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Short asymmetric synthesis of a model monomeric unit for α/γ peptide foldamers based on β-amino linked *D*-leucine – *cis*-γ-butyrolactone γ-acetic acid conjugate

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Abstract: This synthetic work reports a concise, asymmetric synthesis of β-amino-γ-butyrolactone acetic acid – *D*-leucine hybrid **1** which will serve as model starting template sub-unit for preparing α/γ foldameric oligopeptides containing unnatural γ-amino acid residues. The synthesis of *cis*-substituted **1** was successfully achieved in six linear steps with very good stereoinduction.

Keywords: cis-γ-butyrolactone; foldamer; oligopeptide; asymmetric epoxide opening; γ-amino acid.

1. Introduction

Naturally-derived peptides and proteins constitute important structural and biochemical functions in many living organisms. These oligomeric scaffolds feature unique structural attributes through a display of functionalities arranged in three-dimensional space with well-defined folding architectures. Their potential in exhibiting excellent binding affinity and selectivity are desirable facets in relation to the discovery and development of new pharmacologically interesting materials. However, in many cases, these compounds do not hold much potential in drug discovery due to poor cell-interacting properties and susceptibility to proteolysis.[1] Thus, studies toward unnatural peptides that can complement protein secondary structures have found niche in chemical research.

Foldameric structures based on aliphatic oligoamides are regarded as homologs of natural α-peptides, where additional carbon atoms are introduced into the side-chain, furnishing β–, γ–, δ–, and other types of peptides.[2-3] In these systems, the amide bonds are bonded to aliphatic carbon chains. Most studied motifs have been mostly based on biologically active β-peptides. Interestingly, mixed

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sequences such as α/β– and β/γ-peptide sequences have also been prepared for medicinal chemistry applications.[4-6] While cyclopentane constrained β-amino acids has been reported to effect stability on certain helical motifs,[7-8] there is little literature evidence known for the preparation of foldameric oligoamides joined by γ–butyrolactone units. The incorporation of a lactone may presumably lead to more stabilized helical structures owing to additional H-bonding interaction of the carboxyl moiety and rigidity conferred by a oxo-tetrahydrofuran unit. Considering the value of peptide-based foldamers in structural chemistry and drug discovery, and as part of our continued interest to come up with novel, biologically active oligoamides, we hereby report a concise strategy for the asymmetric synthesis of a protected β-amino linked *D*-leucine–cis-γ-butyrolactone γ–acetic acid hybrid as model monomeric unit for α/γ foldameric structures.

2. Experimental

Infrared (IR) spectra (KBr) were recorded on a Perkin Elmer 1700X FTIR spectrophotometer instrument (KBr pellet). ^{1}H NMR spectra were recorded with a Bruker Avance 300 MHz spectrometer at 300 K, using TMS as an internal standard. Chemical shifts are expressed in parts per million (ppm, units). Coupling constants are in units of hertz (Hz). The spin multiplicities are indicated by the symbols s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), and br (broad). MS spectra were recorded on a Agilent GC–7890A MS 5975 and Agilent LCMS QTof 6520. TLCs and preparative thin-layer chromatography were performed on silica gel GF/UV 254, and the chromatograms were performed on silica gel (100-200 mesh) visualized under UV light at 254 nm and 365 nm. All solvents were of reagent grade and, when necessary, were purified and dried by standard methods.

