Synthetic approaches towards a new class of strained “lactenediynes”

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Dedicated to Prof. Giuseppe Bartoli on his 65th Anniversary

Abstract
The paper described two alternative synthetic approaches towards a new class of strained “lactenediynes”, compounds where a 10-membered enediyne ring is fused with a β-lactam. Although the two alternative syntheses were successful up to the last step, the cyclization to give the desired products failed in both cases, probably because of excessive steric strain in the products. In one of the two approaches (which was the most efficient one in terms of overall yield) a mixture of diastereoisomeric cyclodimers was isolated in moderate yield. A certain degree of stereoselectivity was observed. These compounds may be interesting as new supramolecular systems.

Keywords: Enediynes, beta-lactams, azetidinones

Introduction
The natural enediyne antibiotics are among the most potent anti-cancer substances known to date.1 They act through a unique mechanism involving direct radical attack on cellular DNA that provokes single or double cleavage of the DNA chain.2 The high potency of these compounds (e.g., Calicheamicin)3 is counterbalanced by their poor selectivity. This problem has recently been solved partly by conjugation with a selective antibody. The resulting conjugate (Mylotarg™) has shown very promising activity towards some types of previously intractable tumors.4 However, the quest for simpler analogues of the highly complex (and often unstable) natural enediynes, endowed with higher selectivity, is still very active.5,6
The design of “artificial” enediynes is based on the special mechanism of action of these compounds, which involves a triggering event that converts a stable prodrug into a reactive drug. The reactive moiety is typically a 3-ene-1,5-diyne moiety embedded in a 10-membered ring. While it has been demonstrated that this system can easily undergo the Bergman rearrangement, producing a diradical, the presence of suitable additional steric elements may prevent this reaction. These elements may therefore be regarded as a “safety-catch”. In order for the drug to work, they should be easily removable under physiological conditions. Along these lines, about ten years ago we introduced a new class of bicyclic enediynes, characterized by the fusion of the 10-membered enediyne ring with a β-lactam. The latter acts as the “safety-catch”, preventing cycloaromatization. On the other hand, opening of the β-lactam (that is removal of the safety-catch) represents the needed "triggering event" that converts the stable prodrug into a drug.

In our previous papers we reported the synthesis of two types of lactenediynes, represented by the general formulas 1 and 2 (Scheme 1). In particular, compounds 1 have proved to be very promising. They are very stable prodrugs (even in the dry state), but readily undergo cycloaromatization after β-lactam opening. While normally the β-lactam is too stable to undergo spontaneous opening under physiological conditions, the incorporation of appropriate activating substituents affords compounds that undergo the cascade of reactions leading to DNA damage under mild conditions. Taking advantage of this fact, and using the “handles” present on the starting lactenediyne for attaching DNA-complexing substructures, we have recently prepared a series of lactenediynes characterized by their ability to induce single- and double strand DNA cleavage at concentrations as low as $10^{-7}$ M.

Scheme 1. Various types of lactenediynes.

This kind of activation is however unselective, thus bringing the same drawbacks as the natural enediynes. Thus, we are now trying to develop a controlled mechanism for β-lactam opening, by tethering an amino group to the nitrogen in order to induce an intramolecular transamidation reaction. After a thorough study of this acyl transfer process we have been able to demonstrate the feasibility of this approach, by preparing the protected amines 3 that are able to cleave DNA only after removal of the protecting group. Coupled with the use of enzymatically or photochemically removable protecting groups, this result might lead to the development of lactenediyne prodrugs which could be made active at will.
However, the DNA-cleaving activity of the free amines 4 turned out to be disappointingly lower than that of unselectively activated lactenediyynes. Further studies have shown that the main problem is the slowness of the intramolecular transamination process that converts the stable lactenediyne-aminos of general formula 4 into enlarged lactams that then undergo fast cycloaromatization. Surprisingly, in these compounds the \( \beta \)-lactam ring was found to be even more stable towards hydrolysis than in the simple monocyclic \( \beta \)-lactam models used in our preliminary studies.\(^{16}\)

Lactenediyynes of type 1 were not expected to be more strained than simple monocyclic \( \beta \)-lactams. As evidenced by force-field calculations carried out with CSC Chem3D, \textit{trans} fusion with the cyclodeca-3-ene-1,5-diyne produces only minor changes in the conformation of the 10-membered ring (Figure 1). Thus, the \( \beta \)-lactam may preserve its planarity.

In seeking analogues of 1 endowed with higher reactivity at the \( \beta \)-lactam site, we were attracted to the isomeric lactenediyynes of type 5 (Scheme 1), where the azetidinone is \textit{trans} fused to carbons 7 and 8 of the parent enediyne ring. In this case, it is clear that the fusion must bring about a greater change in the conformation of the 10-membered ring. Figure 1 shows the minimized (Chem3D) conformations of unsubstituted cyclodeca-3-ene-1,5-diyne compared to those of simple representatives of the general structures 1 and 5. While in 1 the conformation of the 10-membered ring is quite similar to that of the parent monocyclic compound, in the case of 5, there is a remarkable deviation from the preferred conformation of the parent compound. Especially noteworthy is the modification of the indicated dihedral angle. This distortion also causes a deviation from planarity for the \( \beta \)-lactam ring (the characteristic N-C-C-C=O dihedral angle becomes 8.5°). As a result, 5 experiences an increase of calculated steric strain of about 6.5 Kcal/mole, compared to the isomeric 1.
We argued that the increase in steric strain in this new class of lactenediynes could overcome
the low reactivity in the intramolecular transamidation of the β-lactam experienced for
compounds 4 and therefore permit the development of more efficient selectively activated
lactenediyne prodrugs. Therefore, we embarked on the synthesis of these new types of strained
lactenediynes.

Results and Discussion

In the total synthesis of lactenediynes it is mandatory to assemble the enediyne ring after the
azetidinone: the latter is necessary as a “safety-catch” in order to stabilize the former. There are
two main general strategies: the conjugated enediyne may be constructed during cyclization of
the 10-membered ring or, alternatively, it may be assembled in a previous step. We have studied
both approaches. Scheme 2 summarizes the designed retrosyntheses. The final formation of the
10-membered ring was expected to be the most difficult step, in view of the steric strain of the bicyclic system. Therefore, we selected the two methodologies that, in our personal experience and according to literature data, seemed most likely to meet success. In both cases, the first stages entail the preparation of a highly functionalized β-lactam precursor. For this task, the Staudinger reaction is probably the most useful method thanks to its high convergence. For the preparation of 5, the presence of a triple bond directly attached to carbon-4 of the azetidinone is an advantage, since Staudinger reactions are known to give good results when non-enolizable imines are employed.

Scheme 2. Retrosyntheses.

For route A, we needed a diyne 6, in order to exploit the Stille-type coupling developed by Danishefsky. It should be noted that this is the cross-coupling method of choice for cyclic enediyne synthesis, whereas other types of cross-coupling reactions (Sonogashira, Suzuki, metathesis) have so far met no success in the final cyclization to 10-membered enediynes. This compound could derive from manipulation of the enyne 9. Our plan was to prepare it by steroselective allylation of β-lactam 10, in turn prepared by Staudinger reaction of the imine 11. We decided to use the common intermediate 9 also for the second approach (route B). In this case, the allyl group should be manipulated by removing one carbon atom. After construction of the acyclic enediyne, we planned to accomplish the ring formation through the Nozaki reaction, which is probably the most general and efficient reaction employed in enediyne synthesis for the crucial closure of the mesocyclic ring.

Before starting the synthesis we had to select the R and R groups. Our choice was dictated by the compatibility with the planned reaction sequences. Moreover, we wanted a group R that could be removed at the end of the synthesis. Finally, R should preferably be a protected alcohol for two reasons: (a) because the Staudinger reaction is particularly efficient with alkoxyacetic
acids; (b) in order to have an additional handle to be exploited for attaching activating or DNA-complexing substituents. Thus we selected two orthogonal oxidatively removable protecting groups: the \textit{p}-methoxyphenyl (PMP) and the \textit{p}-methoxybenzyl (PMB).

The required alkoxyacetic acid 12 was prepared straightforwardly on a multigram scale starting from \textit{p}-methoxybenzyl alcohol (Scheme 3).\textsuperscript{20} Although the aldehyde 15,\textsuperscript{21} as well as the alcohol 14,\textsuperscript{22} were known compounds, the reported preparations turned out to be, in our hands, unsatisfactory for large scale synthesis. We preferred a slightly longer route that was, however, well suited for our purposes thanks to the high yields and the possibility of purifying the intermediates by distillation. Thus, propargyl alcohol 13 was converted, according to a literature procedure, into the THP derivative,\textsuperscript{23} silylated, and deblocked to give 14 in excellent yields. After oxidation, the volatile aldehyde 15 was converted directly into the crude imine 11 which, without purification, was subjected to a Staudinger condensation with 12, under the conditions developed by Palomo.\textsuperscript{24} The yield was rather good for this kind of reaction, while the selectivity favoring the \textit{cis}- isomer 16a over the \textit{trans}- one 16b was slightly lower than usual. The \textit{cis}- and \textit{trans}- isomers could easily be separated by chromatography and/or crystallization. Obviously, these \(\beta\)-lactams were obtained in racemic form. Thus, all the compounds described in this paper were racemic, although, for the sake of clarity, just one enantiomer is shown.

The next step turned out to be one of the most troublesome. We had previously alkylated, with high stereoselectivity, azetidinones similar to 16 by reaction of the corresponding lithium enolate with a propargyl bromide. We expected the same behavior in this case. However, while the diastereoselectivity was also complete in this case, the yield was lowered by the formation of considerable amounts of self-condensation products. The main difference between 16 and the analogues previously used is the triple bond (instead of a styryl unit) positioned at carbon- 4. The low steric requirements of the alkyne are most likely the cause of this unexpected behavior. After careful optimization we could raise the yield to 59\%, but were unable completely to suppress the self-condensation by-process. Interestingly, by replacing the PMBO group with a methoxy group an even worse result was obtained: we could isolate only 20-25\% of the expected allylated product. Even more surprisingly, starting from the \textit{trans}- epimer of 16, no adduct 17 was obtained, but only self-condensation products.
Scheme 3. Reagents ad conditions: a) NaH, ClCH₂CO₂H, DMF b) DHP, CH₂Cl₂, H⁺ c) nBuLi, Me₃SiCl, THF d) p-TSA, MeOH e) (COCl)₂, DMSO, Et₃N, CH₂Cl₂ f) p-anisidine, CH₂Cl₂, MgSO₄ g) 11, PhOPCl₂, Et₃N, CH₂Cl₂ h) 1. LDA; 2. allyl-Br, THF.

