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Synthesis of 14-membered enediyne-embedded macrocycles†

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A concise and practical strategy towards a novel class of 14-membered macrocycles containing an enediyne (*Z*-3-ene-1,5-diyne) structural unit is described. A highly modular assembly of various precursors *via* sequential Ugi/Sonogashira reactions allowed the preparation of hybrid enediyne-peptide macrocycles in most cases as single diastereoisomers. Selected macrocyclic compounds showed moderate antiproliferative activity, and can be considered as templates suitable for further diversification in terms of ring size, shape, and stereochemistry.

Introduction

Macrocyclic compounds cover a relatively underexplored area of chemical space between traditional small-molecule drugs and antibodies.[1,2] Owing to the ability to combine the cell permeability and oral bioavailability of small molecules with the potency and selectivity of biologics, macrocyclic compounds have emerged as promising candidates to modulate challenging targets such as protein–protein interactions (PPIs).[3–6] The combination of conformational preorganization with a certain degree of flexibility enabling non-covalent interactions with biomolecules is the main structural advantage of macrocyclic compounds compared with acyclic molecules of similar molecular weight. Even though more than 100 marketed drugs are derived from macrocycles, this class of molecules is still underexplored, largely because of the concerns about the difficulty in their synthesis, and the synthesis of their derivatives.[7]

The chemical complexity of natural macrocycles makes them poorly amenable for lead optimization *via* typical medicinal chemistry tools, and often hampers structural diversification.[5] Macrocyclic compounds with considerable peptidic character are typically too polar to obtain the necessary pharmacokinetic profile. Nevertheless, the approach based on natural product-inspired macrocycles garnered quite a lot of attention as a relatively simple method to access structurally diverse macrocyclic compounds.[8] Thus, a macrolactone inhibitor of the Sonic Hedgehog pathway has been developed exploiting a rich family of polyketides,[9] and diverse macrocyclic compounds with anticancer properties were designed inspired by benzoquinone ansamycins,[10] while a rapamycin-inspired macrocyclic library led to the discovery of a nucleoside uptake inhibitor (Fig. 1A).[11] In addition, macrocyclic libraries comprising cyclophane,[12] tetrahydropyran,[13] succinimide, pyrrolidine, spirooxindole, and pyrrolidinyl-spirobarbiturate[14] motifs have been successfully prepared following the same approach.

In this regard, we found our inspiration in highly potent natural products containing the *cis*-enediyne (*Z*-3-hexene-1,5-diyne) structural motif embedded in a 9- or 10-membered ring (Fig. 1B).[15] The biological activity of enediyne compounds is attributed to the DNA cleavage caused by 1,4-benzenoid diradicals formed by the Bergman cycloaromatization of the enediyne core.[16] Many efforts have been devoted to the synthesis and activity elucidation of structurally simple, acyclic and cyclic enediyne derivatives.[17] Since 12-membered and larger enediyne rings are stable toward Bergman cycloaromatization,[16] they are almost neglected in the research, with no data about their biological profile. At the same time, many macrocyclization strategies faced difficulties in the preparation of medium sized rings of 11–14 atoms.[5] Having in mind that natural product-inspired macrocycles showed potential as chemical probes and drug leads for new protein targets,[8,11] we were interested in merging peptide-like structures with a rigid enediyne scaffold into a new class of hybrid macrocyclic compounds (Scheme 1). In addition, its *Z* planar conformationally preorganized structure would facilitate ring closing by the macrolactonization reaction. Macrolactone precursors can be prepared in just a few steps from easily accessible starting materials, with the key step being the multicomponent Ugi reaction[18] for the introduction of structural diversity. We presume that such hybrid structures could be interesting for probing various biological targets, and therefore, here present

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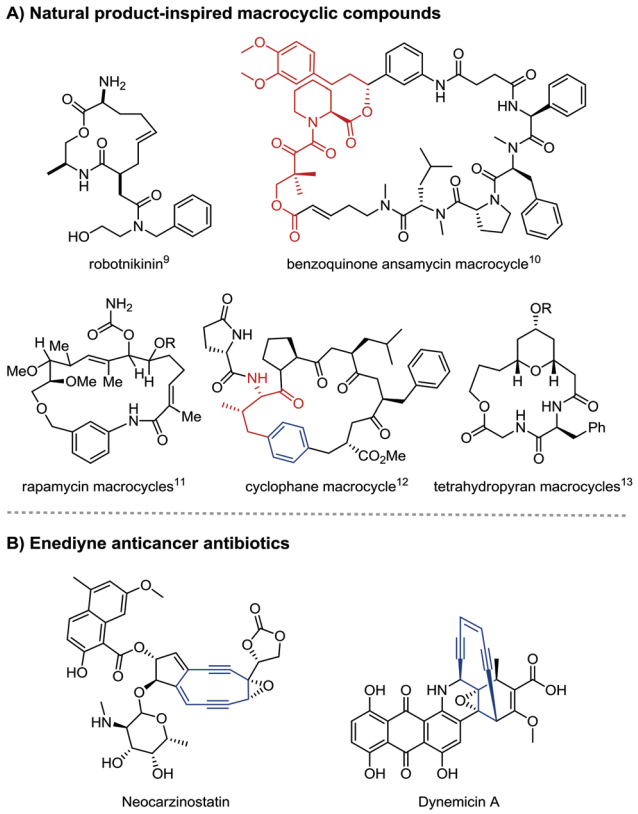
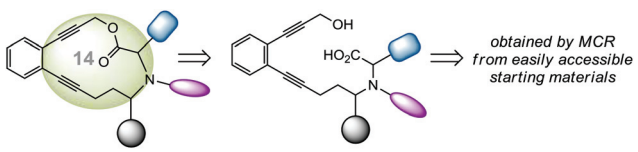


Fig. 1 (A) Representative examples of natural product-inspired macrocyclic compounds; (B) representative examples of enediyne anticancer antibiotics.



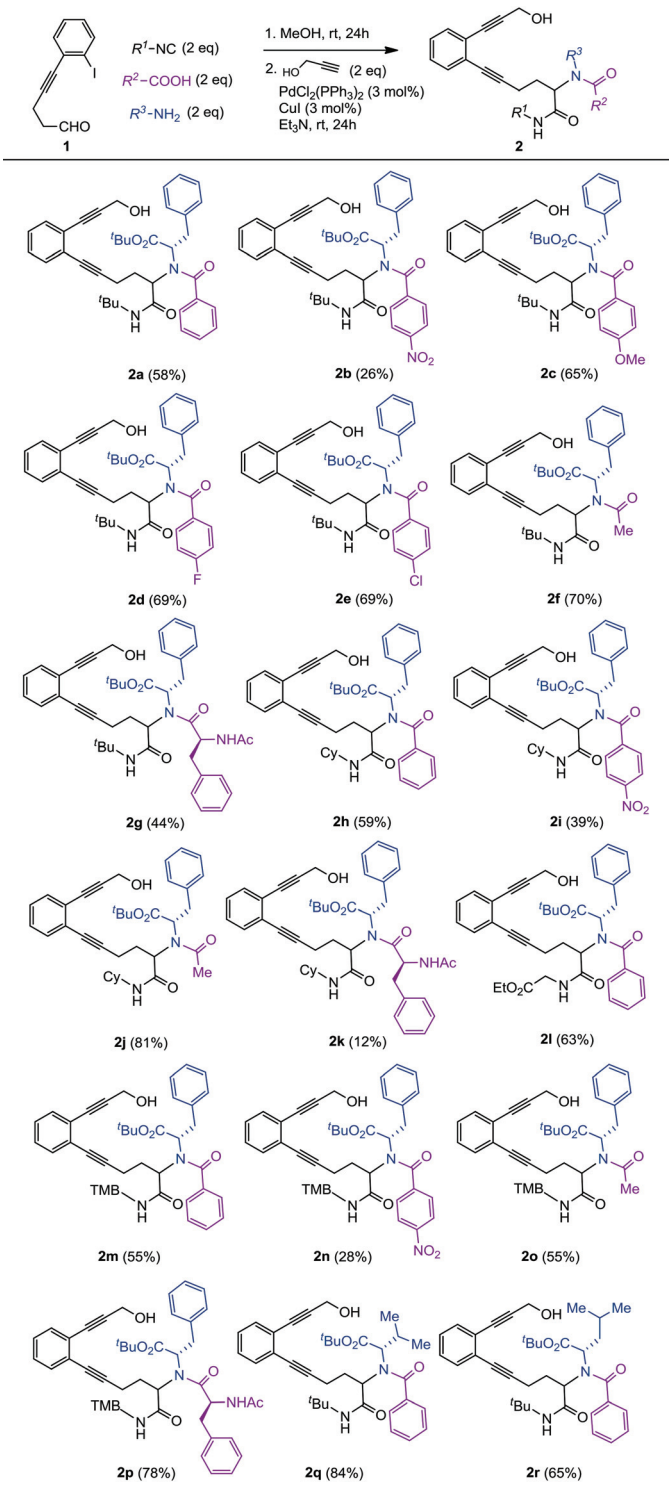
Scheme 1 Strategy towards 14-membered enediyne macrolactones.

a general strategy for the synthesis of 14-membered enediyne-embedded macrocycles.

Results and discussion

We started our investigations by performing the Ugi reaction between aldehyde **1**[19] (prepared in two steps from commercially available compounds, see the ESI† for details), a carboxylic acid, an amine, and an isocyanide, followed by the Sonogashira reaction with propargyl alcohol (Table 1). Firstly, various carboxylic acids were combined with aldehyde **1**, *tert*-butyl isocyanide and phenylalanine *tert*-butyl ester as an amine component. When benzoic acid was employed, the Ugi–Sonogashira sequence product was obtained in fair yield over two steps (**2a**, 58%). The reaction maintained its effectiveness when electron-donating and electron-withdrawing substituents

Table 1 Substrate scope: the Ugi–Sonogashira reaction sequence[a]



