Research Article

Synthesis of Hybridpetide (β-α-α-α-) Comprising NDA-Val-Ala-Gly

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ABSTRACT

The construction of a tetrapeptide comprising natural α-amio acids valine, alanine, glycine and β amino acid (AZT) in β-α-α-α- fashion employing operationally simple reaction protocols has been described.

Keywords: oligomer, foldamer, coupling reaction, tetrapeptide, β amino acid.

1. INTRODUCTION

The diverse functions performed by proteins generally require distinctly folded peptide conformations, which will be governed by the α-amino acid sequence. The correlation between α-amino acid sequence and molecular structure among the α-peptides has inspired many researchers to explore unnatural oligomers that can adopt specific conformation.[1-3] In this context, importance of the heterogeneous or mixed backbones (containing natural and unnatural amino acids) as replacements for the α-amino acid backbone has been highlighted with an objective to conquer the susceptibility to proteolytic degradation and enhancing stability.[4-5]

Many research groups have tried to explore several unnatural oligomers to expand the foldamer chemistry by synthesizing backbones containing two different types of subunits, like, combination of α-amino acid residues with β-amino acid residues,[6-13] or with α-aminoxy acid residues,[14] or with γ-amino acid residues.[15-16] Few groups have explored the folding behavior of α/β-peptides (less than ten residues) present in 1:1 fashion.[3-6] Oligomers that contain both α- and β-amino acid residues, in regular patterns throughout the backbone are emerged as structural mimics of α-helix forming conventional peptides.[17] Vaijayanthi *etal.* have shown that the 1:1 α/NDA peptides exhibit binding affinity for DNA/RNA strands.[18] In order to know the conformational preferences, stability and spatial arrangement of new oligomers for subsequent design and development, we have taken up the synthesis of tetrapeptide **1** (Figure **1**). Herein, we have reported the synthesis of tetrapeptide **1** using natural α-amio acids (Valine, Alanine and Glycine) and β amino acid (AZT) in β-α-α-α- fashion.

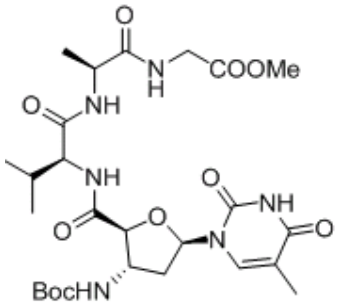
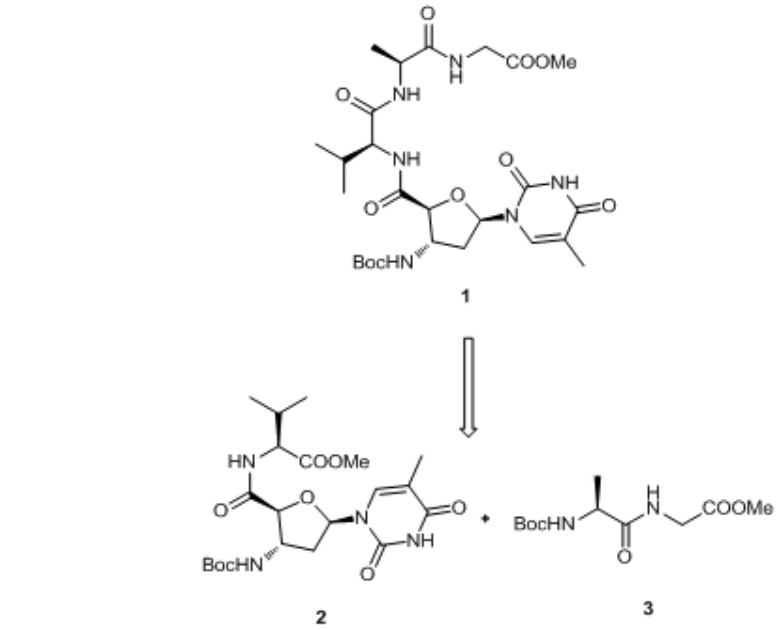
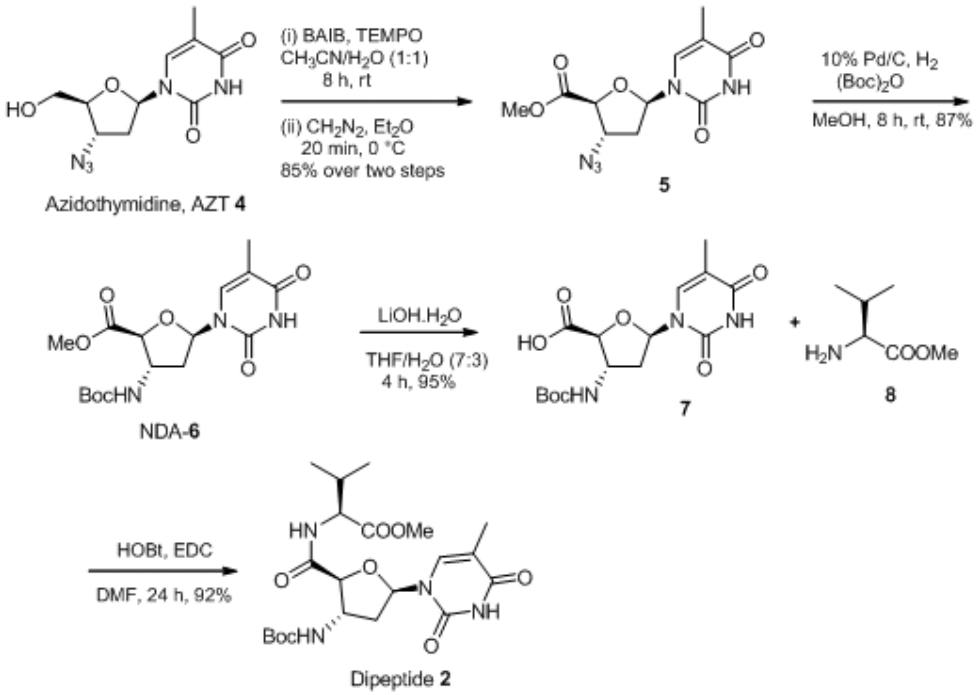