Since the trans- isomer should obviously form the same enolate, this striking behavior reflects the fact that the balance between the rate of enolization and self-condensation is crucial. The trans- isomer is probably enolized more slowly because the proton is more encumbered. It is interesting to note that the obtained self-condensed adducts are not allylated, so self-condensation must take place only before allylation. In order to improve the yield we tried to use other N-protecting groups (e.g., silylated protection) but without success. Anyhow, the stereoselectivity of allylation was remarkably high: we could observe only one diastereoisomer, whose relative configuration was unambiguously established as trans- by NOE experiments. Despite the moderate yield of the allylation, 17 was easily prepared on > 10 g scale.

As mentioned before, the intermediate 17 was used for both synthetic routes. We will describe route A first. Transformation of 17 into an homologated alkyne required a regioselective hydroboration-oxidation, which was successfully achieved with 9-BBN (Scheme 4). The resulting primary alcohol was oxidized to the corresponding aldehyde and then subjected to the Corey-Fuchs protocol.⁵,²⁵ The first step (formation of the vinylic dibromide) worked well with a 72% yield. More problematic was the subsequent elimination step with n-BuLi that, probably because of reactivity of the azetidinone, furnished in the best cases only a 30-40% yield of the desired alkyne 19. In order to by-pass this homologation we also tried to react directly the lithium enolate of 16 with 4-iodo-1-trimethylsilylbut-1-yn. However, only the elimination product (4-trimethylsilylbut-1-ene-3-yn) was detected. Desilylation of 19 and di-iodination afforded in 52% unoptimized yield the required substrate 20 for the final Danishefsky cyclization. To our disappointment, by using the conditions we had employed previously several times in the syntheses of Dynemicin analogs,⁵,¹⁸ we were unable to detect even traces of the desired lactenediyne 21. We observed instead a rather unstable product whose structure was
tentatively assigned as the bis-stannyl derivative 22. We thought that this failure might be because Danishefsky reaction is quite sensitive to conformational factors.

**Scheme 4.** Reagents and conditions: a) 1. 9-BBN 2. KHCO₃, H₂O₂ b) (COCl)₂, DMSO, Et₃N, CH₂Cl₂ c) CBr₄, PPh₃, Et₃N, CH₂Cl₂ d) n-BuLi, –78°C, THF e) 1. AgNO₃, EtOH 2. KCN f) I₂·morpholine, benzene g) (Z)-(Me₃Sn)CH=CH(SnMe₃), Pd(PPh₃)₄, LiCl, DMF, 70°C.

We hoped that route B, based on the more general Nozaki cyclization, might be more successful (Scheme 5). In this case, the double bond of 17 had to be oxidatively degraded. Previous work in our laboratory has shown the possibility of ozonizing selectively a terminal double bond in the presence of a terminal (non-silylated) triple bond. Thus, 17 was desilylated to 23 and subjected to an ozonolysis–reduction to give the alcohol 24. This alcohol has a remarkable tendency to undergo intramolecular transacylation under basic catalysis, affording the corresponding lactone 26. Therefore, it is important to perform the NaBH₄ reduction at low temperature.

The alcohol 24 could, in principle, be subjected directly to Castro–Stephens–Sonogashira coupling with (Z)-1-chloro-4-trimethylsilylbut-1-ene-3-yne in order to install the acyclic enediyne moiety. However, by using the usual method [Pd(PhCN)₂Cl₂, CuI, piperidine, THF, “sacrificial” alkyne], we observed extensive intramolecular transacylation to give the enediyne derivative of lactone 26. Evidently, piperidine at room temperature is sufficient to promote this intramolecular process. Thus, we had to protect the primary hydroxyl before carrying out the coupling. In this way, enediyne 27 was obtained in excellent yields. Having installed all the needed carbon atoms, a series of high-yielding functional group transformations allowed conversion of 27 into the iodoaldehyde 29 in 77% yield for four steps. The final oxidation of 28 to 29 was not trivial. Swern oxidation or other attempted methods gave unsatisfactory yields.
Eventually the best results were achieved with IBX.\textsuperscript{27} The synthesis from 17 of this aldehyde, which is the substrate for the final Nozaki cyclization, turned out to be very efficient (49\% for 8 steps).

\textbf{Scheme 6.} Reagents and conditions: a) NaHCO\textsubscript{3}, MeOH b) 1. O\textsubscript{3}, –78°C. 2. NaBH\textsubscript{4}, MeOH c) TBDMSOTf, 2,6-lutidine, CH\textsubscript{2}Cl\textsubscript{2} d) (Z)-1-chloro-4-trimethylsilylbut-1-en-3-yne, Pd(PhCN)\textsubscript{2}Cl\textsubscript{2}, Cul, piperidine, Me\textsubscript{3}SiC≡CH, THF e) I\textsubscript{2}-morpholine, benzene f) HF, CH\textsubscript{3}CN-H\textsubscript{2}O g) IBX, DMSO-THF h) CrCl\textsubscript{2}, NiCl\textsubscript{2}, THF.

We then subjected the iodoaldehyde 29 to the usual Nozaki conditions (CrCl\textsubscript{2}, NiCl\textsubscript{2}, THF). The starting material was completely consumed, and we could detect only three spots (A, B, and C) on TLC. They were isolated and accounted for an overall yield of only 27\%. During work-up, however, we noticed a remarkable amount of poorly soluble gummy products, that were highly polar on TLC, and did not migrate, even with pure AcOEt. We think that these could be either decomposition products (deriving from opening of the $\beta$-lactam ring) or linear oligomers. Examination of the three spots A, B, and C in HPLC-MS indicated that none of the desired
product 30 was present: they were all composed of cyclodimers, according to the molecular mass. While A and B were single products (from HPLC and NMR), C contained at least three isomeric products. It is worth noting that, since the starting aldehyde is racemic, and because of the formation of two new stereogenic centers, several stereoisomeric cyclodimers 31 are possible. In 31 there are six asymmetric centers, but two relative configurations are fixed (the β-lactams are trans-). Therefore, according to the vant' Hoff rule, 16 stereoisomers (8 diastereoisomers) are expected. However, for symmetry reasons, some of them coincide and therefore there are only six possible diastereoisomers. In some of them, the two halves are identical by NMR (homotopic or enantiotopic). The major compound obtained (corresponding to spot A) accounts, alone, for 43% of the whole mixture. Thus, the cyclization displays a certain degree of stereoselectivity. A gives in the NMR a single set of signals for the two halves. Therefore, it should be a symmetric compound, with the same relative configuration between the stereogenic centers 20, 1, and 3 in the two halves. It was not possible, however, to establish unambiguously which is the relative configuration, nor whether the absolute configurations of the two halves are the same (meso- compound) or not (C2 symmetry). A and B are white solids, poorly soluble in most solvents, but quite stable in the dry state. Thus the Nozaki reaction, which was so efficient for the synthesis of lactenediynes of type 1 (with yields ranging from 60 to 80% and with no formation of cyclodimers) in this case completely failed to afford the desired monomer.

The results obtained show that lactenediynes of general formula 5 are probably destined to remain a chimera. The feature that particularly attracted us, that is their expected steric strain, is most probably the very reason for the failure to obtain them through closure of the 10-membered ring. Maybe this time we expected too much, but we reasonably hoped that the steric strain would not completely prevent monomer formation. This study has demonstrated that the cost to be paid in terms of strain is, in this case, too high for cyclization to occur. However, apart from the last step—which can be optimized—the overall synthesis (route B) to 31 is efficient and these cyclodimers may find other interesting application (e.g., as host-guest systems). The partial stereoselectivity in the dimerization process is a positive feature toward this goal.

**Experimental Section**

**General Procedures.** NMR spectra were recorded in CDCl3 at 200 or 300 MHz (1H), and 50 or 75 MHz (13C), using TMS as internal standard. Chemical shifts are reported in ppm (δ scale), coupling constants are reported in Hertz. Peak assignment in 1H- NMR spectra was also made with the aid of double resonance and COSY experiments. In AB systems, the proton A is considered downfield and B upfield. Peak assignment in 13C- spectra was made with the aid of DEPT experiments.

GC-MS were carried out on a HP-5971A instrument, using an HP-1 column (12 m long, 0.2 mm wide), electron impact at 70 eV, and a 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, and (unless otherwise stated) with an initial temperature of 100°C, initial time 2 min, rate 20°C/min, final temp. 260°C, final time 4 min, injection temperature 250°C, detector temp. 280°C. Rt are in min HRMS was carried out on a hybrid q-TOF geometry tandem mass spectrometer (Q-STAR XL MS/MS system - Applied Biosystems MSD Sciex, Toronto, Canada) equipped with a MALDI ion source. 2,5-Dihydroxybenzoic acid at a final conc. of 10 mg/ml in 70:30 0.1% TFA in H₂O/0.05% TFA in CH₃CN was used as matrix. All the measurements were carried out by mixing 500 fmoles of peptides obtained in-house (ALELFR, MW=747.4272, LFTGHPETLEK, MW=1270.6550) with 500 fmoles of sample. Internal instrument calibration was performed using the main singly charged matrix fragment at m/z 137.0239 (from DHB) and singly charged ions at m/z 748.4352 and 1271.6630) of the above mentioned hexapeptides. The main peak obtained is that of the mono-sodium or potassium adduct.

IR spectra were measured with a Perkin-Elmer 881 instrument as CHCl₃ solutions. Melting points were measured on a Büchi 535 apparatus and are uncorrected. TLC analyses were carried out on silica gel plates and developed with U.V. or with molybdate reagent (21 g (NH₄)MoO₄·4H₂O, 1 g Ce(SO₄)₂, 469 ml H₂O, 31 ml H₂SO₄). RF values were measured after an elution of 7-9 cm. Chromatography was carried out on 220-400 mesh silica gel using the “flash” methodology. Petroleum ether (40-60°C) is abbreviated as PE. In extractive work-up, aqueous solutions were always re-extracted thrice with the appropriate organic solvent. Organic extracts were washed with brine, dried over Na₂SO₄ and filtered before evaporation of the solvent under reduced pressure. Dry solvents were purchased from Fluka, with the exception of THF, which was freshly distilled from K/benzophenone. All reactions employing dry solvents were carried out under a nitrogen (or argon when specified) atmosphere. The purity of all compounds was established by TLC, ¹H- and ¹³C- NMR, and by GC-MS or HPLC or HPLC-MS.

