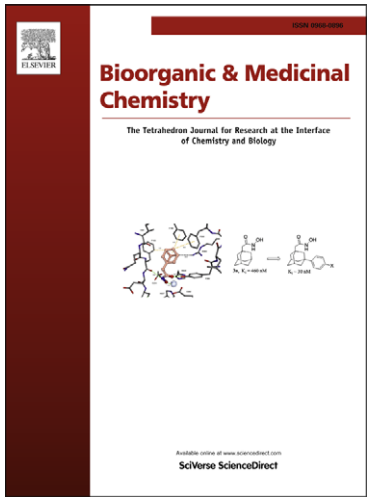


A Chiron Approach Towards the Synthesis of 3-Hydroxy Lysine and Its Derivatives

K.S. Ajish Kumar

PII: S0968-0896(13)00137-5
DOI: http://dx.doi.org/10.1016/j.bmc.2013.02.013
Reference: BMC 10608

To appear in: *Bioorganic & Medicinal Chemistry*

Please cite this article as: Ajish Kumar, K.S., A Chiron Approach Towards the Synthesis of 3-Hydroxy Lysine and Its Derivatives, *Bioorganic & Medicinal Chemistry* (2013), doi: http://dx.doi.org/10.1016/j.bmc.2013.02.013

A Chiron Approach Towards the Synthesis of 3-Hydroxy Lysine and Its Derivatives

K. S. Ajish Kumar*
Bio-organic Division, Bhabha Atomic Research Centre,
Trombay, Mumbai- 400085
Email: ajish@barc.gov.in

Abstract

A general and efficient route towards the synthesis of three derivatives of structurally and functionally important amino acid, lysine is reported. Chemoselective reduction of aldehydic functionality in C-3-azido conjugated aldehyde **4**, under Luche condition, is the key step in the synthetic sequence. The lysine derivative, (2S,3R)-2,6-diazido-3-hydroxy-hex-4-ene-oic acid **9** could be used to prepare switch peptide using Staudinger reaction, while the unprotected 3-hydroxy lysine **10** is a proven reaction intermediate towards the synthesis of natural product (−)-Balanol.

Introduction

Synthesis of modified amino acids and its application in the construction of proteins or molecules of biological importance, has been the subject of interest for last few decades.[1] Ever since the discovery of native chemical ligation (NCL),[2] wherein, unmodified amide bonds are constructed in aqueous conditions, it is possible to synthesize peptides with few tons of amino acid residues having modification at a specific site. The ability to obtain peptides/proteins in workable quantities, as homogeneous and pure product, is the biggest gain of this unique method. Considering the importance of modified amino acids, which is also an important tool in synthesis and study of lantibiotics[3] there has been particular interest in the synthesis, biosynthesis, and activity studies of such amino acids.[4] Among the unnatural amino acids, the side chain modified with hydroxy derivative have received considerable attention (Figure 1). Some of the commercially available hydroxy incorporated amino acids include, 3-hydroxy derivatives of phenylalanine **1a**, leucine **1b**, valine **1c**, lysine **1d** and 4-hydroxy derivatives of glutamine **2a**, valine **2b**, and lysine **2c**. In addition to 3- and 4-hydroxy lysines, **1d**/**2c**, 5-hydroxy lysine, **2d** is well known and is used for various purposes.[5] Recently, two different groups[6] independently employed this amino acid or its masked derivative as the starting material for the synthesis of δ-mercaptolysine, an amino acid used for isopeptide chemical ligation (ICL).[7] In NCL, for cysteine free ligations, the presence of suitably positioned sulphydryl moiety in the amino acid side chain is one of the criteria to be fulfilled. The above mentioned hydroxy amino acids, not only serve to incorporate interesting modification in peptides and proteins, but also it could be utilized as

an important starting material for the synthesis of corresponding mercapto derivatives, a tool for NCL.[8]

