Hydrazide of rhodamine B [36]. Rhodamine B (1.19 g, 2.62 mmol) was dissolved in 15 ml of MeOH, then hydrazine hydrate (6 ml, 124 mmol) was added, and the mixture was stirred for 24 h at room temperature. The product was filtered off, washed with cold MeOH, dissolved in minimal amount of MeOH, and precipitated by water. Then it was filtered off and washed with cold MeOH. Yield: 0.44 g (37%); TLC: Rf (CHCl3–MeOH, 9 : 1) 0.63, Rf (CHCl3–EtOH–AcOH, 85 : 10 : 5) 0.83, Rf (CHCl3–MeOH, 4 : 1) 0.84. UV (70% H2O–30% MeCN): λmax = 554 nm; LC-MS, m/z calculated for C28H32N4O2+= 457.3, found 457.6.

Rho-Tyl (I) (Fig. S1). Tylosin (26 mg, 0.028 mmol) was dissolved in 1.2 ml of 0.4 M sodium acetate buffer (pH 4.7) and mixed with the solution of rhodamine B hydrazide (13.2 mg, 0.028 mmol) in 2.2 ml DMSO. The mixture was kept overnight at room temperature, whereupon it was diluted with 20 ml of water, titrated with 5% NaHCO3 to pH 8.5, and extracted with CHCl3 (3 × 15 ml). Then the organic layer was dried over anhydrous MgSO4, filtered, and evaporated on a rotary evaporator. Resulting crimson oil was purified by column chromatography in the solvent systems: CHCl3–MeOH, 9 : 1 and CHCl3–MeOH–AcOH, 60 : 10 : 1. Yield: 30.4 mg (80%); TLC: Rf (CHCl3–MeOH, 9 : 1) 0.45, Rf (CHCl3–MeOH–CH3COOH, 60 : 10 : 1) 0.25, Rf (CHCl3–MeOH, 8 : 1) 0.67; τ (HPLC) = 19.2 min (gradient of 20–80% MeCN in H2O (0.01% TFA) for 30 min); fluorescence (H2O): λex = 552 nm, λem = 578 nm; MALDI MS, m/z calculated for C29H34N4O2+, 457.3, found 457.6.

Rho-Des (II) was obtained similarly to Rho-Tyl (I) starting from the following reagents: desmycosin (200 mg, 0.26 mmol) and rhodamine B hydrazide (120 mg, 0.26 mmol). The product was purified by column chromatography in the solvent system: CHCl3–MeOH, 8 : 1. Yield: 90.0 mg (30%); TLC: Rf (CHCl3–MeOH–H2O, 65 : 25 : 4) 0.70, Rf (CHCl3–MeOH, 6 : 1) 0.52, Rf (CHCl3–MeOH, 8 : 1) 0.28; τ (HPLC) = 16.0 min (gradient of 20–80% MeCN in H2O (0.01% TFA) for 30 min); UV (70% H2O–30% MeCN): λmax = 554, 278, 238 nm; ε (554 nm, 70% H2O–30% MeCN) = 1.87·105 M–1·cm–1; fluorescence (H2O): λex = 563 nm, λem = 584 nm; MALDI MS m/z calculated for C67H91N4O21S+, 1319.6, found 1317.5.

Flu-Tyl (III). Tylosin (12 mg, 0.013 mmol) was dissolved in 0.6 ml of 0.4 M sodium acetate buffer (pH 4.7) and mixed with the solution of fluorescein-5-thiosemicarbazide (6 mg, 0.014 mmol) in 0.6 ml of DMSO. The mixture was kept at 50°C for 12 h. Then the solution was titrated with 5% NaHCO3 to pH 8.5 and extracted with ethyl acetate. Then organic layer was dried over anhydrous Na2SO4, filtered and evaporated on a rotary evaporator. The resulting yellow oil was purified by column chromatography in the solvent system: CHCl3–MeOH, 4 : 1. Yield: 12 mg (70%); TLC: Rf (CHCl3–MeOH, 4 : 1) 0.41, Rf (CHCl3–MeOH, 5 : 1) 0.24, Rf (CHCl3–MeOH–AcOH, 60 : 10 : 1) 0.09; τ (HPLC) = 14.5 min (gradient of 20–80% MeCN in H2O (0.01% TFA) for 30 min); fluorescence (0.1 M Tris, pH 9.0): λex = 492 nm, λem = 516 nm; MALDI MS, m/z calculated for C67H98N5O15S+, 1212.7, found 1213.0.

