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Simplified dynemicin analogues: diastereoselective synthesis and evaluation of their activity against plasmid DNA

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The total synthesis of two diastereoisomeric simplified dynemicin analogues is reported. The key steps involved are: the regio- and diastereoselective functionalisation of an appropriate racemic quinoline precursor and the ring closure to give the 10-membered enediyne moiety through a Pd(0)-catalysed Stille reaction. After the successful conversion of one of these derivatives into a compound more readily activable under nearly physiological conditions, the activity against plasmid DNA was evaluated.

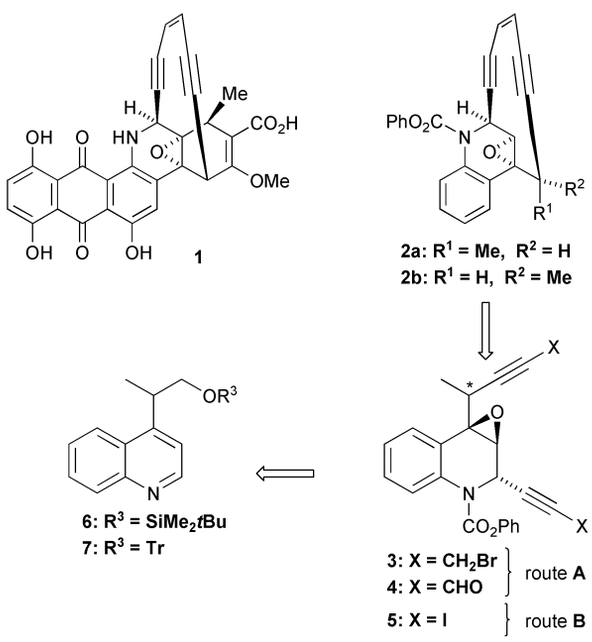
Introduction

Natural enediyne antibiotics (*e.g.* calicheamicin, esperamicin, dynemicin **1**) constitute a small family of cyclic derivatives, all characterised by the presence of a 10-membered 3-ene-1,5-diyne cyclic moiety. They are among the most powerful anti-tumoral compounds known to date.[1,2] All these compounds are distinguished by a unique mode of action: they are actually natural prodrugs, their biological properties being displayed only after removal of an appropriate "safety lock". This is represented, in dynemicin and analogues, by an epoxide, whose relative configuration is *trans* with respect to the 10-membered unsaturated ring.[1,3–7] The opening of the oxirane ring in dynemicin, which is followed by Bergman cycloaromatisation leading to a high reactive diradical responsible for DNA damage, is a consequence of a series of cascade events, arising from an initial bioreduction of the anthraquinone moiety.[6] Since **1** is characterized by a rather complex structure, several groups decided to synthesise simpler derivatives equipped merely with the moieties essential to display the desired biological activity. However, when these simplified analogues lack the anthraquinone portion, alternative triggering mechanisms had to be exploited. The most widely used is based on the protection of the nitrogen (for example as carbamate). As soon as this blocking group is removed, the restored nucleophilic power of the nitrogen causes epoxide opening under physiological conditions.

Within our project in the enediyne field, that has also witnessed various synthetic approaches to the family of artificial enediynes called "lactenediynes",[8,9] we decided to synthesise new simplified analogues of dynemicin **1**, namely diastereoisomeric derivatives **2a**,**b**, and to explore their behaviour in DNA cleavage experiments.[10]

Results and discussion

Although derivatives with a structure resembling **2a**,**b** have already been prepared by Isobe and coworkers,[5,11] we planned a completely different strategy, depicted in Scheme 1. We envisaged two different routes (A and B), having in common the starting material [4-(1-hydroxyethyl)quinoline] and the ring-closure of the enediyne ring at the double bond site. The major difference between routes A and B is that, in the first case, the required 6-carbon atom skeleton of the enediyne moiety is already present on the acyclic precursor **3** or **4**. The ring closure may be realized through two different strategies:



Scheme 1 Retrosynthetic analysis.

1) the base-induced coupling of the bis(propargyl)bromide **3**[12] or 2) the intramolecular pinacol coupling performed on the bis(aldehyde) **4**.[9] On the other hand, following route B, only the acetylenic carbons are present on the acyclic derivative **5** and the remaining two sp^2-carbon unit is joined directly during the coupling reaction.[3,13–15]

The preparation of **3**–**5** requires the introduction of two suitable acetylenic moieties, one at position 2 of the starting quinoline nucleus, and the other in the side chain bonded at C_4. The selection of the most efficient order of introduction of these two triple bonds was not trivial. Initial attempts to transform the formyl group of 2-(quinolin-4-yl)propanal into a terminal triple bond, using several homologation procedures, failed:[16,17] only in one case[16] did we isolate a modest amount of a homologation product, but it was the allene derived from isomerization of the triple bond.

From these preliminary experiments it was clear that the triple bond directly attached at C_2 of the quinoline nucleus should be introduced first, thus transforming the side chain into the second alkyne group at the level of a 1,2-dihydroquinoline system. The regio- and diastereoselective addition of acetylides

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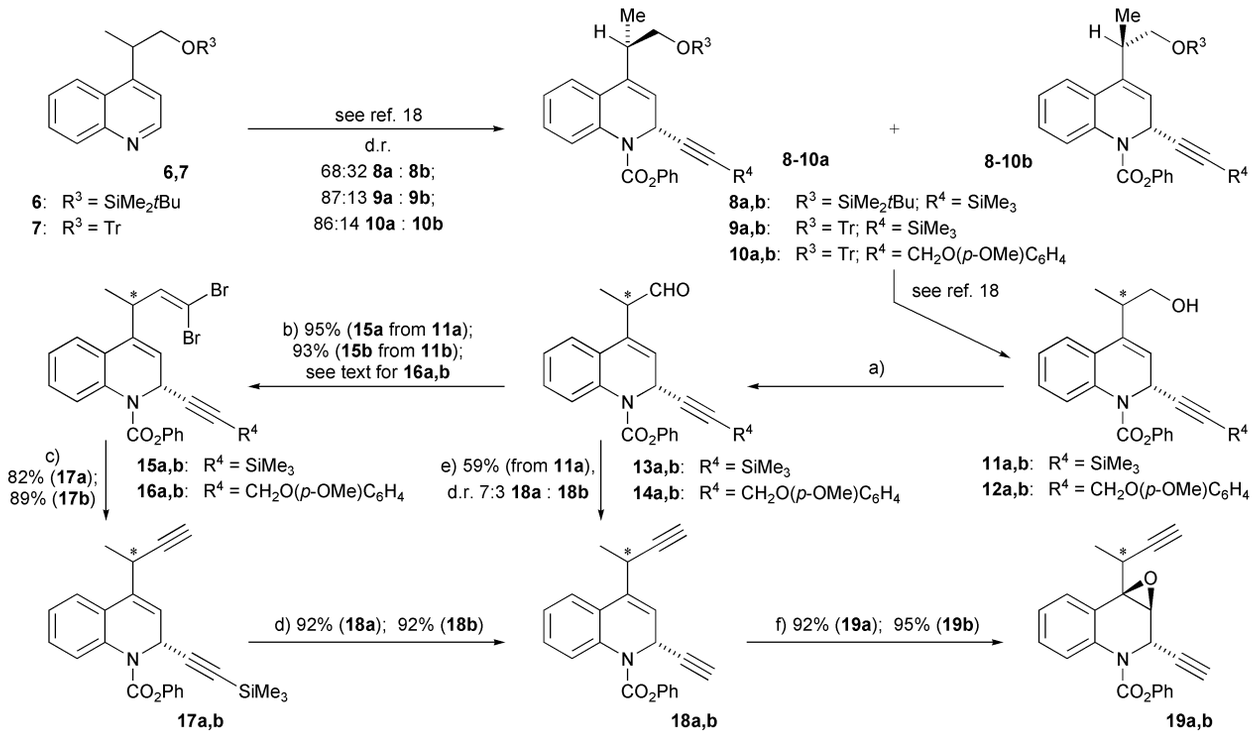


Scheme 2 *Reagents and conditions*: a) $(COCl)_2$, DMSO, iPr$_2$EtN, −78 °C; b) CBr_4, PPh_3, −78 ⟶ −50 °C; c) *n*-BuLi, toluene, −78 °C, argon; d) $NaHCO_3$, MeOH, 60 °C; e) $MeCOCN_2P(O)(OMe)_2$, K_2CO_3, MeOH, 0 °C ⟶ r.t.; f) *m*-CPBA, CH_2Cl_2, 0 °C.

to quinolines **6** or **7** has been thoroughly studied by us and recently reported.[18] Applying this methodology, all derivatives **8**–**10a**,**b** may be prepared with moderate to good stereoselectivity, depending upon the nature of the primary alcohol protecting group.[18]

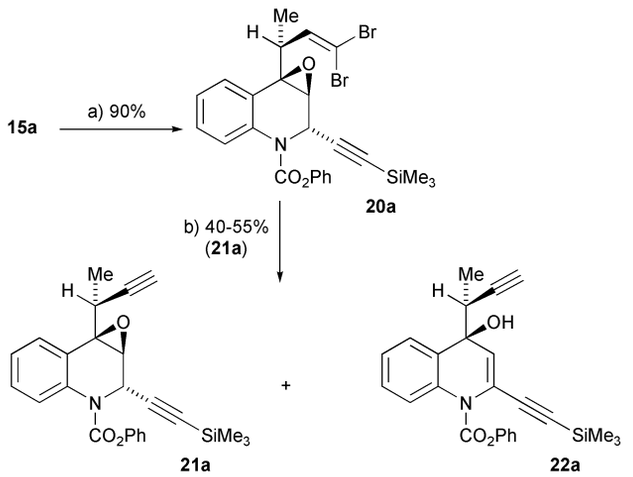
We chose to explore first route A, using **10a**,**b**, in which a protected propargylic hydroxymethyl group is already present, as starting material. After trityl removal under acid conditions, the inseparable diastereomeric mixture of **12a**,**b** was oxidised to the aldehydes **14a**,**b** under slightly modified Swern conditions.[19] However, treatment of the aldehydes with CBr_4/PPh_3, following the Corey–Fuchs protocol,[16] did not furnish the expected dibromoolefins **16a**,**b**, probably because of competitive electrophilic reactions on the highly activated propargylic *p*-methoxybenzyl ether (Scheme 2).

Therefore we decided to introduce later the protected hydroxymethyl group and carried out the same sequence on trimethylsilyl substituted alkynes **11a** or **11b**.[20] In this case the two pure diastereoisomers could be readily separated by chromatography, and were submitted independently to the following transformations. The preparation of the aldehyde was quite troublesome, due to the propensity of these derivatives to undergo epimerisation. While alternative oxidants, like for example Dess–Martin reagent,[21] did not work well, a modified Swern procedure allowed us to suppress epimerisation, provided that a rapid work-up under neutral or slightly acidic conditions was performed and that aldehydes **13a** or **13b** were used without chromatographic purification just after isolation. Initially we tried the direct conversion of the aldehydes into triple bonds, using Ohira's protocol.[17] Under these conditions also the desired *C*-desilylation occurred. Although the overall yield of **18a**,**b** from **11a** was satisfactory (59%), we observed an extended epimerisation: starting from pure **11a**[22] we isolated a 7:3 mixture of **18a** and **18b**. This is probably a consequence of the required basic conditions and of the working temperature, which must be higher than 0 °C in order to allow *in situ* generation of the reagent.