Fig. 1: Structure of tetrapeptide 1

2. RESULTS AND DISCUSSION



Scheme. 1: Retrosynthetic analysis of tetrapeptide 1

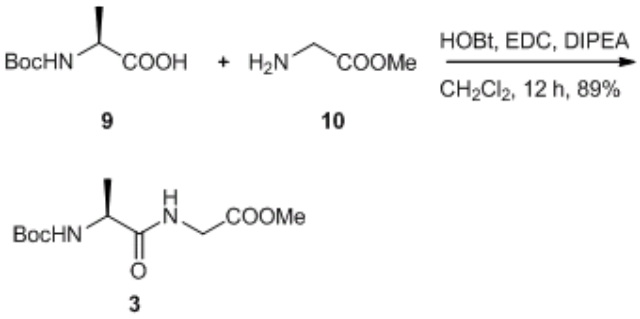


Scheme. 2: Synthesis of dipeptide 2

As a part of our ongoing research in the synthesis of peptide helical foldamers,[19-21] we have synthesized tetrapeptide **1**, comprised of *β* (unnatural) and *α* (natural) amino acids using standard peptide coupling conditions. Tetrapeptide **1** was retrosynthetically divided into two main dipeptide fragments **2** and **3** (Scheme 1).

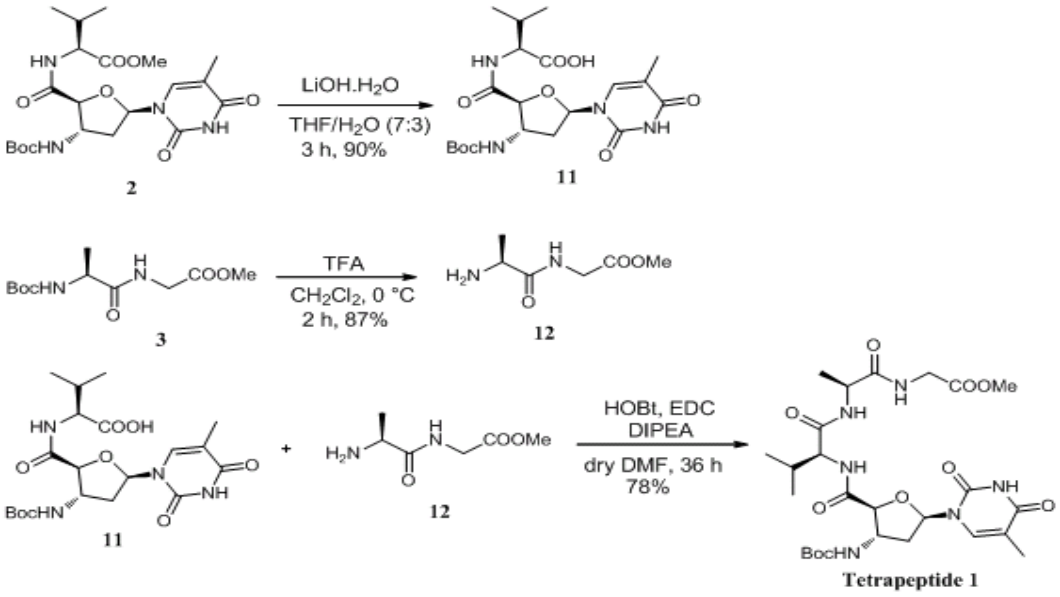
Synthesis of dipeptide **2** was commenced from commercially available 3′azido-3′-deoxythymidine (AZT) **4**. Compound **4** was oxidized to azido acid in CH_3CN/H_2O (1:1) with BAIB and TEMPO and esterified using diazomethane to furnish azido methyl ester **5** in 85% yield. This azido ester **5** was reduced to amino ester using 10% Pd/C in methanol and further protected as carbamate with $(Boc)_2O$ to provide NDA (Nucleoside Derived Aminoester) **6** (87% yield). Hydrolysis of compound **6** using LiOH afforded corresponding acid **7** in 95% yield. Dipeptide Boc-NDA-Val-OMe **2** was synthesized by coupling Boc-NDA acid **7** with (*S*)-valine methyl ester **8** in presence of EDC and HOBt as coupling agents in DMF in 92% yield (Scheme **2**).

Dipeptide **3** was synthesized by the coupling of Boc-(*S*)-alanine **9** to (*S*)-glycine methyl ester **10** in the presence of EDC and HOBt in CH_2Cl_2 in 89% yield (Scheme **3**).



Scheme. 3: Synthesis of dipeptide 3

Tetrapeptide **1** was synthesized using dipeptide fragments **2** and **3**. Dipeptide **2** was hydrolyzed to its corresponding acid **11** and was taken further for coupling reaction without purification. Later, deprotection of Boc group of dipeptide **3** was achieved using TFA in CH_2Cl_2 to afford amine **12**. Coupling acid **11** with amine **1**2 in the presence of HOBt, EDC and DIPEA in DMF resulted the required tetrapeptide in 78% yield.



