Efficient Synthesis of Conformationally Constrained, Aminotriazoloazepinone-containing Di- and Tripeptides via a One-Pot Ugi-Huisgen tandem Reaction

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1. General Information
\(^1\)H and \(^{13}\)C NMR spectra (25°C) were recorded at 250 MHz and 63 MHz on a Bruker Avance DRX 250 spectrometer or at 500 MHz and 126 MHz respectively on a Bruker Avance II 500 spectrometer. Chemical shifts are in parts per million (ppm). The assignments were made using one dimensional (1D) \(^1\)H and \(^{13}\)C spectra. HRMS data was recorded with a Micromass QTOF-micro system. Mass spectra were recorded with a LCMS-MS triple-quadrupole system. Analytical HPLC was performed on an Agilent 1100 Series system with a Supelco Discovery BIO Wide Pore RP column (25 cm × 4.6 mm, 5 µm). Flow rates of 1 ml/min were used and detection was done at 215 nm. The solvent system consisted of 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B). The gradient consisted of a 20 min. run from 3% B to 97% B. Flash chromatography was performed with silica gel 60 (Davisil, 0.040-0.063 mm) from Merck. Glass plates with silica gel 60 F254 (Merck) were used for thin layer chromatography. Visualization of the products on TLC plates was realized using UV light (254 nm), KMnO₄ spray. Melting points were determined on a Büchi B-540 apparatus and are uncorrected. Reactions were performed using a Biotage® Initiator® Microwave Synthesiser. All commercial
reagents and solvents were used without further purification. Diastereomeric ratios were calculated based on the integration of the Boc-proton resonances for all compounds except where noted in the manuscript.

2. General Procedure for synthesis of \(N\)-Boc-2-amino-3-azidopropanoic acid (12)

To a mixture of sodium azide (9.6 eq.) in water-methanol (5:8 v/v) at 0 °C was added triflic anhydride (1.86 eq., max. 2.5 ml) dropwise. The mixture was stirred at 0 °C for a further 2 hours after which time the layers were partitioned, and the aqueous layer was washed with dichloromethane. Combined organics were washed with a 10% solution of sodium bicarbonate.

To a mixture of Boc-diaminopropanoic acid (1.0 eq.) in water-methanol (1:2 v/v) was added sodium bicarbonate (10 eq.) and copper(ii) sulfate pentahydrate (0.013 eq.). The crude triflic azide mixture was then added dropwise at room temperature and the mixture allowed to stir overnight. Methanol was then removed under reduced pressure and the mixture acidified to pH 6 with HCl 1 N. Phosphate buffer (pH 6.2 was then added) and the resulting mixture was extracted with ethyl acetate. The resulting aqueous layers were further acidified to pH 2 and extracted again with ethyl acetate. Combined organics were washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo to give product as a viscous yellow-green oil.

3. General Procedure for Ugi reaction under heating in a sealed vial

In a microwave vial was combined \(N\)-Boc-2-Amino-3-azidopropanoic acid (0.4 mmol), propargylamine (0.4 mmol), aldehyde (0.4 mmol) and isocyanide (0.4 mmol) in MeOH (4 ml). The vial was sealed and the mixture was stirred at room temperature for 24 hours. Reaction conversion was monitored by HPLC analysis and after full conversion the vial was heated at 70 °C in an oil bath for 36 h. Upon reaction completion the methanol was removed from the mixture under reduced pressure. The crude products were purified either by precipitation from apolar solvents (hexane or heptane) with sonication or were purified directly by flash chromatography. Purity was determined by reversed phase HPLC, using UV detection (215 nM).
4. Characterisation of Compounds

(S)-tert-Butyl (5-(2-(tert-Butylamino)-2-oxo-1-phenylethyl)-6-oxo-5,6,7,8-tetrahydro-4H-triazolo[1,2,3]diazepin-7-yl)carbamate (14)


HPLC Rf: 16.4, 16.6 min; 

1H NMR (250 MHz, CDCl3): δ 7.59-7.40 (5H, m), 7.39-7.29 (4H, m), 7.26-7.21 (1H, m), 7.11 (2H, d, J = 11.0 Hz), 6.13 (2H, d, J = 13.8 Hz), 6.00 (2H, d, J = 8.3 Hz), 5.63-5.50 (2H, m), 5.30-5.13 (2H, m), 4.97 (2H, dt, J = 5.5, 13.8 Hz), 4.76-4.56 (3H, m), 4.40-4.10 (3H, m), 1.48 (18H, s), 1.45 (9H, s), 1.40 (9H, s); 