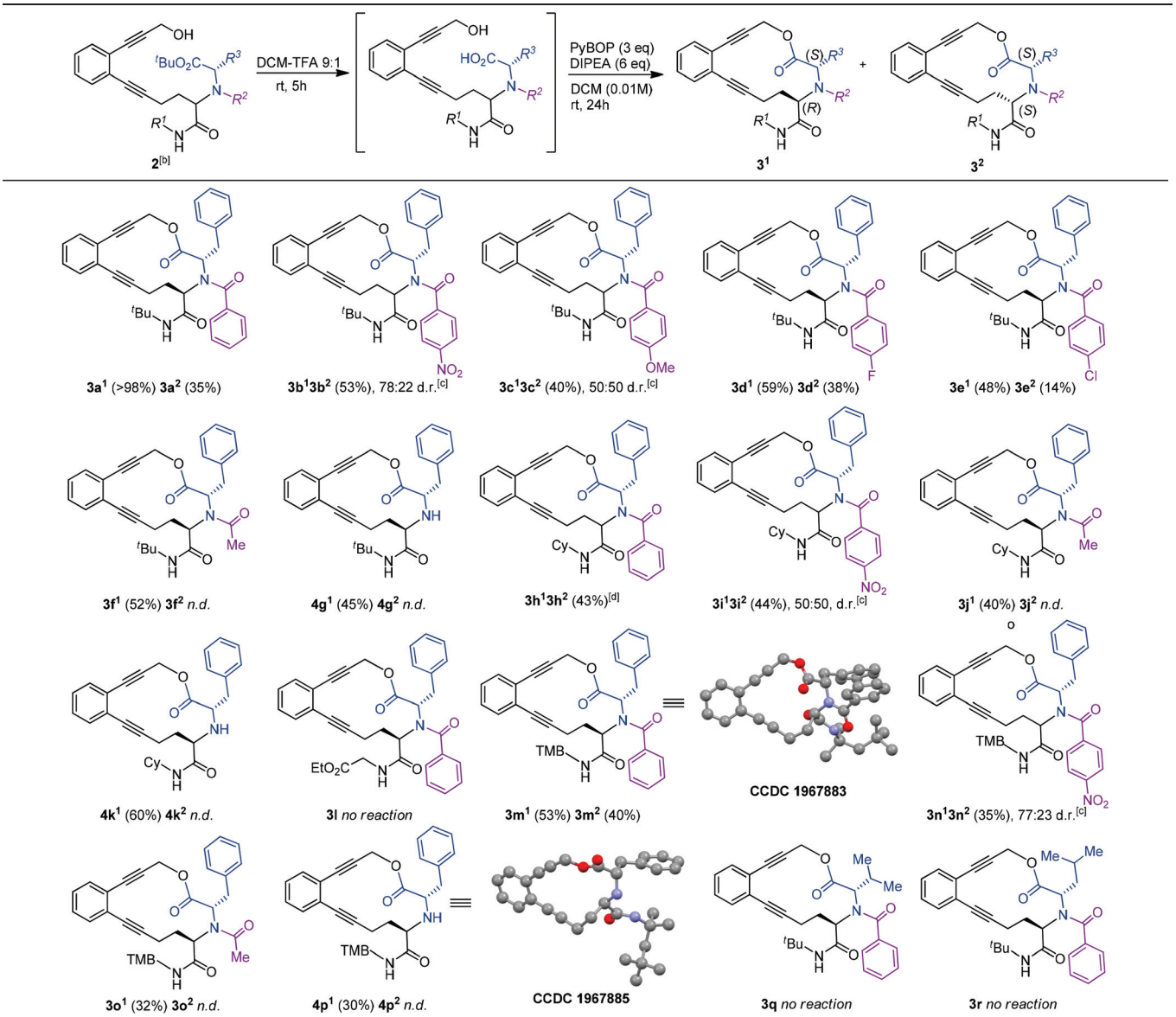
[a] Reactions performed on a 0.35 mmol scale. Isolated yields after 2 steps. Products are isolated as a 1 : 1 mixture of diastereomers. TMB = 1,1,3,3-tetramethylbutyl.

were placed on the aryl ring of benzoic acid (**2b**–**2e**), as well as with aliphatic carboxylic acids (**2f**, acetic acid, 70%, and **2g**, *N*-acetyl phenylalanine, 44%). Various isocyanides reacted

efficiently with aldehyde **1**, phenylalanine *tert*-butyl ester, and several different carboxylic acids, yielding products **2h–2p** in moderate to very good yields. Finally, valine and leucine *tert*-butyl esters were investigated as amine components in the sequence reaction with benzoic acid, *tert*-butyl isocyanide and aldehyde **1**. Both reactions proceeded smoothly and yielded products **2q** and **2r** in 84% and 65% isolated yields, respectively. All products **2a–2r** were isolated as a 1 : 1 inseparable mixture of diastereomers.

With acyclic enediynes in hand, we turned our attention to their macrocyclization *via* the macrolactonization reaction. The cyclization step is an enduring challenge in macrocyclic synthesis, regularly plagued by low yields and often impeded by epimerization, cyclodimerization, and oligomerisation.[20] After performing optimization investigations (see the ESI†), the best results were obtained in the reaction sequence that employs the hydrolysis of *tert*-butyl ester with trifluoroacetic acid in dichloromethane (1 : 9 mixture) for 5 h at room temperature, followed by lactone formation with PyBOP (3 eq.) and DIPEA (6 eq.) in dichloromethane (0.01 M solution) for 24 h at room temperature (Table 2). Under the chosen reaction conditions, acyclic enediyne **2a** afforded two macrocyclic products

Table 2 Substrate scope: macrolactonization[a]



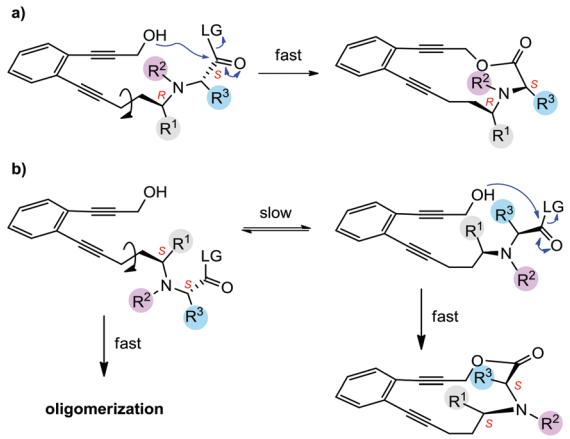
[a] Reactions were performed on a 0.15 mmol scale for **2**. Isolated yields after 2 steps. n.d. – not detected. Major (*R,S*) diastereomer drawn. TMB = 1,1,3,3-tetramethylbutyl. [b] Acyclic enediyne **2** is a 1 : 1 mixture of two diastereoisomers. Where individual macrolactone diastereoisomers are separated during purification, the yield is given for each of them, having in mind that the theoretical yield for each diastereoisomer is 50%mmol of the corresponding precursor **2**. Where the macrolactone is isolated as a mixture of two diastereoisomers, overall yield is given. [c] d.r. determined from the ^{1}H NMR of the isolated product. [d] Major diastereoisomer **3h¹** was partially isolated in a pure form (see the Experimental section), however overall yield is given.

which were separated and characterized as two diastereoisomers, **3a**^{1} and **3a**^{2} (Table 2). Given that the macrocyclization reaction was performed with acyclic enediyne **2a** present as a 1 : 1 mixture of two diastereoisomers, from the amount of isolated products it can be calculated that **3a**^{1} was formed in an almost quantitative yield from the corresponding acyclic precursor, while **3a**^{2} was formed in 35% yield. Encouraged by this result, we subjected all enediynes **2** to the chosen macrocyclization reaction.

Macrocycles **3b** and **3c** were isolated as inseparable mixtures of two diastereomers in 53% and 40% overall yield. Macrocycles bearing halogen atoms on the aryl ring of a tertiary amide were isolated as separated diastereomers (**3d**^{1} 59%, **3d**^{2} 38%, and **3e**^{1} 48%, **3e**^{2} 14%). Only one diastereomer of enediyne **2f** (originating from the Ugi–Sonogashira sequence with acetic acid as a carboxylic acid component) underwent macrocyclization, providing macrolactone **3f**^{1} in 52% isolated yield. Interestingly, rather than yielding the expected product **3g**, the macrocyclization of enediyne **2g** under the chosen reaction conditions was accompanied by the hydrolysis of the tertiary amide, thus generating macrolactone **4g**^{1} (45%) as the sole product. Cyclization of acyclic enediynes **2h** and **2i** resulted in mixtures of diastereomers **3h** (43%) and **3i** (44%). When **2j** was subjected to macrolactonization conditions, only one diastereomer was isolated (**3j**^{1}) in 40% yield. The cyclization of phenylalanine amide-containing **2k** ended with the same result as the macrolactonization of its counterpart **2g**; hydrolysed macrolactone **4k**^{1} was isolated in 60% yield as the only product. Interestingly, the cyclization of enediyne **2l** did not proceed under the optimized reaction conditions. The cyclization of acyclic precursors **2m–2p** proceeded complementarily to thus far observed results: substrate **2m** possessing a benzamide group yielded both diastereomers **3m**^{1} and **3m**^{2} in 53% and 40% yield, respectively, substrate **2n** with 4-nitrobenzamide provided an inseparable mixture of diastereomers **3n**, only one diastereomer **2o** was prone to cyclization, while **2p** under standard cyclization conditions resulted in the hydrolysed product **4p**^{1}. Finally, acyclic enediynes comprising valine (**2q**) and leucine (**2r**) did not yield respective macrolactones under the applied cyclization conditions. The absolute configurations of **3m**^{1} and **4p**^{1} were unambiguously assigned to be (*R*,*S*), and the absolute configurations of the remaining products were assigned by analogy.

For insight into the obtained results, the comparison of cyclization precursors **2** and isolated macrolactones **3** and **4** indicates that the macrocyclization outcome is highly influenced, and can be to some extent predicted by the nature of the tertiary amide group in the cyclization precursor (formed during the Ugi reaction). Thus, compounds **2** with benzamide or substituted benzamide groups afforded both diastereoisomers of the corresponding macrocyclic compounds. Compounds **2f**, **2j** and **2o** with an acetamido group yielded a single macrocyclic diastereoisomer, while cyclization of precursors **2g**, **2k** and **2p** with a phenylalanine amide group resulted in a single diastereoisomer of hydrolysed macrolactones **4**. The HPLC analysis of acyclic enediyne **2g** subjected to deprotection with TFA in DCM pointed out that a partial hydrolysis of the tertiary amide bond occurred during the *tert*-butyl ester deprotection (see the ESI†). An analysis of the macrocyclization reaction revealed the disappearance of all precursors, but the predominant formation of a single macrocyclic product, isolated and characterized as **3g**^{1}. For comparison, the deprotection of acyclic enediyne **2a** was not accompanied by noticeable tertiary amide hydrolysis (ESI†), thus indicating that the extent of hydrolysis differs between precursors and is associated with their structure. Propensity of an open-chain precursor molecule to adopt a favourable preorganization with a short end-to-end distance is a key factor determining the efficiency of macrocyclization.[21] This intrinsic favourable preorganization can be considered either conformational or configurational.[22] Configurational preorganization is a particular case of conformational preorganization in which the conformational preferences are associated with the stereochemical configurations of the chiral centres. A common feature of cyclization precursors **2** is the rigid, planar, achiral enediyne scaffold which favors the folded preorganization of the open-chain molecule. The observed difference in the stereochemistry of the macrocyclic products can be mainly, but not exclusively, attributed to the preorganization of precursors determined by the configuration of the chiral center formed in the Ugi reaction. The predominat formation of (*R*,*S*) macrocycles indicates that the conformation of the heterochiral precursor is more favorable for macrocyclization or it adopts a favourable conformation faster than homochiral ones, as schematically presented at Scheme 2. The conformational preorganization of homochiral precursors is a slow process and is therefore hampered by competitive reactions such as oligomerization.

A lot of research has been done in structural fine-tuning enabling the transformation of macrocyclic compounds into good pharmaceutical candidates. However, despite great progress in these areas, cell permeability, oral bioavailability, and metabolic stability still remain a challenging task. It has been emphasized that an increased hydrophobicity is a major determinant of cellular penetrance along with pI and α-helicity.[23] The enediyne motif contributes most to the hydrophobicity of the prepared macrocyclic systems, and we were keen to get some preliminary insights into the biological potential of enediyne-embedded macrocycles. We selected macrocycles bearing different R^1 groups (*t*Bu, Cy or TMB), with or without an R^2 group (macrocycles **3** *vs.* **4**) and a single *vs* mixture of diastereoisomers to elucidate the role of other structural elements on biological activity. The effect of selected compounds was tested on the proliferation of two cell lines, HCT116 (colon carcinoma) and HEK293T (embryonic kidneys), Table 3. Macrolactones **3e**^{1}, **4g**^{1}, **3h**^{1} and **3m**^{1} showed modest inhibitory activity towards the colon carcinoma cell line HCT116 (GI_{50} values 14–30 μM), and prominent inhibitory effect towards the human embryonic kidney cell line HEK293T with GI_{50} values 2–3 μM. Only macrolactone **3i**, tested as a mixture of diastereoisomers, showed no activity on the two cell lines.



