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Article

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# Synthesis, antibacterial and anticancer evaluation of novel spiramycin-like conjugates containing C(5) triazole arm

Katarzyna Klich,[1] Krystian Pyta,[1] Marcelina M. Kubicka,[2] Piotr Ruszkowski,[3] Lech Celewicz,[1] Marzena Gajecka,[2,4] Piotr Przybylski[1]*

[1]*Faculty of Chemistry, Adam Mickiewicz University, Umultowska 89b, 61-614 Poznan, Poland*

[2]*Department of Genetics and Pharmaceutical Microbiology, University of Medical Sciences, Swiecickiego 4, 60-781 Poznan, Poland*

[3]*Department of Pharmacology, University of Medical Sciences, Rokietnicka 5a, 60-806 Poznan, Poland*

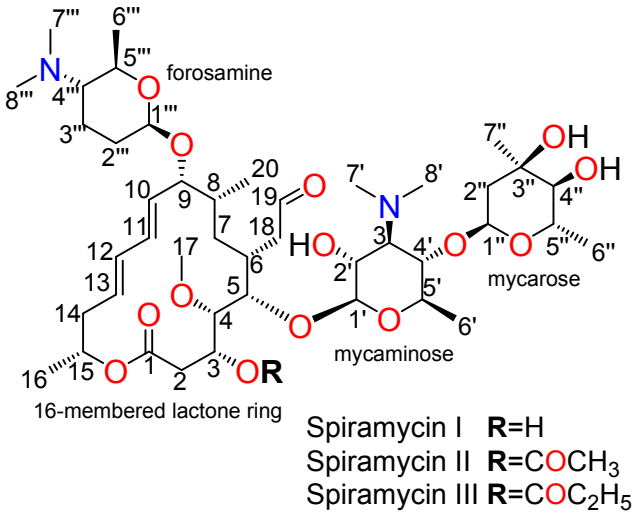
[4]*Institute of Human Genetics, Polish Academy of Science, Strzeszynska 32, 60-479 Poznan, Poland*

ABSTRACT: Huisgen cycloaddition allowed obtaining novel triazole-bridged antibiotics (**6-16**) with the reconstructed C(5) arm of spiramycin. $^{1}$H-$^{1}$H NOESY couplings indicated the structure of novel derivatives in solution and demonstrated that the rebuilt C(5) arm is slightly differently oriented relative to the aglycone part, if compared to that of spiramycin (**1**). Combined analysis of biological data together with experimentally determined lipophilicity (clogP) and solubility shown the importance of chemical nature of the newly introduced triazole C(5) arm in the presence of attractive antibacterial and anticancer potency. The most cytotoxic active triazole conjugates having hydrophobic and bulky C(5) arm showed higher selectivity towards cancer cell lines (HeLa, KB, MCF-7, Hep-G2 and U87) relative to HDF normal cells than that of the parent spiramycin. Our studies have demonstrated that the aldehyde group is not crucial for the presence of interesting antibacterial [$MIC_{(S.\ pneumoniae)}$ ~ 1.2 μM] and anticancer [$IC_{50\ (HepG2)}$ ~ 6 μM] properties of 16-membered lactone macrolides, based on spiramycin's aglycone.

Keywords: 16-membered lactone macrolides; *click* chemistry; spectroscopy, clogP and solubility, antibacterial and anticancer.

## INTRODUCTION

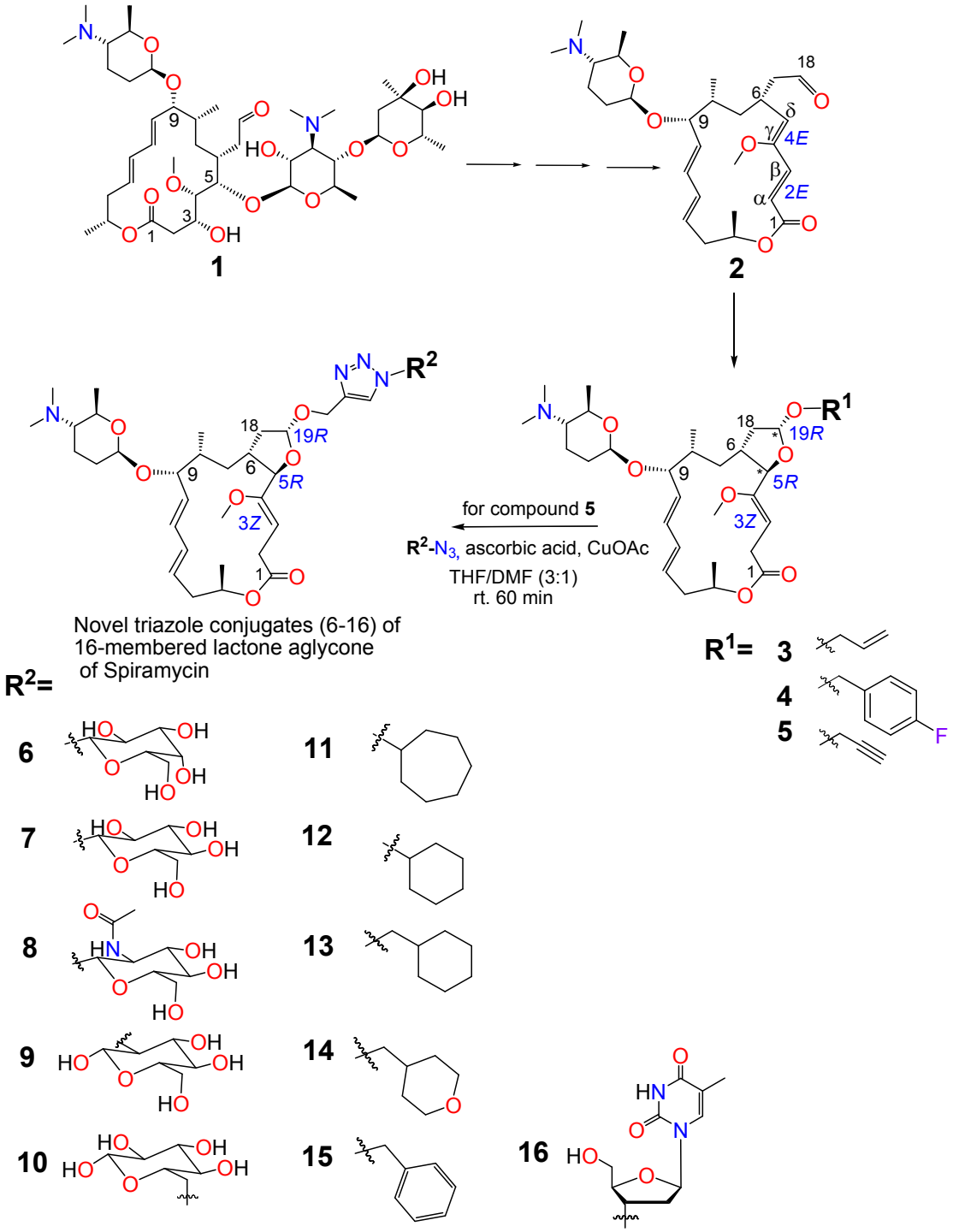
Spiramycin (Scheme 1) is a natural antibiotic produced by *Streptomyces ambofaciens* in the form of a mixture of three compounds, the so-called spiramycins I-III.[1,2] Spiramycin I, having hydroxyl substituent at C(3) atom, is dominant (~80 %) in the mixture produced by the bacteria.[3] Spiramycins are structurally similar to leucomycins in the presence of a common 16-membered lactone aglycone and mycaminosyl-mycarose moiety attached at C(5). However, spiramycins, in contrast to leucomycins, additionally possess forosamine at C(9) position. The spectrum of spiramycins' biological properties comprises mainly bacteriostatic activity against most of Gram-(+) cocci and rods, mycoplasmas and *Toxoplasma gondii* .[4,5] Comparison of spiramycins' activity against different Gram-(+) bacteria strains *in vitro* with that determined for 14-membered lactone macrolides as erythromycins indicates that it is at least twice lower.[6] However, the advantage of spiramycins over erythromycins is their good gastrointestinal tolerance, higher affinity to tissues and a fewer adverse effects.[7,8] To the best of our knowledge, the activity of spiramycins in cancer cells has never been studied up to now, although literature provides some examples of other-group macrolides showing properties of this type e.g. maytansine /ansamacrolide/ or marine 22-membered lactone macrolide (-)-dictyostatin.[9–12] The target site of spiramycins' action is well




**Scheme 1** Structures of Spiramycins I-III together with the carbon atom numbering.

recognized from X-ray studies as the exit of the ribosomal tunnel near the peptidyl transferase loop and the loop of domain II of 23S rRNA, belonging to the large subunit 50S.[13–15] Spiramycin and related 16-membered lactone macrolides have a common mechanism of activity which involves binding to 50S subunit and steric blocking of the peptide exit tunnel, which contributes to inhibiting peptide synthesis at different stages. The stage of peptide synthesis inhibition is related to the length and chemical nature of the arm attached at C(5) position of the macrolide's aglycone.[16] It is also known that bacteria resistance to 16-membered lactone macrolides is achieved when e.g. nucleotides A2058 /*E. coli*/ or G2099 /*H. marismortui*/ undergoes *N*-methylation because of the steric clashing between the *N*,*N*-dimethyl group of the nucleotide and the mycaminose part.[17,18] Among whole group of mutations, the replacement of A2103 with G in *H. marismortui* ribosomes (A2062 in *E. coli*) seems to be the most important and confers resistance against spiramycin or tylosin.[19] Therefore as reported earlier the modifications of the aldehyde group, which is usually reversibly bonded to the amine group of A2103 of ribosome with formation of hemiaminal, for this-type macrolides gave derivatives at least 100-1000 fold

less active over unmodified parent antibiotics.[20] Up to know, many modifications, both within spiramycin and leucomycin groups, have been proposed and tested to obtain comparably or more active alternative agents able to fight the growing bacterial resistance. Most of the modifications were related to transformation of: the aldehyde *via* reduction, nucleophilic addition,[21–23] hydroxyl groups within the aglycone and saccharides parts *via* etherification, acylation or sulphonylation,[24,25] diene moiety *via* epoxidation, reduction, Diels-Alder reaction or metathesis with contraction of the lactone ring,[26–29] forosamine and/or mycaminose *N*-oxidation or *N*-substitution[30,31] and incorporation of the nitrogen into the lactone macrocyclic ring.[32–34] Interesting platform to synthesis and drug discovery among group of macrolide lactone antibiotics, has been recently proposed by Seiple et al.[35] Convenient synthetic strategy leading to obtain of new chemical entities characterized by attractive biological properties is the use of *click* chemistry reactions.[36–38] Interesting approach to modification of these structurally complexed macrolides, in view of their known mechanism of action, has been described by Omura and Sharpless et al.[39] They proposed the functionalization of terminal hydroxyls of mycarose saccharide with alkyne followed by conversion into respective triazole moieties *via* Fokin-Huisgen cycloadditions. However, as a result of this transformation, the obtained derivatives were significantly less active than the parent leucomycin due to the presence of a too long arm at C(5) of the aglycone. Recently, we have proposed a novel cascade approach to modification of the aglycone of spiramycins, which opened a possibility of another pathway to construct the saccharide part at the aglycone.[40] As a continuation of these studies, in the present work we used a Huisgen cycloaddition to rebuilt spiramycin's arm at C(5) of the aglycone and to evaluate the influence of the modification on antibacterial and anticancer potency of novel triazole conjugates **6-16**.




**Scheme 2** Synthetic route leading to novel triazole conjugates of spiramycin aglycone **6-16** *via* previously obtained compounds **2** and **5** [Ref. 39].

## RESULTS AND DISCUSSION

In order to rebuilt spiramycin arm at C(5), the earlier obtained compound **2**,[40] *via* sequence of cascade transformations from spiramycin was further subjected to Huisgen dipolar cycloaddition with the use of different azides containing hydrophobic and hydrophilic substituents in the presence of CuOAc in THF/DMF mixture of solvents. Novel triazole conjugates of spiramycin's aglycone **6-16** were obtained with the total yields 18-60 % after purification. The structures of all triazole conjugates **6-16** in solution were determined *via* 1D and 2D NMR (Fig. 1, Supporting Information) and FT-IR (Fig. 49S, Supporting Information) spectroscopic analysis. The use of HSQC method enabled the assignment of H23 and C23 of triazole ring bonded to the aglycone structure (Fig. 1a). In turn, application of long-range heteronuclear couplings (HMBC) to structural analysis of conjugates **6-16** (Fig. 1b and 1c) revealed simultaneous correlations of carbon and proton signals between the newly formed triazole ring and those of tetrahydrofuran bicyclic moiety and $R^2$ terminal substituent (Scheme 2). Furthermore, the FT-IR spectrum of intermediate **2** shows two separated bands at 1722 and 1702 $cm^{-1}$, assigned to carbonyl stretching vibrations of aldehyde and double unsaturated lactone, respectively (Fig. 49S). In the spectra of novel triazole products **6-16** one of these bands /$\nu(C=O)_{aldehyde}$/ vanishes, whereas the other one is shifted towards higher wavenumbers /to 1728 $cm^{-1}$/. This result clearly indicated the lack of aldehyde group within structures of **6-16** and the fact that the lactone of the macrocyclic ring is no longer strongly π-π conjugated with the two double bonds, in contrast to **2**. Additionally, for **6-10** and **16** conjugates the presence of complex bands assigned to ν(O-H) stretching vibrations confirmed successful introduction of the saccharide part into the structure of new-type antibiotics (Fig. 49b). Application of $^1H$-$^1H$ NOESY method revealed mutual arrangement of newly introduced triazole ring relative to the modified aglycone part in solution (Fig. 2).