So we turned our attention to the Corey–Fuchs methodology. While the first step, that is the preparation of dibromoolefins **15a** and **15b**, worked well, the following transformation into the corresponding alkynes **17a**,**b** was more problematic, representing probably the most crucial step of the whole synthesis. Initial experiments using the standard conditions (*n*-BuLi) under nitrogen for the dehydrobromination/debromination step furnished unsatisfactory (not higher than 40%!) and poorly reproducible yields. The use of different bases (*t*-BuLi, LDA) gave even worse results. We tried also a two step sequence, passing through the formation of bromoalkyne promoted under mild conditions by sodium bis(trimethylsilylamide) (NaHMDS):[23] however this method led only to partial desilylation of the preexisting alkyne as well as an elimination reaction at positions 1–2 with the consequent formation of quinoline derivatives. This latter experiment indicated that the H in position 2 of dihydroquinoline was rather prone to base-mediated deprotonation reactions.

Suspecting that this might also be the main reason for the unsatisfactory yields of the *n*-BuLi mediated reaction, and hoping that removal of the double bond could make this hydrogen less acidic, we tried to carry out the dehydrobromination step after introduction of the epoxide. We first attempted to prepare the epoxy alcohol by oxidation of **8a**,[24] followed by TBDMS removal. However, deprotection[24] gave the desired epoxy alcohol only in moderate yield. Moreover this compound turned out to be unstable, rapidly forming a by-product which lacked both the epoxide and the hydroxymethyl groups and which possessed an hexocyclic double bond. So we carried out epoxidation of the dibromoalkene **15a** (Scheme 3). This reaction was found to be selective furnishing **20a** in excellent yield. However, treatment of **20a** with *n*-BuLi gave again only a moderate yield of **21a** together with up to 5–10% of a by-product identified as **22a**. The formation of **22a** is once again due to the unsuspected high acidity of the propargylic proton and this was confirmed also by treatment of **20a** with NaHDMS. Actually, instead of the expected bromoalkyne corresponding to **21a**, we isolated only the analogue of **22a**, having the dibromoolefin moiety instead of the terminal triple bond, in 45% yield, thus demonstrating that proton abstraction from the propargylic carbon is preferred to dehydroalogenation.

Due to the difficulties encountered with these alternative routes, we again turned our attention on the *n*-BuLi promoted



Scheme 3 *Reagents and conditions*: a) *m*-CPBA, CH_2Cl_2, 0 °C; b) *n*-BuLi, toluene, −78 °C.

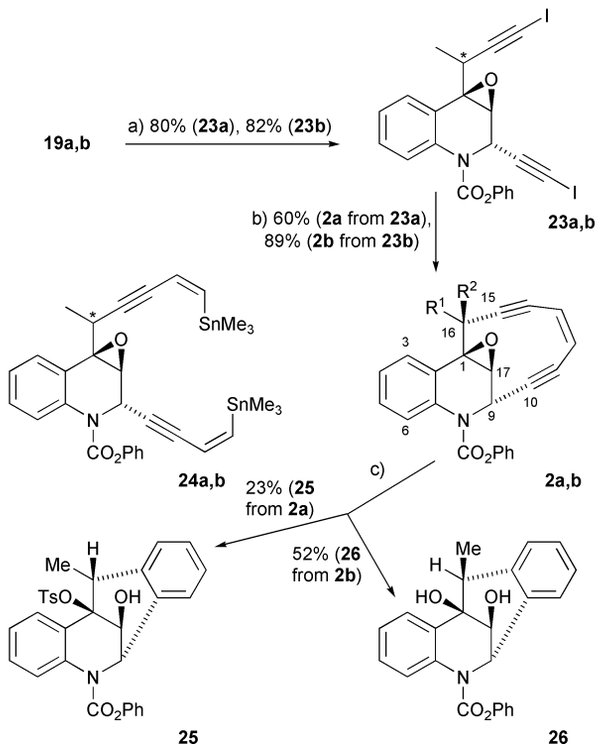
transformation of **15a**,**b** into **17a**,**b** searching for a way to optimize it. We eventually found that careful control of temperature (−78 °C), of the reaction time (15 min), and of the amount of *n*-BuLi (2.2 eq) was beneficial. Most of all, performing the reaction under high purity argon instead of nitrogen, the isolated yield was dramatically increased to 82–89%!

The following *C*-desilylation took place under unusually mild conditions,[25] thus affording **18a** and **18b** with an overall yield of 72% and 76% from **11a** and **11b** respectively. Although this route was longer than Ohira's method, the yield was better and, most importantly, no epimerisation was detected.

Diacetylenic derivatives **18a**,**b** could be suitable for the accomplishment of the synthesis following route A, by the introduction of two hydroxymethyl groups at the alkyne termini. For this purpose we tried several methods. Deprotonation of alkynes with various bases (*n*-BuLi, LDA), followed by trapping of the anions with different electrophiles [2-(trimethylsilyl)ethoxymethyl] chloride (SEM-Cl), (*p*-methoxy)benzyloxymethyl chloride (PMBOM-Cl), ethyl chloroformate] did not succeed. Moreover, when SEM-Cl was used, we isolated a small amount of a new compound in which the electrophile was bonded to the carbon α to nitrogen, thus demonstrating again the acidity of proton in position 2. Also an attempt to treat **20a** with *n*-BuLi and trapping *in situ* the acetylide with PMBOM-Cl failed.

For this reason we finally turned our attention to route B, having in mind to build the cyclic enediyne through a Stille double coupling reaction involving a bis(iodide) and (*Z*)-bis-(trimethylstannyl)ethylene under Pd(0) catalysis. Our initial reluctance toward this strategy was due to the fact that it has been only seldom used for assembling these systems.[3,13–15] More importantly, in the reported examples the reaction was performed only on systems conformationally more rigid than ours. In order to check this strategy we first epoxidised the double bond under standard conditions and, according to a previous report by Isobe,[5] isolated only one diastereoisomer in nearly quantitative yield.

For the Stille coupling we had to transform **19a** and **19b** into the bis(iodides) **23a** and **23b** and the best found conditions employed the *N*-iodosuccinimide/$AgNO_3$ system (Scheme 4), for both stereoisomers.[26] From molecular mechanics calculations[27] we were able to predict **23b** (derived from **8b** or **9b**) as the best suited diastereoisomer for the coupling reaction, owing to the proximity of the iodine–bearing carbons. Actually, for both **23a**,**b** we found about 8 favoured conformations characterised by similar energy; however, the average distance between the two sp hybridised carbons is 5.0–6.0 Å in the conformations of **23a** and only 4.1–4.4 Å in those of **23b**. Moreover, the most favourable conformations of **23b** are those with the hydrogen (bonded at the same carbon as the methyl) directed toward



Scheme 4 *Reagents and conditions*: a) NIS, $AgNO_3$, THF;. r.t., dark; b) (*Z*)-$Me_3SnCH{=}CHSnMe_3$, $Pd(PPh_3)_4$, LiCl, DMF, 70 °C; c) i. *p*-TSA (0.5 M sol. in THF), benzene, cyclohexa-1,4-diene, r.t., 30 min; ii. Et_3N, r.t. 24 h.

the aromatic ring of the bicyclic system, as in the enediyne derivative **2b**, obtained from it.

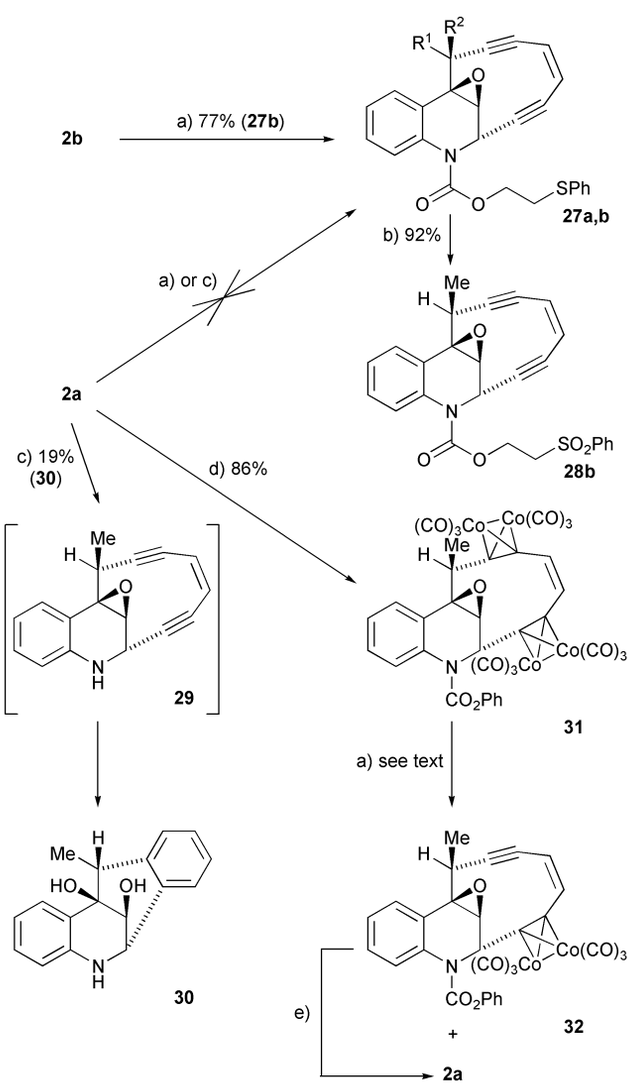
Initially our results for the coupling reaction were disappointing and we had to optimise a series of parameters in order to succeed. In particular a correct choice of the Pd(0) source was important. The *in situ* generation of Pd(0) by reduction of more stable $Pd(OAc)_2$ by means of diphenylphosphinoethane (dppe), did not work. In our hands also tris(dibenzylideneacetone)dipalladium chloroform complex, successfully used for an enediyne ring formation,[13] did not give the coupling reaction. Finally, we found the best catalyst to be tetrakis(triphenylphosphine)palladium but, in contrast with literature data,[28] good results were obtained only when working under the strict exclusion of water and oxygen, with the catalyst weighed in a glove box in an inert atmosphere. Moreover high dilution conditions were important in order to minimise the double attack of the tin reagent molecule to both iodine-bearing carbons. Finally, the addition of LiCl also had a beneficial effect, as previously reported by Nantz.[14] The experimental data confirmed the theoretical predictions. Stereoisomer **23b** gave an excellent 89% overall yield of **2b**, under optimized conditions, and **24b** was never recovered. On the contrary, **2a** was obtained in 46–60% yield, variable amounts of **24a** (16–23%) being always isolated.

In the ^{1}H NMR spectra of **2a** and **2b** some diagnostic signals showed the same trend previously observed for the tricyclic lactones derived from **9a** and **9b**, which allowed the demonstration of their relative stereochemistry.[18] Actually, the chemical shifts of H-16 in **2a** (3.04 ppm *vs.* 3.97 ppm in **2b**) or of the methyl group in **2b** (1.25 ppm *vs*. 1.77 ppm in **2a**) are strongly shifted upfield for isomer **b** since this group is directed toward the aromatic ring, thus providing further evidence for the relative stereochemistry of the adducts **8**–**10a**,**b**.

