Fluorinated analogues of lipidic dialkynylcarbinol pharmacophores: synthesis and cytotoxicity in HCT116 cancer cells

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Lipidic alkynylcarbinols (LACs) have been identified as potential antitumor compounds, and a thorough understanding of their pharmacophoric environment is now required to elucidate their biological mode of action. In the dialkynylcarbinol (DAC) series, a specific study of the pharmacophore potential has been undertaken by focusing on the synthesis of three fluorinated derivatives followed by their biological evaluation. This work highlights the requirement of an electron-rich secondary carbinol center as a key structure for cytotoxicity in HCT116 cells.

Introduction

Originally identified as natural bioactive compounds extracted from marine sponges or constitute a class of potential antitumoral compounds [2]. Over the past few years, efforts have been devoted to the delineation of both optimal structural features [3] and cellular targets [4] of LAC derivatives exhibiting *in vitro* anti-proliferative activity against the HCT116 colon tumor cell line. Noteworthy is a significant and systematic chirality effect, *i.e.* a dependence of the cytotoxicity on the absolute configuration of The IC_{50} value of a racemic LAC sample is thus expected to be roughly two times higher than that of the corresponding eutomer: results in the racemic series are however relevant prior to optimization

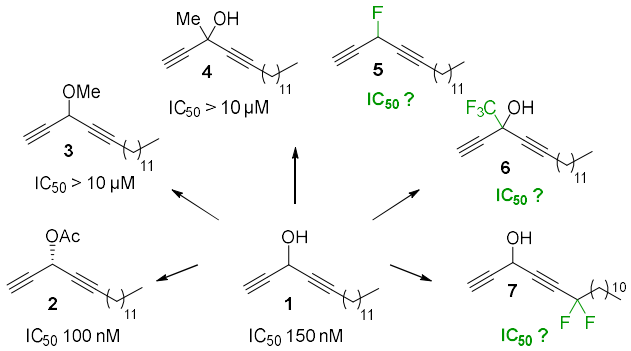
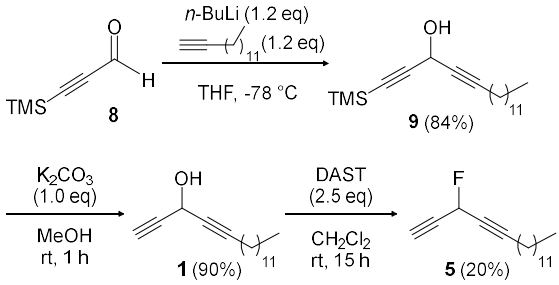


Figure 1. Insight into structure-activity relationships for the cytotoxicity of DACs through systematic pharmacophore modulation.

in the eutomeric series. In the search for the highest potency among various LAC pharmacophore models [3b], the dialkynylcarbinol (DAC) **1** was selected as a reference for its high bioactivity (IC_{50} = 150 nM), structural simplicity and ready synthetic accessibility (**Figure 1**) [5]. The impact on cytotoxicity of further alterations of the DAC warhead and lipidic environment of **1** was then explored (**Figure 1**). We reported that the acetylated analogue (*S*)-**2** displayed a cytotoxicity in HCT116 similar to that of (*S*)-**1**, probably due to *in cellulo* hydrolysis of the acetate moiety, releasing the free DAC [5]. In contrast, the *O*-methylated derivative **3** proved to be inactive (IC_{50} > 10 µM) [6]. This dramatic effect of a slight modification (H → Me) could be attributed to the stability of the methyl ether group in biological medium, persistently masking the alcohol moiety. Furthermore, the *C*-methylated tertiary alcohol **4** displayed the same lack of activity. Overall, the results for **3** and **4** may thus reflect the carbinol inability to be oxidized, which may be important for its cytotoxicity. Further investigations were thus envisaged through the impoverishment of the DAC warhead by fluorination, the targeted fluorinated analogues being expected to provide a new panel to the systematic study of the DAC pharmacophore.