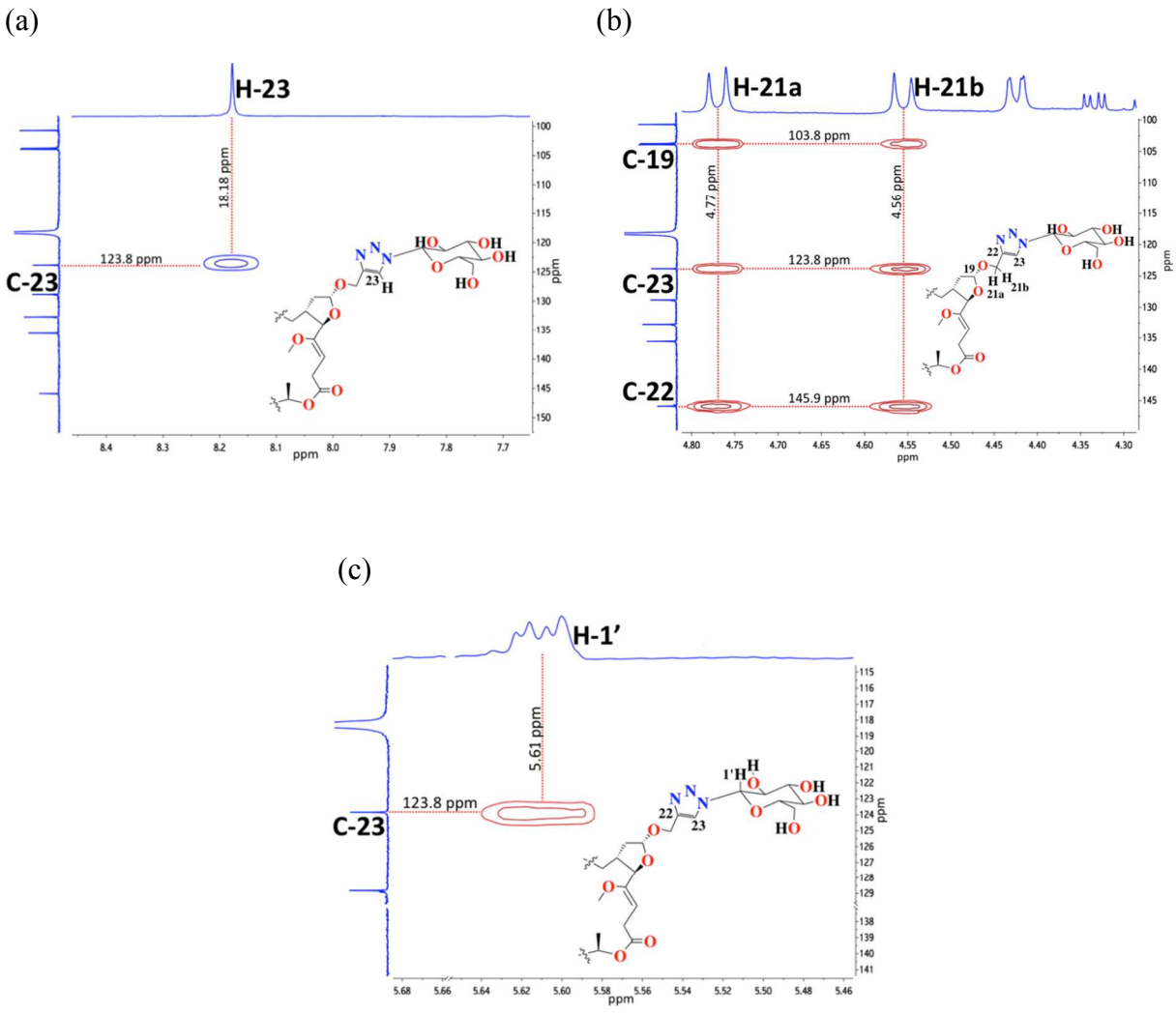



**Fig. 1** Exemplary 2D NMR correlation spectra of derivative **7**, indicating the presence of triazole bridge between terminal substituent and the newly formed tetrahydofuran bicyclic moiety: (a) $^{1}$H-$^{13}$C HSQC revealing H(23)proton-C(23)carbon assignment of triazole; (b) $^{1}$H-$^{13}$C HMBC showing a connection between the newly formed triazole ring and bicyclic moiety; (c) $^{1}$H-$^{13}$C HMBC revealing a connection between triazole and the terminal substituent, introduced *via* a respective azide.

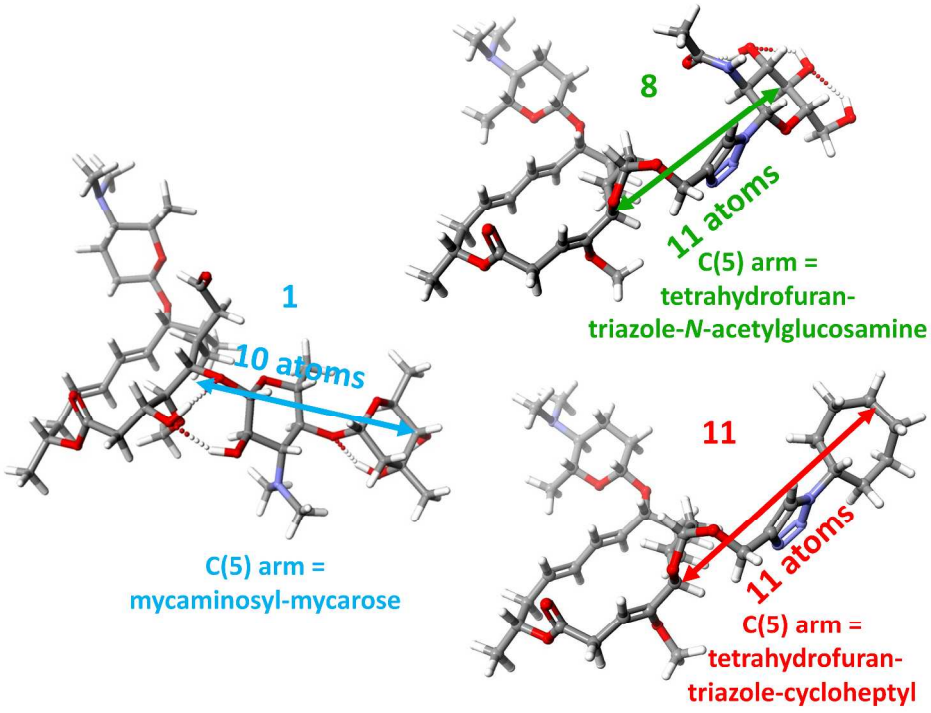



**Fig. 2** The lowest-energy structures of compounds: **1** ($H_f$°= − 608.2 kcal/mol), **8** ($H_f$°= − 539.27 kcal/mol) and **11** ($H_f$°= − 494.2 kcal/mol) showing the orientation of the reconstructed C(5) arm relative to the aglycone part, calculated by B88-LYP (GGA) DFT /*Scigress Explorer* package FJ 2.4. EU 3.1.8/.

Generally, $^{1}$H-$^{1}$H NOESY contacts recorded for **6-16**, assigned to 16-membered aglycone part, can be divided into two groups i.e. those assigned to the protons oriented above the aglycone (marked by green - Fig. 50S) and the others assigned to the protons oriented to the bottom of the aglycone ring (blue color - Fig. 50S). Taking into consideration of the following group of contacts: H(3)-H(6), H(6)-H(11), H(6)-H(19), H(9)-H(11), H(11)-H(13) and H(13)-H(15) all these protons are suggested to be at same face of the aglycone /above the aglycone/ whereas the second group of contacts: H(2)-H(14), H(2)-H(17), H(5)-H(17), H(10)-H(12), and H(12)-H(14) is assigned to protons which are directed to the bottom of the aglycone (Fig. 50S). Mutual $^{1}$H-$^{1}$H contacts between H(5), H(17) and H(21) show their close vicinity and orientation of the -

(21)$CH_2$- methylene protons to the bottom of the aglycone. As concluded from the presence of $^1$H-$^1$H contacts found between H(23) and H(19) and H(1') in NOESY spectra, the arrangement of the triazole ring relative to bicyclic tetrahydrofuran moiety is similar for all obtained derivatives irrespectively of the type of terminal substituent (Fig 2). Comparison of C(5) arm structures between spiramycin and those of synthesized triazole conjugates **6-16** shows that they are of comparable length, whereas the mutual arrangement of C(5) arms relative to the macrocyclic lactone ring is slightly different in each case (Fig. 2).

**Antibacterial studies.** Antibacterial test results as well as experimentally determined lipophilicity and solubility of **1-16** compounds are collected in Tables 1 and 1S (Supporting Information). Analysis of these data shows that **11-15** derivatives, containing bicyclic-triazole bridged aglycones, in general have no activity against all tested bacteria. First of all the lack of aldehyde group (no reversible covalent bonding) and limited possibility of stabilization of the terminal substituent at C(5) arm *via* H-bonds are the "weak points" of **11-15** compounds in view of the earlier published models.[17, 41] Additionally, although compounds **11-15** show the most favourable lipophilicity from among all other derivatives (Table 1S), they have the lowest solubility, which seems to be a very important factor taking into regard the polar walls of the ribosomal tunnel and filling the tunnel with water molecules (transport to the target site of action). Compounds **3-5** also have poor solubility and therefore they show no antibacterial activity. Similarly, low solubility of triazole conjugate containing AZT (compd. **16**) can be an explanation of its no antibacterial activity. The importance of the role of reversible bonding of the aldehyde in the presence of antibacterial activity can be concluded from biological data of derivative **2** (Table 1). The presence of antibacterial activity against Gram-positive microorganisms on the level 4-33 μM is mainly a result of forosamine interactions and the aldehyde bonding with the target site at ribosomes as well as quite well-balanced solubility and

lipophilicity of compound **2**. However, it should be underlined here, that the activity of **2** is about 8-28 times lower than that of the parent antibiotic **1**, when MICs are expressed in μM. This result is understandable because compound **2**, in contrast to **1**, does not have saccharide part involved in hydrogen bonding with PTC loop nucleotides as G2099 and G2540 /*H. marismortui*/. Interesting results were obtained for some of derivatives **6-10** containing terminal saccharides at the reconstructed C(5) arm (Table 1 and Table 1S). Taking into regard just their not favourable lipophilicity and the best of all solubility parameters, no explanation of different antibacterial properties among them is possible, especially in view of **1**. This experimental result suggests slightly different binding modes among this group of triazole-saccharide conjugates. Further comparison of physico-chemical parameters of compounds **6-10**, having relatively hydrophilic C(5) arm, shows that at good solubility of all these derivatives, just compound **8** has lipophilicity close to that of **1**, which contributes to its generally comparable activity with that of **1** /is 2-4 less active than **1** [μM]/. It should be underlined that conjugate **8** is also characterized by much higher activity than that of **2** having aldehyde (Table 1). All these results clearly show that 16-membered macrolide derivatives, even not containing aldehyde group, but having additionally functionalized saccharide can be active against Gram-positive bacteria at a level comparable to that of **1**.

**Anticancer studies.** To the best of our knowledge the leucomycin-type derivatives showed some anticancer activity.[28] However, the anticancer potency of spiramycin and its analogs has not been studied up to now. Cytotoxic activity determined in six human cell lines: cervical (HeLa), nasopharyngeal (KB), breast (MCF-7), liver (HepG2), glioblastoma (U87) normal human dermal fibroblast (HDF) of novel triazole conjugates, compared to those of AZT and cytarabine standards, are shown in Table 2. Analysis of these data revealed that **1** showed only limited

cytotoxicity, irrespectively of the type of the cancer cell line. Much lower activities /> 100 μM/ in comparison to that of the parent compound **1**, displayed derivatives **2**, **8** and **9**. It should be mentioned here that the most active against Gram-positive bacteria, triazole conjugate **8**, has very weak anticancer activity. The other studied derivatives (compounds **10**-**16**) show significantly greater potency than that found for **1** in all cancer cell lines (Table 2). Anticancer properties of the triazole conjugates containing within the C(5) arm terminal saccharides (**8**-**10**) are varied. Derivative **10**, having 6'-substituted saccharide revealed medium anticancer activity in HeLa, KB and U87 cell lines ($IC_{50}$ ~ 12 μM), in contrast to the other ones of this group, having saccharide attached *via* C(1') atom. Incorporation of AZT into the C(5)-triazole arm resulted in increased activity of **16** in HeLa and KB cancer cell lines ($IC_{50}$ ~ 15 μM), however, lower than the activity of AZT itself. Interestingly, all triazole conjugates **11**-**15**, containing aliphatic or aromatic C(5)-terminal substituents, displayed interesting cytotoxic potency in all cancer cell lines (Table 2). Among relatively hydrophobic triazole conjugates of the favourable logP values, the derivative bearing cycloheptyl substituent (compound **11**) is characterized by the highest potency in the range $IC_{50}$ = 6.02 - 8.63 μM. $IC_{50}$ values determined for **11** are only 2-3 times higher than those determined for cytarabine and are up to 5-time lower than for **1**. Taking into account structures of **10**-**15** and their anticancer activity it can be concluded that there is some relationship between the activity and the bulkiness of the C(5)-arm terminal substituent. The activity order beginning from the most active is following: **11** with cycloheptyl > **13** with methylene cyclohexyl > **12** with cyclohexyl > **15** with benzyl group. Another favourable factor of novel hydrophobic triazole conjugates is their selectivity indexes (SI). Comparison of these indexes for **1** and all synthesized compounds (Table 3), revealed that those containing hydrophobic triazole arm (compounds **11**-**15**) have SI >1, whereas all others have SI ~ 1 or SI < 1. A comparison of SI values determined for **11**-**15** with those of **CYT**, **FUra** and **FdU** standards demonstrates that the most active **11** has

SI comparable or even higher than those of the standards, whereas also active compound **13** has even better SI than the standards used (except for HeLa cell line). It should be added that the most potent against bacteria derivative **8**, among all novel triazole conjugates, is 5 fold less toxic (less active in HDF cell line) than slightly more active parent antibiotic spiramycin (compound **1**).

Results of these studies clearly showed that for anticancer activity of this-group of macrolides the presence of the aldehyde is not so important, whereas the occurrence the hydrophobic and bulky structure of C(5) arm is crucial. It has been demonstrated also that the use of combined cascade and *click* approaches to modification of spiramycin allowed to obtain of the new class of macrolide derivatives having interesting cytotoxic properties (comparable with **FUra** and even better than those of **FdU**).

## CONCLUSION

Novel bicyclic-conjugates bearing the reconstructed arm at C(5) of the aglycone have been synthesized by a combined cascade and *click* chemistry approach. 1D and 2D NMR and FT-IR spectroscopic studies confirmed the obtaining of these derivatives and revealed their structures in solution. As indicated by $^{1}$H-$^{1}$H NOESY studies the reconstructed C(5)-triazole arm within novel derivatives has the length comparable with that of spiramycin but is slightly reoriented due to the presence of bicyclic moiety. Antibacterial activity studies demonstrated that the most active among novel triazole conjugates is that containing the terminal *N*-acetylsaccharide moiety (compound **8**). Comparison of the antibacterial activity and physico-chemical parameters of this-group of macrolides revealed that for antibacterial potency comparable with that of **1** the presence of aldehyde is not so important. Furthermore, our studies indicated that for the presence

of interesting anticancer potency and selectivity toward cancer cells of novel spiramycin-like conjugates containing C(5) triazole arm, even more attractive than cytotoxic standard **FdU** or comparable with **FUra**, the aldehyde group is not necessary. Comparison of cytotoxic data within group of new triazole conjugates containing different nature of the rebuilt C(5) arm, revealed that the most active ones are those bearing hydrophobic and relatively bulky substituents at the end of the arm.

## EXPERIMENTAL SECTION

**General Experimental:** Spiramycin was purchased from LKT Laboratories. $CH_3CN$ and $CD_3CN$ for spectroscopic measurements as well as NaH, methanol, pirrydynium *p*-toluenosulfonate, trimethyl orthoformate, propargyl alcohol, allyl alcohol, 4-fluorobenzyl alcohol, 1-Azido-1-deoxy-β-*D*-galactopyranoside, 1-Azido-1-deoxy-β-*D*-glucopyranoside, 2-Acetamido-2-deoxy-β-*D*-glucopyranosyl azide, 2-Azido-2-deoxy-*D*-glucose, 6-Azido-6-deoxy-*D*-galactose, sodium azide, benzyl bromide, bromocyclohexane, (bromomethyl)cyclohexane, bromocycloheptane, 4-(bromomethyl)tetrahydropyran, AZT, acetonitrile, difluoroacetate acid, acetic anhydride, THF, DMF used for the syntheses of new Spiramycin derivatives were purchased from Aldrich. $CH_3COOC_2H_5$, $Et_2O$, NaCl were purchased from POCH S.A. Gliwice (Poland). $H_2O$ HPLC gradient grade and $CH_3CN$ HPLC gradient grade were purchased from J.T. Baker.

