Exploring the chemical reactivity of triisopropylsilyl dialkynyl methanol for the synthesis of dialkynylcarbinol-related compounds

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Abstract: Prompted by the pharmacological relevance of lipidic alkynylcarbinols, 1-(triisopropyl)silyl)penta-1,4-diyn-3-ol was exploited as a versatile C5 organic framework. Selective functionalization of this dissymmetrical dialkynylcarbinol precursor was achieved via the creation of either a Csp-Csp (alkyne), a Csp-Csp^2 (alkene and aryl) or a Csp-Csp^3 (RCHOH) bond. This allowed to access a novel racemic series of bioinspired acetylenic lipids that revealed amongst the most potent to date, with IC_{50} down to 27 nM on osteosarcoma U2OS cells. Click-type 1,3-dipolar cycloadditions such as a copper-catalyzed reaction with a long-chain alkyl azide (CuAAC) and a base-promoted reaction with a lipidic nitrile oxide were then first described in a racemic version. Preparation of both enantiomers of 1-(triisopropyl)silyl)penta-1,4-diyn-3-ol by kinetic enzymatic resolution with CAL-B immobilized on acrylic resin was carefully optimized. Finally, the use of resolved samples in 1,3-dipolar cycloaddition reactions led to the enantioenriched cycloadducts without significant epimerization of the carbinol center.

Introduction

The alkynylcarbinol group is an important functional unit in organic synthesis that is found in the structure of several naturally occurring compounds[1] from both sea[2] and land[3]. Representative examples of such secondary metabolites are (3*S*)-eicos-(4*E*)-en-1-yn-3-ol (from marine sponge *Cribrochalina vasculum*), [4] strongylodiol D (from marine sponge *Petrosia* sp.),[5] lembehyne A (from marine sponge *Haliclona* sp.)[6] as well as falcarindiol (from carrot root Apiaceae),[7] and panaxytriol (from Panax ginseng C. A. Meyer)[8] (Figure 1). These natural products, defined as Lipidic AlkynylCarbinols (LACs), are known to display various biological properties such as antibacterial[9] and antiproliferative[10] activities.

In 2013, our teams launched the first systematic structure-activity relationship study in the LAC series. Step-by-step pharmacomodulation of the prototypical natural product (3*S*)-eicos-(4*E*)-en-1-yn-3-ol (Figure 1)[4] involved the replacement of the internal C=C bond by a C≡C bond, the inversion of the carbinol absolute configuration and the shortening of the aliphatic chain. This led to the identification of the original DiAlkynylCarbinol (DAC) **1** with significantly enhanced cytotoxicity (IC_{50} = 0.2 μM for *rac*-**1** *vs.* IC_{50} = 10 μM for (3*S*)-eicos-(4*E*)-en-1-yn-3-ol on HCT116 cancer cells).[11] Of note, the chiral molecule **1** showed a strong eudismic ratio in favor of the (*S*)-enantiomer, displaying significantly higher antiproliferative activity than the (*R*)-enantiomer (IC_{50} = 90 nM for (*S*)-**1** *vs.* 3000 nM for (*R*)-**1**).[11a] This property was later explained by the finding that these molecules behave as prodrugs enantiospecifically oxidized by a

cellular enzyme, HSD17B11, into protein reactive dialkynylketones that mediate DAC cytotoxic activity.[12] The various analogues previously studied were typically accessed by a linear synthetic sequence based on the addition of a suitable lipidic lithium acetylide onto trialkylsilyl propiolaldehyde[13] followed by a *C*-trialkylsilyl deprotection (Figure 2).

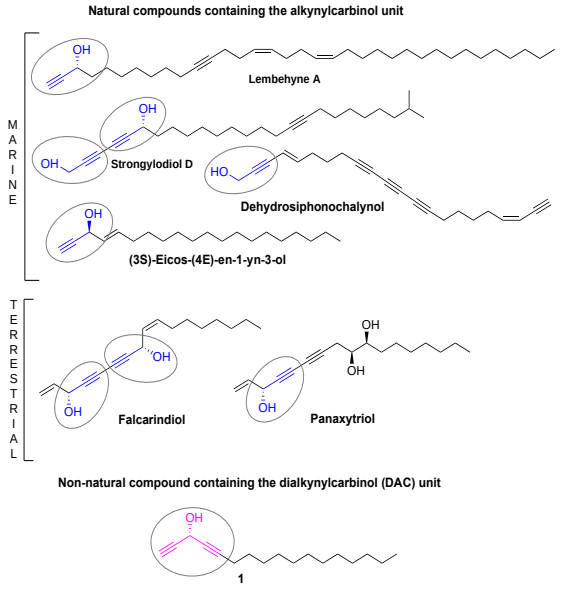


Figure 1. Examples of natural products embedding an alkynyl carbinol unit and structure of the non-natural dialkynylcarbinol (DAC) (*S*)-**1**.

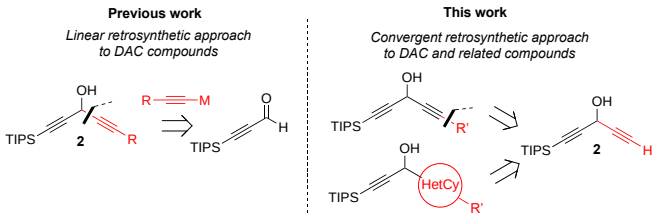
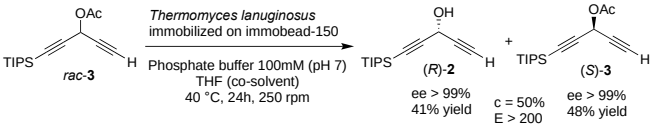


Figure 2. Linear (left) and convergent (right) retrosynthetic approaches for DAC derivatives.

As a continuation of our synthetic efforts, we were interested in exploiting a minimal dissymmetrical dialkynylcarbinol unit to provide a unified access to a broader panel of DAC-containing compounds. The chiral mono *C*-protected dialkynylcarbinol 1-(triisopropyl)silyl)penta-1,4-diyn-3-ol (**2**) (Figure 2) is a highly functionalized C_5 organic framework readily available in either racemic[14] or enantiopure forms.[15] In 2020, a lipase-mediated enzymatic kinetic resolution (EKR) was successfully implemented to produce enantioenriched **2** from the acetate precursor *rac*-**3** (Scheme 1).[15] According to this hydrolytic approach, (*R*)-**2** and its acetylated enantiomer (*S*)-**3** were obtained with an enantiomeric excess (*ee*) >99%, a conversion (*c*) of 50% and an enantioselectivity (E) >200, thanks to the use of a lipase from *Thermomyces lanuginosus* immobilized on immobead-150 (TLL).



Scheme 1. Previously reported enzymatic kinetic resolution (EKR) of *rac*-**2** *via* acetate hydrolysis.[15]

The present study aims at exploring the potential of **2** as a versatile chemical platform for the convergent synthesis of new DAC-related lipidic derivatives through the investigation of several transformations exploiting the reactivity of the terminal alkyne (Figure 2). In-depth optimization of the lipase-mediated EKR of *rac*-**2** via acylation approach is also included to this study in the prospect of applying a complementary approach to produce enantiopure (*S*)-**2**.

