Synthesis and aromatisation of cyclic enediyne-containing amino acids†

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A series of cyclic enediyne-containing amino acids with ring sizes varying from 10 to 12 atoms have been prepared starting from propargylglycine and homopropargylglycine. Their reactivity towards Bergman cyclisation under elevated temperatures has been explored. The enediynes displayed marked differences in cyclisation half-lives depending on the olefinic substituent and the ring size. A potential candidate for incorporation into peptides has been identified.

Introduction

Enediyne cytotoxins rank among Nature's most powerful antitumour and antibiotic agents. First discovered in the mid 1980's,[1] they triggered a considerable interest in the enediyne chemistry and biology. Upon thermal activation, the enediyne ((*Z*)-3-ene-1,5-diyne) moiety (coined "warhead")[2] contained in these compounds undergoes a symmetry-allowed rearrangement, the so-called Bergman cyclisation reaction (BC), causing conversion into the corresponding benzene derivative.[3] The intermediate benzenoid σ,σ-1,4-diradical (sometimes called *para*-benzyne) is capable of abstracting hydrogen radicals from nearby DNA, ultimately resulting in single and double strand cleavage.[1]

All natural enediynes have their "warhead" pre-activated by being incorporated in a strained ring system thereby increasing the reactivity towards cycloaromatisation. A few enediynes have been approved for chemotherapeutic use, the first ever being neocarzinostatin (Japan, 1996).[4] Mylotarg®, a conjugate of a monoclonal antibody and the natural enediyne calicheamicin γ_1^1, has been approved by the FDA in 2006 for the treatment of acute myeloid leukemia.[5] Due to the complexity and toxicity of the naturally occurring enediynes, the design and synthesis of readily accessible model systems mimicking their chemical and biological function has become an important research target.[6] Examples of some model enediynes related to the current study are shown in Fig. 1. The group of Basak prepared the cyclic nitrogen-containing enediyne **1** and conducted kinetic studies.[7] Banfi *et al.* addressed the general problem of selectively triggering the BC by fusing a cyclic enediyne to a β-lactam moiety (**2**) which serves as a locking device that can be opened in a slightly basic environment.[8]

The first cyclic enediyne-containing amino acids published in literature were assembled by Du *et al.*,[9] but our group has also conducted research into this compound class.[10] Du *et al.* reported the synthesis of a series of 10-membered benzofused enediynes (**3**) and studied their half-lives. Additionally, the tosyl-protected variant **4** was shown to be capable of cleaving supercoiled DNA (Φ-X DNA) upon incubation at 37 °C for 24 h at concentrations of 10 μM and higher.

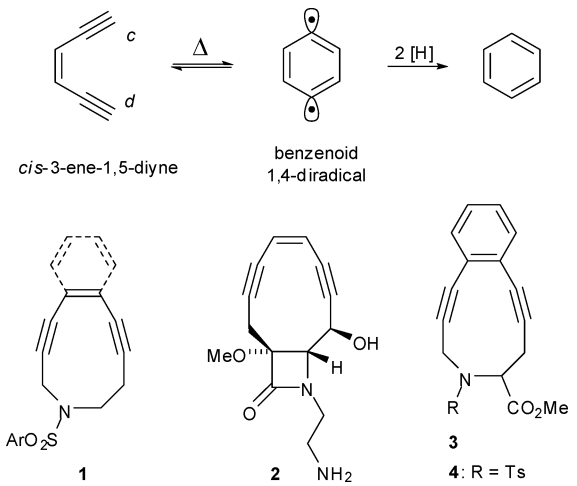

Fig. 1 The Bergman cyclisation and some *N*-heterocyclic enediynes.

Owing to the powerful DNA cleaving and thus potential antitumour activities of the enediynes, expanding the series of readily accessible enediyne-containing amino acids could be of general interest, especially in combination with suitable DNA targeting probes. The versatile and well-described chemistry of amino acids would ensure multiple possibilities for incorporation of the amino acids in peptides or other targeting devices. In this report, we disclose the synthesis and evaluation of a series of 10-, 11- and 12-membered cyclic enediyne-containing amino acids **5** (Scheme 1) as well as their cycloaromatisation products. Furthermore, we report the unexpected racemisation of the title compounds (specifically **4**) occurring in the final step of their syntheses even under mild conditions.

Retrosynthetically, the target compounds **5** should be accessible *via* intramolecular C–N bond formation from the open chain enediynes **6**.[9] We envisaged to assemble these cyclisation precursors by means of Sonogashira coupling[11] of the appropriate acetylenic amino acids **7**[12] with enynes **8**. The latter compounds,

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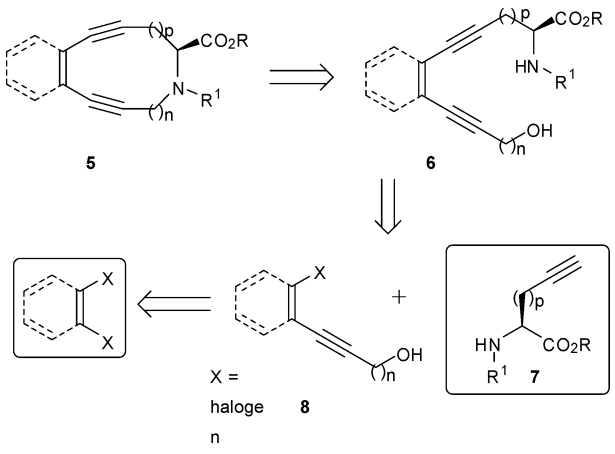
† Electronic supplementary information (ESI) available: NMR spectra of compounds **14–19**, **23–26**, **28**, **29**, **31–36**; crystal data and structure refinements for compounds **4**, **19**, **29** and **34**. CCDC reference numbers 237705 (**4**), 698982 (**29**), 698983 (**19**) and CCDC 698984 (**34**). For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b815176h

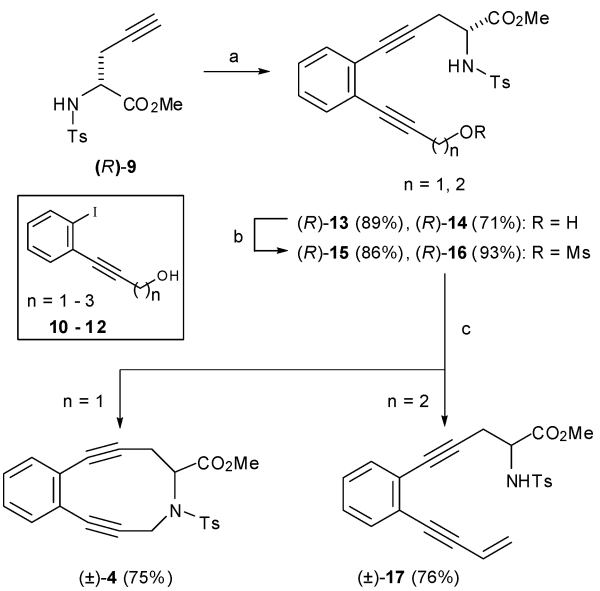


Scheme 1 Retrosynthesis of the title compounds.

in turn, would be constructed by Sonogashira reactions starting from (*Z*)-configured vicinal vinyl dihalides.

Results and discussion

Initially, we studied the ring closure *via* intramolecular mesylate displacement (Scheme 2).[13]



Scheme 2 Mesylate displacement strategy. *Reagents and conditions:* (a) Haloenynol **10** (n = 1) or **11** (n = 2), $PdCl_2(PPh_3)_2$ (cat.), CuI (cat.), Et_2NH, Et_2O, rt; (b) MsCl, Et_3N, CH_2Cl_2, 0 °C; (c) K_2CO_3, DMF, (high dilution), rt.

Aryliodides **10** and **11** were prepared from 1,2-diiodobenzene and propargyl alcohol or homopropargyl alcohol under Sonogashira conditions.[14] Coupling of these enynes with enantiopure *N*-tosyl-protected propargylglycine methyl ester[15] (*R*)-**9** proved to be straightforward leading to the acyclic enediynes (*R*)-**13** and (*R*)-**14** in 89% and 71% yield, respectively.[16] Reaction with methanesulfonyl chloride (MsCl) afforded the corresponding mesylates **15** and **16**, setting the stage for base-induced cyclisation reactions. Treatment of highly diluted solutions (6 mM) of these mesylates in DMF with potassium carbonate (5 equiv.) resulted in a clean conversion of the starting materials into products within 3 h according to TLC. In case of n = 1, the desired 10-membered cyclic enediyne **4** could be isolated as a crystalline solid. Its 11-membered homologue, however, could not be prepared using the latter reaction since elimination rather than ring closure took place, and the linear dienediyne **17** was produced instead. Interestingly, neither **4** nor **17** showed optical activity in the polarimeter as opposed to their respective parent compounds.

The racemic nature of product **4** was confirmed by its crystal structure (see Fig. 2), showing both enantiomers in the unit cell. Supposedly, the racemisation takes place after cyclisation during which the sulfonamide proton is substituted. Due to the lack of a more acidic *NH*, the α-proton of the now cyclic structure is prone to deprotonation by a relatively mild base such as potassium carbonate. Following this reasoning, the racemisation of **17** can best be explained by assuming initial formation of the desired 11-membered ring, followed by racemisation and finally ring strain-promoted eliminative ring fission[17] to give the acyclic expanded conjugated system.