**HPLC, 1D and 2D NMR and FT-IR measurements:** The elemental analysis of new derivatives of Spiramycin was carried out on Vario ELIII (Elementar, Germany). The purity of the final compounds, found in all cases as ≥ 95%, was determined by HPLC method using Dionex Ultimate 3000 equipped with an LPG-3400 SD gradient pump using Thermo GOLD C18 150×4.6 mm (5 μm) and Accucore XL column, TCC-3000SD thermostat to columns (column

temp. equal 25 ºC) and Dionex VWD- 3400RS variable wavelength UV-vis detector (detection at $\lambda_{max}$=232 nm); the flow rates were 0.5 and 1 mL/min with injection volumes of 10 µL in acetonitrile mixtures and the mobile phase: 35:55:10 $H_2O/CH_3CN$/0.01M ammonium acetate buffer prepared in 50:50 mixture $CH_3CN:H_2O$.

The FT-IR spectra of new derivatives were recorded in in $CH_3CN$ solution. All FT-IR spectra were recorded on an *iS50* FT-IR spectrophotometer (Thermo, Nicolet, US) equipped with a DTGS detector and two-columnar purge gas generator (Parker, Balston, US); resolution 1 $cm^{-1}$, NSS = 150, range 4000-400 $cm^{-1}$. The Happ-Genzel apodization function was used.

The $^1H$ and $^{13}C$ measurements of new derivatives of Spiramycin were performed in $CD_3CN$ using Varian Mercury 400 MHz and Bruker Avance 600 MHz spectrometers. The operating frequencies for $^1H$ measurements were 400.075 and 600.08 MHz; pulse width corresponding to the flip angle of 450; spectral width, swh = 9842.5 Hz; acquisition time at=0.2 sec; relaxation delay $d_1$=1.0 s; T = 293.0 K, TMS was used as the internal standard. No window function or zero filling were used. Digital resolution was 0.2 Hz/point. $^{13}C$ NMR spectra were recorded at the operating frequency 150.454 MHz; pulse width corresponding to the flip angle of 600; sw = 19000 Hz; at = 1.8 s; d1=1.0 s; T = 293.0 K and TMS as the internal standard. Line broadening parameters of 0.5 or 1 Hz were applied. $^1H$ and $^{13}C$ NMR resonances unambiguously assigned on the basis of the $^1H$-$^{13}C$ HMBC, $^1H$-$^{13}C$ HSQC, $^1H$-$^1H$ COSY couplings.

**Antibacterial assays:** The antimicrobial activity of novel spiramycin derivatives was studied against the standard Gram-positive cocci: *Micrococcus luteus* ATCC 10240, *Staphylococcus epidermidis* ATCC 12228, Staphylococcus epidermidis ATCC 49134, *Streptococcus pneumoniae* ATCC 49619, *Bacillus subtilis* ATCC 6635 and two clinical strains of *Staphylococcus aureus*

(MRSA strain I and II). The microorganisms used were bought from Argenta company, LGC Standards or were obtained (MRSA strains) from the collection of the Department of Genetics and Pharmaceutical Microbiology at the University of Medical Sciences in Poznan, Poland. The presence of MRSA resistance mechanism among clinical strains was confirmed using a disc diffusion method with the cefoxitin disc (30 μg), in accordance with the EUCAST recommendation. In addition, PCR (*polymerase chain reaction*) reactions were also performed to verify the presence of the *mecA* gene, that encodes the protein PBP2A (*penicillin binding protein* 2A), responsible for the resistance to all β-lactams antibiotics (The PCR method is the gold standard by the EUCAST). Minimal Inhibitory Concentration (MIC μg/mL) values were determined independently for 1-16 compounds according to the recommendations of the European Society of Clinical Microbiology and Infectious Diseases (EUCAST) using a serial microdilution method on polystyrene plates with Mueller Hinton II broth MHB (Becton Dickinson), as a medium. Concentrated solutions of the tested compounds were dissolved in DMSO (POCH, Gliwice) and diluted in MHB to obtain the required concentration. The concentrations of the tested compounds in liquid medium ranged from 0.064 to 64 μg $mL^{-1}$. The final inoculum of all studied organisms was approximately $5 \cdot 10^5$ CFU $mL^{-1}$ (Colony Forming Units per mL). Minimal inhibitory concentration (MIC - data shown in Tables 1 and 1S) was determined after 18 h of incubation at 35 ± 2 °C.

**Cytotoxicity assays:** Human cancer cells HeLa (cervical cancer cell line) and KB (carcinoma nasopharynx) were cultured in RPMI 1640 medium and human cancer cells MCF-7 (breast cancer cell line) were cultured in DMEM medium. HepG2 (liver cancer cell line) were cultured in MEM medium. Each medium was supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin/streptomycin solution. The cell lines were kept in the incubator at

37°C. The optimal plating density of cell lines was determined to be $5 \times 10^4$. All the cell lines were obtained from The European Collection of Cell Cultures (ECACC) supplied by Sigma-Aldrich. The protein-staining SRB (Sigma-Aldrich) microculture colorimetric assay, developed by the National Cancer Institute (USA) for in vitro antitumor screening was used in this study, to estimate the cell number by providing a sensitive index of total cellular protein content, being linear to cell density. The monolayer cell culture was trypsinized and the cell count was adjusted to $5 \times 10^4$ cells. To each well of the 96 well microtiter plate, 0.1 mL of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was washed out and 100 μL of six different compound concentrations (0.1, 0.2, 1, 2, 10, and 20 μM) were added to the cells in microtitre plates. The tested com-pounds were dissolved in DMSO (containing 10% of water) (100 μL) and the content of DMSO did not exceed 0.1%; this concentration was found to be nontoxic to the cell lines. The cells were exposed to compounds for 72 hours at 37 °C in a humidified atmosphere (90% RH) containing 5% $CO_2$. After that, 25 μL of 50% trichloroacetic acid was added to the wells and the plates were incubated for 1 hour at 4°C. The plates were then washed out with the distilled water to remove traces of medium and next dried by the air. The air-dried plates were stained with 100 μL of 0.4% sulforhodamine B (prepared in 1% acetic acid) and kept for 30 minutes at room temperature. The unbound dye was removed by rapidly washing with 1% acetic acid and then air dried overnight. The protein-bound dye was dissolved in 100 μL of 10 mM unbuffered Tris base (pH 10.5) for optical density determination at 490 nm. All cytotoxicity experiments were performed three times. Cell survival was measured as the percentage absorbance compared to the control (nontreated cells). Cytarabine and AZT (Sigma-Aldrich) were used as the internal standards. Results of anticancer studies of novel triazole conjugates are shown in Table 2. SI index was calculated from equation $SI = IC_{50\ \text{normal cell line HDF}}/IC_{50\ \text{respective cancerous cell line}}$ (Table 3). A beneficial

SI > 1.0 indicates a compound with efficacy against tumour cell greater than the toxicity against normal cells.

**Determination of logP and water solubility: 1** and derivatives **2-16** were dissolved in HPLC gradient grade water (pH=7, T=25°C) and the calibration curves were determined by measuring absorbance as a function of their concentration with Metertech Inc. UV-VIS SP-80001 spectrophotometer. Analytical wavelength for the determination of calibration curves A f(c) and the concentrations $c_{H2O}$ and $c_{octanol}$ on the basis of UV-vis measurements was $\lambda_{max}$=244 nm. To determine $logP_{exp}$ the known amounts of **1** and **2-16** were dissolved in 10 mL of octanol to which 10 mL of $H_2O$ was added. The mixture was shaken, vigorously stirred for 1 hour and then separated. In order to determine the concentration of the compound in the aqueous layer, the respective measurement of absorbance was performed. Experimental $logP_{exp}$ values were calculated according to the following equation: $logP_{exp} = log(c_{octanol}/c_{H2O})$ and shown in Table 1 and Table 2S. To determine the solubility in the gradient grade water, a known amount of each of **1** and **6-16** derivatives was weighted and respective volumes of $H_2O$ were added (by 0.1 mL) upon vigorous stirring of the solution. The solubility test was performed at least three times for the derivative studied and its result is given in Table 1 and Table 2S.

**Syntheses and spectral characterization of novel triazole conjugates 6-16:** Synthetic procedures of spiramycin derivatives **2-5** together with the analytical data of them (FT-IR, HRMS, HPLC and Elemental data) are included in ref. 39.

General synthetic procedure of novel triazole conjugates of spiramycin aglycone 6-16 together with the analytical data (FT-IR, HRMS, HPLC and Elemental data):

180 mg (0.33 mmol) of **5** was dissolved in 6mL mixture of THF/MeOH (3:1) and respective mixtures were prepared with each of the following compounds taken separately (0.33 mmol): 1-azido-1-deoxy-β-D-galactopyranoside (**6**), 1-azido-1deoxy-β-D-glucopyranoside (**7**), 2-Acetamido-2-deoxy-β-D-glucopyranosyl azide (**8**), 2-Azido-2-deoxy-D-glucose (**9**), 6-Azido-6-deoxy-D-galactose (**10**), azidocycloheptane (**11**), azidocyclohexane (**12**), (azidomethyl)cyclohexane (**13**), 4-(azidomethyl)tetrahydropyran (**14**), benzil azide (**15**), AZT (**16**). Then, to the each mixture 45mg (0.37 mmol) of $CH_3COOCu(I)$ and 124.5mg (0.71 mmol) of ascorbic acid were added.

The mixtures were stirred at room temperature for an hour and after that to the **6-10** diethyl ether was added and extracted twice with 25 ml of water and saturated $NaHCO_3$. In the next step the water layer was extracted three times with 25ml of ethyl acetate. The organic layer was evaporated giving the products **6-10**.

The mixtures were stirred at room temperature for an hour and after that to the **11-16** diethyl ether was added and extracted three times with 25 ml of water and saturated $NaHCO_3$. The separated organic layer was evaporated and the synthesized derivatives **11-16** were next purified by column chromatography with silica gel (25 cm × 1 cm, silica gel 60, 0.040-0.063 mm/230-400 mesh ASTM, Fluka) with ethyl acetate as an eluent.

New triazole conjugates of spiramycin's aglycone **6-11**, **15** and **16** were obtained as a white powders whereas derivatives **12-14** as a colorless oils.

**6**: (2*R*,3a*R*,5*R*,6*R*,7*E*,9*E*,12*R*,16*Z*,17a*R*)-6-(((2*R*,5*S*,6*R*)-5-(dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)-17-methoxy-5,12-dimethyl-2-((1-((2*S*,3*S*,4*R*,5*S*,6*S*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)-1*H*-1,2,3-triazol-4-yl)methoxy)-3a,4,5,6,11,12,15,17a-octahydro-2*H*-furo[2,3-*f*][1]oxacyclohexadecin-14(3*H*)-one (122mg, Yield

49.4%) mp 93-95°C. HPLC $R_t$= 3.480 min. Elemental analysis $C_{37}H_{58}N_4O_{12}$: calculated: C, 59.18; H, 7.79; N, 7.46; measured: C, 59.21; H, 7.77; N, 7.47; HRMS (ESI - TOF) m/z: $[M+H]^+$ = 751.4104. FT-IR ($CH_3CN$): ν(O-H) – 3488 $cm^{-1}$, ν(C-H) – 2877 $cm^{-1}$, $\nu(C=O)_{lactone}$ -1724 $cm^{-1}$, ν(C=C) -1673 $cm^{-}1$, $\nu(C-O)_{lactone}$ -1226 $cm^{-1}$, ν(C-O) -1125 $cm^{-1}$, ν(C-O) -1092 $cm^{-1}$, $^1$H NMR (600 MHz, $CD_3CN$, 25°C), δ= 0.91 (d, $^3J_{H8,H20}$=6.7 Hz, 3H, 20-H), 1.11 (d, $^3J_{H5'''',H6''''}$=6.1 Hz, 3H, 6'''-H), 1.14 (m, 1H, 7a-H), 1.25 (d, $^3J_{H15,H16}$=6.4 Hz, 3H, 16-H), 1.32 (m, 1H, 2'''a-H), 1.42 (m, 1H, 7b-H), 1.43 (m, 1H, 3'''b-H), 1.70 (dt, $^3J_{H18a,H19}$=5.0 Hz, $^3J_{H6,H18a}$=5.1 Hz, $^2J$=12.4 Hz, 1H, 18a-H), 1.78 (m, 1H, 3'''a-H), 1.78 (m, 1H, 2'''b-H), 1.95 (m, 1H, 8-H), 2.11 (m, 1H, 14b-H), 2.11 (m, 1H, 18b-H), 2.11 (m, 1H, 4'''-H), 2.15 (s, 6H, 7'''-H, 8'''-H), 2.25 (m, 1H, 6-H), 2.46 (m, 1H, 14a-H), 2.87 (ddd, $^4J$=1.4 Hz, $^3J_{H2b,H3}$=6.0 Hz, $^2J$=15.3 Hz, 1H, 2b-H), 3.18 (dd, $^3J_{H2a,H3}$=7.8 Hz, $^2J$=15.3 Hz, 1H, 2a-H), 3.43 (dq, $^3J_{H4''',H5'''}$=9.5 Hz, $^3J_{H5''',H6'''}$=6.1 Hz, 1H, 5'''-H), 3.61 (s, 3H, 17-H), 3.68 (m, 1H, 6'-H), 3.68 (m, 1H, 3'-H), 3.79 (t, $^3J_{H4',H5'}$=5.8 Hz, $^3J_{H5',H6'}$=5.8 Hz, 1H, 5'-H), 3.95 (m, 1H, 4'-H), 3.99 (d, $^3J_{H5,H6}$=9.5 Hz, 1H, 5-H), 4.09 (dd, $^3J_{H8,H9}$=4.6 Hz, $^3J_{H9,H10}$=9.3 Hz, 1H, 9-H), 4.24 (t, $^3J_{H1',H2'}$=9.6 Hz, $^3J_{H2',H3'}$=9.6 Hz, 1H, 2'-H), 4.42 (dd, $^3J_{H1''',H2a''}$=9.4 Hz, $^3J_{H1''',H2b''}$=2.0 Hz, 1H, 1'''-H), 4.56 (d, $^2J$=11.9 Hz, 1H, 21b-H), 4.77(d, $^2J$=11.9 Hz, 1H, 21a-H), 4.84 (dd, $^3J_{H2a,H3}$=7.8 Hz, $^3J_{H2b,H3}$=6.0 Hz, 1H, 3-H), 5.20 (m, 1H, 15-H), 5.20 (d, $^3J_{H18a,H19}$=5.0 Hz, 1H, 19-H), 5.54 (d, $^3J_{H1',H2'}$=9.6 Hz, 1H, 1'-H), 5.55 (m, 1H, 13-H), 5.60 (dd, $^3J_{H10,H11}$=15.2 Hz, $^3J_{H9,H10}$=9.3 Hz, 1H, 10-H), 6.01 (ddd, $^3J_{H12,H13}$=15.2 Hz, $^3J_{H11,H12}$=10.5 Hz, $^4J$=1.6 Hz, 1H, 12-H), 6.11 (dd, $^3J_{H10,H11}$=15.2 Hz, $^3J_{H11,H12}$=10.5 Hz, 1H, 11-H), 8.15 (s, 1H, 23-H), $^{13}$C NMR (600 MHz, $CD_3CN$, 25°C), δ= 15.7 (20-C), 18.8 (3'''-C), 19.5 (6'''-C), 20.5 (16-C), 32.1 (2'''-C), 32.2 (2-C), 34.5 (8-C), 36.1 (7-C), 39.1 (6-C), 39.3 (18-C), 40.9 (7''', 8'''-C), 41.7 (14-C), 58.5 (17-C), 60.9 (21-C), 62.2 (6'-C), 65.9 (4'''-C), 69.9 (4'-C), 70.0 (15-C), 70.8 (2'-C), 74.2 (5'''-C), 74.7 (3'-C), 79.0 (5'-C), 80.5 (9-C), 83.7 (5-C), 89.1 (1'-