13C NMR (125 MHz, CDCl3): δ 172.9, 170.6, 170.3, 168.1, 166.8, 154.9, 134.2, 131.8, 131.6, 130.4, 129.5, 129.3, 128.8, 81.0, 80.9, 61.3, 61.2, 52.4, 52.0, 50.2, 49.0, 48.9, 37.9, 37.5, 28.8, 28.6, 28.4; HRMS (m/z) calcd. for C23H32N6O4 (M+H)+: 457.2558, found: 457.2540.

(S)-tert-Butyl (5-(2-(cyclohexylamino)-2-oxo-1-phenylethyl)-6-oxo-5,6,7,8-tetrahydro-4H-triazolo[1,2,3]diazepin-7-yl)carbamate (15)


HPLC Rf: 17.21 (1H, m), 7.11 (2H, d, J = 6.00 (2H, d, J = 172.9, 170.6, 170.3, 168.1, 166.8, 154.9, 134.2, 131.8, 131.6, 130.4, 129.5, 129.3, 128.8, 81.0, 80.9, 61.3, 61.2, 52.4, 52.0, 50.2, 49.0, 48.9, 37.9, 37.5, 28.8, 28.6, 28.4; HRMS (m/z) calcd. for C23H32N6O4 (M+H)+: 457.2558, found: 457.2540.

(S)-tert-Butyl (5-(2-(benzylamino)-2-oxo-1-phenylethyl)-6-oxo-5,6,7,8-tetrahydro-4H-triazolo[1,2,3]diazepin-7-yl)carbamate (16)

Yield: 237 mg (76%). Brown solid, mp: 132-133 °C. Purification: column 30% EtOAc in cyclohexane; dr: 50/50. 

HPLC Rf: 15.5 min; 

1H NMR (250 MHz, CDCl3): δ 7.50-7.40 (4H, m), 7.38-7.27 (12H, m), 7.25-7.06 (6H, m), 6.72 (1H, s), 6.26 (2H, s), 6.04 (4H, dt, J = 7.1, 34.8 Hz), 5.31-5.12 (2H, m), 4.97 (2H, dd, J = 4.2, 14.4 Hz), 4.78 (1H, t, J = 17.2 Hz), 4.67 (1H, d, J = 4.2 Hz), 4.60-4.51 (2H, m), 4.40-4.33 (3H, m), 4.18 (2H, q, J = 14.4 Hz). 1.49 (9H, s), 1.48 (9H, s); 

13C NMR (125 MHz, CDCl3): δ 170.6, 170.4, 168.9, 168.1, 155.0, 137.7, 133.9, 133.8, 129.8, 127.9, 125.1, 81.0, 61.2, 61.1, 60.58, 50.1, 50.0, 49.0, 43.8, 43.6, 37.8, 37.5, 28.4, 21.1, 14.3; HRMS (m/z) calcd. for C26H30N6O4 (M+H)+: 491.2401, found: 491.2410.
(S)-tert-Butyl (5-(1-(4-bromophenyl)-2-(tert-Butylamino)-2-oxoethyl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (17)

Yield: 171 mg (74%). White solid, mp: 152-154 °C. Purification: 30% EtOAc in cyclohexane; dr: 53:47. HPLC Rf: 16.9, 17.1 min; 1H NMR (250 MHz, CDCl3): δ 7.57 (2H, d, J = 8.3 Hz), 7.49 (1H, s), 7.43 (2H, d, J = 8.3 Hz), 7.25-7.22 (2H, s), 7.01 (2H, d, J = 8.3 Hz), 6.81 (1H, s), 6.07 (2H, d, J = 8.3 Hz), 5.94 (2H, t, J = 8.3 Hz), 5.48 (2H, d, J = 13.5 Hz), 5.31-5.12 (2H, m), 5.04-4.98 (2H, dd, J = 8.3, 16.5 Hz), 4.81 (1H, d, J = 16.5 Hz), 4.72 (1H, d, J = 8.3 Hz), 4.60 (1H, d, J = 8.3 Hz), 4.39 (1H, d, J = 16.5 Hz), 4.29-4.11 (2H, m), 1.48 (18H, s), 1.39 (9H, s), 1.19 (9H, s); 13C NMR (125 MHz, CDCl3): δ 170.6, 170.3, 167.5, 166.2, 154.7, 133.1, 132.9, 132.5, 132.4, 131.9, 131.5, 131.2, 130.4, 130.3, 130.3, 132.6, 132.3, 81.0, 80.9, 60.4, 60.1, 52.4, 52.0, 50.1, 50.0, 48.9, 37.8, 37.3, 29.7, 28.7, 28.4, 28.3, 26.9; HRMS (m/z) calcd. for C23H13BrN6O4 (M+H)+: 535.1663, 537.1646, found: 535.1650.

