Supplement to: A. G. Tereshchenkov, A. V. Shishkina, V. V. Karpenko, V. A. Chertkov, A. L. Konevega, P. S. Kasatsky, A. A. Bogdanov, and N. V. Sumbatyan, New Fluorescent Macrolide Derivatives for Studying Interactions of Antibiotics and Their Analogs with the Ribosomal Exit Tunnel (ISSN 0006-2979, *Biochemistry (Moscow)*, 2016, Vol. 81, No. 10, pp. 1163-1172)

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: R_f ($CHCl_3$–MeOH, 9 : 1) 0.65, R_f($CHCl_3$–EtOH–AcOH, 85 : 10 : 5) 0.83, R_f ($CHCl_3$–MeOH, 4 : 1) 0.84. UV (70% H_2O–30% MeCN): λ_{max} = 554 nm; LC-MS, m/z calculated for $C_{28}H_{32}N_4O_2^+$ 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 (0.4 M sodium acetate, 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% $NaHCO_3$ to pH 8.5, and extracted with $CHCl_3$ (3 × 15 ml). Then the organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated on a rotary evaporator. Resulting crimson oil was purified by column chromatography in the solvent systems: $CHCl_3$–MeOH, 9 : 1 and $CHCl_3$–MeOH–AcOH, 60 : 10 : 1. Yield: 30.4 mg (80%); TLC: R_f ($CHCl_3$–MeOH, 9 : 1) 0.45, R_f ($CHCl_3$–MeOH–CH_3COOH, 60 : 10 : 1) 0.25, R_f ($CHCl_3$–MeOH, 8 : 1) 0.67; τ (HPLC) = 19.2 min (gradient of 20-80% MeCN in H_2O (0.01% TFA) for 30 min); fluorescence (H_2O): λ_{ex} = 552 nm, λ_{em} = 578 nm; MALDI MS, m/z calculated for $C_{74}H_{108}N_5O_{18}^+$, 1354.8, found 1354.9; ^{1}H NMR (COSY, HSQC, HMBC, 600 MHz, $CDCl_3$) δ 0.94 (3H, t, J 7.3, H17), 0.97 (3H, br s, H18), 1.16 (12H, dt, J 8.3, 7.1, CH_3^R), 1.26 (6H, m, H21, H5‴CH_3), 1.33 (6H, d, J 5.9, H5′CH_3, H5″CH_3), 1.44 (4H, br s, H3″CH_3, H7a), 1.58-1.68 (3H, m, H16a, H4, H7b), 1.75 (1H, dd, J 14.5, 3.8, H2″a), 1.79 (9H, br s, H22, $N(CH_3)_2$), 1.89 (1H, ddd, J 14.3, 7.3, 2.6, H16b), 2.06 (1H, d, J 14.5, H2″b), 2.28 (1H, d, J 11.3, H19a), 2.43 (2H, br s, H2a,b), 2.55 (2H, m, H6, H3′), 2.85-2.97 (2H, br s, H14, H8), 3.02 (1H, dd, J 7.8, 2.8, H2‴), 3.06 (1H, d, J 9.0, H3) 3.18 (1H, td, J 10.1, 9.4, 3.2, H4‴), 3.24-3.28 (3H, m, H19b, H4′, H5′), 3.33 (8H, q, J 7.1, CH_2^R), 3.48 (3H, s, H2‴OCH_3), 3.52 (1H, m, H5‴), 3.54 (1H, dd, J 9.7, 6.4, H23a), 3.60-3.66 (6H, m, H3‴OCH_3, H2′, H5, H5″), 3.75 (1H, t, J 3.2, H3‴), 3.98 (1H, dd, J 9.7, 3.8, H23b), 4.17 (1H, br s, H4″), 4.45 (1H, br s, H1′), 4.55 (1H, d, J 7.8, H1‴), 5.01 (1H, td, J 9.6, 2.7, H15), 5.18 (1H, br s, H1″), 5.90 (1H, br s, H13), 6.18 (1H, br s, H10), 6.29 (2H, dd, J 8.2, 1.8, H6^R), 6.38 (2H, d, J 1.8, H7^R), 6.55 (2H, d, J 8.2, H5^R), 6.99 (1H, d, J 7.3, H4^R), 7.26 (1H, m, H11), 7.35-7.45 (2H, m, H2^R, H3^R), 7.92 (1H, br s, H1^R), 8.08 (1H, br s, H20); ^{13}C NMR (151 MHz, $CDCl_3$) δ 9.70 (2C), 12.64, 17.78 (2C), 18.22 (2C), 26.13, 29.71, 33.62, 39.13, 41.09, 44.29, 44.33 (2C), 45.08 (2C), 59.67, 61.80, 69.25, 70.67, 71.33, 72.70, 75.28, 77.24, 79.80, 81.95, 94.09, 98.12, 101.09, 108.19, 117.76, 120.31, 123.30, 123.61, 127.70, 128.09, 133.32, 142.28, 147.97, 152.72, 165.37.