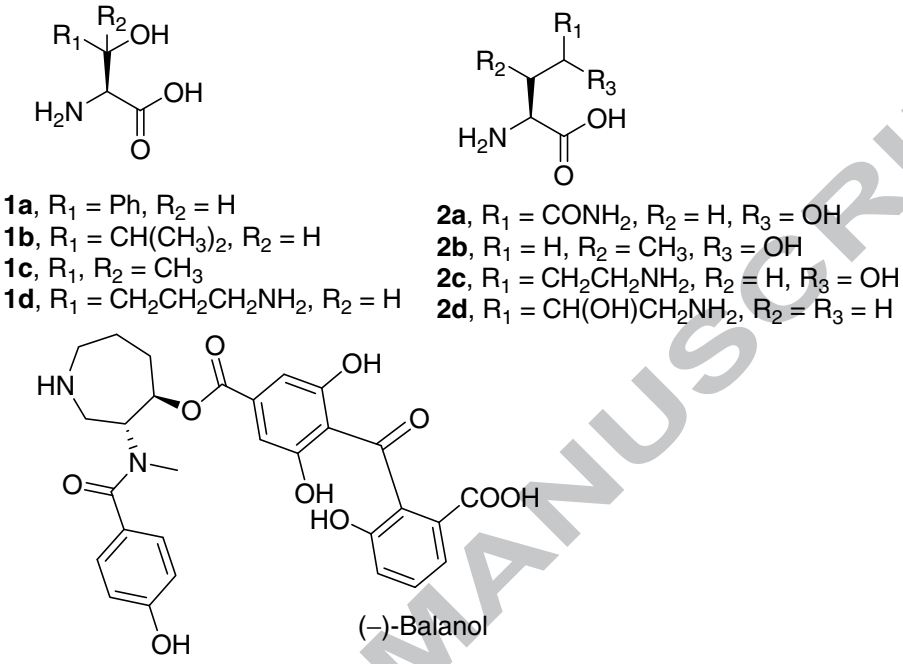


Figure 1: Hydroxy amino acids and (–)-Balanol

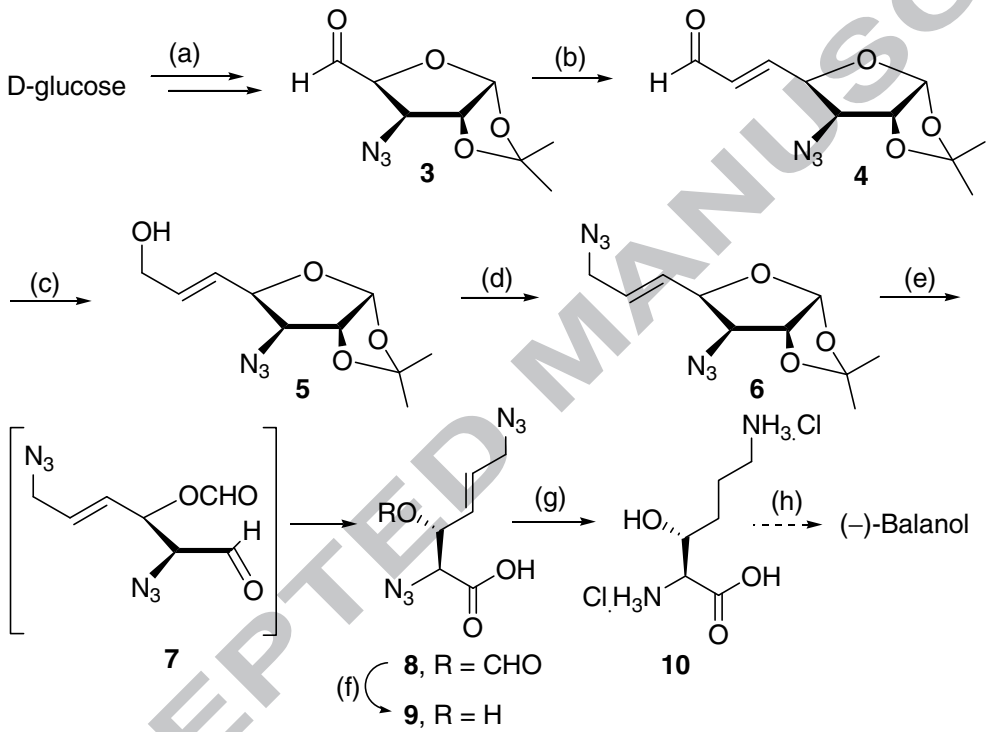
In addition, some of these amino acids are fragments of natural products for eg. stereo isomers of 3-hydroxy leucine. (2S,3S)-3-hydroxy leucine, is an important amino acid present in several peptide antibiotics like, azinthricin,[9] A g3586c,[10] and telomycin[11] while, isomer (2R,3S)-3-hydroxy leucine is present in lactacystin.[12] Another isomer of leucine, (2S,3R)-3-hydroxy leucine, is found to be a constituent of a macrocyclic peptide lactone antibiotic lysobactin[13] isolated from fermentations of Lysobacter Sp. ATCC 53042. 3-Hydroxy lysine, is an interesting residue that has got wide applicability as protein marker for studying radical induced protein oxidation.[14] 3-Hydroxy lysine is also as an important reaction intermediate towards the synthesis of natural product (–)-Balanol,[15] a potent protein kinase C (PKC) inhibitor which is involved in cell growth, metabolism and differentiation.[16] In short, these

unnatural hydroxy amino acids are either present in natural product or could easily be transformed into moieties that are present in natural products/could be used in protein synthesis. Citing the multiple application of 3-hydroxy lysine **1d**, we here report, the synthesis of different derivatives of this amino acid based on chiron approach, with D-glucose as starting material. The synthetic strategy is planned, taking into account of the multiple nucleophilic functionalities in hydroxy lysine, so that we can perform orthogonal protection of choice at specific functional group required for Boc/Fmoc strategy. With this objective, as a preliminary study, synthesis of (2S,3R)-2,6-diazido-3-hydroxy-hex-4-ene-oic acid **9**, (2S,3R)-2,6-diamino-3-hydroxy-hexanoic acid hydrochloride **10**, and, (2S,3R)-6-azido-2-*N*-benzyloxy carbonyl-3-*O*-formylo-hexanoic acid **13**, is reported.

