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Skeletal optimization of cytotoxic lipidic dialkynylcarbinols

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Abstract: In line with a recent study of the pharmacological potential of bio-inspired synthetic acetylenic lipids, after identification of the terminal dialkynylcarbinol (DAC) and butadiynyl alkynylcarbinol (BAC) moieties as functional antitumor pharmacophoric units, this work specifically addresses the issue of the carbon backbone length. A systematic variation of the aliphatic chain was thus carried out in both the DAC and BAC series. The critical impact of the length of the lipidic skeleton was first confirmed in the racemic series, with the highest cytotoxic activity for C17 to C18 backbones. Enantiomerically enriched samples were prepared by asymmetric synthesis of the optimal C18 DAC and C17 BAC derivatives. Samples with upgraded enantiomeric purity were alternatively produced by enzymatic kinetic resolution. Eutomers possessing the (*S*) configuration displayed IC_{50} of cytotoxicity against HCT 116 cancer cells down to 15 nM, the highest level of activity reached to date in this series.

Introduction

Acetylenic lipids represent an unusual class of metabolites combining a circumscribed structural diversity with a rich spectrum of biological activities, including anticancer properties.[1] More than 100 such natural products were notably isolated from various species of marine sponges over the past two decades.[2] Whilst the presence of a terminal alkynylcarbinol unit has previously been correlated with the antitumor activity of natural representatives, it is only recently that the structure-activity relationships of acetylenic lipids started being addressed in detail.[3] A systematic study based on the prototypical natural product (*S*,*E*)-icos-4-en-1-yn-3-ol (**1**) isolated from the sponge *Cribrochalina vasculum* was recently reported.[4,5] Not only were basic structural requirements for the cytotoxicity delineated, but, as a result of several structural modifications, the potency of the fully synthetic derivative (*S*)-**2** was increased more than 100 times as compared to **1** (Fig. 1).

Two key structural alterations were (i) the inversion of configuration of the carbinol center and (ii) the replacement of the original internal double bond by a second triple bond. This led to the identification of the (*S*)-configured terminal dialkynylcarbinol (so-called DAC) unit as non-natural bioinspired pharmacophore for antitumor activity (this series is viewed as (*R*)-like in reference to the natural compound **1**, the formal retention of the *S* configuration being due to a change in CIP priority).[4] The third variable structural feature considered in the initial study was the length of the linear lipidic skeleton. Indeed, truncating the C20 backbone of the reference natural product **1** by three carbon atoms led to a six-fold increase in cytotoxicity in both optical series.[4]

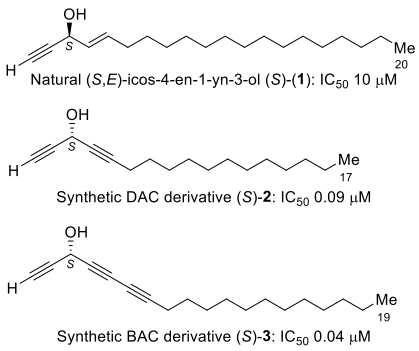
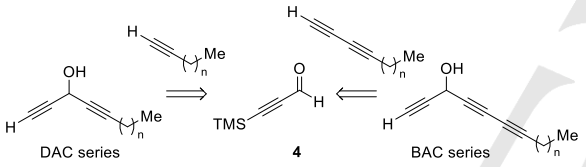


Figure 1. Natural alkynylcarbinol **1**[5] and previously described synthetic analogues **2**[4] and **3**.[6]

The C17 skeleton, originally selected for the sake of availability of the tetradecyne starting material, was retained as a standard backbone for further structural modifications.[7] More recently, the extension of the pharmacophore was envisaged by means of an ethynylogation approach.[6] Insertion of an additional acetylenic unit at the internal position of **2** gave rise to the butadiynyl alkynylcarbinol (so-called BAC) function in the C19-derivative (*S*)-**3** displaying a promising IC_{50} value of 40 nM on the human colon carcinoma cell line HCT 116. This result motivated a systematic study of the impact of the linear carbon chain length on the cytotoxic activity, with the aim of addressing two key questions: is C17 the optimal length of the linear skeleton in the DAC series? Is it also the case for BAC derivatives? A first set of results along this line is presented below.

Results and Discussion

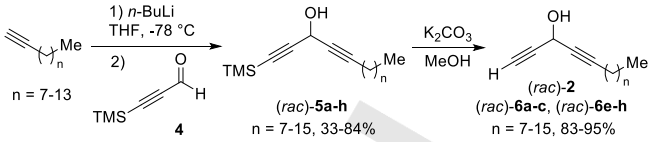
In order to assess the influence of the aliphatic backbone on cytotoxicity, it was decided to first run the study with readily accessible racemic samples of DAC and BAC lipids. A straightforward synthetic route was based on the use of the ynal **4** as precursor of the DAC terminus (Scheme 1). This substrate can indeed undergo smooth addition of a variety of alkynyl metals carrying the target lipophilic appendage. Such an approach thus offers a unified access to both DAC and BAC series.



