SUPPLEMENTARY MATERIAL

Synthesis and Analysis

All reactions were performed under argon using oven-dried glassware and dry solvents. Dry tetrahydrofuran (THF) was obtained by standard methods and freshly distilled under argon from sodium benzophenone ketyl prior to use. All of the reagents were used as purchased from commercial suppliers without further purification. The –100 °C bath was prepared by addition of liquid nitrogen to a pre-cooled (–78 °C) mixture of 1:1 ethanol/methanol. Microwave-assisted reaction was performed in a sealed glass vial by using a temperature- and pressure-controlled single-mode microwave reactor (CEM, Discover LabMate) equipped with a 300 W power source. Temperature was set at 150 °C, pressure at 150 psi, and power source at 150 W. Such values were reached within 5 min and were maintained for 30 min by IR sensor thermal control and pressure feedback control. Preloaded (0.25 mm) glass supported silica gel plates (Kieselgel 60, Merck) were used for TLC analysis, and compounds were visualized by exposure to UV light and by dipping the plates in 1 % Ce(SO₄)·4H₂O, 2.5% (NH₄)₆Mo₇O₂₄·4H₂O in 10% sulfuric acid followed by heating on a hot plate. Melting points were measured in open capillary tubes on a Stuart SMP30 Melting Point apparatus. Optical rotations were determined at +20 °C on a Perkin-Elmer 241 polarimeter and are expressed in 10⁻¹ deg cm² g⁻¹. ¹H and ¹³C NMR spectra were recorded on a Bruker Avance-400 MHz spectrometer. Chemical shifts (δ) are reported in ppm and were calibrated to the residual signals of the deuterated solvent.¹ Multiplicity is given as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad signal; coupling constants (J) are given in Hz. Two-dimensional NMR techniques (COSY, HMBC, HSQC) were used to aid in the assignment of signals in ¹H and ¹³C spectra. Particularly, in the ¹³C spectra, the signal of the boron-bearing carbon atom tends to be broadened, often beyond the detection limit;

¹ H. E. Gottlieb V. Kotlyar, A. Nudelman (1997) J. Org. Chem. 62, 7512-7515.
however, its resonance was unambiguously determined by HSQC. NMR spectra of all intermediates are reported in Supplementary Figure 1. High-resolution mass spectra were recorded on an Agilent Technologies 6520 Accurate-Mass Q-TOF LC/MS.

SM23 was synthesized as already described. Regarding S02030 (see Figure S1), the initial intermediates 1, 2 and 3 were obtained following literature procedure.

(+)−Pinanediol (1R)-2-azido-1-(N-bis(trimethylsilyl)amino)ethaneboronate (4) Lithium bis(trimethylsilyl)amide (1 M solution in THF, 9.87 mL, 9.87 mmol) was added dropwise to a solution of 3 (2.80 g, 9.87 mmol) in anhydrous THF (25 mL) at −100 °C under argon flow. The mixture was allowed to warm to room temperature overnight. The resulting solution was concentrated under reduced pressure, and the crude was treated with light petroleum (150 mL). The white inorganic precipitate (LiCl) was filtered off on a MgSO₄ pad and washed with abundant light petroleum. The solvent was evaporated in vacuo to afford 4 as a pale yellow oil (3.23 g, 80%), used as such for the subsequent reaction. [α]D = +4.2 (c 1.6, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 0.15 (18H, s, [Si(CH₃)₂]₂), 0.83 (3H, s, pynanyl CH₃), 1.10 (1H, d, J = 10.9, pinanyl Hendo), 1.28 (3H, s, pinanyl CH₃), 1.39 (3H, s, pinanyl CH₃), 1.84−2.31 (5H, m, pinanyl protons), 2.85 (1H, dd, J = 8.5, 5.9, BCH), 3.29 (1H, dd, J = 12.3, 8.5, BCH-CH₂), 3.37 (1H, dd, J = 12.3, 5.9, BCH-CH₂), 4.31 (1H, dd, J = 8.7, 1.7, CHOB). ¹³C NMR (100 MHz, CDCl₃): δ 2.5, 2.7, 24.0, 26.3, 27.0, 28.3, 35.2, 38.2, 39.4, 43.4 (br, CB), 51.4, 56.1, 78.6, 86.1.

