Supporting Information

Design and Synthesis of a Potential Activity-Based Probe for Protein Kinases

Santosh Keshipeddy, Yu Shi, Nathan Hnatiuk, Martha Morton, Xudong Yao,
Amy R. Howell*

Department of Chemistry, University of Connecticut, Storrs, Connecticut 06269-3060
amy.howell@uconn.edu

General Experimental. $^1$H NMR spectra were recorded at 300 MHz, 400 MHz and/or 500 MHz and calibrated to the CDCl$_3$ peak at 7.27 ppm. $^{13}$C NMR spectra were recorded at 75 MHz, 100 MHz and/or 125 MHz and calibrated to the CDCl$_3$ peak at 77.23 ppm. $^{31}$P NMR was recorded at 202 MHz using 85% H$_3$PO$_4$ as external standard. Chemical shifts are reported in units of parts per million (ppm). IR spectra were recorded on an FT-IR spectrometer. Mass spectrometric analysis was performed on a quadrupole time-of-flight tandem mass spectrometer (QTOF) equipped with an in-house modified electrospray (ES) source. All of the reactions were performed under N$_2$. Column chromatography was performed using silica gel, 40 microns flash silica. Thin layer chromatography was performed on silica gel (Silica gel 60 F$_{254}$) glass plates, and the compounds were visualized by UV and phosphomolybdic acid stain. High-performance liquid chromatography (HPLC) was performed using a reversed-phase column (1.9 µm, 100 × 1.0 mm). The column temperature was set at 40 °C. Solvent A was 1% CH$_3$CN in 10 mM ammonium acetate (pH 6.86), and solvent B was CH$_3$CN. Drying of the samples was performed either on a SpeedVac or on a lyophilyzer.
1-(tert-Butyldimethylsiloxy)-2-[(4-hydroxymethyl)phenyl]-2-propene (6).

4-(Hydroxymethyl)phenylboronic acid (2.50 g, 16.8 mmol), 1-(tert-butyldimethylsiloxy)-2-iodo-2-propene 5¹ (5.00 g, 16.8 mmol), and Cs₂CO₃ (5.50 g, 16.8 mmol) were added to anhydrous EtOH (85 mL). Pd(PPh₃)₄ (0.6 g, 0.5 mmol) was added to the slurry, and the mixture was heated to 60 °C and stirred for 2 h. The reaction mixture was allowed to cool to rt, diluted with H₂O (200 mL) and extracted with ether (2 x 125 mL). The organic layers were combined, washed with brine (125 mL), dried (MgSO₄) and concentrated. The resultant oil was purified by flash chromatography on silica gel (petroleum ether/EtOAc 17:3) to give 1-(tert-butyldimethylsiloxy)-2-[(4-hydroxymethyl)phenyl]-2-propene (6) as a clear oil (4.1 g, 88%): IR (KBr) 3366, 2955, 2929, 2857, 2360, 1078 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.41 (d, J = 8.3 Hz, 2H), 7.34 (d, J = 8.2 Hz, 2H), 5.44 (dt, J = 1.6 Hz, 1.6 Hz, 1H), 5.40 (dt, J = 1.6 Hz, 1.6 Hz, 1H), 4.70 (d, J = 5.8 Hz, 2H), 4.53 (t, J = 1.6 Hz, 2H), 1.66 (t, J = 5.9 Hz, 1H), 0.94 (s, 9H), 0.11 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 146.9, 140.5, 138.7, 127.1, 126.4, 111.7, 65.2, 64.9, 26.1, 18.6, -5.1; HRMS (TOF) calcd for C₁₆H₂₇O₂Si (M + H)⁺ m/z 279.1780. Found: 279.1790.

1-(tert-Butyldimethylsiloxy)-2-[(4-bromomethyl)phenyl]-2-propene.