Results and Discussion

In the synthesis, as shown in scheme 1, D-glucose was transformed to azido aldehyde **3** as reported by Tronchet et.al.[17] The Wittig olefination of aldehyde **3** with (Triphenylphosphoranylidene) acetaldehyde afforded the conjugated aldehyde **4** as the major product in 79% yield. The aldehyde **4** possess multiple reaction sites sensitive to reduction in conjugated olefin, azide, and aldehyde, therefore the selective reduction of aldehyde group is required to incorporate the desired C-6 amino functionality of lysine. This key step of chemoselective reduction of aldehydic functionality in C-3 azido conjugated aldehyde **4** was achieved under Luche condition[18] to give C-3-azido allyl alcohol **5**. Subsequently, activation of allylic hydroxy group in **5** using mesyl chloride and refluxing the resultant mesylate with sodium azide in DMF yielded allylic azide **6** in 78% yield, over two steps. In order to generate the required carboxylic acid functionality at C-1 position of lysine, the 1,2-acetonide

group in allylic azide **6** was deprotected using TFA-H_2O (3:2) and the resultant hemiacetal was subjected to periodate cleavage to yield azido aldehyde **7**, that on oxidation using $NaClO_2$, NaH_2PO_4, and 30% H_2O_2 afforded the highly functionalized lysine derivative **8** in 68% yield (over three steps). The product **8** could serve as an interesting conformationally constrained lysine, to study the folding of protein as well as in peptidomimetics design/foldamers.[19]

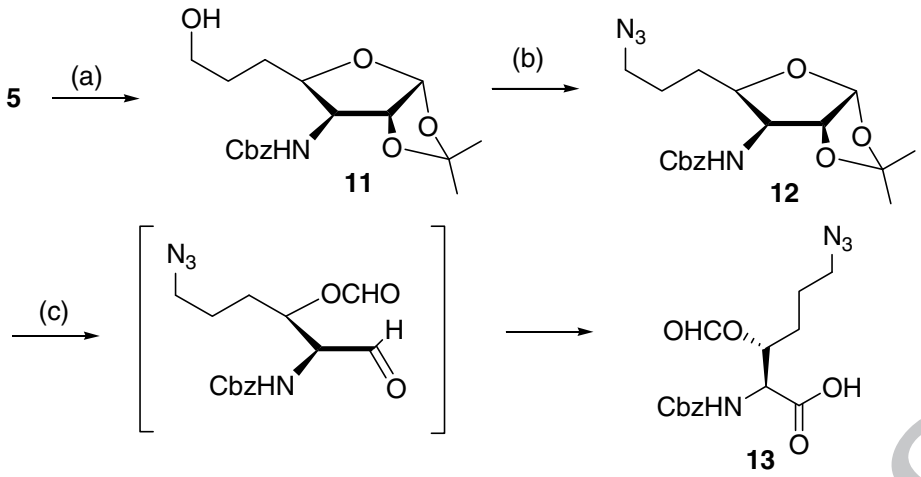


Scheme 1: Synthesis of β-hydroxy lysine: (a) ref 17; (b) PPh_3=CHCHO, CH_2Cl_2, 0 °C to 30 °C, 12 h; (c) $NaBH_4$, $CeCl_3.7H_2O$, THF, -40 °C, 30 min; (d) i) MsCl, TEA, CH_2Cl_2, 0 °C to 10 °C, 30 min, ii) NaN_3, DMF, 80 °C, 1. 5 h; (e) i) TFA-H_2O (3:2), 0 °C-20 °C, 6 h, ii) $NaIO_4$, acetone-water (4:1), 0 °C to 15 °C, 30 min, iii) $NaClO_2$, NaH_2PO_4, 30% H_2O_2, 0 °C to15 °C, 10 h; (f) LiOH (0.3 M), THF-H_2O (4:1), 0 °C, 1.5 h; (g) H_2 (80 Psi), 10% Pd-C, MeOH-HCl, 25 °C, 12 h; (h) ref 15.

To avoid the possible migration of formyl group during the conversion of azide **8** to amine **10** during hydrogenation it was thought to unmask the C-3 hydroxy group. Thus, Saponification

of amino acid **8** in the presence of 0.3 M LiOH in THF furnished (2S,3R)-2,6-diazido-3-hydroxy-hex-4-ene-oic acid **9** in quantitative yield. Amino acid **9** is an interesting derivative of lysine that could be used in the synthesis of switch peptide[20] using Staudinger reaction. Finally, hydrogenation of **9** (10% Pd-C, 80 psi) in methanol for 12 h at room temperature, followed by purification, yielded hydroxy lysine **10** as a white solid in 59% yield.[21] The spectral and analytical data of **10** were in agreement with that reported, [observed $[\alpha]_D^{25}$ +17.41 (c 1.87, CH_3OH), reported $[\alpha]_D^{25}$ +16.90 (c 1.83, CH_3OH)].[21a] Lampe et. al. reported the synthesis of (−)-Balanol from amino acid **10** by transforming it to suitably protected azepane.[15] The structural confirmation of amino acid **10** also fixes the structural integrity of the amino acid **8**.

For Boc/Fmoc strategies of peptide synthesis the side chain functionalities of the hydroxyl lysine should be orthogonally protected to make it useful for solid phase peptide synthesis (SPPS). Hence, the utility of present strategy in the synthesis of orthogonally protected lysine was also explored; as a proof of this a model amino acid **13** was targeted. Thus, as shown in Scheme 2, the C-3-azido-allylic alcohol **5** was subjected to hydrogenation, thereafter *insitu* protection of the resultant amine with benzyloxycarbonyl group afforded *N*-protected amino alcohol **11**, in 78% yield. The C-7 hydroxy functionality in **11** was converted to azide **12**, through mesylate pathway, in 76% yield.



Scheme 2: Synthesis of orthogonally protected β-hydroxy lysine: (a) i) H_2, 10% Pd-C, MeOH-HCl, 25 ^oC, 12 h, ii) CbzCl, $NaHCO_3$, MeOH-H_2O (5:2), 5 h; (b) i) MsCl, TEA, CH_2Cl_2, 0 ^oC to 10 ^oC, 30 min, ii) NaN_3, DMF, 80 ^oC, 2 h; (c) i) TFA-H_2O (3:2), 0 ^oC-20 ^oC, 6 h, ii) $NaIO_4$, acetone-water (4:1), 0 ^oC to 15 ^oC, 30 min, iii) $NaClO_2$, NaH_2PO_4, 30% H_2O_2, 0 ^oC to15 ^oC, 10 h.

