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Synthesis of a Photoactivatable 9-*Z*-Oleic Acid For Protein Kinase C Labeling

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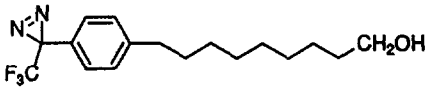
Abstract: A convenient synthesis of (Z)-18-[4-[3(trifluoromethyl)diazirinyl]phenyl]-9-octadecenoic acid (13) for photoaffinity labeling is described. Photochemical experiments on 13 and its precursor 11 in ethanol solution as well as in an argon matrix at 12 K demonstrate the formation of diazo compounds and ultimately carbene-derived products.

Photoaffinity labeling (PAL) is a method used to elucidate the binding sites on enzymes and other biological molecules.[1-3] Protein kinase C (PKC) is a remarkable enzyme which is known to play a central role in signal transmission across membranes in animal cells.[4-7] A unique property of this protein is that its catalytic activity is enhanced by a lipid component of the cell membrane, namely phosphatidylserine.[8] This activity is further enhanced by *sn*-1,2-diacylglycerol. Previous studies have shown that oleic acid activates the enzyme in the presence of *sn*-1,2-diacylglycerol and thus mimics phosphatidylserine.[9] With the purpose of investigating the binding site for oleic acid and hence phosphatidylserine on PKC, we describe here the multi-step synthesis of a diazirine-labeled oleic acid **13**. Since unsaturated fatty acids are important components of lipid membranes, such compounds may have broader applicability in the study of membrane binding proteins. The diazirine group was chosen as a carbene-generating photoactivatable substituent in **13** because azides can sometimes be disadvantageous due to hydrogen abstraction and tar formation from triplet nitrenes; furthermore, aryl azides require photoactivation near 250 nm, which is too close to the UV absorption of proteins (λ < 280 nm).[1-3] In contrast, aryldiazirines absorb at long wavelengths (368 nm for **13**), remote from protein damaging regions. Preliminary photochemical experiments on **13** and one of its precursors (**11**) are also reported.



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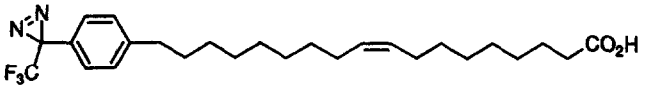
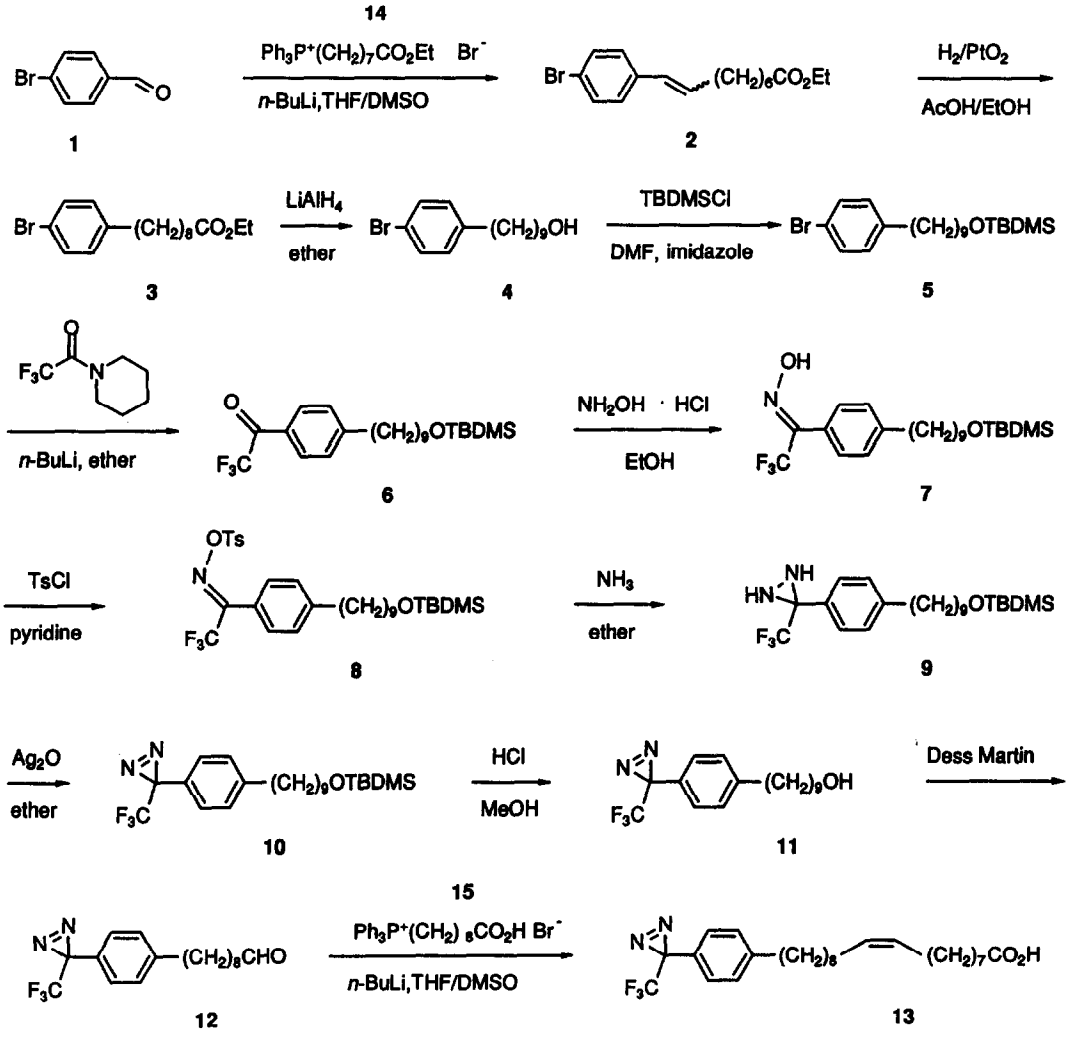


13

The synthesis of photoaffinity labeled fatty acid analogues has previously been described by Khorana et al.[10,11] and Brunner et al.[12] Here, we report the use of two-stage Wittig chain elongations to build up the oleic acid analogue **13**. The first Wittig reaction converted *p*-bromobenzaldehyde (**1**) to the olefin **2** (Scheme 1), islolated in 47% yield as a 2:1 *cis/trans* mixture.