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: $CHCl_3$–MeOH, 8 : 1. Yield: 90.0 mg (30%); TLC: R_f ($CHCl_3$–MeOH–H_2O, 65 : 25 : 4) 0.70, R_f ($CHCl_3$–MeOH, 6 : 1) 0.52, R_f ($CHCl_3$–MeOH, 8 : 1) 0.28; τ (HPLC) = 16.0 min (gradient of 20-80% MeCN in H_2O (0.01% TFA) for 30 min); UV (70% H_2O–30% MeCN): λ_{max} = 554, 278, 238 nm; ε (554 nm, 70% H_2O–30% MeCN) = $1.87 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$; fluorescence (H_2O): λ_{ex} = 563 nm, λ_{em} = 584 nm; MALDI MS m/z calculated for $C_{67}H_{98}N_5O_{15}^+$, 1212.7, found 1213.0.

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% $NaHCO_3$ to pH 8.5 and extracted with ethyl acetate. Then organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated on a rotary evaporator. The resulting yellow oil was purified by column chromatography in the solvent system: $CHCl_3$–MeOH, 4 : 1. Yield: 12 mg (70%); TLC: R_f($CHCl_3$–MeOH, 4 : 1) 0.41, R_f ($CHCl_3$–MeOH, 5 : 1) 0.24, R_f ($CHCl_3$–MeOH–AcOH, 60 : 10 : 1) 0.09; τ (HPLC) = 14.5 min (gradient of 20-80% MeCN in H_2O (0.01% TFA) for 30 min); fluorescence (0.1 M Tris, pH 9.0): λ_{ex} = 492 nm, λ_{em} = 516 nm; MALDI MS, m/z calculated for $C_{67}H_{91}N_4O_{21}S^+$, 1319.6, found 1317.5.

BODIPY-Tyl (IV) (Fig. S1). To a solution of BODIPY FL C5 succinimide ester (2.0 mg, 4.8 μmol) in 200 μl $CHCl_3$ 30 μl of 1 M anhydrous hydrazine (30 μmol) in THF was added, and the mixture was stirred at the room temperature for 40 min. Then it was diluted with $CHCl_3$ (300 μl), washed with water (3 × 100 μl) and evaporated *in vacuo*. TLC: R_f($CHCl_3$–MeOH, 9 : 1) 0.49. The result-