Scheme 1. Lipidic DACs and BACs retrosynthetic route.

Since the early reports of its preparation,[8] numerous syntheses of the ynal precursor **4** have been described.[9] Aldehyde **4** can be prepared by direct formylation of TMS-acetylene.[8b,9a-h] A robust alternative procedure, relying on the oxidation of a propargylic alcohol precursor, was applied here.[8a,9i-t]

Racemic dialkynylcarbinols synthesis. Lipidic skeletons varying by the progressive addition of one carbon from C14 to C21 were first targeted in the DAC series in order to systematically browse a large range of aliphatic chain lengths. Synthesis of the DAC derivatives proceeded readily through a two-step sequence starting from commercially available terminal alkynes (see SI for experimental details). Lithiation with *n*-BuLi followed by addition of the corresponding acetylides to ynal **4** led to trimethylsilyl-protected DAC intermediates **5a-h** in moderate to high yields. These primary products were desilylated by treatment with K_2CO_3 in MeOH to deliver the targets DAC derivatives (Scheme 2).



Scheme 2. Synthesis of racemic DACs **2**, **6a-c** and **6e-h**.

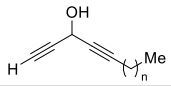
With this series of eight racemic samples in hand, the impact of aliphatic chain length on antitumor activity was evaluated by measuring their IC_{50} of cytotoxicity at 72 h in HCT 116 colon cancer cells (Table 1). A clear influence of the lipidic carbon skeleton on the cytotoxic behavior was observed. In comparison to the C17 derivative of reference (*rac*)-**2**, typically displaying an IC_{50} of 150 nM, the cytotoxicity was found to decrease dramatically upon shortening of the aliphatic chain. Chopping of two methylene units led to a 30-fold loss in cytotoxicity, and the IC_{50} was superior to 5 μM for the C14 derivative (*rac*)-**6a** (Table 1). Increasing the length of the acetylenic lipid backbone of **2** gave rise to a milder effect. A *ca.* 3-fold decrease in activity was observed upon addition of 3 to 4 methylene fragments. On the other hand, the direct homologue of the (*rac*)-**2** reference compound, the C18 lipidic DAC (*rac*)-**6e**, was found to be slightly more cytotoxic with an IC_{50} value of 100 nM, suggesting some room for improvement in the corresponding enantioenriched form. Overall, this first part of the study confirmed that a carbon skeleton of C17-C18 was indeed optimal in the DAC acetylenic lipid sequence.

Enantioselective dialkynylcarbinols synthesis. In order to fully assess the cytotoxic activity of the C18 DAC derivative **6e**, enantio-enriched samples were prepared in both the (*R*) and (*S*) series. Asymmetric synthesis of the targeted DACs was accomplished using the previously developed modified Carreira procedure for enantioselective addition of terminal alkynes onto ynals **4**.[4,10] Treatment of **4** with pentadecyne in the presence of $Zn(OTf)_2$, Et_3N and (+)- or (–)-*N*-methyl-ephedrine (NME) in CH_2Cl_2 led to (*S*)- or (*R*)-**5e**, respectively, with high yields (Scheme 3). Absolute configurations were assigned by analogy with previous results,[4,5] in agreement with Carreira's precedents.[11]

Enantiomeric excesses (e.e.) were measured by chiral supercritical fluid chromatography (SFC) of the corresponding 1-naphthyl carbamate derivatives. Final protodesilylation of the enantio-enriched adducts (*S*)- and (*R*)-**5e**, was carried out smoothly by the action of K_2CO_3 in MeOH. The sign of the optical rotations of the DACs (*R*)-(–)- and (*S*)-(+)-**6e** was in agreement with our previous work,[4,5] while the absolute value remained very low, as expected from the weak local chiral character of the DAC center ($[\alpha]_D$ < 10° for e.e. ≥ 80 %).

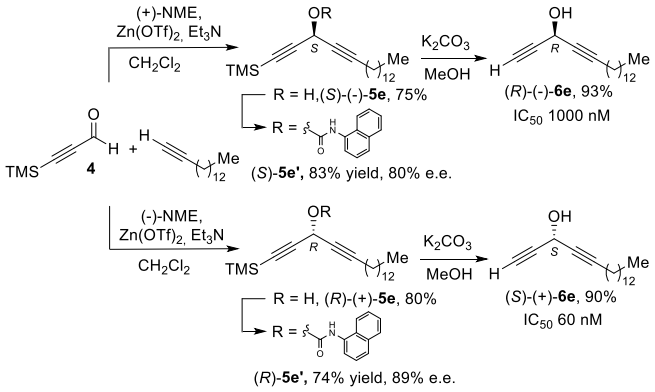
Evaluation of the antitumor potency of the enantiomers (*R*)- and (*S*)-**6e** confirmed the dramatic influence of the carbinol absolute configuration on the cytotoxicity, revealing an eudismic ratio (obtained by dividing the IC_{50} of the eutomer by that of the distomer) of 0.06. In addition, the IC_{50} value of the eutomeric C18 DAC (*S*)-**6e** reached 60 nM, comparing favorably with the value of 90 nM observed in the same assay for (*S*)-**2**, the eutomeric C17 reference compound.

