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ORIGINAL ARTICLE

5-*O*-Mycaminosyltylonolide antibacterial derivatives: design, synthesis and bioactivity

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Tylosin is a 16-membered macrolide broad-spectrum antibiotic that has an important role in veterinary medicine, active against Gram-positive and a restricted range of Gram-negative bacteria. We synthesized 15 types of tylosin-related derivatives by chemical modification and evaluated them against mastitis pathogens. Among them, 20-deoxy-20-{*N*-methyl-*N*-[1-(3-quinolyl)-1*H*-1,2,3-triazol-4-yl]methylamino}-5-*O*-mycaminosyltylonolide 2f and 20-deoxy-20-{*N*-benzyl-*N*-[1-(3-quinolyl)-1*H*-1,2,3-triazol-4-yl]methylamino}-5-*O*-mycaminosyltylonolide 2k were found to not only expand their antibacterial impact to include Gram-negative bacteria, such as *Escherichia coli* and *Klebsiella pneumoniae*, but also to retain or increase antibacterial activity against Gram-positive bacteria, such as *Staphylococcus aureus* and *Streptococcus uberis* in comparison with the parent tylosin.
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INTRODUCTION

Macrolide antibiotics are recognized as being essential medicines in both human and animal health worldwide.[1] In particular, the 16-membered macrolide, tylosin (TYL, TYLAN®, Figure 1), which was developed by Eli Lilly (Indianapolis, IN, USA), has a significant role in the treatment of infectious diseases, for example, respiratory diseases, mastitis, etc. in animal health.[2] Our research group has been striving to create such antibiotics through chemical modification of naturally occurring microbial metabolites.[3] Thirty years ago, our collaborative research with Eli Lilly resulted in the development of a novel antibiotic, tilmicosin (TLM, MICOTIL®),[4] (http://www.elanco.us/products-services/beef/cattle-brd.aspx) a tylosin derivative, for respiratory diseases of animals. More recently, the Institute of Microbial Chemistry and Merck Animal Health developed a novel antibiotic tildipirosin (ZUPREVO®)[5] (http://www.zuprevo.com) for prevention of bovine respiratory disease, which possesses potent antibacterial activity against *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni*. However, the antibacterial activity of these compounds against *Escherichia coli* and *Klebsiella pneumoniae*, which occasionally cause mastitis, are still unsatisfactory. Mastitis is an infectious disease, found in domestic animals and humans, caused by bacteria, such as *Staphylococcus aureus*, *Streptococcus uberis*, *E. coli* and *K. pneumoniae*. In the dairy industry it is a major problem, leading to lost milk production and substantial economic losses for farmers.[6] Although some antibiotics such as cephem[7] and lincosamide[8] have been used, novel antibiotics are still urgently needed. Therefore, our continuing research efforts have been focused on creating antibiotic macrolides with an expanded spectrum of activity, in particular against Gram-negative bacteria such as *E. coli*.

In this study, we report that 16-membered macrolide tylosin derivatives, 20-deoxy-20-{*N*-methyl-*N*-[1-(3-quinolyl)-1*H*-1,2,3-triazol-4-yl]methylamino}-5-*O*-mycaminosyltylonolide **2f** and 20-deoxy-20-{*N*-benzyl-*N*-[1-(3-quinolyl)-1*H*-1,2,3-triazol-4-yl]methylamino}-5-*O*-mycaminosyltylonolide **2k**, were found to not only exhibit significant activity against *E. coli* and *K. pneumoniae* but also maintained or increased their antibacterial activity against Gram-positive bacteria.

RESULTS AND DISCUSSION

Our primary approach was to introduce a functionalized amino moiety, based on 5-*O*-mycaminosyltylonolide (OMT),[9] in order to increase antibacterial activity, as our structure–activity relationships analyses of 16-membered macrolides were as follows (Table 1, we re-evaluated antibacterial activity of these natural products and their derivatives using our assay system): (1) OMT (MIC 128 μg ml^{-1} against *E. coli*, 64 μg ml^{-1} against *K. pneumoniae*) shows slightly better antibacterial activity against *E. coli* and *K. pneumoniae* than that of tylosin (MIC >128 μg ml^{-1} against *E. coli*, >128 μg ml^{-1} against *K. pneumoniae*), along with similar antibacterial activity against Gram-positive bacteria, for example, *S. aureus*; (2) introduction of amino groups (tildipirosin; MIC 8 μg ml^{-1} against *E. coli*, 16 μg ml^{-1} against *K. pneumoniae*) shows increased antibacterial activity against *E. coli* and *K. pneumoniae* compared with that of OMT (MIC 128 μg ml^{-1} against *E. coli*, 64 μg ml^{-1} against *K. pneumoniae*).

However, tildipirosin (MIC 8–16 μg ml^{-1} against *S. aureus*) does not show stronger activity against Gram-positive bacteria than OMT (MIC 1 μg ml^{-1} against *S. aureus*). This means that introduction of an amino group to OMT loses antibacterial activity against Gram-positive bacteria. With initial structure–activity relationshi in mind, we decided

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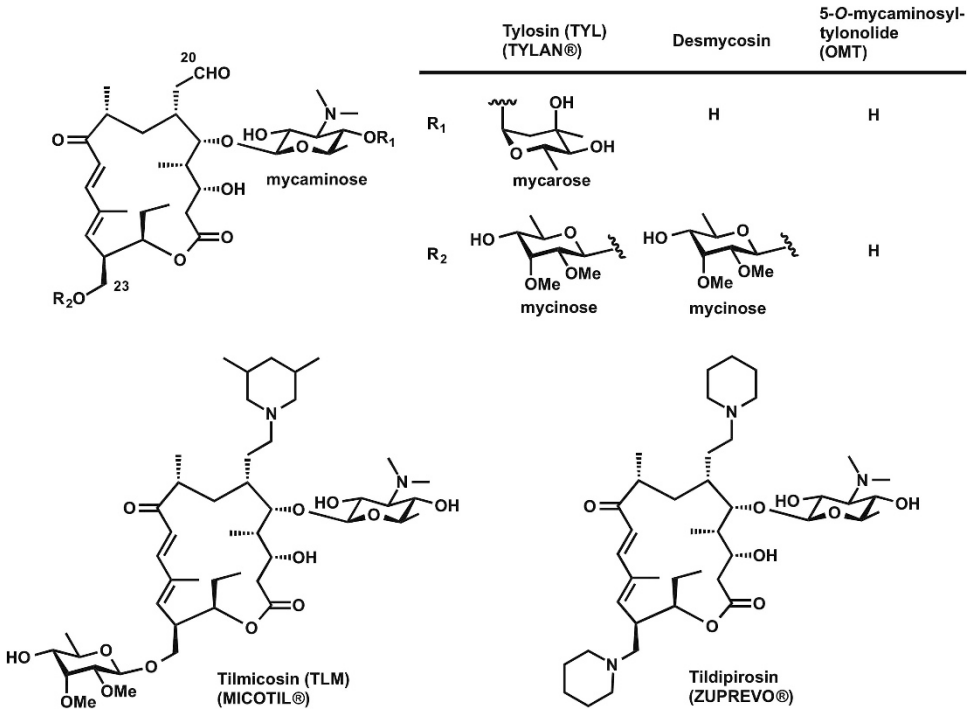