Scheme 1

Hydrogenation with PtO_2 in EtOH/AcOH furnished **3** in 89% yield. Further reduction with lithium aluminum hydride in THF gave alcohol **4** in 96% yield. Protection with TBDMS chloride in imidazole/DMF afforded the TBDMS ether **5** in 96% yield. Subsequent introduction of the diazirine group[13-16] via the trifluoromethyl ketone **6** (49%), oxime **7** (54%), *p*-toluenesulfonyloxime **8** (54%), and

diaziridine **9** (90%) produced the desired diazirine **10** (90%) using oxidation of **9** with silver oxide. The tosylation of **7** with *p*-toluenesulfonyl chloride in refluxing pyridine may be susceptible to further optimization as under the conditions employed, undesirable byproducts were formed. Adventitious removal of the protecting group led to the corresponding primary alcohol and chloride as well as the tosylate.

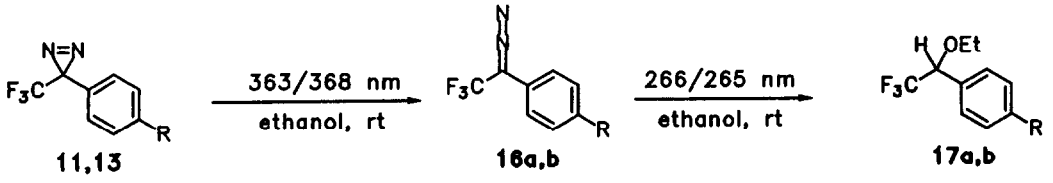
However, **8** could be isolated without purification of the intermediates in an overall yield of 27%, starting with silyl ether **5**. For further characterization, compounds **6-8** were purified by column chromatography (see Experimental Section).

The progress of each reaction step from compound **1** to **9** was monitored by GC. The compounds bearing a diazirine-group (**10-13**) were found to be unstable under GC conditions.

Removal of the protecting group in **10** in acidified methanol and oxidation of the alcohol **11** (80%) with periodinane reagent[17,18] permitted the synthesis of the crucial aldehyde **12** in 52% yield. Swern oxidation as well as other oxidation reagents failed in this case.[19] In the last step, the side chain of **12** was further extended to the final product **13** using the Wittig reagent **15**. ^{13}C NMR revealed that the Z-isomer of **13** was formed exclusively in this reaction. In comparison with natural Z-oleic acid (C-8, C-11: 27.41, 27.41; C-9, C-10: 129.60, 129.90),[20,21] Z-**13** showed the expected chemical shift values (C-8, C-11: 27.19, 27.22; C-9, C-10: 129.82, 129.96) for the olefinic carbon and the adjacent methylene carbon atoms. (For comparison, the ^{13}C resonances of *E*-oleic acid are as follows:[20] C-8, C-11: 32.71, 32.71; C-9, C-10: 130.36, 130.71.) The olefinic hydrogen atoms of **13** appear at δ = 5.33 ppm as a multiplet of a triplet with J = 4.5 Hz, in agreement with the chemical shifts reported for several fatty acid analogues in the literature.[22,23] The FT-IR spectrum shows, beside the strong band due to the carbonyl stretching at 1710 cm^{-1}, two absorption frequencies of medium intensity at 1620 cm^{-1} and 1520 cm^{-1} characteristic of all the compounds bearing a diazirine group (**10-13**). The UV spectrum of **13** reveals a flat broad band at 368 nm (ε 591), ascribed to the diazirine moiety. The main absorption wavelengths at 223 nm (ε 17986) and 201 nm (shoulder; ε 13671) are assigned to the aromatic ring system.

Photochemical experiments were performed on compounds **11** and **13** in ethanol solution in a standard UV cuvette (d = 1 cm) at room temperature.

Monochromatic irradiation at 363 nm and 368 nm, respectively, generated the diazo valence isomers **16** (Scheme 2).



16a,17a: R = $-(CH_2)_9OH$
16a,17b: R = $-(CH_2)_8CH{=}CH(CH_2)_7CO_2H$

Scheme 2

Subsequent irradiation in the main absorption bands (266 nm for **11**, and 265 nm for **13**) caused disappearance of **16** and presumably formation of the corresponding carbenes. In the case of **11**, the ethyl ether **17a**, resulting from an O-H insertion of this reactive intermediate into ethanol, could be isolated and characterized by ^{1}H NMR spectroscopy (δ 1.13 (t, 3 H, J = 7.0 Hz, -CH_3); 3.45 (q, 2 H, J = 7.0 Hz, -OCH_2); 4.46 (q, 1 H, J = 6.5 Hz, H-C-CF_3)). The AA'BB' pattern for the aromatic hydrogen atoms of **17a** (δ 7.18) was shifted 0.13 ppm to lower field in comparison with **11** (δ 7.05) (see Experimental Section).[24]

11 and **13** were converted to the diazo isomers **16** with first order kinetics and half-lives of 376 s and 158 s respectively. The decrease in the bands at 363 nm (**11**) or 368 nm (**13**) was accompanied by increases in a set of bands at 266 (s) and 434 nm (w) for **11** (Figure 1), and at 265 (s) and 434 nm (w) for **13**.

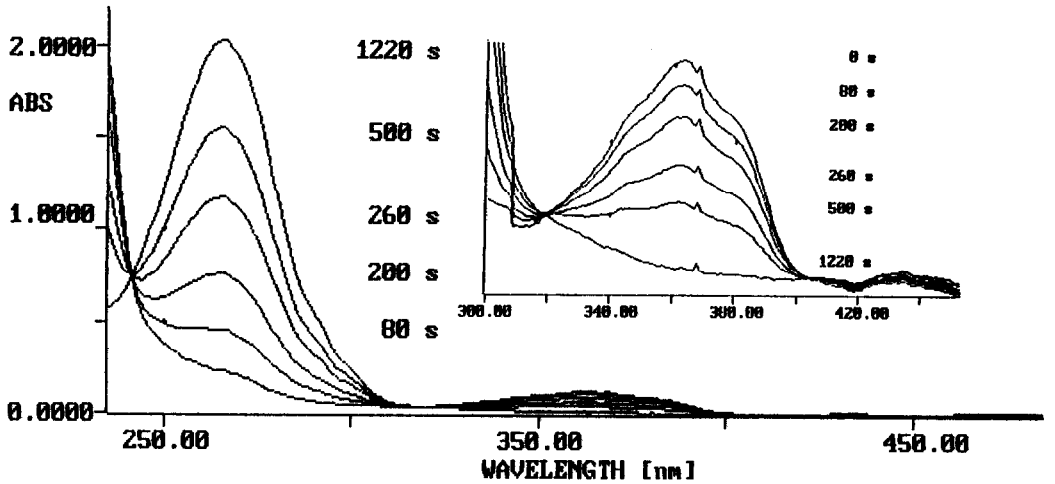


Figure 1. Disappearance of the UV absorption maximum of diazirine **11** in ethanol (c = 0.20 mM) at λ_{max} = 363 nm (inset) and appearance of diazo compound **16a** (λ_{max} = 266 nm and 434 nm (inset)). Similar results were obtained for **13** (see text).

