DOI: 10.1002/cmdc.201200366

A DNA Methyltransferase Modulator Inspired by Peyssonenyne Natural Product Structures

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A novel epigenetic modulator that displays a DNMT1 inhibition and DNMT3A activation profile was characterized (compound **8**). This compound is a derivative of palmitic acid that incorporates the putative reactive functional group (diynone) of the peyssonenyne natural products. Other analogues containing the diynone or an acetoxyenediyne did not show the same biological profile. In U937 human leukemia cells, diynone **8** induced cell differentiation and apoptosis, which correlated with the expression of Fas protein. Very surprisingly, diynone **8** was toxic to normal human fibroblasts (BJ) and mouse embryo fibroblasts (MEF), but not to immortalized human fibroblasts (BJEL); this unique effect was not observed with the classical DNMT inhibitor 5-azacytidine. Therefore, compound **8** interferes in a very specific manner with signaling pathways, the activities of which differ between normal and immortalized cell types. This toxicity is reminiscent of the effects of *Dnmt1* ablation on mouse fibroblasts. In fact, some of the genes deregulated by the loss of *Dnmt1* are similarly deregulated by **8**, but not by the DNMT inhibitor SGI-1027.

Introduction

Epigenetic aberrations have been well established in different types of cancer[1] but also in an increasing number of different diseases such as diabetes,[2] lupus,[3] asthma,[4] multiple neurological disorders,[5] cardiovascular disease,[6] and fibrogenesis.[7] Methylation of DNA at the C5 position of cytosine in CpG nucleotide islands (and the so-called DNA shores)[8] is the most widely studied epigenetic modification in humans.[9] This molecular signature fulfills the criteria of an epigenetic molecular signal, namely propagation, transmission, and effects on gene expression.[10] Symmetrical methylation on both DNA strands generates two hemi-methylated double strands upon replication, which are then transformed into fully methylated strands by maintenance DNA methyltransferases (DNMT1). Moreover, methylation remains stable after several rounds of DNA replication in vivo. Finally, DNA methylation causes transcriptional responses by either preventing or promoting the recruitment of transcription factors to target-binding sites.[10] Certain CpG island promoters become methylated during development, resulting in long-term transcriptional silencing.[11] DNA methylation is also important in X-chromosome inactivation in females to compensate for the single dose in males, as well as in the parent-specific monoallelic expression of genes, known as genomic imprinting.[12]

The family of DNMT enzymes catalyzes the transfer of the methyl group of *S*-adenosyl-L-methionine (*S*-AdoMet, SAM) to DNA.[13] Five DNMTs have been reported in mammals: DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L, but only DNMT1, DNMT3A, and DNMT3B possess methyltransferase activity. DNMT3A and DNMT3B are de novo methyltransferases that establish embryonic methylation patterns which are then copied to daughter cells during S phase by the maintenance DNMT1.[14] DNMT3L lacks intrinsic catalytic activity but modulates the activity of DNMT3A and DNMT3B, to which it is physically associated, and is required for establishing maternal genomic imprinting. DNMT1 is the most abundant DNMT in the cell and, although it also has de novo activity, maintains the methylation pattern in DNA and its copy in the replicated strand.[9] Finally, DNMT2 is a relative small protein which lacks the N-terminal regulatory domain. It has been reported that DNMT2 does not methylate DNA but instead methylates cytosine 38 (C38) in the anticodon loop of aspartic acid transfer RNA ($tRNA^{asp}$).[15] DNMT enzymes contribute to keep the genome in the "epimutated" state and are also found to be overexpressed in several tumors.[9,16]

Cancer cells are characterized by a global loss of DNA methylation (hypomethylation) and genomic instability,[9b] which is usually accompanied by increased de novo methylation (hyper-

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201200366.

methylation) of a subset of promoters of tumor suppressor genes contained within normally unmethylated CpG nucleotide islands.[9,16,17] Thus, DNA hypomethylation plays a causal role in tumor formation, possibly by promoting chromosomal instability. Similarly, loss of imprinting in embryonic stem cells predisposes them to tumorigenesis.[18] However, the effects of DNA hypomethylation can be opposite at different stages of tumorigenesis,[19] and deletion of *Dnmt3b* has even been reported to exert a tumor suppressive effect.[20] While the enzymatic reversal of this epigenetic modification, DNA demethylation, remains to be demonstrated—oxidation of 5-methylcytosine to 5-hydroxymethylcytosine catalyzed by TET1-3 has been shown,[21] but the mechanism of its conversion into unmethylated cytosine still remains unknown—current therapeutic intervention protocols focus on inhibition of DNMT enzymes.

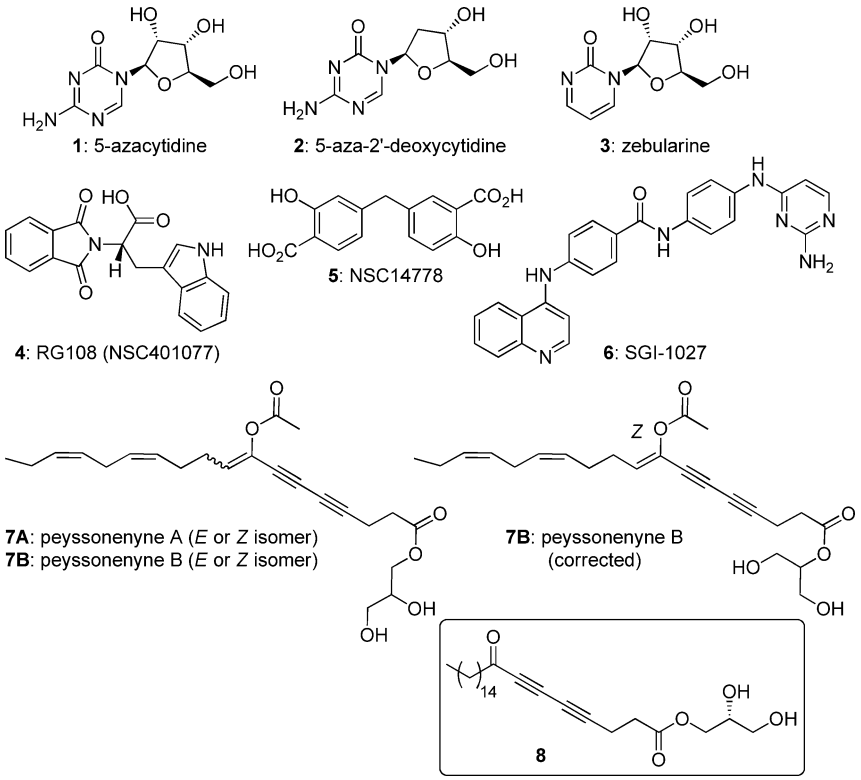


Figure 1. Adenosine analogues and other small-molecule DNMTis.

DNMT inhibitors (DNMTis) can be broadly divided in two groups, nucleoside and non-nucleoside inhibitors.[22] The first group is formed by 5-azacytidine **1** (5-aza-CR, azacitidine, Vidaza), 5-aza-2′-deoxycytidine **2** (5-aza, 5-aza-CdR, decitabine, Dacogen), and their deoxyribonucleoside analogues, such as 5-fluoro-2′-deoxycytidine (FCDR) and zebularine **3**. These drugs function as suicide inhibitors after their phosphorylation and incorporation into DNA (also RNA for 5-azacytidine **1**), and are captured by the invariant active-site Cys residue of the DNMTs after base-flipping.[23] However, the azanucleosides also act through nonspecific mechanisms, which contribute to their effectiveness in the clinic. They are approved for the treatment of patients with high-risk myelodisplastic syndromes (MDS) and are in clinical trials for other indications alone (AML, CMML) or in combination (advanced solid cancer, refractory solid tumors) with HDAC inhibitors.[9b]

The azanucleoside drugs **1** and **2** sequester DNMTs at low doses, leading to global demethylation as cells divide and DNA is repaired, but at higher doses they are cytotoxic. Other limitations of these drugs are the requirement for metabolic transformation into the corresponding triphosphates and the lack of specificity of **1**, which is also incorporated into RNA and affects protein synthesis. Both compounds **1** and **2** are unstable in neutral aqueous solutions, complicating the treatment regimens.[24]

The second, more diverse group includes the local anesthetic procaine, the antiarrythmic drug procainamide, hydralazine, and other small molecules, as well as natural products (Figure 1). RG108 (**4**)[25] and the more potent and selective (DNMT1 versus DNMT3B) analogue diarylmethane NSC14778 (**5**)[26] were discovered by virtual ligand screening using the homology-built DNMT1 model. The quinoline-based SGI-1027 (**6**) is a SAM competitor, and was found to reactivate tumor suppressor genes by blocking DNMT1 (as well as DNMT3A and DNMT3B) activity and inducing its degradation.[27]

Among the natural compounds, DNMT inhibitory activities have been reported for the green tea polyphenol epi-gallocatechin-3-gallate (EGCG), nanaomycin (a selective DNMT3B inhibitor)[28] from *Streptomyces*, and several marine natural products: the pyridoacridine dercitin,[29] the halogenated monoterpene halomon from the red alga *Portieria hornemannii*,[30] several psammaplins and bisaprasin from *Verongida* sponges,[31] and the glycerol fatty acids peyssonenynes (**7**) from the red alga *Peyssonelia caulifera*.[32]

The mechanism of inhibition of these structurally diverse ligands is unclear. Binding to CpG-rich DNA sequences, thus perturbing the interactions between DNMTs and their target sequences, or binding to the target enzyme are two possible mechanisms. In addition, for those inhibitors with electrophilic groups, inactivation of the invariant Cys residue is also likely. Binding modes have been proposed for EGCG,[33] as well as for hydralazine, procainamide, and procaine[34] using a DNMT1 enzyme homology model. In the latter case, the obtained binding poses are the same as previously suggested for 5-azacytidine (**1**) and 5-aza-2′-deoxycytidine (**2**), in which the deoxyribose oxygen stabilizes the intermediate formed after the nucleophilic attack of AdoMet.[34] RG108 (**4**) is believed to selec-

tively block the active domain of DNMT1, occupying the pocket of the native cytosine close to the Cys residue but with no covalent binding.[25b] More accurate insight will be obtained from docking studies using the recently reported crystal structure of the complex with DNA of both human and mouse DNMT1 bound to *S*-adenosyl-L-homocysteine.[35]

We recently reported the total synthesis of the purported structures of peyssonenynes A and B (compounds **7**, *E* or *Z* geometric isomers) in both enantiomeric forms, determined the configuration of the enol acetate conjugated to the diyne functional group, and corrected the structure of peyssonenyne B (**7b**), which was shown to correspond to the *sn*-2 positional isomer of the *sn*-1/3 counterpart with *Z* geometry (peyssonenyne A). All four compounds are roughly equipotent DNMT1 inhibitors as determined by a radioactive methyl transfer assay,[36] and therefore, neither the absolute configuration nor the geometry of the enol acetate appear to play a significant biological role on this epigenetic inhibitory activity.

