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A Click Chemistry Approach to Pleuromutilin Derivatives, Part 2: Conjugates with Acyclic Nucleosides and Their Ribosomal Binding and Antibacterial Activity

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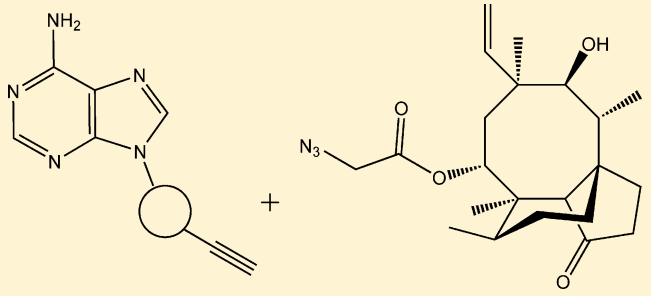
Supporting Information

ABSTRACT: Pleuromutilin is an antibiotic that binds to bacterial ribosomes and thereby inhibit protein synthesis. A new series of semisynthetic pleuromutilin derivatives were synthesized by a click chemistry strategy. Pleuromutilin was conjugated by different linkers to a nucleobase, nucleoside, or phenyl group, as a side-chain extension at the C22 position of pleuromutilin. The linkers were designed on the basis of the best linker from our first series of pleuromutilin derivatives following either conformational restriction or an isosteric methylene to oxygen exchange. The binding of the new compounds to the *Escherichia coli* ribosome was investigated by molecular modeling and chemical footprinting of nucleotide U2506, and it was found that all the derivatives bind to the specific site and most of them better than pleuromutilin itself. The effect of the side-chain extension was also explored by chemical footprinting of nucleotide U2585, and the results showed that all the compounds interact with this position to varying degrees. Derivatives with a conformational restriction of the linker generally had a higher affinity than derivatives with an isosteric exchange of one of the carbons in the linker with a hydrophilic oxygen. A growth inhibition assay with three different bacterial strains showed significant activity of several of the new compounds.



INTRODUCTION

Few new antibiotics reach the market, and at the same time, the problem with bacterial resistance to many antibiotics is increasing. Therefore, there is a need for developing new antibiotics. Natural products or semisynthetic derivatives of natural products have so far been the most successful way for developing new antibiotics. The natural compound pleuromutilin[1] **1** (Figure 1) has a modest antibacterial activity and inhibits bacterial protein synthesis by binding to the ribosomes. Modification of the C14 position of pleuromutilin has led to some more active compounds, such as retapamulin **2**, tiamulin **3**, and valnemulin **4** (Figure 1). Retapamulin is the first antibiotic in the pleuromutilin class to be approved for human use; it is used in the treatment of topical skin infections.[2] Tiamulin and valnemulin are used in veterinary medicine for pigs and poultry. The interaction of tiamulin and valnemulin with the bacterial ribosome has been investigated and it was shown that the two drugs bind to domain V of 23S rRNA in the peptidyltransferase center (PTC), thereby inhibiting peptide bond formation by hindering a correct location of the tRNA.[3] Chemical footprinting of various pleuromutilin derivatives suggests a similar mode of binding of the tricyclic mutilin core of all pleuromutilin derivatives in the PTC but that the side-chain extension at the C14 position can assume distinct conformations in the binding pocket.[4] The detailed interactions of 23S rRNA and four pleuromutilin derivatives have been determined by X-ray structures of antibiotic−ribosome complexes.[5−7]

Previous work in our group has led to the synthesis and analysis of binding of 19 semisynthetic pleuromutilin derivatives.[8] The derivatives were synthesized by use of a parallel click chemistry strategy, where the C22 hydroxy-group of pleuromutilin was substituted by an azide group and then used in a standard Cu(I)-catalyzed alkyne−azide [3 + 2] cycloaddition (CuAAC reaction)[9,10] with a series of 19 terminal alkynes. The alkynes were linked to different nucleobases, nucleosides, or a phenyl group with a variation of the linker length. The binding affinities of the 19 derivatives to *Escherichia coli* ribosomes were evaluated by chemical footprinting. This method shows how well the derivatives protect accessible Us in 23S rRNA against reaction with CMCT [*N*1-cyclohexyl-*N*3-(2-morpholinoethyl)carbodiimide *p*-toluenesulfonate]. Previous footprinting on pleuromutilin derivatives shows two clear

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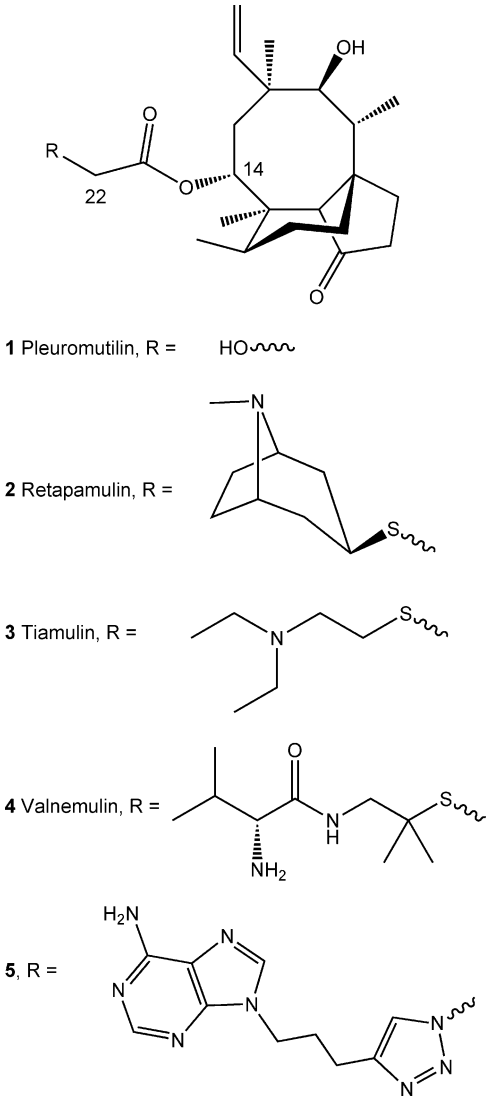


Figure 1. Chemical structure of pleuromutilin and derivatives thereof.

footprints at U2506 and U2585.[4] The protection at U2506 is believed to correlate to binding affinity, while the effect at U2585 tells whether the extension from the mutilin core is placed in the neighborhood of U2585. All the derivatives were found to bind in the PTC, and 11 showed stronger affinity for the PTC than pleuromutilin itself. Compound **5** (Figure 1) was the best binding derivative from the series.[8] A common feature for the four best derivatives was a three-carbon linker between the triazole and the nucleobase. Another trend was that adenine is favored over thymine or the simple phenyl group. Footprinting at nucleotide U2585 demonstrated that all 19 derivatives also interact with this position, a position that is unaffected by pleuromutilin itself. The two best derivatives from the previous series, that is, **5** and a derivative with a phenyl instead of adenine, were investigated by molecular docking. This modeling suggested that the mutilin core binds to the PTC in the same way as for tiamulin. It also suggested that the side chain is extended into the peptide exit tunnel and that a stacking interaction is taking place between the adenine/phenyl group and U2586 or A2062. Finally, a stacking interaction between the triazole and nucleobase U2585 was indicated.

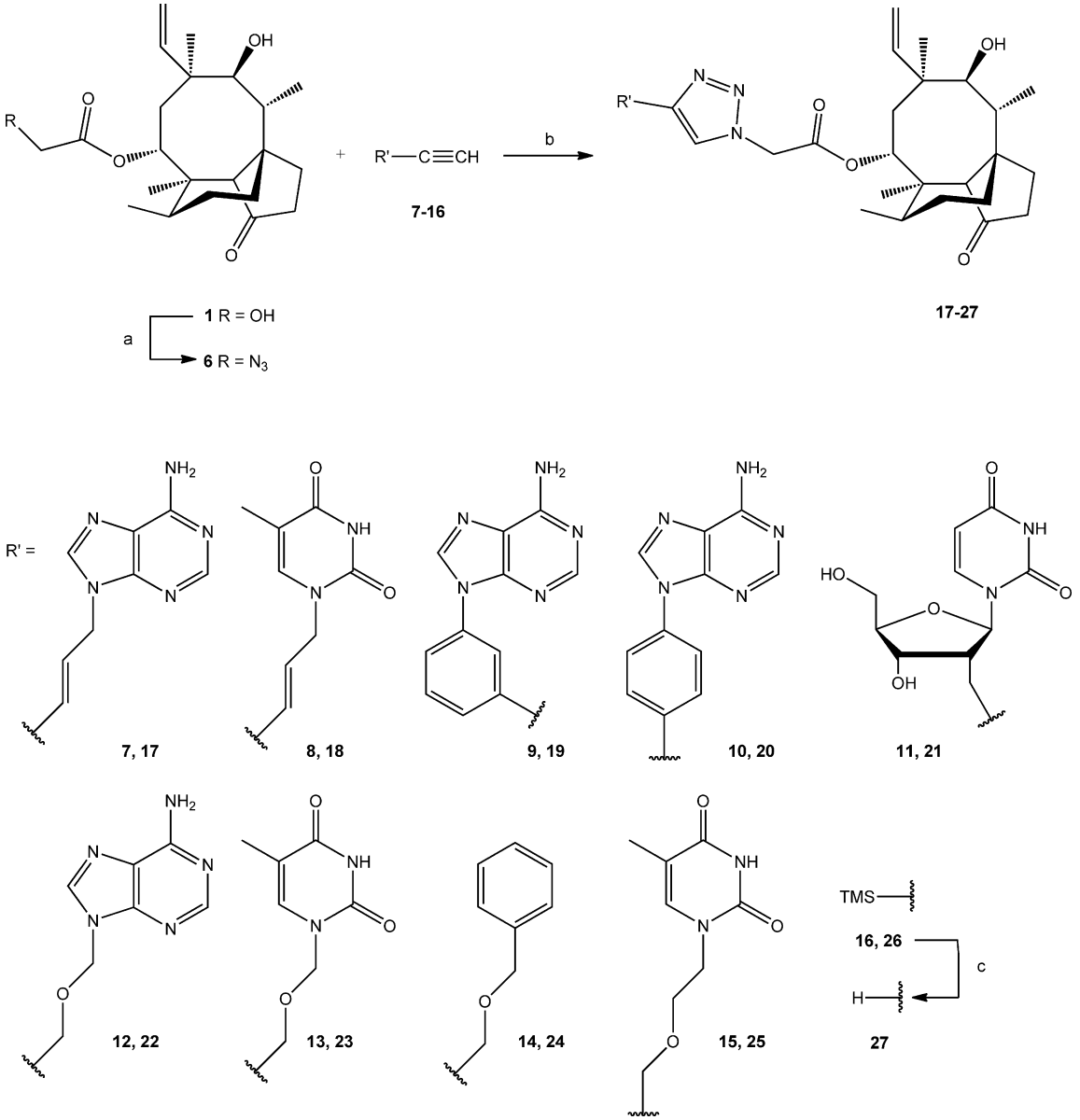
For the present study, we decided to elaborate on **5** by varying the three-carbon linker, as well as to increase our knowledge of the binding site by synthesis of other analogues in combination with further molecular docking studies. Binding to the *E. coli* ribosome is investigated by chemical footprinting, and the potential biological activity is tested by determining the inhibition on growth of three different bacteria.

