Supporting Information

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Thiacycloalkynes for Copper-Free Click Chemistry**

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General Procedures

All chemical reagents were purchased from Sigma-Aldrich, Acros, or TCI chemicals and used without purification unless noted otherwise. Solvents were purified as described by Pangborn et al.[1] In all cases, magnesium sulfate or sodium sulfate were used as drying agents and solvent was removed by reduced pressure with a Buchi Rotovapor R-114 equipped with a Welch self-cleaning dry vacuum. Non-volatile products were further dried by reduced pressure with an Edwards RV5 high vacuum. Thin layer chromatography was performed with EMD 60 Å silica gel plates. Unless otherwise specified, Rf values are reported in the solvent system the reaction was monitored in. Flash chromatography was performed using Silicycle® 60 Å 230-400 mesh. All 1H, 13C, and 19F NMR spectra are reported in ppm and referenced to solvent peaks. Spectra were obtained on Bruker AV-300, AVB-400, AVQ-400, DRX-500, or AV-500, AV-600 instruments. High resolution electron ionization (EI) and electrospray ionization (ESI) mass spectra were obtained from the UC Berkeley Mass Spectrometry Facility.

Experimental Procedures

(Hepta-1,6-dien-4-yloxy)triisopropylsilane (12).

NaH (60 % w/w in mineral oil, 0.46 g, 12 mmol, 3.3 equiv) was dissolved in THF (5.0 mL, anhydrous) and cooled to 0 °C. To this mixture, hepta-1,6-dien-4-ol (0.50 mL, 3.5 mmol, 1.0 equiv) was added dropwise and the mixture was stirred at 0 °C. After 30 min, triisopropylsilyl chloride (1.5 mL, 7.0 mmol, 2.0 equiv) was added to the reaction mixture. The reaction was warmed to rt and stirred overnight. The following day, the reaction was quenched with an aqueous solution of saturated ammonium chloride (8 mL). The product was extracted into dichloromethane (3 x 10 mL) and the combined organics were dried with MgSO4, decanted and evaporated to dryness. The crude product was purified by silica gel chromatography (hexane) to give desired product in quantitative yield (930 mg, 3.5 mmol). Rf = 0.95 in 3:1 hexanes/EtOAc. 1H NMR (500 MHz, CDCl3): δ 5.84 (ddd, J = 16.3, 13.9, 7.2 Hz, 2H), 5.06-5.03 (m, 4H), 3.91 (p, J = 5.8 Hz, 1H), 2.34-2.28 (m, 4H), 1.07-1.04 (m, 21H). This compound was previously reported by Livinghouse and coworkers.[2]

Triisopropyl((1-(oxiran-2-yl)pent-4-en-2-yl)oxy)silane (13).

(Hepta-1,6-dien-4-yloxy)triisopropylsilane 12 (2.4 g, 9.0 mmol, 1.0 equiv) was dissolved in dichloromethane (74 mL). To this solution, an aqueous solution of saturated sodium bicarbonate (124 mL) and acetone (7.4 mL, 0.10 mol, 11 equiv) was added and the reaction mixture was cooled to 0 °C. To the vigorously stirring reaction mixture, oxone (22 g, 36 mmol, 4.0 equiv) dissolved in water (87 mL) was added dropwise using an addition funnel. The reaction mixture was slowly warmed to rt and stirred overnight. The organic layer was separated from the aqueous layer. The aqueous layer was extracted with dichloromethane (2 x 100 mL). The combined organics were dried with MgSO4, decanted and evaporated to dryness to give crude mixture of unreacted starting material, desired product, and diepoxidized byproduct. The crude product was purified by silica gel
chromatography (100:1 to 10:1 hexane/EtOAc). This procedure yielded desired product as a 1:0.8 mixture of two diastereomers (1.1 g, 2.9 mmol, 32%) plus some recovered starting material and ((1,3-di(oxiran-2-yl)propan-2-yl)oxy)triisopropylsilane. Rf = 0.8 in 3:1 hexanes/EtOAc. $^1$H NMR (500 MHz, CDCl$_3$): major diastereomer δ 5.86-5.73 (m, 1H), 5.08-5.02 (m, 2H), 4.11-4.08 (m, 1H), 3.07-3.03 (m, 1H), 2.77 (t, $J = 4.9$ Hz, 1H), 2.46-2.31 (m, 3H), 1.76-1.61 (m, 2H), 1.07-1.03 (m, 21H); minor diastereomer δ 5.86-5.73 (m, 1H), 5.08-5.02 (m, 2H), 4.11-4.08 (m, 1H), 3.07-3.03 (m, 1H), 2.73 (t, $J = 4.9$ Hz, 1H), 2.46-2.31 (m, 3H), 1.76-1.61 (m, 2H), 1.07-1.03 (m, 21H). $^{13}$C NMR (125 MHz, CDCl$_3$): major diastereomer δ 134.8, 117.8, 70.5, 50.0, 47.3, 42.8, 40.1, 18.5, 13.0; minor diastereomer δ 135.0, 117.8, 70.6, 49.6, 48.1, 42.0, 39.7, 18.6, 12.9. HRMS (ESI): calcd for C$_{16}$H$_{32}$O$_2$NaSi$^+$ [M + Na]$^+$, 307.2064; found, 307.2061.

5-(oxiran-2-yl)-4-(((triisopropylsilyl)oxy)pentyl ethanethioate (14).

Triisopropyl((1-(oxiran-2-yl)pent-4-en-2-yl)oxy)silane 13 (660 mg, 2.3 mmol, 1.0 equiv) was dissolved in dichloroethane (20 mL). To this solution, thioacetic acid (550 µL, 7.7 mmol, 3.3 equiv) and azobisisobutyronitrile (AIBN) (130 mg, 0.79 mmol, 0.34 equiv, recrystallized from methanol) were added. The reaction mixture was heated to reflux for 2 h at which point the mixture was cooled to rt. The organic layer was washed with an aqueous solution of saturated sodium bicarbonate (2 x 20 mL) and water (20 mL). The aqueous layer was extracted with dichloromethane (25 mL) and the combined organics were dried with MgSO$_4$, decanted and evaporated to dryness. The crude product was purified by silica gel chromatography (90:1 to 25:1 hexane/EtOAc) to yield the desired product as a 1:0.4 mixture of two diastereomers (720 mg, 2.0 mmol, 87%). Rf = 0.6 in 4:1 hexanes/EtOAc. $^1$H NMR (500 MHz, CDCl$_3$): major diastereomer δ 4.05-4.01 (m, 1H), 3.03-3.01 (m, 1H), 2.87-2.85 (m, 2H), 2.77 (t, $J = 4.5$, 1H), 2.48-2.43 (m, 2H), 2.32 (s, 3H), 1.65-1.58 (m, 6H), 1.07-1.03 (m, 21H); minor diastereomer δ 4.05-4.01 (m, 1H), 3.03-3.01 (m, 1H), 2.87-2.85 (m, 2H), 2.75 (t, $J = 4.7$, 1H), 2.48-2.43 (m, 2H), 2.32 (s, 3H), 1.83-1.65 (m, 6H), 1.07-1.03 (m, 21H). $^{13}$C NMR (150 MHz, CDCl$_3$): major diastereomer δ 195.9, 70.1, 49.6, 47.4, 39.9, 36.6, 30.6, 29.3, 25.0, 18.1, 12.6; minor diastereomer δ 195.5, 70.0, 48.9, 46.8, 39.4, 35.7, 30.6, 29.3, 24.7, 18.1, 12.5. HRMS (ESI): calcd for C$_{18}$H$_{36}$O$_3$NaSSi$^+$ [M + Na]$^+$, 383.2047; found, 383.2048.

5-((triisopropylsilyl)oxy)thiocan-3-ol (15).

NaH (60% w/w in mineral oil, 20 mg, 0.50 mmol, 8.9 equiv) was added to a round-bottom flask followed by slow addition of ethanol (10 mL). 5-(oxiran-2-yl)-4-(((triisopropylsilyl)oxy)pentyl ethanethioate 14 (20 mg, 0.056 mmol, 1.0 equiv) dissolved in ethanol (5.0 mL) was added dropwise and the reaction mixture was heated to reflux. After 3 h, the reaction mixture was cooled to rt and the ethanol was removed by rotary evaporation. The viscous liquid was dissolved in dichloromethane (20 mL), and the organic layer was washed with an aqueous solution of saturated ammonium chloride (25 mL). The aqueous layer was extracted with dichloromethane (2 x 20 mL) and the combined organics were dried with MgSO$_4$, decanted and evaporated to dryness. The crude product was purified by silica gel chromatography (12:1 hexane/EtOAc). This procedure resulted in 13 mg of desired product as a 1:0.85 mixture of two diastereomers.
(0.040 mmol, 72 %). Rf = 0.5 and 0.45 in 3:1 hexanes/EtOAc. 1H NMR (500 MHz, CDCl3): mixture of diastereomers δ 4.24-4.19 (m, 1H, 1H'), 4.17-4.13 (m, 1H), 3.93 (bs, 1H'), 3.51 (bs, 1H'), 3.03 (dd, J = 15.1, 5.6 Hz, 1H'), 2.89 (qd, J = 15.2, 4.4 Hz, 2H), 2.73-2.67 (m, 2H, 1H'), 2.64-2.53 (m, 1H, 1H'), 2.36 (dt, J = 14.9, 2.6 Hz, 1H'), 2.25 (dd, J = 14.4, 9.0, 2.2 Hz, 1H), 2.20-2.13 (m, 1H'), 2.08-1.98 (m, 1H, 2H'), 1.96-1.84 (m, 2H), 1.80-1.50 (m, 2H, 3H'), 1.03 (m, 21H, 21H'). 1H NMR (500 MHz, CDCl3): major diastereomer δ 4.21 (m, 1H), 4.15-4.16 (m, 1H), 2.89 (qd, J = 15.2, 4.4 Hz, 2H), 2.72-2.68 (m, 2H), 2.60-2.56 (m, 1H), 2.25 (dd, J = 12.0, 7.5 Hz, 1H), 2.03 (dd, J = 12.1, 3.2 Hz, 1H), 1.93-1.89 (m, 2H), 1.74-1.58 (m, 2H), 1.07-1.03 (m, 21H); 13C NMR (150 MHz, CDCl3): major diastereomer δ 68.5, 67.1, 40.7, 39.4, 34.8, 33.3, 24.6, 18.1, 12.3 HRMS (ESI): calcd for C16H34O2NaSSi+ [M + Na]+, 341.1941; found, 341.1941.