(4-Methoxybenzyl)oxyacetic acid (12). NaH, (60% in mineral oil; 10.99 g, 275 mmol) was placed under nitrogen in a flask equipped with internal thermometer and dropping funnel. Dry DMF (200 ml) and dry THF (100 ml) were added and the resulting suspension cooled in ice. A solution of 4-methoxybenzyl alcohol (15.82 g, 114.49 mmol) in dry THF (20 ml) was added through the dropping funnel during ca. 10 min, keeping the internal temperature < 7-8°C. The ice bath was removed and the suspension stirred for further 15 min, cooled to below 4°C, and a solution of bromoacetic acid (17.50 g, 125.94 mmol) in dry THF (20 ml) was added through the dropping funnel during ca. 10 min, keeping the internal temperature < 7-8°C. The ice bath was removed and the suspension stirred for further 15 min, cooled to below 4°C, and a solution of bromoacetic acid (17.50 g, 125.94 mmol) in dry THF (20 ml) was added through the dropping funnel during ca. 10 min, keeping the internal temperature < 7-8°C. After stirring at R.T. for 20 h, the reaction was quenched with H₂O (250 ml), stirred for 20 min, diluted with H₂O (250 ml) and washed with PE/Et₂O 1:1 (150 ml). The aqueous phase was acidified with 4 M HCl (40 ml) to pH 1, and saturated with NaCl. Extraction with AcOEt (1 x 100 ml), AcOEt/EtOH 9:1 (1 x 100 ml) and AcOEt/EtOH 8:2 (4 x 80 ml), followed by evaporation at 20 mbar, and then at 0.1 mbar, gave an oil (25.80 g). This was taken up in Et₂O and treated with NaOH (4.94 g, 122.5 mmol in H₂O, 150 ml). The layers were separated and the aqueous one washed twice with AcOEt (70+50 ml). The combined organic layers were extracted again with satd aq. NaHCO₃ (50 ml). The united
aqueous layers were acidified with conc. HCl (18 ml) to pH 1 and extracted with AcOEt (4 x 80 ml). The organic layer gave upon evaporation, and stripping at 0.1 mbar, a yellow-brown solid (18.458 g). Trituration with Et<sub>2</sub>O/PE afforded pure 12 as a white solid (17.221 g, 77%). M.p.: 50.8-51.8°C. <sup>1</sup>H- N.M.R. (CDCl<sub>3</sub>, 200 MHz.): δ 9.78 [1 H, broad s, O<sub>H</sub>]; 7.29 [2 H, d, H meta to OMe, J 8.8]; 6.89 [2 H, d, H ortho to OMe, J 8.8]; 4.58 [2 H, s, CH<sub>2</sub>Ar]; 4.11 [2 H, s, CH<sub>2</sub>CO<sub>2</sub>H]; 3.81 [3 H, s, CH<sub>3</sub>].

3-(Trimethylsilyl)prop-2-yn-1-ol (14). A solution of O-(tetrahydropyranyloxy)-but-2-yn-1-ol (20.46 g, 146 mmol) in dry THF (160 ml) was cooled to –65°C, and treated with n-BuLi (1.6 M in hexanes) (100 ml, 160 mmol) added from a dropping funnel, in 10 min. After warming to –28°C in 30 min, Me<sub>3</sub>SiCl (22.0 ml, 174 mmol) in dry THF (20 ml) was added during 5 min. The temperature was allowed to rise to +5°C during 2 h. After quenching with saturated aq. NaHCO<sub>3</sub>, the mixture was extracted thrice with Et<sub>2</sub>O. Evaporation to dryness furnished crude O-(tetrahydropyranyloxy)-3-trimethylsilyl-but-2-yn-1-ol, yellow oil (31.00 g), R<sub>f</sub> 0.61 (ETP/Et<sub>2</sub>O 5:1). GC-MS (60°C for 2 min; then 20°C/min): R<sub>t</sub> 6.35; m/z 211 (M<sup>+</sup> – 1, 0.7), 197 (M<sup>+</sup> – 15, 1.0), 173 (8.6), 128 (7.5), 113 (14.7), 112 (10.7), 111 (32.2), 103 (39.4), 101 (50.5), 97 (18.0), 96 (10.0), 85 (100), 84 (12.9), 83 (65.4), 81 (12.9), 79 (10.4), 77 (11.3), 75 (40.2), 73 (60.0), 67 (18.4), 59 (12.4), 57 (14.4), 55 (50.3), 53 (12.6), 45 (18.7), 43 (38.0), 41 (28.8). This compound was not purified, but directly taken up in abs. MeOH (150 ml) and treated at 0°C with p-toluenesulfonic acid monohydrate (1.50 g, 7.88 mmol). After 20 min, the cooling bath was removed, and the solution stirred for 80 min at R.T. Solid NaHCO<sub>3</sub> (840 mg, 10 mmol) was added and most MeOH evaporated at 20 mbar. The residue was taken up in Et<sub>2</sub>O and washed with brine. The organic phase was dried, and then distilled at 22 mbar. After a head fraction containing mostly 2-methoxytetrahydropyran (as well as some 14) between 40°C and 60°C, pure 14 (by GC-MS and <sup>1</sup>H NMR) distilled at 74-78°C as a colorless liquid (15.3 g, 82%). R<sub>f</sub>: 0.22 (PE/Et<sub>2</sub>O 5:1). Found: C, 62.1; H, 9.7. C<sub>11</sub>H<sub>20</sub>O<sub>2</sub>Si requires: C, 62.21; H, 9.49%. GC-MS (60°C for 2 min; then 20°C/min): R<sub>t</sub> 2.75; m/z 128 (M<sup>+</sup>, 0.58), 113 (62), 87 (16), 85 (100); 83 (5.4); 75 (30); 73 (18); 69 (5.1); 61 (28); 55 (6.0); 53 (9.8); 45 (41); 43 (13).<sup>1</sup>H- n.m.r. (CDCl<sub>3</sub>, 200 MHz.): δ 4.27 [2 H, s, CH<sub>2</sub>]; 1.70 [1 H, br. s, O<sub>H</sub>]; 0.18 [9 H, s, CH<sub>3</sub>].

(3R<sup>*</sup>, 4S<sup>*</sup>)-3-(4-Methoxybenzyloxy)-1-(4-methoxyphenyl)-4-[2-(trimethylsilyl)ethyn-1-yl]-2-azetidinone (16a). DMSO (11.34 ml) in dry CH<sub>2</sub>Cl<sub>2</sub> (250 ml) was cooled to –70°C and treated, during 5 min, with a solution of (COCl)<sub>2</sub> (11.28 ml, 129.36 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (30 ml). After further 10 min, a solution of alcohol 14 (9.76 g, 76.1 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 ml) was introduced. After 10 min, Et<sub>3</sub>N (43.5 ml, 311.97 mmol) was added to the suspension. The temperature was allowed to rise to −25°C in 45 min. The mixture was poured into a suspension of (NH<sub>4</sub>)<sub>2</sub>PO<sub>4</sub> (20 g) in H<sub>2</sub>O (250 ml). Conc. HCl (7 ml) (resulting pH = 2-3) and PE (150 ml) were added. The phases were separated and the aqueous one re-extracted twice with Et<sub>2</sub>O. The organic layer was washed with brine (100 ml) containing aq. saturated NaHCO<sub>3</sub> (10 ml), and dried. This solution, as evidenced by GC-MS, contained nearly pure 3-(trimethylsilyl)propynal 15. It was treated directly with anhydrous MgSO<sub>4</sub> (50 g) and p-anisidine (8.005 g, 65 mmol). The solution was
stirred at r.t. overnight and evaporated to dryness to give a crude oil (17.50 g). GC-MS showed that it was mainly composed of the expected imine 11. It was taken up under nitrogen in dry CH₂Cl₂ (250 ml) and cooled to –30°C. The solution was treated with Et₃N (40.77 ml, 292.5 mmol) and with a solution of acid 12 (15.94 g, 81.25 mmol) in CH₂Cl₂ (50 ml). Then a solution of PhOPCl₂ (13.60 ml, 91 mmol) in CH₂Cl₂ (30 ml) was slowly added, through a dropping funnel, during 3 h. After further 2 h, the temperature was allowed to rise to 0°C. The suspension was stirred for 2 h at 0°C and overnight at r.t. Water (60 ml) was added and the mixture stirred for 2 h. Then it was poured into saturated aq. NaHCO₃ (150 ml), the phases separated, and the aqueous one re-extracted once with Et₂O. The reunited organic layers were washed with a solution of (NH₄)H₂PO₄ (20 g), and conc. HCl (8 ml) in H₂O (200 ml). An oil separated at this point (it is poorly soluble in both phases), but it is not the desired product, and could be discarded. After a final washing with brine, drying, and evaporation, the crude product (that, by GC, contains a 91:9 cis-trans- mixture) was chromatographed through 300 g of silica (PE/CH₂Cl₂ 1:1 to PE/CH₂Cl₂/ Et₂O 45:45:10) to give a fraction containing both trans- and cis-lactams (6.13 g) and a fraction containing only the cis-isomer 16a (13.74 g). Chromatography of the first fraction on 200 g of silica (PE/ AcOEt 82:18 to 72:18) gave pure trans isomer (1.72 g, 6.5%) 16b and pure cis-isomer 16a (4.33 g). The overall yield of cis-isomer is therefore 18.07 g (68%). The overall yield is 74%. An analytical sample of cis- 16a may be obtained by crystallization (CH₂Cl₂ / Et₂O / PE). M.p. 104.5-105.1°C. An analytical sample of trans isomer 16b was obtained by trituration (Et₂O / PE): m.p. 86.6-87.6°C.

