

Bergman Cyclization of Acyclic Amino Acid Derived Eneidyne Leads to the Formation of 2,3-Dihydrobenzo[*f*]isoindoles

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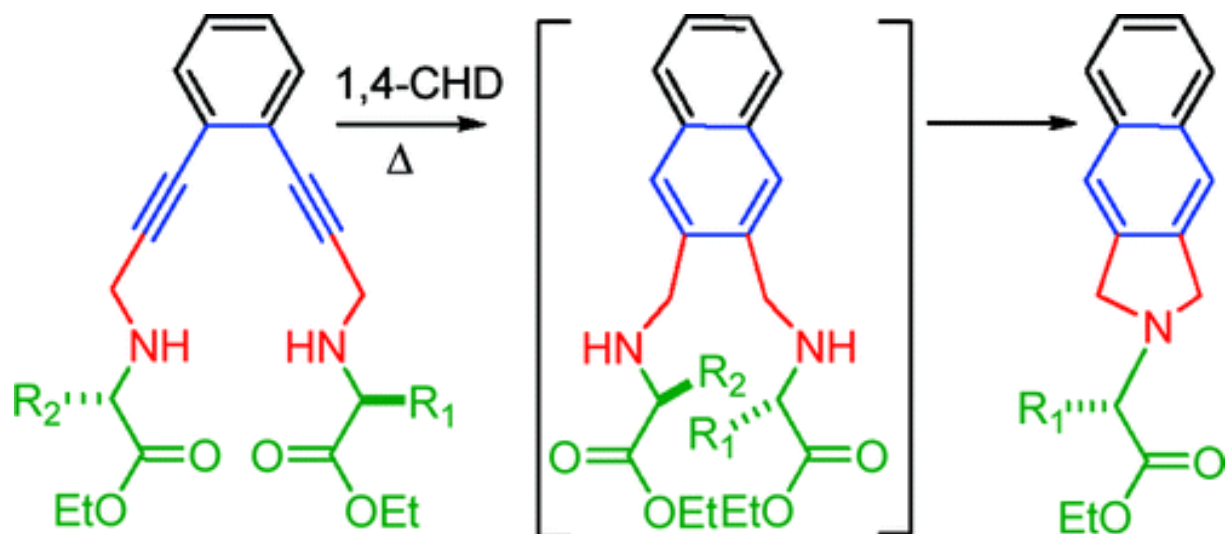
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Abstract



Eneidyne–peptide conjugates are recently recognized as useful tools in targeting various proteins, while the mechanism underlying the observed activity remains somewhat unclear. Addressing these issues, we have prepared acyclic amino acid derived enediynes and disclosed a novel thermally induced cyclization–elimination pathway. Initial formation of 1,4-benzene diradical and H-atom abstraction from an external donor is followed by S_N2 substitution leading to 2,3-dihydrobenzo[*f*]isoindoles. The proposed mechanism is supported by experimental and computational data. Additionally, we showed that amino acid side chains, although placed three bonds away from acetylene terminuses, have an appreciable influence on the reactivity of studied enediynes. These results demonstrate that amino acid or peptide parts of enediyne–peptide conjugates cannot be considered as recognition elements exclusively but may also participate in various reactions through amine functionality.

Introduction

Knowledge gathered in the field of enediynes chemistry has greatly influenced research in different areas of organic chemistry, spectroscopy, and material-oriented chemistry. Isolated from soil bacteria in the 1980s, enediynes compounds soon became recognized as important synthetic targets from numerous points of view. Antitumor properties of naturally occurring enediynes are attributed to the embedded enediyne ((Z)-3-hexene-1,5-diyne) moiety.(1) After initial triggering through intramolecular nucleophilic addition, the enediyne group undergoes Bergman cyclization (BC).(2) Stabilization of thus formed 1,4-benzene diradical is achieved by the abstraction of two protons from the DNA backbone, resulting in the cleavage of the DNA and consequently death of a cell. Total syntheses of natural enediynes brought solutions for many challenging molecular architectures and made a distinct contribution to the progress of synthetic methodologies.(3-6) Apart from their biological activity, enediynes are key building blocks in the synthesis of helical polycyclic aromatic hydrocarbons, interesting due to their optical and electronic properties.(7) Also, it was suggested that enediynes are potentially useful mechanophores, stress-sensitive units applied to the synthesis of polymers whose properties can be altered by mechanical energy.(8)

Structural complexity, nonselectivity, and poor stability of natural enediynes urge scientists to find new molecules with advantages of natural occurring ones but having less drawbacks. With this aim, a number of cyclic and acyclic enediyne-related molecules have been prepared and their activity has been tested upon thermal activation,(9) photochemical activation,(10) or complexation with metals.(11)

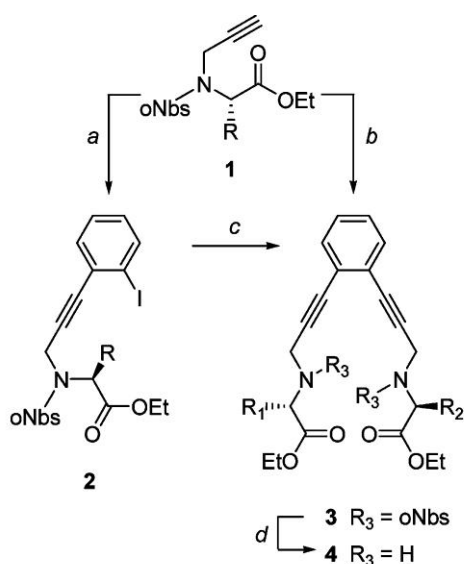
Our scope of research is the study of acyclic amino acid derived enediynes. Amino acids, as carriers of the enediyne moiety, have multiple advantages: they are good complexation ligands; an array of side chains provides easy change of molecule properties (e.g., lipophilicity, acidity) and versatility of functional groups enables binding of other molecules. Therefore, enediyne–amino acid conjugates are lately recognized as important tools in studies of Bergman cyclization. A series of photoactivated lysine–enediyne conjugates have been used to demonstrate nonrandom double-stranded DNA cleavage and the role of amino acid residue on the selectivity of DNA cleavage.(12) Also, some of these conjugates were capable of recognizing the site of single-stranded DNA cleavage and induce conversion of this damage into double-stranded DNA cleavage. Moreover, the activity of lysine–enediyne conjugates toward pH-controlled light-induced Bergman cyclization was emphasized recently.(12) It was shown that enediyne–amino acid conjugates effectively target α -chymotrypsin and inhibit its activity,(13) while enediyne–(Asp)₃ conjugate showed high affinity binding to the H1 histone, causing its degradation upon photoactivation.(14) Cyclic analogues with an enediyne moiety bonded to the amino acid α -carbon have also been reported and their activity evaluated.(15) Contrary to that, our approach was to retain properties of natural amino acids by placing the enediyne moiety at the N-terminus and benefit from the neighboring side chains (compounds **4a–e**, Scheme 1). Thus, C-terminuses can be easily modified in defined steps of the synthesis (coupling with amino acids or polyamines to enhance solubility or binding to biomolecules), while the enediyne skeleton will direct conformation of peptide grown on both C-terminuses. We were also driven by the question: can side chains distinctively influence the rate of Bergman cyclization? Here, we answer this question and provide evidence about the process following initial Bergman cyclization.

Results and discussion

Synthesis

Derivatives **4a–e** were prepared according to the synthetic path shown in Scheme 1. The Sonogashira coupling of fully protected N-propargylated derivatives of alanine, valine, and leucine **1** (16) with 1,2-diiodobenzene was performed in THF in the presence of piperidine, CuI, and Pd(PPh₃)₄. When coupled with an equimolar amount of 1,2-diiodobenzene (Scheme 1, path a), corresponding monosubstituted derivatives **2** were obtained in around 50% yield (slight excess of 1,2-diiodobenzene was used to ensure complete consumption of acetylene component and facilitate purification by column chromatography). Second, Sonogashira coupling with either the same or different derivative **1** gave enediynes-bridged symmetric Ala-Ala (**3a**), Val-Val (**3b**), Leu-Leu (**3c**), and asymmetric Ala-Val (**3d**) and Val-Gly (**3e**) sequences in good yields. Symmetric enediynes **3a–c** were also prepared according to path b, by coupling of **1** with 1.2 equiv of 1,2-diiodobenzene. Deprotection of *o*-nitrobenzenesulfonyl group (*o*Nbs) with PhSH and K₂CO₃ in DMF gave aromatic enediynes **4** in good yields. All compounds were characterized by NMR spectroscopy, mass spectrometry, HRMS, and FT-IR spectroscopy (Experimental Section).

SCHEME 1. Synthetic Path to 4a–e^a



^aReagents and conditions: (a) *o*-C₆H₄I₂ (3.6 mmol), piperidine (3 mmol), CuI (0.3 mmol), Pd(PPh₃)₄ (0.03 mmol) in THF, 30 min, then **1** (3 mmol) and piperidine (3 mmol) in THF, 2 h; (b) *o*-C₆H₄I₂ (0.5 mmol), piperidine (1 mmol), CuI (0.1 mmol), Pd(PPh₃)₄ (0.01 mmol) in THF, 30 min, then **1** (1 mmol) and piperidine, 10 h; (c) **2** (1 mmol), piperidine (1 mmol), CuI (0.1 mmol), and Pd(PPh₃)₄ (0.01 mmol) in THF, 30 min, then **1** (1 mmol) and piperidine (1 mmol), 10 h; (d) **3** (0.5 mmol), K₂CO₃ (1 mmol) and PhSH (1 mmol), DMF, 1 h.