(5S)-tert-Butyl (5-(2-(tert-Butylamino)-1-(1H-indol-3-yl)-2-oxoethyl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (18)

Yield: 87 mg (40%). Orange solid, mp: 143-145 °C. Purification: 90% EtOAc in cyclohexane; dr: 58:42; HPLC Rf: 15.2 min; 1H NMR (250 MHz, CDCl3): δ 8.96 (2H, d, J = 6.1 Hz), 10.9, 11.1 (1H, m), 7.48-7.41 (4H, m), 7.30-7.27 (1H, m), 7.24-7.23 (2H, m), 7.20-7.14 (1H, m), 7.09-7.04 (2H, m), 6.89 (1H, t, J = 8.3 Hz), 6.44 (1H, s), 6.38 (1H, s), 6.07 (1H, s), 6.05 (1H, s), 5.90 (1H, s), 5.68 (1H, s), 5.28-5.05 (2H, m), 4.99-4.89 (2H, dd, J = 5.5, 22.0 Hz), 4.82-4.62 (4H, dd, J = 5.5, 22.0 Hz), 4.28-4.18 (1H, m), 4.13-4.00 (1H, m), 1.49 (18H, s), 1.40 (9H, s), 1.17 (9H, s); 13C NMR (125 MHz, CDCl3): δ 172.0, 170.4, 170.2, 168.7, 167.6, 155.2, 136.6, 136.3, 132.1, 131.9, 130.1, 130.0, 126.7, 126.6, 126.2, 125.3, 125.2, 123.6, 121.1, 120.9, 119.0, 118.8, 117.7, 112.2, 112.1, 109.5, 108.3, 81.3, 81.2, 55.4, 55.1, 53.8, 52.4, 52.0, 50.4, 49.3, 49.2, 37.6, 36.6, 28.8, 28.7; HRMS (m/z) calcd. for C23H13N6O4 (M+H)+: 496.2667, found: 496.2646.

(5R)-tert-Butyl (5-(2-(tert-Butylamino)-1-(4-(dimethylamino)phenyl)-2-oxoethyl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (19)

Yield: 237 mg (76%). Yellow solid, mp: 145-146 °C. Purification: 40% EtOAc in cyclohexane; dr: 52:48; HPLC Rf: 11.9, 12.6 min, 12.507 min; 1H NMR (250 MHz, CDCl3): δ 7.44 (1H, s), 7.18 (2H, d, J = 11.0 Hz), 6.95 (2H, d, J = 8.3 Hz), 6.74 (3H, t, J = 8.3 Hz), 6.54 (2H, d, J = 8.3 Hz), 6.04 (1H, s), 5.97 (1H, s), 5.46 (1H, s), 5.33 (1H, s), 5.27-5.06 (2H, m), 5.01-4.94 (2H, dd, J = 8.3, 19.3 Hz), 4.78-4.58 (4H, dd, J = 8.3, 19.3 Hz), 4.32-4.08 (4H, m), 3.00 (6H, s), 2.93 (6H, s), 1.48 (18H, s), 1.39 (9H, s), 1.22 (9H, s); 13C NMR (125 MHz, CDCl3): δ 170.3, 169.9, 168.6, 167.3, 154.8, 150.8, 131.8, 131.7, 131.5, 130.4, 130.1, 129.8, 120.5, 120.2, 112.5, 112.2, 80.7, 61.8, 61.2, 52.0, 51.8, 50.1, 50.1, 48.9, 40.3, 40.2, 37.5, 37.0, 28.7, 28.5, 28.3; HRMS (m/z) calcd. for C25H33N4O4 (M+H)+: 500.2980, found: 500.2963.
**tert-Butyl ((7S)-5-(1-(tert-Butylamino)-1-oxopropan-2-yl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (20)**

