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Synthesis of Variously Functionalized Azabicycloalkane Scaffolds by Domino Metathesis Reactions

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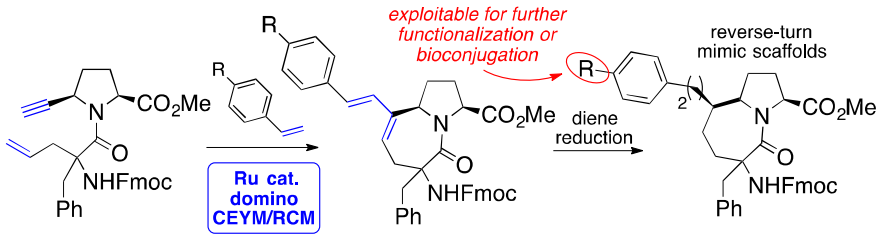
Abstract: 7,5-fused azabicycloalkane scaffolds, carrying a quaternary stereocenter at C3 position of the lactam ring, can act as effective reverse-turn mimics and have proved to be useful intermediates for the preparation of Arg-Gly-Asp (RGD)-based cyclopentapeptides (cRGD) with nanomolar activity as $\alpha_v\beta_3/\alpha_v\beta_5$ integrin antagonists. Here we report the synthesis of new azabicycloalkane scaffolds endowed at C6 position with a *p*-substituted phenethyl side chains, which could be exploited to obtain cRGD-based bioconjugates that may find promising applications in anticancer therapy. By performing a domino Cross Enyne Metathesis/Ring Closing Metathesis (CEYM/RCM) in the presence of styrene derivatives, followed by catalytic hydrogenation of the diene system, we easily converted a dipeptide precursor into the desired C6-functionalized azabicycloalkane scaffolds. The presence of a suitably protected *p*-amino group on the styrene moiety could be exploited, after deprotection, either to directly conjugate a bioactive compound or to introduce a suitable spacer between the cRGD unit and the bioactive compound.



Introduction

One of the main issues concerning anticancer therapy is the selectivity of the active species with respect to the target. Most chemotherapeutics, albeit being potent cytotoxic, cytostatic or antiangiogenic agents, often exhibit low selectivity for their respective targets causing severe side effects, which highly reduce the efficacy and sustainability of the therapy. In light of this, significant efforts are being pursued in order to develop targeted delivery systems[1].

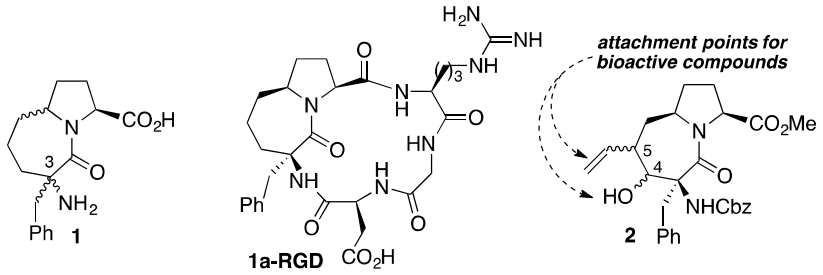
By exploiting the presence of a homing device, it is possible to deliver cytotoxic drugs selectively to the target, allowing a reduction of the active ingredient doses and side effects. In this field, integrin antagonists may play a pivotal role because of their overexpression on many tumour cell types and on neoangiogenic vessels with respect to their healthy counterparts. Through binding with extracellular matrix (ECM) components, integrins regulate a wide range of processes like cell adhesion, proliferation and differentiation[2]. In particular integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ were found to be interesting targets because of their key role in metastatic spreading and neoangiogenesis[3], which are the most harmful processes for diffusion and progression in colon cancer, melanoma, breast cancer, and brain cancers, such as glioblastoma (GBM)[4].

The common recognition motif for the interaction of these receptors with their endogenous ligands is the tripeptide sequence Arg-Gly-Asp (RGD)[5]. Cilengitide, a cyclic RGD-based pentapeptide, was the first $\alpha_v\beta_3/\alpha_v\beta_5$ integrin antagonist to be tested in clinical trials for the treatment of GBM[6]. Even though its use as an anticancer therapeutic has been recently discontinued due to the failure in phase III clinical trials[7], the important biological roles played by $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin subtypes and the low toxicity of RGD-based antagonists stimulated the development of new high affinity ligands[8] suitable for conjugation with bioactive compounds. These RGD-based bioconjugates may find promising application for targeted drug delivery, theranostic, and general cancer-cell labeling[9].

Our continuous efforts in this field have been focused on the synthesis of azabicycloalkane scaffolds, which can act as effective reverse-turn mimetics in such a way that a properly embedded RGD sequence could be forced to adopt conformations suitable for receptor binding[10].

In particular 7,5-fused bicyclic systems of the type **1** (Figure 1), carrying a quaternary stereocenter at C3 position of the lactam ring, proved to be useful intermediates for the synthesis of RGD-based cyclopentapeptides such as **1a-RGD**, a nanomolar inhibitor of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin receptors[11]. Moreover, **1a-RGD** is one of the few RGD-like antagonists capable of inhibiting cell migration and attachment, and inducing anoikis in glioblastoma cell lines[12]. The relevant biological activity shown by **1a-RGD** prompted us to develop a synthetic protocol that would allow further functionalization of the bicyclolactam scaffold, in order to obtain bioconjugates that may find application for targeted drug delivery systems, as theranostic agents or for cancer cell labeling.

Figure 1.

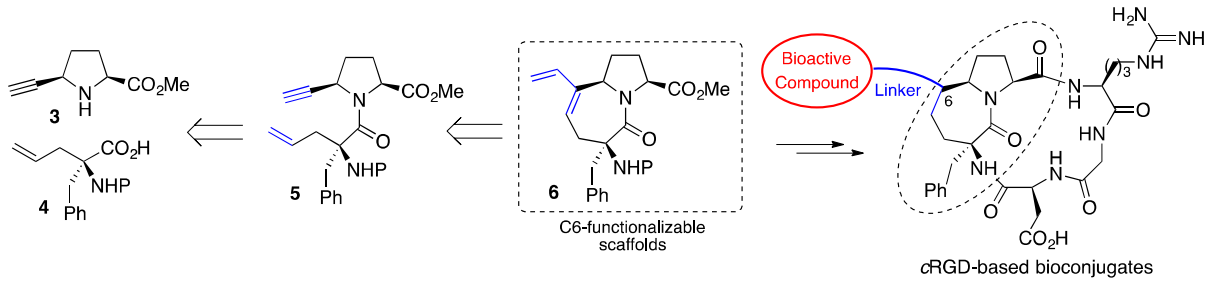


In a recent work we reported a synthetic strategy that allowed the attainment of 7,5-fused azabicycloalkane scaffolds of type **2** endowed with an exocyclic double bond. The C5 vinyl moiety turned out to be highly reactive towards Heck-coupling reactions, thus demonstrating its usefulness and versatility for the introduction of suitable linkers to which bioactive compounds could be conjugated[13].

As a part of our research project, we were interested in extending our studies towards the synthesis of C6-functionalizable azabicycloalkane scaffolds (Scheme 1) in order to broaden the scope of our library of structures. Indeed, the preparation of a set of further functionalizable dipeptide mimics could be useful to gain detailed information about the conformation specifically required by the peptide to effectively bind and activate the receptor[14]. Literature precedents by Lubell et al. reported

the synthesis of a series of C6-functionalized azabicyclo[5.3.0]alkanone amino acids exploiting a diastereoselective S_N1 displacement of an iodide□precursor[15]. This synthetic approach, which allowed also the introduction of a hydroxyl and an azido group at C6, has as its main limitation the possibility of introducing only *O*- or *N*-linked side-chains. To the best of our knowledge, no examples of installation of *C*-linked side-chains at 6-position onto azabicyclo[5.3.0]alkanone amino acids have been previously reported. These considerations, together with the need of a more rapid synthetic protocol to prepare "easy-to-functionalize" azabicycloalkane scaffolds, led us to consider the synthetic approach depicted in Scheme 1, exploiting as a key reaction a ring closing enyne metathesis (RCEYM). The intramolecular bond reorganization involved in the reaction would allow to convert in one step the dipeptide **5** into the corresponding cyclic diene **6**, endowed with an exocyclic vinyl moiety at C6 position of the seven-membered lactam ring. As highlighted by the retrosynthetic analysis, the dipeptide **5** could be obtained by a condensation reaction between the α-allyl quaternary α-amino acid **4** and the 5-ethynylproline derivative **3**. After a regioselective functionalization of the exocyclic double bond with an appropriate linker, followed by reduction of the diene system, it would be possible to use the functionalized azabicycloalkane scaffolds as templates for the attainment of cRGD-based bioconjugates.

Scheme 1. RCEYM approach for the preparation of C6-functionalized azabicycloalkane scaffolds

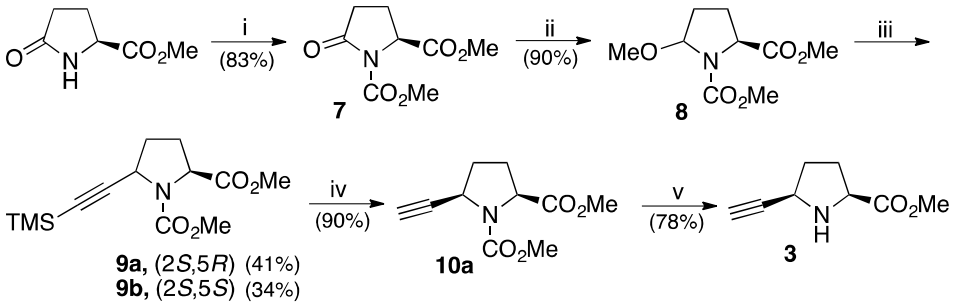


Results and discussion

The synthesis of the first building block, namely the (2*S*,5*R*)-5-ethynylpyrrolidine-2-methyl ester **3**, was performed following the synthetic route depicted in Scheme 2. The readily available methyl (*S*)-pyroglutamate was *N*-protected as methyl carbamate[16] and converted into the corresponding methoxylated derivative **8** by reduction with Super-Hydride ($LiBEt_3H$) followed by treatment with trimethyl orthoformate in the presence of pyridinium p-toluenesulfonate (PPTS). This optimized procedure allowed us to obtain the desired methoxylated carbamate **8** in yields higher than those previously reported in literature[17].

The Lewis acid-catalyzed bistrimethylsilylacetylene addition to the *N*-acyliminium intermediate derived from **8** afforded a *cis/trans* mixture of alkynylated derivatives **9**[18]. The chromatography purification of the diastereomeric mixture allowed the isolation of the *cis* stereoisomer **9a** which, after removal of the trimethylsilyl group with tetrabutylammonium fluoride and subsequent deprotection of the methyl carbamate with iodotrimethylsilane, gave the desired 5-ethynylproline methyl ester **3** in good overall yield.