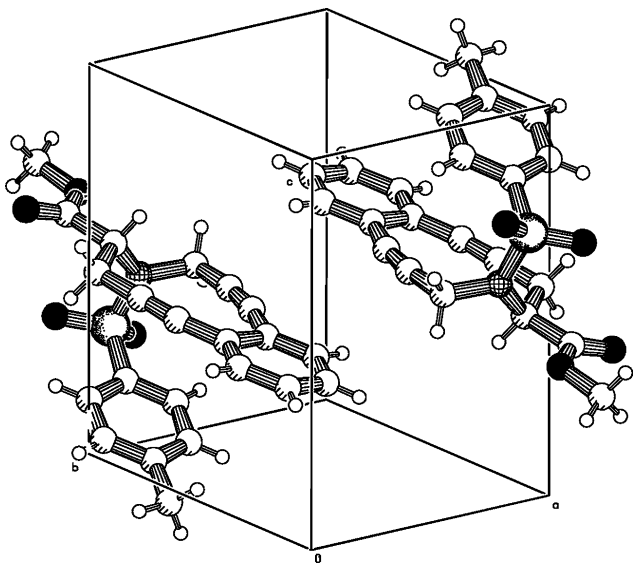
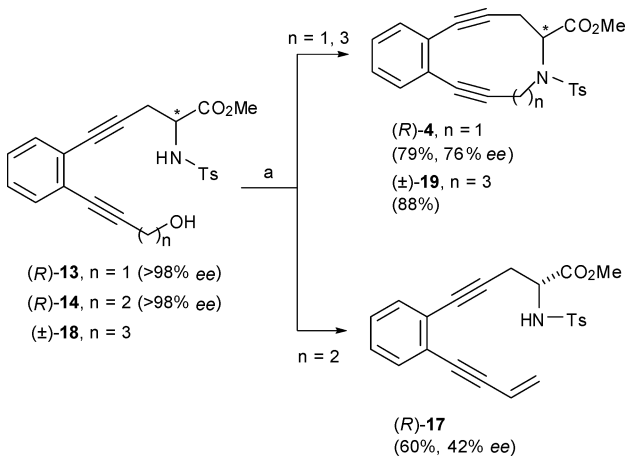


Fig. 2 Both enantiomers of enediyne **4** are present in its unit cell.[18]

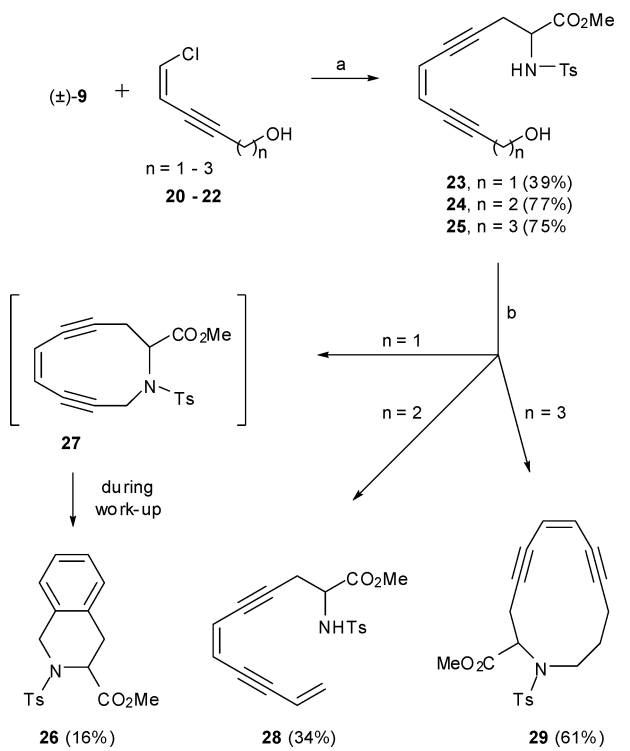
Clearly, the racemisation and elimination problems in the cyclisation step called for conditions as gentle as possible to promote this transformation in the desired sense.[19] The Mitsunobu reaction[20] has been recognised as a mild and efficient way to *N*-alkylate sulfonamides or sulfonamide-protected amino acids, especially when applied for intramolecular bond formation.[21] This strategy has also been applied by Du *et al.* in the synthesis of the cyclic enediyne-containing amino acids **3**.[9] Following this concept, acyclic enediynes **13** and **14** were treated with DEAD and PPh_3 under high dilution (~5 mM) in THF (Scheme 3). To our dismay, the outcome very much resembled the mesylate displacement approach. Starting from (*R*)-**13** (>98% *ee*), the 10-membered enediyne (*R*)-**4** was obtained with a substantially diminished enantiomeric excess (76% *ee*). Surprisingly, even under the mild conditions applied, elimination and also partial racemisation took place with homopropargylic enynol (*R*)-**14** (>98% *ee*). The *ee* of the elimination product (*R*)-**17** was determined to be as low as 42%. It has to be assumed that racemisation proceeds



Scheme 3 Synthesis *via* Mitsunobu-type reaction. *Reagents and conditions:* (a) PPh_3, DEAD, THF (high dilution), rt.

via the cyclic enediyne as in the mesylate approach. These observations, once more, clearly show the acidic nature of the α-proton of the cyclic amino acids. Finally, the racemic homologue (±)-**18** was cyclised under identical conditions, furnishing the corresponding cyclic 12-membered enediyne **19** in a good yield of 88%. These results emphasise the more general applicability of the Mitsunobu reaction for preparing cyclic enediynes, although the previously unaddressed[9] issue of racemisation could not be fully circumvented.

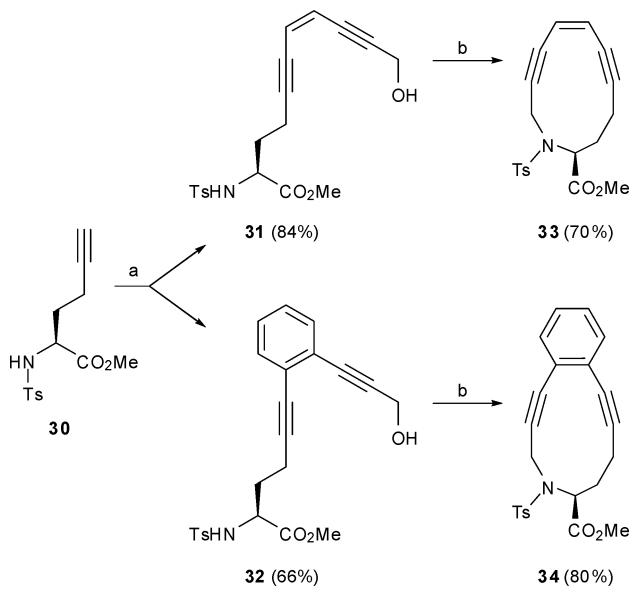
Along the same line, we set out to assemble an analogous series of non-benzofused enediynes (Scheme 4). Synthesis of the cyclisation substrates from racemic propargylglycine (±)-**9** and haloenynols **20–22**[14] proceeded *via* Sonogashira coupling as anticipated, giving rise to enediynols **23–25**. Subjection of precursor **23** to Mitsunobu conditions led to the consumption of starting material with concomitant appearance of a single new spot on TLC which, however, had disappeared after work-up. No enediyne could be isolated, instead the tetrahydroisoquinoline derivative **26** was obtained, showing a different retention factor on TLC.



Scheme 4 Synthesis of olefinic cyclic enediynes. *Reagents and conditions:* (a) Haloenynol (**20**, **21**, or **22**), $PdCl_2(PPh_3)_2$ (cat.), CuI (cat.), *n*-$BuNH_2$, Et_2O, rt; (b) PPh_3, DEAD, THF (high dilution), rt.

Supposedly, the enediyne **27** had been formed, but underwent Bergman cyclisation at ambient temperature within 30 minutes under work-up conditions. In contrast to this behaviour, its benzofused counterpart **4** was stable in crystalline form at room temperature for months and could be kept in a chloroform solution for several days without deterioration.[22] The higher homologues reacted like their benzofused counterparts: the homopropargylic alcohol **24** led to the elimination product **28** in 34%, while **25** was transformed into the 12-membered cycle **29** in a reasonable yield (61%).

In order to gain access to the thus far elusive 11-membered cyclic enediynes, it was decided to start from protected (*S*)-homopropargylglycine[15] **30**. In this strategy, β-elimination of the amine is no longer possible in the cyclisation step. Acyclic enediynes **31** and **32** were readily formed by Sonogashira couplings. Their intramolecular condensation under high dilution Mitsunobu conditions furnished the cyclic 11-membered enediynes **33** and **34** in 70% and 80% yield, respectively, with both products displaying optical activity (Scheme 5).[23]



Scheme 5 Assembly of 11-membered cyclic enediynes. *Reagents and conditions:* (a) Haloenynol (**10** or **20**), $PdCl_2(PPh_3)_2$, CuI (cat.), *n*-$BuNH_2$, Et_2O, rt; (b) PPh_3, DEAD, THF (high dilution), rt.