C), 100.8 (1’’’-C), 103.8 (19-C), 104.0 (3-C), 124.0 (23-C), 128.9 (10-C), 132.6 (12-C), 132.7 (13-C), 135.5 (11-C), 145.8 (22-C), 157.7 (4-C), 174.0 (1-C),

**7**: (2*R*,3a*R*,5*R*,6*R*,7*E*,9*E*,12*R*,16*Z*,17a*R*)-6-(((2*R*,5*S*,6*R*)-5-(dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)-17-methoxy-5,12-dimethyl-2-((1-((2*S*,3*S*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)-1*H*-1,2,3-triazol-4-yl)methoxy)-3a,4,5,6,11,12,15,17a-octahydro-2*H*-furo[2,3-*f*][1]oxacyclohexadecin-14(3*H*)-one (54 mg, Yield 21.8%) mp 84-86°C. HPLC $R_t$= 3.373min. Elemental analysis $C_{37}H_{58}N_4O_{12}$: calculated: C, 59.18; H, 7.79; N, 7.46; measured: C, 59.22; H, 7.80; N, 7.45; HRMS (ESI - TOF) m/z: $[M+H]^+$ = 751.4130. FT-IR ($CH_3CN$): ν(O-H) -3494 $cm^{-1}$, ν(C-H) -2930 $cm^{-1}$, $\nu(C=O)_{lactone}$ -1726 $cm^{-1}$, ν(C=C) -1675 $cm^{-1}$, $\nu(C-O)_{lactone}$ -1230 $cm^{-1}$, ν(C-O) -1125 $cm^{-1}$, ν(C-O) -1068 $cm^{-1}$, 1H NMR (600 MHz, CD3CN, 25°C), δ= 0.91 (d, 3JH8,H20=6.7 Hz, 3H, 20-H), 1.12 (d, 3JH5```,H6```=6.1 Hz, 3H, 6’’’-H), 1.15 (m, 1H, 7a-H), 1.25 (d, 3JH15,H16=6.3 Hz, 3H, 16-H), 1.32 (m, 1H, 2’’’a-H), 1.42 (m, 1H, 3’’’b-H), 1.43 (m, 1H, 7b-H), 1.70 (m, 1H, 18a-H), 1.79 (m, 1H, 3’’’a-H), 1.79 (m, 1H, 2’’’b-H), 1.94 (m, 1H, 8-H), 2.10 (m, 1H, 4’’’-H), 2.12 (m, 1H, 18b-H), 2.12 (m, 1H, 14b-H), 2.15 (s, 6H, 7’’’-H, 8’’’-H), 2.26 (m, 1H, 6-H), 2.46 (m, 1H, 14a-H), 2.87 (ddd, 4J=1.5 Hz, 3JH2b,H3=6.0 Hz, 2J=15.3 Hz, 1H, 2b-H), 3.18 (dd, 3JH2a,H3=7.9 Hz, 2J=15.1 Hz, 1H, 2a-H), 3.44 (m, 1H, 5’’’-H), 3.48 (m, 1H, 4’-H), 3.54 (m, 1H, 5’-H), 3.55 (m, 1H, 3’-H), 3.61 (s, 3H, 17-H), (α) 3.63 (m, 1H, 6’-H), (β) 3.77 (dd, 3JH5’,H6’=2.4 Hz, 2J =12.0 Hz, 1H, 6’-H), 3.99 (d, 3JH5,H6=9.7 Hz, 1H, 5-H), 4.00 (m, 1H, 2’-H), 4.09 (dd, 3JH8,H9’=4.6 Hz, 3JH9,H10’=9.3 Hz, 1H, 9-H), 4.43 (dd, 3JH1’’’,H2a’’’=9.3 Hz, 3JH1’’’,H2b’’’=1.9 Hz, 1H, 1’’’-H), 4.56 (d, 2J`=11.9 Hz, 1H, 21b-H), 4.77 (d, 2J`=11.9 Hz, 1H, 21a-H), 4.83 (dd, 3JH2a,H3=7.9 Hz, 3JH2b,H3=5.9 Hz, 1H, 3-H), 5.20 (m, 1H, 15-H), 5.20 (m, 1H, 19-H), 5.55 (ddd, 3JH12,H13=14.9 Hz, 3JH13,H14a=10.8 Hz, 3JH13,H14b=4.2 Hz, 1H, 13-H), 5.59 (d,

3JH1',H2'=10.3 Hz, 1H, 1'-H), 5.61 (m, 1H, 10-H), 6.02 (ddd, 3JH12,H13=14.9 Hz, 3JH11,H12=10.5 Hz, 4J=1.6 Hz, 1H, 12-H), 6.11 (dd, 3JH10,H11=15.1 Hz, 3JH11,H12=10.5 Hz, 1H, 11-H), 8.18 (s, 1H, 23-H), 13C NMR (600 MHz, CD3CN, 25°C), δ= 15.7 (20-C), 18.8 (3'''-C), 19.5 (6'''-C), 20.5 (16-C), 32.1 (2'''-C), 32.1 (2-C), 34.4 (8-C), 36.1 (7-C), 39.1 (6-C), 39.2 (18-C), 40.9 (7''', 8'''-C), 41.7 (14-C), 58.5 (17-C), 60.9 (21-C), 62.2 (6'-C), 65.9 (4'''-C), 70.0 (15-C), 70.6 (4'-C), 73.3 (2'-C), 74.0 (5'''-C), 77.9 (3'-C), 80.2 (5'-C), 80.5 (9-C), 83.7 (5-C), 88.6 (1'-C), 100.7 (1'''-C), 103.8 (19-C), 103.9 (3-C), 123.8 (23-C), 128.8 (10-C), 132.7 (12-C), 132.7 (13-C), 135.6 (11-C), 145.9 (22-C), 157.7 (4-C), 173.5 (1-C),

**8**: *N*-((2*S*,3*S*,4*S*,5*R*,6*S*)-2-(4-((((2*R*,3a*R*,5*R*,6*R*,7*E*,9*E*,12*R*,16*Z*,17a*R*)-6-(((2*R*,5*S*,6*R*)-5-(dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)-17-methoxy-5,12-dimethyl-14-oxo-3,3a,4,5,6,11,12,14,15,17a-decahydro-2*H*-furo[2,3-*f*][1]oxacyclohexadecin-2-yl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl)acetamide (65 mg, Yield 25%) mp 109-111°C. HPLC $R_t$= 3.353 min. Elemental analysis $C_{39}H_{61}N_5O_{12}$: calculated: C, 59.15; H, 7.76; N, 8.84; measured: C, 59.17; H, 7.78; N, 8.82; HRMS (ESI - TOF) m/z: $[M+H]^+$ = 792.4367. FT-IR ($CH_3CN$): ν(O-H) -3494 $cm^{-1}$, $\nu(N\text{-}H)_{amide}$ -3366 $cm^{-1}$, ν(C-H) -2931 $cm^{-1}$, $\nu(C{=}O)_{lactone}$ -1728 $cm^{-1}$, $\nu(C_{7'}{=}O)$ -1687 $cm^{-1}$, ν(C=C) -1642 $cm^{-}1$, δ(N-H) -1537 $cm^{-1}$, $\nu(C\text{-}O)_{lactone}$ -1240 $cm^{-1}$, ν(C-O) -1122 $cm^{-1}$, ν(C-O) -1068 $cm^{-1}$, γ(N-H) 570 $cm^{-1}$, $^1$H NMR (600 MHz, $CD_3CN$, 25°C), δ= 0.91 (d, $^3J_{H8,H20}$=6.8 Hz, 3H, 20-H), 1.12 (d, $^3J_{H5'''',H6''''}$=6.2 Hz, 3H, 6'''-H), 1.14 (m, 1H, 7a-H), 1.25 (d, $^3J_{H15,H16}$=6.5 Hz, 3H, 16-H), 1.32 (m, 1H, 2'''a-H), 1.42 (m, 1H, 7b-H), 1.42 (m, 1H, 3'''b-H), 1.71 (m, 1H, 18a-H), 1.72 (s, 3H, 8'), 1.79 (m, 1H, 3'''a-H), 1.79 (m, 1H, 2'''b-H), 1.94 (m, 1H, 8-H), 2.10 (m, 1H, 4'''-H), 2.10 (m, 1H, 18b-H), 2.15 (s, 6H, 7'''-H, 8'''-H), 2.16 (m, 1H, 14b-H), 2.27 (m, 1H, 6-H), 2.44 (m, 1H, 14a-H), 2.85 (m, 1H, 2b-H), 3.16 (dd, $^3J_{H2a,H3}$=8.2 Hz, $^2J$=15.4 Hz, 1H, 2a-H), 3.43 (m, 1H, 5'''-H), 3.57 (m, 1H, 5'-

H), 3.57 (m, 1H, 4'-H), 3.61 (s, 3H, 17-H), (α) 3.70 (d, $^{2}J$ =12.0 Hz, 1H, 6'-H), 3.71 (m, 1H, 3'-H), (β) 3.80 (d, $^{2}J$ =12.0 Hz, 1H, 6'-H), 3.97 (d, $^{3}J_{H5,H6}$=9.4 Hz, 1H, 5-H), 4.12 (dd, $^{3}J_{H8,H9}$=4.6 Hz, $^{3}J_{H9,H10}$=9.3 Hz, 1H, 9-H), 4.28 (m, 1H, 2'-H), 4.43 (dd, $^{3}J_{H1'',H2a''}$=9.3 Hz, $^{3}J_{H1'',H2b''}$=1.7 Hz, 1H, 1'''-H), 4.56 (d, $^{2}J$=11.9 Hz, 1H, 21b-H), 4.72 (d, $^{2}J$=12.2 Hz, 1H, 21a-H), 4.83 (dd, $^{3}J_{H2a,H3}$=8.0 Hz, $^{3}J_{H2b,H3}$=5.7 Hz, 1H, 3-H), 5.18 (m, 1H, 15-H), 5.15 (d, $^{3}J_{H18a,H19}$=5.2 Hz, 1H, 19-H), 5.61 (m, 1H, 10-H), 5.61 (m, 1H, 13-H), 5.82 (d, $^{3}J_{H1',H2'}$=10.0 Hz, 1H, 1'-H), 6.02 (ddd, $^{3}J_{H12,H13}$=15.0 Hz, $^{3}J_{H11,H12}$=10.5 Hz, $^{4}J$=1.5 Hz, 1H, 12-H), 6.22 (dd, $^{3}J_{H10,H11}$=15.0 Hz, $^{3}J_{H11,H12}$=10.5 Hz, 1H, 11-H), 8.08 (s, 1H, 23-H), $^{13}$C NMR (600 MHz, $CD_3CN$, 25°C), δ= 15.8 (20-C), 18.8 (3'''-C), 19.5 (6'''-C), 20.5 (16-C), 23.1 (8'-C), 32.1 (2-C), 32.2 (2'''-C), 34.7 (8-C), 36.1 (7-C), 38.7 (6-C), 39.2 (18-C), 40.9 (7''', 8'''-C), 41.6 (14-C), 56.0 (2'-C), 58.6 (17-C), 60.8 (21-C), 62.2 (6'-C), 65.9 (4'''-C), 69.9 (15-C), 71.1 (4'-C), 74.2 (5'''-C), 75.3 (3'-C), 80.1 (5'-C), 80.6 (9-C), 83.9 (5-C), 87.2 (1'-C), 100.9 (1'''-C), 103.4 (19-C), 104.2 (3-C), 123.7 (23-C), 128.9 (10-C), 132.9 (12-C), 132.9 (13-C), 135.5 (11-C), 145.5 (22-C), 157.3 (4-C), 171.4 (7'-C), 173.1 (1-C),

**9**: (2*R*,3a*R*,5*R*,6*R*,7*E*,9*E*,12*R*,16*Z*,17a*R*)-6-(((2*R*,5*S*,6*R*)-5-(dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)-17-methoxy-5,12-dimethyl-2-((1-((2*S*,3*S*,4*S*,5*R*,6*S*)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl)-1*H*-1,2,3-triazol-4-yl)methoxy)-3a,4,5,6,11,12,15,17a-octahydro-2*H*-furo[2,3-*f*][1]oxacyclohexadecin-14(3*H*)-one (131 mg, Yield 53%) mp 114-116°C. HPLC $R_t$= 3.347 min. Elemental analysis $C_{37}H_{58}N_4O_{12}$: calculated: C, 59.18; H, 7.79; N, 7.46; measured: C, 59.17; H, 7.82; N, 7.44; HRMS (ESI - TOF) m/z: $[M+H]^+$ = 751.41317. FT-IR ($CH_3CN$): ν(O-H) -3482 $cm^{-1}$, ν(C-H) -2928 $cm^{-1}$, $\nu(C=O)_{lactone}$ -1726 $cm^{-1}$, ν(C=C) -1675 $cm^{-1}$, $\nu(C-O)_{lactone}$ -1228 $cm^{-1}$, ν(C-O) -1121 $cm^{-1}$, ν(C-O) -1088 $cm^{-1}$, $^{1}$H NMR (600 MHz, $CD_3CN$, 25°C), δ= 0.91 (d, $^{3}J_{H8,H20}$=6.8 Hz, 3H, 20-H), 1.09 (d,

