Design and synthesis of covalent inhibitors of FabA

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**ABSTRACT:** There is an urgent need for the development of new therapeutics with novel modes of action to target Gram-negative bacterial infections, due to resistance to current drugs. Previously, FabA, an enzyme in the bacterial type II fatty acid biosynthesis pathway, was identified as a potential drug target in *Pseudomonas aeruginosa*, a Gram-negative bacteria of significant clinical concern. A chemical starting point was also identified. There is a cysteine, Cys15, in the active site of FabA, adjacent to where this compound binds. This paper describes the preparation of analogues containing an electrophilic warhead with the aim of covalent inhibition of the target. A wide variety of analogues were successfully prepared. Unfortunately, these analogues did not increase inhibition, which may be due to a loop within the enzyme partially occluding access to the cysteine.

### INTRODUCTION

*Pseudomonas aeruginosa* (*P. aeruginosa*) is a Gram-negative, rod-shaped bacteria that is capable of infecting humans. *P. aeruginosa* is an opportunistic infection, which causes an array of life-threatening infections in immunocompromised patients. *P. aeruginosa* has developed resistance to multiple classes of antibiotics and is emerging as a worldwide public health threat, so new treatments are urgently needed. To overcome issues with resistance, there is a need for novel classes of antibacterials which work by mechanisms differentiated from current antibiotics.

Fatty acids are an essential component of all cells; however, the bacterial type II fatty acid biosynthesis pathway (FASII) is sufficiently different from the type I pathway used in eukaryotic cells that FASII has become an attractive target for antibiotic research. In the FASII pathway, fatty acids are synthesized in a stepwise manner attached to acyl-carrier-protein (ACP) by a series of enzymes as shown in Figure 1.

First, FabD converts malonyl-coenzyme A (malonyl-CoA) 1 into malonyl-acyl carrier protein (malonyl-ACP) 2. The product from this is then used in one of two steps, either FabH catalyzes the initiation step where malonyl-ACP 2 is condensed with acetyl-CoA, or FabB or FabF condenses malonyl-ACP 2 with the growing fatty acid chain 3 to give the ketone 4. Regardless, the next step involves the reduction of the ketone 4 to the alcohol 5 by FabG using NADPH. Either FabA or FabZ are able to dehydrate this alcohol 5 to the alkene 6. Finally, FabL or FabV reduces the alkene 6 to the alkane 4 using NADH, and the cycle repeats to further lengthen the fatty acid chain. Many of the enzymes involved in this pathway have previously been investigated as potential antibacterial targets including FabH, FabG, and FabZ. FabI, and FabF. FabA is also able to isomerize E fatty acids to the corresponding Z isomer as shown in Figure 2.

Although both FabZ and FabA are able to catalyze the dehydration shown in Figure 1, only FabA is able to catalyze this isomerization due to subtle changes in the structure of the binding site. FabA is therefore an essential enzyme in the synthesis of unsaturated fatty acids.

A covalent inhibitor 7 of FabA has been reported, which is able to react with a histidine residue, His70, in the active site of the enzyme as shown in Figure 3.

First the alkyne 7 is converted to the allene 8 by FabA; this intermediate is then able to alkylate His70 via a Michael addition to give 9 and inactivate the enzyme. Although this compound covalently inhibits FabA, it was designed as a research tool, not a drug, and likely lacks the properties required to reach the site of action in vivo due to its high molecular weight and lipophilicity. The goal of this work was to investigate the development of a covalent inhibitor of *P. aeruginosa* FabA that can be used as a tool but also has the physiochemical properties that would make it more suited as a precursor to a novel antibacterial drug.
Previously, an in-house, high-throughput screen had identified compound 10 (Figure 4) as a potent inhibitor of FabA, IC$_{50}$ = 2.3 $\mu$M. The crystal structure of compound 10 bound to FabA revealed that the chlorine atom sat approximately 5 Å from Cys15, which provided a potential target for a covalent warhead (Figure 4).

To take advantage of this, several compounds were designed where the left-hand side of the compound, featuring the furan ring and the triazole ring of 10, was retained and the right-hand side altered with the intention of positioning a covalently...
reactive group in the correct orientation to react with Cys15. This synthesis is shown in Scheme 1.

Furan carbohydrazide 11 was reacted with methylisourea sulfate 12 in aqueous sodium hydroxide to give 13. The intermediate was suspended in water and heated to 140 °C under microwave conditions to cyclize the intermediate and give the triazole scaffold 14 in quantitative yield. Several compounds were synthesized as shown above including the example benzylamide (Scheme 1) and saturated analogues shown in Table 1 via a reductive amination to give 15 followed by deprotection (16) and addition of an acrylamide (17) as a covalent warhead, or an unreactive isostere of this.

The final compounds were designed to hold the covalent warhead in a slightly different orientation relative to the triazole core and to position it in the right area to form a covalent bond to the target cysteine residue - Cys15. These compounds were screened against FabA and the results are shown in Table 1.

The only compounds which showed significant inhibition of FabA were the known FabA inhibitor 10 and the closely related compound 19 where the chlorine atom had been replaced with a nitrile group. To investigate if compound 19 was able to covalently bind to the target, mass spectrometry was used. Formation of a covalent bond would result in an increase in the mass measured for the appropriate peptide, but no MS-fragment corresponding to this compound bound to a peptide was detected, suggesting there was no covalent binding.

The three most potent compounds, 10, 18, and 19, all contained a benzene ring, and it is known from the crystal structure that this benzene occupies space close to the target cysteine residue - Cys15. Attaching a more reactive warhead to a benzene core may allow covalent bond formation. The vinyl sulfonamide warhead was selected as it is more reactive than the acrylamides previously investigated.

Treating 2-chloroethane sulfonyl chloride with a hindered pyridine derivative gave the required vinyl sulfonyl chloride, which was used to synthesize the vinyl sulfonamides 28 and 29 (Scheme 2). Compound 29 was an analogue of the vinyl sulfonamide which could not form a covalent link to the FabA protein, acting as a control.

Compounds 28 and 29 were screened against FabA but were found to be inactive (IC₅₀ > 30 μM). This is unlikely to be due to the compound not being reactive enough to form a covalent bond, suggesting that either the target cysteine residue, Cys15, is not reactive or that these types of compounds are not placing the covalent warhead in the correct orientation and/or space to form a covalent bond.

In in silico modeling and docking experiments, it was observed that all predicted interactions between the known inhibitor and the protein, based on the crystal structure, occurred with the triazole ring and the furan ring as shown in Figure 5.
Scheme 2. Synthesis of 2-Amino-2-N-(4-vinylsulfonylaminobenzyl)-5-furyl-1,3,4-triazole 28 and 2-Amino-2-N-(4-ethylsulfonylaminobenzyl)-5-furyl-1,3,4-triazole 29

Scheme 3. Preparation of a Range of Cores (42–45)

Figure 5. Interactions between known inhibitor and FabA based on the obtained crystal structure (PDB 4CL6).
To take advantage of this, it was proposed that developing a set of compounds with flexible linkers of a variety of lengths should allow the covalent warhead to adopt a wide variety of conformations in the binding site. This should better facilitate the formation of a covalent bond. However, these compounds would have a lot of rotational freedom on binding and, therefore, a larger entropic penalty to binding than compounds synthesized thus far. Any of these compounds that seemed to be binding covalently would need to be studied using X-ray crystallography to understand how they bind. Using this information, the linker would be constrained to reduce the entropy while maintaining the ability to form a covalent bond.

**FLEXIBLE LINKERS TO COVALENT WARHEAD**

Previously, directly forming amines on this core via a reductive amination was successful so this method was employed to access these intermediates. The required aldehydes (34−37) were synthesized (Scheme 3) and used to add appropriate alkyl chains to the triazole core to give intermediates (38−41) which were subsequently deprotected (42−45).

Finally, the covalent warheads (46−49, 54−57) and an unreactive isostere (50−53, 58−61) of each were installed on the amine as shown in Scheme 4.

The resulting compound library was screened against FabA; however, in all cases, no significant inhibition of the enzyme was found (IC₅₀ > 30 μM). This suggested that it was extremely challenging to covalently inhibit FabA with this scaffold.

**PROBING THE BINDING SITE**

To investigate why FabA could not be covalently inhibited by compounds based on the triazole scaffold, a molecular dynamics simulation of the protein was performed using the crystal structure of the known inhibitor bound to FabA. This revealed that there is a loop in the active site which sits between the cysteine residue being targeted, Cys15, and the binding site. This loop was observed to not significantly move at any point during the simulation, as shown in Figure 6.

To investigate if this loop was blocking the cysteine residue, a small set of compounds was designed in order to probe the binding site. These were designed to put groups of various sizes and polarity in the active site in approximately the position of the loop and observe any flexibility of the protein.

Two simple alkane compounds 62 and 63 were synthesized from the corresponding aldehydes (Scheme 5). Two alcohols were also synthesized (Scheme 6).

The diols were reacted with tert-butyl(chloro)diphenylsilane to give the protected alcohols 66 and 67 in high yield. A Swern oxidation was used to convert these to the aldehydes 68 and 69 which were used in reductive aminations with the amino triazole core to give the amines 70 and 71. The silyloxy
protecting group was removed in the final step to obtain the desired alcohols 72 and 73. Another route was required to synthesize the desired carboxylic acids and esters (Scheme 7). The lactone 74 was treated with methanol to give the alcohol 75, and a Swern oxidation was used to convert this to the aldehyde 76. Performing a reductive amination with this aldehyde and the amino triazole core gave the required ester 77. However, attempting to hydrolyze this ester to the carboxylic acid 79 using lithium hydroxide instead gave the lactam 78. Subsequently this was converted to the desired straight chain carboxylic acid 79 by treating with aqueous acid. For the shorter chain ester, the acetal was converted to the aldehyde using Amberlite acidic resin, and the reductive amination of the amino triazole core with the aldehyde gave the desired ester (Scheme 8).
The resulting library of potential inhibitors was screened against FabA (Table 2).