A) Yield: 22 mg (26%) White powder, mp: 79-80 °C. Purification: preparative-HPLC; dr: 58/48. HPLC Rₜ: 13.2 min; ¹H NMR (250 MHz, CDCl₃): δ 7.55 (1H, s), 5.90 (1H, d, J = 6.5 Hz), 5.44 (1H, s), 5.26-5.16 (1H, m), 5.10-5.07 (1H, m), 5.00 (1H, dd, J = 2.8, 8.5), 4.84 (1H, d, J = 19.0 Hz), 4.61 (1H, d, J = 19.0 Hz), 4.16 (1H, t, J = 12.5), 1.48 (9H, s), 1.37 (3H, d, J = 7.5 Hz), 1.03 (9H, s); ¹³C NMR (125 MHz, CDCl₃): δ 170.44, 168.1, 155.13, 132.3, 131.1, 81.2, 52.6, 51.6, 50.2, 48.6, 35.4, 28.4, 13.7; HRMS (m/z) calcd. for C₁₈H₂₃N₂O₄ (M+H)⁺: 395.2401, found: 395.2408.

B) Yield: 24 mg (28%). White powder, mp: 100-102 °C. Purification: preparative-HPLC; dr: 58/48. HPLC Rₜ: 13.8 min; ¹H NMR (250 MHz, CDCl₃): δ 7.56 (1H, s), 5.92 (1H, d, J = 6.5 Hz), 5.65 (1H, s), 5.25-5.15 (1H, m), 5.05-4.98 (2H, m), 4.91 (1H, d, J = 16 Hz), 4.65 (1H, d, J = 19.3 Hz), 4.17 (1H, t, J = 12 Hz), 1.49 (9H, s), 1.36 (9H, s), 1.25 (3H, d, J = 7.8 Hz); ¹³C NMR (125 MHz, MeOH-d₄): δ 171.3, 170.9, 156.0, 80.2, 53.7, 51.1, 49.6, 19.1, 36.0, 27.6, 27.4, 14.6; HRMS (m/z) calcd. for C₁₈H₂₃N₂O₄ (M+H)⁺: 395.2401, found: 395.2414.

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**tert-Butyl ((7S)-5-(2-(tert-Butylamino)-1-cyclohexyl-2-oxoethyl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (21)**

Yield: 89 mg (44%). Brown oil. Purification: preparative-HPLC; dr: 64/36. HPLC Rₜ: 11.9, 12.2 min; ¹H NMR (250 MHz, CDCl₃): δ 7.55 (1H, s), 5.91 (1H, d, J = 8.3 Hz), 5.56 (1H, s), 5.26-5.16 (1H, m), 5.10-4.94 (4H, m), 4.84 (1H, d, J = 17.3 Hz), 4.69-4.58 (1H, m), 4.16 (1H, t, J = 13.0 Hz), 1.48 (9H, s), 1.39 (1H, s), 1.36 (1H, s), 1.35 (4H, s), 1.25 (4H, s), 1.04 (8H, s); ¹³C NMR (125 MHz, CDCl₃): δ 173.6, 170.3, 169.6, 154.9, 132.2, 132.1, 131.2, 81.2, 53.4, 52.7, 51.6, 50.4, 50.3, 48.7, 48.6, 36.3, 35.4, 28.8, 28.5, 28.4, 15.2, 13.8; HRMS (m/z) calcd. for C₂₃H₃₈N₂O₄ (M+H)⁺: 463.3027, found: 463.3027.