5-((triisopropylsilyl)oxy)thiocan-3-yl pivalate.

5-((triisopropylsilyl)oxy)thiocan-3-ol 15 (231 mg, 0.726 mmol, 1.0 equiv) was dissolved in pyridine (11.5 mL, anhydrous). To this solution, pivaloyl chloride (350 µL, 2.9 mmol, 3.9 equiv) was added. The reaction mixture was then warmed to 35 °C and stirred overnight. The reaction was cooled to rt and evaporated to dryness. The crude product was purified by silica gel chromatography (25:1 hexane/EtOAc) to afford the desired product in 80 % yield as a 1:1 mixture of diastereomers (243 mg, 0.581 mmol). Rf = 0.8 in 3:1 hexane/EtOAc. 1H NMR (500 MHz, CDCl3): δ 5.25-5.19 (m, 1H), 4.83-4.79 (m, 1H), 4.32-4.25 (m, 1H), 2.95 (dd, J = 15.1, 4.8 Hz, 1H), 2.79-2.63 (m, 6H), 2.61-2.55 (m, 1H), 2.30 (ddd, J = 14.6, 8.6, 2.8 Hz, 1H), 2.21 (dt, J = 14.3, 10.1 Hz, 1H), 2.15 (ddd, J = 14.7, 7.1, 1.9 Hz, 1H), 2.08-2.03 (m, 1H), 1.99-1.76 (m, 6H), 1.74-1.61 (m, 2H), 1.14 (s, 18H) 1.04-1.01 (s, 21H). 13C NMR (125 MHz, CDCl3): δ 177.9, 1778.8, 71.9, 70.5, 68.8, 68.7, 42.0, 39.1, 39.0, 37.6, 37.0, 36.4, 34.6, 34.1, 33.9, 33.3, 27.5, 27.4, 24.6, 23.2, 18.5, 18.6, 12.8, 12.7. HRMS (ESI): calcd for C21H42O3NaSSi+ [M + Na]+, 425.2516; found, 425.2513.

5-hydroxythiocan-3-yl pivalate (16).

5-((triisopropylsilyl)oxy)thiocan-3-yl pivalate (243 mg, 0.581 mmol, 1.0 equiv) was dissolved in THF (5.6 mL, anhydrous). The solution was cooled to 0 °C and tetrabutyammonium fluoride (TBAF) (1.70 mL of 1.0M solution in THF, 1.70 mmol, 2.93 equiv) was added dropwise to this solution. The reaction was then warmed to rt and stirred for 2 h. The reaction was evaporated to dryness, and the crude product was purified by silica gel chromatography (3:1 hexane/EtOAc) to give 118 mg of the desired product as a 1:1 mixture of two diastereomers (0.48 mmol, 83%). Rf = 0.2 in 3:1 hexane/EtOAc. 1H NMR (600 MHz, CDCl3): δ 5.07 (tdd, J = 8.1, 4.1, 1.5 Hz, 1H), 4.97-4.89 (m, 1H), 4.22-4.18 (m, 1H), 4.04-4.00 (m, 1H), 2.91-2.78 (m, 3H), 2.77-2.68 (m, 3H), 2.67-2.56 (m, 3H), 2.34-2.22 (m, 2H). 1C NMR (150 MHz, CDCl3): δ 178.2, 177.5, 71.5, 70.5, 68.0, 68.1, 39.2, 39.0, 38.7, 38.6, 36.1, 36.0, 33.9, 33.1, 32.7, 32.6, 27.1, 27.0, 24.1, 23.4. HRMS (ESI): calcd for C12H22O3NaS+[M + Na]+, 269.1182; found, 269.1184.
5-oxothiocan-3-yl pivalate (17).

5-hydroxythiocan-3-yl pivalate 16 (75 mg, 0.31 mmol, 1.0 equiv) was dissolved in dichloromethane (1.8 mL). To this solution, Dess-Martin periodinane (195 mg, 0.460 mmol, 1.50 equiv) was added, and the reaction mixture was stirred vigorously. A small amount of water (6 µL) was solvated in dichloromethane (6 mL) through pipetting, and the resulting wet dichloromethane was slowly added to the reaction mixture. The addition of the wet dichloromethane resulted in precipitation to form a cloudy mixture. After 30 min, the reaction was complete and dichloromethane was evaporated off. The reaction mixture was diluted in ether (10 mL), and the organics were washed with 1:1 sodium bicarbonate/10 % sodium thiosulfate aqueous solution (12 mL). The organic layer was washed with water (10 mL) and saturated sodium chloride solution (10 mL), dried with MgSO₄, decanted and evaporated to dryness. This procedure resulted in 55 mg of desired product (0.23 mmol, 74 %). Rf = 0.25 in 3:1 hexane/EtOAc. ¹H NMR (500 MHz, CDCl₃): δ 5.19 (tt, J = 10.2, 3.9 Hz, 1H), 2.93 (dd, J = 14.4, 3.7 Hz, 1H), 2.95-2.76 (m, 2H), 2.60 (dd, J = 12.7, 3.7 Hz, 1H), 2.45-2.53 (m, 3H), 2.41-2.36 (m, 1H), 2.22-2.12 (m, 2H), 1.14 (s, 9H). ¹³C NMR (150 MHz, CDCl₃) δ 208.7, 177.3, 72.4, 45.0, 42.9, 38.7, 34.7, 32.7, 27.0. HRMS (ESI): calcd for C₁₂H₂₀O₃NaS⁺ [M + Na]⁺, 267.1025; found, 267.1027.

(E)-5-(((trifluoromethyl)sulfonyl)oxy)-3,6,7,8-tetrahydro-2H-thiocin-3-yl pivalate (18).

5-oxothiocan-3-yl pivalate 17 (20 mg, 0.082 mmol, 1.0 equiv) was dissolved in THF (2.8 mL, anhydrous), and the reaction was cooled to -78 °C. To this solution, sodium bis(trimethylsilyl)amide (NaHMDS) (82 µL of 2M in THF, 0.16 mmol, 2.0 equiv) was added. After stirring at -78 °C for 30 min, N-phenyl-bis(trifluoromethanesulfonimide) (33 mg, 0.092 mmol, 1.1 equiv) was added. After 20 additional minutes, the reaction was quenched with methanol (1 mL) and evaporated to dryness. The crude product was purified by silica gel chromatography (10:1 hexane/EtOAc) to give the desired product (20 mg, 0.053 mmol, 64 %). Rₑ = 0.7 in 3:1 hexane/EtOAc. ¹H NMR (600 MHz, CDCl₃): δ 5.88 (t, J = 8.6 Hz, 1H), 5.15-5.18 (m, 1H), 3.05-3.07 (m, 1H), 2.90-2.96 (m, 2H), 2.74 (dd, J = 15.2, 7.7 Hz, 1H), 2.62-2.70 (m, 2H), 2.57-2.61 (m, 1H), 2.46-2.51 (m, 1H), 1.16 (s, 9H). ¹³C NMR (150 MHz, CDCl₃) δ 177.5, 149.4, 122.4, 118.4 (q, J = 318 Hz), 73.5, 38.7, 34.0, 33.7, 32.2, 30.0, 26.9. ¹⁹F NMR (376 MHz, CDCl₃): δ -73.12 (s, 3F). HRMS (ESI): calcd for C₁₃H₁₉O₅F₃NaS₂⁺ [M + Na]⁺, 399.0519; found, 399.0519.

2,6,7,8-tetrahydro-4,5-didehydro-2H-thiocin-3-ol (thiaOCT, 8).