Scheme 2 Macrocyclization of (a) heterochiral and (b) homochiral enediyne precursors.

Table 3 GI_{50} values of selected macrocycles on two cell lines

GI_{50}[a] (μM)		
	Cell lines	
Compound	HTC116	HEK293T
3e[1]	18 ± 12	2 ± 1
4g[1]	14 ± 1	3 ± 2
3h[1]	30 ± 1	3 ± 2
3i	≥100	≥100
3m[1]	21 ± 1	3 ± 1
Doxorubicin	0.04 ± 0.004	0.01 ± 0.001

[a] GI_{50}; the concentration that causes 50% growth inhibition. Doxorubicin was tested as a reference compound. The stability of macrolactones in phosphate buffer (pH 7.2) was tested and they were stable for 24 h at 37 °C, and even at 65 °C for an additional 24 h (ESI†).

These preliminary results indicate that some other mechanism, and not the Bergman cycloaromatization is responsible for the observed activity of macrocycles. However, additional chemical and biological studies are needed to elucidate whether cell disruption or interaction with intracellular target is responsible for the antiproliferative effect of macrocycles. The lack of selectivity in this first generation of hybrid enediyne-peptide macrocycles can be due to a small number of recognition elements, as improved target selectivity has been shown to be often achieved by increasing the structural complexity.[23,24] Therefore, we intend to enlarge and further diversify the library of enediyne macrocycles (ring size, stereochemistry, presence and type of tertiary amide group) to better understand the structure–activity relationship and mechanism of action of this class of macrocycles.

Conclusions

In conclusion, we described a novel class of 14-membered macrocycles containing an enediyne (*Z*-3-ene-1,5-diyne) structural unit. A highly modular assembly of various precursors *via* sequential Ugi/Sonogashira reactions allowed the preparation of enediyne embedded macrocycles in most cases as single diastereoisomers. Our findings lay the ground for future efforts directed at the diversification of enediyne macrocycles in terms of ring size, shape, and stereochemistry.

Experimental

General information

Unless otherwise indicated, solvents were used as supplied (analytical or HPLC grade) without further purification. Reagents were used directly as supplied by major chemical suppliers. Flash column chromatography was carried out using silica gel (Merck, 40–63 μm particle size). Analytical thin-layer chromatography was carried out on a Merck Kieselgel 60 F254 0.25 mm precoated aluminium plates. Visualization was carried out under ultra-violet irradiation (254 nm) and by appropriate heating with potassium permanganate solution. RP-HPLC analysis was performed on a Shimadzu 20AT liquid chromatography system equipped with an M20A diode array detector. Separation was performed on a Zorbax RF XDB-C18 column (3,5 μm, 4,6 × 75 mm). NMR spectra were recorded on a Bruker Avance 600 MHz and 300 MHz spectrometers, operating at 150.92 or 75.47 MHz for ^{13}C and 600.13 or 300.13 MHz for ^{1}H nuclei. Chemical shifts are quoted in ppm, and are referenced to the residual non-deuterated solvent peak. Spectra were acquired at 298 K. Infrared spectra were recorded on a Varian UV/vis Cary 4000 spectrometer equipped with an attenuated total reflectance attachment with internal calibration. Absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}). Mass spectrometry measurements were performed on an Agilent 6420 Triple Quad mass spectrometer, operating in positive electrospray ionization (ESI) mode. High-resolution mass spectrometry (HRMS) was performed on a MALDI TOF/TOF 4800 Plus Analyzer operating in reflection mode. Calibrant and analyte spectra were obtained in positive ion mode. Calibration type was internal with the calibrant produced by matrix ionization (monomeric, dimeric and trimeric CHCA), with azithromycin and angiotensin II dissolved in the α-cyano-4-hydroxycinnamic acid matrix in the mass range *m*/*z* 190.0499 to 749.5157 or 1046.5417. Accurately measured spectra were internally calibrated and elemental analysis was performed on the Data Explorer v. 4.9 software with a mass accuracy better than 5 ppm.

General procedure for synthesis of macrocyclic precursors 2. Aldehyde **1**[19] (100 mg, 0.35 mmol) was dissolved in methanol (0.1 M) under argon, and the amino component (2 eq.) was added. After 2 h, carboxylic acid (2 eq.) and isocyanide (2 eq.) were added. The reaction mixture was stirred at room temperature for 24 h. The solvent was concentrated under reduced pressure and the residue dissolved in triethylamine (3 mL) and $PdCl_2(PPh_3)_2$ (3 mol%) was added under argon. After 15 min CuI (3 mol%) and after an additional 5 min prop-2-yn-1-ol (2 eq.) were added. The reaction mixture was stirred for 24 h at

room temperature and quenched with saturated NH_4Cl. The product was extracted with ethyl-acetate and the organic layer was washed with brine and water, dried over Na_2SO_4 and concentrated under reduced pressure and the product was obtained by flash column chromatography. Products were isolated and characterized as a mixture of diastereoisomers, although in some cases the separation of two diastereoisomers was achieved on TLC. However, the separation on a column was incomplete and unsatisfactory, ending with a loss of material. Therefore, both diastereoisomers were collected together during the purification. We also observed that in some cases the ratio of diastereoisomers changed in favour to one of them, but this is solely due to column chromatography purification and not the diastereoselectivity of the reaction.

(2*S*)-*tert*-Butyl 2-(*N*-(1-(*tert*-butylamino)-6-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-1-oxohex-5-yn-2-yl)benzamido)-3-phenylpropanoate (2a). Yield 58%; yellow oil; R_f (d_1) = 0.31; R_f (d_2) = 0.23 (petroleum ether/ethyl acetate = 2 : 1). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.63–8.40 (m, 1H), 7.46–7.28 (m, 6H), 7.22–7.13 (m, 6H), 7.04–6.92 (br s, 2H), 4.60–4.26 (m, 4H), 4.13–3.90 (m, 1H), 3.22–2.93 (m, 2H), 2.75 (br s, 1H), 2.59–2.34 (m, 2H), 1.39–1.34 (m, 18H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 171.3, 169.9, 169.7, 131.7, 131.6, 131.5, 129.9, 129.2, 128.8, 128.4, 127.4, 126.7, 126.35, 126.3, 92.7, 91.8, 83.7, 83.1, 66.8, 66.5, 53.4, 51.2, 37.3, 36.2, 30.9, 29,7, 28.6, 27.8, 16.7. HRMS: $C_{39}H_{45}N_2O_5$ ([M + H]$^+$) found: 621.3335, calculated 621.3328.

(2*S*)-*tert*-Butyl 2-(*N*-(1-(*tert*-butylamino)-6-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-1-oxohex-5-yn-2-yl)-4-nitrobenzamido)-3-phenylpropanoate (2b). Yield 26%; white oil; R_f (d_1) = 0.33; R_f (d_2) = 0.23 (petroleum ether/ethyl acetate = 2 : 1). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.30–7.90 (m, 2H), 7.60–7.32 (m, 3H), 7.25–6.96 (m, 8H), 4.70–4.45 (m, 2H), 4.26–3.92 (m, 2H), 3.32–3.14 (m, 1H), 3.09–2.95 (m, 1H), 2.73 (br s, 1H), 262–2.32 (m, 3H), 1.44–1.33 (m, 18H). ^{13}C NMR (151 MHz, $CDCl_3$): δ = 172.1, 171.2, 170.1, 168.7, 167.7, 148.2, 141.6, 136.9, 132.2, 131.7, 131.3, 131.1, 129.6, 129.3, 128.9, 128.7, 128.6, 128.5, 127.7, 127.6, 127.3, 127.2, 127.1, 126.8, 125.8, 123.7, 120.2, 109.4, 93.2, 92.5, 86.7, 86.5, 85.2, 85.167.4, 64.4, 59.5, 54.7, 54.2, 51.3, 36.3, 35.0, 28.8, 28.7, 28.2, 17.9, 16.2. HRMS: $C_{39}H_{44}N_3O_7$ ([M + H]$^+$) found: 666.3185, calculated 666.3179.

(2*S*)-*tert*-Butyl 2-(*N*-(1-(*tert*-butylamino)-6-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-1-oxohex-5-yn-2-yl)-4-methoxybenzamido)-3-phenylpropanoate (2c). Yield 65%; white oil; R_f (d_1) = 0.25; R_f (d_2) = 0.15 (petroleum ether/ethyl acetate = 2 : 1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.45–7.04 (m, 12H), 6.78 (d, J = 8.5 Hz, 2H), 4.52 (br s, 2H), 4.33 (br s, 3H), 3.79 (br s, 3H), 3.20–3.02 (m, 1H), 2.81–2.63 (m, 1H), 1.68 (br s, 4H), 1.50–1.32 (m, 18H); ^{13}C NMR (75 MHz, CD_3OD): δ = 171.1, 161.8, 160.9, 131.7, 131.6, 131.4, 129.2, 128.8, 128.5, 128.4, 128.3, 128.1, 127.8, 127.4, 126.6, 126.5, 125.8, 113.8, 92.9, 88.1, 69.4, 55.3, 51.3, 51.1, 36.0, 33.1, 29.7, 28.63, 28.57, 27.8, 16.7. HRMS: $C_{40}H_{47}N_2O_6$ ([M + H]$^+$) found: 651.3439, calculated 651.3434.

(2*S*)-*tert*-Butyl 2-(*N*-(1-(*tert*-butylamino)-6-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-1-oxohex-5-yn-2-yl)-4-fluorobenzamido)-3-phenylpropanoate (2d). Yield 69%; colorless oil; R_f (d_1) = 0.66; R_f (d_2) = 0.59; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.70 (s, 0.41H), 8.46 (s, 0.65H), 7.48–6.82 (m, 14H), 4.68–4.27 (m, 4H), 4.06–3.85 (m, 1H), 3.30 (br s, 1H), 2.98 (br s, 1H), 2.78–2.33 (m, 3H), 1.4–1.31 (m, 18H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.0, 169.8, 165.1, 161.7, 132.1, 131.8, 131.7, 131.3, 129.3, 128.8, 128.7, 128.6, 128.4, 127.7, 127.5, 127.4, 126.7, 126.6, 115.7, 115.4, 92.5, 92.2, 84.0, 83.4, 80.5, 67.0, 65.8, 60.4, 51.2, 50.9, 35.8, 29.7, 28.6, 27.8, 16.7 ppm. HRMS: $C_{39}H_{44}FN_2O_5$ ([M + H]$^+$) found: 639.3230, calculated 639.3234.

