D-Glucose based syntheses of \( \beta \)-hydroxy derivatives of L-glutamic acid, L-glutamine, L-proline and a dihydroxy pyrrolidine alkaloid†

K. S. Ajish Kumar* and Subrata Chattopadhyay

The \( \beta \)-hydroxy derivatives of L-glutamic acid, L-glutamine and L-proline, useful for peptide/protein studies, were synthesized starting from D-glucose. The C2 carbon in D-glucose provided the carboxylic acid functionality, while the amino and \( \beta \)-hydroxy groups of the amino acids were amenable from the C3 and C4 hydroxy groups of the sugar, respectively. The key intermediate with appropriate carbon framework of the target molecules was constructed by homologation of a suitable azido-D-glucofuranose derivative using the Arndt–Eistert reaction.

Introduction

Amino acids and monosaccharides constitute the major building blocks of the complex molecular systems that are vital for life. There has been a constant effort to understand the structures and functions of such systems by synthesizing them either chemically or biologically.† Hence the importance of modified amino acids as ligating agents, in the synthesis of natural proteins has received considerable attention over the years.‡ These amino acids are also the key ingredients for the synthesis of modified proteins, that help in understanding the structure–activity relationship,§ and lantibioticanth and study of the peptides of interest, besides providing peptidomimetic drugs.¶ Native chemical ligation (NCL) forms the basis of modern chemical synthesis of native, modified or cyclic peptides and proteins of moderate sizes,‖ and is extensively used to synthesize complex protein targets.¶‖

Although the NCL approach has enriched peptide ligation chemistry, the required thiol/selenol-containing amino acids, which are essential, are accessible only through lengthy syntheses. The nonproteinogenic amino acids, possessing suitably placed (at the \( \beta \)/\( \gamma \)-position) hydroxy group(s) along the side chain are useful precursors of the corresponding thiol and selenol derivatives, required for NCL. A few commercially available hydroxy derivatives of natural amino acids, such as, \( \beta \)-hydroxy phenylalanine, \( \beta \)-hydroxy valine, and \( \beta \)-hydroxy leucine have been transformed to the corresponding thiol intermediates and used in NCL.‖ Meanwhile, the \( \beta \)/\( \gamma \)-hydroxy derivatives of glutamic acid, glutamine, lysine, arginine, and aspartic acid have also been synthesized in different laboratories, and their mercapto derivatives are proven residues for the assembly of peptides using NCL.¶ Moreover, many of these hydroxy amino acids are constituents of several natural products with intrinsic biological function.§ Overall, both as unnatural building blocks and target compounds, the \( \beta \)/\( \gamma \)-hydroxylated amino acids are attractive synthetic targets. Consequently, several target-specific§ as well as multi-target oriented‖ syntheses of the hydroxy amino acids have been reported.

Designing a common strategy, for various bioactive molecules has vital significance in organic synthesis. This can provide an economically accessible pathway to an array of discrete compounds from a single starting molecule.‖ The natural amino acids glutamic acid (Glu), glutamine (Gln) and proline (Pro) possess a similar five-carbon skeleton. It was hypothesized that the synthesis of suitable hydroxy derivatives of these may be realized using a common strategy. Hence, in view of our interest in modified amino acid synthesis, applicable for protein synthesis and study,‖‖ we formulated a general strategy for synthesizing the \( \beta \)-hydroxy derivatives of Glu (1a), Gln (1b) and Pro (1c) starting from inexpensive D-glucose. The corresponding \( \beta \)-hydroxy azido acids were also synthesized as the masked amino acids, because similar compounds are proven candidates for Staudinger ligation in peptides/proteins syntheses.‖‖ In addition, several derivatives of 1a–1c, possessing different orthogonal ester protections (Me/allyl/benzyl) were synthesized so that they can be converted to free acids under different reaction conditions. Finally, in view of our interests on iminosugars,‖‖ we have transformed one of the intermediates into a biologically important pyrrolidine alkaloid 2. The chemical structures of the target compounds are shown in Fig. 1. Amongst the chosen targets, L-glutamate is an important nutrient in biochemical pathways like gluconeogenesis and ammonia detoxification,‖ and also plays a major role
in learning, memory and neuronal development in mammalian central nervous system.\textsuperscript{15a}

