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# Synthesis and DNA-cleaving activity of lactenediynes conjugated with DNA-complexing moieties

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**Abstract**—Lactenediynes are compounds characterized by the fusion of a β-lactam with a cyclodeca-3-ene-1,5-diyne. In this work the most promising members of this family have been activated by attaching a carbalkoxy or a carbamoyl group to the azetidinone nitrogen, and conjugated to various DNA-complexing moieties, either acting by intercalation or through groove binding. These conjugated artificial enediynes have been demonstrated to possess in vitro ability to produce single and double strand cleavage of plasmid DNA. As potency and capacity to induce double cut, they rank among the best simple enediyne analogues ever prepared. A thorough investigation was carried out in order to develop the best suited linkers for assembling these conjugates.


## 1. Introduction

The natural enediyne antibiotics are among the most potent anti-cancer chemotherapeutic agents known to date.[1,2] They act through an unique mechanism, involving direct radical attack on cellular DNA. Particularly noteworthy is the ability to induce not only single but also simultaneous double strand DNA cleavage, leading to apoptosis. The high potency of the natural compounds (e.g., Calicheamicin, Fig. 1)[3] is counterbalanced by their poor selectivity, making them unsuitable for clinical use as such. In order to solve this problem, Calicheamicin has been conjugated to a selective antibody. The resulting conjugate (Gemtuzumab, Mylotarg™) has shown very promising activity towards some types of previously intractable tumours.[4]

However, the high structural complexity of natural enediynes makes highly desirable the development of simpler analogues operating through a similar mode of action.[5] Towards this goal some years ago we designed an original class of simplified 'artificial' enediynes, called *lactenediynes*, and characterized by the fusion of a 10-membered enediyne ring with a β-lactam. Three different classes of lactenediynes have been prepared[6] or approached[7] so far. Among them, the most useful seems to be the one characterized by the *trans* fusion of a β-lactam with atoms 8,9 of a 10-membered cyclodeca-3-ene-1,5-diyne, depicted in Scheme 1 as general formula **1**. First of all these compounds are very stable in the dry state, contrary to what often happens with simple enediyne compounds, including other type of lactenediynes. In these compounds, the β-lactam ring behaves as a

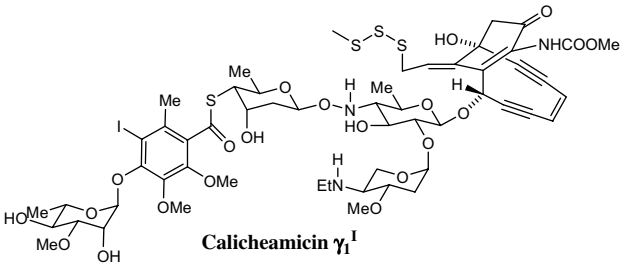


**Figure 1.**

*Keywords*: Enediynes; Antitumor; DNA cleavage; β-Lactams; DNA intercalating agents; DNA groove binders; Camptothecin.
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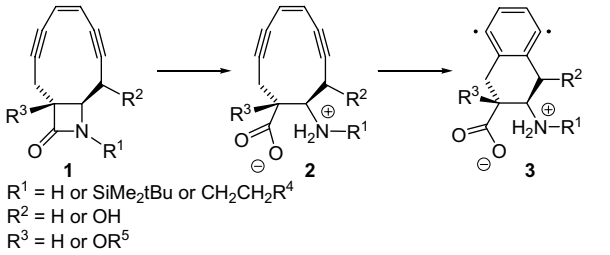



**Scheme 1.**

safety-catch, completely suppressing cycloaromatization of the 10-membered enediyne moiety, whereas opening of the β-lactam ring to **2** unlashes the typical reactivity of monocyclic cyclodeca-3-ene-1,5-diynes, leading to the formation of diradical **3**.

During the past 10 years, we have prepared several compounds of general formula **1**[8–13] and validated the chemical principle that lays behind their design. An important difference among the various substances prepared so far is represented by the number of 'handles', that is the attachment points that can be used for joining appropriate substituents or substructures. These handles can be used to append 'activating' substituents, 'triggering' devices, or DNA-complexing structures ('delivery units'). We have prepared compounds provided with 1–3 handles, represented by substituents $R^1$, $R^2$ and $R^3$.

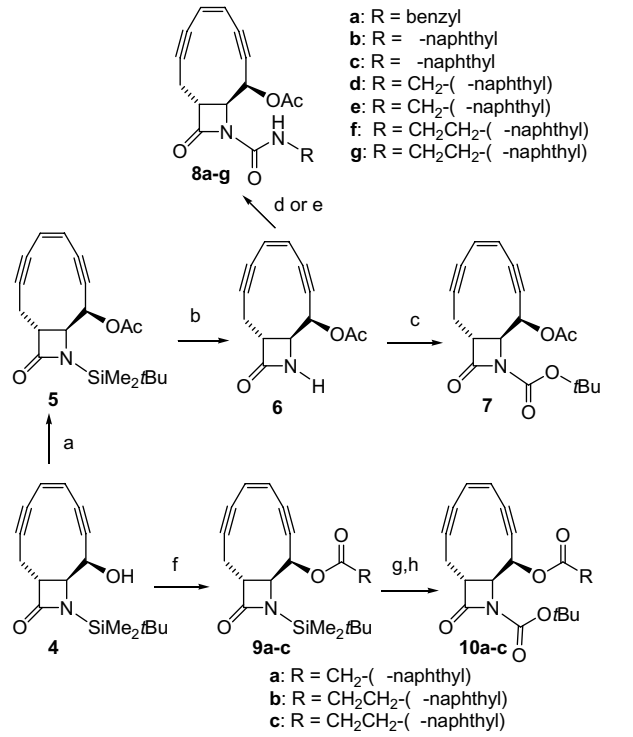
The β-lactam is normally too stable to undergo spontaneous opening under physiological conditions. In order to circumvent this limitation we are following two alternative routes.

In the first one we take advantage of an intramolecular opening of the β-lactam by a suitable nucleophile (an amino group) attached to one of the handles. In particular, compounds **1** with $R^1 = CH_2CH_2NH_2$ were demonstrated to be effective DNA-cleaving agents, whereas protection of the amino group completely suppresses this activity. Although the potency was not impressive, those results were promising in view of selective prodrug activation.[11,13]

In this paper, we report instead the results of a second, alternative, approach, that involves the attachment of appropriate activating substituents to the β-lactam nitrogen. Moreover, the cleaving activity has been improved by joining also DNA-complexing moieties to the basic scaffold. This study has led to very efficient enediyne prodrugs, capable to induce both single and double strand DNA cleavage at concentrations as low as $10^{-7}$ M.

## 2. Results and discussion

In principle, activation of a β-lactam towards hydrolysis can be achieved by placing on the lactam nitrogen an electron withdrawing group. This activation has been exploited, for example, in the development of monocyclic β-lactam antibiotics, such as monobactams[14] or




**Scheme 2.** Reagents: (a) $Ac_2O$, pyridine, DMAP, 96%; (b) 40% aq HF/MeCN, 94%; (c) $Boc_2O$, DMAP, MeCN, 91%; (d) R–N=C=O, $Et_3N$, DMAP, $CH_2Cl_2$. Yields: **8a**: 90%, **8b**: 45%, **8d**: 99%, **8e**: 71%, **8f**: 69%, **8g**: 36%; (e) *N*-(*p*-nitrophenyloxycarbonyl) β-naphthyl-amine, DMAP, $CH_3CN$. Yield: **8c**: 30%; (f) RCOCl, pyridine. Yields: **9a**: 84%, **9b**: 97%, **9c**: 100%; (g) 40% aq HF/MeCN 1:40. Yields: from **9a**: 75%, from **9b**: 100%, from **9c**: 98%; (h) $Boc_2O$, DMAP, MeCN. Yields: **10a**: 93%, **10b**: 79%, **10c**: 100%.

oxamazins.[15] Preliminary studies on simple monocyclic β-lactams have, however, shown that this type of activation was not strong enough for our purposes. We therefore turned our attention to carbonyl derivatives, such as urethane-type compounds (see **7** in Scheme 2) or urea-type compounds (see **8** in Scheme 2). The former activation has been widely employed in the use of β-lactams as acylating agents, for example, in the synthesis of Taxol derivatives or ACE inhibitors.[16] On the other hand, urea-type compounds were previously employed in medicinal chemistry in the field of protease inhibitors.[17,18] These previous reports have shown that urea-type compounds, such as **8**, should be hydrolytically less reactive than urethane-type adducts like **7**.[18] This was confirmed by us, working on model systems.

Therefore, we prepared two simple members of the two classes, namely compounds **7** and **8a**. The synthesis (Scheme 2)[19] started from the previously described racemic lactenediyne **4**.[9] Since the last step of the total synthesis affords an 86:14 ratio of the two diastereoisomers, we did our first exploration work on the major isomer, which has the hydroxy group in a *pseudo-axial* position, and that was available in higher amount. The preparation of **7** and **8a** involved a three step sequence: acetylation of the hydroxy group, removal of the silylated protection, and activation, by reaction with di-*tert*-butyl dicarbonate or with benzyl isocyanate. The overall se-

**Table 1.** Results of cleavage of plasmid DNA with compounds **7**, **8**, **10**, **14**, **15**[a]

| Compound | $CS_{50}$[b] | $CS_{min}$[c] | $CD_{min}$[d] | D/S[e] |
|---|---|---|---|---|
| **7** | 40 | 5 | 25 | 1:15 |
| **8a** | 70 | 8 | 40 | 1:15 |
| **8b** | 25 | 1 | 8 | 1:10 |
| **8c** | 200 | 10 | >200 | No D[f] |
| **8d** | **25** | **1** | **5** | **1:8** |
| **8e** | 70 | n.d. | n.d. | 1:20 |
| **8f** | 40 | 5 | 8 | 1:10 |
| **8g** | 150 | 10 | >100 | No D[f] |
| **10a** | 80 | 10 | 100 | 1:20 |
| **10b** | 80 | 10 | 100 | 1:20 |
| **10c** | 150 | 10 | 100 | 1:20 |
| **14** | 30 | 5 | 10 | 1:12 |
| **15** | 25 | 1 | 5 | 1:8 |

[a] For conditions see Section 4.

[b] $CS_{50}$, concentration of lactenediyne (μM) producing a form ratio of form I/(form II + form III) = 50:50.

[c] $CS_{min}$, minimum concentration of lactenediyne producing detectable quantity of form II.

[d] $CD_{min}$, minimum concentration of lactenediyne producing detectable quantity of form III.

[e] D/S, ratio of form III/form II at 100 μM.

[f] Form III was not visible at 100 μM.

quences proceeded in very high yield, confirming once again the stability of this class of enediynes.

Compounds **7** and **8a**, despite the presence of activating groups, were found to be quite stable. They could be conveniently chromatographed and even stored in freezer as DMSO solution for several months. Moreover, they were demonstrated to be stable in plasma. In order to assess their ability to cleave DNA, we incubated them, at various concentrations, with supercoiled plasmid pBR 322 for 24 h at 37 °C. After gel electrophoresis (see Table 1) we were able to determine the relative ratio of forms I (native, supercoiled), II (relaxed cyclic) and III (linear). Both compounds were found to give single and double strand breaks at concentrations much lower than those required by simple 10-membered monocyclic enediynes.[20] For example, **7** gave 50% of cleavage at 40 μM (Table 1), while typically monocyclic cyclodeca-3-ene-1,5-diynes display the same effect at 500 μM.[20,21] Most importantly, while simple monocyclic enediynes do not provoke the formation of form III (deriving from double cut), **7** afforded also detectable amounts of form III, although the main mechanism involved single cleavage, with a single/double ratio of about 15:1.

These results first indicated that the presence of the β-lactam is beneficial in improving the ability of these compounds to bind to DNA. We strongly believe that DNA cleavage takes place only after opening of the azetidinone. In fact, analogous incubation with an unactivated compound **1** ($R^1$ = β OMe, $R^2$ = Me, $R^3$ = H)[8] gave no cleavage at all even at 1 mM concentration. Urea-type compound **8a** was about 50% less potent than **7**, probably because of slower hydrolysis of the azetidinone. By incubating **7** and **8ab** for 24, 48 and 72 h, we found indeed a slight increase of form II with time for **8a** but not for **7**.

These promising results led us to test the cytotoxicity of **7** (Table 2). The most interesting results are evidenced in bold. As can be seen, on some tumour cell lines, $GI_{50}$ values in the nanomolar range were obtained. For two cell lines (SF-539 and CAKI-1) even a 50% decrease of cell count was observed at concentrations between 10 and 100 μM. Reduction of tumour mass was also demonstrated in vivo on murine models (see Section 4.35).

These simple activated lactenediynes proved to be superior to other simple artificial enediynes, but definitely less potent than natural compounds, and in particular Calicheamicin, which is able to cleave 50% of plasmid DNA at concentrations in the low nanomolar range. Most importantly, Calicheamicin induces a higher percentage of double strand break (2:1 compared to single break!). However, it has been demonstrated that this high level of potency is mainly due to the oligosaccharide part, which acts as the 'delivery system' of this potent warhead. The aglycon itself is indeed 1000 times less potent, affording only a small percentage of double cut (1:30 single/double),[22] and being therefore not any better than **7** or **8a**.

This fact led us to reason that we might be able to increase the potency of our activated lactenediynes and to improve the percentage of double cut, by conjugating them with suitable 'delivery systems', that is groups able to complex tightly (and maybe selectively) with DNA through intercalation or groove binding. These moieties should be however structurally simpler than the highly complex oligosaccharide unit of Calicheamicin! This strategy was previously proved to be successful for other simple artificial enediynes[20,21,23] or for other type of radical generating species.[24]

We first studied the incorporation of naphthyl groups, as very simple intercalating moieties. Since lactenediyne **4** possesses two 'handles', we attached this group alternatively on both, varying also the spacer. For the preparation of compounds **8a**–**g**, various carboxylic acids containing a naphthyl group have been converted into the corresponding isocyanates, which were in turn used for the preparation of the urea-type adducts. Only in the case of β-naphthyl urea **8c** this approach, which started from β-naphthoic acid, was not successful. We started therefore from β-naphthylamine and prepared the corresponding *p*-nitrophenyl carbamate as the acylating agent. In the case of esters **10a**–**c** we used the second handle instead, placing the naphthyl containing group on the hydroxy group to give **9a**–**c**. After removal of the silyl protection, the nitrogen was finally activated as the *t*-butyl urethane to give **10a**–**c**.