(2*S*)-*tert*-Butyl 2-(*N*-(1-(*tert*-butylamino)-6-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-1-oxohex-5-yn-2-yl)-4-chlorobenzamido)-3-phenylpropanoate (2e). Yield 69%; yellow oil; R_f (d_1) = 0.46; R_f (d_2) = 0.37 (petroleum ether/ethyl acetate = 2 : 1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.66 (s, 1H), 8.44 (s, 1H), 7.45–7.19 (m, 21H), 7.10–6.88 (m, 5H), 4.75–4.32 (m, 8H), 4.06–3.89 (m, 2H), 3.31–2.35 (m, 8H), 1.46–1.35 (m, 36H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 136.2, 134.5, 132.1, 131.9, 131.8, 131.4, 129.4, 128.9, 128.8, 128.6, 127.9, 127.7, 126.9, 92.6, 84.1, 83.6, 60.5, 51.4, 35.8, 29.8, 28.7, 27.9, 16.8. HRMS: $C_{39}H_{44}ClN_2O_5$ ([M + H]$^+$) found: 655.2940, calculated 655.2939.

(2*S*)-*tert*-Butyl 2-(*N*-(1-(*tert*-butylamino)-6-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-1-oxohex-5-yn-2-yl)acetamido)-3-phenylpropanoate (2f). Yield 70%; yellow oil; R_f (d_1) = 0.43; R_f (d_2) = 0.35 (petroleum ether/ethyl acetate = 2 : 1). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.85–7.81 (m, 1H), 7.44–7.30 (m, 2H), 7.25–7.14 (m, 6H), 7.02–6.95 (m, 1H), 4.46 (br t, 0.5H), 4.08 (t, J = 6.3 Hz, 0.6H), 3.98 (br d, 0.7H), 3.61–3.49 (m, 0.4H), 3.37 (d, J = 7.2 Hz, 1.4H), 3.21–3.08 (m, 0.5H), 2.68–2.20 (m, 4H), 2.17, 2.10 (s, 2H), 2.04, 1.95 (s, 3H), 1.41 (br s, 6H), 1.35, 1.34 (s, 12H). ^{13}C NMR (151 MHz, $CDCl_3$): δ = 170.8, 170.7, 168.6, 168.4, 138.3, 138.1, 132.1, 132.0, 129.8, 129.4, 128.9, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 127.4, 127.2, 127.19, 126.1, 126.1, 100.5, 100.2, 93.0, 92.5, 83.9, 83.7, 82.6, 65.4, 50.9, 50.8, 36.0, 34.9, 29.3, 29.1, 28.2, 28.8, 27.9, 27.5, 22.2, 22.2, 18.0, 17.7, ppm. HRMS: $C_{34}H_{43}N_2O_5$ ([M + H]$^+$) found: 559.3179, calculated 559.3172.

(2*S*)-*tert*-Butyl 2-(2-acetamido-*N*-(1-(*tert*-butylamino)-6-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-1-oxohex-5-yn-2-yl)-3-phenylpropanamido)-3-phenylpropanoate (2g). Yield 44%; yellow oil; R_f (d_1, d_2) = 0.13 (petroleum ether/ethyl acetate = 2 : 1). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.99–7.55 (M, 2h), 7.50–6.90 (m, 15H), 6.17 (d, J = 8.5 Hz, 0.65H), 5.92 (d, J = 8.8 Hz, 0.4H), 5.36–5.08 (m, 1H), 4.73–4.50 (m, 1H), 4.30–4.05 (m, 1H), 3.76–3.45 (m, 1H), 3.24–2.16 (m, 6H), 1.97, 1.87, 1.71 (s, 6H), 1.41–1.16 (m, 18H). ^{13}C NMR (151 MHz, $CDCl_3$): δ = 171.4, 169.3, 169.1, 168.9, 168.7, 167.3, 139.2, 138.6, 138.5, 137.1, 136.1, 133.2, 132.6, 130.2, 130.1, 129.8, 129.6, 129.5, 129.3, 129.2, 128.9, 128.7, 128.6, 128.3, 128.1, 127.7, 127.5, 127.2, 126.9, 126.8, 126.0, 101.1, 93.1, 92.4, 84.5, 83.7, 83.4, 81.4, 77.4, 77.0, 76.6, 60.6, 59.5, 52.2, 51.4, 51.1, 50.6, 49.6, 39.9, 39.0, 36.6, 30.2, 28.6, 28.4, 28.1, 27.9, 27.8, 23.3, 23.1, 17.1, 16.6. HRMS: $C_{43}H_{52}N_3O_6$ ([M + H]$^+$) found: 706.3865, calculated 706.3856.

(2*S*)-*tert*-Butyl 2-(*N*-(1-(cyclohexylamino)-6-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-1-oxohex-5-yn-2-yl)benzamido)-3-phenylpropanoate (2h). Yield 59%; yellow oil; R_f (d_1) = 0.34; R_f (d_2) = 0.26 (petroleum ether/ethyl acetate = 2 : 1). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.97, 8.48 (br s, 1H), 7.42–7.27 (m, 6H), 7.21–7.03

(m, 6H), 7.01–6.82 (m, 2H), 4.65–4.48 (br d, 3H), 4.41–4.26 (m, 1H), 4.10–3.98 (m, 1H), 3.90–3.50 (m, 2H), 3.37–2.88 (m, 3H), 2.78–2.27 (m, 4H), 1.94–1.76 (m, 2H), 1.62 (s, 3H), 1.46 (s, 2H), 1.39 (br s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 171.1, 170.5, 169.5, 169.0, 136.0, 131.7, 131.5, 130.1, 129.8, 128.9, 128.8, 128.6, 128.4, 128.3, 127.7, 127.3, 126.7, 126.6, 126.2, 92.7, 83.4, 81.3, 77.2, 77.0, 76.8, 66.6, 65.1, 63.1, 61.5, 60.4, 51.2, 51.0, 49.2, 48.1, 37.6, 35.5, 32.8, 32.6, 31.9, 29.6, 27.9, 25.6, 25.0, 24.5, 21.0, 17.0, 16.8. HRMS: $C_{41}H_{47}N_2O_5$ ($[M + H]^+$) found: 647.3499, calculated 647.3485.

(2*S*)-*tert*-Butyl 2-(*N*-(1-(cyclohexylamino)-6-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-1-oxohex-5-yn-2-yl)-4-nitrobenzamido)-3-phenylpropanoate (2i). Yield 39%; orange oil; R_f (d_1, d_2) = 0.13 (petroleum ether/ethyl acetate = 2 : 1). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.09 (br s, 0.8H), 7.96 (br d, 1.4H), 7.80 (br s, 0.6H), 7.71 (br d, 1.2H), 7.54 (br d, 1H), 7.46–7.26 (m, 6H), 7.13–6.90 (m, 3H), 4.54 (s, 2H), 4.64, 4.54 (br t, 1H), 4.26 (br s, 1H), 3.76–3.54 (m, 2H), 3.30–3.17 (m, 1H), 2.71–2.51 (m, 1H), 2.44–2.22 (m, 1H), 2.17, 2.04 (s, 2H), 1.98–1.58 (m, 8H), 1.48 (br. s, 9H), 1.41–1.31 (m, 4H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 170.4, 169.5, 167.6, 148.3, 147.8, 141.4, 139.1, 138.5, 132.5, 131.9, 130.1, 129.1, 128.7, 128.1, 127.5, 127.4, 126.9, 123.9, 100.9, 96.9, 92.7, 91.4, 84.7, 83.5, 62.1, 61.4, 49.1, 48.7, 36.8, 36.1, 33.1, 32.8, 29.6, 28.9, 28.1, 27.9, 25.5, 24.9, 24.6, 16.7. HRMS: $C_{41}H_{46}N_3O_7$ ($[M + H]^+$) found: 692.3329, calculated 692.3336.

(2*S*)-*tert*-Butyl 2-(*N*-(1-(cyclohexylamino)-6-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-1-oxohex-5-yn-2-yl)acetamido)-3-phenylpropanoate (2j). Yield 81%; yellow oil; R_f (d_1, d_2) = 0.18 (petroleum ether/ethyl acetate = 2 : 1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.84, 8.69 (br s, 1H), 7.46–7.43 (m, 2H), 7.31–7.28 (m, 4H), 4.58–4.46 (m, 5H), 4.34 (br s, 1H), 3.79–3.60 (m, 1H), 3.52–3.20 (m, 1.7H), 3.05–2.90 (m, 0.4H), 2.05, 2.03, 1.98 (s, 3H), 1.95–0.51 (m, 9H), 1.45, 1.42, 1.36 (s, 9H), 1.23–1.04 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.1, 172.2, 171.6, 171.4, 169.8, 169.5, 138.7, 137.5, 132.1, 131.9, 131.6, 129.4, 128.7, 128.5, 128.5, 128.1, 128.0, 127.8, 127.6, 127.4, 126.7, 126.6, 126.5, 126.1, 125.5, 93.2, 92.9, 91.8, 83.6, 83.3, 83.1, 81.2, 69.7, 65.3, 60.8, 51.4, 51.2, 51.1, 49.1, 48.9, 48.3, 36.1, 32.6, 32.5, 32.2, 30.3, 28.7, 27.9, 27.9, 27.8, 25.5, 24.9, 24.8, 24.7, 23.3, 22.9, 22.4, 18.1, 16.6. HRMS: $C_{36}H_{45}N_2O_5$ ($[M + H]^+$) found: 585.3337, calculated 585.3328.

(2*S*)-*tert*-Butyl 2-((2*S*)-2-acetamido-*N*-(1-(cyclohexylamino)-6-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-1-oxohex-5-yn-2-yl)-3-phenylpropanamido)-3-phenylpropanoate (2k). Yield 12%; orange oil; R_f (d_1, d_2) = 0.08 (petroleum ether/ethyl acetate = 2 : 1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.67–6.96 (m, 15H), 6.60 (d, J = 9.1 Hz, 0.65H), 6.20 (br s, 0.4H), 5.50 (br s, 0.6H), 5.26–5.17 (m, 0.85H), 4.82–4.72 (m, 0.6H), 4.62–4.30 (m, 3H), 4.12–3.92 (m, 1H), 3.72–3.41 (m, 2H), 3.16–2.81 (m, 2H), 2.74–2.10 (m, 6H), 1.97, 1.87 (s, 3H), 1.81–1.53 (m, 7H), 1.34 (s, 9H), 1.18–0.82 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$): δ = 174.3, 170.9, 170.1, 169.7, 168.9, 168.8, 139.0, 137.1, 136.6, 136.3, 131.9, 131.8, 131.6, 129.9, 129.5, 129.4, 128.7, 128.6, 128.3, 128.0, 127.9, 127.7, 127.4, 126.8, 126.6, 126.2, 126.0, 125.7, 93.1, 92.4, 91.5, 84.0, 83.4, 81.8, 80.5, 80.2, 60.1, 51.4, 51.1, 50.8, 48.8, 48.3, 39.9, 38.6, 36.7, 32.9, 32.6, 32.4, 29.7, 27.9, 27.8, 25.5, 24.9, 24.7, 23.1, 22.9, 17.1, 16.2. HRMS: $C_{45}H_{54}N_3O_6$ ($[M + H]^+$) found: 732.4026, calculated 732.4013.

