.81	
15e	16	44.60	37.27	0.99	
15f	20	45.47	39.35	0.83	
15g	24	44.50	39.28	0.70	Average **2a–g**: 0.80

[a] Experimental error = ±1; average $\Delta\delta$ = 2.62; average r^2 = 0.98.
[b] Experimental error = ±1; average $\Delta\delta$ = 2.50; average r^2 = 0.98.
[c] ΔÅ (C_I–C_{II}): 7.34 Å.

Table 3
Calculated polarity of carbons C-I and C-II of methyl alkyl diesters **17a–g** intercalated within the liposomal bilayer

Compound	Carbons in R′	Carbonyl-I[a] (kcal/mol)	Carbonyl-II[b] (kcal/mol)	$\Delta E_T/\Delta Å_{I-II}$[c] (kcal/mol Å)	
17a	5	42.40	31.61	1.06	
17b	6	42.57	34.89	0.75	
17c	8	43.48	35.28	0.80	
17d	12	43.67	35.04	0.84	
17e	16	43.79	33.54	1.00	
17f	20	43.54	34.80	0.85	Average **4a–f**: 0.88
17g	24	43.33	n.d.[d]	–	

[a] Experimental error = ±1; average $\Delta\delta$ = 2.40; average r^2 = 0.96.
[b] Experimental error = ±1; average $\Delta\delta$ = 2.35; average r^2 = 0.95.
[c] ΔÅ (C_I–C_{II}): 10.23 Å.
[d] Signal not detected.

up 11-fold to 22) causes C-1 of the intercalant to plummet from E_T = 58.8 – not far from the water–lipid interface, down to E_T = 31.6 – presumably near or in the lipid slab.

However, despite the amphiphilic nature of the various intercalants, at first blush, the E_T values of Tables 1–3 would seem to strongly suggest that these molecules do not lie perpendicularly to the interface as was expected. Indeed, in the case of dienones **1**, for example, we anticipated that the E_T values for C-1 would be significantly higher than those of C-3 and C-4. This would have indicated that the C-1 carbonyl is situated closer to the polar interface, while C-3 and the adjacent lipophilic C-4 would be substantially deeper within the bilayer. Instead, the results of Table 1 show that carbons C-1 and C-3 are very close to each other, while C-4 is only slightly lower, depending on the substituent. Unexpectedly, in compound **1h**, C-1 is even lower than C-3, which is lower still than C-4; this would suggest that the carbonyl is actually *deeper* in the liposome than the long chain substituents! These results suggest either that an unexpected phenomenon is occurring, or alternatively that some of our fundamental premises are in error.

All the above data lead us to the following conclusions regarding the dienone **1** system:

(1) The longer the chains in the dienone system, the more the molecule becomes lipophilic and, therefore, the deeper it plunges into the bilayer.
(2) It seems that the charge on C-1, which develops as the environment at C-1 becomes more polar, distributes itself between C-1 and C-3—this is a result of the resonance built into the conjugated enone system. Thus the amount of charge that develops on C-3 is not totally or *primarily* a function of the microenvironmental polarity at C-3, but rather is highly dependant on the polarity experienced at C-1! It is not completely surprising; therefore, that both C-1 and C-3 can give similar E_T results. Hence, we conclude that in the case of dienones **1**, the values of C-3, are unreliable.
(3) As far as C-4 is concerned, the numbers here, too, are problematic since no charge development is expected. The chemical shifts and calculated E_T values observed reflect not some inherent charge developing at C-4—because of the microenvironmental polarity C-4 feels. Rather, they reflect the induction transmitted to it from C-3, which in turn depends on the charge developing at C-1.

Therefore, there is a fundamental problem in using the cyclohexadienones in developing a chemical ruler. In fact, it is possible that indeed some of the dienone derivatives lie perpendicular to the interface, as we originally expected; however, we have no way of proving or measuring this.