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**(S)-tert-Butyl (5-(1-(benzo[d][1,3]dioxol-5-yl)-2-(tert-Butylamino)-2-oxoethyl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (22)**

Yield: 146 mg (67%). Brown oil. Purification: 2% MeOH in dichloromethane; dr: 45/55. HPLC Rₜ: 15.6, 15.8 min; ¹H NMR (250 MHz, CDCl₃): δ 77.48 (1H, s), 7.27 (1H, s), 6.84 (3H, t, J = 7.5 Hz), 6.72-6.52 (3H, m), 6.05-5.92 (8H, m), 5.58 (1H, s), 5.48 (1H, s), 5.29-5.09 (2H, m), 5.02-4.99 (2H, dd, J = 7.5, 17.5 Hz), 4.78-4.65 (2H, m), 4.39-4.08 (4H, m), 1.48 (9H, s), 1.42 (9H, s), 1.39 (9H, s), 1.21 (9H, s); ¹³C NMR (125 MHz, CDCl₃): δ 170.5, 170.2, 168.2, 166.9, 154.9, 148.6, 148.4, 132.0, 131.8, 130.4, 127.9, 127.8, 122.7, 122.6, 109.1, 109.1, 108.9, 108.7, 101.7, 80.9, 61.0, 60.7, 52.2, 51.9, 50.2, 50.1, 49.0, 48.9, 37.6, 37.3, 29.8, 28.7, 28.5, 28.4, 27.0; HRMS (m/z) calcd. for C₂₄H₃₂N₂O₆ (M+H)⁺: 501.2456, found: 501.2439.
(S)-tert-Butyl (5-(2-(tert-Butylamino)-1-(naphthalen-2-yl)-2-oxoethyl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (23)

Yield: 152 mg (69%). Light brown solid, mp: 184-186 °C. Purification: precipitation from hexane; dr: 53/47. HPLC Rf: 16.9 min; 1H NMR (250 MHz, CDCl3): δ 7.96-7.86 (2H, m), 7.81-7.73 (4H, m), 7.68-7.65 (2H, m), 7.60 (2H, d, J = 7.8 Hz), 7.59-7.51 (4H, m), 7.738 (1H, s), 7.35 (1H, d, J = 11.0 Hz), 6.99 (1H, dd, 2.8, 11.0 Hz), 6.49 (1H, s), 6.29 (2H, d, J = 5.5 Hz), 6.02 (2H, d, J = 5.5 Hz), 5.52 (2H, m), 5.32-5.14 (2H, m), 5.00 (2H, dd, J = 5.5, 13.8 Hz), 4.80-4.66 (2H, m), 4.34-4.20 (2H, m), 1.50 (18H, s), 1.44 (9H, s), 1.25 (9H, s); 13C NMR (125 MHz, CDCl3): δ 171.1, 171.0, 168.5, 166.6, 155.0, 133.3, 132.0, 131.9, 131.7, 131.6, 131.5, 131.3, 130.6, 129.8, 129.4, 128.6, 128.4, 128.1, 128.0, 127.9, 127.4, 127.3, 127.1, 125.8, 125.7, 122.6, 121.1, 80.9, 61.6, 52.5, 52.1, 50.3, 50.2, 49.0, 38.0, 37.6, 28.9, 28.6, 28.4; HRMS (m/z) calcd. for C27H34N6O4 (M+H)+: 507.2714, found: 507.2715.

(5)-tert-Butyl (5-(2-(tert-Butylamino)-1-(m-tolyethyl)-2-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (24)

Yield: 154 mg (75%). Light brown solid, mp: 126-128 °C; Purification: precipitation from heptane; dr: 51/49. HPLC Rf: 16.5 min; 1H NMR (250 MHz, CDCl3): δ 7.47 (1H, s), 7.35-7.16 (6H, m), 6.93-6.87 (2H, m), 6.68 (1H, s), 6.04 (4H, dd, J = 11, 27.5 Hz), 5.64 (1H, s), 5.49 (1H, s), 5.32-5.10 (2H, m), 0.2-4.93 (2H, m), 4.83-4.56 (3H, m), 4.39-4.10 (3H, m), 2.40 (3H, s), 2.23 (3H, s), 1.48 (18H, s), 1.41 (9H, s), 1.26 (9H, s); 13C NMR (125 MHz, CDCl3): δ 172.2, 170.9, 170.8, 168.7, 168.1, 139.2, 138.9, 135.6, 133.3, 133.1, 131.6, 130.4, 129.9, 129.8, 129.6, 129.4, 129.2, 128.9, 126.3, 80.2, 63.5, 61.7, 61.2, 54.5, 52.7, 51.7, 51.5, 49.8, 49.5, 49.3, 37.8, 37.5, 28.2, 28.1, 27.9, 25.0, 20.9, 20.7; HRMS (m/z) calcd. for C24H33N6O4 (M+H)+: 471.2714, found: 471.2714.

