Ethynylogation approach in antitumor lipid pharmacochemistry: from dialkynyl-carbinols to trialkynyl-carbinols

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A recently proposed "ethynylogation" pharmacomodulation approach, first envisaged in the series of anticancer lipidic dialkynylcarbinols (DACs) H–C≡C–CH(OH)–C≡C–R at the levels of the H–O)C–H bond. The so-devised mono-lipidic trialkynylcarbinol (TAC) target $(HC{\equiv}C)_2C(OH)–C{\equiv}CR$ and its bis-lipidic counterpart $HC{\equiv}C–C(OH)(C{\equiv}CR)_2$ were synthesized in 4 steps with 33 % and 23 % overall yield, respectively. Their antitumor cytotoxicity has been evaluated towards HCT116 cells: while the latter doubly lipidic TAC is totally inactive (IC_{50} > 120 µM), the former DAC-ethynylogous TAC still exhibits a significant toxicity with an IC_{50} of 40 µM.

Introduction

With the view to setting the title terms in a proper context, scholar emphasis is first given to the basic notion of structure-activity relationships (SARs), which correlate the presence of a generic chemical unit with particular biological/therapeutic effects [1]. The first-key SAR unit can be a *global* molecular skeleton that can be modified/ decorated by various second-key functional units (e.g. norsteroids, corticosteroids or macrolides, related to contraceptive, anti-inflammatory or antibiotic effects, respectively). On the other hand, the first-key SAR unit can be restricted to a *local* well-defined substructure, called a *pharmacophore* [2], that can be embedded in various second-key environments (e.g. beta-lactams, benzodiazepines or arylpropionic acids related to antimicrobial, anxiolytic or analgesic effects, respectively). It is worth noting that SARs can be more or less specific: the number of identified antitumor pharmacophores is thus actually as large as the number of targetable biochemical pathways, and almost as large as the number of marketed anticancer drugs [3].

Classical optimization approaches around a given pharmacophore consist in sequential modifications of the local environment by isomerization (e.g. enantiomerization), reduction/oxidation (e.g. CH–OH → C=O), analogation (e. g. O→S, CH→N), C-H fluorination or general C-H substitution. Homologation (by CH_2-insertion) and vinylogation (by –HC=CH- insertion) are also established pharmacomodulation approaches, within the pharmacophoric unit itself [4] or at its periphery. An alternative "ethynylogation" approach has been recently proposed [5,6]: it is based on the assumption that a third-key SAR criterion would be a distance constraint between the pharmacophore and any component of its surrounding, independently from any relative spatial orientation constraint between them (so regardless conformational effects).

The ethynylogation approach has been first implemented in the series of synthetic lipidic dialkynylcarbinols (DACs) of general formula HC≡C–CH(OH)–C≡CR, inspired from natural lipidic alkenyl-alkynylcarbinols (AACs) (*E*)-HC≡C–CH(OH)–CH=CHR extracted from marine sponges [7]. Beyond efforts aiming at the total synthesis of natural AACs [8], synthesis and biological evaluation of both simplified and modified congeners thereof allowed the identification of non-natural DACs as potent *in vitro* antitumor cytotoxic agents, in particular against the HCT116 cell-line [9]. Starting from the recently disclosed lead **1** (R = *n*-$C_{12}H_{25}$, IC_{50} ≈ 0.10 μM; Figure 1) [5,9a], the C_5OH DAC unit itself, C≡C–C(OH)–C≡C, has been formally separated from either two components of its surrounding, the terminal acetylenic H and the lipidic chain R, by a distance of *ca* 2.4 ± 0.1 Å through the insertion of a C_2 ethyndiyl unit. The corresponding independent ethynylogations thus defined the external and internal butadiynyl-

alkynylcarbinols (BACs) **2** and **3**, respectively, which were synthesized in either racemic or 90 % ee-scalemic forms [5]. Compared to **1**, while the external BAC **2** was found to display a decreased cytotoxicity ($IC_{50} \approx 10$ µM for *rac*-**2**), the internal BAC **3** showed an enhancement thereof: $IC_{50} = 0.12$ µM for *rac*-**3** and $IC_{50} = 0.04$ µM for (*S*)-(+)-**3**, *vs* $IC_{50} = 0.10$ µM for (*S*)-(+)-**1**. A dramatic absolute configuration effect, previously observed for the DAC **1** [9a], was thus also evidenced for the BAC **3**, with an eudismic ratio of *ca* one order of magnitude.