Figure 1 Structures of TYL and related analogues, Desmycosin, OMT, Tilmicosin and Tildipirosin.

to introduce not only some amino groups but also some hydrophobic groups[10] into macrolides.

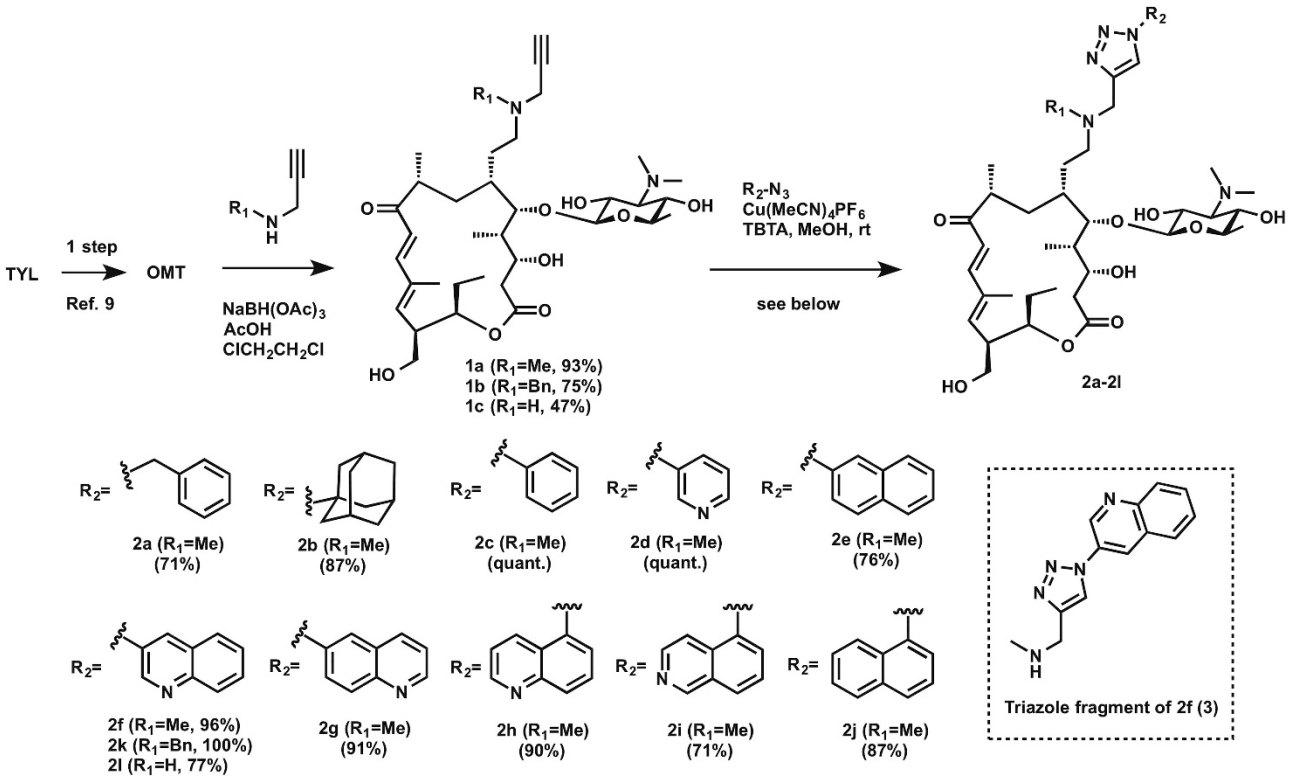
Synthesis and biological evaluation

At the outset, our efforts focused on making derivatives that introduced functionalized amino groups at the C20 position of OMT. We also envisioned that the copper-catalyzed triazole formation[11,12] of an *N*-propargyl compound would allow us to readily search for an appropriate functional group, despite the fact that tylosin has many reactive functional groups, such as alcohol, enone, ester and dimethylamino groups.[13,14] Consequently, we began to investigate appropriate functional groups via a triazole linker, utilizing copper-catalyzed triazole formation, in order to increase antibacterial activity against Gram-positive and -negative bacteria. Reductive amination ($NaBH(OAc)_3$, AcOH, $ClCH_2CH_2Cl$) of OMT, synthesized under acidic condition (0.5 M trifluoroacetic acid (TFA) in H_2O, reflux) from tylosin,[9] with *N*-methylpropargylamine (commercially available), afforded **1a** in 93% yield (Scheme 1). Copper-catalyzed triazole formation (tetrakis(acetonitrile) copper(I) hexafluorophosphate, tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine, MeOH, room temperature) of **1a** with benzyl azide (commercially available), adamantylazide (commercially available), phenyl azide (commercially available), 3-azidopyridine,[15] 2-azidonaphthalene[16] and 3-azidoquinoline[17] readily led to the corresponding triazole products **2a** (71% yield), **2b** (87% yield), **2c** (100% yield), **2d** (100% yield), **2e** (76% yield) and **2f** (96% yield), respectively (Scheme 1). Although we will discuss about biological evaluation later, Table 2 shows that **2f** displayed better antibacterial activity, concentrating our attention on investigating the quinoline moiety position, utilizing copper-catalyzed triazole formation, in order to introduce various quinoline and naphthalene derivatives. As mentioned above, copper-catalyzed triazole formation (tetrakis(acetonitrile) copper(I) hexafluorophosphate, tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine, MeOH, room temperature) of **1a**