tert-Butyl ((7S)-5-(2-(tert-Butylamino)-2-oxo-1-(pyridin-4-yl)ethyl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (25)

Yield: 169 mg (85%). Off-white solid, mp: 128-129 °C; Purification: 10% MeOH in dichloromethane; dr: 54/46; HPLC Rf: 11.3, 12.1 min; 1H NMR (250 MHz, CDCl3): δ 8.63 (2H, s), 8.47 (2H, s), 7.47 (1H, s), 7.26-7.22 (1H, m), 7.12 (1H, d, J = 5.6 Hz), 6.97 (2H, d, J = 4.9 Hz), 6.78-6.74 (1H, m), 6.03 (2H, s), 5.88 (2H, t, J = 5.6 Hz), 5.68 (2H, d, J = 17.8 Hz), 5.26-5.14 (2H, m), 4.95 (2H, dd, J = 4.6, 13.6 Hz), 4.76 (2H, dd, J = 7.3, 18.2 Hz), 4.63-4.43 (2H, m), 4.23-4.09 (2H, m), 1.41 (18H, s), 1.35 (18H, s); 13C NMR (125 MHz, CDCl3): δ 170.9, 166.9, 154.9, 150.4, 150.0, 149.7, 144.0, 143.6, 132.3, 131.7, 131.4, 130.9, 130.6, 123.5, 123.2, 121.6, 81.1, 59.9, 52.6, 50.3, 49.1, 38.4, 28.8, 28.5; HRMS (m/z) calcd. for C22H31N3O4 (M+H)+: 458.2510, found: 458.2511.
**tert-Butyl ((7S)-5-((tert-Butylamino)-2-oxo-1-(o-tolyl)ethyl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (26)**

 Yield: 191 mg (93%). Light brown solid, mp: 123-125 °C; Purification: precipitation from hexane; dr: 52/48. HPLC Rf: 16.0 min; 1H NMR (250 MHz, MeCN-d3): δ 7.38-7.03 (10H, m), 6.52 (2H, d, J = 12.3 Hz), 5.99 (2H, dd, J = 5.8, 12.8 Hz), 5.61 (1H, s), 5.32 (1H, m), 5.23-5.09 (2H, m), 5.08-4.94 (2H, m), 4.80 (1H, d, J = 17.0 Hz), 4.47 (1H, d, J = 17.0 Hz), 4.29 (2H, t, J = 12.5 Hz), 4.11 (2H, t, J = 12.5 Hz), 2.27 (3H, s), 1.74 (3H, s), 1.50 (18H, s), 1.40 (9H, s), 1.25 (9H, s); 13C NMR (125 MHz, CDCl3): δ 170.7, 169.1, 167.2, 154.9, 154.9, 138.3, 133.8, 132.3, 132.0, 131.8, 131.8, 131.6, 131.3, 131.2, 130.6, 130.2, 130.1, 129.6, 129.6, 129.4, 129.0, 128.5, 128.4, 127.0, 126.6, 125.5, 101.8, 80.8, 60.6, 59.3, 53.1, 52.8, 52.2, 51.9, 50.2, 50.0, 49.1, 48.9, 37.6, 36.9, 28.8, 28.6, 28.4; HRMS (m/z) calced. for C24H34N6O4 (M+H)+: 471.2714, found: 471.2716.

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**((S)-methyl 6-((benzoylcarbonyl)amino)-2-((S)-7-((tert-butoxycarbonyl)amino)-6-oxo-7,8-dihydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-5(6H)-yl)acetamido)hexanoate (28)**