Upon reflecting on the mechanism of DNMT1 inhibition by these oxylipin natural products, we reasoned that compounds **7** might act as pro-drugs of a highly reactive diynone that is disguised in the structure of the enol acetate.[37] In vivo saponification by hydrolases would release the Michael acceptor diynone, which could then react with the DNMT enzyme, perhaps capturing the invariant Cys residue. The thiol addition process, which we have mimicked in a model experiment in the present study, might be reversible in vivo and therefore transient.[38]

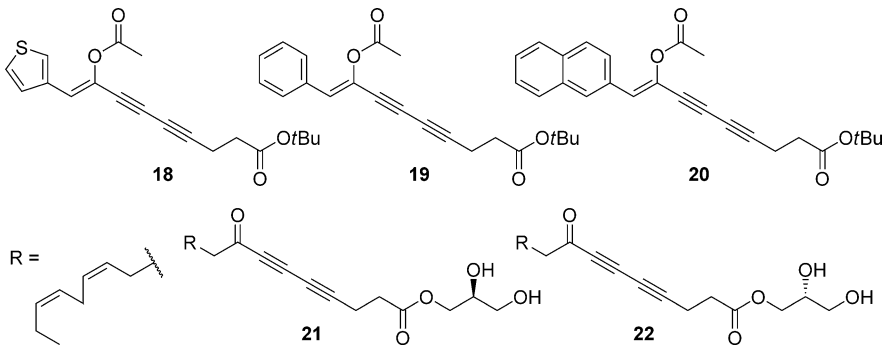


Figure 2. Structures of analogues **18**–**22** (see Supporting Information for synthesis).

Based on these assumptions, we prepared the putative diynones corresponding to the structure of the natural products, as well as additional analogues that were synthetically more accessible but differ in the nature of the group (alkyl, aryl, or heteroaryl) attached to either the diyne enol acetate or the diynone as well as in the carboxylate group. Notable among those is DNMT1 inhibitor **8**, derived from palmitic acid, which exhibited remarkable biological activities and may correspond to a lead compound for the treatment of fibrosis.

Results and Discussion

Synthesis

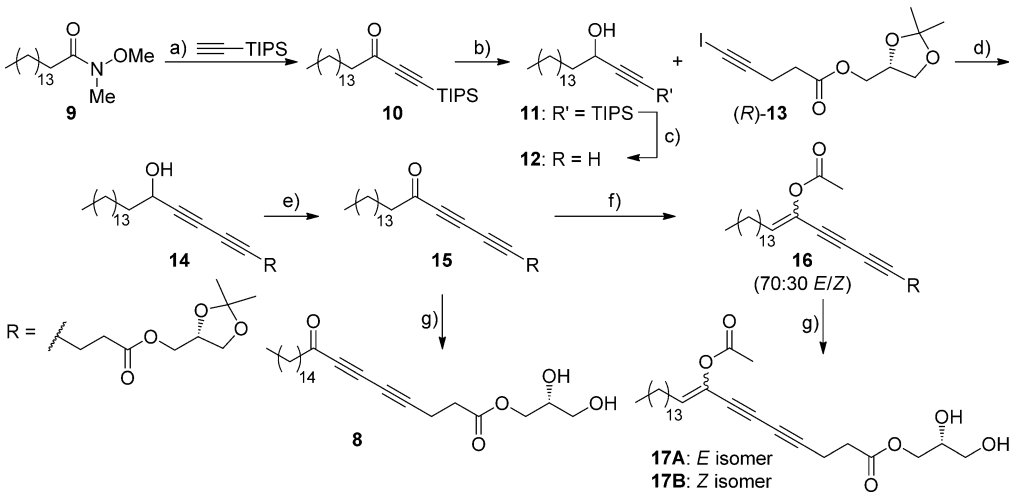
Weinreb amide **9**,[39] prepared from palmitic acid by treatment with *N*,*O*-dimethylhydroxylamine hydrochloride, EDC, and DMAP,[40] was transformed into the propargylic ketone **10** by addition of the anion of TIPS-acetylene. Reduction of the ketone to the corresponding alcohol **11** using sodium borohydride in methanol was required for a successful Cadiot–Chodkiewicz coupling using CuI and piperidine[41] after TBAF deprotection of the TIPS-protected alkyne. Condensation of propargylic alcohol **12** and previously described iodoalkyne **13**[36] provided diynol **14**, which was oxidized to diynylketone **15** using Swern conditions. Ketal hydrolysis in the presence of catalytic quantities of cerium(IV) ammonium nitrate (CAN) at 70 °C gave diynone **8** in good yield.[42] In addition, enol acetate formation from unsaturated ketone **15** using Et_3N, DMAP, and Ac_2O[43] provided a mixture of *E*/*Z* isomers of **16** in 88% yield. Deprotection of the dioxolane as described for **8**[42] afforded a mixture of geometric isomers of the glycerol-derived fatty acid **17**. These acetoxyenediyne isomers (**17A** and **17B**) were separated by RP-HPLC (Nova-Pak C_{18}, 6 μm, 300×19 mm, acetonitrile/water 80:20, 20 mL min^{-1}) and were identified as *E* and *Z*, respectively, based on the magnitude of the $^3J_{C\text{-}H}$ coupling constants determined on an EXSIDE NMR experiment.[44]

Following a similar synthetic sequence, the *tert*-butyl ester analogues of **17** in which the fatty acid is replaced by a thiophene (**18**), a benzene (**19**), or a naphthalene (**20**) ring were also prepared (see Supporting Information). In these cases, the extended conjugation of the acetoxyenediyne favored the exclusive formation of the more stable *Z* isomers (Figure 2), as confirmed by EXSIDE experiments (see Supporting Information).[44a] Diynones **21** and **22** (Figure 2), corresponding to the natural products (*S* and *R* enantiomers, respectively) were also included in the biological screening, as they are readily available by deprotection of the previously described dioxolanes,[36] similarly to the transformation of **15** to **8** (Scheme 1).

Biological evaluation

Activity on human DNMT enzymes

The ability of acetoxyenediynes **17A**, **17B**, and **18**–**20**, and diynones **8**, **21**, and **22** to influence human DNMT1 activity was studied using a radioactive methyl transfer assay after immunoprecipitation of K562 human cells treated with anti-DNMT1 antibodies. At 50 μM, these compounds inhibited DNMT1 activity (Figure 3a) at least as efficiently as RG108 **4**[25a] but less potently than SGI-1027 **6**[27] at the same concentration. Surprisingly, in contrast to RG108, which did not exert a significant effect, compounds **17A** and **17B**, and diynones **8**, **21**, and **22** resulted in up to 2.5-fold stimulation of the intrinsic methyl-



Scheme 1. Synthesis of acetoxyenediynes **17A** and **17B** and diynone **8**. *Reagents and conditions:* a) *n*BuLi, THF, −40→25 °C, 92%; b) $NaBH_4$, CH_3OH, 0 °C, 80%; c) TBAF, THF, 25 °C, 82%; d) CuCl, piperidine, 0 °C, 64%; e) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, −60→25 °C, 99%; f) Et_3N, DMAP, Ac_2O, CH_2Cl_2, 25 °C, 88%; g) CAN (3 mol%), CH_3CN/H_2O, 70 °C; 78% yield for **8**; 74% yield for **17A** and **17B**.

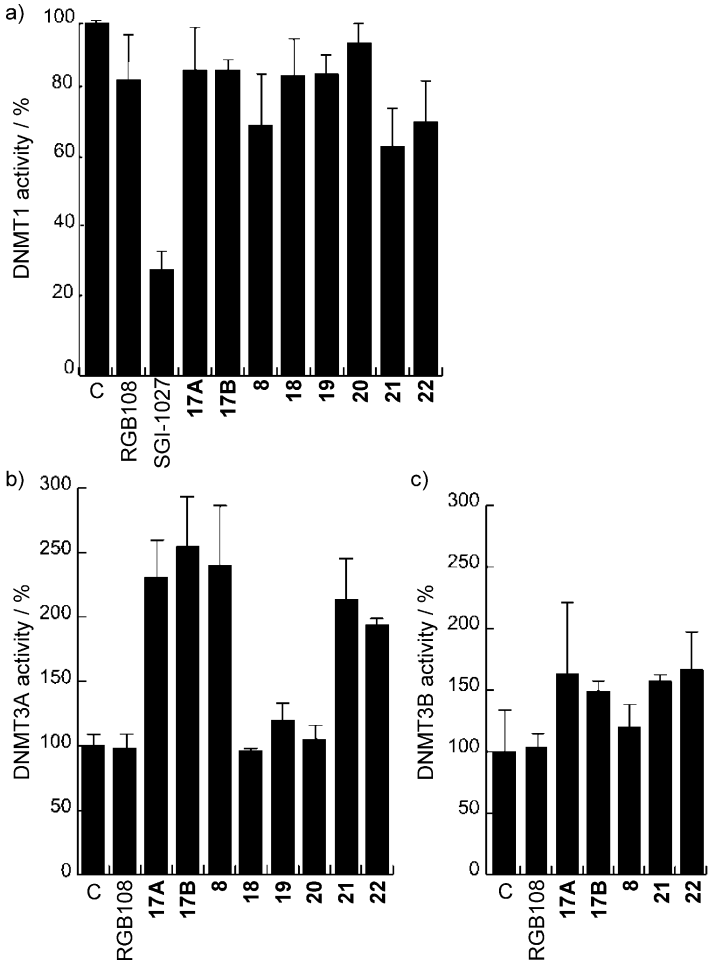


Figure 3. In vitro radioactive assays to evaluate the effect of compounds on a) DNMT1, b) DNMT3A, and c) DNMT3B activity, relative to reference compounds RG108 (**4**) and SGI-1027 (**6**). All compounds assayed at a final concentration of 50 μM; C = vehicle control. Data represent the average values of independent triplicates; error bars represent SD of biological triplicates.

transferase activities of human DNMT3A (Figure 3b) under identical conditions. Analogues **18**–**20** were considerably less potent in this assay. A moderate activating effect was also noticed in DNMT3B (Figure 3c). As **17**, **8**, **21**, and **22** exerted unexpected inhibitory and stimulatory effects on the activities of DNMT1 and DNMT3A respectively, which appeared to be related to their common long carbon chain, we decided to study the effects of these compounds in cell-based assays.

Effects on U937 cell cycle and differentiation

We analyzed the U937 cell cycle by FACS following exposure to the compounds. Two HDAC inhibitors, SAHA[45] and MS-275,[46] both at 5 μM, were selected as reference compounds, as these compounds have well-characterized activity on U937 cells. After treatment for 24 h, compounds **17A** and **21** (50 μM each) induced a slight increase in the pre-G_1 peak, **8** produced considerable apoptosis, which was also noticed at lower concentration, and **17B** and **22** did not induce sub-G1 particles, indicative of nuclear fragmentation. Notably, the pro-apoptotic compounds increased U937 cell death in a dose-dependent manner (Figure 4, and data not shown).