16a. Rf: 0.55 (PE/CH₂Cl₂/ AcOEt 5:5:1). HRMS: found: 432.1568 (M+Na⁺). Calc. for C₂₃H₂₇NO₄SiNa: 432.1607 (accuracy: 9 ppm). GC-MS: Rf: 11.82; m/z 409 (M⁺, 2.3), 352 (M⁺ – 57, 1.1), 288 (2.7), 232 (4.9), 149 (2.9), 134 (2.6), 121 (100), 78 (2.7), 77 (3.5), 73 (4.5). ¹H-N.M.R. (CDCl₃, 300 MHz.): δ 7.51 [2 H, d, aromatics, J 9.0]; 7.41 [2 H, d, aromatics, J 8.7]; 6.92 [4 H, d, aromatics, J 8.7]; 4.92 [1 H, d, H-3, J 4.3]; 4.85 [1 H, d, CHHar, J 11.0]; 4.76 [1 H, d, CHHar, J 11.0]; 4.75 [1 H, d, H-4, J 4.3]; 3.84, 3.83 [2 x 3 H, 2 s, OCH₃]; 0.20 [9 H, s, Si(CH₃)₃]. ¹³C- N.M.R. (CDCl₃, 50 MHz.): δ 162.44 [C-=O]; 159.53, 156.61 [C-OMe]; 130.50, 128.73 [quat. aromatics]; 129.82, 118.68, 114.30, 113.79 [CH aromatic]; 97.73, 95.07 [C≡]; 81.81 [CHO]; 72.25 [ArCH₂]; 55.49, 55.30 [OCH₃]; 50.12 [CHN]; –0.31 [Si(CH₃)₃]. I.R. (CHCl₃): vₘₐₓ. 2999, 2957, 2938, 2910, 2837, 2180 (w), 1751 (vs), 1612, 1504, 1462, 1442, 1385, 1299, 1171, 1116 cm⁻¹.

16b. Rf: 0.60 (PE/CH₂Cl₂/ AcOEt 5:5:1). GC-MS: Rf: 11.57; m/z 409 (M⁺, 3.5), 352 (M⁺ – 57, 1.2), 288 (3.5), 232 (5.7), 230 (2.9), 149 (2.7), 134 (2.8), 121 (100), 91 (2.2), 78 (3.5), 77 (4.4), 73 (5.3). ¹H- N.M.R. (CDCl₃, 200 MHz.): δ 7.45 [2 H, d, aromatics, J 9.1]; 7.34 [2 H, d, aromatics, J 8.8]; 6.90 [2 H, d, aromatics, J 8.7]; 6.87 [2 H, d, aromatics, J 9.1]; 4.80 [1 H, d, CHHar, J 11.3]; 4.78 [1 H, d, H-3, J 1.8]; 4.65 [1 H, d, CHHar, J 11.3]; 4.30 [1 H, d, H-4, J 1.8]; 3.82, 3.79 [2 x 3 H, 2 s, OCH₃]; 0.16 [9 H, s, Si(CH₃)₃]. ¹³C- N.M.R. (CDCl₃, 50 MHz.): δ 162.50 [C-=O]; 159.76, 156.58 [C-OMe]; 130.44, 128.44 [quat. aromatics]; 130.23, 118.69, 114.24, 114.00 [CH aromatic]; 99.24, 93.80 [C≡]; 87.57 [CHO]; 72.92 [ArCH₂]; 55.47, 55.27 [OCH₃]; 50.82 [CHN]; –0.35 [Si(CH₃)₃].
(3R*, 4S*)-3-Allyl-3-(4-methoxybenzoyloxy)-1-(4-methoxyphenyl)-4-[2-(trimethylsilyl)ethyn-1-yl]-2-azetidinone (17). A solution of diisopropylamine (1.447 ml, 10.32 mmol) in dry THF (30 ml) containing few crystals of 2,2'-bipyridyl, was cooled to –30°C, and treated with a 1.6 M solution of n-BuLi in hexanes (5.76 ml, 9.21 mmol). The resulting purple solution was stirred for 5 min at –30°C, for 10 min at 0°C, and then cooled to –81°C. The azetidinone 16a (3.020 g, 7.37 mmol) was dissolved in dry THF (5 ml) and this solution was added through a syringe to the LDA solution during 30 seconds. The flask containing the substrate 16a, and the syringe were washed, recovering in this way 50 mg of 16a, that were not added to the LDA solution. After being stirred at –81°C for 20 minutes the solution containing the β-lactam enolate (still purple) was treated with allyl bromide (743 µL, 10.318 mmol). The temperature was allowed to rise to –50°C during 1 h. After further 15 minutes the reaction was quenched with saturated aqueous NH₄Cl (30 ml), allowed to warm to R.T., and stirred for 1 h. Extraction with Et₂O (3x) gave a crude product that was chromatographed at once on 90 g of silica gel, eluting with PE/AcOEt 82:18 containing 0.5% of Et₃N. Pure 17 was obtained as a yellow solid (1.92 g, 59% taking into account the 50 mg recovered by washing the flask and the syringe). An analytically pure sample was obtained by trituration with Et₂O/pentane.

M.p.: 104.4-104.6°C; Rf 0.55 (PE/AcOEt 78:22); HRMS: found: 472.1900 (M+Na⁺). Calc. for C26H31NO4SiNa: 472.1920 (accuracy: 4 ppm).

1H- N.M.R. (CDCl₃, 300 MHz.): δ 7.54 [2 H, d, aromatics, J 9.0]; 7.37 [2 H, d, aromatics, J 8.7]; 6.92 [2 H, d, aromatics, J 9.0]; 6.87 [2 H, d, aromatics, J 8.7]; 5.87 [1 H, ddt, C=CH₂, Jt = 7.2, Jd = 10.0 and 17.2]; 5.10-5.30 [2 H, m, CH=C=CH₂]; 5.16 [1 H, d, CH=CH₂Ar, J not det.]; 4.83 [1 H, d, CH=CH₂Ar, J 9.9]; 4.60 [1 H, s, H-4]; 3.81, 3.80 [2 x 3 H, 2s, OC₃H₃]; 2.73 [2 H, d, CH₂CH=, J 7.2]; 0.13 [9 H, s, Si(CH₃)₃]. NOEDIFF: on irradiating the signal at 4.60: 4.75% NOE on the two H-ortho to N- of anisyl at 7.54; 3.1% NOE on C=CH₂, 3.6% NOE on CH₂CH=CH₂; on irradiating the CH₂CH=CH₂ signal at 2.73: 6.6% NOE on H-4. ¹³C N.M.R. (CDCl₃, 50 MHz.): δ 164.39 [C=O]; 159.17, 156.65 [C–OMe]; 130.42, 129.93 [quat. aromatics]; 131.19, 129.15, 118.70, 114.31, 113.61 [CH aromatics and CH=CH₂]; 119.93 [CH=CH₂]; 98.29, 95.91 [C=]; 91.21 [C-3]; 68.91 [ArCH₂]; 55.48, 55.25 [OCH₃]; 53.14 [CHN]; 38.84 [CH₂CH=CH₂] –0.43 [Si(CH₃)₃]. I.R. (CHCl₃): vmax. 3031, 2995, 2957, 2836, 2817, 1749 (vs), 1612, 1586, 1503, 1463, 1344, 1383, 1299, 1220, 1172, 1100, 1024 cm⁻¹.

(3R*, 4S*)-(3-Hydroxypropyl)-3-(4-methoxybenzoyloxy)-1-(4-methoxyphenyl)-4-[trimethylsilyl-ethyn-1-yl]-2-azetidinone (18). A solution of 17 (710 mg, 1.58 mmol) in dry THF (15 ml) was cooled to 0°C and treated with 9-borabicyclononane (9-BBN)(590 mg, 590 mg, 4.83 mmol). After 140 min, the temperature was allowed to rise to R.T. After further stirring at R.T. for 70 min, the flask was cooled again in ice and the solution treated in succession with H₂O (2.25 ml), solid KHCO₃ (1.87 g) and 35% H₂O₂ (2.25 ml). After stirring for 1 h at R.T. the mixture was diluted with 5% aqueous NaHCO₃ and extracted with AcOEt. The organic phases were washed with sat. NaCl + 10% NaHSO₃ and evaporated to dryness. Chromatography (PE/AcOEt 3:2 to 1:1) gave pure 18 as a white solid (559 mg, 76%). M.p.: 83.8-84.5°C. Rf: 0.37
(PE/AcOEt 1:1). Found: C, 66.60; H, 7.09, N, 3.03. C₂₅H₃₃NO₅Si requires: C, 66.78; H, 7.11; N, 3.00%. ¹H N.M.R. (CDCl₃, 200 MHz.): δ 7.56 [2 H, d, aromatics, J 8.9]; 7.37 [2 H, d, aromatics, J 8.3]; 6.92 [2 H, d, aromatics, J 8.3]; 6.87 [2 H, d, aromatics, J 8.9]; 5.19 [1 H, d, CHḤAr, J 10.0]; 4.81 [1 H, d, CHḤAr, J 10.0]; 4.57 [1 H, s, H-4]; 3.82, 3.80 [2 x 3 H, 2 s, OCH₃]; 3.69 [2H, broad t, CH₂OH, J 5.9]; 2.15-1.65 [5 H, m, CH₂CH₂ and OH]; 0.14 [9 H, s, Si(CH₃)₃]. ¹³C-N.M.R. (CDCl₃, 50 MHz.): δ 164.61 [C=O]; 159.22, 156.66 [C–OMe]; 130.59, 129.77 [quat. aromatics]; 129.28, 118.66, 114.35, 113.67 [CH aromatic]; 98.09, 96.26 [C=]; 91.67 [CO]; 69.08 [ArCH₂]; 62.27 [CH₂OH]; 55.52, 55.27 [OCH₃]; 54.77 [CHN]; 31.63, 27.02 [CH₂]; –0.41 [Si(CH₃)₃]. I.R. (CHCl₃): ν_max. 3490 (br), 3040, 2955, 2178 (vw), 1745 (vs), 1612, 1584, 1506, 1435, 1384, 1299, 1193, 1170, 1106, 1029 cm⁻¹.