1-(tert-Butyldimethylsiloxy)-2-[(4-hydroxymethyl)-phenyl]-2-propene (6) (4.20 g, 15.1 mmol) was dissolved in CH₂Cl₂ (75 mL). The solution was cooled to 0 °C. CBr₄ (5.50 g, 16.6 mmol) and PPh₃ (4.30 g, 16.6 mmol) were added successively, and the solution
was stirred for 3 h (longer reaction times resulted in deprotection). The reaction mixture was preloaded on flash silica gel (40 mL). Purification by flash chromatography on silica gel (petroleum ether/EtOAc 97:3) provided 1-( tert-butyldimethylsiloxy)-2-[(4-bromomethyl)phenyl]-2-propene as a colorless oil (5.40 g, 98%): IR (KBr) 2954, 2856, 1254, 1077 cm⁻¹; ¹H NMR (400 MHz, CDCl₃)  δ 7.39 (d,  J = 8.4 Hz, 2H), 7.36 (d,  J = 8.3 Hz, 2H), 5.45 (m, 1H), 5.42 (m, 1H), 4.52 (m,  2H), 4.51 (s, 2H), 0.97 (s, 9H), 0.11 (s, 6H); ¹³C NMR (75 MHz, CDCl₃)  δ 146.6, 139.5, 137.3, 129.2, 126.7, 112.3, 64.9, 33.5, 26.1, 18.6, -5.1; HRMS (TOF) calcd for C₁₆H₂₅OSi (M - Br)⁺ m/z 261.1675. Found: 261.1701.

\[
\begin{align*}
\text{OTBS} & \quad \text{N}_3 \\
\text{1-(tert-Butyldimethylsiloxy)-2-[(4-azidomethyl)phenyl]-2-propene (7).}
\end{align*}
\]

NaN₃ (0.64 g, 9.84 mmol) was added in one portion to a stirred solution of 1-(tert-butyldimethylsiloxy)-2-[(4-bromomethyl)phenyl]-2-propene (2.80 g, 8.20 mmol) in DMF (10 mL). After 14 h the solution was diluted with H₂O (40 mL) and extracted with CH₂Cl₂ (3 x 20 mL). The organic layers were combined, washed with brine (100 mL), dried (MgSO₄) and concentrated. The resulting residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc 95:5) to afford 1-(tert-butyldimethylsiloxy)-2-[(4-azidomethyl)phenyl]-2-propene (7) as a colorless oil (2.3 g, 92%): IR (neat) 2954, 2929, 2885, 2857, 2098 cm⁻¹; ¹H NMR (300 MHz, CDCl₃)  δ 7.42 (d,  J = 8.3 Hz, 2H), 7.27 (d,  J = 8.5 Hz, 2H), 5.44 (td,  J = 1.4, 1.4 Hz, 1H), 5.41 (td,  J = 1.7, 1.7 Hz, 1H), 4.52 (dd,  J = 1.6, 1.6 Hz, 2H), 4.31 (s, 2H), 0.93 (s, 9H), 0.10 (s, 6H); ¹³C NMR (100 MHz, CDCl₃)  δ 146.7, 139.4, 134.9, 128.4, 126.7, 112.1, 64.9, 54.8, 26.1, 18.6, -5.1; HRMS (TOF) calcd for C₁₆H₂₆N₃OSi (M + H)⁺ m/z 304.1845. Found: 304.1855.
**2-[(4-Azidomethyl)phenyl]-2-propen-1-ol.**

TBAF (1.0 M in THF, 7.6 mL, 7.6 mmol), was added drop-wise to a solution of 1-(tert-butyldimethylsiloxy)-2-[(4-azidomethyl)phenyl]-2-propene (7) (2.3 g, 7.6 mmol) in THF (40 mL) at 0 °C. The solution was stirred at 0 °C for 1 h. H₂O (20 mL) was added, and the solution was extracted with EtOAc (2 x 50 mL). The organic layers were combined, washed with brine (50 mL), dried (MgSO₄) and concentrated. The resulting residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc 75:25) to afford 2-[(4-azidomethyl)phenyl]-2-propen-1-ol as a clear oil (1.2 g, 84%): IR (neat) 3357(br), 2917, 2099 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.49 (d, J = 8.3 Hz, 2H), 7.23 (d, J = 8.4 Hz, 2H), 5.52 (m, 1H), 5.40 (m, 1H), 4.57 (d, J = 5.9 Hz, 2H), 4.36 (s, 2H), 1.60 (t, J = 6.0 Hz, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 146.9, 138.8, 135.1, 128.5, 126.7, 113.1, 64.9, 54.6; HRMS (FAB) calcd for C₁₀H₁₁N₃O (M)⁺ m/z 189.0902. Found: 189.0905.