Finally, treatment of **12** with TFA-H_2O (3:2) yielded corresponding hemiacetal that on metaperiodate cleavage, followed by oxidation, *vide supra,* yielded the required amino acid **13** in 69 % yield over 3 steps from azide **12**. Similarly, one can incorporate Fmoc group in the place of benzyloxy carbonyl group at the C-2 amine to make it a useful precursor for the synthesis of amino acid derivative for Fmoc-SPPS. The lysine derivative **13** is suitably protected to serve as an interesting intermediate towards the synthesis of (−)-Balanol.[15]

Conclusions

In summary, an efficient route towards the synthesis of three derivatives of hydroxy functionalized lysine is reported, which has got wide range of application in the synthesis and study of peptides/proteins and natural products. Work is in progress to synthesize other possible orthogonally protected lysines, useful for both Boc and Fmoc SPPS, and also its

mercapto variant, useful for peptide ligation, which will be reported elsewhere. Hydroxy lysine **10/13** can easily be transformed to biologically relevant natural product (−)-Balanol, which completes its formal synthesis.

Experimental

General.

The IR spectra were recorded as a thin film (liquids) or as KBr pellets (solids) with a Shimadzu FTIR-8400 spectrometer. The ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR spectra, were recorded with a Brooker Oxford instrument in $CDCl_3$ or D_2O. The elemental analyzes were carried out with a Thermo-Electron Corporation CHNS analyzer model Flash-EA 1112. The optical rotations were measured using a Jasco P1020 polarimeter. The thin layer chromatography was performed on pre-coated plates (0.25 mm, silica gel 60 F254), and the spots were visualized by UV light or spraying with 3.5% solution of 2,4-dinitrophenylhydrazine in ethanol/H_2SO_4 or with basic aqueous $KMnO_4$ solution followed by heating.

3-Azido-1,2-*O*-isopropylidene-3,5,6,7-tetradeoxy-α-D-xylo-5-ene-hept-7-dialdose (4).

To a stirred solution of the azido aldehyde **3** (2.00 g, 9.38 mmol) in CH_2Cl_2 (60 mL) was added PPh_3=CHCHO (4.18 g, 12.19 mmol) at 0 ºC to 30 ^{o}C for 12 h. After completion of reaction (*cf.*TLC), the reaction mixture was evaporated in vacuo to give a residue, which on column chromatography afforded **4** (1.77 g, 79%) as a thick liquid. R_f = 0.48 (EtOAc/hexane, 2:3); $[\alpha]_D^{25}$–23.5 (c 1.93, $CHCl_3$); IR: 2104, 1727 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.27

(s, 3H), 1.44 (s, 3H), 3.98 (d, *J* = 3.4 Hz, 1H), 4.67 (d, *J* = 3.7 Hz, 1H), 5.01-4.84 (m, 1H), 5.91 (d, *J* = 3.7 Hz, 1H), 6.38 (ddd, *J* = 15.8, 7.7, 1.6 Hz, 1H), 6.75 (dd, *J* = 15.8, 4.5 Hz, 1H), 9.54 (d, *J* = 7.7 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 26.4, 26.8, 67.7, 79.0, 83.9, 104.9, 112.8, 134.2, 148.9, 193.1. Anal.Calcd. for $C_{10}H_{13}N_3O_4$: C, 50.21; H, 5.48; N, 17.56. Found: C, 50.24; H, 5.52; N, 17.64.

3-Azido-1,2-*O*-isopropylidene-7-hydroxy-3,5,6-trideoxy-α-D-xylo-hept-5-ene-furanose (5).

To a solution of aldehyde **4** (1.10 g, 4.60 mmol) in THF (25 mL) at 30 °C was added $CeCl_3.7H_2O$ (0.08 g, 2.30 mmol) and stirred for 10 min at same temperature. The reaction mixture was cooled to –40 °C and a solution of $NaBH_4$ (0.17 g, 4.60 mmol) in water (3 mL) was added in intervals for a peroid of 20 min. After completion of reaction (*cf*.TLC) the reaction mixture was decomposed using 1 N HCl (2 mL) and concentrated in vacuo, and extracted with EtOAc (3 × 15 mL). The organic extract was washed with H_2O (2 × 10 mL) and brine (1 × 5 mL), and dried. Concentration of solvent followed by column chromatography of the residue furnished **5** as a colourless thick liquid (0.94 g, 85%). R_f = 0.59 (EtOAc/hexane, 1:1); $[\alpha]_D^{25}$–65.5 (c 1.95, $CHCl_3$); IR: 2106, 3410 (broad) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.30 (s, 3H), 1.49 (s, 3H), 2.00 (bs, exchangeable with D_2O, 1H), 3.83 (d, *J* = 3.2 Hz, 1H), 4.18 (d, *J* = 4.8 Hz, 2H), 4.63 (d, *J* = 3.7 Hz, 1H), 4.73 (dd, *J* = 6.4, 3.1 Hz, 1H), 5.77 (ddt, *J* = 15.6, 6.5, 1.5 Hz, 1H), 5.89 (d, *J* = 3.7 Hz, 1H), 6.11 (dtd, *J* = 15.6, 4.9, 0.8 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 26.8, 27.2, 63.2, 68.5, 80.5, 84.3,

105.0, 112.7, 124.2, 135.9. Anal. calcd. for $C_{10}H_{15}N_3O_4$: C, 49.79; H, 6.27; N, 17.42. Found: C, 49.81; H, 6.31; N, 17.49.