Results and Discussion

Functionalization of the terminal alkyne of 2 *via* creation of a C-C bond

During the synthesis of natural LACs such as (3*S*)-eicos-(4*E*)-en-1-yn-3-ol (Figure 1), A. Sharma and S. Chattopadhyay [16] previously described the formation of a new Csp-Csp^3 bond through the alkylation of an *O*-THP and *C*-TBS analogue of **2** with 1-bromopentadecane upon treatment with *n*-BuLi in the presence of HMPA (Figure 3). On the other hand, some of us accessed the butadiynylcarbinol motif (Csp-Csp bond creation) (Figure 3), which is characteristic of the falcarin-type natural products (Figure 1),[17] by means of the Cadiot-Chodkiewicz coupling reaction (CuCl, $NH_2OH.HCl$, *n*-$PrNH_2$) between **2** and 1-bromo-1-tetradecyne.

To convert a terminal alkyne into an internal one, the Pd/Cu-promoted Sonogashira cross-coupling reaction is one of the most efficient approaches.[18] This reaction was originally developed for the generation of a Csp-Csp^2 bond with aryl or vinyl (pseudo)halides. It was then successfully extended to alkyl halides allowing the less favorable creation of a Csp-Csp^3 bond.[19] The Sonogashira coupling reaction was thus first investigated to form a new Csp-Csp^3 bond from **2**. However, under the conditions used (palladium complexes, copper salts, large excess of an amine base and alkyl bromide), formation of the expected alkylated compound was not observed. Subsequently, the functionalization of **2** was attempted *via* a more conventional Csp-Csp^2 bond formation reaction, leading to compounds with an enynol motif frequently found in biologically active polyacetylenic natural products[2,3] such as dehydrosiphonochalinol (from the marine sponge *Siphonochalina* sp.) (Figure 1).[20]

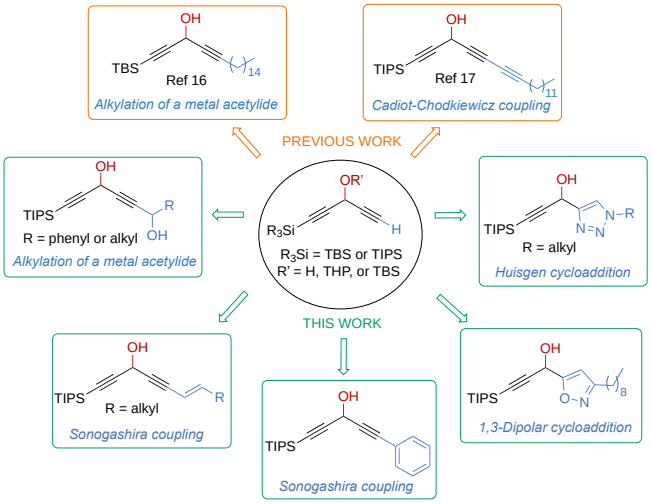


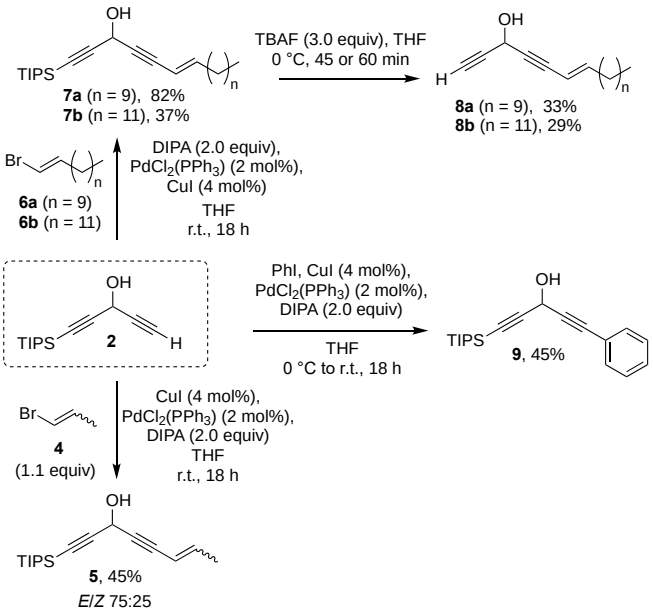
Figure 3. Functionalization of the *C*-silylated dialkynylcarbinol **2** or its closely related *O*-protected derivatives.

A model reaction of **2** with a mixture of *Z*/*E* 1-bromoprop-1-ene (**4**) in the presence of CuI, $PdCl_2(PPh_3)_2$ and diisopropylamine in THF, provided the enynol **5** as a mixture of *E*/*Z* diastereoisomers (75:25) in 45% yield (Scheme 2). This reaction was then carried out using longer chain bromoalkenes such as (*E*)-1-bromo-dodec-1-ene (**6a**) and (*E*)-1-bromo-tetradec-1-ene (**6b**), which were obtained according to the methodology reported by Ney, J. E. *et al.* [21] with 64% and 81% yield, respectively (see SI). The Sonogashira coupling conditions led to the formation of the (*E*)-enynols **7a** and **7b** isolated with 82% and 37% yield, respectively. The TIPS *C*-protecting group of **7a** and **7b** was then cleaved by the action of TBAF in THF to give **8a** and **8b** with ca. 30% yield.[22] Efforts have then been devoted to extending the Sonogashira coupling of **2** with aryl halides. While the use of *para*-bromopentylbenzene, proved unsuccessful, isolation of the arylation product **9** in 45% yield from coupling with iodobenzene highlighted the benefit of using more reactive haloarene precursors (Scheme 2).

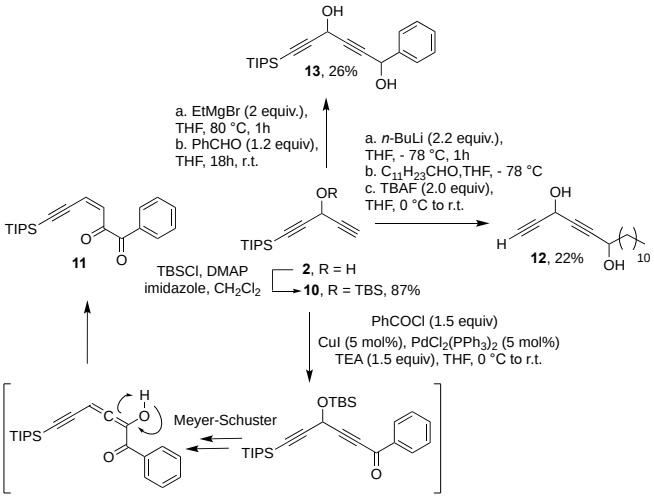
Functionalization of the terminal alkyne of 2 *via* introduction of a C=O or C-OH bond

Introduction of a carbonyl group at the terminal position of the C,O-bis-silylated dialkynyl carbinol platform **10** was tested by addition of the corresponding lithium acetylide onto benzoyl chloride, used as a model reagent. The only product that could be isolated from the complex mixture formed upon disappearance of the starting material was proposed to be **11**, according to the ^{1}H (8.92 ppm (1H, $C_{sp2}H$); 6.30 ppm (1H, $C_{sp2}H$)) and ^{13}C NMR (176.5 ppm (C=O); 162.3 ppm (C=O)) and HRMS data (Scheme 3). Attempts to achieve this transformation *via* a Sonogashira-type coupling led to the same reaction profile with **11** as the only isolated product (Scheme 3). The mechanism described in Scheme 3 may account for the formation of this product. This suggests that the expected reaction does indeed occur, but that the expected product spontaneously evolves through a Meyer-Schuster rearrangement[23] to give **11**.