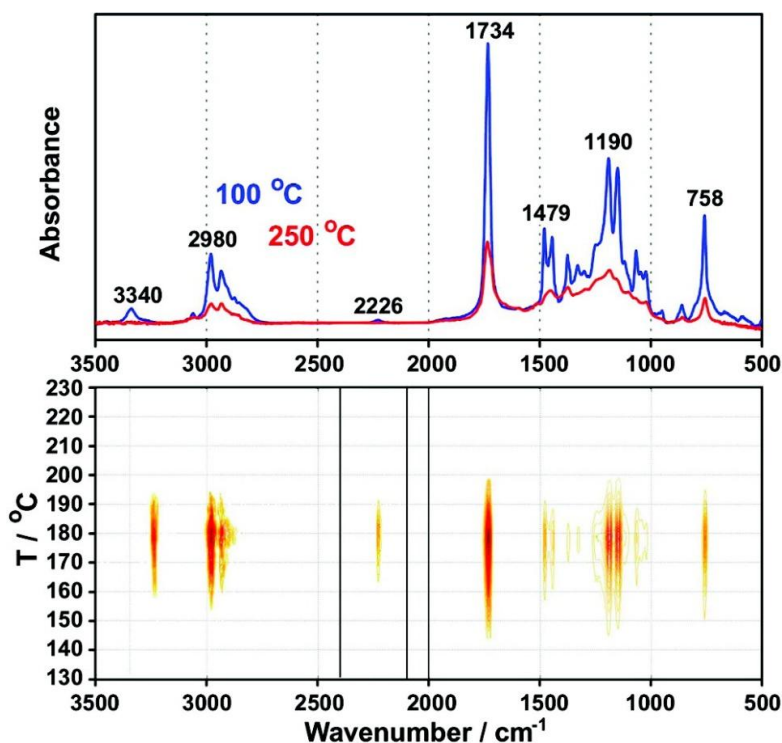
Compound	R ₁	R ₂	Yield/% ^b
4a (Ala-Ala)	—CH ₃	—CH ₃	38
4b (Val-Val)			32
4c (Leu-Leu)			33
4d (Ala-Val)	—CH ₃		19
4e (Val-Gly)		—H	18

Overall yields; two reaction steps for **4a–4c** and three reaction steps for **4d** and **4e**.

Reactivity of Eneidyne 4a–e

Thermal reactivity of enediynes toward the Bergman cyclization is traditionally studied by the differential scanning calorimetry (DSC). It can provide information about onset and maximum temperatures and activation energies of a certain process. However, DSC results have to be interpreted with caution when numerous side reactions occur, capable of lowering activation barriers.⁽¹⁷⁾ Also, the exact structural changes accompanying the process cannot be determined by DSC. Contrary to that, temperature-dependent FT-IR spectroscopy is an established method for simple and rapid monitoring of thermally induced physical and chemical rearrangements that simultaneously provide temperature values and insight into structural changes.⁽¹⁸⁾ IR spectra of enediynes **4a–e** have characteristic vibrational bands of ester and amine groups. The most prominent bands are associated with N–H stretching (3340 cm^{-1}), C=O stretching (1730 cm^{-1}), and C–O–C stretching (1190 cm^{-1}), and they can be used for tracking structural changes within the molecule. The weak band at 2225 cm^{-1} is associated with C≡C stretching vibrations and is essential for monitoring the Bergman cyclization by FT-IR technique. The FT-IR measurements performed within the temperature range of 20–250 °C are consistent for all five enediynes and indicate that a chemical process occurs at approximately 170 °C (Figure 1). Disappearance of an acetylene band at 2225 cm^{-1} is in accordance with anticipated Bergman cycloaromatization. However, substantial intensity changes of all spectral bands and disappearance of the amine band at 3340 cm^{-1} point toward additional or parallel chemical processes occurring.

FIGURE 1.



Temperature-dependent FT-IR measurement of **4a** (Ala-Ala): FT-IR spectra at 100 and 250 °C (top); MW 2D IR correlation analysis as contour plot of the baseline-corrected data (bottom) (analyzed spectral regions, 4000–2400, 2300–2100, 2000–400 cm^{-1} ; window, 10 spectra; window step, 1 spectrum; heating rate, 1 K min^{-1} ; resolution, 4 cm^{-1} ; number of scans per spectrum, 10).

Onset temperatures of cycloaromatization (the temperatures at which process begins), obtained by baseline analysis (see Supporting Information for more details), vary significantly with respect to the bulkiness of side chains (Table 1). Comparison of symmetric compounds **4a**, **4b**, and **4c** (alanine, valine, and leucine side chains, respectively) revealed the temperature difference of 30 °C between compounds **4a** and **4c**. It was demonstrated previously that properties of substituents at the terminal acetylenes may contribute significantly to the reactivity of eneynes.⁽¹⁹⁾ Here, it is evident that substituents placed even three bonds away from reactive centers have a considerable influence on the cyclization process. In this respect, we were interested at looking at asymmetric derivatives **4d** and **4e**. Although not notable, somewhat lower onset temperatures for both compounds can be explained most likely by better spatial accommodation of unequal side chains.

TABLE 1. Results of the FT-IR Measurements of Eneynes **4a–4e**^a

compound	$T_{\text{onset}}/^{\circ}\text{C}$	$T_{\text{max}}/^{\circ}\text{C}$
4a	130	175
4b	135	185
4c	160	210
4d	125	175
4e	125	175

^aMeasurements were performed within the temperature range of 20–250 °C under atmospheric pressure and at the heating rate of 1 K min⁻¹.

Isolation and Characterization of Products

The next step was performing Bergman cyclization in solution and isolation of products. Cycloaromatization of **4a** was performed with 10 equiv of 1,4-cyclohexadiene (1,4-CHD) in DMF at 175 °C (Scheme 2). Reaction mixture was checked by TLC and after 8 h shows consumption of starting compound. ¹³C NMR spectrum of isolated and purified product showed disappearance of acetylenic carbons, a new signal in the aromatic region, and a large downfield shift of CH₂ carbon adjacent to the acetylene (from 37.6 to 55.4 ppm). The ¹H NMR spectrum pointed toward a symmetric molecule with six aromatic protons and only one alanine moiety attached. Also, mass spectrum showed a signal at *m/z* 270 but not *m/z* 387 corresponding to the Bergman product **5a**. Looking at the fragmentation patterns of the molecular [M + H]⁺ ions of both **4a** and the product, we noticed that the [M + H]⁺ ion at *m/z* 270 and its product ions at *m/z* 155 and 196 represent 2 Da mass gain to the product ions of **4a** (*m/z* 268, 153, and 194) (Figure 2). In the MS/MS spectrum of the molecular [M + H]⁺ ion *m/z* 385 of **4a**, the presence of product ion at *m/z* 268 corresponds to the elimination of alanine residue (117 Da).

SCHEME 2. Bergman Cyclization of 4 (10 equiv of 1,4-CHD, DMF, Δ)(20)

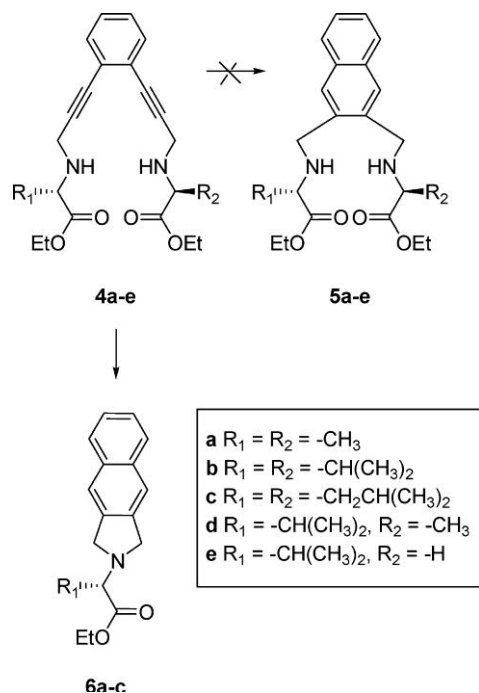
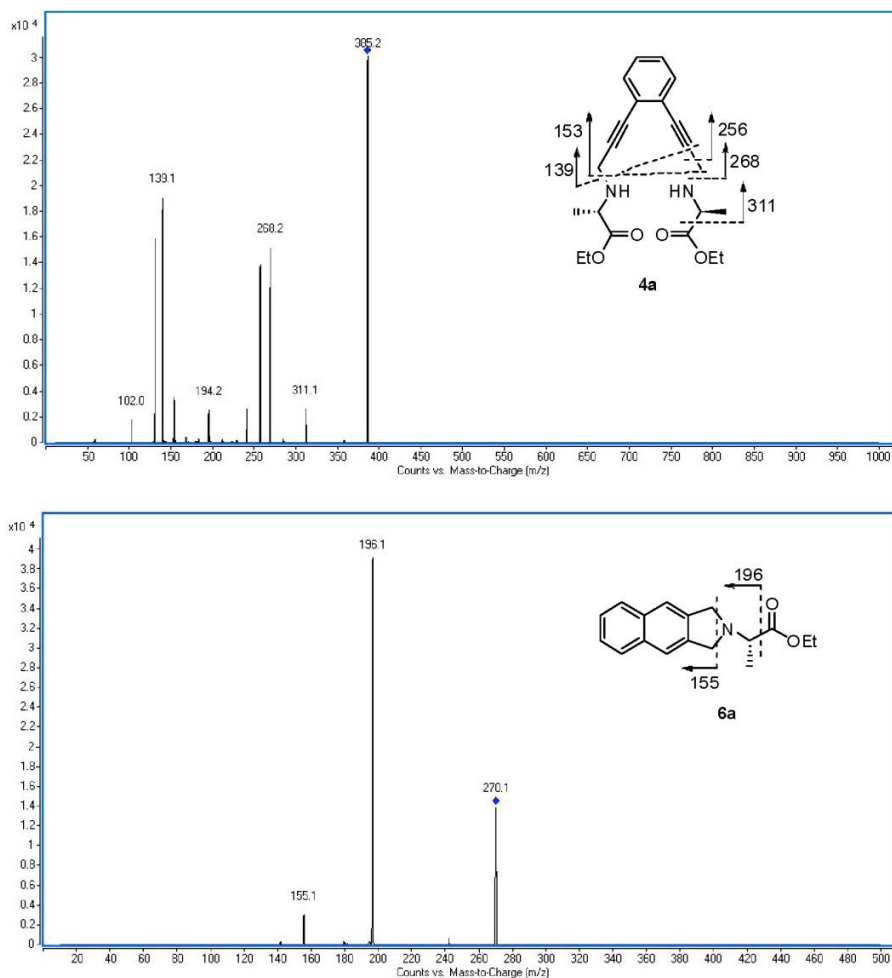


FIGURE 2. MS/MS spectra of the molecular $[M + H]^+$ ions of 4a and 6a.

