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NEW MACROCYCLES WITH POTENT ANTITUBERCULOSIS ACTIVITY ACCESSED BY ONE-POT MULTICOMPONENT REACTIONS

Based on modeling studies we hypothesized that tylosin derivatives without formyl group should rather adopt an erythromycin-like binding mode to the ribosome. Twenty four 16-membered macrocyclic compounds were accessed by multicomponent reactions (Gewald, Ugi) of tylosin and investigated for their antituberculosis activity. The best compound was twice as active as tylosin and might thus be a good starting point for further optimization of biological properties.

Keywords: tylosin, antituberculosis activity, Gewald reaction, macrocycle, multi-component reaction, Ugi reaction.

Approximately one third of the world's population is thought to be infected with *M. tuberculosis* [1]. Once the disease becomes active it is difficult to treat by the current chemotherapeutics and if untreated death rates are as high as 50%. Several reasons are discussed for the difficult disease control including cell division at a very slow rate (once every 16 to 20 h), unusual structure and chemical composition of the mycobacterial cell wall [2], intracellular growth [3], dormant intracellular states and thus evasion from immune system. In recent years more and more outbreaks of multiple drug resistant tuberculosis are reported. Spread and resistance has culminated in extensive drug-resistant tuberculosis (XDR-TB), a form of very drug resistant tuberculosis. In a recent outbreak in South Africa 53 patients in a rural hospital were found to have XDR-TB of whom 52 died [4].

Tylosin is a macrolide bacteriostatic antibiotic (made naturally by the bacterium *Streptomyces fradiae*), active against most gram-positive bacteria, mycoplasma, and certain gram-negative bacteria. It is acting on the 50S ribosome and neither tylosin nor derivatives have in the past been used in tuberculosis chemotherapy. Drug resistance should therefore not been established. We thereof hypothesized that suitably derivatized compounds based on the 16-membered macrocyclic tylosin backbone might be suitable starting points for the discovery of novel antibacterials. In continuation of our interest in neglected tropical and third world diseases and based on our new chemical access to a potential large number of new tylosin derivatives and docking investigation we would like to report the synthesis and our preliminary evaluation of their antituberculosis activity [5–7].

The ribosome is a prime target for antibiotics and many natural products have been described targeting this bacterial key structure, including neamine, ribostamycin, neomycin B, gentamycin, tobramycin, paromomycin, anisomycin, chloramphenicol, lincosamide, streptogramin and the macrocycles viomycin, capreomycin, telitromycin, erythromycin, just to name a few [8–12].

In 2002 the structure of tylosin bound to the large ribosomal substructure of *H. marismortui* has been reported (Fig. 1) [13]. In order to evaluate the potential ribosome binding of tylosin derivatives devoid of a formyl group we studied the structure and modeled some of our attempted synthesis targets in the crystal

structure. As opposed to other macrocyclic ribosome binding antibiotics tylosin binds reversibly covalent to nucleotide residue A2103-NH_2. Despite the different ring size and chemotype tylosin binds remarkably similar to the 14-membered erythromycin. In fact erythromycin and tylosin can be aligned and show similar main contacts to the receptor nucleotides (Fig. 2) [14]. Thus we hypothesized that blocking the formyl group of tylosin by performing chemistry at this functional group will lead to derivative which bind essentially similar to the way erythromycin binds to the ribosome. In fact hypothetic removal of the covalent bond between tylosin and its complex TA2105 and turning the base 45° away similar to the erythromycin receptor frees space on top of the formyl group of tylosin.

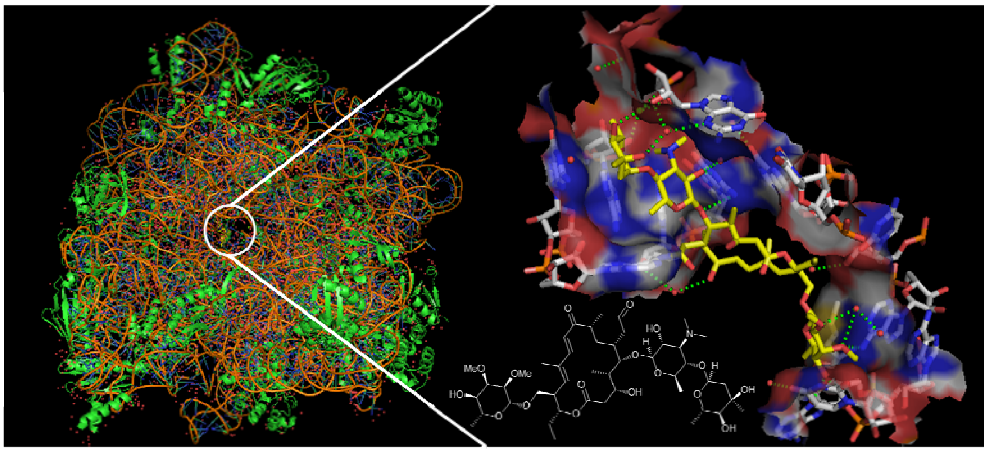


Fig 1. Co-crystal structure of tylosin bound to the 50S ribosomal subunit of *Haloarcula marismortui*. Left: view along the polypeptide exit tunnel which harbors tylosin in its center adjacent to the peptidyl transferase center. Right: close up view of the direct binding site of tylosin (yellow sticks) showing i. a. the extended hydrogen network of the receptor and the sugar hydroxyl groups (PDB ID 1K9M) and insert of the 2D structure of tylosin

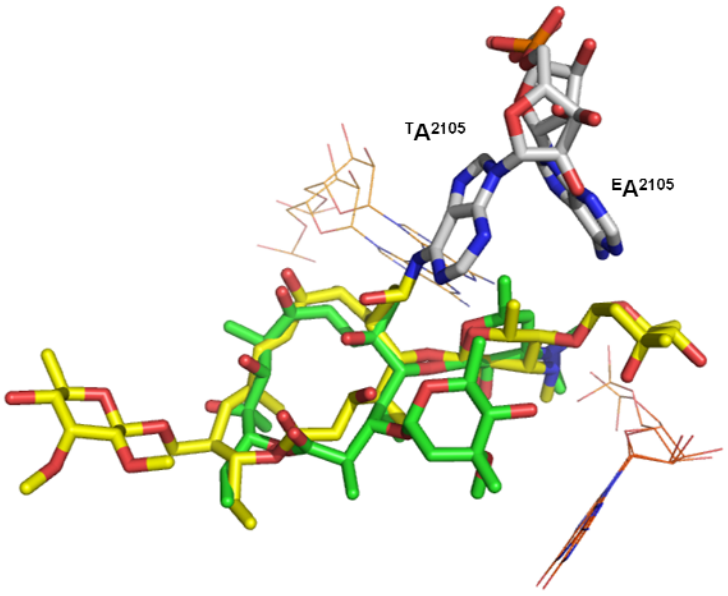


Fig. 2. Alignment of the two macrolide antibiotic tylosin (yellow sticks, PBD 1K9M) and erythromycin (green sticks, PBD 3OHJ) binding to the ribosome (white lines for tylosin, pink lines for erythromycin). $^{T}A^{2105}$ binds covalently to tylosin *via* a hemiaminal (grey sticks). $^{E}A^{2105}$ binds non covalently and is turned 45° away (grey sticks)

