Use of 'Click Chemistry' for the Synthesis of Tetrazole-Containing Analogues of the Neuroprotective Agent Glycyl-L-prolyl-L-glutamic Acid

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Abstract: Tetrazole-containing analogues of glycyl-L-prolyl-L-glutamic acid (GPE) were prepared by coupling of Cbz-glycyl-L-proline with tetrazole-containing glutamic acids followed by hydrogenation of the resultant tripeptide. Synthesis of the tetrazole-containing glutamic acids involved 1,3-dipolar cycloaddition of sodium azide to nitrile derivatives of the corresponding glutamic acids.

Key words: GPE, 1,3-dipolar cycloaddition, tetrazole, TMSI, BoPCl

Glycyl-L-prolyl-L-glutamic acid (GPE, **1**, Figure 1) is a tripeptide derived from insulin-like growth factor I (IGF-1) upon enzymatic hydrolysis.[1] Use of GPE for the treatment of hypoxic-ischemic (HI) brain injury is significant although the poor pharmacological profiles of this agent prompts the development of better analogues.[2] Several analogues of GPE have previously been synthesised with modification at the side chains of either the glutamic acid,[3a,b] glycine,[3c] or proline[3d,e] residues in order to probe the structure–activity relationship (SAR) of GPE and to identify analogues with better neuroprotective activities than the native tripeptide.

The tetrazole moiety (**4a** and **4b**, Figure 2) serves as a surrogate for a carboxylic acid functionality.[4] Pharmaceutical agents containing tetrazole rings have been shown to exhibit improved metabolic stability and oral bioavailability.[5a,b]

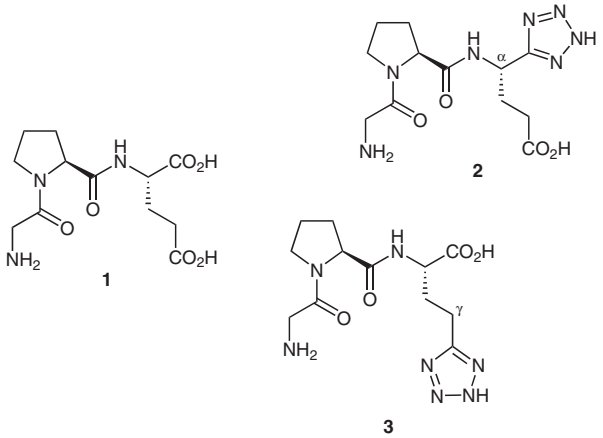
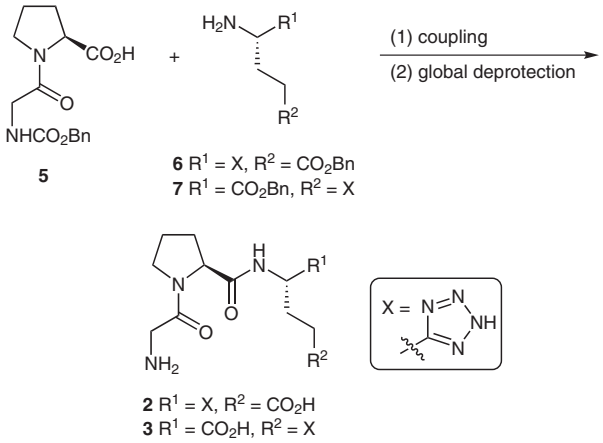


Figure 1 Structures of GPE (**1**) and α- and γ-tetrazole-containing analogues (**2** and **3**) of GPE

Figure 2 Structure of tetrazole

Thus, the preparation of tetrazole-containing analogues of GPE by substituting either the α- or γ-carboxylic acid (**2** or **3**) of glutamic acid with a tetrazole ring may afford a better therapeutic agent for traumatic brain injury.

Our strategy to synthesise tetrazole analogues **2** and **3** of GPE involved coupling of protected glycyl-L-proline **5** to tetrazole-containing glutamic acids **6** and **7**, respectively, thus affording the tripeptide mimics after deprotection by hydrogenolysis of the benzyloxycarbonyl groups (Scheme 1).