In order to demonstrate the propensity of enediynes **2a**,**b** to undergo Bergman cyclisation after epoxide opening, we first simply opened the epoxide in the presence of *p*-toluenesulfonic acid.[5,6] This reaction was very fast and was always complete after 30 min; after neutralisation of the acid with triethylamine,[29] the

solution was stirred at r.t. for 24 h in the presence of cyclohexa-1,4-diene as hydrogen atom donor. Then we isolated two different products: monotosylate **25** from **2a** and diol **26** from **2**.[30] We do not have a sure explanation for this divergent behaviour of very similar compounds that differ only by the configuration of one stereocentre, albeit near to the reaction site. However, we feel that two different competing mechanisms, whose relative rates are influenced by the different conformational equilibria in the two epimers, may be operating.

Once sure that the epoxide opening will promote the cycloaromatisation, we turned our attention on the transformation of **2a**,**b** into analogues that may be triggered under physiological conditions. After a literature examination, we decided to remove the phenyl carbamate, which is too stable,[6,31] and to substitute it with a 2-sulfonylethyl carbamate, previously developed by Nicolaou[32–35] and used also by Unno and Isobe.[31] Such compounds are known to undergo a facile β-elimination process at pH 8–8.5. The nucleophilic substitution promoted by 2-phenylthioethylate worked fine on **2b**, giving **27b** in 77% yield (Scheme 5). The latter was then submitted to oxidation with *m*-CPBA to afford target compound **28b** in excellent yield. Unexpectedly, the same sequence could not be reproduced on **2a**. Actually, treatment of **2a** with 2-phenylthioethylate furnished not even traces of **27a**, while the recovery of starting material was low. Nor by switching to milder conditions (2-phenylthioethanol, Cs_2CO_3, 18-crown-6)[32] did we obtain **27a**.



Scheme 5 *Reagents and conditions*: a) $PhS(CH_2)_2OH$, NaH, THF, 0 °C ⟶ r.t.; b) *m*-CPBA, CH_2Cl_2, 0 °C; c) i. $LiAlH_4$, THF, 0 °C; ii. $PhS(CH_2)_2OCOCl$, $NaHCO_3$–H_2O, r.t.; d) $Co_2(CO)_8$, CH_2Cl_2, r.t.; e) Me_3NO, CH_2Cl_2, r.t.

However, we isolated about 10% of **2b** and a certain amount of the symmetric carbonate derived from 2-phenylthioethanol. Most likely, in this case, after expected nucleophilic attack of the alkoxide, for some unclear reasons, the tetrahedric intermediate prefers to eject the N-heterocycle instead of phenoxide, with the formation of an unsymmetrical carbonate, which is finally converted to the symmetrical one by reaction with another molecule of alkoxide. Once deprotected, enediyne **29** may undergo extended decomposition through Bergman cycloaromatization (in the absence of cyclohexa-1,4-diene, the diradical may follow various degradation processes). On the other hand, the basic reaction conditions can also promote the epimerisation responsible for the formation of **2b**. Another possibility is removal of the phenyl carbamate by reduction with $LiAlH_4$,[6] and to trap the resulting amine **29**, without isolation, with 2-phenylthioethyl chloroformate[36] under classical Schotten–Baumann conditions. In this case also, however, the only identified product was the cycloaromatised derivative **30**, thus demonstrating the instability of free amine **29**.

To avoid these undesired reactions we tried to protect the triple bonds as cobalt complexes.[6] Deep green solid **31** was treated with 2-phenylthioethylate and compounds **31**, **2a** and **32** were isolated in 11, 25 and 43% yields, respectively. The structure of **32** was demonstrated by analogy with literature data and confirmed by treatment with Me_3NO, under conditions known to deprotect these complexes.[6] Actually, **32** was completely transformed into **2a**.

Use of 2-phenylthioethyl chloroformate instead of phenyl chloroformate at the beginning of the synthesis, during the diastereoselective addition to **6**,**7**, seems not to be promising on the basis of our experience with this reaction.[18,37] Moreover the compatibility of this group with the following transformation cannot be foreseen in advance. During the preparation of the epoxide the oxidation to sulfone will take place as well, giving a quite unstable product, which has to be submitted to a series of other reactions. At the moment we have not yet solved the problem of preparation of **27a** or **28a**.

Finally, we turned our attention on the activity evaluation of compounds **2a**,**b** and **28b**. For this purpose we incubated compounds **2a**, **2b** and **28b** for 24 h at 37 °C and pH 8.48 with supercoiled pBR322 plasmid (about 90% in form I) (Fig. 1). As expected **2a** and **2b** did not show cleaving activity at all. With **28b**, in contrast, single-strand breaks were clearly evident at concentrations of the enediyne as low as 1 μM. Moreover, double-strand breaks, the most efficient in damaging DNA, were still present in a 5 μM solution of **28b**, and became much more important when the incubation solution was more concentrated. The concentration that afforded equal quantities of forms I and II was about 32 μM. The presence of DNA scissions is most likely ascribed to carbamate removal through a β-elimination reaction, followed by nitrogen-promoted epoxide opening and final cycloaromatisation.

Compound **28b** showed a very promising activity with respect to DNA cleavage. Comparing its activity with other dynemicin analogues this enediyne appears to be one of the

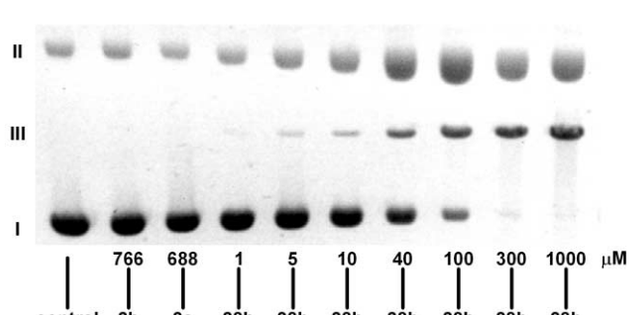


Fig. 1 Results of incubation of compounds **2a**,**b** and **28b** with pBR322 plasmid (67.5 μM bp^{-1}) for 24 h in pH 8.48 buffer at 37 °C.

most active. Interestingly, by analysing structurally related compounds prepared by Nicolaou,[6,32,33,35,38] Isobe[31,39] and Wender[7] it seems that the highest DNA cleavage ability is showed by those compounds bearing a tertiary and not a quaternary carbon in the propargylic position α to the benzylic terminus of the epoxide.[6,7,33,35] Moreover, a hydroxy (and an alkoxy even more) group is usually responsible for a lower activity, while the combination of a quaternary carbon with an alkoxy group represents the worst situation.[31,32,35,38,40]

It's noteworthy that often the dynemicin analogues synthesized so far have a hydroxy or alkoxy group bonded to one propargylic carbon as a consequence of the fact that enediyne ring is formed through a Nozaki reaction. Our derivatives, by contrast, lack this functionality since the cyclic enediyne is built through a Stille coupling. Compound **28b**, having a tertiary carbon and lacking the oxygenated functionality, fits well for the biological activity, as proved also by our experiments.

Conclusion

We have reported here a novel synthesis of dynemicin analogues, characterised by an interesting DNA scission activity. The presence of a consistent double-strand cleavage (about 70 : 30 form II : form III) makes **28b** particularly promising. A possible improvement of the activity is represented by the synthesis of optically active analogues by a chemoenzymatic procedure,[41] having a functionalised group instead of the methyl that will act as a handle for introducing suitable substructures able to give additional interactions with DNA. This strategy will enable us to prepare a series of similar compounds with possibly enhanced activity after the enediyne formation.

Moreover, we are studying different "triggers" that can be more easily introduced into the molecule, in order to promote the epoxide opening through another mechanism.

Our results in this field will be reported in due course.

Experimental

NMR spectra were taken in $CDCl_3$, at 200 MHz (^{1}H), and 50 MHz (^{13}C), using TMS as internal standard. Chemical shifts are reported in ppm (δ scale), coupling constants are reported in Hertz. Peak assignment in ^{1}H NMR spectra was also made with the aid of double resonance experiments. In AB systems, the proton A is considered downfield and B upfield. Peak assignment in ^{13}C spectra was made with the aid of DEPT experiments. GC-MS were carried out on a HP-5971A instrument, using an HP-1 column (12 m long, 0.2 mm wide), electron impact at 70 eV, and a mass temperature of about 170 °C. Unless otherwise indicated analyses were performed with a constant He flow of 0.9 ml min^{-1}, initial temp. 100 °C, initial time 2 min, rate 20 °C min^{-1}, final temp. 260 °C, final time 4 min, initial temp. 250 °C, detector temp. 280 °C. R_t are in min. IR spectra were measured with a Perkin-Elmer 881 instrument as $CHCl_3$ solutions. Melting points were measured on a Büchi 535 apparatus and are uncorrected. TLC analyses were carried out on silica gel plates, which were developed by these detection methods: **A**) UV; **B**) dipping into a solution of $(NH_4)_4MoO_4$·4 H_2O (21 g) and $Ce(SO_4)_2$·4 H_2O (1 g) in H_2SO_4 (31 ml) and H_2O (469 ml) and warming. R_f were measured after an elution of 7–9 cm. Chromatograpy was carried out on 220–400 mesh silica gel (if not otherwise specified) using the "flash" methodology. Petroleum ether (40–60 °C) is abbreviated as PE. In extractive work-up, aqueous solutions were always re-extracted thrice with the appropriate organic solvent. Organic extracts were washed with brine, dried over Na_2SO_4 and filtered, before evaporation of the solvent under reduced pressure. All reactions employing dry solvents were carried out under a nitrogen atmosphere, if not otherwise specified. The purity of all compounds was established by TLC, ^{1}H and ^{13}C-NMR, GC-MS.