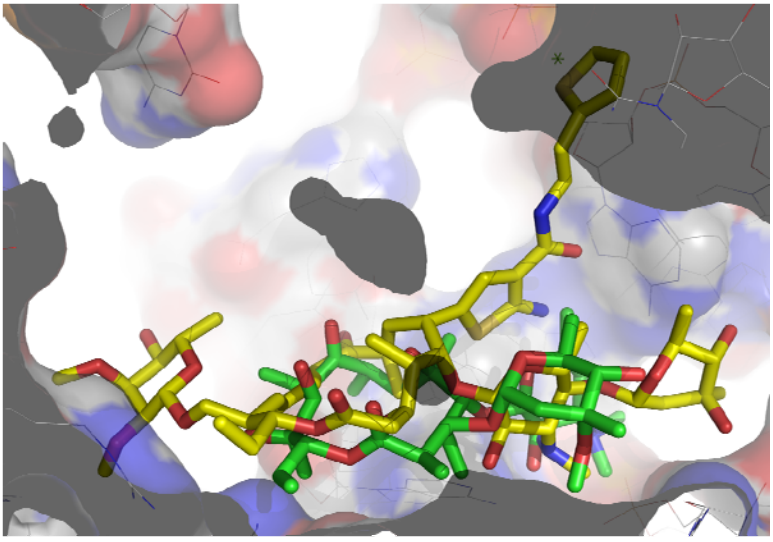


Fig. 3. Cut-away view of compound **6** (yellow sticks) docked into the erythromycin binding site (PDB 3OHJ). The overall macrocyclic backbone of x aligns well with the erythromycin (green sticks) and also in the desosamine and mycaminose basic sugar moiety. E. g. the charge interaction between the dimethylamine sugar moiety and the phosphate ester group of G2505 is recapitulated

The modeling encouraged us to dock some of the compounds into the erythromycin structure. Thus we created different conformers based on several different compounds planned to synthesize and docked those into the erythromycin structure (PBD 3OHJ) using the modeling and docking freeware MOLOC [15, 16]. A typical low energy pose is shown in Fig. 3. In fact the docking shows that tylosin is now adopting an erythromycin-like binding mode. Additionally, the prospective chemistry performed at the formyl group will likely be accommodated by the receptor and eventually new interactions can be picked up. These modeling results encouraged us to synthesize some compounds based on the formyl group of tylosin.

In order to verify or falsify the above computational hypothesis we decided to use fast and efficient multicomponent reaction chemistry (MCR) [17, 18]. MCR chemistry allows for the synthesis of many different scaffolds in very large compound numbers per scaffold. We first investigated the Gewald three-component reaction (Gewald-3CR) of sulfur, α-methylene aldehydes and cyanoacetates and their derivatives [19]. In order to synthesize novel amide derivatives we used our recently published diverse synthesis of cyanoacetamides and subsequent Gewald-3CR [20, 21]. We first investigated the reaction to see if the reaction can be performed in the presence of a wealth of unprotected functional groups in tylosin (hydroxyl, α,β,γ,δ-unsaturated ketone, tertiary amine, lactone, acetale) and to eventually find out optimal reaction conditions. To our delight we could isolated the expected product of the reaction of tylosin, sulfur and cyanoacetamide. Next we synthesized a small library of 20 novel 2-aminothiophene-3-carboxamide derivatives (Table 1).

Additionally we performed some Ugi reactions to evaluate their future usefulness in optimizing antibiotic activities of the target compounds [22]. We used tylosin (**1**) as a carbonyl compound as well as a carboxylic acid component (compound **22**) after oxidation with sulfamic acid and sodium chlorite [23]. Both reactions yielded the expected products **23**–**25** in acceptable yields.

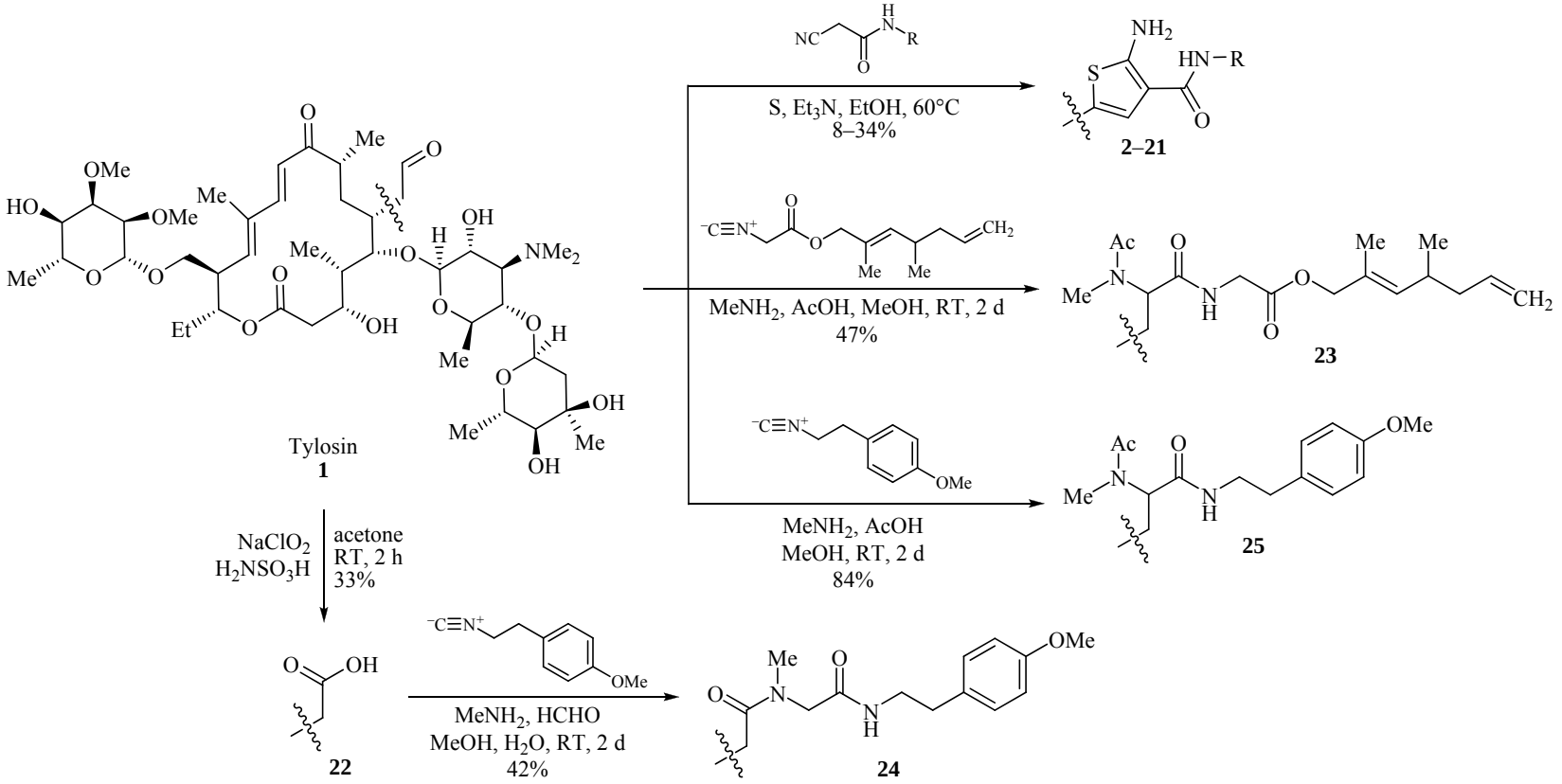
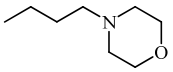
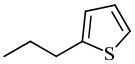
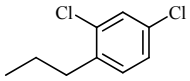