It is well established that the diazirine decomposition is unaffected by acid,[25] whereas the decomposition of diazo compounds is acid catalyzed.[26] As a result, compound **13**, bearing a carboxylic acid function, already showed significant decomposition of the diazo valence isomer after 100 s, indicated by a decreasing absorbance at 265 nm before the diazirine had been fully consumed. In contrast, during the photolysis of **11**, a steady increase of the 266 nm absorbance at the expense of the 363 nm band was observed. After 21 minutes all diazirine was finally consumed, and the 266 nm band (diazo isomer) had reached its maximum absorbance.

Continued irradiation of **11** and **13** at 266 nm and 265 nm, respectively, finally produced UV spectra in which neither the bands around 260 nm and 430 nm (diazo compounds) nor the one near 360 nm (diazirines) were present, i.e. the isomerization of the diazirine to the diazo compound is not reversible.[27]

In biochemical investigations, an instantaneous labeling of the receptor by its ligand on irradiation is desired. Therefore, **11** (c = 0.39 mM in ethanol) was photolyzed in a UV cuvette on broad band irradiation (λ = 335-475 nm) using a 1000 W Hanovia high pressure Xe-Hg lamp. The diazirine **11** now decomposed with a half-life of 17 s, and maximal formation of the diazo isomer (ca. 30%) was observed after 2 min.[28] At this stage, 96% of the diazirine **11** had reacted. The diazo compound disappeared with a half-life of ca. 55 min under these conditions.

The intermediate formation of the diazo valence isomer **16a** from **11** was also confirmed by FT-IR spectroscopy by photolysis of **11** in an argon matrix at 12 K. Irradiation at 363 nm produced a growing band at 2093 cm^{-1} (C=N=N). Further irradiation at 266 nm caused complete and irreversible disappearance of this diazo compound.

Preliminary biochemical experiments with **13** have demonstrated that it does activate PKC in a phosphorylation reaction of histone and that this activation is enhanced by diacylglycerol as in the case of oleic acid itself. An activation of ca 80% of that of oleic acid was achieved (c = 0.12 mM for **13**). These experiments will be reported elsewhere.[29]

Conclusions. (*Z*)-18-[4-[3(trifluoromethyl)diazirinyl]phenyl]-9-octadecenoic acid (**13**) is a photolabile analogue of natural oleic acid. On irradiation with wavelengths > 335 nm (i.e. far away from protein damaging regions), it rapidly eliminates N_2. Although about 30% of the diazirine rearranges to the corresponding diazo valence isomer, this may not be crucial, since the latter is photochemically destroyed as well. The trifluoromethyl group might be useful as a reporter group using ^{19}F NMR technique to follow the biological (noncovalent) and photochemical (covalent) binding of ligands containing oleic acid to receptor proteins. The synthesis of **13** can in principle be modified to incorporate ^{14}C in the carboxylic acid function.

EXPERIMENTAL

IR spectroscopy was performed using a Perkin Elmer 1720-X FT-IR spectrometer. ^1H NMR (200 MHz),^{13}C NMR (50 MHz), ^{19}F NMR (188 MHz) spectra were recorded on a Bruker ACF-200 FT-NMR spectrometer with internal $CDCl_3$ and external $CFCl_3$ standards. High resolution UV spectra were obtained on a Varian Cary 1 UV-Visible spectrophotometer. Photolyses were performed using a 1000 W Hanovia high pressure Xe-Hg lamp coupled with a Schoeffel GM250 monochromator. Kinetic experiments used 1 cm quartz cuvettes at room temperature (25 °C) (c = 0.20 mM for **11**, and 0.05 mM for **13**). The Ar matrix isolation equipment was as previously described.[30] The mass spectrometer was a Kratos MS25RFA. GC analyses were run on a Shimadzu GC-14A gas chromatograph, which was fitted with a 25 m BP5 SGE column and a flame ionization detector. Thin-layer chromatography and column chromatography were performed on precoated Silica gel 60 F_{254} plates and Silica gel 60 (270-430 mesh), respectively (Merck, Darmstadt, FRG). All solvents were distilled and when necessary dried in the appropriate manner.