(3R*, 4S*)-3-(But-3-yn-1-yl)-4-(ethyn-1-yl)-3-(4-methoxybenzyl)-1-(4-methoxyphenyl)-2-azetidinone (19). A solution of dry DMSO (340 µL, 4.79 mmol) in dry CH₂Cl₂ (5 ml) was cooled to –78°C and treated with a 2.22 M solution of (COCl)₂ in CH₂Cl₂ (1.35 ml, 3.00 mmol). After 15 min, a solution of 18 (557 mg, 1.19 mmol) in dry CH₂Cl₂ (5 ml) was slowly added. After 10 min, Et₃N (1.00 ml, 7.17 mmol) was added. The temperature was allowed to rise to –35°C during 2 h. Then the mixture was poured into 5% NH₄H₂PO₄ and extracted with Et₂O. The organic phase was evaporated, taken up with dry toluene, and evaporated again in order to remove most water. The resulting oily crude aldehyde (555 mg) was dissolved in dry CH₂Cl₂ (5 ml), and treated with Et₃N (250 µL, 1.79 mmol). Meanwhile, CBr₂ (917 mg, 2.76 mmol) was dissolved in dry CH₂Cl₂ (10 ml), cooled to –78°C, and treated with PPh₃ (1.160 g, 4.42 mmol). After 20 min, the solution containing the aldehyde and Et₃N was added into the other yellow-orange solution. The temperature was allowed to rise to –30°C. When the reaction was judged complete by tlc, 10% NaHSO₃ was added. Extraction with Et₂O gave, after evaporation and chromatography (PE/CH₂Cl₂ 2:8 to pure CH₂Cl₂) to give the pure vinylic dibromide as a thick oil (531 mg, 72%). Rf 0.44 (PE/AcOEt 8:2). ¹H N.M.R. (CDCl₃, 200 MHz.): δ 7.56 [2 H, d, aromatics, J 9.2]; 7.37 [2 H, d, aromatics, J 8.6]; 6.95-6.86 [2 4, m, aromatics]; 6.47 [1 H, t, CH=CBr₂, J 7.2]; 5.17 [1 H, d, CHḤAr, J 10.2]; 4.80 [1 H, d, CHḤAr, J 10.0]; 4.54 [1 H, s, H-4]; 3.82, 3.81 [2 x 3 H, 2 s, OCH₃]; 2.50-2.35 [2H, m, CH₂C=]; 2.20-2.00 [2 H, m, CH₂CH₂C=]; 0.14 [9 H, s, Si(CH₃)₃]. ¹³C N.M.R. (CDCl₃, 50 MHz.): δ 164.36 [C=O]; 159.21, 156.73 [C–OMe]; 137.25 [CH=]; 130.57, 129.80 [quat. aromatics]; 129.21, 118.70, 114.38, 113.67 [CH aromatic]; 97.82, 96.44 [C=]; 91.14, 89.97 [CO and CBR₂]; 69.07 [ArCH₂]; 62.27 [CH₂OH]; 55.53, 55.30 [OCH₃]; 54.83 [CHN]; 32.94, 27.61 [CH₂]; –0.40 [Si(CH₃)₃]. I.R. (CHCl₃): ν_max. 2957, 2837, 2173 (vw), 1746 (vs), 1612, 1586, 1504, 1439, 1384, 1299, 1196, 1168, 1109, 1024 cm⁻¹.

This dibromide (0.854 mmol) was taken up in dry THF (20 ml), cooled to –78°C, and treated with 1.6 M n-BuLi in hexanes (1.12 ml, 1.79 mmol). After 5 min, a solution of AcOH (250 µL) in Et₂O (2.5 ml) was added. After stirring for 10 min at –78°C, the solution was poured into sat. NH₄Cl, and extracted with Et₂O. Evaporation and chromatography (PE/AcOEt 85:15 to 80:20) gave (3R*,4S*)-3-(but-3-yn-1-yl)-3-(4-methoxybenzyl)-1-(4-methoxyphenyl)-4-[(trimethylsilyl)-ethyn-1-yl]-2-azetidinone as an oil, not completely pure by tlc and NMR (157 mg). The
estimated purity was 85-90%. Rf: 0.40 (PE/AcOEt 8:2). 1H N.M.R. (CDCl3, 200 MHz.): δ 7.56 [2 H, d, aromatics, J 9.0]; 7.37 [2 H, d, aromatics, J 8.5]; 6.95-6.85 [4 H, m, aromatics]; 5.17 [1 H, d, CHHAr, J 10.0]; 4.79 [1 H, d, CHHAr, J 10.0]; 4.71 [1 H, s, H-4]; 3.82, 3.81 [2 x 3 H, 2 s, OCH3]; 2.60-2.45 [CH2C=]; 2.25-2.15 [2 H, m, CH2CH2]; 1.96 [1 H, t, C=CH, J 2.6]; 0.13 [9 H, s, Si(CH3)3].

This monoalkyne was dissolved in 96% EtOH (8 ml), cooled to 0°C, and treated with a 2M aqueous AgNO3 solution (530 µL). A sudden precipitation took place. After 2h and 30 min, a solution of KCN (200 mg, 3.07 mmol) in H2O (1.5 ml) was added and the mixture stirred vigorously at 0°C for 15 min. After dilution with H2O (15 ml) and extraction with Et2O, the organic layers were washed with a 1:1 mixture of 1M NaH2PO4 and sat. NaCl. Evaporation and chromatography (PE/AcOEt 8:2 to 7:3) gave pure 19 as a foam (92 mg, 20% from 18). Rf: 0.40 (PE/AcOEt 75:25). Found: C, 73.85; H, 6.00; N, 3.5. C24H23NO4 requires: C, 74.02; H, 5.95; N, 3.60%. GC-MS: R: 12.26; m/z 389 (M+, 2.2), 231 (3.5), 160 (19.4), 121 (100), 78 (4.0), 77 (5.4), 53 (7.3) 1H- N.M.R. (CDCl3, 200 MHz.): δ 7.54 [2 H, d, aromatics, J 9.1]; 7.37 [2 H, d, aromatics, J 8.7]; 6.97-6.84 [4 H, m, aromatics]; 5.12 [1 H, d, CHHAr, J 10.0]; 4.81 [1 H, d, CHHAr, J 10.0]; 4.77 [1 H, d, H-4, J 2.3]; 3.82, 3.81 [2 x 3 H, 2 s, OCH3]; 2.70 [1 H, d, C=CH, J 2.3]; 2.54 [2 H, dt, CH2C=CH, J= 6.8, Jd= 2.6]; 2.35-2.12 [2 H, m, CH2CH2]; 1.99 [1 H, t, C=CH, J 2.6]. 13C- N.M.R. (CDCl3, 50 MHz.): δ 164.21 [C=O]; 159.30, 156.83 [C=O-OMe]; 130.30, 129.67 (quat. aromatics); 129.42, 118.68, 114.47, 113.73 [CH aromatic]; 90.73 [CO]; 83.20, 78.53, 78.48, 78.42 [C=C]; 69.21 [ArCH2]; 55.54, 55.28 [OCH3]; 53.91 [CHN]; 33.80, 13.15 [CH2]. I.R. (CHCl3): νmax. 3303, 3007, 2958, 2836, 1749 (vs), 1612, 1505, 1463, 1437, 1384, 1300, 1194, 1107, 1025 cm⁻¹.

(3R*,4S*)-3-(4-Iodobut-3-yn-1-yl)-4-(2-iodoethyn-1-yl)-3-(4-methoxybenzylxylo)-1-(4-methoxyphenyl)-2-azetidinone, 20. Iodine (891 mg, 3.51 mmol) was suspended in dry benzene (10 ml) and treated, in the dark, with morpholine (957 µL, 10.97 mmol). An abundant orange precipitate is soon formed. After 40 min, a solution of 19 (171 mg, 439 µmol) in dry benzene (7 ml) is added. The suspension was stirred at r.t. in the dark for 47 h. Then it was poured into 25 ml of 5% NH4H2PO4, added with 0.5 ml of conc. HCl and extracted with Et2O. The organic phases were washed with 10% Na2S2O3 and sat. NaCl, evaporated, and chromatographed (PE/AcOEt 75:25 to 7:3) to give pure 20 as a foam (215 mg, 76%). Rf: 0.38 (PE/AcOEt 75:25). Found: C, 45.45; H, 3.55, N, 2.25. C24H21I2NO4 requires: C, 44.95; H, 3.30; N, 2.18%. 1H N.M.R. (CDCl3, 200 MHz.): δ 7.51 [2 H, d, aromatics, J 9.1]; 7.36 [2 H, d, aromatics, J 8.7]; 6.93 [2 H, d, aromatics, J 9.0]; 6.89 [2 H, d, aromatics, J 8.8]; 5.05 [1 H, d, CHHAr, J 10.0]; 4.85 [1 H, s, H-4]; 4.79 [1 H, d, CHHAr, J 10.0]; 3.81 [6 H, s, OCH3]; 2.75-2.63 [2 H, m, CH2C=CH]; 2.30-2.00 [2 H, m, CH2CH2]. 13C N.M.R. (CDCl3, 50 MHz.): δ 164.20 [C=O]; 159.38, 156.91 [C=OMe]; 130.37, 129.55 (quat. aromatics); 129.68, 118.73, 114.55, 113.82 [CH aromatic]; 93.06, 91.18, 87.47 [C= and CO]; 69.48 [ArCH2]; 55.50 [OCH3]; 55.31 [CHN]; 33.72, 15.57 [CH2]; 15.48, 8.03 C=CI-I.

(3R*, 4S*)-3-Allyl-4-(ethyn-1-yl)-3-(4-methoxybenzylxylo)-1-(4-methoxyphenyl)-2-azetidinone, 23. A suspension of azetidinone 17 (6.857 g, 15.25 mmol) in anhydrous MeOH (140 ml)
was treated with solid NaHCO₃ (1.60 g). After being stirred for 6 h at R.T., the suspension was diluted with CH₂Cl₂ (50 ml) and Et₂O (50 ml), and filtered, washing with CH₂Cl₂. The filtrate was evaporated nearly to dryness, taken up with Et₂O/CH₂Cl₂ 1:1, and washed with brine (50) containing 5 ml of saturated aqueous NH₄Cl. The organic extracts were evaporated to dryness to give a slightly yellow solid. Trituration with Et₂O/pentane afforded pure 23 as a white solid (5.058 g). The mother liquors were evaporated and chromatographed on silica gel (PE/AcOEt 75:25) affording further 479 mg of pure 23. Overall yield: 5.537 g (96%).