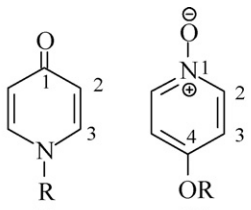
The analysis regarding dienones **1** is in fact true for most conjugated systems where developing charge can be transmitted several carbons away via the π-system. A related analysis resolves analogous results *mutatis mutandis* observed for two other systems we studied briefly: *N*-alkyl pyridones **12** and 4-alkoxypyridine *N*-oxides **13** (Scheme 5). In these cases, the $E_T(30)$ values calculated for C-3 or C-4, respectively, reflect not the inherent polarity experienced at that carbon because of the microenvironment polarity, but rather the charge build-up transferred to it in part from C-1 or C-2, respectively (Cohen, 2005).

The above data lead us clearly to one important conclusion. In developing a chemical ruler, we can only use molecules composed of polar carbons which are not strongly conjugated, even though they would seem preferable due to their large $\Delta\delta$. Instead, we require systems which have at least two polar moieties probes (e.g. carbonyls) – at a known distance from each other – whose charge development in each case is dependant exclusively on its *own* microenvironment. Therefore, an additional demand for the molecular ruler is that the polar carbon bond must not be part of a strongly conjugated system.

3.3. Unconjugated diesters ***15*** and ***17***

3.3.1. Synthesis

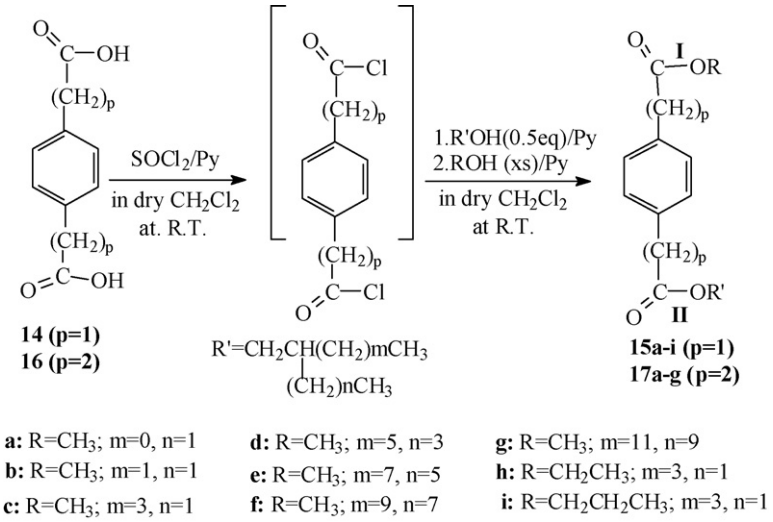
In order to meet the above new criterion, we attempted to engineer compounds that would have the following properties: (a) they would contain two carbonyls as reporter carbons, separated by a known distance one from the other, which are in no way conjugated to each other or any other moiety. (b) The molecules would contain an aromatic ring to give it linearity and allow for easy detection by TLC. (c) The functional groups would permit the preparation of derivatives of varying lipophilicity, for varying penetration of the



12 **13**

a:R=CH_3 **e**:R=$(CH_2)_{11}CH_3$
b:R=$(CH_2)_3CH_3$ **f**:R=$(CH_2)_{14}CH_3$
c:R=$(CH_2)_7CH_3$ **g**:R=$(CH_2)_{17}CH_3$
d:R=$(CH_2)_9CH_3$

Scheme 5. Derivatives of heterocycles **12** and **13**.



Scheme 6. Synthetic route to dicarboxylic esters **15** and **17**.

bilayer. To this end, we turned to benzenediacetic esters **15** and the corresponding dipropionic esters **17**. Of the two independent carbonyls, one is a methyl ester which will serve as the polar head group, while the other is a long chain, highly lipophilic, ester which will hopefully be anchored in or near the lipid slab.

As outlined in Scheme 6, esters **15** and **17** were prepared from the corresponding dicarboxylic acids **14** and **16**, respectively.

