Supporting Information

Strain-Promoted Reaction of 1,2,4-Triazines with Bicyclononynes

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Supporting information

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Figure S1 Rate of reaction of triazinylalanine (TrzAla) 13 (1 mM) with bicyclononyne (BCN) 19 in 10% H₂O/MeCN determined by HPLC measurement of formation of products 20a and 20b. Rate data were globally fitted to 2nd order kinetics (Rate = \( k_2[\text{BCN}][\text{TrzAla}] \)) under the assumption of low substrate consumption during the measured time-course.
General experimental

All reagents were purchased from Sigma Aldrich, Alfa Aesar, Merck or Fisher Scientific and all were used without further purification. All solvents used were HPLC grade and mixtures are v/v. NMR data were collected using a Bruker DPX300 and analysed using MestReNova software. The following abbreviations are used to describe the multiplicity of signals: s = singlet, d = doublet, dd = doublet of doublets, qn = quintet, m = multiplet. J-values are given in Hz. IR spectra were recorded using a PerkinElmer spectrum one FTIR spectrometer, Vibrational frequencies are reported in wavenumbers (cm\(^{-1}\)). Optical rotations were measured using a Schmidt+Haensch polartronic H532, \([\alpha]_D\) values are given in 10\(^{-1}\) deg cm\(^2\) g\(^{-1}\). High resolution mass spectrometry (HRMS) was carried out on a Bruker Daltonics micrOTOF using electrospray ionisation or on a Micromass GCT Premier, using electron impact ionisation (EI). Column chromatography was carried out using silica gel and TLC was performed on silica gel 60-F\(_{254}\) (Merck) with detection by fluorescence upon irradiation with UV light or staining by immersion in a solution of potassium permanganate or ninhydrin and heating. HPLC analyses were carried out on a Dionex HPLC system using an Ace UltraCore 2.5 Super C18, (50 x 2.1mm) and diode array as a detector; either on gradient A: mobile phase (H\(_2\)O/MeCN, 0.1% TFA), gradient (5-95% MeCN) or B: mobile phase (H\(_2\)O/MeCN), gradient (5-95% MeCN), at a flow rate of 0.5 ml/min. Melting points were determined on a Stuart SMP3 melting point apparatus. Melting points obtained were uncorrected.
Synthesis of 5a-c, 6,7,Fmoc-Ser-OMe, Fmoc-Ser(OTs)-OMe, 11, 14, 17a, 18 & 19

3-Amino-1,2,4-triazine 5a

\[
\text{H}_2\text{N} \quad \text{N} \quad \text{N} \quad \text{N}
\]

Aminoguanidine bicarbonate (3.00 g, 22 mmol, 1 eq) was stirred in H\textsubscript{2}O (40 ml), the pH was dropped to 3-4 on addition of small portions of 18% HCl (~5 ml) until CO\textsubscript{2} gas evolution seized and the solution became clear.\textsuperscript{[43]} Glyoxal (40% in H\textsubscript{2}O) (2.5 ml, 22 mmol, 1 eq) was added and the pH increased to 12 with addition of 50% KOH (~5 ml). The mixture was stirred for 2 hours and lyophilised. The resultant brown powder was triturated in 1:3 hexanes/EtOAc and hot filtered. The solution was concentrated and the crude product was recrystallised from 1:3 hexanes/EtOAc to give the title compound (365 mg, 3.91 mmol, 17%) as pale orange needles. m.p. 179.2-179.9 °C; \( R_F \) (5% MeOH in DCM) 0.17; \( \delta_H \) (300 MHz, CDCl\textsubscript{3}) 8.50 (1H, d, \( ^3J_{H-H} \) 2.4, \( H_6 \)), 8.20 (1H, d, \( ^3J_{H-H} \) 2.4, \( H_5 \)), 7.17 (2H, s, \( NH_2 \)); \( \delta_C \) (125 MHz, CDCl\textsubscript{3}) 163.3 (C\textsubscript{3}), 149.88 (C\textsubscript{5}), 140.63 (C\textsubscript{6}); \( \nu_{\text{max}} \) (solid)/cm\textsuperscript{-1}: 3016 & 2970 (NH stretch); m/z (EI) Found: M(+H) 93.0, C\textsubscript{3}H\textsubscript{5}N\textsubscript{4} requires 93.0436); HPLC (5-95% A) retention time 0.43 min, 100%.

6-Methyl-3-amino-1,2,4-triazine and 5-methyl-3-amino-1,2,4-triazine 5b

\[
\text{H}_2\text{N} \quad \text{N} \quad \text{N} \quad \text{N}
\]

The reaction procedure was similar to that for 5a. The Crude product was recrystallized twice from boiling isopropanol to give either a mixture of 6-Methyl-3-amino-1,2,4-triazine and 5-methyl-3-amino-1,2,4-triazine (2:1) (53.1 mg, 0.74 mmol, 18%) as a yellow solid or 6-Methyl-3-amino-1,2,4-triazine: m.p. 122.0-125.6 °C; \( R_F \) (5% MeOH in DCM) 0.17; \( \delta_H \) (500 MHz, DMSO): 8.15 (1H, s, \( H_5 \)), 6.90 (2H, s, \( NH_2 \)), 2.38 (3H, s, \( CH_3 \)), \( \delta_C \) (125 MHz, CDCl\textsubscript{3}) 162.0 (C\textsubscript{3}), 150.2 (C\textsubscript{5}), 147.8 (C\textsubscript{6}), 17.9 (CH\textsubscript{3}); \( \nu_{\text{max}} \) (solid)/cm\textsuperscript{-1}: 3160 (NH\textsubscript{2}); m/z (EI) Found: M(+H) 110.1 C\textsubscript{4}H\textsubscript{7}N\textsubscript{4} requires 110.0592. HPLC (5-95% A): retention time 1.11 min, 100%. (6-methyl-3-amino-1,2,4-triazine & 5-methyl-3-amino-1,2,4-triazine (2:1): \( \delta_H \) (500 MHz, MeOD): 8.46 (1H, s, 5-methyl-\( H_6 \)), 8.23 (1H, s, 6-methyl-\( H_5 \)), 2.48 (3H, s, 6-methyl-\( CH_3 \)), (3H, s, 5-methyl-\( CH_3 \)).