Tylosin
1
NaClO2
H2NSO3H
acetone
RT, 2 h
33%
22
S, Et3N, EtOH, 60°C
8–34%
2–21
MeNH2, AcOH, MeOH, RT, 2 d
47%
23
MeNH2, AcOH
MeOH, RT, 2 d
84%
25
MeNH2, HCHO
MeOH, H2O, RT, 2 d
42%
24

Table 1

Yields of 2-aminothiophene-3-carboxamide derivatives 2–21

Compound	R	Yield, %	Compound	R	Yield, %
2	Piperidino	17	**12**	*n*-Bu	9
3	CH_2-*t*-Bu	21	**13**	Allyl	11
4	Cyclohexyl	24	**14**	Propargyl	11
5		27	**15**	CH_2CH_2OH	22
6		18	**16**	$CH_2CH(OH)CH_2OH$	34
7	CH_2CH_2OEt	25	**17**	CH_2Ph	25
8	$CH_2CH_2CH_2OMe$	16	**18**	CH_2CH_2Ph	32
9	CH_2-3-Py	18	**19**	CH_2CHPh_2	19
10	Cyclopropyl	27	**20**	$CH_2CH_2CH_2Ph$	12
11		13	**21**		8

The compounds were screened in an microplate alamar blue assay versus BACTEC 460 system to determine the minimal inhibitory concentration (MIC) as described previously (Fig. 4) [24].

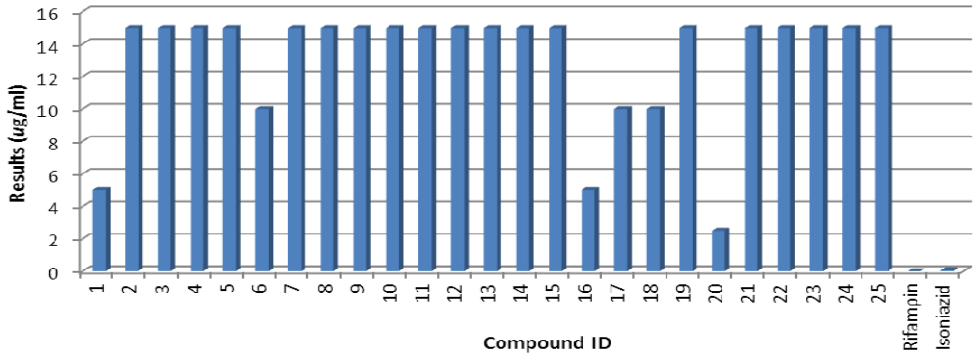


Fig. 4. Plot of tuberculosis MIC results

Table 2

Antituberculosis activity of compounds 6, 16–18, 20 compared with tylosin (1)

Compound	MIC (μg/ml)	IC_{50}	IC_{90}
Tylosin (**1**)	5	1.47	3.43
6	10	5.38	6.09
16	5	3.23	4.75
17	10	8.68	7.37
18	10	4.78	5.32
20	2.5	1.21	1.36

The IC_{50} and IC_{90} of the best compound **20** are slightly better than tylosin (**1**). There is a trend of augmenting activity with increasing chain length between the amide and the aryl group (benzyl < C_2-phenyl ~ C_2-thiophene < C_3-phenyl). Bulky branched biphenyl compounds are not tolerated. 1-Aminoglycol derived derivative **16** is similar active to tylosin (**1**).

We have prepared 24 novel macrocyclic compounds based on modeling studies performed in the macrolide binding site of the bacterial ribosome. We reasoned that chemistry performed at the aldehyde group of the 16-membered tylosin could yield compounds adopting a erythromycin-like binding mode. The erythromycin binding mode allows for the accommodation of substitutents imposed on the formyl group of tylosin. In order to test this hypothesis we synthesized 24 different derivatives by reacting the aldehyde group of tylosin in a one-pot Gewald-3CR and an Ugi MCR. Surprisingly all reactions could be performed starting form unprotected tylosin and the expected products could be isolated albeit in low yields. The compounds were tested for their antituberculosis activity. The most active compound was twice as active as tylosin and a structure–activity relationship could be established. The compounds synthesized herein form the basis for an ongoing optimization aiming towards potent antituberculosis drugs.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 600 spectrometer (600 and 150 MHz, respectively) in CD_3OD, residual solvent peaks were used as standard (3.30 ppm for ^1H nuclei, 49.0 ppm for ^{13}C nuclei). High resolution mass spectra were obtained at the University of Pittsburgh Mass Spectrometry facility. LC-MS analysis was performed on an Shimadzu instrument, using an analytical C18 column (Dionex Acclaim 120 Å, 2.1 × 50 mm, 3.0 μm, flow rate 0.2 ml/min). Analytical thin-layer chromatography (TLC) was preformed on silica gel plates available from Whatman, visualization was accomplished by UV irradiation at 254 nm, or by staining with one of the following reagents: iodine, ninhydrin (0.3% w/v in glacial AcOH – *n*-BuOH, 3:97), Vaughn's reagent (4.8 g of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ and 0.2 g of $Ce(SO_4)_2\cdot 4H_2O$ in 10 ml of conc. H_2SO_4 and 90 ml of H_2O). Preparative TLC was conducted using preparative silica gel TLC plates (1000 μm, 20 × 20 cm). Flash column chromatography was performed using silica gel 60 (particle size 0.040–0.055 mm, 230–400 mesh, EM science distributed by Bioman).

Starting cyanoacetamides are prepared according to the procedure described in [21]. All other reagents and solvents are commercially available and were used without additional purification. All reactions were carried out under air atmosphere.

Preparing compounds 2–21 by Gewald-3CR (General Method). A 20 ml vial with stir bar is charged with tylosin (**1**) (0.916 g, 1.0 mmol), *N*-substituted 2-cyanoacetamide (1.0 mmol), sulfur (0.032 g, 1.0 mmol), and Et_3N (0.101 g, 1.0 mmol) in EtOH (5 ml, 0.2 M solution). The reaction is heated at 60°C in an oil bath for 10 h. A chromatotron is utilized with appropriate solvents (50–100% EtOAc in hexanes) to extract appropriate tylosin derivatives as a light-yellow solid.

Compound 2. ^1H NMR spectrum, δ, ppm (*J*, Hz): 0.92 (3H, d, *J* = 6.8); 0.98 (3H, t, *J* = 7.2); 1.21 (3H, d, *J* = 6.8); 1.21 (3H, s); 1.23 (3H, d, *J* = 6.8); 1.24 (3H, d, *J* = 6.8); 1.26 (3H, d, *J* = 6.8); 1.91 (6H, s); 2.03 (3H, s); 3.52 (3H, s); 3.59 (3H, s); 4.60 (1H, d, *J* = 8.0); 5.05–5.12 (2H, m); 6.00 (1H, d, *J* = 10.8); 6.58 (1H, d, *J* = 14.4); 6.70 (1H, s); 7.27 (1H, d, *J* = 15.6). ^{13}C NMR spectrum, δ, ppm: 8.7; 11.7; 16.7; 17.4; 18.0; 18.2; 23.1; 24.3; 24.7; 25.2; 30.5; 34.0; 40.0; 40.5; 40.9; 41.2; 41.9; 44.8; 56.4; 58.2; 60.8; 65.2; 67.0; 68.8; 69.5; 69.7; 69.8; 71.1; 72.5; 73.2; 75.0; 76.4; 80.2; 81.4; 81.5; 83.7; 96.3; 100.9; 101.0; 102.9; 105.0; 119.2; 120.7; 126.0; 127.3; 135.0; 142.7; 148.3; 161.7; 164.8; 173.2; 205.4. Found, *m*/*z*: 1119.5698. $C_{54}H_{88}N_4NaO_{17}S$. Calculated, *m*/*z*: 1119.5763.