(E)-5-(((trifluoromethyl)sulfonyl)oxy)-3,6,7,8-tetrahydro-2H-thiocin-3-yl pivalate 18 (75 mg, 0.20 mmol, 1.0 equiv) was dissolved in THF (6.0 mL, anhydrous). The reaction mixture was cooled to 0 °C and lithium diisopropylamide (LDA) (0.2 M in THF, 2.1 mL, 0.42 mmol, 2.1 equiv) was added dropwise. The addition of LDA resulted in a color change from pale yellow to brown. After 10 min, the reaction was quenched with methanol (2 mL) and evaporated to dryness. The crude product was purified by silica gel chromatography (10:1 to 3:1 hexane/EtOAc) to yield the desired cyclooctyne 6 (15 mg, 0.11 mmol, 53 %). Rₑ = 0.3 in 3:1 hexane/EtOAc. ¹H NMR (400 MHz, CDCl₃): δ 4.37-
4.34 (m, 1H), 3.34 (dd, $J = 10.0$, 4.8 Hz, 1H), 3.28 (d, $J = 7.0$ Hz, 1H), 3.14 (dt, $J = 9.4$, 2.8 Hz, 1H), 2.92-2.88 (m, 1H), 2.76 (d, $J = 10.0$ Hz, 1H), 2.52-2.49 (m, 1H), 2.43-2.38 (m, 3H). $^{13}$C NMR (150 MHz, CDCl$_3$) δ 91.2, 90.8, 71.5, 42.4, 41.1, 29.2, 23.5. HRMS (EI): calcd for C$_7$H$_{10}$OS$^+$ [M]$^+$, 142.0452; found, 142.0449; [M-H$_2$O]$^+$, 124; [M-CHO]$^+$, 113; [M-C$_2$H$_3$O]$^+$, 99; [M-C$_5$H$_5$O]$^+$, 61.

**Figure S1**

The reaction of thiaOCT (8) and benzyl azide was monitored by $^1$H NMR for 1500 min at rt. ThiaOCT and benzyl azide were separately dissolved in CD$_3$CN and mixed together in a 1:1 ratio at a concentration of 19 mM. The percent conversion was calculated by the disappearance of thiaOCT and benzyl azide relative to the formation of product as determined by integration. The second-order rate constant was determined by plotting $1/[$8$]$ versus time. The plot was fit to a linear regression and the slope corresponds to the second-order rate constant. Shown are data from three replicate experiments. The three lines had an average slope of $0.00032 \pm 7.7 \times 10^{-6}$ M$^{-1}$s$^{-1}$. 

![Second-order rate constant of thiaOCT and benzyl azide](image_url)
Scheme S1. Synthesis of thiaDIFBO

\[
\begin{array}{c}
\text{S1} \quad \begin{array}{c}
\text{O} \\
\text{LDA} \\
\text{NFSI} \quad 84\% \quad \text{ii. NFSI} \\
\end{array}
\end{array}
\quad \begin{array}{c}
\text{S2} \quad \begin{array}{c}
\text{O} \\
\text{LDA} \\
\text{NFSI} \quad 44\% \\
\end{array}
\end{array}
\quad \begin{array}{c}
\text{19} \\
\text{AlMe}_3, \\
\text{TMSCHN}_2
\end{array}
\]

\[
\begin{array}{c}
\text{9} \quad \text{CsF, quant.} \\
\text{TMS} \\
\text{OTf} \\
\text{7.5\% (2 steps)}
\end{array}
\quad \begin{array}{c}
\text{S3} \\
\text{NaHMDS} \\
\text{Tf}_2\text{O}
\end{array}
\quad \begin{array}{c}
\text{S4} \\
\text{i. LDA} \\
\text{ii. NFSI}
\end{array}
\]

NFSI = \text{N-fluorobenzensulfonimide}

\text{S1} \text{ was sequentially difluorinated using LDA and N-fluorobenzensulfonimide (NFSI) to yield 19. This intermediate was homologated using AlMe}_3 \text{ and TMSCHN}_2 \text{ to yield \alpha-silyl ketone S3. This step was far less efficient than achieved with the all-carbon cycloheptane analog, perhaps due to Lewis acid/base pairing of the sulfur atom and the trimethylaluminum. Due to the labile nature of the \alpha-silyl ketone, crude S3 was immediately converted to vinyl triflate S4, which was then quantitatively converted to thiaDIFBO (9) by treatment with CsF.}

\text{4-fluoro-3,4-dihydrobenzo[b]thiepin-5(2H)-one (S2).}

A flame-dried flask was charged with 3,4-dihydrobenzo[b]thiepin-5(2H)-one S1 (1.2 g, 6.6 mmol, 1 equiv). The flask was evacuated and backfilled with nitrogen twice. THF (33 mL, anhydrous) was added to the flask and the solution cooled to -78 °C. LDA (4.0 mL of 2M solution in heptane/THF/ethylbenzene, 8.0 mmol, 1.2 equiv) was added and the solution warmed to 0 °C and allowed to stir for 1 h. Separately, a dry flask was charged with N-fluorobenzensulfonimide (NFSI) (2.7 g, 8.6 mmol, 1.3 equiv) and evacuated and backfilled with nitrogen twice. THF (30 mL, anhydrous) was added and the solution cooled to -78 °C. The solution of base was slowly added to the NFSI solution over 12 min via syringe and the mixture was allowed to warm to rt over 30 min, at which point it was quenched with saturated ammonium chloride (50 mL). The organic layer was separated and the aqueous layer extracted with ethyl acetate (2 x 100 mL). The organic layers were combined, dried with sodium sulfate, filtered, and concentrated under reduced pressure. The crude oil was purified using silica gel chromatography (93:7 hexanes/EtOAc) to yield 1.1 g (5.6 mmol, 85%) of the desired product as a yellow oil. R_f = 0.48 in 9:1 hexane/EtOAc. ^1H NMR (300 MHz, CDCl_3): δ 8.02-7.98 (dd, J = 7.6,1.5 Hz, 1H), 7.47-7.44 (d, J = 7.8 Hz, 1H), 7.41-7.36 (td, J = 7.5, 1.5 Hz, 1H), 7.33-7.28 (t, J = 7.1 Hz, 1H), 5.81-5.76 (dd, J = 7.2, 9 Hz, 0.5 H), 5.65-5.60 (dd, J = 7.2, 9 Hz, 0.5H),
3.24-3.16 (m, 1H), 2.89-2.70 (m, 2H), 2.52-2.37 (m, 1H). $^{13}$C NMR (130 MHz, CDCl$_3$): $\delta$ 197.5-197.4 (d, $J = 16.6$), 141.7, 134.9, 131.8, 131.1, 130.1, 126.2, 93.8-92.5 (d, $J = 185$), 36.4-36.3 (d, $J = 24$), 29.7 (d, $J = 12$). $^{19}$F NMR (375 MHz, CDCl$_3$): $\delta$ -189.4 (dt, $J_1 = 4, 45$ Hz, 1F). HRMS (EI): calc for C$_{10}$H$_9$FOS$^+$ [M]$^+$, 196.0358; found, 196.0357; [M-CO]$^+$, 168; [M-CH$_3$S]$^+$, 149.

4,4-difluoro-3,4-dihydrobenzo[b]thiepin-5(2H)-one (19).

A flame-dried flask was charged with 4-fluoro-3,4-dihydrobenzo[b]thiepin-5(2H)-one S2 (800 mg, 4.1 mmol, 1 equiv). The flask was evacuated and backfilled with nitrogen twice. THF (20 mL, anhydrous) was added to the flask and the solution cooled to $-78$ ºC. LDA (2.5 mL of 2 M solution in heptane/THF/ethylbenzene, 4.9 mmol, 1.2 equiv) was added and the solution warmed to 0 ºC and allowed to stir for 1 h. Separately, a dry flask was charged with NFSI (1.7 g, 5.3 mmol, 1.3 equiv) and evacuated and backfilled with nitrogen twice. THF (20 mL, anhydrous) was added and the solution cooled to $-78$ ºC. The solution of base was slowly added to the NFSI solution over 10 min via syringe and the reaction then allowed to warm to rt. Saturated ammonium chloride (40 mL) was added to the reaction followed by ethyl acetate (50 mL). The organic layer was separated and the aqueous layer extracted with ethyl acetate (2 x 50 mL). The organic layers were combined, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude oil was purified using silica gel chromatography (93:7 hexanes/EtOAc) to yield 390 mg (1.8 mmol, 45%) of a light yellow oil that solidified upon cooling. R$_f$ = 0.55 in 9:1 hexane/EtOAc. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.65-7.63 (d, $J = 7.8$, 1H), 7.39-7.30 (m, 2H), 7.26-7.24 (t, $J = 8$ Hz, 1H), 3.05-3.03 (t, $J = 6$ Hz, 2H), 2.76-2.68 (m, 2H). $^{13}$C NMR (150 MHz, CDCl$_3$): $\delta$ 193, 138.5, 135, 132, 131.2, 130, 126.4, 118.6 (t, $J = 243$), 39.6 (t, $J = 25$), 28. $^{19}$F NMR (375 MHz, CDCl$_3$): $\delta$ -99 (t, $J = 19$ Hz, 1F). HRMS (EI): calc for C$_{10}$H$_8$F$_2$OS$^+$ [M]$^+$, 214.0264; found, 214.0270; [M-CO]$^+$, 186; [M-CH$_2$S]$^+$, 168.