Scheme 1 Synthesis of tetrazole-containing analogues **2** and **3**

As reported previously, the synthesis of *N*-benzyloxycarbonyl-glycyl-L-proline (**5**, Scheme 2) was carried out by coupling *N*-benzyloxycarbonyl-glycine (**8**) with *N*-hydroxysuccinimide (**9**) under N_2 at 0 °C.[6] Without purification, the resultant compound **10** was then coupled to L-proline (**11**) at room temperature followed by acidification to afford dipeptide **5** in 77% yield.[7]

α-Tetrazole glutamic acid **12** was obtained by 1,3-dipolar cycloaddition of nitrile **15** with NaN_3 (Scheme 3). Nitrile

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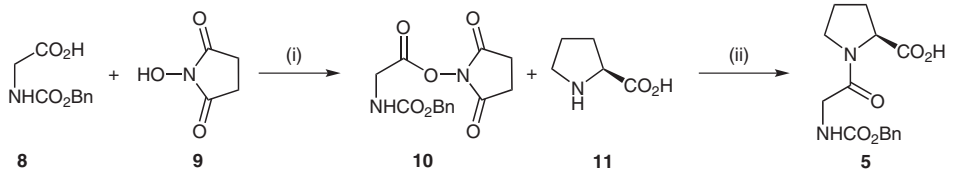
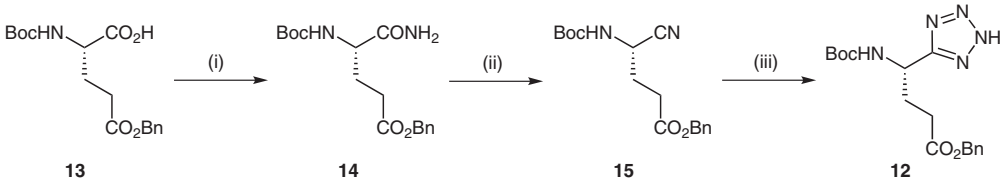
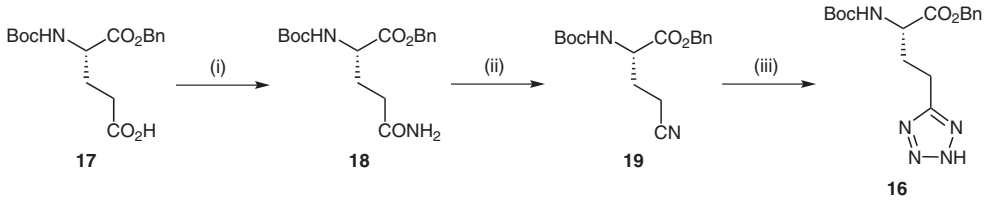


Scheme 2 Reagents and conditions: (i) DCC, DME, N_2, 0 °C, 3 h; (ii) $NaHCO_3$, H_2O, DME, 4 h then HCl (77% over 2 steps).



Scheme 3 *Reagents and conditions*: (i) Boc_2O, NH_4HCO_3, pyridine, dioxane, N_2, r.t., 18 h (93%); (ii) cyanuric chloride, DMF, 0 °C to r.t., 22.5 h (98%); (iii) Et_3N, AcOH, NaN_3, dry toluene, N_2, reflux, 16 h (88%).



Scheme 4 *Reagents and conditions*: (i) Boc_2O, NH_4HCO_3, pyridine, dioxane, N_2, r.t., 19 h (79%); (ii) cyanuric chloride, DMF, 0 °C to r.t., 22.5 h (26%); (iii) Et_3N, AcOH, NaN_3, dry toluene, N_2, reflux, 29 h (82%).

15 in turn was obtained in excellent yield from acid **13** via amide **14**. Initial synthesis of tetrazole **12** was carried out using NaN_3 and $ZnBr_2$ in a mixture of H_2O–*i*-PrOH conditions reported by Sharpless et al.[8] The synthesis of the tetrazole-containing glutamic acid using these conditions was unsuccessful in our hands despite the synthesis of an analogous Fmoc-protected tetrazole-containing glutamic acid having been reported previously.[9]

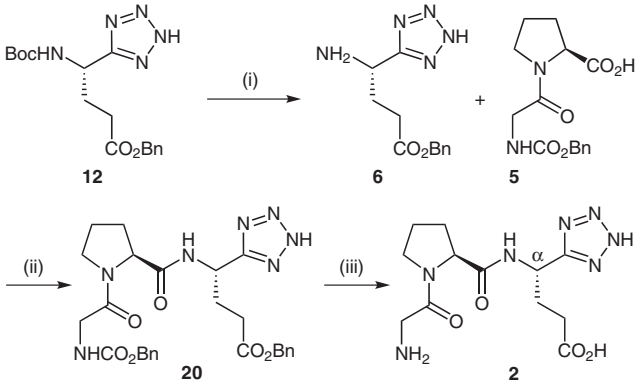
It was suspected in our case that hydrolysis of the ester group by water was occurring under these conditions. This was confirmed by the observation of benzyl alcohol in the ^1H NMR spectrum of the crude product. Use of H_2O was therefore avoided by using Et_3N, AcOH, and NaN_3 in dry toluene under reflux affording α-tetrazole glutamic acid **12** in 88% yield after purification by flash column chromatography.[10]