A longer alkyl chain in the molecule gave better potency, e.g., 63 compared to 62. Adding a polar group to this resulted in a decrease in the potency, e.g., 73 vs 63. The log D was calculated in Stardrop and found not to be correlated well with the potency meaning, this is not just related to the lipophilicity. Ultimately, these data suggest that these compounds are binding in a hydrophobic pocket. A covalent warhead has heteroatoms which induce polarity, and even if the loop sitting in the binding site in front of the target cysteine residue, Cys15, can move it seems unlikely that a polar covalent inhibitor of FabA will be defined as 100% inhibitory activity, whereas of the complete reaction mixture as 0% inhibitory activity. Curve fittings and calculations of IC50 values were performed using ActivityBase XE version 9.2.0.106 from IDBS with a four-parameter logistics model.

**EXPERIMENTAL SECTION**

**FabA RapidFire High-Throughput Mass Spectrometry Assay.** Details of FabA RapidFire high-throughput mass spectrometry (HTMS) assay development will be published separately. An overview is shown in Figure 7. Briefly, compounds in DMSO stock were dispensed into 384-well assay plate (Greiner 781101) through an ECHO 550 acoustic liquid-handling system (LabCyte). Assays were performed by adding 5 μL 40 nM FabA protein solution in reaction buffer (50 mM Tris, pH 7.5, 1 mM DTT, 0.1% BSA, 0.005% NP40), and the reaction was initiated with the addition of a 5 μL 720 μM substrate 3-OH decanoyl-N-acetylcysteamine (3OH-NAC) in assay buffer. The plates were incubated in the plate shaker at 300 rpm at room temperature for 30 min, followed by addition of 90 μL of 1% formic acid to quench enzyme reaction. The reaction mixture was subjected to RapidFire HTMS analysis.

RapidFire HTMS was performed using a RapidFire 365 system (Agilent) coupled to a triple quadrupole mass spectrometer 6470 (Agilent). The samples were loaded onto a C4 cartridge (Agilent) using deionized water containing 5 mM ammonium formate at flow rate of 1.5 mL/min and eluted to the mass spectrometer using acetonitrile/deionized water (90/10, v/v) containing 5 mM ammonium formate in at a flow rate of 1.25 mL/min. The sipper was washed to minimize carryover with deionized water followed by acetonitrile. Aspiration time, load/wash time, elution time and re-equilibration time were set to 600, 3000, 5000, and 500 ms, respectively, with a cycle time of approximately 10 s. The triple-quadrupole mass spectrometer with electro-spray ion source was operated in positive multiple reaction monitoring (MRM) mode. The detailed setting for the mass spectrometer parameters was as follows: capillary voltage: 3000 V; gas temperature: 350 °C; gas flow: 7 l/min; nebulizer: 40 psi; sheath gas temperature 300 °C; sheath gas flow: 11 l/min; and nozzle voltage 1500 V. The MRM transitions (Q1 and Q3) for 2-decenyloyl-N-acetylcysteamine (2DE-NAC), as a reaction product, were set as 272.1/153.1. The mass resolution window for both parental and daughter ions was set at as unit (0.7 Da). The dwell time, fragmentor, and collision energy for each transition were 50 ms, 100 V and 8 eV, respectively.

The inhibitory activity was calculated using the reaction product peak area. The peak area of the reaction product without enzyme was defined as 100% inhibitory activity, whereas of the complete reaction mixture as 0% inhibitory activity. Curve fittings and calculations of IC50 values were performed using ActivityBase XE version 9.2.0.106 from IDBS with a four-parameter logistics model.

**General Methods.** Chemicals and solvents were purchased from commercial sources and were used without any further purification unless noted otherwise. Air and water sensitive reactions were carried out under an inert nitrogen atmosphere in oven-dried glassware. Analytical thin-layer chromatography (TLC) was performed on precoated TLC plates (layer 0.20 mm silica gel 60 with fluorescent indicator UV254, from Merck). Developed plates were air-dried and analyzed under a UV lamp (UV254/365 nm) and by staining with permanganate or ninhydrin. Flash column chromatography was performed on prepacked silica gel cartridges (230–400 mesh, 40–63 μm, from SiliCycle) using a Teledyne ISCO Combiflash Rf or Combiflash Rf 200i. 1H (500 MHz), 13C (125 MHz), 1H (400 MHz), 13C (100 MHz), and 2D NMR spectra were recorded in DMSO-d6, MeOD-d4, or CDCl3 using a Bruker Avance spectrometer. Proton chemical shifts are reported in ppm relative to the residual DMSO peak (δ = 2.50 ppm), methanol peak (δ = 3.31 ppm), or chloroform peak (δ = 7.26 ppm). Multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), sept (septet), m (multiplet), brs (broad singlet), dd (doublet of doublets), or as a

**Table 2. Final Library of Compounds Was Screened against FabA**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>IC50 (µM)</th>
<th>% inhibition at 100 µM</th>
<th>cLogD</th>
</tr>
</thead>
<tbody>
<tr>
<td>62</td>
<td>R'</td>
<td>&gt;100</td>
<td>48</td>
<td>0.31</td>
</tr>
<tr>
<td>63</td>
<td>R'</td>
<td>1.4</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>R'-OH</td>
<td>&gt;100</td>
<td>26</td>
<td>-0.75</td>
</tr>
<tr>
<td>73</td>
<td>R'-OH</td>
<td>50</td>
<td>72</td>
<td>-0.33</td>
</tr>
<tr>
<td>82</td>
<td>R'-O</td>
<td>&gt;100</td>
<td>0</td>
<td>0.09</td>
</tr>
<tr>
<td>77</td>
<td>R'-O</td>
<td>9.2</td>
<td>94</td>
<td>0.24</td>
</tr>
<tr>
<td>79</td>
<td>R'-O</td>
<td>&gt;100</td>
<td>0</td>
<td>-1.43</td>
</tr>
</tbody>
</table>

*Where a compound was not potent enough to measure a pIC50, the percent inhibition at 100 µM is reported.*
combination of these. Coupling constants (J) are quoted to the nearest 0.1 Hz. 13C chemical shifts are reported in ppm relative to the residual DMSO peak (δ = 39.51 ppm), methanol peak (δ = 49.00 ppm), or chloroform peak (δ = 77.16 ppm). High-resolution electrospray measurements were performed on a Bruker Daltonics MicrOTOF mass spectrometer.

1-(Furan-2-carbonyl)amino)guanidine 13. Furan-2-carbonylhydrazide (5.0 g, 39.64 mmol, 1 equiv) and o-methylisourea bisulfate (20.5 g, 119 mmol, 5 equiv) were added to a solution of sodium hydroxide (7.93 g, 198 mmol, 8 equiv) in water (100 mL) and the reaction was stirred at room temperature. After 6 h the resultant precipitate was filtered, washed with water, diethyl ether and dried to give 2-amino-5-furyl-1,3,4-triazole (5.1 g, 99%) as an off-white powder.

After 6 h the reaction was evaporated to dryness to give 2-amino-5-furyl-1,3,4-triazole (5.1 g, 99%) as a gray powder. 1H (500 MHz, DMSO-d6): δ 10.81 (1H, brs), 7.54 (1H, q, J = 0.8 Hz), 6.91 (2H, brs), 6.76 (2H, brs), 6.63 (1H, d, J = 2.9 Hz), 6.44 (1H, dd, J = 3.2, 1.8 Hz); 13C (125 MHz, DMSO-d6): δ 155.19, 152.78, 141.87, 110.80, 108.28; MS (ESI): m/z = 169.1 [M + H]+. Analysis is in agreement with the literature.

2-Amino-5-furyl-1,3,4-triazole 14. 1-(Furan-2-carbonyl)amino)guanidine 13 (5.78 g, 34.39 mmol, 1 equiv) was suspended in water (15 mL) and heated in the microwave to 140 °C, 6 bar, for 1 h. The reaction was evaporated to dryness. The residue was purified by flash chromatography (0–10% MeOH in DCM) to give 2-amino-5-furyl-1,3,4-triazole (5.1 g, 99%) as an off-white powder. 1H (500 MHz, DMSO-d6): δ 12.11 (1H, brs), 7.68 (1H, s), 6.69 (1H, s), 6.54 (1H, s), 6.05 (2H, brs); 13C (125 MHz, DMSO-d6): δ 142.74, 111.32, 107.63; MS (ESI): m/z = 151.1 [M + H]+. Analysis is in agreement with the literature.

2-Amino-5-furyl-1,3,4-triazole 15. 2-Amino-5-furyl-1,3,4-triazole 14 (400 mg, 2.66 mmol, 1 equiv), tert-butyl N-(4-formylphenyl)carbamate (884 mg, 4.00 mmol, 1.5 equiv) and sodium triacetoxycarbonylhydrizide (1130 mg, 5.33 mmol, 2 equiv) were dissolved in THF (25 mL) under nitrogen and acetic acid (305 μL, 5.33 mmol, 2 equiv) was added. The reaction was stirred overnight at room temperature. LCMS indicated the reaction reached 50% conversion, so a second portion of sodium triacetoxycarbonylhydrizide (1130 mg, 5.33 mmol, 2 equiv) and acetic acid (305 μL, 5.33 mmol, 2 equiv) were added, and the reaction stirred overnight at room temperature. LCMS indicated no further conversion occurred, so 10 mL saturated sodium hydrogen carbonate solution was added, and the mixture stirred vigorously, the layers were separated, and the aqueous layer extracted 2x with 20 mL ethyl acetate. The combined organic layers were dried over MgSO4 passed through a phase separator and evaporated to dryness. The residue was purified by flash chromatography (0–10% MeOH in DCM) to give 2-amino-2-N-(4-(Boc)aminobenzyl)-5-furyl-1,3,4-triazole (200 mg, 1.33 mmol, 1 equiv) and 4-chlorobenzaldehyde (281 mg, 2.00 mmol, 1.5 equiv) were dissolved in dry methanol (15 mL) under nitrogen. Acetic acid (0.15 mL, 2.66 mmol, 2 equiv) was added, and the reaction stirred at room temperature. After 20 min, sodium cyanoborohydride (167 mg, 2.66 mmol, 2 equiv) was added and the reaction stirred overnight.