M.p.: 100.3-101.3°C; HRMS: found: 400.1542 (M+Na⁺). Calc. for C₂₃H₂₅NO₄Na: 400.1525 (accuracy: 4 ppm). Rₚ 0.46 (PE/AcOEt 75:25); GC-MS (150°C for 2 min; then 25°C/min to 280°C): Rₜ 8.16; m/z 377 (M⁺, 1.1), 280 (0.9), 219 (3.5), 160 (18.9), 159 (1.9), 149 (3.0), 144 (2.3), 134 (2.8), 121 (100), 91 (3.6), 78 (6.3), 77 (7.2), 41 (6.6).¹H N.M.R. (CDCl₃, 300 MHz.): δ 7.53 [2 H, d, aromatics, J 9.0]; 7.37 [2 H, d, aromatics, J 8.7]; 6.92 [2 H, d, aromatics, J 9.0]; 6.87 [2 H, d, aromatics, J 8.4]; 5.89 [1 H, ddt, CH=C=CH₂, J₁= 7.2, J₂= 10.0 and 16.9]; 5.15-5.30 [2 H, m, CH=CH₂]; 5.11 [1 H, d, CHHAr, J 10.0]; 4.85 [1 H, d, CHHAr, J 10.0]; 4.60 [1 H, d, H-4, J 2.1]; 3.81, 3.80 [2 x 3 H, 2 s, OC=O]; 2.77 and 2.72 [2 H, AB part of ABX syst., CH₂CH=, J_{AB}= 14.2, JAX = 7.2, JBX= 7.7]; 2.67 [1 H, d, C=CH, J 2.1]. NOEDIFF: on irradiating the signal at 4.60: 4.45% NOE on the two H ortho to N of anisyl at 7.54; 2.6% NOE on CH=CH₂, 3.2% NOE on CH₂CH=CH₂. ¹³C N.M.R. (CDCl₃, 50 MHz.): δ 164.14 [C=O]; 159.24, 156.75 [C-OMe]; 130.15, 129.75 [quat. aromatics]; 131.03, 129.38, 118.66, 114.42, 113.69 [CH aromatics and CH=CH₂]; 120.03 [CH=CH₂]; 91.15 [C-3]; 78.31, 77.08 [C=CH₂]; 69.03 [ArCH₂]; 55.53, 55.27 [OCH₃]; 52.45 [CHN]; 38.62 [CH₂CH=CH₂]. I.R. (CHCl₃): νmax 3300, 3004, 2952, 2934, 2835, 1753 (vs), 1612, 1504, 1384, 1299, 1192, 1099, 1028 cm⁻¹.

(3R*,4S*)-4-(Ethen-1-yl)-3-(2-hydroxyethyl)-3-(4-methoxybenzyloxy)-1-(4-methoxyphenyl)-2-azetidinone (24). A solution of 23 (1.545 g, 4.093 mmol) in dry CH₂Cl₂ (40 ml) was treated with dry MeOH (20 ml) and Sudan III (Solvent Red 23) dye (12 mg). The red solution was cooled to –78°C, and ozonized for about 10 min (at maximum power and at a flow of 90 l/h), until the red color started to fade. Ozonolysis was interrupted, the oxygen flow was substituted by a nitrogen flow, and the solution was treated with 0.5 ml of Me₂S + 0.5 ml of cyclohexene in 2 ml of CH₂Cl₂. All these operations were carried out as quickly as possible. After 5 min, Et₃N (500 µL), and NaBH₄ (774 mg, 20.47 mmol) were added and the apparatus put under a static nitrogen atmosphere. The temperature was allowed to rise slowly to –10°C in 3 h and 30 min, and the solution stirred at this temperature until conversion of the intermediate aldehyde to 24 was complete by tlc (30 min). In some instances it was necessary to add further NaBH₄ in order to reach completion. It is not recommended to allow the temperature to rise above –10°C in order to avoid formation of lactone 26 deriving from intramolecular lactam opening by the alcohol. The mixture was finally quenched by pouring into an Erlenmeyer flask containing 70 ml of 5% (NH₄)H₂PO₄ and 20 ml of 1 M HCl (caution: vigorous gas evolution!). Extraction with Et₂O (3 times), washing with saturated NaCl, and evaporation afforded a crude product that was chromatographed at once through silica gel (75 g) eluting with PE/AcOEt 1:1 containing 1% of 96% EtOH. Pure 24 was obtained as a slightly yellow oil (1.343 g, 86%).
R f 0.29 (PE/AcOEt 60:40); 1H N.M.R. (CDCl 3, 200 MHz): δ 7.55 [2 H, d, aromatics, J 8.8]; 7.37 [2 H, d, aromatics, J 8.8]; 6.93 [2 H, d, aromatics, J not det.]; 6.89 [2 H, d, aromatics, J not det.]; 5.14 [1 H, d, CHHAr, J 10.2]; 4.83 [1 H, d, CHHAr, J 10.0]; 4.68 [1 H, d, H-4, J 2.2]; 4.10-3.85 [2 H, m, CH 2 OH]; 3.82, 3.81 [2 x 3 H, 2 s, OCH 3 ]; 2.75 [1 H, d, C=CH, J 2.2]; 2.55 [1 H, dd, OH, J 4.8, 7.4]; 2.22 [2 H, t, CH 2 CH 2 OH, J 5.1]. 13C N.M.R. (CDCl 3, 50 MHz.): δ 164.84 [C=O]; 159.48, 157.03 [C–OMe]; 130.18, 129.44 [quat. aromatics]; 129.60, 118.80, 114.52, 113.85 [aromatics CH]; 90.84 [C-3]; 78.88, 77.23 [C=]; 69.46 [ArCH 2 ]; 58.48 [CH 2 OH]; 55.56, 55.50 [OCH 3 ]; 54.45 [CHN]; 37.48 [CH 2 CH 2 OH]. I.R. (CHCl 3):  vmax 3503 (broad), 3302 (s), 1754 (vs), 1586 (m), 1383 (w), 1299, 1246, 1165, 1105, 1027 cm -1.

(3R*,4S*)-4-(Ethyn-1-yl)-3-(2-((tert-butyldimethylsilyloxy)ethyl)-3-(4-methoxybenzylonyloxy)-1-(4-methoxyphenyl)-2-azetidinone (25). A solution of 24 (1.305 g, 3.421 mmol) in dry CH 2 Cl 2 (15 ml) was cooled to −10°C, and treated with 2,6-lutidine (1.19 ml, 10.26 mmol) and tert-butyldimethylsilyl triflate (1.18 ml, 5.13 mmol). After 5 min the solution was warmed to 0°C and stirred at this temperature until complete by tlc (30 min). It was poured into saturated aqueous NaHCO 3 (30 ml) and diluted with Et 2 O (30 ml). The phases were separated, and the aqueous one re-extracted with Et 2 O (2x). The united organic layers were washed with a mixture of 1M NaH 2 PO 4 (30 ml) and 0.5 M citric acid (10 ml), and then with saturated NaCl. drying, evaporation and chromatography on 65 g of silica (PE/AcOEt 80:20) gave, after extensive stripping at 10 −2 mbar, pure 25 as a solid (1.527 g, 90%). An analytically pure sample was obtained by trituration (pentane/Et 2 O 1:1).

M.p.: 87.1-87.7°C; HRMS: found: 518.2376 (M+Na +). Calc. for C 28 H 37 NO 5 SiNa: 518.2339 (accuracy: 7 ppm). R f 0.38 (PE/AcOEt 85:25; GC-MS: R, 14.55 min; m/z 495 (M +, 0.4%), 438 (M +−57, 2.1), 302 (1.4), 280 (3.1), 160 (21.1), 145 (4.0), 121 (100), 89 (6.8), 77 (4.2), 75 (4.3), 73 (9.9). 1H N.M.R. (CDCl 3, 200 MHz.): δ 7.53 [2 H, d, aromatics, J 9.1]; 7.36 [2 H, d, aromatics, J 8.7]; 6.92 [2 H, d, aromatics, J 9.0]; 6.87 [2 H, d, aromatics, J 8.7]; 5.15 [1 H, d, CHHAr, J 10.2]; 4.96 [1 H, d, H-4, J 2.2]; 4.80 [1 H, d, CHHAr, J 10.2]; 3.96 [1 H, ddd, CHHOSi, J 4.3, 8.6, 10.1]; 3.81 [1 H, dt, CHHOSi, J 5.0, J 6 = 10.1]; 3.81, 3.80 [2 x 3 H, 2 s, OCH 3 ]; 2.64 [1 H, d, C=CH, J 2.2]; 2.23 [1 H, dt, CHCH 2 O, J 4.5, J 5 = 14.4]; 2.08 [1 H, ddd, CHHCH 2 O, J 5.4, 8.6, 14.4]. 13C N.M.R. (CDCl 3, 50 MHz.): δ 164.90 [C=O]; 159.17, 156.68 [C–OMe]; 130.56, 130.09 [quat. aromatics]; 129.26, 118.68, 114.41, 113.64 [CH aromatics]; 89.90 [C-3]; 78.10, 78.00 [C=]; 68.84 [ArCH 2 ]; 58.31 [CH 2 Os]; 55.54, 55.26 [OCH 3 ]; 53.93 [CHN]; 36.82 [CH 2 CH 2 O]; 25.83 [(CH 3 ) 2 C]; 18.25 [(CH 3 ) 3 C]; −5.25, −5.61 [(CH 3 ) 2 Si]. I.R. (CHCl 3):  vmax 3302, 2954, 2928, 2855, 1745 (vs), 1612, 1586, 1497, 1462, 1441, 1383, 1299, 1246, 1165, 1105, 904 cm −1.