 Yield: 66 mg (51%). White powder, mp: 71-72 °C; Purification: Preparative HPLC (5-100% MeCN in 20 mins); HPLC Rf: 15.1 min; 1H NMR (500 MHz, CDCl3): δ 8.05 (1H, s), 7.58 (1H, s), 7.33 (5H, s), 6.84-6.82 (1H, m), 6.00-5.99 (1H, m), 5.24-5.14 (2H, m), 5.09 (2H, m), 5.01-4.93 (2H, m), 4.51-4.45 (2H, m), 4.27-4.20 (2H, m), 4.19-4.06 (1H, d, J = 16 Hz), 3.70-3.69 (3H, s), 3.16-3.15 (2H, m), 1.82-1.75 (1H, m), 1.67-1.58 (1H, m), 1.46 (9H, s), 1.25 (2H, m); 13C NMR (125 MHz, CDCl3): δ 172.6, 170.6, 167.2, 156.9, 156.9, 155.0, 136.7, 131.5, 130.9, 128.7, 128.3, 128.1, 81.1, 66.8, 52.7, 52.3, 51.8, 50.2, 48.8, 42.3, 40.4, 31.4, 29.3, 28.4, 22.1; HRMS (m/z) calcd. for C26H39N3O5Na (M+Na)+: 624.2752, found: 624.2747.

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**Methyl-2-((S)-7-((tert-butoxycarbonyl)amino)-6-oxo-7,8-dihydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-5(6H)-yl)-2-phenylacetamido)acetate (30)**

 Yield: 43% (over four steps). Brown powder, mp: 91-92 °C; Purification: Preparative HPLC (5-100% MeCN in 20 mins); HPLC Rf: 13.2, 13.7 min; 1H NMR (500 MHz, CDCl3): δ 7.48-7.28 (10H, m), 7.18 (2H, d, J = 9.3 Hz), 6.78 (1H, s), 6.37 (2H, s), 6.22 (1H, s), 5.98 (2H, s), 5.29-5.16 (2H, m), 4.99 (1H, d, J = 18.0 Hz), 4.82 (1H, d, J = 18.0 Hz), 4.61 (2H, t, J = 18.0 Hz), 4.42 (2H, t, J = 18.0 Hz), 4.38-4.28 (1H, m), 4.24-4.19 (2H, m), 4.07-3.88 (3H, m), 3.79 (3H, s), 3.71 (3H, s), 1.49 (18H); 13C NMR (125 MHz, CDCl3): δ 170.6, 169.8, 169.1, 168.2, 155.0, 133.3, 133.0, 131.8, 131.4, 130.4, 129.7, 129.4, 129.1, 81.3, 81.1, 61.3, 61.1, 52.7, 50.3, 49.0, 48.9, 48.9, 41.5, 41.3, 37.9, 37.6, 28.4; HRMS (m/z) calcd. for C22H30N6O6 (M+H)+: 473.2143, found: 473.2132
5. HPLC Chromatograms

(S)-tert-Butyl (5-(2-(tert-Butylamino)-2-oxo-1-phenylethyl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (14)

(S)-tert-Butyl (5-(2-(cyclohexylamino)-2-oxo-1-phenylethyl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (15)
(S)-tert-Butyl (5-(2-(benzylamino)-2-oxo-1-phenylethyl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (16)

(5)-tert-Butyl (5-(1-(4-bromophenyl)-2-(tert-Butylamino)-2-oxoethyl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (17)
(S)-tert-Butyl (5-(2-(tert-Butylamino)-1-(1H-indol-3-yl)-2-oxoethyl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (18)

(S)-tert-Butyl (5-(2-(tert-Butylamino)-1-(4-(dimethylamino)phenyl)-2-oxoethyl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (19)
First diastereoisomer of tert-Butyl ((7S)-5-(1-tert-Butylamino)-1-oxopropan-2-yl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (20)

Second diastereoisomer of tert-Butyl ((7S)-5-(1-(tert-Butylamino)-1-oxopropan-2-yl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (20)
**tert-**Butyl ((7S)-5-(2-(**tert**-Butylamino)-1-cyclohexyl-2-oxoethyl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (21)

![Chemical structure of 21](image1)

**Chemical structure of 21**

**HPLC chromatogram of 21**

**Minutes**

0.00  0.10  0.20  0.30  0.40  0.50  0.60  0.70

**AU**

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**(S)-tert-**Butyl (5-(1-(benzo[d][1,3]dioxol-5-yl)-2-(**tert**-Butylamino)-2-oxoethyl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (22)

![Chemical structure of 22](image2)

![Chemical structure of 22](image2)