Table 1 Antibacterial activity of 16-membered macrolides against 27 type strains

Strain/compound	TYL	Desmycosin	OMT	TLM	Tildipirosin
			MIC μg ml^{-1}		
S. aureus FDA209P[a]	0.5	0.5	1	0.5	8
S. aureus Smith[a]	2	1	1	1	16
MRSA KUB853[b]	>128	>128	>128	>128	>128
MRSA KUB854[b]	>128	>128	>128	>128	>128
MRSA 70[b]	>128	>128	>128	>128	>128
MRSA 92–1191[b]	>128	>128	>128	>128	>128
S. aureus KUB857[c]	1	1	2	0.5	8
S. aureus KUB858[d]	>128	>128	>128	>128	>128
S. aureus KUB859[e]	64	>128	>128	>128	>128
S. aureus KUB860[f]	>128	>128	>128	>128	>128
S. epidermidis KUB795[g]	1	1	2	0.5	4
M. luteus ATCC9341[h]	⩽0.25	⩽0.25	⩽0.25	⩽0.25	⩽0.25
E. faecalis ATCC29212[h]	1	1	1	4	16
E. faecalis NCTC12201[i]	>128	>128	>128	>128	>128
E. faecium NCTC12204[j]	>128	>128	>128	>128	>128
E. coli NIHJ JC-2[h]	>128	>128	128	128	8
C. freundii ATCC8090[h]	>128	>128	>128	>128	32
K. pneumoniae NCTC9632[h]	>128	>128	64	64	16
P. mirabilis IFO3849[h]	>128	>128	>128	>128	>128
P. vulgaris OX-19[h]	>128	>128	>128	>128	>128
M. morganii IID Kono[h]	>128	>128	>128	>128	>128
S.marcescens IFO12648[h]	>128	>128	>128	>128	64
E. cloacae IFO13535[h]	>128	>128	>128	>128	32
E. aerogen NCTC10006[h]	>128	>128	>128	>128	16
P. aeruginosa 46001[h]	>128	>128	128	>128	>128
P. aeruginosa E-2[h]	>128	>128	128	>128	>128
A. calcoaceticus IFO12552[h]	>128	128	>128	64	32

Abbreviation: OMT, 5-*O*-mycaminosyltylonolide.
[a]*S. aureus* FDA209P and Smith: susceptible strains.
[b]MRSA KUB853, MRSA KUB854, MRSA 70, and MRSA 92–1191: MRSA strains isolated from clinical patients.
[c]*S. aureus* KUB857: macrolide resistant strain, encoded by *erm* gene.
[d]*S. aureus* KUB858: macrolide resistant strain, encoded by *erm* gene.
[e]*S. aureus* KUB859: encoded by *erm* gene.
[f]*S. aureus* KUB860: encoded by *erm* and *mef* gene.
[g]*S. epidermidis* KUB795: strains isolated from clinical patients.
[h]Standard strain.
[i]*Enterococcus faecalis* NCTC12201: encoded by *van A* gene.
[j]*E. faecium* NCTC12204: encoded by *van A* gene.



Scheme 1 Synthesis of triazole derivatives at the C20 position of OMT.

with 6-azidoquinoline,[18] 5-azidoquinoline,[19] 5-azidoisoquinoline[20] and 1-azidonaphthalene[21] afforded the corresponding triazole products **2g** (91% yield), **2h** (90% yield), **2i** (71% yield) and **2j** (87% yield), respectively.

We tested analogues **2a–2j** for *in vitro* activity against 27 types of bacteria, including Gram-positive and -negative strains, as well drug-susceptible and drug-resistant organisms (Table 2).[22] The biological evaluation revealed that alkyne **1a** (MIC 64 μg ml^{-1} against *S. aureus*) was significantly less potent against Gram-positive bacteria, compared with OMT (MIC 1 μg ml^{-1} against *S. aureus*). Pleasingly, a triazole derivative, 3-quinolyl **2f** (MIC 0.25 μg ml^{-1} against *S. aureus*, 4 μg ml^{-1} against *E. coli*) was found to be more potent than OMT against both Gram-positive and -negative bacteria. However, the triazole fragment of **2f** (**3**, see Experimental procedure for synthesis.) did not show any activity against all bacteria (MIC >128 μg ml^{-1}), whereas phenyl derivatives **2a**, **2c**, adamantyl **2b**, pyridinyl **2d** and nitrogen-deficient 2-naphthyl **2e** displayed decreased antibacterial activity. The antibacterial activity of **2f** did not increase against resistant strains, for example, MRSA (MIC >64 μg ml^{-1} against methicillin resistant staphylococcus aureus (MRSA)). The quinoline and naphthyl derivatives, **2g–2j**, showed four- to eightfold less activity than **2f**. Consequently, 3-quinolyl **2f** was found to be the most potent derivative.

Our interest then focused on investigation of *N*-substituted derivatives, instead of the *N*-Me group of **2f**. Reductive amination ($NaBH(OAc)_3$, AcOH, $ClCH_2CH_2Cl$) of OMT with *N*-benzylpropargylamine and propargylamine gave **1b** and **1c** in 75% and 47% yields, respectively. *N*-Bn and *N*-H derivatives **2k** and **2l** were prepared with **1b** and **1c** through copper-catalyzed triazole reaction in 100% and 77% yields, respectively (Scheme 1). Bioactivity data indicated that **2k** and **2l** showed almost the same MIC values as **2f**. However, **2l** showed slightly less antibacterial activity, especially against *S. aureus*. Consequently, we concluded that **2f** and **2k** have the best balance in terms of antibacterial activity as a lead compound (Table 2).