3,7-Diazido-1,2-*O*-isopropylidene-3,5,6,7-tetradeoxy-α-D-xylo-hept-5-ene-furanose (6). To an ice cooled solution of **5** (1.00 g, 4.14 mmol) in CH_2Cl_2 (40 mL) was added triethyl amine (0.75 mL, 5.39 mmol) followed by dropwise addition of methane sulphonyl chloride (0.35 mL, 4.55 mmol), stirred for 15 min at the same temperature, brought to room temperature, stirred for additional 10 min. The reaction mixture was diluted with cold water, and extracted with CH_2Cl_2 (3 x 15 mL). The organic layer was dried over Na_2SO_4, and was purified by flash column chromatography (15% EtOAc/hexane) to afford the corresponding mesylate. To the mesylate dissolved in DMF was added sodium azide (0.67 g, 10.35 mmol) and heated at 80 ^oC for 1.5 h. After completion of reaction (*cf*.TLC), DMF was removed under vacuo, and the residue was extracted with EtOAc (3 x 15 mL). The combined organic layer was dried over Na_2SO_4, concentrated and purified using column chromatography to give azide (**6**) as thick liquid (0.86 g, 78% over two steps); R_f = 0.50 (EtOAc/hexane, 3:7); $[\alpha]_D^{25}$–30.15 (c 1.97, $CHCl_3$); IR: 2102 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.31 (s, 3H), 1.48 (s, 3H), 3.80-3.86 (m, 3H), 4.65 (d, *J* = 3.7 Hz, 1H), 4.74 (dd, *J* = 5.2, 3.6 Hz, 1H), 5.91 (d, *J* = 3.5 Hz, 1H), 5.75-6.05 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 26.8, 27.3, 52.8, 68.4, 80.1, 84.4, 105.1, 112.9, 128.3, 129.4. Anal. calcd. for $C_{10}H_{14}N_6O_3$: C, 45.11; H, 5.30; N, 31.56. Found: C, 45.13; H, 5.27; N, 31.66.

(2S,3R)-2,6-diazido-3-*O*-formylo-hex-4-ene-oic acid (8).

A solution of **6** (0.45 g, 1.69 mmol) in TFA-H_2O (7.00 mL, 3:2) was stirred at 0 ºC to 30 ^oC for 6 h. TFA was removed azeotropically with toluene in vacuo to afford the crude hemiacetal, was purified using flash column chromatography to afford hemi acetal (0.34 g, 89%). To the hemiacetal solution (0.34 g, 1.50 mmol) in acetone/water (10.00 mL, 9:1) at 0 ºC was added $NaIO_4$ (0.36 g, 1.65 mmol). After stirring for 30 min, excess $NaIO_4$ was decomposed with ethylene glycol (0.10 mL), the mixture concentrated in vacuo, and the residue was extracted with $CHCl_3$ (3 × 10 mL) to get the crude α-aminal (0.38 g) **7** as a thick liquid.

To a cooled (0 ºC) and stirred solution of α-aminal in CH_3CN (20 mL) was added a solution of NaH_2PO_4 (0.05 g, 0.30 mmol) in H_2O (2 mL) and 30% H_2O_2 (0.22 mL, 1.65 mmol). The mixture was cooled to 0 ºC, and $NaClO_2$ (0.22 g, 2.40 mmol) in H_2O (5 mL) was added dropwise over a period of 0.5 h. The reaction mixture was stirred at 15 ºC and monitored from the evolution of gas with a bubbler connected to the apparatus. After 10 h, the reaction was decomposed by addition of a small amount of Na_2SO_4 (0.25 g) and extracted with EtOAc (3 × 10 mL). Evaporation of solvent and column purification with EtOH/$CHCl_3$ (5%) gave **8** (0.27 g, 68% from **6**) as a sticky gum. R_f = 0.30 (EtOAc); $[\alpha]_D^{25}$–56.30 (c 1.89, $CHCl_3$); IR: 3352, 2105, 1737 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 3.85 (d, *J* = 5.0 Hz, 2H), 4.04 (d, *J* = 3.2 Hz, 1H), 5.85 (s, 1H), 5.80-6.17 (m, 2H), 7.80-8.01 (bs, exchangeable with D_2O, 1H), 8.10 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 52.2, 64.5, 73.4, 127.8, 131.3, 160.1, 172.4. Anal. calcd. for $C_7H_8N_6O_4$: C, 35.01; H, 3.36; N, 34.99. Found: C, 34.99; H, 3.39; N, 35.08.

(2S,3R)-2,6-diazido-3-hydroxy-hex-4-ene-oic acid (9).