Results and discussion

In the retrosynthetic analysis, we conceived that the C2 carbon in D-glucose would furnish the carboxylic acid functionality, while the C3 and C4 hydroxy groups would provide the required amino/azido and \(\beta\)-hydroxy groups, respectively, of the targeted \(\beta\)-hydroxy amino acid derivatives. The synthesis commenced with the known D-glucose-derived azido aldehyde \textsuperscript{3,15c,16} which was subjected to Pinnick oxidation (NaClO\(_2\)/NaH\(_2\)PO\(_4\)/30\% H\(_2\)O\(_2\))\textsuperscript{17} to furnish the azido acid \textsuperscript{4} in 91\% yield. The acid \textsuperscript{4} was activated as a mixed anhydride using ethyl chloroformate, and subsequently reacted with CH\(_2\)N\(_2\) in Et\(_2\)O to give the \(\alpha\)-diazo ketone \textsuperscript{5} in 80\% yield. Wolff rearrangement\textsuperscript{18} of \textsuperscript{5} in the presence of PhCO\(_2\)Ag and Et\(_3\)N in MeOH afforded the homologated methyl ester \textsuperscript{6} (55\%) that served as the common intermediate for all the target amino acid derivatives.

As the first application of \textsuperscript{6}, we attempted its conversion to the \(\beta\)-hydroxy glutamic acid derivatives. To this end, its 1,2-acetonide group was deprotected using aqueous trifluoroacetic acid (TFA), and the resultant hemiacetal was subjected to cleavage with NaIO\(_4\) to yield the intermediate azido aldehyde. This on Pinnick oxidation afforded the glutamic acid derivative \textsuperscript{7}, containing a formylated C-3 hydroxy group (79\%, over three steps). The formyl group in \textsuperscript{7} could be selectively de-masked with aqueous saturated NaHCO\(_3\) in THF to obtain the hydroxy acid \textsuperscript{8} (89\%). Compound \textsuperscript{7} was also transformed to a fully masked Glu derivative \textsuperscript{9} (84\%) by reacting with allyl bromide in the presence of NaHCO\(_3\) in anhydrous DMF. As above, the formyl group in \textsuperscript{9} could be selectively removed with NaHCO\(_3\) in THF at room temperature to obtain the \(\beta\)-hydroxy diester \textsuperscript{10} in 87\% yield.

Amino acid \textsuperscript{10} is not suitable for Fmoc-SPPS as the side chain methyl ester is not easily cleavable under acidic/reduction condition. Hence it was thought of synthesizing a benzyl ester derivative \textsuperscript{14} which would also serve as an ideal starting material for the various C3-substituted glutamic acid derivatives. For this, the ester function in \textsuperscript{6} was hydrolyzed using LiOH in aqueous THF to afford the carboxylic acid \textsuperscript{11} (86\%), which on treatment with benzyl chloroformate (CbzCl) in the presence of Et\(_3\)N and 4-dimethylaminopyridine (DMAP) afforded the benzyl ester \textsuperscript{12} in 63\% yield. The ester \textsuperscript{12} was directly transformed to the hydroxy azido acid \textsuperscript{13} by a one-pot four-steps reaction sequence. Thus, acidic hydrolysis of the 1,2-acetonide function of \textsuperscript{12}, NaIO\(_4\) cleavage of the resultant diol to the intermediate aldehyde, followed by Pinnick oxidation and alkaline hydrolysis furnished the desired C-3 hydroxy acid \textsuperscript{13} in 71\% yield. This was esterified with allyl bromide and NaHCO\(_3\) to afford another glutamic acid precursor \textsuperscript{14} in 87\% yield. Compound \textsuperscript{14} is a template on which all the functionalities except the \(\beta\)-hydroxy group is protected and thus is a suitable precursor for the synthesis of C-3 substituted glutamic acid derivatives, e.g. \(\beta\)-mercapto glutamic acid. Such a transformation has already been established from similar hydroxy derivatives of various amino acids.

This approach was further extended to synthesis of a template, in which all functionalities except the \(\beta\)-hydroxy group is protected. Thus, it was possible to synthesize a benzyl ester derivative \textsuperscript{14} which could be used for the synthesis of C-3 substituted glutamic acid derivatives.
yield (Scheme 1). The spectral and analytical data of
agreement with that reported.

Synthesis of
Scheme 2
Synthesis of \( \beta \)-hydroxy glutamine derivatives.