(*E*+*Z*)-9-(4-Bromophenyl)-8-nonenoic Acid Ethyl Ester (2). The phosphonium salt **14** was prepared in a two step synthesis by esterification[31] of ω-bromooctanoic acid with dry ethanol and TMS chloride, followed by quaternization with triphenylphosphine in absolute benzene under reflux.[32] To a chilled solution of **14** (2.79 g, 5.4 mmol) in 24 mL of dry THF/DMSO (1:1, v/v,) 2.2 mL of 2.5 M *n*-butyllithium in hexane was added dropwise at 0 °C and the resulting brick red solution was left stirring for a further 30 min at room temperature. *p*-Bromobenzaldehyde (**1**) (1.00 g, 5.4 mmol) in 10 mL of dry THF was added at 0 °C and the reaction was then stirred at ambient temperature for 15 h before being poured into saturated NH_4Cl solution. After ether extraction, the solution was dried ($MgSO_4$) and the solvent evaporated in vacuo. The crude product was purified by column chromatography to afford **2** (0.86 g, 2.5 mmol; 47% yield) as a colourless oil: *Z*-isomer: FT-IR (neat) 2930 (m), 2860 (m), 1735 (vs), 1590 (w), 1180 (s), 1070 (s), 1010 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20-1.64 (m, 8 H, H-3, H-4, H-5, H-6), 1.24 (t, 3 H, J = 7.1 Hz, $-CH_3$), 2.27 (t, 2 H, J = 7.2 Hz, H-2), 2.27 (dt, 2 H, J = 7.2 Hz, 7.1 Hz, H-7), 4.11 (q, 2 H, J = 7.1 Hz, $-OCH_2$), 5.66 (dt, 1 H, J = 11.6 Hz, 7.3 Hz, H-8), 6.32 (d, 1 H, J = 11.6 Hz, H-9), 7.27 (AA'BB', 4 H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 14.21 (q, $-CH_3$), 24.82 (t, C-3), 28.42 (t), 28.85 (t), 28.88 (t), 29.56 (t), 34.23 (t, C-2), 60.09 (t, $-OCH_2$), 120.21 (s), 127.66 (d), 130.28 (d), 131.14 (d), 133.72 (d), 136.54 (s), 173.67 (s, C-1); UV (EtOH) $\lambda_{max}(\varepsilon)$ 252 (14596), 207 nm (15448); MS *m/z* (rel intensity) 340 (16, $[M]^+$), 338, (16, $[M]^+$), 294 (8), 292 (7), 250 (3), 213 (4), 197 (22), 195 (23), 184 (54), 182 (49), 169 (26), 143 (15), 129 (22), 116 (100), 88 (51), 55 (34); HRMS calcd for $C_{17}H_{23}BrO_2$ 338.0881/340.0861, found 338.0859/340.0826; R_f 0.29 (*n*-hexane/diethyl ether, 20:1); GC R_t 12.20 min.

9-(4-Bromophenyl)nonanoic Acid Ethyl Ester (3). To a pre-reduced suspension of 70 mg PtO_2 in 17 mL of dry EtOH/AcOH (7:1, v/v) in a Parr apparatus **2** (1.45 g, 4.3 mmol) was added and hydrogenated at room temperature (60 psi) for 5 d. After filtration, the filtrate was concentrated on a rotary evaporator. Ester **3** (1.30 g, 3.8 mmol; 89% yield) was isolated as a colourless liquid by column chromatography: FT-IR (neat) 2930 (vs), 2855 (s), 1740 (vs), 1490 (s), 1180 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (t, 3 H, J = 7.1 Hz, $-CH_3$), 1.29 (m, 8 H, H-4, H-5, H-6, H-7), 1.54-1.66 (m, 4 H, H-3, H-8), 2.28 (t, 2 H, J = 7.5 Hz, H-2), 2.54 (t, 2 H, J = 7.6 Hz, H-9), 4.12 (q, 2 H, J = 7.1 Hz, $-OCH_2$), 7.21 (AA'BB', 4 H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 14.29 (q, $-CH_3$), 24.98 (t, C-3), 2 · 29.11 (t), 29.18 (t), 29.26 (t), 31.30 (t), 34.40 (t, C-2), 35.35 (t, C-9), 60.20 (t, $-OCH_2$), 119.28 (s), 130.18 (d), 131.28 (d), 141.80 (s), 173.92 (s, C-1); UV (EtOH) $\lambda_{max}(\varepsilon)$ 258 sh (2532), 224 nm (9039); MS *m/z* (rel intensity) 342 (5, $[M]^+$), 340 (7, $[M]^+$), 297 (11), 295 (12), 261 (20), 215 (16), 197 (21), 184 (11), 182 (12), 171 (91), 169 (91), 131 (17), 119 (25), 101 (49), 91 (96), 88 (100), 77 (9), 73 (17); HRMS calcd for $C_{17}H_{25}BrO_2$ 340.1038/342.1017, found 340.1038/342.1016; R_f 0.74 (*n*-hexane/diethyl ether, 4:1); GC R_t 12.36 min.

9-(4-Bromophenyl)nonanol (4). To a solution of 0.5 g (13.2 mmol) of lithium aluminum hydride in 7 mL of absolute THF, **3** (1.3 g, 3.8 mmol) was added slowly via a syringe at 0 °C. After removal of the cooling bath, the reaction was kept stirring for a further 12 h at 70 °C. After appropriate work-up procedure of the mixture, column chromatography afforded **4** (1.1 g, 3.7 mmol; 96% yield) as a colourless liquid: FT-IR (neat) 3360 (m), 2930 (vs), 2855 (s), 1490 (s), 1070 (s), 1010 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.29 (m, 10 H, H-3, H-4, H-5, H-6, H-7), 1.51-1.54 (m, 4 H, H-2, H-8), 1.75 (br, 1 H, -OH), 2.54 (t, 2 H,

J = 7.6 Hz, H-9), 3.61 (t, 2 H, J = 6.5 Hz, H-1), 7.30 (AA'BB', 4 H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 25.71 (t), 29.13 (t), 29.36 (t), 29.39 (t), 29.49 (t), 31.28 (t), 32.73 (t), 35.31 (t, C-9), 62.94 (t, C-1), 119.22 (s), 130.14 (d), 131.22 (d), 141.77 (s); UV (EtOH) $\lambda_{max}(\varepsilon)$ 259 sh (2784), 224 nm (9939); MS *m/z* (rel intensity) 300 (11, $[M]^+$), 298 (11, $[M]^+$), 280 (3), 197 (19), 184 (84), 182 (92), 171 (100), 169 (98), 151 (19), 149 (18), 131 (39), 117 (22), 104 (16), 91 (39); HRMS calcd for $C_{15}H_{23}BrO$ 298.0932/300.0913, found 298.0937/300.0903; R_f 0.45 ($CHCl_3$); GC R_t 11.82 min.