All these compounds were incubated with plasmid pBR 322 and compared with **7**. The results are reported in Table 1. As can be seen, the cleaving activity was heavily dependent on: (a) the type of handle used for attaching the intercalating moiety; (b) the type of attachment of the naphthyl group (α or β). On the other hand, the length of the spacer was less important. The best results were achieved with compound **8d** which was about 1.5–2 times more active as overall cleaving agent than **7**. Most

**Table 2.** Cytotoxicity of compound **7** on various tumour cell lines[a]

| Cellular line | $GI_{50}$ | TGI | $LC_{50}$ |
|---|---|---|---|
| *Leukemias* | | | |
| **MOLT-4** | **−6.39** | **>−4.00** | **>−4.00** |
| RPMI-8226 | −4.75 | >−4.00 | >−4.00 |
| *Lung tumours* | | | |
| A549/ATCC | −4.55 | >−4.00 | >−4.00 |
| EKVX | >4.00 | >−4.00 | >−4.00 |
| HOP-62 | −5.30 | −4.42 | >−4.00 |
| NCI-H226 | −4.40 | >−4.00 | >−4.00 |
| NCI-H23 | −4.77 | −4.15 | >−4.00 |
| NCI-H322M | >−4.00 | >−4.00 | >−4.00 |
| **NCI-H460** | **−7.03** | **>−4.00** | **>−4.00** |
| *Colon tumors* | | | |
| COLO 205 | −4.40 | >−4.00 | >−4.00 |
| HCC-2998 | −4.46 | >−4.00 | >−4.00 |
| HCT-116 | −4.59 | >−4.00 | >−4.00 |
| HCT-15 | −4.33 | >−4.00 | >−4.00 |
| HT 29 | >−4.00 | >−4.00 | >−4.00 |
| KM12 | >−4.00 | >−4.00 | >−4.00 |
| SW-620 | −4.87 | >−4.00 | >−4.00 |
| *CNS tumours* | | | |
| SF-295 | −4.51 | >−4.00 | >−4.00 |
| **SF-539** | **−6.14** | **−5.27** | **−4.59** |
| SNB-19 | −4.58 | >−4.00 | >−4.00 |
| SNB-75 | −4.42 | >−4.00 | >−4.00 |
| U251 | −4.79 | >−4.00 | >−4.00 |
| *Melanomas* | | | |
| M14 | −4.40 | >−4.00 | >−4.00 |
| SK-MEL-2 | −4.32 | >−4.00 | >−4.00 |
| SK-MEL-28 | −4.06 | >−4.00 | >−4.00 |
| UACC-257 | −4.74 | >−4.00 | >−4.00 |
| UACC-62 | −4.67 | >−4.00 | >−4.00 |
| *Ovarian tumours* | | | |
| IGROV1 | −4.71 | >−4.00 | >−4.00 |
| OVCAR-3 | −4.21 | >−4.00 | >−4.00 |
| OVCAR-4 | >−4.00 | >−4.00 | >−4.00 |
| OVCAR-5 | >−4.00 | >−4.00 | >−4.00 |
| OVCAR-8 | −4.84 | 4.05 | >−4.00 |
| SK-OV-3 | −4.06 | >−4.00 | >−4.00 |
| *Kidney tumours* | | | |
| 786–0 | −4.94 | −4.16 | >−4.00 |
| A498 | −4.77 | −4.01 | >−4.00 |
| ACHN | −5.58 | −4.59 | >−4.00 |
| **CAKI-1** | **−6.45** | **−5.19** | **−4.22** |
| RXF 393 | −4.69 | −4.16 | >−4.00 |
| TK-10 | −4.45 | >−4.00 | >−4.00 |
| UO-31 | −5.23 | >−4.00 | >−4.00 |
| *Prostate tumours* | | | |
| DU-145 | −4.79 | −4.25 | >−4.00 |
| *Breast tumours* | | | |
| **MCF7** | **−5.96** | **>−4.00** | **>−4.00** |
| NCI/ADR-RES | −4.54 | >−4.00 | >−4.00 |
| MDA-MB-231/ATCC | >−4.00 | >−4.00 | >−4.00 |
| HS 578T | −4.27 | >−4.00 | >−4.00 |
| MDA-MB-435 | >−4.00 | >−4.00 | >−4.00 |
| MDA-N | −4.06 | >−4.00 | >−4.00 |
| BT-549 | −4.61 | >−4.00 | >−4.00 |
| T-47D | −4.00 | >−4.00 | >−4.00 |

[a] Values are log[concn] in M units. If PG (% growth) is the percentage of cell growth compared to the control experiment, $GI_{50}$: concn of **7** at which PG = 50; TGI: concn at which PG = 0; $LC_{50}$: concn at which PG = −50.

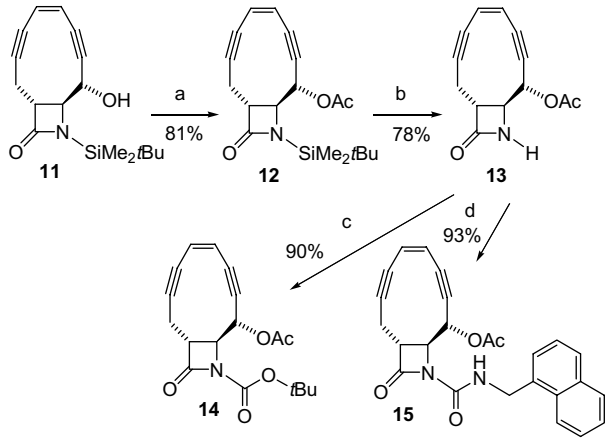
importantly, however, the amount of double cut increased remarkably, and double cut was still visible at 1 μM! We guess that stronger complexation with DNA leads to increased probability of simultaneous double cut.

In order to check the effect of the relative stereochemistry, we prepared also **14** and **15**, that is the epimers of **7** and **8d**, starting from the minor *pseudo-equatorial* isomer **11** (Scheme 3). The last two lines of Table 1 clearly show that relative stereochemistry has a negligible effect.

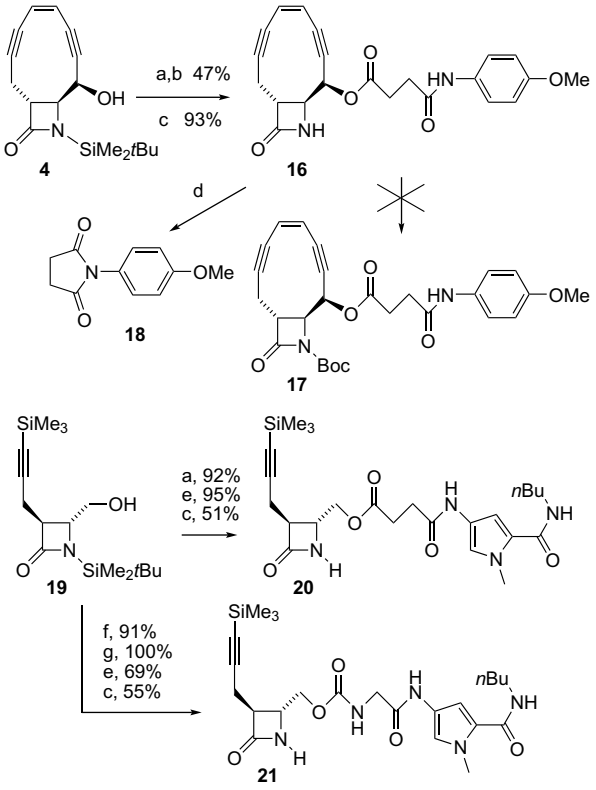
For further improving the activity of these conjugated lactenediynes, we decided to attach to them also minor groove binders, in particular polypyrroles of the netropsin–distamycin family.[21,25] In order to do that we needed to develop appropriate linkers. The methodology used for attaching the naphthyl groups has indeed some limitations, since, in order to vary the length of the spacer, a different substrate must be prepared each time. While this was not a big problem for the synthesis of **8** and **10** (we just needed to prepare naphthylacetic or naphthylpropionic acids) this was anticipated to be problematic for more complex substructures, especially when the function to be attached is a primary amine.

A first logical way to build a general flexible linker is to use the hydroxy group at *C*-9 as handle. Towards this goal we treated lactenediyne **4** with succinic anhydride and the resulting monosuccinate was coupled with a model amine (Scheme 4). Deprotection of the silyl group afforded **16** in acceptable overall yields. However, our attempt to introduce the activating substituent at nitrogen to give **17** failed. Despite the mildness of the basic catalyst used (DMAP), complete intramolecular acyl substitution to release succinimide **18** occurred.

In order to spare the precious compound **4**, this approach was thoroughly studied using the simplified model compound **20**, obtained from **19**[9] in three steps (Scheme 4). However we never succeeded to avoid the unwanted intramolecular process leading to the succinimide, either by changing the acylating reagent (using




**Scheme 3.** Reagents: (a) $Ac_2O$, pyridine, DMAP; (b) 40% aq HF/MeCN; (c) $Boc_2O$, DMAP, MeCN; (d) R–N=C=O, $Et_3N$, DMAP, $CH_2Cl_2$.

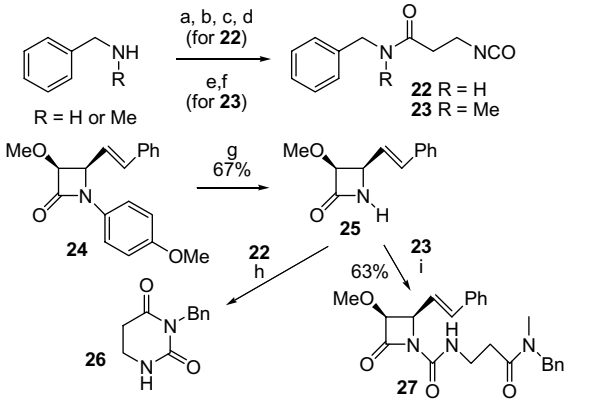



**Scheme 4.** Reagents: (a) succinic anhydride, DMAP, $CH_2Cl_2$; (b) *p*-anisidine, DCC; (c) HF, $CH_3CN$–$H_2O$; (d) $Boc_2O$, DMAP, $CH_2Cl_2$; (e) *N*-methyl 4-amino-1-methyl-pyrrolocarboxyamide, Py-BOP, $CH_2Cl_2$, *N*-methyl morpholine; (f) ethyl isocyanatoacetate, $Et_3N$, DMAP, $CH_2Cl_2$; (g) *Candida antarctica* lipase.

$Boc_2O$, Boc-ON, benzyl isocyanate) or by using other bases instead of DMAP ($Et_3N$, pyridine). We also prepared **21**, the carbamate analogue of **20**, reasoning that an urethane moiety should be more resistant to nucleophilic acylic substitution reactions. However, also in this case it was not possible to introduce the Boc group on the *β*-lactam nitrogen: under the usual conditions ($Boc_2O$, DMAP) the only observed product was indeed an imidazolidinedione deriving from intramolecular attack by the secondary amide nitrogen onto the urethane carbonyl.

A possible alternative solution that avoids this problem would be to join a linker to the hydroxy group at *C*-9 through an ether bond. However, all our attempts to perform a Williamson reaction on **4** failed, because of the already observed easy silyl transfer from nitrogen to oxygen under basic conditions.[13]

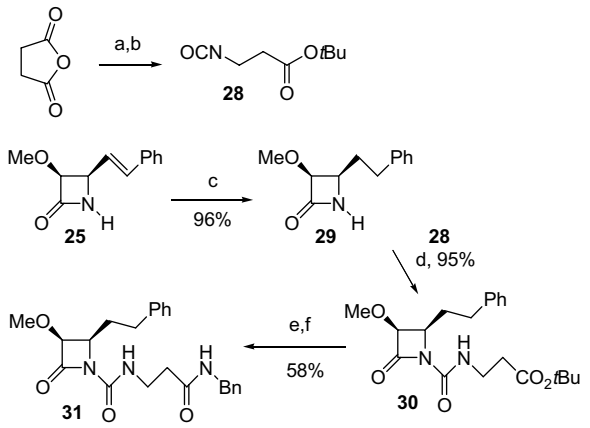
Therefore we decided to use the other handle and to append our linker to the azetidinone nitrogen through an activating urea-type function, like that present in compounds **8**. There are two possibilities: (a) to attach the linker to the DNA-complexing moiety first and then join it to the lactenediyne; (b) to attach the linker to the azetidinone first and then join the DNA-complexing structure. We initially thought that the first strategy had more chances to be successful, because of the anticipated reactivity of the *N*-carbamoyl azetidinone towards intramolecular nucleophilic acyl substitution.




**Scheme 5.** Reagents and condition: (a) *Z*-β-alanine, EDC, HOBT, $CH_2Cl_2$–DMF, 90%; (b) $H_2$, Pd–C, MeOH; (c) HCl; (d) $COCl_2$, $NaHCO_3$, toluene, 33%; (e) succinic anhydride, pyridine, $CH_2Cl_2$; (f) 1—$Ph_2PON_3$; 2—Δ; (g) CAN, $CH_3CN$, $CH_2Cl_2$, $H_2O$; (h) DMAP, $CH_2Cl_2$; (i) DMAP, DBU, $CH_2Cl_2$.

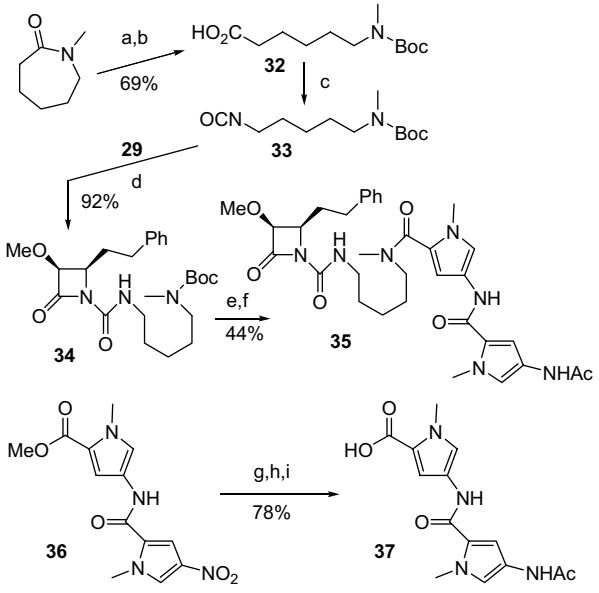
This time we used, as a model azetidinone, known compound **25**,[26] which was conveniently obtained by us through deprotection of **24** (Scheme 5).[12] We converted both a model primary amine (benzylamine) and a model secondary amine (*N*-methylbenzylamine) into the corresponding isocyanates **22** and **23**. In the second case we used a shorter route, proceeding through Curtius rearrangement of a monosuccinic amide. In the first case, however, this protocol was not successful, because of the formation of diketopiperazine **26** during Curtius rearrangement. Therefore we employed a longer route, which involved acylation with a protected β-alanine, followed by deprotection and reaction of the resulting amine with phosgene. The use of the conditions reported by Nowick, was essential, in order to avoid dihydropyrimidinedione formation.[27] When we attempted to join **22**–**25** as usual, once again we could not isolate the expected azetidinyl urea, because of the formation of dihydropyrimidinedione **26**. While one can argue that this product may be formed directly from isocyanate **22**, it should be noted that, when the same crude isocyanate was reacted with *N*-methylaniline, formation of the urea took place smoothly, with negligible formation of dihydropyrimidinedione. Probably the isocyanate attacks regularly the azetidinone, but then, under the reaction conditions, intramolecular displacement by the secondary amide occurs. This process is obviously not possible when the amide is tertiary. Therefore isocyanate **23** gives the expected conjugated azetidinone **27** without problems. Interestingly, in this case the reaction was slow under the usual conditions (DMAP as catalyst), but was strongly accelerated in the presence of DBU.

Although successful, this last method requires the use of a secondary amine, and we were not very happy with that, because of the lack of generality, and the anticipated difficulties in the coupling of secondary aromatic amines. Thus we explored, with some concern, also the second strategy, involving attachment of the linker to the azetidinone first (Scheme 6). For this purpose we prepared, in two high yielding steps, isocyanate **28**, by opening of succinic anhydride with *tert*-butanol,[28] fol-




**Scheme 6.** Reagents and condition: (a) $^{t}$BuOH, NIS, DMAP, $Et_3N$, toluene; (b) 1—$Ph_2PON_3$; 2—Δ; (c) $H_2$, Pd–C, EtOH; (d) DMAP, DBU, $CH_2Cl_2$; (e) $CF_3CO_2H$, $CH_2Cl_2$; (f) $BnNH_2$, DCC, $CH_2Cl_2$.

lowed by Curtius rearrangement. This isocyanate was coupled with model azetidinone **25** in high yield. However, acidic deblocking of the *tert*-butyl ester brought about opening of the azetidinone as well. This opening appears to involve breaking of *N*-C4 bond, probably facilitated by the styryl double bond. Actually, when we used the saturated analogue **29** instead, *tert*-butyl ester cleavage with $CF_3CO_2H$ took place uneventfully, and the β-lactam resisted. The resulting carboxylic acid was coupled with a model primary amine (benzylamine) affording in good (unoptimized) yields the desired adduct. Interestingly, and somehow surprisingly, this time dihydropyrimidinedione **26** was not formed, despite the basic conditions used for the coupling ($Et_3N$). Thus, this linker appears to be very good for joining primary amines to our lactenediynes.



