Synthesis of Intramolecularly Activated Lactenediynes and Evaluation of Their Activity Against Plasmid DNA

Luca Banfi*[a] and Giuseppe Guanti[a]

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The synthesis of new "selectively activated" enediyne prodrugs is reported. These compounds, belonging to the "lactenediyne" family, each possess a protected primary amine tethered at the β-lactam nitrogen. This, once deblocked, can act as a trigger, provoking a cascade of events probably terminating with Bergman cycloaromatization of the enediyne moiety. In vitro experiments on plasmid DNA have shown that the protected compounds are inactive, while the unprotected amine is able to provoke single-strand scissions. These results open the way towards development of enzymatically activated lactenediyne prodrugs.

Introduction

Natural enediyne antibiotics are a small family of compounds endowed with potent cytotoxic and antitumour activities.[1] These properties are due to single- and double-strand cleavage of DNA, probably provoked by a benzenoid diradical formed through cycloaromatization of the enediyne moiety. The most fascinating aspect of these compounds is that their reactivity is regulated by complex triggering mechanisms. They are therefore prodrugs, being transformable in vivo into active species. This conversion is achieved by a cascade of events that can take place, usually without site-selectivity, both in tumour and in healthy cells.

The main drawbacks of natural enediynes are their lack of site-selectivity and their complex structures, which make their total synthesis quite difficult. The first problem has in part been solved by coupling calicheamicin, the most active natural enediyne, with specific antibodies that deliver it selectively to tumour cells.[2] However the development of simpler structures[3] endowed with similar activity but characterised by more selective triggering mechanisms is still the most desirable goal in this area. The ideal enediyne prodrug should be simple, easy to synthesise, highly stable (also in the dry state) and amenable to selective activation in tumour tissues.[4] If the activation (triggering event) can be promoted by a suitable enzyme, strategies such as antibody-directed enzyme prodrug therapy (ADEPT),[5] gene-directed enzyme prodrug therapy (GDEPT)[6] and prodrug monotherapy (PMT)[7] should be exploitable for achieving specific activation. Finally, ideal enediyne prodrugs should also possess handles usable for the attachment of DNA-complexing substructures. The activity of natural[8] and unnatural enediynes[9] can indeed be strongly influenced by the presence of such complexing agents.

Towards this goal we have recently introduced a new class of artificial enediynes, characterised by the fusion of a ten-membered enediyne moiety with a β-lactam.[10−12] Among these, those with *trans* fusion between carbons 3 and 4 of the lactam and carbons 8 and 9 of the enediyne ring (general formula **1**) have emerged as the most useful, thanks to their exceptional chemical stability. Unlike many enediynes known so far, they are white solids, perfectly stable for years, including in the dry state. Opening of the β-lactam (which acts a stabilizing element against cycloaromatization) unleashes the reactivity of the enediyne: fast cycloaromatization was demonstrated to take place easily after this triggering event.

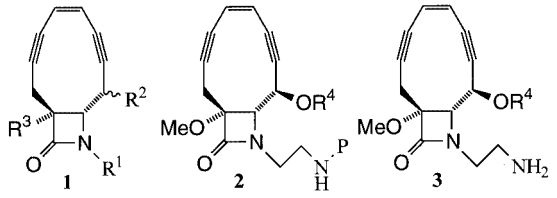
Base-catalysed opening of the azetidinone in inactivated lactenediynes requires conditions that cannot be attained in vivo. It was therefore no surprise that incubation of simple inactivated lactenediynes of general formula **1** (for example, the compounds with R^1 = Me, R^2 = MeO, R^3 = H) with plasmid DNA showed no cleavage at all even at concentrations as high as $5 \cdot 10^{-3}$ M.

We therefore looked for a method through which to induce opening of the β-lactam in a controlled fashion under physiological conditions. We reasoned that intramolecular opening by an amino group to give a larger, more flexible ring, could serve well for this purpose. The amino group responsible for the triggering event could be blocked by an enzymatically[13] or photochemically[14] removable protecting group to give a stable prodrug to be specifically activated only when and where needed.

On the basis of a preliminary study on the rate of intramolecular transamidation carried out on model compounds,[15] we selected prodrugs **2** as our targets

[a] Dipartimento di Chimica e Chimica Industriale dell'Università di Genova,
via Dodecaneso 31, 16146 Genova, Italy
E-mail: banriv@chimica.unige.it, guanti@chimica.unige.it

(Scheme 1). Deprotection of the amino group should give rise to the free amine **3**, which was expected to undergo a cascade of reactions resulting in intramolecular transamidation with ring-enlargement, followed by Bergman cycloaromatization and DNA cleavage. Molecular mechanics calculations suggested that the resulting seven-membered ring should no longer be able to prevent cycloaromatization.

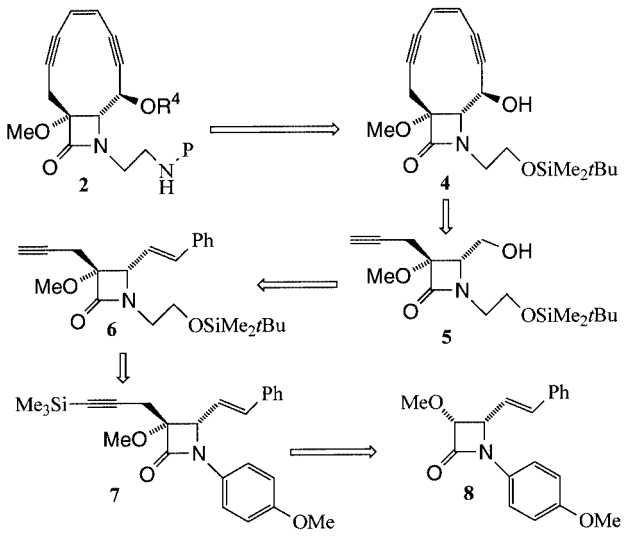


Scheme 1

The synthesis of racemic intermediate **4** has been already described in a preliminary communication.[16] Here we report a full account of the preparation of **4**, the accomplishment of the second part of the synthesis, providing racemic **2**, and the results of DNA cleavage experiments.

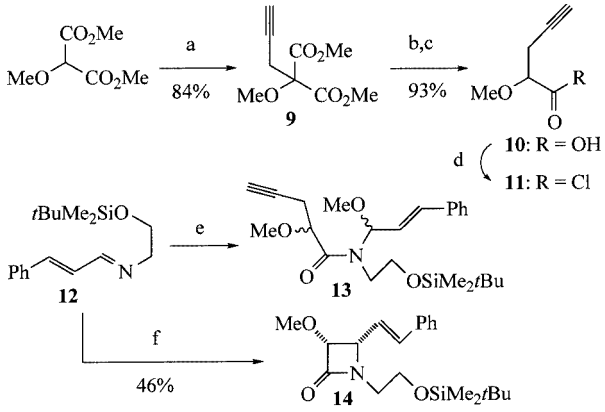
Results and Discussion

Scheme 2 shows the retrosynthetic approach followed by us. The very good yields obtained in previous syntheses of lactenediynes **1** by the use of an intramolecular Nozaki−Hiyama condensation for the final ten-membered ring-closure[10,11] prompted us also to use this method in this case. In order to shorten the synthetic route, we chose to introduce the side chain tethered at N-1 before construction of the unsaturated ring. These considerations identified alcohol **5** as a key intermediate. The preparation of the latter required the development of a strategy completely different from that previously employed for lactenediynes **1** (R^3 = H), which started from aspartic acid. However, the presence of the methoxy group at C-3 of **5** hinted at the use of a Staudinger reaction for the assembly of the β-lactam ring. This highly convergent cycloaddition is actually known to be especially efficient when α-alkoxyketenes are employed.[17] Preliminary Staudinger condensations carried out by us with methoxyacetyl chloride and imines derived from 2-benzyloxyacetaldehyde or 2-benzyloxymethoxyacetaldehyde showed that these reactions were not efficient, affording low yields of products that were difficult to purify. We therefore decided to take advantage of the styryl group as a synthetic equivalent of CH_2OH, hoping to be able to cleave it oxidatively in the presence of the triple bond.



Scheme 2

The most convergent route to trisubstituted β-lactam **6** would be Staudinger condensation between acyl chloride **11** and imine **12** (Scheme 3). We did not find any precedent for this type of reaction in the literature. However, condensation between 2-fluoro-2-phenylacetyl chloride and *N*-cinnamilydene *p*-anisidine has been reported to give the β-lactam − although in low yield − with high stereoselectivity favouring the isomer with F and styryl in a *cis* relationship.[18] This induction was interpreted in terms of stereoelectronic effects. We therefore hoped that chloride **11** would also furnish **6** with the desired *cis* stereochemistry.

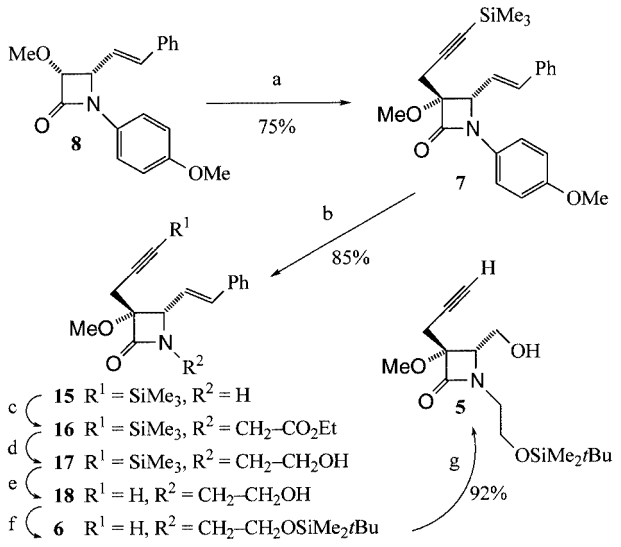


Scheme 3. a) *t*BuOLi, propargyl bromide, *t*BuOH, room temp., 20 h, b) KOH, McOH, room temp, c) H^+, Δ, d) $(COCl)_2$, CH_2Cl_2, benzene, e) **11**, Et_3N, CH_2Cl_2, f) $McOCH_2COCl$, Et_3N, CH_2Cl_2

The synthesis of **11** was carried out from dimethyl 2-methoxymalonate as described in Scheme 3, while imine **12** was prepared in two steps and 98% yield from cinnamaldehyde, 2-aminoethanol and *t*$BuMe_2SiCl$ as described in the Exp. Sect.[19,20] However, condensation between **12** and **11** was sluggish and, after methanolic workup, we were not able to isolate any of the desired lactam **6**, but only modest amounts of hemiaminals **13**. In contrast, treatment of imine **12** with methoxyacetyl chloride worked reasonably well to give the disubstituted *cis* azetidinone **14**. Introduction of the propargyl group at C-3 could in principle be achieved stereoselectively by treatment of the lithium enolate derived from **14** with 3-bromo-1-trimethylsilylpropyne. However, all attempts to perform this reaction failed, showing that the presence of the side chain tethered at nitrogen is not compatible with enolisation, probably because of β-elimination

reactions (we were able to isolate the *N*-vinyl β-lactams in some cases).