4-{(*S)-[(1-Methyl-2-oxo)ethyl]}-2-{(*R**) [(trimethylsilyl)-ethynyl]}-2*H*-quinoline-1-carboxylic acid phenyl ester 13a and its epimer 13b**

A solution of dry DMSO (1.06 ml, 15.04 mmol) in dry CH_2Cl_2 (12 ml) was cooled to −78 °C. A solution of oxalyl chloride (2.22 M in CH_2Cl_2, 4.23 ml, 9.40 mmol) was added, followed, after 10 min, by a solution of **11a** or **11b** (1.52 g, 3.76 mmol) in dry CH_2Cl_2 (18 ml). After additional 10 min, ethyl diisopropylamine (5.89 ml, 33.84 mmol) was added and the resulting slurry was stirred overnight at −78 °C (about 15 h). Quenching with 5% aq $(NH_4)H_2PO_4$ was followed by extractive work-up with ether from pH 7. The combined organic layers were extracted again with $(NH_4)H_2PO_4$, and were finally washed with brine with a small amount of 5% aq $NaHCO_3$ solution added. After drying over Na_2SO_4, the solution was concentrated. The residue was dissolved in ether and dried again over Na_2SO_4. After solvent removal crude aldehyde, as a pale yellow foam, was used as such for the following step. **Characterization of 13a**: R_f 0.33 (toluene/CH_2Cl_2/Et_2O 70:29:1, **A**, **B**); GC-MS R_t 11.14; *m*/*z* 403 (M^+, 10), 403 (10), 374 (6.8), 347 (6.1), 346 (20), 326 (14), 310 (9.2), 282 (8.9), 267 (5.3), 266 (16), 253 (6.0), 252 (16), 208 (8.2), 180 (7.8), 151 (5.3), 144 (10), 77 (28), 75 (9.5), 74 (8.3), 73 (100), 59 (5.3), 51 (6.0), 45 (9.4), 43 (6.6). **Characterization of 13b**: R_f 0.42 (toluene/CH_2Cl_2/Et_2O 70:29:1, **A**, **B**); GC-MS R_t 11.17; *m*/*z* 403 (M^+, 24), 310 (13), 294 (8.5), 292 (6.6), 282 (18), 267 (12), 266 (28), 253 (6.3), 252 (24), 236 (6.2), 208 (9.6), 180 (6.0), 172 (6.7), 151 (5.2), 139 (9.1), 130 (8.6), 83 (7.9), 77 (20), 75 (14), 74 (8.5), 73 (100), 45 (7.9), 43 (6.1).

4-{(*R)-[(3,3-Dibromo-1-methyl)allyl]}-2-{(*R**) [(trimethylsilyl)ethynyl]}-2*H*-quinoline-1-carboxylic acid phenyl ester 15a and its epimer 15b**

A mixture of CBr_4 (2.49 g, 7.52 mmol) and PPh_3 (3.95 g, 15.04 mmol) was cooled to −78 °C and treated with dry CH_2Cl_2 (20 ml) for 10 min. The suspension was then allowed to stir at −20 °C for 10 min and an orange solution was obtained. After cooling again to −78 °C, a solution of crude aldehyde (3.75 mmol) in dry CH_2Cl_2 (18 ml) was added and the mixture was stirred at the same temperature for 20 min. Then the temperature was allowed to raise to −50 °C. After 1.5–2 h the reaction was usually complete and the brown solution was concentrated *in vacuo* and directly purified by chromatography with PE/Et_2O 95:5 ⟶ 8:2 to give 2.00 g of **15a** or 1.96 g of **15b** in 95% and 93% overall yield from **11a** or **11b** respectively as white foams. Both compounds have been crystallised from Et_2O/PE mixtures to give white crystals. **Characterization of 15a**: R_f 0.41 (PE/Et_2O 9:1, **A**, **B**); Mp 117.5–118.3 °C (PE/Et_2O); found: C, 53.4; H, 4.55; N, 2.55. $C_{25}H_{25}Br_2NO_2Si$ requires: C, 53.68; H, 4.50; N, 2.50%; IR: ν_{max} 2964, 2166, 1713, 1382, 1302, 1246, 1018, 842; GC-MS: GC-MS (usual method but with final temp. 290 °C) R_t 12.34; *m*/*z* 482 [M^+(^{79}Br/^{81}Br) −77, 9.5], 466 (6.9), 438 (6.7), 347 (57), 346 (62), 290 (6.2), 233 (7.6), 226 (12), 213 (6.2), 204 (9.1), 180 (10), 151 (7.0), 139 (11), 137 (8.6), 94 (5.9), 77 (34), 75 (8.4), 74 (9.6), 73 (100), 65 (5.3), 51 (7.6), 45 (7.7), 44 (9.3), 43 (5.6), 40 (12); ^{1}H NMR: δ 0.046 [9 H, s, –Si(CH_3)$_3$], 1.37 [3 H, d, >CHCH_3, *J* = 6.8], 3.82 [1 H, centre of m, >C*H*CH$_3$], 5.98 and 5.98 [2 H, AB system, H_2 and H_3, *J* = 7.2], 6.19 [1 H, d, –C*H*=CBr$_2$, *J* = 9.4], 7.17–7.44 [8 H, m, aromatics], 7.74 [1 H, broad d, H_8, *J* = 7.0]; ^{13}C NMR: δ −0.23 [3 C, –Si(*C*H$_3$)$_3$], 17.03 [>CH*C*H$_3$], 38.34 [>*C*HCH$_3$], 45.17 [C_2], 89.00 and 101.15 [2 C, –*C*≡*C*TMS], 89.95 [=*C*Br$_2$], 121.28, 123.60, 124.84, 124.98 and 127.96 [5 C, C_3 and C_{5-8}], 121.58 [2 C, *C ortho* of Ph], 125.68 [*C para* of Ph], 127.15 [C_{4a}], 129.32 [2 C, *C meta* of Ph], 134.35 and 136.88 [2 C, C_4 and C_{8a}], 141.71 [–*C*H=CBr$_2$], 151.06 [*C ipso* of Ph], 151.80 [>*C*O]. **Characterization of 15b**: R_f 0.37 (PE/Et_2O 9:1, **A**, **B**). Mp 152.8–153.2 °C (PE/Et_2O); found: C, 53.8; H, 4.6; N, 2.6. $C_{25}H_{25}Br_2NO_2Si$ requires: C, 53.68; H, 4.50; N, 2.50%; IR: ν_{max} 2966, 2171, 1715, 1383, 1329, 1302, 1193; GC-MS (usual method but with final

temp. 290 °C) R_t 12.54; *m/z* 484 [M^+(^{79}Br/^{81}Br) −77, 5.5], 482 (11), 480 (5.5), 466 (7.3), 347 (17) 346 (62), 290 (5.5), 233 (7.5), 226 (12), 213 (5.9), 204 (8.6), 180 (9.9), 151 (6.8), 139 (13), 137 (11), 77 (38), 75 (6.1), 74 (7.9), 73 (100), 65 (5.6), 51 (6.2), 45 (72), 43 (5.2); ^{1}H NMR: δ 0.099 [9 H, s, –Si(CH_3)$_3$], 1.33 [3 H, d, >CHCH_3, J = 7.0], 3.78 [1 H, centre of m, >CHCH$_3$], 6.00 and 6.00 [2 H, AB system, H_2 and H_3, J = 7.2], 6.55 [1 H, d, –CH=CBr$_2$, J = 8.8], 7.22–7.47 [8 H, m, aromatics], 7.79 [1 H, broad d, H_8, J = 7.0]; ^{13}C NMR: δ −0.22 [3 C, –Si(CH_3)$_3$], 18.85 [>CHCH$_3$], 38.92 [>CHCH$_3$], 45.04 [C_2], 89.20 and 100.99 [2 C, –C≡CTMS], 89.93 [=CBr$_2$], 121.12, 123.19, 124.97, 125.06 and 127.87 [5 C, C_3 and C_{5-8}], 121.57 [2 C, *C ortho* of Ph], 125.72 [*C para* of Ph], 127.37 [C_{4a}], 129.34 [2 C, *C meta* of Ph], 134.26 and 137.08 [2 C, C_4 and C_{8a}], 141.57 [–CH=CBr$_2$], 150.94 [*C ipso* of Ph], 151.81 [>CO].

4-{(*R)-[(1-Methyl)prop-2-ynyl]}-2-{(*R**) [(trimethylsilyl)-ethynyl]}-2*H*-quinoline-1-carboxylic acid phenyl ester 17a and its epimer 17b**

To a solution of **15a** or **15b** (2.07 g, 3.70 mmol) in dry toluene (20 ml) under argon and cooled to −78 °C a solution of *n*-BuLi (5.09 ml, 1.6 M in hexanes, 8.14 mmol) was added dropwise. After 15 min acetic acid (1.6 ml) was added, followed by water (10 ml). After usual extraction with Et_2O the solvent was evaporated and crude product was purified by chromatography with PE/Et_2O 95:5 ⟶ 8:2 to give **17a** (1.21 g, 82%) or **17b** (1.31 g, 89%) as a pale yellow foam. Both compounds have been crystallised from Et_2O/PE mixtures to give white crystals. **Characterization of 17a**: R_f 0.31 (PE/Et_2O 9:1, **A**, **B**); Mp 133.9–134.4 °C (PE/Et_2O); found: C, 74.9; H, 6.4; N, 3.45. $C_{25}H_{25}NO_2Si$ requires: C, 75.15; H, 6.31; N, 3.51%; IR: ν_{max} 3304, 2962, 2170, 1717, 1382, 1328, 1245, 960, 842; GC-MS: R_t 10.69; *m/z* 399 (M^+, 16), 347 (19), 346 (65), 323 (7.8), 322 (29), 307 (5.7), 306 (22), 291 (5.5), 290 (13), 278 (11), 276 (5.4), 262 (6.4), 232 (7.0), 226 (8.6), 217 (5.2), 204 (5.0), 180 (5.8), 151 (6.1), 97 (5.4), 77 (32), 73 (100), 59 (6.5), 53 (6.0), 51 (6.3), 45 (11), 43 (7.4), 39 (5.4); ^{1}H NMR: δ 0.051 [9 H, s, –Si(CH_3)$_3$], 1.58 [3 H, d, >CHCH_3, J = 6.9], 2.18 [1 H, d, –C≡CH, J = 2.5], 3.79 [1 H, broad dq, >CHCH$_3$, J = 2.2, 6.8], 5.99 [1 H, d, H_2, J = 7.0], 6.16 [1 H, dd, H_3, J = 0.9, 6.7], 7.16–7.44 [7 H, m, aromatics], 7.52 [1 H, dd, H_5, J = 1.6, 7.6], 7.76 [1 H, broad d, H_8, J = 7.7]; ^{13}C NMR: δ −0.15 [3 C, –Si(CH_3)$_3$], 20.57 [>CHCH$_3$], 27.32 [>CHCH$_3$], 45.24 [C_2], 70.14 [–C≡CH], 86.11 [–C≡CH], 89.04 and 101.16 [2 C, –C≡CTMS], 121.64, 123.40, 124.68, 124.87 and 127.82 [5 C, C_3 and C_{5-8}], 121.64 [2 C, *C ortho* of Ph], 125.69 [*C para* of Ph], 126.48 [C_{4a}], 129.33 [2 C, *C meta* of Ph], 134.28 and 135.74 [2 C, C_4 and C_{8a}], 150.99 [*C ipso* of Ph], 151.88 [>CO]. **Characterization of 17b**: R_f 0.34 (PE/Et_2O 9:1, **A**, **B**). Mp 161.5–161.9 °C (PE/Et_2O); found: C, 75.4; H, 6.45; N, 3.6. $C_{25}H_{25}NO_2Si$ requires: C, 75.15; H, 6.31; N, 3.51%; IR: ν_{max} 3305, 2962, 2169, 1714, 1381, 1328, 1303, 905; GC-MS: R_t 10.62; *m/z* 399 (M^+, 1.6), 346 (9.6), 306 (6.3), 323 (23), 77 (26), 75 (7.3), 74 (8.8), 73 (100), 59 (6.1), 53 (5.9), 51 (6.0), 45 (11), 43 (6.1); ^{1}H NMR: δ 0.084 [9 H, s, –Si(CH_3)$_3$], 1.45 [3 H, d, >CHCH_3, J = 7.2], 2.42 [1 H, d, –C≡CH, J = 2.5], 3.91 [1 H, broad q, >CHCH$_3$, J = 6.5], 6.01 [1 H, d, H_2, J = 6.6], 6.46 [1 H, d, H_3, J = 6.8], 7.19–7.46 [8 H, m, aromatics], 7.80 [1 H, broad d, H_8, J = 7.0]; ^{13}C NMR: δ −0.20 [3 C, –Si(CH_3)$_3$], 21.30 [>CHCH$_3$], 27.56 [>CHCH$_3$], 45.27 [C_2], 72.14 [–C≡CH], 85.29 [–C≡CH], 88.92 and 100.99 [2 C, –C≡CTMS], 122.20, 123.01, 124.85, 125.06 and 127.80 [5 C, C_3 and C_{5-8}], 121.62 [2 C, *C ortho* of Ph], 125.71 [*C para* of Ph], 126.29 [C_{4a}], 129.35 [2 C, *C meta* of Ph], 134.57 and 135.61 [2 C, C_4 and C_{8a}], 151.03 [*C ipso* of Ph], 151.83 [>CO].