Crystals suitable for X-ray structural analysis could be obtained from compounds **4**, **19**, **29**, and **34** (Fig. 3).[24]

A number of parameters are relevant with respect to the rate of the Bergman cyclisation of cyclic enediynes: It has been proposed that the distance between the acetylene termini (c–d distance, *viz.* Fig. 1) should be in the range of 2.9–3.4 Å in order for the reaction to proceed at physiological temperatures.[25] More recent studies attribute a greater role to the strain difference between

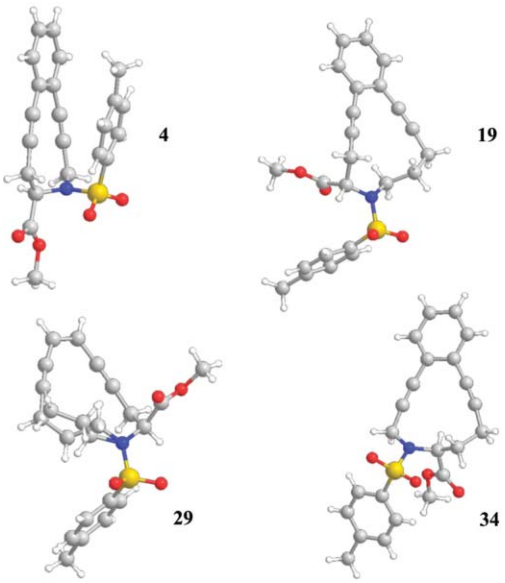
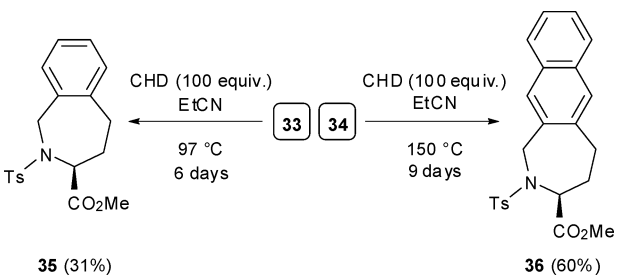


Fig. 3 X-ray crystal structures of cyclic enediynes **4**, **19**, **29**, and **34**.

the ground state and the transition state of the reaction.[26] The electronic influences on the BC are probably the least studied, but it is known that benzannulation of the olefinic portion of cyclic enediynes slows down the cyclisation process.[27] Furthermore, for some cyclic, benzannulated enediynes the rate-determining step is the quenching of the benzenoid diradical, and the reaction rate therefore can be dependent on the concentration of the radical trap.[28]

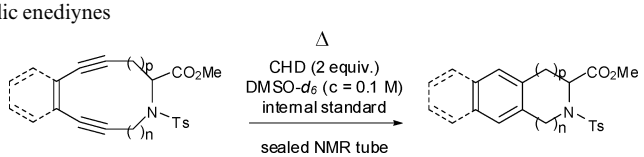
With our series of enediynes in hand we set out to study their behaviour in the BC. Enediynes **4**, **19**, **29**, **33**, and **34** were dissolved in deuterated DMSO (c = 0.1 M), 1,4-cyclohexadiene (CHD, 2 equiv.) and an internal standard were added, and the samples were sealed under an argon atmosphere before being subjected to elevated temperatures. The formation of product and the consumption of starting material were monitored by ^{1}H-NMR. Table 1 summarises the results.

The 10-membered olefinic enediyne **27** (entry 1) underwent cycloaromatisation spontaneously (Scheme 4). Its benzofused analogue **4** (entry 2) is the only structure with a confirmed c–d distance within the "reactive window" of 2.9–3.4 Å, and it displayed a half-life of 59 min at 80 °C under the applied conditions. For the same compound, Du *et al.*[9] reported a half-life of 9.5 h at 55 °C under somewhat different conditions (c = 10^{-4} M in DMF, 100 equiv. CHD).[29] Significantly higher temperatures and reaction times were required to induce BC of the 11-membered rings (entries 3–7), which followed our expectations. From the available crystal structure data of **34** it is apparent that its c–d distance is well outside the proposed reactive range of 2.9–3.4 Å. It is therefore safe to assume that this is also true for its olefinic counterpart **33**. Indeed, only a trace amount of the cyclised product was detected (^{1}H-NMR) after having subjected **33** to temperatures of 80 °C for 24 h. Increasing the temperature to 90 °C caused the BC to proceed with a half-life of 26 h. Even higher temperatures were needed for benzene-fused **34** to aromatise efficiently: after 24 h at 90 °C only small amounts of the corresponding product could be detected. Finally, at 120 °C a half-life of 175 h was measured (entry 7). Both 12-membered species **19** and **29** with c–d distances of 3.77 Å and 3.88 Å, respectively, proved to be thermally stable at a temperature of 90 °C as anticipated. Upon subjecting samples to a temperature of 120 °C, a trace amount of the cyclised **29** could be detected after 24 h while **19** remained unchanged (entries 9 and 10).



Scheme 6 Cycloaromatisation of 11-membered enediynes **33** and **34**.

Table 1 Half-life determination of cyclic enediynes



Entry	Enediyne	Benzo-fused	n, p	Ring size	c–d (Å)[a]	T (°C)	$t_{1/2}$
1	**27**	No	1, 1	10	n.d.	rt–30[b]	<30 min
2	**4**	Yes	—	—	3.23	80	59 min
3	**33**	No	1, 2	11	n.d.	80	Trace[c]
4	—	—	—	—	—	90	26 h
5	**34**[d]	Yes	—	—	3.76	80	Stable[e]
6	—	—	—	—	—	90	Trace[c]
7	—	—	—	—	—	120	175 h
8	**29**	No	3, 1	12	3.88	90	Stable
9	—	—	—	—	—	120	Trace[c]
10	**19**	Yes	—	—	3.77	120	Stable[e]

[a] Obtained by crystal structure elucidation. [b] Compound **27** cyclised during the work-up procedure. [c] A small amount of the aromatisation product was observed by ^{1}H-NMR after 24 h at the indicated temperature. [d] c = 0.05 M. [e] No product was observed by ^{1}H-NMR after 24 h at the indicated temperature.

In order to fully characterise the novel aromatisation products of **33** and **34**,[30] both enediynes were heated in the presence of 100 equivalents of 1,4-cyclohexadiene in proprionitrile (Scheme 6). After 6 days at reflux temperature (97 °C), enediyne **33** had been consumed completely and the tetrahydrobenzazepine **35** was isolated in a mediocre yield of 31%. Similarly, the benzene-fused derivative **34** was converted into the tetrahydronaphthoazepine **36** in a yield of 60% upon heating at 150 °C for 9 days in a sealed tube.

Conclusions

In summary, the synthesis of a series of 10-, 11-, and 12-membered cyclic enediyne-containing amino acids has been described, proceeding *via* readily available acetylene-containing amino acid building blocks. During the cyclisation into the targeted enediyne structures, in several instances an unexpected (partial) racemisation took place, even under virtually neutral Mitsunobu conditions. While the synthesis of 11-membered ring systems failed starting from propargylglycine due to β-elimination after ring closure, isomeric 11-membered ring enediynes could be successfully synthesized starting from homopropargylglycine.

Half-life determination experiments of the cylic enediynes showed that, in line with previous studies, all benzannulated enediynes reacted significantly slower than their unsubstituted counterparts. Moreover, it is evident that ring strain plays a major role: An increase in ring size goes along with a distinct decrease of the rate of cycloaromatisation, *e.g.* the discrepance in reactivity between 11-membered cycle **34** and 12-membered cycle **19**, both showing almost equal c–d distances in the solid state, can best be explained by the higher strain in the smaller ring.

Of the enediynes presented in this study, the 10-membered benzannulated system **4** has the highest potential utility: it is stable at room temperature but can be activated at modestly elevated temperatures. Investigations concerning further structural optimisation and conjugation of **4** with peptides and targeting devices are currently ongoing.

Experimental

General information

Solvents were distilled from appropriate drying agents prior to use and stored under nitrogen. Enantiopure propargyl- and homopropargylglycine were purchased from Chiralix B.V. All other chemicals were purchased from Sigma-Aldrich or Acros and used as received, unless stated otherwise. Reactions were carried out under an inert atmosphere of dry nitrogen or argon. Reactions were followed and R_f values are obtained using thin layer chromatography (TLC) on silica gel-coated plates (Merck 60 F_{254}) with the indicated solvent mixture. IR spectra were recorded on an ATI Mattson Genesis Series FTIR spectrometer, or a Bruker Tensor 27 FTIR spectrometer. NMR spectra were recorded on a Bruker DPX 200 (200 MHz), a Bruker DMX 300 (300 MHz), and a Varian Inova 400 (400 MHz). Chemical shifts are given in ppm with respect to tetramethylsilane (TMS) as internal standard. Coupling constants are reported as *J*-values in Hz. Flash chromatography was carried out using ACROS silica gel (0.035–0.070 mm, 6 nm pore diameter). Optical rotations were determined with a Perkin Elmer 241 polarimeter; concentrations (*c*) are given in g/100 mL. The *ee* values were obtained by chiral HPLC on a Shimadzu LC-2010C apparatus equipped with a Daicell AD-H column (eluent: hexane/2-propanol). High resolution mass spectra were recorded on a MAT900 (EI, CI and ESI).

3-(2-Iodophenyl)prop-2-yn-1-ol (10)

1,2-Diiodobenzene (1.010 g, 3.062 mmol) was dissolved in Et_2O (10 mL). $Pd(PPh_3)_4$ (140 mg, 0.121 mmol) and CuI (53 mg, 0.280 mmol) were added to the stirred solution, followed by *n*-$BuNH_2$ (1.5 mL, 15.2 mmol). Once a clear, homogeneous solution had been formed, prop-2-yn-1-ol (0.175 mL, 3.006 mmol) was added *via syringe* and stirring was continued for 6 h. One aliquot of a saturated solution of NH_4Cl was poured into the reaction flask, the phases were separated, and the aqueous layer was extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with brine, dried over $mgSO_4$, and concentrated *in vacuo.* The crude product was purified by flash chromatography (EtOAc/heptane, 2:7) yielding **10** as a yellowish oil that solidified on cooling (401 mg, 1.554 mmol, 52%). The characterisation data were consistent with those in ref. 14a.