For the synthesis of the \( \beta \)-hydroxy glutamine 1b, the benzyl amide of compound 11 was envisaged to serve as the masked amino equivalent of glutamine. Hence, compound 11 was coupled with benzylamine using HBTU and HOBt in the presence of diisopropylethylamine (DIEA) in DMF to afford the desired amide 15 (62%). This was transformed to the acid 16 (72%, over 4 steps), following the same sequence of reactions used to transform 12 to 13. The acid 16 was converted to the \( N \)-benzyl azido analogue of \( \beta \)-hydroxy glutamine ester 17 (79%) by a base-catalyzed reaction with allyl bromide. However, catalytic hydrogenation of 16 over 10% Pd–C in MeOH even under a pressurized (80 psi \( \text{H}_2 \)) condition led to reduction of the azide functionality only, and furnished the hydrochloride of \( \beta \)-hydroxy glutamyl benzamide 18 instead of the fully unprotected \( \beta \)-hydroxy glutamine hydrochloride 1b. Our attempts to transform 16 to the desired product 1b with HCO\( \text{NH}_4 \)/10% Pd–C/MeOH at room temperature as well as under reflux were also unsuccessful.

In an alternative method, the acid 11 was converted to the amide 19 (90%) with di-tert-butyl dicarbonate ([Boc]\( _2 \)O), (NH\(_4 \)) HCO\(_3 \)-NH\(_2\text{CO}_2\text{NH}_4\) and pyridine in MeCN. This was converted to the acid 20 (\textit{vide supra}), which on catalytic hydrogenation afforded 1b in 90% yield. Azido acids, similar to 13, 16, and 20 are reported to be candidates for Staudinger ligation\(^{14}\) in peptide/protein synthesis (Scheme 2).

Next, we focused our attention to the synthesis of the \( \beta \)-hydroxy proline hydrochloride 1c and its derivative 23. It was also realized that the intermediates, generated in the process may be transformed to the pyrrolidine derivatives such as 1c that are of our own interest as bioactive iminosugars.\(^{14d,v}\) In this direction, compound 6 was subjected to a catalytic transfer hydrogenation (HCO\( \text{NH}_4 \)/10% Pd–C/MeOH) to afford the bicyclic lactam 21 in 91% yield \textit{via} a tandem azide reduction and cyclization. The lactam 21 was reduced with LiAlH\(_4\) in THF under refluxing conditions, and the resultant amine functionality protected with CbzCl to furnish the \( N \)-Cbz protected bicyclic intermediate 22 in 57% yield (over two steps). The carbamate 22 was subsequently transformed to 1c as reported earlier.\(^{16}\) We also synthesized the allyl ester of \( \beta \)-hydroxy proline from 22 without any purification of the intermediates. For this, compound 22 was sequentially subjected to an acid-catalyzed ketal hydrolysis, NaIO\(_4\) cleavage, Pinnick oxidation and alkaline hydrolysis to obtain the crude acid. After drying \textit{in vacuo}, the acid was subjected to a base-catalyzed allylation to furnish the \( \beta \)-hydroxy proline allyl ester 23 in 65% yield (over five steps). It is worth noting that the \( \beta \)-hydroxy esters 14, and 23 could also serve as precursors for functional group transformations at the free hydroxy amino acids.\(^{7,6}\) Next, to confirm the stereochemistry at \( \alpha \) and \( \beta \)-carbon in 14 it is necessary to convert it to a known derivative of \( \beta \)-hydroxy glutamic acid. For this azido acid 13 was opted as suitable substrate thus, a one pot reduction of azide functionality and debenzylation of ester using 10% Pd/C in MeOH suitable substrate thus, a one pot reduction of azide function-
ality and debenzylation of ester using 10% Pd/C in MeOH.
group, because the subsequent deallaylation can be accomplished under neutral and non-reducing conditions using a Pd(II) catalyst (Scheme 3).

For the synthesis of the pyrrolidine iminosugar 2, the carbamate 22 was treated with aqueous TFA to unmask the acetone group, and the resultant diol cleaved with NaIO₄ to yield an aldehyde, which on NaBH₄ reduction afforded the N-Cbz protected pyrrolidine 24. In the final step, the amino functionality in 24 was deprotected by catalytic hydrogenation over 10% Pd-C in MeOH to afford the desired dihydroxypyrrolidine 2 in 84% yield. Compound 2 is a versatile precursor for the 3,4-cis-substituted azo-sugars that show a wide range of biological activity. To our surprise unlike its enantiomer, only a few synthesis of 2 have been reported.¹⁹

Conclusions

In summary, we have devised an important strategy for the synthesis of β-hydroxy derivatives of glutamic acid, proline, glutamine, and a dihydroxy pyrrolidine alkaloid. Using this pathway different orthogonally protected hydroxy equivalent of glutamic acid, glutamine and proline are achievable. Noticeably, similar hydroxy amino acids with their functionalities protected as in 14, and 23 have been used for the synthesis of corresponding thiol derivatives and has been used for peptide ligation (NCL). Inexpensive reagents, cheap starting materials, and simple chemical transformations make this strategy a useful one for the synthesis of various protecting group variants of glutamine, glutamic acid and proline. Our efforts to transform the hydroxy derivatives to mercapto variants and their application in peptide synthesis are in progress and will be reported elsewhere.