(2*S*)-*tert*-Butyl 2-(*N*-(6-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-1-((2-methoxy-2-oxoethyl)amino)-1-oxohex-5-yn-2-yl)benzamido)-3-phenylpropanoate (2l). Yield 63%; while oil; R_f (d_1, d_2) = 0,26 (petroleum ether/ethyl acetate = 2 : 1). ^{1}H NMR (600 MHz; $CDCl_3$): δ = 7.88–7.68 (m, 1H); 7.51–7.10 (m, 12H); 7.01–6.84 (m, 2H); 4.81–4.15 (m, 2H); 4.09–3.49 (m, 6H); 3.46–2.99 (m, 2H), 2.82–1.97 (m, 4H), 1.63–1.28 (m, 12H). ^{13}C NMR (151 MHz; $CDCl_3$): δ = 172.0, 169.7, 169.2, 137.9, 135.8, 132.1, 129.4, 128.7, 128.4, 128.3, 128.03, 127.6, 127.1, 126.1, 125.9, 83.8, 82.1, 61.9, 60.6, 59.9, 51.7, 40.8, 39.9, 36.3, 35.2, 28.9, 28.6, 27.6, 27.4, 16.4, 13.7. HRMS: $C_{39}H_{43}N_2O_7$ ($[M + H]^+$) found: 651.3064, calculated 651.3070.

(2*S*)-*tert*-Butil 2-(*N*-(6-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-1-oxo-1-((2,4,4-trimethylpentan-2-yl)amino)hex-5-yn-2-yl)benzamido)-3-phenylpropanoate (2m). Yield 55%; white oil; R_f (d_1) = 0.40; R_f (d_2) = 0.29 (petroleum ether/ethyl acetate = 2 : 1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.45 (br d, 1H), 7.49–7.27 (m, 6H), 7.20–6.95 (m, 6H), 4.82–3.90 (m, 6H), 3.47–2.20 (m, 6H), 1.51–1.19 (m, 1H), 0.97 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.7, 169.2, 168.9, 168.9, 168.7, 135.5, 130.4, 130.4, 129.4, 128.9, 128.1, 127.9, 127.9, 126.7, 126.5, 126.2, 125.7, 124.5, 92.5, 92.1, 85.8, 84.5, 79.4, 54.8, 53.8, 53.4, 35.1, 31.1, 27.9, 27.4, 15.9. HRMS: $C_{43}H_{53}N_2O_5$ ($[M + H]^+$) found: 676.3881, calculated 676.3876.

(2*S*)-*tert*-Butyl 2-(*N*-(6-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-1-oxo-1-((2,4,4-trimethylpentan-2-yl)amino)hex-5-yn-2-yl)-4-nitrobenzamido)-3-phenylpropanoate (2n). Yield 28%; yellow oil; R_f (d_1, d_2) = 0.64 (petroleum ether/ethyl acetate = 2 : 1); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.21–8.09 (m, 1.3H), 8.03–7.87 (m, 0.8H), 7.64–7.51 (m, 1H), 7.46–7.17 (m, 9H), 7.13–6.98 (m, 2H), 4.61–3.95 (m, 4H), 3.36–3.11 (m, 1H), 3.06–2.88 (m, 0.6H), 2.80–2.50 (m, 1.7H), 2.44–2.21 (m, 1H), 1.83–1.56 (m, 3H), 1.50–1.40 (m, 10H), 1.34 (s, 5H), 1.02, 0.99 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$): δ = 170.9, 170.7, 169.2, 148.3, 148.0, 141.9, 140.6, 138.9, 136.9, 131.8, 130.9, 129.3, 128.7, 128.4, 127.9, 127.8, 127.6, 127.4, 126.9, 126.8, 125.2, 124.9, 123.7, 123.5, 92.1, 90.7, 84.2, 83.8, 82.6, 81.9, 66.8, 65.8, 63.3, 61.6, 60.3, 56.4, 55.7, 53.6, 51.3, 50.9, 50.3, 37.1, 35.6, 31.7, 31.6, 31.5, 31.4, 29.9, 29.6, 28.8, 28.7, 28.5, 27.9, 27.8, 16.9, 16.8, 14.2. HRMS: $C_{43}H_{52}N_3O_7$ ($[M + H]^+$) found: 722.3821, calculated 722.3805.

(2*S*)-*tert*-Butyl 2-(*N*-(6-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-1-oxo-1-((2,4,4-trimethylpentan-2-yl)amino)hex-5-yn-2-yl)acetamido)-3-phenylpropanoate (2o). Yield 55%; yellow oil; R_f (d_1) = 0.51; R_f (d_2) = 0.41 (petroleum ether/ethyl acetate = 2 : 1); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.44–7.14 (m, 10H), 4.77–4.41 (m, 3H), 3.93–3.79 (m, 0.7H), 3.51–3.21 (m, 1.4H), 3.09–2.90 (m, 0.4H), 2.70–2.30 (m, 2.6H), 2.22–1.93 (m, 4H), 1.99 (br. s, 3H), 1.46 (br. s, 8H), 1.42–1.38 (m, 18H), 1.36 (s, 2H), 1.63 (s, 1H), 1.50–1.28 (m, 16H), 0.97, 0.95 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$): δ = 173.0, 171.3, 171.3, 169.3, 138.8, 137.6, 131.9, 131.8, 131.5, 131.5, 129.4, 129.4, 128.7, 128.5, 128.5, 128.4, 127.9, 127.9, 127.7, 127.6, 127.5, 127.3, 126.7, 126.6, 126.1,

125.6, 93.2, 92.9, 91.89, 83.9, 83.4, 83.2, 82.9, 80.9, 80.2, 66.5, 60.3, 55.7, 55.5, 52.0, 51.3, 51.1, 50.6, 36.9, 35.9, 31.6, 31.5, 31.4, 31.3, 31.3, 30.3, 29.1, 28.9, 28.4, 28.2, 27.9, 27.8, 23.3, 23.0, 22.4, 21.0, 18.4, 16.7, 14.1. HRMS: $C_{38}H_{51}N_2O_5$ ($[M + H]^+$) found: 615.3811, calculated 615.3798.

(2*S*)-*tert*-Butyl 2-((2*S*)-2-acetamido-*N*-(6-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-1-oxo-1-((2,4,4-trimethylpentan-2-yl)amino)hex-5-yn-2-yl)-3-phenylpropanamido)-3-phenylpropanoate (2p). Yield 78%; white oil, R_f (d_1, d_2) = 0.18 (petroleum ether/ethyl acetate = 2 : 1). ^{1}H NMR (600 MHz; $CDCl_3$): δ = 7.87–7.79 (m, 1H), 7.63–7.28 (m, 5H), 7.25–7.08 (m, 8H); 7.03–6.94 (m, 1H); 6.12 (d, J = 8.3 Hz, 0.5H); 5.90 (d, J = 8.9 Hz, 0.5H), 5.15 (br s, 0.65H), 4.65–4.51 (m, 1H), 4.29–4.20 (m, 0.5H), 3.70–3.55 (m, 1H), 3.26–2.24 (m, 8H), 2.17, 2.05 (s, 2H), 1.93 (s, 2H), 1.86, 1.80 (s, 3H), 1.44–1.30 (m, 15H), 1.01, 0.94 (s, 9H). ^{13}C NMR (151 MHz; $CDCl_3$): δ = 170.4, 168.9, 167.5, 139.3, 137.2, 136.5, 136.3, 131.8, 131.7, 131.6, 129.6, 129.5, 129.4, 128.5, 128.4, 128.3, 128.0, 127.9, 127.5, 127.4, 127.0, 126.7, 126.3, 125.9, 125.7, 92.9, 92.2, 91.6, 83.9, 83.6, 83.2, 82.2, 80.3, 60.4, 59.9, 59.6, 56.2, 55.5, 51.9, 51.4, 51.3, 51.1, 39.1, 36.9, 36.7, 31.5, 30.3, 28.8, 28.6, 28.4, 28.1, 27.9, 27.7, 22.9, 16.9, 16.4. HRMS: $C_{47}H_{60}N_3O_6$ ($[M + H]^+$) found: 7624485, calculated 762.4482.

(2*S*)-*tert*-Butyl 2-(*N*-(1-(*tert*-butylamino)-6-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-1-oxohex-5-yn-2-yl)benzamido)-3-methylbutanoate (2q). Yield 84%; yellow oil; R_f (d_1) = 0.37; R_f (d_2) = 0.21 (petroleum ether/ethyl acetate = 2 : 1). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.62 (br s, 1H), 7.52–7.30 (m, 5H), 7.22–7.16 (m, 2H), 4.41–4.29 (m, 2H), 4.06–3.70 (m, 2H), 2.84–2.32 (m, 4H), 1.65 (s, 1H), 1.45 (s, 9H), 1.37, 1.35 (s, 9H), 0.85, 0.81 (br d, 6H). ^{13}C NMR (151 MHz, $CDCl_3$): δ = 170.7, 170.6, 169.9, 169.9, 168.9, 136.1, 131.7, 131.7, 131.6, 131.5, 130.2, 128.5, 127.7, 127.4, 127.2, 126.9, 125.8, 93.3, 91.9, 83.1, 82.6, 80.1, 77.6, 77.2, 77.0, 76.8, 70.7, 69.7, 64.8, 60.4, 51.4, 51.2, 50.9, 29.7, 28.8, 28.7, 28.6, 28.0, 27.9, 19.84, 19.3, 17.1, 14.2. HRMS: $C_{35}H_{45}N_2O_5$ ($[M + H]^+$) found: 573.3321, calculated 573.3328.

(2*S*)-*tert*-Butyl 2-(*N*-(1-(*tert*-butylamino)-6-(2-(3-hydroxyprop-1-yn-1-yl)phenyl)-1-oxohex-5-yn-2-yl)benzamido)-4-methylpentanoate (2r). Yield 65%; yellow oil; R_f (d_1) = 0.43; R_f (d_2) = 0.25 (petroleum ether/ethyl acetate = 2 : 1). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.91, 8.71 (br s, 1H), 7.47–7.30 (m, 7H), 7.22–7.14 (m, 2H), 4.63–4.40 (m, 3H), 4.24 (br d, 1H), 3.87 (br d, 1H), 3.01 (br s, 1H), 2.76–2.71 (m, 1H), 2.54–2.44 (m, 2H), 1.98–1.91 (m, 1H), 1.64 (br s, 1H), 1.37, 1.34 (s, 17H), 0.91 (br s, 3H), 0.91 (d, J = 6.4 Hz, 3H), 0.53 (br d, 3H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 174.6, 171.1, 170.1, 136.1, 131.6, 130.5, 128.6, 127.6, 127.2, 126.7, 126.1, 125.9, 92.9, 92.3, 83.4, 83.2, 82.8, 80.1, 65., 63.90, 60.4, 51.1, 50.9, 38.7, 29.9, 28.7, 28.6, 27.9, 27.8, 24.3, 22.9, 22.1, 21.0, 20.9, 16.8, 14.2. HRMS: $C_{36}H_{47}N_2O_5$ ($[M + H]^+$) found: 587.3478, calculated 587.3485.