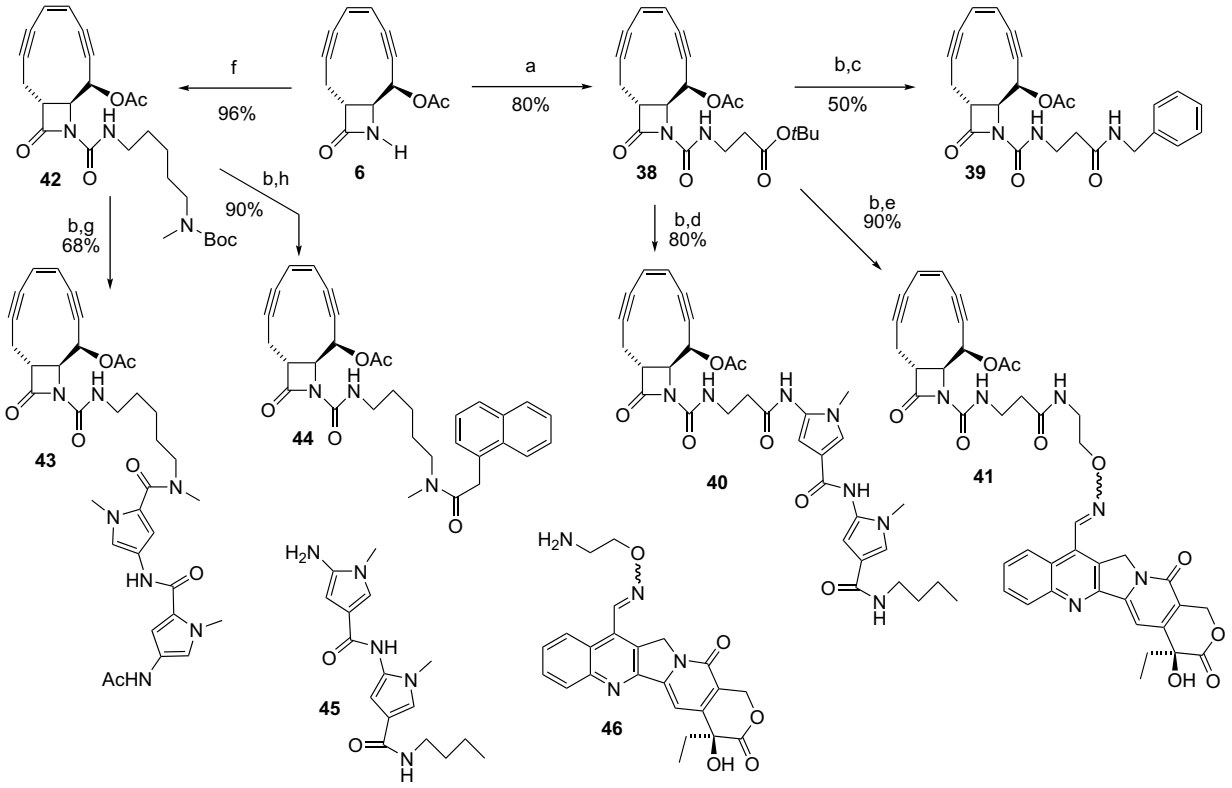

**Scheme 7.** Reagents and conditions: (a) $H_2O$, $H_2SO_4$; (b) $Boc_2O$, $K_2CO_3$, dioxane–$H_2O$; (c) 1—$Ph_2PON_3$; 2—Δ; (d) DMAP, DBU, $CH_2Cl_2$; (e) $CF_3CO_2H$, $CH_2Cl_2$; (f) **37**, PyBOP, $Et_3N$, $CH_2Cl_2$; (g) $H_2$, Pd–C, DMF; (h) $Ac_2O$, pyridine; (i) NaOH, MeOH–$H_2O$, 50 °C.

We developed also a linker useful for binding structures containing a carboxy group (Scheme 7). This time we started from *N*-methyl caprolactam, which was converted, through the known protected amino-acid **32**,[29] into isocyanate **33**. The isocyanate could be coupled in good yields with the model azetidinone **29** to give the azetidinyl urea **34**. Now acid catalysed deblocking was followed by one pot coupling with carboxylic acid **37**, corresponding to a truncated netropsin analogue. The required acid **37**[30] was prepared by us in three steps from derivative **36**.[31–34] Reduction of the nitro group was carried out through hydrogenation[31,33] to give the corresponding amine,[35] which was directly acetylated and saponified to afford **37**.

Having successfully demonstrated, working on model systems, the effectiveness of these two linkers, we implemented their use on the lactenediyne structure itself. Scheme 8 shows all the conjugated compounds that we have prepared and tested. Using the linker suited for primary amines we obtained conjugated compounds **40** and **41** containing again a truncated netropsin analogue and a camptothecin derivative. In order to prepare these two adducts we utilized amines **45** and **46**. Compound **46** was prepared at the Dipartimento di Scienze Molecolari Agroalimentari as previously reported,[36] whereas amine **45** was prepared following essentially the literature procedures.[37] We also synthesized the derivative of benzylamine **39**, which served as a control, since benzylamine was not anticipated to have a particular affinity to DNA. We also prepared conjugated adducts **43** and **44**, containing a truncated netropsin analogue and a naphthylacetic acid, using compound **42**, endowed with the linker appropriate for joining carboxylic acids.

The results of incubation with plasmid pBR 322 are shown in Table 3. As a comparison, the results with **7** and **8d** are also included. All compounds were found to be more active than the parent derivative **7**. It is interesting to note that the benzylamine derivative **39** had an activity identical to the naphthyl derivative **8d**, which had been selected as the best one by the results of Table 1. This means that the linker itself has a positive effect, because **39** is considerably more active than **8a**, which also contains a benzylamine, but without any linker.

However the most striking results were found with compounds **40** and **41**, conjugated with a netropsin analogue and with camptothecin. Not only they were 2–3 times more active than **8d**, but also the amount of double cleavage increased significantly. It is very difficult to achieve this level of double cut, Calicheamicin being a real exception. Most other natural anticancer agents acting through radical attack to DNA afford a lower percentage of double strand cleavage. For example, with Bleomycin the ratio is only 1:9.[22,38] Figure 2 shows the electrophoresis gel after incubation with compounds **40** and **41** at 15 μM concentration.




**Scheme 8.** Reagents: (a) **28**, DMAP, DBU, $CH_2Cl_2$; (b) $CF_3CO_2H$, $CH_2Cl_2$; (c) benzylamine, Py-BOP, $Et_3N$, $CH_2Cl_2$; (d) **45**, Py-BOP, $Et_3N$, $CH_2Cl_2$; (e) **46**, Py-BOP, $Et_3N$, $CH_2Cl_2$; (f) **33**, DMAP, DBU, $CH_2Cl_2$; (g) **37**, Py-BOP, $Et_3N$, $CH_2Cl_2$; (h) 1-naphthylacetic acid, Py-BOP, $Et_3N$, $CH_2Cl_2$.

**Table 3.** Results of cleavage of plasmid DNA with conjugate compounds **7**, **8d**, **38**–**40**, **42**, **43**[a]

| Compound | $CS_{50}$ | $CS_{min}$ | $CD_{min}$ | D/S |
|---|---|---|---|---|
| **7** | 40 | 5 | 25 | 1:15 |
| **8d** | 25 | 1 | 5 | 1:8 |
| **39** | 25 | 1 | 5 | 1:8 |
| **40** | **15** | **0.3** | **1** | **1:6** |
| **41** | **10** | **0.3** | **1** | **1:5** |
| **43** | 35 | 5 | 15 | 1:10 |
| **44** | 25 | 1 | 10 | 1:10 |

[a] For the meanings of the column headers, see Table 1.

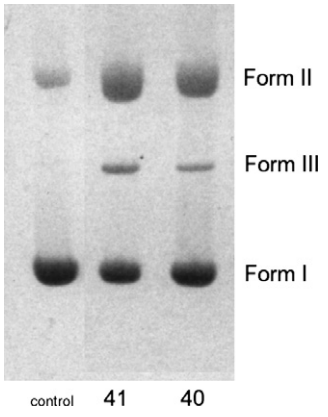



**Figure 2.** Result of incubation of compounds **40** and **41** (15 μM) with pBR 322 plasmid (67.5 μM/bp) for 24 in, pH 7.5, buffer at 37 °C.

## 3. Conclusions

In conclusion, this study has allowed to develop an efficient activation strategy able to transform highly stable lactenediyne derivatives into reactive prodrugs, which have shown a remarkable in vitro activity as DNA-cleaving agents, as well as cytotoxicity in the micromolar (or high nanomolar range) against some tumour cell lines.

A thorough study on model compounds has allowed the development of suitable linkers for attaching DNA-complexing moieties, such as aromatic intercalators or groove binders. Analysis of a small collection of conjugated compounds has permitted the discovery of potent DNA-cleaving agents, which have in particular demonstrated a remarkable ability to induce simultaneous double strand break. These results rank them among the most effective simplified artificial enediynes synthesized so far.[1] Further in vitro and in vivo tests on these conjugated lactenediynes are in progress.

## 4. Experimental

### 4.1. General

NMR spectra were taken in $CDCl_3$ at 200 MHz ($^1H$), and 50 MHz ($^{13}C$), or, when stated, at 300 MHz ($^1H$) and 75 MHz ($^{13}C$), using TMS as internal standard for $^1H$ NMR and the central peak of $CDCl_3$ (at

77.02 ppm) for $^{13}C$ NMR. Chemical shifts are reported in ppm ($\delta$ scale), coupling constants are reported in hertz. Peak assignment in $^{13}C$ spectra was made with the aid of DEPT experiments. In ABX systems, proton A is the one upfield. 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. Only $m/z > 33$ were detected. All analyses were performed with a constant He flow of 0.9 mL/min with initial temperature of 100 °C, init. time 2 min, rate 20 °C/min, final temperature 280 °C, final time 4 min, inj. temperature 250 °C, det. temperature 280 °C. $t_R$ are in minutes. IR spectra have been measured 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 and developed at UV (when not otherwise stated) or with 'molibdic' reagent (21 g $(NH_4)MoO_4{\cdot}4H_2O$, 1 g $Ce(SO_4)_2$, 469 mL $H_2O$, 31 mL $H_2SO_4$) or with ninhydrin. $R_f$ were measured after an elution of 7–9 cm. Chromatographies were carried out on 220–400 mesh silica gel using the 'flash' methodology. Petroleum ether (40–60 °C) is abbreviated as PE. All reactions employing dry solvents were carried out under a nitrogen atmosphere. Abbreviations used are listed in a note.[19]

### 4.2. (1*R**,9*R**,10*S**)(*Z*)-9-Acetoxy-11(*tert*-butyldimethylsilyl)-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (5)

A solution of lactenediyne **4**[9] (100 mg, 0.332 mmol) in dry $CH_2Cl_2$ (1.2 mL) was sequentially treated with pyridine (1.2 mL), 4-dimethylaminopyridine (DMAP) (6 mg, 0.053 mmol) and acetic anhydride (157 μL, 1.66 mmol). After stirring for 75 min at rt, the mixture was evaporated to dryness, taken up with *n*-heptane and evaporated again. This procedure was repeated once again and finally the crude product was chromatographed (PE/AcOEt 8:2 → 6:4) to give pure **5** as a white solid (96%). $R_f$ 0.48 (PE/$Et_2O$ 1:1). Found: C, 66.6; H, 7.4, N, 4.0. $C_{19}H_{25}NO_3Si$ requires C, 66.44; H, 7.34; N, 4.08%. GC–MS: $t_R$ 9.57 min. *m/z*: 286 (M−57, 7.4); 144 (10.2); 126 (4.7); 117 (100); 115 (17.0); 100 (5.5); 75 (29.3); 73 (17.6); 57 (6.9); 43 (21.7). IR: $\nu_{max}$ 3060, 2995, 2970, 2940, 2870, 2305, 1745, 1605 (w), 1425, 1370, 1325, 1265, 1230, 1200, 1155, 1090, 1010, 920 $cm^{-1}$. $^1H$ NMR: $\delta$ 6.00, 5.90 [2H, AB syst., C*H*=C*H*, $J = 9.7$]; 5.61 [1H, t, C*H*–OAc, $J = 1.9$]; 3.90–3.73 [2H, m, C*H*–N and C*H*–C=O]; 2.93 [1H, ddd, C*H*H–C≡C, $J = 1.8$, 3.9, 17.9]; 2.61 [1H, dd, C*H*H–C≡C, $J = 12.5$, 17.9]; 2.12 [3H, s, $CH_3$C=O]; 0.95 [9H, s, $(CH_3)_3C$]; 0.25 and 0.20 [2× 3H, 2 s, $(CH_3)_2Si$].

### 4.3. (1*R**,9*R**,10*S**)(*Z*)-9-Acetoxy-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (6)

A solution of **5** (105.2 mg, 0.306 mmol) in $CH_3CN$ (4.75 mL) was cooled to −20 °C, and treated with a 40% aqueous HF solution (0.250 mL, 5.74 mmol). After 20 min, the temperature was allowed to reach 0 °C and the solution stirred at this temperature for 2 h. After further 1.5 h at rt, the reaction was complete and the solution was poured into a saturated $NaHCO_3$ aqueous solution. Extraction with AcOEt (three times), drying ($Na_2SO_4$), evaporation and chromatography (PE/AcOEt 3:7 + 1% MeOH) gave the pure product **6** as a white solid (66 mg, 94%). $R_f$ 0.35 (PE/AcOEt 1:1). Found: C, 68.0; H, 4.95, N, 6.05. $C_{13}H_{11}NO_3$ requires C, 68.11; H, 4.84; N, 6.11%. GC–MS: $t_R$ 8.23 min. *m/z*: 229 ($M^+$, 30.9); 187 (23.9); 186 (38.1); 170 (7.5); 159 (14.5); 158 (33.9); 144 (16.1) 142 (12.4); 141 (14.2); 140 (9.4); 130 (71.1); 115 (57.9); 114 (18.7); 112 (55.1); 103 (14.6) 91 (11.8); 89 (15.5); 84 (15.8); 77 (17.1); 63 (23.9); 51 (14.1); 43 (100). $^1H$ NMR: $\delta$ 6.05–5.80 [4H, m, C*H*=C*H*, C*H*–OAc, N*H*]; 3.98 [1H, t, C*H*–N, $J = 2.4$]; 3.76 [1H, ddd, C*H*–C=O, $J = 2.6$, 3.9, 12.5]; 2.93 [1H, ddd, C*H*H–C≡C, $J = 1.8$, 4.0, 18.0]; 2.67 [1H, dd, C*H*H–C≡C, $J = 12.7$, 18.0]. 2.15 [3H, s, $CH_3$C=O]. $^{13}C$ NMR (50 MHz): $\delta$ 170.3 and 167.4 [*C*=O]; 127.0, 121.9 [*C*H=*C*H]; 100.4, 93.2, 88.2, 84.0 [*C*≡*C*]; 61.6, 58.1; 53.5 [*C*H–OAc, *C*H–N, *C*H–C=O]; 20.7 [$CH_3$C=O]; 19.3 [$CH_2$–C≡C].

### 4.4. (1*R**,9*R**,10*S**)(*Z*)-9-Acetoxy-11-(*tert*-butoxycarbonyl)-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (7)

A solution of compound **6** (101.5 mg, 0.443 mmol) in dry $CH_3CN$ (5 mL) was treated sequentially with 4-dimethylaminopyridine (32.7 mg, 0.268 mmol) and di-*tert*-butyl dicarbonate (190 mg, 0.871 mmol). After 30 min the mixture was poured into saturated aqueous $NH_4Cl$ and extracted with ethyl acetate. After drying ($Na_2SO_4$) and evaporation, chromatography (PE/$Et_2O$ 70:30) gave pure **7** as a white solid (133 mg, 91%). $R_f$: 0.60 (PE/$Et_2O$ 1:1). Found: C, 65.8; H, 5.8, N, 4.2. $C_{18}H_{19}NO_5$ requires C, 65.64; H, 5.81; N, 4.25%. GC–MS: $t_R$ 9.35 min. *m/z*: 329 [$M^+$, 6.1%]; 231 [11.4]; 187 [11.1]; 186 [15.0]; 171 [5.9]; 159 [6.5]; 158 [11.9]; 144 [9.2]; 130 [18.6]; 116 [7.0]; 115 [19.1]; 114 [6.5]; 112 [7.0]; 63 [6.3]; 57 [100]; 56 [8.0]; 44 [10.6]; 43 [71.9]; 41.[23.3] $^1H$ NMR: $\delta$ 6.08 [1H, t, C*H*–OAc, $J = 1.9$]; 6.01 and 5.92 [2H, AB syst., C*H*=C*H*, $J_{AB} = 9.6$]; 4.23 [1H, t, C*H*–N, $J = 2.6$]; 3.80 [1H, dt, C*H*–C=O, $J_d = 12.5$, $J_t = 3.6$]; 2.96 [1H, ddd, C*H*H–C≡C, $J = 1.3$, 4.0, 17.9]; 2.71 [1H, dd, C*H*H–C≡C, $J = 12.5$, 17.9]; 2.12 [3H, s, $CH_3$–C=O]; 1.51 [9H, s, $(CH_3)_3C$].

### 4.5. General procedure for the preparation of *N*-carbamoyl derivatives 8a,b,d–g: (1*R**,9*R**,10*S**)(*Z*)-9-acetoxy-11-[(1-naphthylmethyl)carbamoyl]-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one 8d

A solution of (1-naphthyl)acetic acid (372 mg, 2.00 mmol) in dry toluene (35 mL) was treated with $Et_3N$ (1.00 mL, 6.8 mmol) and diphenyl phosphoryl azide (1.20 mL, 6.0 mmol). After stirring at rt for 30 min, TLC showed complete conversion of the acid into the corresponding acyl azide. The solution was then heated at reflux, monitoring the Curtius rearrangement through IR. When the azide band at 2180 $cm^{-1}$ disappeared (about 30 min) being replaced by an isocyanate band at 2250 $cm^{-1}$, the solution was cooled, diluted with a, pH 7, buffer solution (phosphate) and extracted three times with $Et_2O$. After drying ($Na_2SO_4$) and evaporation, the resulting yellow oil was taken up in dry $CH_2Cl_2$

(8 mL). This solution was approximately 0.25 M in the isocyanate.