4-(2-Iodophenyl)but-3-yn-1-ol (11)

The title compound was prepared following the same procedure as described for **10** starting from 1,2-diiodobenzene (669 mg, 2.027 mmol) in Et_2O (10 mL), $PdCl_2(PPh3)_2$ (70 mg, 0.010 mmol), CuI (40 mg, 0.210 mmol), Et_2NH (1.05 mL, 10 mmol), and but-3-yn-1-ol (0.190 mL, 2.510 mmol). Flash chromatography (EtOAc/heptane, 1:10 → 1:3) furnished **11** as a light brown oil (318 mg, 1.169 mmol, 58%). The characterisation data were consistent with those in ref. 14b.

5-(2-Iodophenyl)pent-4-yn-1-ol (12)

The title compound was prepared following the same procedure as described for **10** by letting react a solution of 1,2-diiodobenzene (2.34 g, 7.09 mmol) in Et_2O (20 mL) with $Pd(PPh_3)_4$ (218 mg, 0.188 mmol), CuI (61 mg, 0.320 mmol), *n*-$BuNH_2$ (3.8 mL, 38.4 mmol), and pent-4-yn-1-ol (0.66 mL, 7.09 mmol) over a period of 12 h. Work-up and purification by flash chromatography (EtOAc/heptane, 1:10 → 2:3) gave **12** as a yellow oil (1.081 g, 3.78 mmol, 53%). The characterisation data were consistent with those in ref. 14a.

(*R*)-5-[2-(3-Hydroxy-prop-1-ynyl)-phenyl]-2-(toluene-4-sulfonylamino)-pent-4-ynoic acid methyl ester (13)

A stirred solution of 4-(2-iodophenyl)prop-3-yn-1-ol (**10**) (335 mg, 1.298 mmol) in Et_2O (15 mL) was treated with $PdCl_2(PPh_3)_2$ (40 mg, 0.057 mmol), CuI (22 mg, 0.116 mmol), and Et_2NH (0.57 mL, 5.5 mmol). Stirring was continued until a clear, homogeneous solution had been formed and propargylglycine[15] (*R*)-**9** (309 mg, 1.098 mmol) was added. After 4 h at ambient temperature, the reaction mixture was poured into an aliquot of a saturated solution of NH_4Cl, the phases were separated and the aqueous layer was extracted with EtOAc (33 × 8 mL). The combined organic layers were washed with brine, dried over $mgSO_4$, and all volatiles were removed *in vacuo.* Purification

by flash chromatography (EtOAc/heptane, 2:3) provided **13** as a yellowish oil (401 mg, 0.975 mmol, 89%). R_f 0.48 (EtOAc/heptane, 1:1); *ee* = 98.5%; ν_{max} (neat) 3483, 3276, 2952, 1737, 1635, 1592, 1477, 1439; ^{1}H-NMR and ^{13}C-NMR spectra were consistent with those published in ref. 9; HRMS (EI) calcd. for $C_{22}H_{21}NO_5S$ (M^+) 411.1140, found 411.1136.

4-Tosyl-1,2,7,8-tetradehydro-3,4,5,6-tetrahydro-4-benzazecine-5-carboxylic acid methyl ester (4)

i) ***via*** **mesylate displacement.** To a cooled solution (0 °C) of alcohol (*R*)-**13** (338 mg, 0.821 mmol) in CH_2Cl_2 (15 mL), MsCl (0.13 mL, 1.67 mmol) and Et_3N (0.23 mL, 1.66 mmol) were added. The reaction mixture was allowed to reach room temperature and was stirred for 4 h after which it was poured into CH_2Cl_2/water (80 mL, 1:1). The layers were separated, and the organic layer was washed with water (23 × 40 mL), dried over $mgSO_4$ and concentrated *in vacuo.* Purification by flash chromatography (EtOAc/heptane, 2:3) afforded the mesylate (*R*)-**15** as a yellow oil (347 mg, 0.709 mmol, 86%). R_f 0.22 (EtOAc/heptane, 2:3); δ_H (300 MHz, $CDCl_3$) 7.75 (2 H, d, *J* = 8.2 Hz), 7.46–7.40 (1 H, m), 7.36–7.22 (5 H, m), 5.68 (1 H, d, *J* = 8.4 Hz), 5.22 (2 H, dd, *J* = 15.9, 17.4 Hz), 4.18–4.13 (1 H, m), 3.62 (3 H, s), 2.97 (2 H, d, *J* = 4.8 Hz), 2.38 (3 H, s).

A dilute solution of the mesylate (*R*)-**15** (334 mg, 0.682 mmol) in DMF (115 mL; c = 6 mM) was treated with K_2CO_3 (470 mg, 3.40 mmol) and stirred for 2 h at room temperature. The mixture was poured into EtOAc/water (600 mL, 1:1), and the layers were separated. The organic layer was washed with water (33 × 300 mL), dried over $mgSO_4$, and concentrated *in vacuo* at room temperature. After flash chromatography (EtOAc/heptane, 1:4), enediyne (±)-**4** was obtained as a white solid 201 mg, 0.511 mol, 75%). Crystals suitable for X-ray analysis could be grown from EtOAc/heptane. $[\alpha]^{22}_D = 0°$ (*c* 0.5, CH_2Cl_2).

ii) ***via*** **Mitsunobu reaction.** Triphenylphosphine (262 mg, 0.999 mmol) was dissolved in a dilute solution (c = 5 mM) of alcohol (*R*)-**13** (207 mg, 0.503 mmol) in THF (100 mL). DEAD (40% in toluene; 0.150 mL, 0.953 mmol) was added, and the reaction mixture was stirred for 80 min at ambient temperature followed by removal of all volatiles *in vacuo* at room temperature. Flash chromatography (EtOAc/heptane, 1:10 → 1:3) afforded (*R*)-**4** as a white solid (156 mg, 0.396 mmol, 79%). *ee* = 73%.

General characterisation data. R_f 0.19 (EtOAc/heptane, 1:4); ν_{max} (neat) 2958, 1735, 1593, 1459, 1433; ^{1}H-NMR and ^{13}C-NMR spectra were consistent with those published in ref.9; HRMS (EI) calcd. for $C_{22}H_{19}NO_4S$ (M^+) 393.1035, found 393.1038.

(*R*)-5-[2-(4-Hydroxy-but-1-ynyl)phenyl]-2-(toluene-4-sulfonylamino)-pent-4-ynoic acid methyl ester (14)

The title compound was synthesised following the same procedure as described for **13** by allowing to react 4-(2-iodophenyl)but-3-yn-1-ol (**11**) in Et_2O (15 mL) with $PdCl_2(PPh_3)_2$ (18 mg, 0.025 mmol), CuI (10 mg, 0.052 mmol), Et_2NH (0.26 mL, 2.5 mmol), and propargylglycine (*R*)-**9** over a period of 12 h. Work-up and purification by flash chromatography (EtOAc/heptane, 2:3) afforded **14** as a light yellow oil (151 mg, 0.355 mmol, 71%). R_f 0.15 (EtOAc/heptane, 2:3); *ee* > 99%; ν_{max} (neat) 3495, 3266, 2955, 1736, 1584, 1473, 1438; δ_H (300 MHz, $CDCl_3$) 7.74 (2 H, d, *J* = 8.4 Hz), 7.38–7.35 (1 H, m), 7.29–7.14 (5 H, m), 6.12 (1 H, d, *J* = 8.7 Hz), 4.20–4.14 (1 H, m), 3.89–3.82 (2 H, m), 3.61 (3 H, s), 2.98 (2 H, dd, *J* = 5.0, 3.4 Hz), 2.86 (2 H, dt, *J* = 5.8, 2.7 Hz), 2.55 (1 H, t, *J* = 6.3 Hz), 2.34 (3 H, s); δ_C (75 MHz, $CHCl_3$) 169.9, 143.3, 136.8, 131.8, 131.6, 129.4, 127.7, 127.3, 126.9, 125.8, 125.0, 91.1, 86.5, 83.0, 81.3, 60.9, 54.3, 52.9, 25.2, 24.0, 21.7; HRMS (EI) calcd. for $C_{23}H_{23}NO_5S$ (M^+) 425.1297, found 425.1309.

5-(2-But-3-en-1-ynyl-phenyl)-2-(toluene-4-sulfonylamino)-pent-4-ynoic acid methyl ester (17)

i) ***via*** **mesylate displacement.** Whilst stirring, a solution of alcohol (*R*)-**14** (460 mg, 1.081 mmol) in CH_2Cl_2 (20 mL) at 0 °C was treated with MsCl (0.17 mL, 2.18 mmol) and Et_3N (0.30 mL, 2.16 mmol). The mixture was allowed to come to room temperature and stirred for additional 3 h after which time it was poured into CH_2Cl_2/water (100 mL, 1:1). The layers were separated, and the organic layer was washed with water (23 × 50 mL), dried over $mgSO_4$ and concentrated *in vacuo.* Purification by flash chromatography (EtOAc/heptane, 2:3) produced mesylate (*R*)-**16** as a yellow oil (495 mg, 0.983 mmol, 93%). R_f 0.15 (EtOAc/heptane, 2:3); δ_H (300 MHz, $CDCl_3$) 7.73 (2 H, d, *J* = 8.4 Hz), 7.40–7.37 (1 H, m), 7.31–7.16 (5 H, m), 5.60 (1 H, d, *J* = 9.0 Hz), 4.46 (2 H, t, *J* = 6.6 Hz), 4.20–4.13 (1 H, m), 3.62 (3 H, s), 3.08 (3 H, s), 3.02 (2 H, t, *J* = 6.6 Hz), 2.93 (2 H, t, *J* = 4.5 Hz), 2.38 (3 H, s).