To assess if the induction of apoptosis was a consequence of terminal differentiation, we measured the expression of the CD11c differentiation marker by FACS (Figure 5). After treating U937 cells for 24 h with the compounds at 5 and 50 μM, only the 50 μM concentration of dienones showed an increase in CD11c-positive cells in comparison with the untreated control and reference compound MS-275 (Figure 5). The apoptogenic activity of **8** was not confined to U937 cells. Indeed, compound **8** was cytotoxic for several normal human cells (fetal amniocytes

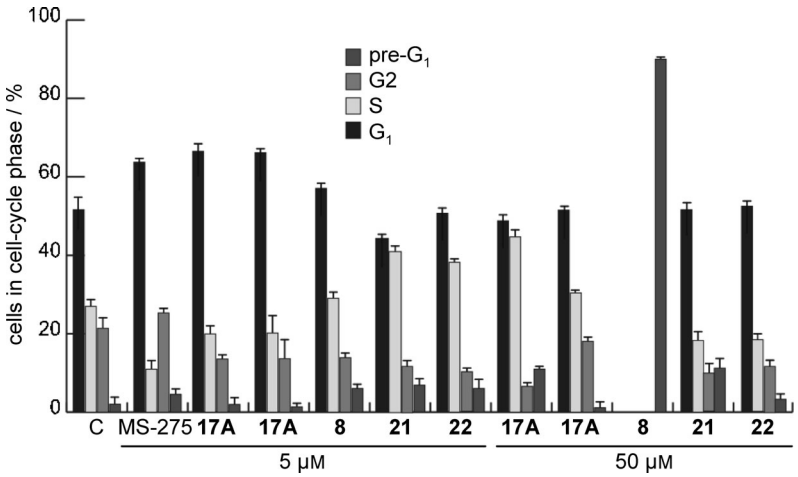


Figure 4. Cell-cycle analysis by FACS after stimulation of U937 cells with the indicated compounds for 24 h. MS-275 was used as control; C = vehicle control. Data represent the average values ± SD of $n = 3$ experiments carried out in duplicate.

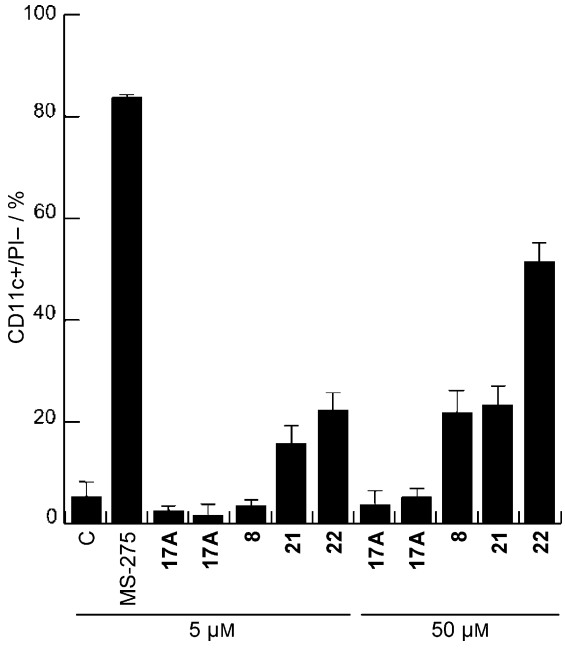


Figure 5. U937 differentiation analysis: CD11c expression of U937 cells treated with the indicated compounds for 24 h. MS-275 was used as control; C = vehicle control. Data represent the average values ± SD of $n = 3$ experiments carried out in duplicate.

from amniotic fluid and CD34+ hematopoietic progenitors), even when used at 25 μM (data not shown).

The impact of the apoptotic effect of these substances on the expression of p21, Bcl-2, and Fas was also evaluated by immunoblotting (see Supporting Information figures S1 A–C). At the highest concentration used, compound **8** induced Fas expression, while induction of p21 expression was already observed at 5 μM. These effects may explain the apoptogenic action of **8**. Moreover, to determine if these compounds can exert additional activities, we performed tubulin (figure S2 A) and histone H3 (figure S2 B) acetylation assays, which would reveal class I and class II HDAC inhibitory activity, with negative results (see Supporting Information).

Differential effects on the viability of normal and immortalized fibroblasts

With the aim of establishing a cellular readout of the activities of acetoxyenediynes **17** and dienones **8**, **21**, and **22**, we used an assay based on the report that Cre-mediated deletion of DNMT1 causes demethylation of cultured primary fibroblasts and p53-dependent cell death, which could be blocked by inactivation of p53.[47] For this, we tested the effects of the various compounds on the viability of normal (BJ) and immortalized (BJEL) human fibroblasts, using the stepwise cellular tumorigenesis model developed by Hahn and Weinberg.[48] In this model, human primary BJ fibroblasts are immortalized by introduction of the early region of SV40, which encompasses large T, resulting in the repression of p53.

Monitoring viable [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide positive; MTT+)] cells revealed that, at a concentration of 10 μM, compound **8** killed normal BJ but not the immortalized BJEL fibroblasts (Figure 6 a). In contrast, the DNA methylation inhibitor 5-aza-2′-deoxycitidine (5-aza; **2**) inhibited growth of the immortalized cells with little, if any, effect on normal BJ cells. At a higher concentration, compound **8** displayed high toxicity and killed both normal and immortalized cells (Figure 6 b).

The effect on human fibroblasts was reproduced with mouse embryo fibroblasts (MEFs). Indeed, diynone **8** completely inhibited the viability of normal primary MEFs, while 5-aza affected immortalized MEFs to a greater extent than normal MEFs (Figure 6 c). Phase contrast views of the corresponding cultures are shown in Figure S3. A comparison with SGI-1027 (**6**)[27] revealed that a significant differential toxic effect for normal fibroblasts was observed only for diynone **8** (Figure 6 c).

Diynone 8 induces genes that are induced by genomic demethylation

Genomic demethylation induced by Cre-mediated *Dnmt1* ablation in MEFs induces a set of 66 genes that show a significant change of expression.[47] Given that **8** recapitulates some of the features of *DNMT1* ablation, we questioned if the same set of genes would be deregulated. Notably, a comparative analysis of the expression of a set of genes strongly induced by *DNMT1* ablation (*Iap*, *Dnmt3a*, *Csk*, *Gjb2*, *Nmyc*, *Slpi*) revealed that the same genes were also deregulated by **8** at 1 or 5 μM, but not

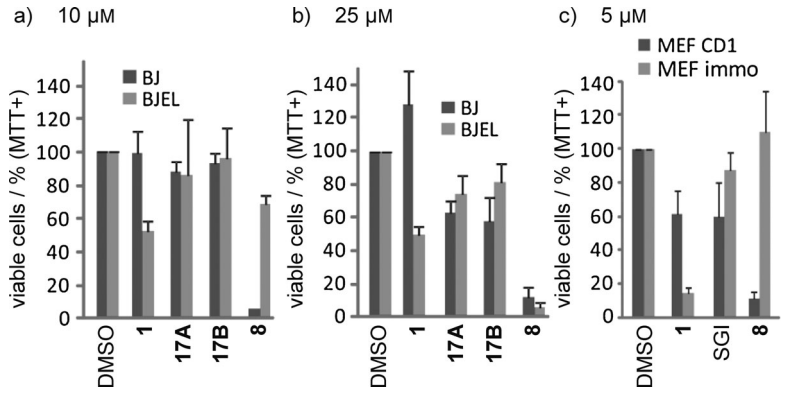


Figure 6. Diynone **8** exhibits the greatest impact on the viability of normal human and mouse fibroblasts: a,b) Effects on cell viability after treatment for 72 h with test compounds along with 5-aza-2′-deoxycytidine (**1**) relative to vehicle control (DMSO) on normal (BJ) and immortalized (BJEL) human fibroblast cell lines; c) effect on cell viability after treatment with compounds SGI-1027 (SGI), **8**, and **1** relative to vehicle control (DMSO) on normal ("MEF CD1") or immortalized ("MEF immo") mouse fibroblasts. Determination of cell viability was performed by MTT assay six days after treatment with the indicated compounds. Data represent the average values ± SD of $n = 2$ experiments carried out in duplicate.

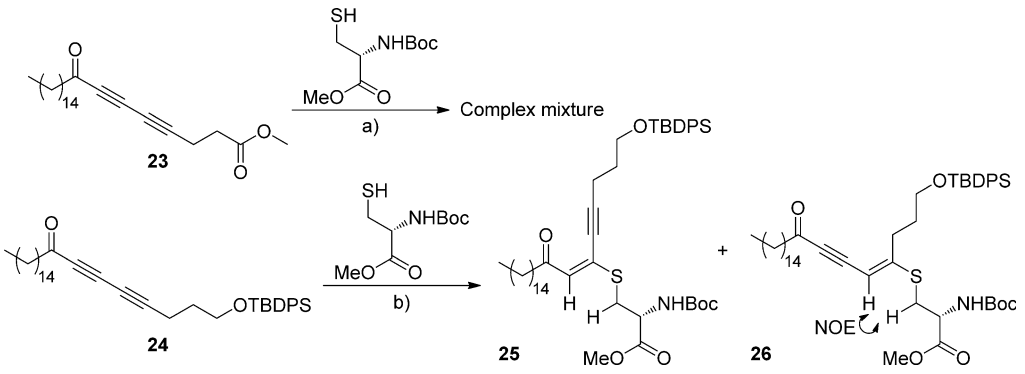
by SGI-1027 (**6**) at the same concentrations (Figure 7). However, while *Iap*, *Csk*, and *Slpi* were similarly induced by *Dnmt1* ablation and exposure to **8**, *Gjb2* and *Nmyc* were upregulated by **8**, while *Dnmt1* ablation resulted in strong suppression of expression from these genes. No significant effects of **8** were observed for the *Dnmt1* ablation-regulated genes *Pea3* and *Scya2* (data not shown), while expression of *Dnmt3a* was increased by diynone **8** (Figure 7).

Mechanistic insight into the possible mechanism of DNMT1 inhibition by 8

In principle, both ester functionalities of the natural product peyssonenynes **7** (and, by analogy, compounds **17**) are expected to undergo in vivo hydrolysis, followed by tautomerization of the enol group to generate a diynone Michael acceptor.[37] The active diynone (**8**, in the case of the acetoxyenediyne **17**) can then react with the thiol[49] of the invariant DNMT Cys residue,[23] thus leading to irreversible covalent attachment to the enzyme.

To provide evidence that supports such a mechanism of action for these compounds, diynone **8** was treated with *N*-Boc-L-cysteine methyl ester in the presence of catalytic quantities of either *p*-TSA or DBU. Whereas no reaction took place in the presence of acid, substrate **8** underwent extensive decomposition when DBU (0.1 equiv) was added, together with the trapping agent, in a toluene/THF solvent mixture. The synthetically more accessible compound **23**, which is the methyl ester analogue of **8** (see Supporting Information for synthesis), yielded a mixture of products when treated with the same thiol and DBU (10 mol%) in toluene at 0 °C or −20 °C, or with benzenethiol in the same conditions or upon heating at 37 °C in an acetonitrile/water mixture.[38]

As some of the products in the above mixture could correspond to thioesterification reactions, the silyl-protected alcohol **24** (Scheme 2) with the same reactive diynone was synthesized (see Supporting Information). Treatment of **24** with *N*-Boc-L-cysteine methyl ester and Et_3N in CH_2Cl_2 at room temperature gave a mixture of products that, after separation by HPLC, were identified and characterized as the highly labile 1,4- and 1,6-Michael-type adducts **25** and **26** (9% and 5% yield, respectively). The geometry of the vinylsulfide in **26** was assigned as *E* by analysis of NOE experiments. Neither NOE/NOESY experiments nor theoretical calculations allowed determination of the geometry of the double bond of the constitutional isomer **25**, which was assigned as *E* by analogy. To our knowledge, this is the first report of the isolation and full characterization of adducts resulting from the irreversible addition of thiols to a diynone and indirectly confirm the anticipated reactive nature of 2,4-diynones (Scheme 2).



Scheme 2. Reactivity of diynones with *N*-Boc-L-cysteine methyl ester. *Reagents and conditions:* a) toluene, DBU (cat); b) Et_3N, CH_2Cl_2, 25 °C, 27%.