Results and discussion

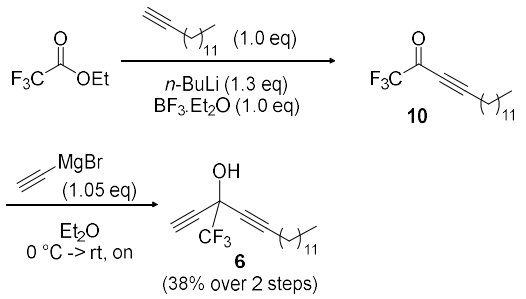
The racemic bis-propargylic fluoride **5**, deoxyfluorinated analogue of **1**, was readily obtained by direct treatment of **1** with diethylaminosulfur trifluoride (DAST, **Scheme 1**) [7].



Scheme 1. Synthesis of the deoxyfluorinated analogue **5** of the reference DAC **1**.

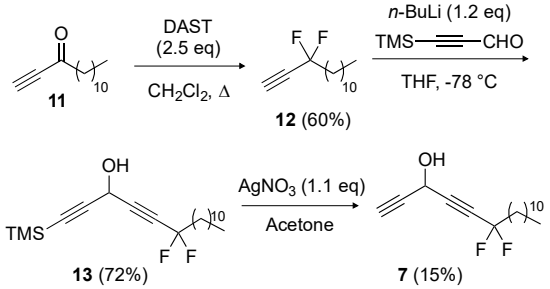
While full conversion of the alcohol was observed by TLC monitoring, **5** was isolated in low yield. This outcome can be attributed to the particularly high volatility of the hydrocarbon fluoride **5**, impeding efficient purification steps and drying procedures. The trifluoro analogue **6** of the tertiary alcohol **4** was secured by addition of ethynylmagnesium bromide onto the trifluoromethylated ynone **10** (**Scheme 2**) [8], itself obtained by reaction of a single equivalent of tetradecynyllithium on commercial ethyl trifluoroacetate in the presence of a

stoichiometric amount of boron trifluoride etherate [9].



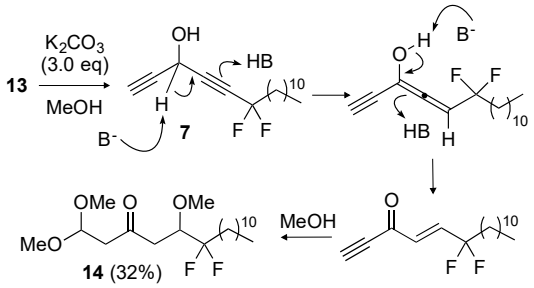
Scheme 2. Synthesis of trifluoromethylated tertiary dialkynylcarbinol **6**.

The difluorinated analogue **7** with a CF_2 group in place of the propargylic methylene group of **1** was obtained through a concise three-step sequence (**Scheme 3**). 3,3-Difluorotetradec-1-yne **12** was prepared by DAST-mediated deoxofluorination of the corresponding ynone **11** [10], and its lithium salt was in turn added to TMS-propynal to give **13** with 72% yield (**Scheme 3**). Desilylation was not observed even under fluorination conditions with an over-stoichiometric amount of DAST.



Scheme 3. Synthesis of the difluorinated dialkynylcarbinol **7**.

Removal of the TMS group, first attempted under standard basic conditions (K_2CO_3/MeOH), led to the formation of an unexpected compound. The latter was identified as the product resulting from the conjugate addition of three molecules of MeOH onto the Michael acceptor formed by a base-induced isomerization of the difluorinated alkynylcarbinol **7** [11]. This structural assignment was notably based on several key data from mass spectrometry (*m/z* = 380), ^{1}H NMR (3 OC*H*$_3$ signals at 3.49, 3.40 and 3.39 ppm, absence of the characteristic terminal acetylenic proton) and ^{13}C NMR (C=O signal at 205 ppm). Even though several isomers of this structure may be formed, under basic conditions, the structure **14** appears the most likely (**Scheme 4**).



Scheme 4. Base-induced isomerization of **7** and subsequent transformation into the triple Michael adduct **14** upon desilylation of **13**.

The final desilylation was smoothly accomplished using $AgNO_3$ (**Scheme 3**). The highly sensitive difluoro-DAC **7** was isolated with 15% yield after column chromatography. It gave characterization data in full agreement with the expected structure.