Scheme 2. Synthesis of the 5-ethynylproline methyl ester



Reagents and conditions: (i) NaHMDS, $ClCO_2CH_3$, THF, -78 °C then 0 °C; (ii) a) $LiEt_3BH$, THF, -78 °C; b) $CH(OCH_3)_3$, PPTS, MeOH; (iii) bistrimethylsilylacetylene, $SnCl_4$, $AlCl_3$, CH_2Cl_2, -45 °C; (iv) TBAF, THF, -20°C then 0 °C; (v) TMSI, CH_2Cl_2, 65 °C

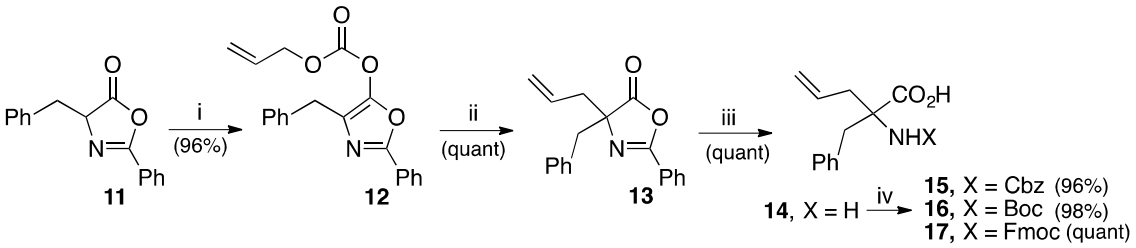
On the basis of our long term interest on the study of the reactivity of 5-alkyl-oxazol-5-(4H)-ones[19] to establish quaternary stereocenters in α,α-disubstituted α-amino acids, we designed the synthesis

of the *N*-protected α-allyl-α-benzyl amino acid **4** (Scheme 1) starting from the corresponding 2-phenyl-4-benzyl-oxazol-5-(4H)-one through an electrophilic allylation of the C4 position of the oxazolone. In addition, this procedure should have allowed an enantioselective version by only marginal modification of the synthetic protocol. It follows that a reaction promoted by a modifiable catalyst would meet this need. Several endeavors in this direction have been reported in the literature, *e.g.* Trost et al. performed the direct installation of an allyl moiety at the C-4 position of the oxazolone ring by exploiting an asymmetric intermolecular allylic alkylation reaction in the presence of chiral palladium catalysts[20]. The highest levels of ee's (≥90%) were obtained with substituted allyl derivatives, while the use of allyl acetate gave the desired 4-alkyl-4-allyl oxazolones with ee's not higher than 40%. A different strategy made use of a diazaphosphorane (BEMP) as a base to deprotonate various oxazolones and subsequent allylation of the resulting enolates with allyl bromide to give racemic quaternary allyl oxazolones[21]. The same products were enantioselectively obtained using *Cinchona*-derived dimeric ammonium salts (80% ee)[22], or chiral tetraaminophosphonium salts (91% ee)[23] as phase-transfer organocatalysts.

Among the various methods able to introduce an allyl group on the oxazolone C-4 stereocenter, we opted for a protocol based on a Tsuji decarboxylative allylation[24]. Four main considerations directed our efforts towards this choice: a) the catalytic nature of the process; b) the possibility to exploit readily accessible and cheap starting materials; c) the opportunity of rapidly obtaining the racemic quaternary amino acid in order to test the feasibility and the "robustness" of our synthetic strategy; d) the perspective to devise, as a future goal, an enantioselective variant of this transformation by the use of chiral Pd catalysts. It is worth mentioning that even though a catalytic enantioselective Tsuji allylation of simple enolizable carbonyl derivatives was reported by Stoltz et al.[25], this reaction has remained essentially unexplored on oxazol-5-(4H)-ones. To the best of our knowledge only 4-allyl-4-methyl-oxazol-5-one was prepared in a very low 2% ee employing (*S*)-*t*-Bu-phosphinooxazoline as chiral ligand[26].

Racemic phenylalanine-derived azlactone **11** was easily converted to the allyl enol carbonate **12** by formation of the corresponding enolate and subsequent treatment with allyl chloroformate (Scheme 3). For the key Tsuji allylation step, we opted for a catalytic system previously utilized by us in the study of asymmetric allylic alkylation reaction for the synthesis of quaternary α-D-*C*-mannosyl-(*S*)-amino acids[19a]. Adding a Pd/diphosphine 1:4 complex, prepared by mixing in a separate flask $Pd_2(dba)_3$ with 1,2-bis(diphenylphosphino)ethane (dppe), to a solution of **12**, we obtained the desired 4-allyl-oxazolone **13** in quantitative yields. The subsequent opening of the oxazolone ring with trifluoroacetic acid afforded the free quaternary amino acid **14**, which was converted into the corresponding *N*-protected derivatives **15**-**17**. Notably, this protocol was successfully applied to the gram-scale synthesis of the zwitterionic amino acid **14** without any decrease in the overall yield.

Scheme 3. Synthesis of the *N*-protected quaternary amino acids **15**-**17**



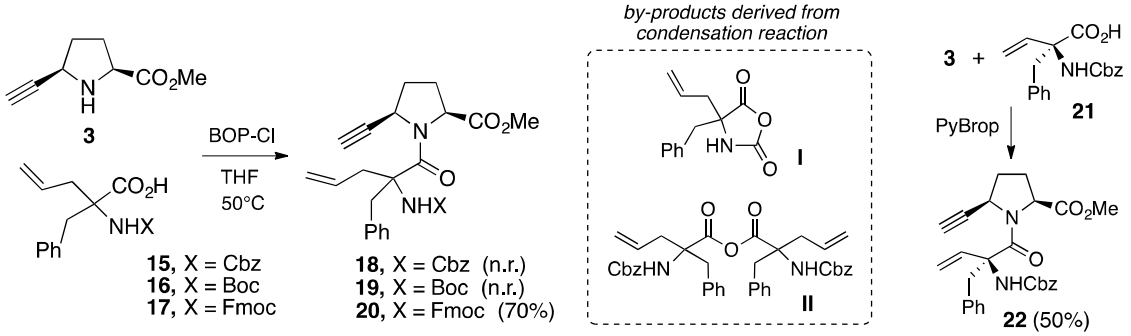
Reagents and conditions: (i) allyl chloroformate, Et_3N, THF, 0 °C; (ii) $Pd_2(dba)_3$*$CHCl_3$, 1,2-bis(diphenylphosphino)ethane (dppe), THF; (iii) TFA, 100 °C; (iv) compound **15**: $(Cbz)_2O$, CH_3CN; compound **16**: $(Boc)_2O$, THF; compound **17**: Fmoc-Cl, THF/H_2O.

The subsequent condensation reaction turned out to be troublesome. To the best of our knowledge no examples of condensation between 5-alkynyl proline esters and quaternary *N*-protected α-amino acids have been previously reported in literature, whereas *N*-Boc-α-cyclopentyl glicine and a *N*-Boc-valine were shown to couple in good yield under standard conditions[27]. Extending the search to 5-substituted proline derivatives, apart from our works on 5-allyl proline[10a, 13], no examples of condensation with quaternary amino acids were known, while tertiary amino acids carrying an allyl or a benzyl moiety at Cα were shown to couple both with 5-vinyl and 5-allyl proline esters[28].

Following our previous experience on similar substrates[13], the first experiments were carried out by performing a condensation reaction between (2*S*,5*R*)-5-ethynylpyrrolidine-2-methylester **3** and N-Cbz-α-allyl-α-benzyl amino acid **15** in the presence of PyBrop as condensing agent (Scheme 4). Despite the screening of various solvents [CH_2Cl_2, tetrahydrofuran (THF), *N,N*-dimethylformamide (DMF)], the application of conventional and microwave-assisted heating, and attempts to force the reaction conditions by increasing the temperature (from room temperature to 100 °C), and prolonging the reaction time (up to 48 hours), it has never been possible to isolate the desired dipeptide **18**. We recovered only unreacted starting material along with variable amounts (8–40%) of by-products ***I*** and ***II***. The *N*-carboxyanhydride ***I*** was likely formed by nucleophilic attack of the carbamate carbonyl oxygen onto the activated carboxylic group of **15** followed by water addition on the intermediate imminium ion and final elimination of benzyl alcohol, while the symmetric anhydride ***II*** results from self-condensation of the quaternary amino acid. These by-products turned out to be stable towards flash chromatography on silica gel, and fully unreactive in our attempts to separately condense them with 5-ethynyl proline **3**.

To overcome these drawbacks, we performed an extensive screen of condensing agents, such as *N*,*N*-dicyclohexylcarbodiimide (DCC) alone or in the presence of *N*-hydroxysuccinimide (NHS), isobutyl chloroformate (IBCF), 1-hydroxybenzotriazole (HOBt), 1-hydroxy-7-azabenzotriazole (HOAt), 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU), bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl), and (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU). Unfortunately, the outcome of the reaction remained unchanged, since only DCC afforded traces (< 4%) of a product whose 1H-NMR, albeit complicated by the presence of conformers, was in agreement with the structure of dipeptide **18**.

Scheme 4. Condensation reaction



In order to understand whether the difficulties encountered in the condensation reaction were ascribable to the quaternary amino acid **15** or to the 5-alkynylproline **3**, the latter derivative was reacted with enantiopure (*S*)-*N*-Cbz-α-vinyl phenylalanine **21**[29]. Applying our standard reaction condition, *i.e.* the use of PyBrop in CH_2Cl_2 at rt, the reaction proceeded smoothly, allowing the isolation of the dipeptide **22** in unoptimized 50% yield. This result indicates that probably the presence of the allyl moiety could make the amino acid **15** less prone to condensation by shielding the carbonyl face from attack of the *N*-nucleophile. By reasoning that different protecting groups on the amine function could also affect the conformational properties of the quaternary amino acid, we synthesized the *N*-(*tert*-butoxycarbonyl)- and *N*-(fluorenyl-9-methoxycarbonyl)-protected derivatives **16** and **17**.

Although the *N*-Boc derivative **16** was shown to be unreactive towards the condensation with 5-alkynyl proline **3**, giving only the *N*-carboxyanhydride side product ***I***, the first attempt to react the *N*-Fmoc-protected amino acid **17** allowed the isolation of the desired dipeptide **20** with an encouraging 42% yield. By carrying out a study of the reaction parameters similar to that previously reported, we eventually found experimental conditions that allowed to improve the yield of the condensation up to 70% by the use of BOP-Cl as condensing agent and a reaction temperature of 50 °C.

The following key reaction, *i.e.* the ring closing enyne metathesis of dipeptide **20** turned out to be a further obstacle to overcome (Scheme 5). An extensive experimentation was carried out by the

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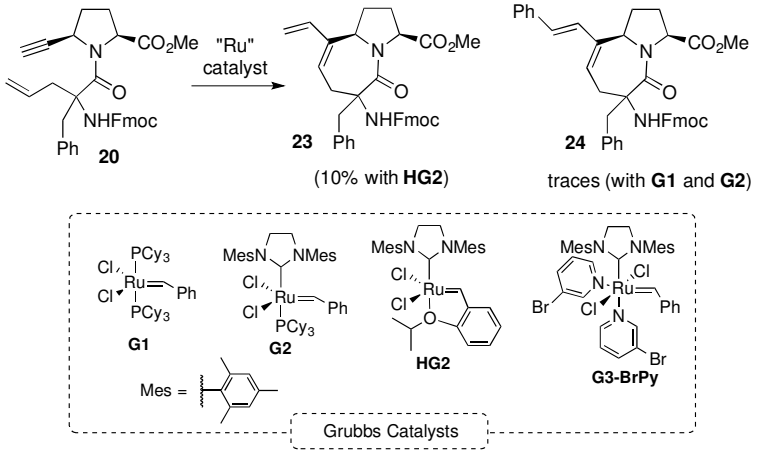
screening of the Grubbs catalysts highlighted in Scheme 5, varying reaction parameters such as catalyst loading (5–30%), temperature (from rt to 120 °C), heating mode (conventional or MW-assisted), solvent, and concentration (0.01–0.1M). Moreover, in order to preserve the catalyst activity, especially when heating the reactions in high-boiling aprotic solvents (dichloroethane, toluene), a slow addition of the catalyst to the reaction mixture by the use of a syringe pump was tested. Unfortunately, we have never been able to isolate the desired cyclic diene **23** in yields higher than 10%, achieved only by the use of the phosphine-free Hoveyda-Grubbs catalyst **HG2** in refluxing toluene.