RESULTS

Chemical Synthesis. A new series of pleuromutilin derivatives was synthesized based on the design used in our former study, by the click chemistry approach.[8] The derivative with the higest affinity from our previous series, **5**, is used as a lead compound. The aim was first of all to make conformational restrictions of the three-carbon linker between the triazole ring and the nucleobase, as well as to make an isosteric exchange of one of the carbons in the linker with oxygen in order to increase the hydrophilicity of the linker. Thus pleuromutilin **1** was converted to the azide **6**[8,11] and reacted with a series of alkynes **7**−**16** by the CuAAC reaction[9,10] to give a series of 10 new pleuromutilin triazole derivatives **17**−**25** and **27** (Scheme 1). The compounds were chosen on the basis of lead compound **5**, the previous study, and ease of synthesis. The compounds **17**, **19**, and **20** were made as conformationally restricted analogues of **5**, and **18** was made in order to compare the effect of two different nucleobases with the same restricted linker. Compounds **22**−**24** were made as more hydrophilic analogues of **5** and its corresponding thymine and phenyl counterparts included in our first study.[8] The compounds **21** and **25** were made to follow up on the conformational restriction of a uracil analogue of **5** as well as on extension of the linker with an oxygen atom. The unsubstituted triazole compound **27** was included as a reference compound to investigate the general effect of side-chain extensions at the triazole. All CuAAC reactions proceeded in medium to good yields to give the pure target compounds after simple chromatographic purification. Finally, also a 1,5-disubstituted 1,2,3-triazole isomer of the lead derivative **5** was synthesized, that is, **28** (Scheme 2). To give this regioisomer, a ruthenium catalyst[12] was applied in the cycloaddition reaction between the azide derivative of pleuromutilin **6** and the terminal alkyne *N*9-(pentyn-5-yl)adenine[8] **29**.

The alkyne building blocks **7**−**15** used in the click reaction were synthesized in a few steps from simple nucleobase or nucleoside precursors. The alkynylated nucleobases with a conformational restriction in the linker, **7** and **8**, were synthesized by Mitsunobu reactions with 2-penten-4-yn-1-ol and *N*6-bis-Boc-adenine **30**[13] or *N*3-benzoylthymine **31**,[14] respectively (Scheme 3), followed by hydrolysis with either hydrochloric acid or sodium methoxide in methanol to remove the protecting groups. The alkyne building blocks **9** and **10**, which contain a more bulky aromatic but still conformationally restricted linker, were also synthesized from **30** (Scheme 4). The protected nucleobase was used in a copper-mediated Chan−Lam−Evans-modified Ullmann condensation with either (3-iodophenyl)boronic acid or (4-iodophenyl)boronic acid according to a known procedure[15] to give compounds **32** and **33**.[15] These were used in a Sonogashira coupling with trimethylsilylacetylene to introduce the triple bond and provide compounds **34** and **35**. This was followed by a basic deprotection of the TMS- and Boc-protecting groups in one step to give compounds **9** and **10** (Scheme 4). Nucleoside **11** was synthesized from the TBS-protected 2′-*C*-allyl-2′-deoxyur-

Scheme 1. Overview of Click Chemistry Approach and Chemical Structure of the 10 Derivatives of Pleuromutilin and Applied Terminal Alkynes[a]



[a]Reagents and conditions: (a) (i) TsCl, DMAP, CH_2Cl_2; (ii) NaN_3, acetone, H_2O (52%, two steps).[8,11] (b) Sodium ascorbate, $CuSO_4$, *t*-BuOH, H_2O or THF, H_2O; **17** (29%), **18** (45%), **19** (64%), **20** (31%), **21** (62%), **22** (53%), **23** (65%), **24** (71%), **25** (76%). (c) TBAF, THF, **27** (58%, two steps).

idine derivative **36**,[16] which was protected at the nucleobase with a benzoyl group to give compound **37** (Scheme 5). This was followed by an oxidative cleavage to give the aldehyde **38**. The aldehyde was then converted to an alkyne by use of the Ohira–Bestmann reagent[17,18] and debenzoylated to give **39**, followed by desilylation to give nucleoside **11**. The two alkynes **12** and **13**, containing a three-atom linker with an oxygen, were synthesized by Vorbrüggen coupling to bis(propargyloxy)-methane[19] involving either the *N6*-formamidine-protected adenine **40**[20] or unprotected thymine **41** (Scheme 6). The benzyl propargyl ether **14** was synthesized according to a known procedure from benzylbromide and propargyl alcohol.[21] Alkyne **15** was synthesized from 3-(benzyloxymethyl)-1-(2-hydroxyethyl)thymine **42**,[14,22] which was alkylated with propargyl bromide to give **43** (Scheme 7). Compound **43** was first deprotected with BCl_3 in dichloromethane (DCM), which gave a mixture of two products, 3-(hydroxymethyl)-1-[2-(propargyloxy)ethyl]thymine and the target 1-[2-(propargyloxy)ethyl]thymine. The mixture was treated with NaOH in THF and water to give the pure alkyne **15**. Compound **27** was synthesized from the cycloaddition between the azide derivative **6** and trimethylsilylacetylene to give compound **26**, followed by a desilylation with TBAF to give **27** (Scheme 1).

Chemical Footprinting of Pleuromutilin Derivatives in PTC. The affinities of the 11 new derivatives (**17**–**25**, **27**, and **28**) as well as for the lead derivative from the previous series **5** and the derivatives tiamulin **3** and valnemulin **4** for the binding site in the PTC of ribosomes from *E. coli* were examined by chemical footprinting at nucleotide U2506 as described

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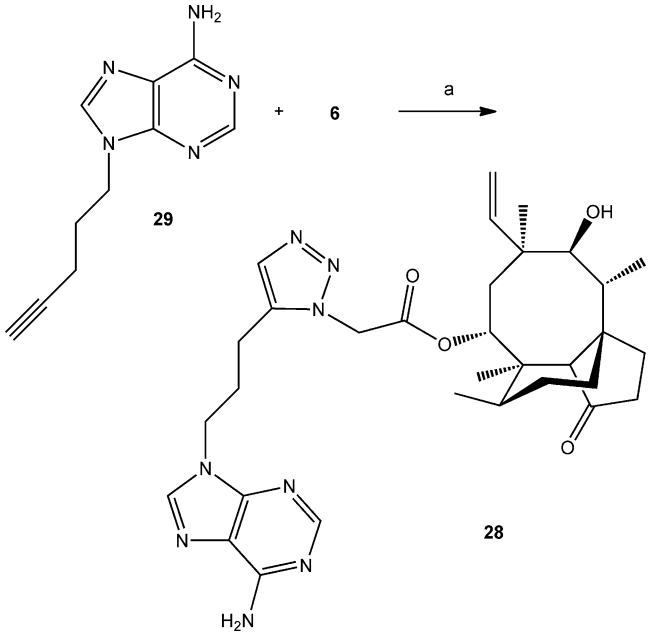
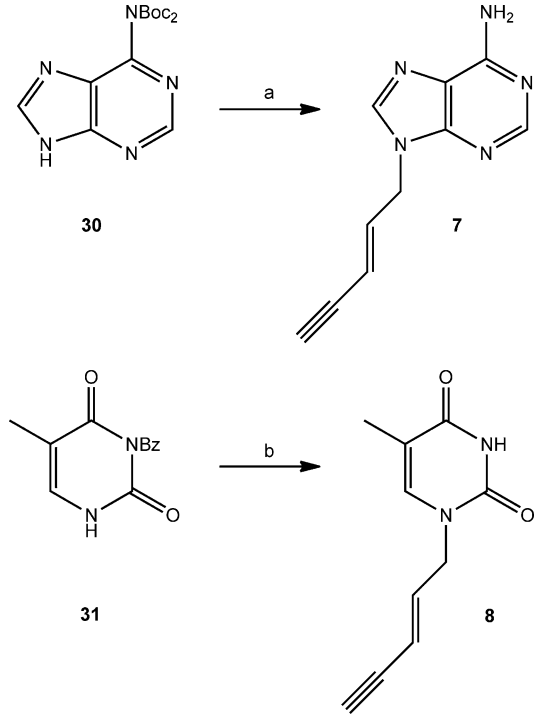
Scheme 2. Synthesis of 1,5-Disubstituted Isomer of 5[a]



[a]Reagents and conditions: (a) CpRuCl(COD), toluene, DMF (25%).