The antitumoral potential of the fluorinated DAC analogues **5-7** was then assessed by *in vitro* cell viability assays in cancer cells (**Table 1**). The HCT116 cell line was selected as a model. This representative human colon carcinoma cell line is frequently used in the biological evaluation of potential antitumor compounds. In addition,

cytotoxicity data against HCT116 allow direct comparison with our previous work [2], [3], [4].

Table 1. Cytotoxic activity of DAC analogues in HCT 116 cancer cells.

DAC derivatives	IC_{50} [μM]	DAC derivatives	IC_{50} [μM]
rac-**1**	0.15[a,2a]	*rac*-**5**	>10[a]
(*S*)-**2**	0.10[a,5]	*rac*-**6**	>10[a]
rac-**3**	>10[a,6]	*rac*-**7**	>10[a]
rac-**4**	>50[b,6]		

[a]Cells were seeded in 96-well plates and were treated with concentrations ranging from 10 nM to 10 μM; after 48 or 72 h, the number of live cells was evaluated by a standard MTT test. [b]Cells were seeded in 96-well plates and were treated with concentrations ranging from 10 nM to 50 μM; after 96 h, the number of live cells was evaluated by a WST test.

None of the fluorinated electron-impoverished DAC analogues **5**, **6** and **7** displayed any detectable activity at concentrations up to 10 μM (**Table 1**). These results indicate a correlation between the oxidability of DACs or analogues and their cytotoxicity (see Introduction and Figure 1): deoxyfluorination or proximal fluorination of the carbinol center of **1** indeed induces an increase of the oxidation potential in **5-7**. These results point to the requirement of an electron-rich carbinol center for inducing cytotoxicity against HCT116 cells.

Conclusions

The disclosed results highlight key features for cytotoxicity of DAC pharmacophores, *i.e.* the actual presence, secondary character and electron-richness of the carbinol center. Although the corresponding dialkynylketone was described as non-cytotoxic [6], the essential need for a readily oxidizable carbinol pharmacophore provides a guideline for the design of DACs with improved cytotoxicity. While a prominent criterion for cytotoxic drug candidates is their selectivity towards tumor cells *vs.* normal cells, the very first criterion is their intrinsic activity. The disclosed fluorinated LACs being basically not cytotoxic, they serve as "negative guidelines" for the design of non-fluorinated candidates, for which this concern will be addressed in due course after optimization.

Experimental part

General.

All reagents were obtained from commercial suppliers and used without any further purification. Dichloromethane and tetrahydrofuran (THF) were obtained by filtration through a drying column on a filtration system. Thin-layer chromatography (TLC) analyses were performed on silica gel precoated plates (Merck 60 F254). Visualization of the developed chromatogram was performed by UV light (254 nm) and using 10 % phosphomolybdic acid in EtOH, or aqueous potassium permanganate ($KMnO_4$) stain. Flash column chromatography purifications were performed using flash silica gel (SDS 35-70 mm). Nuclear magnetic resonance spectra were recorded in $CDCl_3$ on a Bruker Advance 300 MHz spectrometer. Chemical shifts for ^1H NMR spectra are recorded in parts per million (ppm) with the solvent resonance as the reference

$CDCl_3$ (δ = 7.26 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad), coupling constant in Hz and integration. Chemical shifts for ^{13}C NMR spectra are recorded in parts per million using the central peak of $CDCl_3$ (δ = 77.16 ppm) as the reference. All ^{13}C NMR spectra were obtained with complete proton decoupling. Chemical shifts for ^{19}F NMR spectra are recorded in parts per million (ppm) with an internal standard resonance as the reference fluorobenzene (δ = -113.15 ppm). IR analysis were run on a Thermo-Nicolet Diamond ATR (4 cm^{-1} of resolution, 16 scans) equipped with a DTGS detector. High-resolution mass spectrometry (HRMS) was performed on a Thermo-Finnigan MAT 95 XL instrument.

Experimental procedures and characterizations.

3-fluorohexadeca-1,4-diyne (**5**). DAST (0.1 mL, 0.8 mmol, 2.0 equiv.) was added to a solution of previously described [5] heptadeca-1,4-diyn-3-ol (100 mg, 0.4 mmol) in CH_2Cl_2 (27 mL) at -10 °C. The reaction mixture was warmed up to rt and was stirred overnight. Pentane and water (1:1) were added. The aqueous phase was extracted with pentane and the organic layer was separated. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was submitted to flash chromatography over silica gel (up to pentane/Et_2O, 9 : 1) to give compound **5** (20 mg, 20%) as a colorless oil: $\boldsymbol{R_f}$ = 0.61 (100% pentane). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 5.68 (dq, J = 46.9, 2.1 Hz, 1H), 2.74 (dd, J = 5.3, 2.3 Hz, 1H), 2.27 (qd, J = 7.0, 2.1 Hz, 2H), 1.6-1.45 (m, 2H), 1.42-1.22 (m, 18H), 0.95-0.80 (m, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 86.3, 83.3 (d, J = 252.0 Hz), 77.1, 72.3, 68.2, 52.3, 29.7, 29.6, 29.2, 29.0, 28.9, 28.6, 28.4, 18.8, 18.5. **^{19}F NMR** (282 MHz, $CDCl_3$) δ -160.4 (m). **MS** (DCI-NH_3 neg.): *m/z* 249 (M-1). **FTIR** (cm^{-1}) (neat): 3300, 2922, 2853, 2319, 2242, 2135, 1467, 1313, 1154, 999, 934.

3-(trifluoromethyl)heptadeca-1,4-diyn-3-ol (**6**). An ethynylmagnesium bromide solution (1.88 mL, 1.05 equiv., 0.5 M in THF) was added to a solution of 1,1,1-trifluorohexadec-3-yn-2-one **10** (260 mg, 0.90 mmol) in THF (5 mL) under N_2 atmosphere at -78 °C. The mixture was stirred at rt overnight. A saturated aqueous NH_4Cl solution was added, the aqueous layer was extracted with Et_2O and the organic layer was separated. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was submitted to flash chromatography over silica gel (up to pentane/Et_2O, 8 : 2) to give trifluoromethylated dialkynylcarbinol **6** (108 mg, 38% over two steps) as a brown oil: $\boldsymbol{R_f}$ = 0.5 (pentane/Et_2O, 8 : 2). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 2.94 (s, 1H), 2.68 (s, 1H), 2.26 (t, J = 7.1 Hz, 2H), 1.63-1.53 (m, 2H), 1.43-1.27 (m, 18H), 0.92 (t, J = 6.7 Hz, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 122.0 (q, J = 285.0 Hz), 88.9, 74.4, 72.8, 68.1, 32.1, 29.8, 29.8, 29.7, 29.6, 29.5, 29.1, 28.9, 27.9, 22.8, 18.7. **HRMS** (DCI-

CH_4): calcd for $C_{18}H_{28}F_3O$ $[M+H]^+$: 317.2092 *m/z*, found: 317.2102 *m/z*. **FTIR** (cm^{-1}) (neat): 3408 (br), 3307, 2924, 2854, 2246, 2129, 1266, 1193, 1173, 1073, 998.

6,6-difluoroheptadeca-1,4-diyn-3-ol (**7**). 6,6-Difluoro-1-(trimethylsilyl)heptadeca-1,4-diyn-3-ol **13** (320 mg, 0.9 mmol) was stirred overnight with $AgNO_3$ (168 mg, 0.99 mmol, 1.1 equiv.) in wet acetone (5 mL). The reaction was quenched with water and Et_2O was added. The aqueous layer was extracted with Et_2O (3 x 4 mL) and the organic layer was separated. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was submitted to flash chromatography over SiO_2 (with a gradient elution up to pentane/Et_2O, 9 : 1) to give the desired product **7** (38 mg, 15%) as a brown oil: R_f = 0.5 (pentane/Et_2O 9:1). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 5.22 – 5.16 (m, 1H), 2.62 (d, J = 2.4 Hz, 1H), 2.31 (d, J = 8.0 Hz, 1H), 2.14 – 1.91 (m, 2H), 1.62 – 1.45 (m, 2H), 1.28 (d, J = 12.2 Hz, 16H), 0.88 (t, J = 6.7 Hz, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 114.8 (t, J = 233.4 Hz), 83.1 (t, J = 6.9 Hz), 79.4 (t, J = 2.3 Hz), 77.8, 74.2, 51.9, 39.1 (t, J = 25.5 Hz), 32.1, 29.7, 29.6, 29.5, 29.4, 29.1, 22.8, 22.7 (t, J = 3.5 Hz), 14.3. **^{19}F NMR** (282 MHz, $CDCl_3$) δ -83.99 (td, J = 15.1, 3.8 Hz). **HRMS** (DCI-CH_4): calcd for $C_{17}H_{27}F_2O$ $[M+H]^+$: 285.2030 *m/z*, found: 285.2027 *m/z*. **FTIR** (cm^{-1}) (neat): 3364 (br), 3310, 2925, 2855, 2262, 2126, 1031.

1,1,1-trifluorohexadec-3-yn-2-one (**10**). According to previously reported reaction conditions [9], to a stirred solution of 1-tetradecyne (1.73 mL, 7.04 mmol) in THF (11 mL) was added *n*-butyllithium (3.7 mL, 9.15 mmol, 2.5 M in hexanes) dropwise at -78 °C. The resulting solution was stirred at the same temperature for 30 min, at which time a solution of ethyl trifluoroacetate (0.84 mL, 7.04 mmol) and boron trifluoride diethyl etherate (0.87 mL, 7.04 mmol) in THF (2 mL) was introduced dropwise. After reaction completion (ca. 1 h), a saturated aqueous NH_4Cl solution was added. The aqueous layer was separated and extracted with Et_2O. The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure to give the crude product, which was purified by flash chromatography on silica gel (up to pentane/Et_2O, 8 : 2) to afford desired trifluoromethylketone **10** (260 mg, 13%). Spectral data were comparable with those previously reported in the literature [9b] and are not described in detail.

Tetradec-1-yn-3-one (**11**). Dess-Martin periodinane reagent (202 mg, 0.48 mmol, 1 equiv.) was added to a solution of tetradec-1-yn-3-ol (100 mg, 0.48 mmol) in CH_2Cl_2 (2 mL) under N_2 atmosphere. The mixture was stirred at rt overnight. A saturated aqueous $NaHCO_3$ solution was added, the aqueous layer was extracted with CH_2Cl_2 and the organic layer was separated. The combined organic layers were

dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was submitted to flash chromatography over silica gel (up to pentane/Et_2O, 8 : 2) to give ketone **11** (86 mg, 87%) as a yellow oil: R_f = 0.57 (pentane/Et_2O, 8 : 2). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 3.19 (s, 1H), 2.58 (t, J = 7.4 Hz, 1H), 1.67 (t, J = 7.2 Hz, 2H), 1.57 (s, 1H), 1.33-1.22 (m, 16H), 0.95 (t, J = 6.0 Hz, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 187.8, 81.6, 78.4, 45.6, 32.0, 29.7, 29.5, 29.4, 29.0, 23.9, 22.8, 14.3. **FTIR** (cm^{-1}) (neat): 3299, 3255, 2853, 2924, 2093, 1683, 1466.

3,3-difluorotetradec-1-yne (**12**). Tetradec-1-yn-3-one **11** (1.5 g, 7.2 mmol) and DAST (2.23 mL, 18 mmol, 2.5 equiv.) were heated neat at 56 °C and stirred under nitrogen atmosphere over 5 h. The crude product was submitted to flash chromatography over silica gel (up to pentane /Et_2O, 95 :5) to give the difluorinated product **12** (1.0 g, 60%) as a yellow oil: R_f = 0.57 (pentane/Et_2O, 8 : 2). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 2.74 (t, J = 4.9 Hz, 1H), 2.10-1.94 (m, 2H), 1.60-1.50 (m, 2H), 1.39-1.22 (m, 16H), 0.93 (t, J = 5.4 Hz, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 114.6 (t, J = 232.0 Hz), 75.1 (t, J = 6.8 Hz), 39.2 (t, J = 25.4 Hz), 32.1, 29.9, 29.7, 29.6, 29.5, 29.1, 23.0, 14.3. **^{19}F NMR** (282 MHz, $CDCl_3$) δ -83.9 (td, J = 15.1 Hz, 5.0 Hz). **FTIR** (cm^{-1}) (neat): 3305, 2923, 2854, 2134, 2095, 1685, 1466.

6,6-difluoro-1-(trimethylsilyl)heptadeca-1,4-diyn-3-ol (**13**). *n*-Butyllithium solution (2.6 mL, 0.65 mmol, 1.2 equiv., 2.5 M in hexanes) was added to a solution of 3,3-difluorotetradec-1-yne **12** (125 mg, 0.54 mmol) in THF (15 mL) under N_2 atmosphere at -78 °C. After 45 min of stirring, 3-(trimethylsilyl) propiolaldehyde (0.1 mL, 3.17 mmol, 5.8 equiv.) was added at -78 °C. Temperature and stirring were maintained during 30 min. A saturated aqueous NH_4Cl solution was added, the aqueous layer was extracted with Et_2O and the organic layer was separated. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was submitted to flash chromatography over silica gel (up to pentane/Et_2O, 9 : 1) to give dialkynylcarbinol **13** (140 mg, 72%) as a brown oil: R_f = 0.47 (pentane/Et_2O, 9 : 1). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 5.17 (dt, J = 7.5, 3.7 Hz, 1 H), 2.26 (d, J = 7.3 Hz, 1H), 2.12-1.96 (m, 2H), 1.60-1.49 (m, 2H), 1.37-1.22 (m, 16H), 0.94 (t, J = 13.4 Hz, 3H), 0.20 (s, 9H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 127.2, 117.8, 114.7, 111.6, 100.0, 91.2, 83.5 (t, J = 7.0 Hz), 77.3, 52.3, 39.1 (t, J = 25.5 Hz), 31.9, 29.6, 29.5, 29.4, 29.0, 23.3, 14.1, 0.0. **^{19}F NMR** (282 MHz, $CDCl_3$) δ -83.79 (td, J = 15.0 Hz, 3.6 Hz). **FTIR** (cm^{-1}) (neat): 3390 (br), 2926, 2856, 1467, 1252, 1043, 845, 761.

6,6-difluoro-1,1,5-trimethoxyheptadecan-3-one (**14**): 6,6-Difluoro-1-(trimethylsilyl)heptadeca-1,4-diyn-3-ol **13** (140 mg, 409 µmol) was stirred overnight with K_2CO_3 (169 mg, 1.2 mmol, 3 equiv.) and MeOH (2 mL). A saturated aqueous NH_4Cl solution was added, the aqueous layer was extracted with Et_2O (3 x 5 mL) and the organic layer was separated. The

combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was submitted to flash chromatography over SiO_2 (up to pentane/Et_2O, 9 : 1) to give the triple Michael adduct **14** (50 mg, 33%) as a brown oil: R_f = 0.5 (pentane/Et_2O 9:1). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 4.84 (t, J = 5.6 Hz, 1H), 4.05 –3.92 (m, 1H), 3.49 (s, 3H), 3.40 (s, 3H), 3.39 (s, 3H), 2.81 (dd, J = 5.6, 2.0 Hz, 2H), 2.76 (d, J = 5.8 Hz, 2H), 2.07 – 1.72 (m, 2H), 1.59 – 1.47 (m, 2H), 1.33-1.19 (m, 16H), 0.97 (t, J = 6.7 Hz, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 204.5 (C=O), 125.7 (t, J = 245.9 Hz, CF_2), 101.4, 77.4 (dd, J = 30.4, 27.8 Hz), 60.0 (OMe), 54.2 (OMe), 53.8 (OMe), 47.4, 43.7 (dd, J = 3.8, 1.9 Hz), 32.7 (t, J = 24.1 Hz), 32.0, 29.7, 29.6, 29.6, 29.5, 29.5, 22.8, 21.4 (t, J = 4.3 Hz), 14.3. **MS** (DCI-NH_3 neg.): *m/z* 280 (M-1).

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