Scheme. 4: Synthesis of tetrapeptide 1

3. CONCLUSION

In conclusion, we have achieved the synthesis of tetrapeptide comprising NDA-Val-Ala-Gly following simple and standard peptide coupling reaction protocols. Further investigations toward the study of turn properties and other applications are currently being studied and will be published accordingly.

4. ACKNOWLEDGMENTS

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5. Experimental Section

Spectral data for selected compounds

(2*S*,3*R*,5*R*)-Methyl-3-azido-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1-(2*H*)yl)-tetrahydrofuran-2carboxylate (5)

To a stirred solution of azidothymidine alcohol **4** (5 g, 18.7 mmol) in CH_3CN/H_2O (1:1, 120 mL), were added BAIB (12.05 g, 37.4 mmol) and TEMPO (932 mg, 5.6 mmol) at 0 °C and allowed to stir at room temperature. After 6 h, the reaction mixture was quenched with saturated solution of $Na_2S_2O_3$ (80 mL) and extracted with ethyl acetate (2 x 50 mL). The combined extract was dried over Na_2SO_4 and concentrated under reduced pressure to give acid (4.4 g, 85%). The crude acid (4.4 g, 15.9 mmol) was dissolved in dry ether (50 mL) (methanol was added till it become clear solution (10 ml)) and treated with ethereal solution of diazomethane (3.2 g, 31.3 mmol) at 0 °C. After 30 min, aqueous NH_4Cl solution was added and extracted with ethyl acetate. The combined organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Silica gel column chromatography (30% ethyl acetate/hexane) of the residue gave azido ester **5** (4.2 g, 90% yield) as a liquid.