Compound 3. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.81 (9H, s); 0.92 (3H, d, *J* = 6.8); 0.98 (3H, t, *J* = 7.2); 1.21 (3H, d, *J* = 6.8); 1.21 (3H, s); 1.23 (3H, d, *J* = 6.8); 1.24 (3H, d, *J* = 6.8); 1.26 (3H, d, *J* = 6.8); 1.91 (6H, s); 2.03 (3H, s); 3.52 (3H, s); 3.59 (3H, s); 4.48 (1H, d, *J* = 8.0); 4.91–5.02 (2H, m); 5.89 (1H, d, *J* = 10.8); 6.48 (2H, d, *J* = 14.4); 6.62 (1H, s); 7.16 (1H, d, *J* = 15.6); 7.26 (1H, t, *J* = 6.4). ^{13}C NMR spectrum, δ, ppm: 8.8; 10.9; 11.8; 13.2; 16.7; 16.8; 17.4; 17.5; 18.1; 18.2; 19.6; 24.4; 24.7; 26.5; 26.7; 32.3; 33.7; 40.0; 40.5; 41.0; 41.2; 41.8; 44.8; 45.1; 49.5; 58.3; 60.2; 60.9; 65.3; 67.0; 68.8; 69.4; 69.7; 69.8; 71.1; 72.4; 73.2; 75.1; 76.4; 80.2; 81.5; 83.7; 96.4; 101.0; 102.9; 107.3; 119.2; 121.2; 126.2; 135.0; 142.7; 148.3; 160.5; 167.0; 171.6; 173.3; 205.4. Found, *m*/*z*: 1106.6819. $C_{54}H_{89}N_3NaO_{17}S$. Calculated, *m*/*z*: 1106.6810.

Compound 4. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.92 (3H, d, *J* = 6.8); 0.98 (3H, t, *J* = 7.2); 1.21 (3H, d, *J* = 6.8); 1.21 (3H, s); 1.23 (3H, d, *J* = 6.8); 1.24 (3H, d, *J* = 6.8); 1.26 (3H, d, *J* = 6.8); 1.91 (6H, s); 2.03 (3H, s); 3.52 (3H, s); 3.59 (3H, s); 5.05–5.10 (2H, m); 6.00 (1H, d, *J* = 10.8); 6.58 (1H, d, *J* = 14.4); 6.77 (1H, s); 7.27 (1H, d, *J* = 15.6). ^{13}C NMR spectrum, δ, ppm: 8.7; 11.7; 13.1; 16.7; 17.4; 18.1; 19.5; 24.3; 25.2; 25.3; 32.8; 33.0; 40.1; 41.0; 41.1; 44.8; 60.2; 60.8; 65.3; 68.8; 69.5; 69.7; 69.8; 71.1; 72.5; 73.2; 75.1; 76.5; 80.2; 81.5; 96.3; 101.0; 135.0; 148.3; 166.1; 171.7; 173.3; 205.6. Found, *m*/*z*: 1096.5998. $C_{55}H_{90}N_3O_{17}S$. Calculated, *m*/*z*: 1096.5991.

Compound 5. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.92 (3H, d, *J* = 6.8); 0.98 (3H, t, *J* = 7.2); 1.21 (3H, d, *J* = 6.8); 1.21 (3H, s); 1.23 (3H, d, *J* = 6.8); 1.24 (3H, d, *J* = 6.8); 1.26 (3H, d, *J* = 6.8); 1.91 (6H, s); 2.03 (3H, s); 3.52 (3H, s); 3.59 (3H, s); 4.49 (1H, d, *J* = 8.0); 4.91–4.98 (2H, m); 5.87 (1H, d, *J* = 10.8); 6.46 (1H, d, *J* = 14.4); 6.48 (1H, s); 7.15 (1H, d, *J* = 15.6). ^{13}C NMR spectrum, δ, ppm: 8.8; 11.5; 11.8; 13.1; 16.8; 17.5; 18.2; 19.6; 24.4; 24.7; 25.9; 34.4; 37.4; 40.1; 40.7; 41.0; 41.2; 41.9; 44.8; 44.9; 53.5; 56.0; 56.7; 58.3; 60.2; 60.8; 65.3; 66.4; 67.1; 68.8; 69.5; 69.7; 69.8; 71.1; 72.5; 73.2; 75.0; 75.1; 76.5; 80.2; 81.5; 83.9; 96.3; 101.0; 102.9; 106.6; 119.4; 121.3; 126.2; 135.0; 142.5; 148.2; 160.9; 167.0; 171.6; 173.5; 205.3. Found, *m*/*z*: 1163.5978. $C_{56}H_{92}N_4NaO_{18}S$. Calculated, *m*/*z*: 1163.6025.

Compound 6. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.92 (3H, d, *J* = 6.8); 0.98 (3H, t, *J* = 7.2); 1.21 (3H, d, *J* = 6.8); 1.21 (3H, s); 1.23 (3H, d, *J* = 6.8); 1.24 (3H, d, *J* = 6.8); 1.26 (3H, d, *J* = 6.8); 1.91 (6H, s); 2.03 (3H, s); 3.52 (3H, s); 3.59 (3H, s); 4.61 (1H, d, *J* = 8.0); 5.04–5.11 (2H, m); 6.00 (1H, d, *J* = 10.8); 6.59 (1H, d, *J* = 14.4); 6.65 (1H, s); 6.92 (1H, d, *J* = 3.2); 6.97 (1H, dd, *J* = 8.8, *J* = 3.2); 7.25 (1H, d, *J* = 4.4); 7.26 (1H, d, *J* = 15.6). ^{13}C NMR spectrum, δ, ppm: 11.4; 11.7; 11.8; 13.1; 16.8; 17.4; 18.0; 19.5; 24.4; 24.7; 28.9; 29.8; 34.3; 40.1; 40.7; 41.0; 41.2; 44.8; 47.0; 58.2; 60.2; 65.3; 67.1; 68.8; 69.5; 69.7; 69.8; 71.1; 72.6; 73.2; 75.0; 75.2; 76.5; 80.2; 81.5; 84.0; 96.4; 101.0; 106.8; 123.3; 124.8; 125.1; 126.0; 126.5; 135.0; 141.6; 142.5; 148.1; 161.0; 166.9; 173.3; 205.3. Found, *m*/*z*: 1146.5172. $C_{55}H_{85}N_3NaO_{17}S_2$. Calculated, *m*/*z*: 1146.5218.

Compound 7. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.92 (3H, d, *J* = 6.8); 0.98 (3H, t, *J* = 7.2); 1.21 (3H, d, *J* = 6.8); 1.21 (3H, s); 1.23 (3H, d, *J* = 6.8); 1.24 (3H, d, *J* = 6.8); 1.26 (3H, d, *J* = 6.8); 1.91 (6H, s); 2.03 (3H, s); 3.52 (3H, s); 3.59 (3H, s); 4.61 (1H, d, *J* = 8.0); 5.04–5.11 (2H, m); 6.00 (1H, d, *J* = 10.8); 6.59 (1H, d, *J* = 14.4); 6.65 (1H, s); 7.26 (1H, d, *J* = 15.6). ^{13}C NMR spectrum, δ. ppm: 8.7; 11.7; 14.1; 16.7; 17.4; 18.1; 24.4; 38.6; 40.1; 41.0; 44.8; 58.2; 60.7; 65.4; 66.0; 68.8; 69.0; 69.6; 69.7; 72.5; 73.2; 75.0; 76.4; 80.2; 81.5; 84.0; 96.3; 101.0; 106.7; 126.0; 135.0; 148.2; 161.0; 167.0; 173.2; 205.4. Found, *m*/*z*: 1108.5571. $C_{53}H_{87}N_3NaO_{18}S$. Calculated, *m*/*z*: 1108.5603.