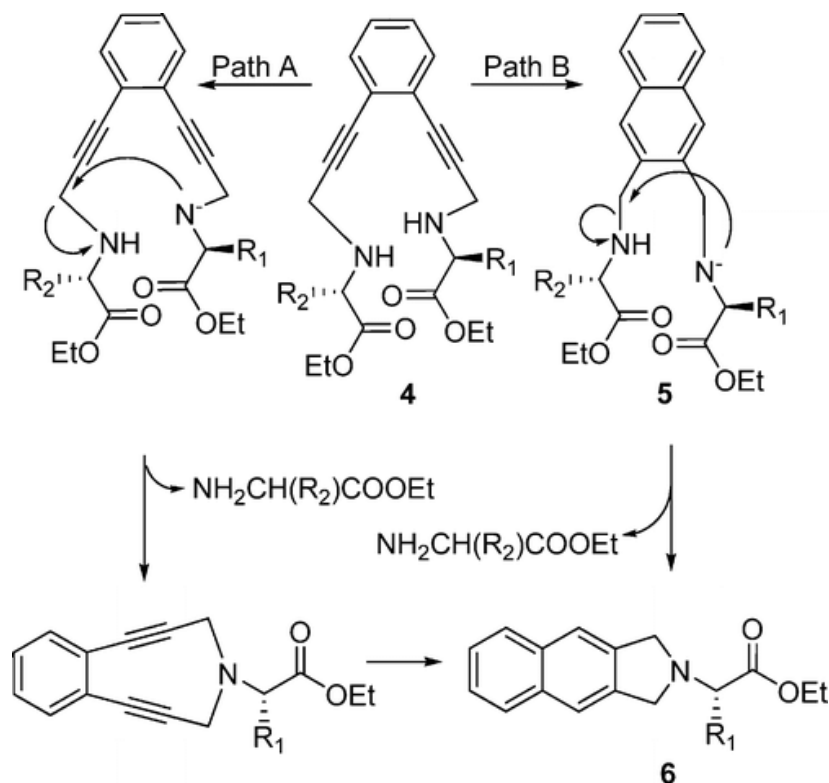


All together, FT-IR, NMR, and MS analysis undoubtedly confirmed the structure of 2,3-dihydrobenzo[f]isoindole **6a**. The very same result was obtained with other two symmetric derivatives, **4b** and **4c**. Formation of isoindoles was accompanied by polymerization reactions (poorly soluble material) that account for low yields of isolated products (around 20%). It was demonstrated previously by using IR, solid-state NMR, UV-vis spectroscopy, and MS that formation of different polymer products in the Bergman cycloaromatization reaction of simple aromatic enediyne can be explained by a set of intermolecular radical reactions.(21)

Isoindole-related compounds are not unknown in enediyne chemistry; they have been obtained from the cobalt complex of a strained nine-membered enediyne ring.(22) Upon deprotection, this compound underwent a spontaneous Bergman cyclization at 0 °C. Benzo[f]isoindoles display some interesting properties; they are farnesyl transferase inhibitors(23) and EP4 receptor agonists.(24) To the best of our knowledge, this is the first example of isoindoles obtained by the Bergman cyclization of acyclic enediynes.

Two paths of the mechanism, both of them based on S_N2, are possible (Scheme 3). In path A, the amino group of one amino acid performs nucleophilic attack on the methylene carbon atom placed between acetylene and the amino group of the second amino acid. Newly formed nine-membered ring is highly unstable and spontaneously undergoes Bergman cyclization resulting in **6**. In path B, Bergman cyclization is the first step; it brings amines closer, facilitating S_N2 attack. Methylene carbon atom placed between acetylene and the amino group (path A) or attached to the aromatic ring (path B) is deficient in electrons and, consequently, susceptible to nucleophilic attack. To prove which path is genuine, **4a** was heated under the same conditions as before, but without 1,4-CHD. If path A is probable, then the result should be decomposition of substrate since nine-membered rings are extremely unstable(19) and there is no H-atom donor to quench the Bergman diradical. If the mechanism goes by path B, the substrate should remain intact. After 48 h of heating, substrate **4a** remains intact based on TLC and MS and was quantitatively retrieved. Furthermore, it survived heating up to 240 °C for the same time period. This led us to conclude that the formation of the isoindole derivative takes place through path B, the Bergman cyclization followed by intramolecular nucleophilic substitution with consequent amino acid elimination.(25)

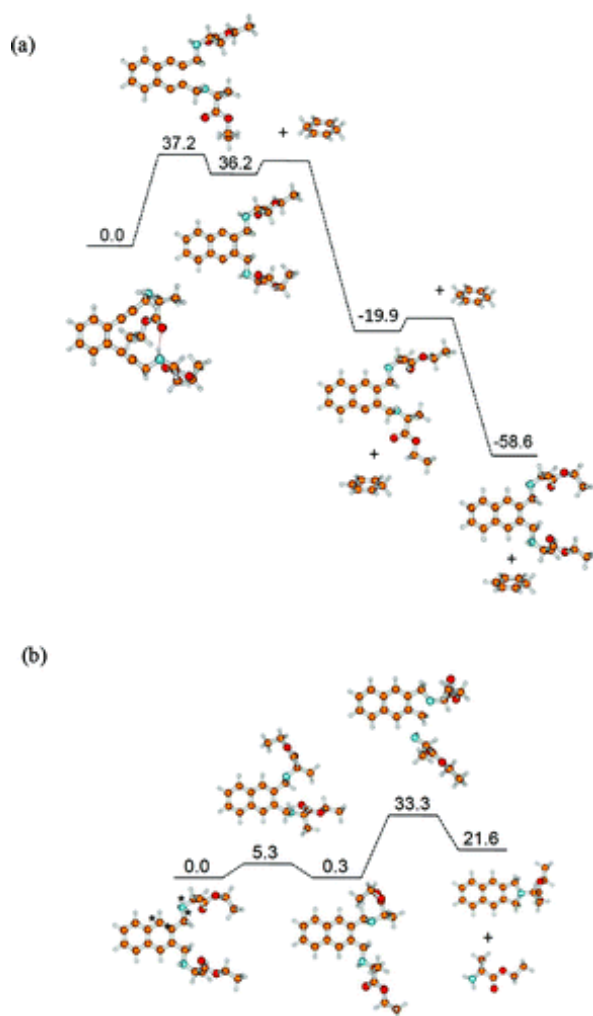
SCHEME 3. Two Possible Mechanism Paths to 6



Additional support was searched from quantum chemistry approaches as a complement to experimental studies. Gas phase geometries of intermediates, transition states, reactants, and products in paths A and B for the formation of isoindole-related compounds were obtained using the density functional theory (DFT) with the UB3LYP(26) and UBLYP(27) exchange-correlation functional and the 6-31G(d,p) basis set as implemented in the Gaussian03 quantum chemical software package.(28) Computed energy profiles for two paths are shown in Figures 3 and 4. As proposed above, the first step in path B is the formation of a diradical followed by two hydrogen abstraction steps from the 1,4-CHD as a H-atom donor. The activation barrier for the cyclization step is calculated to be 37.2 kcal/mol at the UB3LYP/6-31G(d,p) level of theory and 29.1 kcal/mol at the UBLYP/6-31G(d,p) level (Figure 3a). The energy of the transition state is just slightly above the energy of the diradical intermediate, which is less stable for 36.2 kcal/mol (27.1 0 kcal/mol at UBLYP/6-31G(d,p) level) than the eneidyne reactant **4a**. Calculated energy of diradicals should be regarded with care as it is known that higher multiconfigurational levels of theory are needed to accurately describe multiconfigurational electronic structure of singlet diradicals.(29) However, an unrestricted broken-symmetry approach has proven to be a practical tool for the study of eneidyne and enyne-allene cyclizations,(30, 31) and thus the UDFT method should provide a correct qualitative picture of a Bergman cyclization process. Two highly exothermic hydrogen abstraction steps follow the endothermic cyclization with energy gains of 56.1 and 38.1 kcal/mol, and the overall Bergman cycloaromatization is an exothermic process. Unfortunately, all attempts to locate the transition states in the two hydrogen abstraction steps failed and led to either a reactant or product structure. However, previous detailed theoretical

studies of the Bergman cyclization mechanism demonstrated that barriers to hydrogen abstraction are only a few kcal/mol less than one-tenth of the overall energy change.(30) The second step in path B (Figure 3b) includes a nucleophilic substitution reaction resulting in the elimination of the amino acid and the formation of isoindole-related product **6a**. Here, we should point out that several conformers of the Bergman product **5a**, as well as of the enediyne **4a**, were located due to the significant floppiness of amino acid chains. The schemes in Figure 4 include only the ones with the largest stability. The most stable conformer of structure **5a** is the one in which the C–C–N dihedral angle (in Figure 4b denoted with an asterisk) is 2.7°. However, the nucleophilic attack by the amino group on the methylene carbon atom occurs in the conformer with the C–C–N dihedral angle of 56.5°. So, the final step in path B proceeds only after the rotation of the amino acid backbone around the C–C bond by 53.8°. The barrier for this rotation is only 5.3 kcal/mol, while the substitution step has a barrier of 33.3 kcal/mol. In the course of the Bergman cycloaromatization reaction, 58.6 kcal/mol of energy was released and the overall reaction is still exothermic.