BODIPY-Tyl (IV) (Fig. S1). To a solution of BODIPY FL C5 succinimide ester (2.0 mg, 4.8 μmol) in 200 μl CHCl3, 30 μl of 1 M anhydrous hydrazine (30 μmol) in THF was added, and the mixture was stirred at room temperature for 40 min. Then it was diluted with CHCl3 (300 μl), washed with water (3 × 100 μl) and evaporated in vacuo. TLC: Rf (CHCl3–MeOH, 9 : 1) 0.49. The result-
ing BODIPY FL C5 hydrazide (1.6 mg, 4.8 μmol) was dissolved in 50 μl of DMF and added to the tylosin (4.4 mg, 4.8 μmol) solution in 250 μl of sodium acetate buffer (0.4 M sodium acetate, pH 5.7). The mixture was stirred for 30 min at the room temperature, diluted with water, the product was extracted with CHCl₃, and the combined organic extracts were evaporated in vacuo after drying. The resulting product was purified by HPLC (gradient of 20-80% MeCN in H₂O (0.01% TFA) for 30 min).

Yield: 2.2 mg (37%); TLC: R_f (CHCl₃–MeOH, 9 : 1) 0.27, R_f (CHCl₃–MeOH, 4 : 1) 0.60, R_f (CHCl₃–MeOH–H₂O, 65 : 25 : 4) 0.75; τ (HPLC) = 15.7 min (gradient of 20-80% MeCN in H₂O (0.01% TFA) for 20 min); UV (MeOH): λ_max = 505, 284 nm; fluorescence (BIND buffer): λ_ex = 504 nm, λ_em = 512 nm; LC-MS, m/z calculated for C₆₂H₉₇BF₂N₅O₁₇+, 1232.7, found 1233.5; 1H NMR (600 MHz, CDCl₃), δ: 0.87 (3H, t, J 7.3, H₁₇), 0.96 (3H, dd, J 7.6, 6.8, H₁₈), 1.13 (3H, d, J 6.6, H₅CH₃), 1.19-1.22 (12H, m, H₃’CH₃, H₂₁, H₅’CH₃, H₅’’CH₃), 1.26 (2H, dd, J 6.7, 6.2, H₄⁸), 1.51-1.64 (4H, m, H₄, H₇, H₁₆a), 1.66 (1H, dd, J 14.5, 3.7, H₂”a), 1.68-1.77 (7H, m, H₂₂, H₂₈, H₃₈), 1.78-1.97 (3H, m, H₁₆b, H₂a, H₂”b), 2.13-2.29 (5H, m, H₆, H₁₉a, N-CH₃), 2.34-2.42 (1H, m, H₂b), 2.48 (3H, t, J 4.7, N-CH₃), 2.54 (1H, m, H₃’), 2.60 (1H, m, H₈), 2.81 (3H, s, CH₃), 2.88 (3H, s, CH₃), 2.89-2.98 (5H, m, H₁₉b, H₁₄, H₂”, H₁₈), 3.10 (1H, m, H₄”), 3.21-3.36 (2H, m, H₄”, H₅”), 3.41 (3H, s, H₂”OCH₃), 3.42-3.50 (3H, m, H₂”, H₅”), H₂₃a), 3.55 (4H, m, H₅”, H₃”OCH₃), 3.64 (1H, m, H₅), 3.68 (1H, t, J 3.0, H₃”), 3.81 (1H, m, H₃), 3.91 (1H, dt, J 9.4, 3.8, H₂₂b), 4.19-4.26 (2H, m, H₄”, H’”), 4.48 (1H, dt, J 7.9, 4.8, H’”), 4.92 (1H, m, H₁₅), 5.05 (1H, br s, H’”), 6.02 (1H, dd, J 12.2, 4.6, H₁₃), 6.16 (1H, d, J 15.8, H₁₀), 6.20-6.27 (1H, m, H₅”), 6.82-6.85 (2H, m, H₆”, H₈”), 7.22-7.33 (1H, m, H₁₁), 7.95 (1H, s, H₇”), 8.23 (1H, br s, H₂₀).

**NBD-TyI (V)** (Fig. S1).

*N-(7-nitro-2,1,3-benzoxadiazol-4-yl)ethane-1,2-diamine (Vα)* [37]. 4-Chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) (100 mg, 0.50 mmol) was dissolved in 10 ml of MeCN and slowly added to a solution of ethylenediamine (67 μl, 1.0 mmol) in MeCN (10 ml) at 0°C in
the darkness. The solution was stirred at 0°C for 30 min and then 1 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure; the residue was dissolved in 30 ml of water, extracted with CH₂Cl₂, dried over anhydrous Na₂SO₄ and evaporated on a rotary evaporator. The product was purified by column chromatography in the solvent system: CH₂Cl₂–MeOH, 2 : 1. Yield: 18 mg (16%); TLC: Rₜ(CH₂Cl₂–MeOH, 4 : 1) 0.10, Rₜ(CH₂Cl₂–MeOH, 2 : 1) 0.14, Rₜ(CH₂Cl₂–MeOH–aqNH₃, 65 : 25 : 4) 0.63; UV (H₂O): λₘₐₓ = 465, 335 nm; LC-MS, m/z calculated for C₁₇H₁₀N₅O₃⁺, 224.1, found 224.2.