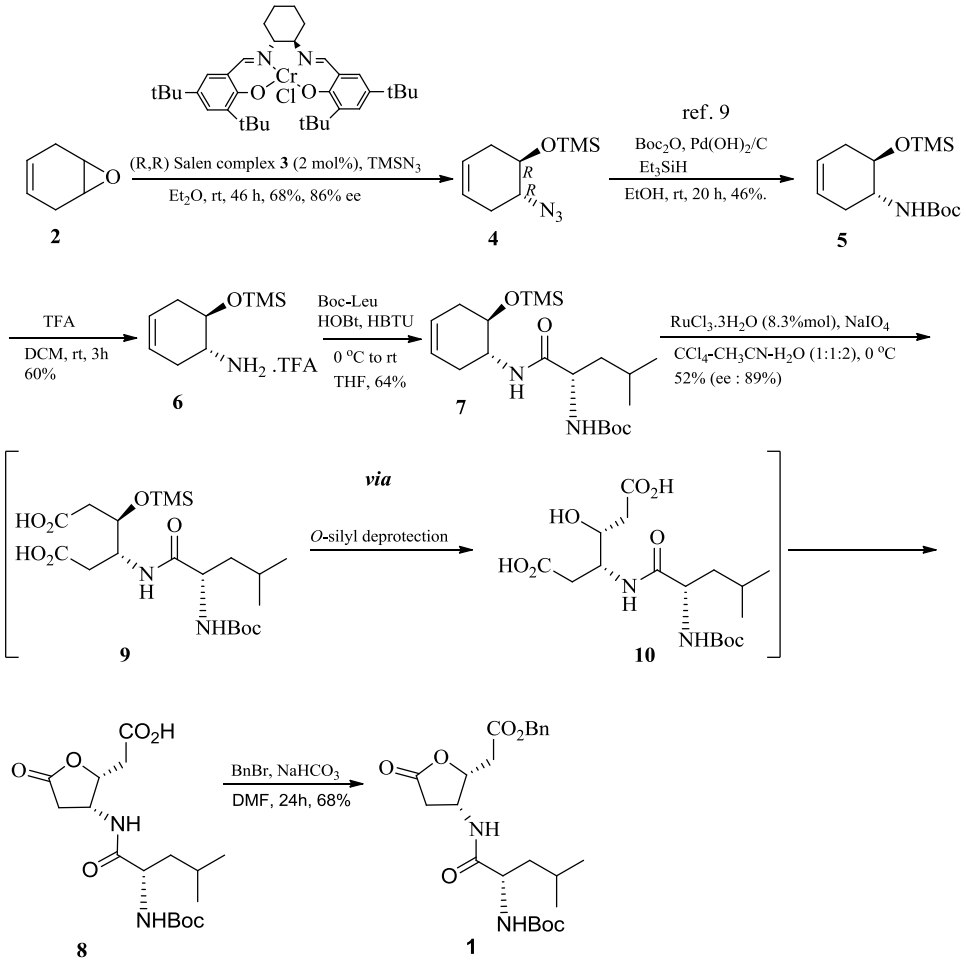
2.1 General procedure for synthesis of (1R,6R)-6-azidocyclohex-3-enyloxy trimethylsilane ***(4):*** To a mixture of epoxide **2** (0.61 g, 6.32 mmol, 1 equiv) in 2.1 ml of Et_2O was added catalyst complex **3** (7.99 mg, 0.126 mmol, 2 mol %). The mixture was stirred for 15 minutes and subsequently trimethylsilylazide (0.88 ml, 6.63 mmol, 1.05 equiv) was added slowly. After the mixture was stirred for 46 hours at room temperature then the solvent was evaporated under reduced pressure to give yellowish crude product, which was purified by column chromatography on silica gel (petroleum ether: ethyl acetate, 9:1) to yield 0.84g (68 %) of **4** as yellowish oil. R_f = 0.83 (SiO_2, hexanes/ethylacetate 9:1); $[\alpha]_D^{20}$=-14.8 (c = 0.4, DCM), 86% ee (chiral HPLC). IR (Film): $\tilde{\nu}$ = 2957, 2905, 2107, 1438, 1250, 1140, 881, 840, 748, 667 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 5.48-5.61 (m, 2H, H-1,H-2), 3.73-3.84 (m, 1H), 3.49-3.59 (m, 1H), 2.32-2.48 (m, 2H), 2.08-2.20 (m, 1H), 1.90-2.03 (m, 1H), 1.98 (s, 9-H, TMSO); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ = 124.6, 123.9, 71.9, 62.9, 34.7, 30.9, 0.0 (TMS); MS [CI, NH_3] : *m/z* (%) = 212.1 (11) $[M+H]^+$.

*2.2 Synthesis of 2-hydroxy-cyclohexyl)-carbamic acid tert-butyl ester (****5****):* To a stirred mixture of azido trimethylsiloxy cyclohexene **4** (0.2 g, 1.02 mmol, 1 equiv) in 6 mL ethanol, was added *tert*-butoxycarbonyl (Boc_2O) (0.33 g, 1.5 mmol, 1.5 equiv) and 20% $Pd(OH)_2/C$ (10.4 mg) at room temperature. Triethylsilane (0.28 mL, 1.74 mmol, 1.7 equi.) was added and the mixture stirred for 20 h under a N_2 atmosphere. The mixture was filtered through Celite and the filtrate was concentrated under reduce pressure to give yellowish solid which was purified by column chromatography on silica gel (petroleum ether: ethyl acetate, 15:1) to yield 0.13 g (46%) of compound **5** as yellowish solid. R_f = 0.27 (SiO_2, hexane/ethylacetate 15:1); M. pt. 79-81 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 5.52-5.57 (m, 2H), 3.48-3.59 (d, 1H), 3.84 (s, 1H), 2.36-2.465 (m, 2H), 2.05-2.09 (m, 2H), 1.38 (s, 9H), 0.85 (s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) (δ/ppm): 159.2, 124.3, 76.6, 65.1, 30.9, 28.4, 24.7; IR (v/cm^{-1}): 2982.14, 1738.28, 1446.43, 1235.04. IR (v/cm^{-1}): 3340.1, 2976.5, 1689.4, 1531.0, 1366.9, 1249.5, 1102.6, 887. MS [Cl, NH_3]: *m/z* = 285.24, HRMS-ESI, *m/z* 285.1758 (calcd for $C_{14}H_{27}NO_3Si$, 285.1760).