(+)−Pinanediol (1R)-2-azido-1-(2-thienylacetylamino)ethaneboronate (5) A solution of 4 (3.23 g, 7.91 mmol) in THF (8 mL) was added to anhydrous methanol (2.5 M solution in THF, 3.16 mL, 7.91 mmol) at −10 °C under argon. After 10 min the cooling bath was removed and the

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² Morandi, F., Caselli, E., Morandi, S., Focia, P. J., Blazquez, J., Shoichet, B. K., and Prati, F. (2003) *J Am Chem Soc* 125, 685-695.

³ (a) Davoli P., Fava R., Morandi S., Spaggiari A., Prati F. (2005) *Tetrahedron* 61, 4427-4436. (b) Matteson D.S., Maliakal D., Fabry-Asztalos L. (2008) *Journal of Organometallic Chemistry* 693, 2258-2262.
solution was stirred at room temperature for an additional hour. Thereafter, the temperature was lowered to –10 °C again and 2-thiophenacetyl chloride (1.07 mL, 8.70 mmol) in THF (5 mL) was slowly dropped in. After 10 min the cooling bath was removed and the resulting solution was allowed to react at room temperature for 3 h. Reaction was monitored by TLC (light petroleum/ethyl ether 8:2 with triethylamine 2%), that revealed a total conversion of starting 4. The reaction mixture was then partitioned between ethyl acetate (100 mL) and water (30 mL), the aqueous phase re-extracted with ethyl acetate (2 x 40 mL) and the combined organic phases were washed with saturated NaHCO₃ (30 mL), dried over MgSO₄, filtered and concentrated in vacuo. The crude was crystallized from diethyl ether and n-hexane to give compound 5 as a beige solid (1.90 g, 62%). M.p. 139-140 °C. [α]D = –61.5 (c 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 0.85 (3H, s, pynanyl CH₃), 1.28 (3 H, s, pinanyl CH₃), 1.34 (1H, d, J = 10.5, pinanyl Hendo), 1.39 (3H, s, pinanyl CH₃), 1.77-2.36 (5H, m, pinanyl protons), 2.95 (1H, td, J = 10.1, 3.4, BCH), 3.42 (1H, dd, J = 12.9, 10.1, BCH-CH₂), 3.60 (1H, dd, J = 12.9, 3.4, BCH-CH₂), 3.91 (2H, s, CH₂CO), 4.24 (1H, dd, J = 8.7, 2.1, CHO-B), 6.59 (1H, br, NH), 6.96-6.97 (1H, m, HArom), 7.02 (1H, dd, J = 5.2, 3.5, HArom), 7.30 (1H, dd, J = 5.2, 1.1, HArom). ¹³C NMR (100 MHz, CDCl₃): δ 24.2, 26.6, 27.3, 28.9, 34.0, 36.3, 38.2, 39.9, 41.4 (br, CB), 52.0, 53.9, 77.3, 84.6, 126.3, 127.6, 128.2, 133.4, 174.4. HRMS (ESI-TOF) m/z: [M–H]⁻ Calcd for C₁₈H₂₄BN₄O₃S 387.1671; Found 387.1656.

(+)-Pinanediol (1R)-2-(4-carboxy-[1,2,3]triazol-1-yl)-1-(2-thienylacetylamino)ethaneboronate (6) In a microwave glass vial the starting compound 5 (100 mg, 0.26 mmol) and propiolic acid (17 µL, 0.28 mmol) were dissolved in 1,4-dioxane (3 mL) and copper in charcoal 3 wt% (55 mg, 0.026 mmol) was added. The vessel was sealed and heated under microwave irradiation at 150 °C for 30 min. The reaction mixture was filtered
through a pad of celite to remove the catalyst and washed with ethyl acetate. The solution was concentrated in vacuo and the crude was crystallized from diethyl ether and n-hexane to afford 6 as a beige solid (71 mg, 60%) M.p. 112-114 °C dec. [α]D = −71.6 (c 1.3, MeOH). 1H NMR (400 MHz, DMSO-d6): δ 0.82 (3H, s, pinanyl CH3), 1.23 (3H, s, pinanyl CH3), 1.24 (3H, s, pinanyl CH3), 1.37 (1H, d, J = 9.9, pinanyl Hendo), 1.64-2.23 (5H, m, pinanyl protons), 3.05 (1H, d, J = 8.6, BCH), 3.89 (2H, s, CH2CO), 4.06 (1H, d, J = 7.1, CHO), 4.35 (1H, dd, J = 14.4, 10.6, BCH-CH2), 4.43 (1H, dd, J = 14.4, 3.9, BCH-CH2), 6.95-6.99 (2H, m, HArom), 7.43 (1H, d, J = 4.9, HArom), 8.69 (1H, s, CTriaz), 9.76 (1H, s, CONH), 13.06 (1H, br, COOH). 13C NMR (100 MHz, DMSO-d6): δ 24.0, 26.2, 27.2, 29.2, 31.6, 36.5, 37.6, 39.3, 42.5 (br, CB), 52.0, 52.3, 75.5, 82.4, 125.7, 126.8, 126.9, 129.3, 134.6, 139.5, 161.9, 175.1. HRMS (ESI-TOF) m/z: [M–H]− Calcd for C21H26BN4O5S 457.1726; Found 457.1747.