6-Phenyl-3-amino-1,2,4-triazine 5c

\[
\text{H}_2\text{N} \quad \text{N} \quad \text{N} \quad \text{N}
\]

The reaction procedure was similar to that of 5a. The crude product was recrystallized twice from boiling isopropanol to give 6-Phenyl-3-amino-1,2,4-triazine
(100 mg, 0.58 mmol, 45%) as a flocculent orange solid; \( R_F \) (5% MeOH in DCM) 0.10; \( \delta_H \) (500 MHz, CDCl\(_3\)): 9.34 (1H, s, 5-H), 8.19 (2H, dd, \(^3J_{H-H} 1.4, 4 J_{H-H} 8.2\), benzyl-\( H_{2+5} \)), 7.63-7.56 (3H, m, benzyl-\( H_{3-5} \)), 7.25 (2H, s, NH\(_2\)); \( \delta_C \) (125 MHz, CDCl\(_3\)): 163.8 (\( C_3 \)), 150.4 (\( C_5 \)), 135.7 (\( C_6 \)), 130.3 (benzyl-\( C_1 \)), 126.7 (benzyl-\( C_2-6 \)); \( \nu_{\text{max}} \) (solid)/cm\(^{-1}\): 3301 (NH stretch); \( m/z \) (ES) Found: \( M(\text{+H}) \) 173.0822, C\(_9\)H\(_9\)N\(_4\) requires 173.1866; HPLC: retention time 1.20 min, 90%.

**3-((Benzyloxy)carbonyl)propanoic acid 6**

A mixture of succinic anhydride (2.00 g, 20 mmol, 1 eq), benzyl alcohol (2.1 ml, 20 mmol, 1 eq), DMAP (2.48 g, 20 mmol, 1 eq) and triethylamine (1 ml) in DCM (~30 ml) was stirred at room temperature for 16 hours.\(^{[44]}\) The reaction was quenched with 5% Na\(_2\)CO\(_3\) (~30 ml) and the layers were separated. The aqueous layer was acidified with 1M HCl (~pH 3-4), extracted with EtOAc (3 × 30 ml) and the combined organics washed with brine (2 × 20 ml), dried and evaporated to give the title compound 3-((Benzyloxy)carbonyl)propanoic acid (2.52 g, 12.1 mmol, 61%) as a colourless amorphous solid; \( R_F \) (2:1 hexanes:EtOAc) 0.21; \( \delta_H \) (500 MHz, CDCl\(_3\)): 7.39-7.26 (5H, m, Ar-\( H \)), 5.16 (2H, s, Ar-\( CH_2 \)), 2.84-2.56 (4H, m, OC\(_H_2\)C\(_H_2\)O); \( \delta_C \) (125 MHz, CDCl\(_3\)): 178.0 (COOH), 172.0 (OCO\(_CH_2\)), 135.7 (Ar-\( C_1 \)), 128.6, 128.3 & 128.2 (Ar-\( C_2-6 \)), 66.7 (Ar\(_CH_2\)), 28.9 (OCH\(_2\)CH\(_2\)O); \( \nu_{\text{max}} \) (solid)/cm\(^{-1}\): 3181 (OH); 1736 (CO); \( m/z \) (ES): Found \( M(\text{+Na}) \) 231.0628. C\(_{11}\)H\(_{12}\)O\(_4\)Na requires 231.0555; elemental analysis (Found: C, 62.5; H, 5.8; O, 30.7; C\(_{11}\)H\(_{12}\)O\(_4\) requires C, 63.4; H, 5.8; O, 30.7); HPLC (5-95% A): retention time 2.14 min, 100%.

**Benzyl 3-(6-methyl-1,2,4-triazin-3-yl carbamoyl)propanoate and Benzyl 3-(5-methyl-1,2,4-triazin-3-ylcarbamoyl)propanoate 7**

