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Stereoselective Synthesis of (2*R*)-6-Benzyloxycarbonylamino-2-*tert*-butoxycarbonylaminohex-4-ynoic Acid

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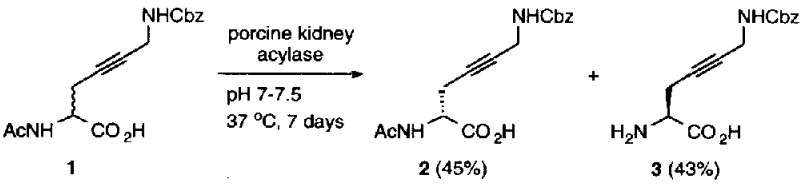
Abstract: A stereoselective synthesis of a constrained analog of D-lysine is presented.

A stereoselective synthesis of (2*R*)-2,6-diaminohex-4-ynoic acid, suitably protected for peptide synthesis is presented. This non-natural amino acid derivative can serve as a useful constrained D-lysine mimic. It can also potentially be incorporated into a substrate of interest and reduced with tritium to provide a radiolabeled D-lysine-containing compound. The corresponding L-lysine mimic was prepared based on the



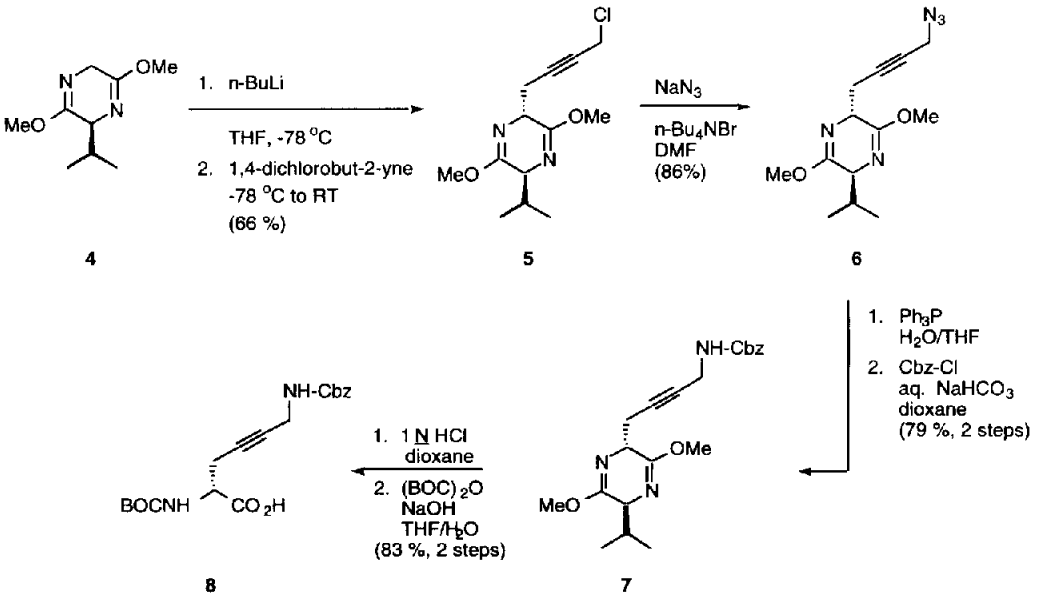
1 **2** (45%) **3** (43%)

procedures of Sasaki *et al.* or Jansen *et al.*[1] Briefly, this entails carrying out an enzymatic resolution of 6-benzyloxycarbonylamino-2-acetylaminohex-4-ynoic acid (**1**) using porcine kidney acylase to give (2*R*)-6-benzyloxycarbonylamino-2-acetylaminohex-4-ynoic acid (**2**) and (2*S*)-6-benzyloxycarbonylamino-2-