We therefore turned to a longer and less convergent route (Scheme 4) involving electrophilic propargylation on *N*-*p*-anisyl-2-azetidinone **8**, followed by deprotection and stepwise introduction of the side chain to give **6**. The known[20] starting azetidinone **8** was obtained (Scheme 4) in moderate yield (46%) but with high *cis* stereoselectivity (92:8) from the inexpensive methoxyacetyl chloride and *N*-cinnamilydene *p*-anisidine. Various attempts to improve this yield were unfruitful. Phenyl dichlorophosphate-promoted condensation of methoxyacetic acid[21] gave a very similar yield (45%; *cis*/*trans* = 86:14). A recent report[22] claiming a very high yield (99%) in the related 2-chloro-1-methylpyridinium iodide-mediated condensation between cinnamilydene *p*-anisidine and phenoxyacetic acid prompted us to attempt that method too, but in our hands the yields were once again around 43−45%.



Scheme 4. a) 1) LDA; 2) $Me_3Si-C\equiv C-CH_2-Br$; b) $Ce(NH_4)_2(NO_3)_6$, H_2O/CH_3CN, c) $LiN(SiMe_3)_2$, $Br-CH_2CO_2Et$, THF, 76%; d) $Ca(BH_4)_2$, THF/EtOH, 72%; e) $AgNO_3$, KCN, 95%, f) $Me_2tBuSiCl$, imidazole, DMF, 98%, g) 1) O_3, CH_2Cl_2, MeOH, Solvent Red 23; 2) Me_2S; 3) $NaBH_4$

Propargylation of the lithium enolate derived from **8** afforded − with very high stereocontrol (98:2)[23] − the *cis* isomer **7**, the relative configuration of which was unambiguously determined by NOE experiments. On irradiation of the OC*H*$_3$ group, a 2.9% NOE was detected for the C*H*=CHPh, while no increase was observed for C*H*−N. In contrast, irradiation of the propargylic C*H*$_2$ provoked an 8.9% NOE on C*H*−N and no effect on C*H*=CHPh. To obtain a good yield, the order of reagent addition in the deprotonation step was crucial. Contrary to our expectations, best yields were achieved by adding LDA to a cold (−78 °C) solution of lactam **8**.

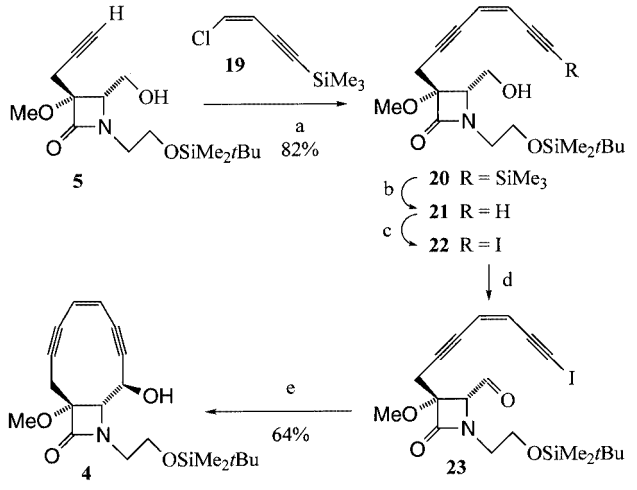
After oxidative removal of the nitrogen protection, the side chain was introduced at *N*-1 by alkylation with ethyl bromoacetate, followed by $Ca(BH_4)_2$ reduction. The resulting alcohol **17** was smoothly protected as its silyl ether **6** after removal of the *C*-TMS substituent.[24] The overall yield of the conversion of **7** into **6** was good (43%).

At this point our strategy involved selective oxidative degradation of the styryl residue in the presence of the triple bond. We were able to find only two examples of this kind of chemoselective transformation in the literature, both involving terminal double bonds.[25,26] We first tried ozonolysis of the trimethylsilyl alkyne (obtained by protecting the OH group of **17** as the TBDMS ether), working up the reaction with $NaBH_4$ to obtain the primary alcohol. Although a certain degree of selectivity was observed, we were not able to obtain the desired product (deriving from attack only on the double bond) in yields better than 50%. In the main by-product, not only had the styryl group been converted into CH_2OH, but the $R-C\equiv C-SiMe_3$ group had also undergone an oxidative degradation to give an ester ($R-CO_2Me$). Ozone is known to attack triple bonds, but this reaction has very rarely been utilized, because its course is often complex, giving rise to mixtures of products.[27] In this case, however, the conversion of silylated alkyne into the methyl ester definitely seems clean, and therefore we think that this unprecedented transformation may find useful synthetic applications, as it makes the anion of trimethylsilylacetylene a synthetic equivalent of the $[CO_2Me]^-$ acylanion. On the other hand, it was much easier to control the reaction when the terminal alkyne **6** was employed, and alcohol **5** was obtained in excellent yields. In order to monitor the ozonolysis, we used Solvent Red 23 as internal indicator.[25]

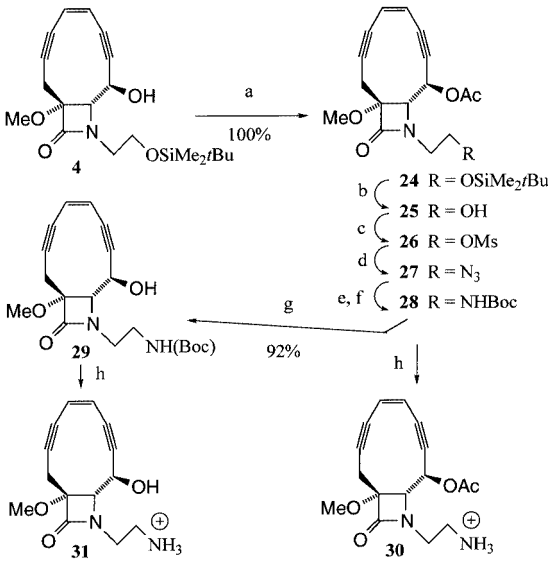
The key intermediate **5** was then converted into iodoaldehyde **23** by a four-step sequence (Scheme 5). The first stage was the Castro−Stephens−Sonogashira coupling with chloroenyne **19**,[28] which was best carried out with $Pd(PhCN)_2Cl_2$ and using a "sacrificial" alkyne.[11] Conversion of the silylated alkyne into iodoalkyne was achieved by a two-step procedure. Finally, Swern oxidation gave iodoaldehyde **23**. The intramolecular variant[29] of Nozaki−Hiyama−Kishi coupling, mediated by $CrCl_2$ and $NiCl_2$,[30] proceeded smoothly, affording lactenediyne **4** in a yield particularly noteworthy for the formation of a strained medium-size ring. The condensation also turned out to be highly stereoselective: only the 9,10-*trans* alcohol **4** could be detected in the reaction mixture by TLC or ^1H NMR. The diastereoisomeric ratio was thus estimated to be higher than 95:5. The relative configuration was unambiguously assigned on the basis of the high (8.8 Hz.) H_9-H_{10} coupling constant. These systems should be conformationally rigid and the 9,10-*cis* isomer would be expected to have a small (around 2 Hz.) H_9-H_{10} coupling constant.

This stereochemical course is particularly surprising in view of the cyclization results previously reported by us[11] for the synthesis of lactenediyne **1** (R^1 = $SiMe_2tBu$, R^2= OH, R^3 = H). In that case, the major isomer, formed in 86:14 ratio, had the 9,10-*cis* configuration. A possible explanation of this behaviour has already been published in our preliminary communication.[16]

Compound **4** is a stable, white solid that we have prepared on the gram scale. The side chain bonded at N-1 can



Scheme 5. a) $Pd(PhCN)_2Cl_2$, CuI, piperidine/THF, b) $AgNO_3$, KCN, 83%, c) I_2·morpholine, benzene, room temp., 95%, d) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, 96%, e) $CrCl_2$, $NiCl_2$ cat., THF, room temp.



Scheme 6. a) Ac_20, pyridine, b) HF, CH_3CN/H_20, 96%, c) MsCl, Et_3N, CH_2Cl_2, −30 °C, d) NaN_3, DMF, 60 °C, 87% (from **25**), e) PPh_3, THF/H_20, 8 h, room temp., f) Boc-ON, Et_3N, 85%, g) MeONa, MeOH, 0 °C, h) CF_3C0_2 H, CH_2C1_2

be employed for the introduction of internal nucleophiles capable of opening the β-lactam, while the secondary hydroxyl can be used for appending DNA-complexing substructures. We first converted **4**, as shown in Scheme 6, into the ammonium salts **30** and **31**. After acetylation of the secondary alcohol and removal of the TBDMS group, the primary alcohol **25** was converted into the corresponding azide **27**. All these steps proceeded in high yield, once again confirming the robustness of this type of lactenediynes. Unlike some acyclic precursors, such as **21**, **22**, and **23**, that tend to decompose in the dry state or during GC analysis (especially **21**), **4** and its derivatives **24**−**29** were completely stable. They are white compounds (in most cases solid) with no tendency to become coloured on standing. They can all be analysed by GC at oven temperatures of over 200 °C!

Conversion of azide **27** into the protected amine **28** was more problematic than the previous steps. Attempts to isolate the free amine by chromatography after Staudinger reduction afforded unsatisfactory yields. We therefore tried a one-pot procedure,[31] using $(Boc)_2O$ as the acylating agent. To our surprise, the main product under these conditions was the dimeric urea of amine **30**. We also found the same behaviour with simple monocyclic model azides, prepared as already described.[15] A possible explanation for this unexpected behaviour would be provided by addition of Boc_2O to the intermediate phosphazene, followed by a rearrangement (through a six-membered TS) to give Ph_3PO, $(tBuO)_2CO$ and an isocyanate. Reaction between the last of these and free amine originating from phosphazene hydrolysis would account for urea formation. This mechanism suggested that the problem might be solvable by replacement of Boc_2O with a different acylating reagent. Indeed, when Boc-ON was used, urea formation was suppressed. The best yields were anyway obtained by allowing the azide to react for a certain time with Ph_3P and H_2O prior to addition of Boc-ON.

Compound **28** was finally deacetylated to give **29** in good yield. The overall yield of **29**, starting from the known β-lactam **8**, was 7.8% over 18 steps (87% average yield for each step). Treatment of **28** or **29** with trifluoroacetic acid removed the protection to give the ammonium salts **30** and **31**. These were characterised by reprotection (Boc−ON, Et_3N) to give back the starting Boc derivatives **28** and **29** in good overall yield. Ethanolic solutions of these ammonium salts were also found to be stable for one day at room temperature; therefore no transamidation occurs when the amino groups are still in the protonated form.

Protected compounds **28**−**29** and unprotected compounds **30**−**31** were incubated for 24 h at 37 °C and pH 7.5 with supercoiled pBR322 plasmid (90% in form I) (Figure 1). As expected, **28** and **29** showed no cleaving activity at all. With **30** and **31**, on the contrary, single-strand breaks were clearly evident. The acetylated compound **30** seems to

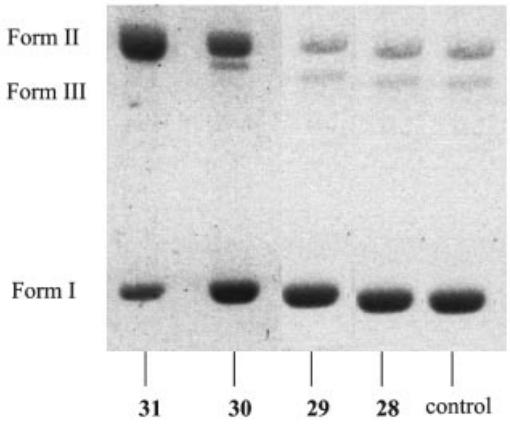


Figure 1. Results of incubation of compounds **28**−**31** (10^{-3} M) with pBr322 plasmid (67.5 μM/bp) for 24 h in pH 7.5 buffer at 37 °C

be about 4−5 times less active than the alcohol **31**. With the latter we also carried out experiments at different concentrations. Single-strand scissions were still evident at concentrations as low as 40 μM. The concentration that afforded equal quantities of form I and form II was about 300 μM. No double-strand break (that is, form III formation) was detected at any concentration.