3-((benzyloxy)carbonyl)propanoic acid 6 (297 mg, 2.7 mmol, 1 eq), 5/6-methyl-1,2,4-triazin-3-amine 5b (561 mg, 2.7 mmol, 1 eq), 4-dimethylaminopyridine (660 mg, 5.4 mmol, 2 eq) and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.03 g, 5.4 mmol, 2 eq) were stirred at 40°C under a nitrogen atmosphere in DMF (10 ml) for 32 hours. The reaction was quenched with H\(_2\)O (20 ml) and the aqueous layer extracted with EtOAc (4 × 30 ml). The combined organics were washed with H\(_2\)O (1 × 30 ml) and brine (2 × 40 ml), dried (MgSO\(_4\)) and concentrated \textit{in vacuo}. The crude yellow oil was purified by column chromatography on silica gel (2:1 hexanes/EtOAc) to give a mixture of Benzyl 3-(6-methyl-1,2,4-triazin-3-ylcarbamoyl)propanoate and Benzyl 3-(5-methyl-1,2,4-triazin-3-ylcarbamoyl)propanoate (3:1) (45 mg, 0.15 mmol, 7%) as an orange solid. \( R_F \) (2:1 hexanes/EtOAc) 0.27; \( \delta_H \) (500 MHz, CDCl\(_3\)): 8.87 (1 H, s, 5-
methyl- \textit{H}_6), 8.36 (1 H, s, 6-methyl-\textit{H}_5), 7.38-7.35 (5 H, m, Ar-\textit{H}), 3.22 – 3.18 (2 H, m, 5-methyl-OCH\textsubscript{2}C\textsubscript{H}\textsubscript{2}OH), 3.12 (2 H, t, \textit{J}_{\text{H-H}} 6.5, 6-methyl-OCH\textsubscript{2}C\textsubscript{H}\textsubscript{2}OH), 2.86 – 2.81 (2 H, m, OCH\textsubscript{2}C\textsubscript{H}\textsubscript{2}OH), 2.68 (3H, s, 6-methyl-\textit{CH}_3), 2.54 (3 H, s, 5-methyl-\textit{CH}_3); \textit{\delta}_C (125 MHz, CDCl\textsubscript{3}): 173.0 (O\textsubscript{C}OCH\textsubscript{2}), 160.9 (5/6-methyl-Tz-C\textsubscript{5}/C\textsubscript{6}), 157.4 (Tz-C\textsubscript{3}), 149.9 & 145.9 (5/6-methyl-Trz-C\textsubscript{5}/C\textsubscript{6}), 135.8 (Ar-C\textsubscript{1}), 128.6, 128.3 & 128.2 (Ar-C\textsubscript{2}-6), 66.7 (ArC\textsubscript{H}2), 32.6 & 32.4 (OCH\textsubscript{2}C\textsubscript{H\textsubscript{2}OH}), 2.86 – 2.81 (2 H, m, OC\textsubscript{H}2CH\textsubscript{2}OH), 2.68 (3H, s, 6-methyl-C\textsubscript{H}3), 2.54 (3 H, s, 5-methyl-C\textsubscript{H}3);

\nu_{\text{max}} (solid)/cm\textsuperscript{-1}: 3430 (NH stretch), 1709 (CO);

\textit{m/z} (ES): Found: \textit{M(\textit{+H})} 301.1294, C\textsubscript{15}H\textsubscript{17}N\textsubscript{4}O\textsubscript{3} requires 301.1295; HPLC (5-95\% A): retention time 1.88 min, 84%.

\textbf{Fmoc-Ser-OMe}

\textbf{(9H-Fluoren-9-yl)methyl (S)-1-(methoxycarbonyl)-2-hydroxyethylcarbamate}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{Fmoc-Ser-OMe.png}
\caption{Fmoc-Ser-OMe}
\end{figure}

Sodium bicarbonate (6.7 g, 60 mmol, 2.5 eq) was added to a vigorously stirred solution of L-serine methyl ester hydrochloride \textbf{10} (5 g, 32 mmol, 1 eq) in H\textsubscript{2}O (~60 ml) at 0°C,\textsuperscript{29} the turbid solution immediately became clear. Fmoc N-hydroxysuccinimide ester (10.8 g, 32 mmol, 1 eq), dissolved in H\textsubscript{2}O (~60 ml) was added dropwise and a colourless precipitate formed. The solution was allowed to warm to room temperature and stirred for 4 hours, at which time TLC showed complete consumption of both reagents. The reaction was quenched with H\textsubscript{2}O (1 × 50 ml) and extracted with EtOAc (3 × 60 ml). The combined organics were washed with H\textsubscript{2}O (2 × 50 ml) and brine (2 × 50 ml); dried (MgSO\textsubscript{4}) and concentrated on the cold finger. The crude colourless solid was recrystallised from EtOAc/hexanes, cooled to -20°C, and the resultant crystals collected at the pump, before being washed with ice-cold hexanes (2 × 50 ml) to yield \textit{Fmoc-Ser-Ome} (9.66g, 28 mmol, 88\%) as large, colourless crystals. m.p. 128.2-130.6 °C; \textit{[\alpha]}\textsubscript{D}\textsuperscript{27} +4.35 (c = 0.12, CHCl\textsubscript{3}); \textit{R}\textsubscript{F} (3:1 hexanes/EtOAc) 0.17; \textit{\delta}\textsubscript{H} (500 MHz, CDCl\textsubscript{3}): 7.70 (2H, d, \textit{J}_{\text{H-H}} 7.5, Fmoc-\textit{H}_4), 7.57-7.50 (2H, m, Fmoc-\textit{H}_1), 7.34 (2H, t, \textit{J}_{\text{H-H}} 7.5, Fmoc-\textit{H}_3), 7.25 (2H, t, \textit{J}_{\text{H-H}} 7.5, Fmoc-\textit{H}_2), 5.66-5.59 (1H, m, NH), 4.45-4.27 (3H, m, Fmoc-CH\textsubscript{2}CH\textsubscript{2} & H\textsubscript{\alpha}), 4.16 (1 H, t, \textit{J}_{\text{H-H}} 6.8, Fmoc-CH\textsubscript{2}CH\textsubscript{2}), 3.94 (1 H, d, \textit{J}_{\text{H-H}} 8.9, H\beta), 3.86 (1 H, d, \textit{J}_{\text{H-H}} 10.2, H\beta), 3.73 (3 H, s, OCH\textsubscript{3}); \textit{\delta}_C (125 MHz, CDCl\textsubscript{3}): 171.1 (COOCH\textsubscript{3}), 156.2 (OC\textsubscript{ONH}), 143.7 (Fmoc-C\textsubscript{4a}), 141.4 & 141.3 (Fmoc-C\textsubscript{4a}), 127.77 (Fmoc-C\textsubscript{3}), 127.1 (Fmoc-C\textsubscript{2}), 125.1 (Fmoc-C\textsubscript{1}), 120.0 (Fmoc-C\textsubscript{4}), 67.2 (Fmoc-CH\textsubscript{2}CH\textsubscript{2}), 63.3 (C\beta), 56.03 (C\textsubscript{\alpha}), 52.8 (OCH\textsubscript{3}), 47.2 (Fmoc-CH\textsubscript{2}CH\textsubscript{2}); \textit{\nu}_{\text{max}} (solid)/cm\textsuperscript{-1}: 3470 & 2431 (NH stretch), 3314 (OH) 1749 (CO); \textit{m/z} (ES): Found: \textit{M(\textit{+H})} 342.1339, C\textsubscript{19}H\textsubscript{20}NO\textsubscript{5} requires 342.1336; HPLC (5-95\% B): retention time 2.94 min, 100\%.
Fmoc-Ser(OTs)-OMe
(9H-Fluoren-9-yl)methyl (S)-1-(methoxycarbonyl)-2-(p-toluenesulfonate)ester