IR (KBr): ν_{max} 3185, 3048, 2109, 1747, 1695, 1470, 1274, 1100, 761 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.90 (br, 1H), 7.93 (s, 1H), 6.32 (dd, *J* = 7.6, 5.9 Hz), 4.5 (d, *J* = 1.6 Hz, 1H), 4.39 (dt, *J* = 5.9, 2.5 Hz, 1H), 3.86 (s, 3H), 2.47 (ddd, *J* = 13.5, 5.9, 2.5 Hz, 1H), 2.18 (ddd, *J* = 14.4, 5.9, 2.5 Hz, 1H), 1.96 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.5, 163.9, 150.4, 135.7, 111.4, 86.4, 81.5, 63.7, 52.9, 36.4, 12.5; HRMS: Calculated for $C_{11}H_{13}N_5O_5Na$ $[M+Na]^+$ 318.0809; found 318.0825; $[\alpha]^{20}_D$: + +75.7 (*c* = 2.0, $CHCl_3$).

(2*S*,3*R*,5*R*)-Methyl-3-(*tert*-butoxycarbonylamino)-5-(5-methyl-2,4-dioxo-3,4-di hydropyrimidin-1(2*H*)-yl)-tetrahydrofuran-2-carboxylate (6)

To a stirred solution of azido ester **5** (4 g, 13.5 mmol) in methanol (30 mL), was added 10% Pd/C (120 mg) and $(Boc)_2O$ (3.7 g, 17.3 mmol) at room temperature. To the reaction mixture was applied hydrogen pressure (atmospheric) for 5 h. The reaction was diluted with $CHCl_3$ (50 mL), filtered through a small pad of celite, methanol was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using ethyl acetate and hexane (40%) to give Boc-protected amine **6** (4.3 g, 87%, over two steps) as a white solid.

IR (KBr): ν_{max} 3318, 3192, 2981, 2109, 1746, 1698, 1471, 1273, 1072, 757 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 11.34 (br, 1H), 7.87 (s, 1H), 7.56 (d, *J* = 7.1 Hz, 1H), 6.23 (t, *J* = 5.9 Hz, 1H), 4.29-4.23 (m, 2H), 3.68 (s, 3H), 2.27-2.18 (m, 2H), 1.79 (s, 3H), 1.39 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.3, 163.9, 155.2, 150.5, 136.3, 109.5, 85.2, 81.3, 78.7, 53.9, 52.5, 35.7, 28.2, 12.5; HRMS: Calculated for $C_{16}H_{23}N_3O_7Na$ $[M+Na]^+$ 392.1428; found 392.145; $[\alpha]^{20}_D$: -6.0 (*c* = 0.2, $CHCl_3$).

(*S*)-Methyl-2-((2*S*,3*S*,5*R*)-3-(*tert*-butoxycarbonylamino)-5-((*S*)-5-methyl-2,6-dioxo-1,2,3,6-tetrahydropyridin-3-yl)-tetrahydrofuran-2-carboxamido)-3-methyl butanoate (3)

(*S*)-Valine (0.5 g, 4.2 mmol) was added to an ice cooled solution of acetyl chloride (0.45 mL, 6.4 mmol) in methanol (10 mL) under nitrogen atmosphere. The reaction mixture was refluxed for 3 h and then, solvent was removed under reduced pressure. The residue was dissolved in dry DMF (2 mL) and basified (pH = 8-9) with DIPEA at 0 °C to get free amine **8** (0.45 g, 82% yield).

In another round bottom flask, to a stirred solution of *N*-Boc monomer acid (NDA acid) **7** (1.24 g, 3.5 mmol) in dry DMF (10 mL), were added HOBt (0.47 g, 3.5 mmol) and EDC (1.33 g, 7 mmol) at 0 °C. After 15 min, the basified solution of (*S*)-valine methyl ester **8** (0.45 g, 3.5 mmol) was added to the reaction mixture at 0 °C and stirred for 24 h at room temperature. The reaction mixture was diluted with ethyl acetate (20 mL), washed with aqueous NH_4Cl (30 mL), aqueous $NaHCO_3$ (20 mL) and brine (20 mL). The combined organic layer was dried over Na_2SO_4, concentrated in *vacuo* and purified by silica gel column chromatography using ethyl acetate/hexane (4:1) to afford Boc-NDA-Val-

OMe dipeptide **2** (1.5 g, 92% yield) as a white solid.

