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A synthetic route to enediyne-bridged amino acids

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Abstract—A general strategy for the preparation of enediyne-bridged amino acids has been disclosed. A cross-coupling reaction between amino- and carboxyl-modified amino acids under Sonogashira reaction conditions gave a protected enediyne–peptide conjugate. The model obtained can be used as a template in studies of Bergman cycloaromatization or peptide conformational preferences induced by the presence of the enediyne moiety.

1. Introduction

Structural diversity in natural products represents an invaluable source of inspiration for organic and medicinal chemists in their attempts to create first-line drug candidates. Naturally occurring enediyne anticancer antibiotics possess an exceptional biological profile due to their unique molecular structure, striking mode of action and high potency.[1,2] They are among the most effective anticancer drugs acting as DNA cleaving agents. On-site triggered Bergman cycloaromatization and subsequent proton abstraction from sugar phosphate skeletons leads to scission of the DNA double helix.[3,4] There is a growing body of research, theoretical and experimental, dedicated to the design and synthesis of structurally less demanding and more selective models of enediyne antibiotics, with focus on three main components: the active 'warhead',[5] the delivery system[6,7] and the triggering 'device'.[8] The enediyne moiety has been embedded within differently sized rings,[9,10] and activated through ligand-to-metal charge transfer[11] or photochemical triggering.[12] Also, enediynes equipped with a polyamine module as the DNA binding group showed potent DNA damaging ability.[13] All these findings prompted us to consider amino acids and peptides as enediyne carriers. Installing the enediyne functional group into the peptide framework is expected to introduce interesting properties to novel enediyne–peptide adducts. Peptides can increase solubility, alter acid–base properties and provide binding sites for metal complexation. On the other hand, rigidity of the enediyne unit is expected to influence peptide conformation. Although syntheses of enediyne moieties with amino- and carboxyl functional groups (enediynyl amino acids) have been reported,[14] we were interested in finding a *general procedure* for modification of *any amino acid* at the *N*- and *C*-terminus in a way suitable for the construction of an aliphatic or aromatic type of enediyne moiety (Fig. 1). This Letter reports the first example of C→N enediyne-bridged dipeptide unit.

The synthetic approach for amino group modification was examined on the commercially available lysine derivative Boc-Lys[Z(2-Cl)]–OH. To avoid undesired side-reactions, the carboxyl group was converted into an ester group (compound **1**, Scheme 1). The Boc group was removed and the compound obtained subjected to the next reaction without purification. To increase the amino group reactivity, a 2-nitrobenzenesulfonyl (oNbs)

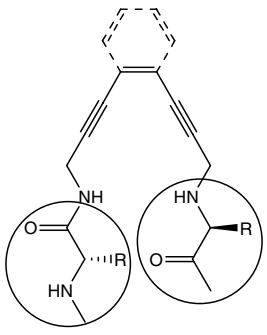

Figure 1.