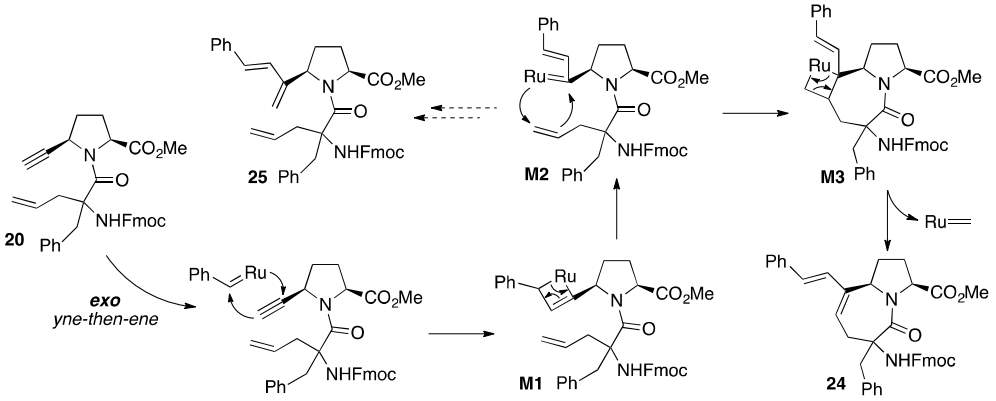
Scheme 5. RCEYM experimentation



The use of third-generation catalyst **G3-BrPy**, reported to be a fast metathesis initiator[30], resulted in the complete recovery of unreacted starting material, whilst **G1** and **G2** afforded only traces of aryl-functionalized diene **24**. The isolation of this side-product led us to reason about a plausible mechanism leading to its formation. Though the diene **24** could formally be obtained by a cross metathesis reaction of the desired scaffold **23** with the benzylidene residue of **G1** and **G2** Grubbs catalysts, the low reactivity showed by the dipeptide **20** in the RCEYM step suggested us an alternative synthetic pathway (Scheme 6). We speculated that the side-product **24** could also be generated by an initial conversion of the dipeptide **20** into the more reactive triene **M2** through an

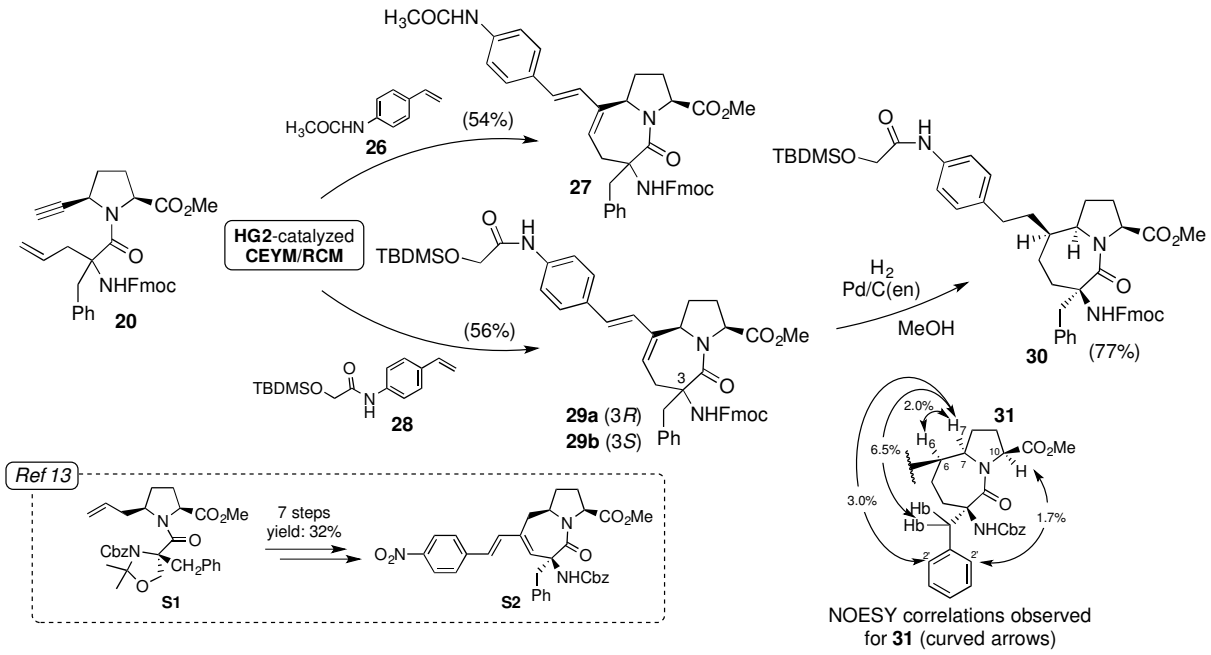
"*exo*" cross enyne metathesis (CEYM) reaction[31] between the proline triple bond and the benzylidene moiety of the catalyst. The hypothesized pathway was in agreement with the results reported by Lee and co-workers in the tandem CM (cross metathesis)/RCM reaction of alkynyl silyloxy-tethered enynes[32]. They observed that, employing sterically hindered mono-, di-, and trisubstituted alkenes, the initial CM reaction was shown to occur on the alkyne moiety. This literature precedent could be an indication that the allyl group in dipeptide **20** is sterically hindered, plausibly by some conformational effect. On the other hand, one can not exclude that the conformation of the peptide bond in **20** could turn from the usually more stable s-trans to s-cis after the addition of the Ru-carbene on the double bond, thus inhibiting or slowing down the following ene-yne metathesis and favoring the alternative yne-ene mechanism. The subsequent RCM of the intermediate **M2**, probably more prone to cyclize than the starting dipeptide **20**, could allow the attainment of **24**. In order to get some insight into this hypothesis, we reacted **20** in the presence of 10% Hoveyda-Grubbs catalyst **HG2** and a stoichiometric amount of styrene in order to maximize the formation of the benzylidene Ru catalyst as the active species of the catalytic cycle. It was indeed fruitful that this control experiment was performed, as the TLC monitoring of the reaction, after few minutes, showed the formation of the aryl-functionalized derivative **24**, which was isolated in 42% yield. The reaction produced also a small amount of diene **23** (<10%), unreacted starting material (15%), and 1,2-diphenylethene (12%), which arises from homocoupling of styrene, while the expected triene **25** was not isolated, thus supporting our speculation that once formed, it immediately reacts to give the RCM product **24**.

Scheme 6. Hypothesized mechanism for the formation of compound **24**



In the light of these findings we next tried to extend the scope of the reaction to *p*-functionalized styrene derivatives. If successful, this could pave the way to a direct preparation of seven-membered lactam rings endowed with variously substituted aromatic linkers that could be conjugated with differently functionalized compounds. As a proof of concept we repeated the same procedure with 4-vinylaniline derivatives, which can be regarded as models of "versatile linkers". Indeed, an opportunely protected *p*-amino group on the newly introduced aromatic moiety could be exploited, after deprotection, both to directly conjugate a bioactive compound or to introduce a suitable spacer between the RGD-based unit and the bioactive compound. Alternatively, the aromatic amine could be easily converted into an azide and submitted to a Huisgen azide-alkyne 1,3-dipolar cycloaddition[33]. We initially decided to explore the feasibility of the domino CEYM/RCM protocol in the presence of *N*-(4-vinylphenyl)acetamide as a test compound. Applying our optimized reaction conditions, *i.e.* slow addition of the **HG2** catalyst (0.05 eq.) to a solution of dipeptide **20** in the presence of stoichiometric amount of the styrene derivative **26**, we isolated the desired functionalized azabicycloalkane scaffold **27** in 54% yield (Scheme 7). This result is particularly remarkable, since the preparation of our previously reported 5-functionalized scaffold **S2**[13], differing substantially only for the position of the diene system, required 7 synthetic linear steps, and was obtained in 32% overall yield starting from the corresponding dipeptide precursor **S1**[34].

Scheme 7. Domino CEYM/RCM protocol for the preparation of functionalized azabicycloalkane scaffolds



Eventually, in order to explore the feasibility of introducing a spacer between the RGD-based azabicycloalkane scaffolds and the bioactive compounds, the experimentation was extended to the use of the *O*-protected 2-hydroxy-*N*-(4-vinylphenyl)acetamide derivative **28**. This choice was mainly dictated by the prospect of modulating the length of the spacer and, at the same time, changing the nature of the functional group acting as a handle for further synthetic manipulations. Reaction of 4-vinylaniline with *O*-TBS-α-hydroxy acetyl chloride gave the *N*-acetyl derivative **28**, which reacted smoothly with dipeptide **20** using the same experimental conditions as before giving the desired scaffolds **29**[35] in almost unchanged yields. In order to study the reactivity of the diene system towards the reduction reaction required for the attainment of the corresponding saturated bicyclolactam scaffolds, compound **29a** was submitted to catalytic hydrogenation. Given the high synthetic value of **29a**, to avoid the known undesiderable deprotection of the *O*-TBDMS protecting group in the presence of Pd/C[36], we performed the reaction using Pd/C poisoned with ethylenediamine (Pd/C(en)). This Pd/C–ethylenediamine complex, which was successfully

employed in the chemoselective reduction of various reducible functionalities leaving intact the TBDMS group[37], allowed the isolation of compound **30**, as a single stereoisomer, with 77% yield. The configurational assignment of the newly created C6 stereocenter was carried out on the *N*-Cbz derivative **31**, which was synthesized, exclusively for analytical purpose, in order to get an easier analysis of NOESY spectrum. The magnetization transfer between H_7 and H_b and H_7 and $H_{2'}$ allowed to verify the absolute *R*-configuration at the ring fusion carbon (Scheme 7). In a similar way, the NOE between H_{10} and $H_{2'}$ confirmed the *S* absolute configuration at C10. Diagnostic for the absolute configuration at C6 is the presence of an NOE between H_6 and H_7 (see supporting information for details). This data was in agreement with the results obtained by Lubell et al. on similar azabicycloalkane scaffolds[15]. Indeed, they observed that a magnetization transfer between vicinal protons only occurred when the vicinal protons were oriented towards the same side of the seven-membered lactam ring.

Computational studies

Computational studies designed to investigate the ability of the C6-functionalized azabicycloalkane scaffolds to adopt reverse-turn conformations were performed on the N-acetyl-N'-methylamide dipeptide analogues **32a** and **32b**, differing for the absolute configuration at C6 (Supporting Information, Figure S4). Both compounds were characterized by the presence of capping groups on the N- and C-termini suitable for the definition of the various turns and H-bond parameters. The preferred conformations of each stereoisomeric scaffold were compared to the structures of the parent 7,5-fused azabicyclolactam derivative **33**.

The quantitative characterization of the turn propensity of the functionalized azabicycloalkane scaffolds showed that these systems are more effective as reverse-turn mimetics than as β- or γ-turn mimics, in agreement with the results obtained for the unsubstituted bicyclic lactam **33**. In fact, the percentages of conformers with a torsion angle β (absolute value) of less than 60° are within the range 90-97%, whereas the percentages of conformers forming intramolecular hydrogen bonds

(stabilizing either a γ-turn or a β-turn) are significantly lower (0-25% for γ-turn, 0-2% for β-turn, see Table S2). The analysis of the $C\alpha_i$-$C\alpha_{i+3}$ distance (dα) confirms that the reverse-turn mimetic scaffolds induce rather 'open' turns. Accordingly, the lowest energy conformer of **33**, **32a** and **32b** features an open turn, which induces the peptide backbone to reverse direction (Figure S5).