The presence of DNA scissions is probably due to β-lactam opening, followed by cycloaromatization of the resulting seven-membered ring. Reactivity tests were carried out on **30** and **31** in EtOH with a Et_3N/AcOH buffer. Disappearance of **31** was quite slow (it was still clearly evident at TLC after 8 days at 37 °C), suggesting that the only moderate activity towards DNA may be due to an insufficient rate of transamidation. We were, however, unable to identify the reaction products. In the case of **30**, substrate disappearance was faster (about 1 day), but the main product, isolated and characterised, was the hydroxyacetamide derived from transacetylation. Evidently the amino group prefers to attack the acetyl group instead of the β-lactam. This unwanted reaction may explain the lower cleavage activity of **30** than of **31**.

Conclusions

The results reported here demonstrate for the first time the potential to activate a lactenediyne prodrug through deprotection of a tethered amino group. Since several enzymatically[13] or photochemically removable[14] amine protecting groups are known, the achievement of enzymatically or photochemically triggered prodrugs for use in ADEPT, GDEPT or PMT strategies seems to be at hand.

The moderate activity and the absence of double-strand breaks, however, indicate that improvements are needed. We are currently trying to increase the rate of intramolecular ring-enlargement, through suitable structural modifications. Moreover, we aim to succeed in improving activity and at the same time to favour simultaneous double-strand breaks, through the incorporation of substituents that bind strongly with DNA.

Experimental Section

Abbreviations: PE: petroleum ether; LDA: lithium diisopropylamide; Boc−ON: 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile.

General Remarks: ^{1}H NMR and ^{13}C NMR spectra were taken in $CDCl_3$, at 200 MHz and 50 (or 20) MHz, respectively. Chemical shifts are reported in ppm (δ scale), with TMS as an internal standard. Coupling constants are reported in Hz. In ABX systems, the proton A is considered downfield and B upfield. Peak assignment in ^{13}C spectra was also made on the basis of DEPT experiments. GC-MS was carried out on a HP-5971A instrument, with an HP-1 column (12 m long, 0.2 mm wide), electron impact at 70 eV, a mass temperature of about 167 °C, and starting the mass range from m/z = 33. Analyses were performed with a constant He flow of 0.9 mL/min, starting at 100 °C for 2 min (unless otherwise noted) and then raising the temperature by 20 °C/min. R_t values are measured in minutes from injection. IR spectra were measured as $CHCl_3$ solutions. TLC analyses were carried out on silica gel plates, which were viewed either by U.V. or by dipping into a solution of $(NH_4)_4MoO_4{\cdot}4H_2O$ (21 g) and $Ce(SO_4)_2{\cdot}4H_2O$ (1 g) in H_2SO_4 (31 cc) and H_2O (469 cc) and warming. R_f values were measured after an elution of 7−9 cm. Chromatography was carried out on 220−400 mesh silica gel by the "flash" methodology. All reactions employing dry solvents were carried out under nitrogen (or argon, where indicated) atmosphere. During extractions, the aqueous phases were always reextracted twice with the indicated organic solvent. Organic extracts were always dehydrated with Na_2SO_4 and filtered before evaporation to dryness.

Dimethyl 2-Methoxy-2-(2-propynyl)malonate (9): *n*BuLi (1.6 M in hexanes, 5.0 mL, 8.0 mmol) was slowly added to dry *t*BuOH (25 mL), while cooling with an ice bath when necessary. After 10 min from the end of addition, dimethyl 2-methoxymalonate (1.00 mL, 7.23 mmol) was added dropwise, again cooling when necessary. After 15 min, propargyl bromide (80% in toluene, 0.886 mL, 7.95 mmol) was added. The mixture was allowed to react at room temp. overnight and was then quenched with saturated aqueous NH_4Cl. Extraction with Et_2O, evaporation and chromatography (PE/EtOAc, 80:20), gave pure malonate **9** as a low-melting solid (1.22 g, 84%). R_f = 0.37 (PE/EtOAc, 80:20). $C_9H_{12}O_5$ (200.19): calcd. C 54.00, H 6.04; found C 53.7, H 6.2. GC−MS: R_t = 3.34, m/z: 185 [$M^{+\cdot}$ − 15] (0.1%), 170 (21.5), 161 (16.8), 142 (8.0), 141 (100), 133 (6.6), 113 (5.9), 109 (22.5), 82 (6.8), 81 (23.8), 75 (16.2), 68 (5.3), 67 (9.7), 66 (6.8), 59 (37.7), 53 (9.6), 45 (5.8), 43 (6.3), 41 (6.7), 39 (28.9). IR: $\tilde{\nu}_{max}$ = 3307, 2999, 2955, 2839, 1746, 1435, 1296, 1246, 1127, 1095, 1050, 976, 952, 864 cm^{-1}. ^{1}H NMR: δ = 3.84 (s, 6 H, C*H*$_3$OC=O), 3.46 (s, 3 H, C*H*$_3$O), 3.03 (d, J = 2.7 Hz, 2 H, C*H*$_2$), 2.09 (t, J = 2.7 Hz, 1 H, ≡C*H*) ppm.

(+/−) 2-Methoxy-4-pentynoic Acid (10): A solution of malonate **9** (1.86 g, 9.27 mmol) in absolute MeOH (32 mL) was treated with a solution of KOH (2.60 g, 46.3 mmol) in H_2O (4.63 mL) and MeOH (810 mL). The solution was stirred overnight at room temp. and quenched with aqueous KH_2PO_4 (1 M, 18.5 mL) and aqueous HCl (2 M, 21 mL). After evaporation of most of the MeOH, the mixture was diluted with H_2O and the pH was adjusted to 1. Saturation with NaCl followed by extraction with EtOAc and evaporation to dryness gave the malonic diacid. It was heated under N_2 for 1 h at 100 °C and for 3 h at 120 °C. After further drying under high vacuum, compound **10** was obtained as a solid (933 mg, 78%). It was considered pure enough by TLC and NMR and used as such for the preparation of acyl chloride **11**, which was carried out by treatment with $(COCl)_2$ for 4 h at room temp. in CH_2Cl_2/benzene. Data for **10**: R_f = 0.67 (PE/EtOAc/AcOH, 76:19:5). ^{1}H NMR: δ = 10.15 (broad s, 1 H, CO_2H), 3.99 (dd, J = 5.1, 5.9 Hz, 1 H, C*H*−CO_2H), 3.56 (s, 3 H, OC*H*$_3$), 2.79 and 2.72 (AB part of ABXY syst., J_{AB} = 17.0, J_{AX} = 4.8, J_{BX} = 6.1, J_{AY} = J_{BY} = 2.7, 2 H, C*H*$_2$−C≡), 2.09 (t, J = 2.7 Hz, 1 H, ≡C*H*).

(*E*)-[2-(*tert*-Butyldimethylsilyloxy)ethyl](3-phenylpropenylidene)-amine (12): A solution of cinnamaldehyde (8.14 g, 61.6 mmol) in dry CH_2Cl_2 (70 mL) was cooled to 0 °C and treated with freshly activated powdered molecular sieves (3 Å, 7 g) and with ethanolamine (4.71 mL, 6.67 mmol). The suspension was stirred for 30 min at 0 °C and for 4 h at room temp. At this point, GC showed completion of the reaction. After filtration, the solution was evaporated to dryness to give an oil (9.67 g). This was taken up in dry CH_2Cl_2 (80 mL), cooled to 0 °C, and treated with Et_3N (15.38 mL, 110.4 mmol) and Me_2tBuSiCl (8.73 g, 57.9 mmol). After stirring for 30 min at 0 °C and 8 h at room temp., the solution was poured

into a suspension of $NaHCO_3$ (7 g) in H_2O (90 mL). The phases were separated and the aqueous phase was reextracted with CH_2Cl_2. The organic extracts were washed with saturated aqueous NaCl and evaporated to dryness. Further stripping overnight at 0.1 mbar gave pure (GC) imine **12** as a yellow liquid (15.65 g, 98%). It was used as such, without further purification, for the synthesis of **14** and for the unsuccessful attempt to prepare **6**. GC−MS: R_t = 9.67 (initial temp.: 70 °C), *m*/*z*: 274 [$M^{+\cdot}$ − 15] (2.9%), 233 (19.9), 232 (100), 144 (6.6), 115 (27.3), 89 (11.7), 77 (3.8), 75 (13.8), 73 (40.9), 59 (28.6), 45 (6.4). ^{1}H NMR: δ = 8.01 (dd, *J* = 6.8, 1.3 Hz, 1 H, C*H*=N), 7.50−7.30 (m, 5 H, aromatics), 7.03−6.80 (m, 2 H, C*H*=C*H*), 3.87 (t, *J* = 5.7, CH_2), 3.64 (t, *J* = 5.7, CH_2), 0.87 [s, 9 H, $(CH_3)_3$], 0.05 [s, 6 H, $(CH_3)_2$].

(3*R,4*S**,*E*)-1-[2-(*tert*-Butyldimethylsilyloxy)ethyl]-3-methoxy-4-(3-phenylethenyl)azetidin-2-one (14):** A solution of methoxyacetyl chloride (7.41 mL, 81.1 mmol) in dry CH_2Cl_2 (150 mL) was cooled at −78 °C. Et_3N (22.6 mL, 162.1 mmol) was added by dropping funnel over 15 min. Abundant precipitation of a white solid took place. The temperature was allowed to rise to −40 °C over 1 h, and a solution of imine **12** (15.65 g) in CH_2Cl_2 (50 mL) was added over 30 min. The mixture was stirred at −20 °C for 2 h, at 0 °C for 5 h, and at room temp. overnight. Water (20 mL) was added and the mixture was stirred vigorously for 10 min. After dilution with aqueous $(NH_4)H_2PO_4$ (5%, 120 mL) and HCl (2 M, 40 mL, pH = 4), the phases were separated. The organic extracts were washed with saturated aqueous $NaHCO_3$ and saturated NaCl, and evaporated to dryness. Immediate chromatography (PE/CH_2Cl_2/EtOAc, from 59:41:0 to 45:30:25) gave pure lactam **14** as an oil (8.937 g, 46%). GC analysis of the crude product showed a *cis*/*trans* ratio of 97:3. The main by-product was *N*-[2-(*tert*-butyldimethylsilyloxy)ethyl]-2-methoxyacetamide. R_f = 0.44 (PE/CH_2Cl_2/EtOAc, 50:35:15). $C_{20}H_{31}NO_3Si$ (361.55): calcd. C 66.44, H 8.64, N 3.87; found C 66.7, H 8.5, N 3.7. GC−MS: R_t = 10.08, *m*/*z*: 361 [$M^{+\cdot}$] (2.1), 346 (1.9), 304 (96.3), 272 (19.0), 244 (11.8), 232 (10.3), 228 (9.3), 198 (13.3), 187 (12.3), 186 (7.8), 160 (100), 159 (37.6), 144 (31.1), 129 (30.1), 128 (23.5), 127 (10.6), 117 (33.4), 115(49.7), 100 (82.9), 91 (23.0), 89 (19.5), 75 (27.5), 73 (62.1), 59 (31.6), 45 (19.7). ^{1}H NMR: δ = 7.47−7.25 (m, 5 H, aromatics), 6.71 (d, *J* = 15.9 Hz, 1 H, PhC*H*=CH), 6.23 (dd, *J* = 9.1, 15.9, 1 H, PhCH=C*H*), 4.62 (d, *J* = 4.4 Hz, 1 H, C*H*−OMe), 4.42 (dd, *J* = 4.4, 9.1 Hz, 1 H, C*H*−N), 3.82−3.62 (m, 2 H, CH_2OSi), 3.53 (dt, *J* = 14.0, 4.8, 1 H, C*H*H−N), 3.46 (s, 3 H, OCH_3), 3.07 (ddd, *J* = 5.0, 7.4, 14.0, 1 H, C*H*−N), 0.90 [s, 9 H, $(CH_3)_3$], 0.05 [s, 6 H, $(CH_3)_2$] ppm.