**2-(4-(Azidomethyl)phenyl)oxiran-2-yl)methanol.**

Freshly prepared anhydrous DMDO² (0.33 M, 28.0 mL, 9.12 mmol) was added dropwise to a solution of 2-[(4-azidomethyl)phenyl]-2-propen-1-ol (1.20 g, 6.34 mmol) in CH₂Cl₂ (30 mL) at 0 °C. The reaction mixture was stirred for 1 h, while allowing the reaction temperature to warm to rt. Solvent removal provided (2-(4-(azidomethyl)phenyl)oxiran-2-yl)methanol as a pale yellowish oil (1.30 g, 100%): IR (neat) 3438, 2990, 2929, 2876, 2100, 1251, 1041 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.40 (d, J = 8.1
Hz, 2H), 7.31 (d, J = 8.1 Hz, 2H), 4.33 (s, 2H), 4.10 (m, 1H), 4.05 (m, 1H), 3.25 (d, J = 5.3 Hz, 1H), 2.80 (d, J = 5.3 Hz, 1H), 2.17 (br s, 1H); \(^1^3^C\) NMR (100 MHz, CDCl\(_3\)) \(\delta\) 137.8, 135.5, 128.5, 126.7, 63.3, 60.4, 54.6, 52.8; Anal. Calcd for C\(_{10}\)H\(_{11}\)N\(_3\)O\(_2\): C, 58.53; H, 5.40; N, 20.48. Found: C, 58.35; H, 5.77; N, 20.66.

![Structure](image)

**2-(4-(Azidomethyl)phenyl)oxirane-2-carbaldehyde (4).**

Dess-Martin periodinane (0.7 g, 1.8 mmol) was added to a solution of (2-(4-(azidomethyl)phenyl)oxiran-2-yl)methanol (0.3 g, 1.5 mmol) in CH\(_2\)Cl\(_2\) (50 mL). The reaction mixture was stirred for 6 h at rt, then diluted with CH\(_2\)Cl\(_2\) (20 mL) and aqueous saturated NaHCO\(_3\) (15 mL). The layers were separated, and the organic layer was washed with brine (30 mL), then dried (MgSO\(_4\)). Removal of the solvent, followed by a flash chromatography on silica gel (petroleum ether/EtOAc 92:8) afforded 4 as colorless oil (0.2 g, 67%): IR (neat) 2927, 2828, 2100, 1731, 1255 cm\(^{-1}\); \(^1^H\) NMR (400 MHz, CDCl\(_3\)) \(\delta\) 9.13 (s, 1H), 7.50 (d, J = 8.8 Hz, 2H), 7.34 (d, J = 8.8 Hz, 2H), 4.35 (s, 2H), 3.39 (d, J = 5.4 Hz, 1H), 3.16 (d, J = 5.4 Hz, 1H); \(^1^3^C\) NMR (100 MHz, CDCl\(_3\)) \(\delta\) 196.6, 136.2, 132.0, 128.3, 127.8, 61.1, 54.5, 53.1; Anal. Calcd for C\(_{10}\)H\(_9\)N\(_3\)O\(_2\): C, 59.11; H, 4.46; N, 20.68. Found: C, 59.51; H, 4.26; N, 20.29.
1-(2-(4-(Azidomethyl)phenyl)oxiran-2-yl)prop-2-en-1-ol.