IR (KBr): ν_{max} 3587, 3338, 3187, 2976, 1655, 1438, 1148, 1073, 783 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.33 (s, 1H), 8.30 (s, 1H), 7.55 (d, *J* = 8.6 Hz, 1H), 6.90 (s, 1H), 6.56 (dd, *J* = 8.3, 5.2 Hz, 1H), 4.62 (s, 1H), 4.51 (q, *J* = 8.6, 4.3 Hz, 1H), 4.34 (t, *J* = 5.7 Hz, 1H), 3.76 (s, 3H), 2.41 (dd, *J* = 13.4, 4.5Hz, 1H), 2.31 (m, 1H), 2.05 (m, 1H), 1.97 (s, 3H), 1.51 (s, 9H), 1.02 (d, *J* = 6.7 Hz, 3H), 0.98 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.1, 171.1, 163.6, 156.5, 150.9, 136.1, 111.8, 86.6, 84.0, 80.9, 57.2, 56.5, 52.1, 35.4, 30.3, 28.3, 19.1, 17.3, 12.5; HRMS: Calculated for $C_{21}H_{33}N_4O_8$ $[M+H]^+$ 469.2293 found 469.2315; $[\alpha]^{20}_D$: + 15.4 (*c* =2, MeOH).

(*S*)-Methyl-2-(2-(*tert*-butoxycarbonyl amino)propanamido)acetate(3)

To a solution of (*S*)-alanine (0.5 g, 5.6 mmol) in dioxane (10 mL) and water (10 mL), were added sequentially NaOH (0.22 g, 5.6 mmol) and $(Boc)_2O$ (2.44 mL, 11.2 mmol) at 0 °C and stirred at the room temperature for 5 h. Reaction mixture was cooled to 0 °C and acidified using citric acid until pH becomes acidic. Reaction mixture was extracted with ethyl acetate (3 x 20 mL), dried over sodium sulphate, and concentrated under reduced pressure to get compound *N*-Boc-alanine **9** (1 g, 94%).

In another round bottom flask, (*S*)-glycine (1 g, 13.3 mmol) was added to an ice cooled solution of acetyl chloride (1.42 mL, 19.9 mmol) in methanol (10 mL) under nitrogen atmosphere and refluxed for 3 h. Solvent was removed under reduced pressure and basified (pH = 8-9) with DIPEA at 0 °C to get (*S*)-glycine methyl ester **10** (0.96 g, 81% yield).

To a solution of (*S*)-Boc-alanine **9** (1.06 g, 5.6 mmol) in dry CH_2Cl_2 (10 mL) were added added HOBt (0.75 g, 5.6 mmol) and EDC (2.14 g, 11.2 mmol) at 0 °C and stirred for 15 min. To this reaction mixture, a solution of (*S*)-glycine methyl ester **10** (0.5 g, 5.6 mmol) in dry CH_2Cl_2 (10 mL) was added at 0 °C and stirred at room temperature for 12 h. The reaction mixture was diluted with dichloromethane (20 mL), washed with aqueous NH_4Cl (30 mL), aqueous $NaHCO_3$ (15 mL) and brine (15 mL). The combined organic layer was dried over Na_2SO_4, concentrated in *vacuo* and purified by silica gel column chromatography (ethyl acetate/hexanes, 3:7) to afford Boc-Ala-Gly-OMe dipeptide **3** as a liquid (1.22 g, 89%).