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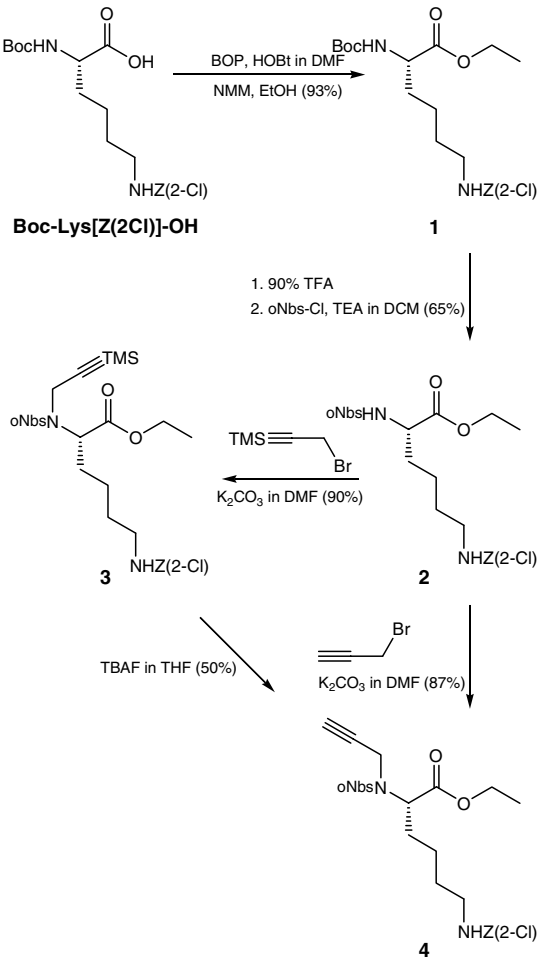
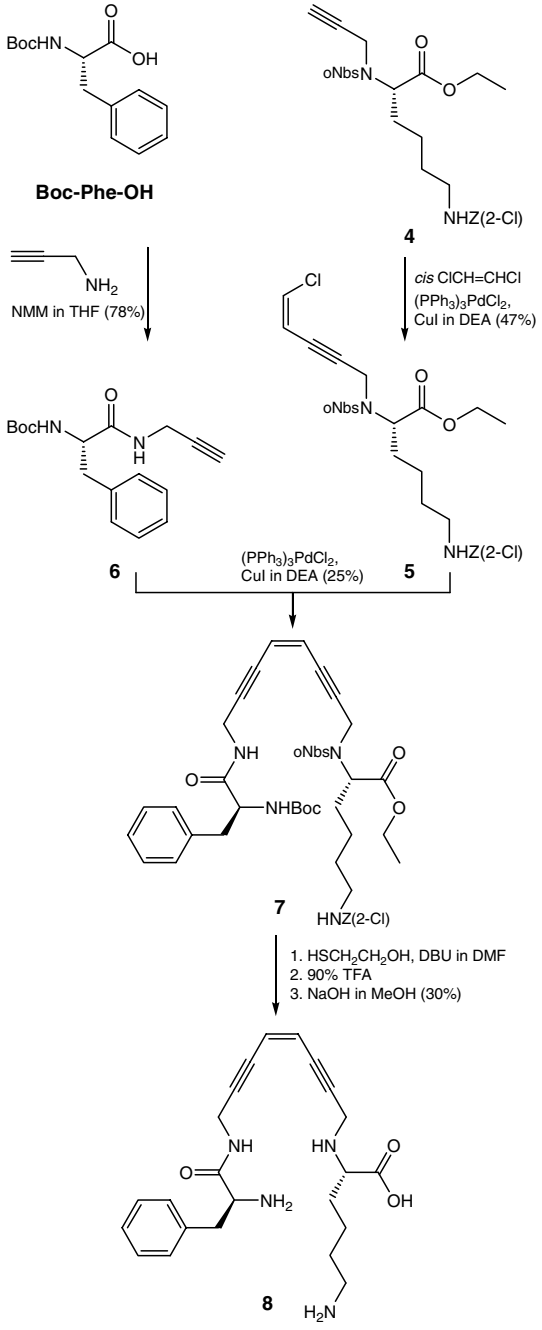


Scheme 1.

group was introduced by reaction with oNbs–Cl at room temperature in the presence of TEA to afford **2** in 65% yield. N-Alkylation of **2** with trimethylsilylpropargyl bromide in the presence of K_2CO_3 gave **3** in 90% yield. Attempts to remove the TMS group in **3** with a 1:1 mixture of THF and methanol containing a few drops of 1 M NaOH[15] revealed that this deprotection step proceeds slowly and was incomplete; hydrolysis of the ester bond also took place. An alternative procedure involved tetrabutylammonium fluoride (TBAF) in THF, which gave secondary amine **4** in an improved 50% yield. To simplify the reaction scheme, *N*-terminal activated lysine derivative **2** was treated with propargyl bromide under the same conditions as described for the preparation of **3**, and the N-alkylated derivative **4** was obtained in 87% yield.

Syntheses of enediyne moieties are well described in the literature. Generally, the cross-coupling reaction of sp^2-C halides and terminal acetylenes occurs through Pd–Cu catalyzed Sonogashira reaction.[16] However, a number of examples show that the actual reaction conditions strongly depend on characteristics of the aryl or alkenyl halide and acetylene compound involved in the cross-coupling reaction.[17] We have examined a number of different approaches, varying the type and quantity of base, solvent, catalyst and order of addition of reactants, and found optimal conditions for the construction of the chloroenyne derivative of lysine **5** (Scheme 2). Generally, *cis*-dichloroethene, CuI and Pd catalyst were dissolved in diethylamine and stirred at room temperature under argon for 15 min. A solution of acetylene **4** in diethylamine was added dropwise and the reaction mixture stirred for further 3 h. The chloroenyne derivative of lysine **5** was obtained in 47% yield after the column chromatography. The second part of an enediyne bridge was prepared from Boc-Phe–OH by converting the carboxyl group into a propargylamide group (Scheme 2), amide **6** was obtained in 78% yield using the mixed anhydride method. The intermolecular Pd–Cu catalyzed cross-coupling of terminal acetylene **6**

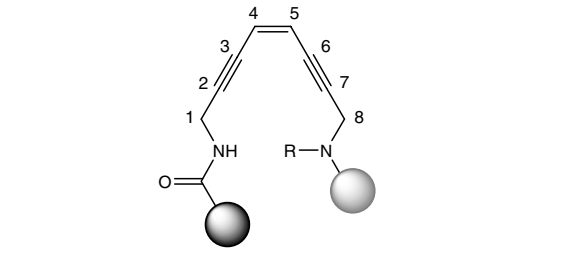


Scheme 2.

