Bioconjugation by Native Chemical Tagging of C–H Bonds

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SUPPORTING INFORMATION – PROCEDURES

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General Experimental Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Yields refer to chromatographically and spectroscopically (1H NMR) homogeneous material, unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm E. Merck silica plates (60F-254), using UV light as the visualizing agent and KMnO$_4$ or acidic solution of p-anisaldehyde and heat as a developing agent. Flash silica gel chromatography was performed using E. Merck silica gel (60, particle size 0.043–0.063 mm). Preparative HPLC was performed using a Waters Atlantis dC$_{18}$ OBD 10 m column with dimension 30 x 250 mm, unless otherwise noted. NMR spectra were recorded on Bruker DRX-600, DRX-500, AMX-400, and Varian INOVA-399 instruments and were calibrated using residual undeuterated solvent as an internal reference (CDCl$_3$ @ 7.26 ppm 1H NMR, 77.2 ppm $^{13}$C NMR; CD$_3$OD @ 3.31 ppm 1H NMR, 49.0 ppm $^{13}$C NMR; CD$_3$CN @ 1.94 ppm 1H NMR, 1.3 ppm $^{13}$C NMR). The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, sept= septet, m = multiplet, br = broad. Gas chromatography was performed on an Agilent Technologies 7890A instrument using a 30 meter DB-5 column with an internal diameter of 0.250 millimeters; reaction species were calibrated against tetradecane as an internal standard. In situ reaction calorimetry was performed using an Omnical Insight-CPR-220 calorimeter. High-resolution mass spectra (HRMS) were recorded on an Agilent LC/MSD TOF mass spectrometer by electrospray ionization time-of-flight reflectron experiments. IR experiments were recorded on a Perkin-Elmer Spectrum BX FTIR spectrometer. Melting points were recorded on a Fisher-Johns 12-144 melting point apparatus and were uncorrected.

Synthesis of sodium 7-azido-1,1-difluoroheptane-1-sulfinate (DAAS-Na; 3):

\[
\begin{align*}
\text{Cl} & \quad \text{NaN}_3 \\
\text{DMF, 65°C, 6 h} & \quad 100% \\
\end{align*}
\]

To a solution of 4$^{[1]}$ (4.77 g, 15.3 mmol, 1.0 equiv) in DMF (40 mL) was added NaN$_3$
(4.7 g, 72.3 mmol, 4.7 equiv) and NaI (40 mg, 2.67 mmol, 0.17 equiv), and the mixture was stirred at 65 °C for about 10 h under Ar. After completion, the reaction mixture was diluted with ethyl acetate (100 mL), and washed with H₂O (3 × 100 mL) and brine, dried over Na₂SO₄. Upon concentration in vacuo, the crude product 5 (4.87 g, 100%) was obtained as a light yellow oil, which could be used without further purification.

**CAUTION!** This reaction uses sodium azide, which is an explosive and toxic compound. It is important to not expose this reagent to heavy metals (because even more explosive metal azides can form) or to acid (formation of toxic hydrazoic acid). We have successfully run this reaction in up to 7.7-gram scale (of compound 4), and we have conducted this reaction numerous times on ~5-gram scale without any explosions. However, a safety assessment should be performed before attempting this reaction on greater than 10 grams of starting material.

**Physical state:** light yellow oil;

*Rf* = 0.70 (silica gel, 1:1.5 EtOAc:hexanes);

**¹H NMR (400 MHz, CDCl₃):** δ 8.86 (ddd, *J* = 4.7, 1.8, 0.9 Hz, 1 H), 8.16 (dt, *J* = 7.9, 1.0 Hz, 1 H), 8.03 (td, *J* = 7.8, 1.7 Hz, 1H), 7.66 (ddd, *J* = 7.7, 4.7, 1.2 Hz, 1 H), 3.26 (t, *J* = 6.9 Hz, 2 H), 2.46 – 2.33 (m, 2 H), 1.71 – 1.64 (m, 2 H), 1.63 – 1.56 (m, 2 H), 1.47 – 1.36 (m, 4 H) ppm;

**¹³C NMR (151 MHz, CDCl₃):** δ 152.4, 151.0, 138.4, 128.7, 126.5, 125.3 (t, *J*<sub>CF</sub> = 287.5 Hz), 51.4, 30.1 (t, *J*<sub>CF</sub> = 19.8 Hz), 28.7, 28.7, 26.4, 20.8 (t, *J*<sub>CF</sub> = 3.3 Hz) ppm;

**¹⁹F NMR (376 MHz, CDCl₃):** δ –102.4 ppm;

**IR (neat):** ν = 2938, 2090, 1342, 1167, 734, 597, 565 cm⁻¹;

**HRMS (ESI-TOF):** calc’d for [C<sub>12</sub>H₁₄F₂N₄O₂S⁺H⁺] 319.1035; found 319.1034.
EtSH (1.3 mL, 1.3 equiv) was added slowly to a suspension of NaH (95%; 502 mg, 19.9 mmol, 1.3 equiv) in THF (40 mL) at 0 °C under Ar (Caution: EtSH has a strongly disagreeable odor, so it should be handled in a well-ventilated hood!) After stirring at 0 °C for 5 min, a THF (30 mL) solution of 2-[(7-azido-1,1-difluoroheptylsulfonyl)pyridine (5) (4.87 g, 15.3 mmol, 1.0 equiv) was added. The mixture was stirred at 0 °C for 12 h. After the removal of solvent in vacuo, the residue was diluted with H₂O (20 mL) and neutralized to pH = 7 with H₂SO₄ (1 M). The resultant mixture was then extracted with Et₂O (3 × 30 mL) to remove 2-(ethylthio)pyridine and EtSH. The aqueous phase was concentrated, and the residue was purified by column chromatography (1:6 MeOH:DCM as eluent) yielding DAAS-Na (3) (3.5 g, 87% yield).

Physical state: white solid (m.p. 134 – 136 °C);

\( R_f = 0.40 \) (silica gel, 1:3 MeOH:DCM);

\(^1\)H NMR (400 MHz, D₂O): \( \delta 3.32 \ (t, J = 6.9 \text{ Hz}, 1 \text{ H}), 2.06 – 1.93 \ (m, 2 \text{ H}), 1.65 – 1.53 \ (m, 4 \text{ H}), 1.44 – 1.36 \ (m, 4 \text{ H}) \) ppm;

\(^13\)C NMR (151 MHz, D₂O): \( \delta 128.3 \ (t, J_{\text{CF}} = 284.4 \text{ Hz}), 51.9, 28.9, 28.7 \ (t, J_{\text{CF}} = 21.2 \text{ Hz}), 28.5, 26.4, 21.0 \ (t, J_{\text{CF}} = 4.0 \text{ Hz}) \) ppm;

\(^19\)F NMR (376 MHz, D₂O): \( \delta –115.4 \) ppm;

IR (neat): \( \nu = 2932, 2360, 2340, 2087, 1655, 1419, 1160, 1029, 824, 727 \text{ cm}^{-1} \);

Elem. Anal.: C₇H₁₂F₂N₃NaO₃S (263.24), calc’d: C, 31.94; H, 4.59; S, 12.18; N, 15.96; found: C, 32.05; H, 4.54; S, 12.46; N, 15.66.

Functionalization of bioactive molecules with DAAS-Na (3), standard procedure:

To a solution of bioactive molecule (0.05 mmol, 1.0 equiv), DAAS-Na (3; 39.6 mg, 0.15 mmol, 3.0 equiv) and ZnCl₂ (10.2 mg, 0.075 mmol, 1.5 equiv) in DMSO (or DCM) (0.2 mL) and H₂O (0.08 mL) was added TsOH•H₂O (9.5 mg, 0.05 mmol, 1.0 equiv) or occasionally, TFA (4 µL, 0.05 mmol, 1.0 equiv) instead. The reaction mixture was cooled in an ice bath and TBHP (70% solution in water, 0.035 mL, 0.25 mmol, 5.0 equiv) was added dropwise with vigorous stirring and the stirring was continued at this temperature for 5 min. The reaction was warmed to room
temperature or 50 °C and monitored by TLC and LC-MS until completion. For substrates that were not completely consumed in 12 h, a second addition of ZnCl₂ (10.2 mg, 0.075 mmol, 1.5 equiv), DAAS-Na (3; 39.6 mg, 0.15 mmol, 3.0 equiv) and TBHP (0.035 mL, 0.25 mmol, 5.0 equiv) was performed. Upon consumption of starting material, the reaction mixture was partitioned between hexanes (2.0 mL) and water (2.0 mL). The aqueous phase was basified with saturated aqueous NaHCO₃ (2.0 mL), then extracted with ethyl acetate (2 × 2.0 mL). The combined organic layers were washed with brine and dried over Na₂SO₄, concentrated in vacuo and purified with column chromatography or PTLC.

**NOTE:** If the addition of TBHP is performed too rapidly, the resulting exotherm can result in reduced yield and selectivity. This is especially important on larger scales, where a syringe pump may be used.
Camptothecin analog (1a)

On a 0.05 mmol scale, the standard procedure was followed with a reaction time of 29 h at room temperature in DCM:H₂O (0.2:0.08 mL) to provide 1a (10.9 mg, 42% yield).