IR (KBr): ν_{max} 3324, 2979, 2929, 1752, 1673, 1522, 1218, 1169, 772 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ δ 6.95 (br, 1H), 5.23 (d, *J* = 6.9 Hz, 1H), 4.30-4.15 (m, 1H), 4.01 (d, *J* = 5.2 Hz, 2H), 3.72 (s, 3H), 1.41 (s, 9H), 1.35 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ δ 173.2, 170.1, 155.4, 79.9, 52.1, 49.7, 40.9, 28.1, 18.3; HRMS: Calculated for calculated for $C_{11}H_{20}N_2O_5Na$ $[M+Na]^+$ 283.1264 found 283.129; $[\alpha]^{20}_D$: -19.5 (*c* = 2, $CHCl_3$).

Methyl-2-((*S*)-2-((*S*)-2-((2*S*,3*S*,5*R*)-3-(*tert*-butoxycarbonylamino)5(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1-(2*H*)-yl) tetrahydro-furan-2-carboxamido)-3-methyl butanamido) propanami-do) acetate(1)

To a solution of Boc-NDA-Val-OMe **2** (0.6 g, 1.2 mmol) in THF:H_2O (7:3, 10 mL) was added $LiOH.H_2O$ (0.161 g, 3.8 mmol) at 0 °C and stirred for 3 h. Reaction mixture was acidified with potassium bisulphate, extracted with ethylacetate (2 x 20 mL). Combined organic layer was dried over sodium sulphate and concentrated under reduced pressure to give Boc-NDA-Val-acid **11** (0.52 g, 90%).

In another round bottom flask, to a solution of Boc-Ala-Gly-OMe **3** (0.33 g, 1.2 mmol) in CH_2Cl_2 (5 mL) was added TFA (0.3 mL, 3.8 mmol) at 0 °C and stirred at the room temperatue for 2 h. Solvent was evoparated and washed with toluene (2 x 5 mL) to provide salt of dipeptide which was further basified with DIPEA to afford free amine NH_2-Ala-Gly-OMe **12** (0.184 g, 87%).

To a solution of Boc-NDA-Val-acid **11** (0.52 g, 1.1 mmol) in dry DMF (15 mL) were added HOBt (0.15 g, 1.1 mmol) and EDC (0.44 g, 2.3 mmol) at 0 °C and stirred for 20 min. To this reaction mixture, the basified solution of NH_2-Ala-Gly-OMe **12** (0.61 g, 2.3 mmol) in dry DMF (10 mL) was added at 0 °C and stirred at room temperature for 36 h. The reaction mixture was diluted with ethyl acetate (30 mL), washed with saturated solution of aqueous NH_4Cl (30mL), aqueous $NaHCO_3$ (20 mL) and brine (20 mL). The combined organic layer was dried over Na_2SO_4, concentrated in *vacuo* and purified by silica gel column chromatography (2% methanol /chloroform) to furnish the required Boc-NDA-Val-Ala-Gly-OMe tetrapeptide **1** as a solid (0.53 g, 78%).

IR (KBr): ν_{max} 3294, 2926, 1678, 1533, 1219, 1167, 772 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 11.30 (br, 1H), 8.24-8.14 (m, 3H), 7.99 (s, 1H), 7.55 (d, *J* = 7.1 Hz, 1H), 6.26 (t, *J* = 6.6 Hz, 1H), 4.37-4.29 (m, 2H), 4.28-4.23 (m, 1H), 4.22-4.17 (m, 1H), 3.90-3.84 (dd, *J* = 6.0, 5.7 Hz, 1H), 3.82-3.77 (dd, *J* = 6.0, 5.7 Hz, 1H), 3.6 (s, 3H), 2.34-2.26 (m, 1H), 2.20-2.13 (m, 1H), 2.03-1.95 (m, 1H), 1.75 (s, 3H), 1.38 (s, 9H), 1.23 (d, *J* = 7.1 Hz, 3H), 0.87 (d, *J* = 6.6 Hz, 3H), 0.82 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.3, 170.8, 170.0, 169.5,

163.7, 155.5, 150.2, 163.5, 135.9, 109.7, 85.3, 83.0, 78.8, 67.3, 58.3, 54.0, 51.3, 48.0, 38.0, 31.1, 27.9, 18.7, 17.4, 12.0; HRMS: Calculated for calculated for $C_{26}H_{41}N_6O_{10}$ $[M+H]^+$ 597.2825, found 597.2898; $[\alpha]^{20}_D$: -8.7 (*c* = 0.7, MeOH).

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