The reaction was evaporated to dryness and the residue dissolved in 20 mL ethyl acetate, filtered through Celite, and washed with 10 mL water. The aqueous layer was extracted 2x with 10 mL ethyl acetate and the combined organic layers washed with 15 mL saturated sodium hydrogen carbonate solution, dried over MgSO4 passed through a phase separator, and evaporated to dryness. The residue was purified by flash chromatography (0–8% MeOH in DCM) to give 2-(4-chlorobenzyl)amino-5-furyl-1,3,4-triazole (72.6 mg, 19%) as a white powder. 1H (400 MHz, MeOD-d4): δ 7.60–7.32 (4H, m), 6.92 (1H, d, J = 3.2 Hz), 6.56 (1H, dd, J = 3.3, 1.8 Hz), 4.49 (2H, s); 13C (100 MHz, MeOD-d4): δ 145.83, 131.96, 129.87, 129.56, 112.46, 54.79, 49.85, 47.16; HRMS m/z (ESI+): calcd for C12H12N2O2 [M + H]+: 275.0705, found 275.0711 (6.2 ppm).

2-(4-Chlorobenzyl)amino-5-furyl-1,3,4-triazole 16. 2-Amino-5-furyl-1,3,4-triazole 14 (100 mg, 0.67 mmol, 1 equiv) was dissolved in methanol (15 mL) at room temperature, and 4-fluorobenzaldehyde (165 mg, 1.33 mmol, 2 equiv) and 3 Å molecular sieves were added. The mixture was heated to reflux and stirred gently overnight. TLC confirmed complete conversion of the starting material, so the reaction was cooled to 0 °C, and sodium cyanoborohydride (84 mg, 1.33 mmol, 2 equiv) was added and allowed to slowly warm to room temperature overnight. The mixture was evaporated to dryness, dissolved in ethyl acetate and filtered through Celite, washed with saturated sodium hydrogen carbonate solution, brine, dried over MgSO4 and evaporated.
to dryness. The residue was purified by flash chromatography (2–10% MeOH in DCM) to yield 2-(4-flurobenzyl)-1,3,4-triazole (25.2 mg, 14%) as a white powder. \(^1\)H (400 MHz, MeOD-d\(_4\)) \(\delta\) 7.59 (1H, s), 7.38 (2H, dd, \(\delta J = 8.6, 5.4\) Hz), 7.05 (2H, \(\delta J = 8.8\) Hz), 6.90 (1H, s), 6.54 (1H, s), 4.46 (2H, s); HRMS \(m/z\) (ESI\(^+\)) calcd for \(C_{15}H_{12}N_3O\) [M + H]\(^+\): 259.0990, found 259.0984 (2.4 ppm).

2-(4-Cyanobenzyl)-1,3,4-triazole 19. 2-Amino-5-furyl-1,3,4-triazole (70.4 mg, 30%) as a white powder.

The residue dissolved in 20 mL ethyl acetate, filtered through Celite and washed with 10 mL water. The aqueous layer was extracted 2× with 10 mL ethyl acetate, and the combined organic layers were washed with 15 mL saturated sodium hydrogen carbonate solution, dried over MgSO\(_4\), passed through a phase separator, and evaporated to dryness. The residue was purified by flash chromatography (3–8% MeOH in DCM) to yield 2-(4-cyanobenzyl)-1,3,4-triazole (32 mg, 16%) as a white powder. \(^1\)H (400 MHz, MeOD-d\(_4\)) \(\delta\) 7.69 (4H, m), 7.60 (1H, \(\delta J = 1.0\) Hz), 7.53 (4H, m), 6.90 (1H, \(\delta J = 3.3\) Hz), 6.54 (1H, dd, \(\delta J = 3.4, 1.8\) Hz), 4.58 (2H, s); \(^13\)C (100 MHz, MeOD-d\(_4\)) \(\delta\) 148.93, 144.69, 133.39, 133.25, 129.00, 128.28, 112.51, 110.43, 47.73; HRMS \(m/z\) (ESI\(^+\)) calcd for \(C_{15}H_{12}N_3O\) [M + H]\(^+\): 266.1036, found 266.1034 (1.1 ppm). Analysis is in agreement with the literature.\(^17\)

2-Amino-2-(1-(Boc)piperidin-4-yl)-5-furyl-1,3,4-triazole 83. 2-Amino-5-furyl-1,3,4-triazole (100 mg, 0.67 mmol, 1 equiv) and tert-butyl 4-formylpiperidine-1-carboxylate (697 mg, 3.50 mmol, 1.5 equiv) were dissolved in DCM (5 mL), TFA (202 \(\mu\)L, 2.63 mmol, 15 equiv) was added, and the reaction was stirred for 2 h. The residue was dissolved in DCM (5 mL), and triethylamine (73 \(\mu\)L, 0.53 mmol, 3 equiv) and propanoyl chloride (17 \(\mu\)L, 0.19 mmol, 1.1 equiv) were added. After 30 min, water (5 mL) was added, the layers separated, and the aqueous layer extracted 2× with 5 mL DCM, the combined organic layers were dried over MgSO\(_4\) and evaporated to dryness. The residue was purified by flash chromatography (0–10% MeOH in DCM) to give 2-amino-2-(1-(Boc)piperidin-4-yl)-5-furyl-1,3,4-triazole (17 mg, 26%) as a white powder. \(^1\)H (500 MHz, DMSO-d\(_6\)) \(\delta\) 7.80 (1H, \(\delta J = 1.0\) Hz), 6.91 (1H, \(\delta J = 3.3\) Hz), 6.62 (1H, dd, \(\delta J = 3.4, 1.8\) Hz), 4.38 (1H, dd, \(\delta J = 12.8\) Hz), 3.85 (1H, \(\delta J = 13.3\) Hz), 3.11 (2H, \(\delta J = 6.8\) Hz), 2.95 (1H, \(\delta J = 12.3\) Hz), 2.50 (1H, m), 2.29 (2H, \(q, \delta J = 7.4\) Hz), 1.77 (3H, m), 1.09 (1H, m), 0.98 (4H, \(t, \delta J = 7.4\) Hz); \(^13\)C (125 MHz, DMSO-d\(_6\)) \(\delta\) 170.97, 143.98, 111.66, 109.66, 48.11, 44.56, 40.82, 36.63, 29.81, 29.03, 25.56, 9.46; MS (ESI): \(m/z\) = 304.2 [M + H]\(^+\).

tert-Butyl 4-Oxopiperidine-1-carboxylate 84. Piperidin-4-one (500 mg, 5.04 mmol, 1 equiv) and di-tert-butyl dicarbonate (2202 mg, 10.09 mmol, 2 equiv) were dissolved in methanol (20 mL). Triethylamine (1.05 mL, 7.57 mmol, 1.5 equiv) was added and the reaction stirred overnight at room temperature. The reaction was evaporated to dryness, the residue partitioned between ethyl acetate and saturated sodium hydrogen carbonate solution, the layers separated, and the aqueous layer extracted 2× with ethyl acetate. The combined organic layers were dried over MgSO\(_4\) and evaporated to dryness. The residue was purified by flash chromatography (0–5% MeOH in DCM) to give tert-butyl 4-oxopiperidine-1-carboxylate (318 mg, 32%) as a white solid. \(^1\)H (400 MHz, CDCl\(_3\)) \(\delta\) 3.64 (4H, \(t, \delta J = 6.2\) Hz), 2.36 (4H, \(t, \delta J = 6.2\) Hz), 1.41 (9H, s); \(^13\)C (100 MHz, CDCl\(_3\)) \(\delta\) 207.64, 154.43, 80.34, 41.12, 28.42. Analysis is in agreement with the literature.\(^18\)

2-Amino-2-(1-(Boc)piperidin-4-yl)-5-furyl-1,3,4-triazole 85. 2-Amino-5-furyl-1,3,4-triazole (100 mg, 0.67 mmol, 1 equiv), tert-butyl 4-oxopiperidine-1-carboxylate 84 (697 mg, 3.50 mmol, 1.5 equiv) and sodium triacetoxyborohydride (988 mg, 4.66 mmol, 2 equiv) were dissolved in THF (25 mL) under nitrogen, and acetic acid (267 \(\mu\)L, 4.66 mmol, 2 equiv) was added. The reaction was stirred at room temperature overnight.

LCMS indicated the reaction was incomplete, so a second portion of sodium triacetoxyborohydride (988 mg, 4.66 mmol, 2 equiv) and acetic acid (267 \(\mu\)L, 4.66 mmol, 2 equiv) were added, and the reaction stirred overnight at room temperature. LCMS indicated no further conversion occurred, so 10 mL saturated sodium hydrogen carbonate solution was added, and the mixture stirred vigorously, the layers were separated, and the aqueous layer extracted 2× with 20 mL ethyl acetate. The combined organic layers were dried over MgSO\(_4\), passed through a phase separator, and evaporated to dryness. The residue was purified by flash chromatography (0–5% MeOH in DCM) to give 2-amino-2-(1-(Boc)piperidin-4-yl)-5-furyl-1,3,4-triazole (350 mg, 2.33 mmol, 1 equiv), tert-butyl 4-oxopiperidine-1-carboxylate 84 (697 mg, 3.50 mmol, 1.5 equiv) and sodium triacetoxyborohydride (988 mg, 4.66 mmol, 2 equiv) were dissolved in THF (25 mL) under nitrogen, and acetic acid (267 \(\mu\)L, 4.66 mmol, 2 equiv) was added. The reaction was stirred at room temperature overnight.
with DCM, the combined organics were dried over MgSO₄. TLC indicated no intermediate remained. Water (3 mL) was added, the layers separated, and the aqueous layer extracted 2× with DCM, the combined organics were dried over MgSO₄, and evaporated in vacuo. The residue was purified by flash chromatography (0–5% MeOH in DCM) to give 2-amino-2-N-(1-acylpyrrolidin-4-yl)-5-furyl-1,3,4-triazole (13 mg, 83%) as a white powder. \(^1\)H (400 MHz, CDCl₃): \(\delta 7.50 \ (1H, dd, J = 1.8, 0.8 \ Hz), 6.93 \ (1H, dd, J = 3.4, 0.6 \ Hz), 6.59 \ (1H, dd, J = 16.8, 10.6 \ Hz), 6.52 \ (1H, dd, J = 3.4, 1.8 \ Hz), 6.27 \ (1H, dd, J = 16.8, 19.9 \ Hz), 5.69 \ (1H, dd, J = 10.6, 19.9 \ Hz), 4.47 \ (2H, m), 3.91 \ (2H, m), 2.96 \ (1H, m), 2.16 \ (2H, m), 1.44 \ (2H, m); MS (ESI): \(m/z = 288.2 \ [M + H]^+\).