General procedure for the synthesis of macrocyclic compounds 3 and 4. Compounds **2** were dissolved in DCM/TFA (9 : 1) and the reaction mixture was stirred until the starting compound disappeared (typically 5 h). The solvent evaporated, the residue was dissolved in MeOH and 2 mL of 2 M sodium hydroxide was added. The reaction mixture was stirred for 24 h, the solvent was evaporated, and the residue portioned between 10% citric acid and ethyl-acetate. The organic layer was washed with brine and water, dried over Na_2SO_4 and concentrated under reduced pressure. The product was dissolved in dichloromethane (0.01M), and PyBOP (6 eq.) and DIPEA (3 eq.) were added. The reaction mixture was stirred for 24 h and the solvent was evaporated. The residue was extracted with ethyl-acetate and saturated NH_4Cl, the organic layer was washed with brine and water, dried over Na_2SO_4 and concentrated under reduced pressure. The product was purified by flash column chromatography. Acyclic enediynes **2** are 1 : 1 mixture of two diastereoisomers. Where individual macrolactone diastereoisomers are separated during purification, the yield is given for each of them, having in mind that theoretical yield for each diastereoisomer is 50%mmol of the corresponding precursor **2**. Where macrolactone is isolated as a mixture of two diastereoisomers, the overall yield is given.

Macrocycle 3a[1]. Yield quant (43 mg), colorless oil, R_f = 0.28 (petroleum ether/ethyl acetate = 2 : 1); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.49 (s, 1H), 7.41 (d, J = 7.6 Hz, 1H), 7.38–7.34 (m, 2H), 7.28–7.23 (m, 3H), 7.23–7.16 (m, 4H), 7.07–6.86 (m, 4H), 5.13 (d, J = 15.1 Hz, 1H), 4.94 (d, J = 15.1 Hz, 1H), 4.73 (dd, J = 9.8 Hz, 3.7 Hz, 1H), 3.86–3.79 (m, 1H), 3.47 (s, 1H), 3.23 (dd, J = 14.9 Hz, 9.9 Hz, 1H), 3.10–2.95 (m, 1H), 2.88–2.75 (m 1H), 2.54–2.43 (m, 2H), 1.40 (br. s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 169.0, 168.8, 135.4, 131.5, 130.4, 129.3, 129.9, 128.0, 127.9, 127.8, 126.7, 126.5, 126.2, 125.7, 92.5, 85.8, 79.4, 66.6, 53.9, 50.5, 34.9, 28.2, 15.8; υ_{max} (neat): 3358, 2950, 1740, 1660, 1650, 1536, 1415, 1310, 1260, 1240, 1195, 1030, 961, 755, 710, 520 cm^{-1}; HRMS: $C_{35}H_{34}N_2O_4$ ($[M + Na^+]$) found 547.2597, calculated 547.2615.

Macrocycle 3a[2]. Yield 35% (15 mg), colorless oil, R_f = 0.25 (petroleum ether/ethyl acetate = 2 : 1); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.42–7.37 (m, 3H), 7.33 (t, J = 7.7 Hz, 2H), 7.28–7.22 (m, 2H), 7.19–7.16 (m, 4H), 6.97–6.90 (m, 3H), 5.17 (d, J = 15.4 Hz, 1H), 5.01 (d, J = 15.4 Hz, 1H), 4.98–4.94 (m, 1H), 4.59 (dd, J = 11.0 Hz, J = 2.8 Hz, 1H), 3.51 (dd, J = 14.5 Hz, 6.8 Hz, 1H), 3.15 (dd, J = 14.5 Hz, 6.8 Hz, 1H), 2.91–2.85 (m, 1H), 2.76 (t, J = 6.8 Hz, 2H), 2.04–1.99 (m, 1H), 1.34 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 172.6, 170.2, 167.8, 136.8, 135.3, 131.2, 130.8, 129.4, 128.9, 128.7, 128.2, 128.0, 127.9, 126.8, 126.2, 125.9, 125.6, 123.9, 93.3, 86.1, 84.8, 80.6, 63.7, 58.6, 53.3, 50.6, 36.5, 28.2, 27.3, 17.4; υ_{max} (neat): 3360, 2950, 1730, 1655, 1652, 1534, 1421, 1312, 1260, 1220, 1195, 1026, 961, 753, 698, 549, 497 cm^{-1}; HRMS: $C_{35}H_{34}N_2O_4$ ($[M + H^+]$) found 547.2575, calculated 547.2597.

Macrocycle 3b. Yield 53% (27 mg); yellow oil; R_f = 0.45 (petroleum ether/ethyl acetate = 2 : 1). Chemical shifts are given for both diastereoisomers. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.11 (br s, 1H), 8.08–8.05 (m, 2H), 7.46–7.35 (m, 2H), 7.33–7.19 (m, 6H), 7.14–7.01 (m, 3H), 5.21 (dd, J = 15.3 Hz, J = 5.1 Hz, 1H), 5.07 (d, J = 15.5 Hz, 0.5H), 4.91 (d, J = 15.2 Hz, 0.5H), 4.66–4.59 (m, 1H), 4.41 (dd, J = 10.4 Hz, J = 2.7 Hz, 0.5H), 3.85 (dd, J = 12.0, J = 3.5 Hz, 0.5H), 3.53–3.42 (m, 1H), 3.35–3.21 (m, 1H), 3.13–2.92 (m, 0.7H), 2.91–2.66 (m, 2H), 2.56–2.44 (m, 1.2H), 1.59 (br s, 1H), 1.44 (br. s, 5H), 1.34 (br. s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.1, 171.2, 170.1, 168.7, 168.0, 148.2,

141.6, 136.9, 132.2, 129.6, 129.3, 128.9, 128.7, 128.6, 128.5, 127.7, 127.6, 127.3, 127.2, 127.2, 127.1, 126.8, 123.7, 120.2, 109.4, 93.2, 92.5, 86.7, 86.5, 85.2, 85.1, 81.3, 80.0, 67.4, 64.4, 59.5, 54.7, 54.2, 51.3, 51.3, 36.3, 35.0, 29.7, 28.7, 28.7, 28.2, 17.9, 16.2; υ_{max} (neat): 3305, 2959, 2955, 1738, 1664, 1526, 1430, 1315, 1303, 1259, 1222, 1172, 852, 751, 701 cm^{-1}; HRMS: $C_{35}H_{33}N_3O_6$ ([M + Na^+]) found 614.2267, calculated 614.2267.

Macrocycle 3c. Yield 40% (50 mg); yellow oil; R_f = 0.48 (petroleum ether/ethyl acetate = 2 : 1). Chemical shifts are given for both diastereoisomers. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.26–8.09 (m, 2H), 7.55–7.25 (m, 5H), 7.21–7.15 (m, 3H), 7.10–76.80 (m, 3H), 6.49, 6.31 (br s, 1H), 5.26–4.68 (m, 3H), 3.95, 3.86, 3.84, 3.81 (s, 3H), 3.61–3.41 (m, 1H), 3.37–2.86 (m, 2H), 2.81–2.47 (m, 2H), 2.16–1.91 (m, 1H), 1.73–1.58 (m, 1H), 1.38, 1.29, 1.08 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 172.6, 171.4, 136.6, 132.6, 131.5, 131.4, 130.6, 130.4, 128.8, 128.5, 128.2, 128.1, 127.9, 127.8, 127.7, 126.9, 126.8, 126.6, 126.3, 126.1, 124.2, 123.8, 120.0, 114.1, 113.3, 113.2, 107.9, 92.6, 92.2, 85.9, 85.2, 85.0, 79.9, 79.7, 61.8, 59.5, 57.9, 55.2, 54.9, 54.8, 52.5 52.1, 51.9, 51.7, 39.4, 38.4, 29.5, 28.2, 28.1, 27.9, 16.1; υ_{max} (neat): 3323, 2930, 2913, 1734, 1671, 1601, 1508, 1359, 1250, 1165, 1093, 975, 754, 698, 496 cm^{-1}; HRMS: $C_{36}H_{36}N_2O_5$ ([M + H^+]) found 577.2717, calculated 577.2702.

Macrocycle 3d^1. Yield 59% (20 mg); colourless oil; R_f = 0.51 (petroleum ether/ethyl acetate = 2 : 1); ^{1}H NMR (300 MHz; $CDCl_3$): δ = 8.45 (s, 1H); 7.43–7.36 (m, 2H); 7.24–7.17 (m, 5H); 7.02–6.98 (m, 2H); 6.94 (d, J = 7.1 Hz, 4H); 5.17 (d, J = 15.2 Hz, 1H); 4.93 (d, J = 15.2 Hz, 1H); 4.68 (dd, J = 10.1 Hz, J = 3.5 Hz, 1H); 3.85–3.79 (m, 1H); 3.51–3.44 (m, 1H); 3.29–3.21 (m, 1H); 2.99–2.80 (m, 2H); 2.55–2.42 (m, 2H); 1.40 (bs, 9H). ^{13}C NMR (151 MHz; $CDCl_3$): δ = 169.3, 169.2, 164.2, 162.5, 136.9, 132.1, 131.9, 130.9, 129.4, 128.5, 128.4, 127.2, 126.9, 126.8, 115.7, 115.5, 92.9, 86.4, 85.6, 79.9, 53.4, 35.3, 28.7, 28.6, 16.3; υ_{max} (neat): 3251, 3057, 2949, 1745, 1670, 1625, 1540, 1430, 1360, 1320, 1225, 1160, 1155, 958, 908, 847, 755, 708, 570 cm^{-1}; HRMS $C_{35}H_{33}FN_2O_4$ ([M + H^+]) found 565.2500, calculated. 565.2503.

Macrocycle 3d^2. Yield 38% (13 mg); colourless oil; R_f = 0.44 (petroleum ether/ethyl acetate = 2 : 1); ^{1}H NMR (300 MHz; $CDCl_3$): δ = 7.42–7.28 (m, 4H); 7.25–7.23 (m, 1H); 7.21–7.12 (m, 5H); 7.02–6.99 (m, 1H); 6.98–6.94 (m, 2H); 6.92 (s, 1H); 5.20 (d, J = 15.5 Hz, 1H); 5.03 (d, J = 15.5 Hz, 1H); 4.94–4.89 (m 1H); 4.55 (dd, J = 10.6 Hz, J = 2.8 Hz, 1H); 3.54–3.47 (m, 1H); 3.21–3.14 (m, 1H); 2.91–2.80 (m, 1H); 2.80–2.72 (m, 2H); 2.05–2.00 (m, 1H); 1.32 (bs, 9H). ^{13}C NMR (75 MHz; $CDCl_3$): δ = 172.5, 170.8, 168.3, 137.2, 131.9, 131.3, 129.2, 128.9, 128.8, 128.6, 128.5, 127.6, 126.9, 115.7, 93.7, 86.7, 85.4, 81.3, 64.5, 59.5, 51.2, 36.9, 28.8, 28.5, 28.1, 18.0; υ_{max} (neat): 3275, 3077, 2993, 1732, 1710, 1675, 1532, 1465, 1350, 1282, 1206, 1145, 965, 886, 736, 712 cm^{-1}; HRMS $C_{35}H_{33}FN_2O_4$ ([M + H^+]) found 565.2500, calculated 565.2503.