The diacids were converted to diacyl chlorides with excess thionyl chloride. Upon removal of the latter, the diacyl chlorides were reacted with 0.5 equiv. of the long chain alcohol (R′OH) yielding the corresponding monoester; the latter was then treated with excess methanol (for analogs **a**–**g**), ethanol (for analog **15h**) or isopropanol (for analog **15i**) creating the desired mixed ester. Identification of the two carbonyls (dubbed C-I for the methyl ester carbonyl, and C-II for that of the long chain ester) was accomplished by ^{13}C NMR 2D HMQC and HMBC techniques.

Focusing on the polarizable carbonyls C-I and C-II alone, NMR spectra of derivatives **15a**–**i** and **17a**–**g** were run in five pure deuterated solvents [$E_T(30)$ values in kcal/mol in parentheses]: methanol (55.4), acetonitrile (45.6), acetone (42.2), chloroform (39.1), and benzene (34.3). Correlation graphs (exemplified by Figs. 2 and 3) between solvent polarity ($E_T(30)$ values) and chemical shift (δ) were prepared. We then intercalated the diesters into dimyristoylphosphatidylcholine (DMPC) liposomes and observed the NMRs of C-I and C-II. The correlation graphs were then used to determine the calculated polarity ($E_T(30)$) values experienced by carbons C-I and C-II within the liposomal bilayer for each derivative. These calculated $E_T(30)$ values give us, in turn, insight as to where these carbons

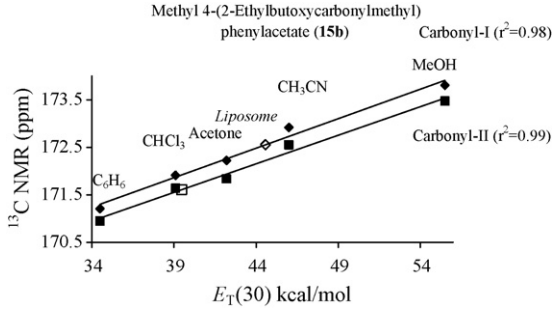


Fig. 2. Plots of the ^{13}C NMR chemical shift (ppm) for C-I (diamond) and C-II (square) of **15b** vs. solvent polarity ($E_T(30)$).

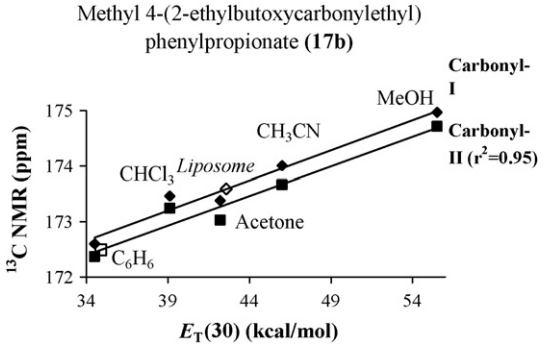


Fig. 3. Plots of the ^{13}C NMR chemical shift (ppm) for C-I (diamond) and C-II (square) of **17b** vs. solvent polarity ($E_T(30)$).

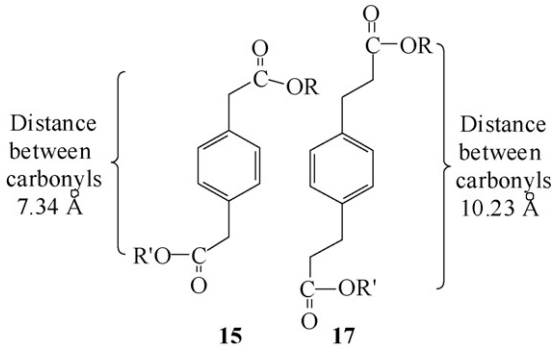
are located along the continuum of polarities ranging from highly polar at the interphase down to non-polar at the lipid slab. The results are summarized in Tables 2 and 3, respectively. Table 4 compares the results for **15c**, **h** and **i** and gives us insight into the effect of changing the head group lipophilicity.