Conclusions

We have reported a new synthetic approach to obtain 7,5-fused 2-oxo-1-azabicycloalkane scaffolds characterized by the presence of a *p*-substituted phenethyl side-chain at C6 position of the seven-membered lactam ring. The synthetic sequence is based on a domino CEYM/RCM reaction that allowed the one-pot conversion of the dipeptide **20** into the corresponding C6-functionalized bicyclic systems **27** and **29** in 54-56% overall yield. As a proof of concept, compound **29a**, endowed with a further functionalizable side-chain, was submitted to catalytic hydrogenation and converted to the saturated azabicyclolalkane derivative **30**. Computational studies proved that the functionalization of the seven-membered lactam ring at C6 position does not alter the conformational properties of the scaffolds. Thus the newly synthesized azabicycloalkane scaffolds can be viewed as useful templates for the synthesis of *c*RGD-based bioconjugates that may find promising applications for targeted drug delivery, theranostic or cancer cell labelling.

Experimental Section

General remarks

All chemicals were of reagent grade and were used without further purification. Solvents were purified according to the guidelines in Purification of Laboratory Chemicals[38]. All solvents were freshly distilled from the appropriate drying agent. THF, and toluene were distilled from sodium/benzophenone ketyl; TEA and DCM from CaH_2. Reactions requiring anhydrous conditions were performed under N_2. Yields were calculated for compounds purified by flash chromatography and judged homogeneous by thin-layer chromatography, NMR, and mass spectrometry. Thin layer

chromatography was performed on Kieselgel 60 F_{254} (Merck) glass Plate eluting with solvents indicated, visualized by a 254 nm UV lamp, and stained with aqueous ceric molybdate solution or iodine and a solution of 4,4'-methylenebis-*N,N*-dimethylaniline, ninhydrin, and KI in an aqueous ethanolic solution of AcOH. Flash chromatography was performed on Merck Kieselgel 60 (230–400 mesh). Optical rotations $[\alpha]_D$ were measured in a cell of 5 cm path length and 1 mL capacity with a Jasco DIP-1000 polarimeter. Infrared spectra were recorded on a Perkin–Elmer ATR-FTIR 1600 series spectrometer using neat samples. Mass spectra were acquired with a Thermo Finnigan Q Exactive (API- HESI) FT Orbitrap mass spectrometer. Glassware for all reactions was oven-dried at 110 °C and cooled in a desiccator, or flame-dried and cooled under inert atmosphere prior to use. Liquid reagents and solvents were introduced by oven-dried syringes through septa-sealed flasks under an inert atmosphere.

NMR spectroscopic methods

Nuclear magnetic resonance spectra were acquired using a Bruker Avance 400 MHz spectrometer equipped with Bruker's TopSpin 1.3 software package and a Bruker Avance III HD 500 MHz spectrometer equipped with Bruker's TopSpin 3.5 (patch level 7) and provided with a high-sensitivity 5mm TCI cryoprobe. The abbreviatons s, d, t, q, br s, and m stand for the resonance multiplicities singlet, doublet, triplet, quartet, broad singlet, and multiplet, respectively. In the peak listing of 13C spectra abbreviations s and t refer to zero and two protons attached to the carbons, as determined by DEPT-135 experiments. Phase-sensitive 2D-NOESY experiments were performed at 298K by using noesygpph pulse program from the Bruker library (mixing time of 0.5s or 0.8s). Sample temperatures were controlled with the variable-temperature unit of the instrument.

Synthesis of (2S)-dimethyl 5-oxopyrrolidine-1,2-dicarboxylate (7)

(*S*)-methyl 5-oxopyrrolidine-2-carboxylate (4.00 g, 28.0 mmol) was solubilized in dry THF (70.0 mL, 0.40 M) under nitrogen atmosphere and the reaction mixture was cooled at -78 °C. Methyl chloroformate (2.38 mL, 30.8 mmol) and a 1 M solution of LiHMDS in THF (30.8 mL, 30.8 mmol) were added in three portions at 40 minutes intervals. Formation of the product was monitored by TLC analysis, R_f = 0.58 (AcOEt/MeOH 90:10). After the last addition, the mixture was stirred for 15 minutes at -78 °C and then was warmed to 0 °C. Afterward 56 mL of a 10% HCl aqueous solution were added at 0 °C and the mixture was allowed to warm to room temperature. The organic solvent was evaporated under reduced pressure, 84 mL of 10% HCl solution were added and the aqueous phase was extracted three times with AcOEt. The organic phases were collected, dried with anhydrous Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The crude mixture was purified by flash chromatography (AcOEt/hexane, 70:30) to obtain pure compound **7** as a yellow oil (4.67 g, 83 %). *R*f = 0.27; $[\alpha]_D^{22}$ –37.0 (c = 0.5, $CDCl_3$); IR (neat, cm-1) 2957, 1796, 1716, 1200, 1179, 1047, 1032; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.11 (dddd, *J* = 2.6, 3.2, 9.4, 13.3 Hz, 1H), 2.39 (dddd, *J* = 9.3, 9.4, 10.6, 13.3 Hz, 1H), 2.54 (ddd, *J* = 3.2, 9.3, 17.6 Hz, 1H), 2.68 (ddd, *J* = 9.4, 10.6, 17.6 Hz, 1H), 3.81 (s, 3H), 3.89 (s, 3H), 4.72 (dd, *J* = 2.6, 9.4 Hz, 1H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 22.2 (t), 31.5 (t), 53.2, 54.3, 59.0, 152.3 (s), 171.9 (s), 173.1 (s); HRMS (ESI) calculated for $C_8H_{12}NO_5$ $[M+H]^+$ 202.0710, found 202.0709 (Δ = 0.7 ppm).

Synthesis of dimethyl (2*S*)-5-methoxypyrrolidine-1,2-dicarboxylate (8)

Compound **7** (6.43 g, 32.0 mmol) was solubilized in dry THF (80.0 mL, 0.40 M) under nitrogen atmosphere and the reaction mixture was cooled at -78 °C. A 1 M solution of $Li(Et)_3BH$ in THF (35.2 mL, 35.2 mmol) was added dropwise. Formation of the product was monitored by TLC analysis, R_f = 0.25 (Toluene/AcOEt 60:40). After 10 minutes the reaction was warmed to 0 °C and 45 mL of a $NaHCO_3$ saturated aqueous solution and 7.80 mL of a 30% wt H_2O_2 aqueous solution were added. Afterwards the mixture was allowed to warm to room temperature and was stirred for

30 minutes. The organic solvent was evaporated under reduced pressure, the residue was recovered with a NaCl saturated solution and was extracted three times with AcOEt. The organic phases were collected, dried with anhydrous Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure to obtain the product as a colorless oil without further purification.

The crude mixture (5.54 g, 27.3 mmol) was dissolved in dry MeOH (90.9 mL, 0.30 M) under nitrogen atmosphere and added with trimethyl orthoformate (22.4 mL, 205 mmol) and Pyridinium p-toluenesulfonate (PPTS) (6.87 g, 27.3 mmol). Formation of the product was monitored by TLC analysis, R_f = 0.35 (hexane/AcOEt 60:40). After reaction completion the solvent was evaporated under reduced pressure, the residue was recovered with a $NaHCO_3$ saturated aqueous solution and was extracted three times with Et_2O. The organic phases were collected, dried with anhydrous Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The crude product **8** was reacted in the next step without further purification. (5.62 g, 81 % over two steps). *R*f = 0.35.

Synthesis of (2*S*)-dimethyl 5-((trimethylsilyl)ethynyl)pyrrolidine-1,2-dicarboxylate (9)

Compound **8** (4.18 g, 19.2 mmol) and bis(trimethylsilyl)acetylene (6.56g, 38.5 mmol) were solubilized in dry DCM (96.2 mL, 0.20 M) under nitrogen atmosphere and the reaction mixture was cooled at -45 °C. A 1M solution of $SnCl_4$ in DCM (25.0 mL, 25.0 mmol) and $AlCl_3$ (3.60 g, 26.9 mmol) were added. The reaction mixture was allowed to warm gradually to room temperature. Formation of the products was monitored by TLC analysis, R_f = 0.35, R_f = 0.26 (hexane/AcOEt 70:30). After 12 hours the mixture was slowly added to a $NaHCO_3$ saturated aqueous solution (128 mL). Afterwards 64 mL of a 1 M HCl solution were added and the aqueous phase was extracted three times with AcOEt. The organic phases were collected, dried with anhydrous Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The crude mixture was purified by flash chromatography (hexane/AcOEt, 70:30) to obtain diastereoisomers **9**.

Diastereoisomer 9a (2S,5R): Colorless oil (2.24 g, 41%). R_f = 0.26 (AcOEt/hexane 70:30); $[\alpha]_D^{22}$ +22.4 (c = 1.0, $CDCl_3$); IR (neat, cm-1) 2956, 2900, 2174, 1741, 1704, 1195, 1171, 1038; ^{1}H NMR

(dmso-d_6, 90 °C, 400 MHz) δ 0.15 (s, 9H), 1.93 (m, 1H), 2.03 (m, 1H), 2.16 (m, 1H), 2.27 (m, 1H), 3.63 (s, 3H), 3.67 (s, 3H), 4.30 (app t, *J* = 7.2 Hz, 1H), 4.57 (dd, *J* = 3.4, 7.3 Hz, 1H); $^{13}C\{^1H\}$ NMR (dmso-d_6, 90 °C, 100 MHz) δ 0.8 (3C), 29.6 (t), 33.4 (t), 50.5, 52.5, 53.0, 60.2, 87.4 (s), 107.1 (s), 154.9 (s), 172.7 (s); HRMS (ESI) calculated for $C_{13}H_{21}NNaO_4Si$ $[M+Na]^+$ 306.1132, found 306.1130 (Δ = 0.8 ppm). **Diastereoisomer 9b (2S,5S):** Colorless oil (1.85 g, 34%). R_f = 0.35 (AcOEt/hexane 70:30); $[\alpha]_D^{22}$ –41.5 (c = 1.0, $CDCl_3$); IR (neat, cm-1) 2956, 2900, 2171, 1748, 1706, 1192, 1173, 1069; ^1H NMR (dmso-d_6, 90 °C, 400 MHz) δ 0.15 (s, 9H), 1.92 (m, 1H), 2.01 (m, 1H), 2.17 (m, 1H), 2.38 (m, 1H), 3.63 (s, 3H), 3.66 (s, 3H), 4.35 (dd, *J* = 1.5, 8.9 Hz, 1H), 4.62 (dd, *J* = 1.4, 7.9 Hz, 1H); $^{13}C\{^1H\}$ NMR (dmso-d_6, 90 °C, 100 MHz) δ; 0.7, 29.4 (t), 32.4 (t), 50.2, 52.7, 53.0, 59.6, 87.2 (s), 107.1 (s), 154.8 (s), 172.9 (s); HRMS (ESI) calculated for $C_{13}H_{21}NNaO_4Si$ $[M+Na]^+$ 306.1132, found 306.1129 (Δ = 0.9 ppm).