A solution of **8** (0.13 g, 0.54 mmol) in THF at 0 ^{o}C was hydrolyzed with 0.3 M LiOH (0.09 g, 2.16 mmol), LiOH solution was added in small portions over a period of 30 min, after 1h of reaction at same temperature, sat. citric acid solution (4 mL) was added and extracted using EtOAc (4 x 10 mL). The extract was dried over sodium sulphate, concentrated, and purified using column chromatography to afford **9** as a thick liquid (0.11 g, 93%). $[\alpha]_D^{25}$–0.12 (c 1.00, $CHCl_3$); IR: 3323, 2108, 1745 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.84 (d, *J* = 4.3 Hz, 2H), 4.06 (d, *J* = 2.8 Hz, 1H), 4.76 (t, *J* = 3.1 Hz, 1H), 5.85-5.92 (m, 2H), 7.06 (bs, 2H, exchangeable with D_2O); ^{13}C NMR (50 MHz, $CDCl_3$): δ 52.5, 66.5, 72.9, 127.9, 132.5, 173.1; Anal. Calcd. for $C_6H_8N_6O_3$: C, 33.97; H, 3.80; N, 39.61. Found: C, 33.96; H, 3.85; N, 39.68.

(2S,3R)-2,6-diamino-3-hydroxy-hexanoic acid. 2HCl (10).

The formyl free product **9** (0.10 g, 0.41 mmol) and 10% Pd/C (0.045 g) in MeOH: HCl (4:1 mL) was stirred under a H_2 atmosphere at 80 psi for 12 h at 25 ºC. The catalyst was filtered through a pad of Celite 545. The filterate was contentrated, washed using EtOAc and purified using coloumn chromatography using (5% MeOH-H_2O) to afford **10** as a white solid (0.065 g, 59%); mp 187-191 ^{o}C [reported 188-192 ^{o}C][20]; $[\alpha]_D^{25}$ +17.41 (c 1.87, CH_3OH), reported +16.90 (c 1.83, CH_3OH)[20]; IR: 3350, 1733 cm^{-1}; ^{1}H NMR (200 MHz, D_2O): δ 1.59-1.95 (m, 4H), 3.07 (t, *J* = 6.9 Hz, 2H), 4.06 (s, 1H), 4.14-4.34 (m, 1H); ^{13}C NMR (50 MHz, D_2O): δ 23.3, 29.9, 38.9, 57.7, 68.4, 170.5. Anal. Calcd. for $C_6H_{16}Cl_2N_2O_3$: C, 30.65; H, 6.86; N,

11.92. Found: C, 30.63; H, 6.89; N, 12.02.; ESI-MS: Calcd. for $[C_6H_{13}N_2O_3 + 2Na]^+$: 207.06 Da, Observed: 206.98 Da.

3-*N*-benzyloxycarbonyl-7-hydroxy-1,2-*O*-isopropylidene-3,5,6-trideoxy-α-D-xylo-hept-furanose (11).

To a stirred solution of azido alcohol **5** (1.75 g, 7.25 mmol) and 10% Pd/C (0.17 g) in MeOH (10 mL) was stirred under a H_2 (80 psi) atmosphere for 12 h at 25 ºC. The catalyst was filtered through a pad of Celite 545. The filterate was contentrated and redissolved in MeOH-H_2O (15 mL, 4:1), and cooled to 0 ^oC, to it $NaHCO_3$ (1.71 g, 20.44 mmol) and CbzCl (60% solution in toluene) (2.00 mL, 14.50 mmol), was added sequentially and stirred to room temperature for 5 h. After completion of reaction on tlc, reaction mixture was concentrated and extracted using EtOAc (3 x 10 mL). The combined organic layer was kept over anhydrous Na_2SO_4, concentrated under vacuo, and purified using column chromatography to afford **11** as a thick liquid. (1.98 g, 78%). R_f = 0.40 (EtOAc/hexane, 3:2); $[\alpha]_D^{25}$–12.7 (c 1.15, $CHCl_3$); IR: 3326, 1690 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.24 (s, 3H), 1.48 (s, 3H), 1.50-1.70 (m, 4H), 2.15 (s, 1H, exchangeable with D_2O), 3.60 (s, 2H), 4.07-4.29 (m, 2H), 4.48 (d, *J* = 3.7 Hz, 1H), 5.05 (s, 3H, one exchangeable with D_2O), 5.75 (d, *J* = 3.8 Hz, 1H), 7.33 (s, 5H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 24.9, 26.4, 26.7, 27.5, 58.5, 63.0, 67.9, 79.1, 85.3, 104.5, 112.4, 128.9, 129.0, 129.1, 136.6, 156.5. Anal. Calcd for $C_{18}H_{25}NO_6$: C, 61.52; H, 7.17; N, 3.99. Found: C, 61.55; H, 7.22; N, 4.10; ESI-MS: Calcd for.$[C_{18}H_{25}NO_6 + Na]^+$: 374.14 Da, Observed: 373.94 Da.

7-azido-3-*N*-benzyloxy carbonyl-1,2-*O*-isopropylidene-3,5,6,7-tetradeoxy-α-D-xylo-hept-furanose (12).