**Chemical structure of 22**

**HPLC chromatogram of 22**

**Minutes**

0.00  0.10  0.20  0.30  0.40  0.50  0.60  0.70  0.80  0.90  1.00

**AU**
(S)-tert-Butyl (5-(2-(tert-Butylamino)-2-oxo-1-(m-tolyl)ethyl)-6-oxo-5,6,7,8-tetrahydro-4\textit{H}-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (24)

![Chemical Structure](image1)

(S)-tert-Butyl (5-(2-(tert-Butylamino)-1-(naphthalen-2-yl)-2-oxoethyl)-6-oxo-5,6,7,8-tetrahydro-4\textit{H}-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (23)

![Chemical Structure](image2)
tert-Butyl ((7S)-5-(2-(tert-Butylamino)-2-oxo-1-(pyridin-4-yl)ethyl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (25)

![Graphical representation of compound 25]

tert-Butyl ((7S)-5-(2-(tert-Butylamino)-2-oxo-1-(o-tolyl)ethyl)-6-oxo-5,6,7,8-tetrahydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-7-yl)carbamate (26)

![Graphical representation of compound 26]
(S)-methyl 6-(((benzyloxy)carbonyl)amino)-2-(2-((S)-7-((tert-butoxycarbonyl)amino)-6-oxo-7,8-dihydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-5(6H)-yl)acetamido)hexanoate (28)

Methyl-2-(2-((S)-7-((tert-butoxycarbonyl)amino)-6-oxo-7,8-dihydro-4H-[1,2,3]triazolo[1,5-a][1,4]diazepin-5(6H)-yl)-2-phenylacetamido)acetate (30)
6. NMR Spectral Data

14 - \(^1\)H NMR (250 MHz, CDCl\(_3\))

```
8.5  8.0  7.5  7.0  6.5  6.0  5.5  5.0  4.5  4.0  3.5  3.0  2.5  2.0  1.5  1.0  0.5 ppm
```

14 - \(^{13}\)C NMR (125 MHz, CDCl\(_3\))

```
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm
```
15 - $^1$H NMR (250 MHz, CDCl$_3$)

15 - $^{13}$C NMR (125 MHz, CDCl$_3$)
16 - $^1$H NMR (250 MHz, CDCl$_3$)

16 - $^{13}$C NMR (125 MHz, CDCl$_3$)
17 - $^1$H NMR (250 MHz, CDCl$_3$)

17 - $^{13}$C NMR (125 MHz, CDCl$_3$)
19 - ¹H NMR (250 MHz, CDCl₃)

19 - ¹³C NMR (125 MHz, CDCl₃)
19A - $^1$H NMR (250 MHz, CDCl$_3$)

![1H NMR spectrum](image)

19A - $^{13}$C NMR (125 MHz, CDCl$_3$)

![13C NMR spectrum](image)
19B - $^1$H NMR (250 MHz, CDCl$_3$)

19B - $^{13}$C NMR (125 MHz, MeOH-d$_4$)
21 - $^1$H NMR (250 MHz, CDCl$_3$)

21 - $^{13}$C NMR (125 MHz, CDCl$_3$)
22 - $^1$H NMR (250 MHz, CDCl$_3$)

22 - $^{13}$C NMR (125 MHz, CDCl$_3$)
23 - $^1$H NMR (250 MHz, CDCl$_3$)

![$^1$H NMR spectrum](image)

23 - $^{13}$C NMR (125 MHz, CDCl$_3$)

![$^{13}$C NMR spectrum](image)
24 - $^1$H NMR (250 MHz, CDCl$_3$)

- 2.4 - 1.3 ppm

24 - $^{13}$C NMR (125 MHz, CD$_3$CN)

- 190 - 10 ppm
25 - $^1$H NMR (250 MHz, CDCl$_3$)

25 - $^{13}$C NMR (125 MHz, CDCl$_3$)
26 - $^1$H NMR (250 MHz, MeCN-d$_6$)

![NMR spectrum image]

26 - $^{13}$C NMR (125 MHz, CDCl$_3$)

![NMR spectrum image]
28 - $^1$H NMR (500 MHz, CDCl$_3$)

31 - $^{13}$C NMR (125 MHz, CDCl$_3$)
30 - $^1$H NMR (500 MHz, CDCl$_3$)

31 - $^{13}$C NMR (125 MHz, CDCl$_3$)