\[\text{Fmoc\textsubscript{H}N} \quad \text{O} \quad \text{OMe} \quad \text{O} \quad \text{S} \quad \text{O} \quad \text{O} \]

p-Toluenesulfonyl chloride (16.4 g, 86 mmol, 2 eq) was added to a solution of Fmoc-Ser-OMe (14.7 g, 43 mmol, 1 eq) in pyridine (~40 ml) at 0°C.\[29]\) The colourless solution immediately turned pale orange. The reaction mixture was allowed to warm to room temperature and stirred for 16 hours. The resultant clear red solution was quenched with H\(_2\)O (1 × 40 ml) and extracted with EtOAc (3 × 60 ml). The combined organics were washed with 1M citric acid solution (3 × 50 ml), saturated NaHCO\(_3\) (2 × 50 ml), brine (1 × 40 ml), 1M HCl solution (3 × 50 ml), saturated NaHCO\(_3\) (2 × 50 ml) and brine (2 × 40 ml). The organic layer was dried (MgSO\(_4\)) and concentrated on the cold finger. The colourless solid was recrystallised from 1:1 EtOH/Petroleum ether and collected at the pump. The resultant pale yellow crystals were triturated in ice-cold petroleum ether for 30 minutes and collected at the pump to yield Fmoc-Ser-OMe (13.38 g, 27 mmol, 63%) as colourless crystals. m.p. 119.7-120.2 °C; [\(\alpha\)]\(_D\)\(^{27}\) +3.25 (c = 0.14, CH\(_2\)Cl\(_2\)); \(R_F\) (3:1 hexanes/EtOAc) 0.29; \(\delta_H\) (500 MHz, CDCl\(_3\)) : 7.74 – 7.64 (4 H, m, Fmoc-H\(_4\) & OTs-H\(_2\)), 7.56 – 7.49 (2 H, m, Fmoc-H\(_3\)), 7.34 (2 H, t, \(^3J_{\text{H-H}}\) 7.3, Fmoc-H\(_3\)), 7.29-7.23 (2 H, m, Fmoc-H\(_2\)), 7.21 (2 H, d, \(^3J_{\text{H-H}}\) 8.2, OTs-H\(_3\)), 5.55 (1 H, d, \(^3J_{\text{H-H}}\) 7.9, NH), 4.53 – 4.48 (1 H, m, H\(_d\)), 4.39 (1 H, d, \(^2J_{\text{H-H}}\) 7.0, H\(_\beta\)), 4.35 (1H, d, \(^2J_{\text{H-H}}\) 7.5, H\(_\beta\)), 4.32 – 4.15 (2 H, m, CHCH\(_2\)), 4.12 (1 H, t, \(^3J_{\text{H-H}}\) 7.2, CHCH\(_2\)), 3.67 (3 H, s, OCH\(_3\)), 2.29 (3 H, s, OTsCH\(_3\)). \(\delta_C\) (125 MHz, CDCl\(_3\)) : 168.7 (COOCH\(_3\)) 155.6 (OC\(_{\text{ONH}}\)), 145.2 (OTs-C\(_4\)) 143.6 (Fmoc-C\(_{1\alpha}\)) 141.3 (Fmoc-C\(_{1\beta}\)) 132.3 (OTs-C\(_2\)), 130.0 (OTs-C\(_3\)), 128.0 & 127.8 (Fmoc-C\(_3\)), 127.2 (Fmoc-C\(_2\)), 125.2, &125.1 (Fmoc-C\(_1\)), 120.0 (Fmoc-C\(_4\)), 69.0 (C\(_\beta\)), 67.5 (CHCH\(_2\)), 53.4 (C\(_\alpha\)), 53.1 (OCH\(_3\)), 47.02 (CHCH\(_2\)), 21.58 (OTs-CH\(_3\)); \(\nu_{\text{max}}\) (solid)/cm\(^{-1}\): 3428 & 3338 (NH stretch), 1749 (CO); \(m/z\) (ES): Found: \(M^+\mathrm{H}\) 496.1428, C\(_{26}\)H\(_{26}\)NO\(_7\)S requires 496.1424; HPLC (5-95% B): retention time 4.10 min, 61%.