To an ice cooled solution of **11** (1.53 g, 4.35 mmol) in CH_2Cl_2 (40 mL) was added triethyl amine (0.79 mL, 5.66 mmol) followed by dropwise addition of methane sulphonyl chloride (0.37 mL, 4.78 mmol), stirred for 15 min at the same temperature, brought to room temperature, stirred for additional 10 min. The reaction mixture was diluted with cold water, and extracted with CH_2Cl_2 (3 x 15 mL). The organic layer was washed sequentially with 1N HCl (15 mL) and with brine (15 mL), dried over Na_2SO_4, and was purified by flash column chromatography (20% EtOAc/hexane) to afford corresponding mesylate. To the mesylate solution in DMF (7 mL) was added sodium azide (0.70 g, 10.87 mmol) and heated at 80 ^oC for 2 h. After completion of reaction (*cf.*TLC), DMF was removed under vacuo, and the residue was extracted with EtOAc (3 x 15 mL). The combined organic layer was dried over Na_2SO_4, concentrated and purified using column chromatography (10% EtOAc/hexane) to give azide **12** as a thick liquid (1.24 g, 76%); R_f = 0.40 (EtOAc/hexane, 3:7); $[\alpha]_D^{25}$ –9.80 (c 1.60, $CHCl_3$); IR: 2109, 1695 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.26 (s, 3H), 1.50 (s, 3H), 1.57-1.83 (m, 4H), 3.26 (d, *J* = 5.5 Hz, 2H), 4.16 (d, *J* = 7.4 Hz, 2H), 4.49 (d, *J* = 3.8 Hz, 1H), 4.81 (d, *J* = 9.2 Hz, 1H, exchangeable with D_2O), 5.10 (s, 2H), 5.76 (d, *J* = 3.8 Hz, 1H), 7.34 (s, 5H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 25.3, 25.4, 26.8, 27.1, 51.9, 58.6, 67.9, 78.7, 85.3, 104.5, 112.5, 128.9, 129.1, 129.3, 136.7, 156.5. Anal. calcd. for $C_{18}H_{24}N_4O_5$: C, 57.44; H, 6.43; N, 14.88. Found: C, 57.48; H, 6.48; N, 14.95. ESI-MS: Calcd. for $[C_{18}H_{24}N_4O_5 + Na]^+$: 399.15 Da, Observed: 399.04 Da.

(2S,3R)-6-azido-2-*N*-benzyloxy carbonyl-3-*O*-formylo-hexanoic acid (13).

The procedure was similar to that followed for synthesis of **8** from **6**. After column purification of the extract afforded **13** as a thick liquid (0.22 g, 69% from 0.35 g of **12**). R_f = 0.30 (EtOAc); $[\alpha]_D^{25}$+14.71 (c 1.75, $CHCl_3$); IR: 3352, 1733 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.58-1.78 (m, 4H), 3.27 (t, *J* = 6.2 Hz, 2H), 4.64 (dd, *J* = 9.7, 1.6 Hz, 1H), 5.13 (s, 2H), 5.52 (t, *J* = 5.7 Hz, 1H), 5.63 (d, *J* = 9.7 Hz, 1H), 7.35 (s, 5H), 7.97 (s, 1H), 8.72 (bs, 1H, exchangeable with D_2O); ^{13}C NMR (50 MHz, $CDCl_3$): δ 25.3, 29.1, 51.4, 56.6, 68.3, 73.7, 128.8, 129.1, 129.3, 136.5, 157.4, 160.8, 173.7. Anal.calcd. for $C_{15}H_{18}N_4O_6$: C, 51.43; H, 5.18; N, 15.99. Found: C, 51.46; H, 5.23; N, 16.07; ESI-MS: Calcd. for $[C_{15}H_{18}N_4O_6 +Na]^+$: 373.10 Da, Observed: 372.99 Da.

Acknowledgment

AKS is thankful to Prof. S Chattopadhyay, Head, Bio-Organic Division, BARC, for his constant support and encouragement. AKS gratefully acknowledges BRNS-DAE (Mumbai, INDIA) for the award of K. S. Krishnan Research Associateship.

References

1. (a) *Amino acids, Peptides, and Proteins*; Sheppard, R. C., Ed.; The Chemical Society: London, 1972-1981 (b) *Amino acids, Peptides, and Proteins*; Young, G. T., Ed.; The Chemical Society: London, 1968-1971.Okeley, N. M.; Zhu, Y.; van der Donk W. A. *Org. Lett.* **2000**, *2*, 3603; (c) Zhou, H.; van der Donk W. A. *Org. Lett.* **2001**, *3*, 593 (d) M. Haj-Yahya, K. S. A. Kumar, L. A. Erlich, A. Brik, *Biopolymers (Pept Sci),* **2010**, *94*, 504; (e) K. S. A. Kumar, L. Spasser, L. Erlich, S. N. Bavikar, A. Brik, *Angew. Chem. Int. Ed.* **2010**, *49*, 9126; (f) K. S. A. Kumar.; A. Brik. *J. Pep. Sci*. **2010**, *16*, 524; (g) C. T. Walsh, *Post translational modifications of protiens: Expanding natures inventory*, Roberts and Company, Englewood, **2006**.
2. Dawson P. E.; Muir, T. W.; Clark-Lewis, l.; Kent, S. B. H. Science **1994**, *266*, 776.
3. (a) Levengood, M. R.; Knerr, P. J.; Oman T. J.; van der Donk W. A. *J. Am. Chem. Soc.* **2009**, *131*, 12024; (b) Knerr P. J.; van der Donk W. A. *J. Am. Chem. Soc.* **2012**, *134*, 7648.
4. (a) Walsh, C. T.; *Antibiotics: Actions, Origins, Resistance*; ASM Press: Washington, DC, 2003. (b) Williams, R. M. *Synthesis of optically active amino acids*; Pergamon: Oxford, 1989.
5. Herbert K. R.; Williams, G. M.; Cooper, G. J. S.; Brimble, M. A. *Org. Biomol. Chem.* **2012**, *10*, 1137.
6. (a) Kumar, K. S. A.; Haj-Yahya, M.; Olschewski, D.; Lashuel, H. A.; Brik, A. *Angew. Chem., Int. Ed*. **2009**, *48*, 8090; (b) Merkx, R.; Ekkebus, R.; Hameed, D. S.; Smit, J. J.; Jong, A-de.; Hilkmann, H.; Sixma, T. K.; Ovaa, H. *Angew. Chem., Int. Ed*. **2010**, *49*, 10149.