Table 1. Cytotoxic activity of racemic lipidic DACs in HCT 116 cancer cells.



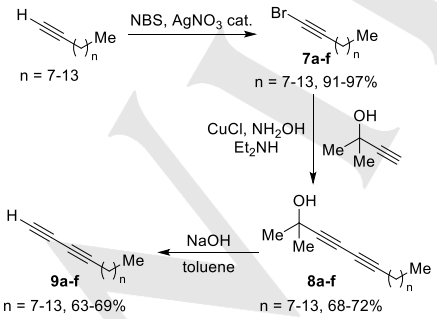
Backbone length	C14 (n = 8)	C15 (n = 9)	C16 (n = 10)	**C17 (n = 11)**	**C18 (n = 12)**	C19 (n = 13)	C20 (n = 14)	C21 (n = 15)
compound	(*rac*)-**6a**	(*rac*)-**6b**	(*rac*)-**6c**	(*rac*)-**2**	(*rac*)-**6e**	(*rac*)-**6f**	(*rac*)-**6g**	(*rac*)-**6h**
IC_{50} (nM)[a]	>5000	5000	500	**150**	**100**	200	500	400

[a] Cells were seeded in 96-well plates and were treated with concentrations ranging from 10 μM to 5 nM; after 72 h, cells were incubated with the MTT reagent, and the absorbance was measured.



Scheme 3. Asymmetric synthesis of (*R*)- and (*S*)-**6e**.

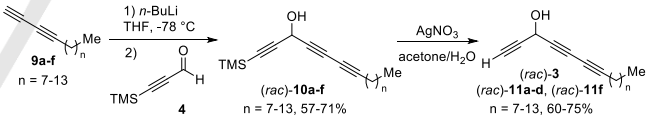
Racemic butadiynyl alkynylcarbinols synthesis. The BAC series, comprising the prototypical C19 derivative (*S*)-**3** displaying a remarkable IC_{50} of 40 nM,[6] was then considered. In order to gain insights into the optimal carbon backbone length, a series of racemic samples of varying chain lengths were, there again, first secured according to the general synthetic route described in scheme 1. Based on the observations made in the DAC series, a smaller collection of six lipidic skeletons was selected with a length ranging from C15 to C21, with a focus on the C17-C18 region. The 1,3-diyne precursors **9a-f** carrying the target aliphatic appendage were readily obtained from the corresponding terminal alkynes (Scheme 4) (see SI for experimental details).



Scheme 4. Synthesis of 1,3-diynes **9a-f**.

A three-step reaction sequence relying on the Cadiot-Chodkiewicz coupling with 2-methylbut-3-yn-2-ol, used as a protected form of acetylene, was implemented (Scheme 4). $AgNO_3$-catalyzed bromination of the alkynes with NBS was followed by Cu(I)-mediated cross coupling in Et_2NH in the presence of hydroxylamine. Deprotection of the terminal position was finally ensured by elimination of acetone in alkaline conditions. The expected 1,3-diynes **9a-f** were thus obtained in 39-48% overall yields.

The stage was then set for the construction of the series of racemic BAC lipids. Lithiation of the terminal 1,3-diynes **9a-f** by treatment with *n*-BuLi at low temperature and subsequent addition of the resulting alkylacetylides onto the pivotal ynal **4** led to the *C*-silylated precursors **10a-f** in 57-71 % yields (Scheme 5). Use of the mild K_2CO_3/MeOH protodesilylation conditions proved unexpectedly inadequate, giving rise to extensive degradation in the BAC series. The sensitivity of the reactant to basic medium can be attributed to the presence of the 1,3-diyne moiety. On the other hand, alternative treatment with $AgNO_3$ in acetone/H_2O smoothly delivered the desired desilylated BACs **11a-d**,**f** in 60-75 % yields (Scheme 5).

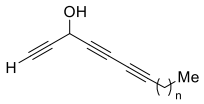


Scheme 5. Synthesis of racemic BACs **3**, **11a-d** and **11f**.

The series of racemic BACs derivatives was subjected to cytotoxicity evaluation in HCT 116 cancer cells (Table 2). A milder effect of the carbon chain length on antitumor potency was observed as compared to the previous DAC series, the shorter C15 and the longer C21 derivatives displaying equivalent cytotoxicity with an IC_{50} in the 220-250 nM range. Truncating the lipid backbone by one carbon at a time from C19 to C17 led to a gradual enhancement of potency, reaching an IC_{50} value of 60 nM for (*rac*)-**11c**. This finding indicated that C17 was the optimal length for the lipidic BAC skeleton.