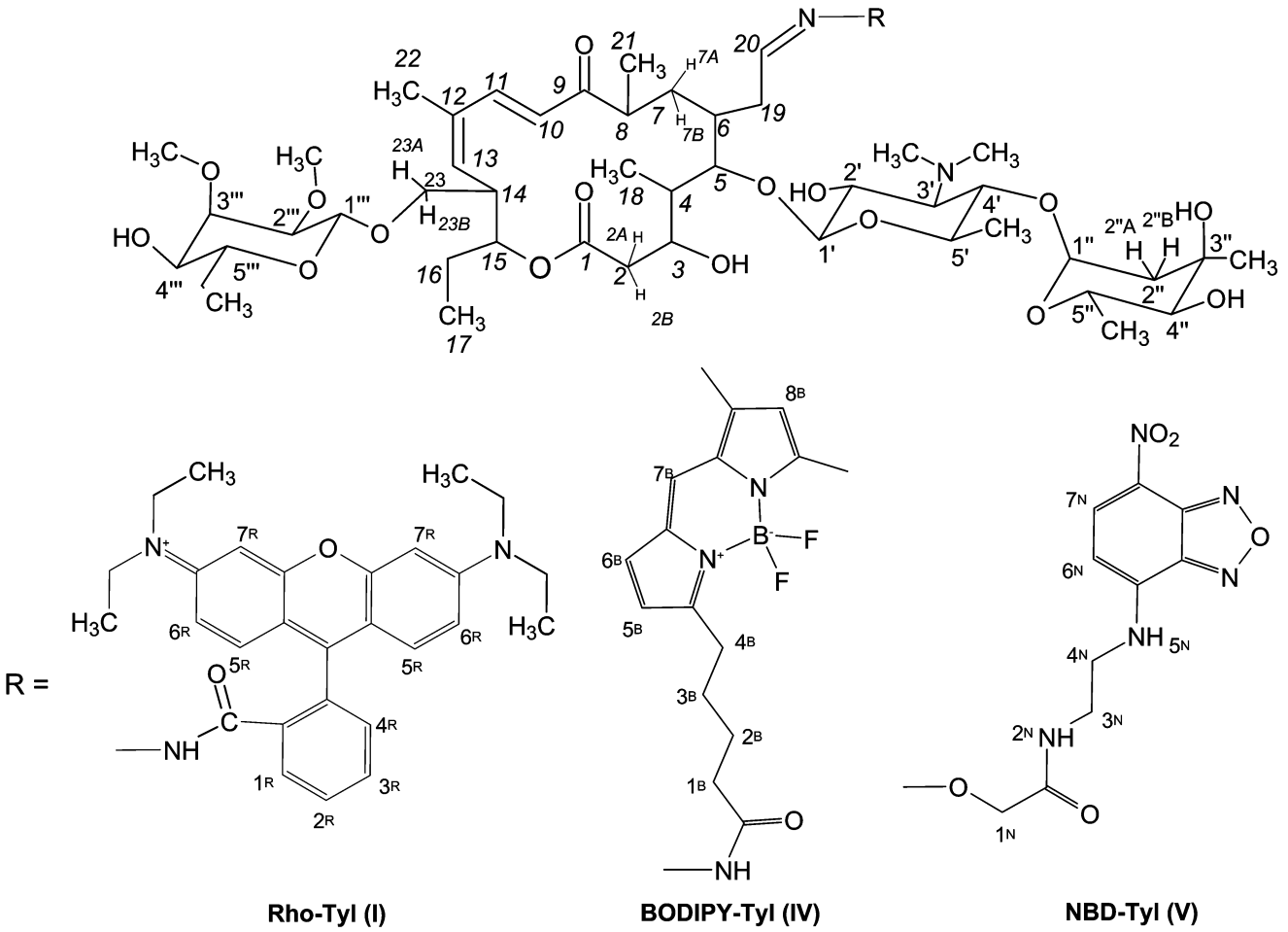

Fig. S1. Chemical structures of fluorescent derivatives of tylosin. Numbering of atoms used in the NMR spectral data is shown.