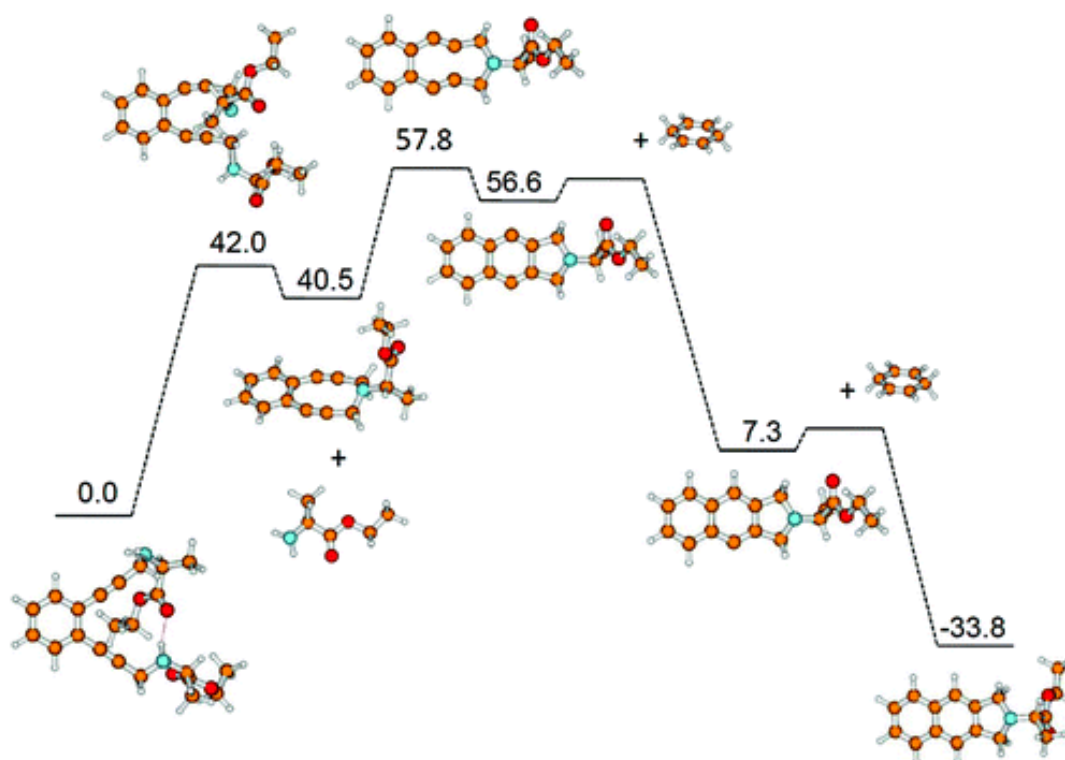
Figure 3. Path B.



(a) Reaction energy pathway for the Bergman cycloaromatization of enediyne **4a** with 1,4-cyclohexadiene as a H-atom donor ending with the Bergman product **5a**. (b) Reaction energy pathway for the elimination reaction of Bergman product **5a**, giving the isoindole product **6a**. All energies are calculated at the UB3LYP/6-31G(d,p) level of theory and given in kcal/mol relative to the energy of the **4a** (a) and **5a** (b).

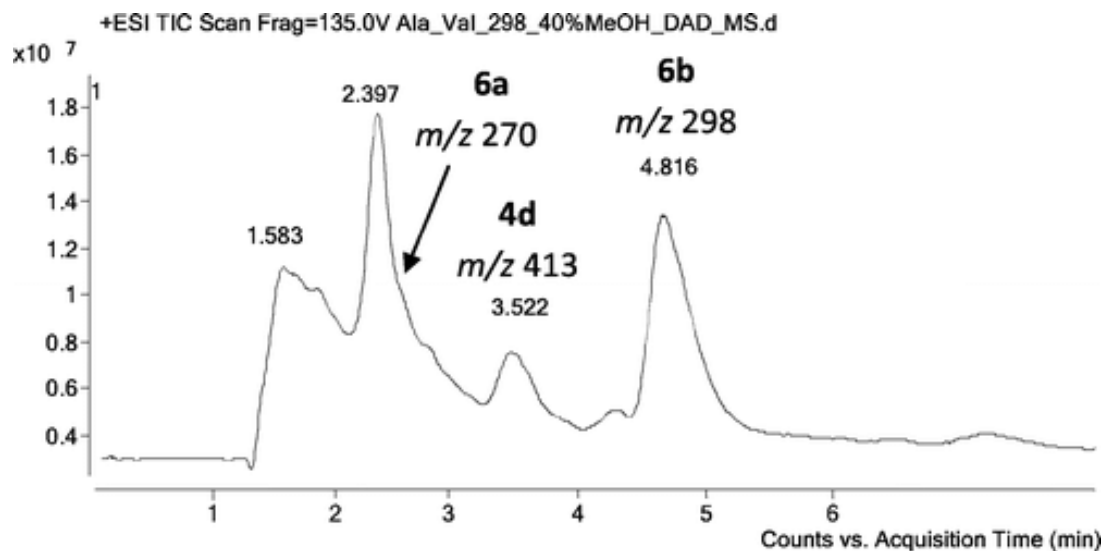
In path A, the elimination reaction precedes the Bergman cyclization. The calculated energy barrier for the nucleophilic attack in enediyne **4a** is 42.0 kcal/mol (Figure 4). The substitution step is followed by the formation of a diradical with the barrier of 17.3 kcal/mol. The diradical formation step is also highly endothermic and thus reversible. We believe that two energetically unfavorable starting steps in path A can explain the experimental finding that 2,3-dihydrobenzo[*f*]isoindoles form via mechanism B where only one small barrier exists.

Figure 4. Path A. Reaction energy pathway calculated at the UB3LYP/6-31G(d,p) level of theory. All energies are given in kcal/mol and relative to the energy of the enediyne **4a**.



With the cycloaromatization–elimination mechanism of enediynes **4a–c** proven, we turned our attention to asymmetric derivatives. Therefore, compounds **4d** and **4e** were heated in the presence of 1,4-CHD, and they both yielded (based on TLC and MS) only valine-related isoindole **6b**, although in the latter, the major event was decomposition of substrate, accompanied by traces of **6b**. Reaction mixture of Ala-Val enediyne **4d** was also checked by HPLC-MS. TIC chromatogram (Figure 5) clearly points to the formation of valine-related isoindole **6b** ($R_T = 4.82$ min), while alanine-related isoindole **6a** is detected only in traces among numerous components eluted at $R_T = 2.40$ min (mass spectra shown in Supporting Information).

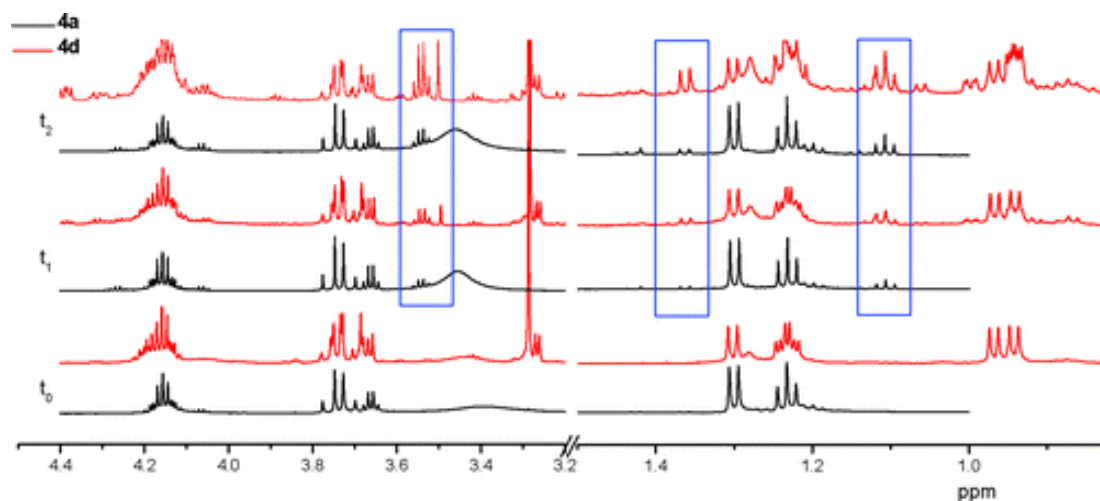
FIGURE 5. TIC chromatogram of the reaction mixture of enediyne **4d**.



Finally, further insight into cyclization–elimination processes of asymmetric enediynes was sought by using NMR spectroscopy. We were aware of its limitations in this particular case: formation of multiple compounds present in low quantities and polymerization reaction hamper conclusions about kinetics. Therefore, we focused on parallel experiments: enediynes **4a** and **4d** were dissolved in deuterated DMF (0.043 M) with 2 equiv of 1,4-CHD and heated at 175 °C. NMR spectra were recorded at different time points (every 5 h). If formation of isoindole-related Bergman products **6a** and **6b** is accompanied by the release of H-Ala-OEt, as we have proposed, NMR spectra should reflect this resemblance in the mechanism. Indeed, aliphatic region of spectra recorded after 5 and 10 h showed the appearance of new signals that can be assigned as α and β protons of alanine and CH_3 protons of the ethyl ester moiety (Figure 6). Unfortunately, protons of enediynes and products have quite similar chemical shifts in $\text{DMF-}d_7$; the aromatic part of the spectrum is covered by benzene formed upon proton transfer from 1,4-CHD to the diradical, and formation of numerous products is visible in broadening of the present signals and appearance of new ones, especially after 10 h of heating. However, the presence of the same pattern in symmetric **4a** and asymmetric enediyne **4d** supported our initial findings about products of the Bergman cyclization of asymmetric enediynes.