Physical state: yellow solid (m.p. = 185 – 190 °C, decomposed);

R₇ = 0.80 (silica gel, 1:20 MeOH:DCM);

¹H NMR (400 MHz, CDCl₃): δ 8.27 (dd, J = 12.8, 8.8 Hz, 1 H), 7.86 (t, J = 8.0 Hz, 1 H), 7.72 (t, J = 8.0 Hz, 1 H), 7.68 (s, 1 H), 5.76 (d, J = 16.4 Hz, 1 H), 5.44 (s, 2 H), 5.31 (d, J = 16.4 Hz, 1 H), 3.78 (brs, 1 H), 3.22 (t, J = 6.7 Hz, 2 H), 2.49 – 2.36 (m, 2 H), 1.98 – 1.83 (m, 2 H), 1.58 – 1.50 (m, 4 H), 1.37 – 1.33 (m, 4 H), 1.05 (t, J = 7.3 Hz, 4 H) ppm;

¹³C NMR (151 MHz, CDCl₃): δ 174.0, 157.5, 152.7, 150.2, 150.1, 145.7, 137.5 (t, J CF = 27.6 Hz), 131.1, 130.7, 129.0, 126.1, 124.9, 124.1, 123.9 (t, J CF = 245.2 Hz), 119.3, 98.2, 72.9, 66.5, 51.5 (t, J CF = 9.2 Hz), 51.4, 39.1 (t, J CF = 25.5 Hz), 31.7, 28.8, 28.7, 26.5, 22.2, 8.0 ppm;

¹⁹F NMR (376 MHz, CDCl₃): δ –91.9 (ABq, 2F, Δδ AB = 0.2, J AB = 258.5 Hz) ppm;

IR (neat): ν = 2925, 2360, 2083, 1746, 1658, 1593, 1149, 766 cm⁻¹;

HRMS (ESI-TOF): calc’d for [C₂₇H₂₇F₂N₅O₄+H⁺] 524.2104; found 524.2105.

Buspirone analog (2a)

On a 0.10 mmol scale, the standard procedure was followed. The reaction mixture was stirred at 50 °C for 28.5 h in DMSO:H₂O (0.40:0.16 mL) (second addition of
reagents was performed after 20.5 h) to provide 2a (2.4 mg, 9% yield, 78% brsm).

Physical state: colorless oil;

$R_f = 0.17$ (silica gel, 1:20 DCM:MeOH);

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.41 (d, $J = 4.8$ Hz, 1 H), 6.73 (d, $J = 4.8$ Hz, 1 H), 3.84 – 3.78 (m, 6 H), 3.26 (t, $J = 7.2$ Hz, 1 H), 2.59 (s, 4 H), 2.26 – 2.14 (m, 2 H);

$^{13}$C NMR (151 MHz, CDCl$_3$): $\delta$ 172.4, 163.2 (t, $J_{CF} = 28.4$ Hz), 161.6, 159.5, 121.0 (t, $J_{CF} = 241.6$ Hz), 104.8, 58.4, 53.1, 51.5, 45.1, 43.8, 39.7, 39.5, 37.7, 35.5 (t, $J_{CF} = 23.9$ Hz), 29.9, 29.0, 28.8, 26.6, 26.2, 24.3, 21.9 (t, $J_{CF} = 3.8$ Hz) ppm;

$^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ –102.0 ppm;

IR (neat): $\nu = 2931, 2858, 2360, 2094, 1668, 1343, 1130, 819$ cm$^{-1}$;

HRMS (ESI-TOF): calc’d for [C$_{28}$H$_{42}$F$_2$N$_8$O$_2$+H$^+$] 561.3472; found 561.3482.

Sceptrin analog (6a)

On a 0.05 mmol scale, the standard procedure was followed with a reaction time of 1 h in DMSO:H$_2$O (0.2:0.08 mL) at 50 °C. Then, the reaction mixture was directly purified by HPLC to provide 6a (17.3 mg, 35% yield).

Physical state: yellow solid (m.p. = 129 – 132 °C);

$[\alpha]_D^\circ = –12.9$ (c = 0.58, MeOH)

$R_f = 0.33$ (silica gel, 1:6:20 H$_2$O:MeOH:CHCl$_3$, saturated with NH$_3$);

$^1$H NMR (400 MHz, CD$_3$OD): $\delta$ 6.91 (d, $J = 1.6$ Hz, 1 H), 6.85 (s, 1 H), 6.70 (d, $J = 1.6$ Hz, 1 H), 6.60 (s, 2H), 3.53 – 3.48 (m, 4 H), 3.30 – 3.27 (m, 2 H), 3.04 – 3.00 (m, 4 H), 2.40 – 2.37 (m, 2 H), 1.74 – 1.70 (m, 2 H), 1.62 – 1.59 (m, 2 H), 1.45 – 1.42 (m, 4 H) ppm;

$^{13}$C NMR (151 MHz, D$_2$O): $\delta$ 192.2, 163.1, 162.0, 149.0, 132.1, 130.8, 128.6, 128.6,
127.1, 123.1, 116.8, 113.4, 110.4, 103.4, 97.6, 52.4, 49.6, 44.6, 44.0, 42.9, 42.3, 41.3, 39.3, 29.8, 29.8, 27.6, 25.1 ppm;

**IR (neat):** $\nu = 2926, 2854, 2096, 1669, 1566, 1185, 1131, 719$ cm$^{-1}$;

**HRMS (ESI-TOF):** calc’d for [C$_{29}$H$_{35}$Br$_2$N$_{13}$O$_3$+H$^+$] 772.1425; found 772.1442.

**Papaverine analogs (7a and 7b)**

On a 0.1 mmol scale, the standard procedure was followed, employing TFA (8 µL, 0.10 mmol, 1.0 equiv) instead of TsOH•H$_2$O as an acid additive. The reaction was conducted in DMSO:H$_2$O (0.4:0.16 mL) at 60 ºC for 11 h to yield 7a (8.5 mg, 17% yield) and 7b (8.0 mg, 16% yield).

**Analog 7a**

**Physical state:** colorless oil;

$R_f = 0.60$ (silica gel, 1:1 EtOAc:hexanes);

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.76 (s, 1 H), 7.35 (s, 1H), 7.12 (s, 1H), 6.84 (d, $J = 1.6$ Hz, 1 H), 6.78 (dd, $J = 6.8$, 1.6 Hz, 1 H), 6.75 (d, $J = 6.8$ Hz, 1 H), 4.54 (s, 2 H), 4.01 (s, 3 H), 3.92 (s, 3 H), 3.82 (s, 3 H), 3.77 (s, 3 H), 3.23 (t, $J = 5.6$ Hz, 1 H), 2.48 – 2.38 (m, 2 H) ppm;

$^{13}$C NMR (151 MHz, CDCl$_3$): $\delta$ 158.2, 152.9, 150.7, 149.1, 147.7, 146.3 (t, $J_{CF} = 28.4$ Hz), 133.4, 132.1, 123.2, 122.7 (t, $J_{CF} = 241.6$ Hz), 120.6, 115.1 (t, $J_{CF} = 5.4$ Hz), 112.0, 111.2, 106.2, 104.3, 56.3, 56.1, 56.0, 55.8, 51.5, 42.2, 36.5 (t, $J_{CF} = 25.7$ Hz), 29.0, 28.8, 26.7, 22.5 (t, $J_{CF} = 3.8$ Hz) ppm;

$^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ –98.7 ppm;

**IR (neat):** $\nu = 2929, 2855, 2092, 1508, 1424, 1252, 1026$ cm$^{-1}$;

**HRMS (ESI-TOF):** calc’d for [C$_{27}$H$_{32}$F$_2$N$_4$O$_4$+H$^+$] 515.2464; found 515.2475.
**Analog 7b**

**Physical state:** colorless oil;

$R_f = 0.42$ (silica gel, 1:1 EtOAc:hexanes);

$^1\text{H NMR (400 MHz, CDCl}_3$: $\delta$ 8.51 (s, 1 H), 7.44 (s, 1 H), 7.42 (s, 1 H), 6.82 (s, 1 H), 6.76 – 6.81 (m, 2 H), 4.55 (s, 2 H), 4.01 (s, 3 H), 3.91 (s, 3 H), 3.83 (s, 3 H), 3.78 (s, 3 H), 3.24 (t, $J = 6.8$ Hz, 1 H), 2.43 – 2.31 (m, 2 H) ppm;

$^{13}\text{C NMR (151 MHz, CDCl}_3$: δ 160.5, 152.8, 149.8, 149.2, 147.8, 139.0 (t, $J_{\text{CF}} = 6.0$ Hz), 131.8, 129.9, 124.2 (t, $J_{\text{CF}} = 25.7$ Hz), 123.9 (t, $J_{\text{CF}} = 241.6$ Hz), 123.3, 120.7, 111.9, 111.3, 105.0, 103.3, 56.1, 56.0, 56.0 (2C), 51.5, 42.7, 38.4 (t, $J_{\text{CF}} = 27.2$ Hz), 28.9, 28.8, 26.6, 22.7 (t, $J_{\text{CF}} = 3.8$ Hz) ppm;

$^{19}\text{F NMR (376 MHz, CDCl}_3$: δ –92.3 ppm;

IR (neat): $\nu$ = 2933, 2858, 2359, 2091, 1511, 1419, 1254, 1026, 850 cm$^{-1}$;

HRMS (ESI-TOF): calc’d for [C$_{27}$H$_{32}$F$_2$N$_4$O$_4$+H$^+$] 515.2464; found 515.2462.