(1R)-2-(4-Carboxy-[1,2,3]triazol-1-yl)-1-(2-thienylacetylamino)ethaneboronic acid (7, S02030) To a solution of the (+)-pinanediol boronate 6 described above (255 mg, 0.56 mmol) in acetonitrile (3 mL) were sequentially added phenylboronic acid (68 mg, 0.56 mmol), n-hexane (3 mL) and 3N HCl (0.56 mL, 1.68 mmol). The resulting biphasic solution was vigorously stirred at room temperature. After 30 min the n-hexane layer, containing the pinanediol phenylboronate, was removed and fresh n-hexane (3 mL) was added. This last procedure was repeated several times until a TLC analysis revealed no remaining pinanediol boronate. The acetonitrile phase was concentrated and the residue crystallized from acetonitrile, affording the expected boronic acid 7 as beige solid (178 mg, 98%). M.p. 89-91 °C. [α]D = −85.0 (c 0.94, MeOH). 1H NMR (400 MHz, CD3OD): δ 3.25 (1H, dd, J = 10.3, 3.8, BCH), 4.04 (2H, s, CH2CO), 4.44 (1H, dd, J = 14.5, 10.3, BCH-CH2), 4.54 (1H, dd, J = 14.5, 3.8, BCH-CH2), 6.97 (1H, dd, J = 5.0, 3.6, HArom), 7.01-7.02 (1H, m, HArom), 7.33 (1H, d, J = 5.0, HArom), 8.53 (1H, s, CTriaz). 13C NMR (100 MHz,
CD$_3$OD): $\delta$32.1, 47.2 (br, CB), 53.7, 126.9, 128.2, 128.9, 130.2, 134.5, 141.0, 163.2, 179.2.

HRMS (ESI-TOF) $m/z$: [M–H]$^-$ Calcd for C$_{11}$H$_{12}$BN$_4$O$_5$S 323.0629; Found 323.0619.
Supplementary Figures

Figure S1. NMR spectra of intermediates 4 (a), 5 (b), 6 (c), and final product S02030 (d).

a.

Compound 4

NMR spectra showing peaks at various ppm values.
b. Compound 5

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
Compound 6

$^1$H NMR (400 MHz, DMSO-d$_6$)

$^{13}$C NMR (100 MHz, DMSO-d$_6$)
d.

Compound 7 (S02030)
Figure S2. A. Representative, two-state thermal denaturation of ADC-7 (filled circles) and ADC-7 with *meta*-carboxyphenyl chiral cephalothin analog SM23 (empty circles) as monitored by far-UV CD at 221 nm. The melting temperature for ADC-7 was $T_m \sim 61^\circ C$, and for the complex of ADC-7 with SM23, $T_m \sim 64^\circ C$. B. CD spectrum ADC-7 (filled circles) and ADC-7 with SM23 (empty circles). The differences in signal ellipticity suggest subtle conformational changes induced by ligand upon binding.
**Figure S3.** Isothermal titration calorimetric measurements (ITC) of the binding of ADC-7 β-lactamase (11 µM) and the chiral *meta*-carboxyphenyl cephalothin analog (SM23) (250 µM). The trace represents the recorded changes in heat upon binding, in (μcal/sec) as a function of time for successive injections of the inhibitor (upper row). Integrated heats (lower row) are plotted against the molar ratio of the binding reaction, and fitted with a non-linear least squares equation using a One Site binding model. ($\Delta H = -5.4 \text{ kcal/mol}, \Delta S = +14 \text{ cal/mol}^*K, K_D = 90 \text{ nM}$). The data are reported in Table 1.