It is known that branching of the amino acid α -carbon atom increases electron density in the system (branched alkanes are better electron donors than linear ones) and makes amine more nucleophilic. Therefore, it is reasonable to assume that increased nucleophilicity of amines carrying branched side chains accounts for the formation valine-related product **6b** with the elimination of amino acid carrying less branched side chains. Of course, two asymmetric derivatives are not enough to allow more than hypothesis, and additional studies are undertaken to bring more general conclusions.

Figure 6. Parts of NMR spectra of **4a** and **4d** obtained after heating at 175 °C at different time points ($t_1 = 5$ h, $t_2 = 10$ h).



It is known that branching of the amino acid α -carbon atom increases electron density in the system (branched alkanes are better electron donors than linear ones) and makes amine more nucleophilic. Therefore, it is reasonable to assume that increased nucleophilicity of amines carrying branched side chains accounts for the formation valine-related product **6b** with the elimination of amino acid carrying less branched side chains. Of course, two asymmetric derivatives are not enough to allow more than hypothesis, and additional studies are undertaken to bring more general conclusions.

Eneidyne-derived 1,4-diyl radical can react in several ways; abstraction of protons from DNA is only one of them. It has been proposed that self-decomposition of C-1027 includes H-abstraction from apoprotein and subsequent formation of glycy radical.(32) Glycy radical can further undergo different reactions, depending on environment, leading to peptide scission, formation of cross-linking, or adducts. Therefore, formation of diyl radical can be considered as a trigger for numerous fragmentation and rearrangement reactions. Recently, a detailed study on model eneidyne mimicking members of esperamicine and calchamicine families revealed the importance of intramolecular H-atom abstraction and provided evidence about different products formed, depending on reaction conditions and substitution pattern.(33) Our results made a contribution to an array of Bergman cyclization products and had some enlightening new moments. Absence of an external H-atom donor hampers both cyclization and fragmentation in eneidyne **4a–e**, contrary to eneidyne bearing tetrahydropyran and tetrahydrofuran rings, where fragmentation products were detected in 30–40% yields.(32) However, the amino acid moiety took part in a further process, nucleophilic substitution reaction. Formation of 2,3-dihydrobenzo[*f*]isoindoles triggered by the Bergman cyclization is the first such example and raises questions about reactivity and the role of amino groups in eneidyne–peptide conjugates. Addressing these questions is important in light of recent increased usage of such conjugates in targeting proteins instead of DNA.(13, 14)

Conclusion

In summary, we have synthesized a series of acyclic amino acid derived eneidyne and tested them under conditions of the Bergman cyclization. FT-IR analysis clearly showed that the bulkiness of the amino acid side chain increases the temperature of the Bergman reaction and also indicated significant structural changes during the process. Experimental and computational data support the mechanism comprising cycloaromatization followed by the nucleophilic substitution with subsequent amino acid elimination yielding 2,3-dihydrobenzo[*f*]isoindoles. Also, the structure of amino acid determines the structure of isoindole formed. The influence of amine properties on the formation of 2,3-dihydrobenzo[*f*]isoindoles and the importance of activation energies and amino acid side chain will be investigated to better understand the mechanism. Finally, the presented results opened two important subjects: (a) amino acids with different side chains can be considered for fine-tuning of reactivity, especially in unstable eneidyne systems, due to the established correlation between bulkiness and onset temperature of Bergman cyclization; and (b) reactivity and participation of amino groups in reactions following initial Bergman cyclization is to be taken into account when dealing with eneidyne-peptide conjugates.

Experimental section

The synthesis and characterization data of products are provided below. Synthesis and characterization of amino acid derivatives **1** have been published previously.⁽¹⁶⁾ For further details on general methods, computational details, FT-IR, NMR, and MS spectra, consult the Supporting Information.

General Procedure for the Synthesis of **2**

1,2-Diiodobenzene (3.6 mmol), piperidine (3 mmol), CuI (0.3 mmol), and Pd(PPh₃)₄ (0.03 mmol) are mixed in THF under argon at room temperature for 30 min. **1** (3 mmol) and piperidine (3 mmol) are mixed in THF under argon and added dropwise to the initial solution. The solution is stirred until all substrate is consumed, approximately 2 h. Solvent is evaporated and the product extracted with EtOAc, washed with brine and water, and purified by flash column chromatography.

(*o*Nbs)[3-(2-Iodophenyl)propargyl]-l-alanine ethyl ester (**2a**):

Yield 911 mg (56%) (yellow oil); *R_f* 0.71 (petrol ether/EtOAc 2:1); [α _D] -45.0 (*c* 1.0, EtOAc); ¹H NMR (600 MHz, CDCl₃) δ 8.25–8.23 (m, 1H, H3 *o*Nbs), 7.79–7.77 (m, 1H, H6 *o*Nbs), 7.65–7.60 (m, 3H, H3 2-iodophenyl; H4, H5 *o*Nbs), 7.26–7.24 (m, 2H, H5, H6 2-iodophenyl), 7.00–6.97 (m, 1H, H4, 2-iodophenyl), 4.87 (q, 1H, α Ala, ³*J* _{$\alpha\beta$} = 7.4 Hz), 4.40, 4.74 (d, 2H, CH₂ propargyl, ²*J*_{HH} = 18.7 Hz), 4.12 (q, 2H, CH₂ OEt, ³*J*_{HH} = 7.2 Hz), 1.74 (d, 3H, β Ala, ³*J* _{$\alpha\beta$} = 7.4 Hz), 1.20 (t, 3H, CH₃ OEt, ³*J*_{HH} = 7.2 Hz); ¹³C NMR (150 MHz, CDCl₃) δ 170.8 (CO Ala), 147.5 (C2 *o*Nbs), 138.2 (C3 2-iodophenyl), 133.5 (C1 *o*Nbs), 132.4, 133.0 (C6 2-iodophenyl, C3 *o*Nbs), 131.1, 131.3 (C4, C5 *o*Nbs), 128.3 (C1 2-iodophenyl), 127.2, 129.9, (C4, C5 2-iodophenyl), 123.8 (C6 *o*Nbs), 99.8 (C2 2-iodophenyl), 87.5 (C2 propargyl), 86.1 (C3 propargyl), 61.1 (CH₂ OEt), 55.5 (α Ala), 35.1 (C1 propargyl), 16.4 (β Ala), 13.5 (CH₃ OEt); HRMS (MALDI) *m/z* [M + K]⁺ calcd for C₂₀H₁₉IN₂O₆SK, 580.9640; found 580.9639.

(oNbs)[3-(2-Iodophenyl)propargyl]-l-valine ethyl ester (2b):

Yield 775 mg (45%) (yellow oil); R_f 0.59 (petrol ether/EtOAc 2:1); $[\alpha_D]$ -40.0 (c 0.5, EtOAc); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 8.24–8.22 (m, 1H, H3 oNbs), 7.84–7.80 (m, 1H, H6 oNbs), 7.66–7.60 (m, 3H, H3 2-iodophenyl; H4, H5 oNbs), 7.35–7.26 (m, 2H, H5, H6 2-iodophenyl), 7.04–6.98 (m, 1H, H4, 2-iodophenyl), 4.69, 4.72 (d, 2H, CH_2 propargyl, $^2J_{\text{HH}} = 18.6$ Hz), 4.27 (d, 1H, α Val, $^3J_{\alpha\beta} = 10.3$ Hz), 4.04 (q, 2H, CH_2 OEt, $^3J_{\text{HH}} = 7.1$ Hz), 2.53–2.41 (m, 1H, β Val), 1.12 (t, 3H, CH_3 OEt, $^3J_{\text{HH}} = 7.1$ Hz), 1.04, 1.14 (d, 6H, $\gamma\gamma'$ Val, $^3J_{\beta\gamma} = 6.6$ Hz); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 170.2 (CO Val), 147.3 (C2 oNbs), 138.7 (C3 2-iodophenyl), 133.3 (C1 oNbs), 133.1, 133.6 (C6 2-iodophenyl, C3 oNbs), 131.5, 131.6 (C4, C5 oNbs), 129.0 (C1 2-iodophenyl), 127.7, 129.7, (C4, C5 2-iodophenyl), 124.0 (C6 oNbs), 100.3 (C2 2-iodophenyl), 88.4 (C2 propargyl), 86.1 (C3 propargyl), 65.5 (α Val), 61.1 (CH_2 OEt), 35.5 (C1 propargyl), 19.5, 20.2 ($\gamma\gamma'$ Val), 29.0 (β Val), 14.0 (CH_3 OEt); HRMS (MALDI) m/z $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{22}\text{H}_{23}\text{IN}_2\text{O}_6\text{SNa}$, 593.0214; found, 593.0228.