Enantioselective butadiynyl alkynylcarbinols synthesis. The preparation of **11c** in enantio-enriched form was thus envisioned. Its enantioselective preparation paralleled that of the DAC analogue **6e**. The first example of asymmetric addition of a 1,3-diyne nucleophile onto ynal using the modified Carreira's procedure was previously reported.[6] Thanks to this precedent, both enantiomers of the homologue **10c** could be obtained, (+)-

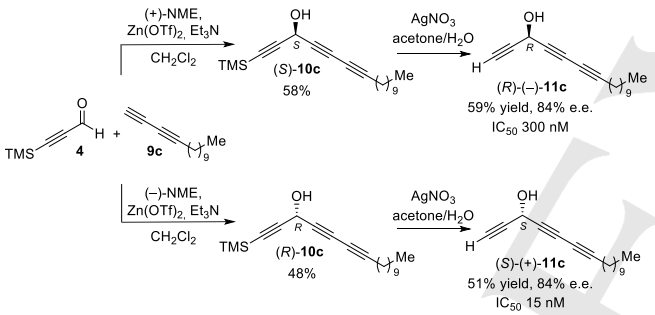
Table 2. Cytotoxic activity of racemic BAC derivatives in HCT 116 cancer cells.



Backbone length	C15 (n = 7)	C16 (n = 8)	**C17 (n = 9)**	C18 (n = 10)	C19 (n = 11)	C21 (n = 12)
compound	(*rac*)-**11a**	(*rac*)-**11b**	(*rac*)-**11c**	(*rac*)-**11d**	(*rac*)-**3**	(*rac*)-**11f**
IC_{50} (nM)[a]	220	150	**60**	90	100	250

[a] Cells were seeded in 96-well plates and were treated with concentrations ranging from 10 μM to 5 nM; after 72 h, cells were incubated with MTT reagent, and the absorbance was measured.

or (–)-NME inducing the formation of the silylated (*S*)- or (*R*)-BAC, respectively (Scheme 6). Treatment with $AgNO_3$ in an acetone/H_2O mixture delivered the desilylated BACs (*R*)- or (*S*)-**11c**, respectively. The optical rotations measured for (*R*)-(–)- and (*S*)-(+)-**11c** were again in agreement with precedent results, corroborating the assignment of the absolute configuration from literature standards.



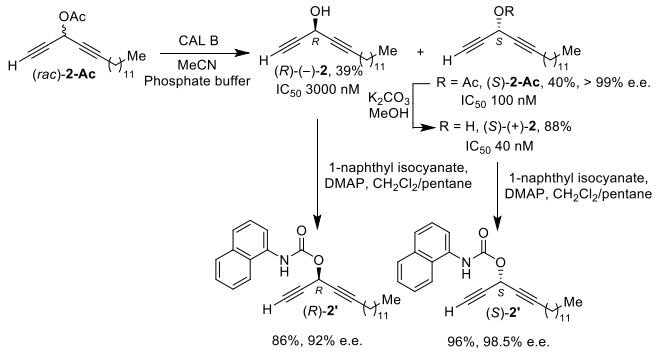
Scheme 6. Enantioselective synthesis of BACs (*R*)-(–)- and (*S*)-(+)-**11c**.

For the sake of comparison, cytotoxicity experiments with the C17 BAC (*R*)- and (*S*)-**11c** were there again carried out on the HCT 116 cancer cell line. The distomeric (*R*)-**11c** displayed an IC_{50} of cytotoxicity of 300 nM. Remarkably, an unprecedented IC_{50} of 15 nM was found for the *S* enantiomer, corresponding to an eudismic ratio of 0.05. This potency was higher than that of the C19 reference compound (*S*)-**3**, displaying an IC_{50} of cytotoxicity of 40 nM in the same bioassay.

The unprecedented level of cytotoxicity reached for (*S*)-**11c**, compared to its enantiomer and the reference lipidic BAC (*S*)-**3**, stimulated efforts for accessing DAC and BAC samples with upgraded enantiomeric purity. Despite its practicality, the asymmetric synthesis approach indeed delivers alkynylcarbinols with e.e. not exceeding 90%.