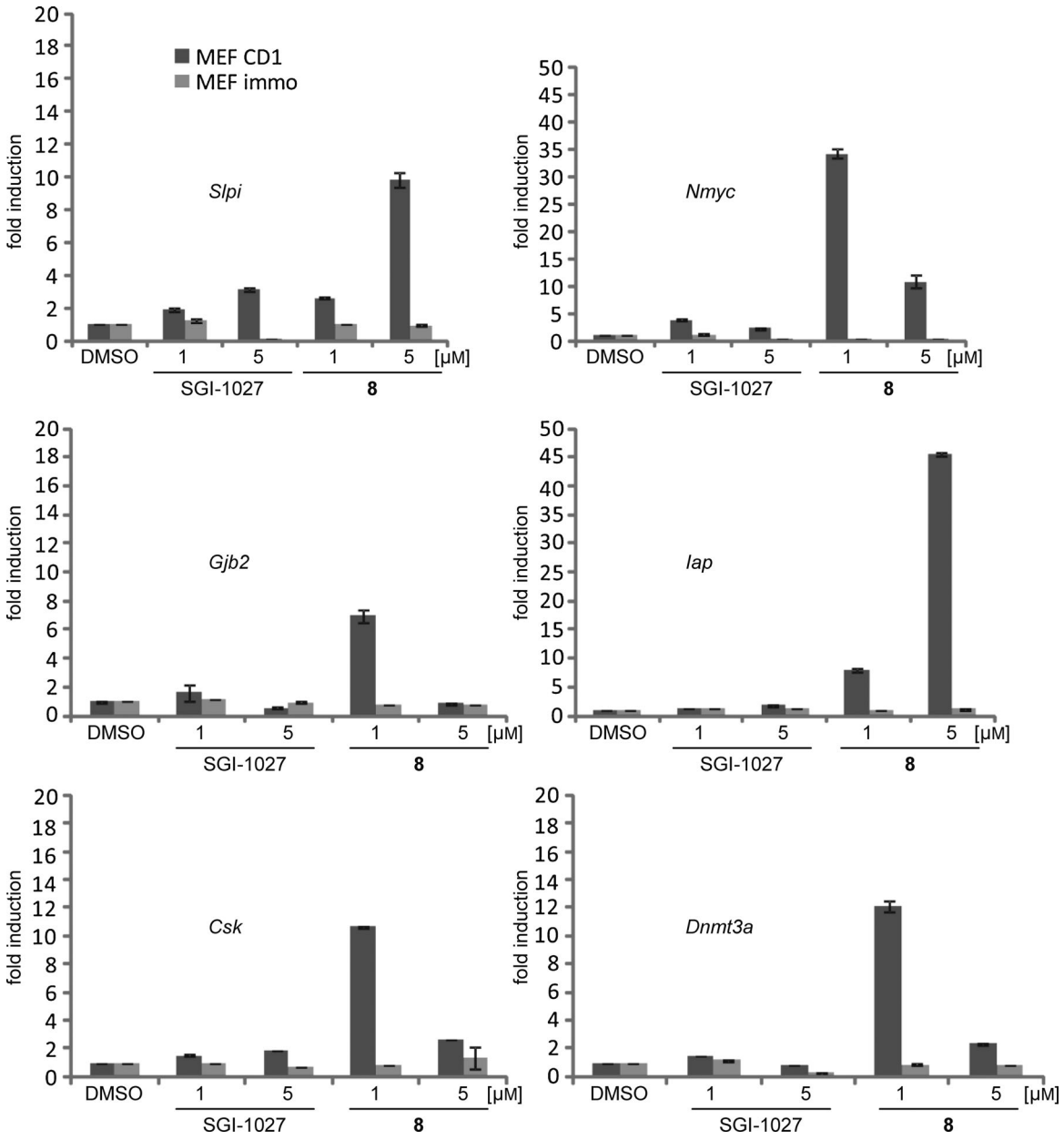


Figure 7. Diynone **8** induces some of the genes that are induced by *Dnmt1* ablation. The expression of the indicated genes in primary MEFs in presence of vehicle or of the indicated compounds was determined by RT-qPCR and normalized to β-actin. The compound-induced induction relative to vehicle is plotted. Data represent the average values ± SD of $n = 2$ experiments performed in duplicate.

Conclusions

Inspired by the structure of the natural products peyssonenynes **7** (Figure 1), we have generated a series of derivatives, some of which display DNMT1-selective inhibitory activity (see Scheme 1 and Figure 2). Notable among these is diynone **8**, a DNMT1 inhibitor with greater potency than RG108 (**4**) and which also leads to DNMT3A activation, while RG108 is inactive in the same assay. Compound **8**, derived from palmitic acid, induced U937 cell differentiation and apoptosis, which correlated with the expression of Fas protein. Trapping experiments using a 2,4-diynone model system (**24**) led to the isolation and characterization of unstable adducts **25** and **26**, which correspond to the conjugate addition of the thiol group of *N*-protected-L-cysteine methyl ester in both the 1,4 and 1,6 modes (Scheme 2). This experiment provides indirect evidence regarding the mechanism of inhibition of DNMT1 by diynone **8** and, by inference, of the precursor acetoxyenediynes present in both **17** and the natural products peyssonenynes **7**. The latter are yet another example of natural products armed with a stable precursor of a disguised highly reactive functional group[37] to favor entry into the cells before release of the chemical weapon.[50]

Our studies reveal a potent toxic activity of diynone **8** on normal human and mouse fibroblasts which is alleviated in immortalized cells, a step that is commonly believed to precede

the final tumorigenic step in human cancer.[51] The fact that **8** is toxic for normal human fibroblasts but does not exhibit significant toxicity for immortalized cells at the same concentration shows that this compound does not display a general toxicity but rather interferes in a very specific manner with pathways that are differently active in the two cell types. This toxicity is reminiscent of the effects of *Dnmt1* ablation on mouse fibroblasts, and we observed that some of the genes deregulated by loss of *DNMT1* are similarly affected by compound **8**. However, several of the genes are divergently regulated, suggesting that compound **8** does not mimic *DNMT1* ablation. Global coupled DNA methylation and transcription profiling studies may reveal which pathways are affected by diynone **8**.

The toxicity for normal cells upon short-term or local application of **8** holds potential for therapy of diseases based on hyperproliferation of fibroblasts, such as renal fibrosis (DNMT1-mediated *RASAL1* hypermethylation has been implicated in kidney fibrosis in the mouse)[7] or other (pathological) scarring diseases.

Experimental Section

Chemistry

General: Solvents (acetone and benzene) were dried according to published methods and distilled before use. THF, CH_2Cl_2, CH_3CN, and CH_3OH were dried using a Puresolv solvent purification system. Et_3N and piperidine were dried by distillation with CaH_2. 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. All solvents and anhydrous solutions were transferred through syringes and cannulae previously dried in the oven for at least 12 h and stored in a dessicator with KOH. For reactions at low temperature, ice-water or CO_2/acetone systems were used. Analytical thin layer chromatography (TLC) was performed on aluminum plates with Merck Kieselgel 60 F_{254} and visualized by UV irradiation (λ 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. UV/Vis spectra were recorded with a Cary 100 Bio spectrophotometer in CH_3OH. IR spectra were obtained on a JASCO IR 4200 spectrophotometer from a thin film deposited onto NaCl glass. 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 countervoltage of 450 V applied to the capillary. Samples were prepared by adding a spray solution of CH_3OH/H_2O/formic acid (70:29.9:0.1, *v/v/v*) to a solution of the sample at a *v/v* ratio of 1 to 5% to give the optimal signal-to-noise ratio. High-resolution mass spectra were taken on a VG AutoSpec instrument. FAB experiments were performed on a VG AutoSpec instrument, using 3-nitrobenzylalcohol or glycerol as matrices. ^{1}H NMR spectra were recorded in $CDCl_3$ and C_6D_6 at ambient temperature on a Bruker AMX-400 spectrometer at 400 MHz with residual protic solvent as the internal reference [$CDCl_3$, δ_H = 7.26 ppm and C_6D_6, δ_H = 7.16 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 $CDCl_3$ (δ_C = 77.16 ppm) or C_6D_6 (δ_C = 128.06 ppm) as the internal reference. The DEPT135 pulse sequence was used to aid in the assignment of signals in the ^{13}C NMR spectra. All compounds tested were purified by RP-HPLC (Nova-Pak HR C_{18} 6 μm, 300×19 mm, CH_3CN/H_2O (80:20) for (*R*)-**17**, Sunfire C_{18} 5 μm, 250×46 mm, CH_3CN for **8**, and Sunfire C_{18} 5 μm, 250×46 mm, CH_3CN/H_2O gradient from 70:30 to 100:0 for **18**, **19**, and **20**) and were shown to have >95% purity (see Supporting Information for (*R*)-**8**).

***N*-methoxy-*N*-methylhexadecanamide (9)**:[44b] *N,O*-dimethylhydroxylamine hydrochloride (1.71 g, 17.55 mmol), EDCI (3.36 g, 17.55 mmol), and DMAP (2.14 g, 17.55 mmol) were added to a solution of palmitic acid (3.0 g, 11.79 mmol) in CH_2Cl_2 (5 mL), 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 **9** as a white solid (3.33 g, 95%): mp: 62–64 °C (hexane/Et_2O); ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 3.66 (s, 3H, OCH_3), 3.16 (s, 3H, NCH_3), 2.39 (t, *J* = 7.6 Hz, 2H, $2H_2$), 1.68–1.52 (m, 2H, $2H_3$), 1.37–1.18 (m, 24H, 12×-CH_2-), 0.86 ppm (t, *J* = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 174.4 (s, C_1), 60.7 (q, OMe), 31.7 (t), 31.5 (t), 29.4 (t, 5×), 29.2 (t, 3×), 29.1 (t), 29.1 (t), 26.8 (q, NCH_3), 24.3 (t), 22.4 (t), 13.8 ppm (q, C_{16}); IR (NaCl): $\tilde{\nu}$ = 2925 (s, C–H), 2855 (m, C–H), 1674 cm^{-1} (m, C=O); MS (FAB$^+$): *m/z* (%) 300 ([*M*+H]$^+$, 100); HRMS (FAB$^+$) calcd for $C_{18}H_{38}NO_2$ ([*M*+1]$^+$): 300.2903, found: 300.2910.

1-(Triisopropylsilyl)-octadec-1-yn-3-one (10):[44b] *n*BuLi (14.20 mL, 1.57 M in THF, 22.28 mmol) was added dropwise to a stirred solution of triisopropylsilyl acetylene (6.09 g, 33.41 mmol, 7.4 mL) in THF (0.2 mmol mL^{-1}) at −40 °C, and the solution was stirred for 1 h. Subsequently, a solution of *N*-methoxy-*N*-methylhexadecanamide **9** (3.33 g, 11.14 mmol) in THF (0.84 mmol) 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. The residue was purified by flash chromatography (reverse phase, CH_2Cl_2/CH_3CN, 10:90) to afford **10** as a colorless oil (4.31 g, 92%): ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 2.54 (t, *J* = 7.4 Hz, 2H, $2H_2$), 1.74–1.63 (m, 2H, $2H_3$), 1.36–1.18 (m, 24H, 12×-CH_2-), 1.17–1.03 (m, 21H, *i*Pr_3-Si), 0.88 ppm (t, *J* = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 188.1 (s), 104.4 (s, C_2), 95.4 (s), 45.8 (t), 32.1 (t), 29.8 (t, 6×), 29.6 (t), 29.5 (t, 2×), 29.1 (t), 24.4 (t), 22.8 (t), 18.6 (q, 6×, *i*Pr_3-Si), 14.2 (q), 11.1 ppm (d, 3×, *i*Pr_3-Si); IR (NaCl): $\tilde{\nu}$ = 2925 (s, C–H), 2855 (m, C–H), 1681 (m, C=O), 1460 cm^{-1} (m); UV (CH_3OH): λ_{max} = 234 nm; MS (EI): *m/z* (%) 421 ([*M*+1]$^+$, 0.2), 420 (*M*$^+$, 0.6), 378 (19), 377 (100), 167 (15); HRMS (EI) calcd for $C_{27}H_{52}OSi$ ([*M*$^+$]): 420.3787, found: 420.3795.