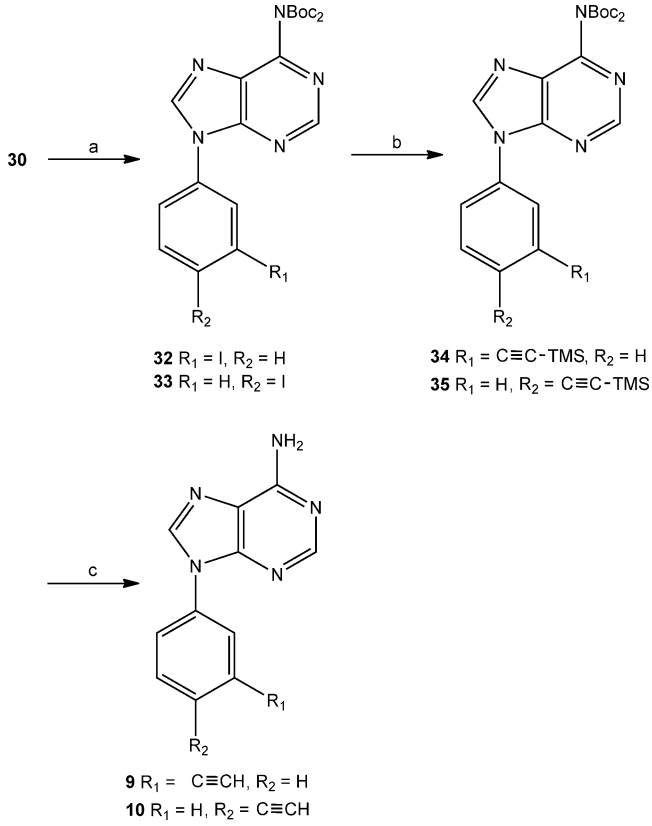
Scheme 3. Synthesis of Alkynes 7 and 8[a]



[a]Reagents and conditions: (a) (i) 2-Penten-4-yn-1-ol, DIAD, PPh_3, THF; (ii) 3 M HCl, MeOH (84% estimated, two steps). (b) (i) 2-Penten-4-yn-1-ol, DEAD, PPh_3, toluene, DCM; (ii) NaOMe, MeOH (17%, two steps).

previously.[8] The rationale for using this protection as an affinity measure came from previous footprinting experiments[4,8] and from the fact that crystal structures have revealed that the position of the tricyclic mutilin core moiety is fixed in a tight binding pocket including position U2506 in 23S rRNA.[5−7] In contrast, the RNA moieties interacting with the pleuromutilin

Scheme 4. Synthesis of Alkynes 9 and 10[a]



[a]Reagents and conditions: (a) (3-Iodophenyl)boronic acid or (4-iodophenyl)boronic acid, $Cu(OAc)_2$, DMF, Et_3N, 3 Å MS; **32** (42%), **33** (51%). (b) TMS-acetylene, $Pd(PPh_3)_2Cl_2$, CuI, DMF, Et_3N; **34** (65%), **35** (86%). (c) KOH, MeOH; **9** (39%), **10** (83%)

side chains can move to accommodate the variable sizes and chemical groups of the drugs.[6] Ribosomes were incubated with and without the drugs and exposed to CMCT that reacts with accessible uridines (including U2506 in 23S rRNA). The difference in reaction with and without drug can then be monitored by primer extension with reverse transcriptase that stops at CMCT-modified Us. The extension product is visualized by gel electrophoresis and the resulting bands are quantified by image analysis to obtain an assessment of the protection against CMCT afforded by the drug. The results are shown in Table 1 for two concentrations of the drug and are ordered by protection at U2506 at 5 μM concentrations, with 1.00 representing no protection (no binding) and 0 representing total protection. All the derivatives were seen to bind in the PTC and eight of them apparently bound better than pleuromutilin itself. It should be noted that the footprinting method is qualitatively good and very specific but not very quantitatively precise. The best derivative **19** showed as good an affinity to the PTC as the two drugs valnemulin **4** and tiamulin **3**. In general, the conformationally restricted derivatives **17**−**20** show a higher affinity for the PTC than the derivatives with an isosteric exchange of one of the carbons in the linker with a more hydrophilic oxygen **22**−**25**. An exception is compound **21**, which has a conformationally restricted linker but displays one of the lowest affinities for the PTC. Again, the trend from the first series[8] of increased binding affinity for adenine derivatives as compared to thymine or phenyl derivatives is observed since **17** binds more strongly

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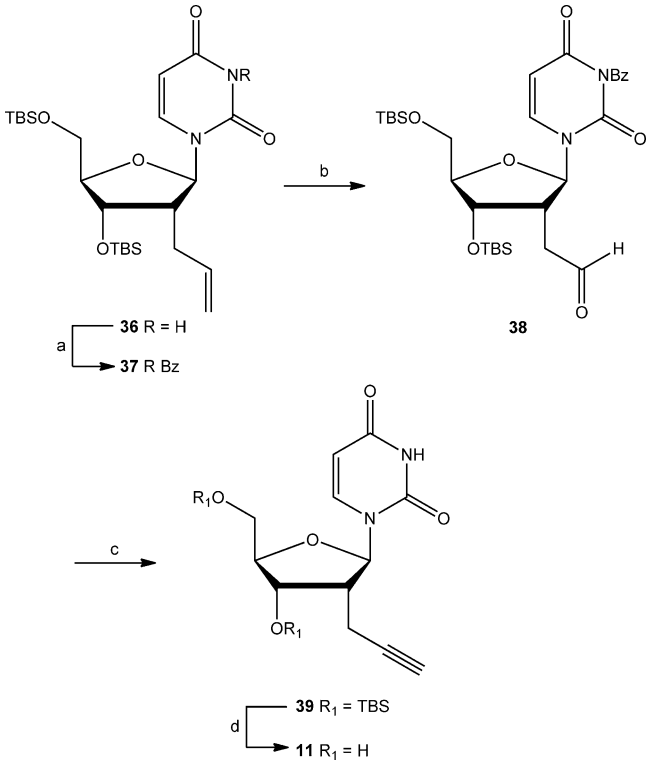
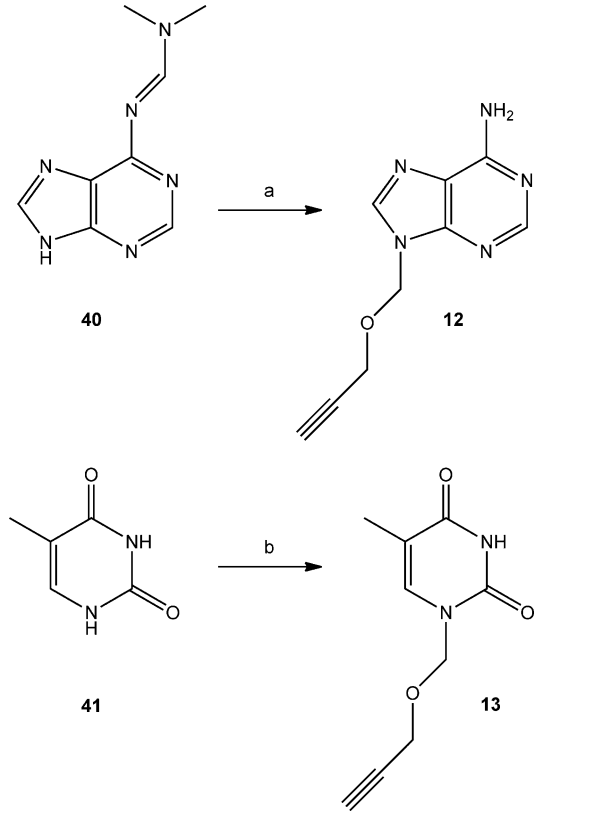
Scheme 5. Synthesis of Nucleoside 11[a]



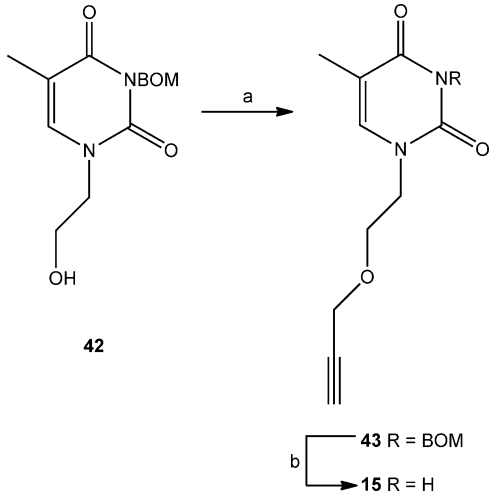
[a]Reagents and conditions: (a) BzCl, pyridine (94%). (b) (i) OsO_4, NMO, acetone, H_2O; (ii) $NaIO_4$, dioxane, H_2O (49%). (c) (i) Dimethyl-2-oxopropylphosphonate, *p*-TsN_3, K_2CO_3, CH_3OH, CH_3CN; (ii) methanolic ammonia (46%). (d) TBAF, THF (57%).

Scheme 6. Synthesis of Alkynes 12 and 13[a]



[a]Reagents and conditions: (a) (i) Bis(propargyloxy)methane, BSA, TMS triflate, $ClCH_2CH_2Cl$, CH_3CN; (ii) NH_3(aq) (14%, two steps). (b) Bis(propargyloxy)methane, BSA, TMS triflate, $ClCH_2CH_2Cl$, CH_3CN (27%).

