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PAPER

Total synthesis of the proposed structures of the DNA methyl transferase inhibitors peyssonenynes, and structural revision of peyssonenyne B†

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The purported structures of the peyssonenynes A and B isolated from *Peyssonnelia caulifera*, and considered to be geometric isomers at the acetoxyenediyne moiety, have been synthesized. The *E* and *Z* geometries of the synthetic compounds were secured by the magnitude of the $^{3}J_{H9-C7}$ values measured using the EXSIDE band-variant of the gradient HSQC pulse sequence and by the chemical shifts of C_6. Comparison of the NMR data of the synthetic and natural products revealed that only those of the *Z* isomers matched, which correspond to peyssonenyne A. Using HPLC analysis it was found that peyssonenyne B must correspond to the *sn*-2 positional isomer of the *Z sn*-1/3 counterpart. The four synthetic *sn*-1/3 diastereomers are roughly equipotent as DNMT1 inhibitors when evaluated on a radioactive methyl transfer enzymatic assay after immunoprecipitation from K562 human leukemia cells with anti-DNMT1 antibody.

Introduction

The oxylipin metabolites peyssonenynes A and B (**1**) were isolated in 2004 from the Fijian red marine alga *Peyssonnelia caulifera*.[1] These oxidized monoacylglyceride oxylipins, which derive biogenetically from a ω3 polyunsaturated fatty acid, are a rare occurrence in red algae, and the 1,3-diyne[2] conjugated to the enolacetate functionality of the peyssonenynes is so far unique to these natural products.[3]

In vitro assays showed that these peyssonenynes A and B inhibited DNA methyl transferase 1 (DNMT1) with IC_{50} values of 16 and 9 μM, respectively. DNMT1 is a member of the DNA methyl transferase protein family,[4] which includes in mammals DNMT2 and DNMT3 (A, B and L). DNMT1 is responsible for maintenance of the DNA methylation pattern during chromosome replication.[5] DNMT2 has no defined role, whereas DNMT3A and DNMT3B are *de novo* methyltransferases that establish embryonic methylation patterns. DNMT3L lacks intrinsic catalytic activity, but modulates the activity of DNMT3A and DNMT3B, to which it is physically associated.[4]

The transfer of a methyl group from *S*-adenosylmethionine (SAM) to DNA cytosine C5 position within CpG dinucleotide-rich regions (CpG islands) is the best known of the covalent modifications of the epigenome. The patterns and extension of DNA methylation impacts on key biological activities, among them the control of gene expression, the regulation of parental imprinting, the stabilization of X-chromosome inactivation and the maintenance of genome integrity in eukaryotes.[5] Aberrant patterns of DNA methylation are associated with several diseases, including the onset and progression of cancer.[4] Cancer cells show global hypomethylation of CpG islands and site-specific hypermethylation of DNA promoter regions (in particular, of tumor suppressor genes) which are normally unmethylated.[6]

Targeting enzymes that modify chromatin (the DNA and its associated histones) with epigenetic drugs is considered a novel and promising anticancer strategy.[7] Selective DNMT inhibitors (DNMTis) might rapidly reactivate the expression of epigenetically-silenced tumour suppressor genes, and this reactivation could lead to growth inhibition of tumour cells or alteration of their sensitivity to other anticancer therapies.[7] Two DNMTis (5-aza-cytidine, Vidaza®, 5-aza-2′-deoxycytidine, Dacogen®) are already in the clinic for the treatment of myelodysplastic syndrome. However, these drugs are cytotoxic azanucleosides, and novel inhibitors with alternative mechanisms of action are actively sought.[7]

Our interest in the anticancer activities of natural product-based epigenetic modulators[8] led us to address the total synthesis of the peyssonenynes, determine their DNMT inhibitory profile and validate these modulators in cell-based assays. Added to this aim was the need to authenticate the gross structure by unambiguous characterization of the enolacetate geometry and determination of the absolute configuration of the glycerol stereocenter. These issues

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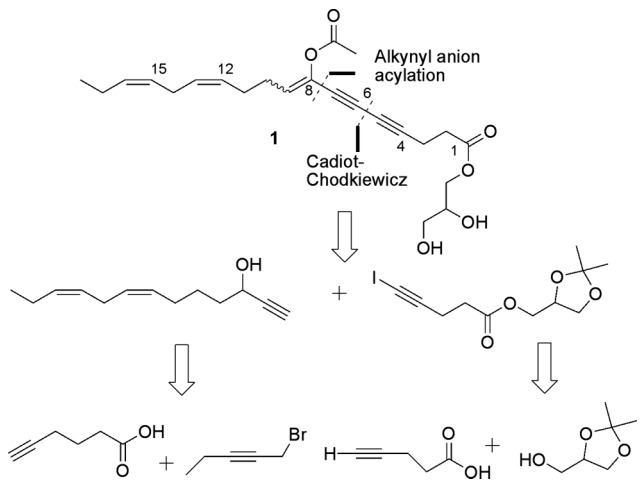
have remained unsolved due to the instability of the peyssonenynes A and B and the minute amounts of these acetoxyenediyne oxylipins that were isolated from the natural source (1.3 and 0.4 mg, respectively, from 365 g of dry alga).[1]

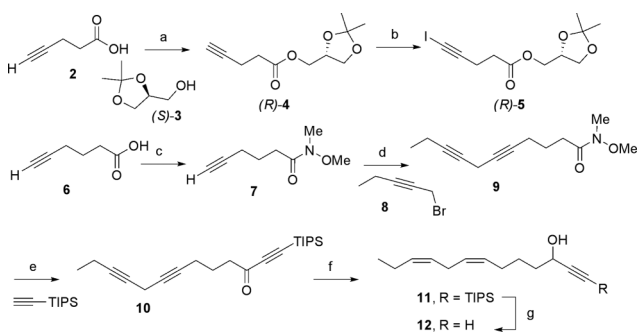
Results and discussion

In our retrosynthetic analysis the generation of the sensitive enolacetate of the peyssonenynes was postponed to a late stage of the sequence after construction of the diyne moiety[9] by the Cadiot–Chodkiewicz coupling[10] of a 1-iodoalkyne and a propargylic alcohol (Scheme 1). These fragments could be traced back to appropriate combinations of glycerol, two terminal alkynoic acids and a propargylic bromide.



Scheme 1 Retrosynthetic analysis of the peyssonenynes.

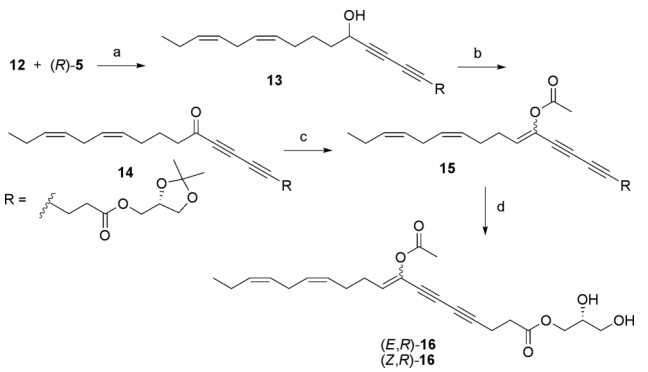
Scheme 2 outlines the synthetic sequence that has been optimized for the preparation of the required building blocks. The chiral fragments of each enantiomer of **1** were derived from enantiopure glycerol isopropylidene ketal **3**, itself obtained from either *D*-mannitol or *L*-gulono-1,4-lactone (only the *R* series is shown in Scheme 2, which starts from (*S*)-**3**) as described.[11] DCC-induced coupling of (*S*)-**3** with pent-4-ynoic acid **2** gave (*R*)-**4** and iodination of the latter provided enantiopure ester (*R*)-**5** in good yield.



Scheme 2 Reagents and reaction conditions: (a) DCC, DMAP, CH_2Cl_2, 25 °C, 12 h, 98%. (b) NIS, $AgNO_3$, acetone, 12 h, 25 °C (69%). (c) EDC, DMAP, HN(OMe)Me·HCl, CH_2Cl_2, 25 °C (100%). (d) Cs_2CO_3, NaI, CuCl, DMF, 25 °C. (e) *n*-BuLi, THF, –40 to 25 °C. (f) $Ni(OAc)_2·4H_2O$, $NaBH_4$, ethylenediamine, EtOH, 1 atm H_2 (75% over the three steps). (g) TBAF, THF, 25 °C, 0.5 h (81%).

Among the alternative routes to the skipped 1,4-diene unit of alkyne **12**,[12] we selected the Cs_2CO_3-promoted coupling between the terminal alkyne **7** and 1-bromopent-2-yne **8** (Scheme 2)[13] followed by semihydrogenation. Hex-5-ynoic acid **6** was converted into the Weinreb amide[14] **7** by treatment with *N*,*O*-dimethylhydroxylamine hydrochloride, EDC and DMAP.[15] Upon Cu(I)-promoted condensation of alkyne **7** and propargylic bromide **8** in the presence of NaI and Cs_2CO_3, diyne **9** was obtained (Scheme 2). Since acetylenic intermediates were difficult to handle because of their instability in solution, they were used in the next reactions without purification. Addition of the anion of TIPS-acetylene to the Weinreb amide **9** afforded the propargylic ketone **10**. Hydrogenation of **10** in the presence of Lindlar catalyst failed to provide the desired product. We turned our attention to P2-Ni catalyst, which is generated *in situ* by reduction of nickel acetate with sodium borohydride in ethanol.[16] Given the sensitivity of the reaction to the steric environment of the unsaturation, the hydrogenation was selective for the skipped diyne (the alkynylsilane remained unaffected) although the reagent also caused the reduction of the ketone to the corresponding alcohol and provided compound **11** (75%, 3 steps).[17] Deprotection of **11** using TBAF afforded propargylic alcohol **12** in good yield.

The conditions reported by Alami and Ferri (CuI, piperidine)[18] were selected for the key cross-coupling reaction[19] given their experimental simplicity in comparison with the standard Cadiot–Chodkiewicz protocol (CuCl, $HONH_2·HCl$, Et_2NH, MeOH). Condensation of propargylic alcohol **12** and iodoalkyne (*R*)-**5** provided diynol **13**, which was oxidized to diynylketone **14** using the Swern conditions (Scheme 3).



Scheme 3 Reagents and reaction conditions: (a) CuCl, piperidine, 0 °C, 6 h (71%). (b) i. DMSO, $(COCl)_2$, CH_2Cl_2, –60 °C; ii. Addition of **13** at –60 °C; iii. Et_3N, –60 to 25 °C (75%). (c) Et_3N, DMAP, Ac_2O, CH_2Cl_2, 25 °C, 4 h (83%). (d) CAN (3 mol%), CH_3CN/H_2O, 70 °C (86%; (*E*,*R*)-**16**, 35%; (*Z*,*R*)-**16**, 16%, after RP-HPLC separation).

A survey of methods for enol acetate formation starting from the unsaturated ketone **14** revealed moderate *E*/*Z* stereoselectivities and low yields in most cases. However, the use of Et_3N, DMAP and Ac_2O[20] provided a 1 : 2 mixture of enolacetate isomers **15** in 83% yield. Without separation the ketal protecting group of **15** was removed using catalytic quantities of cerium(IV) ammonium nitrate (CAN) at 70 °C[21] to afford acylglycerol **16** as a mixture of geometric isomers. These compounds were separated using RP-HPLC (Nova-Pak-C18, 60 Å, 300 × 19 mm, 3 : 1 MeOH/H_2O,

5 mL min^{-1}) to yield the desired (*E*,*R*)-**16** and (*Z*,*R*)-**16** isomers in 35% and 16% yield, respectively. The same sequence (not shown) starting from (*R*)-**3** afforded synthetic material matching (identical ^{1}H- and ^{13}C-NMR spectra) those described above, but with (*S*) configurations.[22]

Separation and purification of the peyssonenynes

The determination of the enantiomeric purity of the peyssonenynes (undisclosed in the original publication)[1] was complicated by the known configurational instability of monoacylglycerols.[23] Monoacylglycerols consist of three isomers, two enantiomers termed *sn*-1/*sn*-3 and their achiral *sn*-2 regioisomer. It is known that rapid 1,2-acyl migrations from the *sn*-1 to the *sn*-3 position *via* the achiral *sn*-2 regioisomer cause significant erosion of their enantiomeric purity.[24] RP-chiral HPLC[25] (Chiralpak® IA, 250 × 10 mm, 100% MeOH, 1.5 mL min^{-1}) was very effective in the separation of the isomers of (*E*,*R*)-**16** and (*E*,*S*)-**16** and the enantiomer excess was determined as *ee*(*R*) = 72% and *ee*(*S*) = 69%. Although traces of acids and bases are reported to induce 1,2-acyl migration,[23] the loss of configurational integrity of **16** is likely traced back to the thermal conditions (70 °C) of the dioxolane deprotection step using CAN, a transformation that could not be induced at lower reaction temperatures. When the same conditions were applied to (*Z*,*R*)-**16** and (*Z*,*S*)-**16**, a baseline separation of *sn*-1/*sn*-3 enantiomers and *sn*-2 regioisomer could not be achieved using a variety of eluents in both normal or reverse phase modes (*n*-hexane/2-propanol, *n*-hexane/2-propanol/CH_2Cl_2, *n*-hexane/AcOEt, *n*-hexane/AcOEt/CH_2Cl_2, *n*-hexane/THF, THF/H_2O) or other chiral columns recommended for the separation of monoacylglycerols of fatty acids like Chiracel OD-H or Chiralpak® IC.[26] Despite this failure, it is reasonable to assume that the enantiomeric excess for both *E* and *Z* geometric isomers is the same since they underwent the same sequence of chemical transformations and purification steps.

Structural assignment of the synthetic and natural peyssonenynes

Attempts to determine the geometry of the enol acetate moiety using either analysis of ^{1}H-NMR/^{13}C-NMR chemical shifts or NOE-difference experiments proved unsuccessful. Gerwick *et al.* could not complete the assignment of the peyssonenynes' geometries due to the paucity of protons in the vicinity of the enolacetate functional group. Although they noted that the geometric isomers showed a most significant *ca.* 0.5 ppm chemical shift difference for the C6 and C8 atoms (C6: δ 74.1 ppm and 73.6 ppm; C8: δ 129.5 ppm and 128.9 ppm, respectively), the low amounts of the natural specimens that remained left (80 μg) precluded the use of 2D-ROESY experiments for structural purposes.[1] In our hands 2D-ROESY was also inconclusive despite having access to larger amounts of synthetic material. Our attention was then turned to a method for the determination of olefin geometry based on the magnitude of the long-range ^{1}H–^{13}C coupling constant ($^{n}J_{H\text{-}C}$) that uses a band-variant of the gradient HSQC pulse sequence termed EXSIDE (Excitation-sculptured Indirect Detection Experiment).[27] The $^{3}J_{H\text{-}C}$ values for the pair of double bond isomers ($^{3}J_{H\text{-}C}$ = 5.24 Hz for *Z*; $^{3}J_{H\text{-}C}$ = 12.68 Hz for *E*; see Fig. 1) fall within the range measured for isomers in series of acetoxyendiynes and acetoxyenynes ($^{3}J_{H\text{-}C}$ varies from 2.6 to 5.2 Hz for *Z*, and from 10.5 to 12.7 Hz for *E*).[28] We also showed that the values of the chemical shifts of the C_{sp}-β carbons of the alkyne attached to the enolacetate are a more straightforward criteria for double bond configurational assignment in acetoxyenediynes (values for C-6 of δ 75.35 and δ 80.00 ppm for (*Z*,*R*)-**16** and (*E*,*R*)-**16**, respectively, see Fig. 2).

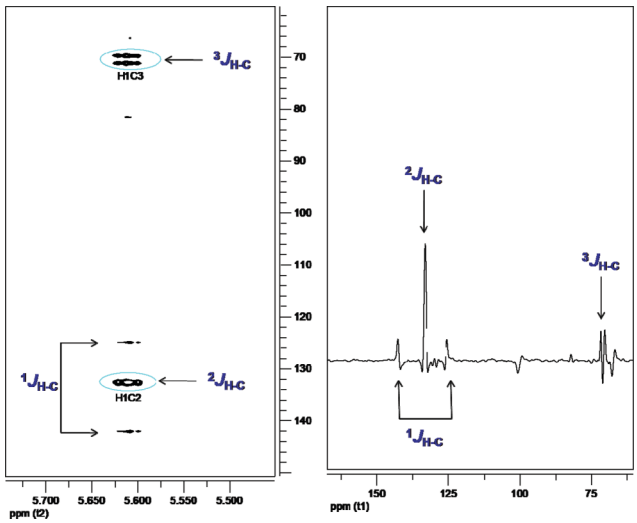


Fig. 1 (Left) 2D EXSIDE of compound (*Z*,*R*)-**16**. (Right) The corresponding 1D vertical trace extracted from the 2D EXSIDE. The relevant signals for the extraction of the $^{1}J_{H\text{-}C}$, $^{2}J_{H\text{-}C}$ and $^{3}J_{H\text{-}C}$ scalar coupling constants are indicated.

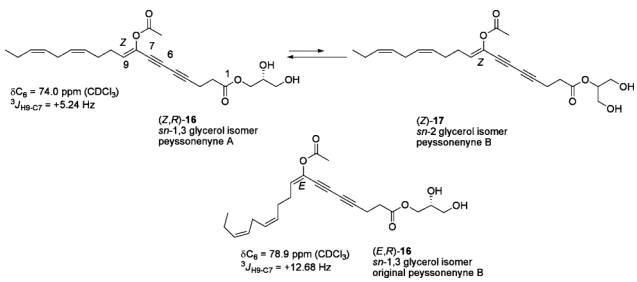


Fig. 2 Dynamic interconversion of the monoacylglycerol positional isomers of peyssonenyne A, and structure of peyssonenyne B (original and corrected).

These spectroscopic evidences allowed us to conclusively assign the enolacetate geometries of the (*E*,*R*)-**16** and (*Z*,*R*)-**16** isomers. Having secured the structure of the synthetic compounds, we proceeded to confirm the identity of the natural peyssonenynes A and B.[1] However, some discrepancies between the NMR chemical shifts in $CDCl_3$ and C_6D_6 of the natural and synthetic compounds were noted (see Tables 1 and 2, E.S.I.). In the original publication it was shown that peyssonenynes A and B displayed nearly identical ^{1}H-NMR spectra in $CDCl_3$ and some minor differences in the ^{13}C-NMR chemical shifts of C-6 (A, δ 74.1 ppm; B, δ 73.6 ppm) and C-8 (A, δ 129.5 ppm; B, δ 128.9).[1] Comparison of the data for the synthetic compounds (*Z*,*R*)-**16** and (*E*,*R*)-**16** revealed significant differences. In addition, we found that the diagnostic signal for C-6 at around δ 79.0 ppm of synthetic (*E*,*R*)-**16** was absent in the data of the products isolated from *Peyssonnelia caulifera.* Puzzled by the inconsistencies, and reflecting on the stereochemical lability of monoacylglycerols, we entertained the possibility that the natural

peyssonenyne B could be the *sn*-2 achiral isomer of peyssonenyne A, namely compound (*Z*)-**17** (Fig. 2).

Using the conditions reported in the original publication for separation of peyssonenynes A and B by RP-HPLC (Sunfire-C18, 5 µm, 250 × 4.6 mm, 3 : 1 MeOH/H_2O, 0.7 mL min^{-1}), only two partially resolved peaks were obtained from (*Z*,*S*)-**16**. However, changing to a chiral HPLC column (Chirlpak® IA, 250 × 10 mm, 45 : 45 : 10 *n*-hexane/AcOEt/CH_2Cl_2, 1.2 mL min^{-1}) we isolated the putative *sn*-2 regioisomer (*Z*)-**17** accompanying the however inseparable *sn*-1 and *sn*-3 enantiomers (an almost racemic sample kept at −78 °C for two years was used). The ^{1}H-NMR spectra of the minor component matched those published for peyssonenyne B (see E.S.I.). Strikingly, however, the integration of the glycerol region of (*Z*)-**17** did not reflect the number of magnetically equivalent protons expected for that fragment. Reinjection of the independent fractions obtained above confirmed the dynamic equilibrium between the glycerol isomers: the *sn*-2 isomer (*Z*)-**17** rapidly afforded a mixture of *sn*-1,3/*sn*-2 (*ca.* 1 : 1 ratio), whereas the *sn*-1,3 fraction showed a slower conversion to *sn*-2 (*Z*)-**17**, probably due to the greater stability of the chiral relative to the achiral isomer (Fig. 3).

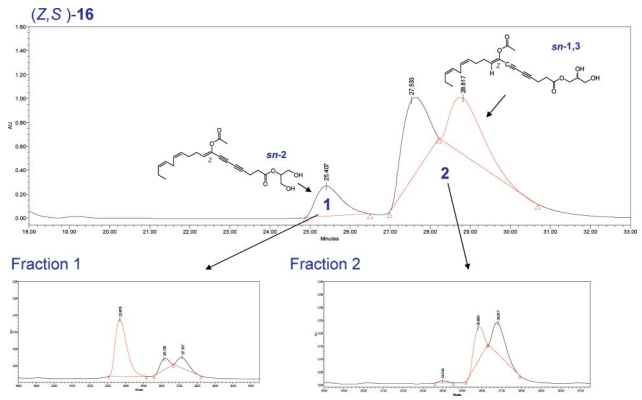


Fig. 3 HPLC traces (Chiralpak® IA, 250 × 10 mm, 45 : 45 : 10 *n*-hexane/AcOEt/CH_2Cl_2 , 1.2 mL min^{-1}) showing the interconvertion of glycerol positional isomers in the peyssonenynes series.

Biological characterization of the synthetic peyssonenynes

Having prepared the enantioenriched and geometrically homogeneous *Z* and *E* isomers of the peyssonenynes, we proceeded to confirm their biological activities. The ability of the synthetic stereoisomers **16** to influence human DNMT1 activity was interrogated in K562 human cells by immunoprecipitation with anti-DNMT1 antibody followed by a radioactive methyl transfer assay (Fig. 4). At 50 µM all diastereomers rather equally inhibited DNMT1 activity at least as efficiently as the known DNMT1 inhibitor RG108[29] but less potently than the recently described specific inhibitor SGI1027[30] at the same concentration (Fig. 4).

Methylation-specific PCR (MSP) revealed a modest increase of unmethylated *RARβ* promoter (the *RARβ2* is a tumor suppressor gene whose expression is silenced in cancer by methylation of CpG islands at promoters)[31] upon treatment of the HCT116 cell line with the peyssonenynes at 10 µM relative to 5-azacytidine at 5 µM suggesting a possible methylation-inhibitory activity (E.S.I.).

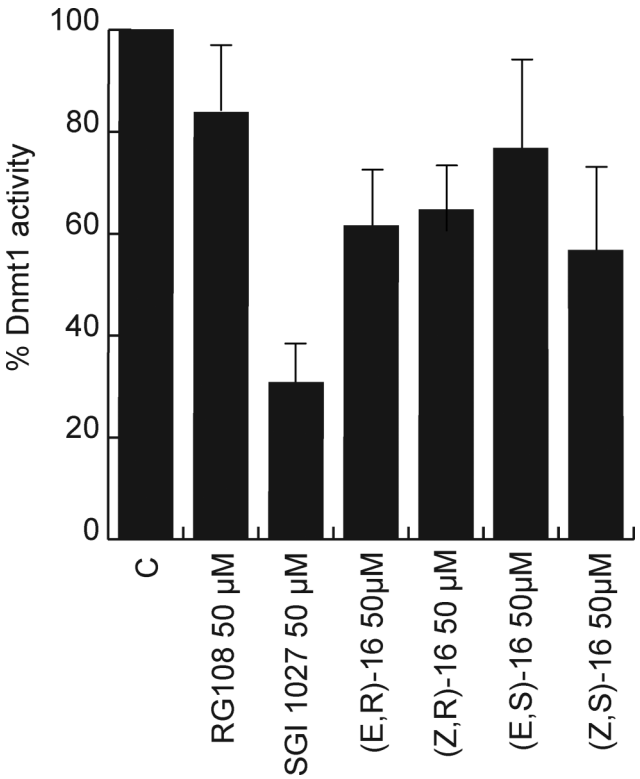


Fig. 4 DNMT1 inhibition activities of synthetic stereoisomers **16** relative to control and to known DNMT1 inhibitors RG108 and SGI1027.

Conclusion

The synthesis of both geometric isomers corresponding to the proposed structures of the peyssonenynes A and B has allowed us to solve the identity puzzle of these natural products. We could confirm that the oxylipins isolated from *Peyssonnelia caulifera* are not geometric isomers of the acetoxy enediyne moiety. The geometry of the enolacetate is *Z* in both compounds, and instead they are, respectively, the *sn*-1,3 (= peyssonenyne A) and *sn*-2 (= peyssonenyne B) positional isomers at the glycerol moiety. This stereochemical lability, due to reversible monoacylglycerol transacylations occurring in solution, makes highly unlikely the isolation of the enantiopure *sn*-1 (*sn*-3) from Nature. Progressive erosion of the enantiopurity is unavoidable, as we have proven with the synthesis of each antipode of the purported natural products.

On DNMT1 inhibition assay the diastereomers are roughly equipotent. The enol acetate geometry and the configuration of the glycerol unit appear to play a minor role on their activity, the latter most likely due to the racemization through formation of the achiral *sn*-2 isomer in solution. Other functional assays are underway in our laboratories to epigenetically profile the peyssonenynes and structural analogues.

Experimental section

General procedures

Solvents were dried according to published methods and distilled before use. All other reagents were commercial compounds of the highest purity available. All reactions were carried out under argon atmosphere, and those not involving aqueous reagents

were carried out in oven-dried glassware. Analytical thin layer chromatography (TLC) was performed on aluminium plates with Merck Kieselgel 60F254 and visualised by UV irradiation (254 nm) or by staining with an ethanolic solution of phosphomolybdic acid or an ethanolic solution of anisaldehyde. Flash column chromatography was carried out using Merck Kieselgel 60 (230-400 mesh) under pressure. IR spectra were obtained on a JASCO IR 4200 spectrophotometer from a thin film deposited onto NaCl glass. Specific rotations were obtained on a JASCO P-1020 polarimeter. Mass spectra were obtained on a Hewlett–Packard HP59970 instrument operating at 70 eV by electron ionization and APEX III FT-ICR MS (Bruker Daltonics, Billerica, MA), equipped with a 7T actively shielded magnet. Ions were generated using an Apollo API electrospray ionization (ESI) source, with a voltage between 1800 and 2200 V (to optimize ionization efficiency) applied to the needle, and a counter voltage of 450 V applied to the capillary. Samples were prepared by adding a spray solution of 70 : 29.9 : 0.1 (v/v/v) CH_3OH/water/formic acid to a solution of the sample at a v/v ratio of 1 to 5% to give the best signal-to-noise ratio. High resolution mass spectra were taken on a VG Autospec instrument. ^{1}H NMR spectra were recorded in $CDCl_3$ or C_6D_6 at ambient temperature on a Bruker AMX-400 spectrometer at 400 MHz with residual protic solvent as the internal reference [$CDCl_3$, $\delta_H = 7.26$ ppm and C_6D_6, $\delta_H = 7.15$ ppm]; chemical shifts (δ) are given in parts per million (ppm), and coupling constants (J) are given in Hertz (Hz). The proton spectra are reported as follows: δ (multiplicity, coupling constant J, number of protons, assignment). ^{13}C NMR spectra were recorded in $CDCl_3$ and C_6D_6 at ambient temperature on the same spectrometer at 100 MHz, with the central peak of $CDCl_3$ ($\delta_C = 77.0$ ppm) or C_6D_6 ($\delta_C = 128.0$ ppm) as the internal reference. The DEPT135 pulse sequence was used to aid in the assignment of signals in the ^{13}C NMR spectra.

(2′*R*)-2,3-*O*-Isopropylidene-2,3-dihydroxy-1-prop-1-yl pent-4-ynoate ((*R*)-4). General procedure for ester formation

To a stirred solution of 5-hexynoic acid **2** (0.37 g, 3.79 mmol), DCC (0.783 g, 3.79 mmol) and DMAP (0.045 g, 0.379 mmol) in CH_2Cl_2 (7 mL) was added a solution of (*S*)-2,3-*O*-isopropylidene-2,3-dihydroxy-1-propanol (*S*)-**3** (0.5 g, 3.79 mmol) in CH_2Cl_2 (5 mL). After stirring the mixture at room temperature overnight, the solvent was evaporated and the residue was purified by flash chromatography (80 : 20 hexane/EtOAc) to afford 0.79 g (98%) of the titled compound as a colourless oil. ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 4.4–4.3 (m, 1H, $H_{2'}$), 4.21 (dd, J = 11.5, 4.6 Hz, 1H, $H_{1'}$), 4.2–4.1 (m, 2H, $H_{1'}$ + $H_{3'}$), 3.76 (dd, J = 8.4, 6.1 Hz, 1H, $H_{3'}$), 2.61 (t, J = 7.5 Hz, 2H, $2H_2$), 2.6–2.5 (m, 2H, $2H_3$), 1.99 (t, J = 2.3 Hz, 1H, H_5), 1.44 (s, 3H), 1.38 (s, 3H) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$): δ 171.3 (s, C_1), 109.6 (s, $C_{1''}$), 82.1 (s, C_4), 73.3 (d, $C_{2'}$), 69.1 (d, C_5), 66.1 (t), 64.7 (t), 33.0 (t, C_2), 26.5 (q), 25.2 (q), 14.1 (t, C_3) ppm. HRMS (ESI$^+$): Calcd for $C_{11}H_{17}O_4$, 213.11237; found 213.11214. IR (NaCl): ν 3286 (w, C≡C–H), 2987 (w, C–H), 2939 (w, C–H), 1741 (s, CO), 1376 (m), 1252 (m), 1215 (m), 1165 (s), 1056 (m) cm^{-1}.

(2′*S*)-2,3-*O*-Isopropylidene-2,3-dihydroxy-1-prop-1-yl pent-4-ynoate ((*S*)-4)

Following the general procedure described above for ester formation, the reaction of 5-hexynoic acid **2** (0.32 g, 3.22 mmol), DCC (0.67 g, 3.22 mmol), DMAP (0.04 g, 0.327 mmol) and (*R*)-2,3-*O*-isopropylidene-2,3-dihydroxy-1-propanol (*R*)-**3** (0.043 g, 3.22 mmol) in CH_2Cl_2 (12 mL), afforded after purification by flash chromatography (80 : 20 hexane/EtOAc), 0.634 g (93%) of the titled compound as a colourless oil.

(2′*R*)-2,3-*O*-Isopropylidene-2,3-dihydroxy-1-prop-1-yl 5-iodopent-4-ynoate ((*R*)-5). General procedure for the synthesis of iodo-alkynes

To a stirred solution of (2′*R*)-2,3-*O*-isopropylidene-2,3-dihydroxy-1-prop-1-yl pent-4-ynoate (*R*)-**4** (0.59 g, 2.80 mmol) in acetone (4 mL), NIS (0.756 g, 3.36 mmol) and $AgNO_3$ (0.475 g, 2.80 mmol) were added and the reaction was stirred overnight at room temperature. The solvent was evaporated and the residue was purified by flash chromatography (80 : 20 hexane/EtOAc) to afford 0.652 g (69%) of the titled compound as a colourless oil. ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 4.3–4.5 (m, 1H, $H_{2'}$), 4.0–4.2 (m, 3H, $2H_{1'}$ + $H_{3'}$), 3.7–3.8 (m, 1H, $H_{3'}$), 2.69 (t, J = 6.8 Hz, 2H, $2H_2$), 2.59 (t, J = 6.7 Hz, 2H, $2H_3$), 1.44 (s, 3H, $H_{1''}$), 1.38 (s, 3H, $H_{1''}$) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$): δ 171.3 (s, C_1), 109.8 (s, $C_{1''}$), 92.1 (s, C_4), 73.4 (d, $C_{2'}$), 66.2 (t), 64.8 (t), 33.0 (t, C_2), 26.6 (q), 25.3 (q), 16.5 (t, C_3), −5.0 (s, C_5) ppm. HRMS (ESI$^+$): Calcd for $C_{11}H_{15}IO_4Na$, 360.9900; found, 360.9907. IR (NaCl): ν 2985 (m, C–H), 2936 (m, C–H), 2888 (w, C–H), 1739 (s, C═O), 1375 (m), 1162 (s) cm^{-1}. The enantiomeric excess was determined using chiral HPLC (Chiralpak® IA, 250 × 10 mm, 5% hexane/*i*-PrOH, 1.5 mL min^{-1}, t_R = 28.1 min; >99% *ee*).

(2′*S*)-2,3-*O*-Isopropylidene-2,3-dihydroxy-1-prop-1-yl 5-iodopent-4-ynoate ((*S*)-5)

Following the general procedure described above for the synthesis of iodo-alkynylderivatives the reaction of (2′*S*)-2,3-*O*-isopropylidene-2,3-dihydroxy-1-prop-1-yl pent-4-ynoate (*S*)-**4** (0.576 g, 2.72 mmol), NIS (0.733 g, 3.23 mmol) and $AgNO_3$ (0.462 g, 2.72 mmol) in acetone (4 mL) afforded, after purification by flash chromatography (80 : 20 hexane/EtOAc), 0.766 g (84%) of the titled compound as a colourless oil. The enantiomeric excess was determined using chiral HPLC (Chiralpak® IA, 250 × 10 mm, 5% hexane/*i*-PrOH, 1.5 mL min^{-1}, t_R = 26.4 min; >99% *ee*).

N-Methoxy-*N*-methylhex-5-ynamide (7)

To a solution of hex-5-ynoic acid **6** (3.0 g, 26.76 mmol) in CH_2Cl_2 (154 mL) was added *N*,*O*-dimethylhydroxylamine hydrochloride (3.91 g, 40.14 mmol), EDCI (7.69 g, 40.14 mmol) and DMAP (4.90 g, 40.14 mmol) and the reaction was stirred overnight at room temperature. A saturated aqueous NaCl solution was added and the mixture was extracted with EtOAc (3×). The combined organic layers were washed with an aqueous HCl solution (5%) and brine, dried over Na_2SO_4 and the solvent was evaporated to afford 4.1 g (100%) of the titled compound as a colourless oil, which was used in the next step without further purification. ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 3.61 (s, 3H, OCH_3), 3.08 (s, 3H, NCH_3), 2.48 (t, J = 6.9 Hz, 2H, $2H_2$), 2.19 (dt, J = 6.8, 2.2 Hz, 2H, $2H_4$), 1.9–1.8 (m, 1H, H_6), 1.8–1.7 (m, 2H, $2H_3$) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$): δ 173.5 (s), 82.8 (s), 68.4 (d), 60.2 (q), 31.1 (q), 29.4 (t), 22.3 (t), 17.0 (t) ppm. IR (NaCl): ν 3294 (m, C≡C–H), 2939 (m, C–H), 1662 (s, C═O), 1420 (m), 1386 (m), 1179 (w), 995

(m) cm^{-1}. HRMS (ESI^+): Calcd for $C_8H_{14}NO_2$, 156.10152; found 156.10191.

(7*Z*,10*Z*)-1-(Triisopropylsilyl)trideca-7,10-dien-1-yn-3-ol (11)

To a stirred suspension of Cs_2CO_3 (0.19 g, 0.55 mmol), NaI (83 mg, 0.55 mmol) and CuCl (0.054 g, 0.55 mmol) in DMF (2 ml), *N*-methoxy-*N*-methylhex-5-ynamide **7** (0.10 g, 0.66 mmol) and propargylic bromide **8** (57 µL, 81 mg, 0.55 mmol) were added and the reaction was vigorously stirred at 25 °C for 3 h. After the addition of a saturated aqueous NH_4Cl solution the mixture was extracted with EtOAc (3×). The combined organic layers were washed with water (4×), dried over Na_2SO_4 and the solvent was evaporated.

To a stirred solution of triisopropylsilyl acetylene (0.20 g, 1.09 mmol, 0.24 mL) in THF (0.2 mmol mL^{-1}) at –40 °C, *n*-BuLi (0.53 mL, 1.57 M in THF, 0.82 mmol) was added dropwise and the solution was stirred for 1 h. Subsequently, a solution of the residue obtained above (0.12 g) in THF (0.84 mmol mL^{-1}) was added dropwise at –10 °C and the reaction was further stirred for 1 h at –10 °C and for 1 h at room temperature. A saturated aqueous solution of NH_4Cl was added and the mixture was extracted with Et_2O (3×). The combined organic layers were washed with brine, dried over Na_2SO_4 and the solvent was evaporated. This reaction afforded 0.2 g of a residue which was used in the next step without further purification.

A solution of sodium borohydride (0.02 g, 0.05 mmol) in ethanol (2 mL) was added to a suspension of nickel acetate tetrahydrate (0.02 g, 0.09 mmol) in ethanol (3 mL) under a H_2 atmosphere and the reaction was vigorously stirred for 30 min. Then a solution of the residue obtained above (0.2 g) in ethanol (3 mL) was added and the mixture was stirred for 4 h, filtered through a small pad of silica gel and the solvent was evaporated. The residue was purified by flash chromatography (silica gel, 95 : 5 hexane/EtOAc) to afford 0.14 g (75% over three steps) of **11** as a colourless oil. ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 5.4–5.2 (m, 4H, $H_7 + H_8 + H_{10} + H_{11}$), 4.4–4.3 (m, 1H, H_3), 2.77 (t, J = 5.8 Hz, 2H, $2H_9$), 2.2–2.0 (m, 4H, $2H_6 + 2H_{12}$), 1.8–1.7 (m, 2H, $2H_4$), 1.6–1.5 (m, 2H, $2H_5$), 1.1–1.0 (m, 21H, *i*-Pr_3Si), 0.97 (t, J = 7.5 Hz, 3H, CH_3) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$): δ 131.9 (d), 129.4 (d), 128.6 (d), 127.2 (d), 108.7 (s), 85.6 (s, C_1), 62.9 (d), 37.5 (t), 26.7 (t), 25.5 (t), 25.1 (t), 20.5 (t), 18.6 (q, 6×, $SiCH(CH_3)_2$), 14.3 (q), 11.1 (d, 3×, $SiCH(CH_3)_2$) ppm. IR (NaCl): ν 3600–3300 (br, OH), 2940 (s, C–H), 2866 (s, C–H), 2168 (w, -C≡C-), 1463 (m), 1000 (m) cm^{-1}. HRMS (ESI^+): Calcd for $C_{22}H_{41}OSi$, 349.29269; found, 349.29312.

(7*Z*,10*Z*)-Trideca-7,10-dien-1-yn-3-ol (12)

To a stirred solution of (7*Z*,10*Z*)-1-(triisopropylsilyl)trideca-7,10-dien-1-yn-3-ol **11** (1.40 g, 4.01 mmol) in THF (15 mL) was added *n*-Bu_4NF (4.41 mL, 1 M in THF, 4.41 mmol) and the reaction was stirred for 30 min at 25 °C. A saturated aqueous solution of $NaHCO_3$ was added and the mixture was extracted with Et_2O (3×). The combined organic layers were washed with brine, dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by flash chromatography (silica gel, 85 : 15 hexane/EtOAc) to afford 0.62 g (81%) of the titled compound as a colourless oil. ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 5.4–5.2 (m, 4H, $H_7 + H_8 + H_{10} + H_{11}$), 4.4–4.3 (m, 1H, H_3), 2.78 (t, J = 5.7 Hz, 2H, $2H_9$), 2.47 (d, J = 2.0 Hz, 1H, H_1), 2.2–2.0 (m, 4H, $2H_6 + 2H_{12}$), 1.8–1.7 (m, 2H, $2H_4$), 1.6–1.5 (m, 2H, $2H_5$), 0.97 (t, J = 7.5 Hz, CH_3) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$): δ 131.9 (d), 129.3 (d), 128.7 (d), 127.2 (d), 84.9 (s), 73.0 (d), 62.2 (d, C_3), 37.2 (t), 26.7 (t), 25.6 (t), 25.0 (t), 20.6 (t), 14.3 (q) ppm. IR (NaCl): ν 3600–3300 (br), 3303 (s, -C≡C–H), 3009 (m, C–H), 2935 (s, C–H), 2868 (m, C–H), 2114 (w, -C≡C-) cm^{-1}. HRMS (ESI^+): Calcd for $C_{13}H_{21}O$, 193.15815; found, 193.15869.

(12*Z*,15*Z*)-(2′*R*)-2,3-*O*-Isopropylidene-2,3-dihydroxy-1-prop-1-yl 8-hydroxyoctadeca-12,15-dien-4,6-diynoate ((*R*)-13). General procedure for the Cadiot–Chodkiewicz cross-coupling reaction

To a stirred solution of (2′*R*)-2,3-*O*-isopropylidene-2,3-dihydroxy-1-prop-1-yl 5-iodopent-4-ynoate (*R*)-**5** (1.09 g, 3.24 mmol) and (7*Z*,10*Z*)-trideca-7,10-dien-1-yn-3-ol **12** (0.62 g, 3.24 mmol) in degassed piperidine (2 mL), at 0 °C, was added copper chloride (31 mg, 0.32 mmol). After stirring for 2 h, a saturated aqueous NH_4Cl solution was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under vacuum. The residue was purified by flash chromatography (silica gel, 80 : 20 hexane/EtOAc) to provide 0.92 g (71%) of a colourless oil that was identified as a 1 : 1 mixture of diastereomers. ^{1}H-NMR (400.13 MHz, C_6D_6): δ 5.4–5.2 (4H, 4H, $H_{12} + H_{13} + H_{15} + H_{16}$), 4.19 (t, J = 6.4 Hz, 1H, H_8), 4.0–3.8 (m, 3H, $2H_{1'} + H_{2'}$), 3.7–3.6 (m, 1H, $H_{3'}$), 3.45 (dd, J = 8.3, 5.9 Hz, 1H, $H_{3'}$), 2.74 (t, J = 6.1 Hz, 2H, $2H_{14}$), 2.22 (t, J = 7.0 Hz, 2H, $2H_2$ or $2H_3$), 2.10 (t, J = 7.0 Hz, 2H, $2H_2$ or $2H_3$), 2.0–1.9 (m, 4H, $2H_{11} + 2H_{17}$), 1.7–1.5 (m, 2H, $2H_9$), 1.5–1.4 (m, 2H, $2H_{10}$), 1.35 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 0.90 (t, J = 7.5 Hz, 3H, CH_3) ppm. ^{13}C-NMR (100.62 MHz, C_6D_6): δ 170.9 (s), 131.9 (d), 129.7 (d), 128.8 (d), 127.7 (d), 109.8 (s), 79.4 (s), 78.6 (s), 73.8 (d), 69.9 (s), 66.3 (t), 66.1 (s), 65.0 (t), 62.5 (d), 37.4 (t), 32.7 (t), 27.0 (t), 26.9 (q), 25.9 (t), 25.6 (q), 25.4 (t), 20.9 (t), 15.3 (t), 14.5 (t) ppm. IR (NaCl): ν 3600–3300 (br, OH), 2936 (m, C–H), 2870 (w, C–H), 2254 (w, -C≡C-), 1740 (s, CO), 1376 (m), 1163 (s), 1053 (s) cm^{-1}. HRMS (ESI^+): Calcd for $C_{24}H_{34}O_5Na$, 425.22978; found, 425.22985.

(12*Z*,15*Z*)-(2′*S*)-2,3-*O*-Isopropylidene-2,3-dihydroxy-1-prop-1-yl 8-hydroxyoctadeca-12,15-dien-4,6-diynoate ((*S*)-13)

Following the general procedure described above for the Cadiot–Chodkiewicz cross-coupling, the reaction of (7*Z*,10*Z*)-trideca-7,10-dien-1-yn-3-ol **5** (0.46 g, 2.39 mmol), (2′*S*)-2,3-*O*-isopropylidene-2,3-dihydroxy-1-prop-1-yl 5-iodopent-4-ynoate (*S*)-**5** (0.77 g, 2.26 mmol) and copper chloride (23 mg, 0.239 mmol) in piperidine (4 mL) at 0 °C afforded, after purification by flash chromatography (silica gel, 80 : 20 hexane/EtOAc), 0.70 g (73%) of the titled compound as a colourless oil.

(12*Z*,15*Z*)-(2′*R*)-2,3-*O*-Isopropylidene-2,3-dihydroxy-1-prop-1-yl 8-oxooctadeca-12,15-dien-4,6-diynoate ((*R*)-14). General procedure for the Swern oxidation

To a stirred solution of oxalyl chloride (93 µL, 0.14 g, 1.07 mmol) in CH_2Cl_2 (7 mL) at –60 °C, DMSO (0.14 mL, 0.14 g, 1.83 mmol) was added dropwise and the reaction was stirred for 5 min at this temperature. Then, a solution of (12*Z*,15*Z*)-(2′*R*)-2,3-*O*-isopropylidene-2,3-dihydroxy-1-prop-1-yl 8-hydroxyoctadeca-12,

15-dien-4,6-diynoate (*R*)-**13** (0.31 g, 0.76 mmol) in CH_2Cl_2 (7 mL) was added. After stirring for 30 min, Et_3N (0.70 mL, 0.51 g, 5.04 mmol) was added and the reaction was stirred for 10 min at –60 °C. The mixture was allowed to warm to room temperature, poured into water and extracted with CH_2Cl_2 (3×). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by flash chromatography (silica gel, 75 : 25 hexane/EtOAc) to afford 0.23 g (75%) of the titled compound as a colourless oil. ^{1}H-NMR (400.13 MHz, C_6D_6): δ 5.5–5.3 (m, 3H), 5.2–5.1 (m, 1H), 4.0–3.8 (m, 3H, $2H_{1'}$ + $H_{2'}$), 3.61 (dd, J = 8.3, 6.2 Hz, 1H, $H_{3'}$), 3.37 (dd, J = 8.4, 5.8 Hz, 1H, $H_{3'}$), 2.72 (t, J = 6.8 Hz, 2H, $2H_{14}$), 2.2–2.1 (m, 2H, $2H_2$ or $2H_3$), 2.1–1.8 (m, 8H), 1.6–1.4 (m, 2H, $2H_{10}$), 1.35 (s, 3H, CH_3), 1.24 (s, 3H, CH_3), 0.92 (t, J = 7.5 Hz, 3H, CH_3) ppm. ^{13}C-NMR (100.62 MHz, C_6D_6): δ 185.7 (s), 170.3 (s), 132.1 (d), 129.5 (d), 128.9 (d), 127.5 (d), 109.8 (s), 88.0 (s), 74.8 (s), 73.7 (d), 73.6 (s), 66.2 (t), 65.2 (t), 64.8 (s), 44.8 (t), 32.0 (t), 26.9 (q), 26.4 (t), 25.8 (t), 25.5 (q), 23.8 (t), 20.9 (t), 15.3 (t), 14.5 (q) ppm. IR (NaCl): ν 2933 (m, C–H), 2235 (m, -C≡C-), 2145 (w, -C≡C-), 1741 (s, CO), 1671 (s, CO), 1375 (m), 1251 (m), 1165 (s) cm^{-1}. HRMS (ESI$^+$): Calcd for $C_{24}H_{33}O_5$, 401.23294; found, 401.23225. The enantiomeric excess was determined using chiral HPLC (Chiralpak® IA, 250 × 10 mm, 5% hexane/*i*-PrOH, 1.5 mL min^{-1}, t_R = 47.6 min; >99% *ee*).

(12*Z*,15*Z*)-(2′*S*)-2,3-*O*-Isopropylidene-2,3-dihydroxy-1-prop-1-yl 8-oxooctadeca-12,15-dien-4,6-diynoate ((*S*)-14)

Following the general procedure described above for the Swern oxidation, the reaction of (12*Z*,15*Z*)-(2′*S*)-2,3-*O*-isopropylidene-2,3-dihydroxy-1-prop-1-yl 8-hydroxyoctadeca-12,15-dien-4,6-diynoate (*S*)-**13**. (0.24 g, 0.59 mmol), oxalyl chloride (72 µL, 0.10 g, 0.82 mmol), DMSO (0.11 mL, 0.11 g, 1.41 mmol), Et_3N (0.54 mL, 0.39 g, 3.87 mmol) in CH_2Cl_2 (11 mL) afforded, after purification by flash chromatography (silica gel, 75 : 25 hexane/EtOAc), 0.21 g (88%) of the titled compound as a colourless oil. The enantiomeric excess was determined using chiral HPLC (Chiralpak® IA, 250 × 10 mm, 5% hexane/*i*-PrOH, 1.5 mL min^{-1}, t_R = 46.1 min; >99% *ee*).

(8*E*,12*Z*,15*Z*)- and (8*Z*,12*Z*,15*Z*)-(2′*R*)-2,3-Dihydroxyprop-1-yl 8-acetoxyoctadeca-8,12,15-trien-4,6-diynoate ((*R*)-16). General procedure for the synthesis of enolacetates

To a stirred solution of (12*Z*,15*Z*)-(2′*R*)-2,3-*O*-isopropylidene-2,3-dihydroxy-1-prop-1-yl 8-oxooctadeca-12,15-dien-4,6-diynoate (*R*)-**14** (0.18 g, 0.45 mmol) in CH_2Cl_2 (11 mL) at 0 °C were added Et_3N (0.31 mL, 0.23 g, 2.23 mmol), DMAP (0.03 g, 0.22 mmol) and acetic anhydride (0.25 mL, 0.27 g, 2.68 mmol) and the reaction mixture was stirred for 2 h at room temperature. A saturated NH_4Cl aqueous solution was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by flash chromatography (silica gel, 80 : 20 hexane/EtOAc) to afford 0.16 g (83%) of the titled compound as a 2 : 1 mixture of *E*/*Z* isomers, which was used in the next step without further separation.

General procedure for ketal deprotection

A stirred solution of the mixture of enolacetates (0.16 g, 0.37 mmol) in acetonitrile/water (2 mL, 1 : 1 v/v) was heated to 70 °C and solid CAN (6 mg, 0.01 mmol) was added. The resulting slightly yellow solution was stirred for 3 h at this temperature. After cooling to room temperature, the reaction mixture was extracted with ether (3×). The combined organic layers were dried over Na_2SO_4 and the solvents were removed *in vacuo*. The residue was purified by flash chromatography (silica gel, 30 : 70 hexane/EtOAc) to give 0.13 g (86%) of the titled compound as a 2 : 1 mixture of *E*/*Z* isomers, which were separated by RP-HPLC (Nova-Pak HR C_{18} 6 µm, 19 × 300 mm, MeOH–H_2O 3 : 1, 5 mL min^{-1}) to yield 52 mg (35%) of the *E* isomer (t_R = 37.4 min) and 23 mg (16%) of the *Z* isomer (t_R = 41.7 min) of (*R*)-**16**.

(8*E*,12*Z*,15*Z*)-(2′*R*)-2,3-Dihydroxyprop-1-yl 8-acetoxyoctadeca-8,12,15-trien-4,6-diynoate ((*E*,*R*)-16)

^{1}H-NMR (400.13 MHz, C_6D_6): δ 5.65 (t, J = 8.1 Hz, 1H, H_9), 5.5–5.2 (m, 4H, H_{12} + H_{13} + H_{15} + H_{16}), 4.0–3.9 (m, 2H, $2H_{1'}$), 3.6–3.5 (m, 1H, $H_{2'}$), 3.37 (dd, J = 11.0, 3.6 Hz, 1H, $H_{3'}$), 3.29 (dd, J = 11.3, 6.0 Hz, 1H, $H_{3'}$), 2.72 (t, J = 6.5 Hz, 2H, $2H_{14}$), 2.3–2.2 (m, 2H, $2H_3$), 2.10 (t, J = 7.0 Hz, 2H, $2H_{17}$), 2.0–1.9 (m, 6H, $2H_2$ + $2H_{10}$ + $2H_{11}$), 1.56 (s, 3H, OC(O)CH_3), 0.91 (t, J = 7.4 Hz, 3H, CH_3) ppm. ^{13}C-NMR (100.62 MHz, C_6D_6): δ 171.1 (s), 168.2 (s), 132.1 (d), 131.5 (d), 130.7 (s), 129.7 (d), 128.4 (d), 127.5 (d), 85.5 (s), 79.5 (s), 70.2 (d), 68.5 (s), 65.9 (s), 65.7 (t), 63.4 (t), 32.4 (t), 28.2 (t), 26.7 (t), 25.9 (t), 20.9 (q), 20.0 (t), 15.5 (t), 14.5 (q) ppm. IR (NaCl): ν 3600–3300 (br, OH), 2921 (s, C–H), 2852 (m, C–H), 2361 (w, -C≡C-), 2237 (w, -C≡C-), 1736 (w, CO), 1462 (w), 1219 (w) cm^{-1}. HRMS (ESI$^+$): Calcd for $C_{23}H_{31}O_6$, 403.20955; found. 403.21152. UV (MeOH): λ_{max} 239, 252, 266, 281 nm. The enantiomeric excess was determined using RP-chiral HPLC (Chiralpak® IA, 250 × 10 mm, 100% MeOH, 1.5 mL min^{-1}, t_R = 13.1 min; 72% *ee*).