We should note that, in the NMR studies described throughout this paper, the molar ratio of intercalant:DMPC was generally 1:5. This rather high concentration of intercalant was necessary in order to observe the desired reporter peaks via NMR. Interestingly, however, in the ^{13}C NMR spectra of the very long chain derivatives **2f**, **2g**, **4f** and **4g** of the unconjugated diesters, where the carbons in R′ range from 20–24, we observed double carbonyl peaks. One pair was sharp and located at relatively high field (e.g., in **2g**, they appeared at 170.58 and 170.98 ppm), while the other pair was broad and situated at lower field (e.g., in **2g**, they were observed at 171.60

Table 4
Calculated $E_T(30)$ of carbonyls C-I, and C-II in compounds **15c**, **15h** and **15i** intercalated within the liposomal bilayer

Compound	Head group	Carbonyl I[a] (kcal/mol)	Carbonyl II[a] (kcal/mol)	$\Delta E_T/\Delta$Å$_{I-II}$[b] (kcal/mol Å)
15c	MeO–	44.44	39.30	0.69
15h	EtO–	40.80	39.15	0.22
15i	$(CH_3)_2CHO$–	38.31	40.81	−0.34

[a] Experimental error = ±1; average $\Delta\delta$: OMe = 2.58; OEt = 2.52; $OCH(CH_3)_2$ = 2.45. Average r^2 = 0.98.
[b] Experimental error = ±1; ΔÅ (C_I–C_{II}): 7.34 Å.



Scheme 7. Calculated distance between the carbonyls of diesters **15** and **17**.

and 172.49 ppm). The doubling results from two populations in these samples: one group includes those molecules which are intercalated within the liposome and feel the polarity corresponding to the liposomal microenvironment; while the other consists of aggregates of these long chain derivatives, and, hence, they feel the microenvironment polarity created by themselves, and should be essentially phospholipid independent (Cohen et al., 2008b).

3.3.2. Location within the bilayer

From the data of Tables 2–4, we can glean several important pieces of information.

(1) Firstly, we see that within each diester family (diacetate **15a**–**g** or dipropionate **17a**–**g**), the location of the C-I carbonyl for each derivative is essentially the same, independent of the length/lipophilicity of the tail. The same is true for the lower lying C-II carbonyl for each derivative. The very lipophilic values of C-II (hexane has an $E_T(30)$ value of 31 kcal/mol), suggests that the lipophilic tail in all cases rapidly reaches into the lipid slab. As a result, lengthening the fatty acid chain further does not pull the molecule into a more lipophilic region solvent. With the lipophilic tail well solvated in the lipophilic slab, the location of C-I and C-II in compounds **15** and **17** is pretty much determined by the solvation needs of these carbonyls and the distance between them.

(2) In this light, we now compare between the families, and see that the C-I carbonyl in both series are anchored close together—at ~44.5 kcal/mol for the diacetates and a tad lower at 43.5 for the dipropionates. Not surprisingly, though, the micropolarity felt by the respective C-II carbonyls in these two families is substantially different. Thus, in the diacetate family **15a**–**g**, the lower carbonyl C-II, which is 7 bonds away from, is 6.5 E_T units lower than that of C-I, centered at $E_T(30) = 38$ kcal/mol. In the dipropionate family **17a**–**g**, C-II which is 9 bonds away from C-I, is 9 E_T units lower than C-I, centered at 33 kcal/mol, near or in the lipid slab.