2.3 Synthesis of 4-amino-4-(6-hydroxy-cyclohex-3-enylcarbamoyl)-2-methyl-butyric acid tert-butyl ester (7): To a stirred solution of compound **5** (31 mg, 0.15 mmol, 1 equiv) in 1 mL DCM was treated with TFA (1 ml) at room temperature for 2 h. The solvent was removed *in vacuo*, diluted with EtOAc, washed with saturated aq Na_2CO_3, and with brine. The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to provide **6** (60%) which was used further for the next reaction without purification. Solution of Boc-*L*-Leu (30.0 mg, 0.16 mmol, 1 equiv) was stirred in dry THF (2 mL). HOBt (11.2 mg, mmol, 1.1 equiv), HBTU (30.0 mg, mmol, 1.0 equiv.) and ammonium salt **6** (20.0 mg, mmol, 1.1 equiv.) were added in dry THF at 0°C. The ice bath was removed and the reaction mixture was stirred for 24 hours at room temperature. The solvent was removed by rotary evaporator. The reaction mixture was dissolved in EtOAc (5 mL) and washed with 1M HCl (5 mL), 5% $NaHCO_3$ (5 mL) and brine (5 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (hexane: ethyl acetate as a solvent) to yield **7** as white solid (30.0 mg, 64%). R_f = 0.375 (SiO_2, hexane/ethylacetate 15:1); M. pt. 173-175 °C. 89% ee (chiral HPLC). ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 5.0 (m, 2H), 4.25-4.38 (m, 1H), 3.33-3.48 (m, 2H), 1.9-2.1 (m, 2H), 1.67-1.76 (m, 2H), 1.48 (s, 9H), 0.9-1.0 (m, 6H); ^{13}C NMR (75.5 MHz, $CDCl_3$) (δ/ppm): 156.0, 124.5, 81.6, 33.6, 28.3, 24.8, 22.9; IR (v/cm^{-1}): 3301.3, 2958.9, 1738.4, 1466.6, 1240.8, 1054.8, 848.5. MS [Cl, NH_3]: *m/z* (%) 399.3 $[M+H]^+$, HRMS-ESI (positive mode; *m/z* 399.2850 (calcd for $C_{20}H_{38}N_2O_4Si$, 399.2523).

*2.4 Synthesis of [3-(2-tert-butoxycarbonylamino-5-methyl-hexanoylamino)-5-oxo-tetrahydro-furan-2-yl]-acetic acid (8):*To a stirred solution of compound **7** (30.0 mg, 0.075 mmol, 1.0 equiv) in 4 ml of biphasic solution consisting of CCl_4:MeCN:H_2O (1:1:2) was added $RuCl_3.3H_2O$ (1.8 mg, 8.3 mol%) at 0 ºC, followed by $NaIO_4$ (65.8 mg, 0.31 mmol, 4.1 equiv.) portion wise. The reaction mixture was stirred for 8 hrs at 0°C. Then reaction mixture was diluted with 10 ml of distilled water and extracted with 15 ml DCM (3x). The organic layer was collected, combined, dried over anhydrous $MgSO_4$ and the solvent removed slowly under ambient temperature under reduced pressure to yield 14.6 mg (52%) of compound **8** as yellowish oil. R_f = 0.33 (SiO_2, ethyl acetate/methanol 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 6.85-6.95 (d, 1H, *J*= 7.8 Hz, NH),5.10-5.19 (m, 1H), 4.90-5.05 (m, 1H), 4.42-4.56 (m, 1H), 2.45-2.83 (m, 4H), 1.80-1.93 (m, 2H), 1.67-1.70 (m, 1H), 1.46-1.57 (s, 9H), 0.77-1.04 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) (δ/ppm): 173.1, 172.6, 171.3, 170.5, 156.4, 79.9, 78.9, 58.0, 47.9, 41.0, 37.4, 35.1, 28.3, 24.7, 23.9. IR (v/cm^{-1}): 3365.2, 2958.6, 1736.3, 1466.6, 1258.7, 1045.9, 844.6; MS [Cl, NH_3]: *m/z* (%) 373.3 $[M+H]^+$, HRMS-ESI (positive mode) *m/z* 373.1959 (calcd for $C_{17}H_{28}N_2O_7$, 373.1897).