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 $CHCl_3$, and the combined organic extracts were evaporated *in vacuo* after drying. The resulting product was purified by HPLC (gradient of 20-80% MeCN in H_2O (0.01% TFA) for 30 min). Yield: 2.2 mg (37%); TLC: R_f ($CHCl_3$–MeOH, 9 : 1) 0.27, R_f ($CHCl_3$–MeOH, 4 : 1) 0.60, R_f ($CHCl_3$–MeOH–H_2O, 65 : 25 : 4) 0.75; τ (HPLC) = 15.7 min (gradient of 20-80% MeCN in H_2O (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 $C_{62}H_{97}BF_2N_5O_{17}^+$, 1232.7, found 1233.5; ^{1}H NMR (600 MHz, $CDCl_3$), 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, H5′CH_3), 1.19-1.22 (12H, m, H3″CH_3, H21, H5″CH3, H5‴CH_3), 1.26 (2H, dd, J 6.7, 6.2, H4^B), 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, H2^B, H3^B), 1.78-1.97 (3H, m, H16b, H2a, H2″b), 2.13-2.29 (5H, m, H6, H19a, N-CH_3), 2.34-2.42 (1H, m, H2b), 2.48 (3H, t, J 4.7, N-CH_3), 2.54 (1H, m, H3′), 2.60 (1H, m, H8), 2.81 (3H, s, CH_3^B), 2.88 (3H, s, CH_3^B), 2.89-2.98 (5H, m, H19b, H14, H2‴, H1^B), 3.10 (1H, m, H4‴), 3.21-3.36 (2H, m, H4′, H5′), 3.41 (3H, s, H2‴OCH_3), 3.42-3.50 (3H, m, H2′, H5‴, H23a), 3.55 (4H, m, H5″, H3‴OCH_3), 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, H23b), 4.19-4.26 (2H, m, H4″, H1′), 4.48 (1H, dt, J 7.9, 4.8, H1‴), 4.92 (1H, m, H15), 5.05 (1H, br s, H1″), 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^B), 6.82-6.85 (2H, m, H6^B, H8^B), 7.22-7.33 (1H, m, H11), 7.95 (1H, s, H7^B), 8.23 (1H, br s, H20).

NBD-Tyl (V) (Fig. S1).

***N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)ethane-1,2-diamine (Va)** [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 CH_2Cl_2, dried over anhydrous Na_2SO_4 and evaporated on a rotary evaporator. The product was purified by column chromatography in the solvent system: CH_2Cl_2–MeOH, 2 : 1. Yield: 18 mg (16%); TLC: R_f(CH_2Cl_2–MeOH, 4 : 1) 0.10, R_f (CH_2Cl_2–MeOH, 2 : 1) 0.14, R_f (CH_2Cl_2–MeOH–aqNH_3, 65 : 25 : 4) 0.63; UV (H_2O): λ_{max} = 465, 335 nm; LC-MS, m/z calculated for $C_8H_{10}N_5O_3^+$, 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-aminooxy)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 CH_2Cl_2 (3 × 5 ml), washed with 0.05 M solution of H_2SO_4 (3 × 5 ml), water (5 ml), 5% solution of $NaHCO_3$ (3 × 5 ml), and saturated NaCl (2 ml). Then the organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated on a rotary evaporator. The product was purified by column chromatography in the solvent system: CH_2Cl_2–MeOH, 9 : 1 and dried in a desiccator over $CaCl_2$. Yield: 21.3 mg (69%); TLC: R_f(CH_2Cl_2–MeOH, 9 : 1) 0.62; UV (70% H_2O–30% MeCN): λ_{max} = 469,