(Z)-4,4-difluoro-6-(trimethylsilyl)-3,4-dihydro-2H-benzo[b]thiocin-5-yl trifluoromethanesulfonate (S4).

A flame-dried flask was charged with 4,4-difluoro-3,4-dihydrobenzo[b]thiepin-5(2H)-one 19 (214 mg, 1 mmol, 1 equiv) and evacuated and backfilled with nitrogen twice. Dry dichloromethane (12.5 mL) was added and the solution was cooled to 0 ºC. Trimethylsilyl diazomethane (TMSCHN$_2$) (600 µL of 2M solution in dichloromethane, 1.2 mmol, 1.2 equiv) was added via syringe immediately followed by trimethylaluminum (AlMe$_3$) (600 µL of 2M solution in toluene, 1.2 mmol, 1.2 equiv). The reaction was stirred at 0 ºC for 10 min, at which point the reaction was quenched with saturated ammonium chloride (5 mL) followed by saturated Rochelle’s salt (5 mL). Three times, dichloromethane (3 x 25 mL) was added and the organic layer was separated. The organic layers were combined, dried over sodium sulfate, filtered, and concentrated to an oil that still contained 4,4-difluoro-6-(trimethylsilyl)-3,4-dihydro-2H-benzo[b]thiocin-5(6H)-one (S3) and toluene was obtained.
The oil was transferred to a dry flask that was evacuated and backfilled with nitrogen twice. Dry THF (10 mL) was added and the reaction cooled to –78 °C. NaHMDS (600 μL of 2M solution in THF, 1.2 mmol, 1.2 equiv) was added and the reaction stirred for 2 h at –78 °C. Trifluoromethane sulfonic anhydride (200 μL, 1.2 mmol, 1.2 equiv) was then added and the reaction stirred for 1 h at –78 °C. Methanol (1 mL) was added and the reaction was then allowed to warm to rt and concentrated. The oily solid was taken up in dichloromethane and filtered. The filtrate was concentrated and purified via HPLC on a 100 Å C18 column, (70% to 100% acetonitrile in water over 30 minutes). The desired product eluted at 17 minutes. Concentration of the desired fraction yielded 16 mg (0.037 mmol, 3.8%) of (Z)-4,4-difluoro-6-(trimethylsilyl)-3,4-dihydro-2H-benzo[b]thiocin-5-yl trifluoromethanesulfonate $S_4$ as a clear oil. $R_f = 0.82$ in 9:1 hexane/EtOAc. $^1$H NMR (500 MHz, CDCl$_3$): δ 7.67-7.65 (d, $J = 8$, 1H), 7.40-7.37 (t, $J = 7.5$, 1H), 7.29-7.26 (m, 1H), 7.16-7.14 (d, $J = 7.5$ Hz, 1H), 3.00-2.96 (m, 1H), 2.83-2.76 (m, 1H), 2.35-2.23 (m, 1H), 2.12-2.05 (m, 1H), 0.019 (s, 9H). $^{13}$C NMR (125 MHz, CDCl$_3$): δ 144.1(t, $J = 30$), 143 (d, $J = 24$), 136.6, 130.3, 129.3, 128.2, 126.5, 122.6, 120, 119.9, 117.5, 116, 34.2 (t, $J = 26.3$ Hz), 29.8 (t, $J = 5$ Hz), -1.3. $^{19}$F NMR (375 MHz, CDCl$_3$): δ -70 (t, $J = 19$ Hz, 3F), -90 (dp, $J = 262$, 22.5 Hz, 1H), -92 (bd, $J = 274$ Hz, 1H) . HRMS (EI): calc for C$_{15}$H$_{17}$F$_5$O$_3$S$_2$Si$^+$ [M$^+$], 432.0309; found, 432.0316, [M-CO$_3$F$_3$S]$^+$, 283; [M-C$_6$H$_4$O$_3$F$_3$S]$^+$, 207.

4,4-difluoro-3,4-dihydro-5,6-didehydro-2H-benzo[b]thiocine (ThiaDIFBO, 9).

(Z)-4,4-difluoro-6-(trimethylsilyl)-3,4-dihydro-2H-benzo[b]thiocin-5-yl trifluoromethanesulfonate $S_4$ (16 mg, 0.037 mmol, 1 equiv) was dissolved in deuterated acetonitrile (1 mL) and cesium fluoride (34 mg, 0.22 mmol, 6 equiv) was added. The reaction was stirred for 1 h at rt. It was then filtered, and the filtrate concentrated and purified by silica gel chromatography (99:1 hexanes/ EtOAc) to yield 7.7 mg (0.036 mmol, 99%) of the desired product as a clear oil. $R_f = 0.86$ in 9:1 hexane/EtOAc. $^1$H NMR (600 MHz, CD$_3$CN): δ 7.56-7.54 (d, $J = 9$ Hz, 1H), 7.47-7.45 (d, $J = 8$ Hz, 1H), 7.42-7.39 (t, $J = 7.2$ Hz, 1H), 7.35-7.33 (t, $J = 7.5$ Hz, 1H), 3.17-3.16 (m, 1H), 2.86-2.79 (m, 2H). $^{13}$C NMR (125 MHz, CD$_3$CN): δ 148, 131.4, 130.9, 128.8, 127.4, 122.4, 120.6, 119.9, 106.3 (t, $J = 9$ Hz), 46.1 (t, $J = 27.5$ Hz), 28.8 (t, $J = 3.8$ Hz). $^{19}$F NMR (375 MHz, CD$_3$CN): -86 (bt, 2F). HRMS (EI): calc for C$_{11}$H$_8$F$_2$S$^+$ [M$^+$], 210.0315; found, 210.0321, [M-C$_2$H$_3$]+, 183; [M-CF$_2$]+, 160.
Figure S2.
The reaction of thiaDIFBO 9 and benzyl azide was monitored by \(^1\)H NMR for 20 min at rt. ThiaDIFBO and benzyl azide were separately dissolved in CD\(_3\)CN and mixed together in a 1:1 ratio at a concentration of 7 mM. The percent conversion was calculated by the disappearance of thiaDIFBO and benzyl azide relative to the formation of product as determined by integration. The triazole isomers were formed in approximately a 3:1 ratio and could be separated by HPLC (1.86:1 H\(_2\)O/MeCN to 1:3 H\(_2\)O/MeCN, 34.5 min, C\(_{18}\) column). The second-order rate constant was determined by plotting 1/[9] versus time. The plot was fit to a linear regression and the slope corresponds to the second-order rate constant. Shown are data from three replicate experiments. The three lines had an average slope of 0.015 ± 0.001 M\(^{-1}\)s\(^{-1}\).

Scheme S2. Synthesis of TMTH
3,3'-thiobis(2,2-dimethylpropanoic acid) (S6).

Sodium carbonate (4.0 g, 37 mmol, 0.5 equiv) was dissolved in water (13 mL) and the solution was poured into a vessel containing chloropivalic acid S5 (10 g, 74 mmol, 1 equiv). Once bubbling subsided, sodium sulfide nonahydrate (17.8 g, 185 mmol, 2.5 equiv) dissolved in water (25 mL) was added. The reaction was stirred for 72 h at rt. The pH was adjusted to 1 by addition of 50% sulfuric acid. The suspension was filtered and the solid dissolved in hot ethanol and filtered. The filtrate was concentrated and recrystallized out of hot water with about 10% ethanol. The solid was dried under high vacuum to yield 6.0 g (26 g, 69%) of a white powder. $^1$H NMR (400 MHz, CDCl₃): δ 2.81 (s, 4H), 1.27 (s, 12H). HRMS (ESI): calc for C₁₀H₁₇O₄S⁻ [M-H]⁻, 233.0853; found, 233.0855. In agreement with reported spectral data.\[3\]

diethyl 3,3'-thiobis(2,2-dimethylpropanoate) (S7).

3,3'-thiobis(2,2-dimethylpropanoic acid) S6 (4.0 g, 17 mmol, 1 equiv) was dissolved in ethanol (45 mL, 770 mmol, 45 equiv) and toluene (45 mL). Concentrated sulfuric acid (300 μL) was added and the flask was equipped with a Dean-Stark apparatus and heated at 90 °C overnight. The next day the reaction was cooled to rt and poured into a separatory funnel containing water (100 mL). The organic layer was separated and washed with saturated sodium bicarbonate (1 x 100 mL). The aqueous layers were combined and back extracted with ethyl acetate (1 x 300 mL). The organic layers were combined, dried over sodium sulfate, filtered, and concentrated. The crude oil was purified via silica gel chromatography (7:1 hexanes/EtOAc) to yield 4.0 g (13 mmol, 81%) of a clear oil. Rₜ = 0.65 in 9:1 hexane/EtOAc. $^1$H NMR (500 MHz, CDCl₃): δ 4.16-4.10 (q, J = 7 Hz, 4H), 2.78 (s, 4H), 1.27-1.24 (t, J = 7 Hz, 6H), 1.23 (s, 12H). $^{13}$C NMR (125 MHz, CDCl₃): δ 177, 61.1, 45.6, 44.6, 25.1, 14.6. FTMS (ESI): calc for C₁₄H₂₆O₄NaS⁺ [M+Na⁺], 313.1444; found, 313.1442. In agreement with reported spectral data.\[3\]

5-hydroxy-3,3,6,6-tetramethylthiepan-4-one (S8).