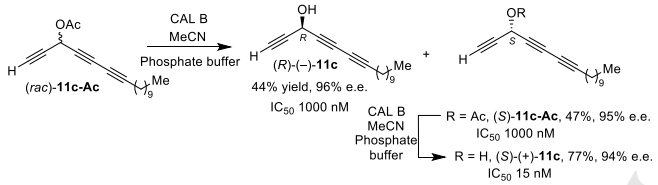
Enzymatic kinetic resolution. The enzymatic kinetic resolution of racemic samples was thus explored as an alternative strategy. Such a chemoenzymatic approach has previously been used in the total synthesis of several marine acetylenic lipids bearing the naturally occurring alkenyl alkynylcarbinol (AAC) moiety. An (*S*)-selective *trans*-acetylation catalyzed by the Novozym 435 lipase thus proved useful for the early synthesis of (*S*)-docosa-(4*E*,15*Z*)-dien-1-yn-3-ol.[12] A similar approach employing Lipase AK (Amano) was later used to access (*S*)-eicos-(4*E*)-en-1-yn-3-ol (**1**),[13] both enantiomers of the two-headed compounds adociacetylene,[14] and duryne,[15] and (*S*,*S*)-dideoxypetrosynol.[16] Lipase PS (Amano) was also reported to catalyze an (*R*)-selective acetylation, exploited for the synthesis and absolute configuration determination of a new two-headed cytotoxic metabolite from the marine sponge *Petrosia sp.* (4*E*,24*E*)-14-methyloctacosa-4,24-diene-1,27-diyne-3,26-diol.[17] The only attempt to resolve a racemic DAC moiety by an enzymatic method was reported in a pioneering publication of Chattopadhyay and coll. who made use of a lipase from *Candida rugosa* in the preparation of an enantio-enriched early intermediate *en route* to (*S*)-eicos-(4*E*)-en-1-yn-3-ol (**1**)[18] and the non-natural 15*E*-isomer of (*R*,*R*)-duryne.[19]

In the present context, the DAC **2** was selected as a reference to implement such a chemoenzymatic approach. In a preliminary round of experiments, a series of lipases were screened for the kinetic resolution of the acetate (*rac*)-**2**-Ac. Only three enzymes out of the 12 tested gave a significant level of resolution, namely Amano lipase PS (*Burkholderia cepacia*) immobilized in diatomaceous earth, *Thermomyces lanuginosus* lipase and *Candida antartica* lipase B (CAL B). The latter gave the optimal results, allowing isolation of (*R*)-**2** in 39% yield and 92% e.e. (as determined by chiral SCF analysis of the corresponding 1-naphthyl carbamate) and (*S*)-**2**-Ac in 40% yield with e.e. >99% (as determined by chiral GC analysis) (Scheme 7) (see SI for experimental details). Further saponification of (*S*)-**2**-Ac (K_2CO_3/MeOH, 88% yield) afforded (*S*)-**2** with 98.5% e.e. (as determined by chiral SCF analysis of the corresponding 1-naphthyl carbamate). Cytotoxicity tests indicated a value of IC_{50} of 40 nM for the enzymatically resolved sample of (*S*)-**2** (*vs.* 3 μM for the corresponding (*R*)-**2**). Upgrading the e.e. led to a more pronounced eudismic ratio reaching 0.013. The corresponding (*S*)-**2**-Ac displayed an IC_{50} of 100 nM possibly due to (*S*)-**2** itself resulting from partial (~40 %) *in cellulo* hydrolysis of the labile acetate in (*S*)-**2**-Ac.



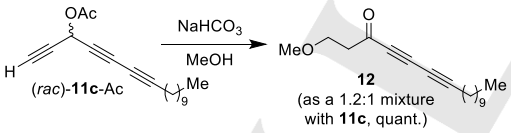
Scheme 7. Kinetic enzymatic resolution of (*rac*)-**2**-Ac.

The chemoenzymatic approach was then applied to the preparation of the most cytotoxic BAC, (*S*)-**11c** (Scheme 8). Treatment of the racemic mixture of the acetate (*rac*)-**11c**-Ac with CAL B delivered the carbinol (*R*)-**11c** (44% yield, 96% e.e., as determined by the chiral SFC) along with the unreacted (*S*)-**11c**-Ac (47% yield, 95% e.e., as determined by chiral GC analysis) (see SI for experimental details).



Scheme 8. Kinetic enzymatic resolution of (*rac*)-**11c**-Ac.

In contrast to the DAC **2**-Ac, the BAC **11c**-Ac turned out to be highly sensitive to standard saponification conditions. Exposure to one equivalent of K_2CO_3 in MeOH led to rapid decomposition (Scheme 9). Changing the base for $NaHCO_3$ slowed down the transformation and allowed identification of the unstable ynone **12** (obtained selectively as a 1.2:1 mixture with the expected carbinol **11c**), likely resulting from a base-induced isomerization in allendiyne ester[20] followed by the terminal addition of a molecule of MeOH. Other hydrolysis conditions proved to be non-productive (MeCN/NH_4OH, Et_2O/dilute aq. AcOH).



Scheme 9. Attempt to saponify acetate (*rac*)-**11c**-Ac.

Finally, the enzymatic approach was attempted for the hydrolysis of (*S*)-**11c**-Ac. Treatment of this acetate with CAL B yielded the expected BAC (*S*)-**11c** in 77% yield and 94% e.e. (as determined by chiral SCF analysis) (Scheme 8) (see SI for experimental details). Enzymatically resolved BAC samples were submitted to cell viability experiments. Unlike its DAC congener (*S*)-**2**-Ac, the eutomeric BAC acetate (*S*)-**11c**-Ac showed a moderate level of cytotoxicity (IC_{50} 1000 nM) that may be correlated to its observed chemical instability. The (*S*)-**11c** however displayed an IC_{50} of 15 nM (*vs.* 1000 nM for the distomeric (*R*)-**11c**, corresponding to an eudismic ration of 0.015) fully confirming the trends observed previously.