**tert-Butyloxycarbonyl-2-(aminooxy)-N-{2-[(7-nitro-2,1,3-benzoxadiazole-4-yl)amino]ethyl}acetamide (Vb).** To a cooled to 0°C solution of (Boc-amino-oxy)acetic acid (15.0 mg, 0.078 mmol) in 1 ml DMF a solution of DCC (26.0 mg, 0.126 mmol) in 300 μl DMF was added with stirring. After 10 min, a solution of Va (17.4 mg, 0.078 mmol) and 14 μl (0.082 mmol) of DIPEA in 1 ml DMF was added. The reaction mixture was stirred for 1.5 h at 0°C and 15 h at room temperature. After filtration of dicyclohexylurea precipitate the reaction mixture was diluted with water (10 ml), extracted with CH₂Cl₂ (3 × 5 ml), washed with 0.05 M solution of H₂SO₄ (3 × 5 ml), water (5 ml), 5% solution of NaHCO₃ (3 × 5 ml), and saturated NaCl (2 ml). Then the organic layer was dried over anhydrous Na₂SO₄, filtered, and evaporated on a rotary evaporator. The product was purified by column chromatography in the solvent system: CH₂Cl₂–MeOH, 9 : 1 and dried in a desiccator over CaCl₂. Yield: 21.3 mg (69%); TLC: Rₜ(CH₂Cl₂–MeOH, 9 : 1) 0.62; UV (70% H₂O–30% MeCN): λₘₐₓ = 469, 335 nm.

**Fig. S2**. Competitive binding of NDB-Tyl and different common antibiotics to 70S ribosomes. a: 1) desmycosin; 2) clarithromycin; 3) azithromycin; b: 4) puromycin; 5) chloramphenicol.
Fig. S3. Competitive binding of fluorescently labeled tylosin and different antibiotics derivatives to 70S ribosomes. a) Displacement of NBD-Tyl by: 1) Phe-Tyl (VII); 2) Boc-βAla-OMT (X); 3) Car-Tyl (VIII). b) Displacement of BODIPY-Tyl by: 4) Phe-Tyl (VII); 5) Car-Tyl (VIII). c) Displacement of BODIPY-Tyl by: 6) Boc-βAla-OMT (X); 7) Boc-γAbu-OMT (XI); 8) Boc-Gly-OMT (IX).
Trifluoroacetate of 2-(aminooxy)-N-2,1,3-benzoxadiazole-4-ylamino[methyl]acetamide (Ve). Vb (21.0 mg, 0.053 mmol) was dissolved in 1 ml of TFA and stirred for 40 min at room temperature. Then TFA was evaporated to dryness on a rotary evaporator, MeOH was added, and the mixture was evaporated again. The product was precipitated with Et2O from MeOH and dried in a vacuum desiccator over CaCl₂. Yield: 21.5 mg (99%); TLC: \( R_f \) (CH₂Cl₂–MeOH, 9 : 1) 0.33; UV (70% H₂O–30% MeCN): \( \lambda_{max} = 475, 341 \) nm; LC-MS, m/z calculated for C₁₀H₁₃N₆O₅⁺, 297.1, found 297.2.

NBD-Tyl (V). Tylosin (47 mg, 0.051 mmol) was dissolved in 3 ml of sodium acetate buffer (0.4 M sodium acetate, pH 5.7) and mixed with the solution of Vc (21 mg, 0.051 mmol) in 8 ml of DMSO. The mixture was kept at 40°C for 17 h, then diluted with water, extracted with CHCl₃, and concentrated on a rotary evaporator. The product was purified by column chromatography on Al₂O₃ in the solvent system: CHCl₃–MeOH, 70 : 1. Yield: 22 mg (36%); TLC: \( R_f \) (CHCl₃–MeOH, 15 : 1) 0.06, \( R_f \) (CH₂Cl₂–MeOH, 3 : 1) 0.28; UV (MeOH): \( \lambda_{max} = 451 \) nm; \( \varepsilon(451 \text{ nm, MeOH}) = 14400 ± 800 \text{ M}^{-1} \cdot \text{cm}^{-1} \); fluorescence (BIND buffer): \( \lambda_{ex} = 488 \) nm, \( \lambda_{em} = 512 \) nm; MALDI MS, m/z calculated for C₆₀H₇₈N₅O₂₃S₂⁺ (M-micarose), 1301.3, found 1301.9.