To a dilute solution of the mesylate (*R*)-**16** (480 mg, 0.953 mmol) in DMF (160 mL; c = 6 mM) was added K_2CO_3 (658 mg, 4.77 mmol) and the mixture was stirred for 2 h at room temperature. The contents of the reaction vessel were poured into EtOAc/water (800 mL, 1:1), and the phases were separated. The organic layer was washed with water (33 × 400 mL), dried over $mgSO_4$, and concentrated in vacuo. After flash chromatography (EtOAc/heptane, 1:4), the title compound was obtained as a colourless oil (295 mg, 0.724 mmol, 76%). $[\alpha]^{22}_D = 0°$ (*c* 0.5, CH_2Cl_2).

ii) ***via*** **Mitsunobu reaction.** A stirred, dilute solution of alcohol (*R*)-**14** (115 mg, 0.270 mmol) in THF (75 mL; c = 4 mM) was treated with PPh_3 (71 mg, 0.271 mmol). As soon as it had been dissolved, DEAD (40% in toluene; 0.043 mL, 0.273 mmol) was added *via syringe* and stirring was continued for 2 h. The volatiles were removed *in vacuo*, and the crude product was subjected to flash chromatography (EtOAc/heptane, 1:10 → 1:3) yielding (*R*)-**17** as a slightly yellowish oil (66 mg, 0.162 mmol, 60%). *ee* = 42%.

General characterisation data. R_f 0.14 (EtOAc/heptane, 1:4); ν_{max} (neat) 3270, 2949, 2915, 1740, 1593, 1480, 1442; δ_H (300 MHz, $CDCl_3$) 7.75 (2 H, d, *J* = 8.4 Hz), 7.44–7.41 (1 H, m), 7.35–7.19 (5 H, m), 6.13 (1 H, dd, *J* = 17.5, 11.2 Hz), 5.80 (1 H, dd, *J* = 17.6, 2.1 Hz), 5.60 (1 H, dd, *J* = 11.1, 2.1 Hz), 5.56 (1 H, d, *J* = 9.1 Hz), 4.22 (1 H, ddd, *J* = 9.1, 5.4, 4.6 Hz), 3.62 (3H, s), 2.99 (1 H, dd, 17, 4.6 Hz), 2.89 (1 H, dd, 17 Hz, 5.4 Hz), 2.38 (3 H, s); δ_C (75 MHz, $CHCl_3$) 169.9, 143.6, 136.9, 132.1, 131.9, 129.6, 127.9 (2 C), 127.6, 127.1, 125.6, 124.9, 117.2, 92.0, 88.7, 86.9, 83.0, 54.5, 53.1, 25.5, 21.8; HRMS (EI) calcd. for $C_{23}H_{21}NO_4S$ (M^+) 407.1191, found 407.1193.

5-[2-(5-Hydroxypent-1-ynyl)phenyl]-2-(toluene-4-sulfonylamino)pent-4-ynoic acid methyl ester (18)

The title compound was synthesised analoguously to the preparation of **13**. Thus, 5-(2-iodophenyl)pent-4-yn-1-ol (**12**) (377 mg, 1.318 mmol) was stirred with $Pd(PPh_3)_4$ (81 mg, 0.070 mmol), CuI (30 mg, 0.158 mmol), and Et_2NH (0.8 mL, 7.7 mmol) in Et_2O (25 mL) before propargylglycine (±)-**9** (405 mg, 1.440 mmol) was added, and stirring was continued over night. Work-up and purification by flash chromatography (EtOAc/heptane, 1:1) afforded **18** as an off-white solid (578 mg, 1.315 mmol, 99%). Crystals suitable for X-ray analysis could be grown from a $CHCl_3$ solution top-layered with heptane. R_f 0.32 (EtOAc/heptane, 1:1); ν_{max} (KBr) 3486, 3118, 2942, 2228, 1752; δ_H (300 MHz, $CDCl_3$) 7.75 (2 H, d, $J = 8.3$ Hz), 7.40–7.37 (1 H, m), 7.30–7.16 (5 H, m), 6.11 (1 H, d, $J = 8.9$ Hz), 4.18 (1 H, td, $J = 9.0$, 5.2 Hz), 3.89 (1 H, q, $J = 5.8$ Hz), 3.64 (3 H, s), 2.98 (1 H, dd, $J = 17.0$, 5.3 Hz), 2.90 (1 H, dd, $J = 17.0$, 5.1 Hz), 2.67 (1 H, dt, $J = 6.6$, 0.8 Hz), 2.34 (3 H, s), 2.30 (1 H, t, $J = 5.6$ Hz), 1.96–1.87 (2 H, m); δ_C (75 MHz, $CHCl_3$) 170.4, 143.6, 137.0, 132.1, 132.0, 128.0, 127.3, 127.2, 126.2, 124.9, 93.8, 86.5, 83.0, 80.1, 61.9, 54.3, 52.8, 31.1, 24.9, 21.5, 16.3; HRMS (CI) calcd. for $C_{24}H_{25}NO_5S$ (M^+) 439.1453, found 439.1441.

5-Tosyl-1,2,9,10-tetradehydro-3,4,5,6,7,8-hexahydro-5-benzazacyclododecine-4-carboxylic acid methyl ester (19)

Triphenylphosphine (87 mg, 1.094 mmol) was dissolved in a solution of enediynic alcohol **18** (401 mg, 0.912 mmol) in THF (110 mL; c = 8 mM). DEAD was added (40% in toluene; 0.500 mL, 1.091 mmol), and the reaction mixture was stirred for 30 min. The volatiles were removed *in vacuo* and the crude product was subjected to flash chromatography (EtOAc/heptane, 1:3) to yield **19** as a colourless oil that solidified on standing (339 mg, 0.804 mmol, 88%). Crystals suitable for X-ray analysis could be grown by slow evaporation of a $CHCl_3$ solution. R_f 0.58 (EtOAc/heptane, 1:1); ν_{max} (KBr) 3442, 3069, 2951, 2920; 2230, 1752, 1598, 1484, 1348; δ_H (400 MHz, $CDCl_3$) 7.68 (2 H, d, $J =$ 8.4 Hz), 7.33–7.25 (4 H, m), 7.21–7.13 (2 H, m), 4.88 (1 H, dd, $J = 6.5$, 2.4 Hz), 4.10 (1 H, ddd, $J = 15.3$, 12.6 Hz, 4.1 Hz), 3.73 (1 H, ddd, $J = 15.0$, 12.3, 4.9 Hz), 3.50 (3 H, s), 3.36 (1 H, dd, $J = 17.7$, 6.5 Hz), 2.83 (1 H, dd, $J = 17.7$, 2.4 Hz), 2.56–2.42 (2 H, m), 2.40 (3 H, s), 2.38–2.30 (1 H, m), 2.08–1.97 (1 H, m); δ_C (100 MHz, $CHCl_3$) 169.1, 143.4, 136.2, 131.1, 129.5, 127.9, 127.2, 126.8, 126.7, 94.5, 88.2, 82.8, 81.4, 57.8, 52.1, 45.8, 30.9, 23.9, 21.4, 17.0; HRMS (CI) calcd. for $C_{24}H_{23}NO_4S$ (M^+) 421.1348, found 421.1329.

(*Z*)-5-Chloropent-4-en-2-yn-1-ol (20)

A stirred solution of (*Z*)-1,2-dichloroethene (1.10 mL, 14.57 mmol) in Et_2O (15 mL) was treated with $Pd(PPh_3)_4$ (386 mg, 0.335 mmol), CuI (206 mg, 1.082 mmol), and Et_2NH (7.20 mL, 69.60 mmol). Once a clear, homogeneous solution had been formed, prop-2-yn-1-ol (0.77 mL, 14.57 mmol) was added and stirring was continued for 3 h at ambient temperature. The reaction mixture was poured into two aliquots of a saturated aqueous solution of NH_4Cl and the phases were separated. The organic layer was extracted with EtOAc (33 × 10 mL), the combined organic phases were washed with 1M $KHSO_4$ (13 × 20 mL) and brine (13 × 20 mL) followed by drying over $mgSO_4$. Removal of the volatiles and purification of the crude product by bulb-to-bulb distillation (110 °C, 5.0 mbar) furnished **20** as a clear, colourless liquid (463 mg, 3.97 mmol, 27%). The characterisation data were consistent with those in ref.7a and ref.14d.

(*Z*)-6-Chlorohex-5-en-3-yn-1-ol (21)

The title compound was prepared using the same methodology as described for **20** by letting react (*Z*)-1,2-dichloroethene (0.395 mL, 5.232 mmol) in Et_2O (10 mL) with $PdCl_2(PPh_3)_2$ (130 mg, 0.112 mmol), CuI (77 mg, 0.404 mmol), Et_2NH (2.00 mL, 19.33 mmol), and but-3-yn-1-ol (0.300 mL, 3.963 mmol) over a period of 4 h. Work-up followed by bulb-to-bulb distillation (60 °C, 0.13 mbar) gave **21** as a clear, colourless liquid (284 mg, 2.175 mmol, 55%). The characterisation data were consistent with those in ref.7a.