With **2k** as a preferred lead compound, we next investigated the effect of mycinose at the C23 position, a neutral sugar and the effect of configuration of the triazole moiety, for example, *anti*-triazole versus *syn*-triazole, with respect to antibacterial activity, respectively (Scheme 2). Reductive amination ($NaBH(OAc)_3$, AcOH, $ClCH_2CH_2Cl$) with desmycosin, prepared from tylosin in one step, and the quinoline triazole **4** (see Experimental procedure for synthesis), synthesized from *N*-benzylpropargylamine via copper-catalyzed triazole formation ($CuSO_4 \cdot 7H_2O$, sodium ascorbate, *t*-BuOH/H_2O), afforded the corresponding triazole derivative **5** in 90% yield, whereas reductive amination ($NaBH(OAc)_3$, AcOH, $ClCH_2CH_2Cl$) with OMT and quinoline *syn*-triazole **6** (see Experimental procedure for synthesis), synthesized from *N*-benzylpropargylamine via thermal condition, afforded the corresponding *syn*-triazole derivative **7** in 96% yield.

Bioactivity data indicated that the mycinose-attached compound **5** possessed significantly reduced antibacterial activity against *E. coli* and *K. pneumoniae*, compared with **2k** (Table 2). Likewise, *syn*-triazole **7** (MIC 4 μg ml^{-1} against *S. aureus*, >64 μg ml^{-1} against *E. coli*) showed less activity than the *anti*-triazole **2k**, suggesting that *anti*-triazole configuration may be necessary for producing antibacterial activity. Taken together, we concluded that the hydroxyl group at the C23 position and an *anti*-triazole moiety are essential.

Overall, structure–activity relationship in this study were highlighted as follows (Figure 2): (1) 3-quinoline has a key role for antibacterial activity against both Gram-positive and -negative bacteria; (2) *anti*-triazole is better than *syn*; (3) *N*-Me or *N*-Bn substitution are more suitable than *N*-H substitution; (4) mycinose removal affects increased activity.

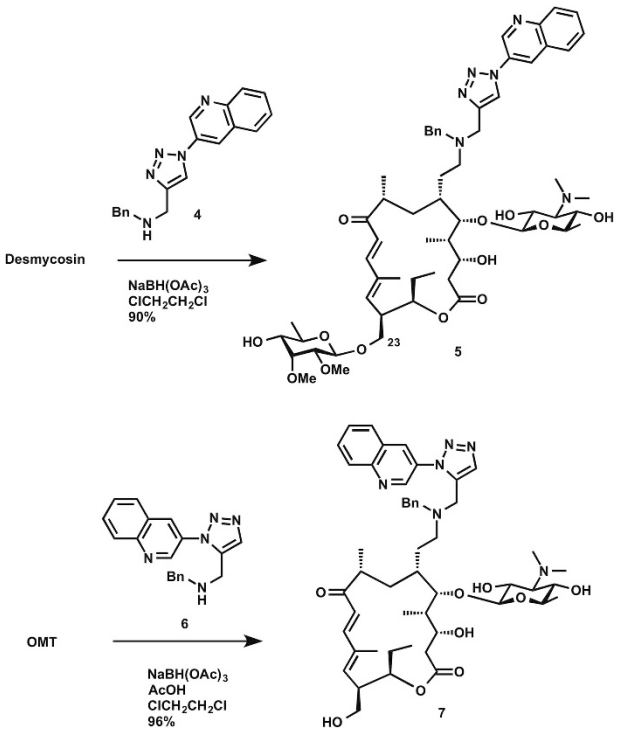
Table 2 Antibacterial activity of 1a–1c, 2a—2l, 3, 5 and 7 against 27 type strains

Strain/compound	***1a***	***1b***	***1c***	***2a***	***2b***	***2c***	***2d***	***2e***	***2f***
					MIC μg ml^{-1}				
S. aureus FDA209P[a]	64	8	32	32	16	2	8	0.5	0.25
S. aureus Smith[a]	32	8	32	16	8	2	8	0.5	0.25
MRSA KUB853[b]	>64	>64	>64	>64	>64	>64	>64	>64	>64
MRSA KUB854[b]	>64	>64	>64	>64	>64	>64	>64	>64	>64
MRSA 70[b]	>64	>64	>64	>64	>64	>64	>64	>64	>64
MRSA 92–1191[b]	>64	>64	>64	>64	>64	>64	>64	>64	>64
S. aureus KUB857[c]	64	4	64	32	64	4	16	1	0.25
S. aureus KUB858[d]	>64	>64	>64	>64	>64	64	>64	>64	>64
S. aureus KUB859[e]	>64	>64	>64	>64	>64	>64	>64	>64	>64
S. aureus KUB860[f]	>64	>64	>64	>64	>64	>64	>64	>64	>64
S. epidermidis KUB795[g]	64	4	64	32	32	2	8	0.5	0.25
M. luteus ATCC9341[h]	4	1	4	1	2	0.25	0.5	⩽0.125	⩽0.125
E. faecalis ATCC29212[h]	>64	16	>64	32	32	2	8	0.25	⩽0.125
E. faecalis NCTC12201[i]	>64	>64	>64	>64	>64	>64	>64	>64	>64
E. faecium NCTC12204[j]	>64	>64	>64	>64	>64	>64	>64	>64	>64
E. coli NIHJ JC-2[h]	>64	>64	>64	32	64	16	32	8	4
C. freundii ATCC8090[h]	>64	>64	>64	>64	>64	32	64	32	16
K. pneumoniae NCTC9632[h]	>64	>64	>64	16	64	8	16	4	1
P. mirabilis IFO3849[h]	>64	>64	>64	>64	>64	>64	>64	>64	64
P. vulgaris OX-19[h]	>64	>64	>64	>64	>64	64	>64	32	8
M. morganii IID Kono[h]	>64	>64	>64	>64	>64	>64	>64	64	32
S. marcescens IFO12648[h]	>64	>64	>64	>64	>64	64	>64	32	32
E. cloacae IFO13535[h]	>64	>64	>64	>64	>64	>64	>64	32	32
E. aerogen NCTC10006[h]	>64	>64	>64	>64	>64	32	64	16	16
P. aeruginosa 46001[h]	>64	>64	>64	>64	>64	>64	>64	>64	>64
P. aeruginosa E-2[h]	>64	>64	>64	>64	>64	>64	>64	>64	>64
A. calcoaceticus IFO12552[h]	>64	>64	>64	64	>64	8	32	8	4