**2-Amino-2-(1-propionylpyrrolidin-4-yl)-5-furyl-1,3,4-triazole 23.** 2-Amino-2-(1-(Boc)pyrrolidin-4-yl)-5-furyl-1,3,4-triazole 85 (70 mg, 0.21 mmol, 1 equiv) was dissolved in DCM (5 mL) and TFA (241 µL, 3.15 mmol, 15 equiv) added. The reaction was stirred for 3 h and evaporated to dryness. The residue was dissolved in DCM (5 mL) and triethylamine (88 µL, 0.63 mmol, 3 equiv) and propanoyl chloride (18 µL, 0.21 mmol, 1 equiv) were added. The reaction was stirred for 3 h and evaporated to dryness. The residue was dissolved in 5 mL DCM and 3 mL water, the layers separated, and the aqueous layer extracted 2× with 5 mL DCM, the combined organics were dried over MgSO₄ and evaporated to dryness. The residue was purified by flash chromatography eluting. The residue was purified by flash chromatography (0–8% MeOH in DCM) to give 2-amino-2-(1-propionylpyrrolidin-4-yl)-5-furyl-1,3,4-triazole (59 mg, 73%) as a yellow solid. \(^1\)H (500 MHz, DMSO-d₆): \(\delta 7.76 \ (1H, d, J = 1.0 \ Hz), 6.83 \ (1H, d, J = 3.3 \ Hz), 6.59 \ (1H, dd, J = 3.3, 1.8 \ Hz), 4.27 \ (1H, d, J = 13.0 \ Hz), 3.83 \ (1H, d, J = 13.5 \ Hz), 3.61 \ (1H, m), 3.10 \ (1H, m), 2.74 \ (1H, t, J = 11.5 \ Hz), 2.32 \ (2H, q, J = 7.4 \ Hz), 1.92 \ (2H, dd, J = 23.0, 12.9 \ Hz), 1.38 \ (1H, q, J = 10.1 \ Hz), 1.29 \ (1H, q, J = 10.1 \ Hz), 0.99 \ (3H, t, J = 7.4 \ Hz); \(^1\)C (125 MHz, DMSO-d₆): \(\delta 171.06, 143.46, 111.50, 108.84, 49.90, 43.44, 40.43, 32.21, 31.43, 25.48, 9.44; MS (ESI): \(m/z = 290.1 \ [M + H]^+\). **Amino-2-N-(4-propionylamidobenzy)-5-furyl-1,3,4-triazole 24.** 2-Amino-2-(4-aminobenzyl)-5-furyl-1,3,4-triazole 27 (10 mg, 0.039 mmol, 1 equiv) was dissolved in THF (5 mL), and triethylamine (11 µL, 0.078 mmol, 2 equiv) was added. The reaction was cooled to 0 °C, and propanoyl chloride (3.4 µL, 0.039 mmol, 1 equiv) was added. After 5 min the reaction was warmed to room temperature and stirred for 30 min. Methanol (5 mL) was added and the reaction evaporated to dryness. The residue was purified by HPLC (5–95% acetonitrile in water (0.1% formic acid)) to give 2-amino-2-N-(4-propionylamidobenzyl)-5-furyl-1,3,4-triazole (6 mg, 44%) as a white powder. \(^1\)H (500 MHz, DMSO-d₆): \(\delta 12.35 \ (1H, brs), 9.74 \ (1H, brs), 7.70 \ (1H, s), 7.26 \ (2H, d, J = 8.4 \ Hz), 7.10 \ (2H, d, J = 8.4 \ Hz), 6.73 \ (2H, dd, J = 16.4, 10.0 \ Hz), 6.55 \ (1H, brs), 6.07 \ (1H, d, J = 16.5 \ Hz), 5.99 \ (1H, d, J = 10.0 \ Hz), 4.31 \ (1H, d, J = 6.4 \ Hz); \(^1\)C (125 MHz, DMSO-d₆): \(\delta 136.30, 127.99, 124.73, 119.88, 111.37, 45.68; HRMS m/z (ESI) calcd for C₁₄H₁₃N₂O₂S [M + H]⁺: 346.0968, found 346.0969 (0.2 ppm).

**2-Amino-2-N-(4-ethylsulfonylamidobenzy)-5-furyl-1,3,4-triazole 28.** 2-Amino-2-N-(4-ethylsulfonylamidobenzy)-5-furyl-1,3,4-triazole 27 (10 mg, 0.039 mmol, 1 equiv) was dissolved in THF (5 mL), and triethylamine (11 µL, 0.078 mmol, 2 equiv) was added. The reaction was cooled to 0 °C, and ethane sulfonyl chloride (3.7 µL, 0.039 mmol, 1 equiv) was added. After 5 min the reaction was warmed to room temperature and stirred overnight. LCMS indicated 50% of the starting material remained, so a second portion of ethane sulfonyl chloride (3.7 µL, 0.039 mmol, 1 equiv) was added and the reaction stirred. After a further 4 h, starting material still remained so a further portion of ethane sulfonyl chloride (3.7 µL, 0.039 mmol, 1 equiv) was added. After a further 2 h, starting material still remained so a fourth portion of ethane sulfonyl chloride (3.7 µL, 0.039 mmol, 1 equiv) was added. After 1 h, starting material still remained so a final portion of ethene sulfonyl chloride (3.7 µL, 0.039 mmol, 1 equiv) was added. After 2 h, 83% of the starting material remained so a final portion of ethene sulfonyl chloride (3.7 µL, 0.039 mmol, 1 equiv) was added. The reaction was stirred overnight at room temperature. The reaction was quenched by the addition of 5 mL methanol and evaporated to dryness. The residue was purified by HPLC (5–95% acetonitrile in water (0.1% formic acid)) to give 2-amino-2-N-(4-ethylsulfonylamidobenzyl)-5-furyl-1,3,4-triazole (25) (500 mg, 3.07 mmol, 1 equiv) was dissolved in ether (5 mL) and cooled to −78 °C. 2,6-Dimethylpyridine (0.43 mL, 3.68 mmol, 1.2 equiv) in ether (2 mL) was added dropwise over 10 min, and the reaction was stirred for 45 min then allowed to warm to room temperature and stirred overnight. The reaction was cooled to 0 °C, and 5 mL 1% H₂SO₄ solution was added. The organic layer was separated, and the aqueous layer extracted 2× with 5 mL ether, dried over MgSO₄, passed through a phase separator, and evaporated to dryness. The residue was purified by distillation using a kugelrohr apparatus (65 °C, 25 mbar) to give vinyl sulfonyl chloride (196.2 mg, 50%) as a colorless oil. \(^1\)H (400 MHz, DMSO-d₆): \(\delta 6.47–6.37 \ (1H, m), 5.63–5.55 \ (1H, m), 5.31–5.23 \ (1H, m).

**Vinyl Sulfonyl Chloride 26.** Chloroethanesulfonyl chloride (25) (500 mg, 3.07 mmol, 1 equiv) was dissolved in ether (5 mL) and cooled to −78 °C. 2,6-Dimethylpyridine (0.43 mL, 3.68 mmol, 1.2 equiv) in ether (2 mL) was added dropwise over 10 min, and the reaction was stirred for 45 min then allowed to warm to room temperature and stirred overnight. The reaction was cooled to 0 °C, and 5 mL 1% H₂SO₄ solution was added. The organic layer was separated, and the aqueous layer extracted 2× with 5 mL ether, dried over MgSO₄, passed through a phase separator, and evaporated to dryness. The residue was purified by distillation using a kugelrohr apparatus (65 °C, 25 mbar) to give vinyl sulfonyl chloride (196.2 mg, 50%) as a colorless oil.
8.46. HRMS m/z (ESI') calc'd for C_{13}H_{18}N_{5}O_{3}S [M + H]^+: 348.1125, found 348.1129 (1.1 ppm).

3-(Boc)aminopropanal 34. Dry DCM (40 mL) was cooled to −78 °C under nitrogen, and oxalyl chloride (0.72 mL, 8.56 mmol, 1.5 equiv) was added. DMSO (1.21 mL, 17.12 mmol, 3 equiv) was added and the reaction stirred at −78 °C for 30 min. 3-(Boc)aminopropanol (31) (1.00 g, 5.71 mmol, 1 equiv) was dissolved in dry DCM (10 mL) and added. The reaction was stirred for 30 min then triethylamine (3.12 mL, 23.01 mmol, 5 equiv) was added and the reaction allowed to slowly warm to room temperature overnight. 40 mL saturated sodium hydrogen carbonate solution was added and stirred vigorously, the layers were separated, and the aqueous layer extracted 2x with 25 mL DCM. The combined organic layers were washed with brine, dried over MgSO₄, passed through a phase separator, and evaporated to dryness. The residue was purified by flash chromatography (0–80% ethyl acetate in heptane) to give 3-(Boc)aminopropanal (0.956 g, 97%) as a colorless oil. The reaction was stirred for 30 min then triethylamine (3.98 mL, 28.53 mmol, 5 equiv) was added and the reaction allowed to slowly warm to room temperature overnight. 40 mL saturated sodium hydrogen carbonate solution was added and stirred vigorously, the layers were separated, and the aqueous layer extracted 2x with 25 mL DCM. The combined organic layers were washed with brine, dried over MgSO₄, passed through a phase separator, and evaporated to dryness. The residue was purified by flash chromatography (0–80% ethyl acetate in heptane) to give 3-(Boc)aminopropanol (0.956 g, 97%) as a colorless oil.