(Z)-7-chlorohept-6-en-4-yn-1-ol (22)

The title compound was synthesised following the method described for **20**. Thus, (*Z*)-1,2-dichloroethene (0.500 mL, 6.622 mmol) in Et_2O (10 mL) was allowed to react with $Pd(PPh_3)_4$ (217 mg, 0.188 mol), CuI (108 mg, 0.563 mmol), Et_2NH (3.00 mL, 29.00 mmol), and pent-4-yn-1-ol (0.560 mL, 6.018 mmol) over a period of 7 h. Work-up and purification by bulb-to-bulb distillation (70 °C, 0.07 mbar) afforded **22** as a clear, colourless liquid (785 mg, 5.429 mmol, 90%). The characterisation data were consistent with those in ref.14c.

(*Z*)-10-Hydroxy-2-(toluene-4-sulfonylamino)dec-6-ene-4,8-diynoic acid methyl ester (23)

The title compound was synthesised by a similar procedure as described for **13**. Thus, (*Z*)-5-chloro-pent-4-en-2-yn-1-ol (**20**) (144 mg, 1.236 mmol) in Et_2O (15 mL) was allowed to react with *n*-$BuNH_2$ (0.6 mL, 6.1 mmol), CuI (23 mg, 0.121 mmol), $PdCl_2(PPh_3)_2$ (42 mg, 0.060 mmol) and propargylglycine (±)-**9** (396 mg, 1.408 mmol) over a period of 5 h. Work-up and purification by flash chromatography (EtOAc/heptane, 2:3) yielded **23** as a yellowish solid (175 mg, 0.484 mmol, 39%). R_f 0.30 (EtOAc/heptane, 1:1); ν_{max} (ATR) 3494, 3274, 2950, 1740, 1329; δ_H (300 MHz, $CDCl_3$) 7.77 (2 H, d, $J = 8.3$ Hz), 7.29 (2 H, d, $J =$ 8.3 Hz), 5.93 (1 H, d, $J = 7.8$ Hz), 5.85 (1 H, td, $J = 10.8$, 1.9 Hz), 5.74 (1 H, td, $J = 10.8$, 1.9 Hz), 5.51 (2 H, d, $J = 6.5$ Hz), 4.17 (1 H, td, $J = 4.5$, 9.0 Hz), 3.64 (3 H, s), 3.03 (1 H, t, $J = 6.5$ Hz), 2.96–2.81 (2 H, m), 2.42 (3 H, s); δ_C (75 MHz, $CHCl_3$) 170.2, 143.8, 136.9, 129.7, 127.1, 119.9, 119.3, 95.8, 90.4, 82.5, 82.0, 53.9, 53.1, 51.4, 25.3, 21.5; HRMS (EI) calcd. for $C_{18}H_{19}NO_5S$ (M^+) 361.0984, found 361.0991.

(*Z*)-11-Hydroxy-2-(toluene-4-sulfonylamino)-undec-6-ene-4,8-diynoic acid methyl ester (24)

The title compound was prepared following a similar procedure as described for **13** by letting react (*Z*)-6-chlorohex-5-en-3-yn-1-ol (**21**) (134 mg, 1.026 mmol) in Et_2O (15 mL) with *n*-$BuNH_2$ (0.51 mL, 5.2 mmol), $PdCl_2(PPh_3)_2$ (38 mg, 0.054 mmol), CuI (0.104 mmol), and propargylglycine (±)-**9** (311 mg, 1.106 mmol) over a period of 4 h. Work-up and flash chromatography

(EtOAc/heptane, 1:10 → 1:1) afforded **24** as a yellow oil (298 mg, 0.794 mmol, 77%). R_f 0.20 (EtOAc/heptane, 1:1); ν_{max} (KBr) 3444, 3265, 2953, 2215, 1753, 1160; δ_H (300 MHz, $CDCl_3$) 7.76 (1 H, d, $J = 8.3$ Hz), 7.28 (1 H, d, $J = 8.3$ Hz), 6.18 (1 H, bs), 5.79 (1 H, td, $J = 10.8$, 2.1 Hz), 5.68 (1 H, td, $J = 10.8$, 1.8 Hz), 4.15 (1 H, t, $J = 4.7$ Hz), 3.80 (1 H, t, $J = 6.2$ Hz), 3.60 (1 H, s), 2.94–2.78 (2 H, m), 2.71 (2 H, dt, $J = 6.2$, 1.5 Hz), 2.40 (1 H, s); δ_C (75 MHz, $CHCl_3$) 169.9, 143.5, 136.8, 129.5, 126.9, 120.1, 118.3, 95.3, 90.1, 81.5, 79.5, 60.6, 54.0, 52.6, 24.8, 23.7, 21.3; HRMS (CI) calcd. for $C_{19}H_{22}NO_5S$ (M^++H) 376.1219, found 376.1206.

(*Z*)-12-Hydroxy-2-(toluene-4-sulfonylamino)-dodec-6-ene-4,8-diynoic acid methyl ester (25)

The title compound was assembled similar to the preparation of **13**. Thus, (*Z*)-7-chlorohept-6-en-4-yn-1-ol (**22**) (88 mg, 0.609 mmol) in Et_2O (15 mL) was coupled to (±)-**9** employing $PdCl_2(PPh_3)_2$ (20 mg, 0.028 mmol), CuI (12 mg, 0.063 mmol), and *n*-$BuNH_2$ (0.30 mL, 3.04 mmol). After stirring over night, work-up and flash chromatography (EtOAc/heptane, 1:10 → 1:1) yielded **25** as a yellowish oil (179 mg, 0.460 mmol, 75%). R_f 0.31 (EtOAc/heptane, 1:1); ν_{max} (ATR) 3525, 3274, 3049, 2950, 2876, 2215, 1744, 1161; δ_H (300 MHz, $CDCl_3$) 7.76 (2 H, d, $J = 8.3$ Hz), 7.28 (2 H, d, $J = 8.3$ Hz), 6.21 (1 H, d, $J = 9.0$ Hz), 5.78 (1 H, td, $J = 10.8$, 2.2 Hz), 5.65 (1 H, td, $J = 10.8$, 2.1 Hz), 4.13 (1 H, td, $J = 9.1$, 5.2 Hz), 3.81 (2 H, t, $J = 6.1$ Hz), 3.60 (3 H, s), 2.95–2.78 (2 H, m), 2.70 (1 H, bs), 2.56 (2 H, dt, $J = 6.7$, 2.2 Hz), 2.40 (3 H, s), 1.88–1.79 (2 H, m); δ_C (75 MHz, $CHCl_3$) 170.1, 143.5, 136.8, 129.5, 126.9, 120.3, 117.9, 97.9, 89.9, 81.4, 78.5, 61.3, 54.0, 52.6, 30.8, 24.8, 21.3, 16.2; HRMS (CI) calcd. for $C_{20}H_{23}NO_5S$ (M^+) 390.1375, found 390.1378.

2-Tosyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methyl ester (26)

Enediynic alcohol **23** (112 mg, 0.310 mmol) and PPh_3 (121 mg, 0.461 mmol) were dissolved in THF (75 mL). Whilst stirring, DEAD (0.075 mL, 0.476 mmol) was added *via syringe* and stirring was continued for 2 h at ambient temperature. All volatiles were removed *in vacuo* (T > 30 °C) and the crude product was purified by flash chromatography (EtOAc/heptane, 1:3 → 1:1) providing **26** as a clear colourless oil (17 mg, 0.049 mmol, 16%). R_f 0.78 (EtOAc/heptane, 1:1); ν_{max} (ATR) 3032, 2950, 2915, 1740, 1459, 1160; δ_H (300 MHz, $CDCl_3$) 7.72 (2 H, d, $J = 8.3$ Hz), 7.28 (2 H, d, $J = 8.3$ Hz), 7.19–7.02 (4 H, m), 5.01 (1 H, t, $J = 4.5$ Hz), 4.70 (1 H, d, $J = 15.5$ Hz), 4.49 (1 H, d, $J = 15.5$ Hz), 3.45 (3 H, s), 3.18 (2 H, d, $J = 4.5$ Hz), 2.41 (3 H, s); δ_C (75 MHz, $CHCl_3$) 170.7, 143.5, 136.0, 131.4, 130.7, 129.5, 128.7, 127.3, 126.8 (2 C), 126.1, 53.8, 52.2, 44.4, 31.9, 21.5; HRMS (EI) calcd. for $C_{18}H_{19}NO_4S$ (M^+) 345.1035, found 345.0985.

(*Z*)-2-(Toluene-4-sulfonylamino)undeca-6,10-diene-4,8-diynoic acid methyl ester (28)

Enediynic alcohol **24** (134 mg, 0.357 mmol) was dissolved in THF (70 mL; c = 5 mM). PPh_3 (142 mg, 0.541 mmol) was added to the stirred solution and once it had dissolved, DEAD (40% in toluene; 0.250 mL, 0.545 mmol) was transferred into the reaction vessel *via syringe*. Stirring was continued for 1 h at ambient temperature followed by removal of all volatiles *in vacuo*. Flash chromatography (EtOAc/heptane, 1:10 → 1:3) of the crude product furnished **28** as a yellowish oil (44 mg, 0.123 mmol, 34%). R_f 0.56 (EtOAc/heptane, 1:1); ν_{max} (KBr) 3470, 3278, 2955, 2208, 1744; δ_H (300 MHz, $CDCl_3$) 7.74 (2 H, d, $J = 8.4$ Hz), 7.28 (2 H, d, $J = 8.4$ Hz), 6.04 (1 H, ddd, $J = 17.5$, 11.1, 2.3 Hz), 5.91 (1 H, dd, $J = 10.8$, 2.2 Hz), 5.77–5.69 (2 H, m), 5.56 (1 H, dd, $J = 11.1$, 2.1 Hz), 5.47 (1 H, d, $J = 9.2$ Hz), 4.15 (1 H, ddd, $J = 9.2$, 5.4, 4.3 Hz), 3.61 (3 H, s), 2.92 (1 H, ddd, $J = 17.6$, 4.3, 2.2 Hz), 2.83 (1 H, ddd, $J = 17.6$, 5.41, 2.2 Hz), 2.41 (3 H, s); δ_C (75 MHz, $CHCl_3$) 170.0, 143.7, 136.9, 129.7, 128.0, 127.1, 119.8, 118.8, 117.1, 95.7, 90.9, 87.2, 81.6, 54.1, 52.8, 25.3, 21.5; HRMS (CI) calcd. for $C_{19}H_{20}NO_4S$ (M^++H) 358.1113, found 358.1103.