1-(Triisopropylsilyl)-octadec-1-yn-3-ol (11): $NaBH_4$ (0.09 g, 2.43 mmol) was added portionwise to a solution of 1-triisopropylsilyloctadec-1-yn-3-one **10** (1.50 g, 3.58 mmol) in CH_3OH (4 mL) at 0 °C, and the mixture was stirred for 1.5 h. The reaction was poured into an aqueous HCl solution (1 N), and the mixture was extracted with Et_2O (3×). The combined organic layers were washed with a saturated aqueous $NaHCO_3$ solution, dried over Na_2SO_4, and the solvent was evaporated. The residue was purified by flash chro-

matography (silica gel, hexane/EtOAc, 95:5) to afford **11** as a colorless oil (1.51 g, 80%): ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 4.38 (dd, J = 12.4, 6.4 Hz, 1H, H_3), 1.77–1.63 (m, 3H, $2H_4$ + OH), 1.52–1.40 (m, 2H, $2H_5$), 1.37–1.20 (m, 24H, 12×-CH_2-), 1.12–1.01 (m, 21H, iPr$_3$Si), 0.88 ppm (t, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 109.2 (s), 85.3 (s), 63.1(d), 38.1 (t), 32.1 (t), 29.9 (t, 5×), 29.8 (t), 29.7 (t, 2×), 29.5 (t), 29.4 (t), 25.3 (t), 22.8 (t), 18.7 (q, 6×, iPr$_3$-Si), 14.2 (q), 11.3 ppm (d, 3×, iPr$_3$-Si); IR (NaCl): $\tilde{\nu}$ = 3600–3300 (br, O–H), 2926 (s, C–H), 2858 (s, C–H), 1462 cm^{-1} (m); MS (FAB$^+$): m/z (%) 422 ([M + 1]$^+$, 3), 421 (M^+, 7), 405 (16), 363 (32), 181 (18), 158 (16), 157 (100); HRMS (FAB$^+$) calcd for $C_{27}H_{53}OSi$ ([M + H]$^+$): 421.3866, found: 421.3858.

Octadec-1-yn-3-ol (12): nBu$_4$NF (2.44 mL, 1 M in THF, 2.44 mmol) was added to a stirred solution of 1-triisopropylsilyloctadec-1-yn-3-ol **11** (0.94 g, 2.21 mmol) in THF (58 mL), 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, hexane/EtOAc/Et_3N, 90:8:2) to afford **12** as a white solid (1.51 g, 82%): mp: 54–56 °C (hexane/Et_2O); ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 4.36 (td, J = 6.6, 1.9 Hz, 1H, H_3), 2.46 (d, J = 2.1 Hz, 1H, H_1), 1.92 (s, 1H, OH), 1.76–1.63 (m, 2H, $2H_4$), 1.50–1.38 (m, 2H, $2H_5$), 1.36–1.17 (m, 24H, 12×-CH_2-), 0.87 ppm (t, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 85.2 (s), 72.9 (d), 62.5 (d), 37.8 (t), 32.1 (t), 29.8 (t, 6×), 29.7 (t, 2×), 29.5 (t), 29.4 (t), 25.2 (t), 22.8 (t), 14.3 ppm (q); IR (NaCl): $\tilde{\nu}$ = 3600–3300 (br, O–H), 2918 (s, C–H), 2850 cm^{-1} (m, C-H); HRMS (ESI$^+$) calcd for $C_{18}H_{34}ONa$ ([M + Na]$^+$): 289.24897, found: 289.25019.

(2′*R*)-2,3-*O*-Isopropylidene-2,3-dihydroxyprop-1-yl 8-hydroxy-tetracosa-4,6-diynoate (14): Copper chloride (0.03 g, 0.26 mmol) was added to a stirred solution of (2′*R*)-2,3-*O*-isopropylidene-2,3-dihydroxyprop-1-yl 5-iodopent-4-ynoate **13** (0.88 g, 2.61 mmol) and octadec-1-yn-3-ol **12** (1.82 g, 6.85 mmol) in degassed piperidine (5 mL) at 0 °C. 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, hexane/EtOAc, 80:20) to provide **14** as a white solid (0.8 g, 64%): mp: 36–38 °C (hexane/Et_2O); ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 4.37 (dd, J = 11.5, 5.9 Hz, 1H, H_8), 4.31 (dd, J = 11.6, 5.7 Hz, 1H, $H_{2'}$), 4.2–4.0 (m, 3H, $2H_{1'}$ + $1H_{3'}$), 3.75 (dd, J = 8.4, 6.2 Hz, 1H, $H_{3'}$), 2.65–2.52 (m, 4H, $2H_2$ + $2H_3$), 1.74–1.60 (m, 2H, $2H_9$), 1.47–1.38 (m, 5H, CH_3 + $2H_{10}$), 1.36 (s, 3H, CH_3), 1.33–1.17 (m, 24H, 12×-CH_2-), 0.87 ppm (t, J = 6.7 Hz, 3H, CH_3); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 171.3 (s), 110.0 (s), 79.0 (s), 77.7 (s), 73.6 (d), 69.6 (s), 66.4 (t), 65.5 (s), 65.1 (t), 62.9 (d), 37.7 (t), 32.9 (t), 32.0 (t), 29.8 (t, 6×), 29.7 (t), 29.6 (t), 29.5 (t), 29.4 (t), 26.8 (q), 25.5 (q), 25.2 (t), 22.8 (t), 15.4 (t), 14.2 ppm (q); IR (NaCl): $\tilde{\nu}$ = 3600–3300 (br, O–H), 2925 (s, C–H), 2854 (m, C–H), 2255 (w, C≡C), 1742 cm^{-1} (m, CO); HRMS (ESI$^+$) calcd for $C_{29}H_{48}O_5Na$ ([M + Na]$^+$): 499.3379, found: 499.3394.

(2′*R*)-2,3-*O*-isopropylidene-2,3-dihydroxyprop-1-yl 8-oxo-tetracosa-4,6-diynoate (15): DMSO (18 μL, 0.02 g, 0.25 mmol) was added dropwise to a stirred solution of oxalyl chloride (13 μL, 0.02 g, 0.15 mmol) in CH_2Cl_2 (1 mL) at −60 °C, and the reaction was stirred for 5 min at this temperature. Then, a solution of (2′*R*)-2,3-*O*-isopropylidene-2,3-dihydroxyprop-1-yl 8-hydroxy-tetracosa-4,6-diynoate **14** (0.47 g, 0.98 mmol) in CH_2Cl_2 (1 mL) was added. After stirring for 30 min, Et_3N (97 μL, 0.07 g, 0.69 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 H_2O, 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, hexane/EtOAc, 80:20) to afford **15** as a yellow oil (0.05 g, 99%): ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 4.34–4.26 (m, 1H, $H_{2'}$), 4.19 (dd, J = 11.5, 4.6 Hz, 1H, $H_{1'}$), 4.11 (dd, J = 11.5, 6.1 Hz, 1H, $1H_{1'}$), 4.07 (dd, J = 8.5, 6.5 Hz, 1H, $H_{3'}$), 3.73 (dd, J = 8.5, 6.0 Hz, 1H, $H_{3'}$), 2.71–2.65 (m, 2H, $2H_2$ or $2H_3$), 2.64–2.58 (m, 2H, $2H_2$ or $2H_3$), 2.52 (t, J = 7.4 Hz, 2H, $2H_9$), 1.68–1.57 (m, 2H, $2H_{10}$), 1.41 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.31–1.17 (m, 24H, 12×-CH_2-), 0.86 ppm (t, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 187.2 (s), 170.9 (s), 110.0 (s), 87.6 (s), 75.4 (s), 73.6 (d), 72.9 (s), 66.3 (t), 65.3 (t), 64.6 (s), 45.6 (t), 32.3 (t), 32.0 (t), 29.8 (t, 4×), 29.7 (t, 2×), 29.5 (t), 29.4 (t, 2×), 29.0 (t), 26.8 (q), 25.4 (q), 24.0 (t), 22.8 (t), 15.6 (t), 14.2 ppm (q); IR (NaCl): $\tilde{\nu}$ = 2925 (s, C–H), 2854 (m, C–H), 2236 (w, C≡C), 1743 (m, C=O), 1673 cm^{-1} (s); HRMS (ESI$^+$) calcd for $C_{29}H_{46}O_5$ ([M + H]$^+$): 475.34129, found: 475.34180.

(8*Z*,2′*R*)- and (8*E*,2′*R*)-2,3-Dihydroxyprop-1-yl 8-acetoxytetracosa-8-en-4,6-diynoate ((*R*)-17): Et_3N (0.54 mL, 0.39 mg, 3.87 mmol), DMAP (47 mg, 0.39 mmol), and acetic anhydride (0.44 mL, 0.47 g, 4.64 mmol) were added to a stirred solution of (2′*R*)-2,3-*O*-isopropylidene-2,3-dihydroxyprop-1-yl 8-oxo-tetracosa-4,6-diynoate **15** (0.37 g, 0.77 mmol) in CH_2Cl_2 (20 mL) at 0 °C, 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, hexane/CH_2Cl_2/EtOAc, 80:20:10) to afford a colorless oil (0.35 g, 88%), identified as a 70:30 mixture of *E*/*Z* isomers, which was used in the next step.

Solid CAN (6 mg, 0.01 mmol) was added to a stirred solution of (2′*R*)-2,3-*O*-methylidene-2,3-dihydroxyprop-1-yl 8-acetoxytetracosa-8-en-4,6-diynoate **16** (0.19 g, 0.36 mmol) in CH_3CN/H_2O (2 mL, 1:1 *v*/*v*) at 70 °C. The resulting slightly yellow solution was stirred for 24 h at this temperature. After cooling to room temperature, the reaction mixture was extracted with Et_2O (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, hexane/EtOAc/Et_3N, 60:38:2) to give a colorless oil (0.13 g,74%) that was identified as a 70:30 mixture of *E*/*Z* isomers. This mixture was separated by RP-HPLC (Nova-Pak HR C_{18} 6 μm, 19×300 mm, CH_3CN/H_2O (80:20), 20 mL min^{-1}) to yield (2*R*′)-2,3-dihydroxyprop-1-yl 8-acetoxytetracosa-8-en-4,6-diynoate (*R*)-**17** (*E* isomer (t_R = 22.7 min): 21.5 mg, 29%; *Z* isomer (t_R = 27.0 min): 53.2 mg, 71%).