Scheme 7. Synthesis of Alkyne 15[a]



[a]Reagents and conditions: (a) NaH, propargyl bromide, THF (82%). (b) (i) BCl_3, DCM; (ii) NaOH, THF, H_2O (13%, two steps).

than **18** and **22** binds more strongly than **23** and **24**. The "1,5-isomer" of **5**, that is, compound **28**, belongs to the strongest binding analogues like the conformationally restricted analogues **17** and **19**. The derivative with the lowest affinity for the PTC is the reference compound **27**, which lacks a side-chain extension at the triazole. Probably, the side-chain extension at the triazole provides better binding affinity to the PTC by making more interactions with the rRNA.

Besides U2506, only U2585 has shown significant changes in accessibility to CMCT upon binding of pleuromutilin derivatives.[4,8] The accessibility of nucleotide U2585 in 23S rRNA, which is positioned relatively close to the binding site of the tricyclic core of pleuromutilin, was therefore also investigated. This protection is not believed to reflect the strength of antibiotic binding (as for position U2506) but merely reflects a closeness of the drug side chain to this position.[4,8] This might thus provide information about whether the side chains of the derivatives are positioned in the same orientation in the PTC. The results in Table 1 show that all the novel derivatives protect U2585 to some extent and all of them provided higher protection than pleuromutilin and (except **27**) tiamulin. In contrast, only derivative **19** gave better protection at nucleotide U2585 than valnemulin.

Docking Studies. Three of the pleuromutilin derivatives with good binding affinities (**5**, **19** and **28**), as well as the derivative showing the smallest binding affinity (**27**), were chosen for a molecular docking investigation. A PTC ribosome model was constructed that consists of all residues within 30 Å from the PTC binding site. Due to the lack of crystal structures of *E. coli* in complex with pleuromutilin derivatives, this model was based on the X-ray structure of *Deinococcus radiodurans* in complex with tiamulin.[5] The docking experiments were performed with Glide;[23−26] details for the setup of the docking experiments are given in the Experimental Section. Redocking of tiamulin **3** into this PTC model placed the drug as in the X-ray structure. The docking results for the four drugs revealed a similar binding pattern as documented for tiamulin with the

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Table 1. Footprinting and MIC Values for Pleuromutilin and Derivatives Thereof

	degree of CMCT mod. relative to positive control				MIC[b] μg/mL		
	U2506,[a] 5 μM	U2506,[a] 50 μM	U2585,[a] 5 μM	U2585,[a] 50 μM	*B. subtilis* 168	*L. innocua*	*E. coli* AS19
Val **4**	0.05	0.04	0.15	0.16	2	1	<0.125
19	0.06	0.06	0.10	0.07	8	>32	1
Tia **3**	0.07	0.03	0.54	0.50	>32	>32	0.5−1
28	0.09	0.04	0.19	0.16	>32	>32	16−32
17	0.10	0.05	0.29	0.16	16	>32	8
5	0.13	0.05	0.23	0.17	>32	>32	8−16
20	0.18	0.08	0.40	0.22	>32	>32	>32
18	0.18	0.06	0.39	0.21	>32	>32	≥32
22	0.21	0.06	0.37	0.22	>32	>32	>32
23	0.23	0.07	0.37	0.22	>32	>32	>32
Pleuro **1**	0.28	0.08	0.89	0.80	32	>32	1−2
25	0.28	0.08	0.43	0.24	>32	>32	≥32
24	0.30	0.08	0.50	0.41	>32	>32	>32
21	0.31	0.09	0.46	0.26	>32	>32	>32
27	0.48	0.18	0.66	0.42	>32	>32	16−32

[a]Selected 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. The numbers represent the relative CMCT accesibility in the presence of the drug, in 5 or 50 μM concentrations, compared to in the absence of the drug. For example, 0.10 means that the intensity of the band on the gel in the presence of the drug is only 10% of the intensity of the corresponding band from samples without the drug. [b]Minimal inhibitory concentration (MIC) of the derivatives determined in a plate assay with three different bacteria, *E. coli* AS19, *Bacillus subtilis* 168, and *Listeria innocua*.

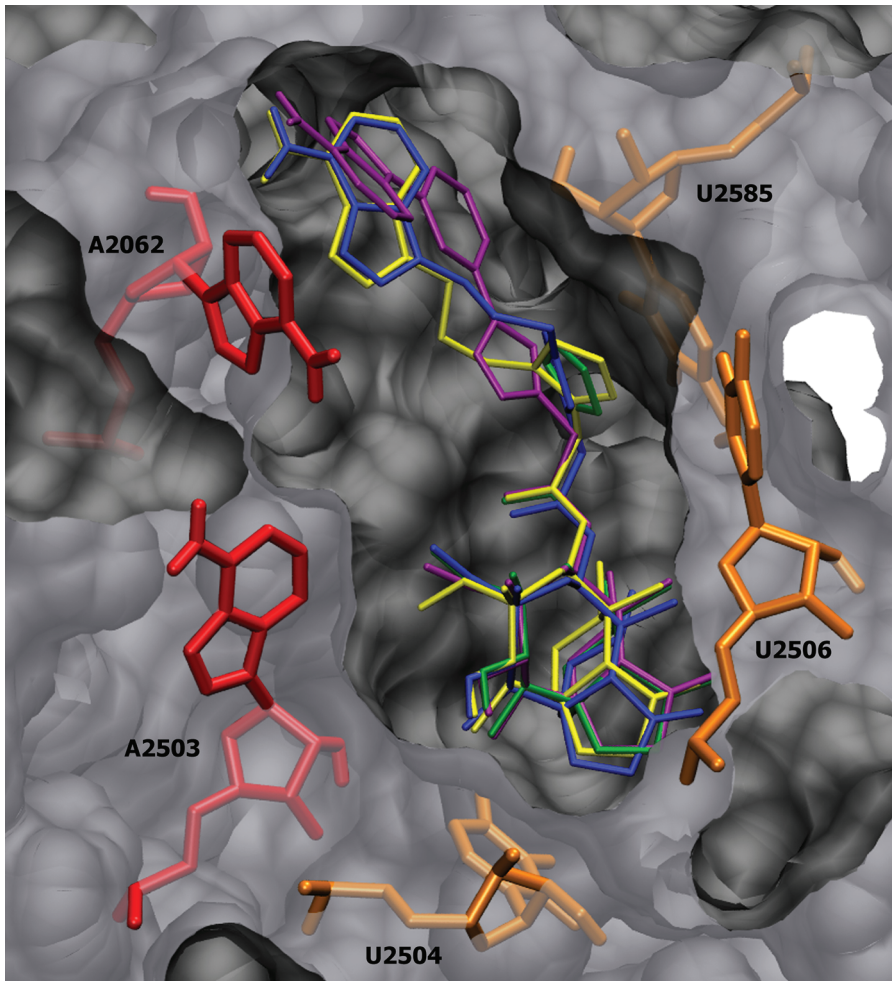

Figure 2. Results of the docking of **5** (blue), **19** (purple), **27** (green), and **28** (yellow) into the PTC model binding site. Residue numbers shown are according to *E. coli* 23S RNA numbering.

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pleuromutilin cores placed close to the U2506 position as presented in Figure 2, which shows a superposition of the four docked drugs. Based on the predicted binding affinities (docking scores), the drugs were found to bind in the sequence **28** > **5** > **19** ≫ **27**, which is in agreement with the footprinting results. Only very small deviations (0.2 kcal/mol) were observed in the binding affinities between the drugs **28**, **5**, and **19**; that is, these three drugs are found to bind with similar strength. In contrast, the scores of the docking procedure clearly distinguish between drugs with high/low binding affinities according to the footprinting results. As observed previously,[8] the results of these docking experiments give indications for a stacking interaction between the triazoles of the drugs and U2585. This stacking interaction seems more pronounced for the more flexible drugs (**5**, **27**, and **28**) than for the very restricted derivative **19** (Figure 2). However, **19** demonstrates the strongest overall binding from the footprinting, and docking of natural pleuromutilin resulted in almost the same docking score as for **27**, indicating that this stacking interaction between the triazole and U2585 is of minor importance for the overall binding of the drugs. An interaction between the side-chain adenines of **5**, **19**, and **28** with A2062 appears to be unlikely on the basis of the docking, and only compound **19** seems partly oriented toward a stacking interaction. However, A2062 is known to be very flexible and the docking might not model this flexibility very well.

Potential Growth Inhibition of Bacteria by Pleuromutilin Derivatives. Although binding affinity for the target is an important parameter for the efficacy of a potential drug, the ultimate criterion is activity against bacterial cells, and the ability to perform in the cells is vital. As a first preliminary step to investigate the biological relevance of the 11 derivatives of pleuromutilin, the minimal inhibitory concentration (MIC) values were determined for three different bacterial strains, a drug-hypersensitive *E. coli* AS19 (Gram-negative), *Bacillus subtilis* 168 (Gram-positive), and *Listeria innocua* (Gram-positive). These are all nonpathogenic strains but if a compound does not have any effect on any of these strains, it is not likely to be useful as a general antibiotic, although the possibility exists that it could be effective against specific strains. The MICs are listed in Table 1, and due to the availability of the synthesized drugs, only relatively low concentrations were tested. *L. innocua* seems very resistant to pleuromutilins in general, as only valnemulin inhibited growth at the concentration tested. For *B. subtilis* 168, valnemulin was also the most potent compound, while compound **19** was second best, followed by **17** and then pleuromutilin. *E. coli* AS19 was also most sensitive to valnemulin, followed by tiamulin, **19**, pleuromutilin, **17**, **5**, and then **27** and **28**. Some of the new compounds are thus comparable in potency to tiamulin, which is the most successful of the commercial pleuromutilin products if measured by usage. For further drug development, promising candidates should be tested against pathogenic bacteria such as *Staphylococcus* and *Streptococcus*.