Macrocycle 3e^1. Yield 48% (30 mg); colourless oil; R_f = 0.68 (petroleum ether/ethyl acetate = 2 : 1); ^{1}H NMR (300 MHz; $CDCl_3$): δ = 8.42 (s, 1H), 8.06–7.96 (m, 1H), 7.96–7.50 (m, 2H), 7.43–7.36 (m, 3H), 7.22 (d, J = 7.7 Hz, 3H), 7.02 (d, J = 5.9 Hz, 2H), 6.83 (d, J = 7.0 Hz, 2H), 5.16 (d, J = 15.2 Hz, 1H), 4.92 (d, J = 15.2 Hz, 1H), 4.63 (dd, J = 10.2 Hz, J = 3.4 Hz, 1H), 3.84–3.79 (m, 1H), 3.52–3.42 (m, 1H), 3.30–3.22 (m, 1H), 2.98–2.78 (m, 2H), 2.55–2.42 (m, 2H), 1.41 (bs, 7H), 1.35 (s, 1H), 1,26 (s, 1H); ^{13}C NMR (151 MHz; $CDCl_3$): δ = 169.2, 169.1, 143.7, 136.9, 136.0, 134.3, 132.1, 130.9, 129.5, 128.7, 128.5, 128.4, 127.9, 127.7, 127.2, 126.9, 126.8, 124.6, 120.3, 120.2, 108.5, 92.9, 86.4, 85.6, 79.9, 67.4, 67.1, 51.1, 35.3, 28.7, 28.6, 28.4, 16.3; υ_{max} (neat): 3343, 2949, 1724, 1625, 1528, 1421, 1312, 1215, 1086, 1014, 832, 754, 697, 546, 499 cm^{-1}; HRMS: $C_{35}H_{33}ClN_2O_4$ ([M + H^+]) found 581.2192; calculated 581.2207.

Macrocycle 3e^2. Yield 14% (9 mg); colourless oil; R_f = 0.50 (petroleum ether/ethyl acetate = 2 : 1); ^{1}H NMR (300 MHz; $CDCl_3$): δ = 7.42–7.36 (m, 3H), 7.31–7.27 (m, 3H), 7.24–7.19 (m, 4H), 7.05 (d, J = 8.4 Hz, 1H), 6.99–6.96 (m, 2H), 6.90 (s, 1H), 5.20 (d, J = 15.5 Hz, 1H), 5.03 (d, J = 15.5, 1H), 4.90–4.84 (m, 1H), 4.59–4.52 (m, 1H), 3.54–3.47 (m, 1H), 3.22–3.15 (m, 1H), 2.91–2.72 (m, 3H), 2.08–1.97 (m, 1H), 1.42 (d, J = 7.7 Hz, 1H), 1.32 (s, 7H), 1.26 (s, 1H); ^{13}C NMR (75 MHz; $CDCl_3$): δ = 172.2, 168.1, 137.1, 136.0, 134.0, 132.0, 131.7, 131.3, 130.4, 129.5, 129.4, 129.2, 128.9, 128.8, 128.7, 128.5, 128.2, 128.1, 127.6, 127.5, 127.3, 126.9, 126.6, 125.5, 124.7, 124.3, 93.6, 86.6, 85.3, 81.2, 64.3, 59.4, 51.1, 36.7, 30.3, 29.7, 28.7, 27.9, 17.9; υ_{max} (neat): 3314, 2982, 1735, 1667, 1543, 1485, 1367, 1224, 1102, 1025, 872, 759, 688, 567 cm^{-1}; HRMS: $C_{35}H_{33}ClN_2O_4$ ([M + H^+]) found 581.2205; calculated 581.2207.

Macrocycle 3f^1. Yield 52% (32 mg); R_f = 0.41 (petroleum ether/ethyl acetate = 2 : 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.38–7.32 (m, 3H), 7.31–7.27 (m, 2H), 7.23–7.20 (m, 4H), 6.34 (s, 1H), 5.10 (d, J = 15.6 Hz, 1H), 4.76 (d, J = 15.6 Hz, 1H), 3.84 (t, J = 7.2 Hz, 1H), 3.58 (dd, J = 10.1 Hz, J = 4.8 Hz, 1H), 3.11–3.04 (m, 1H), 2.97–2.90 (1H), 2.74–2.46 (m, 3H), 2.10–1.99 (m, 2H), 1.73–1.54 (m, 2H), 1.29 (br. s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 173.1, 171.9, 137.1, 131.9, 131.1, 129.0, 128.6, 128.2, 127.4, 126.9, 125.9, 124.3, 93.1, 86.4, 85.5, 80.4, 59.9, 58.4, 52.6, 38.9, 30.0, 28.6, 16.6; υ_{max} (neat): 3323, 2965, 2912, 1737, 1665, 1516, 1441, 1359, 1257, 1217, 1152, 1028, 968, 913, 757, 692, 653 cm^{-1}; HRMS: $C_{30}H_{32}N_2O_4$ ([M + Na^+]) calculated 507.2260; found 507.2253.

Macrocycle 4g^1. Yield 45% (18 mg); white oil; R_f = 0.33 (petroleum ether/ethyl acetate = 2 : 1); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.36 (t, J = 6.7 Hz, 2H), 7.30 (t, J = 7.4 Hz, 2H), 7.24–7.20 (m, 5H), 6.35 (s, 1H), 5.10 (d, J = 15.5 Hz, 1H), 4.76 (d, J = 15.5 Hz, 1H), 3.84 (t, J = 7.2 Hz, 1H), 3.59–3.56 (m, 1H), 3.09–3.05 (m, 1H), 2.96–2.92 (m, 1H), 2.63–2.52 (m, 2H), 2.09–1.99 (m, 2H), 1.68–1.62 (m, 1H), 1.29 (br. s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 173.3, 172.1, 137.2, 132.0, 131.3, 129.2, 128.7, 128.3, 127.5, 126.9, 126.0, 124.4, 93.3, 86.6, 85.7, 80.6, 60.1, 58.5, 52.8, 39.1, 30.2, 28.8, 16.7; υ_{max} (neat): 3331, 2975, 2893, 1767, 1614, 1538, 1452, 1323, 1275, 1238, 1173, 1103, 978, 964, 766, 697 cm^{-1}; HRMS: $C_{28}H_{30}N_2O_3$ ([M + H^+]) calculated 443.2335; found 443.2336.

Macrocycle 3h. Yield 43% (60 mg); white oil; one diastereoisomer was separated from the mixture during the column chromatography (26 mg), while the rest was isolated as a mixture.

Macrocycle 3h^1. 26 mg; white oil; R_f = 0.38 (petroleum ether/ethyl acetate = 2 : 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.53 (s,

1H), 7.44–7.35 (m, 3H), 7.34–7.08 (m, 7H), 7.07–6.80 (m, 4H), 5.07 (dd, J = 36.6 Hz, 15.2 Hz, 2H), 4.81 (d, J = 7.3 Hz, 1H), 3.92 (d, J = 7.6 Hz, 1H), 3.77 (s, 1H), 3.45 (d, J = 17.1 Hz, 1H), 3.28 (dd, J = 15.0 Hz, 10.8 Hz, 1H), 3.11–2.74 (m, 2H), 2.55–2.36 (m, 2H), 1.99–1.86 (m, 1H), 1.70–1.54 (m, 4H), 1.44–1.13 ppm (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.5, 169.6, 169.5, 136.5, 135.9, 131.9, 130.9, 130.0, 129.3, 128.5, 128.3, 127.2, 127.1, 126.7, 126.5, 123.9, 92.9, 86.4, 85.7, 79.9, 66.9, 66.1, 54.5, 47.9, 35.1, 32.9, 32.1, 28.4, 25.6, 24.6, 24.3, 16.3. υ_{max} (neat): 3277, 2929, 2855, 1742, 1662, 1623, 1533, 1430, 1316, 1223, 1175, 753, 698, 661.

Macrocycle 3h. Chemical shifts are given for both diastereoisomers. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.42–7.38 (m, 7H), 7.34–7.33 (m, 3H), 7.32 (s, 1H), 7.30–7.28 (m, 2H), 7.24–7.21 (m, 4H), 7.18–7.17 (m, 7H), 7.03–7.01 (m, 1H), 6.91–6.90 (m, 3H), 5.22 (d, J = 15.4 Hz, 2H), 5.15–5.10 (m, 1H), 5.02–4.98 (m, 3H), 4.83–4.78 (m, 1H), 4.73–4.70 (m, 1H), 3.96–3.86 (m, 1H), 3.76–3.71 (m, 2H), 3.49 (dd, J = 14.8 Hz, J = 6.0 Hz, 2H), 3.45–3.37 (m, 2H), 3.32–3.24 (m, 1H), 3.19 (dd, J = 14.7 Hz, J = 7.9 Hz, 2H), 3.07–2.98 (m, 1H), 2.90–2.84 (m, 2H), 2.75–2.72 (m, 2H), 2.48–2.44 (m, 1H), 2.10–2.04 (m, 2H), 1.95–1.89 (m, 3H), 1.60–1.51 (m, 6H), 1.37–1.31 (m, 4H), 1.21–1.16 (m, 5H); ^{13}C NMR (151 MHz, CDCl3): δ = 174.4, 173.4, 170.7, 169.4 168.3, 137.0, 135.6, 132.1, 131.5, 131.3, 131.1, 130.9, 129.9, 129.5, 129.3, 129.1, 128.8, 128.6, 128.4, 128.3, 127.3, 127.2, 126.7, 126.1, 126.0, 124.5, 94.0, 86.6, 85.3, 81.2, 63.9, 62.3, 59.2, 58.3, 54.4, 53.0, 48.0, 36.3, 32.9, 32.7, 32.6, 32.4, 32.1, 27.9, 25.6, 25.4, 24.6, 24.3, 16.6, 16.3; υ_{max} (neat): 3323, 2908, 2882, 1768, 1632, 1602, 1523, 1412, 1352, 1278, 1122, 1138, 1002, 993, 981, 754, 646, 599 cm^{-1}; HRMS: $C_{37}H_{36}N_2O_4$ ([M + H +]) calculated 573.2753, found 573.2750.

Macrocycle 3i. Yield 44% (28 mg); yellow oil; R_f = 0.57 (petroleum ether/ethyl acetate = 2 : 1). Chemical shiftsa are given for both diastereoisomers. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.33–8.28 (m, 1H), 8.13–8.09 (m, 2H), 7.42–7.37 (m, 2H), 7.32 7.22 (m, 6H), 7.15–6.87 (m, 3H), 5.31–5.15 (m, 1H), 5.09–4.91 (m, 1H), 4.76–4.67 (m, 0.8H), 4.49 (dd, J = 10.8 Hz, J = 3.1 Hz, 0.55H), 3.97–3.73 (m, 1.6H), 3.54–3.22 (m, 2H), 2.89–2.62 (m, 1.8H), 2.54–2.39 (m, 1H), 1.98–1.12 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.2, 171.5, 170.2, 168.9, 168.8, 167.7, 148.4, 148.3, 141.6, 141.5, 136.8, 136.5, 132.1, 131.5, 131.3, 131.1, 129.4, 129.2, 128.8, 128.6, 128.5, 127.7, 127.6, 127.5, 127.3, 127.2, 127.1, 125.8, 124.5, 123.7, 123.6, 93.5, 92.5, 86.7, 86.5, 85.4, 85.1, 81.3, 80.1, 67.4, 66.4, 64.2, 58.7, 54.8, 54.2, 48.3, 48.1, 35.7, 34.8, 32.9, 32.7, 32.3, 28.4, 28.2, 25.6, 25.5, 24.6, 24.4, 17.9, 16.3; υ_{max} (neat): 3318, 2935, 2849, 1739, 1654, 1521, 1430, 1342, 1312, 1235, 1225, 1173, 1014, 852, 755, 701 cm^{-1}; HRMS: $C_{37}H_{35}N_3O_6$ ([M + Na$^+$]) calculated 640.2424, found 640.2446.