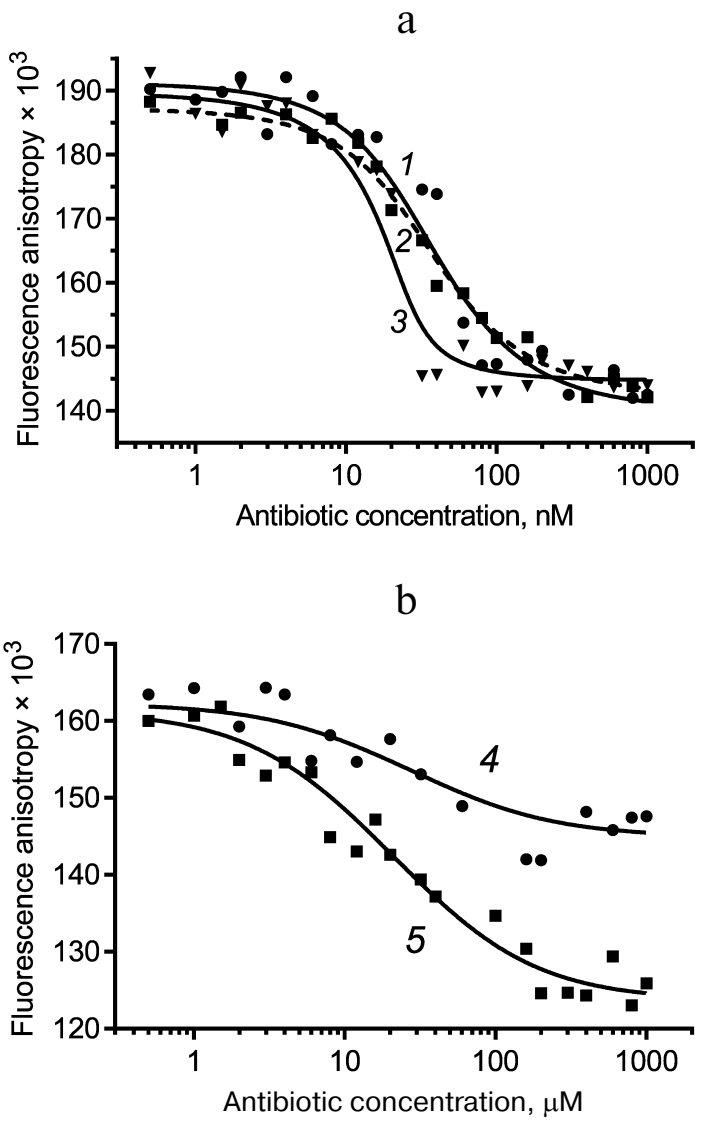

Fig. S2. Competitive binding of NDB-Tyl and different common antibiotics to 70S ribosomes. a: *1*) desmycosin; *2*) clarithromycin; *3*) azithromycin; b: *4*) puromycin; *5*) chloramphenicol.