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was added and the reaction stirred overnight. Four mL 2 N NaOH solution was added and the mixture stirred. The layers were separated, and the aqueous layer extracted 2× with 15 mL ethyl acetate. The combined organic layers were dried over MgSO₄, passed through a phase separator, and evaporated to dryness. The residue was purified by flash chromatography (0–5% MeOH in DCM) to give 2-(4-(Boc)-aminobutylamino)-5-furyl-1,3,4-triazole (554 mg, 48%) as a white solid. ¹H (500 MHz, MeOD-d₄): 6.73–6.76 (1H, s), 6.29–6.32 (1H, dd, J = 5.4, 2.7 Hz), 5.33 (2H, t, J = 6.9 Hz), 3.20 (2H, t, J = 6.9 Hz). LCMS indicated complete conversion, so the residue was purified by flash chromatography (0–6% MeOH in DCM) to give 2-(6-(Boc)aminohexylamino)-5-furyl-1,3,4-triazole (546 mg, 96%). ¹H (500 MHz, MeOD-d₄): 7.53 (1H, d, J = 1.1 Hz), 6.84 (1H, d, J = 3.3 Hz), 6.48 (1H, dd, J = 3.4, 1.8 Hz), 3.20 (2H, t, J = 6.9 Hz), 2.59 (2H, t, J = 7.1 Hz), 2.34 (2H, t, J = 7.1 Hz), 2.02 (2H, t, J = 7.0 Hz), 1.60 (2H, quint, J = 7.3 Hz) 13C (125 MHz, MeOD-d₄): 158.48, 147.86, 144.36, 132.38, 110.00, 79.74, 44.24, 41.23, 30.87, 30.63, 28.79, 27.52, 27.50. MS (ESI): m/z = 350.2 [M + H]⁺.

2-(6-Aminohexylamino)-5-furyl-1,3,4-triazole 45. 2-(6-(Boc)aminohexylamino)-5-furyl-1,3,4-triazole (794 mg, 2.27 mmol, 1 equiv) was dissolved in methanol (20 mL), and hydrogen chloride (2 M in ether) (1.85 mL, 37 mmol, 5 equiv) was added and the reaction stirred at room temperature overnight. The reaction was evaporated to dryness, dissolved in methanol and purified by SCX eluting with 3.5 N NH₃ in methanol to give 2-(4-aminobutylamino)-5-furyl-1,3,4-triazole (554 mg, 48%) as a white solid. ¹H (500 MHz, MeOD-d₄): 6.73–6.76 (1H, s), 6.29–6.32 (1H, dd, J = 3.4, 1.8 Hz), 3.36 (2H, t, J = 6.9 Hz), 2.02 (2H, t, J = 7.0 Hz), 1.60 (2H, quint, J = 7.3 Hz), 1.42 (2H, quint, J = 7.0 Hz), 1.36–1.24 (4H, m); MS (ESI): m/z = 250.2 [M + H]⁺.

2-(3-Acrylamidopropylamino)-5-furyl-1,3,4-triazole 46. 2-(3-Acrylamidopropylamino)-5-furyl-1,3,4-triazole (42 mg, 0.097 mmol, 1 equiv) was dissolved in DCM (2 mL) with triethylamine (40.4 μL, 0.29 mmol, 3 equiv) and cooled to 0 °C. Prop-2-enoyl chloride (7.8 μL, 0.097 mmol, 1 equiv) was added and the reaction allowed to warm to room temperature overnight. Methanol (2 mL) was added, stirred for 30 min. and the reaction evaporated to dryness. The residue was purified by flash chromatography (0–10% MeOH in DCM) to give 2-(5-aminopentylamino)-5-furyl-1,3,4-triazole (11 mg, 41%) as a white powder. ¹H (500 MHz, MeOD-d₄): 7.60 (1H, d, J = 1.2 Hz), 6.89 (1H, d, J = 3.4 Hz), 6.54 (1H, dd, J = 3.4, 1.8 Hz), 3.32 (2H, t, J = 6.9 Hz), 2.34 (2H, t, J = 6.9 Hz), 1.84 (2H, t, J = 6.9 Hz), 1.55 (2H, quint, J = 7.1 Hz), 1.42 (2H, quint, J = 7.0 Hz), 1.36–1.24 (4H, m); MS (ESI): m/z = 236.1517 (22.8 ppm).

2-(3-Acrylamidopropylamino)-5-furyl-1,3,4-triazole 47. 2-(3-Acrylamidopropylamino)-5-furyl-1,3,4-triazole (42 mg, 0.097 mmol, 1 equiv) was dissolved in DCM (3 mL) with triethylamine (40.4 μL, 0.29 mmol, 3 equiv) and cooled to 0 °C. Prop-2-enoyl chloride (7.8 μL, 0.097 mmol, 1 equiv) was added and the reaction allowed to warm to room temperature overnight. Methanol (3 mL) was added, stirred for 30 min. and the reaction evaporated to dryness. The residue was purified by HPLC (5–95% acetonitrile in water (0.1% formic acid)) to give 2-(3-acrylamidopropylamino)-5-furyl-1,3,4-triazole (8 mg, 32%) as a white powder. ¹H (500 MHz, MeOD-d₄): 7.60 (1H, d, J = 1.2 Hz), 6.89 (1H, d, J = 3.4 Hz), 6.54 (1H, dd, J = 3.4, 1.8 Hz), 6.24 (2H, m), 5.66 (1H, dd, J = 8.6, 3.4 Hz), 3.36 (2H, t, J = 6.9 Hz), 3.23 (2H, m), 1.84 (2H, t, J = 6.8 Hz); 13C (125 MHz, MeOD-d₄): 144.37, 132.04, 126.63, 112.43, 110.14, 41.74, 37.79, 30.48. HRMS m/z (ESI⁺) calcd for C₅H₆N₂O₂ [M + H]⁺ = 122.1299, found 122.1306 (2.8 ppm).

2-(3-Acrylamidopropylamino)-5-furyl-1,3,4-triazole 50. 2-(3-(Boc)aminopropylamino)-5-furyl-1,3,4-triazole (20 mg, 0.097 mmol, 1 equiv) was dissolved in DCM (3 mL) with triethylamine (40.4 μL, 0.29 mmol, 3 equiv) and cooled to 0 °C. Propanoyl chloride (8.4 μL, 0.097 mmol, 1 equiv) was added and the reaction allowed to warm to room temperature overnight. Methanol (3 mL) was added, stirred for 30 min. and the reaction evaporated to dryness. The residue was purified by HPLC (5–95% acetonitrile in water (0.1% formic acid)) to give 2-(3-propionamidopropylamino)-5-furyl-1,3,4-triazole (11 mg, 41%) as a white powder. ¹H (500 MHz, MeOD-d₄): 6.68 (1H, d, J = 1.1 Hz), 6.89 (1H, d, J = 3.3 Hz), 6.54 (1H, dd, J = 3.3, 1.8 Hz), 3.33–3.24 (4H, m), 2.21 (2H, q, J = 7.7 Hz), 1.80 (2H, quint, J = 6.8 Hz), 1.13 (3H, t, J = 7.7 Hz); 13C (125 MHz, MeOD-d₄): 177.34, 144.58, 112.51, 110.16, 41.77, 37.78, 30.60, 30.32, 10.60; HRMS m/z (ESI⁺) calcd for C₅H₆N₂O₂ [M + H]⁺ = 264.1455, found 264.1452 (1.0 ppm).
2-(3-(Vinyl)sulfonamidopropylamino)-5-furyl-1,3,4-triazole 54. 2-(3-(Boc)aminopropylamino)-5-furyl-1,3,4-triazole 42 (20 mg, 0.097 mmol, 1 equiv) was dissolved in DCM (2 mL) with triethylamine (0.44 μL, 0.29 mmol, 3 equiv) and cooled to 0 °C. 2-Chloroethanesulfonyl chloride (10.1 μL, 0.097 mmol, 1 equiv) was added and the reaction allowed to slowly warm to room temperature overnight. The residue was purified by HPLC (5–95% acetonitrile in water (0.1% formic acid)) to give 2-(3-(vinyl)sulfonamidopropylamino)-5-furyl-1,3,4-triazole (5 mg, 17%) yield. 1H (500 MHz, MeOD-d4): δ 7.60 (1H, dd, J = 17.6, 0.6 Hz), 6.90 (1H, J = 3.4, 6.7 Hz), 6.37 (1H, dd, J = 16.6, 10.0 Hz), 6.54 (1H, dd, J = 3.4, 1.8 Hz), 6.13 (1H, d, J = 16.6 Hz), 5.94 (1H, d, J = 10.0 Hz), 3.37 (2H, t, J = 6.7 Hz), 3.06 (2H, t, J = 6.7 Hz), 1.83 (2H, quint, J = 6.7 Hz); 13C (125 MHz, MeOD-d4): δ 144.212, 137.74, 126.54, 112.17, 109.81, 42.45, 41.22, 31.11; HRMS m/z (ESI) calcd for C11H8N4O2S [M + H]+; 298.0968, found 298.0965 (1.3 ppm).

2-(3-(Ethyl)sulfonamidopropylamino)-5-furyl-1,3,4-triazole 56. 2-(3-(Boc)aminopropylamino)-5-furyl-1,3,4-triazole 43 (20 mg, 0.097 mmol, 1 equiv) was dissolved in DCM (3 mL) with triethylamine (0.44 μL, 0.29 mmol, 3 equiv) and cooled to 0 °C. Ethanesulfonyl chloride (9.1 μL, 0.097 mmol, 1 equiv) was added and the reaction allowed to warm to room temperature overnight. Methanol (3 mL) was added, stirred for 30 min, and the reaction evaporated to dryness. The residue was purified by HPLC (5–95% acetonitrile in water (0.1% formic acid)) to give 2-(3-(ethyl)sulfonamidopropylamino)-5-furyl-1,3,4-triazole (5 mg, 17%) as a white powder. 1H (500 MHz, MeOD-d4): δ 7.59 (1H, s), 6.90 (1H, s), 6.54 (1H, s), 3.15 (2H, t, J = 6.7 Hz), 3.11–3.01 (4H, m), 1.84 (2H, quint, J = 6.8 Hz), 1.30 (3H, t, J = 7.4 Hz); HRMS m/z (ESI) calcd for C11H8N4O2S [M + H]+; 300.1129, found 300.1125 (0.1 ppm).