γ-Tetrazole glutamic acid **16** was next synthesised from nitrile **19** using the same reaction conditions (Scheme 4). In turn, the synthesis of nitrile from amide **18** proceeded uneventfully.

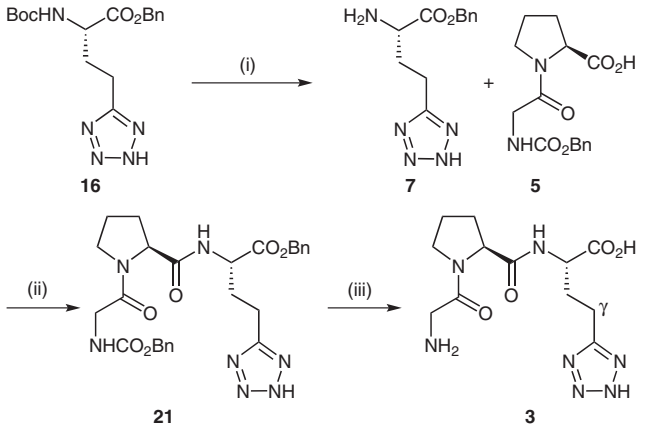
Deprotection of the Boc group from tetrazoles **12** and **16** was next required in order to initiate the subsequent coupling reaction. Sureshbabu et al.[9] reported that deprotection of Boc-Phe tetrazole using TFA was unsuccessful. In the present work, deprotection of the Boc group using TFA led to formation of *tert*-butylated byproducts despite the use of scavengers (H_2O–triisopropylsilane). Fortunately the formation of the *tert*-butyl byproducts was eliminated using trimethylsilyl iodide (TMSI).[11] Deprotection of the Boc group using TMSI proceeds via an S_N2 mechanism giving *tert*-butyl iodide rather than a *tert*-butyl cation as a byproduct. Formation of *tert*-butylated byproduct was eliminated in the absence of the electrophic *tert*-butyl cation.

With the synthesis of tetrazoles **6** and **7** in hand, subsequent coupling with dipeptide **5** using BoPCl in CH_2Cl_2 for 3 hours afforded the protected tripeptides **20** and **21** in 26% and 27% yields, respectively, following purification by reverse-phase HPLC (Scheme 5 and Scheme 6). Tetrazoles **2** and **3** were then obtained via hydrogenation of the protected tripeptides **20** and **21**.

In conclusion, the synthesis of two tetrazole-containing analogues **2** and **3** of the neuroprotective agent GPE (**1**),



Scheme 5 *Reagents and conditions*: (i) TMSI (3 equiv), MeCN, N_2, 18 min (ca. 100%); (ii) BoPCl (2 equiv), CH_2Cl_2, r.t., 3 h (26% over 2 steps from **12**); (iii) H_2, 10% Pd/C, MeOH–H_2O (80:20), r.t., 17.5 h (60%).



Scheme 6 *Reagents and conditions*: (i) TMSI (3 equiv), MeCN, N_2, 18 min (ca. 100%); (ii) BoPCl (2 equiv), CH_2Cl_2, r.t., 3 h (28% over 2 steps from **16**); (iii) H_2, 10% Pd/C, MeOH–H_2O (80:20), r.t., 19.5 h (63%).

is reported herein. Additionally, protected tetrazole-containing glutamic acids **12** and **16** were successfully prepared by 1,3-dipolar cycloaddition of sodium azide with glutamic acid derived nitriles **15** and **19**. Deprotection of the Boc group using TMSI was important to avoid unwanted *tert*-butylation of the deprotected tetrazoles **6** and **7**. Coupling of tetrazoles **6** and **7** with dipeptide **5** gave tripeptides **21** and **22** which then underwent hydrogenation to afford the desired tetrazole-containing analogues **2** and **3**. More importantly, the tetrazole-modified glutamic acids **6** and **7** are useful building blocks for the preparation of bioisosteres of glutamic acid containing peptides thus providing an attractive tool for the generation of peptidomimetics.