Compound **7** (40 mg, 0.174 μmol) was dissolved in dry $CH_2Cl_2$ (0.5 mL) and treated with $Et_3N$ (121 μL, 0.872 mmol), 4-dimethylaminopyridine (3 mg, 0.024 mmol) and 2.8 mL of the above described isocyanate solution (supposed 0.70 mmol). After stirring overnight at rt, the mixture was treated with 5% aqueous $NH_4H_2PO_4$ and extracted three times with $CH_2Cl_2$. Drying ($Na_2SO_4$), evaporation and chromatography (PE/AcOEt 8:2) gave pure **8d** as a white solid (71 mg, 98%). Mp: 167–168 °C (dec). $R_f$ 0.43 (PE/AcOEt 7:3). Found: C, 72.75; H, 4.9, N, 6.75. $C_{25}H_{20}N_2O_4$ requires C, 72.80; H, 4.89; N, 6.79%. IR: $\nu_{max}$ 3372, 3041, 2994, 2956, 2212 (weak), 1769, 1700, 1599, 1511, 1365, 1334, 1295, 1191, 1141, 1102, 1009, 958 $cm^{-1}$. $^1H$ NMR: $\delta$ 8.02 [1H, d, $J$ = 8.4]; 7.91–7.80 [2H, m]; 7.60–7.51 [2H, m]; 7.48–7.42 [2H, m]; 6.70 [1H, t, N*H*–$CH_2$, $J$ = 5.7]; 6.15 [1H, t, C*H*–OCO, $J$ = 2.0]; 5.98 and 5.92 [2H, AB syst., C*H*=C*H*, $J_{AB}$ = 9.5]; 5.00 and 4.85 [2H, AB part of an ABX system, $CH_2$–NH, $J_{AB}$ = 14.5, $J_{AX}$ = 5.8, $J_{BX}$ = 6.1]; 4.35 [1H, t, C*H*–N, $J$ = 2.7]; 3.83 [1H, ddd, $J$ = 4.0, 2.9, 12.4]; 2.90 and 2.69 [2H, AB part of an ABXY system, $CH_2$–C≡C, $J_{AB}$ = 17.8, $J_{AX}$ = 12.8, $J_{BX}$ = 3.8, $J_{AY}$ = 0, $J_{BY}$ = 1.6]; $\delta$ 2.02 [3H, s, $CH_3$–CO].

**4.5.1. (1*R**,9*R**,10*S**)(*Z*)-9-Acetoxy-11-(benzylcarbamoyl)-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (8a).** It was prepared from commercially available benzyl isocyanate following the general procedure described above for **8d**. Yield: 90%. $R_f$ 0.48 (PE/AcOEt 6:4). Found: C, 69.65; H, 5.0, N, 7.65. $C_{21}H_{18}N_2O_4$ requires C, C, 69.60; H, 5.01; N, 7.73%. $^1H$ NMR: $\delta$ 7.40–7.26 [5H, m, aromatics]; 6.74 [1H, t, N*H*, $J$ = 5.6]; 6.10 [1H, t, C*H*–OAc, $J$ = 2.0]; 5.99 and 5.93 [AB system, C*H*=C*H*, $J$ = 9.7]; 4.46 [2H, d, $CH_2$NH, $J$ = 6.0]; 4.34 [1H, t, C*H*–N–C=O, $J$ = 2.6]; 3.86 [1H, ddd, C*H*–C=O, $J$ = 12.4, 4.1, 3.0]; 2.93 and 2.71 [AB part of ABXY system, $CH_2$C≡C, $J_{AB}$ = 17.8, $J_{AX}$ = 12.4, $J_{BX}$ = 3.9, $J_{AY}$ = 0, $J_{BY}$ = 1.6]; 2.09 [3H, s, $CH_3$].

**4.5.2. (1*R**,9*R**,10*S**)(*Z*)-9-Acetoxy-11-(1-naphthylcarbamoyl)-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (8b).** It was prepared from α-naphthoic acid following the general procedure described above for **8d**. Yield: 45%. $R_f$ 0.60 (PE/$Et_2O$ 1:1). Found: C, 72.1; H, 4.65, N, 6.95. $C_{24}H_{18}N_2O_4$ requires C, 72.35; H, 4.55; N, 7.03%. $^1H$ NMR: $\delta$ 8.98 [1H, s, N*H*]; 8.08 [1H, d, $J$ = 7.6]; 7.98–7.84 [2H, m]; 7.69 [1H, d, $J$ = 8.2]; 7.62–7.44 [3H, m]; 6.18 [1H, t, C*H*–OAc, $J$ = 2.0]; 6.03 and 5.96 [AB system, C*H*=C*H*, $J$ = 9.7]; 4.50 [1H, t, C*H*–N–C=O, $J$ = 2.7]; 4.03 [1H, ddd, C*H*–C=O, $J$ = 12.4, 3.0, 4.2]; 3.05 and 2.83 [AB part of ABXY system, $CH_2$C≡C, $J_{AB}$ = 17.8, $J_{AX}$ = 12.4, $J_{BX}$ = 4.2, $J_{AY}$ = 0, $J_{BY}$ = 1.5]; 2.14 [3 H, s, $CH_3$].

**4.5.3. (1*R**,9*R**,10*S**)(*Z*)-9-Acetoxy-11-[(2-naphthylmethyl)carbamoyl]-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (8e).** It was prepared from β-naphthylacetic acid following the general procedure described above for **8d**. Yield: 71%. $R_f$ 0.48 (PE/$Et_2O$ 4:6). Found: C, 72.75; H, 4.9, N, 6.75. $C_{25}H_{20}N_2O_4$ requires C, 72.80; H, 4.89; N, 6.79%. $^1H$ NMR: $\delta$ 7.85–7.74 [3H, m]; 7.50–7.31 [3H, m]; 7.24–7.19 [1H, m]; 6.82 [1H, t, N*H*–$CH_2$, $J$ = 5.9]; 6.12 [1H, t, C*H*–OCO, $J$ = 2.0]; 5.99 and 5.93 [2H, AB syst., C*H*=C*H*, $J_{AB}$ = 9.7]; 4.62 [2H, d, $CH_2$–NH, $J$ = 6.0]; 4.36 [1H, t, C*H*–N, $J$ = 2.7]; 3.86 [1H, ddd, $J$ = 4.0, 2.9, 12.3]; 2.93 and 2.72 [2H, AB part of an ABXY system, $CH_2$–C≡C, $J_{AB}$ = 17.8, $J_{AX}$ = 12.8, $J_{BX}$ = 3.9, $J_{AY}$ = 0, $J_{BY}$ = 1.5]; $\delta$ 2.07 [3H, s, $CH_3$–CO].

**4.5.4. (1*R**,9*R**,10*S**)(*Z*)-9-Acetoxy-11-[(2-(1-naphthyl)-ethyl)carbamoyl]-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (8f).** It was prepared from 3-(α-naphthyl)propanoic acid[39,40] following the general procedure described above for **8d**. Yield: 69%. $R_f$ 0.32 (ETP/$Et_2O$ 1:1). Found: C, 73.15; H, 5.3, N, 6.45. $C_{26}H_{22}N_2O_4$ requires C, 73.23; H, 5.20; N, 6.57%. $^1H$ NMR: $\delta$ 8.08 [1H, d, $J$ = 8.6]; 7.87 [1H, dd, $J$ = 2.1, 7.3]; 7.77 [1H, d, $J$ = 7.8]; $\delta$ 7.61–7.31 [4H, m]; $\delta$ 6.68 [1H, t, N*H*–$CH_2$, $J$ = 4.7]; 6.08 [1H, t, C*H*–OCO, $J$ = 1.9]; 5.99 and 5.92 [2H, AB system, C*H*=C*H*, $J_{AB}$ = 9.7]; 4.31 [1H, t, C*H*–N, $J$ = 2.7]; 3.83 [1H, ddd, C*H*–CO, $J$ = 3.1, 4.1, 12.5]; 3.72–3.59 [2H, m, $CH_2$–NH]; 3.33 [2H, t, $CH_2$–$CH_2$, $J$ = 7.0]; 2.68 and 2.91 [2H, AB part of an ABXY system, $CH_2$–C≡C, $J_{AB}$ = 17.8, $J_{AX}$ = 12.8, $J_{BX}$ = 3.9, $J_{AY}$ = 0, $J_{BY}$ = 1.5]; 2.09 [3H, s, $CH_3$–CO].

**4.5.5. (1*R**,9*R**,10*S**)(*Z*)-9-Acetoxy-11-[(2-(2-naphthyl)-ethyl)carbamoyl]-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (8g).** It was prepared from 3-(β-naphthyl)propanoic acid[40,41] following the general procedure described above for **8d**. Yield: 36%. $R_f$ 0.23 (ETP/$Et_2O$ 1:1). Found: C, 73.1; H, 5.4, N, 6.4. $C_{26}H_{22}N_2O_4$ requires C, 73.23; H, 5.20; N, 6.57%. $^1H$ NMR: $\delta$ 7.86–7.75 [3H, m]; 7.65 [1H, s]; 7.53–7.40 [2H, m]; 7.34 [1H, dd, $J$ = 8.5, 1.0]; 6.47 [1H, t, N*H*, $J$ = 5.7]; 6.05 [1H, t, C*H*–OCO, $J$ = 1.8]; 5.99 and 5.91 [2H, AB system, C*H*=C*H*, $J_{AB}$ = 9.7]; 4.29 [1H, t, C*H*–N, $J$ = 2.5]; 3.83 [1H, dt, C*H*–CO, $J_t$ = 3.1, $J_d$ = 12.5]; 3.62 [2H, q, $CH_2$–NH, $J$ = 6.8]; 3.02 [2H, t, $CH_2$–$CH_2$, $J$ = 7.2]; 2.65 and 2.91 [2H, AB part of an ABX system, $CH_2$–C≡C, $J_{AB}$ = 17.8, $J_{AX}$ = 12.5, $J_{BX}$ = 4.0]; 2.04 [3H, s, $CH_3$–CO].

**4.6. (1*R**,9*R**,10*S**)(*Z*)-9-Acetoxy-11-(2-naphthylcarbamoyl)-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (8c)**

β-Naphthylamine (415 mg, 2.90 mmol) was dissolved in $CHCl_3$ (5 mL) and treated with a solution of *p*-nitrophenyl chloroformate (300 mg, 1.49 mmol) in $CHCl_3$ (1.5 mL). A purple precipitate forms at once. After 15 min the reaction is complete. It was filtered and the mother liquors evaporated to dryness. Crystallization from $CHCl_3$/pentane afforded *N*-(2-naphthyl) *p*-nitrophenyl carbamate (218 mg), as a purple solid (mp 174.8–175.8 °C. $R_f$ 0.60 in PE/AcOEt 7:3), which was used immediately for the next reaction. A solution of compound **7** (37.2 mg, 162 μmol) in dry $CH_3CN$ (2 mL) was treated with DMAP (10.8 mg, 98 μmol) and with the nitrophenyl carbamate (100 mg, 324 μmol). The solution was stirred overnight. Water (200 μL) was added and the mixture stirred for 20 min. The solution

becomes lemon yellow. Extraction (three times) with ACOEt, washing with saturated aqueous NaCl, drying ($Na_2SO_4$), evaporation and chromatography (PE/$CH_2Cl_2$/AcOEt 7:2:1) gave pure **8c** (19 mg, 30%). $R_f$ 0.65 (PE/AcOEt 65:35). Found: C, 72.0; H, 4.45, N, 6.8. $C_{24}H_{18}N_2O_4$ requires C, 72.35; H, 4.55; N, 7.03%. $^1$H NMR: $\delta$ 8.49 [1H, s, N*H*]; 8.10 [1H, d, $J$ = 1.9]; 8.0–7.83 [3H, m]; 7.51–7.37 [3H, m]; 6.16 [1H, t, C*H*–OAc, $J$ = 1.9]; 6.02 and 5.95 [AB system, C*H*=C*H*, $J$ = 9.7]; 4.46 [1H, t, C*H*–N–C=O, $J$ = 2.6]; 3.99 [1H, ddd, C*H*–C=O, $J$ = 12.5, 3.0, 4.2]; 3.02 and 2.79 [AB part of ABXY system, $CH_2$C≡C, $J_{AB}$ = 17.8, $J_{AX}$ = 12.8, $J_{BX}$ = 4.0, $J_{AY}$ = 0, $J_{BY}$ = 1.5]; 2.13 [3H, s, C$H_3$].

### 4.7. (1*R**,9*R**,10*S**)(*Z*)-11(*tert*-butyldimethylsilyl)-9-(1-naphthylacetoxy)-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (9a)

A crude solution (approximately 1.34 M) of 1-naphthylacetyl chloride was prepared by treating 1-naphthylacetic acid (500 mg, 2.69 mmol) in dry $CH_2Cl_2$ (10 mL) with oxalyl chloride (2 mL of a 2.06 M solution, 4.04 mmol). After stirring for 1 h at rt, the solution was evaporated to dryness, taken up with $CH_2Cl_2$ and re-evaporated twice. The resulting yellow solid was taken up with 2 mL of $CH_2Cl_2$. A solution of lactenediyne **4**[9] (30 mg, 99 μmol) in dry $CH_2Cl_2$ (1.0 mL) was treated with pyridine (600 μL) and with the above cited 1.34 M solution of 1-naphthylacetyl chloride in $CH_2Cl_2$ (380 μL, 509 μmol). The solution was stirred overnight and then quenched with 5% aqueous $NH_4H_2PO_4$. Extraction for three times with $CH_2Cl_2$ (resulting pH 5–6), washing with saturated aqueous NaCl, Drying ($Na_2SO_4$), evaporation and chromatography ($Et_2O$/PE 2:8 → 4:6) gave pure **9a** as a white solid (84%). $R_f$: 0.71 ($Et_2O$/PE 1:1). Found: C, 74.1; H, 6.75, N, 2.95. $C_{29}H_{31}NO_3Si$ requires C, 74.16; H, 6.65; N, 2.98%. $^1$H NMR: $\delta$ 7.98 [1H, dd, $J$ = 9.5, 0.6]; 7.86 [1H, dd, $J$ = 7.3, 2.1]; 7.73 [1H, dd, $J$ = 7.2, 2.0]; 7.58–7.49 [2H, m]; $\delta$ 7.46–7.44 [2H, m]; $\delta$ 6.00 and 5.89 [2H, AB system, C$H_2$=C$H_2$, $J_{AB}$ = 9.5]; 5.60 [1H, t, C*H*–OCO, $J$ = 1.8]; 4.15 [2H, s, C$H_2$–CO]; 3.83 [1H, t, C*H*–N, $J$ = 2.3]; 3.69 [1H, ddd, C*H*–C$H_2$, $J$ = 12.6, 3.9, 2.7]; 2.98 and 2.51 [2H, AB part of an ABXY system, C$H_2$–C≡C, $J_{AB}$ = 17.9, $J_{AX}$ = 12.6, $J_{BX}$ = 4.0, $J_{AY}$ = 0, $J_{BY}$ = 1.6]; 0.85 [9H, s, (C$H_3$)$_3$–C]; 0.01 and −0.01 [2× 3H, s, (C$H_3$)$_2$Si].

### 4.8. (1*R**,9*R**,10*S**)(*Z*)-11(*tert*-butyldimethylsilyl)-9-[3-(1-naphthyl)propanoyloxy]-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (9b)

It was prepared in 97% yield from 1-naphthylpropanoic acid and lactenediyne **4** following the same procedure described above for **9a**. $R_f$: 0.74 ($Et_2O$/PE 6:4). Found: C, 74.3; H, 6.9, N, 2.9. $C_{30}H_{33}NO_3Si$ requires C, 74.50; H, 6.88; N, 2.90%. $^1$H NMR: $\delta$ 7.99 [1H, d, $J$ = 8.3]; 7.86 [1H, dd, $J$ = 7.3, 2.1]; 7.73 [1H, dd, $J$ = 7.3, 2.1]; 7.59–7.33 [4H, m]; $\delta$ 6.00 and 5.91 [2H, AB system, C$H_2$=C$H_2$, $J_{AB}$ = 9.7]; 5.64 [1H, t, C*H*–OCO, $J$ = 1.9]; 3.87 [1H, t, C*H*–N, $J$ = 2.4]; 3.73 [1H, ddd, C*H*–C$H_2$, $J$ = 12.6, 3.8, 2.7]; 3.44 [2H, t, C$H_2$CH$_2$CO, $J$ = 8.1]; 2.92 and 2.60 [2H, AB part of an ABXY system, C$H_2$–C≡C, $J_{AB}$ = 17.9, $J_{AX}$ = 12.9, $J_{BX}$ = 3.7, $J_{AY}$ = 0, $J_{BY}$ = 1.7]; 2.88–2.76 [2H, m, C$H_2$CO]; 0.92 [9H, s, (C$H_3$)$_3$–C]; 0.20 and 0.161 [2× 3H, s, (C$H_3$)$_2$Si].