(3*R,4*S**,*E*)-3-Methoxy-1-(4-methoxyphenyl)-4-(3-phenylethenyl)-azetidin-2-one (8):** A solution of *N*-cinnamylidene-*p*-anisidine (m.p. 121.0−121.8 °C, 30.0 g, 126.4 mmol) in dry CH_2Cl_2 (400 mL) was cooled to −30 °C (partial precipitation occurred) and treated with Et_3N (28.19 mL, 202.24 mmol). Methoxyacetyl chloride (16.2 mL, 177 mmol), diluted with CH_2Cl_2, was slowly added to this solution over 150 min. The solution was stirred at −30 °C for 4 h, at −10 °C for 3 h, and at 0 °C for 13 h. After further stirring for 2 h at room temp., the reaction was quenched with H_2O (50 mL). Solid K_2CO_3 (11 g, 79.6 mmol) was added, and the mixture was stirred for 30 min and then cautiously poured into aqueous HCl (2 M, 180 mL) + $(NH_4)H_2PO_4$ (5%, 50 mL). The phases were separated (pH aqueous phase = 1) and the organic extracts were washed with saturated $NaHCO_3$ and brine. Evaporation gave a crude solid. GC-MS analysis of the crude product showed the presence of, apart from **8**, the *trans* isomer (*cis*/*trans* ratio = 92:8) and of *N*-(*p*-methoxyphenyl)methoxyacetamide. This crude product was chromatographed through silica (300 g, CH_2Cl_2/EtOAc, from 100:0 to 85:15) to give a fraction containing nearly pure **8** (16.25 g), and another one (4.87 g) constituted mainly of **8**, contaminated with some *N*-(*p*-methoxyphenyl)methoxyacetamide. This fraction was again chromatographed with PE/CH_2Cl_2/EtOAc, 30:70:0 to 30:65:5, to give a further 3.15 g of nearly pure **8**. Finally, trituration of the recombined product samples (19.40 g) with Et_2O/PE gave pure **8** as a white solid (17.93 g, 46%). M.p.: 149.4−149.7 °C. R_f = 0.53 (PE/EtOAc, 60:40). $C_{19}H_{19}NO_3$ (309.36): calcd. C 73.77, H 6.19, N 4.53; found C 73.8, H 6.2, N 4.5. GC−MS: R_t = 10.94, *m*/*z*: 309 [$M^{+\cdot}$] (5.3), 236 (36.2), 160 (100.0), 159 (23.2), 149 (5.3), 145 (6.0), 134 (9.5), 129 (14.7), 128 (10.7), 117 (12.0), 115 (24.1), 91 (7.4), 77 (7.6). ^{1}H NMR: δ = 7.48−7.24 (m, 7 H, aromatics), 6.87 (d, *J* = 15.9 Hz, 1 H, PhC*H*), 6.83 (d, *J* = 9.0 Hz, 2 H, *H ortho* to OMe), 6.35 (dd, *J* = 8.1, 15.9, C*H*=CHPh), 4.83−4.72 (m, 2 H, C*H*C=O and C*H*−N), 3.76 (s, 3 H, OCH_3), 3.52 (s, 3 H, OCH_3).

(3*R,4*S**,*E*)-3-Methoxy-1-(4-methoxyphenyl)-4-(3-phenylethenyl)-3-[3-(trimethylsilyl)prop-2-ynyl]azetidin-2-one (7):** A solution of β-lactam **8** (5.14 g, 16.6 mmol) in dry THF (100 mL) was cooled to −78 °C and treated over 10 min, by slow addition by dropping funnel, with a solution of LDA in THF/hexanes (0.4 M, prepared in THF from 1.6 M *n*BuLi in hexanes, 41.5 mL, 16.6 mmol). After stirring for 35 min at −78 °C, the solution was treated with 3-bromo-1-trimethylsilylpropyne (2.58 mL, 18.26 mmol). The temperature was allowed to rise to −20 °C over 2 h. After a further 15 min the mixture was poured into aqueous $NH_4H_2PO_4$ (5%, 70 mL) + HCl (1 M, 15 mL) and diluted with Et_2O (100 mL). The phases were separated and the organic one was washed with brine and evaporated to dryness. Chromatography (PE/EtOAc/Et_3N, 89.5:10:0.5, to PE/EtOAc, 80:20) gave nearly pure **7** as a coloured solid (5.683 g). Trituration (Et_2O/PE) afforded pure **7** (5.032 g). Chromatography of mother liquors gave a further 206 mg of pure **7**. Overall yield: 5.238 g, 75%. M.p.: 105.9−106.2 °C. R_f = 0.58 (PE/EtOAc, 85:15). $C_{25}H_{29}NO_3Si$ (419.59): calcd. C 71.56, H 6.97, N 3.34; found C 71.7, H 7.05, N 3.3. GC−MS: R_t = 11.94, *m*/*z*: 419 [$M^{+\cdot}$] (2.9), 404 (1.6), 389 (4.1), 388 (4.6), 308 (18.2), 270 (5.6), 236 (100.0), 201 (15.3), 197 (7.8), 165 (6.5), 158 (6.0), 134 (6.0), 115 (19.4), 89 (9.9), 83 (8.7), 77 (5.5), 73 (37.4), 59 (8.8). ^{1}H NMR: δ = 7.50−7.24 (m, 7 H, aromatics), 6.85 (d, *J* = 9.0 Hz, 2 H, *H ortho* to OMe), 6.84 (d, *J* = 16.0 Hz, 1 H, PhC*H*=), 6.37 (dd, *J* = 8.2, 16.0, 1 H, C*H*=CHPh), 4.83 (dd, *J* = 8.1, 0.7 Hz, 1 H, C*H*−N), 3.78 (s, 3 H, OCH_3), 3.59 (s, 3 H, OCH_3), 2.94 and 2.87 (AB syst., *J* = 17.0, 2 H, CH_2C≡C), −0.01 (s, 9 H, $(CH_3)_3$Si) ppm. ^{13}C NMR (20 MHz): δ = 164.05 (*C*=O), 156.48, 136.07, 135.70 (quat. aromatics), 131.01 and 123.99 (*C*H=*C*H), 128.63, 128.26, 126.73, 118.80, 114.41 (aromatic *C*H), 99.78 and 89.71 (*C*≡*C*), 88.56 (*C*−OMe), 64.88 (*C*−N), 55.60 and 54.74 (OCH_3), 23.56 (CH_2C≡C), −0.18 (CH_3Si). IR: $\tilde{\nu}_{max}$ = 3030, 3001, 2960, 2838, 2177, 1748, 1600, 1506, 1440, 1390, 1297, 1241, 1150, 1112, 1033, 967, 894, 840 cm^{-1}.

(3*R,4*S**,*E*)-3-Methoxy-4-(3-phenylethenyl)-3-[3-(trimethylsilyl)-prop-2ynyl]azetidin-2-one (15):** A solution of lactam **7** (8.40 g, 20.01 mmol) in CH_3CN (210 mL) was cooled to −18 °C and treated, over 10 min, with a solution of $(NH_4)_2Ce(NO_3)_6$ (CAN, 27.45 g) in H_2O (60 mL). The internal temperature remained between −12 °C and −18 °C during addition. After stirring for 15 min at −18 °C, the mixture was quenched with a solution of $Na_2S_2O_5$ (6.60 g, 34.74 mmol) in H_2O (90 mL). The biphasic system was shaken for 3 min and then diluted with Et_2O (200 mL) and filtered through a Celite cake, washing with Et_2O and eventually with EtOAc. The phases were rapidly separated (pH aqueous phase = 2) and the organic extracts were washed with brine/saturated $NaHCO_3$, 4:1, evaporated to dryness and chromatographed (PE/CH_2Cl_2/EtOAc, 35:60:5 to 25:60:15) to give pure **15** as a solid

(5.34 g, 85%). Trituration (Et_2O/pentane) gave an analytically pure sample. M.p.: 102.0°−102.5 °C. R_f = 0.30 (PE/EtOAc, 80:20). $C_{18}H_{23}NO_2Si$ (313.47): calcd. C 68.97, H 7.40, N 4.47; found C 69.1, H 7.6, N 4.4. GC−MS: R_t = 9.41, *m/z*: 313 [$M^{+\cdot}$] (3.9), 298 (5.9), 282 (24.9), 266 (5.4), 202 (38.4), 165 (8.1), 158 (6.0), 139 (34.2), 132 (74.0), 130 (11.6), 115 (100.0), 111 (19.6), 96 (8.1), 89 (21.1), 83 (28.3), 81 (8.6), 77 (7.3), 75 (6.7), 73 (55.8), 59 (16.3), 45 (7.1), 43 (9.8) ^{1}H NMR: δ = 7.50−7.25 (m, 5 H, aromatics), 6.66 (d, *J* = 15.9 Hz, 1 H, PhC*H*=), 6.30 (dd, *J* = 7.6, 15.9, 1 H, C*H*=CHPh), 6.10 (broad s, 1 H, *NH*), 4.45 (dd, *J* = 7.6, 0.7 Hz, 1 H, C*H*−N), 3.54 (s, 3 H, OCH_3), 2.85 (s, 2 H, CH_2C≡C), 0.16 (s, 9 H, (CH_3)$_3$Si) ppm. ^{13}C NMR (50 MHz): δ = 167.76 (*C*=O), 136.16 (quat. aromatic), 134.29 and 124.99 (*C*H=*C*H), 128.64, 128.10, 126.61 (aromatic *C*H), 100.101 and 91.44 (*C*≡*C*), 88.24 (*C*−OMe), 61.08 (*C*−N), 54.36 (O*C*H$_3$), 23.15 (*C*H$_2$C≡C), −0.02 (*C*H$_3$Si). IR: $\tilde{\nu}_{max}$ = 3408, 3007, 2961, 2835, 2176, 1768, 1601, 1505, 1347, 1245, 1097, 1030, 966, 841 cm^{-1}.