Macrocycle 3j[1]. Yield 40% (25 mg); white oil; R_f = 0.25 (petroleum ether/ethyl acetate = 2 : 1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.09 (br s, 1H), 7.42–7.32 (m, 3H), 7.25–7.19 (m, 6H), 5.08–4.97 (m, 2H), 4.67 (dd, J = 10.3 Hz, J = 3.5 Hz, 1H), 3.84–3.69 (m, 2H), 3.63–3.54 (m, 1H), 3.33–3.20 (m, 1H), 3.30–2.92 (m, 1H), 2.80–2.69 (m, 1H), 2.46–2.22 (m, 2H), 1.82 (br. s, 3H), 1.71–1.63 (m, 2H), 1.40–1.12 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.4, 173.2, 169.7, 169.2, 137.2, 131.8, 130.8, 129.1, 128.6, 128.4, 127.2, 126.9, 86.5, 85.7, 54.4, 47.9, 32.9, 32.3, 28.6, 25.6, 24.6, 24.5, 23.6, 16.3; υ_{max} (neat): 3307, 2926, 2896, 1745, 1660, 1621, 1530, 1437, 1370, 1316, 1259, 1178, 1153, 1029, 983, 972, 754, 698, 654, 599, 523, 489 cm^{-1}; HRMS: $C_{32}H_{34}N_2O_4$ ([M + Na$^+$]) calculated 533.2416; found 533.2390.

Macrocycle 4k[1]. Yield 60% (6 mg); colorless oil; R_f = 0.18 (petroleum ether/ethyl acetate = 2 : 1). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.38–7.33 (m, 2H), 7.30 (t, J = 7.3 Hz, 2H), 7.25–7.18 (m, 5H), 6.34 (d, J = 8.0 Hz, 1H), 5.11 (d, J = 15.5 Hz, 1H), 4.76 (d, J = 15.5 Hz, 1H), 3.84 (t, J = 7.1 Hz, 1H), 3.78–3.71 (m, 1H), 3.64 (dd, J = 9.9 Hz, J = 4.8 Hz, 1H), 3.08–3.04 (m, 1H), 2.96–2.92 (m, 1H), 2.63–2.60 (m, 1H), 2.56–2.51 (m, 1H), 2.04–2.00 (m, 1H), 1.83 (s, 2H), 1.76–1.70 (m, 1H), 1.68–1.52 (m, 3H), 1.38–1.21 (m, 3H), 1.17–1.05 ppm (m, 3H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 173.2, 171.6, 136.9, 131.8, 131.1, 129.1, 128.6, 128.2, 127.4, 126.9, 125.9, 124.3, 93.1, 86.5, 85.5, 80.4, 60.0, 58.0, 52.7, 47.8, 39.2, 32.7, 29.9, 25.5, 24.6, 16.7; υ_{max} (neat): 3328, 2935, 2890, 1773, 1628, 1598, 1522, 1421, 1365, 1305, 1259, 1124, 1029, 979, 903, 754, 692, 655, 615, 492 cm^{-1}; HRMS: $C_{30}H_{32}N_2O_3$ ([M + H$^+$]) calculated 469.2491, found 469.2482.

Macrocycle 3m[1]. Yield 53% (28 mg); yellow oil; R_f = 0.60 (petroleum ether/ethyl acetate = 2 : 1); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.38 (s, 1H), 7.42–7.36 (m, 3H), 7.29 (t, J = 7.8 Hz, 2H), 7.25–7.19 (m, 5H), 7.07–6.94 (m, 4H), 5.13 (d, J = 15.1 Hz, 1H), 4.89 (d, J = 15.1Hz, 1H), 4.74 (dd, J = 9.0 Hz, J = 4.6 Hz, 1H), 3.83–3.80 (m, 1H), 3.50 (s, 1H), 3.25–3.21 (m, 1H), 3.06 (s, 1H), 2.82 (s, 1H), 2.57–2.46 (m, 2H), 1.73–1.71 (m, 1H), 1.62–1.59 (m, 1H), 1.50 (br. s, 3H), 1.47 (br. s, 3H), 1.02 (br. s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 168.9, 135.5, 131.6, 130.4, 129.4, 128.9, 128.1, 127.9, 127.8, 126.7, 126.5, 126.2, 125.7, 85.8, 79.4, 54.9, 53.4, 35.1, 31.1, 27.9, 27.4, 15.9; υ_{max} (neat): 3297, 2981, 2865, 1762, 1693, 1614, 1543, 1478, 1315, 1289, 1218, 1192, 1117, 987, 972, 852, 766, 665, 528 cm^{-1}; HRMS: $C_{39}H_{42}N_2O_4$ ([M + H$^+$]) calculated 603.3223, found 603.3212.

Macrocycle 3m[2]. Yield 40% (16 mg); yellow oil; R_f = 0.41 (petroleum ether/ethyl acetate = 2 : 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.46–7.27 (m, 6H), 7.23–7.15 (m, 6H), 7.04, (br s, 1H), 6.95–6.85 (m, 2H), 5.25 (d, J = 15.5 Hz, 1H), 5.01–4.87 (m, 2H), 4.56–4.51 (m, 1H), 3.51 (dd, J = 14.4 Hz, J = 7.0 Hz, 1H), 3.18 (dd, J = 14.4 Hz, J = 6.6 Hz, 1H), 2.98–2.68 (m, 3H), 2.06–1.93 (m, 2H), 1.39 (d, J = 9.9 Hz, 6H), 0.96 (br. s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.1, 169.6, 169.4, 136.1, 131.7, 131.6, 131.5, 129.9, 129.1, 128.5, 128.4, 127.7, 127.4, 126.6, 126.4, 92.6, 83.7, 81.3, 80.4, 66.4, 65.7, 63.6, 61.4, 56.1, 55.5, 51.2, 51.0, 50.3, 37.3, 35.9, 31.5, 31.4, 29.6, 29.0, 28.8, 28.4, 27.8, 17.1, 16.8; υ_{max} (neat): 3323, 2975, 1746, 1682, 1612, 1567, 1492, 1457, 1312, 1298, 1235, 1195, 978, 865, 742, 701, 698, 573, 523, 494 cm^{-1}; HRMS: $C_{39}H_{42}N_2O_4$ ([M + H$^+$]) calculated 603.3223, found 603.3230.

Macrocycle 3n. Yield 35% (19 mg) colorless oil. Chemical shifts are given for both diastereoisomers. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.12–8.09 (m, 2H), 7.48–7.35 (m, 2H), 7.30–7.15 (m,

6H), 7.10–6.98 (m, 3H), 5.30 (d, J = 14.9 Hz, 0.5H), 5.20 (d, J = 14.9 Hz, 0.5H), 5.00, 4.88 (d, J = 15.4 Hz, 1H), 4.69 (br s, 1H), 4.58 (d, J = 8.5 Hz, 0.4H), 4.43 (d, J = 7.9 Hz, 0.6H), 3.85 (dd, J = 11.8 Hz, J = 3.9 Hz, 1H), 3.52–3.46 (m, 1H), 3.34–3.22 (m, 1H), 3.03 (br s, 1H), 2.84–2.78 (b, 2H), 2.54–2.47 (m, 2H), 2.04–1.97 (m, 1H), 1.57–1.52 (m, 6H), 1.40 (d, J = 14.9 Hz, 2H), 1.04, 0.96 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 171.0, 170.4, 168.8, 168.3, 167.3, 148.3, 131.1, 129.5, 129.2, 128.8, 128.7, 128.5, 127.7, 127.5, 127.7, 127.5, 127.1, 125.1, 123.8, 123.7, 120.4, 93.3, 86.5, 85.2, 85.0, 81.4, 59.7, 55.7, 55.3, 54.6, 51.9, 36.4, 31.6, 31.5, 29.1, 28.9, 28.5, 28.1, 27.7, 17.9, 16.4; υ_{max} (neat): 3283, 2946, 1738, 1667, 1628, 1523, 1480, 1434, 1344, 1312, 1222, 1173, 959, 852, 754, 698, 565, 542, 494 cm^{-1}; HRMS: $C_{39}H_{41}N_3O_6$ ([M + H$^+$]) calculated 648.3074, found 648.3065.

Macrocycle 3o[1]. Yield 32% (14 mg); colorless oil; R_f = 0.46 (petroleum ether/ethyl acetate = 2 : 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.38–7.28 (m, 5H), 7.24–7.21 (m, 3H), 7.20–7.18 (m, 1H), 6.47 (s, 1H), 5.08 (d, J = 15.5 Hz, 1H), 4.77 (d, J = 15.5 Hz, 1H), 3.85 (t, J = 7.1 Hz, 1H), 3.59 (dd, J = 10.3 Hz, J = 4.6 Hz, 1H), 3.07 (dd, J = 13.7 Hz, J = 6.7 Hz, 1H), 2.92 (dd, J = 13.6 Hz, J = 7.5 Hz, 1H), 2.64–2.57 (m, 2H), 2.06–1.89 (m, 2H), 1.66–1.58 (m, 4H), 1.36 (d, J = 12.1 Hz, 7H), 0.93 ppm (br. s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.2, 171.5, 136.9, 131.9, 130.9, 129.0, 128.6, 128.1, 127.3, 126.9, 125.9, 124.2, 93.2, 86.4, 85.5, 80.4, 59.7, 58.4, 52.6, 52.5, 39.1, 31.6, 31.5, 29.6, 28.8, 28.4, 16.7; υ_{max} (neat): 3284, 2942, 1722, 1648, 1510, 1474, 1440, 1364, 1304, 1220, 1149, 1012, 975, 832, 760, 723, 694, 657, 648, 463 cm^{-1}; HRMS: $C_{34}H_{40}N_2O_4$ ([M + H$^+$]) calculated 563.2886, found 563.2880.

Macrocycle 4p[1]. Yield 30% (15 mg); colorless oil; R_f = 0.50 (petroleum ether/ethyl acetate = 2 : 1); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.37–7.33 (m, 2H), 7.30 (t, J = 7.4 Hz, 2H), 7.25–7.21 (m, 2H), 7.21–7.18 (m, 3H), 6.47 (br. s, 1H), 5.07 (d, J = 15.5 Hz, 1H), 4.77 (d, J = 15.5 Hz, 1H), 3.85 (t, J = 7.1 Hz, 1H), 3.59 (dd, J = 10.3 Hz, J = 4.6 Hz, 1H), 3.08–3.05 (m, 1H), 2.94–2.91 (m, 1H), 2.63–2.58 (m, 2H), 2.03–1.97 (m, 1H), 1.68–1.58 (m, 4H), 1.38 (br. s, 3H), 1.34 (br. s, 3H), 0.93 (br. s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 173.2, 171.5, 136.9, 131.9, 130.9, 129.0, 128.6, 128.1, 127.9, 127.8, 126.9, 126.0, 124.6, 124.2, 93.2, 86.4, 85.5, 80.4, 59.7, 58.4, 52.6, 52.5, 39.1, 31.5, 31.4, 29.6, 28.8, 28.4, 16.4; υ_{max} (neat): 3311, 2945, 1738, 1667, 1514, 1477, 1443, 1363, 1222, 15, 42, 992, 948, 757, 700, 560, 452 cm^{-1}; HRMS: $C_{32}H_{38}N_2O_3$ ([M + H$^+$]) calculated 499.2961; found 499.2950.

Conflicts of interest

There are no conflicts to declare.

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