aminohex-4-ynoic acid (**3**). The amino acid **3** precipitates from the reaction mixture and after reaction with di-*t*-butyl pyrocarbonate gives (2*S*)-6-benzyloxycarbonyl-amino-2-*tert*-butoxycarbonylaminohex-4-ynoic acid. In theory, the N-acylated by-product from the enzymatic resolution can be used to furnish the corresponding D-lysine mimic. Unfortunately, our attempts to hydrolyse the acetyl group under acidic conditions (6N HCl, reflux) was accompanied by concommitant loss of the 6-Cbz group and varying amounts of HCl addition to the alkyne. Attempts to saponify the acetyl group under basic conditions (aqueous KOH in EtOH or aqueous NaOH in MeOH) was also accompanied by concommitant loss of the 6-Cbz group. Although it is possible to re-acylate the 6-amino group of the diaminoacid via a copper chelate[2], the reported difficulties and low yield of product obtained with the enantiomeric compound made this route unattractive to us.[1(b)] To circumvent these problems, a stereoselective synthesis of this non-natural amino acid was examined.

Deprotonation of the bis-lactim ether **4** with *n*-BuLi followed by adding the resulting anion to excess 1,4-dichloro-2-butyne gave the desired propargylic chloride **5** as the major product (66% yield).[3,4] A small amount (8%) of the diastereomeric alkylation product was also isolated. The reaction of **5** with NaN_3 in the presence of *n*-Bu_4NBr gave the corresponding propargylic azide **6** in excellent yield. *n*-Bu_4NBr was required to ensure reproducible high yields of product.[5] The azide was reduced with triphenylphosphine and the resulting amine was protected with a Cbz-group to give **7** as shown.

Hydrolysis of the chiral auxiliary by the action of dilute acid gave the corresponding amine of **8** and (*S*)-valine methyl ester as their hydrochloride salts. Treatment of this mixture with di-*t*-butyl pyrocarbonate under Schotten-Baumann conditions gave the *t*-butoxycarbonyl protected amines with concomitant hydrolysis



of the methyl esters. Flash chromatography on silica gel afforded enantiomerically pure **8** (>97 %ee).[6] This material can now be used with standard solution or solid phase peptide synthesis techniques compatible with N^{α}-*tert*-butyloxycarbonyl protecting groups.

In conclusion, we have developed an efficient synthesis of a mimic of D-lysine starting from the known bis-lactim ether **4**. The protected amino acid **8** can serve as a D-lysine mimic having a side-chain constrained to an extended conformation. Alternatively, **8** can be substituted for a D-lysine residue in a substrate of interest where the alkyne moiety can subsequently be radiolabled.

Experimental

General. ^{1}H NMR spectra were recorded with a Bruker AMX400 spectra with chemical shifts reported as ppm downfield from internal TMS, with multiplicity, number of protons and coupling constant(s) in Hertz indicated parenthetically. Mass spectra were taken on either VG 70 FE, PE Syx API III or VG ZAB HF instruments. Flash chromatography was carried out using E. Merck Kieselgel 60, 230-400 mesh silica gel.

(3*R*, 6*S*)-3-(4-Chloro-2-butynyl)-2,5-dimethoxy-6-isopropyl-3,6-dihydropyrazine (5). To the bis-lactim ether **4** (0.31 g, 1.70 mmol) in THF (5 mL) at -78 °C was added dropwise *n*-BuLi (0.75 mL of a 2.5 M solution in hexanes, 1.90 mmol). The reaction was stirred at -78 °C for 30 min and then added to a solution of 2,4-dichloro-2-butyne (0.67 mL, 6.80 mmol) in THF (5 mL) at -78 °C. After an additional 1h at -78 °C, the cooling bath was removed and the reaction was stirred for another 17h while warming to RT. The reaction was quenched by adding sat. NH_4Cl (5 mL). The mixture was poured into H_2O (50 mL) and extracted with Et_2O (4 x 50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to give a dark yellow oil. Flash chromatography (10% Et_2O/hexane, silica gel) afforded 0.30 g (66%) of the desired product as a clear oil. The diastereomer of **5** was also isolated (0.04 g, 8%).