Strain/compound	***2g***	***2h***	***2i***	***2j***	***2k***	***2l***	***3***	***5***	***7***
					MIC μg ml^{-1}				
S. aureus FDA209P[a]	1	2	4	1	⩽0.125	0.5	>128	0.5	4
S. aureus Smith[a]	1	2	2	0.5	0.25	0.5	>128	1	4
MRSA KUB853[b]	>64	>64	>64	>64	>64	>64	>128	>128	>64
MRSA KUB854[b]	>64	>64	>64	>64	>64	>64	>128	>128	>64
MRSA 70[b]	>64	>64	>64	>64	>64	>64	>128	>128	>64
MRSA 92–1191[b]	>64	>64	>64	>64	>64	>64	>128	>128	>64
S. aureus KUB857[c]	2	4	8	2	0.25	2	>128	1	4
S. aureus KUB858[d]	>64	>64	>64	>64	>64	>64	>128	>128	>64
S. aureus KUB859[e]	>64	>64	>64	>64	>64	>64	>128	>128	>64
S. aureus KUB860[f]	>64	>64	>64	>64	>64	>64	>128	>128	>64
S. epidermidis KUB795[g]	1	4	8	2	0.25	2	>128	0.5	4
M. luteus ATCC9341[h]	⩽0.125	0.25	0.5	⩽0.125	⩽0.125	0.25	>128	⩽0.25	0.5
E. faecalis ATCC29212[h]	0.5	2	4	1	0.25	0.25	>128	1	4
E. faecalis NCTC12201[i]	>64	>64	>64	>64	>64	>64	>128	>128	>64
E. faecium NCTC12204[j]	>64	>64	>64	>64	>64	>64	>128	>128	>64
E. coli NIHJ JC-2[h]	16	32	32	16	16	8	>128	>128	>64
C. freundii ATCC8090[h]	64	64	>64	64	>64	32	>128	>128	>64
K. pneumoniae NCTC9632[h]	8	16	32	8	8	4	>128	128	>64
P. mirabilis IFO3849[h]	>64	>64	>64	>64	64	>64	>128	>128	>64
P. vulgaris OX-19 [h]	64	>64	>64	64	64	32	>128	>128	>64
M. morganii IID Kono[h]	>64	>64	>64	>64	>64	>64	>128	64	>64
S. marcescens IFO12648[h]	>64	>64	>64	>64	>64	>64	>128	>128	>64
E. cloacae IFO13535[h]	64	>64	>64	>64	64	32	>128	>128	>64
E. aerogen NCTC10006[h]	32	>64	>64	32	32	32	>128	>128	>64
P. aeruginosa 46001[h]	>64	>64	>64	>64	>64	>64	>128	>128	>64
P. aeruginosa E-2[h]	>64	>64	>64	>64	>64	>64	>128	>128	>64
A. calcoaceticus IFO12552[h]	16	32	32	16	16	32	>128	128	>64

[a]*S. aureus* FDA209P and Smith: susceptible strains.
[b]MRSA KUB853, MRSA KUB854, MRSA 70 and MRSA 92–1191: MRSA strains isolated from clinical patients.
[c]*S. aureus* KUB857: macrolide resistant strain, encoded by *erm* gene.
[d]*S. aureus* KUB858: macrolide resistant strain, encoded by *erm* gene.
[e]*S. aureus* KUB859: encoded by *erm* gene.
[f]*S. aureus* KUB860: encoded by *erm* and *mef* gene.
[g]*S. epidermidis* KUB795: strains isolated from clinical patients.
[h]Standard strain.
[i]*E. faecalis* NCTC12201: encoded by *van A* gene.
[j]*E. faecium* NCTC12204: encoded by *van A* gene.

Finally, to create tylosin-based antibiotics for mastitis, we examined the antibacterial spectrum of **2f** and **2k** against pathogens from bovine mastitis (Table 3). In general, **2f** and **2k** were more potent than TLM. With respect to Staphylococci (*S. aureus* and coagulase-negative staphylococci), **2f** showed slightly better activity than TLM, whereas **2k** showed significantly stronger activity than TLM. Especially, the MIC_{90} (<0.03 μg ml^{-1}) of **2k** was *ca.* 30-fold greater than the MIC_{90} (1 μg ml^{-1}) of TLM. In terms of streptococci (*S. uberis*, *Streptococcus dysgalactiae* and *Streptococcus agalactiae*), **2f** and **2k** exhibited almost the same activity, or better, compared with TLM. In terms of *Arcanobacterium pyogenes*, the MIC_{50} (0.004 μg ml^{-1}) of **2f** showed a *ca.* 30-fold increase compared with the MIC_{50} (0.125 μg ml^{-1}) of TLM. Of note, the activity against *E. coli* and *K. pneumoniae* of **2f** was elevated 8- to 16-fold above that of TLM (MIC_{50} 64–128 μg ml^{-1} against *E. coli* and *K. pneumoniae*), whereas **2k** exhibited 4- to 8-fold stronger antibacterial activity against *E. coli* than TLM (MIC_{50}



Scheme 2 Synthesis of **5** and **7**.

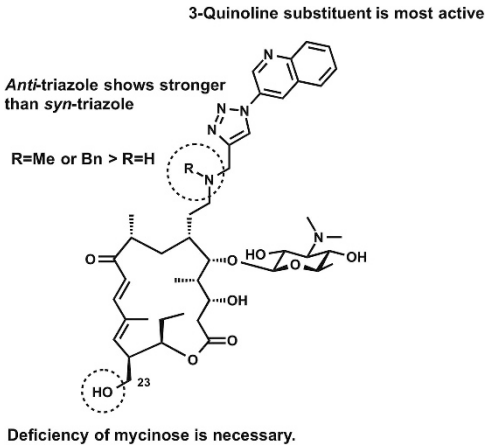


Figure 2 Structure–activity relationship (SAR) maps.