(3) Let us introduce a quantity $\Delta E_T/\Delta Å_{I–II}$, where $\Delta Å_{I–II}$ is the distance in Å between two given "reporter" carbons in a molecule, C-I and C-II, while ΔE_T is the difference in $E_T(30)$ values between these carbons. Thus, $\Delta E_T/\Delta Å_{I–II}$ gives us a measure of how much the $E_T(30)$ changes in going from one point of given depth to another. The latter was derived in each family from the geometric relationships (bond length and angles) between these carbonyls in the optimized (minimum energy) configuration with the aid of PCMODEL calculations. The distances between the reporter carbonyls in diesters **15** and **17** are shown in Scheme 7 and are 7.34 and 10.23 Å, respectively.

The size of the $\Delta E_T/\Delta Å$ presumably depends on two factors. The first is the verticality of the lipid-intercalated compound under study, i.e., to what extent the intercalant is parallel to the lipid chains and perpendicular to the water–lipid interface. As the verticality increases, the polarity difference between the reporting carbons (of fixed $\Delta Å$), as reflected in the ΔE_T observed, is expected to increase as well. Thus, when $\Delta E_T/\Delta Å$ is maximal, we can assume that the intercalated compound lies essentially vertical within the lipid bilayer.

The second factor affecting the size of $\Delta E_T/\Delta Å$ is the depth of the reporter carbon within the bilayer. As noted above, a gradient of polarities exists within the bilayer ranging from water at the interface, down to hexane within the lipid slab. There is no reason to assume, however, that the change in polarity is linear with depth. As a result, $\Delta E_T/\Delta Å$ may well depend on the region within the lipid bilayer that the measurements are taken. For example, change in $\Delta E_T/\Delta Å$ may be moderate in the upper polar region of the bilayer, increase rapidly and peak in the lower polar region, and then slow dramatically as one approaches or enters the lipid slab.

In Tables 2 and 3, we see that for diesters **15**, which lie in the relatively non-polar ΔE_T region of 45–39, the $\Delta E_T/\Delta Å$ is ca. 0.80 ± 0.19. Interestingly, for diesters **17**, lying in the ΔE_T region 44–32, the $\Delta E_T/\Delta Å$ value is 0.94 ± 0.19, not substantially different. The data suggest that in range of ΔE_T values between 45 and 32 there does not seem to be any dramatic changes in alignment of these molecules or in the value of $\Delta E_T/\Delta Å$ with depth.

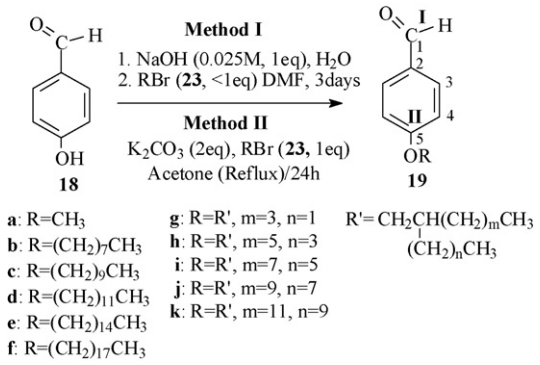
(4) Table 4 lists the results obtained for two additional analogs of **15c**, diesters **15h** and **15i**. The latter differ from **15c** only in that they possess the more lipophilic ethoxy and *iso*-propoxy head groups, respectively, instead of the standard methoxy moiety. Interestingly, Table 4, particularly the value of $\Delta E_T/\Delta Å^{b}_{I–II}$, reveals that the effect of changing the head group even slightly is quite dramatic. As the head group becomes more lipophilic, the lipophilicity difference between the head and the tail rapidly decreases and the diesters quickly orient themselves horizontally within the liposome. Thus, $\Delta E_T/\Delta Å^{b}_{I–II}$ is 0.69 for the methyl ester, but drops to 0.22 and −0.34 for the ethyl and isopropyl analogs, respectively.

All the above results are quite consistent with these methyl alkyl diester families lying vertically within the liposomal bilayer—suggesting them as excellent candidates for the desired molecular ruler. Unfortunately, since all the derivatives in each family lie at the same location, the multiple derivatives are redundant. Nevertheless, one example from each family can supply data points for our ruler, which will be discussed further below.