(8*Z*,12*Z*,15*Z*)-(2′*R*)-2,3-Dihydroxyprop-1-yl 8-acetoxyoctadeca-8,12,15-trien-4,6-diynoate ((*Z*,*R*)-16)

^{1}H-NMR (400.13 MHz, C_6D_6): δ 5.62 (t, J = 7.3 Hz, 1H, H_9), 5.4–5.3 (m, 3H), 5.3–5.2 (m, 1H), 4.0–3.9 (m, 2H, $2H_{1'}$), 3.6–3.5 (m, 1H, $H_{2'}$), 3.37 (dd, J = 11.2, 3.9 Hz, 1H, $H_{3'}$), 3.29 (dd, J = 11.2 and 6.0 Hz, 1H, $H_{3'}$), 2.71 (t, J = 6.8 Hz, 2H, $2H_{14}$), 2.11 (t, J = 7.0 Hz, 2H, $2H_3$), 2.0–1.9 (m, 8H, $2H_2$ + $2H_{10}$ + $2H_{11}$ + $2H_{17}$), 1.57 (s, 3H, OC(O)CH_3), 0.91 (t, J = 7.5 Hz, 3H, CH_3) ppm. ^{13}C-NMR (100.62 MHz, C_6D_6): δ 171.7 (s), 168.0 (s), 132.6 (d), 131.4 (d), 130.6 (s), 130.1 (d), 128.8 (d), 127.8 (d), 84.8 (s), 75.1 (s), 71.0 (s), 70.8 (d), 66.4 (s), 66.1 (t), 64.0 (t), 33.0 (t), 27.1 (t), 26.7 (t), 26.2 (t), 21.3 (t), 20.2 (q), 15.9 (t), 14.8 (q) ppm. IR (NaCl): ν 3600–3300 (br, OH), 2924 (s, C–H), 2854 (m, C–H), 2361 (w, -C≡C-), 2237 (w, -C≡C-), 1736 (m, CO), 1458 (w), 1196 (m) cm^{-1}. HRMS (ESI$^+$): Calcd for $C_{23}H_{31}O_6$, 403.21107; found, 403.21152.

(8*E*,12*Z*,15*Z*)- and (8*Z*,12*Z*,15*Z*)-(2′*S*)-2,3-Dihydroxyprop-1-yl 8-acetoxyoctadeca-8,12,15-trien-4,6-diynoate. (*S*)-16

Following the general procedure described above for the synthesis of enolacetates the reaction of (12*Z*,15*Z*)-(2′*S*)-2,3-*O*-isopropylidene-2,3-dihydroxy-1-prop-1-yl 8-oxooctadeca-12,15-dien-4,6-diynoate (*S*)-**14**. (0.24 g, 0.61 mmol), Et_3N (0.68 mL,

4.86 mmol), DMAP (0.037 g, 0.304 mmol) and acetic anhydride (0.34 mL, 3.64 mmol) in CH_2Cl_2 (15 mL) afforded, after purification by flash chromatography (silica gel, 80 : 20 hexane/EtOAc), 0.23 g (83%) of the title compound as a 2 : 1 mixture of *E*/*Z* isomers, which was used without further separation.

Following the general procedure described above the reaction of the mixture of enolacetates (0.23 g, 0.5 mmol) and CAN (8 mg, 0.015 mmol) in acetonitrile/water (3 ml, 1 : 1 v/v) afforded, after purification by flash chromatography (silica gel, 30 : 70 hexane/EtOAc), 0.187 g (92%) of the title compound as a 2 : 1 mixture of *E*/*Z* isomers, which were separated by RPHPLC (Nova-Pak HR C_{18} 6 μm, 19 × 300 mm, 3 : 1 MeOH/H_2O, 5 mL min^{-1}) to yield 74 g (37%) of the *E* isomer (t_R = 37.4 min) and 29 g (15%) of the *Z* isomer (t_R = 41.7 min) of (*S*)-**16**. The enantiomeric excess of (*S*)-**16** was determined using RP-chiral HPLC (Chiralpak® IA, 250 × 10 mm, 100% MeOH, 1.5 mL min^{-1}, t_R = 14.0 min; 69% *ee*).

Biology

All chemicals used in these experiments were dissolved in DMSO (Sigma-Aldrich): the reference DNMTis RG108 and SGI1027, were used at 50 μM, and the peyssonenynes at 5 μM and 50 μM.

Cell lines. The K562 human leukaemia cell line was grown in RPMI 1640 medium (Euroclone) supplemented with heat-inactivated FBS, 1% glutamine, 1% penicillin/streptomycin and 0.1% gentamycin, at 37 °C in air and 5% CO_2.

DNMT1 immunoprecipitation. The K562 cells were lysed in TAP buffer pH 7–7.5 (50 mM Tris pH 7.0, 180 mM NaCl, 0.15% NP40 v/v, 10% glycerol v/v, 1.5 mM $MgCl_2$, 1 mM $NaMnO_4$, 0.5 mM NaF, 1 mM DTT, 0.2 mM PMSF and protease inhibitor cocktail) for 10 min in ice and centrifuged at 13 000 rpm for 30 min. 650 μg of extracts were diluted in TAP buffer up to 1 mL and pre-cleared by incubating with 20 μL A/G plus agarose (Santa Cruz) for 1 h on a rocking table at 4 °C. The supernatant was transferred to a new tube and 3.25 μg of antibody against DNMT1 (Abcam) was added and IP was allowed to proceed overnight at 4 °C on a rocking table. As negative control the same amount of protein extracts were immunoprecipitated with purified rabbit IgG (Santa Cruz). The following day 50 μL A/G plus agarose were added and incubation was continued for 2 h. The beads were recovered by brief centrifugation and washed with cold TAP buffer several times. At this point the resin was resuspended in 10 μL of DNMT buffer (5 mM EDTA, 0.2 mM DTT, 26 mM NaCl, 20 mM Tris HCl pH 7.4) in order to proceed with the radioactive assay.

DNMT1 radioactive assay. DNMT1 radioactive assay was performed in presence of the peyssonenynes at 50 μM plus a reaction mixture composed of 10 μL of DNMT1-bound resin, 5 μCi of AdoMet (radioactive methyl donor), 0.1 μg of poly dI–dC (methyl acceptor), and DNMT buffer. The reaction was carried out for 2 h at 37 °C with gently stirring and the experiment was performed in duplicate. Subsequently each sample was spread on Whatman DE-81 paper (in quadruplicate) and the papers were washed three times with 5% Na_2HPO_4 and once with distilled water. An the end the papers were transferred in the scintillation vials containing 5 mL of scintillation fluid in order to read the dpm values.

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