Fmoc-IodoAla-OMe 11
(9H-Fluoren-9-yl)methyl (R)-1-(methoxycarbonyl)-2-iodoethylcarbamate

\[\text{Fmoc\textsubscript{H}N} \quad \text{I} \quad \text{O} \quad \text{Me} \]

Sodium iodide (7.20 g, 48 mmol, 3 eq) in acetone (80 ml) was added to a stirred solution of Fmoc-Ser(OTs)-Ome (8.00 g, 16 mmol, 1 eq) in acetone (160 ml).[29] The colourless solution turned yellow. After stirring for 16 hours at room temperature the
mixture was filtered at the pump and concentrated in vacuo to obtain a yellow oil. The oil was dissolved in DCM (40 ml) and washed with water (3 × 50 ml), saturated sodium thiosulfate solution (2 × 50 ml) and brine (2 × 40 ml). The organic layer was dried (MgSO₄), concentrated and purified by column chromatography on silica gel (2:1 DCM/hexanes) to leave a colourless viscous oil. The oil was triturated in an ice-cold solution of 1:1 hexane/EtOH and collected at the pump to yield Fmoc-IodoAla-OMe (3.74 g, 8.2 mmol, 52%) as a flocculent, colourless solid. m.p. 148.8-149.4°C; [α]D²⁷ + 4.85 (c = 0.11, CH₂Cl₂); Rf (2:1 DCM/hexanes) 0.21; δH (500 MHz, CDCl₃): 7.78 (2 H, d, 3JH-H 7.5, Fmoc-H₄), 7.63 (2 H, d, 3JH-H 7.4, Fmoc-H₇), 7.42 (2 H, t, 3JH-H 7.5, Fmoc-H₃), 7.34 (2 H, tdd, 3JH-H 7.5, 4JH-H 3.0, 5JH-H 1.1, Fmoc-H₂), 5.69 (1 H, d, J 7.3, NH), 4.60 (1 H, dt, 3JH-H 7.6, 3JH-H 3.8, Hα), 4.48 – 4.36 (2 H, m, Fmoc-CHCH₂), 4.26 (1 H, t, 3JH-H 7.2, Fmoc-CHCH₂), 3.84 (3 H, s, OCH₃), 3.61 (1 H, d, 3JH-H 3.8, Hβ); δC (125 MHz, CDCl₃); 169.7 (COOCH₃), 155.4 (OCONH), 143.8 & 143.7 (Fmoc-C₄a), 141.3 (Fmoc-C₁a), 127.8 (Fmoc-C₃), 127.1 (Fmoc-C₂), 125.2 & 125.1 (Fmoc-C₄), 120.05 (Fmoc-C₅), 67.4 (CHCH₂), 54.1 (Cα) 53.2 & 53.1 (OCH₃), 45.2 (CHCH₂) 7.4 (Cβ); νmax (solid)/cm⁻¹: 3743 & 3338 (NH stretch), 1749 (CO); m/z (ES) Found: M(+)H, 452.0349, C₁₉H₁₉INO₄ requires 452.0353; HPLC (5-95% B): retention time 3.91 min, 72%.

FITC-Gaba-TrzAla-CONH₂ 14

Rink Amide Novagel™ (0.64 mmol/g) was purchased from Novabiochem, Fmoc-Gaba-OH¹ and fluorescein isothiocyanate isomer 1 (FITC) were purchased from Sigma Aldrich.

FITC-Ga-TrzA-CONH₂ was synthesised manually using standard solid phase synthesis protocol: Rink Amide Novagel™ (0.032 mmol) was swollen in DMF (2-3 ml-the same volume was used for the wash steps) for 30 min with agitation (Stuart Rotator SB2). The solution was then removed by vacuum filtration and the first coupling mixture added. Coupling reactions of Fmoc-TrzAla-OH 13 (3 eq. with respect to resin, 0.096 mmol, 37 mg) and Fmoc-Gaba-OH (5 eq. with respect to resin, 0.16 mmol, 65 mg) were carried out using 5 eq. HCTU and 10 eq. DIPEA in

¹ Gaba = γ-aminobutyric acid
DMF (1 mL) for 60 minutes with agitation. After each coupling reaction the solution was removed by filtration and the resin was washed with DMF (3 × 2 min), 20% piperidine in DMF (v/v, 5 × 2 min) and DMF (5 × 2 min). The peptide N-termini was appended to fluorescein by mixing the resin with a solution containing FITC (5 eq, 0.16 mmol, 60 mg) and DIPEA (5 eq, 0.8 mmol, 165 μl) for 16 h in the dark with agitation. The solution was removed by filtration and the resin was washed with DMF (3 × 2 min), DCM (3 × 2 min) and MeOH (3 × 2 min) and dried for 2 h in vacuo. The peptide was cleaved from the resin by mixing with a cleavage cocktail (2ml) consisting of TFA (95%), H2O (2.5%) and TIS (2.5%). The cleavage cocktail was applied to the resin and mixed for 2 h, then precipitated into ice-cold diethyl ether (30 ml) and the precipitate collected by centrifugation (4,000 × g, 10 minutes). The ethereal supernatant was decanted, the peptide pellet resuspended in ice-cold diethyl ether and centrifuged again. This was repeated 2 more times before residual ether was removed under a stream of nitrogen. The resultant amorphous solid was dissolved in the minimum volume H2O (with the addition of small portions of Dioxane), frozen and lyophilized to give the crude material as a flocculent orange solid. FITC-Ga-TrzA-CONH2 was purified by mass directed HPLC (5-95% MeCN + 0.1% formic acid), the volatiles were removed in vacuo and the remaining solution frozen and lyophilized to leave the title compound (7.52 mg, 0.12 mmol, 37%) as a flocculent orange solid.