In a flame-dried flask, sodium metal (390 mg, 17.2 mmol, 10 equiv) was added portionwise to boiling m-xylene (3.5 mL, anhydrous) while stirring vigorously. The reaction was then capped and positive nitrogen pressure was applied. Diethyl 3,3'-thiobis(2,2-dimethylpropanoate) S7 (500 mg, 1.72 mmol, 1 equiv) in m-xylene (3.5 mL, anhydrous) was added via syringe over 2 h and the reaction was allowed to reflux for 0.5 h after addition. The reaction was then cooled to rt, filtered through celite and washed five times with toluene. The celite layer was carefully quenched with isopropanol (10 mL) and ethanol (2 mL). The filtrate was concentrated and filtered through a silica plug (8:1 EtOAc/MeOH). The combined eluent was concentrated. The resulting oil was then purified via silica gel chromatography (9:1 to 3:1 hexanes/EtOAc) to yield 65 mg (0.32 mmol, 19%) of light yellow crystals. Rₜ = 0.59 in 3:1 hexane/EtOAc. $^1$H NMR (600 MHz,
3,3,6,6-tetramethylthiepane-4,5-dione (S9).

A flame-dried flask was charged with oxalyl chloride (78 μL, 0.89 mmol, 2.5 equiv) and dichloromethane (1.8 mL, anhydrous). The solution was cooled to –78 °C. Dimethyl sulfoxide (110 μL, 1.60 mmol, 4.5 equiv, anhydrous) was added and the reaction stirred for 15 min at –78 °C. 5-hydroxy-3,3,6,6-tetramethylthiepan-4-one S8 (72 mg, 0.36 mmol, 1 equiv) in dichloromethane (1.8 mL, anhydrous) was added and the reaction was stirred for another 15 min at –78 °C. Triethylamine (220 μL, 1.6 mmol, 4.5 equiv.) was added and the reaction stirred for 15 min at –78 °C and warmed to 0 °C for 25 min. Water (1 mL) was added and the organic layer separated. The aqueous layer was washed with dichloromethane (2 x 10 mL) and the organic layers were combined, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The oil was purified on silica gel chromatography (95:5 hexanes/EtOAc) to yield 70 mg (0.35 mmol, 98%) of a light yellow oil. Rf = 0.58 in 9:1 hexane/EtOAc. 1H NMR (400 MHz, CDCl3): δ 2.58 (s, 4H), 1.27 (s, 6H), 1.20 (s, 6H). 13C NMR (150 MHz, CDCl3): δ 151.7, 45.3, 42, 44.6, 27.9, 26.8. FTMS (ESI): calc for C14H21N4S+ [M + H] +, 229.1481; found, 229.1481. In agreement with reported spectral data.[4]

(1Z,1'E)-(3,3,6,6-tetramethylthiepane-4,5-diylidene)bis(hydrazine) (S10).

3,3,6,6-tetramethylthiepane-4,5-dione S9 (150 mg, 0.75 mmol, 1 equiv) was combined with hydrazine sulfate (390 mg, 3.0 mmol, 4 equiv), anhydrous hydrazine (20 drops), and ethanol (15 drops) in ethylene glycol (1.5 mL) and stirred overnight at 150 °C in a sealed vial. The following day, the reaction was cooled to rt, quenched with water (1 mL), and extracted three times with diethyl ether (3 x 2 mL). The organic layers were combined and concentrated to a white solid that was recrystallized out of hexanes to yield 100 mg (0.44 mmol, 59 %) of white crystals. Rf = 0.46 in 1:1 hexane/EtOAc. 1H NMR (400 MHz, CDCl3): δ 5.24 (s, 4H), 2.55-2.45 (q, J = 14.4 Hz, 4H), 1.33 (s, 6H), 1.20 (s, 6H). 13C NMR (150 MHz, CDCl3): δ 151.7, 45.3, 42, 44.6, 27.9, 26.8. FTMS (ESI): calc for C14H21N4S+ [M + H] +, 229.1481; found, 229.1481. In agreement with reported spectral data.[4]
The reaction was quenched with aqueous sodium bicarbonate (1 mL) and extracted with dichloromethane (3 x 3 mL). The organic layers were combined, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was used for kinetics experiments. Purification for analytical purposes was performed by fractional distillation using a Kugelrohr apparatus. 10 mg (0.059 mmol, 14 %) of the desired product were collected at 50 °C, 8 mmHg as a light yellow oil. Rf = 0.75 in 19:1 hexane/diethyl ether. ¹H NMR (600 MHz, CDCl₃): δ 2.80 (s, 4H), 1.22 (s, 12H). ¹³C NMR (150 MHz, CDCl₃): δ 108.6, 52.7, 35, 26.2. HRMS (EI): calc for C₁₀H₁₆S⁺ [M]⁺, 168.0973; found, 168.0973, [M-CH₃]⁺, 153, [M-C₃H₇]⁺, 125, [M-C₂H₅S]⁺, 107. In agreement with reported spectral data.⁴

Figure S3

The reaction of TMTH 10 and benzyl azide was monitored by ¹H NMR for 12 min at rt. TMTH and benzyl azide were separately dissolved in CD₃CN and mixed together in a 1:1 ratio at concentration of 5 mM (blue, green, pink) or 2.9 mM (purple, orange, red). The percent conversion was calculated by the disappearance of TMTH and benzyl azide relative to the formation of product as determined by integration. The second-order rate constant was determined by plotting 1/[10] versus time. The plot was fit to a linear regression and the slope corresponds to the second-order rate constant. Shown are data from three replicate experiments at each concentration. The six lines had an average slope of 4.0 ± 0.4 M⁻¹s⁻¹.
Figure S4

a)

\[
\begin{array}{c}
\text{[Structure]} \\
\rightarrow \\
\text{[Structure]}
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\]

b)

\[
\begin{array}{c}
\text{[Structure]} \\
\text{DCM} \\
\rightarrow \\
\text{[Structure]}
\end{array}
\]
**Figure S4.**  
a) 30 s interval timepoints of NMR kinetics measurements of the reaction between 2.9 mM azide and 2.9 mM 10 in CD$_3$CN starting at 1.63 min through 5.63 min. (Measurements were taken until 15 min from mixing of starting materials.) The singlet at 5.75 ppm corresponds to the triazole benzylic protons, the singlet at 4.39 ppm corresponds to the azide benzylic protons, the singlet at 2.81 ppm corresponds to the methylene protons on TMTH, and the doublet at 2.78 ppm corresponds to the methylene protons on the product.  
b) Direct comparisons of 10 (bottom), the reaction in progress at 2.63 min (middle) and a purified sample of triazole product (top). Peak at 1.9 ppm is CD$_3$CN and at 2.15 ppm is H$_2$O. At 16.13 min, the NMR spectrum shows 84% conversion to product. To obtain pure triazole sample, the kinetics samples were combined after each had been allowed to react for ~16 min. After concentration, the crude mixture was purified by silica gel chromatography (hexanes to 3:1 hexanes/EtOAc) resulting in pure triazole product in 38% isolated yield. 

**Scheme S3. Synthesis of 11**

As with DIFBO and thiaDIFBO, the synthesis of 11 proceeded through a key ring expansion step (Scheme 3). Compound S11 was dimethylated by treatment with KHMD and methyl iodide to produce 20. Homologation of 20 was performed using AlMe$_3$ and TMSCHN$_2$, producing the unexpected silyl enol ether S12, which was readily converted to ketone S13 upon treatment with acid. Compound S13 was then treated with KHMD and trifluoromethane sulfonic anhydride to form vinyl triflate S14. Attempts to eliminate the triflate using LDA or hexamethyldisilylamide bases gave no reaction, perhaps due to unfavorable steric interactions between these large bases and the gem-dimethyl group. However, treatment of S14 with NaH in the presence of benzyl azide gave triazole cycloadducts S15 and S16, suggesting that 11 was formed in situ.
3-methylthiochroman-4-one.

THF (5.5 mL, anhydrous) and LiHMDS (3.61 mL of 1 M solution in THF, 3.61 mmol, 1.2 equiv) were combined and cooled to -78 °C. Thiochromanone S11 (502 mg, 3.05 mmol, 1.0 equiv) was dissolved in THF (2.0 mL, anhydrous) and added to the solution of base over 1 h at -78 °C. After stirring for an additional hour at -78 °C, MeI (0.93 mL, 15 mmol, 4.9 equiv) was added and the mixture was warmed to rt over 3 h at which point, the reaction was quenched with MeOH (2 mL) and evaporated to dryness. Silica gel chromatography (35:1 hexanes/ EtOAc) resulted in pure desired compound (400 mg, 2.24 mmol, 73%). Rf = 0.45 in 6:1 hexane/ethyl acetate. 1H NMR (600 MHz, CDCl₃): δ 8.05 (dd, J = 7.2, 1.0, 1H), 7.33-7.30 (m, 1H), 7.19 (dd, J = 7.9, 0.7 Hz, 1H), 7.13-7.10 (m, 1H), 3.09 (s, 1H), 3.08 (d, J = 3.1 Hz, 1H), 2.88 (dp, J = 8.7, 6.8 Hz, 1H), 1.30 (d, J = 6.8 Hz, 3H). 13C NMR (150 MHz, CDCl₃): δ 196.5, 141.9, 133.0, 130.5, 129.6, 127.4, 124.9, 42.2, 33.1, 15.1. HRMS (EI): Calcd. for C₁₀H₁₀OS+ [M]+ 178.0452, found 178.0452; [M-C₂H₂O]+, 136; [M-C₄H₆O]+, 108.