Experimental section

{[3aR,5S,6R,6aR]-6-Azido-2,2-dimethyltetrahydrofuro[2,3-d][1,3]-dioxole-5-carboxylic acid 4

To a stirred solution of 3 (3.31 g, 15.52 mmol) in MeCN (50 mL) were added NaH₂PO₄ (0.484 g, 3.10 mmol) in H₂O (5 mL) and aqueous 30% H₂O₂ (2.3 mL, 17.1 mmol). The mixture was cooled to 0 °C, NaClO₂ (2.24 g, 24.84 mmol) in H₂O (6 mL) was dropwise added and stirred at 20 °C till completion of the reaction (cf. 12 h, monitored by gas evolution). The reaction mixture was treated with sodium sulphate (1.00 g), and extracted with EtOAc (3 × 30 mL). Evaporation of solvent and column chromatography (silica gel, 10% MeOH/CHCl₃) of the residue gave 4 (3.25 g, 91%) as a thick liquid. Rf = 0.30 (30% MeOH/CHCl₃); [α]D²⁵ − 313.1 (c 1.08, CHCl₃); rmax/cm⁻¹: 3430, 2108, 1683 cm⁻¹; ¹H NMR: δ 8.19 (broad s, D₂O exchangeable, 1H), 6.01 (d, J = 3.4 Hz, 1H), 4.86 (d, J = 3.7 Hz, 1H), 4.67 (d, J = 3.4 Hz, 1H), 4.33 (d, J = 3.7 Hz, 1H), 1.48 (s, 3H), 1.32 (s, 3H); ¹³C NMR: δ 171.2, 113.1, 105.2, 82.9, 78.1, 66.5, 26.6, 26.2. Anal. calcd for C₁₀H₁₄N₂O₂: C, 41.92; H, 4.84; N, 18.33%. Found: C, 41.99; H, 4.90; N, 18.42%.

3-Azido-6-diazo-3,6-dideoxy-1,2-O-isopropylidene-5-keto-α-xylo-1,4-furanose 5

To a cooled (0 °C) and stirred solution of 4 (3.12 g, 13.62 mmol) in THF (45 mL) was sequentially added Et₃N (2.27 mL, 16.33 mmol) and ethyl chloroformate (1.43 mL, 14.97 mmol). After 15 min, the mixture was brought to room temperature and filtered through Celite-545. CH₂N₂ [prepared from N-nitrosomethyl urea (2.00 g, 19.40 mmol) and KOH (5 g)] in Et₂O (50 mL) was dropwise added to the filtrate at 0 °C in 0.5 h. After stirring at room temperature for 2 h, the mixture was concentrated in vacuo, and the residue purified by column chromatography (silica gel, 10% EtOAc/hexane) gave 5 (2.78 g, 80%) as a thick liquid. Rf = 0.35 (20% EtOAc/hexane); [α]D²⁵ − 90.3 (c 1.14, CHCl₃); rmax/cm⁻¹: 2105, 1720 cm⁻¹; ¹H NMR: δ 5.95 (d, J = 3.3 Hz, 1H), 5.81 (s, 1H), 4.72 (d, J = 3.1 Hz, 1H), 4.62 (d, J = 3.3 Hz, 1H), 4.35 (d, J = 3.1 Hz, 1H), 1.48 (s, 3H), 1.32 (s, 3H); ¹³C NMR: δ 191.1, 112.7, 105.1, 82.8, 82.5, 66.7, 54.7, 26.4, 26.0. Anal. calcd for C₇H₁₁N₃O₅: C, 42.69; H, 4.38; N, 27.66%. Found: C, 42.75; H, 4.44; N, 27.74%.

Methyl[[[3aR,5R,6S,6aR]-6-azido-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl]acetate 6

To a stirred solution of 5 (1.00 g, 3.95 mmol) in anhydrous MeOH (15 mL) was dropwise added silver benzoate (0.290 g, 1.26 mmol) in Et₃N (3 mL). After stirring at 25 °C for 20 min, the mixture was concentrated in vacuo, and the residue purified by column chromatography (silica gel, 5% EtOAc/hexane) to obtain 6 (0.560 g, 55%) as a thick liquid. Rf = 0.52 (20% EtOAc/hexane); [α]D²⁵ − 59.1 (c 1.17, CHCl₃); rmax/cm⁻¹: 2105, 1737, 1208 cm⁻¹; ¹H NMR: δ 5.81 (d, J = 3.7 Hz, 1H), 4.63 (d, J = 3.7 Hz, 1H), 4.60–4.44 (m, 1H), 4.08 (d, J = 3.2 Hz, 1H), 3.67 (s, 3H), 2.87–2.59 (m, 2H), 1.47 (s, 3H), 1.28 (s, 3H); ¹³C NMR: δ 170.6, 112.1, 104.1, 83.5, 75.4, 66.7, 51.9, 33.5, 26.5, 26.1; ESI-MS: calcd for [C₁₀H₆N₂O₂+Na]⁺: 288.09 Da. Found: 279.88 Da. Anal. calcd for C₁₀H₁₂N₂O₂: C, 46.69; H, 5.88; N, 16.33%. Found: C, 46.67; H, 5.93; N, 16.43%.

(2S,3R)-2-Azido-3-(formyloxy)-5-methoxy-5-oxopentanoic acid 7

A solution of 6 (0.702 g, 2.73 mmol) in TFA–H₂O (3.00 mL, 3 : 2) was stirred at 0 °C for 6 h. Azeotropic removal of TFA with toluene in vacuo afforded the intermediate hemiacetal (0.700 g, thick liquid), which was taken in acetone/water (10 mL, 9 : 1), cooled to 0 °C and NaIO₄ (0.640 g, 2.99 mmol) added. After stirring for 0.5 h, the reaction mixture was concentrated in vacuo, the residue extracted with CHCl₃ (3 × 10 mL), and the extract concentrated in vacuo to get the crude α-azido aldehyde (0.503 g, thick liquid). This was dissolved in MeCN (5 mL), treated successively with NaH₂PO₄ (0.08 g, 0.53 mmol) in H₂O (1 mL) and 30% H₂O₂ (0.40 mL, 2.95 mmol), cooled to 0 °C, and NaClO₂ (0.39 g, 4.36 mmol) in H₂O (1.5 mL) added into it in 20 min. After stirring at 20 °C till completion of the reaction (~10 h, monitored by gas evolution), the reaction mixture was treated with sodium sulphate (0.20 g), and extracted with EtOAc (3 × 15 mL). Concentration of the extract in vacuo followed by column chromatography (silica gel, 10% MeOH/CHCl₃) of the
residue gave 7 (0.500 g, 79% in three steps) as a thick liquid. Rf = 0.30 (30% MeOH/CHCl3); [α]D25 = −6.00 (c 1.0, CHCl3); rmax/cm−1: 2111, 1701 cm−1; 1H NMR: δ 9.31–8.71 (broad m, 1H, D2O exchangeable), 8.00 (s, 1H), 5.84–5.65 (m, 1H), 4.31 (d, J = 2.2 Hz, 1H), 3.68 (s, 3H), 2.84 (dd, J = 6.8, 1.2 Hz, 2H); 13C NMR: δ 171.6, 170.2, 159.9, 69.5, 62.3, 52.3, 35.2. Anal. calc'd for C7H9N3O6: C, 36.37; H, 3.92; N, 18.18%. Found: C, 36.42; H, 3.98; N, 18.28%.

(2S,3R)-2-Azido-3-hydroxy-5-methoxy-5-oxopentanoic acid 8

To a cooled (0 °C) and stirred solution of 7 (0.141 g, 0.61 mmol) in THF (3 mL) was added aqueous saturated NaHCO3 (1 mL). After stirring for 0.5 h, the reaction mixture was concentrated in vacuo, the residue acidified to pH 1 with aqueous 1 N HCl, and extracted with EtOAc (6 × 10 mL). The combined organic extracts were dried, concentrated, and the residue purified by column chromatography (silica gel, 30% MeOH/CHCl3) to give 8 (0.30 g, 86%) as a thick liquid. Rf = 0.30 (30% MeOH/CHCl3); [α]D25 = −31.4 (c 1.0, CHCl3); rmax/cm−1: 3584, 2102, 1742 cm−1; 1H NMR: δ 6.90–7.81 (broad s, D2O exchangeable, 1H), 5.86 (d, J = 3.7 Hz, 1H), 4.68 (d, J = 3.7 Hz, 1H), 4.62–4.50 (m, 1H), 4.10 (d, J = 3.2 Hz, 1H), 2.94–2.65 (m, 2H), 1.51 (s, 3H), 1.31 (s, 3H); 13C NMR: δ 175.8, 112.3, 104.2, 83.5, 75.1, 66.7, 33.6, 26.5, 26.2. Anal. calc'd for C5H8N3O7: C, 44.44; H, 5.39; N, 17.28%. Found: C, 44.40; H, 5.41; N, 17.39%.

Benzyl[(3R,5R,6S,6aR)-6-azido-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl]acetate 12

To a solution of 11 (0.210 g, 0.86 mmol) in CH2CN (5 mL) was added Et3N (0.27 mL, 1.93 mmol), CbzCl (0.245 mL, 1.72 mmol) and DMAP (0.05 g, 0.43 mmol). After stirring for 2 h at 25 °C, another portion of CbzCl (0.120 mL, 0.86 mmol) and DMAP (0.03 g, 0.22 mmol) were added and the mixture stirred overnight. It was concentrated in vacuo and extracted with EtOAc (3 × 15 mL). The combined organic extracts were sequentially washed with aqueous NaHCO3 (5 mL) and water (5 mL), and dried. Concentration of extract and column chromatography (silica gel, 5% EtOAc/hexane) of the residue afforded 12 (0.180 g, 63%) as a viscous liquid. Rf = 0.51 (10% EtOAc/hexane); [α]D25 = −28.5 (c 1.0, CHCl3); rmax/cm−1: 3110, 1734 cm−1; 1H NMR: δ 6.74 (s, 5H), 5.84 (d, J = 3.7 Hz, 1H), 5.14 (s, 2H), 4.65 (d, J = 3.7 Hz, 1H), 4.59 (ddd, J = 8.2, 6.3, 3.3 Hz, 1H), 4.09 (d, J = 3.3 Hz, 1H), 2.96–2.66 (m, 2H), 1.49 (s, 3H), 1.31 (s, 3H); 13C NMR: δ 170.0, 135.5, 128.6, 128.4, 128.2, 112.2, 104.2, 83.6, 75.5, 66.8, 66.7, 33.9, 26.5, 26.2; ESI-MS: calcd for [C5H12N3O7 + Na]+: 356.12 Da. Found: 355.89 Da. Anal. calc'd for C9H12N3O7: C, 57.65; H, 5.75; N, 12.61%. Found: C, 57.63; H, 5.78; N, 12.70%.

(2S,3R)-2-Azido-5-(benzoxyl)-3-hydroxy-5-oxopentanoic acid 13

As described earlier, 12 (0.100 g, 0.30 mmol) was deacetalized with TFA–H2O (3 mL: 2, the resultant diol cleaved with NaOH (0.072 g, 0.33 mmol) in 10% aqueous acetone (5 mL) followed by oxidation with NaH2PO4 (0.01 g, 0.06 mmol), 30% H2O2 (50 μL, 0.30 mmol) and NaClO2 (0.05 g, 0.49 mmol). The product was then dehydrated with aqueous saturated NaHCO3 (1 mL). Usual workup and column chromatography (silica gel, 2% MeOH/CHCl3) of the residue afforded 13 (0.060 g, 71% over four steps) as a thick liquid. Rf = 0.31 (10% MeOH/CHCl3); [α]D25 = −20.0 (c 1.01, CHCl3); rmax/cm−1: 3112, 1725 cm−1; 1H NMR: δ 7.33 (s, 5H), 6.91 (broad s, D2O exchangeable, 2H), 6.89, 66.7, 64.9, 52.1, 37.8. Anal. calc'd for C9H13N3O7: C, 44.44; H, 5.39; N, 17.28%. Found: C, 44.51; H, 5.46; N, 17.36%.
5-Benzyl 1-prop-2-en-1-yl(2S,3R)-2-azido-3-hydroxypentanedioate 14

Following the procedure used for 9, the acid 13 (0.078 g, 0.27 mmol) was subjected to alkylation using allyl bromide (29.0 µL) and NaHCO3 (0.056 g) in DMF (1 mL). Usual workup and column chromatography (silica gel, 5% EtOAc/hexane) of the residue afforded 14 (0.075 g, 87%) as a thick liquid. Rf = 0.38 (20% EtOAc/hexane); [α]25D +24.3 (c 1.08, CHCl3); vmax/cm⁻¹: 2108, 1742 cm⁻¹; 1H NMR: δ 7.34 (s, 5H), 6.05-5.80 (m, 1H), 5.42-5.29 (m, 2H); 13C NMR: δ 172.4, 171.7, 135.1, 128.6, 128.5, 128.3, 68.7, 67.0, 64.8, 38.0. Anal. calc'd for C12H13N3O5: C, 51.61; H, 4.69; N, 15.05%. Found: C, 51.58; H, 4.72; N, 15.13%.