Compound 8. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.89 (3H, s); 1.00 (3H, t, *J* = 7.2); 1.17 (3H, s); 1.21 (3H, s); 1.20 (3H, d, *J* = 7.2); 1.24 (3H, d, *J* = 7.2); 1.26 (3H, d, *J* = 7.2); 1.63–1.69 (1H, m); 1.91 (6H, s); 2.03 (3H, s); 3.52 (3H, s); 3.59 (3H, s); 4.61 (1H, d, *J* = 8.0); 5.05–5.12 (2H, m); 6.00 (1H, d, *J* = 10.8); 6.59 (1H, d, *J* = 14.4); 6.65 (1H, s); 7.26 (1H, d, *J* = 15.6). ^{13}C NMR spectrum, δ, ppm: 8.8; 11.8; 13.2; 16.8; 14.5; 18.2; 24.4; 29.5; 34.3; 36.3; 40.1; 40.6; 41.0; 41.2; 41.9; 44.8; 57.6; 58.3; 60.2; 60.8; 65.3; 67.0; 68.7; 68.8; 70.5; 71.1; 72.5; 73.2; 75.0; 75.1; 76.5; 80.2; 81.5; 83.9; 96.3; 191.0; 102.9; 106.8; 119.4; 121.3; 125.9; 135.0; 142.6; 148.2; 160.8; 167.0; 173.2; 205.3. Found, *m*/*z*: 1086.5731. $C_{53}H_{88}N_3O_{18}S$. Calculated, *m*/*z*: 1086.5784.

Compound 9. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.92 (3H, d, *J* = 6.8); 0.98 (3H, t, *J* = 7.2); 1.21 (3H, d, *J* = 6.8); 1.21 (3H, s); 1.23 (3H, d, *J* = 6.8); 1.24 (3H, d, *J* = 6.8); 1.26 (3H, d, *J* = 6.8); 1.91 (6H, s); 2.03 (3H, s); 3.52 (3H, s); 3.59 (3H, s); 4.61 (1H, d, *J* = 8.0); 5.04–5.11 (2H, m); 6.00 (1H, d, *J* = 10.8); 6.59 (1H, d, *J* = 14.4); 6.69 (1H, s); 7.24 (1H, d, *J* = 15.6); 7.44 (1H, dd, *J* = 8.0, *J* = 4.8); 7.85 (1H, d, *J* = 8.0); 8.44 (1H, dd, *J* = 7.2, *J* = 2.4); 8.56 (1H, d, *J* = 2.4). ^{13}C NMR spectrum, δ, ppm: 8.7, 11.7; 16.7; 17.5; 18.0; 19.3; 24.4; 24.7; 34.2; 39.7; 40.0, 40.6, 41.0, 41.2, 41.9, 44.8, 44.9, 58.1; 58.2; 60.8, 65.3, 67.0; 68.8; 69.2; 69.5, 69.7; 69.8; 71.0; 72.5; 73.2; 75.0; 76.4; 80.2; 81.5; 84.0; 96.3; 101.0; 102.9; 106.1; 119.3; 121.2; 123.9; 126.1; 127.3; 135.0; 136.2; 136.3; 142.6; 147.2; 148.1; 148.2; 161.6; 166.9; 173.2; 205.4. Found, *m*/*z*: 1105.5630. $C_{55}H_{85}N_4O_{17}S$. Calculated, *m*/*z*: 1105.5574.

Compound 10. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.60 (2H, s); 0.78 (2H, d, *J* = 6.6); 0.87 (3H, s); 0.96 (3H, t, *J* = 7.2); 1.21 (3H, d, *J* = 6.8); 1.21 (3H, s); 1.23 (3H, d, *J* = 6.8); 1.24 (3H, d, *J* = 6.8); 1.26 (3H, d, *J* = 6.8); 1.91 (6H, s); 2.03 (3H, s); 3.52 (3H, s); 3.59 (3H, s); 4.61 (1H, d, *J* = 8.0); 5.01–5.13 (2H, m); 6.00 (1H, d, *J* = 10.8); 6.59 (1H, d, *J* = 14.4); 6.65 (1H, s); 7.26 (1H, d, *J* = 15.6). ^{13}C NMR spectrum, δ, ppm: 5.3; 8.6; 11.8; 16.6; 16.9; 18.1; 21.5; 24.2; 39.6; 40.5; 41.0; 44.3; 52.8; 58.4; 60.8; 65.0; 68.9; 69.3; 70.5; 72.6; 74.7; 75.9; 79.6; 80.9; 84.0; 95.7; 100.5; 106.3; 134.5; 141.5; 147.8; 159.5; 167.9; 205.0. Found, *m*/*z*: 1076.5414. $C_{52}H_{83}N_3O_{17}SNa$. Calculated, *m*/*z*: 1076.5341.

Compound 11. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.27–0.30 (2H, m); 0.51–0.56 (2H, m); 0.90 (3H, s); 0.96 (3H, t, *J* = 7.2); 1.05–1.10 (1H, m); 1.21 (3H, d, *J* = 6.8); 1.21 (3H, s); 1.23 (3H, d, *J* = 6.8); 1.24 (3H, d, *J* = 6.8); 1.26 (3H, d, *J* = 6.8); 1.91 (6H, s); 2.03 (3H, s); 3.52 (3H, s); 3.59 (3H, s); 4.61 (1H, d, *J* = 8.0); 5.05–5.13 (2H, m); 6.00 (1H, d, *J* = 10.8); 6.59 (1H, d, *J* = 14.4); 6.65 (1H, s); 7.26 (1H, d, *J* = 15.6). ^{13}C NMR spectrum (150 MHz, MeOD), δ, ppm: 2.6; 8.7; 10.7; 11.7; 16.7; 17.4; 18.1; 24.3; 24.7; 40.1; 41.0; 41.1; 42.0; 43.3; 44.8; 58.2; 60.8; 65.2; 69.1; 69.5; 69.7; 69.8; 71.7; 72.5; 73.2; 75.0; 76.4; 80.2; 81.5; 84.1; 96.3; 101.1; 103.0; 106.3; 134.5; 141.5; 147.8; 159.5; 167.9; 205.0. Found, *m*/*z*: 1090.5514. $C_{53}H_{85}N_3O_{17}SNa$. Calculated, *m*/*z*: 1090.5498.

Compound 12. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.90 (3H, s); 0.96 (3H, t, *J* = 7.2); 1.21 (3H, d, *J* = 6.8); 1.21 (3H, s); 1.23 (3H, d, *J* = 6.8); 1.24 (3H, d, *J* = 6.8); 1.26 (3H, d, *J* = 6.8); 1.91 (6H, s); 2.03 (3H, s); 3.52 (3H, s); 3.59 (3H, s); 4.61 (1H, d, *J* = 8.0); 5.02–5.17 (2H, m); 6.00 (1H, d, *J* = 10.8); 6.59 (1H, d, *J* = 14.4); 6.65 (1H, s); 7.26 (1H, d, *J* = 15.6). ^{13}C NMR spectrum, δ, ppm: 8.3; 8.7; 11.7; 12.5; 16.9; 17.4; 18.1; 19.9; 24.3; 30.9; 31.8; 37.9; 38.5; 40.1; 41.0; 42.0; 43.3; 44.8; 58.2; 60.8; 65.2; 69.1; 69.7; 71.7; 72.5; 73.2; 75.0; 76.4; 80.2; 81.5; 84.1; 96.3; 101.1; 103.0; 106.3; 134.5; 141.5; 147.8; 159.5; 166.6; 205.0. Found, *m*/*z*: 1092.5675. $C_{53}H_{87}N_3O_{17}SNa$. Calculated, *m*/*z*: 1092.5654.