DISCUSSION

The results presented herein are in line with the outcome from the first series[8] that nucleobases conjugated to the C22 position of pleuromutilin through a triazole linkage are well adopted within the PTC binding pocket. Derivatives conjugated to the nucleobase adenine give a better binding affinity than the derivatives conjugated to thymine, which again is superior to a simple phenyl group. Another general trend is that compounds with a conformationally restricted linker demonstrate better binding affinities to the PTC than the derivatives with oxygen in the linker. Compound **19** is best in both the footprinting and MIC studies, which is also in good agreement with the present docking results. Also, **17** and **28** show a slight improvement in binding affinity as compared to **5**, and this might reflect a small change in the positioning of the adenine in the binding site. The docking studies indicate that the pleuromutilin core is located close to the U2506 position and that the triazole moiety stacks with the position U2585. However, this stacking interaction seems to be of minor importance for the overall binding of the derivatives, since **27** demonstrates a low affinity and since the stacking according to the docking study seems less pronounced for the best binder **19** as compared to other derivatives. Nevertheless, the triazole might have an important role in directing the side chain toward a suitable position.

The relatively low variation in affinity observed is not surprising due to the fact that the designs are based on a reasonably good binder in the form of the pleuromutilin core. Nevertheless, the variations in chemical structure may be important when it comes to in vivo function. Compound **19** is the best derivative in both our assays (Table 1) and is hereby the superior candidate for further studies.

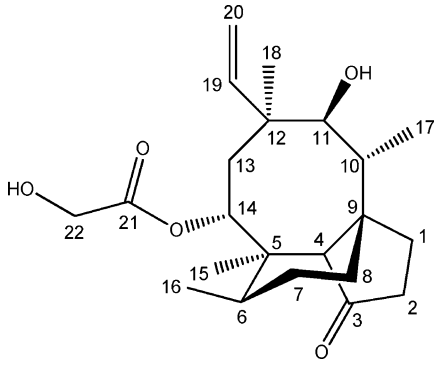
CONCLUSION

A series of 11 new pleuromutilin derivatives has been efficiently synthesized by a parallel click chemistry strategy. The alkyne building blocks were synthesized in relatively few steps, and in combination with the click chemistry strategy, this gives easy access to new active derivatives of pleuromutilin. The chemical footprinting showed that all the new derivatives bind in the PTC in the ribosome and seven of these bind better than pleuromutilin itself. The docking reflected the footprinting results, indicating the potential of this method in future design. Derivative **19** showed binding affinity to the PTC at a level of the known drugs valnemulin and tiamulin and is also the most potent compound in the new series on the basis of MIC experiments. The present close interplay between a straightforward and reliable docking procedure and a convenient semisynthetic strategy opens the door for fast development of new potent antibiotics based on pleuromutilin.

EXPERIMENTAL SECTION

General. All reactions with anhydrous solvents were performed under an atmosphere of either nitrogen or argon. Column chromatography was performed either as flash column chromatography or as standard column chromatography carried out on glass columns with silica gel 60 (particle size 0.040−0.063 mm.). Microwave heated reactions were preformed with an Emrys Creator. NMR spectra were recorded at 300 or 400 MHz for ^{1}H NMR and at 75 or 101 MHz for ^{13}C NMR. The chemical shift values (δ) are reported in parts per million (ppm) relative to tetramethylsilane as internal standard. Two-dimensional (2D) spectra were used to assign NMR spectra, and standard numbering of pleuromutilin and nucleosides was applied. Electrospray ionization mass spectra (ESI-MS) were recorded in positive-ion mode.

General Procedure for Synthesizing Pleuromutilin Derivatives. The pleuromutilin azide **6** (0.07−0.20 mmol), the alkyne derivative (0.07−0.21 mmol), sodium ascorbate (0.01−0.03 mmol), and $CuSO_4 \cdot 5H_2O$ (0.01−0.04 mmol) were dissolved in *t*-BuOH/H_2O (2−3 mL, 1:1 v/v) in a microwave vial. The vial was sealed and heated with magnetic stirring in the microwave reactor at 110 °C for 30 min. The reaction mixture was concentrated under reduced pressure, and the residue was purified by column chromatography (0−10% CH_3OH



in CH_2Cl_2) to give the product as a white foam. Purities ≥95% were secured by HPLC.

*Synthesis of 22-{4-[(E)-3-(Adenin-9-yl)propen-1-yl]-1,2,3-triazol-1-yl}-22-deoxypleuromutilin (**17**).* The general procedure was applied with compound **6** (74 mg. 0.18 mmol), *N*9-[(*E*)-pent-2-en-4-yn-1-yl]adenine **7** (30 mg, 0.15 mmol), sodium ascorbate (3.8 mg, 19 μmol), and $CuSO_4{\cdot}5H_2O$ (5.1 mg, 20 μmol) in *t*-BuOH/H_2O (2 mL, 1:1 v/v). Yield 26 mg (29%); R_f 0.4 (10% CH_3OH in CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 8.39 [1H, s, H2(A)], 7.87 [1H, s, H8(A)], 7.57 [1H, s, H5(triazole)], 6.66 (1H, dt, *J* = 5.8, 16.0 Hz, *N*9-CH_2CH), 6.53 (1H, d, *J* = 16.0 Hz, *N*9-CH_2CHCH), 6.39 (1H, dd, J_{cis} = 11.1 Hz, J_{trans} = 17.4 Hz, H19), 5.81 (1H, d, *J* = 8.5 Hz, H14), 5.75 [2H, s, NH_2(A)], 5.32 (1H, d, J_{cis} = 11.1 Hz, H20), 5.20 (1H, d, J_{trans} = 17.4 Hz, H20), 5.11−4.97 (4H, m, H22, *N*9-CH_2), 3.39−3.32 (1H, m, Hz, H11), 2.30−2.06 (5H, m, H2, H4, H10, and H13), 1.78−1.08 (9H, m, H1, H6, H7, H8, H13, and 11-OH), 1.35 (3H, s, H15), 1.17 (3H, s, H18), 0.88 (3H, d, *J* = 7.0 Hz, H17), 0.70 (3H, d, *J* = 7.0 Hz, H16). ^{13}C NMR ($CDCl_3$) δ 216.7 (C3), 165.1 (C21), 155.6 [C6(A)], 153.4 [C2(A)], 150.2 [C4(A)], 145.0 [C4(triazole)], 140.4 [C8(A)], 138.7 (C19), 125.7 (N9-CH_2CH), 122.2, 122.1 [C5(triazole), N9-CH_2CHCH) 119.7 [C5(A)], 117.8 (C20), 74.7 (C11), 71.2 (C14), 58.1 (C4), 51.6 (C22), 45.5 (C9), 45.2 (N9-CH_2), 44.8 (C13), 44.1 (C12), 42.0 (C5), 36.6 (C6), 36.2 (C10), 34.5 (C2), 30.4 (C8), 26.9 (C7), 26.5 (C18), 24.9 (C1), 16.9 (C16), 14.8 (C15), 11.6 (C17). HRMS (ESI) *m*/*z* 625.3221 [M + Na]$^+$, $C_{32}H_{42}N_8O_4Na$ calcd 625.3221.

*Synthesis of 22-{4-[(E)-3-(Thymin-1-yl)propen-1-yl]-1,2,3-triazol-1-yl}-22-deoxypleuromutilin (**18**).* The general procedure was slightly modified with compound **6** (54 mg, 0.13 mmol), *N*1-[(*E*)-pent-2-en-4-ynyl]thymine **8** (33 mg, 0.17 mmol), sodium ascorbate (5.3 mg, 27 μmol), and $CuSO_4{\cdot}5H_2O$ (4.5 mg, 18 μmol) in *t*-BuOH/H_2O (3.0 mL, 1:1 v/v) and conventional heating at 80 °C for 21.5 h. Yield 35 mg (45%); R_f 0.5 (10% CH_3OH in CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 9.19 [1H, s, NH(T)], 7.64 [1H, s, H5(triazole)], 7.05 [1H, s, H6(T)], 6.62 (1H, d, *J* = 16.0 Hz, N1-CH_2CHCH), 6.49−6.36 (2H, m, N1-CH_2CH, H19), 5.81 (1H, d, *J* = 8.5 Hz, H14), 5.32 (1H, d, J_{cis} = 12.0 Hz, H20), 5.21 (1H, d, J_{trans} = 17.4 Hz, H20), 5.11, 5.03 (2H, AB, *J* = 17.6 Hz, H22), 4.49 (2H, d, *J* = 6.1 Hz, N1-CH_2), 3.37 (1H, dd, *J* = 6.5, 10.3 Hz, H11), 2.31−2.07 (5H, m, H2, H4, H10, and H13), 1.93 [3H, s, CH_3(T)], 1.79−1.09 (9H, m, H1, H6, H7, H8, H13, and 11-OH), 1.36 (3H, s, H15), 1.18 (3H, s, H18), 0.88 (3H, d, *J* = 6.9 Hz, H17), 0.71 (3H, d, *J* = 7.0 Hz, H16). ^{13}C NMR ($CDCl_3$) δ 216.8 (C3), 165.1, 164.3 [C21, C4(T)], 150.9 [C2(T)], 145.1 [C4-(triazole)], 139.8 [C6(T)], 138.7 (C19), 125.5 (N1-CH_2CH), 122.6, 122.2 [N1-CH_2CHCH, C5(triazole)], 117.7 (C20), 111.3 [C5(T)], 74.6 (C11), 71.2 (C14), 58.1 (C4), 51.6 (C22), 49.3 (N1-CH_2), 45.5 (C9), 44.8 (C13), 44.1 (C12), 41.9 (C5), 36.6 (C6), 36.1 (C10), 34.5 (C2), 30.4 (C8), 26.9 (C7), 26.5 (C18), 24.9 (C1), 16.9 (C16), 14.7 (C15), 12.5 [CH_3(T)], 11.6 (C17). HRMS (ESI) *m*/*z* 616.3114 [M + Na]$^+$, $C_{32}H_{43}N_5O_6Na$ calcd 616.3106.