Ethyl (3*R,4*S**,*E*)-2-[3-Methoxy-2-oxo-4-(3-phenylethenyl)-3-{[3-(trimethylsilyl)prop-2-ynyl]azetidinyl}]acetate (16):** A solution of lactam **15** (5.46 g, 17.41 mmol) in dry THF (120 mL), in a two-necked flask equipped with a dropping funnel, was cooled to −30 °C and treated rapidly with ethyl bromoacetate (1.062 mL, 9.58 mmol). A freshly prepared solution of KN(SiMe$_3$)$_2$ in toluene (0.5 M, 19.16 mL, 9.58 mmol) was then added over 5 min from the dropping funnel. Another portion of ethyl bromoacetate (1.062 mL, 9.58 mmol) was then added, followed by dropwise addition of another portion of KN(SiMe$_3$)$_2$ in toluene (19.16 mL, 9.58 mmol) over 5 min. The temperature was allowed to rise to 0 °C over 1 h. The mixture was stirred at this temperature for 2 h and treated again with bromoacetate (0.70 mL, 6.3 mmol) and KN(SiMe$_3$)$_2$ (0.5 M, 12.6 mL). After further stirring for 1 h, the reaction mixture was quenched with saturated NH_4Cl, extracted with Et_2O (2 ×) and EtOAc (1 ×), and evaporated to dryness. Chromatography (PE/EtOAc, 8:2 to 7:3) gave pure **16** as an oil (4.93 g, 71%), as well as recovered **15** (394 mg, 7%). Yield from unrecovered substrate: 76%. R_f = 0.44 (PE/EtOAc, 80:20). $C_{22}H_{29}NO_4Si$ (399.56): calcd. C 66.13, H 7.32, N 3.51; found C 66.35, H 7.3, N 3.4. GC−MS: R_t = 10.53, *m/z*: 399 [$M^{+\cdot}$] (1.3), 398 (2.4), 384 (7.4), 368 (44.9), 367 (5.0), 288 (59.6), 287 (18.6), 286 (18.3), 260 (5.6), 255 (6.8), 242 (7.5), 218 (46.1), 214 (8.4), 201 (15.8), 197 (9.1), 196 (5.9), 192 (6.1), 165 (13.9), 158 (8.3), 144 (20.0), 139 (29.1), 115 (72.2), 113 (13.7), 111 (15.7), 96 (15.0), 91 (17.3), 89 (30.0), 83 (38.8), 75 (15.5), 73 (100.0), 59 (32.6), 43 (20.5), 42 (16.8). ^{1}H NMR: δ = 7.50−7.25 (m, 5 H, aromatics), 6.69 (d, *J* = 16.0 Hz, 1 H, PhC*H*=), 6.28 (dd, *J* = 8.7, 15.9, 1 H, C*H*=CHPh), 4.52 (d, *J* = 8.7 Hz, 1 H, C*H*−N), 4.19 (q, *J* = 7.2 Hz, 2 H, CH_2CH$_3$), 4.14 (d, *J* = 17.9 Hz, 1 H, C*H*HCO$_2$Et), 3.80 (d, *J* = 17.9 Hz, 1 H, C*H*HCO$_2$Et) 3.58 (s, 3 H, OCH_3), 2.93 and 2.87 (AB syst., *J* = 17.3, 2 H, CH_2C≡C), 1.27 (t, *J* = 7.2, 3 H, CH_3CH$_2$), 0.14 [s, 9 H, (CH_3)$_3$Si] ppm. ^{13}C NMR (50 MHz): δ = 167.71 and 167.47 (*C*=O), 136.10 (quat. aromatic), 136.29 and 123.15 (*C*H=*C*H), 128.59, 128.20, 126.66 (aromatic *C*H), 100.22 and 90.65 (*C*≡*C*), 87.96 (*C*−OMe), 66.27 (*C*−N), 61.51 (*C*H$_2$CH$_3$), 54.40 (O*C*H$_3$), 41.03 (N-*C*H$_2$), 22.93 (*C*H$_2$C≡C), 14.05 (*C*H$_3$CH$_2$), −0.07 (*C*H$_3$Si). IR: $\tilde{\nu}_{max}$ = 2963, 2177, 1760, 1649, 1599, 1447, 1405, 1374, 1351, 1298, 1243, 1095, 1024, 967, 840 cm^{-1}.

(3*R,4*S**,*E*)-1-(2-Hydroxyethyl)-3-methoxy-4-(3-phenylethenyl)-3-[3-(trimethylsilyl)prop-2-ynyl]azetidin-2-one (17):** A suspension of finely ground $CaCl_2$ (2.855 g, 25.73 mmol) in dry THF (60 mL) and dry EtOH (30 mL) was cooled to −20 °C and treated portionwise with $NaBH_4$ (1.622 g, 42.87 mmol). After stirring for 50 min, a solution of ester **16** (4.894 g, 12.25 mmol) in dry THF (15 mL) was added. After stirring for 5 h at the same temperature, the mixture was carefully poured into an ice-cooled Erlenmeyer flask charged with aqueous citric acid (0.5 M, 150 mL, internal temperature should not rise over +20 °C). Extraction with Et_2O (2 ×) and EtOAc (1 ×) (pH aqueous phase = 3), followed by washings with saturated aqueous $NaHCO_3$ and brine and by evaporation to dryness, afforded a crude product. It was purified by chromatography (PE/EtOAc, 60:40 to 50:50) to give pure **17** as a white solid (3.149 g, 72%). An analytical pure sample was obtained by trituration with PE/Et_2O. M.p.: 71.3−71.8 °C. R_f = 0.23 (PE/EtOAc, 60:40). $C_{20}H_{27}NO_3Si$ (357.52): calcd. C 67.19, H 7.61, N 3.92; found C 67.15, H 7.6, N 3.9. GC−MS: R_t = 10.36, *m/z*: 357 [$M^{+\cdot}$] (0.8), 356 (1.0), 342 (6.1), 326 (31.0), 270 (8.2), 256 (6.5), 255 (9.9), 246 (100.0), 245 (12.6), 244 (13.3), 201 (30.3), 197 (14.6), 196 (7.0), 176 (47.3), 165 (17.2), 158 (14.3), 154 (6.5), 144 (14.6), 130 (7.2), 115 (65.0), 111 (15.3), 96 (13.4), 91 (16.7), 89 (26.9), 83 (33.6), 81 (11.2), 73 (98.9), 59 (26.7), 45 (20.8), 43 (15.3) 399. ^{1}H NMR: δ = 7.50−7.25 (m, 5 H, aromatics), 6.72 (d, *J* = 16.0 Hz, 1 H, PhC*H*=), 6.23 (dd, *J* = 8.8, 16.0, 1 H, C*H*=CHPh), 4.36 (d, *J* = 8.8 Hz, 1 H, C*H*−N), 3.90−3.55 (m, 3 H, CH_2O*H*), 3.51 (s, 3 H, OCH_3), 3.35 (dt, *J* = 14.5, *J* = 4.7, C*H*H−N), 3.18 (dt, *J* = 14.5, *J* = 5.0, C*H*H−N), 2.90 and 2.80 (AB syst., *J* = 17.0, 2 H, CH_2C≡C), 0.16 [s, 9 H, (CH_3)$_3$Si] ppm. ^{13}C NMR (50 MHz): δ = 167.80 (*C*=O), 135.92 (quat. aromatic), 136.62 and 123.12 (*C*H=*C*H), 128.63, 128.33, 126.67 (aromatic *C*H), 100.12 and 89.26 (*C*≡*C*), 88.23 (*C*−OMe), 65.48 and 59.92 (*C*H$_2$OH and *C*HN), 54.20 (O*C*H$_3$), 45.24 (N-*C*H$_2$), 22.61 (*C*H$_2$C≡C), −0.09 (*C*H$_3$Si). IR: $\tilde{\nu}_{max}$ = 3383 (br), 3000, 2961, 2838, 2176, 1731, 1647, 1599, 1405, 1365, 1344, 1240, 1093, 1042, 968, 840 cm^{-1}.

(3*R,4*S**,*E*)-1-(2-Hydroxyethyl)-3-methoxy-4-(3-phenylethenyl)-3-(prop-2-ynyl)azetidin-2-one (18):** A solution of alcohol **17** (2.98 g, 8.335 mmol) in 96% EtOH (100 mL) was cooled to −15 °C and treated with a solution of $AgNO_3$ (2.83 g, 16.67 mmol) in H_2O (8.5 mL). Immediate precipitation was observed. The suspension was stirred for 70 min while the temperature was allowed to reach 0 °C. At this point, a solution of KCN (3.53 g, 54.18 mmol) in H_2O (20 mL) was added and the mixture was stirred vigorously for 15 minutes. After dilution with H_2O (150 mL, pH = 11.5), the mixture was extracted with Et_2O. The organic extracts were washed with a 1:1 mixture of KH_2PO_4 (1 M) and saturated NaCl, evaporated to dryness, and chromatographed (PE/EtOAc, 3:7 to 2:8) to give pure **18** as an oil (2.27 g, 95%). R_f = 0.23 (PE/EtOAc, 50:50). $C_{17}H_{19}NO_3$ (285.34): calcd. C 71.56, H 6.71, N 4.91; found C 71.35, H 6.6, N 4.85. GC−MS: R_t = 9.08, *m/z*: 285 [$M^{+\cdot}$] (0.5), 284 (2.0), 270 (4.6), 254 (10.2), 246 (29.1), 198 (10.3), 197 (17.7), 183 (8.5), 176 (29.7), 167 (19.3), 165 (19.8), 156 (40.0), 153 (10.6), 152 (7.4), 144 (11.5), 129 (30.4), 115 (100.0), 91 (18.9), 82 (18.8), 77 (12.7), 67 (51.9), 51 (8.4), 45 (12.3), 42 (9.2), 39 (45.1), 81 (12.2), 73 (107.0), 59 (28.8), 45 (22.5), 43 (16.5). ^{1}H NMR: δ = 7.50−7.25 (m, 5 H, aromatics), 6.73 (d, *J* = 15.9 Hz, 1 H, PhC*H*=), 6.25 (dd, *J* = 8.8, 16.0, 1 H, C*H*=CHPh), 4.36 (d, *J* = 8.8 Hz, 1 H, C*H*−N), 3.90−3.72 (m, 2 H, CH_2OH), 3.58−3.48 (m, 1 H, O*H*), 3.55 (s, 3 H, OCH_3), 3.40 (dt, *J* = 14.3, *J* = 4.9, C*H*H−N), 3.20 (ddd, *J* = 4.2, 6.0, 14.3, C*H*H−N) 2.86 and 2.82 (AB part of ABX syst., J_{AB} = 16.9, J_{AX} = 2.5, J_{BX} = 2.6, 2 H, CH_2C≡C), 2.13 (t, *J* = 2.6 Hz, 1 H, C≡C*H*) ppm. ^{13}C NMR (50 MHz): δ = 167.70 (*C*=O), 135.78 (quat. aromatic), 136.87 and 122.77 (*C*H=*C*H), 128.63, 128.40, 126.73 (aromatic *C*H), 88.98 (*C*−OMe), 77.89 and 71.46 (*C*≡*C*), 65.57 and 59.93 (*C*H$_2$OH and *C*HN), 54.21 (O*C*H$_3$), 45.05 (N-*C*H$_2$), 21.30 (*C*H$_2$C≡C). IR: $\tilde{\nu}_{max}$ = 3395 (broad), 3305, 2997, 2963, 2938, 2838, 1745, 1647, 1598, 1448, 1406, 1373, 1345, 1313, 1248, 1146, 1094, 1042, 967, 831 cm^{-1}.