(oNbs)[3-(2-Iodophenyl)propargyl]-l-leucine ethyl ester (2c):

Yield 860 mg (49%) (yellow oil); R_f 0.69 (petrol ether/EtOAc 2:1); $[\alpha_D]$ -35.0 (c 1.0, EtOAc); $^1\text{H NMR}$ (600 MHz, CDCl_3) δ 8.20–8.19 (m, 1H, H3 oNbs), 7.81–7.80 (m, 1H, H6 oNbs), 7.65–7.60 (m, 3H, H3 2-iodophenyl; H4, H5 oNbs), 7.35–7.26 (m, 2H, H5, H6 2-iodophenyl), 7.01–6.98 (m, 1H, H4, 2-iodophenyl), 4.76–4.74 (m, 1H, α Leu), 4.50, 4.65 (d, 2H, CH_2 propargyl, $^2J_{\text{HH}} = 19.0$ Hz), 4.03 (q, 2H, CH_2 OEt, $^3J_{\text{HH}} = 7.0$ Hz), 1.84, 2.01 (m, 2H, $\beta\beta'$ Leu), 1.95 (m, 1H, γ Leu), 1.12 (t, 3H, CH_3 OEt, $^3J_{\text{HH}} = 7.0$ Hz), 0.95, 0.99 (d, 6H, $\delta\delta'$ Leu, $^3J_{\gamma\delta} = 6.7$ Hz); $^{13}\text{C NMR}$ (150 MHz, CDCl_3) δ 170.7 (CO Leu), 147.6 (C2 oNbs), 138.2 (C3 2-iodophenyl), 132.8 (C1 oNbs), 132.5, 133.0 (C6 2-iodophenyl, C3 oNbs), 130.8, 131.1 (C4, C5 oNbs), 128.5 (C1 2-iodophenyl), 127.2, 129.2 (C4, C5 2-iodophenyl), 123.7 (C6 oNbs), 99.9 (C2 2-iodophenyl), 87.9 (C2 propargyl), 85.6 (C3 propargyl), 60.9 (CH_2 OEt), 58.1 (α Leu), 38.6 (β Leu), 35.2 (C1 propargyl), 24.2 (γ Leu), 21.0, 22.3 ($\delta\delta'$ Leu), 13.5 (CH_3 OEt); HRMS (MALDI) m/z $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{23}\text{H}_{25}\text{IN}_2\text{O}_6\text{SNa}$, 607.0370; found, 607.0366.

General Procedure for the Synthesis of 3

2 (1 mmol), piperidine (1 mmol), CuI (0.1 mmol), and $\text{Pd}(\text{PPh}_3)_4$ (0.01 mmol) are mixed in THF under argon at room temperature for 30 min. **1** (1 mmol) and piperidine (1 mmol) are mixed in THF under argon and added dropwise to the initial solution. The solution is stirred overnight. Solvent is evaporated and the product extracted with EtOAc, washed with brine and water, and purified by flash column chromatography. Symmetric compounds **3a–c** can be prepared alternatively: 1,2-diiodobenzene (0.5 mmol), piperidine (1 mmol), CuI (0.1 mmol), and $\text{Pd}(\text{PPh}_3)_4$ (0.01 mmol) are mixed in THF under argon at room temperature for 30 min. **1** (1 mmol) and piperidine (1 mmol) are mixed in THF under argon and added dropwise to the initial solution. The solution is stirred until all substrate is consumed, approximately 2 h. Solvent is evaporated and the product extracted with EtOAc, washed with brine and water, and purified by flash column chromatography.

3,3'-(1,2-Phenylene)bis(prop-2-yne-1-yl)bis[(2-nitrobenzensulfonyl)-l-alanine ethyl ester] (3a):

Yield 377 mg (50%) (yellow oil); R_f 0.37 (petrol ether/EtOAc 2:1); $[\alpha_D]$ -57.0 (c 1.0, MeOH); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 8.23–8.20 (m, 2H, H3 oNbs), 7.64–7.58 (m, 6H, H4,5,6 oNbs), 7.29–7.20 (m, 4H, H3,6, H4,5 phenylene), 4.86 (q, 2H, α Ala, $^3J_{\alpha\beta} = 7.2$ Hz), 4.38, 4.46 (d, 4H, CH_2 propynyl, $^2J_{\text{HH}} = 19.0$ Hz), 4.08 (q, 4H, CH_2 OEt, $^3J_{\text{HH}} = 7.2$ Hz), 1.70 (d, 6H, β Ala, $^3J_{\alpha\beta} = 7.2$ Hz), 1.15 (t, 6H, CH_3 OEt, $^3J_{\text{HH}} = 7.2$ Hz); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 171.1 (CO Ala), 147.9 (C2 oNbs), 133.7 (C1 oNbs), 133.5 (C5 oNbs), 131.4, 131.8, 132.0 (C3, C4 oNbs; C3,6 phenylene), 128.2 (C4,5 phenylene), 124.8 (C1,2 phenylene), 124.2 (C6 oNbs), 88.5 (C22' propynyl), 83.0 (C33' propynyl), 61.6 (CH_2 OEt), 55.9 (α Ala), 35.7 (C11' propynyl), 16.7 (β Ala), 13.9 (CH_3 OEt); HRMS (MALDI) m/z $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{34}\text{H}_{34}\text{NaN}_4\text{O}_{12}\text{S}_2$, 777.1506; found, 777.1471.

3,3'-(1,2-Phenylene)bis(prop-2-yne-1-yl)bis[(2-nitrobenzensulfonyl)-l-valine ethyl ester] (3b):

Yield 390 mg (48%) (yellow oil); R_f 0.69 (petrol ether/EtOAc 1:1); $[\alpha_D]$ -67.0 (c 1.0, MeOH); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 8.24–8.20 (m, 2H, H3 oNbs), 7.64–7.58 (m, 6H, H4,5,6 oNbs), 7.29–7.21 (m, 4H, H3,6, H4,5 phenylene), 4.56–4.74 (m, 2H, α Val), 4.25 (d, 4H, CH_2 propynyl, $^2J_{\text{HH}} = 19.0$ Hz), 4.09–3.96 (m, 2H, β Val), 4.02 (q, 4H, CH_2 OEt, $^3J_{\text{HH}} = 7.1$ Hz), 1.09 (t, 6H, CH_3 OEt, $^3J_{\text{HH}} = 7.1$ Hz), 1.03, 1.14 (d, 12H, $\gamma\gamma'$ Val, $^3J_{\beta\gamma} = 6.7$ Hz); $^{13}\text{C NMR}$ (150 MHz, CDCl_3) δ 170.1 (CO Val), 148.2 (C2 oNbs), 133.6 (C5 oNbs), 133.2 (C1 oNbs), 13.9 (CH_3 OEt), 131.5, 131.6, 132.0, (C3, C4 oNbs; C3,6 phenylene), 128.1 (C4,5 phenylene), 125.0 (C1,2 phenylene), 123.9 (C6 oNbs), 88.8 (C22' propynyl), 82.7 (C33' propynyl), 65.6 (α Val), 61.1 (CH_2 OEt), 35.7 (C11' propynyl), 28.9 (β Val), 19.5, 19.9 ($\gamma\gamma'$ Val); HRMS (MALDI) m/z $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{38}\text{H}_{42}\text{NaN}_4\text{O}_{12}\text{S}_2$, 833.2132; found, 833.2158.

3,3'-(1,2-Phenylene)bis(prop-2-yne-1-yl)bis[(2-nitrobenzensulfonyl)-l-leucine ethyl ester] (3c):

Yield 394 mg (47%) (yellow oil); R_f 0.68 (petrol ether/EtOAc 1:1); $[\alpha_D]$ -16.0 (c 1.0, MeOH); $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ 8.23–8.11 (m, 2H, H3 oNbs), 7.75–7.63 (m, 6H, H4,5,6 oNbs), 7.47–7.35 (m, 4H, H3,6, H4,5 phenylene), 4.78–4.67 (m, 2H, α Leu), 4.36, 4.20 (d, 4H, CH_2 propynyl, $^2J_{\text{HH}} = 19.0$ Hz), 4.03 (q, 4H, CH_2 OEt, $^3J_{\text{HH}} = 7.2$ Hz), 2.06–1.71 (m, 6H, γ Leu, $\beta\beta'$ Leu), 1.13 (t, 6H, CH_3 OEt, $^3J_{\text{HH}} = 7.2$ Hz), 0.83, 0.90 (d, 12H, $\delta\delta'$ Leu, $^3J_{\text{HH}} = 6.5$ Hz); $^{13}\text{C NMR}$ (75 MHz, $\text{DMSO}-d_6$) δ 171.3 (CO Leu), 147.4 (C2 oNbs), 134.8 (C5 oNbs), 131.2 (C1 oNbs), 130.7, 131.5, 132.2 (C3, C4 oNbs; C3,6 phenylene), 128.8 (C4,5 phenylene), 124.5 (C1,2 phenylene), 124.0 (C6 oNbs), 89.8 (C22' propynyl), 81.3 (C33' propynyl), 60.9 (CH_2 OEt), 58.3 (α Leu), 38.0 (β Leu), 35.6 (C11' propynyl), 23.9 (γ Leu), 21.6, 22.6 ($\delta\delta'$ Leu), 13.4 (CH_3 OEt); HRMS (MALDI) m/z $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{40}\text{H}_{46}\text{NaN}_4\text{O}_{12}\text{S}_2$, 861.2451; found, 861.2411.

(2-Nitrobenzensulfonyl)[3-(2-(3-(*N*-((*S*)-1-ethoxy-1-oxopropan-2-yl)-2-nitrophenylsulfonamido)prop-1-ynyl)phenyl)prop-2-ynyl]-l-valine ethyl ester (3d):

Yield 400 mg (51%) (yellow oil); R_f 0.36 (petrol ether/EtOAc 2:1); $[\alpha_D]$ -47.0 (c 1.0, MeOH); $^1\text{H NMR}$ (600 MHz, CDCl_3) δ 8.26–8.18 (m, 2H, H3 oNbs Ala,Val), 7.65–7.59 (m, 6H, H4,5,6 oNbs Ala,Val), 7.27–7.25 (m, 2H, H3,6 phenyl), 7.22–7.20 (m, 2H, H4,5 phenyl), 4.86 (q, 1H, α Ala, $^3J_{\alpha\beta} = 6.9$ Hz), 4.71–4.57, 4.41–4.37 (m, 4H, CH_2 prop-2-ynyl, CH_2 prop-1-ynyl), 4.22 (d, 1H, α Val, $^3J_{\beta} = 10.0$ Hz), 4.11–3.96 (m, 4H, CH_2 OEt, Ala,Val), 2.44–2.37 (m, 1H, β Val), 1.71 (d, 3H, β Ala, $^3J_{\alpha\beta} = 6.9$ Hz), 1.17–1.02 (m, 12H, CH_3 OEt Ala,Val, $\gamma\gamma'$ Val); $^{13}\text{C NMR}$ (150 MHz, CDCl_3) δ 169.5, 170.7 (CO Ala,Val), 147.4, 147.7 (C2 oNbs Ala,Val), 132.9, 133.3 (C1 oNbs Ala,Val), 132.7, 133.1 (C5 oNbs Ala,Val), 131.0, 131.1, 131.3, 131.4, 131.5, 131.6 (C3,4 oNbs Ala,Val, C3,6 phenyl), 127.6, 127.7 (C4,5 phenyl), 124.3, 124.5 (C1,2 phenyl), 123.4, 123.6 (C6 oNbs Ala,Val), 88.0, 88.3 (C2 prop-2-ynyl, C2 prop-1-ynyl), 82.1, 82.6 (C3 prop-2-ynyl, C1 prop-1-ynyl), 65.1 (α Val), 60.6, 61.1 (CH_2 OEt Ala,Val), 55.5 (α Ala), 35.1, 35.2 (C1 prop-2-ynyl, C3 prop-1-ynyl), 28.4 (β Val), 18.9, 19.4 ($\gamma\gamma'$ Val), 16.0 (β Ala), 13.4, 13.5 (CH_3 OEt Ala,Val); HRMS (MALDI) m/z $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{36}\text{H}_{38}\text{N}_4\text{O}_{12}\text{S}_2\text{Na}$, 805.1819; found, 805.1840.