### 4.9. (1*R**,9*R**,10*S**)(*Z*)-11(*tert*-butyldimethylsilyl)-9-[3-(2-naphthyl)propanoyloxy]-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (9c)

It was prepared in 100% yield from 2-naphthylpropanoic acid and lactenediyne **4** following the same procedure described above for **9a**. $R_f$: 0.64 ($Et_2O$/PE 6:4). Found: C, 74.25; H, 6.95, N, 2.8. $C_{30}H_{33}NO_3Si$ requires C, 74.50; H, 6.88; N, 2.90%. $^1$H NMR: $\delta$ 7.82–7.75 [3H, m]; 7.64 [1H, s]; 7.50–7.38 [2H, m]; $\delta$ 7.32 [1H, dd, $J$ = 1.6, 8.4]; $\delta$ 5.97 and 5.86 [2H, AB system, C$H_2$=C$H_2$, $J_{AB}$ = 9.6]; 5.60 [1H, t, C*H*–OCO, $J$ = 1.9]; 3.84 [1H, t, C*H*–N, $J$ = 2.5]; 3.70 [1H, ddd, C*H*–C$H_2$, $J$ = 12.6, 3.8, 2.7]; 3.13 [2H, t, C$H_2$CH$_2$CO, $J$ = 7.6]; 2.88 and 2.58 [2H, AB part of an ABXY system, C$H_2$–C≡C, $J_{AB}$ = 17.9, $J_{AX}$ = 12.8, $J_{BX}$ = 3.7, $J_{AY}$ = 0, $J_{BY}$ = 1.6]; 2.83–2.75 [2H, m, C$H_2$C=O]; 0.91 [9H, s, (C$H_3$)$_3$–C]; 0.17 and 0.11 [2× 3H, s, (C$H_3$)$_2$Si].

### 4.10. (1*R**,9*R**,10*S**)(*Z*)-11-(*tert*-butoxycarbonyl)-9-(1-naphthylacetoxy)-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (10a)

Substrate **9a** (22.2 mg, 47.3 μmol) was dissolved in $CH_3CN$ (2 mL) and cooled to −20 °C. The solution was treated with 40% aqueous HF (40 μL). After 20 min, the solution was brought to 0 °C, and, after 2 h, to rt. After stirring for 3.5 h the mixture was quenched with saturated aq $NaHCO_3$ and extracted three times with AcOEt. after washing with saturated aq NaCl, drying ($Na_2SO_4$) evaporation, and chromatography (PE/AcOEt 6:4), pure *(1R*,9R*,10S*)(Z)-9-(1-naphthylacetoxy)-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one* was obtained (12.6 mg, 75%). $R_f$: 0.11 ($Et_2O$/PE 1:1). $^1$H NMR: $\delta$ 7.93–7.79 [3H, m]; 7.62–7.39 [4H, m]; 6.01 and 5.91 [2H, AB system, C*H*=C*H*, $J_{AB}$ = 9.8]; 5.83 [1H, t, C*H*–OCO, $J$ = 1.93]; 5.64 [1H, s, N*H*–CO]; 4.15 [2H, s, C$H_2$–CO]; 3.86 [1H, t, C*H*–N, $J$ = 2.3]; 3.63 [1H, ddd, C*H*–C=O, $J$ = 2.6, 3.8, 12.6]; 2.88 and 2.62 [2H, AB part of an ABXY system, C$H_2$–C≡C, $J_{AB}$ = 18.0, $J_{AX}$ = 12.8, $J_{BX}$ = 3.7, $J_A$ = 0, $J_{BY}$ = 1.8].

This compound (12.6 mg, 35.4 mmol) was dissolved in dry $CH_3CN$ (2 mL) and treated with 4-dimethylaminopyridine (2.6 mg, 21 μmol) and di-*tert*-butyl dicarbonate (15.2 mg, 71 μmol). After 40 min the reaction was complete. It was treated with saturated aqueous $NH_4Cl$ and extracted three times with AcOEt. After drying ($Na_2SO_4$), evaporation and chromatography (PE/$Et_2O$ 65:25), pure **9a** was obtained (7.5 mg, 93%). $R_f$: 0.58 ($Et_2O$/ETP 1:1). $^1$H NMR: $\delta$ 7.94–7.79 [3H, m]; 7.56–7.40 [4H, m]; 6.09 [1H, t, C*H*–OCO, $J$ = 1.9]; 6.00 and 5.91 [2H, AB system, C*H*=C*H*, $J_{AB}$ = 9.7]; 4.17–4.13 [3H, m, C$H_2$–CO and C*H*–N]; 3.60 [1H, dt, C*H*–C$H_2$, $J_d$ = 12.3, $J_t$ = 3.6]; 2.88 and 2.65 [2H, AB part of an ABXY system, C$H_2$–C≡C, $J_{AB}$ = 17.9, $J_{AX}$ = 12.8, $J_{BX}$ = 3.8, $J_{AY}$ = 0, $J_{BY}$ = 1.4]; 1.32 [9H, s, (CH$_3$)$_3$C].

### 4.11. (1*R**,9*R**,10*S**)(*Z*)-11-(*tert*-butoxycarbonyl)-9-[(1-naphthyl)propanoyloxy-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (10b)

It was prepared in 79% yield (100% and 79% for the two steps) from lactenediyne **9b** following the same procedure described above for **10a**. $R_f$: 0.64 ($Et_2O$/ETP 1:1). Found: C, 74.0; H, 5.85, N, 2.75. $C_{29}H_{27}NO_5$ requires C, 74.18; H, 5.80; N, 2.98%. $^1$H NMR: $\delta$ 7.98 [1H, d, $J$ = 8.05]; 7.86 [1H, dd, $J$ = 7.3, 2.0]; 7.73 [1H, d, 7.4]; 7.59–7.29, [4H, m] 6.12 [1H, t, C*H*–OCO, $J$ = 2.0]; 6.01 and 5.93 [2H, AB system, C*H*=C*H*, $J_{AB}$ = 10.0]; 4.22 [1H, t, C*H*NCO, $J$ = 2.0]; 3.69 [1H, dt, C*H*–$CH_2$, $J_d$ = 12.3, $J_t$ = 3.9]; 3.42 [2H, t, $CH_2CH_2CO$, $J$ = 8.1]; 2.93 and 2.69 [2H, AB part of an ABXY system, $CH_2$–C≡C, $J_{AB}$ = 17.9, $J_{AX}$ = 12.5, $J_{BX}$ = 3.9, $J_{AY}$ = 0, $J_{BY}$ = 1.6]; 2.87–2.77 [2H, m, $CH_2CO$]; 1.49 [9H, s, $(CH_3)_3C$].

### 4.12. (1*R**,9*R**,10*S**)(*Z*)-11-(*tert*-butoxycarbonyl)-9-[(2-naphthyl)propanoyloxy-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (10c)

It was prepared in 98% yield (100% and 98% for the two steps) from lactenediyne **9c** following the same procedure described above for **10a**. $R_f$: 0.62 ($Et_2O$/ETP 1:1). Found: C, 74.15; H, 5.8, N, 2.7. $C_{29}H_{27}NO_5$ requires C, 74.18; H, 5.80; N, 2.98%. $^1$H NMR: $\delta$ 7.82–7.75 [3H, m]; 7.63 [1H, s]; 7.49–7.39 [2H, m]; 7.32 [1H, dd, $J$ = 1.7, 8.4]; 6.07 [1H, t, C*H*–OCO, $J$ = 2.1]; 5.98 and 5.88 [2H, AB system, C*H*=C*H*, $J_{AB}$ = 9.7]; 4.20 [1H, t, C*H*N, $J$ = 2.6]; 3.65 [1H, dt, C*H*–$CH_2$, $J_d$ = 12.4, $J_t$ = 3.9]; 3.12 [2H, t, $CH_2CH_2CO$]; 2.88 and 2.66 [2H, AB part of an ABXY system, $CH_2$–C≡C, $J_{AB}$ = 18.0, $J_{AX}$ = 12.8, $J_{BX}$ = 3.9, $J_{AY}$ = 0, $J_{BY}$ = 1.6]; 2.79 [2 H, t, $CH_2CO$, $J$ = 7.8]; 1.45 [9H, s, $(CH_3)_3C$].

### 4.13. (1*R**,9*S**,10*S**)(*Z*)-9-Acetoxy-11(*tert*-butyldimethylsilyl)-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (12)

It has been prepared in 81% starting from **11**, following the same procedure described for **5**. $R_f$ 0.54 (PE/$Et_2O$ 1:1). Found: C, 66.7; H, 7.45, N, 4.0. $C_{19}H_{25}NO_3Si$ requires C, 66.44; H, 7.34; N, 4.08%. GC–MS: $t_R$ 9.54 min; *m*/*z* 286 (M−57, 1.7); 144 (3.8); 118 (9.9); 100 (4.2); 75 (28.8); 59 (2.7); 43 (19.5). IR: $\nu_{max}$ 3006, 2929, 2857, 2398, 1745, 1602, 1364, 1278, 2214, 1163, 1098, 1007, 952 cm$^{-1}$. $^1$H NMR: $\delta$ 5.90 [2H, s, C*H*=C*H*]; 5.22 [1H, d, C*H*–OCO, $J$ = 9.3]; 3.47 [1H, dt, C*H*–$CH_2$, $J_d$ = 12.2, $J_t$ = 3.5]; 2.95 and 2.66 [2H, AB part of an ABX syst., $CH_2$–C≡C, $J_{AB}$ = 17.7, $J_{AX}$ = 12.3, $J_{BX}$ = 3.3]; 2.15 [3H, s, $CH_3$–CO]; 0.94 [9H, s, $(CH_3)_3C$]; 0.28 and 0.27 [2× 3H, s, $(CH_3)_2Si$]. $^{13}$C NMR: $\delta$ 172.50 [*C*OO]; 169.54 [*C*O–N]; 125.12 and 122.35 [*C*H=*C*H]; 100.44, 93.83, 87.15, 84.32 [*C*≡*C*]; 69.19 [*C*H–OCO]; 59.71 and 56.97 [*C*H–N and C*H*–CO]; 26.15 [$(CH_3)_3C$]; $\delta$ 20.31 [$C(CH_3)_3$]; $\delta$ −5.03 and −5.32 [$(CH_3)_2Si$].

### 4.14. (1*R**,9*S**,10*S**)(*Z*)-9-Acetoxy-11-azabicyclo[8.2.0]-dodec-5-ene-3,7-diyn-12-one (13)

It has been prepared in 78% starting from **12**, following the same procedure described for **6**. Note however that in this case the reaction is much slower requiring 10 h at rt to reach completion. $R_f$ 0.31 (PE/AcOEt 1:1). Found: C, 68.35; H, 4.95, N, 6.0. $C_{13}H_{11}NO_3$ requires C, 68.11; H, 4.84; N, 6.11%. $^1$H NMR: $\delta$ 6.21 [1H, br s, N*H*–CO]; 5.91 [2H, s, C*H*=C*H*]; 5.41 [1H, d, C*H*–OCO, $J$ = 9.3]; 3.52 [1H, ddd, C*H*–$CH_2$, $J$ = 12.2, 4.1, 2.6]; 3.41 [1H, dd, C*H*–NH, $J$ = 9.4, 2.6]; 2.93 and 2.75 [2H, AB part of ABXY syst., $CH_2$–C≡C, $J_{AB}$ = 17.8, $J_{AX}$ = 12.6, $J_{BX}$ = 3.6, $J_{AY}$ = 0, $J_{BY}$ = 1.0]; 2.14 [3H, s, $CH_3$–CO].

### 4.15. (1*R**,9*S**,10*S**)(*Z*)-9-Acetoxy-11-(*tert*-butoxycarbonyl)-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (14)

It has been prepared in 90% yield starting from **13**, following the same procedure described for **7**. $R_f$: 0.60 (PE/$Et_2O$ 1:1). Found: C, 65.75; H, 5.9, N, 4.3. $C_{18}H_{19}NO_5$ requires C, 65.64; H, 5.81; N, 4.25% $^1$H NMR $\delta$ 5.94 [2H, s, C*H*=C*H*]; 5.84 [1H, d, C*H*–OCO, $J$ = 9.1]; 4.33 [1H, dd, C*H*–N, $J$ = 9.16, 3.21]; 3.45 [1H, dt, C*H*–$CH_2$, $J_d$ = 12.0, $J_t$ = 3.7]; 2.95 and 2.75 [2H, AB part of an ABX system, $CH_2$–C≡C, $J_{AB}$ = 17.8, $J_{AX}$ = 12.5, $J_{BX}$ = 3.6]; 2.11 [3H, s, $CH_3$–CO]; 1.51 [9H, s, $(CH_3)_3CO$].

### 4.16. (1*R**,9*S**,10*S**)(*Z*)-9-Acetoxy-11-[(1-naphthylmethyl)-carbamoyl]-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (15)

It was prepared in 67% yield starting from **13**, following the same procedure described for **8d**. $R_f$ 0.57 (PE/AcOEt 7:3). Found: C, 72.6; H, 4.95, N, 6.65. $C_{25}H_{20}N_2O_4$ requires C, 72.80; H, 4.89; N, 6.79%. $^1$H NMR: $\delta$ 7.90–7.78 [2H, m]; 7.62–7.42 [4H, m]; 6.81 [1H, t, N*H*–$CH_2$, $J$ = 5.5]; 5.93 [1H, d, C*H*–OCO, $J$ = 9.0]; 5.93 [2H, s, C*H*=C*H*]; 4.97 and 4.83 [2H, AB part of an ABX system, $CH_2$–NH, $J_{AB}$ = 14.9, $J_{AX}$ = 5.5, $J_{BX}$ = 6.0]; 4.46 [1H, dd, C*H*–N, $J$ = 9.2, 2.9]; 3.52 [1H, dt, C*H*–$CH_2$, $J$ = 12.0, 3.6]; 2.92 and 2.74 [2H, AB part of an ABX system, $CH_2$–C≡C, $J_{AB}$ = 17.9, $J_{AX}$ = 12.4, $J_{BX}$ = 3.4]; 2.21 [3H, s, $CH_3$–CO].

### 4.17. *N*-Benzyl 3-isocyanatopropanamide (22)

*N*-(Benzyloxycarbonyl) β-alanine (824 mg, 3.70 mmol) was dissolved in dry $CH_2Cl_2$ (6 mL) and DMF (6 mL). The solution was cooled to 0 °C and treated with *N*-hydroxybenzotriazole (HOBT) (500 mg, 3.70 mmol), benzylamine (445 μL, 4.07 mmol) and EDC[19] (852 mg, 4.44 mmol). After 10 min the cooling bath was removed and the mixture stirred for 4 h at rt. The reaction was quenched with water, and extracted with AcOEt. The organic extracts were washed with 1 M NaOH and 5% $NH_4H_2PO_4$. Drying ($Na_2SO_4$), evaporation and chromatography ($CH_2Cl_2$/AcOEt 45:55 to 30:70) gave pure *N-benzyl 3-(benzyloxycarbonylamino)propanamide*[42] as a solid (1.044 g, 90%). $R_f$ 0.67 ($CH_2Cl_2$/AcOEt 3:7, ninhydrin). $^1$H NMR: $\delta$ 7.65–7.22 [10H, m, aromatics]; 6.00 [1H, s, N*H*]; 5.49 [1H, s, N*H*]; 5.06 [2H, s, $CH_2O$]; 4.41 [2H, d, NHC$H_2$Ph, $J$ = 5.6]; 3.49 [2H, q, NHC$H_2CH_2$, $J$ = 5.8]; 2.43 [2H, t, $CH_2CH_2CO$, $J$ = 5.8].