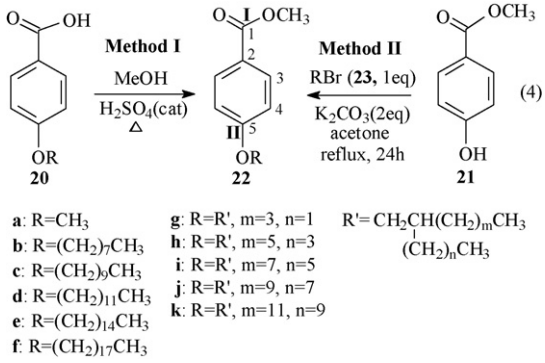
*3.4. 4-Alkoxybenzaldehydes **19** and methyl 4-alkoxybenzoates **22** as ruler candidates*

3.4.1. Synthesis

In an attempt to widen the number of substrates contributing to our ruler, we turned to two aromatic systems bearing polar groups, 4-alkoxybenzaldehydes and methyl 4-alkoxybenzoates. A preliminary study of the change of ^{13}C NMR chemical shifts (δ) with increasing solvent polarity was carried out on the carbons of the commercially available methyl 4-methoxybenzaldehydes (**19a** in Scheme 8) and 4-methoxybenzoates (**22a** in Scheme 9). In both cases, the $\Delta\delta$ values around the ring in going from benzene to methanol (from $E_T(30)$ 34.3 to 55.4 kcal/mol) were negligible except for the exocyclic carbonyl carbon and ring carbon *para* to it, which were large (>2.3). We thus explored the preparation of these families of compounds.



Scheme 8. Synthetic route to 4-alkoxybenzaldehydes **19**.



Scheme 9. Synthetic routes to methyl 4-alkoxybenzoates **22**.

The various benzaldehyde derivatives were synthesized using the century and a half old Williamson reaction (Williamson, 1852), either under the classic strongly basic conditions (Barton and Ollis, 1979) (Scheme 8, method I) or via the more convenient one-pot Hong-bin variation (Hong-bin et al., 2001) (method II).

Methyl benzoates **22b–d** were prepared via the esterification of the corresponding 4-alkoxybenzoic acids (Scheme 9, method I), while analogs **22a** and **22e–k** were prepared from the corresponding phenol **21**, via the Hong-bin variation (Hong-bin et al., 2001) of the Williamson reaction (method II).

In both the aldehyde **19** and methyl ester **22** systems, NMR spectra of the various derivatives were run in four or five pure deuterated solvents selected from methanol, acetonitrile, acetone, chloroform and benzene. Focusing on the exocyclic carbonyl carbon (C-1) and ring carbon *para* to it (C-5), correlation graphs were prepared for each of the derivatives (see typical Figs. 4 and 5 for aldehyde **19h** and ester **22j**) and the compounds were intercalated into the DMPC liposome. The correlation graphs were then used to

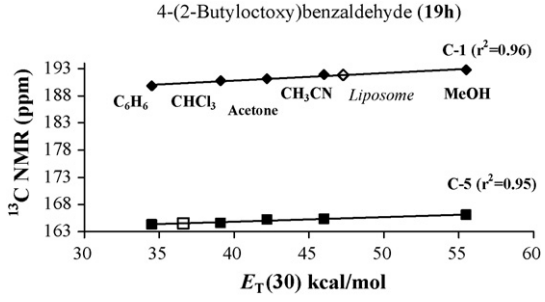


Fig. 4. Plots of the ^{13}C NMR chemical shift (ppm) for C-1 and C-5 of 4-(2-butyloctoxy)benzaldehyde (**19h**) vs. solvent polarity (E_T(30)).