Conclusions

Initial studies had shown that several simple structural modifications were sufficient to enhance by two orders of magnitude the cytotoxicity of the natural lipidic AAC (*S*,*E*)-**1**. Fine optimization of the chiral alkynylcarbinol unit having led to original dialkynyl- and butadiynyl-carbinol (respectively DAC and BAC) pharmacophores, the lipidic backbone was still deserving consideration. This first account confirms that the length of the carbon skeleton is indeed critical, showing that the C17 to C18 backbone is optimal. In particular, the eutomeric C17 BAC derivative (*S*)-**11c** revealed to be the most potent synthetic derivative to date in this sequence of lipidic dialkynylcarbinols.

Experimental Section

Chemistry

General procedure A: Carreira asymmetric alkynylation modified for ynals. A flame dried flask was charged with $Zn(OTf)_2$ (4 equiv) and (–)- or (+)-*N*-methylephedrine (4 equiv) and dried under vacuum for 30 min. After purging with argon, CH_2Cl_2 and triethylamine (4 equiv) were added. The resulting mixture was vigorously stirred for 2 h at rt before addition in one portion of 1-pentadecyne or tetradeca-1,3-diyne **9c** (4 equiv). After 1 h stirring, 3-(trimethylsilyl)-2-propynal **4** (1 equiv) in CH_2Cl_2 was added, and the mixture was stirred at rt overnight under inert atmosphere. After treatment with a saturated aqueous NH_4Cl solution, the aqueous layer was extracted with CH_2Cl_2. The organic phase was then washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel to give the expected enantioenriched DACs **(*R*)-** or **(*S*)-5e** or BACs **(*R*)-** or **(*S*)-10c**.

(–)-(3*S*)-1-(Trimethylsilyl)octadeca-1,4-diyn-3-ol (*S*)-5e: General procedure A was applied to (+)-*N*-methylephedrine (179 mg, 1 mmol), $Zn(OTf)_2$ (363 mg, 1 mmol), 1-pentadecyne (0.25 mL, 1 mmol), Et_3N (0.13 mL, 1 mmol) and 3-(trimethylsilyl)-2-propynal **4** (32 mg, 0.25 mmol, as an 80% solution in CH_2Cl_2). The crude product was purified by flash chromatography on silica gel (petroleum ether/Et_2O: 9/1; R_f 0.15) to give **(*S*)-5e** (63 mg, 75% yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.13 (t, *J* 2.1 Hz, 1H), 2.26 (td, *J* 7.1, 2.1 Hz, 2H), 2.13 (s, 1H), 1.61 – 1.50 (m, 2H), 1.46 – 1.26 (m, 20H), 0.90 (t, *J* 6.6 Hz, 3H), 0.22 (s, 9H).^{13}C NMR (75 MHz, $CDCl_3$) δ (C≡C) 102.57, (C≡C) 88.87, (C≡C) 85.89, (CH-OH) 52.82, (12 CH_2) 31.95, 29.70, 29.69, 29.68, 29.66, 29.56, 29.38, 29.16, 28.89, 28.32, 22.72, 18.77, (CH_3) 14.15, (3C-Si) -0.28. Optical rotation $[\alpha]_D^{20}$ –0.4 (*c* 1, $CHCl_3$). HRMS-DCI (CH_4) m/z $[M+H]^+$ calcd for $C_{21}H_{39}OSi$: 335.2770, found: 335.2761. Enantiomeric excess (ee 80%) was evaluated from the chiral SFC analysis of the corresponding 1-naphtyl carbamate **(*S*)-5'e** (see SI).

(+)-(3*R*)-1-(Trimethylsilyl)octadeca-1,4-diyn-3-ol (*R*)-5e: General procedure A was applied to (–)-*N*-methylephedrine (358 mg, 2 mmol, 4 equiv), $Zn(OTf)_2$ (727 mg, 2mmol), 1-pentadecyne (0.5 mL, 2 mmol), Et_3N (0.27 mL, 2mmol) and 3-(trimethylsilyl)-2-propynal **4** (63 mg, 0.5 mmol, as an 80% solution in CH_2Cl_2). The crude product was purified by flash chromatography on silica gel (petroleum ether/Et_2O: 9/1; R_f 0.15) to give **(*R*)-5e** (66 mg, 80% yield) as a colorless oil. ^{1}H NMR and ^{13}C NMR were identical to that of **(*S*)-5e**. $[\alpha]_D^{20}$ +0.38 (*c* 1, $CHCl_3$). Enantiomeric excess (ee 90%) was evaluated from the chiral SFC analysis of the corresponding 1-naphtyl carbamate **(*R*)-5'e** (see SI).

General procedure B: K_2CO_3-promoted protodesilylation in MeOH. K_2CO_3 (1 equiv) was added to a solution of the TMS-protected DAC **5** (1 equiv) in MeOH (0.2 M). The solution was stirred at rt until complete consumption of the starting material, as judged by TLC monitoring (about

4 hours). After addition of brine, the aqueous layer was extracted with CH_2Cl_2, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel to give **6**.

(–)-(3*R*)-Octadeca-1,4-diyn-3-ol (*R*)-6e: The general procedure B was applied to (*S*)-1-(trimethylsilyl)octadeca-1,4-diyn-3-ol **(*S*)-5e** (22 mg, 0.06 mmol) and K_2CO_3 (8.3 mg, 0.06 mmol). Purification by flash chromatography on silica gel (petroleum ether/Et_2O: 9/1; R_f 0.15) gave **(*R*)-6e** (15 mg, 93% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.10 (d, *J* 2.2 Hz, 1H), 2.54 (d, *J* 2.3 Hz, 1H), 2.22 (td, *J* 7.1, 2.1 Hz, 2H), 1.98 (br s, OH), 1.59 – 1.44 (m, 2H), 1.37 (m, 2H), 1.25 (s, 18H), 0.88 (t, *J* 6.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (C≡C) 86.20, (C≡C) 81.47, (C≡C) 76.91, (C≡C) 72.17, (CH-OH) 52.4, (12 CH_2) 31.91, 29.67, 29.64, 29.60, 29.48, 29.34, 29.09, 28.85, 28.25, 22.68, 18.66, (CH_3) 14.11. $[\alpha]_D^{20}$ – 3.4 (*c* 1.1, $CHCl_3$). HRMS-DCI (CH_4) m/z $[M+H]^+$ calcd for $C_{18}H_{31}O$: 263.2375, found: 263.2374.

(+)-(3*S*)-Octadeca-1,4-diyn-3-ol (*S*)-6e: The general procedure B was applied to (*R*)-1-(trimethylsilyl)octadeca-1,4-diyn-3-ol **(*R*)-5e** (50 mg, 0.15 mmol) and K_2CO_3 (21 mg, 0.15 mmol). Purification by flash chromatography on silica gel (petroleum ether/Et_2O: 9/1; R_f 0.15) gave **(*S*)-6e** (35 mg, 90% yield) as a white solid. ^{1}H NMR and ^{13}C NMR were identical to that of **(*R*)-6e**. $[\alpha]_D^{20}$ +3.3 (*c* 1.2, $CHCl_3$).

(–)-(3*S*)-1-(trimethylsilyl)heptadeca-1,4,6-triyn-3-ol (*S*)-10c: The general procedure A was applied to (+)-*N*-methylephedrine (376 mg, 2.1 mmol, 4 equiv), $Zn(OTf)_2$ (763 mg, 2.1 mmol), tetradeca-1,3-diyne **9c** (0.4 g, 2.1 mmol), Et_3N (0.3 mL, 2.1 mmol) and 3-(trimethylsilyl)-2-propynal **4** (133 mg, 0.525 mmol, 1 equiv, as a 50% solution in CH_2Cl_2). The crude product was purified by flash chromatography on silica gel (petroleum ether/Et_2O: 95/5; R_f 0.1) to give **(*S*)-10c** (96 mg, 58% yield) as an orange oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.17 (d, *J* 6.8 Hz, 1H), 2.38 – 2.22 (m, 3H), 1.65 – 1.49 (m, 2H), 1.47 – 1.29 (m, 14H), 0.97 – 0.86 (m, 3H), 0.22 (s, 9H).^{13}C NMR (75 MHz, $CDCl_3$) δ (C≡C) 100.73, (C≡C) 90.25, (C≡C) 83.18, (C≡C) 71.62, (C≡C) 69.84, (C≡C) 64.20, (CH-OH) 53.00, (9 CH_2) 31.91, 29.57, 29.48, 29.32, 29.08, 28.85, 28.07, 22.70, 19.31, (CH_3)14.14, (3 C-Si) -0.36. $[\alpha]_D^{20}$ –10.1 (*c* 1.0, $CHCl_3$). HRMS-DCI (CH_4) m/z $[M+H]^+$ calcd for $C_{20}H_{33}OSi$: 317.2301, found: 317.2296. The enantiomeric excess (ee 83%) was evaluated by the chiral SCF analysis of **(*R*)-11c** (*vide infra*).

(+)-(3*R*)-1-(trimethylsilyl)heptadeca-1,4,6-triyn-3-ol (*R*)-10c: General procedure A was applied to (–)-*N*-methylephedrine (376 mg, 2.1 mmol, 4 eqiuv), $Zn(OTf)_2$ (763 mg, 2.1 mmol), tetradeca-1,3-diyne **9c** (0.4 g, 2.1 mmol), Et_3N (0.3 mL, 2.1 mmol) and 3-(trimethylsilyl)-2-propynal **4** (133 mg, 0.525 mmol, 1 equiv, as a 50% in CH_2Cl_2). The crude product was purified by flash chromatography on silica gel (petroleum ether/Et_2O: 95/5; R_f 0.1) to give **(*R*)-10c** (79 mg, 48% yield) as a white oil. ^{1}H NMR and ^{13}C NMR were identical to that of 1-(trimethylsilyl)heptadeca-1,4,6-triyn-3-ol **(*S*)-10c**. $[\alpha]_D^{20}$ +10.2 (*c* 1.0, $CHCl_3$). The enantiomeric excess (ee 84%) was evaluated by the chiral SCF analysis of **(*S*)-11c** (*vide infra*).

General procedure C: $AgNO_3$-catalyzed protodesilylation in acetone. $AgNO_3$ (0.1 equiv) was added to a solution of TMS-protected BAC **10** (1 equiv) in acetone (0.1 M) followed by one drop of water. The mixture was stirred until TLC monitoring showed disappearance of the starting material (*ca.* 3 h) and then quenched with a saturated aqueous NaCl solution. The aqueous layer was extracted with diethyl ether and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel to give the corresponding butadiynyl alkynylcarbinols **11**.

(–)-(3*R*)-heptadeca-1,4,6-triyn-3-ol (*R*)-11c: The general procedure C was applied to (–)-(3*S*)-1-(trimethylsilyl)heptadeca-1,4,6-triyn-3-ol **(*S*)-10c** (90 mg, 0.28 mmol) and $AgNO_3$ (5 mg, 0.028 mmol). Purification by flash chromatography on silica gel (pentane/Et_2O: 8/2; R_f 0.15) gave **(*R*)-11c** (40 mg, 59% yield) as an orange viscous product. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.14 (s, 1H), 2.58 (d, *J* 2.3 Hz, 1H), 2.28 (t, *J* 6.4 Hz, 2H), 2.24 (br s, OH), 1.53 (m, 2H), 1.45 – 1.08 (m, 14H), 0.88 (t, *J* 6.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 83.42, 79.95, 73.23, 71.12, 70.13, 63.95, 52.38, 31.87, 29.53, 29.44, 29.28, 29.03, 28.79, 27.99, 22.66, 19.25, 14.10. HRMS-DCI (CH_4) m/z $[M+H]^+$ calcd for $C_{17}H_{25}O$: 245.1905, found: 245.1901. $[\alpha]_D^{20}$ –8.5 (*c* 1.0, $CHCl_3$). Chiral SFC analysis (Chiralpak IA-3 μm (4.6x100 mm), CO_2/MeOH, 1.5 mL/min, PDA detector (λ 242 nm), rt **(*R*)-11c** 3.05 min and **(*S*)-11c** 3.19 min), ee 84%.

(+)-(3*S*)-heptadeca-1,4,6-triyn-3-ol (*S*)-11c: The general procedure C was applied to (+)-(3*R*)-1-(trimethylsilyl)heptadeca-1,4,6-triyn-3-ol **(*R*)-10c** (75 mg, 0.24 mmol) and $AgNO_3$ (4 mg, 0.024 mmol). Purification by flash chromatography on silica gel (pentane/Et_2O: 8/2; R_f 0.15) gave **(*S*)-11c** (30 mg, 51% yield) as an orange viscous product. ^{1}H NMR and ^{13}C NMR were identical to that of heptadeca-1,4,6-triyn-3-ol **(*R*)-11c**. $[\alpha]_D^{20}$ + 6.9 (*c* 1.1, $CHCl_3$). Chiral SFC analysis (Chiralpak IA-3 μm (4.6x100 mm), CO_2/MeOH, 1.5 mL/min, PDA detector (λ 242 nm), rt **(*R*)-11c** 3.13 min and **(*S*)-11c** 3.18 min), ee 84%.

Biology

Cytotoxicity: The drugs' cytotoxicity was determined by standard MTT tests on HCT116 cells. In brief, 5.000 HCT116 cells were distributed in 96 flat bottom well plates in 100 μL of DMEM 10% FCS and 200 microliters in the column destined to receive the drug's highest concentrations, *i.e.* 10 μM. 2 μL of DMSO containing the drugs at 1 mM were then added to each well of that first column. For each drug, duplicates of concentrations ranging from 10 μM to 5 nM were carried out by means of 12 successive 2-fold dilutions using a multi-channel pipette to pass 100 μl to the wells in the adjacent column and mixing thoroughly by pipetting up and down each time. Assays were performed 3 times independently, and controls always included dilutions of the reference drug **(*S*)-2** (IC_{50} 80-100 nM). 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 isopropanol, 0.1 M HCl were then added to each well, and the plates were returned to 37 °C for 90 min before reading the OD at 570nM.

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