and chloroenyne **5** was carried out under the same conditions as described for **5**, and the enediyne-bridged Phe-Lys motif was obtained in 25% yield after the column chromatography. Compound **7** can be further used as a template in peptide chain elongation reactions or for studying the Bergman cyclization. We ran a deprotection scheme to test the susceptibility of various groups toward certain deprotection methods. The procedure was performed in a stepwise manner without isolation of intermediate products, however, the success of each step was checked by NMR analysis. Cleavage of the oNbs group was achieved with DBU/2-sulfanylethanol in DMF, through a Meisenheimer complex with subsequent loss of SO_2 and liberation of the amine. The next step was the cleavage of the acid labile Boc and Z(2-Cl) groups by treatment with 90% TFA. Finally, base-catalyzed ester hydrolysis gave the deprotected Phe-Lys bridged enediyne **8** in 30% overall yield (Scheme 2).

Characterization of all the compounds relied predominantly on 1D and 2D NMR analysis. Propargylation of sulfonamide nitrogen in **4** was evident by the appearance of a terminal acetylene proton at 2.26 ppm and diastereotopic CH_2 protons at 4.14 and 4.37 ppm (Table 1). The structure of compound **5** was confirmed by the loss of the terminal acetylene proton and the appearance of two signals at 5.80 and 6.35 Hz assigned to the *cis*-chloroethene moiety. Also, the signal at 5.80 ppm, assigned to H5 (Table 1) appeared as a weakly resolved td, due to coupling with the H88′ protons achieved through five bonds. NMR spectra of fully protected enediyne-bridged Phe-Lys compound **7** were recorded in DMSO-d_6 and $CDCl_3$. Although the ^1H NMR spectrum in DMSO was better resolved, for comparison purposes the chemical shifts in $CDCl_3$ are listed in Table 1. As for the initial compounds, the H11′ protons adjacent to the phenylalanine amide nitrogen appeared at 4.13 ppm, while the diastereotopic H88′ protons attached to the lysine sulfonamide nitrogen occurred at 3.98 and 4.10 ppm. The vinyl protons H4 and H5 were apparent at 6.15 ppm. In deprotected compound **8** only the H88′ protons proximal to the free amino group were shifted up-field due to the absence of the deshielding effect of the oNbs group.

Table 1. NMR chemical shifts of the enediyne bridge protons in compounds **4**–**8**[a]



	H11′	H3	H4	H5	H6	H88′
4					2.26	4.14
						4.37
5			6.35	5.80		4.35
						4.68
6	3.98	2.18				
7	4.13		6.15	6.15		3.98
						4.10
8	4.10		6.15	6.15		3.12
						3.30

[a] In $CDCl_3$ at room temperature.

In summary, we have reported general route for the synthesis of enediyne-bridged amino acid units. The procedure presented here will be used for the synthesis of peptide-based enediynes for the study of Bergman cycloaromatization and additionally for studying peptide conformational preferences induced by the presence of the enediyne moiety. The results of these studies will be published separately, however a preliminary data on the reactivity of compound **7** in the Bergman reaction is provided here. Treatment of **7**, dissolved in methanol, with 100 equiv of 1,4-cyclohexadiene (1,4-CHD) under anaerobic conditions in a sealed ampoule at 50 °C showed the disappearance of 50% of the starting material after 24 h (followed by NMR analysis), associated with the formation of the Bergman product. The reaction conducted under the same conditions at 100 °C showed complete conversion after 24 h. These initial data imply that peptide-based enediynes undergo Bergman cycloaromatization.