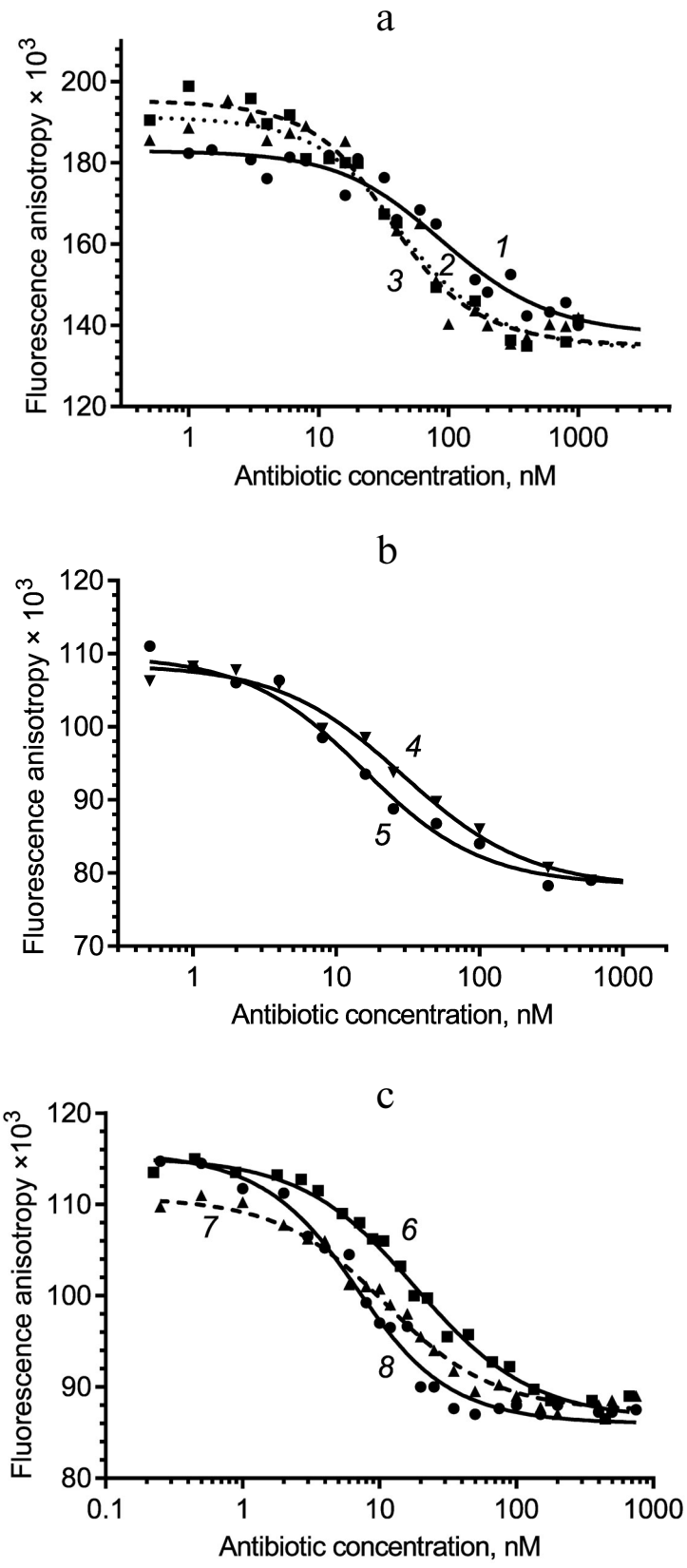

Fig. S3. Competitive binding of fluorescently labeled tylosin and different antibiotics derivatives to 70S ribosomes. a) Displacement of NBD-Tyl by: *1*) Phe-Tyl (**VII**); *2*) Boc-βAla-OMT (**X**); *3*) Car-Tyl (**VIII**). b) Displacement of BODIPY-Tyl by: *4*) Phe-Tyl (**VII**); *5*) Car-Tyl (**VIII**). c) Displacement of BODIPY-Tyl by: *6*) Boc-βAla-OMT (**X**); *7*) Boc-γAbu-OMT (**XI**); *8*) Boc-Gly-OMT (**IX**).

338 nm; LC-MS, m/z calculated for $C_{15}H_{20}N_6NaO_7^+$, 419.1, found 419.3.