Table 3 Antibacterial activity of TLM, 2f and 2k for mastitis pathogens

Strain/compound		*TLM*	***2f***	***2k***
			MIC μg ml^{-1}	
S.aureus ($n=12$)	Range	0.5–1	0.125–2	⩽0.03
	MIC_{50}	0.5	0.25	⩽0.03
	MIC_{90}	1	0.5	⩽0.03
CNS ($n=10$)	Range	0.25–1	⩽0.03–1	⩽0.03
	MIC_{50}	0.5	0.25	⩽0.03
	MIC_{90}	1	0.25	⩽0.03
S.uberis ($n=12$)	Range	2->4	0.015–0.5	0.03–0.25
	MIC_{50}	>4	0.03	0.06
	MIC_{90}	>4	0.06	0.125
S. dysgalactiae ($n=10$)	Range	0.25–1	0.03–0.25	0.06–0.125
	MIC_{50}	0.25	0.03	0.06
	MIC_{90}	0.25	0.06	0.06
S. agalactiae ($n=10$)	Range	2->4	0.004->4	0.03->4
	MIC_{50}	>4	0.015	0.06
	MIC_{90}	>4	>4	>4
A. pyogenes ($n=10$)	Range	0.125–4	0.004->4	0.004->4
	MIC_{50}	0.125	0.004	0.008
	MIC_{90}	1	>4	>4
E. coli ($n=11$)	Range	64–128	4–8	8–16
	MIC_{50}	64	8	16
	MIC_{90}	128	8	16
K. pneumonia ($n=10$)	Range	128	8–16	16–32
	MIC_{50}	128	16	32
	MIC_{90}	128	16	32

Abbreviation: CNS, coagulase-negative staphylococci.

64 μg ml^{-1}). Both the MIC_{50} and MIC_{90} of **2f** tends to be higher than those for **2k** against Gram-negative bacteria.

CONCLUSION

In conclusion, we developed several novel tylosin derivatives with a view to identifying a good lead compound for the development of a novel treatment for veterinary mastitis and clarified structure–activity relationships of the compounds. The antibacterial spectrum of **2f** and **2k** expands with regard to Gram-negative bacteria, such as *E. coli* and *K. pneumoniae*, compared with the parent compound tylosin. In addition, antibacterial activity against Gram-positive bacteria is retained or enhanced. Although the purpose of this study was to develop veterinary medicines, we believe that the results provide a useful insight for drug design, with respect to 16-membered macrolide antibiotics, for both human and animal health.

EXPERIMENTAL PROCEDURES

General methods

Analytical and preparative thin layer chromatography separations were performed using pre-coated silica gel plates with a fluorescent indicator (Merck 60 F254, Merck KGaA, Darmstadt, Germany). Flash column chromatography was performed using Kanto Chemical (60N, spherical neutral, 0.040–0.050 mm, catalog number 37563–84, Tokyo, Japan) or Merck silica gel (60N, 230–400 mesh ASTM 0.040–0.063 mm, catalog number 109385). ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, using a JEOL ECA-500 spectrometer (500 MHz, JEOL Ltd., Tokyo, Japan). Chemical shifts are expressed in p.p.m. using internal solvent peaks for $CDCl_3$ (^{1}H NMR: 7.26 p.p.m.; ^{13}C NMR: 77.16 p.p.m.) and CD_3OD (^{1}H NMR: 3.31 p.p.m.; ^{13}C NMR: 49.0 p.p.m.) as references. *J*-values are given in hertz. Coupling patterns are expressed as s (singlet), d (doublet), dd (double doublet), ddd (double double doublet), t (triplet), dt (double triplet), q (quartet), m (multiplet) or br (broad). All infrared spectra were measured using a Horiba FT-210 spectrometer (Horiba Ltd., Kyoto, Japan). High- and low-resolution mass spectra were acquired using JEOL JMS-700 MStation and JEOL JMS-T100LP instruments. Melting points were determined using a Yanaco Micro Melting Point System MP-500P (Anatec Yanaco Corporation, Kyoto, Japan).

Chemicals

All reagents were directly used as purchased, without further purification, unless otherwise noted. All new compounds were synthesized at the Kitasato Institute for Life Sciences, Kitasato University. ^{1}H and ^{13}C NMR charts of all new compounds are reported in the Supplementary Information.

Antibacterial activity measurement

Antibacterial activities (Tables 1 and 2) of tylosin derivatives against *S. aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Enterococcus faecalis*, *Enterococcus faecium*, *E. coli*, *Citrobacter freundii*, *K. pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Morganella morganii*, *Serratia marcescens*, *Enterobacter cloacae*, *Enterobacter aerogen*, *Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus* were investigated using the National Committee for Clinical Laboratory Standards method. (National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved standard M31-A. NCCLS, Wayne, PA (1999).).

Antibacterial activities (Table 3) of tylosin derivatives against bovine mastitis isolates were determined by microbroth dilution methodology according to Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved standard VET01, Clinical and Laboratory Standards Institute; Wayne, PA, USA).

Experimental procedures and compound characterization

General procedure of reductive amination. To a solution of OMT or desmycosin in 1,2-dichloroethane (0.1 M for the starting materials) at room temperature was added amines (1.5 equiv.), $NaBH(OAc)_3$ (1.5 equiv.) and AcOH (3.0 equiv.). The reaction mixture was stirred at room temperature until the starting material was consumed. To the mixture was added saturated aqueous $NaHCO_3$. The resulting mixture was extracted with $CHCl_3$. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by silica gel chromatography ($CHCl_3$/MeOH/30% NH_3 aq.) to give the corresponding amine derivatives.

General procedure of triazole formation

To a solution of alkyne and azide derivatives (1.2–1.5 equiv.) in MeOH (0.1 M for alkyne) at room temperature were added tetrakis(acetonitrile) copper(I) hexafluorophosphate (0.5 mol%) and tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl) methyl]amine (0.5 mol%). The reaction mixture was stirred at room temperature until the starting material was consumed. To the mixture was added saturated aqueous $NaHCO_3$. The resulting mixture was extracted with $CHCl_3$. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by silica gel chromatography ($CHCl_3$/MeOH/30% NH_3 aq.) to give triazole derivatives.

20-Deoxy-20-(*N*-methyl-*N*-propargylamino)-5-*O*-mycaminosyltylonolide (1a)

According to the general procedure of reductive amination, OMT (1.00 g, 1.67 mmol) and *N*-methylpropargylamine (209 μl, 2.51 mmol) were converted to **1a** (1.01 g, 93%) as a pale yellow solid.