*Synthesis of 22-{4-[3-(Adenin-9-yl)phenyl]-1,2,3-triazol-1-yl}-22-deoxypleuromutilin (**19**).* The general procedure was slightly modified with compound **6** (28 mg, 0.07 mmol), *N*9-(3-ethynylphenyl)adenine **9** (16 mg, 0.07 mmol), sodium ascorbate (1.5 mg, 7.6 μmol), and $CuSO_4{\cdot}5H_2O$ (2.1 mg, 8.4 μmol) in *t*-BuOH/H_2O (2.5 mL, 1:1 v/v) and conventional heating at 80 °C for 18 h. Yield 20 mg (64%); R_f 0.2 (10% CH_3OH in CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 8.44 [1H, s, H2(A)], 8.21 (1H, s, Ph), 8.18 [1H, s, H8(A)], 7.99 [1H, s, H5(triazole)], 7.89 (1H, d, *J* = 7.8 Hz, Ph), 7.75 (1H, d, *J* = 8.9 Hz, Ph), 7,63 (1H, m, Ph), 6.42 (1H, dd, J_{cis} = 11.3 Hz, J_{trans} = 17.4 Hz, H19), 5.98 [2H, br s, NH_2(A)], 5.84 (1H, d, *J* = 8.5 Hz, H14), 5.34 (1H, d, J_{cis} = 11.3 Hz, H20), 5.24−5.09 (3H, m, H20 and H22), 3.40−3,34 (1H, m, H11), 2.33−2.08 (5H, m, H2, H4, H10, and H13), 1.79−1.10 (9H, m, H1, H6, H7, H8, H13, and 11-OH), 1.38 (3H, s, H15), 1.19 (3H, s, H18), 0.89 (3H, d, *J* = 7.0 Hz, H17), 0.74 (3H, d, *J* = 7.0 Hz, H16). ^{13}C NMR ($CDCl_3$) δ 216.7 (C3), 165.1 (C21), 156.0 [C6(A)], 153.9 [C2(A)], 150.1 [C4(A)], 147.2 [C4(triazole)], 139.6 [C8(A)], 138.7 (C19), 135.6, 132.3, 130.6, 125.5, 123.3 (Ph), 121.7 [C5(triazole)], 120.7 (Ph), 120.2 [C5(A)], 117.7 (C20), 74.7 (C11), 71.3 (C14), 58.1 (C4), 51.8 (C22), 45.5 (C9), 44.8 (C13), 44.1 (C12), 42.0 (C5), 36.7 (C6), 36.2 (C10), 34.5 (C2), 30.4 (C8), 26.9 (C7), 26.6 (C18), 24.9 (C1), 17.0 (C16), 14.8 (C15), 11.6 (C17). HRMS (ESI) *m*/*z* 661.3211 [M + Na]$^+$, $C_{35}H_{42}N_8O_4Na$ calcd 661.3221.

*Synthesis of 22-{4-[4-(Adenin-9-yl)phenyl]-1,2,3-triazol-1-yl}-22-deoxypleuromutilin (**20**).* The general procedure was applied with compound **6** (68 mg. 0.17 mmol), *N*9-(4-ethynylphenyl)adenine **10** (31 mg, 0.13 mmol), sodium ascorbate (3.4 mg, 17 μmol), and $CuSO_4{\cdot}5H_2O$ (4.2 mg, 17 μmol) in *t*-BuOH/H_2O (2 mL, 1:1 v/v). Yield 26 mg (31%); R_f 0.3 (10% CH_3OH in CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 8.45 [1H, s, H2(A)], 8.14 [1H, s, H8(A)], 8.06−8.04 (2H, m, Ph), 7.94 [1H, s, H5(triazole)], 7.82−7.80 (2H, m, Ph), 6.44 (1H, dd, J_{cis} = 10.9 Hz, J_{trans} = 17.4 Hz, H19), 5.85 (1H, d, *J* = 8.4 Hz, H14), 5.62 [2H, br s, NH_2(A)], 5.36 (1H, dd, J_{gem} = 1.5 Hz, J_{cis} = 10.9 Hz, H20), 5.26−5.09 (3H, m, H20 and H22) 3.37 (1H, dd, *J* = 6.4 Hz, *J* = 10.4 Hz, H11), 2.36−2.09 (5H, m, H2, H4, H10, and H13), 1.80−1.11 (9H, m, H1, H6, H7, H8, H13, and 11-OH), 1.38 (3H, s, H15), 1.20 (3H, s, H18), 0.89 (3H, d, *J* = 6.8 Hz, H17), 0.75 (3H, d, *J* = 7.2 Hz, H16). ^{13}C NMR ($CDCl_3$) δ 216.8 (C3), 165.2 (C21), 155.9 [C6(A)], 154.0 [C2(A)], 150.2 [C4(A)], 147.4 [C4(triazole)], 139.6 [C8(A)], 138.8 (C19), 134.8, 130.5, 127.4, 124.0 (Ph), 121.4 [C5(triazole)], 120.4 [C5(A)], 117.9 (C20), 74.7 (C11), 71.4 (C14), 58.2 (C4), 51.9 (C22), 45.6 (C9), 44.9 (C13), 44.2 (C12), 42.1 (C5), 36.7 (C6), 36.3 (C10), 34.6 (C2), 30.5 (C8), 27.0 (C7), 26.6 (C18), 25.0 (C1), 17.1 (C16), 14.8 (C15), 11.7 (C17). HRMS (ESI) *m*/*z* 639.3398 [M + H]$^+$, $C_{35}H_{43}N_8O_4$ calcd 639.3401.

*Synthesis of 22-(4-{[2R-(Uracil-1-yl)-4S-hydroxy-5R-hydroxymethyl]tetrahydrofuran-3R-ylmethyl}-1,2,3-triazol-1-yl)-22-deoxypleutomutilin (**21**).* The general procedure was applied with compound **6** (64 mg. 0.16 mmol), nucleoside **11** (35 mg, 0.13 mmol), sodium ascorbate (3.3 mg, 17 μmol), and $CuSO_4{\cdot}5H_2O$ (4.3 mg, 17 μmol) in *t*-BuOH/H_2O (2 mL, 1:1 v/v) was applied. Yield 55 mg (62%); R_f 0.5 (10% CH_3OH in CH_2Cl_2). ^1H NMR (DMSO-d_6) δ 11.22 [1H, br s, NH(U)], 7.81 [1H, d, *J* = 8.1 Hz, H6(U)], 7.78 [1H, s, H5(triazole)], 6.12 (1H, dd, J_{cis} = 11.2 Hz, J_{trans} = 17.8 Hz, H19), 5.99 (1H, d, *J* = 9.1 Hz, H1′), 5.62 [1H, d, *J* = 8.1 Hz, H5(U)], 5.56 (1H, d, *J* = 8.3 Hz, H14), 5.39 (1H, s, H3′-OH) 5.29−5.03 (5H, m, H22, H20, and H5′-OH), 4.53 (1H, d, *J* = 6.0 Hz, H11-OH), 4.12−4.09 (1H, m, H3′), 3.90 (1H, t, *J* = 3.7 Hz, H4′) 3.54 (2H, d, *J* = 3.0 Hz, H5′), 3.42 (1H, t, *J* = 6.0 Hz, H11), 2.99−2.91 (1H, m, H2′-CH_2), 2.62−2.53 (2H, m, H2′, H2′-CH_2), 2.40 (1H, s, H4), 2.23−1.99 (4H, m, H2, H10 and H13), 1.67−1.00 (8H, m, H1, H6, H7, H8, and H13), 1.24 (3H, s, H15), 1.07 (3H, s, H18), 0.81 (3H, d, *J* = 7.0 Hz, H17), 0.62 (3H, d, *J* = 7.0 Hz, H16). ^{13}C NMR (DMSO-d_6) δ 216.9 (C3), 165.6 (C21), 162.9 [C4(U)], 150.8 [C2(U)], 144.4 [C4(triazole)], 140.7, 140.4 [C6(U), C19], 123.8 [C5(triazole)], 115.4 (C20), 102.2 [C5(U)], 87.4 (C4′), 87.0 (C1′), 72.5 (C11), 71.8 (C3′), 70.4 (C14), 61.8 (C5′), 57.1 (C4), 50.9 (C22), 47.6 (C2′), 44.9 (C9), 44.1 (C12), 43.3 (C13), 41.5 (C5), 36.4 (C6), 36.2 (C10), 33.9 (C2), 30.0 (C8), 28.5 (C18), 26.5 (C7), 24.4 (C1), 19.7 (C2′-CH_2), 16.1 (C16), 14.2 (C15), 11.5 (C17). HRMS (ESI) *m*/*z* 692.3258 [M + Na]$^+$, $C_{34}H_{47}N_5O_9Na$ calcd 692.3266.