3,3-dimethylthiochroman-4-one (20).

THF (1.0 mL, anhydrous) and LiHMDS (0.96 mL of 1 M solution in THF, 0.96 mmol, 1.2 equiv) were combined and cooled to -78 °C. 3-methylthiochroman-4-one (144 mg, 0.809 mmol, 1.0 equiv) was dissolved in THF (1.0 mL, anhydrous) and added to the solution of base over 1 h at -78 °C. After stirring for an additional hour at -78 °C, MeI (0.25 mL, 4.0 mmol, 4.9 equiv) was added and the mixture was warmed to rt over 3 h, at which point the reaction was quenched with MeOH (1 mL) and evaporated to dryness. Silica gel chromatography (40:1 hexanes/ EtOAc) resulted in pure 20 (129 mg, 0.672 mmol, 83%). Rf = 0.55 in 6:1 hexane/ethyl acetate. 1H NMR (500 MHz, CDCl₃): δ 8.08 (dd, J = 8.0, 1.2 Hz, 1H), 7.33 (ddd, J = 8.0, 7.2, 1.5 Hz, 1H), 7.20 (dd, J = 8.0, 0.8 Hz, 1H), 7.14 (ddd, J = 8.2, 7.2, 1.2 Hz, 1H), 3.07 (s, 2H), 1.32 (s, 6H). 13C NMR (125 MHz, CDCl₃): δ 198.5, 141.6, 132.9, 130.3, 129.7, 127.3, 124.9, 41.1, 39.3, 23.7. HRMS (EI): Calcd. for C₁₁H₁₂OS+ [M]+ 192.0609, found 192.0604; [M-C₃H₄O]+, 136; [M-C₅H₈O]+, 108.

(3,3-dimethyl-2,3-dihydrobenzo[b]thiepin-5-yloxy)trimethylsilane (S12).

3,3-dimethylthiochroman-4-one 20 (365 mg, 1.90 mmol, 1.0 equiv) was dissolved in CH₂Cl₂ (10 mL, anhydrous) and cooled to -78 °C. AlMe₃ (1.14 mL of 2M solution in toluene, 2.28 mmol, 1.2 equiv) was added and the solution was stirred for 15 min at which point, TMSCN₂ (1.14 mL of 2M solution in CH₂Cl₂, 2.28 mmol, 1.2 equiv) was added. The solution was warmed to rt overnight. The mixture was quenched with aqueous Rochelle’s salt (5 mL) and stirred until two layers formed. The quenched solution was extracted with dichloromethane (3 x 10 mL). The organic layers were combined, dried with MgSO₄, decanted, and evaporated to dryness. Silica gel chromatography (150:1 hexanes/ EtOAc) resulted in pure S12 (160 mg, 0.576 mmol, 30%). Rf = 0.85 in 6:1 hexane/ethyl acetate. 1H NMR (600 MHz, CDCl₃): δ 7.38 (d, J =
3,3-dimethyl-3,4-dihydrobenzo[b]thiepin-5(2H)-one (S13).

Silyl enol ether S12 (90 mg, 0.32 mmol, 1 equiv) was dissolved in MeOH and 12M HCl (1 drop) was added. The mixture was stirred for 30 min at rt and then quenched with saturated sodium bicarbonate (until bubbling ceased). The MeOH was removed by rotary evaporation and the resulting aqueous solution was extracted with dichloromethane (3 x 10 mL). The organic layers were combined, dried with MgSO₄, decanted, and evaporated to dryness. This procedure resulted in pure ketone S13 (65 mg, 0.31 mol, 97% yield). Rf = 0.5 in 4:1 hexane/ethyl acetate. ¹H NMR (400 MHz, CDCl₃): δ 7.46 (dd, J = 7.5, 1.1 Hz, 1H), 7.29 (d, J = 7.3 Hz, 1H), 7.20 (td, J = 7.5, 1.4 Hz, 1H), 7.14 (td, J = 7.5, 1.4 Hz, 1H), 4.03 (s, 2H), 2.83 (s, 2H), 1.32 (s, 6H). ¹³C NMR (150 MHz, CDCl₃): 209.3, 138.2, 136.8, 132.7, 130.6, 128.6, 127.5, 49.3, 47.2, 46.2, 24.3. HRMS (EI): Calcd. for C₁₂H₁₄OS⁺ [M]+ 206.0765, found 206.0764

3,3-dimethyl-2,3-dihydrobenzo[b]thiepin-5-yl trifluoromethanesulfonate (S14).

Ketone S13 (62 mg, 0.30 mol, 1 equiv.) was dissolved in THF (4.5 mL, anhydrous) and cooled to -78 °C. KHMDHS (0.72 mL of 0.5 M solution in toluene, 0.36 mmol, 1.2 equiv) was added and the solution was stirred for 2 h at -78 °C at which point trifluoromethane sulfonic anhydride (70 μL, 0.42 mmol, 1.4 equiv) was added and the reaction was warmed to 0 °C over 2 h. The reaction mixture was quenched with MeOH (1 mL) and evaporated to dryness. Silica gel chromatography with hexanes/ethyl acetate (150:1) resulted in pure S14 (66 mg, 0.20 mmol, 66%). Rf = 0.85 in 4:1 hexane/ethyl acetate. ¹H NMR (500 MHz, CDCl₃): δ 7.47 (dd, J = 7.6, 1.4 Hz, 1H), 7.30-7.24 (m, 2H), 7.20 (td, J = 7.4, 1.7 Hz, 1H), 6.62 (s, 1H), 2.86 (s, 2H), 1.42 (s, 6H). ¹³C NMR (150 MHz, CDCl₃): δ 158.3, 139.2, 133.9, 132.9, 131.5, 128.2, 127.2, 122.0, 118.7 (q, J = 320 Hz), 45.5, 44.5, 27.3. ¹⁹F NMR (565 MHz, CDCl₃): δ -75.63. HRMS (EI): Calcd. for C₁₃H₁₃O₃S₂F₃⁺ [M]+ 338.0258, found 338.0262; [M-C₄H₈]⁺, 150; [M-C₆H₁₂Si]⁺, 136; [M-C₈H₁₈OSi]⁺, 108.

Triazole products (S15 and S16).

Vinyl triflate S14 (10 mg, 0.030 mmol, 1.0 equiv.) was dissolved in THF (1.0 mL, anhydrous). NaH (10 mg, 60% in mineral oil, 0.26 mmol, 8.7 equiv.) was added to this solution followed by benzyl azide (15 μL, 0.12 mmol, 4.0 equiv). The mixture was allowed to stir overnight. The following day the reaction was quenched with MeOH (0.25 mL) and evaporated to dryness. The crude reaction mixture was purified by silica gel chromatography (6:1, 4:1, 1:1 hexanes/ethyl acetate). This procedure resulted in 5 mg of pure S15 and S16 (0.016 mmol, 53%) in a 1:0.6 ratio. Rf = 0.25 in 4:1 hexane/ethyl acetate. ¹H NMR (600 MHz, MeOD): δ 8.21 (d, J = 7.8 Hz, 1H), 7.60 (d, J = 7.8 Hz, 1H), 7.14-7.11 (m, 2H), 7.00 (t, J = 7.1 Hz, 1H), 5.81 (s, 1H), 2.77 (s, 2H), 1.28 (s, 6H), 0.30 (s, 9H). ¹³C NMR (125 MHz, CDCl₃): δ 162.3, 137.4, 137.1, 131.5, 131.0, 126.6, 124.8, 110.1, 46.1, 44.2, 28.0, 0.8. HRMS (EI): Calcd. for C₁₅H₂₂OSSi⁺ [M]+ 278.1161, found 278.1163; [M-C₄H₈]⁺, 222; [M-C₆H₁₂Si]⁺, 165; [M-C₈H₁₄OSi]⁺, 136; [M-C₈H₁₈OSi]⁺, 108.
1H’), 7.56 (d, J = 7.8 Hz, 1H’), 7.52 (d, J = 7.7 Hz, 1H), 7.39-7.35 (m, 3H, 2H’), 7.31-7.22 (m, 2H, 3H’), 7.08 (d, J = 7.7 Hz, 2H), 6.96 (d, J = 6.8 Hz, 2H’), 5.92 (s, 2H), 5.69 (s, 2H’), 2.96 (s, 2H), 2.90 (s, 2H’), 1.50 (s, 6H’), 1.46 (s, 6H). $^{13}$C NMR (150 MHz, MeOD): δ 154.0, 143.5, 143.0, 142.1, 139.5, 138.2, 137.4, 134.8, 134.5, 133.1, 132.4, 131.5, 131.1, 130.2, 130.0, 129.9, 129.9, 129.1, 129.0, 128.8, 128.6, 128.6, 127.8, 127.54, 127.52, 55.0, 54.1, 51.6, 51.2, 49.7, 39.5, 39.0, 30.4, 28.4. HRMS (ESI): Calcd. for C$_{19}$H$_{20}$N$_3$S [M+H]$^+$ 322.1372, found 322.1374.