(2S,3R)-3-Hydroxy-γ-glutamic acid hydrochloride 1a

A mixture of 13 (0.085 g, 0.31 mmol) and 10% Pd–C (0.02 g) in methanolic HCl (10 mL) was stirred for 12 h under H2 (80 psi). The catalyst was filtered through Celite-545 and washed with MeOH (3 × 10 mL), concentrated and the residue dried in vacuo to afford 1a (0.048 g, 95%) as a semisolid. [α]25D +14.7 (c 1.02, H2O); vmax/cm⁻¹: 3577, 1737 cm⁻¹; 1H NMR: δ 4.66-4.58 (m, 1H), 4.10 (d, J = 3.3 Hz, 1H), 2.87 (dd, J = 16.4, 4.0 Hz, 1H), 2.72 (dd, J = 16.4, 8.7 Hz, 1H); 13C NMR: δ 174.1, 170.3, 65.5, 57.3, 38.4; ESI-MS: calc'd for [C4H10NO3 + Na]+: 194.03 Da. Found: 194.06 Da. Anal. calc'd for C12H16N2O5: C, 51.61; H, 5.69; N, 15.43%. Found: C, 51.58; H, 5.69; N, 15.46%.

Prop-2-en-1-yl(2S,3R)-2-azido-5-(benzylamino)-3-hydroxy-5-oxopentanoate 17

Following the procedure described earlier, 16 (0.06 g, 0.22 mmol) was reacted with allyl bromide (20 µL) in the presence of NaHCO3 (0.04 g) in DMF (2 mL). Usual workup and column chromatography (silica gel, 8% EtOAc/hexane) of the residue gave 17 (0.055 g, 79%) as a thick liquid. Rf = 0.41 (30% EtOAc/hexane); [α]25D +16.4 (c 0.55, CHCl3); vmax/cm⁻¹: 2105, 1744 cm⁻¹; 1H NMR: δ 7.38-7.17 (m, 5H), 6.13-5.69 (m, partially D2O exchangeable, 2H), 5.45-5.15 (m, 2H), 4.67 (broad d, J = 5.8 Hz, 2H), 4.62-4.46 (m, 1H), 4.38 (d, J = 5.7 Hz, 2H), 3.75 (d, J = 3.2 Hz, 1H), 2.58 (dd, J = 15.4, 8.9 Hz, 1H), 2.37 (dd, J = 15.4, 3.8 Hz, 1H), 1.58 (broad s, D2O exchangeable, 1H); 13C NMR: δ 170.8, 168.4, 137.6, 131.1, 128.8, 128.7, 127.7, 119.4, 69.6, 66.7, 65.1, 43.7, 39.1; ESI-MS: calc'd for [C15H18N4O5 + Na]+: 341.12 Da. Found: 340.90 Da. Anal. calc'd for C12H16N4O3: C, 56.60; H, 5.70; N, 17.60%. Found: C, 56.55; H, 5.68; N, 17.69%.