2-[(*R)-Ethynyl]-4-{(*R**)-[(1-methyl)prop-2-ynyl]}-2*H*-quinoline-1-carboxylic acid phenyl ester 18a and its epimer 18b**

Starting from **13a**: a solution of crude aldehyde **13a** (155 μmol), prepared as described above from **11a**, and dimethyl (1-diazo-2-oxopropyl)phosphonate (45 mg, 234 μmol), prepared from dimethyl 2-oxopropylphosphonate sodium anion and tosyl-azide[42] through a literature procedure,[43] in dry methanol (2 ml) was cooled to 0 °C and treated with anhydrous K_2CO_3 (22 mg, 156 μmol). After 30 min at 0 °C the mixture was stirred for 7.5 h at r.t. Quenching with saturated aq NH_4Cl was followed by usual extraction with Et_2O. Crude product was purified by chromatography with PE/Et_2O 7:3, to give **18a**,**b** (30 mg, 59% from **11a**) as a 7:3 diastereomeric mixture, as determined by ^{1}H NMR.

Starting from **17a**,**b**: a suspension of **17a** or **17b** (1.15 g, 2.88 mmol) was dissolved in dry methanol (22 ml), treated with $NaHCO_3$ (967 mg, 11.51 mmol) and stirred at 60 °C for 2 h. The mixture was concentrated under vacuum, partitioned between H_2O/Et_2O and extracted as usual. Chromatography with PE/Et_2O 95:5 ⟶ 7:3 gave **18a** or **18b** (867 mg, 92% yield for both) as a pale yellow oil. Both compounds have been crystallised from Et_2O/PE mixtures to give white crystals. **Characterization of 18a**: R_f 0.39 (PE/Et_2O 8:2, **A**, **B**); Mp 138.2–138.7 °C (PE/Et_2O); found: C, 80.95; H, 5.25; N, 4.35. $C_{22}H_{17}NO_2$ requires: C, 80.71; H, 5.23; N, 4.28%; IR: ν_{max} 3302, 2999, 2391, 1719, 1384, 1330, 1302, 1187; GC-MS: R_t 10.09; *m/z* 327 (M^+, 27), 326 (9.2), 283 (7.0), 282 (18), 274 (52), 250 (100), 234 (8.1), 230 (17), 219 (19), 216 (9.8), 206 (34), 205 (21), 204 (40), 192 (17), 191 (56), 190 (41), 189 (18), 180 (13), 179 (7.8), 178 (13), 168 (12), 166 (8.8), 165 (14), 164 (12), 163 (11), 154 (24), 153 (7.1), 152 (10), 139 (8.2), 128 (9.0), 127 (8.3), 77 (49), 65 (16), 63 (10), 53 (7.1), 51 (19.2), 39 (16); ^{1}H NMR: δ 1.58 [3 H, d, >CHCH_3, J = 7.0], 2.19 and 2.24 [2 H, 2d, 2 –C≡CH, J = 2.5 and 2.4], 3.79 [1 H, broad dq, >CHCH$_3$, J = 2.0, 6.5], 6.02 [1 H, dd, H_2, J = 2.3, 6.7], 6.18 [1 H, dd, H_3, J = 1.1, 6.7], 7.17–7.44 [7 H, m, aromatics], 7.55 [1 H, dd, H_5, J = 1.7, 7.7], 7.78 [1 H, broad d, H_8, J = 7.7]; ^{13}C NMR: δ 20.51 [>CHCH$_3$], 27.33 [>CHCH$_3$], 44.23 [C_2], 70.26 and 71.99 [2 C, 2 –C≡CH], 79.83 and 85.93 [2 C, 2 –C≡CH], 121.07, 123.64, 124.78, 124.92 and 128.12 [5 C, C_3 and C_{5-8}], 121.60 [2 C, *C ortho* of Ph], 125.80 [*C para* of Ph], 126.34 [C_{4a}], 129.40 [2 C, *C meta* of Ph], 134.16 and 136.32 [2 C, C_4 and C_{8a}], 150.94 [*C ipso* of Ph], 151.87 [>CO]. **Characterization of 18b**: R_f 0.39 (PE/Et_2O 8:2, **A**, **B**). Mp 171.4–171.7 °C (PE/Et_2O); found: C, 80.45; H, 5.35; N, 4.2. $C_{22}H_{17}NO_2$ requires: C, 80.71; H, 5.23; N, 4.28%; IR: ν_{max} 3303, 2984, 2116, 1722, 1382, 1328, 1301, 1262, 1189, 1024; GC-MS: R_t 10.36; *m/z* 327 (M^+, 22), 282 (17), 275 (8.4), 274 (40), 251 (16), 250 (88), 230 (18), 219 (17), 216 (9.0), 206 (36), 205 (22), 204 (42), 192 (18), 191 (63), 190 (45), 180 (15), 179 (9.6), 178 (16), 168 (12), 165 (18), 164 (15), 163 (15), 154 (30), 153 (10), 152 (15), 151 (24), 139 (13), 128 (15), 127 (12), 126 (11), 115 (10), 89 (11), 78 (9.1), 77 (100), 76 (12), 75 (15), 65 (49), 63 (25), 53 (19), 51 (55), 50 (15), 39 (60); ^{1}H NMR: δ 1.43 [3 H, d, >CHCH_3, J = 7.1], 2.24 and 2.39 [2 H, 2d, 2 –C≡CH, J = 2.3 and 2.3], 3.89 [1 H, broad q, >CHCH$_3$, J = 7.2], 6.01 [1 H, dd, H_2, J = 2.3, 6.7], 6.46 [1 H, dd, H_3, J = 1.3, 6.7], 7.18–7.44 [8 H, m, aromatics], 7.79 [1 H, broad d, H_8, J = 7.8]; ^{13}C NMR: δ 21.21 [>CHCH$_3$], 27.47 [>CHCH$_3$], 44.23 [C_2], 72.02 and 72.29 [2 C, 2 –C≡CH], 79.64 and 85.07 [2 C, 2 –C≡CH], 121.58, 123.13, 124.92, 125.04 and 128.06 [5 C, C_3 and C_{5-8}], 121.54 [2 C, *C ortho* of Ph], 125.79 [*C para* of Ph], 125.99 [C_{4a}], 129.37 [2 C, *C meta* of Ph], 134.31 and 136.04 [2 C, C_4 and C_{8a}], 150.85 [*C ipso* of Ph], 151.77 [>CO].

(2*S,3*S**,4*R**)-3,4-Epoxy-2-ethynyl-4-{(*R**)-[(1-methyl)prop-2-ynyl]}-2*H*-quinoline-1-carboxylic acid phenyl ester 19a and its epimer 19b**

A solution of **18a** or **18b** (778 mg, 2.38 mmol) in dry CH_2Cl_2 (15 ml) was cooled to 0 °C and treated with *m*-CPBA (≈ 80%, 2.05 g, 9.51 mmol). After 4–6 h the reaction is usually complete. Excess *m*-CPBA was removed by treatment with Me_2S (698 μl, 9.51 mmol) at 0 °C. Saturated aq $NaHCO_3$ solution was added (25 ml) and the biphasic system was vigorously stirred at r.t. for

15 min. Usual work-up with Et_2O, followed by chromatography with PE/Et_2O 9:1 ⟶ 1:1 gave **19a** (751 mg, 92%) or **19b** (775 mg, 95%) as a pale yellow foam. Both compounds have been crystallised from Et_2O/PE mixtures to give white crystals. **Characterization of 19a**: R_f 0.45 (toluene/CH_2Cl_2/Et_2O 90:5:5, **A**, **B**); Mp 138.2–138.7 °C (PE/Et_2O); found: C, 76.5; H, 5.05; N, 4.15. $C_{22}H_{17}NO_3$ requires: C, 76.95; H, 4.99; N, 4.08%; IR: ν_{max} 3304, 3008, 2124, 1722, 1381, 1323, 1287, 1025; GC-MS: R_t 10.46; *m/z* 343 (M^+, 64), 290 (12), 250 (33), 222 (18), 207 (11), 206 (18), 205 (27), 204 (61), 194 (38), 193 (16), 192 (23), 191 (69), 190 (20), 183 (11), 180 (16), 179 (18), 178 (33), 170 (12), 167 (16), 166 (12), 165 (17), 156 (11), 155 (15), 154 (69), 152 (15), 146 (15), 141 (17), 140 (24), 128 (22), 127 (18), 115 (23), 90 (20), 89 (11), 77 (100), 65 (26), 63 (18), 53 (39), 51 (52), 50 (11), 39 (36); ^{1}H NMR: δ 1.48 [3 H, d, >CHCH_3, J = 7.0], 2.30 [2 H, d, 2 –C≡CH, J = 2.6], 3.65 [1 H, broad dq, >CHCH$_3$, J = 2.4, 6.7], 4.10 [1 H, d, H_3, J = 3.0], 5.92 [1 H, t, H_2, J = 2.6], 7.11–7.43 [7 H, m, aromatics], 7.59 [1 H, broad d, H_5 or H_8, J = 7.8], 7.80 [1 H, dd, H_5 or H_8, J = 1.2, 8.0]; ^{13}C NMR: δ 18.28 [>CHCH$_3$], 28.05 [>CHCH$_3$], 43.75 [C_2], 56.91 [C_4], 63.46 [C_3], 71.67 and 74.21 [2 C, 2 –C≡CH], 77.35 and 82.70 [2 C, 2 –C≡CH], 121.47, 125.60, 125.69, 127.33, 128.63 and 129.27 [9 C, aromatic CH],[44] 125.50 [C_{4a}], 135.11 [C_{8a}], 150.87 [*C ipso* of Ph], 153.40 [>CO].[45] **Characterization of 19b**: R_f 0.52 (toluene/CH_2Cl_2/Et_2O 90:5:5, **A**, **B**); Mp 101.5–101.8 °C (PE/Et_2O); found: C, 76.55; H, 4.85; N, 4.2. $C_{22}H_{17}NO_3$ requires: C, 76.95; H, 4.99; N, 4.08%; IR: ν_{max} 3304, 2995, 2423, 1722, 1381, 1324, 1122, 1024. GC-MS: R_t 10.42; *m/z* 343 (M^+, 78), 343 (78), 250 (51), 222 (22), 207 (16), 206 (26), 205 (39), 204 (94), 194 (54), 193 (22), 192 (32), 191 (100), 183 (14), 180 (21), 179 (24), 178 (48), 167 (22), 166 (16), 165 (22), 155 (20), 154 (97), 152 (20), 146 (20), 141 (21), 140 (30), 128 (28), 127 (23), 115 (27), 90 (21), 77 (98), 65 (24), 63 (16), 53 (33), 51 (40), 39 (24); ^{1}H NMR: δ 1.46 [3 H, d, >CHCH_3, J = 7.1], 2.20 and 2.26 [2 H, 2 d, 2 –C≡CH, J = 2.2 and 2.5], 3.46 [1 H, centre of m, >CHCH$_3$], 4.10 [1 H, d, H_3, J = 2.6], 5.89 [1 H, t, H_2, J = 2.6], 7.12–7.43 [7 H, m, aromatics], 7.57 [1 H, broad d, H_5 or H_8, J = 7.7], 7.84 [1 H, dd, H_5 or H_8, J = 7.7]; ^{13}C NMR: δ 16.40 [>CHCH$_3$], 27.82 [>CHCH$_3$],[45] 43.79 [C_2], 57.54 [C_4], 64.47 [C_3], 72.09 and 74.22 [2 C, 2 –C≡CH], 77.19 and 83.68 [2 C, 2 –C≡CH], 121.48, 125.68, 125.71, 127.42, 127.77, 128.65 and 129.30 [9 C, aromatic CH], 125.99 [C_{4a}], 135.07 [C_{8a}], 150.93 [*C ipso* of Ph], 153.40 [>CO].[45]

(2*S**,3*S**,4*R**)-4-{(*R**)-[(3,3-Dibromo-1-methyl)allyl]}-3,4-epoxy-2-[(trimethylsilyl)ethynyl]}-2*H*-quinoline-1-carboxylic acid phenyl ester 20a

The same procedure described for the preparation of **19a**,**b** was followed, starting from **15a** (435 mg, 778 μmol) to give **20a** (403 mg, 90%) as an ivory foam after chromatography with PE/Et_2O 95:5 ⟶ 9:1. Crystallization from Et_2O/PE gave **20a** as a white solid. R_f 0.31 (PE/Et_2O 9:1, **A**, **B**); Mp 152.6–153.0 °C (PE/Et_2O); found: C, 52.45; H, 4.35; N, 2.5. $C_{25}H_{25}Br_2NO_3Si$ requires: C, 52.19; H, 4.38; N, 2.43%; IR: ν_{max} 3005, 2180, 1721, 1375, 1322, 1286, 899; GC-MS (usual method but with final temp. 290 °C) R_t 12.84; *m/z* 575 [M^+(^{79}Br/^{81}Br), 2.7], 482 (12), 374 (34), 363 (15), 362 (38), 334 (18), 315 (11), 263 (12), 226 (22), 242 (15), 234 (10), 215 (16), 213 (30), 211 (18), 196 (10), 183 (18), 180 (18), 168 (26), 167 (10), 154 (14), 146 (13), 139 (14), 133 (12), 131 (12), 97 (17), 83 (83), 77 (63), 75 (10), 73 (100), 65 (11), 51 (13); ^{1}H NMR: δ 0.029 [9 H, s, –Si(CH_3)$_3$], 1.22 [3 H, d, >CHCH_3, J = 6.8], 3.66 [1 H, dq, >CHCH$_3$, J = 6.6, 8.8], 3.96 [1 H, d, H_3, J = 3.4], 5.84 [1 H, d, H_2, J = 3.0], 6.50 [1 H, d, –CH=CBr$_2$, J = 8.8], 7.13–7.43 [8 H, m, aromatics], 7.56 [1 H, dd, H_5 or H_8, J = 1.0, 7.6]; ^{13}C NMR: δ −0.43 [3C, –Si(CH_3)$_3$], 15.53 [>CHCH$_3$], 37.74 [>CHCH$_3$] 44.63 [C_2], 57.98 [C_4], 63.83 [C_3], 90.99, 91.84, 98.61 [3C, –C≡CTMS and = CBr$_2$], 121.51, 125.64, 125.84, 127.11, 127.56, 128.51, 129.28 [9 C, aromatic CH], 126.82 [C_{4a}], 135.33 [C_{8a}], 138.30 [–CH=CBr$_2$], 151.01 [*C ipso* of Ph], 153.39 [>CO].[45]

(2*S**,3*S**,4*R**)-3,4-Epoxy-4-{(*R**)-[(1-methyl)prop-2-ynyl]}-2-[(trimethylsilyl)ethynyl]}-2*H*-quinoline-1-carboxylic acid phenyl ester 21a

The same procedure described for the preparation of **17a**,**b**, but working under nitrogen, was followed, starting from **20a** (104 mg, 180 μmol) to give **21a** in 40–45% yield as a yellow oil after chromatography with PE/Et_2O 9:1 ⟶ 85:15. R_f 0.45 (PE/Et_2O 7:3, **A**, **B**); found: C, 72.45; H, 6.10; N, 3.25. $C_{25}H_{25}NO_3Si$ requires: C, 72.36; H, 6.06; N, 3.37%; IR: ν_{max} 3306, 2960, 2178, 1720, 1379, 1323, 1244, 1015; GC-MS: R_t 11.64; *m/z* 415 (M^+, 11), 362 (8.3), 334 (5.3), 322 (19), 278 (5.7), 168 (5.4), 156 (9.8), 154 (19), 146 (6.2), 97 (12), 83 (6.8), 77 30), 75 (8.8), 71 (8.3), 73 (100), 65 (5.1), 59 (7.6), 53 (9.3), 51 (6.9), 45 (8.1), 43 (7.6), 39 (5.3); ^{1}H NMR: δ 0.011 [9 H, s, –Si(CH_3)$_3$], 1.48 [3 H, d, >CHCH_3, J = 6.9], 2.22 [1 H, d, –C≡CH, J = 2.4], 3.68 [1 H, broad dq, >CHCH$_3$, J = 2.2, 6.9], 4.09 [1 H, d, H_3, J = 3.0], 5.85 [1 H, d, H_2, J = 3.0], 7.11–7.42 [7 H, m, aromatics], 7.57 [1 H, broad d, H_5 or H_8, J = 8.0], 7.64 [1 H, dd, H_5 or H_8, J = 1.1, 8.0]; ^{13}C NMR: δ −0.44 [3C, –Si(CH_3)$_3$], 18.47 [>CHCH$_3$], 28.10 [>CHCH$_3$], 44.52 [C_2], 56.78 [C_4], 63.52 [C_3], 71.53 [–C≡CH], 82.62 [–C≡CH], 91.86 and 98.78 [2 C, –C≡CTMS], 121.49, 125.39, 125.59, 127.05, 127.52 128.40 and 129.23 [10 C, aromatic CH and C_{4a}],[44] 135.43 [C_{8a}], 150.97 [*C ipso* of Ph], 153.20 [>CO].[45]

(2*S**,3*S**,4*R**)-3,4-Epoxy-2-iodoethynyl-4-{(*R**)-[(3-iodo-1-methyl)prop-2-ynyl]}-2*H*-quinoline-1-carboxylic acid phenyl ester 23a and its epimer 23b

To a solution of **19a** or **19b** (823 mg, 2.40 mmol) in dry THF (20 ml) silver nitrate (41 mg, 240 μmol) and *N*-iodosuccinimide (1,32 g, 5.75 mmol) were added at r.t. and the suspension was stirred in the dark for 1.5 h. After filtration the solution was partitioned between water and ether and extracted as usual. Chromatography with PE/Et_2O 100:0 ⟶ 0:100 gave desired **23a** (204 mg, 80%) or **23b** (210 mg, 82%) as yellow foams. Both compounds have been crystallised from iPr$_2$O/PE/CH_2Cl_2 mixtures to give pale yellow crystals. **Characterization of 23a**: R_f 0.46 (toluene/CH_2Cl_2/Et_2O 70:29:1, **A**, **B**); Mp 125.8–126.4 °C (dec.) (iPr$_2$O/PE/CH_2Cl_2); IR: ν_{max} 2928, 2194, 1722, 1378, 1324, 1289, 907; GC-MS: unsuitable for this analysis; ^{1}H NMR: δ 1.46 [3 H, d, >CHCH_3, J = 7.0], 3.80 [1 H, q, >CHCH$_3$, J = 6.9], 4.06 [1 H, d, H_3, J = 2.9], 6.04 [1 H, d, H_2, J = 2.9], 7.10–7.42 [7 H, m, aromatics], 7.58 [1 H, broad d, H_5 or H_8, J = 8.0], 7.64 [1 H, dd, H_5 or H_8, J = 1.3, 7.7]; ^{13}C NMR: δ 14.14 and 14.20 [2 C, 2 –C≡CI], 18.30 [>CHCH$_3$], 30.24 [>CHCH$_3$], 45.53 [C_2], 57.09 [C_4], 63.52 [C_3], 87.75 and 92.63 [2 C, 2 –C≡CI], 121.53, 125.31, 125.68, 125.78, 127.31, 128.73, and 129.34 [10 C, aromatic CH and C_{4a}],[44] 135.02 [C_{8a}], 150.91 [*C ipso* of Ph], 153.10 [>CO].[45] **Characterization of 23b**: R_f 0.49 (toluene/CH_2Cl_2/Et_2O 70:29:1, **A**, **B**); Mp 96.6–97.0 °C (dec.) (iPr$_2$O/PE/CH_2Cl_2); IR: ν_{max} 3007, 2394, 2192, 1721, 1379, 1324, 1187, 917; GC-MS: unsuitable for this analysis; ^{1}H NMR: δ 1.43 [3 H, d, >CHCH_3, J = 7.1], 3.60 [1 H, broad q, >CHCH$_3$, J = 7.2], 4.30 [1 H, d, H_3, J = 2.6], 6.01 [1 H, d, H_2, J = 2.9], 7.11–7.43 [7 H, m, aromatics], 7.57 [1 H, broad d, H_5 or H_8, J = 8.1], 7.78 [1 H, broad d, H_5 or H_8, J = 7.3]; ^{13}C NMR: δ 14.06 and 14.11 [2 C, 2 –C≡CI], 16.32 [>CHCH$_3$], 29.99 [>CHCH$_3$], 45.56 [C_2], 57.69 [C_4], 64.35 [C_3], 87.49 and 92.62 [2 C, 2 –C≡CI], 121.46, 125.72, 127.32, 127.69, 128.69, and 129.30 [10 C, aromatic CH and C_{4a}],[44] 134.94 [C_{8a}], 150.89 [*C ipso* of Ph], 153.18 [>CO].[45]

(1*R**,9*S**,16*R**,17*S**)-1,17-Epoxy-16-methyl-8-phenoxycarbonyl-8-azatricyclo[7.7.1.0^{2,7}]heptadeca-2(3),4,6,12-tetraene-10,14-diyne 2a and its epimer 2b