2-(4-Acrylamidobutylamino)-5-furyl-1,3,4-triazole 47. 2-(4-Aminobutylamino)-5-furyl-1,3,4-triazole 43 (20 mg, 0.097 mmol, 1 equiv) and triethylamine (37.8 μL, 0.27 mmol, 3 equiv) were dissolved in DCM (2 mL) and cooled to 0 °C. Prop-2-enoyl chloride (7.3 μL, 0.09 mmol, 1 equiv) was added and the reaction allowed to warm to room temperature overnight. Methanol (2 mL) was added and stirred for 30 min, and the reaction was evaporated to dryness. The residue was purified by HPLC (5–95% acetonitrile in water (0.1% formic acid)) to give 2-(4-acylamidobutylamino)-5-furyl-1,3,4-triazole (6 mg, 23%) as a white solid. 1H (500 MHz, MeOD-d4): δ 7.59 (1H, s), 6.89 (1H, d, J = 3.3 Hz), 6.54 (1H, dd, J = 3.3, 1.8 Hz), 6.22 (2H, m, H1S), 5.64 (1H, dd, J = 8.0, 4.1 Hz), 3.29 (4H, m), 1.65 (4H, m); 13C (125 MHz, MeOD-d4): δ 144.32, 132.07, 126.56, 112.42, 110.00, 43.94, 40.05, 28.11, 27.72; HRMS m/z (ESI) calcd for C11H18N4O2S [M + H]+; 276.1467, 404.58; found 276.1457 (4.4 ppm).

2-(4-Propionaminobutylamino)-5-furyl-1,3,4-triazole 51. 2-(4-Aminobutylamino)-5-furyl-1,3,4-triazole 43 (20 mg, 0.085 mmol, 1 equiv) and triethylamine (35.5 μL, 0.255 mmol, 3 equiv) were dissolved in DCM (2 mL) and cooled to 0 °C. Prop-2-enoyl chloride (6.7 μL, 0.085 mmol, 1 equiv) was added and the reaction allowed to warm to room temperature overnight. Methanol (2 mL) was added and stirred for 30 min, and the reaction was evaporated to dryness. The residue was purified by HPLC (5–95% acetonitrile in water (0.1% formic acid)) to give 2-(4-propionaminobutylamino)-5-furyl-1,3,4-triazole (4 mg, 15%) as a white powder. 1H (500 MHz, MeOD-d4): δ 8.48 (1H, s), 7.59 (1H, brs), 6.89 (1H, brs), 6.54 (1H, brs), 6.21 (1H, d, J = 8.4 Hz), 6.20 (1H, d, J = 3.7 Hz), 5.63 (1H, dd, J = 8.4, 3.7 Hz), 3.27 (2H, td, J = 7.1, 1.6 Hz), 1.54 (6H, m); HRMS m/z (ESI) calcd for C11H18N4O2S [M + H]+; 290.1614, found 290.1616 (1.5 ppm).

Synthesis of 2-(5-(Propionaminopentylamino)-5-furyl-1,3,4-triazole 52. 2-(5-Aminopentylamino)-5-furyl-1,3,4-triazole 44 (20 mg, 0.085 mmol, 1 equiv) and triethylamine (35.5 μL, 0.255 mmol, 3 equiv) were dissolved in DCM (2 mL) and cooled to 0 °C. Propanoyl chloride (7.4 μL, 0.085 mmol, 1 equiv) was added and the reaction allowed to warm to room temperature overnight. Methanol (2 mL) was added and stirred for 30 min, and the reaction was evaporated to dryness.
The residue was purified by HPLC (5–95% acetonitrile in water (0.1% formic acid)) to give 2-((propionaminopentylamino)-5-furyl-1,3,4-triazole (6 mg, 23%) as a white solid. \(^1\)H (500 MHz, MeOD-d$_4$): \(\delta 7.58\) (1H, s), 6.89 (1H, s), 6.54 (1H, dd, \(J = 8.6, 3.5\) Hz), 3.26 (4H, t, \(J = 7.2\) Hz), 1.56 (2H, quint, \(J = 7.2\) Hz), 1.42 (4H, m), 1.24 (3H, t, \(J = 7.2\) Hz). \(1^3\)C (125 MHz, MeOD-d$_4$): \(\delta 168.12, 144.32, 132.11, 126.40, 112.40, 109.99, 44.25, 30.43, 30.65, 30.30, 27.71, 27.51; HRMS m/z (ESI) calcd for C$_4$H$_2$N$_2$O$_3$ [M + H]$^+$; 304.1778, found 304.1778 (3.4 ppm).

**Synthesis of 2-((H-exyl)sulfonylamino)-5-furyl-1,3,4-triazole 56.** 2-((Aminopentylamino)-5-furyl-1,3,4-triazole 44 (20 mg, 0.085 mmol, 1 equiv) was dissolved in DCM (3 mL) with triethylamine (35.5 \(\mu\)L, 0.24 mmol, 3 equiv) and cooled to 0 °C. Ethanesulfonyl chloride (8.1 \(\mu\)L, 0.085 mmol, 1 equiv) was added and the reaction allowed to warm to room temperature overnight. Methanol (2 mL) was added and stirred for 30 min, and the reaction was evaporated to dryness. The residue was purified by HPLC (5–95% acetonitrile in water (0.1% formic acid)) to give 2-((vinyl)sulfonylamino)-5-furyl-1,3,4-triazole (9 mg, 31%) as a white powder. \(^1\)H (500 MHz, MeOD-d$_4$): \(\delta 7.59\) (1H, dd, \(J = 1.7, 0.6\) Hz), 6.89 (1H, d, \(J = 3.2\) Hz), 6.54 (1H, dd, \(J = 3.4, 1.8\) Hz), 3.29 (2H, d, \(J = 7.2\) Hz), 3.04 (2H, m), 2.97 (2H, m), 1.70 (4H, m), 1.49 (2H, m), 1.24 (3H, t, \(J = 7.2\) Hz).

**Synthesis of 2-((6-Aminohexylamino)-5-furyl-1,3,4-triazole 57.** 2-((Aminohexylamino)-5-furyl-1,3,4-triazole 45 (20 mg, 0.080 mmol, 1 equiv) was dissolved in DCM (2 mL) with triethylamine (33.5 \(\mu\)L, 0.24 mmol, 3 equiv) and cooled to 0 °C. 2-Chloroethanesulfonyl chloride (8.4 \(\mu\)L, 0.080 mmol, 1 equiv) was added and the reaction allowed to slowly warm to room temperature overnight. Five mL methanol was added and the reaction evaporated to dryness. The residue was purified by HPLC (5–95% acetonitrile in water (0.1% formic acid)) to give 2-((vinyl)sulfonylamino)-5-furyl-1,3,4-triazole (3 mg, 10%) as a colorless oil. \(^1\)H (500 MHz, MeOD-d$_4$): \(\delta 7.61\) (1H, dd, \(J = 1.8, 0.8\) Hz), 6.92 (1H, dd, \(J = 3.4, 0.8\) Hz), 6.61 (1H, dd, \(J = 16.6, 10.0\) Hz), 6.56 (1H, dd, \(J = 3.5, 1.8\) Hz), 6.11 (1H, d, \(J = 16.6\) Hz), 5.94 (1H, d, \(J = 10.0\) Hz), 3.27 (2H, t, \(J = 7.1\) Hz), 2.94 (2H, t, \(J = 7.0\) Hz), 1.64 (2H, quint, \(J = 7.1\) Hz), 1.56 (2H, quint, \(J = 7.0\) Hz), 1.42 (4H, quint, \(J = 3.7\) Hz); \(^1^3\)C (125 MHz, MeOD-d$_4$): \(\delta 144.71, 137.81, 126.16, 112.50, 110.45, 44.30, 43.76, 30.92, 30.56, 27.39; HRMS m/z (ESI) calcd for C$_3$H$_2$N$_2$O$_3$ [M + H]$^+$; 340.1448, found 340.1438 (3.0 ppm).

**Synthesis of 2-((6-Ethyl)sulfonylamino)-5-furyl-1,3,4-triazole 61.** 2-((Aminohexylamino)-5-furyl-1,3,4-triazole 45 (20 mg, 0.08 mmol, 1 equiv) was dissolved in DCM (3 mL) with triethylamine (33.5 \(\mu\)L, 0.24 mmol, 3 equiv) and cooled to 0 °C. Ethanesulfonyl chloride (7.6 \(\mu\)L, 0.08 mmol, 1 equiv) was added and the reaction allowed to warm to room temperature overnight. Methanol (3 mL) was added, stirred for 30 min, and the reaction evaporated to dryness. The residue was purified by HPLC (5–95% acetonitrile in water (0.1% formic acid)) to give 2-((ethyl)sulfonylamino)-5-furyl-1,3,4-triazole (9 mg, 31%) as a white powder. \(^1\)H (500 MHz, MeOD-d$_4$): \(\delta 7.59\) (1H, s), 6.89 (1H, s), 6.54 (1H, s), 3.27 (2H, t, \(J = 7.1\) Hz), 3.04 (4H, m), 1.64 (2H, quint, \(J = 7.1\) Hz), 1.57 (2H, quint, \(J = 7.0\) Hz), 1.44 (4H, quint, \(J = 3.6\) Hz), 1.30 (3H, t, \(J = 7.4\) Hz); \(^1^3\)C (125 MHz, MeOD-d$_4$): \(\delta 144.05, 112.10, 109.72, 47.01, 44.24, 43.83, 31.35, 30.65, 27.42, 27.39, 8.47; HRMS m/z (ESI) calcd for C$_3$H$_2$N$_2$O$_3$ [M + H]$^+$; 342.1549, found 342.1560 (0.9 ppm).

**Synthesis of 2-((Propionylamino)-5-furyl-1,3,4-triazole 62.** 2-Amino-5-furyl-1,3,4-triazole 14 (100 mg, 0.67 mmol, 1 equiv) was dissolved in THF (7 mL). Propanal (96 \(\mu\)L, 1.33 mmol, 2 equiv) and sodium triacetoxoborohydride (282 mg, 1.33 mmol, 2 equiv) were added and the reaction stirred at room temperature overnight. Five mL water and 10 mL DCM were added and the layers separated. The aqueous layer was extracted 2× with 10 mL DCM, and the combined organic layers were dried over MgSO$_4$ passed through a phase separator, and evaporated to dryness. The residue was purified by flash chromatography (0–100% ethyl acetate in heptane) to give 2-propionylamino-5-furyl-1,3,4-triazole (84 mg, 66%) as a white powder. \(^1\)H (500 MHz, DMSO-d$_6$): \(\delta 12.14\) (1H, s), 7.67 (1H, s), 6.69 (1H, d, \(J = 3.1\) Hz), 6.58 (1H, d, \(J = 5.7\) Hz), 6.53 (1H, m), 3.10 (2H, q, \(J = 6.7\) Hz), 1.53 (2H, sext, \(J = 7.4\) Hz), 0.89 (3H, t, \(J = 7.4\) Hz); MS (ESI): \(m/z = 193.2 [M + H]^+$.