(3R*,4S*,Z)-3-(2-((tert-Butyldimethylsilyloxy)ethyl)-3-(4-methoxybenzylonyloxy)-1-(4-methoxyphenyl)-4-[6-(trimethylsilyl)hex-3-ene-1,5-diyn-1-yl]-2-azetidinone (27). Pd(PhCN) 2 Cl 2 (112.6 mg, 293.5 µmol) and Cul (55.9 mg, 293.5 µmol) were placed under ultrapure argon in a two-necked flask equipped with a dropping funnel, and suspended in dry THF (20 ml). A solution of the substrate 25 (1.435 g, 2.895 mmol) in dry THF (10 ml) was transferred by
cannula into the dropping funnel. Piperidine (4.35 ml, 44.02 mmol) was added to the flask. Oxygen was completely removed from the system by three vacuum/Ar cycles. Trimethylsilylacetylene (236 µL, 1.761 mmol) was added to the flask by syringe. The solution (initially light green) turned to light yellow and soon after to dark brown. After 10 min, (Z)-1-chloro-4-(trimethylsilyl)but-1-ene-3-yne26 (900 µL, 5.283 mmol) was added. The whole apparatus was closed in a glove-bag (Aldrich), filled with nitrogen. After 35 minutes from the addition of the choroenyne, the substrate solution was added through the dropping funnel. The nearly black solution was stirred for 3 h and 30 min, and then poured into saturated aqueous NH₄Cl (50 ml) containing 15 ml of 2M HCl. The mixture was extracted 3 times with Et₂O and the dark brown organic layers washed with saturated NH₄Cl (30 ml) + 1 M K₂HPO₄ (10 ml). After drying, evaporation, and two successive chromatographies on silica gel (PE/AcOEt 85:15), pure 27 was obtained as a dark yellow oil (1.503 g, 84%). Rf 0.50 (PE/AcOEt 85:15); GC-MS: not feasible. HRMS: found: 640.2956 (M+Na+). Calc. for C₃₅H₃₅NO₅S₂Na: 640.2890 (accuracy: 0.5 ppm). ¹H N.M.R. (CDCl₃, 300 MHz.): δ 7.56 [2 H, d, aromatics, J 9.0]; 7.36 [2 H, d, aromatics, J 8.4]; 6.91 [2 H, d, aromatics, J 9.0]; 6.85 [2 H, d, aromatics, J 8.4]; 5.88 [1 H, d, C=H, J 10.2]; 5.82 [1 H, dd, C=H, J = 1.9, 10.9]; 5.18 [1 H, d, CH/HAr, J 9.9]; 5.11 [1 H, d, H-4, J 2.1]; 4.78 [1 H, d, CH/HAr, J 9.9]; 3.94 [1 H, ddd, CH/HOSi, J 4.8, 8.3, 10.2]; 3.82 [1 H, dt, CH/HOSi, J= 5.0, Jd= 10.2]; 3.81, 3.80 [2 x 3 H, 2 s, OCH₃]; 2.23 [1 H, dt, CH/HCH₂O, J= 5.0, Jd= 14.4]; 2.12 [1 H, ddd, CH/HCH₂O, J 5.8, 8.4, 14.4]. ¹³C N.M.R. (CDCl₃, 50 MHz.): δ 164.92 [C=O]; 159.16, 156.65 [C–OMe]; 130.77, 130.03 [quat. aromatics]; 129.60, 118.78, 114.41, 113.59 [CH aromatics]; 120.77, 119.25 [CH=CH]; 103.45, 101.53, 90.86, 90.75, 86.70 [C≡ and C-3]; 69.10 [ArCH₂]; 58.44 [CH₂O]; 55.52, 55.25 [OCH₃]; 54.80 [CHN]; 37.25 [CH₂CH₂O]; 25.96 [(CH₃)₂C]; 18.25 [C(CH₃)]; –0.35 [(CH₃)₂Si]; –5.37, –5.57 [(CH₃)₂Si]. I.R. (CHCl₃): νmax 2952, 2854, 2144 (w), 2066 (w), 1744 (vs), 1611, 1585, 1504, 1463, 1440, 1383, 1332, 1299, 1192, 1101, 1020, 907 cm⁻¹.

(3R*,4S*,Z)-4-[6-Iodohex-3-ene-1,5-diyn-1-yl]-3-(2-hydroxyethyl)-3-(4-methoxybenzyl oxy)-1-(4-methoxyphenyl)-2-azetidinone (28). A solution of 27 (1.414 g, 2.288 mmol) in dry MeOH (20 ml) was treated with NaHCO₃ (1.40 g). The suspension was stirred at 20°C for 7 h. Then it was diluted with Et₂O (25 ml) and filtered through a sintered funnel, washing thoroughly with Et₂O. The filtrate was evaporated to dryness and suddenly taken up with Et₂O (15 ml) and filtered again. After evaporation, the oily residue was chromatographed at one trough 60 g of silica gel (PE/AcOEt 82:18) to give the pure desilylated enediyne as a yellow oil that tends to darken on standing (1.189 g, 95%). Prior to evaporation of the united fractions, 1 mg of 4-(3-tert-butyl-4-hydroxy-5-methylphenylthio)-2-tert-butyl-6-methylphenol (radical stabilizer) was added. Rf 0.43 (PE/AcOEt 80:20); ¹H N.M.R. (CDCl₃, 300 MHz.): δ 7.58 [2 H, d, aromatics, J 9.0]; 7.35 [2 H, d, aromatics, J 8.7]; 6.91 [2 H, d, aromatics, J 9.0]; 6.86 [2 H, d, aromatics, J 8.7]; 5.89 [1 H, dd, CH=CH, J 1.2, 11.0]; 5.84 [1 H, dd, CH=CH, J = 1.9, 11.0]; 5.18 [1 H, d, CH/HAr, J 10.5]; 5.15 [1 H, d, H-4, J 1.5]; 4.79 [1 H, d, CH/HAr, J 10.5]; 3.97 [1 H, ddd, CH/HOSi, J 4.8, 8.3, 10.2]; 3.83 [1 H, dt, CH/HOSi, J= 5.1, Jd= 10.2]; 3.81, 3.80 [2 x 3 H, 2 s, OCH₃]; 3.14 [1 H, d, C≡CH, J 1.8]; 2.23 [1 H, dt, CH/HCH₂O, J= 4.7, Jd= 14.4]; 2.12 [1 H, ddd, CH/HCH₂O, J 5.4,
Iodine (1.674 g, 6.597 mmol) was suspended in dry benzene (30 ml) and treated with morpholine (1.73 ml, 19.79 mmol). An orange solid is formed. The resulting suspension was stirred for 40 min at 20°C and then treated with a solution of freshly prepared desilylated enediyne (1.200 g, 2.19 mmol) in dry benzene (15 ml). After stirring at 20°C for 16 h, the mixture was diluted with Et₂O, and poured into 75 ml of 5% aqueous (NH₄)H₂PO₄ + 40 ml of 0.4M Na₂S₂O₃. After separation of the phases (and re-extraction twice with Et₂O), the organic phases were washed with 30 ml 5% (NH₄)₂SO₄, dried, evaporated, and immediately chromatographed through silica gel (60 g) (PE/AcOEt 80:20); 41 mg. Yield based on recovered starting material: 91%. Prior to evaporation of the united, 1 mg of 4-(3-tert-butyl-6-methylphenylthio)-2-tert-butyl-6-methylphenol (radical stabilizer) was added. Rf 0.39 (PE/AcOEt 80:20); ¹H N.M.R. (CDCl₃, 300 MHz.): δ 7.58 [2 H, d, aromatics, J 9.0]; 7.35 [2 H, d, aromatics, J 8.4]; 6.95 [2 H, d, aromatics, J 9.0]; 6.86 [2 H, d, aromatics, J 8.7]; 5.97 [1 H, d, CH=CH, J 10.5]; 5.77 [1 H, dd, CH=CH, J = 1.8, 10.5]; 5.16 [1 H, d, H-4, J 2.1]; 5.15 [1 H, d, CHHAr, J 10.2]; 4.80 [1 H, d, CHHAr, J 10.2]; 3.96 [1 H, ddd, CHHOSi, J 4.4, 8.8, 10.2]; 3.83 [1 H, dt, CHHOSi, J= 5.2, J= 10.2]; 3.81, 3.80 [2 x 3 H, 2 s, OCH₃]; 2.24 [1 H, dt, CHHCH₂O, J= 4.6, J= 14.4]; 2.11 [1 H, ddd, CHHCH₂O, J 5.4, 8.7, 14.4]; 0.89 [9 H, s, (CH₃)₃]; 0.06, 0.02 [2 x 3 H, 2 s, SiCH₃]. ¹³C N.M.R. (CDCl₃, 75 MHz.): δ 164.95 [C=O]; 159.15, 156.62 [C=O]; 130.72, 130.15 [quat. aromatics]; 129.41, 118.82, 114.56, 113.61 [CH aromatics]; 120.93, 120.79 [CH=CH]; 91.42, 90.93, 90.70, 86.41 [C= and C-3]; 69.04 [ArCH₂]; 58.38 [CH₂OSi]; 55.53, 55.25 [OCH₃]; 54.81 [CHN]; 37.11 [CH₂CH₂O]; 25.98 [(CH₃)₂C]; 18.24 [(CH₃)₃C]; 15.81 [C=I]; –5.33, –5.56 [(CH₃)₂Si]. I.R. (CHCl₃): ν max 2927, 2865, 2809, 1744 (vs), 1612, 1586, 1442, 1381, 1351, 1298, 1147, 1110, 827 cm⁻¹.

A solution of this iodoenediyne (1.291 g, 1.922 mmol) in CH₃CN (10 ml) was cooled to 0°C, and treated with H₂O (250 µL) and 40% aqueous HF (250 µL). After stirring at 0°C for 30 min, and at 20°C for 2 h, the reaction was judged complete by tlc. It was poured into saturated aqueous NaHCO₃ (50 ml) and extracted 3 times with Et₂O. The organic phase was washed with sat. NaCl, dried, evaporated, and immediately chromatographed on 60 g of silica (PE/AcOEt 45:55) to give pure 28 as a nearly colorless foam that tends to become yellowish on standing (1.008 g, 94%). Prior to evaporation of the united fractions, 1 mg of 4-(3-tert-butyl-6-hydroxy-5-methylphenylthio)-2-tert-butyl-6-methylphenol (radical stabilizer) was added. Due to instability
of this product at the dry state, it was conserved in solution and it was not possible to perform elemental analysis. The same applies for the intermediates for the synthesis of 28 from 27. R_f 0.35 (PE/AcOEt 50:50); ^1H N.M.R. (CDCl_3, 300 MHz): δ 7.59 [2 H, d, aromatics, J 9.0]; 7.36 [2 H, d, aromatics, J 9.0]; 6.96 [2 H, d, aromatics, J 9.0]; 6.87 [2 H, d, aromatics, J 8.7]; 6.03 [1 H, d, CH=CH, J 10.8]; 5.82 [1 H, dd, CH=CH, J = 1.8, 10.8]; 5.14 [1 H, d, C-HAr, J 9.9]; 4.88 [1 H, d, CH_2OH]; 4.07-3.85 [2 H, m, C_2OH]; 3.82, 3.80 [2 x 3 H, 2 s, OCH_3]; 2.59 [1 H, dd, O-H, J 4.6, 7.9]; 2.32-2.16 [2 H, m, CH_2CH_2O]. ^13C N.M.R. (CDCl_3, 75 MHz): δ 164.90 [C=O]; 159.38, 156.89 [C–OMe]; 130.29, 129.50 [quat. aromatics]; 129.69, 118.92, 114.61, 113.78 [CH aromatics]; 121.43, 120.51 [CH=CH]; 91.49, 91.37, 89.78, 87.06 [C≡ and C-3]; 69.53 [ArCH_3]; 58.45 [CH_2OH]; 55.53, 55.26 [OCH_3]; 55.29 [CHN]; 37.53 [CH_2CH_3O]; 16.52 [=C–I]. I.R. (CHCl_3): ν_{max} 3487 (broad), 3035, 2953, 2836, 1740 (vs), 1612, 1506, 1383, 1299, 1192, 1103, 1031, 816 cm^{-1}.