$^{3}J_{H5```,H6``}$=5.8 Hz, 3H, 6’’’-H), 1.10 (d, $^{3}J_{H5```,H6``}$=5.8 Hz, 3H, 6’’’-H), 1.14 (m, 1H, 7a-H), 1.24 (d, $^{3}J_{H15,H16}$=6.4 Hz, 3H, 16-H), 1.26 (d, $^{3}J_{H15,H16}$=6.5 Hz, 3H, 16-H), 1.31 (m, 1H, 2’’’a-H), 1.39 (m, 1H, 7b-H), 1.43 (m, 1H, 3’’’b-H), 1.69 (m, 1H, 18a-H), 1.74 (m, 1H, 3’’’a-H), 1.78 (m, 1H, 2’’’b-H), 1.95 (m, 1H, 8-H), 2.09 (m, 1H, 4’’’-H), 2.11 (m, 1H, 18b-H), 2.12 (m, 1H, 14b-H), 2.14 (s, 6H, 7’’’-H, 8’’’-H), 2.27 (m, 1H, 6-H), 2.46 (m, 1H, 14a-H), 2.87 (m, 1H, 2b-H), 3.16 (m, 1H, 2a-H), 3.44 (m, 1H, 5’’’-H), (α) 3.46 (m, 1H, 4’-H), (β) 3.55 (m, 1H, 4’-H), (α) 3.48 (m, 1H, 5’-H), 3.60 (s, 3H, 17-H), 3.62 (s, 3H, 17-H), (α) 3.72 (d, $^{2}J$ =12.0 Hz, 1H, 6’-H), (β) 3.83 (d, $^{2}J$ =12.0 Hz, 1H, 6’-H), (β) 3.88 (m, 1H, 5’-H), 3.98 (d, $^{3}J_{H5,H6}$=9.6 Hz, 1H, 5-H), 4.00 (d, $^{3}J_{H5,H6}$=9.6 Hz, 1H, 5-H), 4.06-4.15 (m, 1H, 9-H), (α) 4.09 (m, 1H, 3’-H), (α) 4.20 (dd, $^{3}J_{H1',H2'}$=8.2 Hz, $^{3}J_{H2',H3'}$=10.5 Hz, 1H, 2’-H), (β) 4.42 (m, 1H, 3’-H), 4.43 (m, 1H, 1’’’-H), 4.52 (d, $^{2}J$=11.6 Hz, 1H, 21b-H), 4.54 (d, $^{2}J$=11.8 Hz, 1H, 21b-H), (β) 4.64 (dd, $^{3}J_{H1',H2'}$=3.3 Hz, $^{3}J_{H2',H3'}$=11.0 Hz, 1H, 2’-H), 4.75 (d, $^{2}J$=11.8 Hz, 1H, 21a-H), 4.78 (d, $^{2}J$=11.6 Hz, 1H, 21a-H), 4.84 (m, 1H, 3-H), (α) 5.14 (d, $^{3}J_{H1',H2'}$=8.1 Hz, 1H, 1’-H), 5.16 (m, 1H, 15-H), 5.19 (d, $^{3}J_{H18a,H19}$=4.9 Hz, 1H, 19-H), 5.25 (ddq, $^{3}J_{H15,H16}$=6.3 Hz, $^{3}J_{H15,H14a}$=10.1 Hz, $^{3}J_{H15,H14b}$=2.8 Hz, 1H, 15-H), (β) 5.32 (d, $^{3}J_{H1',H2'}$=3.3 Hz, 1H, 1’-H), 5.61 (m, 1H, 10-H), 5.68 (ddd, $^{3}J_{H12,H13}$=15.0 Hz, $^{3}J_{H13,H14a}$=10.9 Hz, $^{3}J_{H13,H14b}$=5.5 Hz, 1H, 13-H), 6.04 (m, 1H, 12-H), 6.12 (dd, $^{3}J_{H10,H11}$=15.0 Hz, $^{3}J_{H11,H12}$=10.4 Hz, 1H, 11-H), 6.13 (dd, $^{3}J_{H10,H11}$=15.1 Hz, $^{3}J_{H11,H12}$=10.5 Hz, 1H, 11-H), (β) 7.95 (s, 1H, 23-H), (α) 8.03 (s, 1H, 23-H), $^{13}$C NMR (600 MHz, $CD_3CN$, 25°C), δ= 15.6 (20-C), 15.7 (20-C), 18.8 (3’’’-C), 19.5 (6’’’-C), 20.5 (16-C), 32.1 (2-C), 32.1 (2’’’-C), 32.2 (2’’’-C), 34.0 (8-C), 34.5 (8-C), 35.9 (7-C), 36.1 (7-C), 38.8 (6-C), 39.2 (6-C), 39.2 (18-C), 40.9 (7’’’, 8’’’-C), 41.6 (14-C), 41.8 (14-C), 58.6 (17-C), 60.8 (21-C), 60.9 (21-C), 62.5 (6’-C), (β) 65.8 (2’-C), 65.9 (4’’’-C), (α) 68.7 (2’-C), 70.0 (15-C), (β) 71.1 (3’-C), (α) 71.9 (4’-C), (β) 72.2 (4’-C), (β) 72.8 (5’-C), 74.2 (5’’’-C), (α) 75.1 (3’-C), (α) 77.3 (5’-C), 80.2 (9-C), 80.5 (9-C), 83.6 (5-C), 83.8 (5-C), (β) 92.3 (1’-C), (α) 95.7 (1’-C), 100.4 (1’’’-C), 100.7 (1’’’-C), 103.4 (19-C), 104.0 (3-

C), (β) 124.0 (23-C), (α) 125.6 (23-C), 128.6 (10-C), 129.0 (10-C), 132.5 (13-C), 132.7 (12-C), 132.7 (13-C), 132.9 (12-C), 135.5 (11-C), 135.8 (11-C), 145.2 (22-C), 145.3 (22-C), 157.6 (4-C), 157.8 (4-C), 173.5 (1-C), 173.6 (1-C),

**10**: (2*R*,3a*R*,5*R*,6*R*,7*E*,9*E*,12*R*,16*Z*,17a*R*)-6-(((2*R*,5*S*,6*R*)-5-(dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)-17-methoxy-5,12-dimethyl-2-((1-(((2*S*,3*R*,4*R*,5*S*,6*S*)-3,4,5,6-tetrahydroxytetrahydro-2*H*-pyran-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-3a,4,5,6,11,12,15,17a-octahydro-2*H*-furo[2,3-*f*][1]oxacyclohexadecin-14(3*H*)-one (127 mg, Yield 51.1%) mp 96-98°C. HPLC $R_t$= 3.420 min. Elemental analysis $C_{37}H_{58}N_4O_{12}$: calculated: C, 59.18; H, 7.79; N, 7.46; measured: C, 59.20; H, 7.76; N, 7.48; HRMS (ESI - TOF) m/z: $[M+H]^+$ = 751.4147. FT-IR ($CH_3CN$): ν(O-H) -3482 $cm^{-1}$, ν(C-H) -2932 $cm^{-1}$, $\nu(C=O)_{lactone}$ -1726 $cm^{-1}$, ν(C=C) -1675 $cm^{-1}$, $\nu(C-O)_{lactone}$ -1226 $cm^{-1}$, ν(C-O) -1125 $cm^{-1}$, ν(C-O) -1066 $cm^{-1}$, $^1$H NMR (600 MHz, $CD_3CN$, 25°C), δ= 0.91 (d, $^3J_{H8,H20}$=6.8 Hz, 3H, 20-H), 1.10 (d, $^3J_{H5'''',H6''''}$=6.2 Hz, 3H, 6’’’-H), 1.14 (m, 1H, 7a-H), 1.25 (d, $^3J_{H15,H16}$=6.3 Hz, 3H, 16-H), 1.32 (m, 1H, 2’’’a-H), 1.37 (m, 1H, 7b-H), 1.42 (m, 1H, 3’’’b-H), 1.70 (m, 1H, 18a-H), 1.79 (m, 1H, 3’’’a-H), 1.79 (m, 1H, 2’’’b-H), 1.94 (m, 1H, 8-H), 2.09 (m, 1H, 4’’’-H), 2.09 (m, 1H, 18b-H), 2.09 (m, 1H, 14b-H), 2.14 (s, 6H, 7’’’-H, 8’’’-H), 2.27 (m, 1H, 6-H), 2.42 (m, 1H, 14a-H), 2.85 (dd, $^3J_{H2,H3}$=5.9 Hz, $^2J$=15.2 Hz, 1H, 2b-H), (β) 3.07 (m, 1H, 2’-H), (α) 3.13 (m, 1H, 4’-H), 3.14 (dd, $^3J_{H2a,H3}$=8.1 Hz, $^2J$=15.1 Hz, 1H, 2a-H), (β) 3.18 (m, 1H, 4’-H), (α) 3.27 (dd, $^3J_{H1',H2}$=9.3 Hz, $^3J_{H2',H3}$=3.7 Hz, 1H, 2’-H), (β) 3.35 (t, $^3J_{H2',H3'}$=9.0 Hz, $^3J_{H3',H4'}$=9.0 Hz, 1H, 3’-H), 3.44 (m, 1H, 5’’’-H), 3.60 (s, 3H, 17-H), (α) 3.60 (m, 1H, 3’-H), (β) 3.70 (m, 1H, 5’-H), 3.97 (d, $^3J_{H5,H6}$=9.5 Hz, 1H, 5-H), (α) 4.08 (m, 1H, 5’-H), 4.13 (dd, $^3J_{H8,H9}$=4.6 Hz, $^3J_{H9,H10}$=9.3 Hz, 1H, 9-H), (β) 4.41 (m, 1H, 1’-H), 4.43 (dd, $^3J_{H1''',H2a''}$=9.3 Hz, $^3J_{H1''',H2b''}$=1.7 Hz, 1H, 1’’’-H), (α) 4.46 (m, 1H, 6’b-H), (β) 4.48 (d, $^2J$ =14.4 Hz, $^3J_{H5',H6'}$=3.7 Hz, 1H, 6’-H), 4.53 (d, $^2J$=11.9 Hz, 1H, 21b-H), 4.54 (d,

$^{2}J$=11.9 Hz, 1H, 21b-H), 4.72 (d, $^{2}J$=12.1 Hz, 1H, 21a-H), (α) 4.73 (m, 1H, 6'a-H), (β) 4.77 (m, 1H, 6'-H), 4.81 (m, 1H, 3-H), (α) 5.02 (d, $^{3}J_{H1',H2}$=3.7 Hz, 1H, 1'-H), 5.15 (m, 1H, 15-H), 5.19 (d, $^{3}J_{H18a,H19}$=4.9 Hz, 1H, 19-H), 5.59 (m, 1H, 13-H), 5.61 (m, 1H, 10-H), 6.03 (m, 1H, 12-H), 6.13 (dd, $^{3}J_{H10,H11}$=15.0 Hz, $^{3}J_{H11,H12}$=10.5 Hz, 1H, 11-H), 7.87 (s, 1H, 23-H), $^{13}$C NMR (600 MHz, $CD_3CN$, 25°C), δ= 15.68 (20-C), 15.71 (20-C), 18.8 (3'''-C), 19.52 (6'''-C), 19.55 (6'''-C), 20.5 (16-C), 32.09 (2-C), 32.15 (2-C), 32.09 (2'''-C), 32.15 (2'''-C), 34.35 (8-C), 34.40 (8-C), 35.86 (7-C), 35.95 (7-C), 38.88 (6-C), 38.94 (6-C), 39.2 (18-C), 40.9 (7''', 8'''-C), 41.64 (14-C), 41.69 (14-C), 52.14 (6'-C), 52.20 (6'-C), 58.6 (17-C), 60.86 (21-C), 60.91 (21-C), 65.9 (4'''-C), 69.78 (15-C), 69.84 (15-C), (α) 71.0 (5'-C), (β) 72.4 (4'-C), (α) 72.7 (4'-C), (α) 73.3 (2'-C), 74.2 (5'''-C), (α) 74.4 (3'-C), (β) 75.1 (5'-C), (β) 75.7 (2'-C), (β) 77.2 (3'-C), 80.32 (9-C), 80.36 (9-C), 83.7 (5-C), 83.8 (5-C), (α) 93.3 (1'-C), (β) 97.7 (1'-C), 100.7 (1'''-C), 103.36 (19-C), 103.42 (19-C), 104.18 (3-C), 104.24 (3-C), (β) 125.6 (23-C), (α) 125.74 (23-C), 128.84 (10-C), 128.91 (10-C), 132.61 (13-C), 132.62 (13-C), 132.74 (12-C), 132.79 (12-C), 135.48 (11-C), 135.59 (11-C), 145.34 (22-C), 145.39 (22-C), 157.41 (4-C), 157.47 (4-C), 173.09 (1-C), 173.12 (1-C),

**11**: (2*R*,3a*R*,5*R*,6*R*,7*E*,9*E*,12*R*,16*Z*,17a*R*)-2-((1-cycloheptyl-1*H*-1,2,3-triazol-4-yl)methoxy)-6-(((2*R*,5*S*,6*R*)-5-(dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)-17-methoxy-5,12-dimethyl-3a,4,5,6,11,12,15,17a-octahydro-2*H*-furo[2,3-*f*][1]oxacyclohexadecin-14(3*H*)-one (106 mg, Yield 47%) mp 65-67°C. HPLC $R_t$= 15.027 min. Elemental analysis $C_{38}H_{60}N_4O_7$: calculated: C, 66.64; H, 8.83; N, 8.18; measured: C, 66.63; H, 8.85; N, 8.19; HRMS (ESI - TOF) m/z: $[M+H]^+$ = 685.4512. FT-IR ($CH_3CN$): ν(C-H) -2932 $cm^{-1}$, $\nu(C{=}O)_{lactone}$ -1726 $cm^{-1}$, ν(C=C) -1675 $cm^{-1}$, $\nu(C\text{-}O)_{lactone}$ -1228 $cm^{-1}$, ν(C-O) -1123 $cm^{-1}$, ν(C-O) -1068 $cm^{-1}$, $^{1}$H NMR (600 MHz, $CD_3CN$, 25°C), δ= 0.91 (d, $^{3}J_{H8,H20}$=6.7 Hz, 3H, 20-H), 1.10 (d, $^{3}J_{H5'''',H6''''}$=6.2 Hz, 3H, 6'''-H),

1.14 (m, 1H, 7a-H), 1.25 (d, $^{3}J_{H15,H16}$=6.4 Hz, 3H, 16-H), 1.33 (m, 1H, 2’’’a-H), 1.43 (m, 1H, 7b-H), 1.43 (m, 1H, 3’’’b-H), 1.72-1.51 (m, 2H, 27-H), 1.72-1.51 (m, 2H, 28-H), 1.72-1.51 (m, 2H, 26-H), 1.72-1.51 (m, 2H, 29-H), 1.67 (m, 1H, 18a-H), 1.68 (m, 1H, 25b-H), 1.68 (m, 1H, 30b-H), 1.80 (m, 1H, 3’’’a-H), 1.80 (m, 1H, 2’’’b-H), 1.80 (m, 1H, 25a-H), 1.80 (m, 1H, 30a-H), 1.94 (m, 1H, 8-H), 2.08 (m, 1H, 14b-H), 2.08 (m, 1H, 18b-H), 2.08 (m, 1H, 4’’’-H), 2.15 (s, 6H, 7’’’-H, 8’’’-H), 2.25 (m, 1H, 6-H), 2.46 (m, 1H, 14a-H), 2.88 (ddd, $^{4}J$=1.4 Hz, $^{3}J_{H2b,H3}$=6.1 Hz, $^{2}J$=15.3 Hz, 1H, 2b-H), 3.14 (ddd, $^{5}J$=0.9 Hz, $^{3}J_{H2a,H3}$=7.8 Hz, $^{2}J$=15.3 Hz, 1H, 2a-H), 3.43 (dq, $^{3}J_{H4’’’,H5’’’}$=9.4 Hz, $^{3}J_{H5’’’,H6’’’}$=6.2 Hz, 1H, 5’’’-H), 3.60 (s, 3H, 17-H), 3.97 (d, $^{3}J_{H5,H6}$=9.5 Hz, 1H, 5-H), 4.08 (dd, $^{3}J_{H8,H9}$=4.6 Hz, $^{3}J_{H9,H10}$=9.4 Hz, 1H, 9-H), 4.41 (dd, $^{3}J_{H1’’’,H2a’’’}$=9.3 Hz, $^{3}J_{H1’’’,H2b’’’}$=1.9 Hz, 1H, 1’’’-H), 4.49 (d, $^{2}J$=11.6 Hz, 1H, 21b-H), 4.69 (m, 1H, 24-H), 4.73 (d, $^{2}J$=11.7 Hz, 1H, 21a-H), 4.84 (dd, $^{4}J$=0.8 Hz, $^{3}J_{H2a,H3}$=7.8 Hz, $^{3}J_{H2b,H3}$=6.1 Hz, 1H, 3-H), 5.16 (m, 1H, 15-H), 5.17 (d, $^{3}J_{H18a,H19}$=4.9 Hz, 1H, 19-H), 5.52 (ddd, $^{3}J_{H12,H13}$=14.6 Hz, $^{3}J_{H13,H14a}$=10.3 Hz, $^{3}J_{H13,H14b}$=4.2 Hz, 1H, 13-H), 5.61 (dd, $^{3}J_{H10,H11}$=14.5 Hz, $^{3}J_{H9,H10}$=9.3 Hz, 1H, 10-H), 6.02 (ddd, $^{3}J_{H12,H13}$=14.5 Hz, $^{3}J_{H11,H12}$=10.3 Hz, $^{4}J$=1.5 Hz, 1H, 12-H), 6.09 (dd, $^{3}J_{H10,H11}$=14.5 Hz, $^{3}J_{H11,H12}$=10.5 Hz, 1H, 11-H), 7.94 (s, 1H, 23-H), $^{13}$C NMR (600 MHz, $CD_3CN$, 25°C), δ= 15.8 (20-C), 18.8 (3’’’-C), 19.5 (6’’’-C), 20.5 (16-C), 24.9 (26-C), 24.9 (29-C), 28.4 (27-C), 28.4 (28-C), 32.1 (2’’’-C), 32.2 (2-C), 34.5 (8-C), 36.0 (7-C), 36.2 (25-C), 36.2 (30-C), 39.2 (6-C), 39.3 (18-C), 40.9 (7’’’, 8’’’-C), 41.7 (14-C), 58.6 (17-C), 60.9 (21-C), 63.1 (24-C), 65.9 (4’’’-C), 69.6 (15-C), 74.2 (5’’’-C), 80.6 (9-C), 83.7 (5-C), 100.9 (1’’’-C), 103.4 (19-C), 104.0 (3-C), 122.5 (23-C), 129.1 (10-C), 132.3 (13-C), 132.9 (12-C), 135.5 (11-C), 145.2 (22-C), 157.8 (4-C), 173.0 (1-C),

**12**: (2*R*,3a*R*,5*R*,6*R*,7*E*,9*E*,12*R*,16*Z*,17a*R*)-2-((1-cyclohexyl-1*H*-1,2,3-triazol-4-yl)methoxy)-6-(((2*R*,5*S*,6*R*)-5-(dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)-17-methoxy-5,12-

dimethyl-3a,4,5,6,11,12,15,17a-octahydro-2*H*-furo[2,3-*f*][1]oxacyclohexadecin-14(3*H*)-one (65 mg, Yield 29.4%) HPLC $R_t$= 11.667 min. Elemental analysis $C_{37}H_{58}N_4O_7$: calculated: C, 66.24; H, 8.71; N, 8.35; measured: C, 66.21; H, 8.73; N, 8.34; HRMS (ESI - TOF) m/z: $[M+H]^+$ = 671.4354. FT-IR ($CH_3CN$): ν(C-H) -2932 $cm^{-1}$, $\nu(C=O)_{lactone}$ -1726 $cm^{-1}$, ν(C=C) -1675 $cm^{-1}$, $\nu(C-O)_{lactone}$ -1228 $cm^{-1}$, ν(C-O) -1123 $cm^{-1}$, ν(C-O) -1068 $cm^{-1}$, $^1$H NMR (600 MHz, $CD_3CN$, 25°C), δ= 0.91 (d, $^3J_{H8,H20}$=6.8 Hz, 3H, 20-H), 1.10 (d, $^3J_{H5```,H6```}$=6.1 Hz, 3H, 6’’’-H), 1.14 (m, 1H, 7a-H), 1.26 (d, $^3J_{H15,H16}$=6.4 Hz, 3H, 16-H), 1.32 (m, 1H, 27b-H), 1.32 (m, 1H, 2’’’a-H), 1.45 (m, 1H, 3’’’b-H), 1.45 (m, 1H, 7b-H), 1.45 (m, 1H, 25b-H), 1.45 (m, 1H, 29b-H), 1.51 (m, 1H, 27a-H), 1.71 (m, 2H, 26-H), 1.71 (m, 2H, 28-H), 1.71 (m, 1H, 18a-H), 1.81 (m, 1H, 3’’’a-H), 1.81 (m, 1H, 2’’’b-H), 1.88 (m, 1H, 8-H), 1.88 (m, 1H, 25a-H), 1.88 (m, 1H, 29a-H), 2.08 (m, 1H, 14b-H), 2.08 (m, 1H, 18b-H), 2.08 (m, 1H, 4’’’-H), 2.15 (s, 6H, 7’’’-H, 8’’’-H), 2.26 (m, 1H, 6-H), 2.47 (m, 1H, 14a-H), 2.87 (ddd, $^4J$=1.4 Hz, $^3J_{H2b,H3}$=6.1 Hz, $^2J$=15.3 Hz, 1H, 2b-H), 3.14 (ddd, $^5J$=0.9 Hz, $^3J_{H2a,H3}$=7.9 Hz, $^2J$=15.3 Hz, 1H, 2a-H), 3.43 (dq, $^3J_{H4’’’,H5’’’}$=9.4 Hz, $^3J_{H5’’’,H6’’’}$=6.1 Hz, 1H, 5’’’-H), 3.60 (s, 3H, 17-H), 3.97 (d, $^3J_{H5,H6}$=9.5 Hz, 1H, 5-H), 4.08 (dd, $^3J_{H8,H9}$=4.6 Hz, $^3J_{H9,H10}$=9.3 Hz, 1H, 9-H), 4.41 (dd, $^3J_{H1’’’,H2a’’’}$=9.3 Hz, $^3J_{H1’’’,H2b’’’}$=1.9 Hz, 1H, 1’’’-H), 4.45 (m, 1H, 24-H), 4.50 (d, $^2J$=11.6 Hz, 1H, 21b-H), 4.73 (d, $^2J$=11.7 Hz, 1H, 21a-H), 4.84 (dd, $^4J$=0.8 Hz, $^3J_{H2a,H3}$=7.8 Hz, $^3J_{H2b,H3}$=6.0 Hz, 1H, 3-H), 5.15 (m, 1H, 15-H), 5.18 (d, $^3J_{H18a,H19}$=4.9 Hz, 1H, 19-H), 5.52 (ddd, $^3J_{H12,H13}$=14.7 Hz, $^3J_{H13,H14a}$=10.3 Hz, $^3J_{H13,H14b}$=4.2 Hz, 1H, 13-H), 5.61 (dd, $^3J_{H10,H11}$=14.6 Hz, $^3J_{H9,H10}$=9.3 Hz, 1H, 10-H), 6.02 (ddd, $^3J_{H12,H13}$=14.6 Hz, $^3J_{H11,H12}$=10.4 Hz, $^4J$=1.5 Hz, 1H, 12-H), 6.10 (dd, $^3J_{H10,H11}$=14.5 Hz, $^3J_{H11,H12}$=10.5 Hz, 1H, 11-H), 7.92 (s, 1H, 23-H), $^{13}$C NMR (600 MHz, $CD_3CN$, 25°C), δ= 15.8 (20-C), 18.8 (3’’’-C), 19.5 (6’’’-C), 20.5 (16-C), 25.8 (27-C), 25.9 (26-C), 25.9 (28-C), 32.1 (2’’’-C), 32.2 (2-C), 34.1 (25-C), 34.1 (29-C), 34.5 (8-C), 36.0 (7-C), 39.2 (6-C), 39.3 (18-C), 40.9 (7’’’, 8’’’-C), 41.7 (14-C), 58.6 (17-C), 60.7 (24-C), 60.9 (21-C), 65.9 (4’’’-C), 69.6 (15-C), 74.2 (5’’’-C), 80.5 (9-C), 83.7

(5-C), 100.9 (1’’’-C), 103.4 (19-C), 104.0 (3-C), 122.5 (23-C), 129.1 (10-C), 132.3 (13-C), 132.9 (12-C), 135.5 (11-C), 145.2 (22-C), 157.7 (4-C), 173.0 (1-C),

**13**: (2*R*,3a*R*,5*R*,6*R*,7*E*,9*E*,12*R*,16*Z*,17a*R*)-2-((1-(cyclohexylmethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-6-(((2*R*,5*S*,6*R*)-5-(dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)-17-methoxy-5,12-dimethyl-3a,4,5,6,11,12,15,17a-octahydro-2*H*-furo[2,3-*f*][1]oxacyclohexadecin-14(3*H*)-one (81 mg, Yield 36%) HPLC $R_t$= 15.793 min. Elemental analysis $C_{38}H_{60}N_4O_7$: calculated: C, 66.64; H, 8.83; N, 8.18; measured: C, 66.67; H, 8.81; N, 8.19; HRMS (ESI - TOF) m/z: $[M+H]^+$ = 685.4553. FT-IR ($CH_3CN$): ν(C-H) -2930 $cm^{-1}$, $\nu(C=O)_{lactone}$ -1724 $cm^{-1}$, ν(C=C) -1673 $cm^{-1}$, $\nu(C-O)_{lactone}$ -1226 $cm^{-1}$, ν(C-O) -1123 $cm^{-1}$, ν(C-O) -1066 $cm^{-1}$, $^1$H NMR (600 MHz, $CD_3CN$, 25°C), δ= 0.91 (d, $^3J_{H8,H20}$=6.8 Hz, 3H, 20-H), 1.00 (m, 1H, 26b-H), 1.00 (m, 1H, 30b-H), 1.10 (d, $^3J_{H5’’’,H6’’’}$=6.1 Hz, 3H, 6’’’-H), 1.14 (m, 1H, 7a-H), 1.24 (m, 1H, 27b-H), 1.24 (m, 1H, 29b-H), 1.25 (d, $^3J_{H15,H16}$=6.4 Hz, 3H, 16-H), 1.34 (m, 1H, 2’’’a-H), 1.44 (m, 1H, 3’’’b-H), 1.44 (m, 1H, 7b-H), 1.57 (m, 1H, 26a-H), 1.57 (m, 1H, 30a-H), 1.60 (m, 2H, 28-H), 1.72 (m, 1H, 18a-H), 1.74 (m, 1H, 27a-H), 1.74 (m, 1H, 29a-H), 1.79 (m, 1H, 3’’’a-H), 1.79 (m, 1H, 2’’’b-H), 1.94 (m, 1H, 25-H), 1.95 (m, 1H, 8-H), 2.08 (m, 1H, 14b-H), 2.08 (m, 1H, 18b-H), 2.08 (m, 1H, 4’’’-H), 2.15 (s, 6H, 7’’’-H, 8’’’-H), 2.27 (m, 1H, 6-H), 2.46 (m, 1H, 14a-H), 2.86 (ddd, $^4J$=1.4 Hz, $^3J_{H2b,H3}$=6.1 Hz, $^2J$=15.3 Hz, 1H, 2b-H), 3.15 (ddd, $^5J$=0.9 Hz, $^3J_{H2a,H3}$=8.0 Hz, $^2J$=15.3 Hz, 1H, 2a-H), 3.42 (dq, $^3J_{H4’’’,H5’’’}$=9.4 Hz, $^3J_{H5’’’,H6’’’}$=6.1 Hz, 1H, 5’’’-H), 3.60 (s, 3H, 17-H), 3.97 (d, $^3J_{H5,H6}$=9.5 Hz, 1H, 5-H), 4.09 (dd, $^3J_{H8,H9}$=4.6 Hz, $^3J_{H9,H10}$=9.3 Hz, 1H, 9-H), 4.21 (d, $^3J_{H24,H25}$=7.2 Hz, 2H, 24-H), 4.41 (dd, $^3J_{H1’’’,H2a’’’}$=9.3 Hz, $^3J_{H1’’’,H2b’’’}$=1.9 Hz, 1H, 1’’’-H), 4.52 (d, $^2J$=11.8 Hz, 1H, 21b-H), 4.73 (d, $^2J$=11.8 Hz, 1H, 21a-H), 4.83 (dd, $^4J$=0.8 Hz, $^3J_{H2a,H3}$=8.0 Hz, $^3J_{H2b,H3}$=6.0 Hz, 1H, 3-H), 5.16 (m, 1H, 15-H), 5.17 (d, $^3J_{H18a,H19}$=4.9 Hz, 1H, 19-H), 5.53 (ddd, $^3J_{H12,H13}$=14.6 Hz, $^3J_{H13,H14a}$=10.3 Hz, $^3J_{H13,H14b}$=4.2 Hz, 1H, 13-H), 5.62 (dd, $^3J_{H10,H11}$=14.6

Hz, $^{3}J_{H9,H10}$=9.4 Hz, 1H, 10-H), 6.02 (ddd, $^{3}J_{H12,H13}$=14.6 Hz, $^{3}J_{H11,H12}$=10.4 Hz, $^{4}J$=1.5 Hz, 1H, 12-H), 6.10 (dd, $^{3}J_{H10,H11}$=14.5 Hz, $^{3}J_{H11,H12}$=10.5 Hz, 1H, 11-H), 7.86 (s, 1H, 23-H), $^{13}$C NMR (600 MHz, $CD_3CN$, 25°C), δ= 15.7 (20-C), 18.8 (3’’’-C), 19.5 (6’’’-C), 20.5 (16-C), 26.3 (27-C), 26.3 (29-C), 26.9 (28-C), 31.1 (26-C), 31.1 (30-C), 32.1 (2-C), 32.2 (2’’’-C), 34.5 (8-C), 36.1 (7-C), 39.0 (6-C), 39.3 (18-C), 39.6 (25-C), 40.9 (7’’’, 8’’’-C), 41.7 (14-C), 56.8 (24-C), 58.5 (17-C), 61.0 (21-C), 65.9 (4’’’-C), 69.6 (15-C), 74.2 (5’’’-C), 80.6 (9-C), 83.7 (5-C), 100.9 (1’’’-C), 103.4 (19-C), 104.0 (3-C), 124.9 (23-C), 129.1 (10-C), 132.4 (13-C), 132.9 (12-C), 135.4 (11-C), 145.5 (22-C), 157.7 (4-C), 173.0 (1-C),

**14**: (2*R*,3a*R*,5*R*,6*R*,7*E*,9*E*,12*R*,16*Z*,17a*R*)-6-(((2*R*,5*S*,6*R*)-5-(dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)-17-methoxy-5,12-dimethyl-2-((1-((tetrahydro-2*H*-pyran-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-3a,4,5,6,11,12,15,17a-octahydro-2*H*-furo[2,3-*f*][1]oxacyclohexadecin-14(3*H*)-one (41 mg, Yield 18.1%) HPLC $R_t$= 6.673 min. Elemental analysis $C_{37}H_{58}N_4O_8$: calculated: C, 64.70; H, 8.51; N, 8.16; measured: C, 64.67; H, 8.53; N, 8.18; HRMS (ESI - TOF) m/z: $[M+H]^+$ = 687.4357. FT-IR ($CH_3CN$): ν(C-H) -2932 $cm^{-1}$, $\nu(C{=}O)_{lactone}$ -1726 $cm^{-1}$, ν(C=C) -1675 $cm^{-1}$, $\nu(C{-}O)_{lactone}$ -1226 $cm^{-1}$, ν(C-O) -1121 $cm^{-1}$, ν(C-O) -1092 $cm^{-1}$, $^{1}$H NMR (600 MHz, $CD_3CN$, 25°C), δ= 0.91 (d, $^{3}J_{H8,H20}$=6.8 Hz, 3H, 20-H), 1.10 (d, $^{3}J_{H5```,H6``}$=6.2 Hz, 3H, 6’’’-H), 1.14 (m, 1H, 7a-H), 1.25 (d, $^{3}J_{H15,H16}$=6.4 Hz, 3H, 16-H), 1.31 (m, 1H, 2’’’a-H), 1.32 (m, 1H, 26b-H), 1.32 (m, 1H, 29b-H), 1.39 (m, 1H, 7b-H), 1.41 (m, 1H, 3’’’b-H), 1.45 (m, 1H, 25-H), 1.45 (m, 1H, 26a-H), 1.45 (m, 1H, 29a-H), 1.69 (dt, $^{3}J_{H18a,H19}$=5.1 Hz, $^{3}J_{H6,H18a}$=5.1 Hz, $^{2}J$=12.4 Hz, 1H, 18a-H), 1.76 (m, 1H, 3’’’a-H), 1.80 (m, 1H, 2’’’b-H), 1.95 (m, 1H, 8-H), 2.09 (m, 1H, 4’’’-H), 2.10 (m, 1H, 18b-H), 2.12 (m, 1H, 14b-H), 2.15 (s, 6H, 7’’’-H, 8’’’-H), 2.26 (m, 1H, 6-H), 2.47 (m, 1H, 14a-H), 2.86 (ddd, $^{4}J$=1.5 Hz, $^{3}J_{H2b,H3}$=5.9 Hz, $^{2}J$=15.3 Hz, 1H, 2b-H), 3.15 (dd, $^{3}J_{H2a,H3}$=8.2 Hz, $^{2}J$=15.4 Hz, 1H, 2a-H), 3.30 (m, 1H, 27b-H),

3.30 (m, 1H, 28b-H), 3.43 (dq, $^{3}J_{H4'''',H5''''}$=9.4 Hz, $^{3}J_{H5'''',H6''''}$=6.2 Hz, 1H, 5'''-H), 3.60 (s, 3H, 17-H), 3.88 (m, 1H, 27a-H), 3.88 (m, 1H, 28a-H), 3.98 (d, $^{3}J_{H5,H6}$=9.5 Hz, 1H, 5-H), 4.10 (dd, $^{3}J_{H8,H9}$=4.6 Hz, $^{3}J_{H9,H10}$=9.4 Hz, 1H, 9-H), 4.27 (d, $^{3}J_{H24,H25}$=7.2 Hz, 2H, 24-H), 4.42 (dd, $^{3}J_{H1''',H2a''}$=9.3 Hz, $^{3}J_{H1''',H2b''}$=2.0 Hz, 1H, 1'''-H), 4.53 (d, $^{2}J$=11.9 Hz, 1H, 21b-H), 4.73 (d, $^{2}J$=11.9 Hz, 1H, 21a-H), 4.82 (dd, $^{3}J_{H2a,H3}$=8.0 Hz, $^{3}J_{H2b,H3}$=5.9 Hz, 1H, 3-H), 5.14 (m, 1H, 15-H), 5.18 (d, $^{3}J_{H18a,H19}$=5.0 Hz, 1H, 19-H), 5.53 (ddd, $^{3}J_{H12,H13}$=14.9 Hz, $^{3}J_{H13,H14a}$=10.8 Hz, $^{3}J_{H13,H14b}$=3.9 Hz, 1H, 13-H), 5.62 (dd, $^{3}J_{H10,H11}$=15.0 Hz, $^{3}J_{H9,H10}$=9.3 Hz, 1H, 10-H), 6.03 (ddd, $^{3}J_{H12,H13}$=15.0 Hz, $^{3}J_{H11,H12}$=10.5 Hz, $^{4}J$=1.7 Hz, 1H, 12-H), 6.11 (dd, $^{3}J_{H10,H11}$=15.0 Hz, $^{3}J_{H11,H12}$=10.5 Hz, 1H, 11-H), 7.87 (s, 1H, 23-H), $^{13}$C NMR (600 MHz, $CD_3CN$, 25°C), δ= 15.7 (20-C), 18.7 (3'''-C), 19.5 (6'''-C), 20.5 (16-C), 31.0 (26-C), 31.1 (29-C), 32.1 (2-C), 32.2 (2'''-C), 34.5 (8-C), 36.1 (7-C), 37.0 (25-C), 38.9 (18-C), 39.2 (6-C), 40.9 (7''', 8'''-C), 41.7 (14-C), 56.1 (24-C), 58.5 (17-C), 61.0 (21-C), 65.9 (4'''-C), 67.7 (27-C), 67.7 (28-C), 69.6 (15-C), 74.2 (5'''-C), 80.5 (9-C), 83.7 (5-C), 100.8 (1'''-C), 103.5 (19-C), 104.0 (3-C), 124.9 (23-C), 129.1 (10-C), 132.4 (13-C), 132.8 (12-C), 135.4 (11-C), 145.6 (22-C), 157.6 (4-C), 173.1 (1-C),

**15**: (2*R*,3a*R*,5*R*,6*R*,7*E*,9*E*,12*R*,16*Z*,17a*R*)-2-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methoxy)-6-(((2*R*,5*S*,6*R*)-5-(dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)-17-methoxy-5,12-dimethyl-3a,4,5,6,11,12,15,17a-octahydro-2*H*-furo[2,3-*f*][1]oxacyclohexadecin-14(3*H*)-one (134 mg, Yield 60.1%) mp 63-65°C. HPLC $R_t$= 10.173 min. Elemental analysis $C_{38}H_{54}N_4O_7$: calculated: C, 67.23; H, 8.02; N, 8.25; measured: C, 67.19; H, 8.05; N, 8.27; HRMS (ESI - TOF) m/z: $[M+H]^+$ = 679.4045. FT-IR ($CH_3CN$): ν(C-H)$_{ar}$ -3094 cm$^{-1}$, ν(C-H) -2877 cm$^{-1}$, ν(C=O)$_{lactone}$ -1728 cm$^{-1}$, ν(C=C) -1673 cm$^{-1}$, ν(C-O)$_{lactone}$ -1226 cm$^{-1}$, ν(C-O) -1123 cm$^{-1}$, ν(C-O) -1090 cm$^{-1}$, $^{1}$H NMR (600 MHz, $CD_3CN$, 25°C), δ= 0.91 (d, $^{3}J_{H8,H20}$=6.7 Hz, 3H, 20-H), 1.11 (d, $^{3}J_{H5'''',H6''''}$=6.0 Hz, 3H, 6'''-H), 1.11 (m, 1H, 7a-H), 1.25 (d, $^{3}J_{H15,H16}$=6.3 Hz, 3H, 16-H), 1.32

(m, 1H, 2’’’a-H), 1.37 (m, 1H, 7b-H), 1.42 (m, 1H, 3’’’b-H), 1.68 (dt, $^{3}J_{H18a,H19}$=5.0 Hz, $^{3}J_{H6,H18a}$=5.0 Hz, $^{2}J$=12.4 Hz, 1H, 18a-H), 1.78 (m, 1H, 3’’’a-H), 1.78 (m, 1H, 2’’’b-H), 1.94 (m, 1H, 8-H), 2.09 (m, 1H, 4’’’-H), 2.09 (m, 1H, 14b-H), 2.09 (m, 1H, 18b-H), 2.16 (s, 6H, 7’’’-H, 8’’’-H), 2.26 (m, 1H, 6-H), 2.42 (m, 1H, 14a-H), 2.84 (ddd, $^{4}J$=1.5 Hz, $^{3}J_{H2b,H3}$=5.9 Hz, $^{2}J$=15.3 Hz, 1H, 2b-H), 3.13 (dd, $^{3}J_{H2a,H3}$=7.9 Hz, $^{2}J$=15.3 Hz, 1H, 2a-H), 3.43 (dq, $^{3}J_{H4’’’,H5’’’}$=9.5 Hz, $^{3}J_{H5’’’,H6’’’}$=6.1 Hz, 1H, 5’’’-H), 3.58 (s, 3H, 17-H), 3.97 (d, $^{3}J_{H5,H6}$=9.6 Hz, 1H, 5-H), 4.07 (dd, $^{3}J_{H8,H9}$=4.6 Hz, $^{3}J_{H9,H10}$=9.4 Hz, 1H, 9-H), 4.41 (dd, $^{3}J_{H1’’’,H2a’’’}$=9.4 Hz, $^{3}J_{H1’’’,H2b’’’}$=2.1 Hz, 1H, 1’’’-H), 4.52 (d, $^{2}J$=11.8 Hz, 1H, 21b-H), 4.73 (d, $^{2}J$=11.8 Hz, 1H, 21a-H), 4.81 (dd, $^{3}J_{H2a,H3}$=8.0 Hz, $^{3}J_{H2b,H3}$=5.8 Hz, 1H, 3-H), 5.11 (ddq, $^{3}J_{H15,H16}$=6.5 Hz, $^{3}J_{H15,H14a}$=12.9 Hz, $^{3}J_{H15,H14b}$=3.4 Hz, 1H, 15-H), 5.17 (d, $^{3}J_{H18a,H19}$=4.9 Hz, 1H, 19-H), 5.36 (ddd, $^{3}J_{H12,H13}$=14.7 Hz, $^{3}J_{H13,H14a}$=10.8 Hz, $^{3}J_{H13,H14b}$=3.9 Hz, 1H, 13-H), 5.58 (m, 2H, 24-H), 5.61 (m, 1H, 10-H), 6.02 (m, 1H, 12-H), 6.02 (m, 1H, 11-H), 7.33 (m, 1H, 26-H), 7.33 (m, 1H, 28-H), 7.33 (m, 1H, 30-H), 7.38 (dd, $^{3}J$=8.1 Hz, $^{3}J$=6.5 Hz, 1H, 27-H), 7.38 (dd, $^{3}J$=8.1 Hz, $^{3}J$=6.5 Hz, 1H, 29-H), 7.96 (s, 1H, 23-H), $^{13}$C NMR (600 MHz, $CD_3CN$, 25°C), δ= 15.7 (20-C), 18.8 (3’’’-C), 19.5 (6’’’-C), 20.5 (16-C), 32.2 (2-C), 32.1 (2’’’-C), 34.5 (8-C), 36.1 (7-C), 39.0 (6-C), 39.2 (18-C), 40.9 (7’’’, 8’’’-C), 41.7 (14-C), 54.3 (24-C), 58.5 (17-C), 61.0 (21-C), 65.9 (4’’’-C), 69.6 (15-C), 74.2 (5’’’-C), 80.5 (9-C), 83.7 (5-C), 100.9 (1’’’-C), 103.5 (19-C), 103.9 (3-C), 124.8 (23-C), 128.7 (26-C), 128.7 (30-C), 128.9 (10-C), 129.2 (28-C), 129.8 (27-C), 129.8 (29-C), 132.5 (13-C), 132.7 (12-C), 135.5 (11-C), 137.2 (25-C), 146.1 (22-C), 157.7 (4-C), 173.0 (1-C),

**16**: 1-((2*R*,4*S*,5*S*)-4-(4-((((2*R*,3a*R*,5*R*,6*R*,7*E*,9*E*,12*R*,16*Z*,17a*R*)-6-(((2*R*,5*S*,6*R*)-5-(dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)-17-methoxy-5,12-dimethyl-14-oxo-3,3a,4,5,6,11,12,14,15,17a-decahydro-2*H*-furo[2,3-*f*][1]oxacyclohexadecin-2-yl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-

dione (56 mg, Yield 20.9%) mp 121-123°C. HPLC $R_t$= 4.600 min. Elemental analysis $C_{41}H_{60}N_6O_{11}$: calculated: C, 60.57; H, 7.44; N, 10.35; measured: C, 60.59; H, 7.43; N, 10.34; HRMS (ESI - TOF) m/z: $[M+H]^+$ = 813.4373. FT-IR ($CH_3CN$): ν(O-H) -3496 $cm^{-1}$, ν(N-H) -3265 $cm^{-1}$, ν(C-H) -2930 $cm^{-1}$, $\nu(C=O)_{lactone}$ -1716 $cm^{-1}$, ν(C=O) -1693 $cm^{-1}$, ν(C=C) -1661 $cm^{-1}$, $\nu(C-O)_{lactone}$ -1230 $cm^{-1}$, ν(C-O) -1168 $cm^{-1}$, $^1$H NMR (600 MHz, $CD_3CN$, 25°C), δ= 0.91 (d, $^3J_{H8,H20}$=6.7 Hz, 3H, 20-H), 1.11 (d, $^3J_{H5```,H6```}$=6.2 Hz, 3H, 6’’’-H), 1.15 (m, 1H, 7a-H), 1.24 (d, $^3J_{H15,H16}$=6.3 Hz, 3H, 16-H), 1.32 (m, 1H, 2’’’a-H), 1.39 (m, 1H, 7b-H), 1.43 (m, 1H, 3’’’b-H), 1.70 (dt, $^3J_{H18a,H19}$=5.0 Hz, $^3J_{H6,H18a}$=5.0 Hz, $^2J$=12.4 Hz, 1H, 18a-H), 1.77 (m, 1H, 3’’’a-H), 1.80 (m, 1H, 2’’’b-H), 1.86 (d, $^4J_{H6'',H7''}$=1.2 Hz, 3H, 7’’b-H), 1.95 (m, 1H, 8-H), 2.10 (m, 1H, 4’’’-H), 2.11 (m, 1H, 18b-H), 2.12 (m, 1H, 14b-H), 2.15 (s, 6H, 7’’’-H, 8’’’-H), 2.26 (m, 1H, 6-H), 2.46 (m, 1H, 14a-H), 2.69 (ddd, $^3J_{H1',H2'}$=6.2 Hz, $^3J_{H2'a,H3'}$=8.6 Hz, 1H, 2’a-H), 2.87 (m, 1H, 2b-H), 2.89 (m, 1H, 2’b-H), 3.14 (ddd, $^5J$=0.9 Hz, $^3J_{H2a,H3}$=7.8 Hz, $^2J$=15.4 Hz, 1H, 2a-H), 3.43 (dq, $^3J_{H4```,H5''}$=9.4 Hz, $^3J_{H5```,H6''}$=6.1 Hz, 1H, 5’’’-H), 3.62 (s, 3H, 17-H), 3.74 (dd, $^3J_{H4',H5'}$=3.1 Hz, $^2J$=12.3 Hz, 1H, 5’b-H), 3.86 (dd, $^3J_{H4',H5'}$=2.9 Hz, $^2J$=12.3 Hz, 1H, 5’a-H), 3.99 (d, $^3J_{H5,H6}$=9.3 Hz, 1H, 5-H), 4.10 (dd, $^3J_{H8,H9}$=4.6 Hz, $^3J_{H9,H10}$=9.4 Hz, 1H, 9-H), 4.37 (dt, $^3J_{H3',H4'}$=5.8 Hz, $^3J_{H4',H5'}$=3.0 Hz, 1H, 4’-H), 4.42 (dd, $^3J_{H1```,H2a''}$=9.2 Hz, $^3J_{H1```,H2b''}$=1.9 Hz, 1H, 1’’’-H), 4.56 (d, $^2J$=11.9 Hz, 1H, 21b-H), 4.75 (d, $^2J$=11.9 Hz, 1H, 21a-H), 4.82 (dd, $^3J_{H2a,H3}$=8.2 Hz, $^3J_{H2b,H3}$=5.7 Hz, 1H, 3-H), 5.14 (m, 1H, 15-H), 5.19 (d, $^3J_{H18a,H19}$=5.0 Hz, 1H, 19-H), 5.39 (dt, $^3J_{H2'a,H3'a}$=8.6 Hz, $^3J_{H2',H3'}$=5.6 Hz, $^3J_{H3',H4'}$=5.6 Hz, 1H, 3’-H), 5.54 (ddd, $^3J_{H12,H13}$=14.9 Hz, $^3J_{H13,H14a}$=11.1 Hz, $^3J_{H13,H14b}$=4.3 Hz, 1H, 13-H), 5.61 (dd, $^3J_{H10,H11}$=15.0 Hz, $^3J_{H9,H10}$=9.4 Hz, 1H, 10-H), 6.02 (ddd, $^3J_{H12,H13}$=15.0 Hz, $^3J_{H11,H12}$=10.5 Hz, $^4J$=1.6 Hz, 1H, 12-H), 6.12 (dd, $^3J_{H10,H11}$=15.0 Hz, $^3J_{H11,H12}$=10.5 Hz, 1H, 11-H), 6.43 (t, $^3J_{H1',H2'}$=6.2 Hz, 1H, 1’-H), 7.71 (q, $^4J_{H6'',H7''}$=1.2 Hz, 1H, 6’’b-H), 8.03 (s, 1H, 23-H), $^{13}$C NMR (600 MHz, $CD_3CN$, 25°C), δ= 12.6 (7’’-C), 15.7 (20-C), 18.8 (3’’’-C), 19.5 (6’’’-C), 20.5 (16-C), 32.1 (2-C), 32.2 (2’’’-C),

34.3 (8-C), 36.1 (7-C), 38.7 (2'-C), 39.1 (6-C), 39.2 (18-C), 40.9 (7''', 8'''-C), 41.8 (14-C), 58.4 (17-C), 60.1 (3'-C), 60.9 (21-C), 61.9 (5'-C), 65.9 (4'''-C), 69.8 (15-C), 74.2 (5'''-C), 80.4 (9-C), 83.6 (5-C), 85.6 (4'-C), 86.1 (1'-C), 100.7 (1'''-C), 103.6 (19-C), 103.8 (3-C), 111.2 (5''-C), 124.4 (23-C), 128.9 (10-C), 132.6 (13-C), 132.8 (12-C), 135.4 (11-C), 137.4 (6''-C), 145.9 (22-C), 151.5 (2''-C), 157.7 (4-C), 164.8 (4''-C), 173.3 (1-C),

General procedure for synthesis of azides

Respective bromides 0.5g (3.07 mmol) was dissolved in DMF and next to the mixture (3,07 mmol) $NaN_3$ was added. The mixtures were vigorously stirred at room temperature overnight and after that each mixture was evaporated to dry. In the next step diethyl ether was added and extracted several times with 50 ml of water. The organic layer was evaporated giving the products as a colorless oils.

**AZ1** azidocycloheptane (453.5 mg, 90.7%) Elemental analysis $C_7H_{13}N_3$: calculated: C, 60.40; H, 9.41; N, 30.19; measured: C, 60.37; H, 9.43; N, 30.18; $^1$H NMR $\delta$ (ppm) = 3.55 (1H, tt, 3J=8,6, 3J=4,34, H-1); 1.88 (2H, m, H-2a + H-7a); 1.64-1.58 (2H, m, H-2b + H-7b); 1.58-1.52 (2H, m, H-3a + H-6a); 1.52-1.46 (4H, m, H4 +H5); 1.44-1.34 (2H, m, H-3b + H-6b); $^{13}$C NMR $\delta$ (ppm) = 63.3 (C-1); 34.4 (C-2 + C-7); 28.5 (C-4 + C-5); 24.3 (C-3 + C-6); FT-IR ($CH_3CN$): ν(C-H) -2932 $cm^{-1}$, ν($N_3$) -2097 $cm^{-1}$,

**AZ2** azidocyclohexane (644 mg, 59.3%) Elemental analysis $C_6H_{11}N_3$: calculated: C, 57.57; H, 8.86; N, 33.57; measured: C, 57.60; H, 8.84; N, 33.59; $^1$H NMR $\delta$ (ppm) = 3.36 (1H, m, H-1); 1.82 (2H, m, 2-a + 6-a); 1.67 (2H, m, 3-a + 5-a); 1.27 (4H, m, H-2b + H-3-b + 5-b + 6-b); 1.49 (1H, m, 4-a); 1.19 (1H, m, 4-b); $^{13}$C NMR $\delta$ (ppm) = 60.7 (C-1); 32.2 (C-2 + C-6); 25.9 (C-4); 24.9 (C-3 + C-5); FT-IR ($CH_3CN$): ν(C-H) -2936 $cm^{-1}$, ν($N_3$) -2097 $cm^{-1}$,

**AZ3** (azidomethyl)cyclohexane (322 mg, 95%) Elemental analysis $C_7H_{13}N_3$: calculated: C, 60.40; H, 9.41; N, 30.19; measured: C, 60.42; H, 9.40; N, 30.20; $^1$H NMR $\delta$ (ppm) = 3.15 (2H, d, $^3J$=6,7); 1.72 (4H, m, H3-a, + H-4a + H6-a + H7-a); 1.66 (1H, m, H5-a); 1.54 (1H, m, H5-b); 1.26 (2H, m, H4-b + H6-b); 1.17 (1H, ddt, $^3J$=10,6, $^3J$=7,40, $^3J$=2,0, H-2); 0.97 (2H, m, H-3b + 7-b); $^{13}$C NMR $\delta$ (ppm) = 58.5 (C-1); 38.8 (C-2); 31.2 (C-3 + C-7); 27.0 (C-5); 26.4 (C-4 + C-6); FT-IR ($CH_3CN$): ν(C-H) -2932 cm$^{-1}$, ν($N_3$) -2101 cm$^{-1}$,

**AZ4** 4-(azidomethyl)tetrahydropyran (463.5 mg, 93%) Elemental analysis $C_6H_{11}N_3O$: calculated: C, 51.05; H, 7.85; N, 29.77; measured: C, 51.07; H, 7.82; N, 29.78; $^1$H NMR $\delta$ (ppm) = 3.88 (2H, ddd, $^2J$=13,3, $^3J$=7,2, $^3J$=4,8, H-4a + H-5a); 3.33 (2H, td, , $^2J$=11,9, $^3J$=11,9, $^3J$=2,1, H-4b + H-5b); 3.20 (2H, d, $^3J$=6,7, H-1); 1.79 (1H, m, H-2); 1.60 (2H, m, H3-a + H-6a); 1.27 (2H, m, H3-b + H6-b); $^{13}$C NMR $\delta$ (ppm) = 67.9 (C-4 + C-5); 57.8 (C-1); 36.1 (C-2); 31.1 (C-3 + C-6); FT-IR ($CH_3CN$): ν(C-H) -2938 cm$^{-1}$, ν($N_3$) -2105 cm$^{-1}$, ν(C-O) -1141 cm$^{-1}$, ν(C-O) -1092 cm$^{-1}$,

**AZ5** benzyl azide (470 mg, 94%) Elemental analysis $C_7H_7N_3$: calculated: C, 63.14; H, 5.30; N, 31.56; measured: C, 63.15; H, 5.28; N, 31.57; $^1$H NMR ($\delta$ ppm in DMSO-$d_6$): 7.58 (5H, m); 4.44 (2H, s); $^{13}$C NMR ($\delta$ ppm in DMSO-$d_6$): 135.6, 128.7, 128.4, 128.1, 53.6; FT-IR ($CH_3CN$): ν(C-H)$_{ar}$ -3003 cm$^{-1}$, ν(C-H) -2944 cm$^{-1}$, ν($N_3$) -2105 cm$^{-1}$.

# ASSOCIATED CONTENT

**Supporting Information**

All FT-IR and $^{1}H$, $^{13}C$ NMR spectra of novel triazole conjugates of spiramycin's aglycone **6-16** and azides, full antibacterial activity data (Table 1S), $^{1}H$-$^{1}H$ NOESY contacts detected for **8** (Fig. 50S), structures of all novel chemical entities **6-16** presented in ChemDraw 8.0 format (Fig. 51S).

## AUTHOR INFORMATION

*Corresponding Author: E-mail: *piotrp@amu.edu.pl,* Phone +48 61 8291693

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## ABBREVIATIONS USED

DMSO, Dimethyl sulfoxide; HDF, human dermal fibroplasts; MEM, Minimum essential medium; DMEM, Dulbecoos Modified Eagles medium; SRB, sulforhodamine B ; MRSA, methicillin-resistant *Staphylococcus aureus*.

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Table 1. Selected average MIC values of **1**, **2**, **6**-**8** compounds ($SD_{MIC}$ = 0) expressed in μg/mL and in [μM], evaluated in the 0.25-64 μg/mL range of concentrations, together with experimentally determined $solubility_{exp}$ [mg/mL] and lipophilicity ($clogP_{exp}$).

| Compound | **1** | **2** | **6** | **7** | **8** |
|---|---|---|---|---|---|
| *B. subtilis* | 0.5 | 8 | 64 | 64 | 4 |
| *ATCC6633* | [0.59] | [16.34] | [85.23] | [85.23] | [5.05] |
| *M. luteus* | 0.25 | 4 | 64 | 64 | 4 |
| *ATCC 10240* | [0.30] | [8.17] | [85.23] | [85.23] | [5.05] |
| *S. aureus* | 8 | 16 | >64 | >64 | 16 |
| *MRSAI* | [9.49] | [32.68] | [>85.23] | [>85.23] | [20.20] |
| *S. aureus* | 4 | 16 | >64 | >64 | 16 |
| *MRSAII* | [4.74] | [32.68] | [>85.23] | [>85.23] | [20.20] |
| *S. epidermidis* | 2 | 8 | >64 | 64 | 8 |
| *ATCC12228* | [2.37] | [16.34] | [>85.23] | [85.23] | [10.10] |
| *S. epidermidis* | 2 | 8 | >64 | 64 | 8 |
| *ATCC49134* | [2.37] | [16.34] | [>85.23] | [85.23] | [10.10] |
| *S. pneumoniae* | 0.25 | 2 | 8 | 8 | 1 |
| *ATCC49619* | [0.30] | [4.08] | [10.65] | [10.65] | [1.26] |
| $clogP_{exp}$ | 0.58 | 0.74 | -0.05 | -0.08 | 0.25 |
| $solubility_{exp}$ [mg/mL] | 4.5 | 0.7 | > 11 | > 11 | > 11 |

Table 2. $IC_{50}$ values of **1**, AZT, Cyt, FUra, FdU and selected new triazole conjugates, determined in various human cell cancer lines (HeLa, KB, MCF-7, HepG2, U87) and normal Human Dermal Fibroblasts cell line (HDF); given in μM concentration (± SD).

| Cmpd. | **HeLa** | **KB** | **MCF-7** | **HepG2** | **HDF** | **U87** |
|---|---|---|---|---|---|---|
| **1** | 34.41±1.17 | 31.26 ±0.07 | 33.11 ±0.39 | 30.51 ±0.15 | 31.10 ±0.57 | --- |
| **2** | 127.26 ±0.22 | 132.89 ±0.02 | 130.55 ±0.29 | 134.96 ±0.04 | 139.49 ±0.12 | --- |
| **8** | 149.27 ±0.22 | 150.37 ±0.54 | 136.44 ±0.14 | 146.86 ±0.03 | 159.24 ±0.91 | --- |
| **9** | 214.51 ±0.52 | 202.67 ±0.52 | 227.89 ±0.05 | 172.34 ±0.11 | 171.83 ±0.41 | --- |
| **10** | 12.16 ±0.78 | 15.04 ±0.42 | --- | --- | --- | 12.49±0.91 |
| **11** | 8.63 ±0.25 | 7.33 ±0.07 | 8.54 ±1.30 | 6.02 ±0.12 | 10.70 ±0.64 | --- |
| **12** | 20.75 ±0.02 | 19.51 ±0.27 | 20.08 ±0.06 | 19.04 ±1.36 | 23.89 ±0.12 | --- |
| **13** | 14.72 ±0.61 | 13.37 ±0.03 | 13.37 ±1.10 | 13.94 ±0.72 | 25.10 ±0.18 | --- |
| **15** | 28.06 ±0.04 | 28.56 ±1.30 | 25.81 ±2.93 | 25.15 ±0.65 | 38.58 ±0.09 | --- |
| **16** | 14.40 ±0.09 | 15.17 ±0.22 | --- | --- | --- | --- |
| **AZT** | 10.77 ±2.66 | 9.77 ±4.57 | 7.67±1.09 | --- | 14.41 ±2.58 | --- |
| **Cyt** | 3.54 ±0.16 | 4.07 ±0.08 | 3.82 ±0.25 | 2.86 ±0.09 | 4.99 ±0.84 | --- |
| **FUra** | 6.23 ±0.46 | 4.84 ±0.15 | 6.53 ±0.82 | 6.60 ±0.18 | 7.02 ±0.20 | --- |
| **FdU** | 6.50 ±0.24 | 8.69 ±1.18 | 12.19 ±1.34 | 8.91 ±0.34 | 13.05 ±0.74 | --- |

**Cyt** - cytarabine

**AZT** - 3'-azido-3'-deoxythymidine

**FUra –** 5-fluorouracil

**FdU –** 5-fluoro-2'-deoxyuridine

Table 3. Selectivity index (SI) of **1** and selected new triazole conjugates obtained, determined for various human cell cancer lines (HeLa, KB, MCF-7, HepG2) and normal Human Dermal Fibroblasts cell line (HDF).

| Compound | **HeLa** | **KB** | **MCF-7** | **HepG2** |
|---|---|---|---|---|
| **1** | 0.90 | 0.90 | 0.94 | 1.02 |
| **2** | 1.10 | 1.05 | 1.07 | 1.03 |
| **8** | 1.07 | 1.06 | 1.17 | 1.08 |
| **9** | 0.80 | 0.85 | 0.75 | 1.00 |
| **11** | 1.24 | 1.46 | 1.25 | 1.78 |
| **12** | 1.15 | 1.22 | 1.19 | 1.26 |
| **13** | 1.71 | 1.88 | 1.88 | 1.80 |
| **15** | 1.38 | 1.35 | 1.50 | 1.53 |
| **AZT** | 1.34 | 1.48 | 1.88 | --- |
| **Cyt** | 1.41 | 1.23 | 1.31 | 1.75 |
| **FUra** | 1.13 | 1.45 | 1.08 | 1.06 |
| **FdU** | 2.01 | 1.50 | 1.07 | 1.47 |

# *TOC*

## Synthesis, antibacterial and anticancer evaluation of novel spiramycin-like conjugates containing C(5) triazole arm

Katarzyna Klich, Krystian Pyta, Marcelina M. Kubicka, Piotr Ruszkowski, Lech Celewicz, Marzena Gajecka, Piotr Przybylski