This compound (502 m, 1.61 mmol) was dissolved in MeOH (12 mL) and hydrogenated over 10% Pd/C (54 mg) at room temp. and pressure for 80 min. After filtration of the catalyst, the solvent was evaporated. the resulting amine was taken up in dry $CH_2Cl_2$ (3 mL) and treated with 1 M HCl in diethyl ether (3 mL). A yellowish solid separated. Filtration afforded 264 mg of the solid hydrochloride (76%). This solid (1.23 mmol) was suspended in $CH_2Cl_2$ (12 mL) and treated with saturated aqueous $NaHCO_3$ (12 mL). After vigorously stirring for 1 min, the mixture was cooled to 0 °C and stirring stopped, allowing the phases to separate. Then a 1.93 M toluene solution of phosgene (1.275 mL, 2.46 mmol) was added, via syringe, directly to the organic phase. Then stirring was resumed for 15 min at 0 °C. The phases were separated, and the aqueous one re-extracted three times with $CH_2Cl_2$. Drying ($Na_2SO_4$) and evaporation afforded crude isocyanate **22** (108 mg, 33% from *N*-benzyl 3-(benzyloxycarbonylamino)propanamide) as a yellow oil. This compound was used as such, without further purifications. However, $^1$H NMR showed that it was essentially pure. IR: $\nu_{max}$ 2247, 1665 cm$^{-1}$. $^1$H NMR: $\delta$ 7.39–7.19 [5H, m]; 5.94 [1H, s, N*H*]; 4.47 [2H, d, C$H_2$Ph, $J$ = 5.4]; 3.66 [2H, t, NC$H_2$CH$_2$, $J$ = 5.8]; 2.46 [2H, t, C$H_2$CO, $J$ = 6.2].

### 4.18. (3*R**,4*S**)(*E*)-3-Methoxy-4-styryl-2-azetidinone (25)

To a solution of (3*R**,4*S**)(*E*)-3-methoxy-1-(*p*-methoxyphenyl)-4-styryl-2-azetidinone **24** (608.2 mg, 1.96 mmol) in $CH_2Cl_2$ (6.5 mL) and $CH_3CN$ (14 mL), cooled to −18 °C, a solution of CAN[19] (2.699 g, 4.92 mmol) in $H_2O$ (6 mL) was dropped during 5 min. After further stirring for 15 min, the mixture was dropped into a solution of $Na_2S_2O_5$ (644 mg) in $H_2O$ (9 mL). The resulting pH was 1. Extraction with $Et_2O$ (three times), followed by washing of the united organic layers with 5% aqueous $NaHCO_3$ and with saturated NaCl, and finally drying ($Na_2SO_4$) and evaporation, gave a crude product that was chromatographed with PE/AcOEt 7:3 to 4:6 to give compound **25** as a yellowish solid (266 mg, 67%). $R_f$ 0.20 (PE/AcOEt 1:1). The analytical data were identical with those reported.[26]

### 4.19. (3*R**,4*S**)(*E*)-1-[2-[(*N*-benzyl-*N*-methyl)carbamoyl]-ethylcarbamoyl]-3-methoxy-4-styryl-2-azetidinone (27)

*N*-Methylbenzylamine (301 mg, 2.41 mmol) was dissolved in dry $CH_2Cl_2$ (2 mL). The solution was cooled in cold water and treated with pyridine (0.50 mL) and succinic anhydride (372 mg, 3.72 mmol). After 5 min the cooling bath was removed and the solution allowed to stir for 70 min at rt. The mixture was diluted with saturated aqueous $NH_4Cl$, acidificated with HCl to pH 3, and extracted with $CH_2Cl_2$. The organic extracts were washed with saturated aqueous NaCl, dried ($Na_2SO_4$) and evaporated to dryness. Chromatography (AcOEt + 1% AcOH) afforded a white solid (510 mg). Part of this solid (209.2 mg, supposed 0.946 mmol) was taken up in dry toluene (4 mL) and treated with $Et_3N$ (160 μL, 1.12 mmol) and diphenyl phosphoryl azide (250 μL, 1.12 mmol). After stirring at rt for 135 min, TLC showed complete conversion of the acid into the corresponding acyl azide. The solution was then heated at 80 °C, monitoring the Curtius rearrangement through TLC. When the reaction was complete (140 min), the solution was cooled, diluted with 5% aqueous $NaHCO_3$ and extracted three times with AcOEt. After drying ($Na_2SO_4$) and evaporation, the resulting yellow oil was taken up in dry $CH_2Cl_2$ (7 mL). This solution was approximately 0.135 M in *N-Benzyl-N-methyl-3-isocyanatopropanamide* **23** and was used as such for the following reaction.

Azetidinone **25** (24.9 mg, 122 μmol) was dissolved in $CH_2Cl_2$ (300 μL) and treated with the above prepared isocyanate solution (1.35 mL, 183 μmol), DMAP[19] (7.5 mg, 61 μmol), and DBU[19] (18 μL, 121 μmol). The solution was refluxed for 4 h. The mixture was diluted with AcOEt and 5% $NH_4H_2PO_4$. Extraction with AcOEt (three times), followed by washing with saturated NaCl, drying ($Na_2SO_4$) and evaporation to dryness gave a crude product that was chromatographed (PE/AcOEt 2:8) to give pure **27** as an oil (32.0 mg, 63%). $R_f$ 0.36 (PE/AcOEt 3:7). Found: C, 68.45; H, 6.5, N, 9.9. $C_{24}H_{27}N_3O_4$ requires C, 68.39; H, 6.46; N, 9.97%. $^1$H NMR (2 conformers are present: A (major) and B (minor) in ratio 58:42): $\delta$ 7.45–7.07 [11H, m, aromatics and N*H*]; 6.83 (conf. A) [1H, d, CH=C*H*Ph; $J$ = 15.8]; 6.81 (conf. B) [1H, d, CH=C*H*Ph, $J$ = 15.8]; 6.23 (conf. A) [1H, dd, C*H*=CHPh, $J$ = 8.4, 15.8]; 6.22 (conf. B) [1H, dd, C*H*=CHPh, $J$ = 8.4, 15.8]; 4.81 (conf. A) [1H, dd, *H*-4, $J$ = 5.0, 8.4]; 4.79 (conf B) [1H, dd, *H*-4, $J$ = 5.0, 8.4]; 4.69 (conf. A) [1H, d, *H*-3, $J$ = 5.0]; 4.68 (conf. B) [1H, d, *H*-3, $J$ = 5.0]; 4.59 (conf. A) [2H, s, C$H_2$Ph]; 4.49 (conf. B) [2H, s, C$H_2$Ph]; 3.70–3.54 [2H, m, C$H_2$CH$_2$CON(Me)Bn]; 3.49 [3H, s, OC$H_3$]; 2.96 (conf. B) [3H, s, NC$H_3$]; 2.86 (conf. A) [3H, s, NC$H_3$]; 2.66–2.56 [2H, m, CH$_2$C$H_2$CON(Me)Bn].

### 4.20. (3*R**,4*S**)(*E*)-3-Methoxy-4-(2-phenylethyl)-2-azetidinone (29)

A solution of azetidinone **25** (302.9 mg, 1.49 mmol) in 96% EtOH (6 mL) was hydrogenated over 10% Pd–C (97 mg) for 5 h. The reaction was followed by GC–MS. $t_R$ of **25**: 7.45 min; $t_R$ of **29**: 6.97 min. MS of **29**: *m*/*z*: 162 (M$^+$−43 (–HN=C=O), 6.0); 117 (1.6); 91 (12.8); 71 (100.0); 65 (5.0); 43 (2.7); 41 (12.7); 39 (3.0). Filtration of the catalyst and evaporation afforded compound **29** (294.5 mg, 96%), which was not further purificated, but used as such for further reactions. $^1$H NMR (300 MHz.): $\delta$ 7.40–7.12 [5H, m, aromatics]; 5.97 [1H, br s, N*H*]; 4.50 [1H, dd, *H*-3, $J$ = 2.7, 5.1]; 3.75 [1H, dt, *H*-4, $J_d$ = 8.1, $J_t$ = 5.1]; 3.55 [3H, s, OC$H_3$]; 2.80–2.60 [2H, m, C$H_2$Ph]; 2.08–1.82 [2H, m, C$H_2$CH$_2$Ph].

### 4.21. (3*R**,4*S**)1-[(2-(*tert*-Butoxycarbonyl)ethyl)carbamoyl]-3-methoxy-4-(2-phenylethyl)-2-azetidinone (30)

Succinic acid mono-*tert*-butyl ester was prepared as previously described[28]: succinic anhydride (1.521 g, 15.20 mmol) was dissolved in dry toluene (10 mL) and treated with NIS[19] (532.5 mg, 4.6 mmol), DMAP[19]

(189.3 mg, 1.5 mmol), triethylamine (635 μL, 4.56 mmol) and dry *tert*-butyl alcohol (4.3 mL). The solution was refluxed for 24 h and then cooled, diluted with AcOEt and washed with 0.5 M aqueous citric acid (pH 2). The aqueous phase was re-extracted with AcOEt and the united organic layers were washed with saturated aqueous NaCl, dried ($Na_2SO_4$) and evaporated to give crude succinic acid mono-*tert*-butyl ester as a brown solid (2.409 g). A part of this solid (201 mg, 1.15 mmol) was dissolved in toluene (4.5 mL) and treated with $Et_3N$ (196 μL, 1.41 mmol) and diphenyl phosphoryl azide (295 μL, 1.37 mmol). After stirring for 5 h at rt, the mixture was refluxed for 3.5 h. After cooling and evaporating to dryness, the residue was taken up in AcOEt and washed with 5% aqueous $NaHCO_3$. Drying ($Na_2SO_4$) and evaporation gave crude *tert*-butyl 3-isocyanatopropionate **28**. It was taken up in dry $CH_2Cl_2$ (2.56 mL) in order to have a theoretical 0.45 M solution.

Compound **29** (50.9 mg, 248 μmol) was dissolved in dry $CH_2Cl_2$ (700 μL) and treated with the above prepared solution of isocyanate **28** (1.10 mL, 495 μmol), DMAP (15.2 mg, 124 μmol), and DBU (37 μL, 248 μmol). The solution was stirred for 16 h at rt, diluted with AcOEt, and washed with 5% aqueous $NH_4H_2PO_4$ and saturated aqueous NaCl. Drying ($Na_2SO_4$), evaporation and chromatography (PE/AcOEt 7:3) gave pure **30** as an oil (89.3 mg, 95%). $R_f$ 0.41 (PE/AcOEt 6:4). Found: C, 64.1; H, 7.65, N, 7.5. $C_{20}H_{28}N_2O_5$ requires C, 63.81; H, 7.50; N, 7.44%. $^1$H NMR $\delta$ 7.40–7.12 [5H, m, aromatics]; 6.95 [1H, br t, N*H*, $J$ = 5.9]; 4.54 [1H, d, *H*-3, $J$ = 5.6]; 4.21 [1H, dt, *H*-4, $J_d$ = 7.8, $J_t$ = 5.6]; 3.59 [3H, s, OC$H_3$]; 3.52 [2H, q, NHC$H_2$, $J$ = 6.2]; 2.77 [2H, t, C$H_2$Ph, $J$ = 7.8]; 2.48 [2H, t, C$H_2$CO$_2$$^t$Bu, $J$ = 6.2]; 2.35–2.13 [2H, m, C$H_2$CH$_2$Ph]; 1.46 [9H, s, C(C$H_3$)$_3$].

### 4.22. (3*R**,4*S**)1-[(2-(Benzylcarbamoyl)ethyl)carbamoyl]-3-methoxy-4-(2-phenylethyl)-2-azetidinone (31)

A solution of azetidinyl urea **30** (37.3 mg, 99 μmol) in dry $CH_2Cl_2$ (500 μL) was treated with trifluoroacetic acid (250 μL). After stirring for 30 min at rt, the solvent was rapidly evaporated, taken up with $CH_2Cl_2$ and evaporated again (this process was repeated three times). It was finally taken up in $CH_2Cl_2$ (500 μL), treated with benzylamine (14 μL, 129 μmol) and with DCC[19] (25.3 mg, 123 μmol). After stirring for 45 min at rt, the reaction was complete. Dilution with AcOEt, washing with 5% aqueous $NH_4H_2PO_4$ and with saturated aqueous NaCl, drying ($Na_2SO_4$) and evaporation afforded a crude product that was chromatographed (PE/AcOEt 3:7 to 2:8) to give pure **31** as a white solid (23.4 mg, 58% from **30**). $R_f$ 0.17 (PE/AcOEt 1:1 + 2% AcOH). $R_f$ of intermediate acid (same eluent): 0.32. Found: C, 67.6; H, 6.8, N, 10.0. $C_{23}H_{27}N_3O_4$ requires C, 67.46; H, 6.65; N, 10.26%. $^1$H NMR $\delta$ 7.37–7.12 [10H, m, aromatics]; 7.06 [1H, br t, N*H*, $J$ = 5.8]; 6.05 [1H, br t, N*H*Bn, $J$ = not measurable]; 4.51 [1H, d, *H*-3, $J$ = 5.6]; 4.43 [2H, d, C$H_2$Ph, $J$ = 6.0]; 4.15 [1H, dt, *H*-4, $J_d$ = 7.6 $J_t$ = 5.6]; 3.59 [3H, s, OC$H_3$]; 3.59 [2H, q, NHC$H_2$, $J$ = 6.2]; 2.75 [2H, t, C$H_2$Ph, $J$ = 8.1]; 2.47 [2H, t, C$H_2$CONH, $J$ = 6.2]; 2.35–2.10 [2H, m, C$H_2$CH$_2$Ph].

### 4.23. (3*R**,4*S**)1-[(5-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)pentyl)carbamoyl]-3-methoxy-4-(2-phenylethyl)-2-azetidinone (34)

A 0.45 M solution of crude isocyanate **33** was prepared from know acid **32**[29] following the same procedure described above for the synthesis of isocyanate **28** (see the preparation of **30**).

Azetidinone **29** (92.0 mg, 448 μmol) was dissolved in $CH_2Cl_2$ (5 mL) and treated with the solution of **28** (2.0 mL, 900 μmol), DMAP[19] (27.4 mg, 224 μmol) and DBU[19] (67 μL, 448 μmol). The solution was stirred for 16 h at rt, diluted with AcOEt, and washed with 5% aqueous $NH_4H_2PO_4$ and saturated aqueous NaCl. Drying ($Na_2SO_4$), evaporation, and chromatography (PE/AcOEt 6:4) gave pure **34** as an oil (184.3 mg, 92%). $R_f$ 0.47 (PE/AcOEt 1:1). Found: C, 64.4; H, 8.3, N, 9.35. $C_{24}H_{37}N_3O_5$ requires C, 64.41; H, 8.33; N, 9.39%. $^1$H NMR $\delta$ 7.34–7.15 [5H, m, aromatics]; 6.58 [1H, br t, N*H*, $J$ = 5.7]; 4.55 [1H, d, *H*-3, $J$ = 5.4]; 4.21 [1H, dt, *H*-4, $J_d$ = 7.2 $J_t$ = 5.2]; 3.59 [3H, s, OC$H_3$]; 3.29 [2H, t, C$H_2$N, $J$ = 6.6]; 3.19 [2H, t, C$H_2$N, $J$ = 6.8]; 2.82 [3H, s, NC$H_3$]; 2.78 [2H, t, C$H_2$Ph, $J$ = 8.2]; 2.35–2.10 [2H, m, C$H_2$CH$_2$Ph]; 1.63–1.45 [4H, m, C$H_2$]; 1.44 [9H, s, C(C$H_3$)$_3$]; 1.20–1.10 [2H, m, C$H_2$].

### 4.24. 4-(4-Acetamido-1-methyl-1H-pyrrole-2-carboxamido)-1-methyl-1H-pyrrole-2-carboxylic acid (37)

A solution of known compound **36**[31,32,34] (111 mg, 362 μmol) in dry DMF (4 mL) was hydrogenated over 10% Pd–C (65 mg)[34] for 6 h at rt. The catalyst was rapidly filtered through cotton, washing with a total of 7 mL of DMF. The filtrate was immediately treated with acetic anhydride (61 μL, 651 μmol), and pyridine (52 μL, 651 μmol). After stirring for 100 min at rt the acetylation is complete. Most DMF was evaporated at 1 mbar. The residue was taken up in AcOEt and filtered through a paper filter to remove traces of the hydrogenation catalyst. The clear orange-yellow solution was washed with 5% aqueous $NH_4H_2PO_4$ and saturated NaCl, dried ($Na_2SO_4$) and evaporated to dryness. The crude product was chromatographed with $CH_2Cl_2$/acetone 8:2 to $CH_2Cl_2$/acetone 7:3 + 1% MeOH to give pure *methyl 4-(4-acetamido-1-methyl-1H-pyrrole-2-carboxamido)-1-methyl-1H-pyrrole-2-carboxylate* (89.4 mg, 78%). It is worth noting that this product is not very soluble in most organic solvents. Therefore, introduction to the chromatographic column has been made by dissolving it in $CH_2Cl_2$/MeOH 8:2, adding 500 mg of 40–60 mesh silica, evaporating the solvents and introducing the resulting powder at the head of the column. $R_f$ 0.45 ($CH_2Cl_2$/acetone 70:30 + 1% MeOH). $^1$H NMR ($d_6$-DMSO): $\delta$ 9.89 and 9.83 [2H, 2 s, N*H*]; 7.46 [1H, d, $J$ = 2.0]; 7.15 [1H, d, $J$ = 1.4]; 6.90 [1H, d, $J$ = 1.8]; 6.85 [1H, d, $J$ = 1.8]; 3.84, 3.82, 3.74 [3× 3H, 3 s, C$H_3$O and C$H_3$N]; 1.98 [3H, C$H_3$C=O]. $^{13}$C NMR ($d_6$-DMSO, 75 MHz.): $\delta$ 166.4, 160.7, 158.3 [*C*=O]; 122.8, 122.4, 122.1, 118.4 [aromatic quat.]; 120.7, 118.1, 108.3, 103.8 [aromatic *C*H]; 50.9 [*C*H$_3$O]; 36.1 and 36.0 [*C*H$_3$N]; 23.0 [*C*H$_3$C=O].