Synthesis of (2*S*,5*R*)-dimethyl 5-ethynylpyrrolidine-1,2-dicarboxylate 10a

Compound **9a** (1.50 g, 5.29 mmol) was solubilized in dry THF (88.2 mL, 0.06 M) under nitrogen atmosphere and the reaction mixture was cooled at -20 °C. A 1 M solution of tetrabutylammonium fluoride in THF (5.82 mL, 5.82 mmol) was added and the reaction mixture was warmed to 0 °C in 20 minutes. Formation of the product was monitored by TLC analysis, R_f = 0.41 (hexane/AcOEt 50:50). After 30 minutes 132 mL of an NH_4Cl saturated aqueous solution were added and the mixture was extracted three times with Et_2O. The organic phases were collected, dried with anhydrous Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The crude mixture was purified by flash chromatography (hexane/AcOEt, 60:40) to obtain pure compound **10a** as a colorless oil (1.01 g, 90 %). *R*f = 0.35 (AcOEt/hexane 60:40); $[\alpha]_D^{22}$ +9.3 (c = 1.0, $CDCl_3$); IR (neat, cm-1) 3240, 2955, 2919, 2850, 2117, 1736, 1689, 1189, 1120, 1038; ^1H NMR (dmso-d_6, 90 °C, 400 MHz) δ 1.92–2.08 (m, 2H), 2.15–2.34 (m, 2H), 2.98 (s, 1H, partially masked by H_2O signal), 3.63 (s, 3H), 3.68 (s, 3H), 4.3 (app t, *J* = 6.8 Hz, 1H), 4.57 (m, 1H); $^{13}C\{^1H\}$ NMR (dmso-

d_6, 90 °C, 100 MHz) δ 29.7 (t), 33.1 (t), 49.7, 52.5, 53.1, 60.3, 73.5, 84.5 (s), 154.9 (s), 172.8 (s); HRMS (ESI) calculated for $C_{10}H_{13}NNaO_4$ $[M+Na]^+$ 234.0737, found 234.0736 (Δ = 0.4 ppm).

Synthesis of (2*S*,5*R*)-methyl 5-ethynylpyrrolidine-2-carboxylate (3)

Compound **10a** (1.00 g, 4.73 mmol) was solubilized in dry DCM (4.73 mL, 1.00 M) under nitrogen atmosphere. A solution of iodotrimethylsilane (TMSI) in DCM (14.2 mL, 5.68 mmol) was added and the mixture was heated to 65 °C. Formation of the product was monitored by TLC analysis, R_f = 0.30 (hexane/AcOEt 50:50). After 7 hours the solvent was evaporated under reduced pressure and crude mixture was purified by flash chromatography (hexane/AcOEt, 50:50) to obtain pure compound **3** as a yellow oil (543 mg, 75 %). *R*f = 0.30; $[\alpha]_D^{22}$ +16.0 (c = 1.0, $CDCl_3$); IR (neat, cm-1) 3274, 2952, 2114, 1733, 1621, 1211, 1171, 1076; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.89 (m, 1H), 2.06–2.25 (m, 3H), 2.35 (d, *J* = 2.1 Hz, 1H), 3.10 (s, 1H, exchanges with D_2O), 3.77 (s, 3H), 3.86 (app t, *J* = 6.8 Hz, 1H), 3.94 (m, 1H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 29.8 (t), 33.5 (t), 49.5, 52.8, 59.9, 72.1, 84.9 (s), 175.0 (s); HRMS (ESI) calculated for $C_8H_{12}NO_2$ $[M+H]^+$ 154.0863, found 154.0862 (Δ = 0.3 ppm).

Synthesis of 4-benzyl-2-phenyloxazol-5(4H)-one (**11**)

A solution of *N*-benzoylphenylalanine (10.0 g, 37.2 mmol) in dry DCM (200 mL) and Et_2O (80 mL) was added dropwise over 20 minutes to a stirred solution of DCC (7.67 g, 37.2 mmol) in dry DCM (124 mL, 0.30 M), at 0 °C, under nitrogen atmosphere. Reaction completion was monitored by TLC analysis R_f = 0.55 (hexane/AcOEt 85:15). While keeping the reaction mixture at 0 °C, a vacuum filtration with Gooch apparatus was performed. The residue was washed with Et_2O and the solvent was evaporated under reduced pressure. The crude mixture was purified by crystallization with light petroleum ether to obtain **11** as white solid (7.46 g, 80%). The product was matched with reported data[39].

Synthesis of allyl (4-benzyl-2-phenyloxazol-5-yl) carbonate (12)

To a solution of compound **11** (4.00 g, 15.9 mmol) in dry THF (159 mL, 0.10 M) under nitrogen atmosphere at 0 °C, NEt_3 (2.44 mL, 17.5 mmol) and allyl chloroformate (1.86 mL, 17.5 mmol) were added. Afterwards, the reaction mixture was warmed to room temperature and the formation of the product was monitored by TLC analysis, R_f= 0.25 (hexane/AcOEt 95:05). After 24 hours 80 mL of H_2O were added, the organic solvent was evaporated under reduced pressure and the aqueous layer was extracted three times with Et_2O. The organic phases were collected, dried with anhydrous Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The crude mixture was purified by flash chromatography (hexane/AcOEt, 95:05) to obtain pure compound **12** as an amorphous white solid (5.13 g, 96 %). *R*f = 0.25; IR (neat, cm^{-1}) 3031, 2934, 1781, 1664, 1217, 1119; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.91 (s, 2H), 4.72 (app d, *J* = 5.9 Hz, 2H), 5.39 (dd, *J* = 0.9, 10.4 Hz, 1H), 5.45 (dd, *J* = 0.9, 17.2 Hz, 1H), 5.96 (ddt, *J* = 5.9, 10.4, 17.2 Hz, 1H), 7.19–7.38 (m, 5H), 7.35–7.51 (m, 3H), 7.92–8.03 (m, 2H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz) δ 31.9 (t), 70.9 (t), 120.9 (t), 123.8 (s), 126.4, 126.9, 127.5 (s), 128.9, 129.1, 129.3, 130.6, 130.8, 137.9 (s), 146.7 (s), 151.6 (s), 155.6 (s); HRMS (ESI) calculated for $C_{20}H_{18}NO_4$ $[M+H]^+$ 336.1230, found 336.1229 (Δ = 0.3 ppm).

Synthesis of 4-allyl-4-benzyl-2-phenyloxazol-5(4*H*)-one (**13**)

1,2-bis(diphenylphosphino)ethane (DPPE) (286 mg, 0.725 mmol) and Tris(dibenzylideneacetone) dipalladium(0)-chloroform adduct ($Pd_2(dba)_3CHCl_3$) (250 mg, 0.241 mmol) were solubilized in the least amount of dry THF under nitrogen atmosphere and were maintained under stirring for 30 minutes. Afterwards the mixture was added dropwise to a solution of compound **12** (1.62 g, 4.83 mmol) in dry THF (48.3 mL, 0.10 M) under nitrogen atmosphere. Formation of the product was monitored by TLC analysis, R_f= 0.59 (hexane/AcOEt 85:15). After 30 minutes phosphate buffer was added until the formation of a black precipitate was observed. The organic solvent was evaporated under reduced pressure and the aqueous layer was extracted three times with DCM. The

organic phases were collected, dried with anhydrous Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The crude mixture was purified by flash chromatography (hexane/AcOEt, 90:10) to obtain pure compound **13** as a pale yellow oil (1.41 g, quantitative). The product was matched with reported data[23].

Synthesis of 2-amino-2-benzylpent-4-enoic acid (14)

Compound **13** (1.40 g, 4.80 mmol) was dissolved in TFA/water 90:10 (48.0 mL, 0.10 M) and heated to 100 °C for 12h. After reaction completion, the solvent was evaporated under reduced pressure, the residue was recovered with water and washed three times with DCM. The aqueous phase was evaporated under reduced pressure and the crude mixture was purified by ion-exchange chromatography with AMBERLITE IR-120(PLUS)® resin as stationary phase. The crude mixture was dissolved in H_2O and loaded onto the column. The stationary phase was washed with H_2O until the eluted solution turned from acidic to neutral, then elution with NH_3 10% aqueous solution and evaporation of the solvent under reduced pressure afforded **14** as a white amorphous solid (985 mg, quantitative). IR (neat, cm^{-1}) 3242, 3032, 1607, 1584, 1082; ^1H NMR (CD_3OD, 400 MHz) δ 2.46 (dd, *J* = 8.4, 14.4 Hz, 1H), 2.80 (dd, *J* = 6.3, 14.4 Hz, 1H), 2.99 (d, *J* = 14.1 Hz, 1H), 3.31 (d, *J* = 14.1 Hz, 1H), 5.22–5.30 (m, 2H), 5.90 (dddd, *J* = 6.3, 8.4, 10.3, 16.7 Hz, 1H), 7.28–7.36 (m, 5H); $^{13}C\{^1H\}$ NMR (CD_3OD, 100 MHz) δ 41.1 (t), 42.0 (t), 64.8 (s), 120.2 (t), 127.5, 128.7, 130.5, 131.5, 134.9 (s), 173.6 (s); HRMS (ESI) calculated for $C_{12}H_{16}NO_2$ $[M+H]^+$ 206.1176, found 206.1177 (Δ = 0.4 ppm).

Synthesis of 2-benzyl-2-(((benzyloxy)carbonyl)amino)pent-4-enoic acid (15)

A solution of **14** (60 mg, 0.29 mmol) in dry CH_3CN (2.9 mL, 0.10 M) under nitrogen atmosphere, was added with tetramethyl ammonium hydroxide pentahydrate (105 mg, 0.580 mmol). The reaction mixture was stirred for 45 minutes, then Cbz_2O (166 mg, 0.580 mmol) was added. Formation of the *N*-protected derivative was monitored by TLC analysis, R_f= 0.35 (AcOEt/MeOH

95:05). After 24 hours, the solvent was evaporated under reduced pressure, the residue was recovered with a saturated aqueous solution of Na_2CO_3 and washed three times with Et_2O. The pH of the aqueous phase was adjusted to 1 with a HCl 1N solution and extracted three times with AcOEt. The organic layer was dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to obtain compound **15** as a colorless oil without further purification (94 mg, 96%). The product was matched with reported data[40].

Synthesis of 2-benzyl-2-((tert-butoxycarbonyl)amino)pent-4-enoic acid (16)

To a solution of **14** (40 mg, 0.19 mmol) in dry THF (1.90 mL, 0.10 M) under nitrogen atmosphere, Di-*tert*-butyl dicarbonate (56 mg, 0.25 mmol) and catalytic amount of DMAP were added. Formation of the *N*-protected derivative was monitored by TLC analysis, R_f= 0.45 (hexane/AcOEt 60:40 + 2% AcOH). After 24 hours, the solvent was evaporated under reduced pressure and the crude mixture was purified by flash chromatography (hexane/AcOEt 60:40 + 2% AcOH) to obtain pure compound **16** as a white amorphous solid (57 mg, 98%). The product was matched with reported data[41].

Synthesis of 2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-benzylpent-4-enoic acid (17)

Compound **14** (1.50 g, 7.31 mmol) was suspended in 1:1 THF/H_2O (43.0 mL, 0.17 M) at 0 °C and was added with *N*-(9-Fluorenylmethoxycarbonyloxy)succinimide (2.74 g, 8.04 mmol) and Na_2CO_3 (1.55 g, 14.6 mmol). After 30 minutes the reaction mixture was warmed to room temperature. Formation of the *N*-protected derivative was monitored by TLC analysis, R_f= 0.35 (AcOEt/MeOH 95:05). After 24 hours the pH was adjusted to 2 with a HCl 1N solution and extracted three times with AcOEt. The organic layer was dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude mixture was purified by flash chromatography (AcOEt/MeOH, 95:05) to obtain pure compound **17** as a white amorphous solid (3.12 g, quantitative). *R*f = 0.35. IR (neat, cm^{-1}) 3404, 3067, 3031, 1696, 1686, 1593, 1223, 1076; ^{1}H NMR (dmso-d_6, 90 °C, 400 MHz,

mixture of rotamers) δ 2.94–3.19 (m, 4H, partially masked by H_2O signal), 4.25 (t, *J* = 6.2 Hz, 1H), 4.39–4.51 (m, 2H), 4.95–5.08 (m, 2H), 5.66 (m, 1H), 6.20–6.37 (m, 1H), 6.96–7.08 (m, 2H), 7.15–7.23 (m, 3H), 7.33 (td, *J* = 1.0, 7.4 Hz, 2H), 7.42 (td, *J* = 1.3, 7.4 Hz, 2H), 7.67 (t, *J* = 7.2 Hz, 2H), 7.87 (d, *J* = 7.5 Hz, 2H), the chemical shift of the acid proton could not be unequivocally assigned; $^{13}C\{^1H\}$ NMR (dmso-d_6, 90 °C, 100 MHz, only major rotamer peaks were reported) δ 41.6 (t), 42.7 (t), 47.9, 64.1 (s), 66.2 (t), 109.8 (s), 120.8, 125.8, 127.1, 127.8, 128.4, 128.6, 130.7, 141.7 (s), 144.8 (s), 155.0 (s), 174.1 (s); HRMS (ESI) calculated for $C_{27}H_{24}NO_4$ $[M]^-$ 426.1711, found 426.1717 (Δ = 1.4 ppm).