Figure S5
Figure S5.
A 100 mM solution of TMTH (10) in $d_6$-DMSO was diluted in D$_2$O (A, B, C, D) or deuterated PBS (a solution of PBS was lyophilized and the residue dissolved in D$_2$O) (E, F) to a final concentration of 1 mM. The solution was allowed to sit at room temperature and an NMR taken (Bruker AV-500) at 10 minutes (A, D), 48 h (B, E), and 144 h (C, F). After 48 h, the amount of TMTH has decreased in both samples. The compound still persists after 144 h, though significant amounts are lost.
Figure S6.
TMTH reacts more readily with 2-azidoethanol than glutathione in PBS. All NMR spectra were taken on Bruker AV-500 or Bruker AV-600. A) A 1 mM solution of TMTH in deuterated PBS B) A solution of 1 mM TMTH and 1 mM glutathione in deuterated PBS was allowed to react for 10 min. No reaction is observed. C) A solution of 1 mM TMTH and 1 mM glutathione in deuterated PBS was allowed to react for 26 h. No starting TMTH is present, and the product methyl peaks appear at ~1.1 ppm. D) TMTH was added to a final concentration of 1 mM TMTH to a solution of 1 mM 2-azidoethanol and 1 mM glutathione in deuterated PBS. The NMR taken after 10 min indicates no remaining TMTH and methyl peaks corresponding to those of the cycloadduct with 2-azidoethanol. E) A solution of 1 mM TMTH and 1 mM azidoethanol in deuterated PBS was allowed to react for 10 min. No starting TMTH is present, and the product methyl peaks appear at ~1.3 – 1.4 ppm.

Western Blot Competition Experiments
Western blot analysis was performed on Jurkat cell lysates. Jurkat cells were incubated in the described media containing 25 μM Ac4GalNAz or DMSO vehicle for 3 days. They were then washed in Dulbecco’s modified PBS and lysed in 1% NP-40, NaCl (150 mM), Tris pH 7.4 with protease inhibitors. The cell lysate was then sonicated and cleared by centrifugation. Protein concentration was normalized using a BCA assay.

Figure 2: TMTH (10) was added at varying concentrations for 1.5 hours. Phosphine-FLAG (500 μM final concentration) was added to the protein solutions and the vials agitated overnight.

Figure S8: Phosphine-FLAG (500 μM final concentration) was added to the protein solutions at 0°C. TMTH (10) was then added at varying concentrations and the vials agitated at room temperature overnight.

The following day, 4X loading buffer was added and the proteins were separated on a 4-12% Bis-Tris gradient SDS-PAGE gel at 140 V (Bio-Rad, Criterion system). They were then electroblotted onto nitrocellulose, blocked in 5% bovine serum albumin (BSA, Sigma) in Tris-buffered saline with Tween (TBST,10 mM Tris pH 8.0, 150 mM NaCl, 0.1% Tween-20), and treated with anti-FLAG (M2, Sigma, 1:1000 dilution in 5% BSA from stock) overnight at 4 °C. The blots were washed with TBST three times for ten min then treated with anti-mouse κ light chain-HRP-conjugated secondary antibody (Southern Biotech, 1:5000 dilution in 5% BSA in TBST). The blots were again washed with TBST three times for ten min and analyzed by standard enhanced chemiluminescence immunoblotting methods (Pierce). The blots were stored at 4 °C. India ink staining was performed by washing the blots copiously with deionized water 3x for ten min then TBST for ten min. The blot was then incubated in 1:1000 dilution of India ink in TBST for 1 h then washed for 30 s with water and ten min with TBS.
Figure S7.
India ink staining of the western blot shown in Figure 2

Figure S8.
Simultaneous treatment Ac₄GalNAz-labeled cells with TMTH and phosphine-FLAG inhibits phosphine-FLAG dependent labeling. Jurkat cells were incubated with Ac₄-GalNAz (50μM) or vehicle for 3 d and then lysed. The lysates were treated with phosphine-FLAG (500μM) at 0°C then TMTH (concentrations from 1 nM to 1 mM) and allowed to warm to room temperature and incubate overnight. The lysates were analyzed.
by Western blot using an anti-FLAG antibody then a secondary antibody conjugated to horse radish peroxidase.

**Figure S9**

![Western blot image](image)

**India ink**

**Figure S9.**
India ink staining of the western blot shown in Figure S8.

**Barstar Expression and Purification**

A plasmid (pQE30-Barstar) containing the *Bacillus amyloliquefaciens* protein Barstar as a 6xHis fusion and with two point mutations for improved stability (Cys53Ala and Cys95Ala) in a pQE30 expression vector was obtained from D. Tirrell (California Institute of Technology). For incorporation of the unnatural amino acid azidohomoalanine (AHA), the *E. coli* methionine auxotrophic strain M15-MA was also obtained from D. Tirrell.

To generate both the wild-type Barstar protein (Barstar-MET) and the AHA containing Barstar protein (Barstar-AHA) we followed an expression protocol similar to that reported by Beatty et. al.\[^5\] Briefly, the pQE30-Barstar plasmid was transformed into M15-MA cells and individual transformants were used to inoculate 5 mL of M9 minimal media supplemented with 0.04 mg/mL of each of the 20 amino acids, 1mM MgSO₄, 5 µg/mL thiamine, 0.4% glucose, 200 µg/mL ampicillin and 35 µg/mL kanamycin (i.e., M9 complete media with 20 amino acids). After an overnight incubation at 37 °C with shaking, 1 mL was transferred to 50 mL of M9 complete media with 20 amino acids. After reaching an OD₆₀₀ of 1.0, the cells were pelleted (6100g for 10 min at 4 °C) and washed three times with a sterile solution of ice-cold 0.9% (w/v) NaCl. The culture was resuspended in M9 complete medium with 19 amino acids (no methionine) and divided in half; one sample was supplemented with 1mM methionine (Barstar-MET) while the other was supplemented with 1mM AHA (Barstar-AHA). After 15 min at 37 °C with
shaking, protein expression was induced with 1 mM IPTG. Cultures were clarified by centrifugation 3 h post induction and the Barstar proteins were purified under denaturing conditions using Ni-NTA spin columns according to the manufacturer’s specifications (Qiagen). The purified proteins were verified by ESI-HRMS, calcd for Barstar-MET (C_{52}H_{808}N_{146}O_{156}S_{2}): 11677.1 Da (avg. mass), found 11676.8 Da; calcd for Barstar-AHA (C_{519}H_{802}N_{152}O_{156}): 11667.1 Da (avg. mass), found 11667.4 Da

**Conjugation Reactions**

10 µg of Barstar-MET or Barstar-AHA were acetone precipitated and dissolved in 2.5 µL of PBS. 2.5 µL of 1 mM TMTH in 1% DMSO in PBS were then added to the samples and the samples allowed to incubate at room temperature for 5 d. The proteins were then acetone precipitated and submitted to LC-MS analysis.

**LC-MS Analysis**

Samples were subjected to RP chromatography with an Agilent 1200 LC system that was connected in-line with an LTQ Orbitrap XL hybrid mass spectrometer. External mass calibration was performed prior to analysis. A binary solvent system consisting of buffer A (0.1% formic acid in water (v/v)) and buffer B (0.1% formic acid in acetonitrile (v/v)) was employed.

For the intact Barstar samples, the mass spectrometer was outfitted with an Ion Max electrospray ionization source. The LC was equipped with a Poroshell 300SB-C8 column and a 100 mL sample loop. For each run, after 100 to 200 picomoles of protein was injected onto the column, analyte trapping was performed for 5 min with 0.5% B, followed by a linear gradient from 30% to 95% B over 19.5 min, and finished with a washing step in 95% B for 5 min. The flow rate was maintained at 90 mL min⁻¹. Mass spectra were recorded in positive ion mode over the m/z scan range of 500 to 2000 using the Orbitrap mass analyzer in full-scan, profile mode. Raw mass spectra were processed using Xcalibur (version 4.1, Thermo) and measured charge state distributions were deconvoluted using ProMass (version 2.5 SR-1, Novatia), using the default “small protein” parameters and a background subtraction factor of 1.5.

**Scheme S4**

![Scheme S4](image)

2-thiocyclooct-7-yn-1-(p-nitrophenyl)carbonate (S17).