Data for **(8*E*,2′*R*)-17 (17 A)**: ^{1}H NMR (400.13 MHz, C_6D_6): δ = 5.67 (t, J = 8.0 Hz, 1H, H_9), 4.02 (dd, 1H, J = 11.5, 6.1 Hz, 1H, $H_{1'}$), 3.98 (dd, J = 11.5, 5.0 Hz, 1H, $H_{1'}$), 3.72–3.63 (m, 1H, $H_{2'}$), 3.45 (dd, J = 11.3, 4.0 Hz, 1H, $H_{3'}$), 3.37 (dd, J = 11.3, 6.0 Hz, 1H, $H_{3'}$), 2.25–2.14 (m, 4H, $2H_{10}$ + $2H_2$ or $2H_3$), 2.03 (t, J = 6.9 Hz, 2H, $2H_2$ or $2H_3$), 1.63 (s, 3H, CO_2CH_3), 1.41–1.15 (m, 24H, 12×-CH_2-), 0.91 ppm (t, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100.62 MHz, C_6D_6): δ = 171.2 (s), 168.4 (s), 132.4 (d), 130.4 (s), 85.5 (s), 79.4 (s), 70.4 (d), 68.6 (s), 66.0 (s), 65.8 (t), 63.6 (t), 32.5 (t), 32.4 (t), 30.2 (t, 5×), 30.0 (t), 29.9 (t, 2×), 29.4 (t), 29.2 (t), 28.4 (t), 23.1 (t), 20.1 (q), 15.5 (t), 14.4 ppm (q); IR (NaCl): $\tilde{\nu}$ = 3600–3300 (br, O–H), 2923 (s, C–H), 2853 (m, C–H), 2235 (w, C≡C), 1740 (m, C=O), 1204 cm^{-1} (m); HRMS (ESI$^+$) calcd for $C_{28}H_{44}O_6Na$ ([M + Na]$^+$): 499.30218, found: 499.30301.

Data for **(8*Z*,2′*R*)-17 (17 B)**: ^{1}H NMR (400.13 MHz, C_6D_6): δ = 5.65 (t, J = 7.7 Hz, 1H, H_9), 4.05 (dd, 1H, J = 11.4, 6.1 Hz, 1H, $H_{1'}$), 4.01 (dd, J = 11.5, 4.9 Hz, 1H, $H_{1'}$), 3.74–3.67 (m, 1H, $H_{2'}$), 3.47 (dd, J = 11.3, 4.0 Hz, 1H, $H_{3'}$), 3.40 (dd, J = 11.3, 6.0 Hz, 1H, $H_{3'}$), 2.20 (t, J = 7.2 Hz,

2H, $2H_2$ or $2H_3$), 2.07 (t, $J=7.0$ Hz, 2H, $2H_2$ or $2H_3$), 1.99–1.88 (m, 2H, $2H_{10}$), 1.64 (s, CO_2CH_3), 1.38–1.08 (m, 24H, 12×-CH_2-), 0.92 ppm (t, $J=6.8$ Hz, 3H, CH_3); ^{13}C NMR (100.62 MHz, C_6D_6): $\delta=171.4$ (s), 167.7 (s), 131.9 (d), 130.0 (s), 84.3 (s), 74.6 (d), 70.8 (s), 70.4 (d), 66.1 (s), 65.8 (t), 63.6 (t), 32.6 (t), 32.4 (t), 30.2 (t, 4×), 30.1 (t), 30.0 (t), 29.8 (t, 2×), 29.5 (t), 28.7 (t), 26.6 (t), 23.1 (t), 19.9 (q), 15.5 (t), 14.4 ppm (q); IR (NaCl): $\tilde{\nu}=3600$–3300 (br, O–H), 2922 (s, C–H), 2853 (m, C–H), 2359 (m), 2233 (w, C≡C), 1736 (w, C=O), 1197 cm^{-1} (w); HRMS (ESI$^+$) calcd for $C_{28}H_{45}O_6$ ($[M+H]^+$): 477.32211, found: 477.32107.

(2′*R*)-2,3-Dihydroxyprop-1-yl 8-oxo-4,6-tetracosadiynoate ((*R*)-8): Following the general procedure described above for the deprotection of ketals, the reaction of (2′*R*)-2,3-*O*-isopropylidene-2,3-dihydroxy-1-prop-1-yl 8-oxo-tetracosa-4,6-diynoate **15** (80.2 mg, 0.17 mmol) and CAN (3 mg, 5.1 μmol) in CH_3CN/H_2O (1 mL, 1:1 *v/v*) at 70 °C afforded **(*R*)-8**, after purification by flash chromatography (silica gel, hexane/EtOAc, 70:30), as a white solid (37 mg, 78%): ^{1}H NMR (400.13 MHz, $CDCl_3$): $\delta=4.23$ (dd, $J=11.6$, 4.8 Hz, 1H, $H_{1'}$), 4.19 (dd, $J=11.6$, 6.2 Hz, 1H, $H_{1'}$), 3.99–3.90 (m, 1H, $H_{2'}$), 3.71 (dd, $J=11.3$, 3.7 Hz, 1H, $H_{3'}$), 3.60 (dd, $J=11.3$, 5.9 Hz, 1H, $H_{3'}$), 2.75–2.59 (m, 4H, $2H_2+2H_3$), 2.54 (t, $J=7.4$ Hz, 2H, $2H_9$), 1.70–1.57 (m, 2H, $2H_{10}$), 1.35–1.12 (m, 24H, 12×-CH_2-), 0.87 ppm (t, $J=6.6$ Hz, 3H, CH_3); ^{13}C NMR (100.62 MHz, $CDCl_3$): $\delta=187.6$ (s), 171.5 (s), 87.7 (s), 75.5 (s), 72.9 (s), 70.2 (d), 65.9 (t), 64.7 (s), 63.4 (t), 45.6 (t), 32.4 (t), 32.0 (t), 29.8 (t, 5×), 29.7 (t), 29.5 (t, 2×), 29.4 (t), 29.0 (t), 24.0 (t), 22.8 (t), 15.6 (t), 14.2 ppm (q); IR (NaCl): $\tilde{\nu}=3400$–3100 (br, O–H), 2919 (s, C–H), 2850 (m, C–H), 2239 (w, -C≡C-), 1727 (m, C=O), 1668 (m, C=O), 1173 cm^{-1} (m); HRMS (ESI$^+$) calcd for $C_{26}H_{43}O_5$ ($[M+H]^+$): 435.31192, found: 435.31050.

Methyl-(2*R*,6′*E*)-2-(*tert*-butoxycarbonylamino)-3-(1′-(*tert*-butyldiphenylsilyloxy)-8′-oxotricosa-6′-en-4′-yn-6′-ylthio)propanoate (23), and methyl-(2*R*,4′*E*)-2-(*tert*-butoxycarbonylamino)-3-(1′-(*tert*-butyldiphenylsilyloxy)-8′-oxotricosa-4′-en-6′-yn-4′-ylthio)-propanoate (24): Et_3N (20 μL, 14.6 mg, 0.14 mmol) and *N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester (30 μL, 34 mg, 0.14 mmol) were successively added to a solution of 1-(*tert*-butyldiphenylsilyloxy)-tricosa-4,6-diyn-8-one (84 mg, 0.14 mmol) in CH_2Cl_2 (1.7 mL). The reaction was stirred at room temperature for 72 h and diluted with H_2O, then the mixture was extracted with CH_2Cl_2 (3×). The combined organic layers were dried, and the solvent was evaporated. The residue was purified by flash chromatography (silica gel, hexane/EtOAc, 80:20) to afford **23** and **24** as yellow oils (27 mg, 27%). The mixture of 1,4- and 1,6-Michael adducts was separated by HPLC (Waters Spherisorb 5 μm silica gel, 10×250 mm, hexane/EtOAc (90:10), 2.0 mL min^{-1}) to yield 6.2 mg (5%) of the (*E*)-1,6-Michael adduct ($t_R=30.5$ min) and 11 mg (9%) of the (*E*)-1,4-Michael adduct ($t_R=33.0$ min).

(2*R*,6′*E*)-Methyl 2-(*tert*-butoxycarbonylamino)-3-(1′-(*tert*-butyldiphenylsilyloxy)-8′-oxotricosa-6′-en-4′-yn-6′-ylthio)propanoate (23): ^{1}H NMR (400.13 MHz, C_6D_6): $\delta=7.79$–7.74 (m, 4H, ArH), 7.28–7.23 (m, 6H, ArH), 6.50 (s, 1H, $H_{7'}$), 5.08 (d, $J=8.4$ Hz, 1H, N*H*CO_2-$(CH_3)_3$), 4.80 (dd, $J=13.3$, 7.7 Hz, 1H, H_2), 3.67 (t, $J=5.9$ Hz, 2H, $2H_{1'}$), 3.47 (dd, $J=14.0$, 5.2 Hz, 1H, H_{3A}), 3.19 (s, 3H, CO_2Me), 3.02 (dd, $J=14.0$, 7.3 Hz, 1H, H_{3B}), 2.48 (t, $J=7.1$ Hz, 2H, $2H_{9'}$), 2.13 (t, $J=7.4$ Hz, 2H, $2H_{3'}$), 1.72 (q, $J=7.0$ Hz, 2H, $2H_{2'}$), 1.61–1.50 (m, 2H, $2H_{10'}$), 1.38 (s, 9H, $NHCO_2(CH_3)_3$), 1.36–1.18 (m, 24H, 12×-CH_2-), 1.17 (s, 9H, $SiC(CH_3)$), 0.92 ppm (t, $J=6.8$ Hz, 3H, CH_3); ^{13}C NMR (100.62 MHz, C_6D_6): $\delta=197.2$ (s, $C_{8'}$), 171.2 (s, C_1), 155.3 (s, $NHCO_2C$-$(CH_3)_3$), 139.7 (s), 136.0 (d, 4×), 134.0 (s, 2×), 130.1 (d, 2×), 128.2 (d, 4×), 126.0 (d), 101.6 (s), 79.6 (s), 77.2 (s, $NHCO_2C(CH_3)_3$), 62.7 (t), 54.0 (d, C_2), 52.0 (q, CO_2CH_3), 43.2 (t), 35.7 (t), 32.4 (t), 31.4 (t), 30.2 (t, 6×), 30.0 (t, 2×), 29.9 (t), 29.7 (t), 28.3 (q, $NHCO_2C(CH_3)_3$), 27.1(q, $SiC(CH_3)_3$), 24.8 (t), 23.2 (t), 19.5 (s, $SiC(CH_3)_3$), 16.5 (t), 14.4 ppm (q); IR (NaCl): $\tilde{\nu}=3400$–3200 (br, N–H), 2952 (m, C–H), 2925 (s, C–H), 2854 (m, C–H), 2215 (w, C≡C), 1748 (m, C=O), 1719 (m, C=O), 1658 (w, C=O), 1522 (m), 1167 (m), 1110 (m), 703 cm^{-1} (m); UV (CH_3OH): $\lambda_{max}=264$, 326 nm; HRMS (ESI$^+$) calcd for $C_{48}H_{74}NO_6SSi$ ($[M+H]^+$): 820.50006, found: 820.49937.