**Synthesis of 2-Pentanoylamino-5-furyl-1,3,4-triazole 63.** 2-Amino-5-furyl-1,3,4-triazole 14 (100 mg, 0.67 mmol, 1 equiv) was dissolved in THF (7 mL). Pentanal (142 \(\mu\)L, 1.33 mmol, 2 equiv) and sodium triacetoxoborohydride (282 mg, 1.33 mmol, 2 equiv) were added and the reaction stirred at room...
temperature overnight. Five mL water and 10 mL DCM were added and the layers separated. The aqueous layer was extracted 2× with 10 mL DCM, and the combined organic layers were dried over MgSO₄ passed through a phase separator, and evaporated to dryness. The residue was purified by flash chromatography (0–100% ethyl acetate in heptane) to give 2-pentylamino-5-furyl-1,3,4-triazole (84 mg, 57%) as a white solid. 1H (500 MHz, CDCl₃): δ 7.67 (4H, dt, J = 6.4, 1.5 Hz), 7.48 (1H, dd, J = 1.7, 0.7 Hz), 7.42 (6H, m), 6.88 (1H, dd, J = 3.4, 0.7 Hz), 6.48 (1H, dd, J = 3.4, 1.8 Hz), 3.83 (2H, q, J = 5.7 Hz), 3.45 (2H, t, J = 6.2 Hz), 1.76 (2H, quint, J = 5.9 Hz), 1.11 (9H, s); 13C (125 MHz, CDCl₃): δ 143.27, 135.74, 133.33, 130.10, 128.02, 111.57, 109.43, 61.60, 40.84, 32.47, 27.17, 19.38.

2-N-(3-Hydroxypropyl)amino-5-furyl-1,3,4-triazole 72. 2-N-(3-TBDPSoxypropyl)amino-5-furyl-1,3,4-triazole 70 (366 mg, 0.82 mmol, 1 equiv) was dissolved in THF (10 mL) under nitrogen. TBAF (1 M in THF) (1.64 mL, 1.64 mmol, 2 equiv) was added and the reaction stirred for 3 h when TLC showed no starting material remained. The reaction was evaporated to dryness then dissolved in 10 mL DCM and washed with 10 mL water. The aqueous layer was extracted 2× with 10 mL DCM, and the combined organic layers were dried over MgSO₄ passed through a phase separator, and evaporated to dryness. LCMS revealed product remains in the aqueous layer, so the aqueous layer was evaporated to dryness. The combined residues were purified by flash chromatography (0–10% MeOH in DCM) to give 2-N-(3-hydroxypropyl)amino-5-furyl-1,3,4-triazole (129 mg, 72%) as a white powder. 1H (500 MHz, DMSO-d₆): δ 12.17 (1H, brs), 7.68 (1H, brs), 6.69 (1H, brs), 6.54 (2H, brs), 4.51 (1H, brs), 3.47 (2H, q, J = 5.8 Hz), 3.20 (2H, q, J = 6.5 Hz), 1.67 (2H, quint, J = 6.6 Hz); 13C (125 MHz, DMSO-d₆): δ 142.61, 111.22, 107.50, 58.36, 32.42, 23.02.

5-TBDPSoxypentan-1-ol 67. Pentan-1,5-diol (65) (1.14 mL, 10.92 mmol, 3 equiv) was dissolved in DCM (10 mL) under nitrogen, and triethylamine (0.76 mL, 5.46 mmol, 1.5 equiv) and TBDPSCI (0.95 mL, 3.64 mmol, 1 equiv) were added. The reaction was stirred overnight at room temperature. The reaction was diluted with 30 mL DCM and washed with 15 mL water, 15 mL saturated sodium hydrogen carbonate solution, 15 mL brine, dried over MgSO₄, passed through a phase separator, and evaporated to dryness. The residue was purified by flash chromatography (0–10% MeOH in DCM) to give 5-TBDPSoxypentan-1-ol (1.06 g, 85%) as a colorless oil. 1H (500 MHz, CDCl₃): δ 7.67 (4H, dt, J = 8.0, 1.1 Hz), 7.40 (6H, m), 3.68 (2H, t, J = 6.4 Hz), 3.62 (2H, q, J = 6.2 Hz), 1.58 (4H, m), 1.44 (2H, m), 1.06 (9H, d, J = 0.8 Hz); 13C (125 MHz, CDCl₃): δ 135.73, 134.26, 129.67, 127.74, 63.95, 63.12, 32.64, 32.43, 27.04, 22.14, 19.37. Analysis is in agreement with the literature.

5-TBDPSoxypentanal 69. DCM (15 mL) was cooled to −78 °C, and oxalyl chloride (0.48 mL, 5.46 mmol, 1.5 equiv) was added. DMSO (0.80 mL, 11.28 mmol, 3 equiv) was added and the mixture stirred at −78 °C for 30 min. 5-TBDPSoxypropan-1-ol (1.18 g, 3.76 mmol, 1 equiv) was added to DCM (10 mL) and the mixture stirred at −78 °C for 30 min. Triethylamine (2.62 mL, 18.79 mmol, 5 equiv) was added and the reaction allowed to slowly warm to room temperature overnight. Ten mL saturated sodium hydrogen carbonate solution and 10 mL water were added, stirred vigorously, and the layers separated. The aqueous layer was extracted 2× with 25 mL DCM. The combined organic layers were dried over MgSO₄, passed through a phase separator, and evaporated to dryness. The residue was purified by flash chromatography (0–30% ethyl acetate in heptane) to give 5-TBDPSoxypropan-1-ol (951 mg, 81%) as a colorless oil. 1H (500 MHz, CDCl₃): δ 9.83 (1H, t, J = 2.2 Hz), 7.66 (4H, dd, J = 7.9, 1.4 Hz), 7.42 (6H, m), 4.03 (2H, t, J = 6.0 Hz), 2.61 (2H, td, J = 6.0, 2.2 Hz), 1.05 (9H, s); 13C (125 MHz, CDCl₃): δ 201.92, 135.70, 133.44, 129.96, 127.92, 58.47, 46.45, 26.91, 19.29. Analysis is in agreement with the literature.

Synthesis of 2-N-(3-TBDPSoxypropyl)amino-5-furyl-1,3,4-triazole 70. 2-Amino-5-furyl-1,3,4-triazole 14 (867 mg, 5.77 mmol, 2 equiv), 3-TBDPSoxypropanal 68 (902 mg, 2.89 mmol, 1 equiv), and sodium triacetoxyborohydride (3.67 g, 17.32 mmol, 6 equiv) were dissolved in THF (25 mL) under nitrogen with 3 Å molecular sieves. Acetic acid (0.33 mL, 5.77 mmol, 2 equiv) was added and the reaction stirred for 4 h. TLC indicated no starting material remained, but LCMS seemed to suggest some unreduced imine remained, so sodium borohydride (218 mg, 5.77 mmol, 2 equiv) was added and the mixture stirred for 1 h. Thirty mL saturated sodium hydrogen carbonate solution was added and mixed until the bubbling stopped. Ten mL water was added, and the layers separated, and the aqueous layer extracted 3× with 30 mL DCM. The combined organic layers were washed with brine, dried over MgSO₄, passed through a phase separator, and evaporated to dryness. The residue was purified by flash chromatography (0–6% MeOH in DCM) to give 2-N-(3-TBDPSoxypropyl)amino-5-furyl-1,3,4-triazole (544 mg, 42%) as a white solid. 1H (500 MHz, CDCl₃): δ 7.67 (4H, dt, J = 6.4, 1.5 Hz), 7.48 (1H, dd, J = 1.7, 0.7 Hz), 7.42 (6H, m), 6.88 (1H, dd, J = 3.4, 0.7 Hz), 6.48 (1H, dd, J = 3.4, 1.8 Hz), 3.83 (2H, q, J = 5.7 Hz), 3.45 (2H, t, J = 6.2 Hz), 1.76 (2H, quint, J = 5.9 Hz), 1.11 (9H, s); 13C (125 MHz, CDCl₃): δ 143.27, 135.74, 133.33, 130.10, 128.02, 111.57, 109.43, 61.60, 40.84, 32.47, 27.17, 19.38.
separatorm and evaporated to dryness. The residue was purified by flash chromatography (0–30% ethyl acetate in heptane) to give 5-TBDPS-oxypentanal (560 mg, 95%) as a colorless oil. $^1$H (500 MHz, CDCl$_3$): δ 9.75 (1H, t, $J = 1.7$ Hz), 7.66 (4H, m), 7.41 (6H, m), 3.68 (2H, t, $J = 6.2$ Hz), 2.41 (2H, td, $J = 7.3$, 1.7 Hz), 1.74 (2H, m), 1.60 (2H, m), 1.06 (9H, s); $^{13}$C (125 MHz, CDCl$_3$): δ 202.68, 135.71, 134.06, 129.75, 127.79, 63.45, 43.57, 32.02, 27.02, 19.36, 18.75. Analysis is in agreement with the literature.\(^5\)

**2-N-(5-TBDPS-oxypentyl)aminom-5-furyl-1,3,4-triazole 71.** 2-Amino-5-furyl-1,3,4-triazole 14 (523 mg, 3.48 mmol, 2 equiv), 5-TBDPS-oxypentanal (560 mg, 95%) as a white solid. The mixture was stirred for 1 h. Thirty mL saturated sodium hydrogen carbonate solution was added and mixed until the bubbling was stopped. Ten mL water were added, and this was extracted 3× with 50 mL DCM. The combined organic layers were washed with brine, dried over MgSO$_4$, allowed to slowly warm to room temperature overnight. Ten mL saturated sodium hydrogen carbonate solution was added and the mixture stirred for 30 min at $-78$ °C. Methyl 5-hydroxypentanoate 169 (1 mL, 7.77 mmol, 1 equiv) was added and the mixture stirred at $-78$ °C for 30 min. Triethylamine (5.42 mL, 38.85 mmol, 5 equiv) was added and the reaction allowed to slowly warm to room temperature overnight. Ten mL saturated sodium hydrogen carbonate solution and 10 mL water were added and stirred vigorously, and the layers were separated. The aqueous layer was extracted 2× with 25 mL DCM. The combined organic layers were dried over MgSO$_4$ passed through a phase separator and evaporated to dryness. The residue was purified by flash chromatography (0–50% ethyl acetate in heptane) to give methyl 5-oxopentanoate (812 mg, 80%) as a colorless oil. $^1$H (500 MHz, DMSO-$d_6$): δ 9.66 (1H, t, $J = 1.1$ Hz), 3.59 (3H, s), 2.48 (2H, t, $J = 7.3$, 1.0 Hz), 2.33 (2H, t, $J = 7.4$ Hz), 1.77 (2H, quin, $J = 7.3$ Hz); $^{13}$C (125 MHz, DMSO-$d_6$): δ 200.79, 172.89, 51.16, 42.04, 32.33, 17.03; HRMS (ESI-TOF) calcd for C$_{13}$H$_{10}$O (m/z) = 169.0744, found 169.0747 (7.8 ppm).