Compound 13. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.90 (3H, s); 0.96 (3H, t, *J* = 7.2); 1.21 (3H, d, *J* = 6.8); 1.21 (3H, s); 1.23 (3H, d, *J* = 6.8); 1.24 (3H, d, *J* = 6.8); 1.26 (3H, d, *J* = 6.8); 1.91 (6H, s); 2.03 (3H, s); 3.52 (s, 3H); 3.59 (3H, s); 4.61 (1H, d, *J* = 8.0); 5.03–5.16 (2H, m); 6.00 (1H, d, *J* = 10.8); 6.59 (1H, d, *J* = 14.4); 6.65 (1H, s); 7.26 (1H, d, *J* = 15.6). ^{13}C NMR spectrum, δ, ppm: 8.5; 8.7; 11.7; 12.1; 16.7; 17.4; 17.9; 18.1; 24.3; 24.7; 40.7; 40.9; 45.5; 58.2; 60.8; 65.2; 65.5; 69.1; 69.5; 69.7; 69.8; 71.7; 72.5; 73.2; 75.0; 76.4; 80.2; 81.5; 96.3; 101.1; 114.8; 118.7; 128.6; 134.5; 152.5; 152.8; 205.0. Found, *m*/*z*: 1054.5607. $C_{52}H_{84}N_3O_{17}S$. Calculated, *m*/*z*: 1054.5521.

Compound 14. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.90 (3H, s); 0.96 (3H, t, *J* = 7.2); 1.21 (3H, d, *J* = 6.8); 1.21 (3H, s); 1.23 (3H, d, *J* = 6.8); 1.24 (3H, d, *J* = 6.8); 1.26 (3H, d, *J* = 6.8); 1.91 (6H, s); 2.03 (3H, s); 3.52 (3H, s); 3.59 (3H, s); 4.61 (1H, d, *J* = 8.0); 5.06–5.15 (2H, m); 6.00 (1H, d, *J* = 10.8); 6.59 (1H, d, *J* = 14.4); 6.65 (1H, s); 7.26 (1H, d, *J* = 15.6). ^{13}C NMR spectrum, δ, ppm: 8.7; 11.7; 11.8; 16.7; 17.3; 17.4; 18.2; 24.3; 24.7; 34.3; 40.1; 41.0; 41.1; 44.9; 58.2; 60.8; 65.3; 68.7; 69.5; 69.7; 69.8; 72.5; 73.2; 75.0; 76.4; 80.2; 81.5; 96.3; 101.0; 106.5; 114.5; 135.0; 135.1; 148.2; 152.5; 166.8; 173.26; 205.2. Found, *m*/*z*: 1052.5275. $C_{52}H_{82}N_3O_{17}S$. Calculated, *m*/*z*: 1052.5365.

Compound 15. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.90 (3H, s); 0.96 (3H, t, *J* = 7.2); 1.21 (3H, d, *J* = 6.8); 1.21 (3H, s); 1.23 (3H, d, *J* = 6.8); 1.24 (3H, d, *J* = 6.8); 1.26 (3H, d, *J* = 6.8); 1.91 (6H, s); 2.03 (3H, s); 3.52 (3H, s); 3.59 (3H, s); 4.61 (1H, d, *J* = 8.0); 4.91–4.99 (2H, m); 6.00 (1H, d, *J* = 10.8); 6.59 (1H, d, *J* = 14.4); 6.65 (1H, s); 7.26 (1H, d,

J = 15.6). ^{13}C NMR spectrum, δ, ppm: 10.1; 13.1; 14.5; 18.1; 18.8; 19.4; 19.5; 20.9; 25.7; 26.1; 35.9; 41.6; 42.1; 42.4; 42.6; 42.7; 43.4; 46.2; 59.7; 61.6; 62.2; 62.3; 66.7; 68.4; 70.2; 71.0; 71.1; 71.2; 72.5; 73.9; 74.6; 77.9; 76.5; 81.7; 82.9; 85.3; 97.7; 102.5; 104.3; 107.9; 120.9; 122.9; 127.3; 136.4; 149.6; 162.5; 168.7; 173.0; 174.6; 206.9. Found, *m*/*z*: 1058.2801. $C_{51}H_{84}N_3O_{18}S$. Calculated, *m*/*z*: 1058.2790.

Compound 16. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.90 (3H, s); 0.96 (3H, t, *J* = 7.2); 1.21 (3H, d, *J* = 6.8); 1.21 (3H, s); 1.23 (3H, d, *J* = 6.8); 1.24 (3H, d, *J* = 6.8); 1.26 (3H, d, *J* = 6.8); 1.91 (6H, s); 2.03 (3H, s); 3.52 (3H, s); 3.59 (3H, s); 4.61 (1H, d, *J* = 8.0); 5.04–5.12 (2H, m); 6.00 (1H, d, *J* = 10.8); 6.59 (1H, d, *J* = 14.4); 6.65 (1H, s); 7.26 (1H, d, *J* = 15.6). ^{13}C NMR spectrum, δ, ppm: 7.9; 8.1; 8.6; 16.7; 17.4; 18.1; 24.7; 25.5; 40.1; 41.0; 41.1; 42.0; 43.3; 44.8; 58.2; 60.8; 64.2; 68.3; 68.7; 69.4; 69.8; 71.7; 72.5; 73.2; 75.0; 76.4; 80.2; 81.5; 91.9; 101.4; 101.9; 107.3; 134.7; 143.5; 148.7; 159.5; 167.9; 205.0. Found, *m*/*z*: 1088.5518. $C_{52}H_{86}N_3O_{19}S$. Calculated, *m*/*z*: 1088.5576.

Compound 17. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.90 (3H, s); 0.96 (3H, t, *J* = 7.2); 1.21 (3H, d, *J* = 6.8); 1.21 (3H, s); 1.23 (3H, d, *J* = 6.8); 1.24 (3H, d, *J* = 6.8); 1.26 (3H, d, *J* = 6.8); 1.91 (6H, s); 2.03 (3H, s); 3.52 (3H, s); 3.59 (3H, s); 4.61 (1H, d, *J* = 8.0); 4.91–4.98 (2H, m); 5.87 (1H, d, *J* = 10.8); 6.47 (1H, d, *J* = 14.4); 6.61 (1H, s); 7.11–7.15 (3H, m); 7.21–7.26 (5H, m). ^{13}C NMR spectrum, δ, ppm: 10.2; 13.1; 18.1; 18.9; 19.6; 25.7; 41.5; 42.4; 42.6; 43.6; 46.2; 61.6; 62.2; 66.7; 70.9; 71.1; 71.2; 73.9; 74.6; 76.4; 76.7; 77.9; 81.6; 82.9; 85.5; 97.8; 102.4; 104.4; 108.0; 120.8; 122.8; 127.4; 128.5; 129.2; 129.8; 136.4; 144.1; 149.7; 162.6; 168.2; 174.7; 206.9. Found, *m*/*z*: 1104.5760. $C_{56}H_{86}N_3O_{17}S$. Calculated, *m*/*z*: 1104.5678.