2.5 [3-(2-tert-Butoxycarbonylamino-5-methyl-hexanoylamino)-5-oxo-tetrahydro-furan-2-yl]-acetic acid benzyl ester (1): To a stirred solution of **8** (20 mg, 0.05 mmol) in 4 ml dry DMF was added $NaHCO_3$ (28 mg, 6 equiv.), followed by benzyl bromide (0.1 ml, mmol, 1.6 equiv.) at room temperature and the reaction mixture was stirred for 24 hours. The mixture was diluted with 4 ml EtOAc:H_2O (1:1) and the solution was extracted with 5 ml EtOAc (3x) and the combine organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give yellow crude oil which was purified by column chromatography on silica gel (hexane: ethyl acetate) to give 17.2 mg (68%) of compound **1** as yellowish oil. R_f = 0.375 (SiO_2, ethyl acetate/methanol 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) (δ/ppm): 7.30-7.42 (m, 5H), 6.81-6.71 (d,1H, J=8.4, NH), 5.15-5.22 (s, 2H), 5.05-5.17 (m, 1H), 4.43- 4.50 (m, 1H), 2.53-2.95 (m, 4H), 1.70-2.05 (m, 2H), 1.51-1.60 (m, 1H), 1.45-1.50 (s, 9H), 1.25-1.30 (m, 6H); ^{13}C NMR (75.5 MHz, $CDCl_3$) (δ/ppm): 174.1, 172.4, 169.8, 156.1, 135.3, 128.6, 128.5, 128.4, 80.6 (C-OH), 78.9, 67.1, 58.0, 47.9, 41.1, 37.5, 36.9, 29.6, 24.8, 23.9, 22.6; IR (v/cm^{-1}): 3301.2, 2958.5, 1729.4, 1466.1, 1240.1, 1045.7, 846.6; MS [Cl, NH_3]: *m/z* (%) 480.3 $[M+NH_4]^+$, HRMS-ESI (positive mode) *m/z* 463.2430, calcd for $C_{17}H_{28}N_2O_7$, 463.2366.



Scheme 1. Synthetic pathway en route protected amino acid-β-amino γ–butyrolactone γ–acetic acid **1**

3. Results and Discussion

The asymmetric synthesis of model monomeric amide **1** was over-all carried-out in solution phase (Scheme 1). The synthetic preparation commenced with the asymmetric ring opening of cyclohexene epoxide **2** (prepared from benzene in two steps, 36%) using (*R,R*) Salen catalyst[6] complex **3** and trimethylsilylazide to furnish the *R,R* configurated azido trimethylsiloxy cyclohexene **4** in 68% yield and good enantioselectivity (86% ee using chiral HPLC)[9]. Reduction-protection sequences of azido trimethylsiloxy cyclohexene **4** with $Pd(OH)_2/C$ and triethylsilane in the presence of Boc_2O as trapping agent afforded **5**[10]. Removal of the *tert*-butoxycarbonyl protecting group was achieved with 30% TFA in anhydrous DCM to give the corresponding ammonium salt **6** in 60% yield. In this form, the trifluoroacetate salt is ready for coupling with amino acids. Thus, ammonium salt **6** was coupled with the readily available pre-activated natural amino acid, Boc-*D*-Leucine in the presence of HOBt/HBTU (as coupling reagents in dry THF) and diisopropyl ethyl amine to afford cyclohexene amide **7** in 64% yield. Oxidative lactonization of **7** using ruthenium tetroxide in the presence of sodium periodate as co-oxidant afforded the corresponding leucine-linked oxo-tetrahydrofuran γ–acetic acid **8**. The RuO_4 catalytic oxidative system was generated *in situ* from 8.3 mol % of $RuCl_3.3H_2O$ and 4.1 equivalents of $NaIO_4$ under biphasic conditions (CCl_4:MeCN:H_2O, 1:1:2) generating diacid **9** which readily reacted *in situ* with deprotected secondary alcohol **10**. The oxidative conversion of **7** was completed after 8 hours of stirring at 0 ºC to 5 ºC with improved

enantiomeric purity (89% ee). The geometric configuration of aminated C-3 and acetic acid-linked C-4 in compound 8 was established as *cis* on the basis of mechanistic considerations. Finally, to illustrate the amenability of the model amino acid template to be homologated with amino acids or peptides either at the alpha carboxyl or gamma amino positions after removal of protecting groups in either moieties, the carboxyl group was further protected with a benzyl group. Thus, treatment of compound **8** with benzyl bromide under basic conditions furnished the desired benzyl protected α-carboxyl γ–amino linked *D*-leucine butyrolactone derivative **1**.

Accordingly, we have established a synthetic protocol for preparing protected amino acid-β-amino γ–butyrolactone γ–acetic acid **1** in six linear steps starting from cyclohexene epoxide. Tandem asymmetric epoxide ring-opening/SN_2 and ruthenium-catalyzed oxidative ring served as key steps to induce enantioselectivity in the *cis*-β,γ-substituted lactone motif. Current investigations in our laboratories are geared towards the preparation and biological evaluation of oligopeptides inspired from this peptide sub-unit.

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