Synthesis of (2*S*,5*R*)-methyl 1-(3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-benzylhexa-1,5-dien-2-yl)-5-ethynylpyrrolidine-2-carboxylate (20)

To a solution of **17** (1.00 g, 2.34 mmol) and **3** (358 mg, 2.34 mmol) in dry THF (47.0 mL, 0.05 M), bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) (595 mg, 2.34 mmol) and $NaHCO_3$ (197 mg, 2.34 mmol) were added. The reaction mixture was heated to 50 °C and monitored with TLC analysis, R_f = 0.41 (hexane/AcOEt 60:40). Progressive additions of BOP-Cl (up to 1 eq.) and $NaHCO_3$ (up to 2 eq.) were performed. After 48 hours the solvent was evaporated under reduced pressure and the crude mixture was purified by flash chromatography (hexane/AcOEt 65:35) to obtain pure compound **20** as a white amorphous solid (922 mg, 70%). *R*f = 0.36. IR (neat, cm^{-1}) 3375, 3286, 3030, 2948, 2254, 2116, 1784, 1754, 1719, 1632, 1167, 1077; ^1H NMR ($CDCl_3$, 400 MHz, mixture of diastereoisomers and rotamers) δ 1.59–1.81 (m, 1.3H), 1.90–2.48 (m, 4.7H), 2.52–2.74 (m, 0.6H) 2.84–3.46 (m, 2.4H), 3.54–4.00 (m, 4H), 4.18–4.30 (m, 1H), 4.32–4.57 (m, 2.6H), 4.65–4.76 (m, 0.4H), 5.04–5.32 (m, 2.3H), 5.64–5.93 (m, 1.4H), 6.03–6.25 (m, 0.3H), 7.02–7.15 (m, 1H), 7.16–7.27 (m, 4H), 7.29–7.37 (m, 2H), 7.38–7.47 (m, 2H), 7.54–7.66 (m, 2H), 7.75–7.84 (m, 2H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz, mixture of diastereoisomers and rotamers) δ 26.8 (br, t), 34.6 (br, t), 40.0 (t), 40.3 (t), 40.5 (t), 47.6, 50.1, 50.6, 52.7, 61.9 (br), 65.4 (br, s), 66.8 (t), 74.1 (br), 82.5 (s), 119.8 (t), 119.9 (t), 120.4, 125.4, 125.6, 127.2, 127.4, 127.5 (2C), 128.1, 128.2, 128.4,

128.8, 130.1 (br), 130.9, 132.8, 133.0, 136.1 (br, s), 136.9 (s), 141.7 (s), 144.1 (s), 144.3 (2C, s), 155.1 (s), 170.3 (s), 170.6 (s), 172.3 (s); HRMS (ESI) calculated for $C_{35}H_{34}N_2NaO_5$ $[M+Na]^+$ 585.2360, found 585.2349 (Δ = 1.8 ppm).

Synthesis of methyl (2*S*,5*R*)-1-((*S*)-2-benzyl-2-(((benzyloxy)carbonyl)amino)but-3-enoyl)-5-ethynylpyrrolidine-2-carboxylate (22)

To a solution of **21** (115 mg, 0.354 mmol) and **3** (54 mg, 0.35 mmol) in dry DCM (1.20 mL, 0.30 M), Bromotripyrrolidinophosphonium hexafluorophosphate (PyBrop; 182 mg, 0.396 mmol), *N*,*N*-diisopropylethylamine (DIEA; 0.15 mL, 0.89 mmol) and DMAP (17 mg, 0.14 mmol) were added. After 26 hours the solvent was evaporated under reduced pressure and the crude mixture was purified by flash chromatography (hexane/AcOEt 70:30) to obtain pure compound **22** as a white amorphous solid (83 mg, 50%). *R*f = 0.31 (hexane/AcOEt 70:30); $[\alpha]_D^{22}$ +18.70 (c = 0.25, $CDCl_3$); IR (neat, cm^{-1}) 3292, 2952, 2925, 1722, 1638, 1255, 1197, 1166; ^1H NMR (dmso-d_6, 400 MHz) δ 1.69 (m, 1H), 1.84–2.00 (m, 2H), 2.29 (m, 1H), 3.07 (d, *J* = 13.0 Hz, 1H), 3.22 (d, *J* = 1.8 Hz, 1H), 3.49 (d, *J* = 13.1 Hz, 1H), 3.66 (s, 3H), 4.30 (m, 1H), 4.85 (d, *J* = 12.3 Hz, 1H), 4.86 (m, 1H), 5.03 (d, *J* = 17.8 Hz, 1H), 5.14 (d, *J* = 10.9 Hz, 1H), 5.26 (d, *J* = 12.3 Hz, 1H), 5.84 (dd, *J* = 10.9, 17.7 Hz, 1H), 6.89–7.05 (m, 2H), 7.11–7.25 (m, 3H), 7.29–7.49 (m, 5H), 7.71 (s, 1H); $^{13}C\{^1H\}$ NMR (dmso-d_6, 75 MHz) δ 27.6 (t), 33.9 (t), 42.5 (t), 50.4, 52.4, 61.4, 64.5 (s), 66.2 (t), 74.5, 83.7 (s), 115.1 (t), 127.1, 128.5, 128.7, 129.2, 131.7, 137.1 (s), 138.0 (s), 139.0, 155.0 (s), 170.5 (s), 172.5 (s); HRMS (ESI) calculated for $C_{27}H_{28}KN_2O_5$ $[M+K]^+$ 499.1630, found 499.1625 (Δ = 1.1 ppm).

General Procedure for domino cross-enyne metathesis/ring-closing metathesis

Compound **20** (1 eq.) and the styrene derivative (2 eq.) were solubilized in Toluene (31.0 mL, 0.017 M) and the solution was heated to 80 °C. Hoveyda Grubbs II generation catalyst (33.4 mg, 10% mol) was dissolved in Toluene (22.3 mL) and slowly added to the reaction mixture over 7 hours through the use of a syringe pump. After 1 hour from the end of the addition the solvent was

evaporated under reduced pressure. Formation of the product was monitored by TLC analysis (hexane/AcOEt 60:40).

Synthesis of methyl (3*S*,9a*R*)-6-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-6-benzyl-5-oxo-9-vinyl-2,3,5,6,7,9a-hexahydro-1*H*-pyrrolo[1,2-*a*]azepine-3-carboxylate (23)

The title compound was obtained from compound **20** (100 mg, 0.177 mmol) as a side product of the domino cross-enyne metathesis/ring-closing metathesis reaction. The crude mixture was purified by flash chromatography (hexane/AcOEt 70:30) to obtain **23** as a yellow amorphous solid (10 mg, 10%). *R*f = 0.34 (hexane/AcOEt 70:30); $[\alpha]_D^{22}$ -11.4 (c = 1.0, $CDCl_3$); IR (neat, cm^{-1}) 3371, 2984, 2941, 1720, 1694, 1613, 1241, 1158, 1054; ^1H NMR ($CDCl_3$, 400 MHz, mixture of conformers) δ 1.88–2.05 (m, 3H), 2.32 (m, 1H), 2.42 (dd, *J* = 7.2, 18.3 Hz, 1H), 2.63 (d, *J* = 18.4 Hz, 1H), 3.03 (d, *J* = 14.1 Hz, 1H), 3.62 (d, *J* = 14.1 Hz, 1H), 3.74 (s, 3H), 4.23 (t, *J* = 5.7 Hz, 1H), 4.33 (m,1 H), 4.57–4.70 (m, 2H), 4.75–4.86 (m, 2H), 4.99 (d, *J* = 10.9 Hz, 1H), 5.12 (d, *J* = 17.2 Hz, 1H), 5.56 (d, *J* = 6.4 Hz, 1H), 6.11 (dd, *J* = 11.0, 17.1 Hz, 1H), 6.96–7.07 (m, 2H), 7.22–7.27 (m, 3H), 7.35 (t, *J* = 7.3 Hz, 2H), 7.44 (t, *J* = 7.3 Hz, 2H), 7.63 (app t, *J* = 6.4 Hz, 2H), 7.81 (d, *J* = 7.4 Hz, 2H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz, mixture of conformers) δ 26.6 (t), 33.3 (t, 2C), 40.1 (t), 47.9, 52.8, 59.5, 59.7 (s), 62.0, 66.3 (t), 115.1 (t), 120.4, 121.6, 125.2, 125.3, 127.0, 127.4, 127.5, 128.2 (2C), 128.6, 131.7, 136.9 (s), 137.7, 140.8 (s), 141.8 (s), 141.9 (s), 144.0 (s), 144.1 (s), 155.1 (s), 171.6 (s), 173.3 (s); HRMS (ESI) calculated for $C_{35}H_{34}N_2NaO_5$ $[M+Na]^+$ 585.2360, found 585.2354 (Δ = 1.1 ppm).

Synthesis of (3*S*,9a*R*)-methyl 6-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-6-benzyl-5-oxo-9-((*E*)-styryl)-2,3,5,6,7,9a-hexahydro-1*H*-pyrrolo[1,2-*a*]azepine-3-carboxylate (24)

The title compound was prepared from compound **20** (100 mg, 0.177 mmol) and styrene (37 mg, 0.36 mmol) according to the general procedure. The crude mixture was purified by flash chromatography to obtain diastereoisomers **24** (48 mg, 42%).

Diastereoisomer 24a: Flash chromatography (hexane/AcOEt 65:35). *R*f = 0.27. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.91–2.10 (m, 3H), 2.60 (dd, *J* = 8.7, 13.7 Hz, 1H), 2.68 (dd, *J* = 11.1, 13.7 Hz, 1H), 2.92 (m, 1H), 3.09 (d, *J* = 15.1 Hz, 1H), 3.78 (d, *J* = 15.1 Hz, 1H), 3.79 (s, 3H), 3.91 (dd, *J* = 4.1, 12.2 Hz, 1H), 4.02 (app t, *J* = 9.4 Hz, 1H), 4.17–4.25 (m, 2H), 4.79 (dd, *J* = 5.3, 11.1 Hz, 1H), 4.84 (dd, *J* = 4.9, 11.1 Hz, 1H), 6.19 (d, *J* = 16.7 Hz, 1H), 6.49 (d, *J* = 16.7 Hz, 1H), 7.08–7.25 (m, 10H), 7.29–7.63 (m, 6H), 7.67–7.82 (m, 2H). **Diastereoisomer 24b:** Flash chromatography (hexane/AcOEt 65:35). *R*f = 0.26. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.83–2.16 (m, 4H), 2.30 (dd, *J* = 6.8, 13.8 Hz, 1H), 2.67 (dd, *J* = 8.5, 13.8 Hz, 1H), 2.92 (m, 1H), 3.17 (d, *J* = 15.0 Hz, 1H), 3.65 (d, *J* = 15.0 Hz, 1H), 3.78 (s, 3H), 3.93 (dd, *J* = 4.4, 11.6 Hz, 1H), 4.16 (app t, *J* = 5.1 Hz, 1H), 4.24 (app d, *J* = 8.2 Hz, 1H), 4.66 (dd, *J* = 5.3, 11.0 Hz, 1H), 4.81 (dd, *J* = 6.9, 8.5 Hz, 1H), 4.87 (dd, *J* = 5.0, 11.0 Hz, 1H), 6.15 (d, *J* = 17.0 Hz, 1H), 6.32 (d, *J* = 17.0 Hz, 1H), 7.12–7.25 (m, 10H), 7.27–7.36 (m, 3H), 7.40 (t, *J* = 7.4 Hz, 1H), 7.47 (d, *J* = 7.5 Hz, 1H), 7.58 (d, *J* = 7.5 Hz, 1H), 7.68 (d, *J* = 7.5 Hz, 1H), 7.73 (d, *J* = 7.5 Hz, 1H). ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz, mixture of diastereoisomers 24a and 24b) δ 26.7 (t), 30.1 (t), 33.5 (t), 40.3 (t), 47.9 (t), 52.7, 59.7, 59.8 (s), 62.1, 66.3 (t), 120.4, 120.5, 121.7, 125.2, 125.4, 126.8, 127.1, 127.5 (2C), 128.2 (2C), 128.6, 129.1, 129.3, 130.1, 131.8, 136.9 (s), 137.4 (s), 140.4 8s), 141.7 (s) 141.9 (s), 144.1 (s), 155.1 (s), 171.7 (s), 173.3 (s); IR (neat, cm^{-1}) 2963, 2922, 1725, 1628, 1259, 1078, 1017; HRMS (ESI) calculated for $C_{41}H_{38}N_2NaO_5$ $[M+Na]^+$ 661.2673, found 661.2666 (Δ = 1.0 ppm).

Synthesis of *N*-(4-vinylphenyl)acetamide (**26**)

A solution of vinyl aniline (1.00 g, 8.34 mmol) in dry DCM (83.4 mL, 0.10 M) under nitrogen atmosphere at 0 °C, was added with acetic anhydride (7.86 mg, 83.4 mmol) and DMAP (1.02 g, 8.34 mmol). The pH was monitored with litmus paper and TEA was used to basify the solution when necessary. After 10 minutes the reaction mixture is warmed to room temperature and the formation of the product was monitored by TLC analysis, R_f = 0.37 (hexane/AcOEt 60:40). After 15 minutes the mixture was washed three times with phosphate buffer. The organic layer was dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude mixture was

purified by flash chromatography (hexane/AcOEt 60:40) to obtain compound **26** as a white amorphous solid (1.06 g, 79%). The product was matched with reported data[42].

Synthesis of (3*S*,9a*R*)-methyl 6-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-9-((*E*)-4-acetamidostyryl)-6-benzyl-5-oxo-2,3,5,6,7,9a-hexahydro-1*H*-pyrrolo[1,2-*a*]azepine-3-carboxylate (27)

The title compound was prepared from compound **20** (100 mg, 0.177 mmol) and styrene derivative **26** (57 mg, 0.36 mmol) according to the general procedure. The crude mixture was purified by flash chromatography to obtain diastereoisomers **27**. **Diastereoisomer 27a:** White amorphous solid (30 mg, 24%). R_f= 0.24 (AcOEt/hexane 60:40); $[\alpha]_D^{22}$ +85.22 (c = 1.00, $CDCl_3$) ^{1}H NMR ($CDCl_3$, 400 MHz, mixture of rotamers) δ 2.08–2.22 (m, 6H), 2.62 (m, 1H), 2.85 (d, *J* = 17.4 Hz, 1H), 3.41 (d, *J* = 13.9 Hz, 1H), 3.54 (m, 1H), 3.69 (d, *J* = 13.4 Hz, 1H), 3.75 (s, 3H), 4.17–4.29 (m, 2H), 4.51 (m, 1H), 4.75 (d, *J* = 6.2 Hz, 1H), 4.88 (m,1H), 6.10 (m, 1H), 6.50–6.67 (m, 3H), 6.99–7.11 8m, 2H), 7.16–7.25 (m, 3H), 7.27–7.48 (m, 7H), 7.52 (app t, *J* = 7.6 Hz, 3H), 7.61 (d, *J* = 7.1 Hz, 1H), 7.79 (d, *J* = 7.4 Hz, 2H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz, mixture of rotamers) δ 25.1, 26.7 (t), 30.1 (t), 33.9 (t), 36.1 (t), 47.6, 52.8, 62.3, 62.7, 62.9 (s), 66.8 (t), 124.8, 125.6, 125.8, 127.3, 127.4 (2C), 127.5, 128.0 82C), 128.6, 128.8, 129.3, 129.6, 133.3 (s), 136.8 (s), 137.2 (s, 2C), 137.9 (s), 141.6 (s), 141.7 (s), 144.3 (s), 144.5 (s), 154.7 (s), 168.7 (s), 172.3 (s), 172.7 (s); IR (neat, cm^{-1}) 3349, 2963, 1741, 1716, 1670, 1260, 1075, 1019; HRMS (ESI) calculated for $C_{43}H_{41}N_3NaO_6$ $[M+Na]^+$ 718.2888, found 718.2876 (Δ = 1.6 ppm). **Diastereoisomer 27b**: White amorphous solid (38 mg, 30%). R_f= 0.16 (AcOEt/hexane 60:40); $[\alpha]_D^{22}$ +21.98 (c = 1.00, $CDCl_3$) ^{1}H NMR ($CDCl_3$, 400 MHz, mixture of rotamers) δ 1.94–2.09 (m, 3H), 2.20 (s, 3H), 2.37 (m, 1H), 2.46 (dd, *J* = 6.1, 18.2 Hz, 1H), 2.67 (d, *J* = 18.2 Hz, 1H), 3.05 (d, *J* = 14.1 Hz, 1H), 3.61 (d, *J* = 14.1 Hz, 1H), 3.74 (s, 3H), 4.23 (t, *J* = 5.6 Hz, 1H), 4.39 (br s, 1H), 4.64 (d, *J* = 5.5 Hz, 2H), 4.78–4.90 (m, 2H), 5.66 (d, *J* = 6.1 Hz, 1H), 6.38 (d, *J* = 16.1 Hz, 1H), 6.43 (d, *J* = 16.1 Hz, 1H), 6.97–7.05 (m, 2H), 7.22–7.35 (m, 7H), 7.37–7.45 (m, 2H), 7.46–7.56 (m, 3H), 7.62 (app t, *J* = 6.2 Hz, 2H), 7.77 (app t, J = 6.3

Hz, 2H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz, mixture of rotamers) δ 25.1, 26.7 (t), 30.1 (t), 33.5 (t), 40.3 (t), 47.9, 52.6, 59.7, 59.8 (s), 62.1, 66.3 (t), 120.3, 120.4 (2C), 121.4, 125.2, 125.3, 127.0, 127.3, 127.4, 127.5, 128.2 (2C), 128.4, 128.6, 129.4, 131.7, 133.4 (s), 136.8 (s), 137.9 (s), 140.4 (s), 141.8 (s), 141.9 (s), 144.0 (s), 155.1 (s), 168.8 (s), 171.7 (s), 173.3 (s); IR (neat, cm^{-1}) 3301, 2962, 2923, 1720, 1672, 1629, 1260, 1076, 1021; HRMS (ESI) calculated for $C_{43}H_{41}N_3NaO_6$ $[M+Na]^+$ 718.2888, found 718.2880 (Δ = 1.1 ppm).

Synthesis of 2-((*tert*-butyldimethylsilyl)oxy)-*N*-(4-vinylphenyl)acetamide (28)

To a solution of 2-(tert-Butyldimethylsilyloxy)acetic acid (1.00 g, 5.26 mmol) in dry DCM (6.60 mL, 0.80 M) under nitrogen atmosphere, N-hydroxysuccinimide (667 mg, 5.78 mmol) and DCC (1.20 g, 5.78 mmol) were added. The formation of the product was monitored by TLC analysis, R_f= 0.35 (hexane/AcOEt 70:30). After 1 hour, while keeping the reaction mixture at 0 °C, a vacuum filtration with Gooch apparatus was performed. The solvent was evaporated under reduced pressure and the crude was used in the following reaction without further purification. The crude mixture was solubilized in dry DCM (52.6 mL, 0.10 M) under nitrogen atmosphere. Vinyl aniline (0.616 mL, 5.26 mmol) was added and the reaction mixture was heated to 40 °C. The formation of the product was monitored by TLC analysis, R_f = 0.74 (hexane/AcOEt 70:30). After 16 hours the solvent was evaporated and the crude mixture was purified by flash chromatography to obtain compound **28**. Flash chromatography (hexane/AcOEt 95:05). Pale yellow oil (858 mg, 56%). R_f= 0.35; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.18 (s, 6H), 1.00 (s, 9H), 4.21 (s, 2H), 5.22 (d, *J* = 10.9 Hz, 1H), 5.71 (d, *J* = 17.6 Hz, 1H), 6.70 (dd, *J* = 10.9, 17.6 Hz, 1H), 7.41 (d, *J* = 8.5 Hz, 2H), 7.55 (d, *J* = 8.5 Hz, 2H), 8.50 (s, 1H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz) δ 5.1, 18.6 (s), 26.2, 63.7 (t), 113.5 (t), 119.8, 127.3, 134.3 (s), 136.5, 137.1 (s), 169.4 (s); IR (neat, cm^{-1}) 3385, 2952, 2928, 1694, 1628, 1608, 1253, 1102; HRMS (ESI) calculated for $C_{16}H_{25}NNaO_2Si$ $[M+Na]^+$ 314.1547, found 314.1545 (Δ = 0.4 ppm).