5: ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.35 (t, J = 2.1 Hz, 2H, C$\underline{H}_2$-Cl), 4.19 (t, J = 4.2 Hz, 1H), 4.02 (t, J = 3.4 Hz, 1H), 3.64 (s, 3H, OC$\underline{H}_3$), 3.63 (s, 3H, OC$\underline{H}_3$), 2.70 (m, 2H, C$\underline{H}_2$-C), 2.21 (m, 1H, $(CH_3)_2C\underline{H}$), 1.01 (d, J = 6.8 Hz, 3H, $(C\underline{H}_3)_2CH$), 0.62 (d, J = 6.8 Hz, 3H, $(C\underline{H}_3)_2CH$). MS (ES+) for $C_{13}H_{19}ClN_2O_2$ m/z 271.0 ($M+H^+$).

Diastereomer of **5**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.29 (m, 2H, C$\underline{H}_2$-Cl), 3.94 (m, 2H), 3.61 (s, 3H, OC$\underline{H}_3$), 3.60 (s, 3H, OC$\underline{H}_3$), 2.72 (m, 2H, C$\underline{H}_2$-C), 2.21 (m, 1H, (CH$_3$)$_2$C$\underline{H}$), 1.01 (dd, J = 6.8, 21.7 Hz, 3H), 0.68 (dd, J = 6.8, 5.9 Hz, 3H).

(3*R*, 6*S*)-3-(4-Azido-2-butynyl)-2,5-dimethoxy-6-isopropyl-3,6-dihydropyrazine (6). Compound **5** (0.26 g, 0.95 mmol) in anhydrous DMF (4 mL) was added to a flask containing NaN_3 (0.33 g, 5.00 mmol) and *n*-Bu_4NBr (35 mg, 0.10 mmol). The mixture was stirred at RT for 22h, then poured into 1N HCl (50 mL) and saturated NaCl (50 mL). The aqueous phase was extracted with Et_2O (3 x 50 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated to give a yellow oil. Flash chromatography (10% Et_2O/hexane, silica gel) afforded 0.23 g (86%) of the desired product as an oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.20 (m, 1H), 4.01 (m, 3H), 3.65 (s, 3H, OC$\underline{H}_3$), 3.63 (s, 3H, OC$\underline{H}_3$), 2.74 (m, 2H, C$\underline{H}_2$-C), 2.20 (m, 1H, (CH$_3$)$_2$C$\underline{H}$), 1.01 (d, J = 6.9 Hz, 3H, (C$\underline{H}_3$)$_2$CH), 0.62 (d, J = 6.8 Hz, 3H, (C$\underline{H}_3$)$_2$CH). MS (ES+) for $C_{13}H_{19}N_5O_2$ m/z 278.0 ($M+H^+$).

(3*R*, 6*S*)-3-(4-Benzyloxycarbonylamino-2-butynyl)-2,5-dimethoxy-6-isopropyl-3,6-dihydropyrazine (7). To **6** (0.22 g, 0.78 mmol) in THF (5 mL) was added PPh_3 (0.25 g, 0.94 mmol) and H_2O (50 uL, 2.8 mmol). The mixture was stirred at RT for 19h, then concentrated to give a white solid. This material was dissolved in dioxane (5 mL) and cooled to 0 °C. A solution of $NaHCO_3$ (0.21 g, 2.50 mmol) in H_2O (2.5 mL) was added, followed by benzyl chloroformate (0.36 mL, 2.50 mmol). The reaction mixture was stirred for 4.5h while gradually warming to RT. The reaction mixture was diluted with H_2O (20 mL) and extracted with $CHCl_3$ (4 x 25 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to yield a yellow oil. Flash chromatography (20% EtOAc/hexane, silica gel) afforded 0.24 g (79% yield, 2 steps) of the desired product as a clear oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.61 (m, 1H, N$\underline{H}$), 7.35 (m, 5H, Ar-$\underline{H}$), 5.03 (s, 2H, ArC$\underline{H}_2$CO), 4.15 (m, 1H), 4.05 (m, 1H), 3.69 (m, 2H, C$\underline{H}_2$NH), 3.65 (s, 3H, OC$\underline{H}_3$), 3.63 (s, 3H, OC$\underline{H}_3$), 2.62 (m, 2H, C$\underline{H}_2$-C:::C), 2.23 (m, 1H, (CH$_3$)$_2$C$\underline{H}$), 1.01 (d, J = 6.8 Hz, 3H, (C$\underline{H}_3$)$_2$CH), 0.62 (d, J = 6.8 Hz, 3H, (C$\underline{H}_3$)$_2$CH). MS (ES+) for $C_{21}H_{27}N_3O_4$ m/z 386.2 ($M+H^+$).