Compound 18. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.90 (3H, s); 0.96 (3H, t, *J* = 7.2); 1.21 (3H, d, *J* = 6.8); 1.21 (3H, s); 1.23 (3H, d, *J* = 6.8); 1.24 (3H, d, *J* = 6.8); 1.26 (3H, d, *J* = 6.8); 1.91 (6H, s); 2.03 (3H, s); 2.41–2.56 (2H, m); 3.52 (3H, s); 3.59 (3H, s); 4.61 (1H, d, *J* = 8.0); 5.04–5.11 (2H, m); 5.99 (1H, d, *J* = 10.8); 6.64 (1H, d, *J* = 14.4); 7.19–7.35 (5H, m). ^{13}C NMR spectrum, δ, ppm: 8.7; 11.7; 13.1; 16.8; 17.4; 18.2; 19.5; 24.4; 35.9; 40.1; 40.6; 41.0; 41.2; 44.8; 58.2; 60.1; 60.8; 65.3; 69.5; 69.7; 69.8; 71.1; 72.6; 73.2; 75.0; 75.2; 76.5; 80.2; 81.5; 84.0; 96.4; 101.0; 106.9; 125.9; 128.2; 128.5; 135.0; 139.5; 148.1; 160.8; 166.9; 171.6; 173.2; 205.3. Found, *m*/*z*: 1118.5868. $C_{57}H_{87}N_3O_{17}S$. Calculated, *m*/*z*: 1118.5834.

Compound 19. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.90 (3H, s); 0.96 (3H, t, *J* = 7.2); 1.21 (3H, d, *J* = 6.8); 1.21 (3H, s); 1.23 (3H, d, *J* = 6.8); 1.24 (3H, d, *J* = 6.8); 1.26 (3H, d, *J* = 6.8); 1.91 (6H, s); 2.03 (3H, s); 3.52 (3H, s); 3.59 (3H, s); 4.61 (1H, d, *J* = 8.0); 5.03–5.11 (2H, m); 5.97 (1H, d, *J* = 10.8); 6.47 (1H, d, *J* = 14.4); 6.61 (1H, s); 7.19–7.28 (2H, m); 7.29–7.37 (8H, m). ^{13}C NMR spectrum, δ, ppm: 8.7; 11.1; 11.7; 13.1; 16.7; 17.4; 18.1; 19.4; 19.5; 24.3; 24.6; 25.7; 41.5; 42.4; 42.6; 43.6; 46.2; 61.6; 62.2; 66.7; 70.9; 71.1; 71.2; 73.9; 74.6; 76.4; 76.7; 77.9; 81.6; 82.9; 85.5; 97.8; 102.4; 104.4; 108.0; 120.8; 122.8; 127.4; 128.5; 129.2; 129.8; 136.4; 144.1; 149.7; 162.6; 168.2; 174.7; 205.4. Found, *m*/*z*: 1194.4736. $C_{63}H_{91}N_3O_{17}S$. Calculated, *m*/*z*: 1194.4715.

Compound 20. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.78 (3H, s); 0.86 (3H, t, *J* = 7.2); 1.11 (3H, d, *J* = 6.8); 1.13 (3H, s); 1.18 (3H, d, *J* = 6.8); 1.53 (3H, d, *J* = 6.8); 1.65 (3H, d, *J* = 6.8); 1.78 (6H, s); 2.40 (3H, s); 2.55 (3H, s); 3.59 (3H, s); 4.47 (1H, d, *J* = 8.0); 4.91–4.98 (2H, m); 5.87 (1H, d, *J* = 10.8); 6.47 (1H, d, *J* = 14.4); 6.61 (1H, s); 7.11–7.15 (3H, m); 7.21–7.26 (5H, m). ^{13}C NMR spectrum, δ, ppm: 9.7; 10.2; 13.0; 13.1; 17.8; 18.2; 18.8; 19.4; 19.6; 25.8; 32.1; 33.0; 34.0; 34.2; 35.7; 41.5; 42.4; 42.6; 43.3; 46.3; 46.7; 59.6; 61.6; 62.2; 66.7; 68.5; 70.2; 71.2; 72.5; 73.9; 74.6; 76.4; 77.8; 81.7; 82.9; 85.4; 97.7; 102.5; 104.3; 108.2; 113.1; 115.6; 122.8; 126.9; 127.0; 129.4; 129.6; 143.0; 144.0; 149.7; 162.6; 168.4; 174.8; 206.8. Found, *m*/*z*: 1132.5958. $C_{58}H_{89}N_3O_{17}S$. Calculated, *m*/*z*: 1132.5991.

Compound 21. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.97 (3H, s); 1.08 (3H, t, *J* = 7.2); 1.13 (3H, d, *J* = 6.8); 1.21 (3H, s); 1.23 (3H, d, *J* = 6.8); 1.26 (3H, d, *J* = 6.8); 1.31 (3H, d, *J* = 6.8); 1.91 (6H, s); 2.03 (3H, s); 3.52 (3H, s); 3.59 (3H, s); 4.61 (1H, d, *J* = 8.0); 4.91–4.98 (2H, m); 5.87 (1H, d, *J* = 10.8); 6.47 (1H, d, *J* = 14.4); 6.61 (1H, s); 7.28–7.45 (3H, m). ^{13}C NMR spectrum, δ, ppm: 8.7; 11.8; 16.7; 17.2; 18.0; 24.7; 40.6; 42.0; 47.2; 47.3; 47.5; 58.2; 60.8; 68.8; 69.6; 72.2; 73.2; 80.2; 81.4; 101.2; 127.1; 127.2; 128.8; 132.0; 132.7; 134.5; 135.5; 144.1; 149.7; 162.6; 168.2; 174.7; 206.9. Found, *m*/*z*: 1186.5081. $C_{57}H_{85}Cl_2N_3O_{17}S$. Calculated, *m*/*z*: 1186.5055.

Compound 22. Tylosin (**1**) (0.46 g, 0.5 mmol) dissolved in acetone (3 ml), 0.3 M aq. H_2NSO_3H (4 ml) and 0.2 M aq. $NaClO_2$ (4 ml) were added under ice-cooling. The mixture was stirred for 2 h at room temperature, during the yellow solution turns to be colorless. The reaction mixture was neutralized by adding 7% aq. NH_3 and extracted with $CHCl_3$ (50 ml each). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$ and concentrated to dryness in vacuo. Yield 154 mg (33%), yellow solid. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.87 (3H, t, *J* = 7.2); 0.93 (3H, d, *J* = 6.6); 1.10–1.22 (13H, m); 1.66–2.00 (10H, m); 2.44 (6H, s); 2.85–3.14 (5H, m); 3.21 (3H, s); 3.40 (3H, s); 3.46 (3H, s); 3.53–3.85 (6H, m); 4.03–4.12 (2H, m); 4.48 (1H, d *J* = 7.8); 4.92–5.00 (2H, m); 5.86 (1H, d, *J* = 13.2); 6.39 (1H, d, *J* = 15.6); 7.16 (1H, d, *J* = 15.6); 7.81 (1H, s). ^{13}C NMR spectrum, δ, ppm: 8.2; 8.7; 11.7; 16.6; 16.7; 17.3; 18.3; 24.3; 24.6; 39.1; 41.0; 41.2; 44.8; 58.3; 60.8; 65.3; 68.7; 69.1; 69.6; 69.8; 70.4; 71.2; 72.6; 73.2; 74.9; 76.4; 76.5; 78.1; 80.2; 81.4; 81.9; 82.5; 92.1; 96.1; 101.1; 104.0; 118.2; 134.7; 143.0; 148.2; 172.9. Mass spectrum (t_R 8.72 min), *m/z*: 932.1 $[M+H]^+$. Found, *m/z*: 932.5174. $C_{46}H_{78}NO_{18}$. Calculated, *m/z*: 932.5219.