Bis(iodide) **23a** or **23b** (261 mg, 438 μmol) was poured into a two necked flask equipped with an addition funnel and dissolved in dry DMF (34 ml). All the apparatus was carefully put under an argon atmosphere and kept in dark. Then a solution

of (*Z*)-bis(trimethylstannyl)ethylene [91%, 171 μl, 658 μmol, prepared bubbling acetylene into a dioxane solution of hexamethylditin in the presence of $Pd(Ph_3)_4$[46]] in DMF (30 ml) was transferred into the addition funnel. Bubbling of argon for 30 min into both solutions of reactants was performed using two different needles. Then $Pd(PPh_3)_4$ (61 mg, 52.8 μmol), previously weighed in a glove box under nitrogen, was added to **23a** or **23b** and the resulting slurry was stirred at r.t. for 10 min; finally, dry LiCl (45 mg, 1.06 mmol) was added in the flask and then it was warmed to 70 °C. A slow addition of the tin reagent was then performed over a period of about 2 h.[47] 15 min after the end of the addition the reaction was usually complete. The mixture was poured into crushed ice and extracted as usual with Et_2O performing, if necessary, a filtration over a celite pad. Crude products were usually purified twice by chromatography, using PE/Et_2O 100:0 ⟶ 1:1, to give **2a** (97 mg, 60%) or **2b** (144 mg, 89%) as very pale yellow foams. Compound **2a** was then crystallised from Et_2O to give white crystals. Enediyne **2a** was obtained with a variable yield (46–60%), the other main isolated product being compound **24a** (16–23%) as a pale yellow oil. **Characterization of 2a**: R_f 0.46 (toluene/CH_2Cl_2/Et_2O 70:29:1, **A**, **B**); Mp decomposes at 188 °C without melting (Et_2O); found: C, 78.35; H, 4.75; N, 3.75. $C_{24}H_{17}NO_3$ requires: C, 78.46; H, 4.66; N, 3.81%; IR: ν_{max} 3004, 2968, 2180, 1722, 1319, 1240, 1107, 922; GC-MS: R_t 13.46; *m/z* 367 (M^+, 23), 339 (23), 338 (11), 274 (42), 246 (29), 244 (12), 231 (14), 230 (34), 229 (39), 228 (77), 227 (11), 218 (57), 217 (78), 216 (55), 215 (66), 214 (21), 204 (25), 203 (47), 202 (78), 201 (20), 191 (16), 190 (21), 189 (36), 176 (12), 152 (11), 146 (33), 128 (12), 115 (11), 102 (12), 101 (11), 94 (11), 90 (34), 77 (100), 76 (13), 75 (10), 65 (26), 63 (23), 51 (38), 50 (11), 39 (24); ^{1}H NMR: δ 1.77 [3 H, d, >CHCH_3, J = 7.4], 3.04 [1 H, broad q, H_{16}, J = 7.2], 3.89 [1 H, d, H_{17}, J = 3.0], 5.65 [1 H, dt, H_{12}, J = 1.5, 10.2], 5.81 [1 H, d, H_{13}, J = 10.0], 5.89 [1 H, dd, H_9, J = 1.8, 3.0], 7.14–7.42 [7 H, m, aromatics], 7.56 [1 H, broad d, H_3 or H_6, J = 7.8], 7.92 [1 H, dd, H_3 or H_6, J = 1.8, 8.2]; ^{13}C NMR: δ 15.19 [>CHCH$_3$], 37.42 [C_{16}], 45.98 [C_9], 60.00 [C_1], 70.07 and 70.16 [C_{17}],[48] 87.80, 91.03, 92.04 and 102.68 [4 C, –C≡C–], 121.40, 125.25, 127.05, 128.24, 129.01, and 129.16 [6 C, $C_{3–6}$ and $C_{12–13}$], 121.51 [2 C, *C ortho* of Ph], 125.31 [C_2], 125.74 [*C para* of Ph], 129.35 [2 C, *C meta* of Ph], 135.79 [C_7], 150.97 [*C ipso* of Ph], 153.10 [>CO].[45] **Characterization of 2b**: R_f 0.49 (toluene/CH_2Cl_2/Et_2O 70:29:1, **A**, **B**); found: C, 78.30; H, 4.60; N, 3.9. $C_{24}H_{17}NO_3$ requires: C, 78.46; H, 4.66; N, 3.81%; IR: ν_{max} 3007, 2200, 1720, 1380, 1302, 1281, 1185; GC-MS: R_t 13.65; *m/z* 367 (M^+, 51), 339 (11), 338 (15), 274 (42), 246 (28), 231 (12), 230 (20), 229 (32), 228 (61), 227 (11), 219 (12), 218 (57), 217 (71), 216 (44), 215 (47), 214 (15), 204 (19), 203 (35), 202 (63), 201 (15), 191 (14), 190 (18), 189 (28), 176 (9.5), 146 (29), 115 (9.0), 90 (20), 78 (9.0), 77 (100), 75 (10), 65 (18), 63 (20), 51 (30), 39 (28); ^{1}H NMR: δ 1.25 [3 H, d, >CHCH_3, J = 7.0], 3.97 [1 H, broad q, H_{16}, J = 7.0], 4.17 [1 H, d, H_{17}, J = 2.8], 5.66 [1 H, dd, H_{12}, J = 1.4, 10.0], 5.80 [1 H, dd, H_{13}, J = 1.5, 10.0], 5.96 [1 H, dd, H_9, J = 1.7, 2.7], 7.12–7.41 [7 H, m, aromatics], 7.57 [1 H, broad d, H_3 or H_6, J = 8.2], 7.70 [1 H, dd, H_3 or H_6, J = 1.8, 8.0]; ^{13}C NMR: δ 12.07 [>CHCH$_3$], 26.90 [C_{16}], 45.47 [C_9], 58.92 [C_1], 62.63 and 62.74 [C_{17}],[48] 85.88, 90.82, 92.43 and 103.36 [4 C, –C≡C–], 121.51 [2 C, *C ortho* of Ph], 121.80, 125.66, 126.83, 127.01, 128.73 and 129.38 [9 C, C_2, $C_{3–6}$, $C_{12–13}$ and *C meta* of Ph], 125.78 [*C para* of Ph], 135.07 [C_7], 150.98 [*C ipso* of Ph], 153.21 [>CO].[45] **Characterization of 24a**: R_f 0.34 (PE/Et_2O 9:1, **A**, **B**); found: C, 53.10; H, 5.25; N, 1.9. $C_{32}H_{37}NO_3Sn_2$ requires: C, 53.30; H, 5.17; N, 1.94%; GC-MS: unsuitable for this analysis; ^{1}H NMR: δ 0.10 and 0.22 [18 H, 2 s, 2 –Sn(CH_3)$_3$], 1.44 [3 H, d, >CHCH_3, J = 7.0], 3.75 [1 H, broad q, >CHCH$_3$, J = 7.0], 4.04 [1 H, d, H_3, J = 2.8], 6.06 [1 H, broad t, H_2, J = 2.4], 6.27–6.61 [2 H, m, –CH=CH–] 7.12–7.40 [7 H, m, aromatics], 7.57 [1 H, broad d, H_5 or H_8, J = 8.0], 7.74 [1 H, broad d, H_5 or H_8, J = 7.8]; ^{13}C NMR: δ 9.18 and 8.99 [6 C, 2 –Sn(CH$_3$)$_3$], 17.78 [>CHCH$_3$], 28.58 [>CHCH$_3$], 44.60 [C_2], 57.51 [C_4], 63.66 [C_3], 83.70, 84.43, 86.16 and 89.76 [4 C, –C≡C–], 121.57 [2 C, *C ortho* of Ph], 125.40, 126.61, 127.70 and 128.54 [4 C, $C_{5–8}$], 125.56 [C_{4a}], 125.68 [*C para* of Ph], 129.33 [2 C, *C meta* of Ph], 135.11 [C_{8a}], 146.50 and 148.90 [4 C, –CH = CH–], 150.06 [*C ipso* of Ph], 153.30 [>CO].[45]

(1*R**,9*S**,16*R**,17*S**)-17-Hydroxy-16-methyl-8-phenoxycarbonyl-1-(*p*-toluenesulfonyloxy)-8-azatetracyclo[7.7.1.0^{2,7}.0^{10,15}]-heptadeca-2(3),4,6,10(11),12,14-esaene 25

A solution of **2b** (13.5 mg, 36.7 μmol) in dry benzene (850 μl) was treated, at r.t., with cyclohexa-1,4-diene (150 μl) and *p*-TSA (0.5 M in THF, 77 μl, 38.6 μmol). After 30 min triethylamine (54 μl, 38.6 μmol) was added and the pale pink suspension was stirred again for 24 h. The mixture was partitioned between Et_2O and 5% aq $NaHCO_3$ and extracted as usual. Crude product was purified by preparative TLC, using PE/Et_2O 3:7 as eluent, to give **25** (4.5 mg, 23%) as an oil. R_f 0.37 (PE/Et_2O 3:7, **A**, **B**); GC-MS: R_t 13.29; *m/z* 370 (M^+ −171, 14), 369 (56), 352 (5.1), 340 (14), 261 (11), 249 (17), 248 (100), 247 (53), 246 (67), 234 (5.9), 233 (40), 232 (16), 231 (5.3), 230 (10), 228 (6.2), 220 (16), 218 (14), 217 (12), 216 (5.3), 215 (5.3), 205 (13), 204 (35), 203 (9.8), 202 (7.4), 176 (5.7), 132 (7.9), 128 (5.7), 115 (7.2), 94 (7.7), 91 (6.8), 77 (30), 65 (9.0), 51 (7.8), 40 (5.6), 39 (8.0), 65 (7.6), 63 (6.8), 51 (11), 39 (7.3); ^{1}H NMR: δ 1.51 [3 H, d, >CHCH_3, J = 7.2], 2.49 [3 H, s, CH_3 of Ts], 4.01 [1 H, d (became a s after exchange with D_2O), –OH, J = 4.0], 4.33 [1 H, broad q, H_{16}, J = 6.7], 5.08 [1 H, t, H_{17}, J = 4.2], 6.27 [1 H, d, H_9, J = 5.2], 6.92–7.73 [15 H, m, aromatics], 7.94 [2 H, appar. d, *H ortho* to S, J = 8.6]; ^{13}C NMR: δ 13.73 [>CHCH$_3$], 21.76 [CH$_3$ of Ts], 43.99 [C_{16}], 60.04 [C_9], 67.12 [C_{17}], 95.02 [C_1], 121.78 [2 C, *C ortho* of Ph], 123.13, 123.62, 129.01 and 129.70 [4 C, $C_{3–6}$], 125.75 [*C para* of Ph], 126.14 [2 C, C_{12} and C_{13}], 127.43 [2 C, *C ortho* to S], 128.66 [2 C, C_{11} and C_{14}], 129.45 [2 C, *C meta* of Ph], 129.94 [2 C, *C meta* to S], 132.72 [2 C, C_2 and –SO_2C], 138.73 [C_7], 145.32 [3 C, C_{10}, C_{15} and CH_3C of Ts], 151.22 [*C ipso* of Ph].