2. Synthesis of oNbs-(*cis*-ClCH=CH–C≡C–CH_2)-Lys-[Z(2-Cl)]–OEt (5)

cis-Dichloroethene (70 μl, 0.94 mmol), $(PPh)_3PdCl_2$ (32 mg, 0.047 mmol) and CuI (10 mg, 0.047 mmol) were dissolved in diethylamine (1 ml) and stirred for 15 min under argon. Compound **4** (260 mg, 0.47 mmol) was dissolved in diethylamine (3 ml) and added dropwise via syringe. After 3 h the solvent was evaporated and product extracted with ethyl acetate–water. The organic layer was washed with brine and water and dried over Na_2CO_3. After solvent evaporation the residue was purified by flash column chromatography on silica gel with petrol ether–ethyl acetate (3:2) as eluent to yield compound **5** as a yellow-brown oil (150 mg, 47%). ^1H NMR ($CDCl_3$): 1.13 (t, 3H, OEt, $^3J_{H,H} = 7.14$ Hz), 1.50 (m, 1H, γ Lys), 1.54 (m, 1H, δ Lys), 1.60 (m, 2H, γ′, δ′ Lys), 1.98, 2.04 (m, 2H, ββ′ Lys), 3.19 (m, 2H, ε Lys), 4.05 (q, 2H, OEt, $^3J_{H,H} = 7.10$ Hz), 4.35, 4.58 (dd, 2H, H11′ chloroenyne, $^2J_{H,H} = 19.00$ Hz $^5J_{H,H} = 1.35$ Hz), 4.68 (dd, 1H, α Lys, $^3J_{\alpha,\beta}$ 9.97 Hz, $^3J_{\alpha,\beta'}$ 5.10 Hz), 4.86 (br s, 1H, NHε Lys), 5.21 (s, 2H, CH_2 Z(2-Cl)), 5.80 (td, 1H, H4 chloroenyne, $^3J_{H,H}$ 7.45, $^5J_{H,H} = 1.35$ Hz), 6.35 (d, 1H, H5 chloroenyne, $^3J_{H,H}$ 7.45 Hz), 7.27 (m, 2H, H4,5 Z(2-Cl)), 7.38 (d, 1H, H5 Z(2-Cl), $^3J_{H,H} = 6.79$ Hz), 7.43 (d, 1H, H3 Z(2-Cl), $^3J_{H,H} = 6.69$ Hz), 7.62 (m, 1H, H5 oNbs), 7.70 (m, 2H, H4,6 oNbs), 8.16 (d, 1H, H3 oNbs, $^3J_{H,H} = 7.40$ Hz). Elemental Anal. Calcd for $C_{27}H_{29}Cl_2N_3O_8S$: C, 51.75; H, 4.67; N, 6.71. Found: C, 51.42; H, 4.81; N, 6.73. $R_f = 0.40$ (petrol ether–ethyl acetate 3:2). $[\alpha]_D$ 32.5 (*c* 1.04, MeOH).

3. Synthesis of *cis*-Boc-Phe–NH(CH_2–C≡C–CH=CH–C≡C–CH_2)(oNbs)-Lys[Z(2-Cl)]–OEt (7)

oNbs-(*cis*-ClCH=CH–C≡C–CH_2)-Lys[Z(2-Cl)]–OEt (**5**) (196 mg, 0.31 mmol) was dissolved in 3 ml of diethylamine together with $Pd(PPh_3)_4$ (35.8 mg, 0.031 mmol) and CuI (5.9 mg, 0.031 mmol) under argon. After 15 min acetylene **6** (100 mg, 0.31 mmol) in 3 ml of diethylamine was added dropwise. The reaction mixture was stirred for 5 h at room temperature and then extracted with ethyl acetate and water. After purification by flash column chromatography on silica gel by petrol ether–ethyl acetate 3:2, 70 mg (25%) of product **7** was isolated. ^{1}H NMR (DMSO-d_6): 0.98 (t, 3H, OEt, $^3J_{H,H}$ = 7.03 Hz), 1.28 (s, 9H, CH_3 Boc), 1.32 (m, 2H, γ, δ Lys), 1.34 (m, 2H, γ, δ Lys), 1.61, 1.67 (m, 2H, β Lys), 2.72, (dd, 1H, β Phe, $^2J_{\beta,\beta'}$ = 13.20 Hz, $^3J_{\alpha,\beta}$ = 10.31 Hz), 2.92 (m, 5H, β Phe, ε Lys, H8 bridge), 3.88 (m, 3H, OEt, α Lys), 4.13 (br s, 2H, H1 bridge), 4.12 (m, 1H, α Phe), 5.08 (s, 2H, CH_2 Z(2-Cl)), 6.96 (d, 1H NHα Phe, $^3J_{\alpha,NH}$ = 8.42 Hz), 7.18, 7.25 (d, 2H, H4,5 bridge, $^3J_{H,H}$ = 6.30 Hz), 7.26 (m, 5H Phe aromatic), 7.33 (t, 1H, NHε Lys, $^3J_{H,NH}$ = 5.24 Hz), 7.35, 7.46 (m, 4H, Z(2-Cl) aromatic), 7.84 (m, 2H, C4,5 oNbs), 7.94 (d, 1H, H6 oNbs, $^3J_{H,H}$ = 7.42 Hz), 7.99 (d, 1H, H3 oNbs, $^3J_{H,H}$ = 7.39 Hz), 8.50 (t, 1H, NH Phe amide, $^3J_{H,NH}$ = 4.70 Hz). Elemental Analy. Calcd for $C_{44}H_{50}ClN_5O_{12}S$: C, 58.18; H, 5.55; N, 7.71. Found: C, 57.82; H, 5.77; N, 7.43. R_f = 0.20 (petrol ether–ethyl acetate 3:2). $[\alpha]_D$ 28 (*c* 0.95, MeOH).

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