(*Z*)-1-Tosyl-azacyclododec-6-ene-4,8-diyne-2-carboxylic acid methyl ester (29)

Triphenylphosphine (164 mg, 0.624 mmol) was dissolved in a stirred, dilute solution (c = 5 mM) of enediynic alcohol **25** (161 mg, 0.413 mmol) in THF (90 mL). DEAD (40% in toluene; 0.280 mL, 0.612 mmol) was added and stirring was continued for 30 min at room temperature. Removal of the volatiles followed by flash chromatography (EtOAc/heptane, 1:10 → 1:4) afforded **29** as white, waxy solid (90 mg, 0.252 mmol, 61%). Crystals suitable for X-ray analysis could be grown from a $CHCl_3$ solution top-layered with MeOH. R_f 0.60 (EtOAc/heptane, 1:1); ν_{max} (ATR) 3023, 2949, 2193, 1748, 1333; δ_H (300 MHz, $CDCl_3$) 7.67 (2 H, d, $J = 8.4$ Hz), 7.30 (2 H, d, $J = 8.4$ Hz), 5.72 (2 H, d, $J = 1.5$ Hz), 4.84 (1 H, dd, $J = 6.4$, 2.4 Hz), 4.05–3.94 (1 H, m), 3.84–3.73 (1 H, m), 3.56 (3 H, s), 3.25 (1 H, tdd, $J = 17.6$, 6.4, 1.6 Hz), 2.73 (1 H, dd, $J = 17.6$, 2.4 Hz), 2.51–2.21 (3 H, m), 2.43 (3 H, s), 2.07–1.93 (1 H, m); δ_C (75 MHz, $CHCl_3$) 169.1, 143.5, 136.2, 129.5, 127.2, 121.9, 120.6, 98.4, 91.8, 82.1, 81.2, 57.6, 52.2, 45.6, 30.7, 23.8, 21.4, 17.3; HRMS (EI) calcd. for $C_{20}H_{21}NO_4S$ (M^+) 371.1191, found 371.1181.

(*Z*)-(S)-11-Hydroxy-2-(toluene-4-sulfonylamino)undec-7-ene-5,9-diynoic acid methyl ester (31)

The title compound was prepared using a similar procedure as described for **13**: Protected (*S*)-homopropargylglycine **30** (247 mg, 0.836 mmol) was coupled to (*Z*)-5-chloropent-4-en-2-yn-1-ol (**20**) (100 mg, 0.858 mmol) by the action of $Pd(PPh_3)_4$ 950 mg, 0.043 mmol), CuI (18 mg, 0.095 mmol), and *n*-$BuNH_2$ (0.50 mL, 5.1 mmol) in Et_2O (40 mL) over a period of 5 h. Work-up and flash chromatography (EtOAc/heptane, 1:2 → 1:1) gave access to **31** as a yellowish solid (265 mg, 0.706 mmol, 84%). R_f 0.23 (EtOAc/heptane, 1:1); $[\alpha]^{22}_D$ = +50.0° (*c* 0.11, CH_2Cl_2); ν_{max} (ATR) 3511, 3270, 2949, 1744, 1433, 1338; δ_H (300 MHz, $CDCl_3$) 7.76 (2 H, d, $J = 8.4$ Hz), 7.29 (2 H, d, $J = 8.4$ Hz), 5.82 (1 H, td, $J = 10.9$, 1.8 Hz), 5.76 (1 H, td, $J = 10.9$, 2.0 Hz), 5.34 (1 H, d, $J = 9.2$ Hz), 4.46 (1 H, dd, $J = 6.5$, 1.8 Hz), 4.19–4.11 (1 H, m), 3.59 (3 H, s), 2.58 (1 H, t, $J = 6.5$ Hz), 2.49 (2 H, dt, $J = 6.6$, 2.0 Hz), 2.42 (3 H, s), 2.08–1.97 (1 H, m), 1.94–1.82 (1 H, m); δ_C (75 MHz, $CHCl_3$) 172.0, 143.8, 136.5, 129.7, 127.3, 120.0, 118.7, 95.9, 95.2, 82.6, 79.5, 54.9, 52.8, 51.5, 31.8, 21.6, 15.9; HRMS (ESI) calcd. for $C_{19}H_{21}NNaO_5S$ (M^++Na) 398.1038, found 398.1045.

(*S*)-6-[2-(3-Hydroxy-prop-1-ynyl)phenyl]-2-(toluene-4-sulfonylamino)-hex-5-ynoic acid methyl ester (32)

The title compound was synthesised following a similar procedure as used for **13**. Thus, 3-(2-iodophenyl)prop-2-yn-1-ol (**10**) (130 mg, 0.504 mmol) in Et_2O (25 mL) was allowed to react with $PdCl_2(PPh_3)_2$ (18 mg, 0.026 mol), CuI (10 mg, 0.053 mmol), *n*-$BuNH_2$ (0.26 mL, 2.63 mmol) and protected (*S*)-homopropargylglycine **30** (146 mg, 0.494 mmol) over a period of 5.5 h. Work-up followed by flash chromatography (EtOAc/heptane, 1:10 → 1:1) furnished **32** as a yellow solid (139 mg, 0.327 mmol, 66%). R_f 0.20 (EtOAc/heptane, 1:1); $[\alpha]^{22}_D$ = +26.9° (*c* 1.0, CH_2Cl_2); ν_{max} (ATR) 3499, 3261, 2950, 1735, 1432, 1337; δ_H (300 MHz, $CDCl_3$) 7.74 (2 H, d, *J* = 8.4 Hz), 7.45–7.39 (1 H, m), 7.36–7.30 (1 H, m), 7.26–7.20 (2 H, m), 7.16 (2 H, d, *J* = 8.4 Hz), 5.80 (1 H, d, *J* = 9.1 Hz), 4.55 (2 H, d, *J* = 6.0 Hz), 4.19 (1 H, dt, *J* = 8.6, 5.0 Hz), 3.56 (3 H, s), 3.11 (1 H, t, *J* = 6.0 Hz), 2.54 (2 H, t, *J* = 6.6 Hz), 2.30 (3 H, s), 2.13–2.02 (1 H, m), 1.99–1.87 (1 H, m); δ_C (75 MHz, $CHCl_3$) 172.1, 143.6, 136.4, 132.1, 131.7, 129.5, 127.9, 127.5, 127.2, 125.8, 125.1, 91.8, 91.6, 83.9, 80.6, 54.9, 52.7, 51.3, 31.6, 21.4, 15.7; HRMS (ESI) calcd. for $C_{23}H_{23}NNaO_5S$ (M^++Na) 448.1195, found 448.1194.

(*Z*)-(*S*)-1-Tosyl-azacycloundec-7-en-5,9-diyne-2-carboxylic acid methyl ester (33)

In a stirred solution of enediynic alcohol **31** (103 mg, 0.274 mmol) in THF (55 mL; c = 5 mM) was dissolved PPh_3 (86 mg, 0.380 mmol) followed by the addition of DEAD (40% in toluene; 0.150 mL, 0.327 mmol). Stirring was continued for 15 min at ambient temperature, then all volatiles were removed *in vacuo* at room temperature. Purification of the crude product by flash chromatography (EtOAc/heptane, 1:4) afforded the title compound an off-white solid (69 mg, 0.193 mmol, 70%). R_f 0.62 (EtOAc/heptane, 1:1); $[\alpha]^{22}_D$ = +131.9° (*c* 0.72, CH_2Cl_2); ν_{max} (ATR) 3023, 2950, 2915, 2842, 2198, 1731, 1433, 1333; δ_H (300 MHz, $CDCl_3$) 7.69 (2 H, d, *J* = 8.4 Hz), 7.31 (2 H, d, *J* = 8.4 Hz), 5.83 (1 H, d, *J* = 10.4 Hz), 5.79 (1 H, d, *J* = 10.4 Hz), 5.25 (1 H, dd, *J* = 8.9, 2.5 Hz), 4.49 (1 H, d, *J* = 17.9 Hz), 4.38 (1 H, d, *J* = 17.9 Hz), 3.64 (3 H, s), 2.79–2.69 (1 H, m), 2.63–2.55 (2 H, m), 2.42 (3 H, s), 2.12–2.01 (1 H, m); δ_C (75 MHz, $CHCl_3$) 171.7, 143.6, 137.8, 129.7, 127.0, 122.6, 120.1, 99.4, 91.0, 86.7, 81.8, 57.1, 52.2, 41.9, 33.0, 21.5, 19.1; HRMS (ESI) calcd. for $C_{19}H_{19}NNaO_4S$ (M^++Na) 380.0933, found 380.0960.