**Attempted synthesis of 3-hydroxy-1-(4-methoxybenzylxlo)-11-(4-methoxyphenyl)-11-aza-bicyclo[8.2.0]dodeca-6-en-4,8-diyn-12-ones (30).** A solution of freshly prepared IBX (iodoxybenzoic acid) (m.p.: 233.4°C (dec)) 27 (301 mg, 1.076 mmol) in DMSO (3 ml) was treated with a solution of alcohol 28 (454 mg, 814.5 mmol) in THF (7 ml). The solution was stirred for 21 h at 18-20°C. After a few hours a white precipitate formed. The suspension was diluted with Et_2O (15 ml) and filtered, washing with Et_2O. The filtrate was washed with H_2O and saturated NaCl/H_2O 1:1. After drying and evaporation, the residue was taken up with toluene and evaporated again. Chromatography on 54 g of silica (PE/AcOEt 60:40 to 55:45) gave a yellow oil, which tends to darken. It was azeotroped once with CH_2Cl_2/toluene and twice with benzene. The resulting dark foam was thoroughly dried at 0.05 mbar overnight. The final weight of the resulting aldehyde 29 was 431 mg (95%).

It was taken up in dry THF (10 ml) under Ar. Meanwhile, NiCl_2 (15.8 mg, 122 µmol) and good quality CrCl_2 (it should be off-white; green samples are not well suited for this reaction)(640 mg, 5.207 mmol) were suspended under Ar in dry THF (25 ml). The aldehyde solution was slowly added, with magnetic stirring, to the CrCl_2 suspension, during 1 h at R.T. After further stirring for 3 h, the reaction was judged complete by tlc. The mixture was poured into saturated aqueous NH_4Cl (30 ml) and distilled water (50 ml). After dilution with Et_2O (50 ml), the biphase system was stirred for 40 min at R.T. Between the dark green aqueous layer and the yellow organic layer, a colloidal brown precipitate was evident. It is not easily dissolved in Et_2O, AcOEt, H_2O or CH_2Cl_2. It is partially soluble in acetone, but it gives, at tlc, only a very polar U.V. detectable spot (that does not elute even with pure AcOEt). The phases were separated and the aqueous one re-extracted with AcOEt and CH_2Cl_2. The organic layer was washed with saturated NaHCO_3 (40 ml) + saturated NH_4Cl (10 ml) (reextracting the aqueous layer with CH_2Cl_2).

After drying and evaporation, the crude product (that showed three spots in tlc, called A (R_f 0.61), B (R_f 0.55) and C (R_f 0.45) was chromatoraphed through 50 g of silica with CH_2Cl_2/AcOEt 92:8. This chromatography gave a fraction containing pure A (24.2 mg), a fraction containing A+B (1:1 ratio) (27.6 mg) and a fraction containing C (37.1 mg),
contaminated by a little amount of A and B. The overall yield of A + B + C is therefore 26.7%. A and B are both white solids, that dissolves with some difficulty in CH₂Cl₂ or CHCl₃ and are completely insoluble in Et₂O or AcOEt. They seem to be stable at the dry state. On these fractions, HPLC-MS were carried out with an Agilent 1100 LC/MSD Trap SL instrument (electrospray ion trap analysis) with a C18 reverse phase Polarity column (Waters Corporation, MA, USA). In all cases, before introducing the eluent in the MS, a detection at 230-260 nm was performed using a diode array detector integrated in the system. The MS electrospray ion source parameters were set to maximize, from time to time, the interesting m/z ratios. The separation was performed in linear gradient from 100% H₂O to 100% CH₃CN in 60 min. The column was maintained at 30°C and the flow was 350 µl/min. A T-union was used for post-column infusion (30 µl/min) of aqueous NH₃ (1.3 M) in order to optimize ionization. On HPLC-MS, A gives a single peak (48.14 min) with masses of (in order of increasing intensity) 859.2 [M+H]⁺, 897.2 [M+K]⁺, 991.3 [M+Na]⁺, corresponding to cyclodimer 31. HRMS: 897.2751 (M+K)⁺. Calc. for C₅₂H₄₆O₁₀N₂K: 897.2790 (accuracy: 4 ppm). Under the same conditions, A and B also give a single peak, with the same masses. No peaks corresponding to the monomers 30 were present. HRMS: 897.2716 (M+K)⁺. Calc. for C₅₂H₄₆O₁₀N₂K: 897.2790 (accuracy: 8 ppm). Finally, C gives three UV peaks. One of them (48.14) is probably due to residues of A and B. The other two (46.85, major; 47.76, minor) are probably mixtures of two compounds each, as evident from the ion extracted chromatograms obtained on a single quadrupole instrument (HP 5989 Engine). The detected m/z ratios are the same as for compound A. HRMS: 897.2835 (M+K)⁺. Calc. for C₅₂H₄₆O₁₀N₂K: 897.2790 (accuracy: 5 ppm).

A gives in the NMR a single set of signals, therefore compatible with a symmetric cyclodimer. On the other hand, B (the spectrum was deducted from the one of A/B mixture by subtracting the signals of A) gives two sets of signals (B1 and B2), in a 1:1 ratio. The ratio is the same in the head or tail fractions containing this spot. Since also in HPLC B gives a single peak, we believe that it is given by one of the unsymmetric stereoisomer of cyclodimer 31.

A. ¹H N.M.R. (CDCl₃, 300 MHz.): δ 7.54 [2 H, d, H ortho to N, J 9.3]; 7.33 [2 H, d, H ortho to CH₂, J 9.0]; 6.92 [2 H, d, H ortho to OMe, J 9.0]; 6.82 [2 H, d, H ortho to OMe, J 8.7]; 5.92 [1 H, d, CH=CH, J 10.5]; 5.88 [1 H, d, CH=CH, J = 10.5]; 5.23 [1 H, slightly broad s, H-10]; 5.18 [1 H, broad d, H-3, J 11.1]; 5.07 [1 H, d, CHHAr, J 9.9]; 4.75 [1 H, d, CHHAr, J 9.9]; 3.81, 3.76 [2 x 3 H, 2 s, OCH₃]; 2.49 [1 H, dd, H-2, J 14.0, 2.8]; 2.14 [1 H, dd, H-2, J 14.0, 11.0]. ¹³C N.M.R. (CDCl₃, 75 MHz.): δ 163.47 [C=O]; 159.45, 156.88 [C=OMe]; 130.48, 129.26 [quat. aromatics]; 129.79, 118.74, 114.48, 113.77 [CH aromatics]; 121.00, 119.58 [CH=CH]; 97.90, 90.36, 89.73, 82.49 [C= and C-1]; 69.01 [ArCH₃]; 58.64 [C-3]; 56.01 [C-10]; 55.55, 55.26 [OCH₃]; 39.91 [C-2].

B. ¹H N.M.R. (CDCl₃, 300 MHz.): δ 7.56 [2 H, d, H ortho to N (B2), J 9.0]; 7.54 [2 H, d, H ortho to N (B1), J 9.0]; 7.28 [2 H, d, H ortho to CH₂ (B1 & B2), J 9.0]; 6.98 [2 H, d, H ortho to OMe (B1), J 8.7]; 6.96 [2 H, d, H ortho to OMe (B2), J 8.7]; 6.82 [2 H, d, H ortho to OMe (B1 or B2), J 8.7]; 6.66 [2 H, d, H ortho to OMe (B1 or B2), J 8.4]; 6.02 & 5.96 [2 H, AB system (B1), CH=CH, J 10.8]; 5.95 [1 H, dd, CH=CH (B2), J = 10.8, 0.8]; 5.81 [1 H, dd, CH=CH (B2), J =
10.8, 1.2]; 5.35 [1 H, broad d, H-3 (B1), J 9.0]; 4.93 [1 H, d, H-10 (B2), J 1.5]; 5.09 [1 H, d, CH/Ar (B1 or B2), J 9.9]; 5.06 [1 H, d, CH/Ar (B1 or B2), J 9.0]; 4.86 [1 H, broad dd, H-3 (B2), J 11.4, 6.6]; 4.85 [1 H, slightly broad s, H-10 (B1)]; 4.80 [1 H, d, CH/Ar (B1 or B2), J 9.0]; 4.70 [1 H, d, CH/Ar (B1 or B2), J 9.9]; 4.18 [1 H, d, OCH3]; 3.84, 3.83, 3.77, 3.61 [4 x 3 H, 4 s, OCH3]; 3.22 [1 H, d, OCH3]; 2.66 [1 H, d, H-2 (B1), J 14.7]; 2.37 [1 H, dd, H-2β (B1), J 15.0, 9.3]; 2.16 [1 H, d, H-2β (B2), J 14.3]; 1.36 [1 H, dd, H-2α (B2), J 9.0]; 58.76, 57.96 [ArCH2]; 55.55, 55.26 [OCH3]; 44.18, 40.88 [C-2].

Relevant NOEs: In A and B1 there is a strong NOE between H-2 and H-20 (12.4% in A, 11.6% in B1). In B2 this NOE is much lower (5%). These NOEs demonstrate unequivocally that the configuration of the β-lactam has remained trans (no epimerization). In B2 there is a NOE between H-20 and OH (3.3%). In A and B1 there is no NOE between OH and H-2 or H-20.

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References