Mp: 100.6–102.0 °C; $[\alpha]\ ^{22}_{D}$ −7.4 (*c* 1.0, $CHCl_3$); IR (KBr) cm^{-1}: 3423, 2935, 1736, 1061, 756; ^{1}H NMR (500 MHz, CD_3OD) δ (p.p.m.): 7.30 (d, *J*=15.5 Hz, 1H), 6.48 (d, *J*=15.5 Hz, 1H), 5.93 (d, *J*=10.3 Hz, 1H), 4.94 (m, 1H), 4.28 (d, *J*=7.5 Hz, 1H), 3.77 (d, *J*=10.3 Hz, 1H), 3.69–3.67 (complex m, 2H), 3.60 (d, *J*=9.7 Hz, 1H), 3.37–3.25 (complex m, 4H), 3.13 (m, 1H), 2.86 (m, 1H), 2.64 (t, *J*=2.3 Hz, 1H), 2.62–2.58 (complex m, 2H), 2.51 (s, 6H), 2.47–2.38 (complex m, 3H), 2.27 (s, 3H), 2.05 (d, *J*=16.6 Hz, 1H), 1.89 (m, 1H), 1.86 (s, 3H), 1.80–1.75 (complex m, 2H), 1.69–1.50 (complex m, 4H), 1.42 (m, 1H), 1.26 (d, *J*=5.7 Hz, 3H), 1.22 (d, *J*=6.9 Hz, 3H), 1.03 (d, *J*=6.9 Hz, 3H), 0.95 (t, *J*=7.2 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ (p.p.m.): 206.6, 174.4, 149.6, 144.3, 136.5, 119.7, 105.5, 80.9, 78.8, 76.1, 75.3, 74.3, 72.6, 71.8, 71.7, 68.3, 62.6, 54.2, 48.2, 46.5, 45.6, 42.9, 42.2 (2C), 41.9, 40.6, 35.5, 35.0, 26.7, 26.2, 18.3, 17.9, 13.3, 10.0, 9.6; HRMS (ESI) *m/z*: 651.4202 $[M+H]^+$, calcd. for $C_{35}H_{59}N_2O_9$: 651.4221.

20-Deoxy-20-(*N*-benzyl-*N*-propargylamino)-5-*O*-mycaminosyltylonolide (1b)

According to the general procedure of reductive amination, OMT (1.63 g, 2.72 mmol) and crude *N*-benzylpropargylamine (4.08 mmol) were converted to **1b** (1.59 g, 75%) as a pale yellow solid.

Mp: 87.7–89.1 °C; $[\alpha]\ ^{25}_{D}$ −27.0 (*c* 1.0, $CHCl_3$); IR (Diamond prism) cm^{-1}: 3406 (br), 2927, 1716, 1589, 1057, 741; ^{1}H NMR (500 MHz, CD_3OD) δ (p.p.m.): 7.39–7.34 (complex m, 4H), 7.28 (m, 1H), 7.24 (d, *J*=15.5 Hz, 1H), 6.50 (d, *J*=15.5 Hz, 1H), 6.00 (d, *J*=10.3 Hz, 1H), 5.04 (m, 1H), 4.22 (d, *J*=7.5 Hz, 1H), 3.90 (d, *J*=10.3 Hz, 1H), 3.77–3.65 (complex m, 4H), 3.41 (d, *J*=12.6 Hz, 1H), 3.34 (m, 1H), 3.25–3.14 (complex m, 3H), 3.11 (app t, *J*=9.5 Hz, 1H), 2.88 (m, 1H), 2.76 (m, 1H), 2.69–2.61 (complex m, 2H), 2.57–2.45 (complex m, 3H), 2.51 (s, 6H), 2.38 (app t, *J*=9.7 Hz, 1H), 2.09 (d, *J*=16.6 Hz, 1H), 1.96–1.48 (complex m, 8H), 1.88 (s, 3H), 1.22–1.92 (complex m, 6H), 1.05 (d, *J*=6.9 Hz, 3H), 0.97 (t, *J*=7.2 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ (p.p.m.): 206.6, 174.4, 149.5, 144.0, 139.3, 136.6, 130.7 (2C), 129.5 (2C), 128.2, 119.9, 105.4, 80.4, 78.9, 76.0, 75.3, 74.2, 72.6, 71.71, 71.68, 68.5, 62.5, 58.0, 51.9, 48.0, 46.5, 42.7, 42.2 (2C), 42.1, 40.7, 34.8 (2C), 26.4, 26.2, 18.2, 18.0, 13.4, 10.1, 9.6; HRMS (ESI) *m/z*: 727.4532 $[M+H]^+$, calcd. for $C_{41}H_{63}N_2O_9$: 727.4534.

20-Deoxo-20-*N*-propargylamino-5-*O*-mycaminosyltylonolide (1c)

According to the general procedure of reductive amination, OMT (100 mg, 0.167 mmol) and propargylamine (16.1 μl, 0.251 mmol) were converted to **1c** (50.4 mg, 47%) as a pale yellow solid.

Mp: 106.6–107.8 °C; $[\alpha]\ ^{25}_{D}$ −6.0 (*c* 1.0, $CHCl_3$); IR (Diamond prism) cm^{-1}: 3417(br), 2935, 1716, 1589, 1057, 752; ^{1}H NMR (500 MHz, CD_3OD) δ (p.p.m.): 7.31 (d, *J*=15.5 Hz, 1H), 6.47 (d, *J*=15.5 Hz, 1H), 5.93 (d, *J*=10.9 Hz, 1H), 4.95 (m, 1H), 4.29 (d, *J*=7.5 Hz, 1H), 3.76–3.63 (complex m, 4H), 3.39–3.33 (complex m, 2H), 3.25 (m, 1H), 3.14 (m, 1H), 2.86 (m, 1H), 2.75 (m, 1H), 2.67 (m, 1H), 2.61 (m, 1H), 2.52 (s, 6H), 2.49–2.39 (complex m, 3H), 2.05 (d, *J* =17.2 Hz, 1H), 1.95–1.38 (complex m, 8H), 1.86 (s, 3H), 1.26 (d, *J*=5.7 Hz, 3H), 1.22 (d, *J*=6.9 Hz, 3H), 1.04 (d, *J*=6.9 Hz, 3H), 0.95 (t, *J*=7.5 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ (p.p.m.): 206.7, 174.8, 149.8, 144.3, 136.6, 119.7, 105.4, 82.1, 80.7, 76.3, 74.3, 73.3, 72.6, 71.7 (2C), 68.1, 62.6, 48.3, 46.9, 46.5, 42.4, 42.2 (2C), 40.7, 38.0, 35.8, 34.7, 28.8, 26.2, 18.3, 17.9, 13.2, 10.0, 9.7; HRMS (ESI^+) *m/z*: 637.4073 $[M+H]^+$, calcd. for $C_{34}H_{57}N_2O_9$: 637.4064.