*Synthesis of 22-[4-(Adenin-9-ylmethoxymethyl)-1,2,3-triazol-1-yl]-22-deoxypleuromutilin (**22**).* The general procedure was slightly modified with compound **6** (61 mg, 0.15 mmol), compound **12** (38 mg, 0.19 mmol), sodium ascorbate (4.0 mg, 20 μmol), and $CuSO_4{\cdot}5H_2O$ (10 mg, 40 μmol) in *t*-BuOH/H_2O (3.0 mL, 1:1 v/v)

and conventional heating at 80 °C for 20.5 h. Yield 49 mg (53%); R_f 0.1 (10% CH_3OH in CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 8.37 [1H, s, H2(A)], 8.21 [1H, s H8(A)], 7.82 [1H, s, H5(triazole)], 6.37 (1H, dd, J_{cis} = 11.2 Hz, J_{trans} = 17.2 Hz, H19), 6.15 [2H, br s, NH_2(A)], 5.78 (1H, d, J = 7.6 Hz, H14), 5.67 (2H, s, N9-CH_2O), 5.30−5.07 (4H, m, H20 and H22), 4.74 (2H, s, N9-CH_2OCH_2), 3.40−3.39 (1H, m, H11), 2.25−2.05 (5H, m, H2, H4, H10, and H13), 1.76−1.07 (9H, m H1, H6, H7, H8, H13, and 11-OH), 1.32 (3H, s, H15), 1.15 (3H, s, H18), 0.87 (3H, d, J = 6.4 Hz, H17), 0.67 (3H, d, J = 6.8 Hz, H16). ^{13}C NMR ($CDCl_3$) δ 217.0 (C3), 165.2 (C21), 161.0 [C6(A)], 153.8 [C2(A)], 151.8 [C4(A)], 145.3 [C8(A)], 142.7 [C4(triazole)], 138.9 (C19), 125.4 [C5(triazole)], 117.6 (C20), 111.4 [C5(A)], 74.9 (N9-CH_2O), 74.6 (C11), 71.4 (C14), 60.8 (N9-CH_2OCH_2), 58.1 (C4), 51.8 (C22), 45.6 (C9), 44.8 (C13), 44.2 (C12), 42.0 (C5), 36.7 (C6), 36.2 (C10), 34.6 (C2), 30.5 (C8), 26.9 (C7), 26.7 (C18), 24.9 (C1), 17.0 (C16), 14.6 (C15), 11.7 (C17). HRMS (ESI) m/z 629.3168 [M + Na]$^+$, $C_{31}H_{42}N_8O_5Na$ calcd 629.3171.

*Synthesis of 22-[4-(Thymin-1-ylmethoxymethyl)-1,2,3-triazol-1-yl]-22-deoxypleuromutilin (**23**).* The general procedure was applied with compound **6** (80 mg, 0.20 mmol), 1-(propargyloxymethyl)-thymine **13** (30 mg, 0.15 mmol), sodium ascorbate (3.4 mg, 17 μmol), and $CuSO_4{\cdot}5H_2O$ (5.1 mg, 20 μmol) in t-BuOH/H_2O (2 mL, 1:1 v/v). Yield: 60 mg, 65%; R_f 0.6 (10% CH_3OH in CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 9.73 [1H, br s, NH(T)], 7.92 [1H, s, H5(triazole)], 7.22 [1H, d, J = 1.3 Hz, H6(T)], 6.40 (1H, dd, J_{cis} = 11.1 Hz, J_{trans} = 17.4 Hz, H19), 5.81 (1H, d, J = 8.5 Hz, H14), 5.32 (1H, dd, J_{gem} = 1.4 Hz, J_{cis} = 11.1 Hz, H20), 5.22−5.08 (5H, m, H20, N1-CH_2O, H22), 4.74 (2H, s, N1-CH_2OCH_2), 3.36 (1H, dd, J = 6.5, 10.3 Hz, H11), 2.29−2.02 (5H, m, H2, H4, H10, and H13), 1.94 [3H, d, J = 1.3 Hz, CH_3(T)], 1.78−1.08 (9H, m, H1, H6, H7, H8, H13, and 11-OH), 1.32 (3H, s, H15), 1.16 (3H, s, H18), 0.87 (3H, d, J = 7.0 Hz, H17), 0.70 (3H, d, J = 7.0 Hz, H16). ^{13}C NMR ($CDCl_3$) δ 216.8 (C3), 165.2, 164.1 [C21, C4(T)], 152.0 [C2(T)], 143.5 [C4(triazole)], 139.4 [C6(T)], 138.8 (C19), 125.5 [C5(triazole)], 117.6 (C20), 112.2 [C5(T)], 75.1 (N1-CH_2O), 74.6 (C11), 71.0 (C14), 61.5 (N1-CH_2OCH_2), 58.1 (C4), 51.6 (C22), 45.5 (C9), 44.7 (C13), 44.1 (C12), 41.9 (C5), 36.6 (C6), 36.1 (C10), 34.5 (C2), 30.4 (C8), 26.9 (C7), 26.5 (C18), 24.9 (C1), 16.9 (C16), 14.7 (C15), 12.4 [CH_3(T)], 11.6 (C17). HRMS (ESI) m/z 620.3052 [M + Na]$^+$, $C_{31}H_{43}N_5O_7Na$ calcd 620.3055.

*Synthesis of 22-[4-(Benzyloxymethyl)-1,2,3-triazol-1-yl]-22-deoxypleuromutilin (**24**).* The general procedure was slightly modified with compound **6** (56 mg, 0.14 mmol), benzyl propargyl ether **14** (31 mg, 0.21 mmol), sodium ascorbate (5.9 mg, 30 μmol), and $CuSO_4{\cdot}5H_2O$ (4.5 mg, 18 μmol) in THF/H_2O (3.5 mL, 1:1 v/v), stirring at RT for 20 h, and flash column chromatography (0−100% EtOAc in CH_2Cl_2). Yield 58 mg, 71%; R_f 0.2 (10% EtOAc in CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 7.65 [1H, s, H5(triazole)], 7.36−7.27 (H5, m, Ph), 6.41(1H, dd, J_{cis} = 11.0 Hz, J_{trans} = 17.4 Hz, H19), 5.82 (1H, d, J = 8.5 Hz, H14), 5.34 (1H, d, J_{cis} = 11.0 Hz, H20), 5.21 (1H, d, J_{trans} = 17.4 Hz, H20), 5.10, 5.03 (2H, AB, J = 17.5 Hz, H22), 4.72 (2H, s, PhCH_2O), 4.61 (2H, s, $PhCH_2OCH_2$), 3.35 (1H, dd, J = 6.6, 10.6 Hz, H11), 2.30−2.05 (5H, m, H2, H4, H10, and H13), 1.79−1.09 (9H, m, H1, H6, H7, H8, H13, and 11-OH), 1.35 (3H, s, H15), 1.18 (3H, s, H18), 0.87 (3H, d, J = 7.0 Hz, H17), 0.71 (3H, d, J = 7.0 Hz, H16). ^{13}C NMR ($CDCl_3$) δ 216.7 (C3), 165.2 (C21), 146.0 [C4(triazole)], 138.7 (C19), 137.9, 128.6, 128.0, 127.9 (Ph), 123.9 [C5(triazole)], 117.8 (C20), 74.7 (C11), 72.6 ($PhCH_2OCH_2$), 71.1 (C14), 63.7 ($PhCH_2OCH_2$), 58.1 (C4), 51.7 (C22), 45.5 (C9), 44.8 (C13), 44.1 (C12), 42.0 (C5), 36.7 (C6), 36.2 (C10), 34.5 (C2), 30.5 (C8), 26.9 (C7), 26.5 (C18), 25.0 (C1), 17.0 (C16), 14.8 (C15), 11.6 (C17). HRMS (ESI) m/z 572.3094 [M + Na]$^+$, $C_{32}H_{43}N_3O_5Na$ calcd 572.3096.

*Synthesis of 22-[4-(2-Thymin-1-yl)ethoxymethyl)-1,2,3-triazol-1-yl]-22-deoxypleuromutilin (**25**).* The general procedure was applied with compound **6** (52 mg. 0.13 mmol), 1-[2-(propargyloxy)ethyl]-thymine **15** (25 mg, 0.12 mmol), sodium ascorbate (2.8 mg, 14 μmol) and $CuSO_4{\cdot}5H_2O$ (4.8 mg, 19 μmol) in t-BuOH/H_2O (2 mL, 1:1 v/v). Yield 56 mg, 76%; R_f 0.5 (10% CH_3OH in CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 9.14 (1H, br s, NH), 7.61 [1H, s, H5(triazole)], 7.10 [1H, d, J = 1.2 Hz, H6(T)], 6.41 (1H, dd, J_{cis} = 11.1 Hz, J_{trans} = 17.4 Hz, H19), 5.81 (1H, d, J = 8.5 Hz, H14), 5.32 (1H, dd, J_{gem} = 1.4 Hz, J_{cis} = 11.1 Hz, H20), 5.21 (1H, dd, J_{gem} = 1.4 Hz, J_{trans} = 17.4 Hz, H20), 5.12, 5.05 (2H, AB, J_{AB} = 17.6 Hz, H22), 4.66 (2H, s, N1-$CH_2CH_2OCH_2$), 3.89 (2H, t, J = 4.9 Hz, N1-CH_2CH_2), 3.76 (2H, t, J = 4.9 Hz, N1-CH_2) 3.37 (1H, dd, J = 6.5, 10.4 Hz, H11), 2.31−2.06 (5H, m, H2, H4, H10, and H13), 1.89 [3H, d, J = 1.2 Hz, CH_3(T)], 1.79−1.09 (9H, m, H1, H6, H7, H8, H13, and 11-OH), 1.35 (3H, s, H15), 1.18 (3H, s, H18), 0.88 (3H, d, J = 7.0 Hz, H17), 0.70 (3H, d, J = 7.1 Hz, H16). ^{13}C NMR ($CDCl_3$) δ 216.8 (C3), 165.2, 164.4 [C21, C4(T)], 151.0 [C2(T)], 144.9 [C4(triazole)], 141.8 [C6(T)], 138.7 (C19), 124.0 [C5(triazole)], 117.7 (C20), 110.0 [C5(T)], 74.6 (C11), 71.2 (C14), 68.2 (N1-CH_2), 64.3 (N1-$CH_2CH_2OCH_2$), 58.0 (C4), 51.6 (C22), 48.3 (N1-CH_2CH_2), 45.5 (C9), 44.7 (C13), 44.1 (C12), 41.9 (C5), 36.6 (C6), 36.1 (C10), 34.5 (C2), 30.4 (C8), 26.9 (C7), 26.5 (C18), 24.9 (C1), 16.9 (C16), 14.7 (C15), 12.3 [CH_3(T)], 11.6 (C17). HRMS (ESI) m/z 634.3189 [M + Na]$^+$, $C_{32}H_{45}N_5O_7Na$ calcd 634.3212.