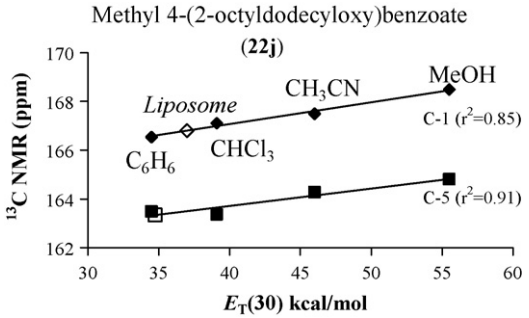


Fig. 5. Plots of the ^{13}C NMR chemical shift (ppm) for C-1 and C-5 of methyl 4-(2-octyldodecyloxy)benzoate (**22j**) vs. solvent polarity (E_T(30)).

determine the calculated polarity (E_T(30)) values experienced by carbons C-1 and C-5 within the liposomal bilayer for each aldehyde or ester derivative. The results are summarized in Tables 5 and 6.

3.4.2. Location within the bilayer

Several important conclusions can be drawn from these data.

(1) The C-1 carbonyl of the benzoate esters **22**, as a group, falls substantially (8–13 E_T units) lower than the corresponding carbonyl in benzaldehydes **19**. Within each of the families, the derivatives tend to break themselves into three subgroups: short-chain unbranched, long-chain unbranched and long-chain branched derivatives. Thus, in the case of the aldehydes, the E_T(30) values for C-1 and C-5 of the unbranched members (**19b–f**) fall at approximately ca. 51 and 38 kcal/mol, respectively, while these carbons in the branched analogs (**19g–k**) lie 2–3 E_T units deeper at values of 48 and 36 kcal/mol, respectively. In the case of esters **5** the differences are much smaller. The E_T(30) values C-1 and C-5 of the unbranched members fall approximately at 38.5 and 35 kcal/mol, respectively; these carbons in the branched analogs lie 1–1.5 E_T units deeper at 37.5 and 33.5 kcal/mol. For the short-chained analogs **19a** and **22a**, while C-1 is located essentially where the unbranched long-chain analogs are, C-5 is situated ca. 3 E_T units higher.

(2) Tables 2 and 3 also records that the difference in E_T (ΔE_T) between carbonyls C-1 and C-5 is substantially smaller in the benzoate esters **22** (3–5 kcal/mol) than it is in the benzaldehydes **19** (12–13 kcal/mol). This is despite the fact that the distance in Angstroms (ΔÅ) between C-1 and C-5 in both the benzoate ester and benzaldehyde systems was determined by PCMODEL calculations to be the same: 4.26 Å.

(3) Tables 2 and 3 reveals that the *para* carbon C-5 for the branched benzaldehydes (**19g–k**), and that of the unbranched benzoates (**22a–f**) lie approximately in the same region (E_T 35–36). Thus, a comparison of the $\Delta E_T/\Delta Å_{1-5}$ values between these families is appropriate. The data reveal that average $\Delta E_T/\Delta Å_{1-5}$ for the branched aldehydes (**19g–k**) is 2.74 (spanning a region from E_T 48 for C-1 to E_T 36 for C-5), as compared to a much smaller value of 0.87 (spanning a region from E_T = 38–35) for the unbranched benzoates (**22a–f**). This despite the fact that the distance from C-1 to C-5 ($\Delta Å_{1-5}$) is the same in both systems.

Two explanations can be suggested to resolve this discrepancy. One is that benzaldehydes lie vertically in the lipid bilayer while the benzoates lie at a substantial angle; thus the ΔE_T of the former is larger. This explanation is unlikely, however, since the average $\Delta E_T/\Delta Å$ values of the benzoates (0.87 for the unbranched analogs and 0.96 for the branched) is consistent with that observed above for diesters **15** (0.80) and **17** (0.94)—which are located in the same

Table 5
Calculated $E_T(30)$ values of carbons C-1 and C-5 of benzaldehydes **19a–k** intercalated within the liposomal bilayer