This ester (78.8 mg, 248 μmol) was dissolved (by warming) in MeOH (2 mL) and treated with a 3 M solution of NaOH in $H_2O$ (1 mL, 3.0 mmol). The solution was stirred overnight. Most MeOH was evaporated, and the residue taken up in AcOEt. The organic phase was washed with 5% aqueous $NH_4H_2PO_4$ (further acidified with HCl so that pH 3). The aqueous phase was extracted five times with AcOEt. Evaporation afforded the crude acid **37** (64.3 mg) that was used as such for the subsequent reactions.

### 4.25. Compound 35

A solution of azetidinyl urea **34** (55.0 mg, 123 μmol) in dry $CH_2Cl_2$ (500 μL) was treated with trifluoroacetic acid (500 μL). After stirring for 90 min at rt, the solvent was rapidly evaporated, taken up with $CH_2Cl_2$/*n*-heptane and evaporated again (this process was repeated three times). It was finally taken up in $CH_2Cl_2$ (1.0 mL), and treated with Py-BOP[19] (46 mg, 104 μmol), crude carboxylic acid **37** (24.0 mg, 80 μmol), and $Et_3N$ (40 μL, 287 μmol). The initial suspension (due to poor solubility of **37**) soon became a yellowish solution. This solution was stirred for 24 h at rt. Then it was diluted with AcOEt and washed with 5% aqueous $NaHCO_3$ and with saturated aqueous NaCl. Drying ($Na_2SO_4$), evaporation and chromatography ($Et_2O$/acetone 4:6) afforded pure **35** (21.8 mg, 44%). $R_f$ 0.41 ($Et_2O$/acetone 4:6). $^1$H NMR ($d_6$-DMSO, 300 MHz): $\delta$ 9.84 and 9.82 [2H, 2 s, N*H*]; 7.32–7.11 [7H, m, aromatics]; 7.00 [1H, t, N*H* urea, $J$ = 5.7]; 6.85 [1H, s, C*H* pyrrole]; 6.39 [1H, s, C*H* pyrrole]; 4.73 [1H, d, *H*-3, $J$ = 5.7]; 4.14 [1H, dt, *H*-4, $J_d$ = 7.2, $J_t$ = 5.6]; 3.82, 3.61 [2× 3H, 2 s, C$H_3$N pyrrole]; 3.50–3.38 [2H, m, C$H_2$N]; 3.47 [3H, s, OC$H_3$]; 3.21–3.06 [2H, m, C$H_2$N]; 3.01 [3H, br s, C$H_3$N tertiary amide]; 2.67 [2 H, t, C$H_2$Ph, $J$ = 7.9]; 2.06–1.80 [2H, m, C$H_2$CH$_2$Ph]; 1.97 [3H, C$H_3$C═O]; 1.62–1.40 [4H, m, C$H_2$]; 1.31–1.15 [2H, m, C$H_2$]. $^{13}$C NMR ($d_6$-DMSO, 75 MHz): $\delta$ 166.6, 166.4, 162.8, 158.2, 150.0 [*C*═O]; 141.4 [quat. of Ph]; 128.3 (×2), 128.1 (×2), 125.8 [*C*H of Ph]; 122.7, 122.6, 122.1, 121.9 [quat. of pyrrole]; 117.9, 115.9, 103.6 (×2) [*C*H of pyrrole]; 82.4 [*C*-3]; 59.1 [O*C*H$_3$]; 56.2 [*C*-4]; 48.0 (very broad) [*C*H$_2$N]; 39.04 [*C*H$_2$N]; 36.0, 34.9 [*C*H$_3$N of pyrroles]; 30.3 and 29.5 [C$H_3$N of tertiary amide (2 conformers)]; 31.4, 29.8 [*C*H$_2$*C*H$_2$Ph]; 28.9, 26.7 (broad), 23.3 [*C*H$_2$*C*H$_2$*C*H$_2$]; 23.0 [*C*H$_3$C═O].

### 4.26. (1*R**,9*R**,10*S**)(*Z*)-9-Acetoxy-11-[(2-(*tert*-butoxycarbonyl)ethyl)carbamoyl]-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (38)

A solution of azetidinone **6** (50.2 mg, 219 μmol) in dry $CH_2Cl_2$ (1.0 mL) was treated with a 0.45 M solution of isocyanate **2**, prepared as above described (see the synthesis of **30**) (980 μL, 441 mol), DMAP[19] (16.4 mg, 134 μmol) and DBU[19] (32 μL, 214 μmol). After stirring for 16 h at rt the mixture was diluted with AcOEt and washed with 5% aqueous $NH_4H_2PO_4$ and aqueous saturated NaCl. Drying ($Na_2SO_4$), evaporation and chromatography (PE/$Et_2O$ 50:50) gave pure **38** as a colourless oil (70.6 mg, 80%). $R_f$ 0.26 (PE/$Et_2O$ 40:60). Found: C, 63.05; H, 6.1, N, 6.95. $C_{21}H_{24}N_2O_6$ requires C, 62.99; H, 6.04; N, 7.00%. $^1$H NMR (300 MHz) $\delta$ 6.80 [1H, t, N*H*, $J$ = 6.1]; 6.04 [1H, t, C*H*OAc, $J$ = 2.0]; 5.98 and 5.92 [2H, AB syst. (with small long range couplings), C*H*═C*H*, $J$ = 9.8]; 4.30 [1H, t, C*H*NC═O, $J$ = 2.5]; 3.85 [1H, ddd, C*H*C═O, $J$ = 3.0, 3.9, 12.6]; 3.57–3.46 [2H, m, C$H_2$N]; 2.93 and 2.70 [2H, AB part of an ABXY system, C$H_2$–C≡C, $J_{AB}$ = 17.8, $J_{AX}$ = 12.6, $J_{BX}$ = 3.9, $J_{AY}$ = 0, $J_{BY}$ = 1.8]; 2.48 [2H, t, C$H_2$CO$_2$$^t$Bu, $J$ = 6.1]; 2.10 [3H, s, C$H_3$CO]; 1.46 [9H, s, C(C$H_3$)$_3$]. $^{13}$C NMR (75 MHz): $\delta$ 170.9, 169.1, 166.5, 149.9 [*C*═O]; 126.5, 122.6 [*C*H═*C*H]; 98.7, 93.7, 87.6, 84.4 [*C*≡*C*]; 81.4 [*C*(CH$_3$)$_3$]; 60.9, 59.5, 52.0 [*C*HOAc, *C*HN, *C*HCO]; 35.6, 35.5 [N*C*H$_2$*C*H$_2$CO$_2$*t*Bu]; 28.1 [C(*C*H$_3$)$_3$]; 20.7 [*C*H$_3$C═O]; 19.1 [*C*H$_2$C≡]. IR: $\nu_{max}$ 3384, 2963, 1773, 1712, 1506, 1368, 1335, 1297, 1258, 1192, 1151, 1103 cm$^{-1}$.

### 4.27. (1*R**,9*R**,10*S**)(*Z*)-9-Acetoxy-11-[(2-(benzylcarbamoyl)ethyl)carbamoyl]-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (39)

A solution of lactenediyne **38** (35.3 mg, 88 μmol) in dry $CH_2Cl_2$ (500 μL) was treated with trifluoroacetic acid (250 μL). After stirring for 30 min at rt, the solvent was rapidly evaporated, taken up with $CH_2Cl_2$ and evaporated again (this process was repeated three times). It was finally taken up in $CH_2Cl_2$ (500 μL), treated with benzylamine (12 μL, 110 μmol) and with DCC[19] (22.3 mg, 108 μmol). After stirring for 100 min at rt, the reaction was complete. Dilution with $CH_2Cl_2$, washing with 5% aqueous $NH_4H_2PO_4$ and with saturated aqueous NaCl, drying ($Na_2SO_4$) and evaporation afforded a crude product that was chromatographed (first with $Et_2O$/AcOEt 1:2 and then with $CH_2Cl_2$/AcOEt 1:1) to give pure **39** as a white solid (19.2 mg, 50% from **38**). $R_f$ 0.28 ($CH_2Cl_2$/AcOEt 1:1). Found: C, 66.25; H, 5.4; N, 9.5. $C_{24}H_{23}N_3O_5$ requires C, 66.50; H, 5.35; N, 9.69%. $^1$H NMR (300 MHz): $\delta$ 7.38–7.21 [5H, m, aromatics]; 6.96 [1H, t, N*H*, $J$ = 6.0]; 6.05 [1H, t, C*H*OAc, $J$ = 1.9]; 6.07–6.04 [1H, br s, N*H*Bn]; 5.99 and 5.91 [2H, AB system, C*H*═C*H*, $J$ = 10.0]; 4.45 [2H, d, C$H_2$Ph, $J$ = 5.7]; 4.27 [1H, t, C*H*NC═O, $J$ = 2.7]; 3.84 [1H, ddd, C*H*C═O, $J$ = 3.0, 3.9, 12.6]; 3.59 [2H, q, NHC$H_2$CH$_2$, $J$ = 6.0]; 2.93 and 2.69 [2H, AB part of an ABXY system, C$H_2$–C≡C, $J_{AB}$ = 17.7, $J_{AX}$ = 12.9, $J_{BX}$ = 3.9, $J_{AY}$ = 0, $J_{BY}$ = 1.8]; 2.57–2.40 [2 H, m, C$H_2$CONH]; 2.03 [3H, s, C$H_3$CO]. IR: $\nu_{max}$: 3438, 3382, 3003, 2928, 2854, 1776, 1755, 1702, 1663, 1509, 1369, 1336, 1298, 1191, 1141, 1102 cm$^{-1}$.

### 4.28. Conjugated lactenediyne (40)

Amine **45** was prepared according to the literature.[37] However the last step (hydrogenation of the corresponding nitro derivative) was carried out in DMF. After filtration of the catalyst, the DMF solution of **45** was directly used for the next coupling. Starting from 60.0 mg of nitro derivative, we obtained a solution of crude **45** (theoric 172 μmol) in 8 mL of DMF. Meanwhile, a solution of lactenediyne **38** (30.8 mg, 77 μmol) in dry $CH_2Cl_2$ (500 μL) was treated with trifluoroacetic acid (500 μL). After stirring for 45 min at rt, the solvent was rapidly evaporated, taken up with $CH_2Cl_2$ and

evaporated again (this process was repeated three times). It was finally taken up in $CH_2Cl_2$ (500 μL), and added to the DMF solution of **45**. $Et_3N$ (40 μL, 272 μmol) and Py-BOP[19] (45 mg, 102 μmol) were added. The resulting solution was stirred for 16 h at rt. After evaporation of DMF at 1 mbar, the residue was taken up in $CH_2Cl_2$, filtered on paper in order to remove traces of hydrogenation catalyst, washed with 5% aqueous $NH_4H_2PO_4$ and with saturated aqueous NaCl. Drying ($Na_2SO_4$) and evaporation afforded a crude product that was chromatographed (AcOEt/acetone 9:1 + 1% MeOH) to give pure **40** as a solid (39.8 mg, 80% from **38**). $R_f$ 0.46 (AcOEt/acetone 9:1). Found: C, 61.5; H, 5.85; N, 14.95. $C_{33}H_{37}N_7O_7$ requires C, 61.58; H, 5.79; N, 15.23%. $^1$H NMR (300 MHz): δ 8.58 [1H, s, N*H*]; 7.78 [1H, s, N*H*]; 7.25 [1H, d, C*H* pyrrole, $J$ = 1.5]; 7.16 [1H, t, urea N*H*$CH_2$, $J$ = 5.9]; 6.88 [1H, d, C*H* pyrrole, $J$ = 1.2]; 6.70 [1H, d, C*H* pyrrole, $J$ = 1.5]; 6.67 [1H, d, C*H* pyrrole, $J$ = 1.8]; 6.48 [1H, br t, N*H*Bu]; 6.22 [1H, t, C*H*OAc, $J$ = 1.9]; 5.98 and 5.88 [2H, ABX system, C*H*═C*H*, $J_{AB}$ = 9.8, $J_{AX}$ = 1.5, $J_{BX}$ = 0]; 4.34 [1H, t, C*H*NC═O, $J$ = 2.7]; 3.90 [6H, s, C$H_3$N]; 3.85 [1H, ddd, C*H*C═O, $J$ = 3.0, 5.1, 11.7]; 3.60–3.43 [2H, m, C$H_2$NH]; 3.36 [2H, q, NHC$H_2$CH$_2$, $J$ = 6.7]; 2.95–2.75 [2H, m, C$H_2$–C≡C]; 2.62–2.40 [2H, m, C$H_2$CONH]; 2.08 [3H, s, C$H_3$CO]; 1.63–1.50 [2H, m, C$H_2$]; 1.39 [2H, hexuplet, CH$_3$C$H_2$, $J$ = 7.5]; 0.94 [3H, t, C$H_3$CH$_2$, $J$ = 7.2]. $^{13}$C (75 MHz): δ 169.9, 168.1, 166.8, 162.0, 159.0, 151.0 [*C*═O]; 126.8, 122.2 [*C*H═*C*H]; 123.7, 123.5, 121.7, 121.5 [quat. of pyrroles]; 118.5, 118.4, 103.8, 103.1 [*C*H of pyrroles]; 99.0, 93.3, 88.0, 84.4 [*C*≡*C*]; 60.8, 59.7, 52.0 [*C*HOAc, *C*HN, *C*HCO]; 39.1, 36.1, 35.8 [NC$H_2$ and C$H_2$CO$_2$$^t$Bu]; 36.6, 36.5 [C$H_3$N]; 31.9, 20.2 [other C$H_2$]; 20.8 [$C_3$C═O]; 18.8 [C$H_2$C≡]; 13.8 [C$H_3$CH$_2$]. IR: $\nu_{max}$: 3438, 3358, 2956, 2930, 1778, 1754, 1654, 1581, 1516, 1464, 1432, 1403, 1337, 1298, 1249, 1191, 1141, 1100 cm$^{-1}$.

### 4.29. Conjugated lactenediyne (41)

A solution of lactenediyne **38** (34.8 mg, 87 μmol) in dry $CH_2Cl_2$ (500 μL) was treated with trifluoroacetic acid (250 μL). After stirring for 80 min at rt, the solvent was rapidly evaporated, taken up with $CH_2Cl_2$ and evaporated again (this process was repeated three times). It was finally taken up in dry $CH_2Cl_2$ (3.5 mL), and treated with compound **46** (20 mg, 46 μmol), $Et_3N$ (43 μL, 308 μmol) and Py-BOP[19] (50 mg, 113 μmol) were added. The resulting solution was stirred for 20 h at rt. After dilution with $CH_2Cl_2$, the solution was washed with $H_2O$. Drying ($Na_2SO_4$) and evaporation afforded a crude product that was chromatographed ($CH_2Cl_2$/acetone 1:1 + 1% MeOH) to give pure **41** as a yellow fluorescent solid (31.1 mg, 90% from **46**). $R_f$ 0.57 ($CHCl_3$/MeOH 9:1). Found: C, 65.15; H, 5.1; N, 10.95. $C_{41}H_{38}N_6O_9$ requires C, 64.90; H, 5.05; N, 11.08%.