*Synthesis of 22-(1,2,3-Triazol-1-yl)-22-deoxypleuromutilin (**27**).* Azide **6** (50 mg. 0.12 mmol), sodium ascorbate (4.6 mg, 23 μmol), and $CuSO_4{\cdot}5H_2O$ (5.0 mg, 20 μmol) were dissolved in t-BuOH/H_2O (4 mL, 1:1 v/v), and the reaction mixture was degassed for 10 min with argon. (Trimethylsilyl)acetylene **16** (0.026 mL, 0.19 mmol) was added and the reaction mixture was heated at 85 °C for 21 h and then concentrated under reduced pressure. The residue was purified by column chromatography (0−1.5% CH_3OH in CH_2Cl_2), which gave a mixture of compounds **26** and **27.** The mixture was dissolved in anhydrous THF (2 mL) and the solution was degassed for 10 min with argon followed by addition of TBAF (0.05 mL, 0.05 mmol, 1 M in THF). The reaction mixture was stirred at RT for 18 h, followed by partitioning in CH_2Cl_2/10% $NaHCO_3$ (10 mL, 2:1 v/v). The aqueous phase was extracted with CH_2Cl_2 (2 × 5 mL). The organic phases were collected and concentrated under reduced pressure. The residue was purified by column chromatography (0−1.5% CH_3OH in CH_2Cl_2). The product was isolated as a white foam: yield 31 mg, 58%; R_f 0.2 (5% CH_3OH in CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 7.76 [1H, s, CH(triazole)], 7.67 [1H, s, CH(triazole)], 6.41 (1H, dd, J_{cis} = 11.0 Hz, J_{trans} = 17.4 Hz, H19), 5.82 (1H, d, J = 8.5 Hz, H14), 5.33 (1H, dd, J_{gem} = 0.9 Hz, J_{cis} = 11.0 Hz, H20), 5.21 (1H, dd, J_{gem} = 0.9 Hz, J_{trans} = 17.4 Hz, H20), 5.14, 5.08 (2H, AB, J = 17.5 Hz, H22), 3.36 (1H, dd, J = 6.6, 10.3 Hz, H11), 2.30−2.06 (5H, m, H2, H4, H10, and H13), 1.79−1.09 (9H, m, H1, H6, H7, H8, H13, and 11-OH), 1.34 (3H, s, H15), 1.18 (3H, s, H18), 0.88 (3H, d, J = 7.0 Hz, H17), 0.71 (3H, d, J = 7.1 Hz, H16). ^{13}C NMR ($CDCl_3$) δ 216.7 (C3), 165.2 (C21), 138.7 (C19), 134.4 [CH(triazole)], 124.9 [CH(triazole)], 117.7 (C20), 74.6 (C11), 71.1 (C14), 58.1 (C4), 51.5 (C22), 45.5 (C9), 44.8 (C13), 44.1 (C12), 41.9 (C5), 36.6 (C6), 36.2 (C10), 34.5 (C2), 30.4 (C8), 26.9 (C7), 26.5 (C18), 24.9 (C1), 16.9 (C16), 14.7 (C15), 11.6 (C17). HRMS (ESI) m/z 452.2521 [M + Na]$^+$, $C_{24}H_{35}N_3O_4Na$ calcd 452.2520.

*Synthesis of 22-{5-[3-(Adenin-9-yl)propyl]-1,2,3-triazol-1-yl}-22-deoxypleuromutilin (**28**).* A solution of Cp*RuCl(COD) (15 mg, 39 μmol) in toluene (1 mL) was degassed for 5 min with argon. *N*9-(Pent-4-yn-1-yl)adenine[8] (49 mg, 0.24 mmol) and a solution of compound **6** (124 mg, 0.31 mmol) in toluene (2 mL), were added. Five drops of DMF was added and the reaction mixture was stirred in the microwave reactor at 110 °C for 15 min. The reaction mixture was concentrated under reduced pressure, and the residue was absorbed onto Celite and purified by column chromatography (0−10% CH_3OH in CH_2Cl_2) to give the product as a white foam: yield 36 mg, 25%; R_f 0.3 (10% CH_3OH in CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 8.34 [1H, s, H2(A)], 7.78 [1H, s, H8(A)], 7.58 [1H, s, H4(triazole)], 6.36 (1H, dd, J_{cis} = 11.0 Hz, J_{trans} = 17.4 Hz, H19), 6.15 [2H, br s, NH_2(A)], 5.76 (1H, d, J = 8.4 Hz, H14), 5.25 (1H, dd, J_{gem} = 1.4 Hz, J_{cis} = 11.0 Hz, H20), 5.16 (1H, dd, J_{gem} = 1.4 Hz, J_{trans} = 17.4 Hz, H20), 5.07, 4.92 (2H, AB, J = 17.7 Hz, H22), 4.27 (2H, t, J = 6.9 Hz, N9-CH_2), 3.39−3.36 (1H, m, H11), 2.64 (2H, t, J = 7.9 Hz, N9-$CH_2CH_2CH_2$), 2.45−1.94 (8H, m, N9-CH_2CH_2, H2, H4, H10, H13, and 11-OH), 1.77−1.08 (8H, m, H1, H6, H7, H8, and H13), 1.35 (3H, s, H15), 1.16 (3H, s, H18), 0.88 (3H, d, J = 7.0 Hz, H17), 0.64 (3H, d, J = 7.0 Hz, H16). ^{13}C NMR ($CDCl_3$) δ 216.7 (C3), 165.3 (C21), 155.8 [C6(A)], 153.2

[C2(A)], 150.2 [C4(A)], 140.2 [C8(A)], 138.9 (C19), 136.5 [C5(triazole)], 132.3 [C4(triazole)], 119.7 [C5(A)], 117.5 (C20), 74.6 (C11), 71.2 (C14), 58.0 (C4), 49.6 (C22), 45.5 (C9), 44.9 (C13), 44.1 (C12), 43.1 (N9-CH_2), 41.9 (C5), 36.5 (C6), 36.1 (C10), 34.5 (C2), 30.4 (C8), 28.2 (N9-CH_2CH_2), 26.9 (C7), 26.7 (C18), 24.9 (C1), 20.5 (N9-$CH_2CH_2CH_2$), 16.8 (C16), 14.7 (C15), 11.6 (C17). HRMS (ESI) m/z 627.3365 $[M + Na]^+$, $C_{32}H_{44}N_8O_4Na$ calcd 627.3378.

Footprinting Experiments. *E. coli* strain MRE600 was used to prepare ribosomes for the chemical footprinting experiments the same way as described previously.[8] The cells were grown in LB medium and lysed, and then ribosomes were isolated on sucrose gradients. Antibiotic binding and chemical modification, as well as primer extension and gel electrophoresis, were performed as described previously,[8] except that a Cy5-labeled primer (in a higher concentration) was used instead of a 5′-^{32}P-labeled deoxyoligonucleotide primer. The gel bands were visualized and the intensities of the modifications were assessed by use of 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 the background value from unmodified samples was subtracted..

Drug Susceptibility Testing. The following strains and media were used: *E. coli* AS19 in LB medium, *B. subtilis* 168 in 25% BHI/75% LB/0.05% Tween, and *L. innocua* in 50% BHI/50% LB. Drug susceptibility testing was performed in a microtiter plate format, where OD values were measured at 450 nm with a microtiter plate reader (Victor 3, Perkin-Elmer). Medium was inoculated with single colonies and incubated overnight. The cultures were diluted to OD_{450} = 0.01, and 100 μL of diluted culture was mixed with 100 μL of drug solutions in a series with 2-fold concentration steps. The tested concentration ranges were 0.125−32 μg/mL. MIC was defined as the drug concentration at which the growth of the cultures was completely inhibited after 24 h for *E. coli* AS19 and *L. innocua* and after 12 h for *B. subtilis* 168 with incubation at 37 °C.

Docking Procedures. The docking calculations were performed by use of the Schrödinger 2010 program package. A PTC model was constructed by truncating the *D. radiodurans* ribosome structure[5] (PDB entry 1XBP) to contain all residues within a spherical cut of 30 Å from the PTC binding site. This ribosome model was prepared by use of the protein preparation wizard in the Schrödinger software. As a control, a redocking of tiamulin into the PTC model placed the drug in the binding pocket identical to its position in the X-ray structure. All docking experiments were based on the Glide module[23−26] in the Schrödinger 2010 program package in the XP (extra precision) mode. Flexibility was modeled by softening of the van der Waals radii with a scaling factor of 0.8 for the nonpolar parts of the receptor atoms and 0.9 for the ligand atoms. In both cases the charge cutoff was set to 0.2. Ranking of the docked ligands was based on the GlideScore (XP GScore).

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures that are not covered in the main text, for compounds **7**−**13**, **15**, **32**, **34**, **35**, **37**−**39**, and **43**, and selected NMR ^{1}H NMR and ^{13}C NMR spectra as well as 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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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS:

PTC peptidyl transferase center; TBS *tert*-butyldimethylsilyl) ; TBAF tetrabutylammonium fluoride; DEAD diethyl azodicarboxylate; DIAD diisopropyl azodicarboxylate; BSA bis-(trimethylsilyl)acetamide; CMCT *N*1-cyclohexyl-*N*3-(2-morpholino)ethylcarbodiimide *p*-toluenesulfonate

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