(3*R,4*S**,*E*)-1-[2-(*tert*-Butyldimethylsilyloxy)ethyl]-3-methoxy-4-(3-phenylethenyl)-3-(prop-2-ynyl)azetidin-2-one (6):** A solution of alcohol **18** (2.622 g, 9.19 mmol) in dry DMF (15 mL) was cooled to 0 °C and treated with imidazole (1.13 g, 16.54 mmol) and *t*BuMe$_2$SiCl (1.80 g, 11.95 mmol). After 15 min the cooling bath was removed and the solution was stirred for 2 h at room temp. and then poured into saturated aqueous NH_4Cl (70 mL) + H_2O (70 mL). After extraction with Et_2O, the organic phases were treated with Et_3N (1 mL), evaporated to dryness and immediately chromatographed (PE/EtOAc, 85:15 + 0.2% of Et_3N, to PE/EtOAc, 75:25) to give pure **6** as a low-melting solid (3.59 g, 98%). R_f = 0.43 (PE/EtOAc, 80:20). $C_{23}H_{33}NO_3Si$ (399.60): calcd. C 69.13, H 8.32, N 3.51; found C 68.9, H 8.5, N 3.35. GC−MS: R_t = 10.57, *m*/*z*: 399 [M^+] (0.4), 384 (3.6), 360 (6.9), 342 (100.0), 310 (11.8), 290 (6.1), 288 (9.9), 270 (27.0), 244 (6.0), 232 (6.2), 226 (38.3), 198 (15.9), 197 (18.1), 183 (9.6), 167 (23.2), 165 (20.2), 158 (9.3), 155 (11.2), 153 (10.1), 144 (21.7), 129 (16.6), 128 (11.2), 115 (91.4), 100 (67.3), 91 (21.4), 89 (16.2), 75 (28.5), 73 (77.1), 67 (31.1), 59 (39.1), 57 (13.7), 45 (14.1), 43 (10.1), 39 (46.7). ^{1}H NMR: δ = 7.50−7.25 (m, 5 H, aromatics), 6.70 (d, *J* = 15.8 Hz, 1 H, PhC*H*=), 6.25 (dd, *J* = 8.6, 16.0, 1 H, C*H*=CHPh), 4.41 (d, *J* = 8.6 Hz, 1 H, C*H*−N), 3.82−3.68 (m, 2 H, CH_2OSi), 3.56 (s, 3 H, OCH_3), 3.53 (dt, *J* = 13.8, *J* = 5.2, C*H*H−N), 3.14 (ddd, *J* = 5.4, 6.8, 14.0, C*H*H−N) 2.85 and 2.78 (AB part of ABX syst., J_{AB} = 17.1, J_{AX} = 2.6, J_{BX} = 2.8, 2 H, CH_2C≡C), 2.07 (t, *J* = 2.7 Hz, 1 H, C≡C*H*), 0.90 [s, 9 H, (CH_3)$_3$], 0.06 [s, 6 H, (CH_3)$_2$Si].

(3*R,4*S**)-1-[2-(*tert*-Butyldimethylsilyloxy)ethyl]-4-hydroxymethyl-3-methoxy-3-(prop-2-ynyl)azetidin-2-one (5):** A solution of alkene **6** (3.277 g, 8.20 mmol) in dry CH_2Cl_2 (80 mL) was treated with dry MeOH (40 mL) and with Sudan Red (Solvent Red 23, 10 mg) and cooled to −78 °C. It was then ozonolysed for about 20 min, until the red colour started to fade. Ozone production was terminated at once, and the solution was immediately treated with Me_2S (1.5 mL) + cyclohexene (1.0 mL) in CH_2Cl_2 (2 mL). After 7 min, $NaBH_4$ (1.24 g, 32.80 mmol) was added and the apparatus was put under nitrogen. The temperature was allowed to rise to 0 °C over 2 h and the solution was carefully poured into saturated NH_4Cl (150 mL) + citric acid (0.5 M, 20 mL), cooled to 0 °C (hydrogen evolution!). Most of the CH_2Cl_2 and methanol were evaporated and the aqueous phase was extracted with Et_2O (2 ×) and EtOAc (2 ×). Evaporation to dryness and chromatography afforded pure **5** as an oil (2.46 g, 92%). R_f = 0.43 (PE/EtOAc, 60:40). $C_{16}H_{29}NO_4Si$ (327.49): calcd. C 58.68, H 8.93, N 4.28; found C 59.0, H 8.75, N 4.1. GC−MS: R_t = 7.92, *m*/*z*: 312 (0.8), 270 (99.7), 242 (13.3), 216 (5.9), 202 (7.7), 144 (15.3), 126 (100.0), 119 (5.0), 118 (6.1), 116 (5.1), 111 (27.3), 109 (36.8), 108 (6.6), 100 (71.2), 97 (19.1), 95 (75.0), 94 (10.1), 89 (16.6), 87 (61.7), 83 (21.0), 75 (63.6), 73 (68.0), 67 (34.0), 66 (26.2), 59 (34.3), 45 (18.6), 43 (40.3), 41 (20.8), 39 (30.5). ^{1}H NMR: δ = 3.96−3.68 (m, 5 H, C*H*N, CH_2O), 3.60 (s, 3 H, OCH_3), 3.41 (t, *J* = 4.9, CH_2−N), 2.99 (t, *J* = 6.6 Hz, 1 H, O*H*), 2.86 and 2.69 (AB part of ABX syst., J_{AB} = 17.2, J_{AX} = 2.6, J_{BX} = 2.6, 2 H, CH_2C≡C), 2.06 (t, *J* = 2.6 Hz, 1 H, C≡C*H*), 0.91 [s, 9 H, (CH_3)$_3$], 0.10 [s, 6 H, (CH_3)$_2$Si] ppm. ^{13}C NMR (50 MHz): δ = 167.04 (*C*=O), 88.03 (*C*−OMe), 77.69 and 71.19 (*C*≡*C*), 64.12, 61.93, 60.53 (*C*H$_2$O and *C*HN), 54.23 (O*C*H$_3$), 43.07 (N-*C*H$_2$), 25.80 [C(*C*H$_3$)$_2$], 21.00 (*C*H$_2$C≡C), 18.22 [*C*(CH$_3$)$_3$], −5.54 (Si*C*H$_3$). IR: $\tilde{\nu}_{max}$ = 3426 (broad), 3305, 2998, 2882, 2858, 1756, 1600, 1462, 1406, 1361, 1313, 1241, 1099, 1046, 1005, 938, 826 cm^{-1}.

(3*R,4*S**,*Z*)-1-[2-(*tert*-Butyldimethylsilyloxy)ethyl]-4-hydroxymethyl-3-methoxy-3-[7-(trimethylsilyl)hept-4-ene-2,6-diyn-1-yl]azetidin-2-one (20):** General remark: maximum care to avoid the presence of oxygen should be taken during this reaction. Palladium bis(benzonitrile)dichloride (168 mg, 0.437 mmol) and CuI (83 mg, 0.437 mmol) were suspended under argon in dry THF (15 mL) and treated with piperidine (6.5 mL) and then with trimethylsilylacetylene (0.185 mL, 1.31 mmol). After 15 min, (*Z*)-1-chloro-4-trimethylsilylbut-1-en-3-yne[28] (1.19 mL, 7.0 mmol) was added. After 30 min, the alkyne **5** (1.430 g, 4.37 mmol) dissolved in THF (5 mL) was introduced. After stirring for 3 h at room temp., the black solution was poured into a mixture of saturated aqueous NH_4Cl (50 mL) and HCl (1 N, 52 mL). After extraction with Et_2O, the organic layer was washed with sat. NH_4Cl, evaporated and chromatographed (PE/EtOAc, 80:20 to 60:40) to give pure **20** as an oil (1.612 g, 82%). R_f = 0.68 (PE/EtOAc, 60:40), 0.26 (CH_2Cl_2/PE/EtOAc, 45:45:10). $C_{23}H_{39}NO_4Si_2$ (449.73): calcd. C 61.42, H 8.74, N 3.11; found C 61.1, H 8.95, N 3.0. GC−MS: R_t = 10.90, *m*/*z*: 449 [M^+] (0.1), 434 (1.8), 392 (27.0), 233 (13.6), 230 (5.2), 217 (9.3), 215 (6.6), 203 (10.5), 202 (22.0), 201 (8.5), 175 (20.8), 159 (8.2), 144 (10.0), 115 (8.6), 100 (22.9), 89 (15.4), 75 (34.5), 73 (100.0), 59 (12.3). ^{1}H NMR: δ = 5.81 (s, 2 H, C*H*=C*H*), 3.94−3.66 (m, 5 H, C*H*N, CH_2O), 3.61 (s, 3 H, OCH_3), 3.39 (t, *J* = 5.2, CH_2−N), 3.00 (t, *J* = 6.7 Hz, 1 H, O*H*), 3.11 and 2.88 (AB part of ABX syst., J_{AB} = 17.4, J_{AX} = 1.3, J_{BX} = 1.6, 2 H, CH_2C≡C), 0.91 [s, 9 H, (CH_3)$_3$], 0.23 [s, 9 H, SiCH_3)$_3$], 0.09 [s, 6 H, (CH_3)$_2$Si] ppm. ^{13}C NMR (50 MHz): δ = 167.28 (*C*=O), 120.08 and 119.67 (*C*H=*C*H), 102. 77, 102. 02, 91.63 and 80.59 (*C*≡*C*), 88.29 (*C*−OMe), 64.32 (*C*HN), 61.75 and 60.64 (*C*H$_2$O), 54.31 (O*C*H$_3$), 43.30 (N-*C*H$_2$), 25.91 [C(*C*H$_3$)$_3$], 22.45 (*C*H$_2$C≡C), 18.31 [*C*(CH$_3$)$_3$], −0.12 [(*C*H$_3$)$_3$Si], −5.42 (Si*C*H$_3$). IR: $\tilde{\nu}_{max}$ = 3407 (broad), 3037, 2953, 2931, 2858, 1749, 1597, 1463, 1406, 1360, 1312, 1193, 1097, 1042, 1015, 938, 837 cm^{-1}.

(3*R,4*S**,*Z*)-1-[2-(*tert*-Butyldimethylsilyloxy)ethyl]-4-hydroxymethyl-3-methoxy-3-[7-(trimethylsilyl)hept-4-ene-2,6-diyn-1-yl]azetidin-2-one (22):** Compound **20** (1.471 g, 3.27 mmol) was desilylated to provide **21** by the same procedure as used for the synthesis of **18**. This time the reaction time was only 20 minutes. Chromatography (PE/EtOAc, 65:35 + 0.5% Et_3N, to PE/EtOAc, 55:45) gave **21**, pure by TLC (1.034 g). Prior to evaporation to dryness, 3-*tert*-butyl-4-hydroxy-5-methylphenylsulfide (5 mg) was added as a stabilizer. Compound **21** is somewhat unstable in the dry state and was therefore kept in solution at −26 °C and used as soon as possible for the next reaction. R_f = 0.45 (CH_2Cl_2/toluene/EtOAc, 1:1:1).