(Z,1S,8R,9r)-9-(Ethoxymethyl)bicyclo[6.1.0]non-4-ene 17a

1,5-cyclooctadiene (35 ml, 283 mmol, 8 eq), rhodium (II) acetate (94 mg, 0.22 mmol, 0.01 eq) were added to a flask which was evacuated and charged with nitrogen three times.[36] The reactants were vigorously stirred in dry DCM (20 ml) and ethyl diazoacetate (85% in DCM, 4.4 ml, 35 mmol, 1 eq) was added dropwise via a syringe pump over 12 hours. The resultant orange solution was concentrated in vacuo, and purified using flash chromatography on silica eluting with hexanes, and after collection of excess 1,5-cyclooctadiene, 1% Et2O in hexanes, to leave (Z,1S,8R,9r)-9-(Ethoxymethyl)bicyclo[6.1.0]non-4-ene (3.17 g, 46%, 18 mmol) (combined isomers: 6.11 g, 34 mmol, 89%) as a colourless oil. [α]D^27 +1.0 (c = 0.07, CH2Cl2); Rf (1 % Et2O in hexanes) 0.24; δ H (500 MHz, CDCl3): 5.68 – 5.60 (2 H, m, H4), 4.14 – 4.07 (2 H, m, CH2CH3), 2.34 – 2.27 (2 H, m, H3), 2.23 – 2.16 (2 H, m, H2), 2.13 – 2.05 (2 H, m, H3), 1.59 – 1.54 (2 H, m, H1), 1.48 (2 H, dddd, 3 JH-H 11.0, 3 JH-H 8.7, 3 JH-H 5.4, 2 JH-H 2.9, H2), 1.25 (2 H, 2, 3 JH-H 7.2, CH2CH3), 1.19 (1 H, t, 3 JH-H 4.6, H1a); δ C (125 MHz, CDCl3): 174.4 (COOEt), 129.9 (C4), 60.2 (CH2CH3), 28.3 (C2), 27.9 (C1a), 27.7 (C1), 26.6 (C3), 14.20 (CH2CH3); νmax (solid)/cm⁻¹: 2934 (alkyne), 1095 (ether); HPLC (5-95% B): retention time 3.77 min, 99%.
(Z,1S,8R,9s)-9-(Ethoxymethyl)bicyclo[6.1.0]non-4-ene 17b

(Z,1S,8R,9s)-9-(ethoxymethyl)bicyclo[6.1.0]non-4-ene was synthesised alongside (Z,1S,8R,9r)-9-(ethoxymethyl)bicyclo[6.1.0]non-4-ene and gave the title compound (2.94 g, 16 mmol, 43%) as a colourless oil. [α]D<sup>27</sup> +3.2 (c = 0.03, CH<sub>2</sub>Cl<sub>2</sub>); R<sub>F</sub> (1 % Et<sub>2</sub>O in hexanes) 0.33; δ<sub>H</sub> (500 MHz, CDCl<sub>3</sub>): 5.65 – 5.58 (2 H, m, Fmoc-H<sub>4</sub>), 4.12 (2 H, q, <sup>3</sup>J<sub>H-H</sub> 7.1, CH<sub>2</sub>CH<sub>3</sub>), 2.55 – 2.46 (2 H, m, Fmoc-H<sub>3</sub>), 2.25 – 2.17 (2 H, m, Fmoc-H<sub>2</sub>), 2.10 – 2.02 (2 H, m, Fmoc-H<sub>3</sub>), 1.88 – 1.79 (2 H, m, Fmoc-H<sub>2</sub>), 1.81 (1 H, t, <sup>3</sup>J<sub>H-H</sub> 8.8, Fmoc-H<sub>1</sub>), 1.44 – 1.35 (2 H, m, Fmoc-H<sub>2</sub>), 1.26 (2 H, t, <sup>3</sup>J<sub>H-H</sub> 7.1, CH<sub>2</sub>CH<sub>3</sub>); δ<sub>C</sub> (125 MHz, CDCl<sub>3</sub>): 172.31 (C<sub>H</sub>2OEt), 129.47 (C<sub>4</sub>), 59.72 (CH<sub>2</sub>CH<sub>3</sub>), 27.09 (C<sub>3</sub>), 24.18 (C<sub>2a</sub>), 22.68 (C<sub>2</sub>), 21.27 (C<sub>1</sub>), 14.41 (CH<sub>2</sub>CH<sub>3</sub>); ν<sub>max</sub> (solid)/cm<sup>-1</sup>: 2937 (alkyne), 1010 (ether); HPLC (5-95% B): retention time 4.00 min, 100%.