Synthesis of (3*S*,9a*R*)-methyl 6-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-6-benzyl-9-((*E*)-4-(2-((*tert*-butyldimethylsilyl)oxy)acetamido)styryl)-5-oxo-2,3,5,6,7,9a-hexahydro-1*H*-pyrrolo[1,2-*a*]azepine-3-carboxylate (29)

The title compound was prepared from compound **20** (300 mg, 0.533 mmol) and styrene derivative **28** (311 mg, 1.07 mmol) according to the general procedure. The crude mixture was purified by flash chromatography to obtain diastereoisomers **29**. **Diastereoisomer 29a:** White amorphous solid (109 mg, 25%). R_f = 0.45 (hexane/AcOEt 60:40); $[\alpha]_D^{22}$ +71.76 (c = 1.00, $CDCl_3$) ^{1}H NMR ($CDCl_3$, 400 MHz, mixture of rotamers) δ 0.19 (s, 6H), 1.01 (s, 9H), 2.08–2.23 (m, 3H), 2.64 (m, 1H), 2.86 (d, *J* = 17.6 Hz, 1H), 3.42 (d, *J* = 14.2 Hz, 1H), 3.55 (dd, *J* = 7.3, 17.6 Hz, 1H), 3.70 (d, *J* = 14.1 Hz, 1H), 3.76 (s, 3H), 4.17–4.28 (m, 4H), 4.51 (m, 1H), 4.75 (m, 1H), 4.89 (br s, 1H), 6.14 (br d, *J* = 6.8 Hz, 1H), 6.53–6.65 (m, 3H), 7.00–7.09 (m, 2H), 7.17–7.25 (m, 3H), 7.29–7.35 (m, 2H), 7.37–7.45 (m, 4H), 7.50–7.63 (m, 4H), 7.78 (app d, *J* = 7.6 Hz, 2H), 8.52 (s, 1H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz, mixture of rotamers) δ 1.4, 18.6 (s), 26.2, 26.8 (t), 30.1 (t), 33.9 (t), 36.1 (t), 47.6, 52.8, 62.3, 62.7, 62.9 (s), 63.7 (t), 66.8 (t), 120.0, 120.3 (2C), 125.0, 125.6, 125.7, 125.8, 127.3, 127.4, 127.5, 128.0 (2C), 128.6, 128.7, 128.8, 129.3, 129.4, 129.6, 133.6 (s), 136.8 (s), 137.1 (s), 137.3 (s), 141.6 (s), 141.7 (s), 144.3 (s), 144.5 (s), 154.6 (s), 169.4 (s), 172.3 (s), 172.6 (s); IR (neat, cm^{-1}) 3377, 2952, 2929, 1742, 1714, 1697, 1630, 1249, 1197, 1103, 1070; HRMS (ESI) calculated for $C_{49}H_{55}N_3NaO_7Si$ $[M+Na]^+$ 848.3702, found 848.3687 (Δ = 1.7 ppm). **Diastereoisomer 29b:** White amorphous solid (136 mg, 31%). R_f = 0.17 (AcOEt/hexane 60:40); $[\alpha]_D^{22}$ +21.29 (c = 1.20, $CDCl_3$) ^{1}H NMR ($CDCl_3$, 400 MHz, mixture of rotamers) δ 0.19 (s, 6H), 1.01 (s, 9H), 1.95–2.08 (m, 3H), 2.38 (m, 1H), 2.48 (dd, *J* = 6.9, 18.5 Hz, 1H), 2.69 (d, *J* = 18.4 Hz, 1H), 3.06 (d, *J* = 14.1 Hz, 1H), 3.62 (d, *J* = 14.1 Hz, 1H), 3.75 (s, 3H), 4.20–4.26 (m, 3H), 4.39 (m, 1H), 4.65 (app d, *J* = 5.8 Hz, 2H), 4.84 (m, 1H), 5.69 (br d, *J* = 6.3 Hz, 1H), 6.41 (d, *J* = 16.2 Hz, 1H), 6.46 (d, *J* = 16.1 Hz, 1H), 6.98–7.07 (m, 2H), 7.23–7.26 (m, 3H), 7.29–7.35 (m, 4H), 7.38–7.45 (m, 3H), 7.56 (d, *J* = 8.3 Hz, 2H), 7.63 (t, *J* = 6.5 Hz, 2H), 7.77 (t, *J* = 7.0 Hz, 2H), 8.52 (s, 1H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz, mixture of rotamers) δ 1.4, 18.6 (s), 26.2, 26.7 (t), 30.1 (t),

33.5 (t), 40.3 (t), 47.9, 52.6, 59.7, 59.8 (s), 62.1, 63.7 (t), 66.3 (t), 120.0, 120.4, 120.5, 121.6, 125.2, 125.3, 125.7, 127.0, 127.4, 127.5, 128.2 (2C), 128.6 (2C), 129.3, 129.4, 131.8, 133.7 (s), 139.9 (s), 137.0 (s), 138.3 (s), 140.4 (s), 141.8 (s), 141.9 (s), 144.1 (s), 155.1 (s), 169.4 (s), 171.7 (s), 173.2 (s); IR (neat, cm^{-1}) 3385, 2948, 2929, 1720, 1699, 1648, 1250, 1197, 1104, 1076; HRMS (ESI) calculated for $C_{49}H_{55}N_3NaO_7Si$ $[M+Na]^+$ 848.3702, found 848.3696 (Δ = 0.6 ppm).

Synthesis of (3*S*,6*R*,9*R*,9a*R*)-methyl 6-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-6-benzyl-9-(4-(2-((*tert*-butyldimethylsilyl)oxy)acetamido)phenethyl)-5-oxooctahydro-1*H*-pyrrolo[1,2-*a*]azepine-3-carboxylate (30)

Preparation of 10% Pd/C(en) (en = ethylenediamine): the catalyst was prepared according to the reported literature procedure[43]. A suspension of commercial 10% Pd/C (0.20 g, 0.19 mmol of Pd metal) and ethylenediamine (0.882 mL, 13.2 mmol) in dry methanol (4.00 mL) under nitrogen atmosphere was stirred for 48 h at room temperature. The solid was filtered, washed with methanol and ether, and dried under vacuum pump at room temperature for 48 h to give the 10% Pd/C(en).

To a solution of **29a** (215 mg, 0.260 mmol) in dry MeOH (5.20 mL, 0.05 M)), under H_2 (balloon), Pd/C(en) was added (43 mg, 20% weight). The reaction mixture was stirred at room temperature for 48 h. Formation of the product was monitored by TLC analysis, R_f = 0.45 (hexane/AcOEt 60:40). The reaction mixture was filtered through a pad of Celite, and the organic phase was evaporated under reduced pressure. The crude mixture was purified by flash chromatography (hexane/AcOEt 65:35) to obtain pure compound **30** as a white amorphous solid (166 mg, 77%). *R*f = 0.35. $[\alpha]_D^{22}$ -11.73 (c = 1.50, $CDCl_3$) ^{1}H NMR (dmso-d_6, 90 °C, 400 MHz, mixture of rotamers) δ 0.15 (s, 6H), 0.96 (s, 9H), 1.60–2.17 (m, 11H), 2.46 (m, 1H), 2.64 (m, 1H), 3.19 (m, 1H), 3.34 (d, *J* = 13.3 Hz, 1H), 3.50–3.63 (m, 3H), 4.13 (dd, *J* = 3.8, 8.5 Hz, 1H), 4.19 (s, 2H), 4.27 (t, *J* = 5.9 Hz, 1H), 4.32–4.45 (m, 2H), 4.54 (dd, *J* = 6.1, 10.6 Hz, 1H), 6.26 (s, 1H), 6.78–6.88 (m, 2H), 7.11–7.23 (m, 5H), 7.30–7.37 (m, 2H), 7.38–7.45 (m, 2H), 7.50 (d, *J* = 8.5 Hz, 2H), 7.65 (t, *J* = 8.0 Hz, 2H), 7.87 (d, *J* = 7.9 Hz, 2H), 9.04 (s, 1H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz, mixture of rotamers) δ -5.1, 1.4, 18.6

(s), 26.0, 26.2, 26.7 (t, 2C), 27.6 (t, 2C), 30.1 (t), 32.8 (t), 38.3 (t), 47.7, 52.5, 63.0, 63.4, 63.7 (t), 65.3 8s), 66.7 (t), 120.1, 120.3, 125.5, 125.7, 127.5 82C), 128.0, 128.7, 129.3, 129.9. 135.4 (s), 136.4 (s), 138.5 (s), 141.7 (s), 144.4 (s), 144.5 (s), 154.8 (s), 169.4 (s), 172.0 (s), 172.6 (s); IR (neat, cm^{-1}) 3378, 3064, 2958, 2929, 1752, 1718, 1697, 1628, 1262, 1106, 1050; HRMS (ESI) calculated for $C_{49}H_{59}N_3NaO_7Si$ $[M+Na]^+$ 852.4015, found 852.4003 (Δ = 1.3 ppm).

Synthesis of methyl (3*S*,6*R*,9*R*,9a*R*)-6-benzyl-6-(((benzyloxy)carbonyl)amino)-9-(4-(2-((*tert*-butyldimethylsilyl)oxy)acetamido)phenethyl)-5-oxooctahydro-1*H*-pyrrolo[1,2-*a*]azepine-3-carboxylate (31)

To a solution of compound **30** (35 mg, 0.042 mmol) in dry THF (0.47 mL, 0.09 M) under nitrogen atmosphere, piperidine (8 µl, 0.08 mmol) was added. Formation of the product was monitored by TLC, R_f = 0.35 (AcOEt/MeOH 95:05). After 6 hours the solvent was evaporated under reduced pressure and the residue was recovered with a HCl 2N solution and washed with Et_2O. The pH of the aqueous phase was adjusted to 9 with a saturated aqueous solution of Na_2CO_3 and extracted three times with AcOEt. The organic phase was dried with anhydrous Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The crude mixture was dissolved in dry DCM (0.42 mL, 0.10 M) under nitrogen atmosphere and cooled to 0 °C. The reaction mixture was added with benzyl chloroformate (12 µl, 0.084 mmol) and DMAP (10 mg, 0.084 mmol). Formation of the product was monitored by TLC, R_f= 0.50 (hexane/AcOEt 60:40). After 1 hour the reaction mixture was allowed to warm to room temperatue. After 4 hours at rt the solvent was evaporated under reduced pressure and the crude mixture was purified by flash chromatography to obtain compound **31**. White amorphous solid (21 mg, 66% over two steps). R_f= 0.35 (AcOEt/hexane 65:35); $[\alpha]_D^{22}$ -40.80 (c = 0.5, $CDCl_3$); ^{1}H NMR (dmso-d_6, 500 MHz, mixture of rotamers) δ -0.03 (s, 1.6H), 0.12 (s, 4.3H), 0.85 (s, 2.5H), 0.92 (s, 6.5H), 1.67–2.20 (m, 11H), 2.40 (m, 1H), 2.65 (m, 1H), 3.30 (d, *J* = 13.6 Hz, 1H, partially masked by H_2O signal), 3.43 (d, *J* = 13.7 Hz, 1H), 3.51 (s, 3H), 3.97 (s, 0.6H), 4.14 (dd, *J* = 3.6, 8.9 Hz, 1H), 4.19 (s, 1.4H), 4.38–4.59 (br, 1H), 5.00 (d, *J* = 12.6 Hz, 1H),

5.17 (d, *J* = 12.6 Hz, 1H), 6.36 (s, 1H), 6.76–6.85 (m, 2H), 7.10–7.23 (m, 5H), 7.32–7.45 (m, 5H), 7.51 (d, *J* = 8.4 Hz, 1.4H), 7.59 (d, *J* = 8.4 Hz, 0.6H), 9.36 (s, 0.7H), 9.55 (s, 0.3H); $^{13}C\{^{1}H\}$ NMR (dmso-d_6, 125 MHz, mixture of rotamers) δ -4.9, -2.7, 18.6(s), 26.1 (t), 26.3 (3C, including two CH_2), 27.1 (t), 30.1 (t), 32.6 (t), 37.8, 52.0, 62.3 (2C, including one CH_2), 63.2, 63.8 (t), 64.2 (s), 65.5 (t), 120.1, 127.0, 128.3, 128.4, 128.8 (2C), 128.9, 130.0, 136.4 (s), 136.5 (s), 136.7 (s), 137.5 (s), 137.7 (s), 137.9 (s), 153.9 (s), 169.2(s), 171.0 (s), 172.2 (s, 2C); IR (neat, cm^{-1}) 3374, 3028, 2950, 2927, 1750, 1717, 1696, 1626, 1102, 1075. HRMS (ESI) calculated for $C_{42}H_{55}N_3NaO_7Si$ $[M+Na]^+$ 764.3702, found 764.3715 (Δ = 1.8 ppm).

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Supporting Information Available: ^{1}H and ^{13}C spectra for all reported compounds and for side products **I** and **II**, 2D-COSY and 2D-NOESY spectra for compounds **29a** and **29b**, 2D-COSY, 2D-NOESY, and 2D-HSQC-DEPT spectra for compounds **31**, details about computational studies. This material is available free of charge via the Internet at http://pubs.acs.org

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