(1*R**,9*S**,16*S**,17*S**)-1,17-Dihydroxy-16-methyl-8-phenoxycarbonyl-8-azatetracyclo[7.7.1.0^{2,7}.0^{10,15}]heptadeca-2(3),4,6,10(11),12,14-esaene 26

The same procedure used for the preparation of **25** was followed, starting from 7.9 mg of **2b** (21.5 μmol). Crude product was purified by preparative TLC, using PE/Et_2O 4:6 as eluent, to give **26** (4.3 mg, 52%) as an oil. R_f 0.31 (PE/Et_2O 4:6, **A**, **B**); GC-MS: R_t 9.59; *m/z* 293 (M^+ −94, 0.85), 248 (9.5), 235 (16), 234 (100), 233 (8.8), 232 (4.7), 217 (16), 204 (5.1), 146 (7.3), 128 (6.0), 116 (14), 115 (9.4), 102 (5.2), 91 (4.8), 90 (5.8), 77 (11), 51 (5.2); ^{1}H NMR: δ 1.43 [3 H, d, >CHCH_3, J = 7.0], 2.60 [1 H, broad d (became a s after exchange with D_2O), C_{17}–OH, J = 4.7] 3.02 [1 H, broad s, C_1-OH], 3.31 [1 H, q, H_{16}, J = 7.2], 4.39 [1 H, t, H_{17}, J = 4.9], 6.06 [1 H, d, H_9, J = 4.7], 6.98–7.71 [13 H, m, aromatics]; ^{13}C NMR: δ 18.65 [>CHCH$_3$], 48.97 [C_{16}], 59.44 [C_9], 64.92 [C_{17}], 72.40 [C_1], 121.72 [2 C, *C ortho* of Ph], 122.93, 125.05, 126.98, 127.96, 128.79, 128.90, and 130.02 [8 C, aromatic CH],[44] 125.81 [*C para* of Ph], 126.14 [2 C, C_{12} and C_{13}], 128.66 [2 C, C_{11} and C_{14}], 129.48 [2 C, *C meta* of Ph], 132.41 [C_2], 134.08 [C_7], 141.10 [2 C, C_{10} and C_{15}], 151.11 [*C ipso* of Ph].

(1*R**,9*S**,16*S**,17*S**)-1,17-Epoxy-16-methyl-8-[(phenylthio)-ethoxy]carbonyl-8-azatricyclo[7.7.1.0^{2,7}]heptadeca-2(3),4,6,12-tetraene-10,14-diyne 27b

NaH (16 mg, 60% in mineral oil, 408 μmol) was suspended in dry THF (2 ml). Then, after cooling to 0 °C, 2-(phenylthio)-ethanol (58 μl, 430 μmol) was added. After 125 min a solution of **2b** (79 mg, 215 μmol) in THF (4 ml) was added and the solution was stirred for 1.3 h at 0 °C and then for 10 min at r.t. The solution was poured into chilled water and extracted with Et_2O. The combined organic layers were rapidly washed

with 1 N NaOH and then with 5% aq $(NH_4)H_2PO_4$ and brine. Chromatography with PE/Et_2O 100:0 ⟶ 7:3 gave **27b** as a white foam (71 mg, 77%), that was crystallised from CH_2Cl_2/Et_2O to give a white solid. R_f 0.44 (toluene/CH_2Cl_2/Et_2O 70:29:1, **A**, **B**); Mp 160.6–161.1 °C (CH_2Cl_2/Et_2O); found: C, 72.75; H, 4.9; N, 3.3. $C_{26}H_{21}NO_3S$ requires: C, 73.04; H, 4.95; N, 3.28%; IR: ν_{max} 2992, 2201, 1704, 1389, 1325, 1302, 815; GC-MS: unsuitable for this analysis; ^{1}H NMR: δ 1.21 [3 H, d, >CHCH_3, J = 7.0], 3.16 [2 H, centre of m, –CH_2CH_2S–], 3.92 [1 H, broad q, H_{16}, J = 7.0], 4.09 [1 H, d, H_{17}, J = 3.8], 4.18–4.45 [2 H, m, –CH_2CH_2S–], 5.64 [1 H, dd, H_{12}, J = 1.4, 10.0], 5.77 [1 H, dd, H_{13}, J = 1.4, 9.8], ≈ 5.80 [1 H, overlapped with previous signal, H_9], 7.16–7.45 [7 H, m, aromatics], 7.47 [1 H, broad d, H_3 or H_6, J = 8.0], 7.65 [1 H, dd, H_3 or H_6, J = 1.6, 8.2]; ^{13}C NMR: δ 12.04 [>CH*C*H$_3$], 26.83 [C_{16}], 32.46 [–CH_2CH_2S–], 45.03 [C_9], 58.83 [C_1], 62.77 and 62.87 [C_{17}],[48] 64.84 [–*C*H$_2$CH$_2$S–], 85.81, 90.55, 92.67 and 103.32 [4 C, –*C*≡*C*–], 121.84, 125.32, 125.54, 126.86, 128.61, 129.11 and 130.03 [11 C, aromatic *C*H and $C_{12–13}$], 127.06 [C_2], 134.94 and 135.12 [C_7 and *C ipso* of Ph], 154.32 [>*C*O].

(1*R,9*S**,16*S**,17*S**)-1,17-Epoxy-16-methyl-8-[(phenylsulfonyl)ethoxy]carbonyl-8-azatricyclo[7.7.1.0^{2,7}]heptadeca-2(3),4,6,12-tetraene-10,14-diyne 28b**

The same procedure described for the preparation of **19a**,**b** was followed, starting from **27b** (70 mg, 164 μmol) and using 106 mg (491 μmol) of *m*-CPBA (≈80%). Quenching with Me_2S (36 μl) was directly followed by extraction with Et_2O, avoiding stirring of the solution in the presence of aq $NaHCO_3$, due to lability of **28b** under basic conditions. After extraction the combined organic layers were very rapidly washed with 5% aq $NaHCO_3$ and then with brine. Chromatography with PE/Et_2O 4:6 ⟶ 0:100 gave sulfone **28b** (69 mg, 91%) as a white foam. R_f 0.26 (PE/Et_2O 6:4, **A**, **B**); found: C, 67.75; H, 4.65; N, 2.95. $C_{26}H_{21}NO_5S$ requires: C, 67.96; H, 4.61; N, 3.05%; IR: ν_{max} 3680, 3006, 1707, 1323, 1303, 1144; GC-MS: unsuitable for this analysis; ^{1}H NMR: δ 1.20 [3 H, d, >CHCH_3, J = 7.0], 3.50 [2 H, t, –$CH_2CH_2SO_2$–, J = 6.0], 3.91 [1 H, broad q, H_{16}, J = 6.9], 4.03 [1 H, d, H_{17}, J = 3.0], 4.37–4.63 [2 H, m, –$CH_2CH_2SO_2$–], 5.64 [1 H, dd, H_{12}, J = 1.4, 9.8], ≈ 5.60 [1 H, overlapped with previous signal, H_9], 5.77 [1 H, dd, H_{13}, J = 1.2, 9.8], 7.12–7.34 [3 H, m, aromatics], 7.52–7.68 [4 H, m, aromatics], 7.92 [2 H, appar. d, *H ortho* to SO_2]; ^{13}C NMR: δ 12.03 [>CH*C*H$_3$], 26.82 [C_{16}], 45.11 [C_9], 55.24 and 59.60 [2 C, –*C*H$_2$*C*H$_2$S–], 58.81 [C_1], 62.53 [C_{17}], 85.85, 90.71, 92.32 and 103.26 [4 C, –*C*≡*C*–], 121.77, 125.55, 125.63, 126.67, 126.86, and 128.69 [6 C, $C_{3–6}$ and $C_{12–13}$], 127.17 [C_2], 128.05 [2 C, *C ortho* of Ph], 129.48 [2 C, *C meta* of Ph], 134.04 [*C para* of Ph], 134.74 [C_7], 139.05 [*C ipso* of Ph], 153.74 [>*C*O].

Compound 31

A solution of **2a** (30 mg, 81.7 μmol) in dry CH_2Cl_2 (3 ml) was treated with $Co_2(CO)_8$ (90%, 78 mg, 204 μmol) and stirred at r.t. for 10 min. After solvent evaporation crude product was directly purified by chromatography using PE/Et_2O 100:0 ⟶ 9:1 to give **31** as a green solid (66 mg, 86%). R_f 0.28 (PE/Et_2O 9:1, **A**, **B**); IR: ν_{max} 3012, 2083, 2061, 2028, 1722; GC-MS: unsuitable for this analysis; ^{1}H NMR: δ 1.99 [3 H, d, >CHCH_3, J = 7.4], 3.32 [1 H, q, H_{16}, J = 7.3], 4.13 [1 H, d, H_{17}, J = 3.0], 6.14 and 6.25 [2 H, AB system, $H_{12–13}$, J = 12.8], 6.65 [1 H, d, H_9, J = 3.0], 7.08–7.42 [7 H, m, aromatics], 7.61 [1 H, broad d, H_3 or H_6, J = 8.4], 7.75 [1 H, dd, H_3 or H_6, J = 1.6, 7.8]; ^{13}C NMR: δ 18.32 [>CH*C*H$_3$], 49.57 and 55.69 [C_9 and C_{16}], 59.02 [C_1], 69.92 [C_{17}], 80.60, 82.90, 87.74 and 95.42 [4 C, –*C*≡*C*–], 121.52 [2 C, *C ortho* of Ph], 123.58, 124.58, 125.00, 127.32, 129.28 and 129.91 [6 C, $C_{3–6}$ and $C_{12–13}$], 124.69 [C_2], 125.88 [*C para* of Ph], 129.50 [2 C, *C meta* of Ph], 134.95 [C_7], 150.96 [*C ipso* of Ph], 153.90 [>*C*O],[45] 198.06 and 199.06 [12 C, *C*O].[45]

Incubation with plasmid DNA and analysis by gel electrophoresis

Working buffer: this was prepared by dissolving TRIS (4.84 g), EDTA (584 mg) and acetic acid (1.142 ml) in distilled water (1 l).

Loading buffer: this was prepared from Ficoll (75 mg), the working buffer (500 μl) and a solution containing 0.25% bromophenol blue and 0.25% xylene cyanol in pH 8.3 TRIS–borate–EDTA buffer (500 μl).

Compounds **2a**, **2b** and **28a** were dissolved in DMSO (7–10 mM sol.) and diluted with additional DMSO to the desired concentrations just before incubation.

Plasmid pBR322 (Fermentas, 90% form I, 500 μg ml^{-1}) was diluted (1 : 10) with a pH 8.48 TRIS (40 mM)/EDTA (4 mM) buffer (prepared with molecular biology water) to provide a concentration of 50 μg ml^{-1}, 75 μM bp^{-1}. This solution (18 μl) was treated with the appropriately diluted solution of enediyne (2 μl). The resulting mixtures were incubated at 37 °C for 24 h. At the end of this period, the solutions (20 μl) were treated with loading buffer (5 μl) and analysed on agarose gel [prepared from agarose (300 mg), working buffer (32 ml) and ethidium bromide (15 μg)], by the submarine methodology. The gel was immersed in working buffer (325 ml) containing ethidium bromide (165 μg) and eluted at 80 mV. After elution, the gel was observed at 302 nm (transilluminator) and photographed.

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