Vinyl magnesium bromide (1.0 M in THF, 1.24 mL, 1.24 mmol) was added to a solution of 2-(4-(azidomethyl)phenyl)oxirane-2-carbaldehyde (4) (210 mg, 1.03 mmol) in THF (10 mL) at -78 °C. The reaction was allowed to stir at -78 °C for 30 min. The reaction mixture was then diluted with EtOAc (20 mL), washed with H₂O (20 mL), saturated aqueous NaHCO₃ (10 mL) and brine (10 mL), dried (MgSO₄) and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 88:12) afforded 1-(2-(4-(azidomethyl)phenyl)oxiran-2-yl)prop-2-en-1-ol (~2:1 diastereomeric ratio) as a yellowish oil (150 mg, 63%): IR (neat) 3447, 2100, 1251 cm⁻¹; major diastereomer ¹H NMR (400 MHz, CDCl₃): δ 7.42 (m, 2H), 7.30 (m, 2H), 5.83 (ddd, J = 17.2, 10.6, 5.6 Hz, 1H), 5.30 (ddd, J = 17.3, 1.4, 1.4 Hz, 1H), 5.19 (ddd, J = 10.5, 1.3, 1.3 Hz, 1H), 4.59 (m, 1H), 4.30 (s, 2H), 3.31 (d, J = 5.4 Hz, 1H), 2.74 (d, J = 5.4 Hz, 1H), 2.01 (br s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 137.6, 136.1, 135.3, 128.2, 127.4, 117.6, 73.2, 62.6, 54.6, 52.6; minor diastereomer ¹H NMR (400 MHz, CDCl₃): δ 7.45 (d, J = 8.1 Hz, 2H), 7.31 (d, J = 8.1 Hz, 2H), 5.83 (ddd, J = 16.9, 10.4, 6.5 Hz, 1H), 5.30 (ddd, J = 17.2, 1.3, 1.3 Hz, 1H), 5.24 (ddd, J = 10.6, 1.1, 1.1 Hz, 1H), 4.48 (m, 1H), 4.34 (s, 2H), 3.21 (d, J = 5.0 Hz, 1H), 2.89 (d, J = 5.0 Hz, 1H), 2.38 (br s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 137.3, 136.1, 135.7, 128.3, 128.2, 118.7, 73.6, 62.7, 54.6, 51.0.
Dess-Martin periodinane (0.08 g, 0.18 mmol) was added to a solution of 1-(2-(4-(azidomethyl)phenyl)oxiran-2-yl)prop-2-en-1-yl)prop-2-en-1-ol (0.02 g, 0.09 mmol) in CH$_2$Cl$_2$ (3 mL) at 0 °C. The reaction mixture was stirred for 1 h while allowing the reaction temperature to warm to rt. Removal of the solvent under reduced pressure at rt followed by flash chromatography on silica gel (petroleum ether/EtOAc 90:10) yielded 3 as yellowish liquid (11 mg, 51%): IR (neat) 2922, 2850, 2099, 1713 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) δ 7.49 (d, $J$ = 8.2 Hz, 2H), 7.31 (d, $J$ = 8.2 Hz, 2H), 6.65 (dd, $J$ = 17.3, 10.4 Hz, 1H), 6.46 (dd, $J$ = 17.3, 1.8 Hz, 1H), 5.83 (dd, $J$ = 10.4, 1.8 Hz, 1H), 4.33 (s, 2H), 3.28 (d, $J$ = 5.5 Hz, 1H), 3.05 (d, $J$ = 5.5 Hz, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 195.2, 136.0, 134.5, 132.0, 130.0, 128.3, 127.7, 62.6, 54.5, 53.5; HRMS (TOF) calcd for C$_{12}$H$_{11}$N$_3$O$_2$Na (M + Na)$^+$ m/z 252.0749. Found: 252.0786.

Thiophenol (2.4 µL, 0.02 mmol) and triethylamine (2.3 µL, 0.02 mmol) were added to a solution of 1-(2-(4-(azidomethyl)phenyl)oxiran-2-yl)prop-2-en-1-yl)prop-2-en-1-yl)prop-2-en-1-one (3) (5 mg, 0.02 mmol) in CH$_2$Cl$_2$ (0.2 mL) at rt. The solution was stirred for 5 min. Removal of the solvent under reduced pressure, followed by flash chromatography on silica gel (petroleum ether/EtOAc 90:10) afforded 8 as colorless oil (5 mg, 75%): IR (neat) 2918,
2099, 1715 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.47 (d, J = 8.2 Hz, 2H), 7.24 (m, 7H), 4.33 (s, 2H), 3.21 (d, J = 5.5 Hz, 1H), 3.13 (t, J = 7.1 Hz, 2H), 2.97 (d, J = 5.5 Hz, 1H), 2.81 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 205.1, 136.2, 135.6, 134.2, 129.9, 129.3, 128.3, 127.9, 126.7, 63.0, 54.6, 53.7, 37.3, 27.6; HRMS (TOF) calcd for C₁₈H₁₈N₃O₂S (M + H)⁺ m/z 340.1119. Found: 340.1107.