In addition to metal-catalyzed formal substitution reactions, the nucleophilic reactivity of the terminal alkyne position of **2** could give rise to nucleophilic addition on a carbonyl group *via* a metal acetylide intermediate. Functionalization of the terminal position of alkyne **2** by a C-OH moiety was thus investigated by adding the lithium acetylide of the C,O-bis-protected dialkynylcarbinol **10** to an aliphatic or an aromatic aldehyde. Under these conditions, the expected addition turned out to be feasible for the aliphatic aldehyde, leading to **12** with 22% yield after a deprotection step with TBAF. For the aromatic aldehyde on the other hand, the use of the lithium acetylide of **2** (generated with 2 equiv. of *n*-BuLi) in the presence of $CeCl_3$,[24] or alternatively 2 equiv. of EtMgBr as a base, was required to obtain the expected product **13** (Scheme 3).



Scheme 2. Sonogashira-type reactions with DAC **2**.

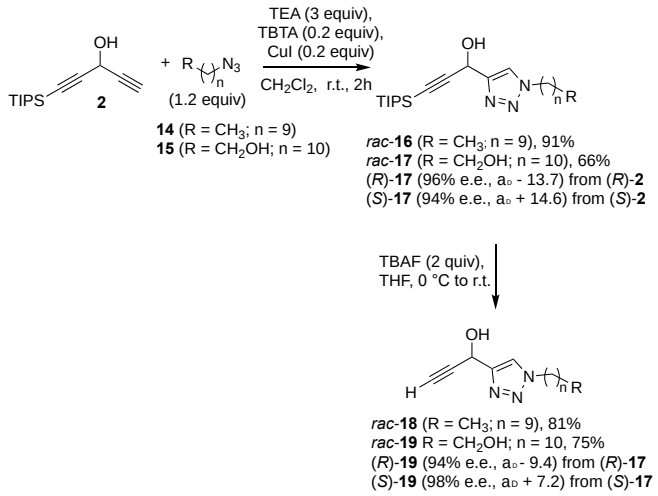


Scheme 3. Selected attempts to react the terminal alkyne of **2** with carbonyl compounds.

Derivatization of the terminal alkyne of 2 *via* a cycloaddition reaction

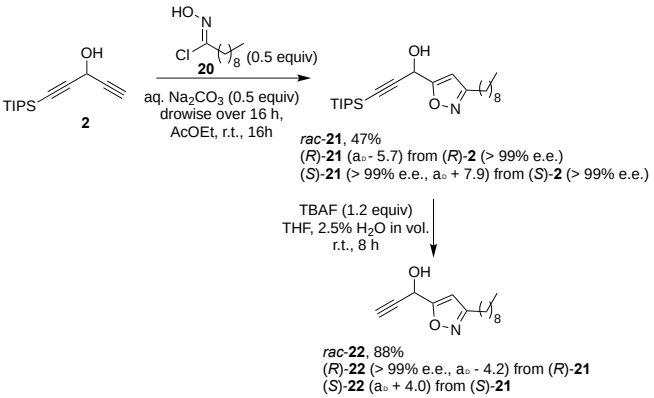
Among the cycloadditions that can be carried out with alkynes, copper-catalyzed 1,3-dipolar cycloaddition reaction stands out as a powerful tool for synthetic organic chemistry. [25] Selective derivatization of the terminal triple bond of **2** was explored *via* a copper catalyzed azide-alkyne cycloaddition (CuAAC) reaction involving the lipidic alkyl azides **14** or **15** (Scheme 4). This

transformation could indeed provide an efficient access to triazolyl alkynylcarbinol skeleton thanks to the complete selectivity arising from the distinct steric accessibility of the two alkyne moieties of **2**. The reaction was performed by adding azide **14** or **15**, prepared from the corresponding alkyl bromide in standard conditions (NaN_3, DMF, 80 °C),[26] to a solution of *rac*-**2**, CuI, TEA and tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) in CH_2Cl_2. The expected 1,2,3-triazoles *rac*-**16** and *rac*-**17** were obtained with 91% and 66% yield, respectively. The *C*-silyl protecting group was then removed by action of TBAF to give the terminal alkynes *rac*-**18** and *rac*-**19**, respectively, in good yields (Scheme 4).



Scheme 4. Derivatization of *rac*-, (*R*)- and (*S*)-**2** via a copper-catalyzed 1,3-cycloaddition reaction to give 1,2,3-triazoles.

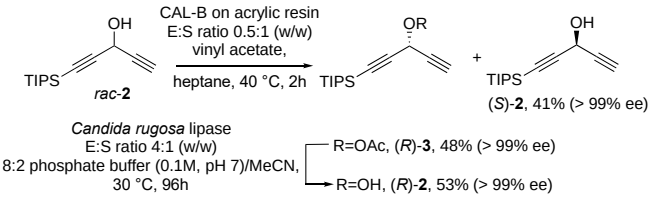
To widen the scope of the tested transformations, we also targeted an isoxazole analogue of *rac*-**21** by means of a 1,3-dipolar cycloaddition between *rac*-**2** and a lipidic nitrile oxide (Scheme 5). Convenient *in situ* formation of this reagent was envisioned by a smooth base-catalyzed dehydrochlorination of the corresponding chlorooxime **20**. The latter was easily prepared by reacting decanal with hydroxylamine hydrochloride followed by chlorination with *N*-chlorosuccinimide (NCS).[27,28] Click-type copper-catalyzed cycloaddition conditions using $CuSO_4$ and sodium ascorbate in the presence of $KHCO_3$ initially delivered the expected isoxazole in 30% yield.[29,30] Screening for optimal procedure, including the standard use of Et_3N in CH_2Cl_2,[31] revealed that dropwise addition of aqueous Na_2CO_3 onto a solution of dipolarophile *rac*-**2** and chlorooxime **20** over 16 h at room temperature in ethyl acetate delivered the targeted cycloadduct *rac*-**21** in 47% isolated yield[32,33] (Scheme 5). The benefit of such mild reaction conditions likely correlates with the sensitivity of the enolizable aliphatic starting chlorooxime that requires long-time storage at low temperature. Subsequent desilylation of the TIPS-protected cycloadduct *rac*-**21** was undertaken (Scheme 5). Standard treatment with TBAF in anhydrous THF at 0 °C for 30 min gave a complex crude mixture from which the expected terminal alkyne *rac*-**22** could be isolated in 64% yield. Addition of 2.5% water in volume as a source of proton to soften the reaction conditions gave a cleaner transformation affording the desilylated product *rac*-**22** in 88% yield.