General Procedure for the Preparation of Tetrazole-Containing Glutamic Acids 12 and 16

To a solution of distilled Et_3N (4 equiv) in dry toluene was added glacial AcOH (4 equiv), and the solution was stirred under N_2 for 5 min. The solution was transferred to a flask containing the appropriate nitrile (1 equiv) and NaN_3 (4 equiv), and the reaction was stirred under N_2 at reflux for 16 h. The solid was filtered, and the filtrate was concentrated under reduced pressure to afford an oil which was purified by flash column chromatography (hexane–EtOAc, 1:1 with 1% AcOH) to afford the desired products as colourless solids.

γ-Benzyl *N*-*tert*-Butyloxycarbonyl-L-glutamate α-Tetrazole (12)

HRMS (EI): *m/z* [M^+] calcd for $C_{17}H_{23}N_5O_4$: 361.1750; found: 361.1752. IR: 3322, 2919, 1716, 1685, 1516, 1438, 1393, 1147, 749, 700 cm^{-1}. Mp 144–146 °C. ^1H NMR (300 MHz, MeOD): δ = 1.43 [9 H, s, $C(CH_3)_3$], 2.11–2.23 (m, *J* = 2.1, 6.9 Hz, 1 H, Gluβ-H), 2.29–2.40 (m, *J* = 6.9 Hz, 1 H, Gluβ-H), 2.51–2.56 (t, *J* = 7.5 Hz, 2 H, Gluγ-H_2), 5.04–5.09 (m, *J* = 4.2 Hz, 1 H, Gluα-H), 5.12 (s, 2 H, OCH_2PH), 7.29–7.37 (m, 5 H, Ph). ^{13}C NMR (75 MHz, MeOD): δ = 27.24 [CH_3, $C(CH_3)_3$], 28.20 (CH_2, Gluβ-C), 29.63 (CH_2, Gluγ-C), 45.27 (CH, Gluα-C), 66.06 (CH_2, OCH_2Ph), 79.60 [q, $C(CH_3)_3$], 127.80 (CH, Ph), 127.92 (CH, Ph), 128.13 (CH, Ph), 136.10 (q, Ph), 156.24 (q, NCO_2), 158.28 (q, C=N), 173.10 (q, Gluγ-*C*O).

α-Benzyl *N*-*tert*-Butyloxycarbonyl-L-glutamate γ-Tetrazole (16)

HRMS (EI): *m/z* [M^+] calcd for $C_{17}H_{23}N_5O_4$: 361.1750; found: 361.1756. IR: 3344, 2979, 1712, 1671, 1523, 1455, 1367, 1158, 754, 698 cm^{-1}. Mp 118–120 °C. ^1H NMR (300 MHz, MeOD): δ = 1.70 [9 H, s, $C(CH_3)_3$], 2.35–2.44 (m, 1 H, Gluβ-H), 2.59–2.63 (m, 1 H, Gluβ-H), 3.26–3.29 (t, *J* = 5.5 Hz, 2 H, Gluγ-H_2), 4.49–4.51 (m, *J* = 3.3 Hz, 1 H, Gluα-H), 5.42–5.44 (s, 2 H, OCH_2PH), 7.64–7.60 (m, 5 H, Ph). ^{13}C NMR (75 MHz, MeOD): δ = 20.36 (CH_2, Gluγ-C), 27.57 [CH_3, $C(CH_3)_3$], 29.35 (CH_2, Gluβ-C), 53.53 (CH, Gluα-C), 66.85 (CH_2, OCH_2Ph), 79.61 [q, $C(CH_3)_3$], 128.10 (CH, Ph), 128.16 (CH, Ph), 128.42 (CH, Ph), 136.05 (q, Ph), 157.50 (q, NCO_2), 158.28 (q, C=N), 172.33 (q, Gluα-*C*O)

General Procedure for the Preparation of Tetrazole-Containing GPE Analogues 2 and 3

TMSI (3 equiv) was added to a solution of tetrazole-containing glutamic acids **6** and **7** in MeCN, and the reaction was stirred under N_2 for 18 min. MeOH was added, and the solution was stirred for 5 min. The solvent was removed under reduced pressure to afford an oil that was reacted with dipeptide **5** (1 equiv), BoPCl (2 equiv), and DIPEA (3 equiv) in CH_2Cl_2 under N_2. After completion of the reaction, the solvent was removed under reduced pressure, and the residue was purified by reverse-phase HPLC (Waters C_{18} Xterra, 19 × 250 mm, 10 mL/min, 1% B to 70% B where B: 0.1% TFA in MeCN; A: 0.1% TFA in H_2O) to afford protected tripeptides as colourless solids. Hydrogenation of the resultant tripeptides afforded the desired analogues **2** and **3**.