ThiaOCT 8 (6.0 mg, 0.042 mmol, 1.0 equiv) was dissolved in dichloromethane (1.6 mL, anhydrous). To this solution, pyridine (21 µL, 0.26 mmol, 6.2 equiv, anhydrous) and p-nitrophenylchloroformate (20 mg, 0.10 mmol, 2.4 equiv) were added. The reaction mixture was stirred overnight and the resulting mixture was evaporated to dryness. The crude product was purified by silica gel chromatography (10:1 to 6:1 hexane/EtOAc) to
give desired product S17 (4.5 mg, 0.015 mmol, 36%). Rf = 0.55 in 3:1 hexane/EtOAc. \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 8.26 (d, \(J = 9.1\) Hz, 2H), 7.37 (d, \(J = 9.5\) Hz, 2H), 5.14-5.09 (m, 1H), 3.21 (d, \(J = 15.6\) Hz, 1H), 3.07 (dt, \(J = 14.4, 3.7\) Hz, 1H), 2.91 (dd, \(J = 15.2, 9.0\) Hz, 1H), 2.81-2.69 (m, 2H), 2.54-2.37 (m, 3H). \(13\)C NMR (150 MHz, CDCl\(_3\)) \(\delta\) 155.3, 151.4, 145.2, 125.3, 121.7, 92.3, 88.0, 82.9, 40.4, 38.5, 25.4, 23.2. HRMS (EI): calcd for \(\text{C}_{14}\text{H}_{13}\text{O}_{5}\text{NS}^+ [M]^+\), 307.0514; found, 307.0514; \([\text{M-NO}_2]^+\), 261; \([\text{M-C}_7\text{H}_5\text{NO}_5]^+\), 124.

2-thiocyclooct-7-yn-1-(Biotin-PEG\(_3\))carbamate (S18).

2-thiocyclooct-7-yn-1-\((p\)-nitrophenyl\)carbonate S17 (4.5 mg, 0.015 mmol, 1.0 equiv) was dissolved in DMF (380 \(\mu\)L, anhydrous). To this solution, triethylamine (5 \(\mu\)L, 0.036 mmol, 2.4 equiv) and Biotin-PEG\(_3\)-amine\([6]\) (7.4 mg, 0.0156 mmol, 1.0 equiv) were added. The reaction was stirred overnight at rt. The following day the reaction mixture was evaporated to dryness and the crude product was purified by silica gel chromatography (10:1 to 4:1 hexanes/EtOAc). This procedure resulted in 4.2 mg of desired product (0.0068 mmol, 45 %). Rf = 0.2 in 3:1 hexane/EtOAc. \(^1\)H NMR (600 MHz, MeOD): \(\delta\) 4.94-4.90 (m, 1H), 4.50-4.48 (m, 1H), 4.31-4.29 (m, 1H), 3.64-3.61 (m, 4H), 3.60-3.57 (m, 4H), 3.53-3.50 (m, 4H), 3.26 (t, \(J = 6.8\) Hz, 2H), 3.22-3.16 (m, 3H), 3.04 (d, \(J = 14.9\) Hz, 2H), 2.93 (dd, \(J = 12.8, 4.9\) Hz, 1H), 2.77 (dd, \(J = 15.1, 9.0\) Hz, 1H), 2.70 (d, \(J = 12.7\) Hz, 2H), 2.59-2.56 (m, 1H), 2.40-2.37 (m, 1H), 2.32-2.21 (m, 2H), 2.20 (t, \(J = 7.4\) Hz, 2H), 1.77-1.68 (m, 8H), 1.43-1.46 (m, 2H). \(13\)C NMR (150 MHz, MeOD) \(\delta\) 174.5, 164.7, 155.6, 88.7, 78.7, 70.1, 69.8, 69.8, 68.5, 68.3, 61.9, 60.2, 55.6, 40.7, 39.6, 38.0, 37.7, 36.4, 35.4, 29.4, 29.0, 28.4, 28.1, 25.4, 24.8, 22.5, 20.1. HRMS (ESI): calcd for \(\text{C}_{28}\text{H}_{47}\text{O}_{7}\text{N}_{4}\text{S}_{2}^+ [M+H]^+\), 615.2881; found, 615.2874.

Cell culture procedures

Jurkat (human T-cell lymphoma) cells were grown in RPMI-1640 media containing 10\% fetal bovine serum, streptomycin (0.1 mg/ mL), and penicillin (100 units/ mL). Cells were grown in the presence of 5\% CO\(_2\) and maintained at densities between 1 \(\times\) \(10^5\) and 2 \(\times\) \(10^6\) cells/ mL.

Cell surface azide labeling

Jurkat cells were incubated in the described media containing 25 \(\mu\)M Ac\(_4\)ManNAz or DMSO vehicle for 3 days. The cells were then pelleted (1500 rpm, 3 min, 4 \(^\circ\)C) and resuspended in 10 mL FACS buffer (PBS with 1\% fetal bovine serum) twice. The cell suspension was then transferred to a 96 well V-bottom plate. The cells were pelleted by centrifugation (3500 rpm, 3 min, 4 \(^\circ\)C). The cells were then resuspended PBS containing 250 \(\mu\)M thiaOCT-biotin, DIMAC-biotin, or vehicle and incubated at rt for 1 h. The cells were washed with FACS buffer (3 \(\times\) 200 \(\mu\)L, 3500 rpm, 3 min, 4 \(^\circ\)C) and resuspended in FACS buffer containing FITC-avidin (100 \(\mu\)L, 1:200 dilution of 1 mg/ mL stock, Sigma-Aldrich) and incubated in the dark at 4 \(^\circ\)C for 15 minutes. The cells were washed with FACS buffer (1 \(\times\) 200 \(\mu\)L, 3500 rpm, 3 min, 4 \(^\circ\)C) and again subjected to FITC-avidin (same conditions as first incubation). The cells were concentrated by centrifugation (3500 rpm, 3 min, 4 \(^\circ\)C) and resuspended in 200 \(\mu\)L cold FACS buffer three times. The cell
suspensions were then diluted to 400 μL for flow cytometry analysis. Flow cytometry was performed on a BD Biosciences FACSCalibur flow cytometer equipped with a 488-nm argon laser.

**Figure S10**

A) Schematic for flow cytometry analysis of live cell labeling with thiaOCT. B) Quantification of cell-surface labeling with thiaOCT-biotin (S18). Cells are grown in the presence (red bars) or absence (blue bars) of 25 μM Ac₄ManNAz for 3 days. The cells were then treated with no reagent, 250 μM thiaOCT-biotin (S18), or 250 μM DIMAC-biotin followed by FITC-avidin. Error bars represent the standard deviation of three replicate experiments.

**Figure S10.**

ThiaOCT can label azides on cell surfaces. A) Schematic for flow cytometry analysis of live cell labeling with thiaOCT. B) Quantification of cell-surface labeling with thiaOCT-biotin (S18). Cells are grown in the presence (red bars) or absence (blue bars) of 25 μM Ac₄ManNAz for 3 days. The cells were then treated with no reagent, 250 μM thiaOCT-biotin (S18), or 250 μM DIMAC-biotin followed by FITC-avidin. Error bars represent the standard deviation of three replicate experiments.
Figure S11.
Representative histograms of fluorescence (x-axis) vs % of total cell counts (y-axis) for the experiment described in Figure S10. Jurkat cells were treated with (+ ManNAz) or without (- ManNAz) 25 μM Ac₄ManNAz for 3 days and then treated with 250 μM thiaOCT-biotin, DIMAC-biotin, or vehicle followed by FITC-avidin.
Representative forward-scatter (x-axis) and side-scatter (y-axis) plots for the experiment described in Figure S10. Jurkat cells were treated with (+ ManNAz) or without (-ManNAz) 25 μM Ac₄ManNAz for 3 days and then treated with 250 μM thiaOCT-biotin, DIMAC-biotin,⁶ or vehicle followed by FITC-avidin.
Figure S13.
Cytotoxicity analysis of thiaOCT-biotin S18. Jurkat cells were treated with (ManNAz, red bars) or without (NoAz, blue bars) 25 μM Ac₄ManNAz for 3 days and then treated with 250 μM thiaOCT-biotin or vehicle followed by FITC-avidin. Prior to flow cytometry analysis, the cells were treated with 7-amino-actinomycin D (7-AAD) following the provided procedure. The samples were diluted and analyzed by flow cytometry. The error bars represent standard deviations from three replicates.

Figure S14.
Representative FL3 vs. FL1 scatter plots for the flow cytometry experiments described in Figure S13. In all plots, the x-axis indicates the degree of cell-surface glycan labeling as measured by FITC fluorescence (FL1), and the y-axis represents the degree of 7-AAD (FL3, cell viability marker) measured. Jurkat cells were treated with (A, B) or without (C) 25 μM Ac₄ManNAz for 3 days and then treated with 250 μM thiaOCT-biotin (A) or
vehicle (B, C) followed by FITC-avidin. Prior to flow cytometry analysis, the cells were treated with 7-AAD following the provided procedure. The samples were diluted and analyzed by flow cytometry.

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mixture of diastereomers

TIPSO

$\text{O}$

1 2 3 4 5 6 7 8

$\text{7}$ $\text{13}$ $\text{3}$ $\text{1}$
mixture of diastereomers
TIPSO

major diastereomer
mixture of diastereomers
mixture of diastereomers
* ethyl acetate
** water
*** dichloromethane
* dichloromethane
* water
** dichloromethane
* dichloromethane
$^{1}H$ starting parameters (zg30)
DRX-500 zBBO probe

![Chemical structure with proton NMR spectrum]

**Major regioisomer**
* water
dichloromethane

**

[Chemical structure diagram]
in CD$_3$CN

* = dichloromethane
** = water
+ regioisomer
S15 + S16

S96