Scheme 5. Derivatization of *rac*-, (*R*)- and (*S*)-**2** *via* base-promoted 1,3-cycloaddition reaction to give an isoxazole.

Preparation of the enantiopure dissymmetrical dialkynyl methanol (*S*)-2, (*R*)-5 and (*R*)-2 by EKR

Following our previous work,[15] we investigated in details the complementary EKR approach based on lipase-catalyzed acylation of **2** that we showed to be a promising approach (Scheme 6).[34] The objective was both to explore in depth the panel of lipases capable of resolving *rac*-**2** and to develop a direct and efficient access to the enantiomer (*S*)-**2**.



Scheme 6: Optimized conditions for the preparation of (*S*)- and (*R*)-**2** by lipase-mediated EKR of *rac*-**2** and lipase-mediated hydrolysis of acetate (*R*)-**3**. Generic screening conditions otherwise used to optimize the lipase-mediated EKR of *rac*-**2**: Enzyme:Substrate **(**E:S) ratio 2:1 (w/w), hexane, 30 °C, 24 h, 250 rpm.

The screening for lipases active on EKR of *rac*-**2** was performed with ten commercial enzymes (four immobilized and six crude preparations, Table S1). The reaction conditions (Enzyme:Substrate (E:S) ratio 2:1 (w/w), hexane, 30 °C, 24 h, 250 rpm) followed the protocol already established for EKR of racemates via the acylation approach [35] (Scheme 6). The enantiomeric excess of the product (*R*)-**3** (ee_p) and the unreacted substrate (*S*)-**2** (ee_s), the conversion (*c*) and the enantioselectivity (E) obtained for each lipase are shown in Table S1**.**

The absolute configurations of both the acylation product [(*R*)-**3**] and the unreacted substrate [(*S*)-**2**] were determined using the authentic enantiopure samples for comparison of their retention times recorded in the GC-FID chromatograms (Table S1).[15] Among the ten lipases tested, immobilized enzymes Amano lipase PS-IM, CAL-B and TLL (Table S1, entries 1-3, respectively), and the crude Amano lipase AK (Table S1, entry 5) showed optimal results of activity (*c* 50%), selectivity (ee_p and ee_s >99%) and enantioselectivity (E >200), thus proving suitable biocatalysts in the EKR of *rac*-**2** *via* acetylation. The influence of the solvent (Table S2), the reaction time, the temperature and the E:S (Table S3) ratio on these EKR experiments were also examined using

these four selected enzymes (see SI). Non-protic polar solvents such as diethyl ether, THF and acetonitrile were found to have a more pronounced influence on the EKR of *rac*-**2** (Table S2, entries 5-7). The use of the two more polar solvents (THF and acetonitrile) yielded low conversion values (*c* 8-39%) in reactions performed with the active enzymes (Table S2, entries 6 and 7).

Among the nonpolar solvents that promoted ideal values of conversion (*c* 50%) and selectivity (ee_p and ee_s >99%; E >200) in the EKR of *rac*-**2** catalyzed by the four selected lipases, heptane stood up as the more eco-compatible solvent to be used in further studies.[36] Regarding the investigated lipases, CAL-B immobilized on acrylic resin and Amano lipase PS-IM featured as keeping the ideal values of conversion and selectivity in five of the seven solvents investigated. Although both immobilized lipases were equally suitable biocatalysts for the investigated EKR, CAL-B on acrylic resin was selected for the subsequent optimization experiments and enzyme recycling since it is more widely used in both academic and industrial contexts.[37] The optimization of the EKR of *rac*-**2** was carried out by evaluating the influence of the temperature (30 and 40 °C), the reaction time and the E:S ratio. The study was performed in heptane, with vinyl acetate as the acyl donor and CAL-B as the biocatalyst. The results of conversion (*c*), enantiomeric excess of the product (ee_p) and the remaining substrate (ee_s) and enantioselectivity (E) are shown in Table S3 (SI). The ideal values of conversion (*c* 50%) and selectivity (ee_p and ee_s >99%; E >200) were achieved within 2 h, at 40 °C and with an E:S ratio of 0.5:1 (Table S3, entry 4), suggesting the great influence of the temperature in the EKR of *rac*-**2**.

Finally, the study of the recycling of CAL-B immobilized on acrylic resin acrylic in the EKR of *rac*-**2** was carried out using heptane as solvent and vinyl acetate as acyl donor. Performing the transformation at 30 °C for 24 h (E:S ratio 2:1, Table S4, entries 1-3) allowed using the enzyme in two consecutive reaction cycles while maintaining the ideal values of conversion (*c* 50%) and selectivity (ee_p and ee_s >99%; E >200). The conversion (c) value decreased to 41% in the third cycle, even though the enzymatic selectivity remained the same (E >200).

Thus, both the alcohol (*S*)-**2** (*ee* >99%) and acetate (*R*)-**3** (*ee* >99%) were produced in 41 and 48% yield, respectively, using the optimized conditions (CAL-B on acrylic resin, heptane, vinyl acetate, 2 h, at 40 °C, E:S ratio of 0.5:1). After separation of the compounds by column chromatography, acetate (*R*)-**3** was subjected to enzymatic hydrolysis catalyzed by *Candida rugosa* lipase (E:S ratio of 4:1) at 30 °C and 96 h, producing alcohol (*R*)-**2** with 53% of yield and *ee* > 99% (Scheme 6).

Exploration of (*S*)-2 and (*R*)-2 for enantiomeric synthesis of the click compounds

The use of (*R*)-**2** (>99% *ee*) and (*S*)-**2** (>99% *ee*) as starting materials for the synthesis of enantiopure or enantioenriched target compounds was explored in the case of the click reactions (Scheme 4 and 5).

The protected triazole products (*S*)-**17** and (*R*)-**17** were prepared in 62% and 65% yield, respectively, and exhibited comparable $[\alpha]_D^{20}$ values of +14.6 and -13.7 (*c* 0.5, $CHCl_3$), respectively (Scheme 4). The final triazole compounds (*S*)-**19** (81%) and (*R*)-**19** (69%), obtained after silyl group deprotection, showed an $[\alpha]_D^{20}$ of +7.2 and -9.4 (*c* 0.5, $CHCl_3$), respectively. An enantiomeric excess of 94% and 98% for (*S*)-**19** and (*R*)-**19**, respectively, was measured by UPC^2 using a Chiralpak® IC column and CO_2/MeOH as mobile phase (Figure S1-S6). This outcome indicates that copper-catalyzed derivatization of enantiopure compound **2** takes place without significant racemization.

The readily accessible enantiopure **2** was also engaged in the 1,3-dipolar cycloaddition with *in situ*-generated nitrile oxide to form an isoxazole (Scheme 5). Previously developed reaction conditions were first applied to (*S*)-**2** to deliver the expected cycloadducts in 56% yield. UPC^2 analysis using Chiralpak® IH column eluted with CO_2/MeCN allowed verifying the absence of racemization (Figure S7-S8). Upon desilylation with TBAF in wet THF, the deprotected alkynol (*S*)-**22** obtained in 83% yield displayed an $[\alpha]_D^{20}$ of +4.0 (*c* 0.8, CH_2Cl_2). The enantiomer (*R*)-**2** was also transformed, according to the same two-step reaction sequence, into the alkynol (*R*)-**22** that showed an $[\alpha]_D^{20}$ of -4.2 (*c* 0.7, CH_2Cl_2). Determination of the enantiomeric excess of the latter, taking into account the eventuality of a racemization during the desilylation step, proved more challenging. After extensive screening of the analytical conditions, chiral resolution of the desilylated cycloadduct proved feasible by UPC^2 analysis using the Chiralpak IC column eluted with CO_2/MeOH and HCOOH (Figure S9-S10). The enantiopure sample of alkynol (*R*)-**22** revealed a complete preservation of the enantiomeric excess of the starting material (*R*)-**2**.