(2-Nitrobenzensulfonyl)[3-(2-(3-(*N*-(2-ethoxy-2-oxoethyl)-2-nitrophenylsulfonamido)prop-1-ynyl)phenyl)prop-2-ynyl]-l-valine ethyl ester (3e):

Yield 453 mg (59%) (yellow oil); R_f 0.60 (petrol ether/EtOAc 1:1); $[\alpha_D]$ -10 (c 0.5, MeOH); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 8.23–8.14 (m, 2H, H3 oNbs Val,Gly), 7.67–7.58 (m, 6H, H4,5,6 oNbs Val,Gly), 7.25–7.21 (m, 4H, H3,4,5,6 phenyl), 4.61, 4.64 (s, 4H, CH_2 prop-2-ynyl, CH_2 prop-1-ynyl), 4.41 (s, 2H, α Gly), 4.26–4.10 (m, 4H, CH_2 OEt Val,Gly), 4.08–3.94 (m, 1H, α Val), 2.45–2.34 (m, 1H, β Val), 1.26–1.01 (m, 12H, CH_3 OEt Val,Gly, $\gamma\gamma'$ Val); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 170.0, 168.5 (CO Val,Gly), 148.0, 148.2 (C2 oNbs Val,Gly), 133.6, 133.7 (C5 oNbs Val,Gly), 133.1, 133.2 (C1 oNbs Val,Gly), 128.0, 128.2, 128.3, 131.1, 131.5, 131.6, 131.9, 132.0 (C4,5, C3,6 phenyl, C3,4 oNbs Val,Gly), 124.5, 125.0 (C1,2 phenyl), 123.9, 124.2 (C6 oNbs Val,Gly), 88.8, 88.9 (C2 prop-2-ynyl, C2 prop-1-ynyl), 82.6, 84.4 (C3 prop-2-ynyl, C1 prop-1-ynyl), 65.5 (α Val), 61.1, 61.6 (CH_2 OEt Val,Gly), 47.7 (α Gly), 35.6, 35.7 (C1 prop-2-ynyl, C3 prop-1-ynyl), 28.9 (β Val), 19.4, 19.8 ($\gamma\gamma'$ Val), 13.9, 14.0 (CH_3 OEt Val,Gly); HRMS (MALDI) m/z $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{35}\text{H}_{36}\text{N}_4\text{O}_{12}\text{S}_2\text{Na}$, 791.1663; found, 791.1673.

General Procedure for the Synthesis of 4

Eneidyne **3** (0.5 mmol), K_2CO_3 (1 mmol), and PhSH (1 mmol) are mixed in DMF at room temperature for 1 h. Solvent is evaporated and the product extracted with EtOAc, washed with brine and water, and purified by flash column chromatography.

3,3'-(1,2-Phenylene)bis(prop-2-yne-1-yl)bis[l-alanine ethyl ester] (4a):

Yield 144 mg (75%) (yellow oil); R_f 0.42 (EtOAc); $[\alpha_D]$ -29.0 (c 1.0, EtOAc); $^1\text{H NMR}$ (300 MHz, CD_3OD) δ 7.43–7.39 (m, 2H, H3,6 phenylene), 7.29–7.27 (m, 2H, H4,5 phenylene), 4.19 (q, 4H, CH_2 OEt, $^3J_{\text{HH}} = 7.0$ Hz), 3.71 (br s, 6H, α Ala, CH_2 propynyl), 1.35 (d, 6H, β Ala, $^3J_{\alpha\beta} = 6.7$ Hz), 1.26 (t, 6H, CH_3 OEt, $^3J_{\text{HH}} = 7.0$ Hz); $^{13}\text{C NMR}$ (75 MHz, CD_3OD) δ 176.0 (CO Ala), 133.1 (C3,6 phenylene), 129.2 (C4,5 phenylene), 126.7 (C1,2 phenylene), 91.2 (C22' propynyl), 83.7 (C11' propynyl), 62.1 (CH_2 OEt), 56.0 (α Ala), 37.6 (C33' propynyl), 18.5 (β Ala), 14.5 (CH_3 OEt); IR (KBr, 25 °C) 3332, 2230, 1732, 1481, 1445, 1194, 1149, 1068, 1022, 760; HRMS (MALDI) m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_4$, 385.2122; found, 385.2117.

3,3'-(1,2-Phenylene)bis(prop-2-yne-1-yl)bis[l-valine ethyl ester] (4b):

Yield 147 mg (67%) (yellow oil); R_f 0.49 (EtOAc/EtOH 10:1); $[\alpha_D]$ -10.0 (c 0.5, MeOH); ^1H NMR (300 MHz, CD_3OD) δ 7.42–7.39 (m, 2H, H3,6), 7.29–7.26 (m, 2H, H4,5 phenylene), 4.17 (q, 4H, CH_2 OEt, $^3J_{\text{HH}} = 7.1$ Hz), 3.67 (d, 4H, CH_2 propynyl, $^2J_{\text{HH}} = 16.9$ Hz), 3.36 (d, 2H, α Val, $^3J_{\alpha\beta} = 5.6$ Hz), 2.07–1.96 (m, 2H, β Val), 2.00 (m, 2H, β Val), 1.23 (t, 6H, CH_3 OEt, $^3J_{\text{HH}} = 7.1$ Hz), 0.97, 0.99 (d, 12H, $\gamma\gamma'$ Val, $^3J_{\beta\gamma} = 2.4$ Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 174.2 (CO Val), 131.7 (C3,6 phenylene), 127.6 (C4,5 phenylene), 125.4 (C1,2 phenylene), 90.4 (C22' propynyl), 82.1 (C11' propynyl), 65.6 (α Val), 60.4 (CH_2 OEt), 37.4 (C33' propynyl), 31.4 (β Val), 17.6, 17.9 ($\gamma\gamma'$ Val), 13.2 (CH_3 OEt); IR (KBr, 25 °C) 3330, 2228, 1730, 1479, 1467, 1439, 1186, 1148, 1024, 756; HRMS (MALDI) m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{26}\text{H}_{36}\text{N}_2\text{O}_4$, 441.2747; found, 441.2739.

3,3'-(1,2-Phenylene)bis(prop-2-yne-1-yl)bis[l-leucine ethyl ester] (4c):

Yield 166 mg (71%) (yellow oil); R_f 0.36 (toluene/EtOAc 1:1); $[\alpha_D]$ -4.5 (c 0.5, MeOH); ^1H NMR (300 MHz, CD_3OD) δ 7.42–7.24 (m, 4H, H3,6, H4,5 phenylene), 4.16 (q, 4H, CH_2 OEt, $^3J_{\text{HH}} = 7.2$ Hz), 3.99 (s, CH_2 , propynyl), 3.62 (br d, 2H, α Leu), 1.80–1.69 (m, 2H, γ Leu), 1.54 (m, 4H, β Leu), 1.25 (t, 6H, CH_3 OEt, $^3J_{\text{HH}} = 7.2$ Hz), 0.95 (d, 12H, $\delta\delta'$ Leu, $^3J_{\gamma\delta} = 4.6$ Hz); ^{13}C NMR (75 MHz, CD_3OD) δ 174.8 (CO Leu), 131.5, (C3,6 phenylene), 127.7 (C4,5 phenylene), 125.4 (C1,2 phenylene), 89.2 (C22' propynyl), 81.5 (C11' propynyl), 60.6 (CH_2 OEt), 59.1 (α Leu), 42.0 (β Leu), 36.7 (C33' propynyl), 24.6 (γ Leu), 21.4, 21.6 ($\delta\delta'$ Leu), 13.2 (CH_3 OEt); IR (KBr, 25 °C) 3336, 2233, 1732, 1481, 1466, 1445, 1194, 1148, 1026, 762; HRMS (MALDI) m/z $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{28}\text{H}_{40}\text{N}_2\text{O}_4\text{Na}$, 491.2880; found, 491.2897.