(Z,1S,8R,9r)-Bicyclo[6.1.0]non-4-ene-9-ylmethanol 18

Under a nitrogen atmosphere, a solution of (Z,1S,8R,9r)-9-(ethoxymethyl)bicyclo[6.1.0]non-4-ene 17a (2.8 g, 14.3 mmol, 1 eq) in DCM (50 ml) was added dropwise to a stirred suspension of lithium aluminium hydride (1.1 g, 28.8 mmol, 2 eq) in DCM (50 ml) at 0°C. The suspension was allowed to warm to room temperature and stirred for 2 hours until TLC showed complete reduction of the ester (3:1 hexanes/EtOAc). The mixture was cooled to 0°C and quenched with sodium sulphate dehydrate (~10 g), filtered through a celite pad, concentrated and azeotroped with toluene to leave a colourless oil. Under a nitrogen atmosphere the reduction product was dissolved in DCM (50 ml) and stirred at 0°C before a solution of bromine (600µl, 13.4 mmol, 1.3 eq) in DCM (9 ml) was added dropwise until a persistent red colour was observed. The reaction was quenched with 10% sodium thiosulphate solution (80 ml), the organics were combined and washed with H<sub>2</sub>O (2 × 80 ml), brine (1 × 70 ml), concentrated and azeotroped with toluene. The resultant yellow oil was dissolved in THF (100 ml) under a nitrogen atmosphere and cooled to 0°C before potassium tert-butoxide (1M in THF, 43 ml) was added dropwise. The solution was heated to reflux and stirred for 16 hours before being cooled to room temperature, quenched with saturated ammonium chloride solution (100 ml), extracted with DCM (3 × 100 ml), brine (1 × 100 ml), dried (MgSO<sub>4</sub>) and concentrated to leave an orange oil. The crude residue was purified by column chromatography on silica gel eluting with 1% Et<sub>2</sub>O in DCM to leave (Z,1S,8R,9r)-bicyclo[6.1.0]non-4-ene-9-ylmethanol (333 mg, 2.21 mmol, 16 %) as a viscous yellow oil. [α]D<sup>27</sup> +2.9 (c = 0.12, CH<sub>2</sub>Cl<sub>2</sub>); R<sub>F</sub> (1 % Et<sub>2</sub>O in hexanes) 0.17; δ<sub>H</sub> (500 MHz, CDCl<sub>3</sub>): 3.56 (2 H, d, <sup>3</sup>J<sub>H-H</sub> 6.3, CH<sub>2</sub>OH), 2.42 (2 H, dd, <sup>2</sup>J<sub>H-H</sub> 13.3, <sup>3</sup>J<sub>H-H</sub> 2.7, H<sub>2</sub>), 2.33 – 2.25 (2 H, m, H<sub>3</sub>), 2.20 – 2.13 (2 H, m, H<sub>3</sub>), 1.45 – 1.35 (2 H, m, H<sub>2</sub>), 0.74
- 0.64 (3 H, m. $H_{2a+1}$). δ$_C$ (125 MHz, CDCl$_3$): 98.8 (C$_4$), 67.2 (CH$_2$OH), 33.4 (C$_2$), 27.3 (C$_1$), 22.6 (C$_{2a}$), 21.4 (C$_3$); $\nu_{\text{max}}$ (solid)/cm$^{-1}$: 3350 (OH stretch), 2923 (alkyne); HPLC (5-95% B): retention time 1.85 min, 86%.

(1S,8R,9r)-9-((Benzoyloxy)methyl)bicyclo[6.1.0]non-4-yne 19 (BCN-Bz)

![Image](image_url)

(Z,1S,8R,9r)-bicyclo[6.1.0]non-4-ene-9-ylmethanol 18 (159 mg, 1.06 mmol, 1 eq) and 4-dimethylaminopyridine (8 mg, 0.06 mmol, 0.06 eq) were stirred in DCM (10 ml) under a nitrogen atmosphere and freshly distilled triethylamine (0.45 ml, 1.32 mmol, 3 eq) was added.$^{[37]}$ The solution was cooled to 0°C and benzoyl chloride (0.24 ml, 2.11 mmol, 2 eq) was added dropwise, the colourless solution became amber. The reaction mixture was allowed to warm to room temperature and stirred for 4 hours until TLC showed complete consumption of starting material (4:1 hexanes/EtOAc). The reaction was quenched with saturated ammonium chloride solution (10 ml) and extracted with DCM (3 × 20 ml), the combined organics were washed with brine (2 × 15 ml), dried (MgSO$_4$) and concentrated to leave a brown oil, which was purified by column chromatography on silica (25:1 hexanes/EtOAc) to leave (Z,1S,8R,9r)-Bicyclo[6.1.0]non-4-ene-9-ylmethanol (144 mg, 0.59 mmol, 57 %) as a colourless solid. $[\alpha]_D^{27} +1.2$ (c = 0.07, CH$_2$Cl$_2$); $R_F$ (25: 1 hexanes/EtOAc) 0.09; δ$_H$ (500 MHz, CDCl$_3$): 8.08 – 8.04 (2 H, m, Bz-$H_2$), 7.59 – 7.55 (1 H, m, Bz-$H_4$), 7.45 (2 H, t, $^3J_{H-H}$ 7.8, Bz-$H_3$), 4.27 (2 H, d, $^3J_{H-H}$ 6.4, COOCH$_2$), 2.48 – 2.41 (2 H, m, Bz-$H_3$), 2.36-2.72 (2 H, m, $H_2$), 2.18 (2 H, dd, $^2J_{H-H}$ 9.4, $^3J_{H-H}$ 5.6, $H_2$), 1.42 (2 H, d, $^2J_{H-H}$ 11.5, $H_3$), 0.90 – 0.80 (3 H, m, $H_{2a+1}$); δ$_C$ (125 MHz, CDCl$_3$): 132.83 (Bz-C$_4$), 130.5 (Bz-C$_1$), 129.6 (Bz-C$_2$), 128.3 (Bz-C$_3$), 98.8 (C$_4$), 69.1 (CH$_2$OH), 33.3 (C$_2$), 23.6 (C$_1$), 23.1 (C$_{2a}$), 21.4 (C$_3$); $\nu_{\text{max}}$ (solid)/cm$^{-1}$: 2227 (alkyne), 1263 (ether); HPLC (5-95% B): retention time 3.93 min, 100%.
General procedure for HPLC assay

Fmoc-TrzAla-OMe 13 (9H-Fluoren-9-yl)methyl (S)-1-(methoxycarbonyl)-2-(1,2,4-triazin-3-yl)ethylcarbamate) (0.73 mg, 1.80 μmol, 1 eq) was dissolved in 900 μl of the required solvent mix (either 100 % MeCN or 10% H2O). BCN-Bz 19 ((1S,8R,9r)-9-((Benzoyloxy)methyl)bicyclo[6.1.0]non-4-yn-1-yl) was dissolved in 900 μl of the required solvent mix ((0.46 mg, 1.8 μmol), (0.92 mg, 3.6 μmol), (1.83 mg, 7.20 μmol) or (3.66 mg (14.3 μmol) with respect to a final concentration (in 1.8 ml) of 1 mM, 2 mM, 4 mM or 8 mM respectively. The two solutions were combined, mixed thoroughly and capped before being immediately incubated at 37 °C in the auto loader of an Agilent technologies 1290 HPLC, fitted with an ACE Ultracore 2.5 Super C18 column (50 × 2.1 mm).