Biological evaluations

Due to the biological relevance of the dialkynylcarbinol fragment in the development of bioinspired anticancer molecules, we assessed the capacity of some of the newly prepared analogues to affect cancer cell viability. Derivatives **8a,b** were selected on the basis of their similarity with benchmark synthetic LACs displaying potent cytotoxicity against HCT116 colon cancer cells. Previous studies have indeed demonstrated the benefit of conjugating the dialkynylcarbinol pharmacophore of LACs with the π-system of an alkynyl or a phenyl moiety. The resulting series called Butadiynyl AlkynylCarbinol (BAC)[17] or Phenyl diAlkynylCarbinol (PAC)[38] led to values of IC_{50} down to the submicromolar range, with both BAC and PAC sharing a similar mechanism of action with the DAC series (Figure 4).[12,38] In agreement with these precedents, compounds **8a,b** displayed IC_{50} of cytotoxicity of 36-42 nM. The 5-time weaker potency of the saturated analogue **23** (IC_{50} 215 nM)[11] compared to that of **8b** substantiates the favorable effect of extending the dialkynylcarbinol pharmacophore through conjugation with an unsaturated fragment. Comparison of the activity of **8a** (IC_{50} 36 nM)) with that of its direct BAC **24** (IC_{50} 60 nM)[11] and PAC **25** (IC_{50} 150 nM)[38] congeners indicates that the conjugated alkenyl moiety has a stronger influence on potency than a phenyl or an alkynyl one. This result may be due to the particular combination of favorable electronic and geometrical effects. Due to the presence of a characteristic enynol fragment in these compounds, we propose to call NAC (eNynyl AkynylCarbinol) this new series of lipidic alkynylcarbinols. Having previously established that the cytotoxic activity of DAC **23**, BAC **24** and PAC **25** depends on their bioactivation by the human enzyme HSD17B11,[12,38] the

cytotoxicity of the NAC **8b** was tested on the human osteosarcoma cell line, U2OS wild-type or knocked-out for HSD17B11. As observed with DAC and PAC, U2OS cells wild-type for HSD17B11 were more sensitive to **8b** than HCT116, with an IC_{50} of 27 nM (*vs.* 36 nM on HCT116). Strikingly, inactivating HSD17B11 in this cell line reduced by 60 times the cytotoxicity of **8b**, with an IC_{50} increased to 1618 nM in U2OS KO HSD17B11, showing that, similarly to DACs, PACs and BACs, NACs cytotoxicity is mediated by HSD17B11 bioactivation.

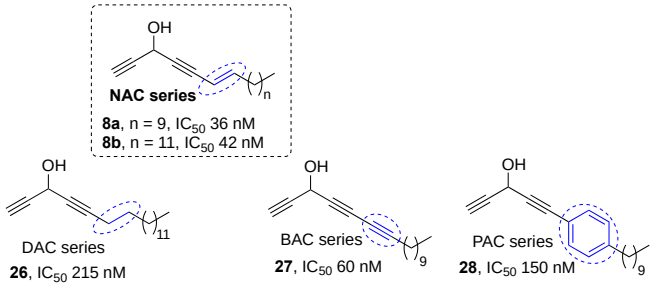


Figure 4. Anticancer potency of NACs **8a,b** in HCT116 cells compared to that of related synthetic LACs.

Conclusion

This work was focused on exploiting the reactivity of a minimal dialkynylcarbinol scaffold to provide a unified access to a broader palette of dialkynylcarbinol-containing lipids and related compounds in racemic and enantiopure versions. Sonogashira reactions and nucleophilic additions to aldehydes, allowing the creation of Csp-Csp^2 (alkene and aryl) and Csp-Csp^3 (RCHOH) bonds, respectively, were successfully carried out with 1-(triisopropyl)silyl)penta-1,4-diyn-3-ol. Selective derivatization of the terminal triple bond of this dissymmetrical dialkynylcarbinol unit was studied *via* click reactions such as a copper-catalyzed 1,3-cycloaddition reaction, affording 1,2,3-triazole compounds, and a 1,3-dipolar cycloaddition with a lipidic nitrile oxide forming an isoxazole derivative. The preparation of (*R*)- and (*S*)-1-(triisopropyl)silyl)penta-1,4-diyn-3-ol by a kinetic enzymatic resolution approach and their use as substrate in click-type reactions illustrated the versatility of this convergent approach to prepare enantiopure or enantioenriched target compounds. Finally, cell viability evaluation showed that the NAC derivatives **8a,b** in which the previously identified dialkynylcarbinol pharmacophore is conjugated with an alkenyl moiety display IC_{50} of cytotoxicity against cancer cells among the lowest recorded to date in this family of bioinspired acetylenic lipids. Similarly to DAC, BAC and PAC, the high cytotoxic activity of NAC in cancer cells was shown to be dependent on HSD17B11, supporting the notion that they also behave as prodrugs bioactivated into cytotoxic species by the action of this human enzyme. Overall, this work confirmed the strong potential for functionalization of this C5 chiral organic framework toward biologically relevant compounds.

Experimental section

THF, diethyl ether, pentane and dichloromethane used in synthetic protocols were dried using a PureSolv system for the purification of solvents. Solvents used in the lipase-mediated kinetic resolution were from Biograde®, HPLC grade solvents were from TEDIA® and vinyl acetate was from Sigma-Aldrich®. All other chemical reagents were used as commercially available. Concentration of commercial solutions of *n*-BuLi were 2.5 M in hexanes, EtMgBr were 3 M in hexanes, TBAF were 1 M in THF, ethynylmagnesium bromide were 0.5 M in THF. The following lipases were purchased from Sigma-Aldrich®: *Candida antarctica* B (CAL-B) immobilized on resin acrylic, Amano lipase AK from *Pseudomonas fluorescens*, lipase from *Rhizopus oryzae*, lipase from *Thermomyces lanuginosus* immobilized on Immobead 150 (TLL), Amano lipase PS from *Burkholderia cepacia*, Amano lipase PS-IM from *Burkholderia cepacia* immobilized on diatomaceous earth, Amano lipase M from *Mucor javanicus*, lipase from *Candida rugosa*, lipase from *Rhizopus niveus* and lipase from the pancreas of the pig. The Lipozyme RM IM from *Rhizomucor miehei* immobilized on anionic resin was from Novo Nordisk® origin. Purifications were proceeded by column chromatography using silica gel (60 P, 70-200 mm) as stationary phase. Analytical silica-gel TLC plates (60 F254, 0.25 mm) were visualized by treatment with an ethanolic solution of phosphomolybdic acid (20%) or with potassium permanganate solution (10%). EKR reactions were monitored using a Shimadzu gas chromatograph (model GC 2010) equipped with an Agilent CP-ChiraSil-DEX CB chiral column, a FID detector and a Shimadzu auto-injector (model AOC-20i). Chiral supercritical fluid chromatographic analyses were performed on a Water UPC^2 Acquity apparatus equipped with a Waters Acquity PDA detector and using the indicated Chiralpak® column. 1H and 13C NMR analyses were performed on Bruker Avance 300 or Avance 400 spectrometer. NMR chemical shifts are in ppm, relative to the tetramethylsilane reference. IR analyses were run on an ATR device (4 cm−1 of resolution, 16 scans) equipped with a DTGS detector. High-resolution mass spectra (HRMS) were performed by desorption chemical ionization DCI or electrospray ionization (ESI) using a TOF mass analyzer.

EKR reaction of *rac*-2 to yield (*S*)-2 and (*R*)-3: *Rac*-**2** (50 mg, 0.211 mmol) was subjected to EKR using CAL-B on acrylic resin (25 mg, (E:S) ratio 0.5:1 (w/w)), heptane (6.75 mL) and vinyl acetate (3 mL) under stirring at 250 rpm for 2 h at 40 °C. After filtration of the enzyme and solvent distillation under reduced pressure, the crude material was purified by liquid absorption chromatography using a mixture of hexane and CH_2Cl_2 (1:1) as mobile phase to deliver alcohol (*S*)-**2** (20.4 mg, 41% yield, > 99% ee according to GC analysis) and acetate (*R*)-**3** (28.2 mg, 48% yield, > 99% ee according to GC analysis using Agilent CP-ChiraSil-DEX CB chiral column). Analytical data for (*S*)-**2** and (*R*)-**3** were in agreement with the literature.[15,34]

Enzymatic hydrolysis of (*R*)-3 to yield (*R*)-2: Acetate (*R*)-**3** (50 mg, 0.180 mmol) was hydrolyzed by treatment with *Candida rugosa* lipase (200 mg, (E:S) ratio 4:1 (w/w)) in a 8:2 mixture of sodium phosphate buffer (0.1 M, pH 7) and MeCN as cosolvent (5mL) during 96 h at 30 °C to furnish (*R*)-**2** (53% yield, > 99% ee according to GC analysis using Agilent CP-ChiraSil-DEX CB chiral column). Analytical data for (*R*)-**2** were in agreement with the literature.[15,34]

(6*E*)-1-[Tris(propan-2-yl)silyl]heptadec-6-en-1,4-diyn-3-ol (7a): To a solution of **2** (200 mg, 0.85 mmol, 1.0 equiv) in dry THF (3 mL), (1*E*)-1-bromododec-1-ene (230 mg, 0.93 mmol, 1.1 equiv), bis(triphenylphosphine)palladium(II) dichloride (12 mg, 0.02 mmol, 2 mol%) and copper(I) iodide (6 mg, 0.03 mmol, 4 mol%) were added at 0 °C. The reaction mixture was stirred for 5 min at the same temperature, then diisopropylamine (236 µL, 1.69 mmol, 2.0 equiv) was added and the mixture was stirred for 18 h at room temperature. After treatment with a saturated aqueous NH_4Cl solution, the aqueous layer was extracted twice with diethyl ether, the organic phase was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel column (cyclohexane/diethyl ether, gradient 100:0 to 95:5) to give **7a** as an orange oil (279 mg, 82% yield). ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ=6.20 (dt, J = 15.7, 7.0 Hz, 1H), 5.49 (dq, J = 15.7, 1.6 Hz, 1H), 5.22 (dd, J = 7.3, 1.8 Hz, 1H), 2.20 (d, J = 7.7 Hz, 1H), 2.10 (qd, J = 7.0, 1.6 Hz, 2H), 1.44 – 1.33 (m, 2H), 1.34 – 1.23 (m, 14H), 1.12 – 1.05 (m, 21H), 0.95 – 0.83 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ=146.8 (CH), 108.6 (CH), 104.4 (C), 86,0 (C), 84.7 (C), 83.3 (C), 53.3 (CH), 33.3 (CH_2), 32.1 (CH_2), 29.7 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.2 (CH_2), 28.7 (CH_2), 22.8 (CH_2), 18.7 (6 x CH_3), 14.3 (CH_3), 11.3 (3 x CH); IR (neat, cm^{-1}) ν_{max}=3373, 2924, 2855, 2219, 2188, 1463, 1031, 882, 674; HRMS (DCI-CH_4): *m/z* calcd for $C_{26}H_{47}OSi$: 403.3396 $[M+H]^+$; found: 403.3404; HRMS (DCI-CH_4): *m/z* calcd for $C_{26}H_{45}OSi_2$ $[M-H]^-$: 401.3240; found: 401.3248.

(6*E*)-Heptadec-6-en-1,4-diyn-3-ol (8a) : To a solution of **7a** (207 mg, 0.51 mmol, 1.0 equiv) in dry THF (2 mL), tetra-*n*-butylammonium fluoride (1 M in THF, 1.54 mL, 1.54 mmol, 3.0 equiv) was added at 0 °C. The reaction mixture was stirred

for 45 min at the same temperature. After treatment with a saturated aqueous NH_4Cl solution, the aqueous layer was extracted with diethyl ether twice, the organic phase was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel column (pentane/diethyl ether, gradient 98:2) to give **8a** as a brown solid (46 mg, 33% yield). M.p. 35 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ=6.23 (dt, *J* = 16.0, 7.1 Hz, 1H), 5.49 (dq, *J* = 16.0, 1.8 Hz, 1H), 5.25 – 5.18 (m, 1H), 2.57 (d, *J* = 2.3 Hz, 1H), 2.32 (d, *J* = 7.4 Hz, 1H), 2.11 (qd, *J* = 7.2, 1.7 Hz, 2H), 1.42 – 1.33 (m, 2H), 1.32 – 1.20 (m, 14H), 0.92 – 0.80 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ=147.3 (CH), 108.3 (CH), 83.9 (2 x C), 81.1 (C), 72.6(CH), 52.6 (CH), 33.3 (CH_2), 32.0 (CH_2), 29.7 (2 x CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.2 (CH_2), 28.7 (CH_2), 22.8 (CH_2), 14.3 (CH_3); IR (neat, cm^{-1}) ν_{max}=3278, 2958, 2921, 2849, 2216, 2124, 1463, 1290, 1163, 1017, 951, 697, 663; HRMS (DCI-CH_4): *m/z* calcd for $C_{17}H_{27}O$ $[M+H]^+$: 247.2062; found: 247.2074.