(2*R*,4′*E*)-Methyl 2-(*tert*-butoxycarbonylamino)-3-(1′-(*tert*-butyldiphenylsilyloxy)-8′-oxotricosa-4′-en-6′-yn-4′-ylthio)propanoate (24): ^{1}H NMR (400.13 MHz, C_6D_6): $\delta=7.81$–7.72 (m, 4H, ArH), 7.32–7.23 (m, 6H, ArH), 5.27 (s, 1H, $H_{7'}$), 5.15 (d, $J=8.4$ Hz, 1H, N*H*CO_2-$(CH_3)_3$), 4.54 (dd, $J=12.8$, 5.5 Hz, 1H, H_2), 3.66 (t, $J=6.2$ Hz, 2H, $2H_{1'}$), 3.17 (s, 3H, CO_2Me), 2.94 (dd, $J=13.6$, 4.9 Hz, 1H, H_3), 2.73–2.63 (m, 3H, $2H_{9'}+H_3$), 2.42 (t, $J=7.4$ Hz, 2H, $2H_{3'}$), 1.91–1.81 (m, 2H, $2H_{2'}$), 1.71–1.62 (m, 2H, $2H_{10'}$), 1.41 (s, 9H, $NHCO_2(CH_3)_3$), 1.37–1.13 (m, 33H, 12×-CH_2-+$SiC(CH_3)$), 0.92 ppm (t, $J=6.8$ Hz, 3H, CH_3); ^{13}C NMR (100.62 MHz, C_6D_6): $\delta=186.4$ (s, $C_{10'}$), 170.5 (s, C_1), 160.9 (s, $C_{4'}$), 155.1 (s, $NHCO_2C(CH_3)_3$), 136.0 (d, 4×), 134.2 (s, 2×), 130.0 (d, 2×), 128.2 (d, 4×), 98.7 (d, $C_{5'}$), 94.2 (s), 87.4 (s), 80.1 (s, $NHCO_2C(CH_3)_3$), 63.5 (t), 52.7 (d, C_2), 52.2 (q, CO_2CH_3), 45.7 (t), 33.6 (t), 32.4 (t, 2×), 30.2 (t, 6×), 30.1 (t), 30.0 (t), 29.9 (t, 2×), 29.4 (t), 28.3 (q, $NHCO_2C(CH_3)_3$), 27.2 (q, $SiC(CH_3)_3$), 24.6 (t), 23.2 (t), 19.5 (s, $SiC(CH_3)_3$), 14.4 ppm (q); IR (NaCl): $\tilde{\nu}=3400$–3200 (br, N–H), 2925 (s, C–H), 2854 (m, C–H), 2166 (m, C≡C), 1750 (w, C=O), 1718 (m, C=O), 1663 (m, C=O), 1522 (m), 1166 (m), 1110 (m), 703 cm^{-1} (m); UV (CH_3OH): $\lambda_{max}=324$ nm; HRMS (ESI$^+$) calcd for $C_{48}H_{74}NO_6SSi$ ($[M+H]^+$): 820.50006, found: 820.49950.

Other compounds: All chemicals used in these experiments were dissolved in DMSO (Sigma–Aldrich); the reference HDAC inhibitors SAHA (Alexis) and MS-275 (Schering AG) were used at 5 μM, while the DNMT inhibitors 5-aza, RG108, and SGI-1027 were used at 50 μM. The peyssonenyne derivatives were also dissolved in DMSO and used at concentrations of 5 μM and at 50 μM.

Biology

Cell cultures: K562 and U937 human leukemia cell lines were 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. BJ cells were grown in DMEM; BJEL were grown in DMEM/199 (4:1) plus hygromycin and G418; DMEM was supplemented with 10% FCS, sodium pyruvate, non-essential amino acids, and gentamycin. Cells were exposed to 1 μm, 5 μM, or 10 μm of the synthetic compounds SGI-1027 or 5-aza-2′-deoxycitidine; stock solutions were in 10 mM DMSO. MEFs were grown in DMEML (4.5 g L^{-1} glucose) with GlutaMAX1, supplemented with 10% FCS, sodium pyruvate, and gentamycin. All experiments with primary MEFs were performed with cultures that had been passaged a maximum of three times.

DNMT1 immunoprecipitation: The K562 cells were lysed in TAP buffer at 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 were centrifuged at 13000 rpm for 30 min. The extracts (650 μg each) were diluted to 1 mL in TAP buffer and were 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, 3.25 μg of antibody against DNMT1 (Abcam) was added, and immunoprecipitation was allowed to proceed overnight at 4 °C on a rocking table. As a 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 assays were performed in the presence of the analogues at 50 μM plus a reaction mixture composed of 10 μL DNMT1-bound resin, 5 μCi *S*-adenosyl-L-[methyl-^{3}H]methionine (radioactive methyl donor; 12–18 Ci mmol^{-1}), 0.1 μg of poly dI-dC (methyl acceptor), and DNMT buffer. The reaction was carried out for 2 h at 37 °C with gentle 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. The papers were then transferred to scintillation vials containing 5 mL scintillation cocktail in order to read the DPM values.

DNMT3A and DNMT3B radioactive assays: DNMT3A and DNMT3B were expressed in bacteria (*E. coli* BL21) and purified following standard procedures. The DNMT radioactive assay was performed in a reaction mixture containing DNMT buffer (5 mM EDTA; 0.2 mM DTT; 26 mM NaCl, 20 mM Tris HCl, pH 7.4), poly dI-dC (0.1 γ/λ), *S*-adenosyl-L-[methyl-^{3}H]methionine (1 μCi), and DNMT3A (30 ng). The reaction was carried out at 37 °C for 2 h on a shaker. The radioactive mixture was then transferred to DE81 paper and washed three times with di-sodium hydrogenphosphate 5% for 5 min and once with sterile water for 2 min. Once dried, the paper was transferred to a scintillation vial containing 5 mL scintillation cocktail. The data represent the average value of independent triplicates; error bars represent standard deviations (SD) of biological triplicates.

Cell-cycle analysis by FACS: 2.5×10^5 U937 cells were collected by centrifugation after 24 h stimulation with the reference compounds or the peyssonenyne analogues. The cells were then resuspended in 500 μL of a hypotonic buffer (0.1% NP-40, 0.1% sodium citrate, 50 μg mL^{-1} PI, RNAse A) and incubated in the dark for 30 min. Analysis was performed with a FACSCalibur (Becton Dickinson) using the Cell Quest Pro software (Becton Dickinson) and ModFit LT version 3 software (Verity). The experiment was performed in duplicate.

Granulocytic differentiation analysis: U937 cells (2.5×10^5) were collected by centrifugation after 24 h stimulation with the reference compounds or the peyssonenyne analogues. The cells were washed with PBS and incubated in the dark at 4 °C for 30 min with 10 μL of PE-conjugated anti-CD11c surface antigen antibody. We also included also a PE control, a sample incubated with 10 μL of PE-conjugated IgG, in order to remove the background signal in the following analysis. At the end of incubation period, the samples were washed again and then resuspended in 500 μL of PBS containing 0.25 μg mL^{-1} protease inhibitors (PI). Analysis was performed with a FACSCalibur (Becton Dickinson) using the Cell Quest Pro software (Becton Dickinson). The experiment was performed in duplicate, and PI positive cells were excluded from the analysis.

Protein extraction: U937 cells were treated with the reference compounds or the peyssonenyne derivatives and were harvested by centrifugation. After washing with PBS, the samples were resuspended in lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% NP40, 10 mM NaF, 1 mM PMSF, and PI), and the lysis reaction was carried out for 15 min at 4 °C. The samples were centrifuged at 13000 rpm for 30 min at 4 °C, and the proteins were quantified by Bradford assay (Bio-Rad).

Histone extraction: Histone extraction was performed according to the Abcam protocol. Briefly, after stimulation with the compounds, the cells were collected by centrifugation and washed two times with PBS. The samples were resuspended in Triton extraction buffer (TEB, PBS containing 0.5% Triton X-100 (*v/v*), 2 mM PMSF, 0.02% (*w/v*) NaN_3), and lysis was performed for 10 min at 4 °C. The samples were centrifuged at 2000 rpm for 10 min at 4 °C, and the pellets were washed in TEB again (this time, in half the volume of buffer). After new centrifugation in the same conditions, the sample were resuspended in 0.2 N HCl, and acid histone extraction was carried out overnight at 4 °C. The following day, the supernatant was recovered by centrifugation, and the proteins were quantified by Bradford assay (Bio-Rad).

Western blots: Total protein samples (50 μg each) were loaded on a 10% polyacrylamide gel in order to evaluate acetyl-tubulin expression (Sigma–Aldrich, 1:500 dilution), while the same amount of proteins were loaded on 15% polyacrylamide gels for p21$^{Cip1/WAF1}$ (Transduction Laboratories, 1:500 dilution), Fas (Cell Signaling, dilution 1:500), and Bcl-2 (Cell Signaling, 1:500 dilution) analysis. ERK1 antibody (Santa Cruz, 1:1000 dilution) was used as a loading control.

Histonic extracts (10 μg each) were loaded on a 15% polyacrylamide gel, and histone H3 acetylation was assessed with an anti-acetyl-histone H3 antibody (Abcam, dilution 1:500). Ponceau Red (Sigma–Aldrich) was used to color the nitrocellulose filter and confirm equal loading.

MTT assays: Cells were treated with compounds in 96-well plates for the times indicated, then were washed and exposed to 0.5 mg mL^{-1} MTT (Sigma–Aldrich) for 4 h at 37 °C. The medium was removed, and 100 μL DMSO was added. After shaking, the plates were read at 550 nm absorbance using an EL800 (Biotek).

Real-time PCR: Total RNA was extracted (DNA and RNA purification kits, Sigma–Aldrich) according to the instructions provided by the manufacturer, and cDNA was synthesized from 2 μg RNA using SuperScript II (Invitrogen) using the following condi-

Table 1. Oligonucleotide primers used for real-time PCR.

Primer	Sense (5′→3′)	Antisense (5′→3′)	PCR product [bp][a]	T_m [°C][b]
CSK	aactatgtccagaagcgtgagg	cacagtgtgtagtctccagggtagt	170	60
DNMT1	agcactaccgcaagtattctgact	gtagaacttgtagagcctcagcttg	152	60
DNMT3A	gccagaactgtaagaactgc	aagagatccacacactcgac	157	55
GJB2	gcctaccggagacatgaaaa	tcaaagatgacccggaagaa	155	60
IAP	cttcacttaggacgcatcac	gcgttgacatcctgtgtt	149	55
NMYC1	gtcactagtgtgtctgttccagctac	gatttcctcctcttcatcttcctc	168	60
SLPI	aagtgcgtgaatcctgttcc	cacatataccctcacagcacttgt	157	60

[a] Product length in base pairs. [b] Duplex melting temperature.

tions: 1 h at 42 °C, 10 min at 95 °C, 4 °C. The cDNA was used for PCR amplification using SYBR Green I (Qiagen) according to standard procedures using ABI770 (Applied Biosystems). PCR primers are listed in Table 1.

Acknowledgements

BJ and BJEL cells were kindly provided by Bill Hahn (Dana–Farber Cancer Institute, Harvard Medical School). We thank Rosaria Benedetti (UNINA2) for technical help in DNMT assays. This work was supported by the European Union LSHC-CT-2005-518417 "Epitron", the Ministerio de Economía y Competitividad–Spain (SAF2010-17935-FEDER; FPU Fellowship to P.G.-D.), the Xunta de Galicia (Grant 08CSA052383PR from DXI+D+i; Consolidación 2006/15 from DXPCTSUG; INBIOMED), the Associazione Italiana per la ricerca contro il cancro (AIRC), the Epigenomics Flagship project EPIGEN (MIUR-CNI), and the Ligue National Contre le Cancer (H.G.; laboratoire labelisé).