Compound	Carbons in R	C-1[a] (kcal/mol)	C-5[b] (kcal/mol)	$\Delta E_T/\Delta Å_{1-5}$[c] (kcal/mol Å)	
19a	1	49.42	41.76	1.80	**19a**: 1.80
19b	8	49.70	38.01	2.74	
19c	10	49.50	38.34	2.62	
19d	12	51.64	37.23	3.38	
19e	15	51.58	38.55	3.06	
19f	18	50.44	38.37	2.83	Average **19b–f**: 2.93
19g	8	47.39	35.45	2.80	
19h	12	47.28	36.61	2.50	
19i	16	47.28	35.84	2.68	
19j	20	47.60	36.06	2.71	
19k	24	48.56	36.77	2.77	Average **19g–k**: 2.70

[a] Experimental error = ±1; average $\Delta\delta$ = 2.99; average r^2 = 0.95.
[b] Experimental error = ±1; average $\Delta\delta$ = 1.72; average r^2 = 0.90.
[c] ΔÅ (C_1–C_5): 4.26 Å.

Table 6
Calculated $E_T(30)$ values of carbons C-1 and C-5 of methyl benzoates **22a–k** intercalated within the liposomal bilayer

Compound	Carbons in R	C-1[a] (kcal/mol)	C-5[b] (kcal/mol)	$\Delta E_T/\Delta Å_{1-5}$[c] (kcal/mol Å)	
22a	1	38.05	38.34	−0.07	**22a**: −0.07
22b	8	38.65	34.93	0.87	
22c	10	38.37	35.87	0.60	
22d	12	38.98	34.75	0.99	
22e	15	38.27	33.99	1.00	
22f	18	38.22	34.40	0.90	Average **22b–f**: 0.87
22g	8	38.83	35.60	0.76	
22h	12	36.93	32.61	1.01	
22i	16	37.52	33.42	0.96	
22j	20	38.49	34.82	0.86	
22k	24	36.39	31.33	1.19	Average **22g–k**: 0.96 Average **22b–k**: 0.91

[a] Experimental error = ±1; average $\Delta\delta$ = 1.96; average r^2 = 0.95.
[b] Experimental error = ±1; average $\Delta\delta$ = 1.34; average r^2 = 0.80.
[c] ΔÅ (C1–C_5): 4.26 Å.

region (E_T = 44–34). A more likely explanation is that the large values in the benzaldehydes **19** are unreliable—and, hence need to be discarded. This is because of resonance of the carbonyl moiety with – and the dispersal of charge into – the aromatic ring. Such resonance is much less pronounced in the case of esters because of the swamping out effect of the alkoxy moiety (see, for example, Russell, 1973).

We conclude, therefore, that only the benzoate **22** data are dependable. Unfortunately, all the members in this group lie in essentially the same region and, hence, we will only be able to use one or two representative (branched and unbranched) for constructing a ruler.

3.5. Conclusions

In this paper, we have developed and applied five criteria for the selection of molecules for the use in the development of a molecular ruler: (1) *Planarity*: the molecule should be close to planar in structure in order to simplify geometric calculations. (2) *Dipolarity*: it should bear a polar head group and a hydrophobic tail group, to force the molecule to align itself in the bilayer perpendicularly to the interface. (3) *Modularity*: the synthesis of the hydrophobic tails of these rulers should be modular allowing varying lipophilicities. (4) *Reliability*: the reporter carbons should show a large change in chemical shift as we proceed to more polar solvents. Finally (5) *Charge localization*: the reporter carbons should not be part of a strongly conjugated system. Using these criteria, we have seen that benzenediacetic esters **15**, benzenedipropionic esters **17**, and methyl 4-alkoxybenzoates **22** are appropriate systems for use in the construction of a molecular ruler, while 4,4-dialkylcyclohexa-2,5-dienones **1**, N-alkyl pyridones **12**, 4-alkoxypyridine N-oxides **13** and 4-alkoxybenzaldehydes **19** are not. Time has come for the actual construction of a molecular ruler. Our efforts in this regard are described in the companion paper (Cohen et al., 2008a).

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