Compound **21** was taken up in dry benzene (15 mL) and added to a preformed I_2−morpholine complex suspension (prepared by treatment of I_2 (3.428 g, 13.5 mmol) with morpholine (3.53 mL, 40.5 mmol) in benzene (25 mL) and stirring at room temp. for 1 h). The mixture was stirred for 9 h at room temp. and poured into a solution of $NH_4H_2PO_4$ (3 g) and HCl (36%, 3.5 mL) in H_2O (50 mL). $Na_2S_2O_5$ solution (0.5 M, 40 mL) was added and the mixture was vigorously shaken and extracted with Et_2O. The organic extracts were washed with $Na_2S_2O_5$ (0.5 M), saturated $NaHCO_3$ and brine. Evaporation followed by immediate chromatography (PE/EtOAc, 65:35 + 0.5% Et_3N, to PE/EtOAc, 50:50) gave pure **22** as an oil (1.298 g, 79% from **20**). It is also recommended to keep **22** in solution at low temperature in the presence of a stabilizer in this case. R_f = 0.49 (CH_2Cl_2/toluene/EtOAc, 1:1:1). This compound was not stable in GC at the required temperatures. ^{1}H NMR: δ = 5.92 (d, *J* = 10.6 Hz, 1 H, C*H*=CH), 5.76 (dt, *J* = 10.6, *J* = 2.2, 1 H, C*H*=CH), 3.94−3.66 (m, 5 H, C*H*N, CH_2O), 3.63 (s, 3 H, OCH_3), 3.41 (t, *J* = 5.3, CH_2−N), 3.01 (dd, *J* = 6.2, 7.8 Hz, 1 H, O*H*), 3.10 and 2.91 (AB part of ABX syst., J_{AB} = 17.5, J_{AX} = 2.0, J_{BX} = 2.2, 2 H, CH_2C≡C), 0.91 (s, 9 H, (CH_3)$_3$], 0.10 (s, 6 H,

(CH_3)$_2$Si) ppm. ^{13}C NMR (50 MHz): δ = 167.27 (*C*=O), 121.72 and 119.62 (*C*H=*C*H), 91.81, 91.66, 80.24 and 14.28 (*C*≡*C*), 88.14 (*C*−OMe), 64.20 (*C*HN), 61.84 and 60.60 (*C*H$_2$O), 54.36 (O*C*H$_3$), 43.20 (N-*C*H$_2$), 25.89 [C(*C*H$_3$)$_3$], 22.37 (*C*H$_2$C≡C), 18.30 [*C*(CH$_3$)$_3$], −5.43 (Si*C*H$_3$). IR: $\tilde{\nu}_{max}$ = 3458 (broad), 3058, 2953, 2858, 1745, 1582, 1405, 1361, 1193, 1097, 928, 816 cm^{-1}.

(1*R,9*R**,10*S**,*Z*)-11-[2-(*tert*-Butyldimethylsilyloxy)ethyl]-9-hydroxy-1-methoxy-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (4):** A solution of dimethyl sulfoxide (0.986 mL, 13.9 mmol) in dry CH_2Cl_2 (35 mL) was cooled to −78 °C and treated with a solution of $(COCl)_2$ in CH_2Cl_2 (2.6 M, 3.33 mL, 8.66 mmol). After 10 min, a solution of alcohol **22** (1.746 g, 3.47 mmol) in CH_2Cl_2 (21 mL) was added over 3 min. After 15 min, the suspension was treated with Et_3N (3.15 mL, 22.6 mmol). The temperature was allowed to rise to −55 °C over 40 min. The reaction mixture was poured into a solution of $NH_4H_2PO_4$ (4.83 g) in H_2O (70 mL). After extraction with PE/Et_2O, 1:1, (1 ×) and Et_2O (2 ×) the organic layers were washed with $NaHCO_3$ (5%) and brine. Evaporation to dryness and chromatography (PE/EtOAc, 60:40) afforded pure aldehyde **23** (1.66 g). This was taken up with dry toluene and evaporated again twice in order to dehydrate it. It was finally taken up in dry THF (35 mL) and treated with freshly activated powdered molecular sieves (4 Å, 50 mg). Meanwhile, $CrCl_2$ (2.016 g, 16.4 mmol) and $NiCl_2$ (63 mg, 0.49 mmol) were suspended under argon in dry THF (70 mL). The solution of aldehyde **23** was added to this suspension by dropping funnel over 1 h at room temp. After stirring for 4 h at room temp., the mixture was poured into H_2O (80 mL) and extracted with Et_2O. The organic phase was washed with 5% $NaHCO_3$ and brine, evaporated, and finally chromatographed (PE/Et_2O, 35:65 to 30:70) to give pure **4** as a white solid (807 mg, 62% from **22**). An analytically pure sample was obtained by trituration with Et_2O/PE. M.p.: 150−151 °C. R_f = 0.40 (PE/Et_2O, 40:60). $C_{20}H_{29}NO_4Si$ (375.53): calcd. C 63.97, H 7.78, N 3.73; found C 64.05, H 7.85, N 3.65. GC−MS: R_t = 10.52, *m*/*z*: 375 [M$^+$] (0.3), 360 (3.0), 318 (100.0), 174 (60.4), 173 (22.8), 159 (10.2), 145 (65.7), 144 (6.9), 141 (11.3), 131 (44.2), 130 (14.4), 115 (30.5), 103 (52.2), 100 (34.4), 89 (21.1), 77 (23.6), 59 (21.9), 45 (12.9). ^{1}H NMR: δ = 5.95−5.80 (m, 2 H, C*H*=C*H*), 4.85 (dd, *J* = 4.8, 8.8 Hz, 1 H, C*H*OH), 4.44 (d, *J* = 4.8 Hz, 1 H, O*H*), 3.97 (d, *J* = 8.8 Hz, 1 H, C*H*N), 3.94−3.64 (m, 3 H, C*H*$_2$O and C*H*HN), 3.59 (s, 3 H, OC*H*$_3$), 3.31 (ddd, *J* = 4.0, 11.0, 15.0, C*H*HN), 3.18 (dd, *J* = 1.4, 18.0, 1 H, C*H*HC≡C), 2.69 (d, *J* = 18.0, 1 H, C*H*HC≡C), 0.93 [s, 9 H, (C*H*$_3$)$_3$], 0.15 [s, 6 H, (C*H*$_3$)$_2$Si] ppm. ^{13}C NMR (50 MHz): δ = 168.02 (*C*=O), 123.91 and 122.72(*C*H=*C*H), 97.76, 96.08, 89.70, 87.59 and 83.83 (*C*≡*C* and *C*−OMe), 70.68 (*C*HOH), 63.24 (*C*HN), 62.80 (*C*H$_2$O), 54.10 (O*C*H$_3$), 44.28 (N-*C*H$_2$), 25.98 [C(*C*H$_3$)$_3$], 21.93 (*C*H$_2$C≡C), 18.52 [*C*(CH$_3$)$_3$], −5.45, −5.52 (Si*C*H$_3$).

(1*R,9*R**,10*S**,*Z*)-9-Acetoxy-11-[2-(*tert*-butyldimethylsilyloxy)ethyl]-1-methoxy-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (24):** A solution of alcohol **4** (112.3 mg, 0.30 mmol) in dry CH_2Cl_2 (1 mL) was treated in sequence with 4-dimethylaminopyridine (6 mg), pyridine (1 mL) and acetic anhydride (85 μL, 0.90 mmol). After the mixture had been stirred for 30 min at room temp., *n*-heptane (15 mL) was added, and the mixture was evaporated to dryness and immediately chromatographed (PE/Et_2O, 40:60) to give pure **24** as a white solid (124.5 mg, 100%). R_f = 0.48 (PE/Et_2O, 40:60). GC-MS: R_t = 10.47, *m*/*z*: 402 [M$^{+\cdot}$ − 15] (2.8), 360 (77.2), 268 (19.7), 174 (34.3), 173 (8.2), 159 (11.4), 146 (9.6), 145 (29.4), 144 (40.5), 141 (5.3), 131 (47.9), 130 (6.4), 128 (6.2), 127 (5.2), 117 (13.8), 116 (8.7), 115 (17.3), 114 (9.0), 113 (8.6), 103 (10.7), 102 (12.1), 101 (10.4), 100 (31.1), 89 (13.3), 77 (9.2), 76 (5.3), 75 (38.9), 74 (7.7), 73 (56.1), 63 (6.5), 59 (18.4), 57 (8.2), 51 (5.3), 45 (10.5), 43 (100), 41 (9.2). ^{1}H NMR: δ = 5.88 (s, 2 H, C*H*=C*H*), 5.63 (d, *J* = 9.6 Hz, 1 H, C*H*−OAc), 4.26 (d, *J* = 9.6 Hz, 1 H, C*H*−N), 3.85−3.55 (m, 3 H, C*H*$_2$OSi, C*H*H−N), 3.59 (s, 3 H, OC*H*$_3$), 3.19 and 2.70 (AB syst., 2 H, *J* = 18.3, C*H*$_2$−C≡C), 3.06 (dt, *J* = 10.2, *J* = 4.6, 1 H, C*H*H−N−C=O), 2.11 (s, 3 H, C*H*$_3$−C=O), 0.90 [s, 9 H, C(C*H*$_3$)$_3$], 0.08 and 0.07 [2 s, 2 × 3 H, (C*H*$_3$)$_2$Si] ppm. ^{13}C NMR (50 MHz.): δ = 169.32 and 167.94 (*C*=O), 124.81 and 122.23 (*C*H=*C*H), 96.43, 94.59, 89.75, 88.31 and 83.74 (*C*≡*C* and *C*OMe), 65.71 and 65.93 (*C*H−OAc and *C*H−N), 59.36 (*C*H$_2$−OSi), 54.21 (O*C*H$_3$), 44.10 (*C*H$_2$−N), 25.81 [C(*C*H$_3$)$_3$], 22.19 (*C*H$_2$−C≡C), 20.90 (*C*H$_3$−C=O), 18.17 [*C*(CH$_3$)$_3$], −5.40 [(*C*H$_3$)$_2$Si]. IR: $\tilde{\nu}_{max}$ 3033, 2955, 2929, 2856, 1753, 1604, 1398, 1372, 1320, 1193, 1094, 1019, 962, 910 cm^{-1}.

(1*R,9*R**,10*S**,*Z*)-9-Acetoxy-11-(2-hydroxyethyl)-1-methoxy-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (25):** A solution of silyl ether **24** (124.5 mg, 0.298 mmol) in acetonitrile (2.4 mL) was cooled to 0 °C and treated with 40% aqueous HF (120 μL). The resulting solution was stirred for 135 min at 0 °C and was then poured into $NaHCO_3$ (5%, 20 mL). Extraction with EtOAc, evaporation, and chromatography (PE/EtOAc, 20:80, to EtOAc) gave pure **25** as a white solid (87.0 mg, 96%). R_f = 0.24 (PE/EtOAc, 1:2). GC-MS: R_t = 9.32, *m*/*z*: 216 (8.8), 201 (10.5), 175 (10.7), 174 (78.2), 173 (14.7), 159 (22.4), 158 (7.5), 156 (8.1), 146 (12.9), 145 (46.4), 144 (65.0), 143 (7.7), 142 (5.9), 140 (6.4), 132 (10.9), 131 (86.8), 130 (13.4), 129 (6.2), 128 (5.6), 127 (7.0), 126 (6.4), 117 (6.2), 116 (11.8), 115 (29.4), 114 (12.7), 113 (12.4), 103 (20.0), 102 (15.6), 91 (6.2), 89 (7.9), 88 (6.0), 87 (6.0), 77 (11.3), 75 (7.4), 63 (16.4), 51 (9.4), 45 (25.2), 44 (7.7), 43 (100). ^{1}H NMR: δ = 5.89 (s, 2 H, C*H*=C*H*), 5.65 (d, *J* = 9.6 Hz, 1 H, C*H*−OAc), 4.22 (d, *J* = 9.6 Hz, 1 H, d, C*H*−N), 3.84 (broad t, *J* = 4.8, 1 H, C*H*$_2$OH), 3.59 (s, 3 H, OC*H*$_3$), 3.54 (dt, *J* = 5.7, *J* = 10.8, 1 H, C*H*H−N), 3.21 and 2.77 (AB syst., *J* = 18.0, 2 H, C*H*$_2$−C≡C), 3.22 (dt, *J* = 10.8, *J* = 4.5, 1 H, C*H*H−N−C=O), 2.12 (s, 3 H, C*H*$_3$−C=O) ppm. ^{13}C NMR (50 MHz.): δ = 169.35 and 168.63 (*C*=O), 124.82 and 122.28 (*C*H=*C*H), 96.20, 94.35, 89.18, 88.42, and 83.96 (*C*≡*C* and *C*OMe), 66.31 and 65.52 (*C*H−OAc and *C*H−N), 59.32 (*C*H$_2$−OH), 54.13 (O*C*H$_3$), 46.64 (*C*H$_2$−N), 22.05 (C*H*$_2$−C≡C), 20.91 (C*H*$_3$−C=O). IR: $\tilde{\nu}_{max}$ = 3408, 3000, 1739, 1600, 1509, 1402, 1372, 1192, 1090 cm^{-1}.