[[9-(4-Bromophenyl)nonyl]oxy]-*tert*-butyldimethylsilane (5). A mixture of **4** (16.5 g, 55 mmol), TBDMS chloride (14.1 g, 94 mmol) and imidazole (9.3 g, 137 mmol) 40 mL of dry DMF was heated to 50 °C and stirred for 48 h. Dilution with pentane and water, extraction with pentane, washing with brine, and removal of the solvent afforded 35 g of crude product. After distillation of the volatile components in vacuo, column chromatography gave **5** (21.7 g, 53 mmol; 96% yield) as a colourless liquid: FT-IR (neat) 2930 (vs), 2855 (s), 1490 (m), 1100 (s), 840 (vs) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.00 (s, 6 H, -Si(CH_3)$_2$), 0.85 (s, 9 H, -C(CH_3)$_3$), 1.24 (m, 10 H, H-3, H-4, H-5, H-6, H-7), 1.42-1.56 (m, 4 H, H-2, H-8), 2.50 (t, 2 H, J = 7.6 Hz, H-9), 3.55 (t, 2 H, J = 6.5 Hz, H-1), 7.16 (AA'BB', 4 H, Ar-H); ^{13}C NMR ($CDCl_3$) δ -5.23 (q, -SiCH_3), 18.40 (s, -$\underline{C}CH_3$), 25.81 (t), 26.02 (q, -C$\underline{C}H_3$), 29.19 (t), 29.42 (t), 29.43 (t), 29.56 (t), 31.34 (t), 32.89 (t), 35.37 (t, C-9), 63.32 (t, C-1), 119.27 (s), 130.17 (d), 131.26 (d), 141.82 (s); UV (EtOH) $\lambda_{max}(\varepsilon)$ 259 (2001), 220 (8631), 207 nm sh (7545); MS *m/z* (rel intensity) 413 (0.3, $[M-H]^+$,$[M+H]^+$), 411 (0.3, $[M-H]^+$), 399 (1), 397 (1), 357 (54), 355 (54), 277 (16), 171 (23), 169 (23), 91 (20), 89 (24), 75 (100); HRMS calcd for $C_{21}H_{36}SiBrO$ ($[M-H]^+$) 411.1719, found 411.1703; R_f 0.85 (*n*-hexane/diethyl ether, 7:3); GC R_t 14.10 min. Anal. Calcd for $C_{21}H_{37}SiBrO$: C, 61.00; H, 9.02. Found: C, 61.40; H, 9.33.

[[9-[4-(2,2,2-Trifluoroacetyl)phenyl]nonyl]oxy]-*tert*-butyldimethylsilane (6). To a stirred solution of **5** (19.14 g, 46 mmol) in 240 mL of dry ether, 19.2 mL of 2.5 M *n*-butyllithium in hexane was added dropwise at -30 °C under an N_2-atmosphere. The reaction mixture was allowed to warm to 0 °C within 2 h and then cooled again to -50 °C. Subsequently, N-(trifluoroacetyl)piperidine (10.50 g, 58 mmol) in 72 mL of dry ether was added. After 3 h stirring at -50 °C, the cooling bath was removed, and the mixture was hydrolyzed with saturated aqueous NH_4Cl at 0 °C. The organic layer was washed three times with saturated aqueous NH_4Cl and once with saturated brine and dried with $MgSO_4$. The solvent and excess of N-(trifluoroacetyl)piperidine were evaporated in vacuo. The crude product (22.30 g) was subjected to column chromatography and gave **6** (9.79 g, 23 mmol; 49% yield) as a colourless yellow oil: FT-IR (neat) 2930 (vs), 2860 (s), 1720 (vs), 1260 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.00 (s, 6 H, -Si(CH_3)$_2$), 0.85 (s, 9 H, -C(CH_3)$_3$), 1.25 (m, 10 H, H-3, H-4, H-5, H-6, H-7), 1.47 (m, 2 H, H-2), 1.60 (m, 2 H, H-8), 2.65 (t, 2 H, J = 7.4 Hz, H-9), 3.55 (t, 2 H, J = 6.4 Hz, H-1), 7.62 (AA'BB', 4 H, Ar-H); ^{13}C NMR ($CDCl_3$) δ -5.24 (q, -SiCH_3), 18.39 (s, -$\underline{C}CH_3$), 25.81 (t), 26.01 (q, -C$\underline{C}H_3$), 29.26 (t), 29.39 (t), 29.43 (t), 29.53 (t), 30.93 (t), 32.90 (t), 36.24 (t, C-9), 63.31 (t, C-1), 116.83 (q, J = 291 Hz, -CF_3), 127.66 (s), 129.19 (d), 130.33 (m, J = 2.1 Hz), 151.92 (s), 180.11 (q, J = 34 Hz, C=O); ^{19}F NMR ($CDCl_3$) δ -71.80; UV (EtOH) $\lambda_{max}(\varepsilon)$ 266 (4199), 217 nm (2395); MS *m/z* (rel intensity) 431 (0.3, $[M+H]^+$), 430 (0.1, $[M]^+$), 429 (0.4, $[M-H]^+$), 415 (1), 373 (32), 323 (25), 277 (9), 229 (28), 211 (23), 201 (21), 152 (23), 117 (20), 91 (28), 77 (100),

75 (75), 73 (41); HRMS calcd for $C_{23}H_{37}SiF_3O_2$ 430.2515, found 430.2496; R_f 0.83 (*n*-hexane/diethyl ether, 7:3); GC R_t 13.08 min. Anal. Calcd for $C_{23}H_{37}SiF_3O_2$: C, 64.15; H, 8.66. Found: C, 64.13; H, 8.95.

[[9-[4-[1-(Hydroxyimino)-2,2,2-trifluoroethyl]phenyl]nonyl]oxy]-*tert*-butyldimethylsilane (7). To a well stirred mixture of NH_2OH·HCl (170 mg, 2.4 mmol) and NaOH (97 mg, 2.4 mmol) in 9 mL of refluxing dry ethanol was added a solution of of **6** (1040 mg, 2.4 mmol) in 1.2 mL of dry ethanol all at once. After refluxing for 16 h, the solvent was evaporated in vacuo and the residue partitioned between ether and water. The organic layer was washed three times with 0.01 N HCl and three times with water and dried over $MgSO_4$. Removal of the solvent and purification of the crude product (900 mg) afforded **7** (580 mg, 1.3 mmol; 54% yield) as a colourless oil, which solidified while standing in the refrigerator: *syn*- or *anti*-oxime: FT-IR (neat) 3310 (m), 2930 (vs), 2860 (vs), 1610 (m), 1515 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.00 (s, 6 H, -Si(CH_3)$_2$), 0.84 (s, 9 H, -C(CH_3)$_3$), 1.22 (m, 10 H, H-3, H-4, H-5, H-6, H-7), 1.45 (m, 2 H, H-2), 1.60 (m, 2 H, H-8), 2.57 (t, 2 H, J = 7.7 Hz, H-9), 3.55 (t, 2 H, J = 6.4 Hz, H-1), 7.31 (AA'BB', 4 H, Ar-H), 8.55 (br, 1 H, -OH); ^{13}C NMR ($CDCl_3$) δ -5.22 (q, -SiCH_3), 18.46 (s, -$\underline{C}$CH_3), 25.79 (t), 26.03 (q, -C$\underline{C}H_3$), 29.35 (t), 29.44 (t), 29.44 (t), 29.55 (t), 31.14 (t), 32.77 (t), 35.95 (t, C-9), 63.62 (t, C-1), 120.84 (q, J = 275 Hz, -CF_3), 123.38 (s), 128.56 (d), 128.67 (d), 145.81 (s), 147.29 (q, J = 32 Hz, C=N); ^{19}F NMR ($CDCl_3$) δ -66.93; UV (EtOH) λ_{max}(ε) 245 (10663), 204 nm (17454); MS *m/z* (rel intensity) 446 (1, $[M+H]^+$), 430 (1), 388 (43), 304 (43), 296 (32), 251 (41), 228 (8), 202 (14), 182 (19), 152 (14), 130 (14), 116 (51), 75 (100); HRMS calcd for $C_{23}H_{39}SiNF_3O_2$ ($[M+H]^+$) 446.2702, found 446.2674; R_f 0.73 (*n*-hexane/diethyl ether, 7:3); GC R_t 15.27 min. Anal. Calcd for $C_{23}H_{38}SiNF_3O_2$: C, 61.99; H, 8.59; N, 3.14. Found: C, 62.09; H, 8.93; N, 2.97.

[[9-[4-[1-[[*p*-Toluenesulfonyl)oxy]imino]-2,2,2-trifluoroethyl]phenyl]nonyl]oxy]-*tert*-butyldimethylsilane (8). To a stirred solution of **7** (0.62 g, 1.4 mmol) in 4 mL of dry pyridine was added *p*-toluenesulfonyl chloride (630 mg, 3.3 mmol) in one step and the mixture was refluxed for 1 h. Immediately after heating the reaction mixture to 115 °C, GC showed disappearance of oxime **7** [t = 15.27 min (6%)] and appearance of two new peaks due to the formation of the unprotected alcohol [t = 13.38 min (28%)] and tosylate **8** [t = 11.48 min (21%)]. The solvent was evaporated in vacuo and the thick slurry was partitioned between water and ether. After removal of the ether, column chromatography gave **8** (0.45 g, 0.8 mmol; 54% yield) as a pale yellow oil: FT-IR (neat) 2930 (vs), 2860 (s), 1610 (m), 1600 (m) cm^{-1}; ^1H NMR ($CDCl_3$) 0.00 (s, 6 H, -Si(CH_3)$_2$), 0.85 (s, 9 H, -C(CH_3)$_3$), 1.25 (m, 10 H, H-3, H-4, H-5, H-6, H-7), 1.46 (m, 2 H, H-2), 1.57 (m, 2 H, H-8), 2.41 (s, 3 H, -CH_3), 2.58 (t, 2 H, J = 7.7 Hz, H-9), 3.55 (t, 2 H, J = 6.5 Hz, H-1), 7.26 (AA'BB', 4 H, Ar-H), 7.54 (AA'BB', 4 H, Ts-H); ^{13}C NMR ($CDCl_3$) δ -5.24 (q, -SiCH_3), 18.39 (s, -$\underline{C}$CH_3), 21.77 (q, -CH_3), 25.81 (t), 26.01 (q, -C$\underline{C}H_3$), 29.32 (t), 29.41 (t), 29.44 (t), 29.53 (t), 31.04 (t, C-8), 32.90 (t, C-2), 35.96 (t, C-9), 63.32 (t, C-1), 119.76 (q, J = 278 Hz, -CF_3), 121.79 (s), 128.54 (d, Ar), 128.81 (d, Ar), 129.29 (d, Ts), 129.86 (d, Ts), 131.32 (s), 146.05 (s), 147.27 (s), 153.96 (q, J = 33 Hz, C=N); ^{19}F NMR ($CDCl_3$) δ -66.95; UV (EtOH) λ_{max}(ε) 259 (12206), 228 (17469), 203 nm (22360); MS *m/z* (rel intensity) 600 (0.3, $[M+H]^+$), 598 (0.1, $[M-H]^+$), 584 (0.4), 542 (21), 372 (73), 302 (76), 229 (100), 155 (36), 130 (31), 116 (47), 91 (69), 75 (87); HRMS calcd

for $C_{30}H_{43}SSiNF_3O_4$ ([M-H]$^+$) 598.2634, found 598.2637; R_f 0.50 (*n*-hexane/diethyl ether, 10:1). GC R_t 11.46 min. Anal. Calcd for $C_{30}H_{44}SSiNF_3O_4$: C, 60.07; H, 7.39; N, 2.34. Found: C, 60.41; H, 7.71; N, 2.23.

[[9-[4-[(Trifluoromethyl)diaziridinyl]phenyl]nonyl]oxy]-*tert*-butyldimethylsilane (9). To a solution of **8** (450 mg, 0.75 mmol) in 7 mL of dry ether in a sealed 100 mL tube was condensed 5 mL of liquid ammonia at -50 °C. The solution was stirred for 20 h at room temperature before being poured into a beaker and digested with ether. Generated *p*-toluenesulfonamide was filtered off and washed with ether. The combined filtrate was extracted three times with half-saturated brine, the ether layer dried ($MgSO_4$) and the solvent evaporated on a rotary evaporator in vacuo. **9** (300 mg, 0.68 mmol; 90% yield) was obtained in pure form by column chromatography: FT-IR (neat) 3250 (w), 2930 (vs), 2860 (vs), 1260 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.00 (s, 6 H, -Si(CH_3)$_2$), 0.85 (s, 9 H, -C(CH_3)$_3$), 1.25 (m, 10 H, H-3, H-4, H-5, H-6, H-7), 1.46-1.55 (m, 4 H, H-2, H-8), 2.16, 2.71 (AB, 2 H, *J* = 8.6 Hz, N-H), 2.56 (t, 2 H, *J* = 7.3 Hz, H-9), 3.55 (t, 2 H, *J* = 6.5 Hz, H-1), 7.30 (AA'BB', 4 H, Ar-H); ^{13}C NMR ($CDCl_3$) δ -5.30 (q, -$SiCH_3$), 18.34 (s, -$\underline{C}CH_3$), 25.79 (t), 25.96 (q, -$C\underline{C}H_3$), 29.26 (t), 2 · 29.41 (t), 29.54 (t), 31.27 (t), 32.86 (t), 35.72 (t, C-9), 57.85 (q, *J* = 36 Hz, C-N), 63.25 (t, C-1), 123.64 (q, *J* = 278 Hz, -CF_3), 127.98 (m, *J* = 1.1 Hz), 128.71 (d), 128.99 (s), 145.13 (s); ^{19}F NMR ($CDCl_3$) δ -76.15; UV (EtOH) λ_{max}(ε) 218 (16393), 201 nm (12015); MS *m/z* (rel intensity) 445 (3, [M+H]$^+$); 429 (3), 387 (100), 304 (21), 275 (24), 228 (41), 199 (16), 181 (73), 144 (19), 130 (33), 116 (63), 91 (21), 75 (83); HRMS calcd for $C_{23}H_{40}SiN_2F_3O$ ([M+H]$^+$) 445.2862, found 445.2862; R_f 0.31 (*n*-hexane/diethyl ether, 10:1); GC R_t 13.40 min. Anal. Calcd for $C_{23}H_{39}SiN_2F_3O$: C, 62.13; H, 8.84; N, 6.30. Found: C, 62.01; H, 8.90; N, 6.29.

[[9-[4-[(Trifluoromethyl)diazirinyl]phenyl]nonyl]oxy]-*tert*-butyldimethylsilane (10). Diaziridine **9** (270 mg, 0.6 mmol) in 3 mL of dry ether was treated with 260 mg of freshly precipitated Ag_2O in the dark. After stirring for 4 h, filtration of the reaction mixture followed by evaporation of the solvent afforded diazirine **10** (240 mg, 5.4 mmol; 90% yield): FT-IR (neat) 3040 (w), 2930 (vs), 2860 (s), 1620 (w), 1520 (w), 1260 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.00 (s, 6 H, -Si(CH_3)$_2$), 0.85 (s, 9 H, -C(CH_3)$_3$), 1.24 (m, 10 H, H-3, H-4, H-5, H-6, H-7), 1.42-1.56 (m, 4 H, H-2, H-8), 2.54 (t, 2 H, *J* = 7.6 Hz, H-9), 3.55 (t, 2 H, *J* = 6.5 Hz, H-1), 7.08 (AA'BB', 4 H, Ar-H); ^{13}C NMR ($CDCl_3$) δ -5.27 (q, -$SiCH_3$), 18.41 (s, -$\underline{C}CH_3$), 25.88 (t), 26.01 (q, -$C\underline{C}H_3$), 28.43 (q, *J* = 40 Hz, C-N), 29.29 (t), 2 · 29.49 (t), 29.61 (t), 31.28 (t), 32.95 (t), 35.69 (t, C-9), 63.31 (t, C-1), 122.33 (q, *J* = 275 Hz, -CF_3), 126.38 (s), 126.42 (m, *J* = 1.4 Hz), 128.91 (d), 144.82 (s); ^{19}F NMR ($CDCl_3$) δ -65.84; UV (EtOH) λ_{max}(ε) 363 (669), 222 (18331), 200 nm (18114); MS *m/z* (rel intensity) 443 (1, [M+H]$^+$), 442 (0.2, [M]$^+$), 441 (0.4, [M-H]$^+$), 415 (1), 385 (3), 246 (15), 245 (84), 153 (100), 107 (23), 75 (43), 73 (40), 69 (24), 55 (40), 41 (32); HRMS calcd for $C_{23}H_{37}SiN_2F_3O$ 442.2627, found 442.2619; R_f 0.79 (*n*-hexane/diethyl ether, 20:1).

9-[4-[3-(Trifluoromethyl)diazirinyl]phenyl]nonanol (11). Diazirine **10** (1.40 g, 3.1 mmol) was dissolved in 2 mL of methanol and 2 drops of concentrated HCl were added via a Pasteur pipette. The

reaction was complete after 30 min.; 2 mL of saturated aqueous Na_2CO_3 was then added and the mixture was extracted with ether. The combined organic layers were washed three times with brine and then dried over $MgSO_4$. Removal of the solvent and purification of the crude product (1.25 g) by column chromatography gave **11** (0.81 g, 2.5 mmol; 80% yield) as a colourless oil: FT-IR (neat) 3350 (m), 2930 (vs), 2860 (s), 1620 (m), 1520 (m), 1180 (vs) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.21 (m, 10 H, H-3, H-4, H-5, H-6, H-7), 1.41-1.51 (m, 4 H, H-2, H-8), 1.71 (br, 1 H, -OH), 2.51 (t, 2 H, J = 7.6 Hz, H-9), 3.53 (t, 2 H, J = 6.6 Hz, H-1), 7.05 (AA'BB', 4 H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 25.74 (t), 28.38 (q, J = 40 Hz, C-N), 29.19 (t), 29.37 (t), 29.40 (t), 29.49 (t), 31.19 (t), 32.74 (t), 35.59 (t, C-9), 62.93 (t, C-1), 122.24 (q, J = 275 Hz, $-CF_3$), 126.34 (s), 126.38 (m, J = 1.3 Hz), 128.86 (d), 144.78 (s); ^{19}F NMR ($CDCl_3$) δ -65.84; UV (EtOH) $\lambda_{max}(\varepsilon)$ 363 (631), 221 (15875), 203 nm (10785); MS *m/z* (rel intensity) 328 (0.1, $[M]^+$), 300 (2), 280 (7), 231 (11), 201 (11), 188 (100), 172 (20), 151 (20), 118 (17), 91 (17), 69 (9), 55 (20), 41 (13); HRMS calcd for $C_{17}H_{23}N_2F_3O$ 328.1763, found 328.1769; R_f 0.37 (*n*-hexane/diethyl ether, 1:1). Anal. Calcd for $C_{17}H_{23}N_2F_3O$: C, 62.18: H, 7.06; N, 8.53. Found: C, 62.47; H, 7.32; N, 8.46.

9-[4-[3-(Trifluoromethyl)diazirinyl]phenyl]nonanal (12). To a solution of periodinane (2.10 g, 5.0 mmol) and 0.65 mL of dry pyridine in 10 mL of dry methylene chloride was added **11** (0.81 g, 2.5 mmol) in 0.5 mL of dry methylene chloride at 0 °C. After stirring for 1 h at 0 °C and 4 h at room temperature, there was added consecutively 7 mL of ether (1 h stirring) and a solution of 7.00 g $Na_2S_2O_3 \cdot 5\ H_2O$ in 10 mL of saturated aqueous $NaHCO_3$ (15 min stirring). After extraction with ether, drying over $MgSO_4$ and removal of the solvent, column chromatography afforded **12** (0.42 g, 1.3 mmol; 52% yield) as a pale yellow oil: FT-IR (neat) 2930 (vs), 2855 (s), 1730 (s), 1180 (vs), 1155 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.23 (m, 8 H, H-4, H-5, H-6, H-7), 1.54 (m, 4 H, H-3, H-8), 2.34 (dt, 2 H, J = 7.3 Hz, 1.8 Hz, H-2), 2.53 (t, 2 H, J = 7.6 Hz, H-9), 7.07 (AA'BB', 4 H, Ar-H), 9.69 (t, 2 H, J = 1.8 Hz, H-1); ^{13}C NMR ($CDCl_3$) δ 22.06 (t, C-3), 28.42 (q, J = 40 Hz, C-N), 2 · 29.14 (t), 29.25 (t), 29.27 (t), 31.18 (t), 35.61 (t, C-9), 43.92 (t, C-2), 122.25 (q, J = 275 Hz, $-CF_3$), 126.39 (s), 126.43 (m, J = 1.1 Hz), 128.89 (d), 144.74 (s), 198.61 (d, C-1); ^{19}F-NMR ($CDCl_3$) δ -65.84; UV (EtOH) $\lambda_{max}(\varepsilon)$ 363 (417), 222 (19152), 201 nm (15866); MS *m/z* (rel intensity) 327 (0.4, $[M+H]^+$), 326 (0.5, $[M]^+$), 298 (10), 270 (5), 229 (31), 199 (71), 186 (45), 172 (100), 151 (53), 145 (25), 131 (41), 117 (25), 91 (21), 69 (31), 55 (72), 41 (72); HRMS calcd for $C_{17}H_{21}N_2F_3O$ 326.1606, found 326.1592; R_f 0.78 (*n*-hexane/diethyl ether, 1:1). Anal. Calcd for $C_{17}H_{21}N_2F_3O$: C, 62.56; H, 6.49; N, 8.58. Found: C, 62.16; H, 6.11; N, 8.52.

(Z)-18-[4-[3-(Trifluoromethyl)diazirinyl]phenyl]-9-octadecenoic Acid (13). The phosphonium salt **15** was prepared by oxidation of ω-bromononanol with PDC[33] in dry methylene chloride, followed by quaternization with triphenylphosphine in dry benzene under reflux.[32] To **15** (184 mg, 0.37 mmol) in 2 mL of dry THF/DMSO (1:1, v/v) was added 0.3 mL of 2.5 M *n*-butyllithium in hexane at 0 °C and the resulting orange-red solution was left stirring for 1 h at room temperature. The ylide solution was cooled to -10 °C and **12** (100 mg, 0.31 mmol) in 0.3 mL of dry THF was added to the solution all at once. The colour turned from orange-red to green and finally to a red solution. After stirring for a further 12 h at room temperature in the dark, the reaction mixture was hydrolyzed with 0.5 mL of saturated aqueous

NH_4Cl at 0 °C. Extraction with ether under neutral conditions gave 170 mg of crude product, which contained the desired compound **13** (17%) (^1H NMR). No product was observed in the 30 mg of crude product isolated from an acidified solution. Purification by column chromatography afforded **13** (20 mg, 0.04 mmol; 14% yield) as a pale yellow oil (purity > 95% by ^1H NMR spectroscopy): FT-IR (neat) 3390 (m), 2930 (vs), 2860 (vs), 1710 (s), 1620 (m), 1520 (m), 1180 (vs), 1160 (vs) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.29, 1.58 (m, 22 H, H-3, H-4, H-5, H-6, H-7, H-12, H-13, H-14, H-15, H-16, H-17), 2.00 (m, 4 H, H-8, H-11), 2.33 (t, 2 H, *J* = 7.4 Hz, H-2), 2.59 (t, 2 H, *J* = 7.6 Hz, H-18), 5.33 (mt, 2 H, *J* = 4.5 Hz, H-9, H-10), 7.13 (AA'BB", 4 H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 24.73 (t, C-3), 27.19 (t, C-8/C-11), 27.22 (t, C-8/C-11), 28.42 (q, *J* = 40 Hz, C-N), 29.07 (t), 29.10 (t), 29.18 (t), 29.22 (t), 29.25 (t), 29.30 (t), 29.44 (t), 29.46 (t), 29.70 (t), 29.76 (t), 31.23 (t, C-2), 35.62 (t, C-18), 122.24 (q, *J* = 275 Hz, $-CF_3$), 126.34 (s), 126.40 (m, *J* = 1.2 Hz), 128.89 (d), 129.82 (d, C-9/C-10), 129.96 (d, C-9/C-10), 144.83 (s), 178.78 (s, C-1); ^{19}F NMR ($CDCl_3$) δ -65.84; UV (EtOH) $\lambda_{max}(\varepsilon)$ 368 (591), 223 (17986), 201 nm (13671); MS *m/z* (rel intensity) 466 (0.1, $[M]^+$), 438 (13), 418 (3), 398 (7), 294 (45), 277 (40), 267 (35), 240 (25), 226 (43), 186 (29), 172 (100), 154 (24), 95 (24), 81 (29), 69 (32); HRMS calcd for $C_{26}H_{37}N_2F_3O_2$ 466.2807, found 466.2785; R_f 0.28 (*n*-hexane/diethyl ether, 1:1).

9-[4-[1-(Hydroxy)-2,2,2-trifluoromethyl]phenyl]nonanol (17a). ^1H NMR δ 1.13 (t, 3 H, *J* = 7.0 Hz, $-CH_3$), 1.19 (m, 10 H, H-3, H-4, H-5, H-6, H-7), 1.45 (m, 4 H, H-2, H-8), 2.50 (t, 2 H, *J* = 7.7 Hz, H-9), 3.45 (q, 2 H, *J* = 7.7 Hz, $-OCH_2$), 3.53 (t, 2 H, *J* = 6.5 Hz, H-1), 4.46 (q, 1 H, *J* = 6.5 Hz, H-C-CF_3), 7.18 (AA'BB', 4 H, Ar-H); UV (EtOH) $\lambda_{max}(\varepsilon)$ 254 (1509), 216 nm (12791).

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