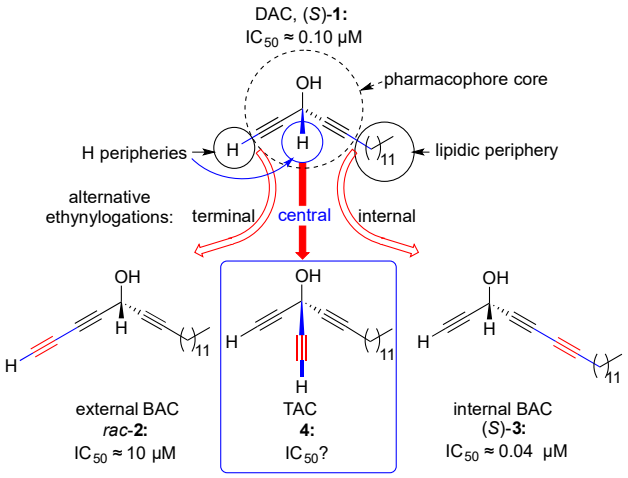
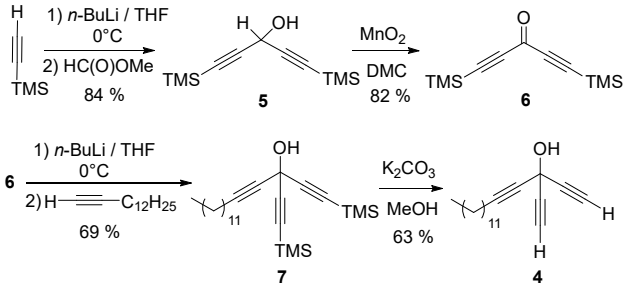


Figure 1. Generalization of the ethynylogation approach in the lipidic DAC series. IC_{50} values correspond to cytotoxicity against HCT116 tumor cells (MTT tests) [5].

The ethynylogation approach is hereafter implemented for the third surrounding component of the C_5OH unit, namely the H atom of the secondary carbinol center of **1**. The so-devised ethynylogous target is thus the trialkynylcarbinol (TAC) **4**. Contrary to the DAC **1** and BAC **3**, the TAC **4** is achiral, thus advantageously allowing disregard of asymmetric synthesis issues.

Results and discussion

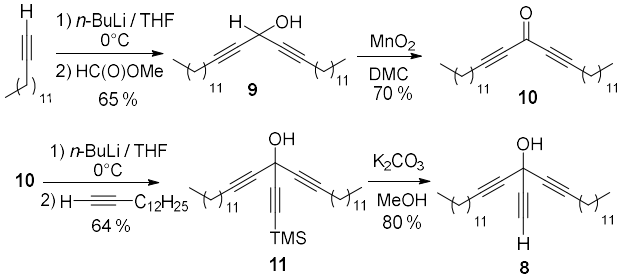
The TAC target **4** was prepared in four steps from trimethylsilylacetylene *via* the previously known DAC **5** and corresponding diynone **6**, obtained sequentially with 84 % and 82 % yield, respectively (Scheme 1) [10]. Reaction of **6** with the lithium salt of 1-tetradecyne gave the disilylated TAC **7** with 89 % yield. Ultimate proto-desilylation of **7** to the TAC target **4** in the presence of K_2CO_3 in methanol proceeded with a 63 % yield. Both the lipidic TACs **7** and **4** happen to be solid products, which were characterized by ^1H, ^{13}C NMR spectroscopy and DCI/CH_4 HRMS.



Scheme 1. Four-step synthesis of the mono-lipidic TAC **4**.

For the sake of comparison, a TAC bearing a single terminal ethynyl moiety and two lipidic alkynyl chains was also envisaged. The so-defined target **8** was prepared in four steps from 1-tetradecyne, sequentially *via* the DAC **9** (65 % yield), the corresponding diynone **10** (70 % yield) and the silylated TAC **11** (64 % yield). Treatment of the latter with K_2CO_3 in methanol afforded the TAC **8** with 80 % yield. These previously unknown doubly lipidic "skipped

diynes" [11] or triynes were obtained as solid products and characterized by ^{1}H, ^{13}C NMR spectroscopy and DCI/CH_4 HRMS.