(1S,2R)-4-(Benzylamino)-1-carboxy-2-hydroxy-4-oxobutan-1-aminocloride 18

Catalytic hydrogenation of 16 (0.048 g, 0.17 mmol) over 10% Pd/C (0.015 g) in MeOH (5 mL) using H2 (80 psi) gave 18 (0.044 g, 89%) as a semi-solid. [α]25D +22.0 (c 1.00, CHCl3); vmax/cm⁻¹: 1713, 1406 cm⁻¹; 1H NMR: δ 7.44 (d, J = 3.9 Hz, 2H), 7.38 (s, 3H), 4.72-4.64 (m, 1H), 4.44 (s, 2H), 4.19 (d, J = 3.7 Hz, 1H), 2.82-2.76 (m, 1H), 2.70 (broad d, J = 14.8, 8.8 Hz, 1H); 13C NMR: δ 173.6, 171.7, 139.5, 130.5, 129.2, 129.0, 67.8, 59.0, 44.8, 41.8; ESI-MS: calc'd for [C11H20N2O4 + H]+: 253.11 Da. Obsd: 253.90 Da. Anal. calc'd for C12H14Cl2N2O4: C, 49.92; H, 5.93; N, 9.70%. Found: C, 49.88; H, 5.96; N, 9.78%.
To a solution of 11 (0.210 g, 0.87 mmol) in CH₂CN (10 mL) was added (Boc)₂O (0.246 g, 1.13 mmol), and NH₄HCO₃ (0.161 g, 2.04 mmol) to give a cloudy mixture. After adding pyridine (0.05 mL, 0.60 mmol), the mixture was stirred at room temperature till completion of the reaction (~for 5 h, cf. TLC). The mixture was concentrated in vacuo, the residue extracted with EtOAc (3 × 15 mL), the organic extract washed with water (5 mL) and dried. Concentration of the extract in vacuo, and column chromatography (silica gel, 30% EtOAc/hexane) of the residue afforded 19 (0.189 g, 90%) as colorless crystals. mp: 122–125 °C; Rₙ = 0.45 (80% EtOAc/hexane); [α]₂⁰⁺ — 23.1 (c 1.18, MeOH); rₚmax/cm⁻¹: 2111, 1665 cm⁻¹; ¹H NMR: δ 5.81 (d, J = 3.7 Hz, 1H), 4.72 (d, J = 3.7 Hz, 1H), 4.56 (dd, J = 6.9, 3.1 Hz, 1H), 4.04 (dd, J = 3.1 Hz, 1H), 2.63 (dd, J = 15.2, 7.3 Hz, 1H), 2.55 (dd, J = 15.2, 6.6 Hz, 1H), 1.45 (s, 3H), 1.29 (s, 3H); ¹³C NMR: δ 172.1, 110.2, 102.7, 81.9, 74.6, 65.6, 33.0, 23.8, 23.4; ESI-MS: calecd for [C₅H₄N₄O₄ + Na]: 265.09 Da. Found: 265.90 Da. Anal. calecd for C₅H₄N₂O₄; C, 44.63; H, 5.83% N, 23.13%. Found: C, 44.59; H, 5.87%; N, 7.10%.

(2S,3R,5)-5-Amino-2-azido-3-hydroxy-5-oxopentanoic acid 20

Following the procedure used for the synthesis of 16, compound 19 (0.08, 0.33 mmol) was deacetalized with TFA–H₂O (3 mL, 3:2), the resultant diol subjected to oxidative cleavage with NaIO₄ (0.08 g, 0.37 mmol) followed by oxidation with NaH₂PO₄ (3a:2b), the resultant diol subjected to oxidative cleavage with NaIO₄ (0.110 g, 0.50 mmol), followed by oxidation with NaH₂PO₄ (0.300 g, 1.16 mmol), 10% Pd/C (0.03 g) and H₂ (80 psi); [α]₁⁰⁰⁺ = -12.3 (c 1.04, H₂O); rₚmax/cm⁻¹: 3576, 1715 cm⁻¹; ¹H NMR: δ 4.86 (t, J = 4.0 Hz, 1H), 4.44 (d, J = 4.0 Hz, 1H), 3.50–3.71 (m, 2H), 2.39–2.29 (m, 1H), 2.26–2.18 (m, 1H); ¹³C NMR: δ 169.2, 70.8, 66.3, 44.0, 32.9. Anal. calecd for C₂H₁₃NO₅: C, 53.83; H, 6.01% N, 8.36%. Found: C, 53.80; H, 6.07%; N, 8.44%.
Prop-2-en-1-yl[25,3R)-N-carboxybenzyl-3-hydroxypyrrolidine-2-carboxylate 23

As described before, 22 (0.064 g, 0.20 mmol) was deacetelated using TFA-H2O (3 mL, 3:2), the resultant dial oxidatively cleaved with NaIO4 (0.047 g, 0.22 mmol) followed by oxidation with Na2P2O7 (0.006 g), 30% H2O2 (21 µL), NaClO2 (0.03 g), and the formyl group unmasked with aqueous NaHCO3 (1 mL) in THF (5 mL). The resultant crude acid was dried in vacuo and allylated with allyl bromide (21.6 µL) and NaHCO3 (0.04 g) in DMF (3 mL). The mixture was concentrated in vacuo, and the residue extracted with EtOAc (3 × 10 mL), the organic extract washed with water (2 × 5 mL) and dried. Concentration of the extract in vacuo, and column chromatography of the residue (silica gel, 15% EtOAc/hexane) gave 23 (0.040 g, 65% over five steps) as a thick liquid. 

References