Compound 23. The mixture of tylosin (**1**) (46 mg, 0.05 mmol), 2,4-dimethylhepta-2,6-dienyl 2-isocyanoacetate (21 mg, 0.10 mmol), AcOH (5.8 μl, 0.10 mmol), 35% $MeNH_2$ solution (8.8 μl, 0.10 mmol) in MeOH (0.2 ml) was stirring at room temperature for 2 days. the product was purified by chromatography on silica gel (MeOH–EtOAc, 1:3). Yield 28 mg (47%), off-white solid. ^{1}H NMR spectrum (mixture of diastereomers), δ, ppm (*J*, Hz): 0.96–0.99 (8H, m); 1.02–1.06 (5H, m); 1.21–1.29 (24H, m); 1.69–1.70 (6H, m); 1.76–1.86 (8H, m); 1.93–2.00 (9H, m); 2.05–2.13 (9H, m); 2.20–2.24 (3H, m); 2.52–2.58 (3H, m); 2.72–2.75 (9H, m); 2.96–3.08 (9H, m); 3.14–3.22 (2H, m); 3.32–3.39 (8H, m); 3.57–3.65 (9H, m); 3.73–3.76 (3H, m); 3.90–3.95 (4H, m); 4.35–4.42 (2H, m); 4.51–4.59 (4H, m); 4.97–5.03 (4H, m); 5.15 (1H, m); 5.33–5.39 (1H, m); 5.74–5.80 (1H, m); 5.94–6.02 (1H, m); 6.46–6.52 (1H, m); 7.23–7.31 (1H, m). ^{13}C NMR spectrum (mixture of diastereomers), δ, ppm: 6.7; 7.4; 7.5; 10.3; 10.4; 11.6; 11.7; 15.0; 15.4; 15.9; 16.5; 16.7; 17.89; 17.92; 19.3; 19.4; 19.6; 21.0; 23.26; 23.30; 24.6; 29.7; 30.8; 30.9; 31.1; 32.0; 38.0; 39.3; 39.4; 39.9; 40.2; 40.5; 43.5; 43.9; 44.1; 52.2; 56.8; 56.9; 59.4; 64.3; 64.5; 64.6; 67.4; 68.2; 68.3; 68.9; 69.1; 69.2; 71.1; 71.9; 73.6; 73.8; 74.6; 78.9; 80.06; 80.10; 94.3; 99.66; 99.71; 101.4; 113.6; 116.5; 127.4; 127.5; 133.1; 134.0; 134.1; 134.2; 135.4; 142.2; 147.0; 168.3; 168.4; 170.7. Found, *m/z*: 1196.7068. $C_{61}H_{102}N_3O_{20}$. Calculated, *m/z*: 1196.7057.

Compound 24. The mixture of acid **22** (23.3 mg, 0.025 mmol), 1-(2-isocyanoethyl)-4-methoxybenzene (8.1 μl, 0.050 mmol), formaldehyde (3.8 μl, 0.050 mmol), 35% $MeNH_2$ solution (4.4 μl, 0.050 mmol) in MeOH (0.25 ml with 50 μl of H_2O) was stirred at room temperature for 2 days. The product was purified by chromatography on silica gel (MeOH–EtOAc, 1:3). Yield 12.5 mg (42%), yellowish solid. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.95–0.97 (4H, m); 1.02–1.03 (3H, m); 1.19–1.30 (14H, m); 1.87–1.95 (10H, m); 2.38–2.43 (2H, m); 2.47–2.51 (8H, m); 3.76–3.04 (10H, m); 3.41–3.58 (13H, m); 3.74–3.78 (6H, m); 3.97 (2H, m); 4.01–4.10 (1H, m); 4.18–4.23 (3H, m); 4.98–5.10 (2H, m); 5.92–6.03 (1H, m); 6.48–6.57 (1H, m); 6.83–6.89 (4H, m); 7.13–7.18 (4H, m); 7.28–7.36 (1H, m). ^{13}C NMR spectrum, δ, ppm: 8.1; 8.6; 11.7; 16.5; 16.7; 17.3; 18.2; 24.3; 32.4; 34.3; 35.6; 40.2; 40.9; 41.0; 41.2; 42.4; 44.9; 50.8; 54.2; 54.3; 57.2; 58.2; 60.7; 65.2; 68.7; 69.0; 69.6; 69.7; 71.3; 72.5; 73.2; 74.6; 74.9; 76.4; 80.3; 80.8; 81.4; 96.3; 101.0; 113.5; 113.6; 129.3; 129.4; 129.5; 130.3; 131.1; 158.4; 158.6; 169.6; 172.0; 172.7; 174.5. Mass spectrum (t_R 9.31 min), *m/z*: 1136.1 $[M+H]^+$. Found, *m/z*: 1136.6451. $C_{58}H_{94}N_3O_{19}$. Calculated, *m/z*: 1136.6482.

Compound 25. The mixture of tylosin (**1**) (46 mg, 0.05 mmol), 1-(2-isocyanoethyl)-4-methoxybenzene (16.1 μl, 0.10 mmol), AcOH (5.8 μl, 0.10 mmol), 35% $MeNH_2$ solution (8.8 μl, 0.10 mmol) in MeOH (0.2 ml) was stirred at room temperature for 2 days. The product was purified by chromatography on silica gel (MeOH–EtOAc, 1:3). Yield 48 mg (84%), off-white solid. ^{1}H NMR spectrum (mixture of diastereomers), δ, ppm (*J*, Hz): 0.95–0.96–1.00 (m, 4H); 1.08 (4H, m); 1.19–1.24 (16H, m); 1.29 (5H, m); 1.47–2.19 (22H, m); 2.52–3.19 (22H, m); 3.32–3.81 (27H, m); 4.32–4.39 (1H, m); 4.49–4.59 (2H, m); 4.96–5.05 (1H, m); 5.12–5.15 (2H, m); 5.40–5.95 (2H, m); 6.39–6.52 (1H, m); 6.86–6.91 (3H, m); 7.13–7.18 (1H, m); 7.20–7.29 (4H, m). ^{13}C NMR spectrum (mixture of diastereomers),

δ, ppm: 8.1; 8.3; 8.6; 8.8; 11.7; 11.8; 11.9; 16.4; 16.7; 17.3; 17.9; 20.7; 21.0; 24.61; 24.64; 30.7; 31.5; 33.3; 34.0; 34.2; 34.3; 39.4; 40.7; 41.0; 41.3; 41.8; 44.6; 45.2; 45.4; 54.3; 54.4; 58.2; 60.8; 65.9; 68.7; 69.4; 69.5; 69.6; 69.7; 70.3; 72.3; 72.4; 73.1; 73.2; 73.3; 74.9; 75.87; 75.92; 76.2; 80.2; 80.3; 81.3; 81.4; 95.7; 101.0; 102.8; 113.6; 117.9; 129.4; 129.6; 129.7; 130.9; 131.3; 134.4; 135.0; 143.6; 148.4; 158.27; 158.32; 158.4; 171.2; 171.7; 172.4; 173.2. Mass spectrum (t_R 9.30 min), *m/z*: 1150.1 $[M+H]^+$. Found, *m*/*z*: 1150.6588. $C_{59}H_{96}N_3O_{19}$. Calculated, *m*/*z*: 1150.6638.

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