Trifluoroacetate of 2-(aminooxy)-*N*-{2-[(7-nitro-2,1,3-benzoxadiazole-4-yl)amino]ethyl}acetamide (Vc). **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 Et_2O from MeOH and dried in a vacuum desiccator over $CaCl_2$. Yield: 21.5 mg (99%); TLC: R_f (CH_2Cl_2–MeOH, 9 : 1) 0.33; UV (70% H_2O–30% MeCN): λ_{max} = 475, 341 nm; LC-MS, m/z calculated for $C_{10}H_{13}N_6O_5^+$, 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 $CHCl_3$, and concentrated on a rotary evaporator. The product was purified by column chromatography on Al_2O_3 in the solvent system: $CHCl_3$–MeOH, 70 : 1. Yield: 22 mg (36%); TLC: R_f ($CHCl_3$–MeOH, 15 : 1) 0.06, R_f (CH_2Cl_2–MeOH, 3 : 1) 0.38, R_f (Al_2O_3, $CHCl_3$–MeOH, 70 : 1) 0.28; UV (MeOH): λ_{max} = 451 nm; ε (451 nm, MeOH) = 14400 ± 800 $M^{-1}\cdot cm^{-1}$; fluorescence (BIND buffer): λ_{ex} = 480 nm, λ_{em} = 547 nm; LC-MS, m/z calculated for $C_{56}H_{88}N_7O_{21}^+$, 1194.6, found 1195.1; ^{1}H NMR (600 MHz, $CHCl_3$), δ: 0.89 (3H, t, J 7.2, H17), 0.96 (3H, t, J 6.8, H18), 1.15 (3H, d, J 6.8, H5′CH_3), 1.16 (3H, s, H3″CH_3), 1.19 (3H, d, J 6.2, H21), 1.19 (3H, d, J 6.2, H5″CH_3), 1.22 (3H, d, J 6.2, H5‴CH_3), 1.52-1.60 (4H, m, H4, H7, H16a), 1.69 (1H, dt, J 14.5, 3.8, H2″a), 1.72 (3H, s, H22), 1.84 (1H, m, H16b), 1.94 (1H, d, J 14.1, H2a), 1.96 (1H, d, J 14.5, H2″b), 2.14-2.21 (2H, m, H6, H19a), 2.42 (8H, m, $N(CH_3)_2$, H2b, H3′), 2.69 (1H, m, H8), 2.87 (1H, d, J 9.6, H19b), 2.97 (1H, td, J 7.8, 2.9, H14), 2.98 (1H, dd, J 7.9, 3.2, H2‴), 3.11 (1H, dt, J 8.9, 4.6, H4‴), 3.21 (2H, m, H4′, H5′), 3.42 (3H, s, H2‴ OCH_3), 3.42-3.53 (7H, m, H2′, H5‴, H23a, $H3^N$, $H4^N$), 3.55-3.58 (1H, m, H5″), 3.54 (3H, s, H3‴ OCH_3), 3.67 (1H, dd, J 8.0, 2.3, H5), 3.69 (1H, t, J 3.1, H3‴), 3.82 (1H, m, H3), 3.94 (1H, dd, J 9.8, 4.0, H23b), 4.15 (1H, dd, 7.6, 4.9, H4″), 4.22 (1H, d, J 7.5, H1′), 4.46 (1H, d, J 7.6, $H1^N$a), 4.49 (1H, d, J 7.6, $H1^N$b), 4.50 (1H, d, J 7.8, H1‴), 4.92 (1H, td, J 9.5, 2.7, H15), 5.00 (1H, d, J 3.8, H1″), 5.88 (1H, d, J 10.8, H13), 6.11 (1H, d, J 8.6, $H6^N$), 6.21 (1H, dd, J 15.5, 11.4, $H2^N$), 6.26 (1H, d, J 15.4, H10), 7.17 (1H, d, J 15.4, H11), 7.37 (1H, t, J 6.5, H20), 7.72 (1H, dd, J 11.7, 7.1, $H5^N$), 8.39 (1H, d, J 8.6, $H7^N$). ^{13}C NMR (151 MHz, $CDCl_3$), δ 9.59, 9.79, 13.12, 17.42, 17.89, 18.40, 19.24, 25.57, 29.84, 31.77, 37.98, 39.47, 40.57, 41.07, 42.09, 43.94, 45.14 (2C), 45.24, 45.57 (2C), 59.75, 61.96, 66.31, 68.87, 68.97, 69.06, 69.61, 70.79, 71.92, 72.55, 73.41, 75.36, 75.52, 75.77, 76.54, 79.95, 82.06, 82.10, 96.77, 101.24, 104.05, 118.24, 124.12, 134.97, 136.50, 136.71, 143.20, 143.68, 144.12, 144.47, 148.91, 154.28, 172.90, 174.61, 204.78.

Alexa-Tyl (VI). Tylosin (0.8 mg, 0.87 μmol) and Alexa Fluor 488 hydrazide (0.5 mg, 0.87 μmol) were dissolved in 0.4 ml of 0.4 M sodium acetate buffer (pH 4.7) and kept for 24 h at 50°C whereupon the mixture was evaporated to dryness on a rotary evaporator. The desired product was separated by HPLC (gradient of 20-80% MeCN in H_2O (0.01% TFA) for 30 min). Yield: 0.6 mg (50%); R_f (^{n}PrOH–H_2O–aqNH_3, 55 : 35 : 10) 0.70; τ (HPLC) = 12.0 min (gradient of 20-80% MeCN in H_2O (0.01% TFA) for 30 min); fluorescence (H_2O): λ_{ex} = 488 nm, λ_{em} = 512 nm; MALDI MS, m/z calculated for $C_{60}H_{78}N_5O_{23}S_2^+$ (M-micarose), 1301.3, found 1301.9.