Scheme 2. Four-step synthesis of the bis-lipidic TAC **8**.

The cytotoxicity of the TACs **4** and **8** was evaluated towards the HCT116 cell-line (Table 1), and compared with that of the parent DAC (*S*)-(+)-**1** (Table 1). Whereas the doubly lipidic TAC **8** was found to have no detectable activity (IC_{50} > 120 μM), the mono-lipidic counterpart **4** turned out to display a sizeable cytotoxicity, with IC_{50} = 40 μM, namely of the same order of magnitude as the BAC *rac*-**2** (10 μM) (Figure 1). With the particular batch of HCT116 cells used for the MTT tests, the reference DAC (+)-**1** showed a reproducible IC_{50} value of 0.10 μM [5].

In the HC≡C–C**R'**(OH)–C≡C$C_{12}H_{25}$ tertiary series (**R'** ≠ H), the decrease in cytotoxicity of the TACs **4** and **8** (**R'** = C≡CH, C≡C$C_{12}H_{25}$) with respect to the secondary DAC **1** (**R'** = H) is *a priori* consistent with a previous observation of complete inactivity of the methylated tertiary DAC (**R'** = CH_3: IC_{50} > 50 μM) [9d]. Nevertheless, the residual activity of **4** shows that the tertiary character of the carbinol center is not a crippling criterion. By comparison to the tertiary methylated DAC [9d] and TAC **8**, the sizeable cytotoxicity of **4** gives formal support to the ethynylogation approach: from **R'** = H in **1** to **R'** = C≡CH in **4**, the structure just undergoes a second-order geometrical modification, *i.e.* a translation of the H atom from the carbinol center by 2.3 Å, resulting in a secondary change of the IC_{50} value. The same translation of another H atom of **1** (the acetylenic H) was observed to have the same effect (in terms of log units) by going from the DAC **1** to the BAC **2** [5].

Table 1. MTT test results against the HCT116 cancer cell-line

Derivative	IC_{50} [nM]
(*S*)-(+)-**1**	0.10
4	40
8	> 120

The cytotoxicity was evaluated for the lipidic TACs **4** and **8** *vs* their parent lipidic DAC **1**. Cells were seeded in 96-well plates and treated with concentrations ranging from 5 nM to 120 μM; after 72 h, the number of live cells was evaluated by standard MTT tests. MTT test of the three compounds were performed in strictly identical conditions (in parallel the same days, with the same biological and auxiliary chemical materials).

The disclosed results are *a priori* consistent with a basic principle of quantitative SAR (QSAR) analysis, stating that continuous variations of a single structural parameter (here a C···H distance, independently from angular parameters and local steric hindrance) entails a "proportional" variation of the biological activity at stake [12].

Conclusions and perspectives

With the view to testing further possible merits of the formal ethynylogation approach, the present results complete the scope of SARs between DACs and anticancer cytotoxicity: beyond the formerly explored BAC series [5], the TAC series is now addressed, providing the first example a tertiary DAC, **4**, with a non-vanishing cytotoxicity. Beyond further ethynylogation of the BAC lead **3**, further prospects are also naturally suggested within the generalized DAC series H–C≡C–C**R'**(OH)–C≡C–**R**, and in particular the systematic variation of the length of the aliphatic chain **R**, and the C-H fluorination of the carbinol center of **1** by going from **R'** = **H** to **R'** = **F**. The F atom should indeed bring less conformational disorder than any alkyl or alkylethynyl **R'** group, and *ca*. a half iso-directional hindrance compared to the second ethynyl group of **4** (**R'** = C≡CH). Beside the limited steric effect, an additional effect of the F substituent should be the enhancement of the OH acidic character.

Experimental part

Synthesis material and methods

The reactions were carried out under an argon atmosphere, in solvents previously dried and distilled: tetrahydrofuran (THF) over sodium/ benzophenone, dichloromethane (DCM) over CaH_2 and methanol (MeOH) over magnesium and iodine. All the other solvents, petroleum ether (PE), ethyl acetate, and reagents were used as commercially available. Commercial solutions of *n*-BuLi were 1.6 M in hexanes. Analytic thin-layer chromatography (TLC) was performed with 0.20 mm silica gel 60F254 plates. Chromatograms were revealed under UV light and/or moistened with 10 % phosphomolybdic acid in EtOH, and visualized on a heating plate. Column chromatography was carried out on silica gel 60A (SDS 35-70 mm). NMR spectra were recorded with a Bruker Avance 300 instrument from solutions of the samples in $CDCl_3$. ^1H and ^{13}C NMR chemical shifts δ are quoted in parts per million (ppm), with positive values to high frequency relative to the tetramethylsilane reference determined from the residual or main solvent peak; coupling constants *J* are given in Hertz. Mass spectra (MS) were obtained on a GCT 1er CAB109 (Waters).

Synthesis

1,5-bis(trimethylsilyl)penta-1,4-diyn-3-ol (**5**). To a flame-dried flask equipped with a condenser, charged with a solution of trimethylsilylacetylene (0.5 mL, 3.61 mmol, 1 equiv.) in THF (20 mL), was dropwise added 1.6 M solution of *n*-BuLi in hexanes (2.2 mL, 3.43 mmol, 0.95 equiv.) at 0°C. After stirring for 20 min at 0 °C, methyl formate (106 mL, 1.73 mmol, 0.48 equiv.) was added, and the resulting solution was gently heated to the limit of reflux with the aid of a hair dryer (until appearance of small bubbles and colour change from yellow to brown). After cooling back to

room temperature and treatment with saturated aqueous NH_4Cl, the aqueous layer was extracted with Et_2O and the combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (PE/Et_2O, 7/3, R_f = 0.7) to give 369 mg of a solid yellow product assigned to the known DAC **5**, with 84 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.13 (d, J = 6.6 Hz, 1H), 2.37 (s, 1H), 0.22 (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (2 C≡C) 101.65, (2 ≡C-Si) 89.73, (C-OH) 53.04, (9 C-Si) -0.34.

1,5-bis(trimethylsilyl)penta-1,4-diyn-3-one (**6**). To a solution of 1,5-bis(trimethylsilyl)penta-1,4-diyn-3-ol **5** (310 mg, 1.38 mmol, 1 equiv.) in DCM (15 mL), was added γ-MnO_2 in one portion (1.8 g, 0.02 mol, 15 equiv.), and the mixture was stirred for 24 h at room temperature (completion of the reaction monitored by TLC). The reaction mixture was then filtered through a pad of Celite®, and the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel chromatography (PE/Et_2O, 90/10, R_f = 0.7) to give an orange solid product assigned to the known diynone **6** with 82 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.29 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (C=O) 160.29, (2 C≡C) 102.51, (2 ≡C-Si) 99.41, (9 C-Si) -0.90, -0.92; MS (DCI-CH_4): *m/z* (%) [MH^+] = 223 (100), [$MC_2H_5^+$] = 251(55).

1-(trimethylsilyl)-3-[2-(trimethylsilyl)ethynyl] heptadeca-1,4-diyn-3-ol (**7**). A flame-dried flask was charged under dry argon with a solution of 1-tetradecyne (200 µL, 0.81 mmol, 1 equiv.) in THF (5 mL). To the stirred solution at 0 °C, was slowly added a 1.6 M solution of *n*-BuLi in hexanes (490 µL, 0.77 mmol, 0.95 equiv.). After stirring for 30 min at the same temperature, a solution of 1,5-bis(trimethylsilyl)penta-1,4-diyn-3-one **6** (162 mg, 0.73 mmol, 0.9 equiv.) in THF (1 mL) was added and the mixture was stirred at -78°C for a further 10 min. After treatment with saturated aqueous NH_4Cl solution, the aqueous layer was extracted with DCM. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (petroleum ether/ethyl acetate, 90/10, R_f = 0.15) to give 209 mg of an orange oil assigned to **7** with 69 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.80 (s, 1H), 2.28 (t, J = 7.1 Hz, 2H), 1.63–1.53 (m, 2H), 1.30 (s, 18H), 0.97–0.85 (m, 3H), 0.24 (s, 19H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (2 C≡C) 102.15, (C≡C) 87.35, (2 C≡C) 84.60, (2 C≡C) 78.11, (C-OH) 54.67, (11 CH_2) 31.94, 29.70, 29.67, 29.65, 29.59, 29.38, 29.17, 28.87, 28.05, 22.72, 18.78, (CH_3) 14.15, (3 C-Si) -0.43; MS (DCI-CH_4): *m/z* [MH^+] = 417; HRMS (DCI-CH_4): *m/z* [MH^+] calcd = 417.3009, found = 417.3008.

3-ethynylheptadeca-1,4-diyn-3-ol (**4**). To a solution of the silylated TAC **7** (109 mg, 0.261 mmol, 1equiv.) in methanol (4 mL) was added K_2CO_3 (36 mg, 0.261 mmol, 1 equiv.). The solution was stirred for 4 h at room temperature (completion of the reaction followed on TLC), before treatment with brine. The aqueous layer was extracted with DCM, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (PE/Et_2O, 90/10, R_f = 0.2) to give 44 mg of a clear viscous oil product, assigned to **4** with 63 % yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.93 (s, 1H), 2.71 (s, 2H), 2.29 (t, J = 7.2 Hz, 2H), 1.64–1.52 (m, 2H), 1.47–1.23 (m, 18H), 0.90 (t, J = 6.75 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (C≡C) 85.31, (C≡C) 81.38, (C≡C) 77.30, (C≡C) 71.00, (C-OH) 53.83, (11 CH_2) 31.94, 29.68, 29.66, 29.62, 29.50, 29.38, 29.09, 28.87, 28.01, 22.72, 18.66, (CH_3) 14.15; MS (DCI-CH_4): *m/z* [MH^+] = 273; HRMS(DCI-CH_4): *m/z* [MH^+] calcd = 273.2218, found = 273.2216.

Nonacosa-13,16-diyn-15-ol (**9**). A flame-dried flask was charged with a solution of 1-tetradecyne (500 µL, 2.03 mmol, 1 equiv.) in THF (15 mL), under dry argon atmosphere. A 1.6 M solution of *n*-BuLi in hexanes (1.32 mL, 2.24 mmol, 1.1 equiv.) was added slowly at 0 °C. The solution was stirred for 30 min at the same temperature, then a solution of methyl formate (62 µL, 1.02 mmol, 0.5 equiv.) in THF (3 mL) was added and the mixture was stirred at 0°C for further 30 min. After treatment with a saturated aqueous NH_4Cl solution and extractions with DCM, the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (PE/ethyl acetate, 95/05, R_f = 0.25) to give 275 mg of a white solid product, assigned to **9** with 65 % yield. Mp = 47°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.1(s, 1H), 2.25 (td, J = 7.1, 2.1 Hz, 4H), 2.13 (s, 1H), 1.55 (q, 4H), 1.42–1.30 (m, 36H), 0.92 (t, J = 6.75 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (2 C≡C) 85.23, (2 C≡C) 78.05, (CH-OH) 52.61, (22 CH_2) 31.95, 29.70, 29.67, 29.66, 29.55, 29.39, 29.16, 28.92, 28.41, 22.72, 18.75 (2 CH_3) 14.16, 14.14; MS (DCI-CH_4): *m/z* [MH^+] = 417, [$MC_2H_5^+$] = 445.

Nonacosa-13,16-diyn-15-one (**10**). To a solution of nonacosa-13,16-diyn-15-ol **9** (104 mg, 0.25 mmol, 1 equiv.) in DCM (10 mL), was added γ-MnO_2 (326 mg, 3.75 mmol, 15 equiv.) in one portion. The mixture was stirred for 24 h at room temperature (completion of the reaction monitored by TLC). The mixture was then filtered through a pad of Celite® using DCM and the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel chromatography (PE/Et_2O, 85/15, R_f = 0.5) to give 72 mg of a white viscous oil

product, assigned to **10** with 70 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.40 (t, J = 7.1 Hz, 4H), 1.67–1.55 (m, 4H), 1.29 (d, J = 4.9 Hz, 36H), 0.94–0.85 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (C=O) 161.50, (2 C≡C) 94.78, (2 C≡C) 82.34, (22 CH_2) 31.94, 29.67, 29.65, 29.62, 29.46, 29.38, 29.05, 28.90, 27.58, 22.72, 19.13, (2CH_3) 14.14; IR (neat): ν (cm^{-1}) = 3440, 2965, 2158, 1622, 1255, 1249, 1163, 879, 846, 760; MS (DCI-CH_4): *m/z* (%) [MH^+] = 415 (100); HRMS (DCI-CH_4): *m/z* [MH^+] calcd = 415.3940, found = 415.3925.