(*S*)-4-Tosyl-1,2,8,9-tetradehydro-4,5,6,7-tetrahydro-3*H*-4-benzazacycloundecine-5-carboxylic acid methyl ester (34)

Enediynic alcohol **32** (126 mg, 0.296 mmol) was dissolved in THF (60 mL; c = 5 mM). Whilst stirring, PPh_3 (93 mg, 0.355 mmol) and DEAD (40% in toluene; 0.165 mL, 0.360 mmol) were added, and stirring was continued for 15 min, then all volatiles were removed *in vacuo*. The crude product was subjected to flash chromatography (EtOAc/heptane, 1:10 → 1:4) to yield **34** as a white solid (96 mg, 0.236 mmol, 80%). Crystals suitable for X-ray analysis could be grown from a $CHCl_3$ solution top-layered with MeOH. R_f 0.70 (EtOAc/heptane, 1:1); $[\alpha]^{22}_D$ = +37.0° (*c* 0.30, CH_2Cl_2); ν_{max} (ATR) 3062, 2950, 2915, 2846, 2228, 1735, 1445, 1329; δ_H (300 MHz, $CDCl_3$) 7.72 (2 H, d, *J* = 8.4 Hz), 7.34–7.18 (6 H, m), 5.23 (1 H, dd, *J* = 8.2, 3.5 Hz), 4.58 (1 H, d, *J* = 17.5 Hz), 4.44 (1 H, d, *J* = 17.5 Hz), 3.65 (3 H, s), 2.76–2.66 (1 H, m), 2.64–2.59 (2 H, m), 2.40 (3 H, s), 2.21–2.11 (1 H, m); δ_C (50 MHz, $CHCl_3$) 171.6, 143.6, 137.7, 129.7, 129.4, 129.3, 128.2, 127.5, 127.1, 126.9, 126.0, 95.1, 87.5, 86.9, 82.0, 57.6, 52.2, 41.7, 32.0, 21.4, 18.5; HRMS (ESI) calcd. for $C_{23}H_{21}NNaO_4S$ (M^++Na) 430.1089, found 430.1092.

(*S*)-2-Tosyl-2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepine-3-carboxylic acid methyl ester (35)

Cyclic enediyne **33** (78 mg, 0.218 mmol) was dissolved in EtCN (10 mL), 1,4-cyclohexadiene (2.06 mL, 21.8 mmol, 100 equiv.) was added, and the reaction mixture was heated to reflux under a protective atmosphere of nitrogen for 6 d. All volatiles were removed and the crude product was purified by flash chromatography (EtOAc/heptane, 1:4) to give **35** as a white solid (24 mg, 0.067 mmol, 31%). R_f 0.60 (EtOAc/heptane, 1:1); $[\alpha]^{22}_D$ = 58.1° (*c* = 0.42, CH_2Cl_2); ν_{max} (ATR) 3031, 2950, 1740, 1437, 1333; δ_H (400 MHz, $CDCl_3$) 7.49 (2 H, d, *J* = 8.4 Hz), 7.21–7.18 (1 H, m), 7.16–7.11 (4 H, m), 7.00–6.96 (1 H, m), 4.81–4.73 (2 H, m), 4.64 (1 H, d, *J* = 16.5 Hz), 3.67 (3 H, s), 2.90 (1 H, dd, *J* = 15.8, 10.1 Hz), 2.59 (1 H, dd, *J* = 15.8, 9.3 Hz), 2.36 (3 H, s), 2.34–2.26 (1 H, m), 2.00–1.92 (1 H, m); δ_C (75 MHz, $CHCl_3$) 171.4, 143.0, 139.9, 137.2, 136.7, 129.4, 129.2, 128.7, 127.6, 127.1, 126.3, 59.2, 52.3, 48.6, 31.4, 29.3, 21.4; HRMS (ESI) calcd. for $C_{19}H_{21}NNaO_4S$ (M^++Na) 382.1089, found 382.1082.

(*S*)-2-Tosyl-2,3,4,5-tetrahydro-1*H*-naphtho[2,3-*c*]azepine-3-carboxylic acid methyl ester (36)

Cyclic enediyne **34** (15 mg, 0.037 mmol), EtCN (3 mL), and 1,4-cyclohexadiene (0.35 mL, 3.70 mmol, 100 equiv.) were enclosed in a sealed tube under a protective atmosphere of nitrogen. The reaction vessel was exposed to a temperature of 150 °C for 9 d after which time the volatiles were removed and the crude product was subjected to preparative TLC (0.5% MeOH in CH_2Cl_2) resulting in **36** as a white solid (9 mg, 0.022 mmol, 60%). R_f 0.49 (EtOAc/heptane, 1:2); $[\alpha]^{22}_D$ = 1.1° (*c* = 0.45, CH_2Cl_2); ν_{max} (ATR) 2936, 1733, 1592, 1420, 1333; δ_H (400 MHz, $CDCl_3$) 7.79–7.75 (1 H, m), 7.73–7.69 (1 H, m), 7.67 (1 H, s), 7.51 (1 H, s), 7.48 (2 H, d, *J* = 2.3 Hz), 7.46–7.42 (2 H, m), 7.03 (2 H, d, *J* = 8.4 Hz), 4.94 (1 H, d, *J* = 16.3 Hz), 4.86 (1 H, t, *J* = 5.1 Hz), 4.72 (1 H, d, *J* = 16.3 Hz), 3.70 (3 H, s), 3.07 (1 H, dd, *J* = 15.3, 11.2 Hz), 2.82 (1 H, dd, *J* = 15.3, 8.1 Hz), 2.48–2.41 (1 H, m), 2.28 (3 H, s), 1.95–1.87 (1 H, m); δ_C (75 MHz, $CHCl_3$) 171.3, 143.1, 138.2, 137.2, 135.1, 132.9, 132.0, 129.2, 127.7, 127.6, 127.5, 127.2, 127.0, 126.1, 125.7, 59.0, 52.4, 49.0, 31.6, 30.1, 21.4; HRMS (ESI) calcd. for $C_{23}H_{24}NO_4S$ (M^++H) 410.1426, found 410.1422.

Half-life determination of the cyclic enediynes

High temperature NMR experiments (T ≤ 80 °C). The NMR probe was heated to a set temperature and allowed to equilibrate until the internal temperature remained constant.[31] A sealed NMR tube containing an enediyne sample (*vide infra*) was inserted and ^{1}H-NMR spectra were recorded at different points in time.

High temperature experiments (T > 80 °C). A sealed NMR tube containing the enediyne sample (*vide infra*) was immersed into an oil bath of constant temperature. In regular intervalls,

the NMR tube was removed from the oil bath and immediately cooled to room temperature by rinsing with heptane and acetone. The conversion of the BC was then controlled by ^{1}H-NMR.

For enediyne 4. A NMR tube (ø = 5 mm) connected to an argon/vacuum manifold was charged with enediyne **4** (20.3 mg, 0.052 mmol) and DMSO-d_6 (450 μL). Of a solution of 1,4-cyclohexadiene in DMSO-d_6 (10% v/v) was added 97 μL (0.103 mmol, 2 equiv.). Cyclohexane was added as internal standard as a solution in DMSO-d_6 (10% v/v) (14 μL, 0.013 mmol). The resulting solution was degassed by three freeze-pump-thaw cycles and the NMR tube was tipped-off by flame under partial vacuum. The concentration of **4** in the thus prepared sample was 0.093 M. Decay of the starting material and formation of product were monitored by high temperature NMR experiments (T = 80 °C). The decay of **4** displayed first-order behaviour. $t_{1/2}$ (80 °C) = 59 min.

For enediyne 19. A sample was prepared as described for **4** from enediyne **19** (21.8 mg, 0.052 mmol) in DMSO-d_6 (450 μL), 1,4-cyclohexadiene in DMSO-d_6 (10% v/v) (98 μL, 0.104 mmol, 2 equiv.), and cyclohexane in DMSO-d_6 (10% v/v) (14 μL, 0.013 mmol) as internal standard. The concentration of **19** in the final solution was 0.093 M. No decay of **19** could be observed up to temperatures of 120 °C.

For enediyne 29. A sample was prepared as described for **4** from enediyne **29** (19.3 mg, 0.052 mmol) in DMSO-d_6 (450 μL), 1,4-cyclohexadiene in DMSO-d_6 (10% v/v) (98 μL, 0.104 mmol, 2 equiv.), and cyclohexane in DMSO-d_6 (10% v/v) (14 μL, 0.013 mmol) as internal standard. The resulting concentration of **29** was 0.093 M. No decay of the enediyne was observed at temperatures of 90 °C. After being subjected to 120 °C for 24 h, a trace amount of a decay product appeared in the ^{1}H-NMR spectra.

For enediyne 33. A sample was prepared as described for **4** from enediyne **33** (17.8 mg, 0.050 mmol) in DMSO-d_6 (500 μL), 1,4-cyclohexadiene (9.5 μL, 0.100 mmol), and a solution of dimethyl oxalate in DMSO-d_6 (2.5 M) (10 μL, 0.025 mmol) as internal standard. The concentration of **33** in the sample was 0.096 M. Decay of **33** and formation of **35** were followed at 90 °C. The decay of **33** displayed first-order behaviour. $t_{1/2}$ (90 °C) = 26 h.

For enediyne 34. A sample was prepared as described for **4** from enediyne **34** (12.3 mg, 0.030 mmol) in DMSO-d_6 (550 μL), 1,4-cyclohexadiene (5.7 μL, 0.060 mmol, 2 equiv.), and MeCN in DMSO-d_6 (10% v/v) (16 μL, 0.031 mmol) as internal standard. The final concentration of **34** was 0.053 M. Decay of **34** and formation of **36** were followed at 120 °C. The decay of **34** displayed first-order behaviour. $t_{1/2}$ (120 °C) = 175 h.

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