Since we started from enantiomerically pure **46** and racemic **38**, this product is obviously a 1:1 diastereoisomeric mixture. Moreover, NMR shows the presence of two geometric isomers in a 62:38 *E*:*Z* ratio. In this latter case the signals are often distinct at NMR. On the contrary, due to the distance between the stereogenic centres, the signals of the two 1:1 diastereoisomers are nearly always superimposed. The signals that can be seen clearly distinct at $^1$H NMR are: *H*-10′ of the *Z* oxime, and C$H_3$C═O of both geometric isomers. $^1$H NMR (300 MHz.) (the main numbers are used for camptothecin, the primed number for the lactenediyne): δ 9.05 [0.62H, s, C*H*═N (*E*)]; 8.23–8.17 [1.62H, m, *H*-9 (*E*) + *H*-12 (*E* + *Z*)]; 8.10 [0.38H, s, C*H*═N (*Z*)]; 7.95 [0.38H, d, $J$ = 8.1, *H*-9 (*Z*)]; 7.83 [1H, t, $J$ = 8.0, *H*-10]; 7.73–7.67 [1H, m, *H*-11]; 7.68 [0.38H, s, *H*-14 (*Z*)]; 7.59 [0.62H, s, *H*-14 (*E*)]; 6.95 [0.62H, t, N*H* (*E*), $J$ = 6.0]; 6.95 [0.38H, t, N*H* (*Z*), $J$ = 6.0]; 6.61 [0.38H, br t, N*H* (*Z*)]; 6.47 [0.62H, br s, N*H* (*E*)]; 6.20–5.85 [3H, m, H-5′, H-6′, H-9′]; 5.72 [1H, d, *H*-17, $J$ = 16.5]; 5.37 [1.24H, s, *H*-5 (*E*)]; 5.32 [0.76H, s, [*H*-5 (*Z*)]; 5.28–5.19 [1H, m, *H*-17]; 4.48 [1.24H, t, OC$H_2$CH$_2$N (*E*), $J$ = 5.4]; 4.40 [0.76H, t, OC$H_2$CH$_2$N (*Z*), $J$ = 4.5]; 4.27 [0.62H, t, H-10′ (*E*), $J$ = 2.7]; 4.23 [0.17H, t, H-10′ (*Z*) (1 diast.), $J$ = 2.7]; 4.22 [0.17H, t, H-10′ (*Z*) (1 diast.), $J$ = 2.7]; 4.00 [0.38H, br s, O*H* (*Z*)]; 3.94 [0.62H, br s, O*H* (*E*)]; 3.88–3.53 [4H, m, C$H_2$N]; 3.52–3.42 [1H, m, H-1′]; 2.96–2.82 [1H, m, H-2′]; 2.74–2.58 [1H, m, H-2′]; 2.53 [1.24H, t, CH$_2$C$H_2$CON (*E*), $J$ = 6.1]; 2.43 [0.76H, t, CH$_2$C$H_2$CON (*Z*), $J$ = 6.1]; 2.10 and 2.09 [1.86H, 2 s, C$H_3$CO (*E*) (2 diast.)]; 2.05 and 2.03 [1.14H, 2 s, C$H_3$CO (*Z*) (2 diast.)]; 1.98–1.80 [2H, m, *H*-19]; 1.03 [3H, t, *H*-18, $J$ = 7.5].

$^{13}$C NMR (75 MHz.): δ 173.8 [(*E*)]; 173.7 [(*Z*)]; 171.4 (*Z*), 171.1 (*E*), 169.23 (*E*), 169.19 (*Z*), 166.4 (*E*), 166.3 (*Z*) [4 *C*═O]; 157.5 (*Z*), 157.4 (*E*) [*C*═O pyridone]; 152.3 (*E*), 152.2 (*Z*), 150.3 (*Z*), 150.2 (*E*), 150.03 (*E*), 149.99 (*Z*), 149.5 (*E*), 148.8 (*Z*), 146.0 (*E* + *Z*) [1 *C*═O +4 quat. aromatics]; 144.5 [*C*H═N (*E*)]; 141.7 [*C*H═N (*Z*)]; 131.7 (*Z*), 130.9 (*E*), 127.4 (*Z*), 125.9 (*E*), 125.3 (*E*), 125.1 (*Z*), 119. 1 (*Z*), 118.8 (*E*) [4 quat. aromatics]; 130.7 (*E* + *Z*), 130.5 (*E* + *Z*), 128.7 (*Z*), 128.6 (*E*), 124.2 (*Z*), 122.8 (*E*), 98.3 (*Z*), 98.0 (*E*) [5 aromatic *C*H]; 126.5 and 122.4 [*C*-5′ and *C*-6′ (*E* + *Z*)]; 98.7, 93.5, 87.7, 84.4 [*C*-3′, *C*-4′, *C*-7′, *C*-8′]; 74.7 (*Z*), 74.2 (*E*) [*C*H$_2$ON]; 72.7 (*E* + *Z*) [*C*-20]; 66.3 (*E*), 66.2 (*Z*) [*C*-17]; 60.8, 59.5 [*C*-9′, *C*-10′]; 52.40 (*E*), 51.39 (*Z*) [*C*H$_2$N]; 51.9 (*E* + *Z*) [*C*-1′]; 39.2 (*Z*), 38.8 (*E*), 36.1 (*E* + *Z*), 35.8 (*E*), 35.4 (*Z*) [2 *C*H$_2$N+*C*H$_2$CON]; 31.6 [*C*-19]; 20.8 [*C*H$_3$CO]; 19.0 [*C*-2′]; 7.8 [*C*-18].

### 4.30. (1*R**,9*R**,10*S**)(*Z*)-9-Acetoxy-11-[(5-(*N*-(*tert*-butoxycarbonyl)-*N*-methylamino)pentyl)carbamoyl]-11-azabicyclo[8.2.0]dodec-5-ene-3,7-diyn-12-one (42)

It was prepared in 96% yield from **6** and **33** using the same procedure used for synthesizing **34**. $R_f$ 0.12 (PE/$Et_2O$ 6:4). Found: C, 63.7; H, 7.1; N, 8.8. $C_{25}H_{33}N_3O_6$ requires C, 63.68; H, 7.05; N, 8.91%. $^1$H NMR: δ 6.38 [1H, t, N*H*, $J$ = 5.7]; 6.05 [1H, t, C*H*OAc, $J$ = 2.0]; 5.99 and 5.93 [2H, AB syst. (with small long range couplings), C*H*═C*H*, $J$ = 10.0]; 4.31 [1H, t, C*H*NC═O, $J$ = 2.6]; 3.85 [1H, ddd, C*H*C═O, $J$ = 3.0, 4.0, 12.4]; 3.37–3.10 [4H, m, C$H_2$N]; 2.94 and 2.72 [2H, AB part of an ABXY system, C$H_2$–C≡C, $J_{AB}$ = 17.9, $J_{AX}$ = 12.7, $J_{BX}$ = 3.8, $J_{AY}$ 0, $J_{BY}$ = 1.6]; 2.83 [3H, s, NC$H_3$];

2.11 [3H, s, $CH_3CO$]; 1.67–1.45 [4H, m, $CH_2$]; 1.45 [9H, s, $C(CH_3)_3$]; 1.38–1.23 [2H, m, $CH_2$].

### 4.31. Conjugated lactenediyne (43)

It was prepared in 68% yield from **42** and carboxylic acid **37** following the same methodology described above for **35**. $R_f$ 0.23 ($CH_2Cl_2$/acetone 1:1). Found: C, 62.1; H, 6.0; N, 14.7. $C_{34}H_{39}N_7O_7$ requires C, 62.09; H, 5.98; N, 14.91%. $^1$H NMR (300 MHz): $\delta$ 8.18 [2H, br s, N*H* of pyrrole]; 7.27 [1H, s, C*H* pyrrole (covered by $CHCl_3$)]; 7.15 [1H, d, C*H* pyrrole, $J$ = 1.8]; 6.48–6.40 [2H, m, N*H* and C*H* pyrrole]; 6.28 [1H, s, C*H* pyrrole]; 6.05 [1H, t, C*H*OAc, $J$ = 2.1]; 5.99 and 5.90 [2H, AB syst. (with small long range couplings), C*H*=C*H*, $J$ = 9.6]; 4.24 [1H, t, C*H*NC=O, $J$ = 2.6]; 3.89 [3H, s, $NCH_3$]; 3.82 [1H, ddd, C*H*C=O, $J$ = 3.0, 3.9, 12.3]; 3.69 [3H, s, $NCH_3$]; 3.60–3.38 [2H, m, $CH_2$N]; 3.38–3.18 [2H, m, $CH_2$N]; 3.07 [3H, s, $NCH_3$]; 2.88 and 2.71 [2H, AB part of an ABXY system, $CH_2$–C≡C, $J_{AB}$ = 17.8, $J_{AX}$ = 12.7, $J_{BX}$ = 3.8, $J_{AY}$ = 0, $J_{BY}$ = 1.8]; 2.11 and 2.10 [2× 3H, 2 s, $CH_3CO$]; 1.72–1.50 [4H, m, $CH_2$]; 1.40–1.27 [2H, m, $CH_2$]. $^{13}$C NMR (75 MHz): $\delta$ 169.3, 167.6, 166.9, 164.0, 159.0, 150.3 [*C*=O]; 126.6, 122.3 [*C*H=*C*H]; 123.2, 123.1, 121.6, 121.4 [aromatic quat.]; 119.4, 117.1, 103.9 (broad), 103.3 [aromatic *C*H]; 98.8, 93.4, 87.8, 84.4 [*C*≡*C*]; 60.8, 59.6 [*C*HN and *C*HOAc]; 52.0 [*C*HC=O]; 49.5 (very broad) [$CH_2$N]; 39.3 [$CH_2$N]; 36.7, 35.6 [pyrrole $CH_3$N]; 30.9 [$CH_3$N]; 29.1, 27.1, 23.4 [other $CH_2$]; 23.5, 20.7 [$CH_3$C=O]; 18.9 [$CH_2$C≡C]. IR: $\nu_{max}$ 3439, 3375, 2989, 2938, 1770, 1752, 1697, 1658, 1609, 1528, 1436, 1399, 1336, 1295, 1253, 1098 cm$^{-1}$.

### 4.32. Conjugated lactenediyne (44)

It was prepared in 90% yield from **42** and 1-naphthylacetic acid following the same methodology described above for **35**. $R_f$ 0.15 (PE/AcOEt 4:6). Found: C, 70.95; H, 6.15; N, 7.65. $C_{32}H_{33}N_3O_5$ requires C, C, 71.22; H, 6.16; N, 7.79%. $^1$H NMR: $\delta$ 8.02–7.91 [1H, m]; 7.90–7.73 [2H, m]; 7.60–7.28 [4H, m]; 6.43–6.28 [1H, m, N*H*]; 6.05 [1H, t, C*H*OAc, $J$ = 1.8]; 5.99 and 5.92 [2H, AB syst., C*H*=C*H*, $J$ = 9.9]; 4.30 [1H, t, C*H*NC=O, $J$ = 2.4]; 4.18–4.05 [2H, m, $CH_2$-naphthyl]; 3.85 [1H, dt, C*H*C=O, $J_d$ = 12.4, $J_t$ = 3.3]; 3.48–3.35 [2H, m, $CH_2$N]; 3.33–3.10 [2H, m, $CH_2$N]; 2.99 and 2.97 [3H, 2 s, $NCH_3$ (2 conformers)]; 2.92 and 2.70 [2H, AB part of an ABXY system, $CH_2$–C≡C, $J_{AB}$ = 17.9, $J_{AX}$ = 12.7, $J_{BX}$ = 3.8, $J_{AY}$ = 0, $J_{BY}$ = 1.6]; 2.10 and 2.07 [3H, 2 s, $CH_3CO$ (2 conformers)]; 1.67–1.10 [6H, m, $CH_2$]. $^{13}$C NMR (several signals are splitted due to conformers at the tertiary amide): $\delta$ 171.0, 166.85, 166.76, 150.1 [4 *C*=O]; 133.8, 132.1, 131.4 [aromatic quat.]; 128.8, 127.6, 126.4, 126.3, 125.8, 125.5, 123.5 [aromatic *C*H]; 126.5, 122.5 [*C*H=*C*H]; 98.7, 96.6, 93.8, 93.7, 87.7, 87.6, 84.4 [*C*≡*C*]; 60.9, 59.5 [*C*HOAc and C*H*N]; 52.0 [*C*HC=O]; 50.3, 47.8, 39.6, 39.5, 39.0, 38.4 [$CH_2$]; 35.9, 33.6 [$CH_3$N (2 conf.)]; 29.41, 29.38, 28.0, 26.7, 23.9, 23.8 [other $CH_2$]; 20.7 [$CH_3$C=O]; 19.04 [$CH_2$C≡C]. IR: $\nu_{max}$ 3380, 2997, 2931, 2859, 1771, 1705, 1633, 1518, 1397, 1370, 1335, 1294, 1262, 1141, 1102 cm$^{-1}$.

### 4.33. Incubation with plasmid DNA and analysis via gel electrophoresis

*Working buffer*: it was prepared dissolving 4.84 g of TRIS, 584 mg of EDTA and 1.142 mL of acetic acid in 1 L of distilled water. *Loading buffer*: it was prepared from 75 mg of Ficoll, 500 μL of working buffer and 500 μL of a solution containing 0.25% bromophenol blue and 0.25% xylene cyanol in, pH 8.3, TRIS–borate–EDTA buffer.

The enediyne derivatives were dissolved in DMSO in order to have a 10 mM solution. These parent solutions 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 using molecular biology water) in order to have a 50 μg/mL, 75 μM/bp concentration. Eighteen microliters of this solution was treated with 2 μL of the appropriately diluted DMSO lactenediyne solution. For example, in order to have a final lactenediyne concentration of 100 μM, 2 μL of a 1 mM 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 15 μL of loading buffer and analysed on agarose gel (prepared from 300 mg agarose, 32 mL working buffer and 1.5 μg ethidium bromide), with the submarine methodology. The gel was immersed in 325 mL of working buffer containing 165 μg of ethidium bromide and eluted at 80 mV. After elution, the gel was observed at 302 nm (transilluminator), and photographed. The intensity ratio between form I and II + III was determined, without corrections (apart from substraction of form II already present in the control), by densitometry. The II/III ratio was determined after incubation with a 100 μM concentration of the enediyne.

### 4.34. Cytotoxicity tests on lactenediyne (7)

These tests were carried out by NCI, Bethesda, Maryland (USA). The cell lines listed in Table 2 were treated for 48 h with various concentrations of **7**. The control tests have been carried out under the same conditions, but without addition of **7**. The cell growth has been estimated through the Sulforhodamine B test[43] followed by colorimetric determination. The percent growth (PG) has been then calculated through the following equations: (a) $PG = 100 \times (OD_{test} - OD_{zero})/(OD_{control} - OD_{zero})$ if $(OD_{test} - OD_{zero}) \geqslant 0$. (b) $PG = 100 \times (OD_{test} - OD_{zero})/(OD_{zero})$ if $(OD_{test} - OD_{zero}) < 0$, where $OD_{test}$ is the absorbance measured after 48 h in the presence of **7**, $OD_{zero}$ is the absorbance measured at the start of the experiment and $OD_{control}$ is the absorbance measured on the cells after 48 h in the absence of **7**. Plotting the $\log_{10}$ of the molar concentrations of **7** versus PG, the following values can be determined: $GI_{50}$: concentration at which PG = 50. TGI: concentration at which PG = 0. $LC_{50}$: concentration at which PG = −50.

### 4.35. In vivo anti-cancer activity tests on lactenediyne (7)

These tests were carried out in a 'hollow fibre assay'[44] by NCI, Bethesda, Maryland (USA). Twelve different cell lines related to human tumours (NCI-H23, NCI-H522, MDA-MB-231, MDA-MB-435, SW-620, COLO 205, LOX IMVI, UACC-62, OVCAR-3, OVCAR-5, U251, SF-295) have been implanted (both intraperitoneally and subcutaneously) in aythmic nude mice. After 3–4 days, the mice have been then subjected to four intraperitoneal treatments (once a day for 4 days) with compound **7**. After collection of the fibres, they have been examined by the MTT test for evaluation of the cellular mass, which has been compared to that of analogues tests where the mice had been treated with the solvent only. In 3 instances out of 12 (intraperitoneal implant) and in 1 instance out of 12 (subcutaneous implant) the test has shown more than 50% reduction of the cellular mass compared to the control test.

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19. Abbreviations used in the text and in the schemes: Boc-ON: 2-(*tert*-butoxycarbonyloxyimino)-2-phenyl-acetonitrile. CAN: cerium ammonium nitrate. DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene. DCC: dicyclohexyl carbodiimide. DMAP: 4-dimethylaminopyridine. EDC: *N*-ethyl-*N*′-(dimethylaminopropyl)carbodiimide. EDTA: ethylenediamino-tetracetic acid. HOBT: *N*-hydroxybenzotriazole. NIS: *N*-iodosuccinimide. Py-BOP: (benzotriazolyloxy) (tripyrrolidino)-phosphonium hexafluorophosphate. TRIS: *tris*(hydroxymethyl)aminomethane.
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