Keywords: DNA methyltransferase · epigenetics · natural products · total synthesis

[1] a) S. E. Johnstone, S. B. Baylin, *Nat. Rev. Genet.* **2010**, *11*, 806 – 812; b) P. A. Jones, S. B. Baylin, *Cell* **2007**, *128*, 683 – 692.
[2] a) C. Ling, L. Groop, *Diabetes* **2009**, *58*, 2718 – 2725; b) L. M. Villeneuve, R. Natarajan, *Am. J. Physiol. Renal. Physiol.* **2010**, *299*, F14 – F25.
[3] B. M. Javierre, A. F. Fernandez, J. Richter, F. Al-Shahrour, J. I. Martin-Subero, J. Rodriguez-Ubreva, M. Berdasco, M. F. Fraga, T. P. O'Hanlon, L. G. Rider, F. V. Jacinto, F. J. Lopez-Longo, J. Dopazo, M. Forn, M. A. Peinado, L. Carreno, A. H. Sawalha, J. B. Harley, R. Siebert, M. Esteller, F. W. Miller, E. Ballestar, *Genome Res.* **2010**, *20*, 170 – 179.
[4] I. M. Adcock, K. Ito, P. J. Barnes, *COPD: J. Chron. Obstruct. Pulmon. Dis.* **2005**, *2*, 445 – 455.
[5] R. G. Urdinguio, J. V. Sanchez-Mut, M. Esteller, *Lancet Neurol.* **2009**, *8*, 1056 – 1072.
[6] C. T. Hang, J. Yang, P. Han, H. L. Cheng, C. Shang, E. Ashley, B. Zhou, C. P. Chang, *Nature* **2010**, *466*, 62 – 67.
[7] W. Bechtel, S. McGoohan, E. M. Zeisberg, G. A. Muller, H. Kalbacher, D. J. Salant, C. A. Muller, R. Kalluri, M. Zeisberg, *Nat. Med.* **2010**, *16*, 544 – 550.
[8] R. A. Irizarry, C. Ladd-Acosta, B. Wen, Z. Wu, C. Montano, P. Onyango, H. Cui, K. Gabo, M. Rongione, M. Webster, H. Ji, J. B. Potash, S. Sabunciyan, A. P. Feinberg, *Nat. Genet.* **2009**, *41*, 178 – 186.
[9] a) A. Portela, M. Esteller, *Nat. Biotechnol.* **2010**, *28*, 1057 – 1068; b) T. K. Kelly, D. D. De Carvalho, P. A. Jones, *Nat. Biotechnol.* **2010**, *28*, 1069 – 1078.
[10] R. Bonasio, S. Tu, D. Reinberg, *Science* **2010**, *330*, 612 – 616.
[11] S. K. T. Ooi, T. H. Bestor, *Cell* **2008**, *133*, 1145 – 1148.
[12] W. Reik, A. Lewis, *Nat. Rev. Genet.* **2005**, *6*, 403 – 410.
[13] R. Z. Jurkowska, T. P. Jurkowski, A. Jeltsch, *ChemBioChem* **2011**, *12*, 206 – 222.
[14] R. Brown, G. Strathdee, *Trends Mol. Med.* **2002**, *8*, S43 – S48.
[15] M. Schaefer, F. Lyko, *Chromosoma* **2010**, *119*, 35 – 40.
[16] a) M. Esteller, *Oncogene* **2002**, *21*, 5427 – 5440; b) M. Rodríguez-Paredes, M. Esteller, *Nat. Med.* **2011**, *17*, 330 – 339.
[17] F. Gaudet, J. G. Hodgson, A. Eden, L. Jackson-Grusby, J. Dausman, J. W. Gray, H. Leonhardt, R. Jaenisch, *Science* **2003**, *300*, 489 – 492.
[18] T. M. Holm, L. Jackson-Grusby, T. Brambrink, Y. Yamada, W. M. Rideout III, R. Jaenisch, *Cancer Cell* **2005**, *8*, 275 – 285.
[19] Y. Yamada, L. Jackson-Grusby, H. Linhart, A. Meissner, A. Eden, H. Lin, R. Jaenisch, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 13580 – 13585.
[20] H. Lin, Y. Yamada, S. Nguyen, H. Linhart, L. Jackson-Grusby, A. Meissner, K. Meletis, G. Lo, R. Jaenisch, *Mol. Cell. Biol.* **2006**, *26*, 2976 – 2983.
[21] a) M. Tahiliani, K. P. Koh, Y. Shen, W. A. Pastor, H. Bandukwala, Y. Brudno, S. Agarwal, L. M. Iyer, D. R. Liu, L. Aravind, A. Rao, *Science* **2009**, *324*, 930 – 935; b) S. Ito, A. C. D'Alessio, O. V. Taranova, K. Hong, L. C. Sowers, Y. Zhang, *Nature* **2010**, *466*, 1129 – 1133.
[22] R. Álvarez, L. Altucci, H. Gronemeyer, A. R. de Lera, *Curr. Top. Med. Chem.* **2011**, *11*, 2749 – 2787.
[23] R. J. Roberts, X. Cheng, *Ann. Rev. Biochem.* **1998**, *67*, 181 – 198.
[24] H.-C. Tsai, H. Li, L. Van Neste, Y. Cai, C. Robert, F. V. Rassool, J. J. Shin, K. M. Harbom, R. Beaty, E. Pappou, J. Harris, R.-W. C. Yen, N. Ahuja, M. V. Brock, V. Stearns, D. Feller-Kopman, L. B. Yarmus, Y.-C. Lin, A. L. Welm, J.-P. Issa, I. Minn, W. Matsui, Y.-Y. Jang, S. J. Sharkis, S. B. Baylin, C. A. Zahnow, *Cancer Cell* **2012**, *21*, 430 – 446.
[25] a) B. Brueckner, R. G. Boy, P. Siedlecki, T. Musch, H. C. Kliem, P. Zielenkiewicz, S. Suhai, M. Wiessler, F. Lyko, *Cancer Res.* **2005**, *65*, 6305 – 6311; b) P. Siedlecki, R. G. Boy, T. Musch, B. Brueckner, S. Suhai, F. Lyko, P. Zielenkiewicz, *J. Med. Chem.* **2006**, *49*, 678 – 683.
[26] D. Kuck, N. Singh, F. Lyko, J. L. Medina-Franco, *Bioorg. Med. Chem.* **2010**, *18*, 822 – 829.
[27] J. Datta, K. Ghoshal, W. A. Denny, S. A. Gamage, D. G. Brooke, P. Phiasivongsa, S. Redkar, S. T. Jacob, *Cancer Res.* **2009**, *69*, 4277 – 4285.
[28] D. Kuck, T. Caulfield, F. Lyko, J. L. Medina-Franco, *Mol. Cancer Ther.* **2010**, *9*, 3015 – 3023.
[29] P. Crews, W. H. Gerwick, F. J. Schmitz, D. France, K. W. Bair, A. E. Wright, Y. Hallock, *Pharm. Biol.* **2003**, *41*, 39 – 52.
[30] E. H. Andrianasolo, D. France, S. Cornell-Kennon, W. H. Gerwick, *J. Nat. Prod.* **2006**, *69*, 576 – 579.
[31] I. C. Piña, J. T. Gautschi, G. Y. S. Wang, M. L. Sanders, F. J. Schmitz, D. France, S. Cornell-Kennon, L. C. Sambucetti, S. W. Remiszewski, L. B. Perez, K. W. Bair, P. Crews, *J. Org. Chem.* **2003**, *68*, 3866 – 3873.
[32] K. L. McPhail, D. France, S. Cornell-Kennon, W. H. Gerwick, *J. Nat. Prod.* **2004**, *67*, 1010 – 1013.
[33] M. Z. Fang, Y. Wang, N. Ai, Z. Hou, Y. Sun, H. Lu, W. Welsh, C. S. Yang, *Cancer Res.* **2003**, *63*, 7563 – 7570.
[34] N. Singh, A. Dueñas-González, F. Lyko, J. L. Medina-Franco, *ChemMedChem* **2009**, *4*, 792 – 799.
[35] J. Song, O. Rechkoblit, T. H. Bestor, D. J. Patel, *Science* **2011**, *331*, 1036 – 1040.
[36] P. García-Domínguez, I. Lepore, C. Erb, H. Gronemeyer, L. Altucci, R. Álvarez, A. R. de Lera, *Org. Biomol. Chem.* **2011**, *9*, 6979 – 6987.
[37] J. C. Kwan, H. Luesch, *Chem. Eur. J.* **2010**, *16*, 13020 – 13029.
[38] A. Sun, Y. J. Lu, H. Hu, M. Shoji, D. C. Liotta, J. P. Snyder, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6627 – 6631.
[39] S. Nahm, S. M. Weinreb, *Tetrahedron Lett.* **1981**, *22*, 3815 – 3818.
[40] S. Aoki, K. Matsui, H. Wei, N. Murakami, M. Kobayashi, *Tetrahedron* **2002**, *58*, 5417 – 5422.
[41] M. Alami, F. Ferri, *Tetrahedron Lett.* **1996**, *37*, 2763 – 2766.
[42] A. Ates, A. Gautier, B. Leroy, J.-M. Plancher, Y. Quesnel, J.-C. Vanherck, I. E. Markó, *Tetrahedron* **2003**, *59*, 8989 – 8999.
[43] F. Compernolle, S. Toppet, T. Brossette, H. Mao, M. Koukni, T. Kozlecki, B. Medaer, M. Guillaume, Y. Lang, S. Leurs, G. J. Hoornaert, *Eur. J. Org. Chem.* **2006**, 1586 – 1592.
[44] a) V. V. Krishnamurthy, *J. Magn. Reson. Ser. A* **1996**, *121*, 33 – 41; b) P. García-Domínguez, M. Martín-Pastor, A. R. de Lera, R. Álvarez, *Magn. Reson. Chem.* **2010**, *48*, 543 – 549.
[45] P. A. Marks, *Oncogene* **2007**, *26*, 1351 – 1356.
[46] R. R. Rosato, S. Grant, *Cancer Biol. Ther.* **2003**, *2*, 30 – 37.
[47] L. Jackson-Grusby, C. Beard, R. Possemato, M. Tudor, D. Fambrough, G. Csankovszki, J. Dausman, P. Lee, C. Wilson, E. Lander, R. Jaenisch, *Nat. Genet.* **2001**, *27*, 31 – 39.
[48] W. C. Hahn, C. M. Counter, A. S. Lundberg, R. L. Beijersbergen, M. W. Brooks, R. A. Weinberg, *Nature* **1999**, *400*, 464 – 468.
[49] J. S. Yadav, B. V. S. Reddy, G. Baishya, *J. Org. Chem.* **2003**, *68*, 7098 – 7100.
[50] J. Singh, R. C. Petter, T. A. Baillie, A. Whitty, *Nat. Rev. Drug Discovery* **2011**, *10*, 307 – 317.
[51] a) W. C. Hahn, R. A. Weinberg, *Nat. Rev. Cancer* **2002**, *2*, 331 – 341; b) W. C. Hahn, R. A. Weinberg, *N. Engl. J. Med.* **2002**, *347*, 1593 – 1603.

Received: July 31, 2012
Revised: September 10, 2012
Published online on October 9, 2012