Glycyl-L-prolyl-L-glutamate α-Tetrazole (2)

HRMS (EI): *m/z* [M^+] calcd for $C_{12}H_{20}N_7O_4$: 326.1571; found: 326.1570. IR: 2947, 1648, 1402, 1353, 1246, 1201, 1119 cm^{-1}. Mp not measured as compound was hydroscopic. ^1H NMR (300 MHz, D_2O): δ = 1.77–2.36 (m, 8 H, Proγ-H_2, Proβ-H_2, Gluγ-H_2, Gluβ-H_2), 3.36–3.48 (m, 2 H, Proδ-H_2), 3.82–3.94 (m, 2 H, Glyα-H_2), 4.32–4.37 (m, 1 H, Proα-H), 5.31–5.18 (m, 1 H, Gluα-H). ^{13}C NMR (75 MHz, D_2O): δ = 21.96* (CH_2, Proγ-C), 24.18 (CH_2, Proγ-C), 29.38 (CH_2, Proβ-C), 31.77* (CH_2, Proβ-C), 28.65* (CH_2, Gluβ-C), 28.90 (CH_2, Gluβ-C), 31.42 (CH_2, Gluγ-C), 31.77* (CH_2, Gluγ-C), 40.19* (CH_2,Glyα-C), 40.38 (CH_2,Glyα-C), 45.25 (CH, Gluα-C), 45.66* (CH, Gluα-C), 46.83 (CH_2, Proδ-C), 47.52* (CH_2, Proδ-C), 59.93* (CH, Proα-C), 60.45 (CH, Proα-C), 145.79 (q, C=N), 165.59 (q, Gly-CO), 165.90* (q, Gly-CO), 172.90* (q, NCO), 173.62 (q, NCO), 179.05 (q, Gluγ-COOH). The product was shown to be a 75:25 mixture of *trans*/*cis* conformers and the chemical shifts for the minor *cis*-conformer are denoted by an asterisk (*).

Glycyl-L-prolyl-L-glutamate γ-Tetrazole (3)

HRMS–FAB: *m/z* [M + H]$^+$ calcd for $C_{12}H_{20}N_7O_4$: 326.1577; found: 326.1570. IR: 2959, 1648, 1536, 1428, 1353, 1246, 1200, 1128 cm^{-1}. Mp not measured as compound was hydroscopic. ^1H NMR (300 MHz; D_2O): δ = 1.91–2.28 (m, 6H, Proγ-H_2, Proβ-H_2, Gluβ-H_2), 2.88–2.99 (m, 2 H, Gluγ-H_2), 3.41–3.49 (m, 2 H, Proδ-H_2), 3.82–3.99 (m, 2 H, Glyα-H_2), 4.12–4.16 (m, 1 H, Gluα-H), 4.37–4.41 (m, 1 H, Proα-H). ^{13}C NMR (75 MHz, D_2O): δ = 19.63 (CH_2, Gluγ-C), 20.09* (CH_2, Proγ-C), 22.03* (CH_2, Gluγ-C), 24.26 (CH_2, Proγ-C), 29.27 (CH_2, Gluβ-C), 29.36 (CH_2, Proβ-C), 29.77* (CH_2, Proβ-C), 31.73* (CH_2, Gluβ-C), 40.24* (CH_2, Glyα-C), 40.40 (CH_2, Glyα-C), 46.92 (CH_2, Proδ-C), 47.54* (CH_2, Proδ-C), 54.10 (CH, Gluα-C), 60.10* (CH, Proα-C), 60.58 (CH, Proα-C), 147.47 (q, C=N), 165.63 (q, Gly-CO), 166.11* (q, Gly-CO), 173.22* (q, NCO), 173.50 (q, NCO), 177.15 (q, Gluα-COOH). The product was shown to be a 80:20 mixture of *trans*/*cis* conformers and the chemical shifts for the minor *cis*-conformer are denoted by an asterisk (*).

Acknowledgment

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