(1*R,9*R**,10*S**,*Z*)-9-Acetoxy-11-(2-azidoethyl)-1-methoxy-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (27):** A solution of alcohol **25** (84 mg, 0.277 mmol) in dry CH_2Cl_2 (2 mL) was cooled to −30 °C and treated in sequence with Et_3N (154 μL, 1.108 mmol) and methanesulfonyl chloride (43 μL, 0.554 mmol). After 50 min the solution was poured into saturated aqueous NH_4Cl. Extraction with Et_2O and evaporation gave crude mesylate **26**. This was taken up in dry DMF (1 mL) and treated with NaN_3 (54 mg, 0.831 mmol). The solution was stirred for 135 min at 50 °C, cooled, poured into a 2:1 mixture of saturated aqueous NH_4Cl and H_2O and extracted with Et_2O. Evaporation and chromatography (CH_2Cl_2/EtOAc, 1:1) afforded pure **27** as an oil (79 mg, 87%). R_f = 0.39 (CH_2Cl_2/EtOAc, 1:1). GC-MS: R_t = 9.52, *m*/*z*: 300 [M$^{+\cdot}$ − 28] (10.2), 201 (6.6), 186 (5.5), 174 (37.2), 173 (9.0), 169 (6.0), 159 (13.5), 156 (7.8), 146 (7.7), 145 (24.9), 144 (47.7), 143 (6.5), 142 (6.7), 141 (6.7), 132 (6.4), 131 (54.0), 130 (8.3), 128 (5.2), 127 (6.4), 116 (9.7), 115 (22.3), 114 (10.0), 113 (10.3), 103 (11.9), 102 (11.9), 89 (8.3), 77 (10.1), 76 (5.0), 75 (7.6), 69 (17.6), 63 (14.6), 62 (6.1), 51 (10.2), 44 (6.3), 43 (100), 42 (20.0). ^{1}H NMR: δ = 5.89 (s, 2 H, C*H*=C*H*), 5.63 (d, *J* = 9.6 Hz, 1 H, d, C*H*−OAc), 4.23 (d, *J* = 9.6 Hz, 1 H, C*H*−N), 3.80−3.40 (m, 3 H, m, CH_2N_3 and C*H*H−N−C=O), 3.59 (s, 3 H, OC*H*$_3$), 3.33−3.12 (m, 1 H,

C*H*H−N−C=O), 3.22 and 2.76 (AB syst., J = 18.0, 2 H, CH_2−C≡C), 2.14 (s, 3 H, CH_3−C=O) ppm. ^{13}C NMR (50 MHz.): δ = 169.24 and 167.69 (*C*=O), 124.87 and 122.26 (*C*H=*C*H), 96.17, 94.23, 89.93, 88.44, and 83.93 (*C*≡*C* and *C*OMe), 66.18 and 65.64 (*C*H−OAc and *C*H−N), 54.20 (O*C*H_3), 48.49 and 41.29 (*C*H_2−N), 21.93 (*C*H_2−C≡C), 20.90 (*C*H_3−C=O). IR: $\tilde{\nu}_{max}$ = 3003, 2963, 2939, 2108, 1760, 1598, 1395, 1372, 1350, 1187, 1092, 1015, 961, 908 cm^{-1}.

(1*R,9*R**,10*S**,*Z*)-9-Acetoxy-11-[2-(*tert*-butoxycarbonylamino)ethyl]-1-methoxy-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (28):** A solution of azide **27** (77 mg, 0.223 mmol) in THF (6 mL) was treated at room temperature with water (1 mL) and triphenylphosphane (92 mg, 0.353 mmol). After stirring for 8 h at room temp., the solution was cooled to 0 °C and treated in sequence with Et_3N (164 μL, 1.176 mmol) and with 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON, 172 mg, 0.706 mmol). After 30 min at 0 °C and 15 min at room temp., the mixture was poured into aqueous $NH_4H_2PO_4$ (5%) and extracted with EtOAc. The organic extracts were washed with brine, evaporated to dryness and chromatographed (PE/EtOAc, 60:40 to 50:50) to give pure **28** as a white solid (80.8 mg, 85%). R_f = 0.43 (PE/EtOAc, 50:50). GC-MS: R_t = 10.88, *m*/*z*: 346 [M$^{+\cdot}$ − 56] (1.5), 241 (4.7), 202 (5.8), 201 (9.9), 175 (7.4), 174 (60.2), 173 (16.2), 159 (22.6), 156 (10.7), 146 (13.1), 145 (48.0), 144 (77.3), 132 (9.5), 131 (87.8), 130 (8.9), 116 (11.7), 115 (22.5), 114 (11.2), 113 (10.2), 103 (13.7), 102 (9.3), 88 (9.2), 77 (9.7), 70 (8.8), 63 (9.7), 59 (16.6), 57 (66.2), 56 (10.0), 44 (24.0), 43 (100), 42 (11.2), 41 (34.7), 39 (11.2). ^{1}H NMR: δ = 5.88 (s, 2 H, C*H*=C*H*), 5.61 (d, J = 9.5 Hz, 1 H, C*H*−OAc), 4.91 (broad s, 1 H, N*H*), 4.31 (d, J = 9.5 Hz, 1 H, C*H*−N), 3.65−3.40 and 3.35−3.08 (2 m, 2 × 2 H, CH_2N), 3.58 (s, 3 H, OCH_3), 3.16 and 2.80 (AB syst., J = 18.4, 2 H, CH_2−C≡C), 2.14 (s, 3 H, CH_3−C=O), 1.44 [s, 9 H, C(CH_3)$_3$] ppm. ^{13}C NMR (50 MHz.): δ = 169.45, 168.08 and 155.95 (*C*=O), 124.79 and 122.27 (*C*H=*C*H), 96.36, 94.39, 89.68, 88.32, and 83.90 (*C*≡*C* and *C*OMe), 79.83 [*C*(CH$_3$)$_3$], 65.73 (*C*H−OAc and *C*H−N), 54.11 (O*C*H_3), 42.85 and 38.14 (*C*H_2−N), 28.38 [C(*C*H_3)$_3$], 21.60 (*C*H_2−C≡C), 20.92 (*C*H_3−C=O).

(1*R,9*R**,10*S**,*Z*)-11-[2-(*tert*-Butoxycarbonylamino)ethyl]-9-hydroxy-1-methoxy-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (29):** A solution of acetate **28** (28.0 mg, 69.6 μmol) in dry THF (1 mL) was treated with dry MeOH (3 mL), cooled to 0 °C, and a solution of MeONa in MeOH (1 M, 80 μL, 80 μmol) was added. After 40 minutes, acetic acid in MeOH (1 M, 120 μL, 120 μmol) was added. Evaporation to dryness and chromatography (PE/EtOAc, 50:50, to PE/EtOAc, 40:60) gave pure **29** as a white solid (23.1 mg, 92%). R_f = 0.33 (PE/EtOAc, 50:50). GC-MS: R_t = 10.66, *m*/*z*: 304 [M$^{+\cdot}$ − 56] (4.1), 241 (5.2), 202 (12.8), 175 (10.9), 174 (87.8), 173 (44.4), 172 (6.3), 160 (5.4), 159 (18.3), 158 (5.6), 146 (16.7), 145 (92.2), 144 (6.5), 143 (12.8), 142 (7.9), 132 (9.4), 131 (83.6), 130 (22.7), 129 (6.5), 117 (10.4), 116 (13.4), 115 (52.9), 114 (12.2), 113 (7.5), 104 (10.1), 103 (99.5), 102 (19.0), 99 (6.8), 91 (7.4), 89 (20.0), 88 (13.7), 87 (7.7), 77 (35.5), 76 (5.6), 75 (6.7), 70 (14.1), 63 (20.0), 59 (26.2), 58 (6.7), 57 (100), 56 (10.6), 55 (9.2), 51 (14.8), 44 (27.4), 43 (14.0), 42 (16.0), 41 (50.6), 39 (19.6). ^{1}H NMR: δ = 5.88 (s, 2 H, C*H*=C*H*), 4.98 (broad s, 1 H, N*H*), 4.81 (dd, J = 6.2, 8.9 Hz, 1 H, C*H*−OH), 4.05 (d, J = 9.2 Hz, 1 H, C*H*−N), 3.57 (s, 3 H, OCH_3), 3.70−3.25 (m, 5 H, CH_2N, O*H*), 3.14 and 2.73 (AB syst., J = 18.3, 2 H, CH_2−C≡C), 1.44 [s, 9 H, C(CH_3)$_3$] ppm. ^{13}C NMR (50 MHz.): δ = 168.87 and 156.63 (*C*=O), 124.18 and 122.52 (*C*H=*C*H), 98.12, 96.46, 89.41, 87.58 and 83.82 (*C*≡*C* and *C*OMe), 80.06 [*C*(CH$_3$)$_3$], 69.88 and 63.20 (*C*H−OH e *C*H−N), 54.16 (O*C*H_3), 42.57 and 38.72 (*C*H_2−N), 28.39 [C(*C*H_3)$_3$], 21.84 (*C*H_2−C≡C).

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).

Boc derivatives **28** and **29** (50 μmol) were dissolved in dry CH_2Cl_2 (0.50 mL) and treated at 0 °C with CF_3CO_2H (0.25 mL) for 60 min. The solvent was evaporated to dryness, and traces of CF_3CO_2H were eliminated by azeotropic evaporation with *n*-heptane. The resulting crude **30** and **31** were dissolved in 96% EtOH (1.00 mL) to provide 0.05 M solutions. These solutions were pretreated, just before incubation, with aqueous NaOH (1 N, 4 μL for every 100 μL). They were then diluted to the desired concentration with EtOH (96%). The fact that these solutions contained compounds **30** and **31** in good purity was assessed by TLC (no Boc derivative was present) and by conversion of an aliquot of each solution (500 μL) back into **28** or **29** by treatment with Boc-ON and Et_3N. This transformation gave the expected products in 80−90% yield. Solutions of compounds **28** and **29** (0.05 M) were obtained by dissolving them in DMSO (they are not very soluble in EtOH). They were diluted with DMSO to the desired concentrations just before incubation.

Plasmid pBR 322 (Fermentas, 90% in form I, 500 μg/mL) was diluted (1:10) with a pH 7.5 TRIS (40 mM)/EDTA (4 mM) buffer (prepared with molecular biology water) to provide a concentration of 50 μg/mL, 75 μM/bp. This solution (18 μL) was treated with the appropriately diluted lactenediyne solution (2 μL). For example, to provide a final lactenediyne concentration of 10^{-3} M, 2 μL of a 10^{-2} M solution was added. The resulting mixtures were incubated at 37 °C for 24 h. At the end of this period, the solutions were treated with loading buffer (15 μL) and analysed on agarose gel (prepared from agarose (300 mg), working buffer (32 mL), and ethidium bromide (1.5 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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