Heptadeca-1,4-diyne-3,6-diol (12): To a round bottom flask containing **2** (100 mg, 0.42 mmol, 1.0 equiv) in THF (4 mL) at -78 °C under a dry N_2 atmosphere with stirring, *n*-butyl lithium (1.6 M in THF, 0.58 mL, 0.93 mmol, 2.2 equiv) was added dropwise. After stirring the solution at the same temperature for 1 h, a solution of dodecanal (78 mg, 0.42 mmol, 1.0 equiv) in THF (2mL) was added. The reaction was monitored by TLC until consumption of the carbonyl compound, quenched with a saturated solution of NH_4Cl. The mixture was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel). (petroleum ether/diethyl ether, 7:3) to give the intermediate **1-(triisopropylsilyl)heptadeca-1,4-diyne-3,6-diol** (46 mg, 26%) as a diastereoisomeric mixture. ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ=5.09 (t, *J* = 1.6, 1H), 4.35 (tdd, *J* = 6.6, 3.4, 1.6, 1H), 2.6 – 2.2 (br s, 1H), 2.2 – 1.7 (br s, 1H), 1.76 – 1.67 (m, 2H), 1.44 – 1.31 (m, 2H), 1.29 – 1.22 (m, 16H), 0.95 – 0.80 (m, 3H).

At 0 °C under N_2 atmosphere, the intermediate **1-(triisopropylsilyl)heptadeca-1,4-diyne-3,6-diol** (46 mg, 0.11 mmol, 1.0 equiv.) was dissolved in anhydrous THF. TBAF (1M in THF, 221 µL, 0.22 mmol, 2.0 equiv.) was added dropwise. The reaction mixture was allowed to reach rt. After completion, the reaction was quenched with a saturated solution of NH_4Cl. The mixture was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel). (petroleum ether/diethyl ether, 7:3) to give **12** (24 mg, 83%) as a diastereoisomeric mixture. ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ=5.16 (t, *J* = 1.8, 1H), 4.42 (tt, *J* = 6.6, 1.9, 1H), 3.51 (br s, 1H), 2.87 (br s, 1H), 2.56 (d, *J* = 2.3, 1H), 1.76 – 1.67 (m, 2H), 1.48 – 1.39 (m, 2H), 1.29 – 1.22 (m, 16H), 0.95 – 0.80 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ=86.0, 81.7, 80.9, 72.9, 62.5, 51.9, 37.5, 32.0, 29.8, 29.8, 29.7, 29.7, 29.5, 29.4, 25.2, 22.8, 14.2. HRMS (DCI-CH_4): *m/z* calcd for $C_{17}H_{29}O_2$: 263.2011 $[M-H]^+$; found: 263.2014.

11-(4-(1-Hydroxy-3-(triisopropylsilyl)prop-2-yn-1-yl)-1*H*-1,2,3-triazol-1-yl)undecan-1-ol (17): Compound **2** (100 mg, 0.423 mmol), azide **15** (108.08 mg, 0.507 mmol), CuI (16.1 mg, 0.085 mmol), tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (44.85 mg, 0.085 mmol), and triethylamine (177 µL, 1.27 mmol) were dissolved in dried CH_2Cl_2 (5 mL) and under argon atmosphere. The reaction was maintained under stirring for 4 h at room temperature. The solvent was distilled under reduced pressure and the residue was purified by column chromatography on silica gel using pentane/EtOAc (7:3) as eluent to give **17** as a white solid (125 mg, 66% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ=7.57 (s, 1H), 5.67 (s, 1H), 4.28 (t, *J* = 7.1 Hz, 2H), 3.56 (t, *J* = 6.7 Hz, 2H), 1.82 (t, *J* = 7.1 Hz, 2H), 1.54 – 1.43 (m, 2H), 1.22 (d, *J* = 18.6 Hz, 15H), 1.02 (s, 21H); ^{13}C NMR (101 MHz, $CDCl_3$, ppm) δ=149.0 (C), 121.6 (CH), 106.0 (C), 86.1 (C), 62.7 (CH_2), 57.0 (CH), 50.5 (CH_2), 32.6 (CH_2), 30.1 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 28.9 (CH_2), 26.3 (CH_2), 25.7 (CH_2), 18.6 (6 x CH_3), 11.4 (3 x CH); IR (neat, cm^{-1}) ν_{max}=3318, 2913, 2848, 2176, 1467, 1227, 1140, 1041, 969, 884, 797, 676, 579, 463; HRMS-DCI (CH_4): *m/z* calcd for $C_{25}H_{48}N_3O_2Si$: 450.3516 $[M+H]^+$; found: 450.3525.

(*S*)-11-(4-(1-Hydroxy-3-(triisopropylsilyl)prop-2-yn-1-yl)-1*H*-1,2,3-triazol-1-yl)undecan-1-ol ((S)-17): Obtained (62% yield, 94% ee as determined by UPC^2 analysis using a Chiralpak® AS column eluted with CO_2/*i*-PrOH) from (*S*)-**2** (ee > 99%) following the procedure described for *rac*-**17**. Physical data were identical to that of *rac*-**17** excepted for optical rotation. $[\alpha]_D^{20}$= +14.6 (c= 0.5 in $CHCl_3$).

(*R*)-11-(4-(1-Hydroxy-3-(triisopropylsilyl)prop-2-yn-1-yl)-1*H*-1,2,3-triazol-1-yl)undecan-1-ol ((*R*)-17): Obtained (65% yield, 96% ee as determined by UPC^2 analysis using a Chiralpak® AS column eluted with CO_2/*i*-PrOH) from (*R*)-**2** (ee > 99%) following the procedure described for *rac*-**17**. Physical data were identical to that of *rac*-**17** excepted for optical rotation. $[\alpha]_D^{20}$= −13.7 (c= 0.5 in $CHCl_3$).

11-(4-(1-Hydroxyprop-2-yn-1-yl)-1*H*-1,2,3-triazol-1-yl)undecan-1-ol (19): To a stirring solution of **17** (0.214 mmol, 96 mg) in dried THF (8 mL) at 0 °C and under argon atmosphere, it was added a solution of tetrabutylammonium fluoride (1M in THF, 0.428 mmol, 428 µL). The mixture was stirred for 4 h at room temperature and product formation was followed by TLC. The reaction was quenched by addition of saturated aqueous solution of NH_4Cl (5 mL), followed by extraction with EtOAc (3 x 5 mL). The combined organic fractions were dried over anhydrous $MgSO_4$, filtrated and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel and using 100% EtOAc as eluent to give **19** as a white solid (47 mg, 75% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ=7.63 (s, 1H), 5.69 (s, 1H), 4.36 (t, *J* = 7.2 Hz, 2H), 3.65 (t, *J* = 6.6 Hz, 2H), 2.63 (d, *J* = 2.3 Hz, 1H), 1.91 (t, *J* = 7.2 Hz, 2H), 1.60 – 1.55 (m, 2H), 1.33 – 1.25 (m, 14H); ^{13}C NMR (101 MHz, $CDCl_3$, ppm) δ=148.1 (C), 121.6 (CH), 82.4 (C), 73.9 (CH), 63.1 (CH_2), 57.0 (CH), 50.7, (CH_2), 32.8 (CH_2), 30.3 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (2 x CH_2), 28.9 (CH_2), 26.5 (CH_2), 25.8 (CH_2); IR (neat, cm^{-1}) ν_{max}=3283, 3128, 2917, 2847, 2119, 1461, 1360, 1226, 1147, 1055, 1004, 940, 795, 670, 581; HRMS-DCI (CH_4) *m/z* calcd for $C_{16}H_{28}N_3O_2$: 294.2182 $[M+H]^+$; found: 294.2172.