(2*R*)-6-Benzyloxycarbonyl-2-*tert*-butoxycarbonylaminohex-4-ynoic acid (8). To **7** (0.23 g, 0.60 mmol) in dioxane (2.4 mL) was added 1N HCl (2.40 mL, 2.40 mmol). The mixture was stirred for 20h at RT, then concentrated to give an oil. This residue was azeotroped to dryness with toluene (5 x 5 mL) and then suspended in dioxane (5 mL). 1N NaOH (5 mL) and di-*tert*-butyl dicarbonate (0.39 g, 1.80 mmol) were added and the mixture was stirred at RT for 22h. The reaction was quenched by adding 1N HCl (10 mL) and H_2O (50 mL). The aqueous phase was extracted with $CHCl_3$ (4 x 50 mL) and the combined organic extracts were dried over Na_2SO_4 and concentrated to give a pink oil. Flash chromatography (50% EtOAc/hexanes + 1%

AcOH, silica gel) afforded 0.19 g (83% yield, 2 steps) of the desired product as a clear oil. $[\alpha]_D^{25}$: -15.0 (c 0.3, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (s, 1H), 7.65 (broad s, 1H), 7.35 (m, 5H, Ph), 7.05 (d, J = 7.9 Hz, 1H), 5.01 (s, 2H, PhC$\underline{H}_2$), 4.05 (m, 1H, α-CH), 3.77 (m, 2H, C$\underline{H}_2$NH)), 2.55 (m, 2H, C$\underline{H}_2$C), 1.38 (s, 9H, BOC). MS (ES+) for $C_{19}H_{24}N_2O_6$ m/z 377.2 ($M+H^+$).

Acknowledgements

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References and Notes

1. (a) Sasaki, A. N.; Morgat, J. L.; Bricas, E. *Pept., Proc. Eur. Pept. Symp., 16th* **1981**, 224. (b) Sasaki, A. N.; Bricas, E. *Tetrahedron Lett.* **1980**, *21(44)*, 4263. (c) Jansen, A. C. A.; Weastink, R. J. M.; Kerling, K. E. T.; Havinga, E. *Recl. Trav. Chim. Pays-Bas* **1969**, *88(8)*, 819.
2. A similar strategy has been employed to selectively protect the 6-amino group of lysine. See Schwzer, R.; Rittel, W. *Helv. Chim. Acta* **1961**, *44*, 159.
3. Schöllkopf, U.; Neubauer, H. -J. *Synthesis* **1982**, 861 and references cited therein.
4. Compound **4** is commercially available from E. M. Merck. It can also be conveniently prepared from valine-glycine diketopiperazine according to the method of Schöllkopf.[2] We found that reproducible, high yields of **4** were only obtained if a freshly prepared solution of Me_3OBF_4 in CH_2Cl_2 was used. The commercially available solution of Me_3OBF_4 from Aldrich or Fluka gave variable low yields of the desired compound. It was also crucial to quench the reaction by pouring it into a phosphate buffer at pH 7.2 and not *vice versa*.
5. (a) Koziara, A. *J. Chem. Res. (S)* **1989**, 296; (b) Koziara, A.; Osowska-Pacewicka, K.; Zawadzki, S.; Zwierzak, A. *Synthesis* **1985**, 202.

6. (a) The enantiomeric excess of **8** was determined by the methodof Kinashita. The protecting groups of **8** were removed by acid hydrolysis and the corresponding diamino acid was derivatized with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate. The diamino acid corresponsing to the enantiomer of **8** was also derivatized with 2,3,4,6-tetra-Oacetyl-β-D-glucopyranosyl isothiocyanate. Reverse phase HPLC analysis of the two diastereomeric thioureas revealed that only the desired antipode was observed within the limits of detection of our HPLC system. (b) Kinashita, T.; Kasahara, Y.; Nimura, N. *J. Chromatography* **1981**, *210*, 77.

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