[3-(2-(3-((S)-1-Ethoxy-1-oxopropan-2-ylamino)prop-1-ynyl)phenyl)prop-2-ynyl]-l-valine ethyl ester (4d):

Yield 140 mg (68%) (yellow oil); R_f 0.34 (petrol ether/EtOAc 1:1); $[\alpha_D]$ -22 (c 0.5 MeOH); ^1H NMR (600 MHz, CDCl_3) δ 7.41–7.39 (m, 2H, H4,5 phenyl), 7.23–7.21 (m, 2H, H3,6 phenyl), 4.22–4.13 (m, 4H, CH_2 OEt Ala,Val), 3.76–3.63 (m, 5H, CH_2 prop-2-ynyl, CH_2 prop-1-ynyl, α Ala), 3.27 (d, 1H, α Val, $^3J_{\alpha\beta} = 5.42$ Hz), 2.03–1.97 (m, 1H, β Val), 1.36 (d, 3H, β Ala, $^3J_{\alpha\beta} = 7.12$ Hz), 1.25, 1.27 (t, 6H, CH_3 OEt Ala,Val, $^3J_{\text{HH}} = 7.02$ Hz), 0.96, 0.99 (d, 6H, $\gamma\gamma'$ Val, $^3J_{\beta\gamma} = 6.75$ Hz); ^{13}C NMR (150 MHz, CDCl_3) δ 174.8, 175.2 (CO Ala,Val), 132.2, 132.3 (C3,6 phenyl), 127.9, 128.0 (C4,5 phenyl), 125.6, 125.8 (C1,2 phenyl), 90.9, 91.4 (C2 prop-2-ynyl, C2 prop-1-ynyl), 82.4, 82.7 (C13' prop-2-ynyl, C1 prop-1-ynyl), 66.2 (α Val), 60.8, 61.1 (CH_2 OEt Ala,Val), 55.2 (α Ala), 37.6, 37.8 (C1 prop-2-ynyl, C3 prop-1-ynyl), 31.9 (β Val), 18.7, 19.2 ($\gamma\gamma'$ Val), 18.4 (β Ala), 14.4, 14.5 (CH_3 OEt Ala,Val); IR (KBr, 25 °C) 3337, 2232, 1730, 1481, 1466, 1445, 1188, 1148, 1026, 760; HRMS (MALDI) m/z $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{24}\text{H}_{32}\text{N}_2\text{O}_4\text{Na}$, 435.2254; found, 435.2251.

[3-(2-(3-(2-Ethoxy-2-oxoethylamino)prop-1-ynyl)phenyl)prop-2-ynyl]-l-valine ethyl ester (4e):

Yield 133 mg (67%) (yellow oil); R_f 0.52 (EtOAc/petrol ether 2:1); $[\alpha_D]$ -5 (c 0.5 MeOH); ^1H NMR (300 MHz, CDCl_3): δ 7.43–7.38 (m, 2H, H4,5 phenyl), 7.24–7.21 (m, 2H, H3,6 phenyl), 4.24–4.11 (m, 4H, CH_2 OEt Val,Gly), 3.77–3.63 (m, 6H, CH_2 prop-2-ynyl, CH_2 prop-1-ynyl, α Gly), 3.27 (d, 1H, α Val, $^3J_{\alpha\beta} = 5.44$ Hz), 2.09–1.97 (m, 1H, β Val), 1.25, 1.28 (t, 6H, CH_3 OEt Val,Gly, $^3J_{\text{HH}} = 7.2$ Hz), 0.96, 0.99 (d, 6H, $\gamma\gamma'$ Val, $^3J_{\beta\gamma} = 6.9$ Hz); ^{13}C NMR (75 MHz, CDCl_3) δ 172.0, 174.6 (CO Val,Gly), 131.9, 132.1 (C4,5 phenyl), 127.7, 127.8 (C3,6 phenyl), 125.4, 125.6 (C1,2 phenyl), 90.5, 91.2 (C2 prop-2-ynyl, C2 prop-1-ynyl), 82.2, 82.9 (C3 prop-2-ynyl, C1 prop-1-ynyl), 66.0 (α Val), 60.6, 60.9 (CH_2 OEt Val,Gly), 49.3 (α Gly), 38.5, 38.6 (C1 prop-2-ynyl, C3 prop-1-ynyl), 18.4, 19.2 ($\gamma\gamma'$ Val), 14.2, 14.3 (CH_3 OEt Val,Gly); IR (KBr, 25 °C) 3336, 2232, 1730, 1481, 1466, 1445, 1190, 1148, 1024, 760; HRMS (MALDI) m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{30}\text{N}_2\text{O}_4$, 399.2278; found, 399.2262.

General Procedure for the Synthesis of 6

4 (0.25 mmol) was dissolved in DMF in a test tube, and 1,4-cyclohexadiene (2.5 mmol) was added. The reaction mixture was degassed, closed under high vacuum, and placed in a preheated oil bath at T_{max} determined by FT-IR spectroscopy for selected derivative. The solution was heated until all substrate was consumed (monitoring by TLC and MS), approximately 24 h. Solvent was evaporated and the product purified by HPLC.

(S)-Ethyl-2-(1*H*-benzo[*f*]isoindol-2(3*H*)-yl)propanoate (6a):

Yield 12 mg (18%); R_f 0.76 (EtOAc/petrol ether 2:1); ^1H NMR (600 MHz, CDCl_3) δ 7.83 (dd, 2H, H3,6, $^3J_{\text{HH}} = 6.1$ Hz, $^4J_{\text{HH}} = 3.4$ Hz), 7.74 (br s, 2H, H2,7), 7.51 (dd, 2H, H4,5, $^3J_{\text{HH}} = 6.2$ Hz, $^4J_{\text{HH}} = 3.3$ Hz), 5.05, 4.88 (br d, 4H, CH_2 isoindole), 4.49 (q, 1H, α Ala, $^3J_{\alpha\beta} = 7.1$ Hz), 4.24 (q, 2H, CH_2 OEt, $^3J_{\text{HH}} = 7.1$ Hz), 1.71 (d, 3H, β Ala, $^3J_{\alpha\beta} = 7.1$ Hz), 1.28 (t, 3H, CH_3 OEt, $^3J_{\text{HH}} = 7.1$ Hz); ^{13}C NMR (150 MHz, CDCl_3) δ 171.3 (CO Ala), 138.1 (C1,8), 133.3 (C9, 10), 128.0 (C3,6), 125.5 (C4,5), 120.8 (C2,7), 70.1 (α Ala), 63.6 (CH_2 OEt), 55.4 (CH_2 isoindole), 17.0 (β Ala), 14.3 (CH_3 OEt); HRMS (MALDI) m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{17}\text{H}_{19}\text{NO}_2$, 270.1488; found, 270.1481.

(S)-Ethyl-2-(1*H*-benzo[*f*]isoindol-2(3*H*)-yl)-3-methylbutanoate (6b):

Yield 12.6 mg (17%); R_t 14.3 min (Eurospher 100 RP C-18 column; 150×4.5 mm i.d.; 5 μm ; flow rate = 0.5 mL/min; mobile phase = 54% MeOH/0.1% TFA); ^1H NMR (600 MHz, CD_3OD) δ 8.12 (br s, 2H, H2,7), 7.90 (br s, 2H, H3,6), 7.54 (dd, 2H, H4,5, $^3J_{\text{HH}} = 6.2$ Hz, $^4J_{\text{HH}} = 3.3$ Hz), 4.87, 4.81 (m, 2H, CH_2 isoindole), 4.41 (d, 1H, α Val, $^3J_{\alpha\beta} = 5.3$ Hz), 4.20 (q, 2H, CH_2 OEt, $^3J_{\text{HH}} = 7.1$ Hz), 2.17 (m, 1H, β Val), 1.27 (t, 3H, CH_3 OEt, $^3J_{\text{HH}} = 7.1$ Hz), 0.94, 0.96 (d, 6H, $\gamma\gamma'$ Val, $^3J_{\beta\gamma} = 7.0$ Hz); ^{13}C NMR (150 MHz, CD_3OD) δ 172.8 (CO Val), 135.2 (C9, 10), 129.3 (C3,6), 128.2 (C4,5), 123.1 (C2,7), 73.7 (α Val), 62.4 (CH_2 OEt), 49.0 (CH_2 isoindole), 32.0 (β Val), 18.2, 19.6 ($\gamma\gamma'$ Val), 14.6 (CH_3 OEt); HRMS (MALDI) m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{19}\text{H}_{23}\text{NO}_2$, 298.1801; found, 298.1797.

(S)-Ethyl 2-(1*H*-benzo[*f*]isoindol-2(3*H*)-yl)-4-methylpentanoate (6c):

Yield 11.7 mg (15%); R_t 17.5 min (Eurospher 100 RP C-18 column; 150 × 4.5 mm i.d.; 5 μm; flow rate = 0.5 mL/min; mobile phase = 54% MeOH/0.1% TFA); ^1H NMR (600 MHz, CD_3OD) δ 8.06, (br s, 2H, H2,7), 7.87 (br s, 2H, H2,7), 7.51 (dd, 2H, H4,5, $^3J_{\text{HH}} = 6.3$ Hz, $^4J_{\text{HH}} = 3.4$ Hz), 4.80, 4.75 (m, 2H, CH_2 isoindole), 4.50 (m, 1H, α Leu), 4.15 (q, 2H, CH_2 OEt, $^3J_{\text{HH}} = 7.2$ Hz), 1.65 (m, 1H, γ Leu), 1.58 (m, 2H, β Leu), 1.24 (t, 3H, CH_3 OEt, $^3J_{\text{HH}} = 7.2$ Hz), 0.93, 0.90 (d, 6H, $^3J_{\gamma\delta} = 6.5$ Hz); ^{13}C NMR (150 MHz, CDCl_3) δ 171.7 (CO Leu), 129.3 (C3,6), 128.3 (C4,5), 123.2, (C2,7), 67.0 (α Leu), 62.5 (CH_2 OEt), 49.0 (CH_2 isoindole), 41.8 (β Leu), 26.1, (γ Leu), 23.3, 22.0 ($\delta\delta'$ Leu), 14.6 (CH_3 OEt); HRMS (MALDI) m/z [$\text{M} + \text{H}$] $^+$ calcd for $\text{C}_{20}\text{H}_{25}\text{NO}_2$, 312.1958; found, 312.1960.

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Supporting information

Materials and methods, computational details, FT-IR measurements, NMR spectra of all compounds, mass spectra of **4a–4e**, and products of Bergman cyclization. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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