Methyl 5-Oxopentanoate 76. DCM (25 mL) was cooled to $-78$ °C, and oxalyl chloride (0.99 mL, 11.66 mmol, 1.5 equiv) was added. DMSO (1.66 mL, 23.31 mmol, 3 equiv) was added and the mixture stirred for 30 min at $-78$ °C. Methyl 5-hydroxypentanoate 169 (1 mL, 7.77 mmol, 1 equiv) was added and the mixture stirred at $-78$ °C for 30 min. Triethylamine (5.42 mL, 38.85 mmol, 5 equiv) was added and the reaction allowed to slowly warm to room temperature overnight. Ten mL saturated sodium hydrogen carbonate solution and 10 mL water were added and stirred vigorously, and the layers were separated. The aqueous layer was extracted 2× with 25 mL DCM. The combined organic layers were dried over MgSO$_4$ passed through a phase separator and evaporated to dryness. The residue was purified by flash chromatography (0–50% ethyl acetate in heptane) to give methyl 5-oxopentanoate (812 mg, 80%) as a colorless oil. $^1$H (500 MHz, DMSO-$d_6$): δ 9.66 (1H, t, $J = 1.1$ Hz), 3.59 (3H, s), 2.48 (2H, t, $J = 7.3$, 1.0 Hz), 2.33 (2H, t, $J = 7.4$ Hz), 1.77 (2H, quin, $J = 7.3$ Hz); $^{13}$C (125 MHz, DMSO-$d_6$): δ 200.79, 172.89, 51.16, 42.04, 32.33, 17.03; HRMS (ESI-TOF) calcd for C$_{13}$H$_{10}$O (m/z) = 169.0744, found 169.0747 (7.8 ppm).

2-N-(O-Methyl 4-carboxybutyl)aminom-5-furyl-1,3,4-triazole 77. 2-Amino-5-furyl-1,3,4-triazole 14 (500 mg, 3.33 mmol, 1 equiv), methyl 5-oxopentanoate 76 (867 mg, 6.66 mmol, 2 equiv) and sodium triacetoxyborohydride (3.53 g, 16.65 mmol, 5 equiv) were dissolved in THF (25 mL) under nitrogen with 3 Å molecular sieves. Acetic acid (0.38 mL, 6.66 mmol, 2 equiv) was added and the reaction stirred overnight. Thirty mL saturated sodium hydrogen carbonate solution was added and stirred until the fizzing stopped. The layers were separated, and the aqueous layer extracted 3× with 25 mL DCM. The combined organic layers were washed with brine, dried over MgSO$_4$, passed through a phase separator, and evaporated to dryness. The residue was purified by flash chromatography (0–10% MeOH in DCM) to give 2-N-(O-Methyl 4-carboxybutyl)aminom-5-furyl-1,3,4-triazole (531 mg, 57%) yield as a white powder. $^1$H (500 MHz, DMSO-$d_6$): δ 12.17 (1H, brs), 7.67 (1H, brs), 6.69 (1H, brs), 6.60 (1H, $J = 5.3$ Hz), 6.53 (1H, brs), 3.58 (3H, s), 3.14 (2H, q, $J = 6.1$ Hz), 2.33 (2H, t, $J = 7.2$ Hz), 1.55 (4H, m); $^{13}$C (125 MHz, DMSO-$d_6$): δ 51.13, 39.23, 28.59, 21.76. Synthesis of 2-N-Piperidin-2-onyl-5-furyl-1,3,4-triazole 78. 2-N-(O-Methyl 4-carboxybutyl)aminom-5-furyl-1,3,4-triazole 77 (100 mg, 0.38 mmol, 1 equiv) was dissolved in methanol (5 mL), and lithium hydroxide hydrate (0.5 M in water) (0.91 mL, 0.45 mmol, 1.2 equiv) was added. The mixture was stirred overnight. LCMS indicated 100% conversion of starting material, so the mixture was evaporated to dryness. The residue was purified by HPLC (5–95% acetonitrile in water (0.1% formic acid)) to give a white powder which NMR revealed to be the cyclized lactam, 2-N-piperidin-2-onyl-5-furyl-1,3,4-triazole (20 mg, 23%). $^1$H (500 MHz, DMSO-$d_6$): δ 13.61 (1H, brs), 7.77 (1H, dd, $J = 1.6$, 0.6 Hz), 6.86 (1H, dd, $J = 3.3$, 0.6 Hz), 6.60 (1H, dd, $J = 3.4$, 1.8 Hz), 3.92 (2H, t, $J = 6.0$ Hz), 2.54 (1H, t, $J = 6.6$ Hz), 1.89 (2H, m), 1.81 (2H, m); $^{13}$C (125 MHz, DMSO-$d_6$): δ 170.01, 143.41, 111.51, 108.56, 47.80, 32.65, 21.86, 19.80.
Synthesis of 2-N-(4-Carboxybutyl)amino-5-furyl-1,3,4-triazole 79. 2-N-Piperidin-2-onyl-5-furyl-1,3,4-triazole 78 (20 mg, 0.086 mmol, 1 equiv) was dissolved in 2 N hydrochloric acid (1.29 mL, 2.58 mmol, 30 equiv) and stirred overnight at room temperature. LCMS showed no conversion so the reaction was heated to 130 °C overnight in a sealed tube. Starting material remained so the reaction was stirred over the weekend at 130 °C. LCMS showed complete conversion so the reaction was evaporated to dryness and the residue purified by HPLC (5–95% acetonitrile in water (0.1% formic acid)) to give 2-N-(4-carboxybutyl)amino-5-furyl-1,3,4-triazole (9 mg, 42%) as a white powder. 1H (500 MHz, DMSO-d6): δ 7.86 (1H, s), 7.17 (1H, d, J = 2.8 Hz), 6.66 (1H, dd, J = 3.3, 1.7 Hz), 3.28 (2H, m), 2.25 (2H, t, J = 6.6 Hz), 1.57 (4H, m); 13C (125 MHz, DMSO-d6): δ 173.24, 144.65, 111.81, 111.19, 42.77, 33.23, 28.25, 21.62; MS (ESI): m/z = 251.1 [M + H]+.

Methyl 3-Oxopropanoate 81. Amberlite IR120 resin hydrogen form (10 g) was suspended in acetone (150 mL) and water (6.1 mL, 337 mmol, 10 equiv) added. Methyl 3,3-dimethoxypropanoate (4.78 mL, 33.75 mmol, 1 equiv) was added and the reaction allowed to stir for 3 days. 4 Å molecular sieves were added and the reaction stirred for an hour to remove the water and any methanol. The mixture was passed through a phase separator to further dry it and filter out the resin. The solvent was then removed in vacuo to give methyl 3-oxopropanoate (4.07 g) as a yellow oil. NMR showed the residue contained a mixture of starting material and product. This material was used directly in the next step. 1H (500 MHz, CDCl3): δ 9.80 (1H, t, J = 2.4 Hz), 3.78 (3H, s), 3.40 (2H, d, J = 2.4 Hz).

2-N-(O-Methyl 2-carboxyethyl)amino-5-furyl-1,3,4-triazole 82. 2-Amino-5-furyl-1,3,4-triazole 14 (1.00 g, 6.66 mmol, 1 equiv) and methyl 3-oxopropanoate 81 (680 mg, 6.66 mmol, 1 equiv) were dissolved in THF (30 mL) over 3 Å molecular sieves. Acetic acid (0.76 mL, 13.32 mmol, 2 equiv) was added followed by sodium cyanoborohydride (13.3 mL, 13.32 mmol, 2 equiv) (1 M in THF) and the reaction stirred over the weekend. The reaction was filtered through Celite and the residue washed with DCM, water, and again with DCM. The filtrate was neutralized with saturated sodium carbonate solution and extracted 3× with 50 mL DCM. The combined organic layers were washed with brine, dried over MgSO4, passed through a phase separator, and evaporated to dryness. The residue was purified by flash chromatography (0–5% MeOH in DCM) to give methyl 3-[(3-[2-furyl]1H-1,2,4-triazol-5-yl)amino]propanoate (839 mg, 53%) as a white powder. 1H (500 MHz, DMSO-d6): δ 12.23 (1H, brs), 7.68 (1H, brs), 6.71 (1H, brs), 6.60 (1H, brs), 6.54 (1H, brs), 3.61 (3H, s), 3.41 (2H, q, J = 6.0 Hz), 2.60 (2H, t, J = 6.9 Hz); 13C (125 MHz, DMSO-d6): δ 172.34, 157.29, 152.22, 147.47, 142.62, 111.23, 107.59, 51.25, 38.80, 33.87; MS (ESI): m/z = 237.1 [M + H]+.

ASSOCIATED CONTENT
Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c08031.

Spectral data for the compounds (PDF)

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Notes
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REFERENCES
(2) Bassetti, M.; Vena, A.; Croxatto, A.; Righi, E.; Guery, B. How to manage Pseudomonas aeruginosa infections. Drugs Context 2018, 7, 212527.

https://doi.org/10.1021/acsomega.2c08031
ACS Omega 2023, 8, 12787−12804
(24) Borg, T.; Tuzina, P.; Somfai, P. Lewis Acid-Promoted Addition of 1,3-Bis(silyl)propenes to Aldehydes: A Route to 1,3-Dienes. Journal of Organic Chemistry 2011, 76, 8070–8075.