2-(4-(Azidomethyl)phenyl)-2-hydroxy-1,5-bis(phenylthio)pentan-3-one (9).

Thiophenol (1.9 µL, 0.02 mmol) and triethylamine (1.8 µL, 0.02 mmol) were added to a stirred solution of 1-(2-(4-(azidomethyl)phenyl)oxiran-2-yl)-3-(phenylthio)propan-1-one (8) (6 mg, 0.02 mmol) in CH₂Cl₂ (0.4 mL) at rt. After 14 h the solvent was removed under reduced pressure. Flash chromatography on silica gel (petroleum ether/EtOAc 95:5) afforded 9 as colorless oil (4 mg, 57%): IR (neat) 2925, 2101, 1696, 1204 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.44 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 7.9 Hz, 2H), 7.2 (m, 10H), 4.32 (s, 2H), 4.11 (s, 1H), 3.85 (d, J = 13.6 Hz, 1H), 3.51 (d, J = 13.6 Hz, 1H), 2.89 (m, 3H), 2.76 (m, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 208.3, 139.4, 135.9, 135.6, 135.2, 131.1, 129.7, 129.3, 129.1, 128.6, 127.4, 126.5, 126.2, 82.3, 54.5, 44.5, 36.7, 27.8; HRMS (TOF) calcd for C₂₄H₂₃N₃O₂S₂Na (M + Na)⁺ m/z 472.1129. Found: 472.1115.
1-(2-(4-(Azidomethyl)phenyl)oxiran-2-yl)-3-
(adenosine 5’-(3-thiotriphosphate)propan-1-one trilithium salt(1).

Adenosine 5’-(3-thiotriphosphate)trilithium salt (5 mg, 0.01 mmol) in H₂O (0.8 mL) was
added to a solution of 1-(2-(4-(azidomethyl)phenyl)oxiran-2-yl)prop-2-en-1-one (3) (11
mg, 0.05 mmol) in THF (1.6 mL). The reaction was monitored by LC-MS. After 6 h the
solvent was removed under vacuum and H₂O (500 µL) and CH₂Cl₂ (500 µL) were
added to redissolve the solid. The aqueous solution was washed with CH₂Cl₂ (3x 500
µL) and purified using hydrophilic/lipophilic balanced materials that were packed in-
house in a spin column (0.8 mL). Deionized H₂O was used as the equilibration and
washing solution and 70% (v/v) CH₃CN/H₂O was used for elution. The eluant was
lyophilized and further purified using hydrophilic/lipophilic materials packed in-house in
TopTip (10-200 µL) using the same equilibration, washing and elution conditions. The
eluant was analyzed by LC-MS. The separation was run at 50 µL/min with a binary
gradient: 1% B at 0 min → 1% B at 5 min → 55% B at 25 min → 70% B at 27 min →
70% B at 30 min → 1% B at 30.1 min → 1% B at 35 min. Pure eluant was lyophilized to
yield probe 1 (1:1 mixture of diastereomers) as white, fluffy solid (2 mg, 30%): ¹H NMR
(500 MHz, D₂O) δ 8.53 (s, 0.5H), 8.52 (s, 0.5H), 8.27 (s, 0.5H), 8.26 (s, 0.5H), 7.42 (m,
4H), 6.13 (d, J = 5.6 Hz, 1H), 4.75 (m, 1H), 4.58 (m, 1H), 4.44 (s, 1H), 4.43 (s, 1H), 4.40
(m, 1H), 4.29 (m, 2H), 3.46 (d, J = 4.7 Hz, 0.5H), 3.45 (d, J = 4.7 Hz, 0.5H), 3.31 (d, J =
4.7 Hz, 0.5H), 3.29 (d, J = 4.7 Hz, 0.5H), 2.97 (m, 4H); ³¹P NMR (202 MHz, D₂O) δ 8.78
(d, J = 29.3 Hz), 8.75 (d, J = 27.6 Hz), -11.48 (d, J = 21.5 Hz), -23.84 (m); HRMS (TOF) calcd for C\textsubscript{22}H\textsubscript{26}N\textsubscript{8}O\textsubscript{14}P\textsubscript{3}S (M - H)\textsuperscript{-} m/z 751.058. Found: 751.045, (M + H)\textsuperscript{+} m/z 753.066. Found: 753.060.

References:
