

A Click Chemistry Approach to Pleuromutilin Conjugates with Nucleosides or Acyclic Nucleoside Derivatives and Their Binding to the Bacterial Ribosome

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Pleuromutilin and its derivatives are antibacterial drugs that inhibit protein synthesis in bacteria by binding to ribosomes. To promote rational design of pleuromutilin based drugs, 19 pleuromutilin conjugates with different nucleoside fragments as side chain extensions were synthesized by a click chemistry protocol. Binding was assessed by chemical footprinting of nucleotide U2506 in 23S rRNA, and all conjugates bind to varying degree reflecting their binding affinity to the peptidyl transferase center. The side chain extensions also show various protections at position U2585. Docking studies of the conjugates with the highest affinities support the conclusion that despite the various conjugations, the pleuromutilin skeleton binds in the same binding pocket. The conjugated triazole moiety is well accommodated, and the nucleobases are placed in different pockets in the 50S ribosomal subunit. The derivative showing the highest affinity and a significantly better binding than pleuromutilin itself contains an adenine-9-ylpropylene triazole conjugate to pleuromutilin C-22.

Introduction

The need for new or better antibiotics increases in parallel to the appearance and spread of bacterial resistance to many antibiotics. Despite synthesis and screening of numerous new compounds, very few new antibiotics have appeared on the market in recent years. Advances in knowledge of binding and mechanism of action of antibiotics in connection to modeling now allow new approaches in the field of drug development. Pleuromutilin¹ **1** (Figure 1) is a natural drug with modest antibacterial activity. Pleuromutilin and derivatives thereof bind to bacterial ribosomes in the peptidyl transferase center and inhibits peptide bond formation.² The semisynthetic pleuromutilin derivative retapamulin **2** has recently been developed as a drug for human use but only for topical treatment of skin infections.³ Other derivatives named tiamulin **3** and valnemulin **4** are used in veterinary medicine to treat diseases in pigs and poultry. Valnemulin **4** had also in extraordinary cases been used in humans⁴ but are probably too toxic for approved use. Resistance to pleuromutilin drugs have been reported⁵ but are so far not very widespread and apparently only slowly developing. All in all, pleuromutilin derivatives have a potential for human use with improvement in pharmacology, binding, and ability to resist development of resistance.

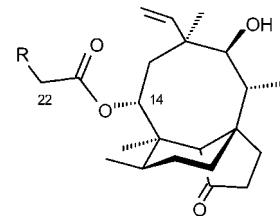
The peptidyl transferase center (PTC)^a where amino acids are joined to produce nascent peptides is composed mainly of the central loop of domain V of 23S rRNA and contains binding sites for various antibiotics of clinical and veterinary importance (Figure 2). Tiamulin **3** and valnemulin **4** have been footprinted

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^a Abbreviations: PTC, peptidyl transferase center; TIPDS, 1,1,3,3-tetraisopropyl-1,3-disiloxanediyl; TBDMS, *tert*-butyldimethylsilyl; TBDPS, *tert*-butyldiphenylsilyl; TBAF, tetrabutylammonium fluoride; NOE, nuclear Overhauser effect; CMCT, N1-cyclohexyl-N3-(2-morpholino)ethylcarbodiimide *p*-toluenesulfonate; AMV, avian myeloblastosis virus; VMD, visual molecular dynamics.



1 Pleuromutilin, R = HO

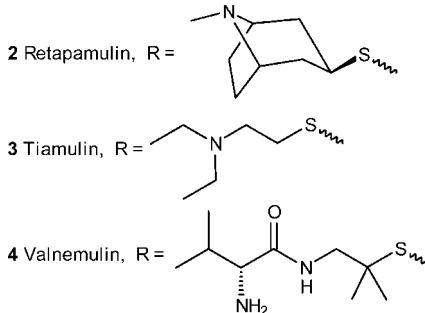


Figure 1. Chemical structure of pleuromutilin and derivatives thereof.

to the PTC.² Numerous data on X-ray structures of 50S ribosomal subunits have revealed the three-dimensional structure of the PTC with drugs bound,⁶ including pleuromutilin derivatives (Figure 2).^{7,8} The crystal structures have also revealed that while the binding site of the pleuromutilin core is constant, the RNA moieties interacting with the side chains move to accommodate the variable sizes and chemical groups of the drugs. This combination of a consistent core binding pocket and a permissible area for side chain binding makes the pleuromutilin family well suited for further modification. A large number of derivatives of pleuromutilin have been investigated, and the most numerous and successful group is semisynthetic analogues like **2–4** with different moieties on the C-14 ester

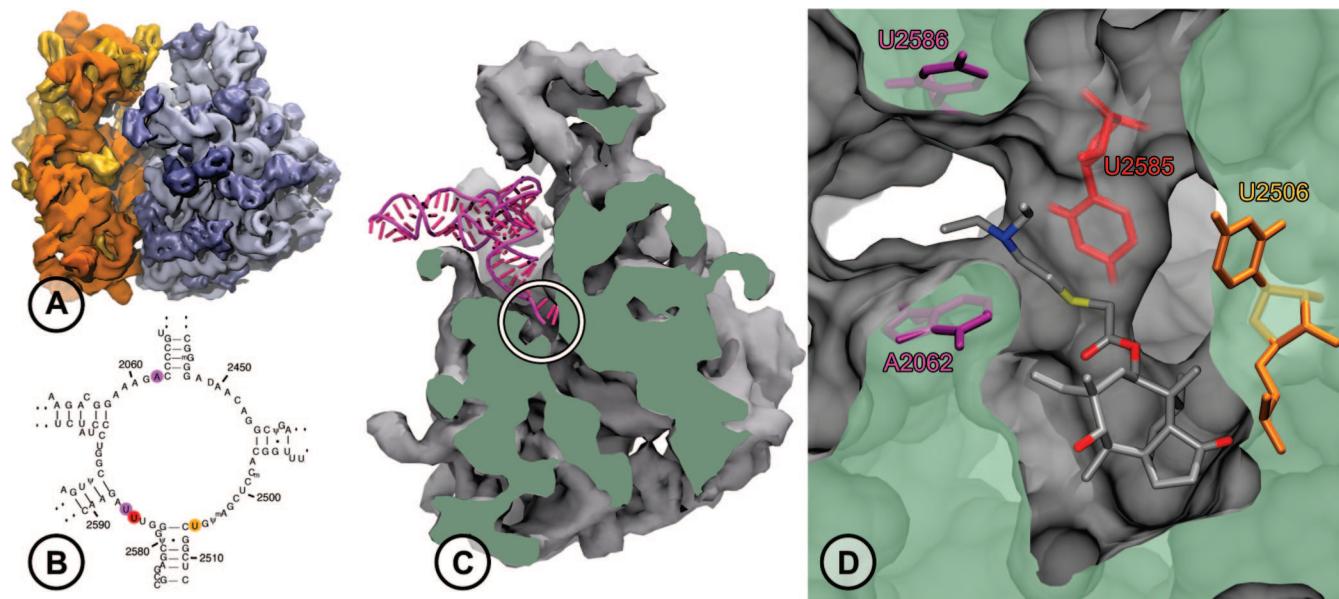


Figure 2. (A) Model of the 70S bacterial ribosome, based on the *E. coli* structure.¹¹ The 50S subunit is shown in blue and gray, and the 30S subunit is shown in yellow and orange. (B) Secondary structure of the central part of domain V of *E. coli* 23S rRNA from the 50S subunit. Footprinted nucleotides are indicated in red and orange, while potential stacking partners for the drugs are indicated in magenta. (C) The 50S subunit of *T. thermophilus*¹² cut in half to reveal the peptide exit tunnel and the pleuromutilin binding site indicated by a circle. tRNA in the P site is also shown. (D) Tiamulin from a *D. radiodurans* 50S–tiamulin complex⁷ aligned into the PTC of the 50S ribosomal subunit of *E. coli*. The drug is shown in stick representation and the surrounding rRNA as gray surface with the nearby 23S rRNA residues U2585 and U2506 indicated in red and orange, respectively, and U2586 and A2062 indicated in magenta.

of pleuromutilin.^{9,10} Most of the pleuromutilin derivatives have been made without any knowledge of their interactions in the ribosome. Isosteric replacements of the thioether of **2–4** have in general led to less active compounds, although 14-carbamates have shown good activity. The presence of amino groups in the side chain, like in **2–4**, in general improves the water solubility significantly.⁹

Since launched by Sharpless in 2001,¹³ the concept of click chemistry has grown in impact within drug discovery.¹⁴ In particular, the Huisgen [3 + 2] cycloaddition reacting terminal alkynes with azides forming 1,2,3-triazoles has been a convenient reaction. The reaction is chemoselective, it accepts almost all other functional groups, it can be performed in aqueous mixtures, and all atoms are used without the production of side products. By use of Cu(I) to catalyze the reaction, a complete regioselectivity for forming only 1,4- over 1,5-disubstituted triazoles has been achieved with a very efficient procedure.¹⁵

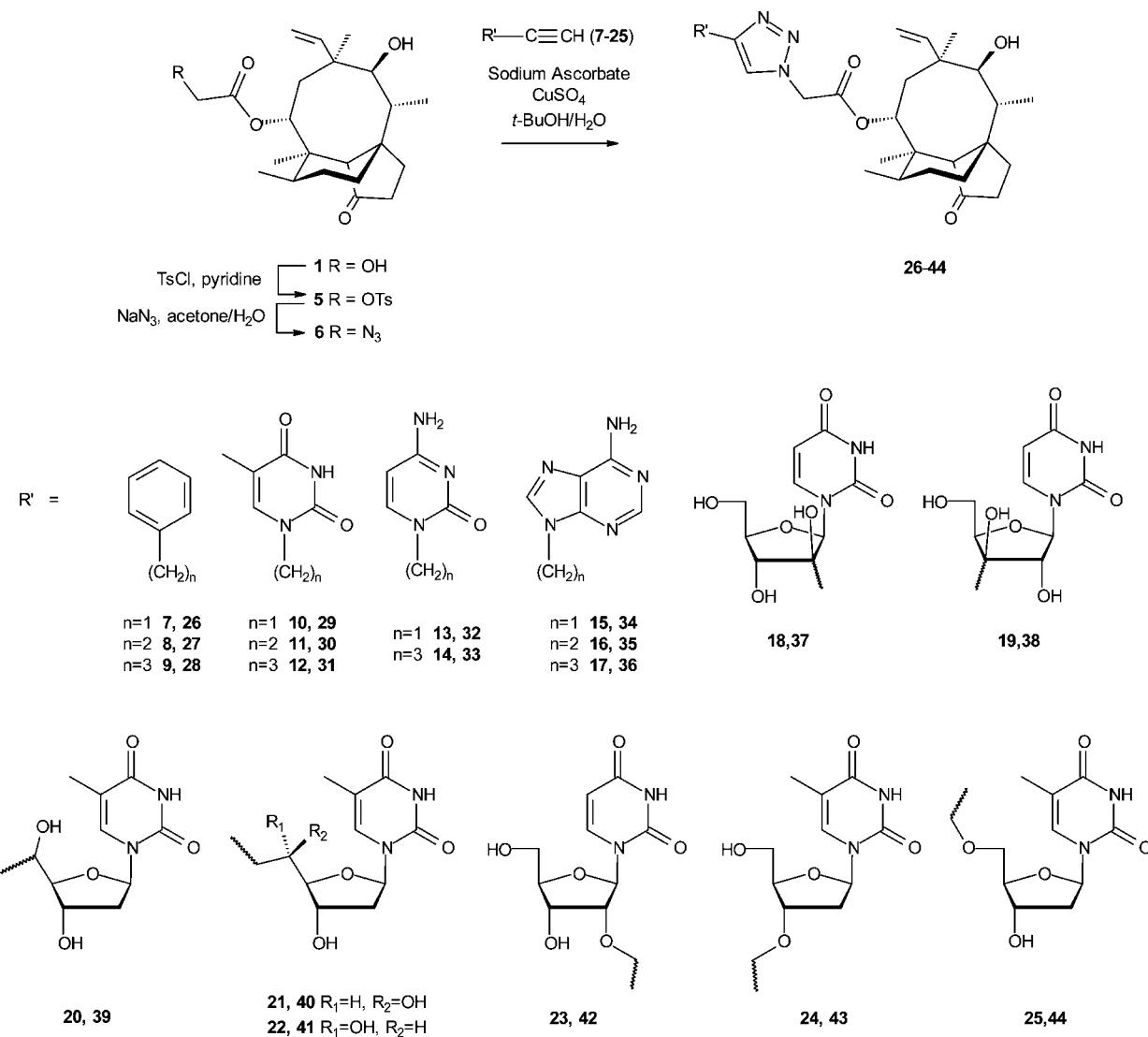
In the present project, we use the pleuromutilin skeleton and its strong and selective binding in the ribosome PTC as our starting point. By converting the primary alcohol of pleuromutilin into an azide, we attach a range of nucleoside derivatives to this position in a parallel synthetic strategy using click chemistry. Hereby we search the binding site of pleuromutilin for further interactions and the nucleobases can potentially bind strong and selectively herein because of their inherent H-bonding properties in combination to stacking properties. Like the amine moieties of **2–4**, the nucleobases are expected also to improve the water solubility of pleuromutilin. A variation of linker length and constitution are explored as well as the presence of the intact ribose or 2'-deoxyribose skeleton. Hence, a combination of structural data from the X-ray structures of pleuromutilin–50S complexes, molecular modeling, parallel synthesis of 19 pleuromutilin conjugates, and biochemical information from chemical footprinting is used to find high-affinity pleuromutilin derivatives and to improve our understanding of drug binding

paving the way for rational design of new successful pleuromutilin derivatives.

Results and Discussion

Design. The design in our approach hinges on two basic criteria: (1) the synthetic availability of the new variants and (2) the available space and chemical environment in the drug binding pocket. In order to obtain a convenient and simple parallel synthetic strategy for a range of pleuromutilin derivatives, we designed a click chemistry based approach for conjugating a range of functionalities at the easily accessible C-22 position of pleuromutilin (Figure 1). A linker containing a triazole as a substitute of the thioether in the established derivatives **2–4** was obtained in all cases. Simple inspection of 50S X-ray crystal structure data reveals that ample space exists for extension of the drug in this direction and for accommodating the triazole (Figure 2). The tricyclic pleuromutilin core is situated in a site pocket of the PTC, while the introduced triazole is expected to be positioned in the narrowest (but sufficiently broad) part of PTC leading into the main peptide exit tunnel and allowing room for the placement of bulky substituents. Because the ribosomal PTC and exit tunnel are rich in interaction possibilities, primarily in the form of RNA backbone, we synthesized the modified drugs based on the space available. A detailed modeling approach was only used on conjugates that showed a good affinity for the ribosome binding site based on the footprinting results. Therefore, the conjugates to be synthesized were chosen to represent a broad spectrum of various nucleobases as well as various linker lengths and constitutions. With respect to synthetic availability, simple acyclic nucleosides displayed the variation on nucleobases, whereas uridine and thymidine derivatives were chosen for the variation in attachment points of nucleoside ribose moieties. A systematic variation of 2',3',5'-O- and 2',3',5'-C-alkynylation of the nucleoside was employed.

Chemical Synthesis. A general click chemistry strategy based on the usual Huisgen-type [3 + 2] cycloaddition reaction

Scheme 1. General Parallel Strategy for the Synthesis of Pleuromutilin Derivatives by Click Chemistry^a

^a Yields: 5 (37%), 6 (92%), 26 (99%), 27 (93%), 28 (91%), 29 (55%), 30 (91%), 31 (38%), 32 (41%), 33 (68%), 34 (48%), 35 (64%), 36 (83%), 37 (81%), 38 (54%), 39 (63%), 40 (96%), 41 (73%), 42 (87%), 43 (78%), 44 (75%).

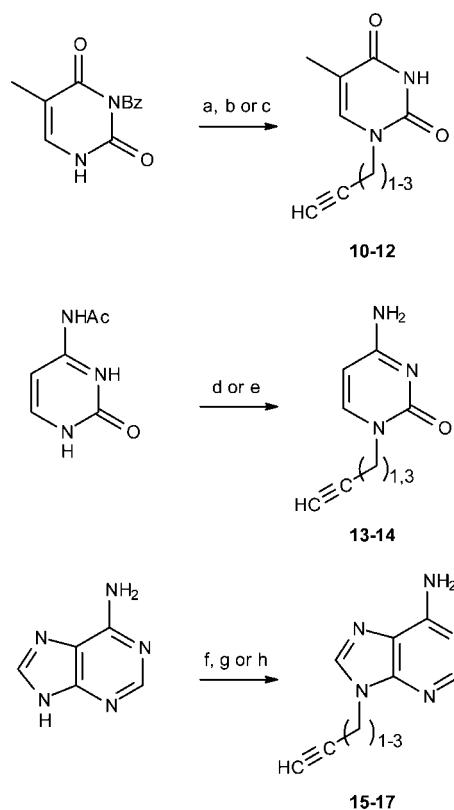
between an azide and a terminal alkyne was used (Scheme 1). Hence, pleuromutin **1** was converted into the known *p*-toluenesulfonyl ester **5**,¹⁶ which by a nucleophilic substitution was further converted into the azide **6**.¹⁶ This azide was reacted with 19 different terminal alkynes **7–25** that were all linked to nucleosides, nucleobases, or a simple aromatic replacement thereof, through a variation of linker lengths. For the cycloaddition, a standard protocol¹⁵ based on the catalysis by Cu(I), which is generated *in situ* by Cu(II)SO₄ and sodium ascorbate, was applied. Microwave heating was used in most cases. Hereby, the pleuromutilin derivatives **26–44** all containing a triazol-1,4-diyli linkage were prepared in mostly very good yields (Scheme 1).

The alkynyl building blocks were prepared in a few steps except for **7**, **8**, and **9**, which were commercially available. The known N1-propargylthymine **10**¹⁷ was made by alkylation of N3-benzoylthymine¹⁸ with propargyl bromide followed by hydrolysis (Scheme 2). The two other N1-alkylated thymine derivatives **11** and **12** were made by Mitsunobu reactions¹⁹ with 3-butyne-1-ol and 4-pentyne-1-ol, respectively, on N3-benzoylthymine followed by hydrolysis. In a similar way, the two N1-alkylated cytosine derivatives **13**¹⁷ and **14** were made from

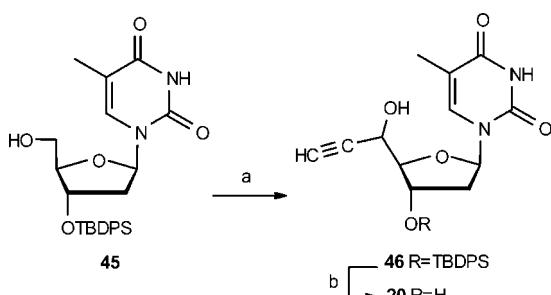
N4-acetylcytosine by using a basic alkylation²⁰ and a Mitsunobu reaction, respectively, followed by hydrolysis. The N9-alkylated adenine derivatives **15**,²¹ **16**, and **17** were made from unprotected adenine using again either basic alkylation with propargyl bromide or Mitsunobu protocols (Scheme 2).

The alkynyl nucleoside derivatives were made in a few steps from either thymidine or uridine. The 2'-C-ethynyl arabino-configured uridine derivative **18** was made from a known stereoselective organolithium reaction on 5',3'-TIPDS-protected and 2'-oxidized uridine,²² followed by global desilylation. The known 3'-C-ethynylxylo-configured uridine derivative **19** was made in a similar way.²³ The 5'-C-ethynylthymidine derivative **20** was made as an inseparable 2.6:1 epimeric mixture from 3'-TBDPS-protected thymidine **45**, which was oxidized with a Swern protocol and alkylated by a Grignard reaction²⁴ to give **46** (Scheme 3). Deprotection afforded **20** in a good overall yield.

The two epimeric 5'-C-propargylthymidine derivatives **21** and **22** were made from 3'-O-TBDMS-protected thymidine **47**, which was oxidized and alkylated using a Barbier-type reaction with zinc dust and propargyl bromide (Scheme 4). After chromatographic separation, **48** and **49** were obtained in good yields from a 2:1 epimeric mixture and deprotected separately

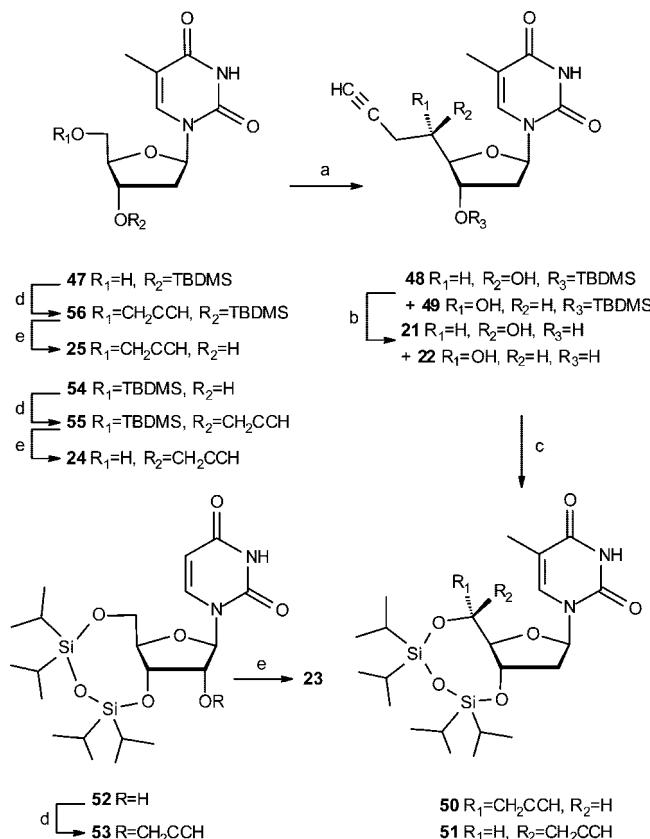
Scheme 2. Synthesis of Alkynylated Nucleobases^a

^a Reagents and conditions: (a) (i) 3-bromopropyne, K_2CO_3 , DMF (100%); (ii) aqueous NH_3 (94% **10**); (b) (i) 3-butyne-1-ol, PPH_3 , DEAD, toluene, DCM (74%); (ii) $NaOCH_3$, CH_3OH (74% **11**); (c) (i) 4-pentyne-1-ol, PPH_3 , DEAD, toluene, DCM (85%); (ii) $NaOCH_3$, CH_3OH (83% **12**); (d) (i) 3-bromopropyne, K_2CO_3 , DMF (40%); (ii) aqueous NH_3 , CH_3OH (97% **13**); (e) (i) 4-pentyne-1-ol, PPH_3 , DEAD, toluene, DCM (9%); (ii) aqueous NH_3 , CH_3OH (100% **14**); (f) 3-bromopropyne, K_2CO_3 , DMF (57% **15**); (g) 3-butyne-1-ol, PPH_3 , DEAD, toluene, DCM (8% **16**); (h) 4-pentyne-1-ol, PPH_3 , DEAD, toluene, DCM (31% **17**).

Scheme 3. Synthesis of 5'-C-Ethynylthymidine^a

^a Reagents and conditions: (a) (i) $CICOOCOCl$, $DMSO$, DCM, Et_3N ; (ii) $HC\equiv CMgBr$, THF (30%); (b) $TBAF$, THF (71%). $TBDPS = tert$ -butyldiphenylsilyl.

to give **21** and **22**. In order to establish the 5'-configuration in the two stereoisomers, each of these were converted to the TIPDS-protected compound, **50** and **51**, respectively, in order to use the decrease in conformational freedom of the bicyclic system in combination with NOE difference 1H NMR spectroscopy. This method has been used in other studies of 5'-C-alkylated thymidine derivatives.^{24,25} Thus, large mutual NOE contacts of 6–8% were detected between H3' and H5' in the NOE difference spectra for compound **50** proving the 5'(R)-configuration, whereas no mutual contacts between H3' and H5' were detected for **51**. On the other hand, strong mutual contacts of 8–9% between H4' and H5' were detected for **51**, whereas

Scheme 4. Synthesis of 5'-C-, 5'-O- and 3'-O-Propargylthymidine as well as 2'-O-Propargyluridine^a

^a Reagents and conditions: (a) (i) Dess–Martin periodinane, DCM; (ii) Zn , 3-bromopropyne, toluene, THF , NH_4Cl (aq) (50%); (b) $TBAF$, THF (92% **21**, 77% **22**); (c) $TIPDSCl_2$, pyridine (52% **50**, 43% **51**); (d) 3-bromopropyne, NaH , THF (40% **53**, 88% **55**, 43% **56**); (e) $TBAF$, THF (69% **23**, 32% **24**, 88% **25**). $TBDMS = tert$ -butyldimethylsilyl.

the corresponding contacts for **50** were only of approximately 1%. This established **50** and thereby **21** to have 5'(R)-configuration and **51** and **22** to have 5'(S)-configuration.

The known 2'-O-propargyluridine **23**²⁶ was made by alkylation of 5',3'-TIPDS-protected but N3-unprotected uridine **52** to give **53** followed by deprotection (Scheme 4). 3'-O-Propargylthymidine **24**²⁷ was made similarly from alkylation of 5'-O-TBDMS-thymidine **54** to give **55** followed by desilylation. Finally, the new 5'-O-propargylthymidine **25** was made with a similar strategy without protecting the nucleobase. Basic alkylation of **47** to give **56** was followed by desilylation to give **25** (Scheme 4).

Chemical Footprinting of Pleuromutilin Derivatives. The interaction of the 19 pleuromutilin derivatives **26–44** with the peptidyl transferase cavity was examined to compare ribosomal binding of the fungal natural product pleuromutilin **1** and the semisynthetic derivative tiamulin **3** with binding of the synthesized derivatives. The drug binding affinities on the ribosome were studied by chemical probing, where accessible rRNA nucleotides were modified by N1-cyclohexyl-N3-(2-morpholino)ethylcarbodiimide *p*-toluenesulfonate (CMCT). After formation of pleuromutilin derivative–70S ribosome complexes, the antibiotic–70S complexes and control samples of 70S ribosomes were treated with CMCT to probe the accessibility of N3 of uridines. Primer extension with reverse transcriptase was then used to identify alterations induced by drug binding. Autoradiograms showing the effect on nucleotides U2506 and U2585 in 23S rRNA are presented in Figure 3, and the quantification

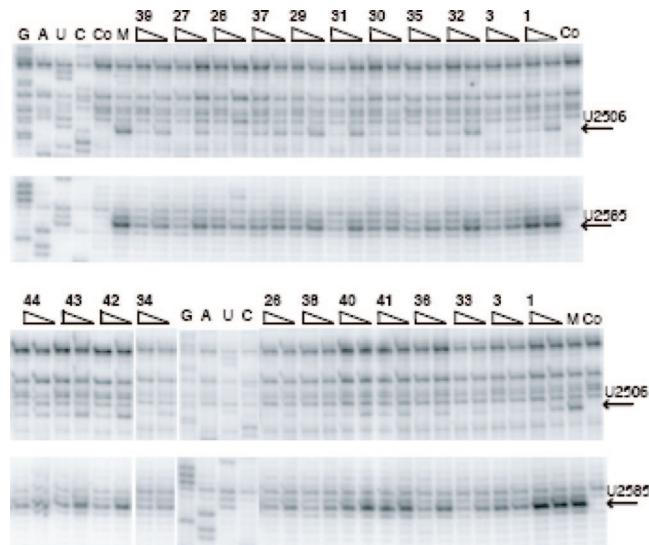


Figure 3. Gel autoradiograms showing footprints of the conjugates on ribosomes modified with CMCT. Nucleotides exhibiting altered reactivities in the presence of the pleuromutilin derivatives are indicated. Dideoxy sequencing lanes are denoted by G, A, U, and C. Lanes are labeled to denote samples from chemically unmodified 70S ribosomes in the absence of drugs (control, Co) and 70S ribosomes modified in the absence of drugs (CMCT, M). Ribosomes modified in the presence of the derivatives are marked with their numbers, and wedges denote a high (50 μ M) or low (5 μ M) drug concentration.

Table 1. Footprinting Data on Pleuromutilin and Derivatives Thereof

compd	length of linker ^a	nucleobase ^b	U2506 ^c		U2585 ^c	
			5 μ M	50 μ M	5 μ M	50 μ M
tiamulin 3						
36	3	A	0.10	0.05	0.59	0.58
28	3	Ph	0.17	0.06	0.33	0.22
38	3	U	0.21	0.08	0.48	0.41
33	3	C	0.24	0.03	0.45	0.18
27	2	Ph	0.27	0.05	0.56	0.26
26	1	Ph	0.29	0.05	0.61	0.35
40	5	T	0.31	0.06	0.54	0.29
41	5	T	0.32	0.09	0.59	0.29
35	2	A	0.33	0.09	0.69	0.35
39	4	T	0.37	0.10	0.40	0.26
31	3	T	0.38	0.10	0.50	0.25
43	5	T	0.41	0.08	0.46	0.24
pleuromutilin 1						
30	5	T	0.42	0.10	0.99	0.98
42	2	T	0.44	0.12	0.73	0.41
37	4	U	0.53	0.10	0.57	0.36
32	2	U	0.54	0.14	0.72	0.29
44	1	U	0.61	0.18	0.74	0.35
29	1	C	0.61	0.16	0.69	0.38
34	6	T	0.62	0.19	0.93	0.36
	1	T	0.69	0.18	0.75	0.37
	1	A	0.73	0.19	0.73	0.37

^a The length of the linker defined as the number of atoms separating the triazole from the nucleobase. ^b A = adenin-9-yl, C = cytosin-1-yl, U = uracil-1-yl, T = thymin-1-yl, Ph = phenyl. ^c Positions in *E. coli* 23S rRNA affected by CMCT modification: U2506 at the binding pocket of the mutilin core and U2585 near the variations in pleuromutilin conjugations (see text). The numbers represent the relative CMCT accessibility in the presence of the drug in 5 or 50 μ M concentrations compared to absence of the drug. For example, 0.10 means that the intensity of the band on the gel in presence of the drug is only 10% of the intensity of the corresponding band from samples without the drug.

of protection against CMCT modification is tabulated in Table 1 ordered by the binding affinity for U2506 as measured at a 5 μ M concentration. The footprinting data show that all derivatives protect U2506 and U2585 when bound to the 70S ribosome. Both footprinting data and X-ray data on binding of pleuromutilin derivatives to the PTC center^{2,7,8,28} indicate that the

conserved tricyclic cores of the compounds are placed identically in a binding pocket involving nucleotide U2506 as illustrated in Figure 2. The differences in the footprints of the drugs at U2506 thus reflect the binding affinity of the compounds. In contrast, the effects at U2585 are considered correlated with placement of the various conjugated substituents because previous data on pleuromutilin derivatives indicated both enhancement and protection effects depending on various side chain extensions.²⁸ The weakness of this particular chemical footprinting assay for assessing affinity of the drug derivatives for the ribosomal binding site is that the footprint at U2506 is relatively weak. Thus, we are quantifying and comparing delicate effects on a weak modification site implying some inaccuracy. The strength of this assay, on the other hand, is that the assayed protection of modification of specific nucleotides ensures that we are looking at specific binding.

The overall picture from the data in Table 1 is that all the tested compounds bind to the pocket in PTC (near U2506) as do tiamulin and pleuromutilin. The CMCT accessibility representing binding affinity varies from 0.17 to 0.73 at 5 μ M, but at 50 μ M all compounds bind strongly with accessibilities from 0.06 to 0.19. In other words, the affinity of the mutilin skeleton is strong and sufficient to allow the entire range of nucleoside derivatives attached to it, as well the triazole linkers, to follow it into the binding pocket. Concerning the interaction with U2585, all the compounds show some interaction though with larger variation. However, in contrast to pleuromutilin, all the other compounds protect well, at least at a 50 μ M concentration. Many of the new compounds demonstrate a stronger interaction with this position than does tiamulin, but tiamulin has the highest measured protection in the binding pocket for the mutilin core as seen at the U2506 footprint.

The best analogues in the series appear to have acyclic linkers of three carbon atoms between the triazole and the nucleobase. However, also a cyclic analogue with the same length, **38**, is among the best binding compounds. The adenine derivative **36** shows the highest affinity, but in general the constitution of the nucleobase does not seem very important, as also the phenyl derivative **28** is binding well. Another general trend is that the compounds with the shortest linkers of only one carbon atom between the nucleobase and the triazole are showing the lowest binding affinities. Then again, there are some deviations to this observation, as compound **26** binds better than expected, whereas the thymine derivative **31** binds with a lower affinity than to be expected. All the remaining compounds bind with intermediate affinities.

Docking Studies. The two pleuromutilin derivatives displaying the best binding affinities, compounds **36** and **28**, were chosen for a molecular docking study. Attempts to place the drugs in the binding pocket by the use of a standard docking protocol were unsuccessful. For agreement with the published drug binding modes, we had to employ a simulated flexible site (explained in details in the Experimental Section) consisting of all residues containing an atom within 25 \AA of 23S rRNA residue 2504. The calculations with the flexible docking protocol placed the drugs correctly into the binding pocket. The criterion for successful docking was set as placement of the pleuromutilin core in the consensus site to be in agreement with the footprinting data on the U2506 position. The final docking results showed similar binding modes for the two drugs, with the conjugated groups extending into the peptide exit tunnel, pointing toward the exit itself (Figure 4). No specific hydrogen bonding with ribosomal bases was detected for the adenine in **36**, and the terminal groups of both **28** and **36** seem to have a

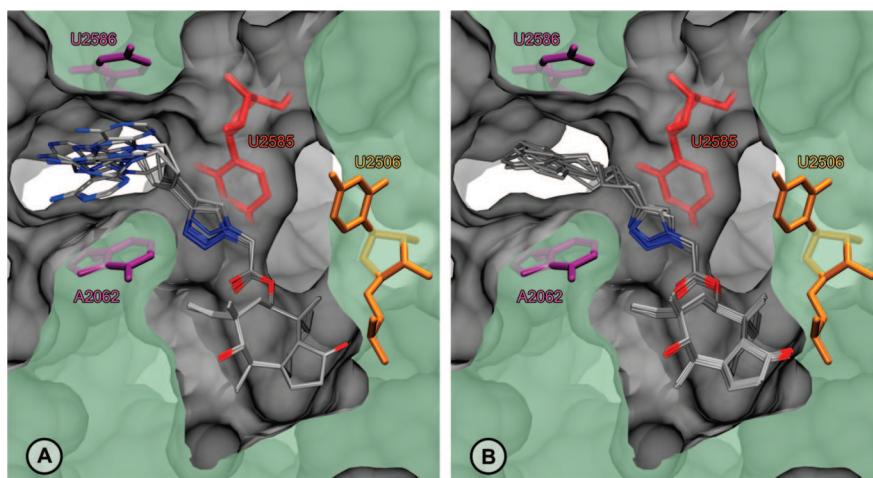


Figure 4. Docking studies of pleuromutilin derivative **36** (A) and **28** (B) in the PTC of the 50S ribosomal subunit. The heavy atoms of the 10 best scoring solutions of each drug are shown as overlaying stick representation. The cut surface is shown in green as in Figure 2. The surrounding rRNA is shown as gray surface with the nearby 23S rRNA residues U2585 and U2506 indicated in red and orange, respectively. Nucleotides U2586 and A2062, possibly implicated in stacking interactions, are shown in magenta.

large degree of freedom, interacting with the ribosome mainly through stacking with either nucleobase A2062 or U2586. A2062 is known to be highly flexible⁷ and might adopt a conformation more favorable for stacking with the conjugates than can be modeled by the flexibility in our docking assay. A stacking interaction between the triazole and U2585 is also indicated.

Discussion. The results of the present study follow the line from the literature demonstrating that the C-14 side chain of pleuromutilin can be voluminously derivatized and thereby improving the binding affinity of the pleuromutilin core for the PTC binding pocket. It is remarkable that all the 19 synthesized derivatives actually bind in the PTC. The present data also prove that nucleobases can be applied in the side chain of C-14 modified pleuromutilin derivatives. Thus, derivatives with simple linkers containing the 1,4-disubstituted triazole in connection to a three-carbon chain show stronger affinities for the PTC than the parent pleuromutilin. The almost similar affinity of the adenine and the phenyl derivatives (**36** and **28**, respectively) indicates that aromatic rings in general can be used to further improve the binding affinity. The study also demonstrates that the triazole is well accepted in the PTC and can replace the typically used thioether or carbamate linkers without compromising the binding affinity significantly. In fact, a stacking interaction with U2585 might favor the binding. Hence, small heterocycles can be adopted near the mutilin core in the binding pocket, and importantly, future drug optimization can be based on the triazole and the click chemistry approach. Conformational restriction of the three-carbon linker in **36** is an obvious target for improvement. Compound **38** can be considered a conformationally restricted derivative of **31**, and the better binding of **38** indicates that a similar or maybe different conformational restriction of the adenine derivative **36** might lead to a further increase in affinity. With a view to further steps toward drug development we also expect the conjugation of natural nucleobases to pleuromutilin to improve water solubility and eventually body distribution as well as being of low toxicity.

The employed click chemistry strategy has made a simple preparation of a series of compounds directly possible in medium to high yields and, importantly, without the need for protecting groups. The cycloaddition of terminal alkynes and an azide is completely chemoselective, thus allowing the presence of unprotected functional groups. Other methods with

the demand of protecting groups and one or more global deprotection steps would have rendered this study impossible. We expect to continue the design of easily available pleuromutilin derivatives with improved affinity in the PTC on the basis of the present click chemistry protocol.

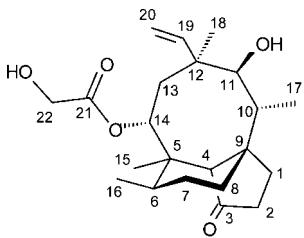
Conclusion

The pleuromutilin derivatives synthesized and examined in this work were all prepared in very few steps by a semisynthesis from pleuromutilin. They all bind to the peptidyl transferase center in the ribosome and most of them with a higher affinity than the natural pleuromutilin antibiotic. Chemical footprints and docking studies in combination rationalized the binding of the mutilin core near the U2506 in the PTC site and the nucleobase conjugated side chains near position U2585 in 23S rRNA. There is seemingly a potential for drug improvement by lengthening and derivitization of the pleuromutilin side chain that can be explored by molecular modeling of drug molecules in the ribosome based on X-ray crystallography models of ribosomes. A click chemistry approach demonstrated a convenient approach toward a library of active pleuromutilin derivatives in the search for future therapeutic antibiotics.

Experimental Section

General. Reactions were performed under an atmosphere of nitrogen when anhydrous solvents were used. Column chromatography was carried out on glass columns using silica gel 60 (0.040–0.063 mm). NMR spectra were recorded at 300 MHz for ¹H NMR and 75 MHz for ¹³C NMR. The δ values are in ppm relative to tetramethylsilane as internal standard. Assignments of NMR spectra are based on 2D spectra and follow the standard numbering of pleuromutilin as shown below as well as standard carbohydrate and nucleoside style; i.e., the carbon atom next to a nucleobase is assigned C-1', etc. HR ESI mass spectra were recorded in positive-ion mode.

General Procedure for Synthesizing the Pleuromutilin Conjugates from Pleuromutilin Azide (6**) and Nucleoside Alkyne Derivatives (**7–25**).** Compound **6** (0.20 mmol) and the alkyne derivative (0.22 mmol) were dissolved in a mixture of *t*-BuOH and water (2.5 mL, 1:1 v/v) in a microwave vial. Sodium ascorbate (4 mg, 0.02 mmol) and CuSO₄·5H₂O (3 mg, 0.01 mmol) were added, and the vial was sealed and heated with stirring in the microwave reactor (Emrys Creator) at 110 °C for 30 min. The residue was concentrated under reduced pressure and purified by



column chromatography (0–10% CH_3OH in CH_2Cl_2) to give the product as a white foam.

Synthesis of 22-(4-Benzyl-1,2,3-triazole-1-yl)-22-deoxyleuromutilin (26). The general procedure was slightly modified using compound **6** (71 mg, 0.176 mmol), 3-phenylpropyne **7** (0.022 mL, 0.157 mmol), $t\text{-BuOH}/\text{H}_2\text{O}$ (1.5 mL), sodium ascorbate (5 mg, 0.025 mmol), and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (2 mg, 0.008 mmol) and conventional heating at 70 °C for 12 h. The mixture was extracted with EtOAc (10 mL), washed with water (2 \times 10 mL), dried (MgSO_4), and concentrated under reduced pressure. The residue was purified by column chromatography (10–30% EtOAc in petrol ether). Yield, 81 mg, 99%; R_f = 0.36 (EtOAc and petrol ether, 2:1 v/v); ^1H NMR (CDCl_3) δ 7.33–7.20 (6H, m, $\text{CH}(\text{triazole})$, Ph), 6.39 (1H, dd, J = 10.8, 16.8, H19), 5.78 (1H, d, J = 8.2, H14), 5.32 (1H, d, J = 10.8, H20), 5.19 (1H, d, J = 16.8, H20), 5.06–4.93 (2H, m, PhCH_2), 4.11 (2H, s, H22), 3.34 (1H, m, H11), 2.40–2.00 (5H, m, H2, H4, H10, H13), 1.80–1.12 (9H, m, H1, H6, H7, H8, 11-OH, H13), 1.28 (3H, s, H15), 1.16 (3H, s, H18), 0.87 (3H, d, J = 6.9, H17), 0.64 (3H, d, J = 7.2, H16); ^{13}C NMR (CDCl_3) δ 216.6 (C3), 165.1 (C21), 148.2 (C(triazole)), 138.7 (C19), 138.5, 128.7, 128.6, 126.5 (Ph), 122.5 (triazol), 117.3 (C20), 74.5 (C11), 70.8 (C14), 58.0 (C4), 51.4 (C22), 45.4 (C9), 44.7 (C13), 44.0 (C12), 41.8 (C5), 36.6 (C6), 36.0 (C10), 34.4 (C2), 32.2 (PhCH_2), 30.3 (C8), 26.8 (C7), 26.3 (C18), 24.8 (C1), 16.6 (C16), 14.6 (C15), 11.5 (C17); HRMS (ESI) m/z (542.3014 [M + Na]⁺, $\text{C}_{31}\text{H}_{41}\text{N}_3\text{O}_4\text{Na}$ calcd 542.3014).

Synthesis of 22-(4-(2-Phenylethyl)-1,2,3-triazole-1-yl)-22-deoxyleuromutilin (27). The general procedure using compound **6** (77 mg, 0.19 mmol), 4-phenyl-1-butyne **8** (29 mg, 0.22 mmol), $t\text{-BuOH}/\text{H}_2\text{O}$ (2.5 mL), sodium ascorbate (4 mg, 0.02 mmol), and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (3 mg, 0.01 mmol) was applied. Yield, 95 mg, 93%; R_f = 0.58 (10% CH_3OH in CH_2Cl_2); ^1H NMR (CDCl_3) δ 7.31–7.19 (6H, m, $\text{CH}(\text{triazole})$, Ph), 6.41 (1H, dd, J = 11.0, 17.4, H19), 5.79 (1H, d, J = 8.4, H14), 5.33 (1H, dd, J = 1.3, 11.0, H20), 5.20 (1H, dd, J = 1.3, 17.3, H20), 5.06–4.94 (2H, m, H22), 3.35 (1H, dd, J = 6.4, 10.4, H11), 3.10–2.97 (4H, m, PhCH_2CH_2), 2.30–2.03 (5H, m, H2, H4, H10, H13), 1.78–1.07 (9H, m, H1, H6, H7, H8, 11-OH, H13), 1.33 (3H, s, H15), 1.17 (3H, s, H18), 0.87 (3H, d, J = 6.9, H17), 0.68 (3H, d, J = 6.9, H16); ^{13}C NMR (CDCl_3) δ 216.6 (C3), 165.2 (C21), 147.8 (C(triazole)), 141.0 (Ph), 138.6 (C19), 128.4, 126.1 (Ph), 122.1 (CH(triazole)), 117.6 (C20), 74.5 (C11), 70.7 (C14), 58.0 (C4), 51.4 (C22), 45.4 (C9), 44.6 (C13), 43.9 (C12), 41.8 (C5), 36.5 (C6), 36.0 (C10), 35.4 (PhCH_2), 34.3 (C2), 30.3 (C8), 27.4 (PhCH_2CH_2), 26.7 (C7), 26.3 (C18), 24.8 (C1), 16.7 (C16), 14.6 (C15), 11.4 (C17); HRMS (ESI) m/z (556.3168 [M + Na]⁺, $\text{C}_{32}\text{H}_{43}\text{N}_3\text{O}_4\text{Na}$ calcd 556.3146).

Synthesis of 22-(4-(3-Phenylpropyl)-1,2,3-triazole-1-yl)-22-deoxyleuromutilin (28). The general procedure using compound **6** (71 mg, 0.18 mmol), 5-phenyl-1-pentyne **9** (29 mg, 0.20 mmol), $t\text{-BuOH}/\text{H}_2\text{O}$ (2.5 mL), sodium ascorbate (4 mg, 0.02 mmol) and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (2 mg, 0.01 mmol) was applied. Yield, 88 mg, 91%; R_f = 0.29 (5% CH_3OH in CH_2Cl_2); ^1H NMR (CDCl_3) δ 7.35 (1H, s, $\text{CH}(\text{triazole})$), 7.31–7.18 (5H, m, Ph), 6.41 (1H, dd, J = 11.0, 17.3, H19), 5.81 (1H, d, J = 8.5, H14), 5.33 (1H, d, J = 11.0, H20), 5.20 (1H, d, J = 17.3, H20), 5.10–4.96 (2H, m, H22), 3.35 (1H, m, H11), 2.77 (2H, t, J = 7.7, PhCH_2), 2.70 (2H, t, J = 7.6, NCH_2), 2.31–1.98 (7H, m, H2, H4, H10, H13, PhCH_2CH_2), 1.79–1.08 (9H, m, H1, H6, H7, H8, 11-OH, H13), 1.34 (3H, s, H18), 1.17 (3H, s, H15), 0.88 (3H, d, J = 6.7, H17), 0.70 (3H, d, J = 6.7, H16); ^{13}C NMR (CDCl_3) δ 216.6 (C3), 165.2 (C21), 148.4 (C(triazole)), 141.8 (Ph), 138.6 (C19), 128.4, 128.3, 125.8 (Ph),

121.9 ($\text{CH}(\text{triazole})$), 117.5 (C20), 74.4 (C11), 70.7 (C14), 57.9 (C4), 51.4 (C22), 45.3 (C9), 44.6 (C13), 43.9 (C12), 41.8 (C5), 36.5 (C6), 36.0 (C10), 35.3 (PhCH_2), 34.3 (C2), 30.9 ($\text{Ph}(\text{CH}_2)\text{CH}_2$), 30.3 (C8), 26.7 (C7), 26.3 (C18), 25.0 (PhCH_2CH_2), 24.7 (C1), 16.7 (C16), 14.6 (C15), 11.4 (C17); HRMS (ESI) m/z (548.3485 [M + H]⁺, $\text{C}_{33}\text{H}_{46}\text{N}_3\text{O}_4$ calcd 548.3483).

Synthesis of 22-(4-(Thymine-1-ylmethyl)-1,2,3-triazole-1-yl)-22-deoxyleuromutilin (29). The general procedure using compound **6** (149 mg, 0.37 mmol), compound **10** (60 mg crude, 0.22 mmol), $t\text{-BuOH}/\text{H}_2\text{O}$ (4 mL), sodium ascorbate (6 mg, 0.03 mmol), and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (4 mg, 0.02 mmol) was applied. Yield, 68 mg, 55%; R_f = 0.48 (15% CH_3OH in CH_2Cl_2); ^1H NMR (CDCl_3) δ 9.36 (1H, m, NH), 7.85 (1H, s, $\text{CH}(\text{triazole})$), 7.32 (1H, d, J = 1.4, H6(T)), 6.38 (1H, dd, J = 11.1, 17.3, H19), 5.77 (1H, d, J = 8.4, H14), 5.31 (1H, d, J = 11.1, H20), 5.20 (1H, d, J = 17.3, H20), 5.12–5.00 (2H, m, H22), 4.97 (2H, AB, J = 15.0, NCH_2), 3.35 (1H, dd, J = 6.5, 10.4, H11), 2.30–2.02 (5H, m, H2, H4, H10, H13), 1.90 (3H, d, J = 1.4, $\text{CH}_3(\text{T})$), 1.85–1.05 (9H, m, H1, H6, H7, H8, 11-OH, H13), 1.27 (3H, s, H15), 1.16 (3H, s, H18), 0.86 (3H, d, J = 7.0, H17), 0.65 (3H, d, J = 6.9, H16); ^{13}C NMR (CDCl_3) δ 216.5 (C3), 164.8, 164.0 (C21, C4(T)), 151.0 (C2(T)), 142.4 (C(triazole)), 140.0 (C6(T)), 138.5 (C19), 125.2 ($\text{CH}(\text{triazole})$), 117.6 (C20), 111.4 (C5(T)), 74.4 (C11), 71.1 (C14), 57.9 (C4), 51.5 (C22), 45.3 (C9), 44.6 (C13), 44.0 (C12), 43.0 (NCH_2), 41.8 (C5), 36.5 (C6), 36.0 (C10), 34.3 (C2), 30.2 (C8), 26.7 (C7), 26.3 (C18), 24.7 (C1), 16.8 (C16), 14.5 (C15), 12.3 ($\text{CH}_3(\text{T})$), 11.4 (C17); HRMS (ESI) m/z (590.2928 [M + Na]⁺, $\text{C}_{30}\text{H}_{41}\text{N}_5\text{O}_6\text{Na}$ calcd 590.2950).

Synthesis of 22-(4-(2-(Thymine-1-yl)ethyl)-1,2,3-triazole-1-yl)-22-deoxyleuromutilin (30). The general procedure using compound **6** (94 mg, 0.23 mmol), compound **11** (33 mg, 0.18 mmol), $t\text{-BuOH}/\text{H}_2\text{O}$ (3 mL), sodium ascorbate (4 mg, 0.02 mmol), and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (3 mg, 0.01 mmol) was applied. Yield, 97 mg, 91%; R_f = 0.68 (20% CH_3OH in CH_2Cl_2); ^1H NMR (CDCl_3) δ 9.39 (1H, s, NH), 7.46 (1H, s, $\text{CH}(\text{triazole})$), 6.95 (1H, d, J = 1.1, H6(T)), 6.36 (1H, dd, J = 10.7, 17.3, H19), 5.77 (1H, d, J = 8.5, H14), 5.30 (1H, dd, J = 1.1, 10.7, H20), 5.18 (1H, dd, J = 1.1, 17.3, H20), 5.09–4.97 (2H, m, H22), 4.07 (2H, t, J = 6.8, NCH_2), 3.34 (1H, m, H11), 3.13 (2H, t, J = 6.8, NCH_2CH_2), 2.29–2.03 (5H, m, H2, H4, H10, H13), 1.82 (3H, d, J = 1.1, $\text{CH}_3(\text{T})$), 1.77–1.05 (9H, m, H1, H6, H7, H8, 11-OH, H13), 1.32 (3H, s, H15), 1.15 (3H, s, H18), 0.86 (3H, d, J = 7.0, H17), 0.68 (3H, d, J = 7.0, H16); ^{13}C NMR (CDCl_3) δ 216.6 (C3), 165.0, 164.4 (C21, C4(T)), 151.0 (C2(T)), 143.8 (C(triazole)), 141.0 (C6(T)), 138.5 (C19), 123.5 ($\text{CH}(\text{triazole})$), 117.5 (C20), 110.3 (C5(T)), 74.4 (C11), 70.9 (C14), 57.9 (C4), 51.4 (C22), 48.0 (NCH_2), 45.3 (C9), 44.6 (C13), 43.9 (C12), 41.7 (C5), 36.4 (C6), 35.9 (C10), 34.3 (C2), 30.2 (C8), 26.7 (C7), 26.3 (C18), 24.9 (NCH_2CH_2), 24.7 (C1), 16.7 (C16), 14.5 (C15), 12.1 ($\text{CH}_3(\text{T})$), 11.4 (C17); HRMS (ESI) m/z (604.3078 [M + Na]⁺, $\text{C}_{31}\text{H}_{43}\text{N}_5\text{O}_6\text{Na}$ calcd 604.3107).

Synthesis of 22-(4-(3-(Thymine-1-yl)propyl)-1,2,3-triazole-1-yl)-22-deoxyleuromutilin (31). The general procedure using compound **6** (129 mg, 0.32 mmol), compound **12** (containing 12% w/w Et_3N , 50 mg, 0.23 mmol), $t\text{-BuOH}/\text{H}_2\text{O}$ (3 mL), sodium ascorbate (5.5 mg, 0.03 mmol), and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (4 mg, 0.02 mmol) was applied. Yield, 52 mg, 38%; R_f = 0.47 (15% CH_3OH in CH_2Cl_2); ^1H NMR (CDCl_3) δ 9.49 (1H, s, NH), 7.58 (1H, s, $\text{CH}(\text{triazole})$), 7.10 (1H, d, J = 1.0, H6(T)), 6.39 (1H, dd, J = 10.6, J = 17.2, H19), 5.78 (1H, d, J = 8.4, H14), 5.30 (1H, dd, J = 1.2, 10.6, H20), 5.08 (1H, dd, J = 1.2, 17.2, H20), 5.14–5.02 (2H, m, H22), 3.77 (2H, t, J = 7.0, NCH_2), 3.34 (1H, dd, J = 6.5, 10.2, H11), 2.78 (2H, t, J = 7.0, NCH_2CH_2), 2.29–1.05 (14H, m, H1, H2, H4, H6, H7, H8, H10, 11-OH, H13), 1.90 (3H, d, J = 1.0, $\text{CH}_3(\text{T})$), 1.31 (3H, s, H15), 1.15 (3H, s, H18), 0.86 (3H, d, J = 7.0, H17), 0.68 (3H, d, J = 6.9, H16); HRMS (ESI) m/z (618.3257 [M + Na]⁺, $\text{C}_{32}\text{H}_{45}\text{N}_5\text{O}_6\text{Na}$ calcd 618.3262).

Synthesis of 22-(4-(Cytosine-1-ylmethyl)-1,2,3-triazole-1-yl)-22-deoxyleuromutilin (32). The general procedure was slightly modified using compound **6** (65 mg, 0.16 mmol), compound **13** (20 mg, 0.14 mmol), $t\text{-BuOH}/\text{H}_2\text{O}$ (4 mL), sodium ascorbate (5.5 mg, 0.03 mmol), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (4.5 mg, 0.02 mmol), and conventional heating at 70 °C for 12 h. Yield, 31 mg, 41%; R_f = 0.45

(20% CH₃OH in CH₂Cl₂); ¹H NMR (CDCl₃) δ 7.94 (1H, s, CH(triazole)), 7.49 (1H, d, J = 6.8, H6(C)), 6.34 (1H, dd, J = 11.3, 17.6, H19), 5.73–5.67 (2H, m, H14, H5(C)), 5.25 (1H, d, J = 11.3, H20), 5.18–4.94 (5H, m, H20, H22, NCH₂), 3.34 (1H, m, H11), 2.65 (2H, br s, NH₂), 2.23–1.87 (5H, m, H2, H4, H10, H13), 1.73–1.07 (9H, m, H1, H6, H7, H8, 11-OH, H13), 1.19 (3H, s, H15), 1.11 (3H, s, H18), 0.84 (3H, d, J = 6.7, H17), 0.60 (3H, d, J = 6.7, H16); ¹³C NMR (CDCl₃) δ 217.0 (C3), 166.2, 165.0 (C2(C), C21), 156.5 (C4(C)), 145.4 (C(triazole)), 143.2 (C6(C)), 138.7 (C19), 125.5 (CH(triazole)), 117.3 (C20), 95.0 (C5(C)), 74.3 (C11), 71.0 (C14), 57.9 (C4), 51.6 (C22), 45.3 (C9), 44.5 (C13), 43.9 (C12), 41.7 (C5), 36.5 (C6), 35.9 (C10), 34.4 (C2), 30.2 (C8), 26.7 (C7), 26.5 (C18), 24.7 (C1), 16.68, 16.61 (NCH₂, C16), 14.4 (C15), 11.4 (C17); HRMS (ESI) m/z (575.2962 [M + Na]⁺, C₂₉H₄₀N₆O₅Na calcd 575.2953).

Synthesis of 22-(4-(3-Cytosine-1-yl)propyl)-1,2,3-triazole-1-yl)-22-deoxypleuromutilin (33). The general procedure using compound **6** (46 mg, 0.11 mmol), compound **14** (15 mg, 0.09 mmol), *t*-BuOH/H₂O (2 mL), sodium ascorbate (2 mg, 0.01 mmol), and CuSO₄·5H₂O (1.2 mg, 0.01 mmol) was applied. Yield, 33 mg, 68%; R_f = 0.66 (20% CH₃OH in CH₂Cl₂); ¹H NMR (CDCl₃) δ 7.51 (1H, s, CH(triazole)), 7.35 (1H, m, H6(C)), 6.37 (1H, dd, J = 11.0, 15.9, H19), 5.76 (1H, d, J = 8.4, H14), 5.28–4.96 (5H, m, H5(C), H20, H22), 3.85–3.76 (2H, m, NCH₂), 3.36 (1H, m, H11), 2.78–2.70 (2H, m, N(CH₂)₂CH₂), 2.52–1.97 (9H, m, H2, H4, H10, H13, NCH₂CH₂, NH₂), 1.86–1.09 (9H, m, H1, H6, H7, H8, 11-OH, H13), 1.29 (3H, s, H15), 1.15 (3H, s, H18), 0.85 (3H, d, J = 6.1, H17), 0.67 (3H, d, J = 6.1, H16); ¹³C NMR (CDCl₃) δ 216.8 (C3), 166.0 (C2(C)), 165.2 (C21), 156.8 (C4(C)), 147.1 (C6(C)), 145.9 (C(triazole)), 138.7 (C19), 122.7 (CH(triazole)), 117.4 (C20), 94.4 (C5(C)), 74.4 (C11), 70.8 (C14), 57.9 (C4), 51.5 (C22), 49.1 (NCH₂), 45.3 (C9), 44.6 (C13), 44.0 (C12), 41.8 (C5), 36.5 (C6), 36.0 (C10), 34.4 (C2), 30.3 (C8), 28.4 (N(CH₂)₂CH₂), 26.7 (C7), 26.5 (C18), 24.7 (C1), 22.2 (NCH₂CH₂), 16.7 (C16), 14.6 (C15), 11.4 (C17); HRMS (ESI) m/z (603.3242 [M + Na]⁺, C₃₁H₄₄N₆O₅Na calcd 603.3266).

Synthesis of 22-(4-(Adenine-9-ylmethyl)-1,2,3-triazole-1-yl)-22-deoxypleuromutilin (34). The general procedure was slightly modified using compound **6** (28 mg, 0.07 mmol), compound **15** (11 mg, 0.06 mmol), *t*-BuOH/H₂O (8 mL), sodium ascorbate (1.5 mg, 0.01 mmol), CuSO₄·5H₂O (1.4 mg, 0.01 mmol), stirring at room temperature for 19 h and then at 85 °C for 19 h. Yield, 17 mg, 48%; R_f = 0.64 (20% CH₃OH in CH₂Cl₂); ¹H NMR (CDCl₃) δ 8.38 (1H, s, H2(A)), 7.99 (1H, s, H8(A)), 7.77 (1H, s, CH(triazole)), 7.26 (2H, br s, NH₂), 6.38 (1H, dd, J = 11.1, 17.3, H19), 5.76 (1H, d, J = 8.6, H14), 5.51 (2H, s, NCH₂), 5.32 (1H, dd, J = 1.5, 11.1, H20), 5.20 (1H, dd, J = 1.5, 17.3, H20), 5.10–4.96 (2H, m, H22), 3.33 (1H, dd, J = 6.8, 10.9, H11), 2.27–1.99 (5H, m, H2, H4, H10, H13), 1.77–1.07 (9H, m, H1, H6, H7, H8, 11-OH, H13), 1.56 (3H, s, H15), 1.15 (3H, s, H18), 0.86 (3H, d, J = 7.0, H17), 0.61 (3H, d, J = 6.8, H16); ¹³C NMR (CDCl₃) δ 217.1 (C3), 165.6 (C21), 156.0 (C6(A)), 152.6 (C2(A)), 149.3 (C4(A)), 142.7 (C(triazole)), 140.7, 140.6 (C8(A), C19), 125.1 (CH(triazole)), 120.8 (C5(A)), 115.5 (C20), 72.6 (C11), 70.5 (C14), 57.1 (C4), 51.1 (C22), 44.9 (C9), 44.2 (C13), 43.3 (C12), 41.5 (C5), 38.0 (NCH₂), 36.5 (C6), 36.2 (C10), 34.0 (C2), 30.1 (C8), 28.6 (C7), 26.6 (C18), 24.5 (C1), 16.1 (C16), 14.2 (C15), 11.6 (C17); HRMS (ESI) m/z (599.3075 [M + Na]⁺, C₃₀H₄₀N₈O₄Na calcd 599.3064).

Synthesis of 22-(4-(2-(Adenine-9-yl)ethyl)-1,2,3-triazole-1-yl)-22-deoxypleuromutilin (35). The general procedure using compound **6** (17 mg, 0.09 mmol), compound **16** (53 mg, 0.13 mmol), *t*-BuOH/H₂O (3 mL), sodium ascorbate (2.2 mg, 0.01 mmol), and CuSO₄·5H₂O (1.5 mg, 0.01 mmol) was applied. Yield, 34 mg, 64%; R_f = 0.73 (20% CH₃OH in CH₂Cl₂); ¹H NMR (CDCl₃) δ 8.35 (1H, s, H2(A)), 7.68 (1H, s, H8(A)), 7.32 (1H, s, CH(triazole)), 6.38 (1H, dd, J = 11.1, 17.4, H19), 6.12 (2H, br s, NH₂), 5.78 (1H, d, J = 8.4, H14), 5.32 (1H, d, J = 11.1, H20), 5.19 (1H, d, J = 17.4, H20), 5.09–4.93 (2H, m, H22), 4.62 (2H, t, J = 6.8, NCH₂), 3.48–3.33 (3H, m, NCH₂CH₂, H11), 2.30–2.20 (3H, m, H2, H10), 2.08–2.06 (2H, m, H4, H13), 1.78–1.05 (9H, m, H1, H6, H7, H8,

H13), 1.30 (3H, s, H15), 1.17 (3H, s, H18), 0.88 (3H, d, J = 7.1, H17), 0.66 (3H, d, J = 7.0, H16); ¹³C NMR (CDCl₃) δ 216.6 (C3), 164.9 (C21), 155.6 (C6(A)), 152.9 (C2(A)), 149.8 (C4(A)), 143.8 (C(triazole)), 140.8 (C8(A)), 138.6 (C19), 123.2 (CH(triazole)), 119.5 (C5(A)), 117.4 (C20), 74.4 (C11), 71.0 (C14), 57.9 (C4), 51.4 (C22), 45.3 (C9), 44.6 (C13), 43.9 (C12), 43.1 (NCH₂), 41.7 (C5), 36.4 (C6), 35.9 (C10), 34.3 (C2), 30.2 (C8), 26.7 (C7), 26.4 (C18), 26.0 (NCH₂CH₂), 24.7 (C1), 16.7 (C16), 14.5 (C15), 11.4 (C17), 36.2 (C10), 34.0 (C2), 30.1 (C8), 28.6 (C7), 26.6 (C18), 24.5 (C1), 16.1 (C16), 14.2 (C15), 11.6 (C17); HRMS (ESI) m/z (613.3210 [M + Na]⁺, C₃₁H₄₂N₈O₄Na calcd 613.3221).

Synthesis of 22-(4-(3-Adenine-9-yl)propyl)-1,2,3-triazole-1-yl)-22-deoxypleuromutilin (36). The general procedure using compound **6** (43 mg, 0.11 mmol), compound **17** (15 mg, 0.08 mmol), *t*-BuOH/H₂O (2 mL), sodium ascorbate (2.4 mg, 0.01 mmol), and CuSO₄·5H₂O (1.8 mg, 0.01 mmol) was applied. Yield, 38 mg, 83%; R_f = 0.30 (10% CH₃OH in CH₂Cl₂); ¹H NMR (CDCl₃) δ 8.32 (1H, s, H2(A)), 7.87 (1H, s, H8(A)), 7.45 (1H, s, CH(triazole)), 6.37 (1H, dd, J = 10.7, 17.4, H19), 6.07 (2H, m, NH₂), 5.78 (1H, d, J = 8.5, H14), 5.29 (1H, d, J = 10.7, H20), 5.18 (1H, d, J = 17.4, H20), 5.12–4.96 (2H, m, H22), 4.28 (2H, t, J = 6.9, NCH₂), 3.35 (1H, m, H11), 2.74 (2H, t, J = 7.2, N(CH₂)₂CH₂), 2.34–2.02 (7H, m, H2, H4, H10, H13, NCH₂CH₂), 1.76–1.04 (9H, m, H1, H6, H7, H8, 11-OH, H13), 1.31 (3H, s, H15), 1.15 (3H, s, H18), 0.86 (3H, d, J = 7.0, H17), 0.68 (3H, d, J = 7.0, H16); ¹³C NMR (CDCl₃) δ 216.6 (C3), 165.1 (C21), 155.5 (C6(A)), 152.9 (C2(A)), 150.0 (C4(A)), 146.6 (C(triazole)), 140.7 (C8(A)), 138.6 (C19), 122.5 (CH(triazole)), 119.5 (C5(A)), 117.4 (C20), 74.4 (C11), 70.9 (C14), 57.9 (C4), 51.4 (C22), 45.3 (C9), 44.7 (C13), 43.9 (C12), 42.9 (NCH₂), 41.8 (C5), 36.5 (C6), 36.0 (C10), 34.3 (C2), 30.2 (C8), 29.3 (N(CH₂)₂CH₂), 26.7 (C7), 26.5 (C18), 24.7 (C1), 22.2 (NCH₂CH₂), 16.7 (C16), 14.6 (C15), 11.4 (C17); HRMS (ESI) m/z (627.3266 [M + Na]⁺, C₃₂H₄₄N₈O₄Na calcd 627.3378).

Synthesis of 22-(4-((2(R)-(Uracil-1-yl)-3(S),4(R)-dihydroxy-5(R)-hydroxymethyl)tetrahydrofuran-3-yl)-1,2,3-triazole-1-yl)-22-deoxypleuromutilin (37). The general procedure using compound **6** (62 mg, 0.15 mmol), compound **18** (33 mg, 0.12 mmol), *t*-BuOH/H₂O (2 mL), sodium ascorbate (3.1 mg, 0.02 mmol), and CuSO₄·5H₂O (3.0 mg, 0.01 mmol) was applied. Yield, 66 mg, 81%; R_f = 0.49 (15% CH₃OH in CH₂Cl₂); ¹H NMR (CH₃OH-d₄) δ 8.04 (1H, d, J = 8.3, H6(U)), 8.02 (1H, s, CH(triazole)), 6.61 (1H, s, H1'), 6.27 (1H, dd, J = 10.9, 18.0, H19), 5.77 (1H, d, J = 8.4, H14), 5.67 (1H, d, J = 8.3, H5(U)), 5.27–5.13 (4H, m, H20, H22), 4.24 (1H, d, J = 3.9, H3'), 4.07 (1H, m, H4'), 3.97–3.83 (2H, m, H5'), 3.48 (1H, d, J = 6.1, H11), 2.36–2.10 (5H, m, H2, H4, H10, H13), 1.82–1.19 (8H, m, H1, H6, H7, H8, H13), 1.36 (3H, s, H15), 1.15 (3H, s, H18), 0.92 (3H, d, J = 7.0, H17), 0.73 (3H, d, J = 6.6, H16); ¹³C NMR (CH₃OH-d₄) δ 219.5 (C3), 167.2 (C21), 166.1 (C4(U)), 152.4 (C2(U)), 147.2 (C(triazole)), 144.7 (C6(U)), 141.0 (C19), 126.8 (CH(triazole)), 116.8 (C20), 101.5 (C5(U)), 88.3 (C1'), 86.0 (C4'), 80.2 (C2'), 79.0 (C3'), 75.4 (C11), 72.6 (C14), 62.4 (C5'), 59.2 (C4), 52.6 (C22), 46.8 (C9), 45.7 (C13), 45.4 (C12), 43.2 (C5), 38.0 (C6), 37.8 (C10), 35.3 (C2), 31.4 (C8), 28.3 (C7), 28.0 (C18), 25.8 (C1), 17.0 (C16), 15.2 (C15), 11.8 (C17); 36.2 (C10), 34.0 (C2), 30.1 (C8), 28.6 (C7), 26.6 (C18), 24.5 (C1), 16.1 (C16), 14.2 (C15), 11.6 (C17); HRMS (ESI) m/z (694.3029 [M + Na]⁺, C₃₃H₄₅N₅O₁₀Na calcd 694.3059).

Synthesis of 22-(4-((2(R)-(Uracil-1-yl)-3(R),4(S)-dihydroxy-5(R)-hydroxymethyl)tetrahydrofuran-4-yl)-1,2,3-triazole-1-yl)-22-deoxypleuromutilin (38). The general procedure using compound **6** (102 mg, 0.25 mmol), compound **19** (53 mg, 0.20 mmol), *t*-BuOH/H₂O (2 mL), sodium ascorbate (4 mg, 0.02 mmol), and CuSO₄·5H₂O (3 mg, 0.01 mmol) was applied. Yield, 72 mg, 54%; R_f = 0.45 (15% CH₃OH in CH₂Cl₂); ¹H NMR (CH₃OH-d₄) δ 8.17 (1H, m, H6(U)), 8.03 (1H, s, CH(triazole)), 6.31 (1H, dd, J = 11.1, 17.4, H19), 5.91 (1H, m, H1'), 5.77 (1H, d, J = 8.1, H14), 5.73 (1H, m, H5(U)), 5.31–5.17 (4H, m, H20, H22), 4.66 (1H, m, H4'), 4.21 (1H, m, H2'), 4.06–3.96 (2H, m, H5'), 3.52 (1H, d, J = 5.1, H11), 2.40–2.14 (5H, m, H2, H4, H10, H13), 1.86–1.16 (8H, m, H1, H6, H7, H8, H13), 1.38 (3H, s, H15), 1.19 (3H, s, H18), 0.95 (3H, d, J = 6.7, H17), 0.77 (3H, d, J = 6.1, H16); ¹³C NMR (CH₃OH-

d) δ 219.4 (C3), 167.1 (C21), 159.1 (C4(U)), 152.3 (C2(U)), 147.8 (C(triazole)), 143.4 (C6(U)), 141.0 (C19), 126.2 (CH(triazole)), 116.7 (C20), 101.4 (C5(U)), 93.9 (C1'), 87.0 (C4'), 83.7 (C2'), 79.6 (C3'), 75.3 (C11), 72.6 (C14), 61.4 (C5'), 59.1 (C4), 52.6 (C22), 46.8 (C9), 45.7 (C13), 45.4 (C12), 43.2 (C5), 38.0 (C6), 37.7 (C10), 35.3 (C2), 31.4 (C8), 28.3 (C7), 28.0 (C18), 25.8 (C1), 17.1 (C16), 15.2 (C15), 11.8 (C17); HRMS (ESI) *m/z* (694.3069 [M + Na]⁺, C₃₃H₄₅N₅O₁₀Na calcd 694.3059).

Synthesis of 22-(4-(Thymidine-5'(R/S)-C-yl)-1,2,3-triazole-1-yl)-22-deoxypleuromutilin (39). The general procedure was slightly modified using compound **6** (19 mg, 0.05 mmol), compound **20** (37 mg, 0.14 mmol), CH₃OH/H₂O (2 mL, 1:1 v/v), sodium ascorbate (2 mg, 0.01 mmol), CuSO₄·5H₂O (1.5 mg, 0.01 mmol), and only 10 min of heating. Yield, 20 mg, 63% (epimeric mixture); *R*_f = 0.46 (15% CH₃OH in CH₂Cl₂); ¹H NMR (CH₃OH-*d*₄) δ 7.95 (1H, br s, H6(T)), 7.87 (1H, br s, CH(triazole)), 6.38–6.18 (2H, m, H19, H1'), 5.73–5.67 (1H, m, H14), 5.22–5.07 (4H, m, H20, H22), 4.56–4.52 (2H, m, H3', H5'), 4.17–4.13 (1H, m, H4'), 3.45–3.39 (1H, m, H11), 2.28–2.09 (7H, m, H2, H2', H4, H10, H13), 1.84 (3H, s, CH₃(T)), 1.78–1.04 (8H, m, H1, H6, H7, H8, H13), 1.23 (3H, s, H15), 1.10 (3H, s, H18), 0.87 (3H, d, *J* = 6.8, H17), 0.64 (3H, d, *J* = 6.5, H16); HRMS (ESI) *m/z* (692.3238 [M + Na]⁺, C₃₄H₄₇N₅O₉Na calcd 692.3266).

Synthesis of 22-(4-(Thymidine-5'(R)-C-ylmethyl)-1,2,3-triazole-1-yl)-22-deoxypleuromutilin (40). The general procedure using compound **6** (75 mg, 0.19 mmol), compound **21** (37 mg, 0.13 mmol), *t*-BuOH/H₂O (3 mL), sodium ascorbate (4 mg, 0.02 mmol), and CuSO₄·5H₂O (3 mg, 0.01 mmol) was applied. Yield, 88 mg, 96%; *R*_f = 0.47 (15% CH₃OH in CH₂Cl₂); ¹H NMR (CDCl₃) δ 8.91 (1H, s, NH), 7.56 (1H, s, H6(T)), 7.33 (1H, s, CH(triazole)), 6.37 (1H, dd, *J* = 11.3, 17.5, H19), 6.16 (1H, t, *J* = 6.9, H1'), 5.79 (1H, d, *J* = 8.7, H14), 5.31 (1H, d, *J* = 11.3, H20), 5.19 (1H, d, *J* = 17.5, H20), 5.13–5.01 (2H, m, H22), 4.61 (1H, m, H3'), 4.27 (1H, m, H5'), 4.13 (1H, m, 5'-OH), 3.83 (1H, m, H4'), 3.36 (1H, m, H11), 3.11–2.92 (2H, m, H6'), 2.35–2.04 (7H, m, H2, H2', H4, H10, H13), 1.90 (3H, s, CH₃(T)), 1.78–1.12 (9H, m, H1, H6, H7, H8, 11-OH, H13), 1.33 (3H, s, H15), 1.16 (3H, s, H18), 0.87 (3H, d, *J* = 6.9, H17), 0.69 (3H, d, *J* = 6.8, H16); HRMS (ESI) *m/z* (706.3391 [M + Na]⁺, C₃₅H₄₉N₅O₉Na calcd 706.3423).

Synthesis of 22-(4-(Thymidine-5'(S)-C-ylmethyl)-1,2,3-triazole-1-yl)-22-deoxypleuromutilin (41). The general procedure using compound **6** (50 mg, 0.12 mmol), compound **22** (29 mg, 0.11 mmol), *t*-BuOH/H₂O (1.5 mL), sodium ascorbate (4 mg, 0.02 mmol), and CuSO₄·5H₂O (3 mg, 0.01 mmol) was applied. Yield, 53 mg, 73%; *R*_f = 0.48 (15% CH₃OH in CH₂Cl₂); ¹H NMR (CDCl₃) δ 8.01 (1H, s, NH), 7.62 (1H, s, H6(T)), 7.52 (1H, s, CH(triazole)), 6.40 (1H, dd, *J* = 11.0, 17.3, H19), 6.29 (1H, t, *J* = 7.7, H1'), 5.81 (1H, d, *J* = 8.4, H14), 5.34 (1H, d, *J* = 11.0, H20), 5.21 (1H, d, *J* = 17.3, H20), 5.12–5.00 (2H, m, H22), 4.60 (1H, m, H3'), 4.19 (1H, m, H5'), 3.92 (1H, m, H4'), 3.77 (1H, m, 5'-OH), 3.35 (1H, dd, *J* = 5.8, *J* = 9.9, H11), 3.16–2.95 (2H, m, H6'), 2.53–2.05 (7H, m, H2, H2', H4, H10, H13), 1.93 (3H, s, CH₃(T)), 1.79–1.10 (9H, m, H1, H6, H7, H8, 11-OH, H13), 1.35 (3H, s, H15), 1.18 (3H, s, H18), 0.88 (3H, d, *J* = 6.7, H17), 0.70 (3H, d, *J* = 6.8, H16); ¹³C NMR (CH₃OH-*d*₄) δ 219.4 (C3), 167.0 (C21), 166.4 (C4(T)), 152.4 (C2(T)), 145.0 (C(triazole)), 141.0, 138.6 (C19, C6(T)), 126.0 (CH(triazole)), 116.7 (C20), 111.6 (C5(T)), 89.5 (C1'), 86.2 (C4'), 75.4 (C11), 73.2 (C3'), 72.5 (C14), 71.5 (C5'), 59.1 (C4), 52.6 (C22), 46.7 (C9), 45.7 (C13), 45.4 (C12), 43.2 (C5), 41.1 (C2'), 38.0 (C6), 37.7 (C10), 35.3 (C2), 31.4 (C8, C6'), 28.3 (C7), 28.0 (C18), 25.8 (C1), 17.0 (C16), 15.2 (C15), 12.6 (CH₃(T)), 11.8 (C17); HRMS (ESI) *m/z* (706.3418 [M + Na]⁺, C₃₅H₄₉N₅O₉Na calcd 706.3423).

Synthesis of 22-(4-(Uridine-2'-O-methyl)-1,2,3-triazole-1-yl)-22-deoxypleuromutilin (42). The general procedure was slightly modified using compound **6** (28 mg, 0.071 mmol), compound **23** (20 mg, 0.071 mmol), *t*-BuOH/H₂O (2 mL), sodium ascorbate (1.4 mg, 0.007 mmol), CuSO₄·5H₂O (0.9 mg, 0.004 mmol), and stirring at room temperature for 4 days and at 75 °C for 24 h. The mixture was extracted with EtOAc (10 mL), and the organic phase was washed with water (2 × 10 mL), dried (MgSO₄), and concentrated

under reduced pressure. The crude product was purified by column chromatography (60–0% petrol ether and then 0–5% CH₃OH in EtOAc). Yield, 26 mg, 87%; *R*_f = 0.04 (EtOAc and petrol ether, 2:1 v/v); ¹H NMR (DMSO-*d*₆) δ 11.32 (1H, br s, NH), 8.03 (1H, s, CH(triazole)), 7.88 (1H, d, *J* = 8.1, H6(U)), 6.12 (1H, dd, *J* = 11.2, 17.7, H19), 5.89 (1H, d, *J* = 4.8, H14), 5.63–5.56 (2H, m, H20), 5.38–5.03 (4H, m, H5(U), H1', 2 × OH), 4.77–4.64 (2H, m, H22), 4.54 (1H, m, H4'), 4.10–3.83 (2H, m, H2', H3'), 3.88 (1H, m, H11), 3.65–3.40 (2H, m, H5'), 2.40–2.00 (5H, m, H2, H4, H10, H13), 1.80–1.09 (9H, m, H1, H6, H7, H8, 11-OH, H13), 1.26 (3H, s, H15), 1.06 (3H, s, H18), 0.81 (3H, d, *J* = 6.6, H17), 0.63 (3H, d, *J* = 6.6, H16); ¹³C NMR (DMSO-*d*₆) δ 218.3 (C3), 167.0 (C21), 164.3 (C4(U)), 151.8 (C2(U)), 145.1 (C(triazole)), 142.0, 141.6 (C19, C6(U)), 126.7 (CH(triazole)), 116.7 (C20), 103.0 (C5(U)), 87.5 (C1'), 86.2 (C4'), 81.9 (C2'), 73.8 (C3'), 71.8 (C11), 69.5 (C14), 64.0 (C22), 61.6 (C4') 58.0 (C5'), 52.2 (OCH₂), 46.2 (C9), 45.4 (C13), 44.5 (C12), 42.8 (C5), 37.7 (C6), 37.5 (C10), 35.2 (C2), 31.3 (C8), 29.8 (C7), 27.8 (C18), 25.7 (C1), 17.4 (C16), 15.5 (C15), 12.8 (C17); HRMS (ESI) *m/z* (708.3184 [M + Na]⁺, C₃₄H₄₇N₅O₁₀Na calcd 708.3215).

Synthesis of 22-(4-(Thymidine-3'-O-methyl)-1,2,3-triazole-1-yl)-22-deoxypleuromutilin (43). The general procedure was slightly modified using compound **24** (94 mg, 0.23 mmol), compound **6** (67 mg, 0.24 mmol), *t*-BuOH/H₂O (1.4 mL), sodium ascorbate (5 mg, 0.025 mmol), CuSO₄·5H₂O (4 mg, 0.016 mmol), and stirring at 75 °C for 24 h. The mixture was extracted with EtOAc (10 mL), and the organic phase was washed with water (2 × 10 mL), dried (MgSO₄), and concentrated under reduced pressure. The crude product was purified by column chromatography (50–0% petrol ether and then 0–5% CH₃OH in EtOAc). Yield, 111 mg, 78%; *R*_f = 0.22 (EtOAc); ¹H NMR (CH₃OH-*d*₄) δ 8.04 (1H, s, H6(T)), 7.81 (1H, s, CH(triazole)), 6.32–6.28 (2H, m, H1', H19), 5.78 (1H, d, *J* = 7.8, H14), 5.29–5.15 (4H, m, H20, OCH₂), 4.71 (2H, s, H22), 4.32 (1H, m, H3'), 4.14 (1H, m, H4'), 3.84–3.71 (2H, m, H5'), 3.49 (1H, d, *J* = 5.7, H11), 2.40–2.10 (7H, m, H2, H2', H4, H10, H13), 1.90 (3H, s, CH₃(T)), 1.84–1.07 (8H, m, H1, H6, H7, H8, H13), 1.33 (3H, s, H15), 1.17 (1H, s, H18), 0.94 (3H, d, *J* = 6.9, H17), 0.73 (3H, d, *J* = 6.6, H16); ¹³C NMR (CH₃OH-*d*₄) δ 219.2 (C3), 166.8 (C21), 165.1 (C2(T)), 152.3 (C4(T)), 146.0 (C(triazole)), 140.9 (C19), 138.1 (C6(T)), 126.7 (CH(triazole)), 116.7 (C20), 111.7 (C5(T)), 86.6, 86.4 (C1', C4'), 80.6 (C3'), 75.4 (C11), 72.5 (C14), 63.2, 63.1 (C5', OCH₂), 59.1 (C4), 52.6 (C22), 46.7 (C9), 45.7 (C13), 45.4 (C12), 43.1 (C2'), 38.4, 38.0, 37.7 (C5, C6, C10), 35.2 (C2), 31.4 (C8), 28.3 (C7), 28.0 (C18), 25.8 (C1), 17.0 (C16), 15.2 (C15), 12.5 (CH₃(T)), 11.8 (C17); HRMS (ESI) *m/z* (706.3445 [M + Na]⁺, C₃₅H₄₉N₅O₉Na calcd 706.3423).

Synthesis of 22-(4-(Thymidine-5'-O-methyl)-1,2,3-triazole-1-yl)-22-deoxypleuromutilin (44). The general procedure was slightly modified using compound **25** (61 mg, 0.23 mmol), compound **6** (92 mg, 0.23 mmol), *t*-BuOH/H₂O (1.4 mL), sodium ascorbate (4.5 mg, 0.023 mmol), CuSO₄·5H₂O (2.8 mg, 0.011 mmol), and stirring at 75 °C for 40 h. The mixture was extracted with EtOAc (10 mL), and the organic phase was washed with water (2 × 10 mL), dried (MgSO₄), and concentrated under reduced pressure. The crude product was purified by column chromatography (40–0% petrol ether and then 0–5% CH₃OH in EtOAc). Yield, 115 mg, 75%; *R*_f = 0.05 (EtOAc); ¹H NMR (CH₃OH-*d*₄) δ 7.92 (1H, s, H6(T)), 7.57 (1H, s, CH(triazole)), 6.24–6.15 (2H, m, H1', H19), 5.64 (1H, d, *J* = 7.8, H14), 5.19–5.03 (4H, m, H20, OCH₂), 4.65 (2H, s, H22), 4.31 (1H, m, H3'), 3.91 (1H, m, H4'), 3.71–3.64 (2H, m, H5'), 3.41–3.31 (1H, m, H11), 2.24–1.96 (7H, m, H2, H2', H4, H10, H13), 1.68 (3H, s, CH₃(T)), 1.79–1.05 (8H, m, H1, H6, H7, H8, H13), 1.19 (3H, s, H18), 1.35, (1H, s, H15), 0.81 (3H, d, *J* = 7, H17), 0.61 (3H, d, *J* = 6, H16); ¹³C NMR (CDCl₃) δ 219.3 (C3), 166.8 (C21), 165.1 (C2(T)), 152 (C4(T)), 145.7 (C(triazole)), 140.9 (C19), 137.8 (C6(T)), 126.6 (CH(triazole)), 116.7 (C20), 111.7 (C5(T)), 87.4, 86.3 (C1', C4'), 75.3, 72.9, 72.5, 71.4 (C3', C5', C11, C14), 65.1 (OCH₂), 59.1 (C4), 52.6 (C22), 46.7 (C9), 45.7 (C13), 45.4 (C12), 43.2 (C5), 41.3 (C2'), 37.9 (C6), 37.7 (C10), 35.2 (C2), 31.4 (C8), 28.2 (C7), 28.0 (C18), 25.9 (C1), 17.1 (C16),

15.2 (C15), 12.6 (CH₃(T)), 11.8 (C17); HRMS (ESI) *m/z* (706.3445 [M + Na]⁺, C₃₅H₄₉N₅O₉Na calcd 706.3423).

Footprinting Experiments. *E. coli* strain MRE600 was used to prepare ribosomes for the chemical footprinting experiments. The cells were grown in LB to an optical density of *A*₄₅₀ = 0.4 and harvested by cooling on ice followed by centrifugation. Cells were washed with TMN (20 mM Tris-HCl (pH 7.8), 10 mM MgCl₂, 100 mM NH₄Cl), centrifuged, and resuspended in 1 mL of TMN. Cells were opened by sconification for 4 × 15 s. The lysate was recovered by centrifugation and layered on a 38 mL 10–40% sucrose gradient prepared in TMN buffer and centrifuged in ultracentrifuge SE90, AH 629 rotor, 19 000 rpm, 18 h. The gradient was analyzed on a Amersham Fraction Collector system. The fractions containing 70S ribosomes were collected, and the ribosomes were recovered by dialysis against TMN and pelleted in ultracentrifuge SE90, Ti50 rotor, 40.000 rpm, 22 h. Ribosomes were resuspended in 200 μL of TMN and stored at –80 °C.

The antibiotic binding and chemical modification were performed as follows: Ribosomes (5 pmol) were incubated with either 5 or 50 μM drug or drug derivative in modification buffer (50 mM Tris-HCl (pH 8.0), 10 mM MgCl₂, 100 mM KCl, and 5 mM dithiotretiol) for 30 min at 37 °C. The modification procedure is described by Ehresmann et al.²⁹ Aliquots (50 μL) of the antibiotic–ribosome complexes were modified with 50 μL of CMCT (42 mg/mL in modification buffer) for 20 min at 37 °C. The CMCT reactions were stopped by precipitating the ribosomes. After centrifugation the ribosomes were resuspended in 0.25 M sodium acetate, pH 6.5, and extracted with phenol and chloroform. The rRNA was precipitated and resuspended in water.

The chemical modifications were monitored by primer extension analysis on 23S rRNA using reverse transcriptase (AMV, Life Sciences) and 5'-[³²P]-labeled deoxynucleotide primers.³⁰ The DNA oligonucleotide 5'-TCCGGTCCTCTCGTACT-3', complementary to nucleotides 2654–2670 of 23S rRNA, was used as primer in the extension reactions. The cDNA products of the primer extension reactions were separated on 6% polyacrylamide sequencing gels. The positions of the stops in cDNA synthesis were identified by reference to dideoxy sequencing reactions on 23S rRNA that were run in parallel. The bands were visualized, and the intensities of the modifications were assessed using a Typhoon trio laser scanner. Experiments were performed at least two times, and the numbers in Table 1 are an average of protections against CMCT modification calculated from samples in the presence of drug relative to control samples without drug and with subtraction of the background value from unmodified samples.

Docking Experiments. Visualization of the original X-ray crystal structure and the docking results was performed in VMD.³¹ The docking experiment was performed using the Shrödinger 2007 package. A flexible drug binding site containing RNA was simulated by creating a truncated version of PTC, consisting of 25 Å of the *E. coli* ribosome¹¹ surrounding the tiamulin binding site. The drugs were then docked using a multistep approach: The first round of docking was performed with all atoms of the drugs scaled to 80% of their WDV radii. The resulting complex with the highest binding score for each drug had the ribosomal residues with atoms within 3 Å of the drug minimized with a 1 kcal/(mol·Å²) restraint, while the drug was unrestrained. Each drug was then redocked into this accommodated site, using the QM polarized ligand docking workflow, assigning the charges in context of the receptor at the B3LYP/3-21G level of theory and a 0.9 scaling of atoms with a partial charge less than ±0.25, using XP docking precision. Minimizations were done using the OPLS2005 forcefield in the MacroModel module, using implicit water, vdW cutoff of 7 Å, electrostatic cutoff of 12 Å, and H-bond cutoff of 4 Å. Initial docking was performed using the Glide module, while the final docking was done using the quantum polarized workflow. The docking procedure was validated by docking tiamulin into the site (Figure 2) showing an identical conformation to the *D. radiodurans*⁷ X-ray crystal structure of tiamulin in PTC (data not shown),

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Supporting Information Available: Experimental procedures that are not covered in the main text, i.e., for the compounds **5**, **6**, **10–18**, **20–25**, **46**, **48–51**, **53**, **55**, and **56**; ¹H NMR spectra, ¹³C NMR spectra, and HPLC profiles for the pleuromutilin conjugates. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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