7. Kumar, K. S. A.; Brik, A. *Isr. J. Chem.* **2011**, *51*, 900.

8. (a) Yan, L. Z.; Dawson, P. E. *J. Am. Chem. Soc*. **2001**, *123*, 526; (b) Crich, D.; Banerjee, A. *J. Am. Chem. Soc*. **2007**, *129*, 10064; (c) Haase, C.; Rohde, H.; Seitz, O. *Angew. Chem. Int. Ed*. **2008**, *47*, 6807; (d) Chen, J.; Wan, Q.; Yuan, Y.; Zhu, J.; Danishefsky, S. J. *Angew. Chem. Int. Ed*. **2008**, *47*, 8521; (e) Harpaz, Z.; Siman, P.; Kumar, K. S. A.; Brik, A. *ChemBio-Chem*, **2010**, *11*, 1232; (f) Tan, Z.; Shang, S.; Danishefsky, S. J. *Angew. Chem. Int. Ed*. **2010**, *49*, 9500; (g) Chen, J.; Wang, P.; Zhu, J.; Wan, Q.; Danishefsky, S. J. *Tetrahedron* **2010**, *66*, 2277; (h) Kumar, K. S. A.; Haj-Yahya, M.; Olschewski, D.; Lashuel, H. A.; Brik, A. *Angew. Chem. Int. Ed*. **2009**, *48*, 8090; (i) Yang, R.; Pasunooti, K. K.; Li, F.; Liu, X.-W.; Liu, C.-F. *J. Am. Chem. Soc*. **2009**, *131*, 13592; (j) Siman, P.; Karthikeyan, S. V.; Brik, A. *Org. Lett*. **2012**, *14*, 1520.

9. Maehr, H.; Liu, C.-M.; Palleroni, N. J.; Smallheer, J.; Todan, L.; Williams T. H.; Blount, J. F. *J. Antibiot.* **1986**. *39, 17.*

10. Smitka,T. A.; Deeter, J. B.; Hunt, A. H.; Mertz, F. P.; Ellis, R. M.; Boeck, L. D.; Yao, R. C. *J. Antibiot.* **1988**, *41,* 726.

11. Sheeham, J. C.; Maeda, K.; Sen, A. K.; Stock, J. A. *J. Am. Chem. Soc.* **1962**, *84,* 1303.

12. (a) Omura, S.; Fujimoto, T.; Otoguro, K.; Koriguchi, R.; Tanaka, H.; Sasaki, *Y. J. Antibiot.* **1991***, 44,* 113; (b) Omuro, S.; Matsuzaki, K.; Fujimoto, T.; Kosuge, K.; Furuya, T.; Fujita, S.; Nakagawa, A. *ibid,* **1991**, *44,* 117.

13. Tymiak, A. A.; McCormick, T. J.; Unger, S. E. *J. Org. Chem.* **1989**, *54,* 1149.

14. Morin, B.; Bubb, W. A.; Davies, M. J.; Dean, R. T.; Fu, S. *Chem. Res. Toxicol*. **1998**, *11*, 1265.

15. Lampe, J. W.; Hughes, P. F.; Biggers, C. K.; Smith, S. H.; Hu, H. *J. Org. Chem*. **1994**, *59*, 5147.

16. (a) Nishizuka, Y. *Science* **1986**, *233,* 305; (b) Nishizuka, Y. *Nature* **1984**, *308,* 693; (c) Farago, A.; Nishizuka, Y. *FEBS Lett.* **1990**, *268,* 350.

17. Tronchet, J. M. J.; Gentile, B.; Ojha-Poncet, J.; Moret, G.; Schwarzanbach, D.; Barblat-Ray, F. *Carbohydr. Res*. **1977**, *59*, 87.

18. Luche, J. L. *J. Am. Chem. Soc.*, **1978**, *100*, 2226.

19. (a) McNaughton-Smith, G.; Hanessian, S.; Lombart, H. G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789; (b) Copeland, T. D.; Wondrak, E. M.; Toszer, J.; Roberts, M. M.; Oraszan, S. *Biochem. Biophys.Res. Commun.* **1990**, *169*, 310; (c) Quibell, M.; Benn, A.; Flinn, N.; Monk, T.; Ramjee, M.; Wang, Y.; Watts, J. *Bio-org. Med. Chem.* **2004**, *12*, 5689.

20. Nepomniaschiy, N.; Grimminger, V.; Cohen, A.; DiGiovanni, S.; Lashuel, H. A.; Brik, A. *Org. Lett*. **2008**, *10*, 5243.

21. (a) Hughes, P. F.; Smith, S. H.; Olson, J. T. *J. Org. Chem*. **1994**, *59*, 5799; (b) Coulon, E.; Ratovelomanana-Vidal, M. C. C-de. A. V.; Genet, J-P, *Tetrahedron Lett*. **1998**, *39*, 6467.

A Chiron Approach Towards the Synthesis of 3-Hydroxy Lysine and Its Derivatives

K. S. Ajish Kumar*
Bio-organic Division, Bhabha Atomic Research Centre,
Trombay, Mumbai- 400085
Email: ajish@barc.gov.in