**Pioglitazone analog (8a)**

On a 0.05 mmol scale, the standard procedure was followed, employing TFA (4 µL, 0.05 mmol, 1.0 equiv) instead of TsOH$\cdot$H$_2$O as an acid additive. The reaction mixture was stirred at 50 ºC for 11 h in DMSO:H$_2$O (0.2:0.08 mL). After neutralizing with saturated aqueous NaHCO$_3$ to pH = 7 at 0 ºC, the reaction mixture was extracted with ethyl acetate (3 × 2.0 mL), then the combined organic layers were washed with brine and dried over Na$_2$SO$_4$. Upon removal of solvents in vacuo, the residue was purified by PTLC to yield 8a (12.9 mg, 48% yield).

**Physical state:** light yellow oil;

$R_f = 0.64$ (silica gel, 1:15 MeOH:DCM);

$^1\text{H NMR (400 MHz, CDCl}_3$: $\delta$ 8.06 (brs, 1 H), 7.55 (d, $J = 8.0$ Hz, 1 H), 7.24 (d, $J = 7.6$ Hz, 1 H), 7.13 (d, $J = 8.8$ Hz, 2 H), 6.85 (d, $J = 8.8$ Hz, 2 H), 4.50 (dd, $J = 9.6$, 4.0 Hz, 1 H), 4.01 (s, 3 H), 3.83 (s, 3 H), 3.87 (s, 3 H), 3.84 (s, 3 H), 3.24 (t, $J = 6.8$ Hz, 1 H), 2.43 – 2.31 (m, 2 H) ppm;
Hz, 1 H), 4.33 (t, J = 6.8 Hz, 2 H), 3.43 (dd, J = 14.4, 4.0 Hz, 1H), 3.26 – 3.20 (m, 4 H), 3.11 (dd, J = 14.4, 9.2 Hz, 1 H), 2.89 – 2.83 (m, 2 H), 2.41 – 2.37 (m, 2 H) ppm;

13C NMR (151 MHz, CDCl3): δ 173.8, 170.1, 158.5, 154.2, 151.0 (t, JCF = 28.7 Hz), 139.4, 136.6, 130.5, 127.8, 124.7, 124.2 (t, JCF = 240.1 Hz), 114.9, 67.1, 53.8, 51.6, 37.9, 37.3, 36.2 (t, JCF = 24.2 Hz), 29.2, 28.9, 26.7, 24.7 (t, JCF = 4.5 Hz), 22.3 (t, JCF = 4.5 Hz), 16.0 ppm;

19F NMR (376 MHz, CDCl3): δ –93.0 ppm;

IR (neat): ν = 2936, 2857, 2092, 1467, 1359, 1016, 759, 585 cm–1;

HRMS (ESI-TOF): calc’d for [C26H31F2N5O3S+H]+ 532.2188; found 532.2186.

Nevirapine analogs (9a, 9b and 9c)

On a 0.1 mmol scale, the standard procedure was followed, employing TFA (8 µL, 0.10 mmol, 1.0 equiv) instead of TsOH•H2O as an acid additive. The reaction was conducted in DMSO:H2O (0.4:0.16 mL) at 50 ºC for 11 h to yield 9a (4.0 mg, 9% yield), 9b (3.7 mg, 8% yield) and 9c (4.1 mg, 9% yield).

Analog 9a

Physical state: white solid (m.p. 156 – 157 ºC, decomposed);

Rf = 0.65 (silica gel, 1:15 MeOH:DCM);

1H NMR (400 MHz, CDCl3): δ 8.23 (d, J = 8.0 Hz, 1 H), 8.16 (d, J = 4.8 Hz, 1 H), 7.42 (s, 1 H), 7.38 (d, J = 8.0 Hz, 1 H), 6.94 (d, J = 5.2 Hz, 1 H), 3.75 – 3.70 (m, 1 H), 3.25 (t, J = 6.8 Hz, 2 H), 2.36 (s, 3 H) ppm;

13C NMR (151 MHz, CDCl3): δ 167.6, 160.0, 157.0 (t, JCF = 30.2 Hz), 153.4, 144.7, 142.1, 138.9, 124.6, 122.3, 121.5 (t, JCF = 240.0 Hz), 120.6, 115.0 (t, JCF = 3.0 Hz),
1H NMR (400 MHz, CDCl₃): δ 8.54 (dd, J = 4.8, 2.0 Hz, 1 H), 8.13 (dd, J = 7.6, 2.0 Hz, 1 H), 7.51 (s, 1 H), 7.27 (s, 1 H), 7.08 (dd, J = 5.2 Hz, 1 H), 3.75 – 3.70 (m, 1 H), 3.25 (t, J = 6.8 Hz, 2 H), 2.39 (s, 3 H);

13C NMR (151 MHz, CDCl₃): δ 168.1, 160.1, 153.0, 152.4, 149.1 (t, J_CF = 30.2 Hz), 140.8, 139.2, 125.4, 121.8 (t, J_CF = 241.6 Hz), 119.9, 119.2, 118.3 (t, J_CF = 3.0 Hz), 51.5, 35.5 (t, J_CF = 24.2 Hz), 29.9, 29.1, 28.8, 26.7, 22.3 (t, J_CF = 3.0 Hz), 18.2, 9.2, 8.9;

19F NMR (376 MHz, CDCl₃): δ -96.9 (ABq, 2F, Δδ_AB = 6.6, J_AB = 255.7 Hz);

IR (neat): ν = 2929, 2856, 2083, 1655, 1385, 790, 598 cm⁻¹;

HRMS (ESI-TOF): calc’d for [C₂₂H₂₄F₂N⁷O+H⁺] 442.2161; found 442.2171.

Analog 9c

Physical state: white solid (m.p. 116 – 119 °C);

R_f = 0.48 (silica gel, 1:15 MeOH:DCM);

1H NMR (400 MHz, CDCl₃): δ 8.49 (d, J = 4.8 Hz, 1 H), 8.14 (d, J = 4.8 Hz, 1 H), 7.56 (s, 1 H), 7.09 (d, J = 5.2 Hz, 1 H), 6.95 (dd, J = 4.8 Hz, 1 H), 3.79 – 3.76 (m, 1 H), 3.27 (t, J = 6.8 Hz, 2 H), 2.72 – 2.56 (m, 1 H), 2.34 (s, 3 H), 0.65 – 0.59 (m, 1 H), 0.48 – 0.42 (m, 1 H) ppm;

13C NMR (151 MHz, CDCl₃): δ 167.6, 162.0, 155.6, 150.2, 148.4 (t, J_CF = 28.7 Hz), 144.7, 140.4, 124.8, 122.7 (t, J_CF = 243.9 Hz), 122.3, 118.0 (d, J_CF = 6.0 Hz), 117.6
(dd, $J_{CF} = 9.1, 4.5$ Hz), 51.5, 38.8 (t, $J_{CF} = 25.7$ Hz), 29.4, 28.9, 28.8, 26.6, 22.0, 17.9, 8.7, 8.6 ppm;

$^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ –94.3 (ABq, 2F, $\Delta \delta_{AB} = 3.4$, $J_{AB} = 244.4$ Hz) ppm;

IR (neat): $\nu = 2923, 2853, 2089, 1664, 1374, 795, 583$ cm$^{-1}$;

HRMS (ESI-TOF): calc’d for [C$_{22}$H$_{24}$F$_2$N$_7$O+H$^+$] 442.2161; found 442.2168.

Milrinone analog (10a)

On a 0.10 mmol scale, the standard procedure was followed with a reaction time of 6 h at 50 $^\circ$C in DMSO:H$_2$O (0.4:0.16 mL) to provide 10a (3.6 mg, 8% yield, 40% brsm).

Physical state: white solid (m.p. = 210 – 215 $^\circ$C, decomposed);

$R_f = 0.60$ (silica gel, 4:1 EtOAc:hexanes);

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.77 – 8.76 (m, 1 H), 7.88 (m, 1 H), 7.54 (s, 1 H), 7.28 – 7.27 (m, 1 H), 3.26 (t, $J = 6.8$ Hz, 2 H), 2.52 (s, 3 H), 2.43 – 2.30 (m, 2 H), 1.61 – 1.47 (m, 4 H), 1.44 – 1.37 (m, 4 H) ppm;

$^{13}$C NMR (151 MHz, CDCl$_3$): $\delta$ 162.4, 156.2 (t, $J_{CF} = 29.6$ Hz), 150.6, 150.3, 149.3, 144.7, 124.7, 121.6 (t, $J_{CF} = 242.2$ Hz), 120.1, 117.9, 114.9, 102.8, 51.5, 36.1 (t, $J_{CF} = 25.1$ Hz), 28.9, 28.8, 26.6, 22.1 (t, $J_{CF} = 3.6$ Hz), 18.9 ppm;

$^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ –99.0 ppm;

IR (neat): $\nu = 2930, 2858, 2097, 1672, 1178$ cm$^{-1}$;

HRMS (ESI-TOF): calc’d for [C$_{19}$H$_{26}$F$_2$N$_6$O+H$^+$] 387.1739; found 387.1712.
Bisacodyl analog (11a)

On a 0.05 mmol scale, the standard procedure was followed with a reaction time of 5 h at 50 °C in DMSO:H₂O (0.2:0.08 mL). The reaction mixture was then diluted with 1 M HCl, and extracted with hexanes. The organic phase was concentrated \textit{in vacuo} to yield a crude mixture containing hydrolyzed side product and desired product. This mixture was treated with acetic anhydride (0.028 mL, 0.3 mmol, 6.0 equiv) and pyridine (0.048 mL, 0.6 mmol, 12.0 equiv) in DCM (0.3 mL). Upon completion of the acetylation, the mixture was concentrated \textit{in vacuo} and purified by PTLC (DCM:acetone = 60:1) to provide 11a (26.8 mg, 50% yield).

\textbf{Physical state}: colorless oil;
\[ R_f = 0.50 \text{ (silica gel, 1:2 EtOAc:hexanes);} \]

\textbf{1H NMR (400 MHz, CDCl}_3): \[ \delta \] 7.72 \text{ (t, } J = 7.8 \text{ Hz, 1 H}), 7.49 \text{ (dd, } J = 7.8, 0.9 \text{ Hz, 1 H}), 7.19 \text{ (d, } J = 7.2 \text{ Hz, 1 H}), 7.19 – 7.16 \text{ (m, 4 H}), 7.02 – 7.00 \text{ (m, 4 H)}, 5.64 \text{ (s, 1 H)}, 3.21 \text{ (t, } J = 7.0 \text{ Hz, 2 H}), 2.28 \text{ (s, 6 H)}, 2.29 – 2.18 \text{ (m, 2 H)}, 1.56 – 1.49 \text{ (m, 1 H)}, 1.33 – 1.24 \text{ (m, 7 H) ppm;} \]

\textbf{13C NMR (151 MHz, CDCl}_3): \[ \delta \] 169.6, 162.2, 154.6 \text{ (t, } J_{CF} = 29.9 \text{ Hz}), 149.4, 140.1, 137.7, 130.4, 124.8, 122.0 \text{ (t, } J_{CF} = 241.6 \text{ Hz}), 121.5, 118.0 \text{ (t, } J_{CF} = 3.8 \text{ Hz}), 57.9, 51.5, 35.8 \text{ (t, } J_{CF} = 24.9 \text{ Hz}), 28.9, 28.8, 26.6, 22.3 \text{ (t, } J_{CF} = 4.5 \text{ Hz}), 21.3 \text{ ppm;} \]

\textbf{19F NMR (376 MHz, CDCl}_3): \[ \delta \] –97.8 ppm;

\textbf{IR (neat)}: \[ \nu = 2935, 2093, 1756, 1503, 1192, 1014, 912 \text{ cm}^{-1}; \]

\textbf{HRMS (ESI-TOF)}: calc’d for \([C_{29}H_{30}F_2N_4O_4+H]^+\] 537.2308; found 537.2322.
Acridine orange analog (12a)

To a solution of acridine orange hemi(zinc chloride) salt (37.0 mg, 0.10 mmol, 1.0 equiv), DAAS-Na (105.3 mg, 0.4 mmol, 4.0 equiv) and ZnCl$_2$ (20.4 mg, 0.15 mmol, 1.5 equiv) in CHCl$_3$ (0.4 mL) and D$_2$O (0.2 mL) was added TsOH•H$_2$O (38.0 mg, 0.2 mmol, 2.0 equiv). The reaction mixture was cooled to 0 °C and TBHP (70% solution in water, 0.077 mL, 0.55 mmol, 5.5 equiv) was added dropwise with vigorous stirring and the stirring was continued at this temperature for 5 min. The reaction mixture was heated to 50 °C. The starting material was consumed in 1 h (monitored by LC-MS). O$_2$ was bubbled into the reaction mixture for 24 h at room temperature to achieve rearomatization. The mixture was then extracted with DCM (2 × 2.0 mL), and the combined organic layers were washed with saturated NaHCO$_3$ then brine, dried over Na$_2$SO$_4$, and concentrated in vacuo. The resultant residue was purified by column chromatography to get 12a (36.0 mg, 82% yield).

Physical state: red solid (m.p. = 75 – 77 °C);

$R_f$ = 0.60 (silica gel, 1:6 MeOH:DCM);

$^1$H NMR (400 MHz, CDCl$_3$): δ 8.13 (d, $J$ = 9.9 Hz, 1 H), 7.25 (d, $J$ = 2.7 Hz, 1 H), 7.05 (dd, $J$ = 10.0, 2.7 Hz, 1 H), 3.25 (s, 12 H), 3.23 (t, $J$ = 6.8 Hz, 2 H), 2.50 – 2.32 (m, 2 H), 1.70 – 1.55 (m, 4 H), 1.45 – 1.34 (m, 4 H) ppm;

$^{13}$C NMR (151 MHz, CDCl$_3$): δ 153.6, 145.5 (t, $J_{CF}$ = 25.1 Hz), 143.1, 128.2 (t, $J_{CF}$ = 10.6 Hz), 124.6 (t, $J_{CF}$ = 247.8 Hz), 115.9, 113.9, 94.7, 51.4, 40.4 (t, $J_{CF}$ = 24.5 Hz), 40.3, 28.8, 28.7, 26.6, 22.2 ppm;

$^{19}$F NMR (376 MHz, CDCl$_3$): δ –83.8 ppm;

IR (neat): ν = 2928, 2859, 2360, 2340, 2093, 1637, 1596, 1505, 1336, 1125 cm$^{-1}$;

HRMS (ESI-TOF): calc’d for [C$_{24}$H$_{30}$F$_2$N$_6$+H$^+$] 441.2573; found 441.2584.
Atazanavir analogs (13a and 13b)

On a 0.05 mmol scale, the standard procedure was followed, employing TFA (8 µL, 0.10 mmol, 2.0 equiv) instead of TsOH•H₂O as an acid additive. The reaction mixture was stirred at 50 °C for 48 h in DMSO:H₂O (0.15:0.15 mL) (second addition of reagents was performed after 24 h) to provide 13a (10.4 mg, 12% yield) and 13b (8.9 mg, 10% yield).

Analog 13a

Physical state: colorless oil;

\( R_f = 0.80 \) (silica gel, 1:10 MeOH:DCM, ran twice);

\(^1\)H NMR (400 MHz, CDCl₃): \( \delta \) 7.99 (d, \( J = 8.1 \) Hz, 2 H), 7.85 (t, \( J = 7.8 \) Hz, 1 H), 7.76 (d, \( J = 7.9 \) Hz, 1 H), 7.57 (d, \( J = 7.7 \) Hz, 1 H), 7.43 (d, \( J = 8.1 \) Hz, 2 H), 7.24 – 7.12 (m, 5 H), 6.59 (brs, 1 H), 6.43 – 6.39 (m, 1 H), 5.39 – 5.28 (m, 1 H), 5.26 – 5.18 (m, 1 H), 4.82 (brs, 1 H), 4.05 (d, \( J = 14.3 \) Hz, 1 H), 4.05 – 4.01 (m, 1 H), 3.95 (d, \( J = 13.9 \) Hz, 1 H), 3.78 (d, \( J = 8.5 \) Hz, 1 H), 3.66 (s, 3 H), 3.63 (s, 3 H), 3.61 – 3.55 (m, 2 H), 3.24 (t, \( J = 6.9 \) Hz, 2 H), 2.94 (d, \( J = 7.6 \) Hz, 2 H), 2.87 (t, \( J = 11.5 \) Hz, 1 H), 2.54 (d, \( J = 12.1 \) Hz, 1 H), 2.48 – 2.34 (m, 2 H), 1.60 – 1.48 (m, 4 H), 1.43 – 1.37 (m, 4 H), 0.86 (s, 9 H), 0.80 (s, 9 H) ppm;

\(^{13}\)C NMR (151 MHz, CDCl₃): \( \delta \) 171.0, 170.9, 157.1, 157.0, 156.4, 154.9 (t, \( J_{CF} = \))
30.0 Hz), 138.4, 138.2, 137.9, 136.8, 129.5, 129.4, 128.5, 127.3, 126.4, 122.0 (t, $J_{CF} = 241.6$ Hz), 121.0, 118.4, 67.4, 63.7, 62.7, 61.6, 61.2, 52.6, 52.5, 52.3, 51.5, 38.8, 35.9 (t, $J_{CF} = 25.2$ Hz), 34.3, 34.0, 29.8, 29.0, 28.8, 26.7, 26.4, 22.2 ppm;

$^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ –98.5 (ABq, 2F, $\Delta \delta_{AB} = 0.4$, $J_{AB} = 253.0$ Hz) ppm;

IR (neat): $\nu$ = 2954, 2360, 2340, 2097, 1707, 1653, 1512, 1361, 1222, 670, 528 cm$^{-1}$;

HRMS (ESI-TOF): calc’d for [C$_{45}$H$_{63}$F$_2$N$_9$O$_7$+H$^+$] 880.4891; found 880.4906.

Analog 13b

Physical state: white solid (m.p. = 60 – 63 ºC);

$R_f$ = 0.70 (silica gel, 1:10 MeOH:DCM, ran twice);

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.76 (d, $J = 5.1$ Hz, 1 H), 7.96 (d, $J = 8.3$ Hz, 2 H), 7.74 (s, 1 H), 7.44 (d, $J = 8.1$ Hz, 2 H), 7.29 (dd, $J = 5.1$, 1.5 Hz, 1 H), 7.23 – 7.12 (m, 5 H), 6.78 (brs, 1 H), 6.51 (d, $J = 9.4$ Hz, 1 H), 5.36 (d, $J = 9.2$ Hz, 1 H), 5.27 (d, $J = 8.5$ Hz, 1 H), 4.08- 4.02 (m, 2 H), 3.96 (d, $J = 13.9$ Hz, 1 H), 3.79 (d, $J = 8.7$ Hz, 1 H), 3.66 (s, 3 H), 3.63 (s, 3 H), 3.61 – 3.56 (m, 2 H), 3.25 (t, $J = 6.9$ Hz, 2 H), 2.93 (d, $J = 7.6$ Hz, 3 H), 2.87 (t, $J = 11.5$ Hz, 1 H), 2.56 (d, $J = 12.3$ Hz, 2 H), 2.36 (t, $J = 7.4$ Hz, 1 H), 2.23 – 2.08 (m, 2 H), 1.70 – 1.43 (m, 4 H), 1.40 – 1.34 (m, 4 H), 0.85 (s, 9 H), 0.78 (s, 9 H) ppm;

$^{13}$C NMR (151 MHz, CDCl$_3$): $\delta$ 171.0, 170.9, 157.8, 157.1, 157.0, 150.3, 146.6 (t, $J_{CF} = 27.8$ Hz), 138.5, 138.2, 137.0, 129.5, 129.5, 128.5, 127.4, 126.4, 122.0 (t, $J_{CF} = 243.2$ Hz), 118.2, 116.3 (t, $J_{CF} = 1.1$ Hz), 67.4, 63.7, 62.66, 61.6, 61.3, 52.6, 52.5, 52.3, 51.4, 38.8, 38.7 (t, $J_{CF} = 26.4$ Hz), 34.3, 34.0, 29.8, 28.8, 28.8, 26.7, 26.4, 22.3 ppm;

$^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ –98.8 ppm;

IR (neat): $\nu$ = 2957, 2361, 2338, 2092, 1706, 1652, 1514, 1362, 1223, 1064 cm$^{-1}$;

HRMS (ESI-TOF): calc’d for [C$_{45}$H$_{63}$F$_2$N$_9$O$_7$+H$^+$] 880.4891; found 880.4909.
On a 0.10 mmol scale, the standard procedure was followed. The reaction mixture was stirred at room temperature for 55 h in DMSO:HO (0.40:0.16 mL) (second addition of reagents was performed after 22 h) to provide 14a (9.2 mg, 16% yield) and 14b (6.6 mg, 12% yield).

Analog 14a

Physical state: colorless oil;
R_f = 0.89 (silica gel, 1:15 MeOH:DCM);

^1^H NMR (400 MHz, CDCl_3): δ 7.62 (d, J = 8.0 Hz, 1 H), 7.45 (d, J = 8.0 Hz, 1 H), 7.18 (s, 1 H), 7.17 (d, J = 8.8 Hz, 1 H), 7.10 (d, J = 8.8 Hz, 1 H), 4.16 (q, J = 7.2 Hz, 2 H), 3.44 – 3.34 (m, 3 H), 3.26 – 3.18 (m, 5 H), 2.96 – 2.88 (m, 1 H), 2.85 – 2.78 (m, 1 H), 1.26 (t, J = 7.2 Hz, 3 H) ppm;

^1^3^C NMR (151 MHz, acetone-d_6): δ 157.3, 152.2 (dd, J_CF = 31.7, 28.7 Hz), 141.4, 139.9, 139.1, 139.1, 136.1, 134.3, 133.4, 131.6, 130.1, 129.7, 126.8, 123.0 (t, J_CF = 241.6 Hz), 119.0 (t, J_CF = 4.5 Hz), 61.6, 51.9, 45.7, 45.6, 36.6 (t, J_CF = 25.7 Hz), 32.1, 31.8, 31.6, 31.5, 29.5, 29.4, 27.2, 23.1 (t, J_CF = 4.5 Hz), 15.0 ppm;

^1^9^F NMR (376 MHz, CDCl_3): δ ~76.1 ppm;

IR (neat): ν = 2927, 2859, 2361, 2095, 1671, 1189, 1139, 723 cm^-1;

HRMS (ESI-TOF): calc’d for [C_29H_33ClF_2N_5O_2+H^-] 558.2442; found 558.2448.

Analog 14b
Physical state: colorless oil;

$R_f=0.38$ (silica gel, 1:15 MeOH:DCM);

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.47 (d, $J = 5.2$ Hz, 1 H), 7.26 (d, $J = 5.2$ Hz, 1 H), 7.12 (d, $J = 1.2$ Hz, 2 H), 7.08 (s, 1 H), 4.15 (q, $J = 6.8$ Hz, 2 H), 3.82 – 3.74 (m, 2 H), 3.41 – 3.37 (m, 2 H), 3.31 – 3.23 (m, 3 H), 3.20 – 3.14 (m, 2 H), 2.96 – 2.87 (m, 1 H), 2.50 – 2.47 (m, 2 H), 2.38 – 2.31 (m, 2 H), 1.25 (t, $J = 6.8$ Hz, 3 H) ppm;

$^{13}$C NMR (151 MHz, acetone-$d_6$): $\delta$ 163.7, 155.7, 148.0, 143.5 (t, $J_{CF} = 27.2$ Hz), 139.9, 137.3, 135.8, 134.9, 133.3, 133.1, 131.4, 131.1, 126.4, 123.9 (t, $J_{CF} = 243.1$ Hz), 119.9 (t, $J_{CF} = 7.6$ Hz), 61.5, 51.8, 45.5, 45.2, 38.9 (t, $J_{CF} = 24.2$ Hz), 33.1, 31.5, 31.1, 29.2 (2C), 27.0, 26.5, 22.9 (t, $J_{CF} = 4.5$ Hz), 15.0 ppm;

$^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ –94.6 ppm;

IR (neat) $\nu = 2931, 2859, 2090, 1693, 1429, 1221, 766$ cm$^{-1}$;

HRMS (ESI-TOF): calc’d for $[C_{29}H_{33}ClF_2N_5O_2]^+H^+$ 558.2442; found 558.2435.

**Bosutinib analog (15a)**

On a 0.05 mmol scale, the standard procedure was followed, employing TFA (16 $\mu$L, 0.20 mmol, 4.0 equiv) instead of TsOH•H$_2$O as an acid additive. The reaction mixture was stirred at 50 °C for 25 h in DMSO:H$_2$O (0.15:0.15 mL) to provide 15a (33.2 mg, 47% yield).

Physical state: yellow oil;

$R_f=0.60$ (silica gel, 1:10 MeOH:DCM);

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.50 (s, 1 H), 7.45 (s, 1 H), 6.92 (brs, 1 H), 6.76 (s, 1 H), 6.34 (s, 1 H), 4.26 (t, $J = 6.7$ Hz, 2 H), 3.65 (s, 3 H), 3.59 (s, 3 H), 3.27 (t, $J = 6.9$ Hz, 2 H), 2.56 (t, $J = 7.1$ Hz, 2 H), 2.53 – 2.40 (m, 8 H), 2.31 (s, 3 H), 2.14 – 2.07 (m, 2 H), 1.70 – 1.58 (m, 4 H), 1.48 – 1.41 (m, 4 H) ppm;
$^{13}$C NMR (151 MHz, CDCl$_3$): $\delta$ 154.5, 154.4, 153.0 (t, $J_{CF} = 29.1$ Hz), 150.8, 150.0, 146.0, 137.7, 130.8, 118.4, 121.5 (t, $J_{CF} = 244.2$ Hz), 117.1, 115.0, 114.6, 110.3, 105.2, 102.1, 92.0, 68.0, 56.7, 56.1, 55.2, 54.8, 53.2, 51.5, 46.1, 36.0 (t, $J_{CF} = 24.5$ Hz), 29.0, 28.8, 26.6, 26.4, 21.9 (t, $J_{CF} = 3.7$ Hz) ppm;

$^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ –95.6 ppm;

IR (neat): $\nu =$ 2937, 2360, 2338, 2091, 1708, 1496, 1442, 1211, 671, 529 cm$^{-1}$;

HRMS (ESI-TOF): calc’d for [C$_{33}$H$_{40}$Cl$_2$F$_2$N$_8$O$_3$+H$^+$] 705.2641; found 705.2648.

**Fasudil analog (16a)**

![Fasudil Analog](image)

On a 0.05 mmol scale, the standard procedure was followed with a reaction time of 11 h in DCM:H$_2$O (0.2:0.08 mL) at room temperature to provide 16a (12.7 mg, 55% yield).

**Physical state:** yellow solid (m.p. 197 – 200 °C);

$R_f = 0.48$ (silica gel, 1:6 MeOH:DCM);

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.86 – 8.83 (m, 1 H), 8.76 (d, $J = 6.0$ Hz, 1 H), 8.72 (d, $J = 6.0$ Hz, 1 H), 8.47 (dd, $J = 6.4$, 1.2 Hz, 1 H), 7.94 (dd, $J = 8.8$, 7.2 Hz, 1 H), 3.59 (t, $J = 6.0$ Hz, 2 H), 3.54 – 3.51 (m, 2 H), 3.39 (t, $J = 6.8$ Hz, 2 H), 2.74 – 2.61 (m, 2 H);

$^{13}$C NMR (151 MHz, acetone-d$_6$): $\delta$ 154.1 (t, $J_{CF} = 30.2$ Hz), 143.0, 137.1, 134.0, 133.1, 131.6 (t, $J_{CF} = 6.0$ Hz), 127.8, 126.8, 125.1 (t, $J_{CF} = 240.1$ Hz), 121.1, 52.2, 51.9, 51.1, 48.4, 48.0, 36.4 (t, $J_{CF} = 24.2$ Hz), 31.8, 30.6, 30.5, 27.2, 22.8 (t, $J_{CF} = 4.5$ Hz) ppm;

$^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ –89.5 ppm;

IR (neat): $\nu =$ 2941, 2863, 2095, 1214, 956, 649 cm$^{-1}$;

HRMS (ESI-TOF): calc’d for [C$_{21}$H$_{28}$F$_2$N$_6$O$_3$S+H$^+$] 467.2035; found 467.2030.
To a solution of varenicline tartrate salt (18.0 mg, 0.05 mmol, 1.0 equiv), DAAS-Na (26.3 mg, 0.10 mmol, 2.0 equiv) and ZnCl₂ (6.8 mg, 0.05 mmol, 1.0 equiv) in CHCl₃ (0.2 mL) and D₂O (0.08 mL) was added TsOH•H₂O (9.5 mg, 0.05 mmol, 1.0 equiv). The reaction mixture was cooled to 0 °C and TBHP (70% solution in water, 0.014 mL, 0.1 mmol, 2.0 equiv) was added dropwise with vigorous stirring and the stirring was continued at this temperature for 5 min, then the reaction mixture was heated to 50 °C for 1 h. After that, the mixture was cooled to room temperature, diluted with 6 M HCl (1.0 mL) and extracted with hexanes (2 × 2.0 mL). The aqueous phase was extracted with CHCl₃ (2 × 2.0 mL), and the combined organic layers were washed with saturated NaHCO₃ then brine, dried over Na₂SO₄, and concentrated in vacuo. The resultant residue was treated with Boc₂O (21.8 mg, 0.1 mmol, 2.0 equiv) in water (0.2 mL). Upon completion in 40 min, the reaction was worked up by diluting with H₂O (1.0 mL) and extraction with ethyl acetate (2 × 2.0 mL). The combined organic layers were washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The resultant residue was purified by PTLC to provide 17a.

To a stirred solution of 17 a in DCM (1 mL) was added TFA (0.2 mL) dropwise at 0 °C under Ar. The reaction was completed in 1 h (monitored by LC-MS and TLC), and the solvent was removed in vacuo to afford 17b (12.8 mg, 53%)

**Physical state:** colorless oil;

\[ R_f = 0.25 \text{ (silica gel, 1:20 MeOH:CH₂Cl₂)} \]

\[ ^1H \text{ NMR (400 MHz, CDCl₃): } \delta \text{ } 9.10 \text{ (s, 1 H), 8.02 (s, 1 H), 8.01 (s, 1 H), 6.76 (brs, 2 H), 3.59 (brs, 2 H), 3.35 (brs, 4 H), 3.26 (t, } J = 6.9 \text{ Hz, 2 H), 2.55 – 2.47 (m, 1 H), 2.46 – 2.32 (m, 2H), 2.14 (d, } J = 11.7 \text{ Hz, 1 H), 1.62 – 1.51 (m, 4 H), 1.50 – 1.37 (m,} \]
Gefitinib analog (18a)

On a 0.10 mmol scale, the standard procedure was followed. The reaction mixture was stirred at 50 °C for 30.5 h in DMSO:H$_2$O (0.40:0.16 mL) (second addition of reagents: ZnCl$_2$ (10.2 mg, 0.075 mmol, 0.75 equiv), DAAS-Na (39.6 mg, 0.15 mmol, 1.5 equiv), TsOH•H$_2$O (9.5 mg, 0.05 mmol, 0.5 equiv) and TBHP (0.035 mL, 0.25 mmol, 2.5 equiv) was performed after 22.5 h) to provide 18a (8.9 mg, 14% yield).

**Physical state:** light yellow solid (m.p. 185 – 188 °C);

$R_f = 0.45$ (silica gel, 1:10 MeOH:DCM);

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.04 (dd, $J = 6.8, 2.4$ Hz, 1 H), 7.64 – 7.60 (m, 1 H), 7.46 (brs, 1 H), 7.40 (s, 1 H), 7.17 (t, $J = 8.8$ Hz, 1 H), 7.14 (s, 1 H), 4.25 (t, $J = 6.4$ Hz, 2 H), 4.00 (s, 3 H), 3.76 (t, $J = 4.8$ Hz, 4 H), 3.22 (t, $J = 6.8$ Hz, 2 H), 2.64 (t, $J = 6.4$ Hz, 2 H), 2.56 – 2.54 (m, 4 H), 2.44 – 2.32 (m, 2 H), 2.19 – 2.12 (m, 2 H) ppm;

$^{13}$C NMR (151 MHz, CDCl$_3$): $\delta$ 157.1 (t, $J_{CF} = 27.2$ Hz), 156.3, 155.6, 154.7 (d, $J_{CF}$ = 164.6 Hz), 149.8, 147.5, 135.5 (d, $J_{CF} = 3.0$ Hz), 123.5, 121.0 (d, $J_{CF} = 18.1$ Hz), 120.9 (d, $J_{CF} = 6.0$ Hz), 120.5 (t, $J_{CF} = 243.1$ Hz), 116.7 (d, $J_{CF} = 22.7$ Hz), 109.0, 108.4, 100.7, 67.8, 66.7, 56.5, 55.4, 53.8, 51.5, 36.1 (t, $J_{CF} = 24.2$ Hz), 29.0, 28.8, 26.6, 25.9, 22.2 (t, $J_{CF} = 3.8$ Hz) ppm;

$^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ –101.1, –121.4 ppm;
IR (neat): v = 2923, 2853, 2093, 1500, 1429, 1051, 794 cm⁻¹;
HRMS (ESI-TOF): calc’d for [C₂₉H₃₅ClF₃N₇O₃⁺H⁺] 622.2515; found 622.2523.

Selumetinib analog (19a)

On a 0.05 mmol scale, the standard procedure was followed with a reaction time of 8 h at 50 °C in CHCl₃:D₂O (0.2:0.08 mL) to provide 19a (7.3 mg, 23% yield).

Physical state: light yellow solid (m.p. = 110 – 114 °C);
R_f = 0.50 (silica gel, 1:10 MeOH:DCM);

¹H NMR (400 MHz, CDCl₃): δ 10.44 (brs, 1 H), 8.10 (s, 1 H), 7.55 (d, J = 2.2 Hz, 1 H), 7.15 (dd, J = 8.7, 2.3 Hz, 1 H), 6.34 (brs, 1 H), 6.31 (d, J = 8.9 Hz, 1 H), 4.05 (s, 3 H), 3.90 (brs, 2 H), 3.62 (brs, 2 H), 3.28 (t, J = 6.8 Hz, 2 H), 2.67 – 2.54 (m, 2 H), 2.36 (t, J = 7.4 Hz, 1 H), 1.77 – 1.58 (m, 4 H), 1.54 – 1.37 (m, 4 H) ppm;

¹³C NMR (151 MHz, CDCl₃): δ 166.0, 150.5 (t, J_CF = 27.9 Hz), 149.6 (d, J_CF = 221.8 Hz), 141.0, 133.2 (d, J_CF = 16.1 Hz), 132.1, 131.5, 131.0, 125.9, 122.6, 120.9 (d, J_CF = 21.3 Hz), 119.4 (t, J_CF = 237.8 Hz), 116.3, 113.2, 108.9, 78.9, 59.5, 51.5, 35.4 (t, J_CF = 22.7 Hz), 32.0, 28.8, 28.8, 26.6, 21.6 ppm.

¹⁹F NMR (376 MHz, CDCl₃): δ –94.9, –133.2 ppm;
IR (neat): v = 2923, 2854, 2091, 1491, 1264, 1045, 871 cm⁻¹;
HRMS (ESI-TOF): calc’d for [C₂₄H₂₆BrClF₃N₇O₃⁺H⁺] 632.0994; found 632.1003.

Aciclovir analog (20a)

On a 0.05 mmol scale, the standard procedure was followed with a reaction time of 2
h at room temperature in DMSO:D₂O (0.2:0.08 mL). The reaction was quenched with NaHCO₃ (12.6 mg) at 0 °C, and extracted with ethyl acetate (3 × 2.0 mL). The combined organic phase was washed with brine and dried over Na₂SO₄. After evaporation of solvent in vacuo, the residue was purified by PTLC to yield 20a (5.4 mg, 27% yield).

Physical state: white solid (m.p. = 179 – 180 °C);

$R_f = 0.40$ (silica gel, 1:10 MeOH:DCM);

$^1$H NMR (400 MHz, acetone-d₆): δ 10.18 (brs, 1 H), 6.35 (s, 2 H), 5.57 (s, 2 H), 3.70 (m, 1 H), 3.66 – 3.60 (m, 4 H), 3.35 (t, $J = 7.0$ Hz, 3 H), 2.53 – 2.41 (m, 2 H), 1.64 – 1.58 (m, 4 H), 1.46 – 1.42 (m, 4 H) ppm;

$^{13}$C NMR (151 MHz, DMSO-d₆): δ 176.2, 156.7, 154.6, 153.1, 139.8 (t, $J_{CF} = 32.2$ Hz), 119.6 (t, $J_{CF} = 237.0$ Hz), 114.9, 72.1, 70.8, 59.9, 50.5, 35.4, 35.2 (t, $J_{CF} = 24.0$ Hz), 35.1, 28.1, 25.9, 21.5 ppm;

$^{19}$F NMR (376 MHz, acetone-d₆): δ –92.9 ppm;

IR (neat): ν = 2924, 2360, 2100, 1693, 1640, 1176, 1116, 1066, 948 cm⁻¹;

HRMS (ESI-TOF): calc’d for [C₁₁₅H₂₂F₂N₈O₃+H⁺] 401.1856; found 401.1862.

Ganciclovir analog (21a)

On a 0.05 mmol scale, the standard procedure was followed with a reaction time of 2 h at room temperature in DMSO:D₂O (0.2:0.08 mL). The reaction was quenched with NaHCO₃ (12.6 mg) at 0 °C, and extracted with ethyl acetate (3 × 2.0 mL). The combined organic phase was washed with brine and dried over Na₂SO₄. After evaporation of solvent in vacuo, the residue was purified by PTLC to get 21a (7.1 mg, 33% yield).

Physical state: white solid (m.p. = 120 – 135 °C, decomposed);
$R_f = 0.20$ (silica gel, 1:10 MeOH:DCM);

$^1$H NMR (400 MHz, acetone-$d_6$): $\delta$ 6.36 (m, 2 H), 5.69 (s, 2 H), 3.86 – 3.80 (m, 1 H), 3.71 (brs, 2 H), 3.63 (dd, $J = 11.4, 4.8$ Hz, 2 H), 3.52 (dd, $J = 11.3, 5.9$ Hz, 2 H), 3.35 (t, $J = 6.9$ Hz, 2 H), 2.61 – 2.33 (m, 2 H), 1.66 – 1.59 (m, 4 H), 1.51 – 1.42 (m, 4 H) ppm;

$^{13}$C NMR (151 MHz, DMSO-$d_6$): $\delta$ 156.7, 154.6, 139.9 (t, $J_{CF} = 31.5$ Hz), 119.6 (t, $J_{CF} = 236.8$ Hz), 115.0, 80.7, 71.7, 60.8, 50.6, 35.3 (t, $J = 23.4$ Hz), 28.1, 28.1, 25.9, 21.5 ppm;

$^{19}$F NMR (376 MHz, acetone-$d_6$): $\delta$ –92.6 ppm;

IR (neat): $\nu = 2944, 2107, 1655, 1214, 955, 649, 557, 526$ cm$^{-1}$;

HRMS (ESI-TOF): calc’d for \([C_{16}H_{24}F_2N_8O_4+H^+] \) 431.1961; found 431.1958.

Chlorothiazide analog (22a)

On a 0.05 mmol scale, the standard procedure was followed with a reaction time of 15 h in acetone:H$_2$O (0.15:0.15 mL) at 50 °C to get 22a (8.2 mg, 35 % yield).

Physical state: white solid (m.p. 220 – 224 °C);

$R_f = 0.56$ (silica gel, 1:10 MeOH:DCM);

$^1$H NMR (400 MHz, acetone-$d_6$): $\delta$ 8.64 (s, 1 H), 8.01 (s, 1 H), 7.22 (s, 1 H), 3.35 (t, $J = 6.8$ Hz, 1 H), 2.65 – 2.52 (m, 2 H) ppm;

$^{13}$C NMR (151 MHz, acetone-$d_6$): $\delta$ 149.4, 141.5, 137.3, 135.5, 128.5, 125.2 (t, $J_{CF} = 246.1$ Hz), 125.0 (t, $J_{CF} = 25.7$ Hz), 122.9, 51.8, 37.5 (t, $J_{CF} = 24.2$ Hz), 29.3, 29.2, 27.1, 22.0 (t, $J_{CF} = 3.2$ Hz) ppm;

$^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ –90.1 ppm;

IR (neat): $\nu = 3426, 3238, 2923, 2857, 2098, 1309, 1151, 787$ cm$^{-1}$;

HRMS (ESI-TOF): calc’d for \([C_{14}H_{14}ClF_2N_6O_4S_2+NH_4^+] \) 488.0748; found 488.0753.
References:

Antibody–Drug Conjugation Procedure

Figure S1 Outline of Antibody–Drug Conjugation:

Methods

Materials

Functionalized bioactive compounds were dissolved in dimethylsulfoxide (DMSO) prior to use and stored at -80 °C thereafter. MOR1 was expressed in 293F cells, purified by protein A chromatography, and stored at -80 °C.

MOR1-DBCO preparation

Monoclonal antibody MOR1 was diluted in DPBS to a final concentration of 5.0 mg/ml. Maleimido-DBCO (Click Chemistry Tools) was prepared as a 10 mM stock in DMSO. Maleimido-DBCO was added to MOR1 at a molar ratio of 3:1. Conjugation was performed at room temperature (RT) with rotating for 4 h. Unreacted maleimido-DBCO was removed by
desalting using coupled HiTrap 26/10 desalting columns (GE Healthcare) on an FPLC (GE Healthcare) with 1X DPBS as running buffer.

**Compound conjugation**

**Conventional method (Method 1)**

500 µL of MOR1-DBCO (diluted to 1.5 mg/mL in DPBS, 30 µM final) was added into 1.5 mL microcentrifuge tubes. 1.5 µL of compound at 10 mM was then added. Final conjugation ratio was 3:1 (compound:MAb). Conjugation was done for 4 h at RT with rotating. Unreacted compound was removed by desalting using Zeba spin desalting columns (Thermo-Fisher Prod #87766, Lot #OB181449), according to the manufacturer’s recommendations.

**High-throughput method (Method 2)**

500 µL of MOR1-DBCO (diluted to 1.5 mg/mL in DPBS, 30 µM final) was added into each well of a 96 well, 2 mL/well plate (Microplate devices, Whatman, Ref 7701-5200). 1.5 µL of compound at 10 mM was then added to individual wells. Final conjugation ratio was 3:1 (compound:MAb). The plate was sealed and conjugation was done for 4 h at RT with shaking on a plate shaker. Unreacted compound was removed by desalting using Zeba desalting spin plate (40K MWCO, 96 well, Thermo-Fisher, Prod #87775, Lot #NB170124), according to the manufacturers recommendations.

**LC-MS analysis**

Samples were reduced with 20 mM dithiothreitol (DTT) for 3 min at 60 °C prior to LC-MS analysis. Separation was performed on a Waters Acquity UPLC fitted with a MassPrep micro desalting column and an on-line switching valve. Mobile phase was 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Column temperature was 65 °C. Flow rate was 0.04 mL/min. MS analysis was done using a Waters Q-TOF Premier in-line with the UPLC fitted with a nano ESI source. Flow split was 20:1. ESI-MS settings were as follows: ESI-MS: Capillary voltage, 4.50 kV, source temp, 100 °C, and collision voltage 6.0 V. Deconvolution was done using MassLynx software, and peak intensities for DBCO-modified MOR1 light chain and compound-DBCO-modified light chain were compared.
Results

Conjugation results

The conjugation of the azido-modified heterocyclic compounds to dicyclobenzyloctyne (DBCO)-modified monoclonal antibody MOR1 using a conventional method was compared to that using a plate-based high-throughput method. The azido group on the modified compounds reacts with the DBCO via a strain-promoted 1,3-dipolar cycloaddition mechanism. Because of the site-specific nature of the conjugation, only one modification per light chain (2 per intact MAb) is possible. No modification sights are present on the heavy chain. This allows efficiency of conjugation to be evaluated using mass spectrometry, whereby the amounts of each modified light chain are compared. The results of this analysis are shown in Table S1. Raw data can be found in Appendix A.

<table>
<thead>
<tr>
<th>Name of small-molecule bioactive agent</th>
<th>Mass of azide containing analog (Da)</th>
<th>Expected mass after bioconjugation (Da)</th>
<th>Observed mass after bioconjugation (Da)</th>
<th>Bioconjugation Efficiency (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>camptothecin (1)</td>
<td>523</td>
<td>24337</td>
<td>24337/24336</td>
<td>100/100</td>
</tr>
<tr>
<td>buspirone (2)</td>
<td>560</td>
<td>24374</td>
<td>24374/24373</td>
<td>19/35</td>
</tr>
<tr>
<td>sceptrin (6)</td>
<td>771</td>
<td>24585</td>
<td>24585/§§</td>
<td>32/§§</td>
</tr>
<tr>
<td>papaverine (7)</td>
<td>514</td>
<td>24328</td>
<td>24329/24327</td>
<td>22/30</td>
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<tr>
<td>pioglitazone (8)</td>
<td>531</td>
<td>24345</td>
<td>24344/24346</td>
<td>35/74</td>
</tr>
<tr>
<td>nevirapine (9)</td>
<td>441</td>
<td>24255</td>
<td>24255/###</td>
<td>54/0</td>
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<td>milrinone (10)</td>
<td>386</td>
<td>24200</td>
<td>24201/24199</td>
<td>95/100</td>
</tr>
<tr>
<td>biscodyl (11)</td>
<td>536</td>
<td>25350</td>
<td>###/###</td>
<td>0/0</td>
</tr>
<tr>
<td>acridine (12)</td>
<td>440</td>
<td>24234</td>
<td>24233/24231</td>
<td>94/100</td>
</tr>
<tr>
<td>atazanavir (13)</td>
<td>879</td>
<td>24693</td>
<td>###/###</td>
<td>0/0</td>
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<tr>
<td>loratadine (14)</td>
<td>557</td>
<td>24371</td>
<td>###/24371</td>
<td>0/7</td>
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<td>bosutinib (15)</td>
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<td>24518</td>
<td>24520/24518</td>
<td>66/88</td>
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<td>fasudil (16)</td>
<td>466</td>
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<td>24280/24279</td>
<td>100/100</td>
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<tr>
<td>varenicline (17)</td>
<td>386</td>
<td>24200</td>
<td>24201/24199</td>
<td>14/19</td>
</tr>
<tr>
<td>gefitinib (18)</td>
<td>621</td>
<td>24435</td>
<td>24436/24435</td>
<td>10/35</td>
</tr>
<tr>
<td>selumetinib (19)</td>
<td>631</td>
<td>24445</td>
<td>24445/§§</td>
<td>10/§§</td>
</tr>
<tr>
<td>aciclovir (20)</td>
<td>400</td>
<td>24214</td>
<td>24214/24214</td>
<td>100/100</td>
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</tbody>
</table>
The two indicated values refer to the mass spectral data arising from two different methods of conjugation (please see the Supporting Information for details); the slight differences in mass between the two methods are deconvolution artifacts. The symbol §§ indicates that the second method of bioconjugation was not conducted. The symbol ## indicates that the bioconjugation was unsuccessful.

<p>| | | | | |</p>
<table>
<thead>
<tr>
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<tr>
<td>ganciclovir (21)</td>
<td>430</td>
<td>2424</td>
<td>24245/24243</td>
<td>99/94</td>
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<tr>
<td>chlorothiazide (22)</td>
<td>470</td>
<td>2428</td>
<td>24285/24284</td>
<td>95/94</td>
</tr>
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</table>
Appendices

Appendix A: ESI-MS of Antibody–Drug Conjugate

Unprocessed data is shown on top, deconvoluted light chain second, and deconvoluted heavy chain on the bottom.

Campothecin Analog (1a)

Campothecin Analog
Chemical Formula: C_{67}H_{42}F_{2}N_{8}O_{4}
Molecular Weight: 523.53
Buspirone analog (2a)

**Chemical Information**

- **Chemical Formula:** C_{36}H_{32}F_{2}N_{6}O_{2}
- **Molecular Weight:** 560.68

**Diagram:**

- Buspirone analog structure
- Chemical formula and molecular weight

**Method 1**

- Mass spectrum data
  - 19.2%

**Method 2**

- Mass spectrum data
  - 35.2%
Sceptrin analog (6a)

Method 1

Sceptrin Analog
Chemical Formula: C_{25}H_{36}Br_{2}N_{13}O_{3}
Exact Mass: 771.1353

LC+Sceptrin 31.8%
Papaverine Analog (7a)
Pioglitazone Analog (8a)
Nevirapine Analog (9a)

Nevirapine Analog
Chemical Formula: C_{22}H_{19}F_{2}N_{7}O
Molecular Weight: 441.48
Milrinone Analog (10a)

Chemical Formula: C_{19}H_{20}F_{2}N_{2}O
Molecular Weight: 386.40
Bisacodyl Analog (11a)

Bisacodyl Analog
Chemical Formula: C_{26}H_{20}F_2N_2O_4
Molecular Weight: 536.57

Method 1

Method 2
Acridine orange analog (12a)
Atazanavir analog (13a)
Loratadine analog (14a)
Bosutinib analog (15a)
Fasudil analog (16a)
Varenicline (Chantix) analog (17a)
Gefitinib analog (18a)

Gefitinib Analog

Chemical Formula: C20H14ClF3N3O3
Molecular Weight: 622.08

Method 1

Method 2
Selumetinib analog (19a)

Selumetinib Analog
Chemical Formula: C_{24}H_{28}BrClF_{3}N_{7}O_{3}
Exact Mass: 631.0921

Method 1
Aciclovir analog (20a)
Ganciclovir analog (21a)

Ganciclovir Analog
Chemical Formula: C₁₈H₂₆F₂N₆O₄
Molecular Weight: 430.41
Chlorothiazide analog (22a)
Appendix B: NMR Spectrum
5

SI-50
C-13 Routine 1b, DCL CryoProbe, 10-26-2006

SI-51
F-19, CDCl₃, 600 MHz NMR Probe. CF₃CO as Ref at 0 ppm.

SI-52
DAAS-Na (3)
Si-54

DAAS-Na (3)
SI-55
F-19, CDCl3, 80X-400 QAP Probe. CF3Cl as Ref at 0 ppm.
F-19, CDCl3, DPX-400 QNP Probe. CF3Cl as Ref at 0 ppm.
SI-63
F-19, CDCl3, BPK-400 QNP Probe. CF3Cl as Ref at 0 ppm.
F-19, CDCl3, BRUKER 400 QNP Probe. CF3Cl as Ref at 0 ppm.
F-19, CDCl3, DNP-400 QNP Probe. CF3Cl as Ref at 0 ppm.

SI-72
F-19, CDCl3, BPK-400 QNP Probe. CF3Cl as Ref at 0 ppm.

SI-75
F-19, CDCl3, BRUKER QNP Probe. CF3Cl as Ref at 0 ppm.
F-19, CDCl3, BRUKER $^{19}$F NMR Probe. CF$_3$Cl as Ref at 0 ppm.
SI-82
C-13 Routine 10b, DCI CryoProbe, 10-26-2006
F-19, CDCl3, DPX-400 QM Probe. CF3Cl as Ref at 0 ppm.
F-19, CDCl3, DEPT-400 QAP Probe. CF3Cl as Ref at 0 ppm.
F-19, CDCl3, 39X-400 NMR Probe, CF3Cl as Ref at 0 ppm.
13a

F-19, CDCl3, 400 MHz. CF3Cl as Ref at 0 ppm.
F-19, CDCl3, D2O-400 QAP Probe. CF3Cl as Ref at 0 ppm.
SI-99
F-19, CDCl3, BRUKER QNP Probe. CF3Cl as Ref at 0 ppm.
$^{19}$F NMR, CDCl$_3$, 500 MHz probe. CF$_3$Cl as ref at 0 ppm.
F-19, CDCl3, BRUKER QNP Probe. CD3C1 as Ref at 0 ppm.
C-13 Routine 1b, DCI CryoProbe, 10-26-2006
F-19, CDCl₃, DPM-400 QAP Probe. CF₃Cl as Ref at 0 ppm.

SI-117
F-19, Acetone, BPH-400 QAP Probe. CDCl₃ as Ref at 0 ppm.
SI-122
F-19, Acetone, BRX-400 QNP Probe. CF3Cl as Ref at 0 ppm.
F-19, CDCl3, BRUKER QNP Probe. CF3Cl as Ref at 0 ppm.