Each 2 μL sample was injected and analysed using a linear gradient (5% H2O, in MeCN to 95% H2O) over 5 min. Typically 4 reactions of varying concentration in respect to BCN-Bz were assayed in parallel. After every analysis, 2 μl of MeCN was injected onto the column and the column washed using the same linear gradient (5% H2O, 0.5% TFA in MeCN to 95% H2O, 0.5% TFA) over 9 min. HPLC analysis was carried out over a 20 hour period. Concentration of the cycloaddition product was measured against a previously measured concentration dilution series of the product. A typical HPLC trace (figure S3) and calibration graph (Figure S2) are shown below.

**Figure S2** Calibration curve for determination of cycloaddition product formation by HPLC.
Figure S3 Representative chromatogram from cycloaddition rate determination. 1. Fmoc-TrzAla-OMe, 3- BCN-Bz, 4/5 20a and 20b
NMR spectra, High-resolution mass spectra and HPLC chromatograms
SampleID: kah176 HRMS
Sample Description: kah176 HRMS_107361_GE1_01_6623.d
Analysis Name: 3a_AccMass_Loop_Positive.m
Submitter: Katherine Horner
Method: maXis impact
Instrument: maXis impact
Source Type: ESI
Ion Polarity: Positive
Acquisition Date: 28/07/2014 19:01:26
Scan Begin: 50 m/z
Scan End: 1500 m/z

The diagram shows the mass spectrum of FmocHN\(\text{OMe}\) with a major peak at 809.3036 and other minor peaks at 101.0043, 179.0855, 405.1560, 526.2299, and 809.3036. The instrument used was Bruker Compass DataAnalysis 4.1, and the analysis was performed on 28/07/2014 at 19:04:39.
Sample Name: 176-6 0.12Mm
Injection Volume: 1.0
Vial Number: P1:A6
Channel: DAD_Signal
Sample Type: standard
Wavelength: n.a.
Control Program: Rapid5-95 Ascentis Express C18
Bandwidth: n.a.
Quantif. Method: MartinH
Dilution Factor: 1.0000
Recording Time: 30/06/2014 14:22
Sample Weight: 1.0000
Run Time (min): 4.92
Sample Amount: 1.0000

| No. | Ret.Time (min) | Peak Name | Height (mAU) | Area (mAU*min) | Rel.Area (%) | Amount (Mm) | Type |
|-----|----------------|-----------|--------------|----------------|--------------|-------------|------|
| 1   | 2.93           | 176       | 207.314      | 5.170          | 100.00       | 0.172       | BMB  |
| Total|                |           | 207.314      | 5.170          | 100.00       | 0.172       |

FmocHN
\[\text{OMe}\]

12
| SampleID       | KAH192       |
|---------------|--------------|
| Analysis Name | KAH192_107362_GE2_01_6624.d |
| Method        | 3a_AccMass_Loop_Positive.m |
| Instrument    | maXis impact |

**Submitter**: Katherine Horner  
**Supervisor**: Stuart Warriner  
**Acquisition Date**: 28/07/2014 19:05:15  
**Scan Begin**: 50 m/z  
**Scan End**: 1500 m/z

![Chemical structure of FmocHN](image)

**Data Analysis**:
- ESI 1500 m/z  
- Ion Polarity: Positive

**Scan Details**:
- Scan Begin: 1 of 22, 28/07/2014 19:08:28  
- Instrument Source Type: Bruker Compass DataAnalysis 4.1

**FmocHN**

![Mass spectrum graph](image)

**Graph Details**:
- +MS, 1.1-1.4 min #67-88, -Peak Bkgmd
### KAH192

| Sample Name: | KAH192 | Injection Volume: | 1.0 |
|--------------|--------|------------------|-----|
| Vial Number: | P1:A4  | Channel:         | DAD_Signal_A |
| Sample Type: | unknown| Wavelength:      | n.a. |
| Control Program: | Rapid5-95 Ace UltraCore C18 | Bandwidth: | n.a. |
| Quantif. Method: | MartinH | Dilution Factor: | 1.0000 |
| Recording Time: | 22/07/2014 08:41 | Sample Weight: | 1.0000 |
| Run Time (min): | 4.91 | Sample Amount: | 1.0000 |

| No. | Ret.Time (min) | Peak Name | Height (mAU) | Area (mAU*min) | Rel.Area (%) | Amount | Type |
|-----|----------------|-----------|--------------|----------------|--------------|--------|------|
| 1   | 2.87           | n.a.      | 321.804      | 18.444         | 100.00       | n.a.   | BMB  |
| Total: | | | 321.804 | 18.444 | 100.00 | 0.000 |

![Graph showing chromatogram for KAH192 sample with peak at 2.87 minutes]
7 KAH242

Sample Name: KAH242  Injection Volume: 2.0
Vial Number: P1:F6  Channel: DAD_Signal A
Sample Type: unknown  Wavelength: n.a.
Control Program: Rapid5-95+TFA Ascentis Express C18  Bandwidth: n.a.
Quantif. Method: MartinH  Dilution Factor: 1.0000
Recording Time: 09/03/2015 12:29  Sample Weight: 1.0000
Run Time (min): 4.90  Sample Amount: 1.0000

| No. | Ret.Time (min) | Peak Name | Height (mAU) | Area (mAU*min) | Rel.Area (%) | Amount (n.a.) | Type |
|-----|----------------|------------|--------------|----------------|--------------|---------------|------|
| 1   | 1.11           | n.a.      | 336.214      | 8.533          | 100.00       | n.a.          | BMB  |
| **Total:** |             |            | **336.214**  | **8.533**      | **100.00**   | **0.000**     |      |