15-[2-(trimethylsilyl)ethynyl]nonacosa-13,16-diyn-15-ol (**11**). A flame-dried flask was charged with a solution of ethynyl-trimethylsilane (80 µL, 0.54 mmol, 1.5 equiv) in THF (5 mL) under dry argon atmosphere. A 1.6 M solution of *n*-BuLi in hexanes (340 µL, 0.54 mmol, 1.5 equiv.) was slowly added at -78 °C. After stirring for 30 min at the same temperature, a solution of nonacosa-13,16-diyn-15-one **10** (150 mg, 0.36 mmol, 1 equiv.) in THF (0.5 mL) was added and the mixture stirred at -78 °C for a further 10 min. After treatment with 5 % aqueous NH_4Cl solution and extractions with DCM, the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (PE/ethyl acetate, 90/10, R_f = 0.15) to give 119 mg a yellow viscous oil product, assigned to **11** with 64 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.77 (s, 1H), 2.27 (t, J = 7.2 Hz, 4H), 1.60-1.55 (m, 4H), 1.47–1.22 (m, 36H), 0.91 (t, J = 6.4, 1.9 Hz, 6H), 0.23 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (C≡C) 102.82, (C≡C) 86.55, (C≡C) 83.96, (C≡C) 78.69, (C-OH) 54.56, (22 CH_2) 31.95, 29.71, 29.67, 29.67, 29.58, 29.39, 29.17, 28.89, 28.14, 22.72, 18.77, (2 CH_3) 14.16, 14.14, (3 C-Si) -0.36; MS (DCI-CH_4): *m/z* [MH^+] = 513; HRMS (DCI-CH_4): *m/z* [MH^+] calcd = 513.4492, found = 513.4481.

15-ethynylnonacosa-13,16- diyn-15-ol (**8**). To a solution of the silylated trialkynylcarbinol **11** (80 mg, 0.16 mmol, 1 equiv) in methanol (2 mL) was added K_2CO_3 (20 mg, 0.16 mmol, 1 equiv.). The solution was stirred for 4 h at room temperature (completion of the reaction followed on TLC) before treatment with brine. The aqueous layer was extracted with DCM, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (PE/Et_2O, 85/15, R_f = 0.15) to give 55 mg of a white viscous oil product, assigned to **8** with 80 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.77 (s, 1H), 2.66 (s, 1H), 2.28 (t, J = 7.2 Hz, 4H), 1.66–1.50 (m, 4H), 1.50–1.21 (m, 36H), 0.97–0.84 (m, 6H).^{13}C NMR (75 MHz, $CDCl_3$) δ (C≡C) 84.29, (C≡C) 82.38, (C≡C) 78.29, (C≡C) 70.09, (C-OH) 54.16, (22 CH_2) 31.95, 29.71, 29.68, 29.65, 29.54, 29.39, 29.13, 28.90, 28.12, 22.72, 18.71,

(2 CH_3) 14.15; MS (DCI-CH_4): *m/z* [MH^+] = 441, [$MC_2H_5^+$] = 469; HRMS (DCI-CH_4): *m/z* [MH^+] calcd = 441.4096, found = 441.4091.

Biological evaluations

MTT tests (MTT = (3-[4,5-diMethylThiazol-2-yl]-2,5-diphenyl Tetrazolium bromide). The drugs' cytotoxicity was determined by standard MTT tests on HCT116 cells. In brief, 10.000 HCT116 cells were distributed in 96 flat bottom well plates in 100 microliters of DMEM 10 % FCS and 1 µL of DMSO containing the drugs dilutions were then added to each well. For each drug, triplicates of concentrations ranging from 10 µM to 5 nM were carried out, by means of 7 successive three fold dilutions of a 1 mM stock solution. Controls always included medium alone, DMSO alone and dilutions of the reference drug (*S*)-(+)-**1** (IC_{50} around 0.1 µM). Plates were then placed in a CO_2 tissue culture incubator for 72 h before the MTT test was performed. This was done by adding 10 µL of MTT stock solution (12 µM, 5 mg/mL in PBS, Sigma) to each well and incubating the plate for 90 min at 37°C. 100 µL of a 0.1 M HCl aqueous solution in isopropanol (v/v 1/9) were then added to each well, and the plates were returned to 37°C for 90 min before reading the OD absorbed at 570 nm.

Supporting Information. ^1H and ^{13}C NMR spectra of the eight compounds **4**-**11**.

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