(*S*)-11-(4-(1-Hydroxyprop-2-yn-1-yl)-1*H*-1,2,3-triazol-1-yl)undecan-1-ol ((*S*)-19): Obtained (81% yield, 94% ee as determined by UPC^2 analysis using a Chiralpak® IC column eluted with CO_2/MeOH) from (*S*)-**17** following the procedure described for *rac*-**19**. Physical data were identical to that of *rac*-**19** excepted for optical rotation. $[\alpha]_D^{20}$= +7.2 (c= 0.5 in $CHCl_3$).

(*R*)-11-(4-(1-Hydroxyprop-2-yn-1-yl)-1*H*-1,2,3-triazol-1-yl)undecan-1-ol ((*R*)-19): Obtained (69% yield, 98% ee as determined by UPC^2 analysis using a Chiralpak® IC column eluted with CO_2/MeOH) from (*R*)-**17** following the procedure described for *rac*-**19**. Physical data were identical to that of *rac*-**19** excepted for optical rotation. $[\alpha]_D^{20}$= −9.4 (c= 0.5 in $CHCl_3$).

1-(3-Nonylisoxazol-5-yl)-3-(triisopropylsilyl)prop-2-yn-1-ol (21): Compound **2** (229 mg, 0,972 mmol, 2.0 equiv.) and *N*-hydroxydecanimidoyl chloride (100 mg, 0.486 mmol, 1.0 equiv.) were dissolved into EtOAc. An aqueous solution of Na_2CO_3 (103 mg, 0.972 mmol, 2.0 equiv.) was added by syringe-pump over 16 h at room temperature. After completion of the reaction, the resulting solution was extracted with EtOAc, dried over $MgSO_4$ and concentrated under reduced pressure. The crude mixture was purified by flash chromatography on silica gel using up to 10% of diethyl ether in pentane. The 3-nonylisoxazole **21** (92 mg, 41%) was isolated as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ=6.20 (d, *J* = 0.7 Hz, 1H), 5.55 (dd, *J* = 7.0, 0.7 Hz, 1H), 2.66 (t, *J* = 7.6 Hz, 2H), 1.70-1.57 (m, 2H), 1.40-1.16 (m, 12H), 1.08 (bs, 21H), 0.91-0.80 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ=169.8, 164.2, 102.9, 101.8, 88.8, 57.7, 31.8, 29.4, 29.3(*2), 29.1, 28.2, 26.0, 22.7, 18.5, 14.1, 11.1; HRMS (DCI-CH_4): *m/z* calcd for $C_{24}H_{44}NO_2Si$: 406.3141 $[MH]^+$; found: 406.3145.

(*S*)-1-(3-Nonylisoxazol-5-yl)-3-(triisopropylsilyl)prop-2-yn-1-ol ((*S*)-21): obtained (56% yield, >99% ee as determined by UPC^2 analysis using a Chiralpak® IH column eluted with CO_2/MeCN) from (*S*)-**2** (ee> 99%) following the procedure described for *rac*-**21**. Physical data were identical to that of *rac*-**21** excepted for optical rotation. $[\alpha]_D^{20}$= + 7.9 (c 0.8, CH_2Cl_2).

(*R*)-1-(3-nonylisoxazol-5-yl)-3-(triisopropylsilyl)prop-2-yn-1-ol ((*R*)-21): obtained (48% yield) from (*R*)-**2** (ee> 99%) following the procedure described for *rac*-**21**. Physical data were identical to that of *rac*-**21** excepted for optical rotation. $[\alpha]_D^{20}$= − 5.7 (c 0.8, CH_2Cl_2).

1-(3-Nonylisoxazol-5-yl)prop-2-yn-3-ol (22): At 0 °C under N_2 atmosphere, **21** (30 mg, 0.074 mmol, 1.0 equiv.) was dissolved in wet THF (2.5 v% H_2O). TBAF (1 M in THF, 89 µL, 0.089 mmol, 1.2 equiv.) was added dropwise. After completion of the reaction, the resulting solution was extracted with diethyl ether, dried over $MgSO_4$ and concentrated under reduced pressure. The crude mixture was purified by flash chromatography on silica gel using up to 10% of diethyl ether in pentane. Compound **22** (15 mg, 88%) was isolated as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ = 6.24 (s, 1H), 5.55 (d, *J* = 2.3 Hz, 1H), 2.71-2.59 (m, 3H), 1.76-1.59 (m, 2H), 1.43-1.20 (m, 12H), 0.92-0.82 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ=169.1, 164.3, 102.0, 79.9, 75.1, 57.1, 31.9, 29.4, 29.3 (*2), 29.2, 28.2,

26.0, 22.7, 14.11; HRMS (DCI-CH_4): *m/z* calcd for $C_{15}H_{24}NO_2$: 250.1807 $[MH]^+$; found: 250.1800.

(*S*)-1-(3-nonylisoxazol-5-yl)prop-2-yn-3-ol ((*S*)-22): obtained (83% yield) from (*S*)-**21** following the procedure described for *rac*-**22**. Physical data were identical to that of *rac*-**25** excepted for optical rotation $[\alpha]_D^{20}$= + 4.0 (c 0.8, CH_2Cl_2).

(*R*)-1-(3-nonylisoxazol-5-yl)prop-2-yn-3-ol ((*R*)-22): obtained (85% yield, > 99% ee according to UPC[2] analysis using the Chiralpak IC column eluted with CO_2/MeOH and HCOOH) from (*R*)-**21** following the procedure described for *rac*-**22**. Physical data were identical to that of *rac*-**22** excepted for optical rotation. $[\alpha]_D^{20}$= − 4.2 (c 0.7, CH_2Cl_2).

Supporting Information

The authors have cited additional references within the Supporting Information.[39,40,41,42]

Acknowledgements

Pr. Remi Chauvin is thanked for his intellectual contribution to this work. We are thankful to Dr. Etienne Joly for initial cell viability data. Brazilian authors thank the Coordenação de Aperfeiçoamento de Ensino Superior (CAPES) for the financial support (Finance Code 001- PROEX 23038.000509/2020-82. AUXPE Nº: 1227/2020), besides the sponsorships of M. V. B. (process: 88887.319063/2019-00) and D. M. B. D. (process: 88887.321412/2019-00). M. C. F. O. (Process: 305148/2023-0) and M. C. de M. (Process: 306289/2021-0) thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for their research grant. French and Brazilian authors thank CAPES/COFECUB (Proc. Nr. 88887.130198/2017-01 and 88887.319063/2019-00) for the financial support of the international collaborative project and Project no Ph-C883–17) for the financial support of the international collaborative project. M. B. thanks the Région Occitanie for her scholarship. S. B. and M. C. acknowledge the support of ANR (ANR- 17-CE18-0002 and ANR-22-CE44-0039-03). Thanks are also due to the CNRS and to the Toulouse University for funding. We are grateful to Isabelle Fabing for scientific and technical expertise on UPC[2] provided by the Integrated Screening Platform of Toulouse (PICT, IBiSA).

Keywords: Alkynes • Lipids • Enzymatic kinetic resolution • Carbinols • Click chemistry

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