20-Deoxy-20-[*N*-methyl-*N*-(1-benzyl-1*H*-1,2,3-triazol-4-yl) methylamino]- 5-*O*-mycaminosyltylonolide (2a)

According to the general procedure of triazole formation, **1a** (100 mg, 0.154 mmol) and azidomethyl benzene (30.8 mg, 0.231 mmol) were converted to **2a** (86.1 mg, 71%) as a pale yellow solid.

Mp: 106.4–108.2 °C; $[\alpha]\ ^{23}_{D}$ −55.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD) δ (p.p.m.): 8.31 (s, 1H), 7.39–7.30 (complex m, 5H), 7.18 (d, *J*=15.5 Hz, 1H), 6.49 (d, *J*=15.5 Hz, 1H), 5.85 (d, *J*=10.3 Hz, 1H), 5.68 (s, 2H), 4.97 (m, 1H), 4.20 (d, *J*=7.5 Hz, 1H), 3.86 (d, *J*=8.6 Hz, 1H), 3.80 (d, *J*=13.8, 1H), 3.66–3.65 (complex m, 2H), 3.54 (d, *J*=10.3 Hz, 1H), 3.36–3.32 (complex m, 2H), 3.18 (m, 1H), 3.12 (m, 1H), 2.86 (m, 1H), 2.80 (m, 1H), 2.64 (m, 1H), 2.51 (s, 6H), 2.49–2.37 (complex m, 2H), 2.22 (m, 1H), 2.09 (d, *J*=18.3 Hz, 1H), 2.07 (s, 3H), 1.91 (m, 1H), 1.85 (s, 3H), 1.83–1.50 (complex m, 7H), 1.21 (d, *J*=6.3 Hz, 3H), 1.19 (d, *J*=5.7 Hz, 3H), 1.02 (d, *J*=6.9 Hz, 3H), 0.96 (t, *J*=7.2 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ (p.p.m.): 206.4, 174.4, 149.6, 145.6, 144.6, 137.2, 136.4, 130.0 (2C), 129.4, 128.8 (2C), 125.8, 119.5, 105.6, 80.4, 76.2, 74.2, 72.6, 71.73, 71.65, 68.4, 62.4, 55.7, 54.8, 52.5, 48.2, 46.6, 43.0, 42.4, 42.2 (2C), 40.4, 34.9, 33.9, 26.1 (2C), 18.2, 17.9, 13.3, 10.1, 9.7; HRMS (ESI^+) *m/z*: 806.4673 $[M+Na]^+$, calcd. for $C_{42}H_{65}N_5NaO_9$: 806.4680.

20-Deoxy-20-{*N*-methyl-*N*-[1-(1-adamantyl)-1*H*-1,2,3-triazol-4-yl] methylamino}-5-*O*-mycaminosyltylonolide (2b)

According to the general procedure of triazole formation, **1a** (100 mg, 0.154 mmol) and 1-azidoadamantane (40.9 mg, 0.230 mmol) were converted to **2b** (111 mg, 87%) as a pale yellow solid.

Mp: 137.0–139.0 °C; $[\alpha]\ ^{26}_{D}$ −10.9 (*c* 1.0, $CHCl_3$); IR (Diamond prism) cm^{-1}: 2916, 1732, 1169, 1057, 748; ^{1}H NMR (500 MHz, CD_3OD) δ (p.p.m.): 8.20 (s, 1H), 7.29 (d, *J*=15.5 Hz, 1H), 6.51 (d, *J*=15.5 Hz, 1H), 5.93 (d, *J*=10.9 Hz, 1H), 4.51 (dt, *J*=2.3, 9.6 Hz, 1H), 4.16 (d, *J*=7.5 Hz, 1H), 3.83 (d, *J*=9.7 Hz, 1H), 3.78 (d, *J*=13.2 Hz, 1H), 3.68 (d, *J*=5.5 Hz, 1H), 3.61 (q, *J*=6.9, 7.5 Hz, 1H), 3.50 (d, *J*=10.3 Hz, 1H), 3.44 (d, *J*=13.2 Hz, 1H),

3.34 (m, 2H), 3.16 (m, 1H), 3.12 (app t, $J=9.2$ Hz, 1H), 2.88 (m, 1H), 2.73 (m, 1H), 2.65 (m, 1H), 2.51 (s, 6H), 2.44 (dd, $J=10.0$, 16.9 Hz, 1H), 2.38 (t, $J=10.0$ Hz, 1H), 2.33–2.16 (complex m, 9H), 2.11 (s, 3H), 2.08 (d, $J=16.6$ Hz, 1H), 1.95–1.45 (complex m, 18H), 1.22 (d, $J=6.9$ Hz, 3H), 1.19–1.16 (complex m, 6H), 1.03 (d, $J=6.9$ Hz, 3H), 0.96 (t, $J=7.5$ Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ (p.p.m.): 206.5, 174.2, 149.6, 144.7, 143.9, 136.4, 122.2, 119.5, 105.7, 80.7, 76.2, 74.3, 72.6, 71.74, 71.69, 68.4, 62.5, 61.2, 55.5, 53.2, 48.3, 46.6, 43.9 (3C), 43.1, 42.2 (2C), 42.1, 40.5, 37.0 (3C), 35.1, 34.3, 31.0 (3C), 26.3, 26.1, 18.3, 17.9, 13.2, 10.1, 9.7; HRMS (ESI) *m/z*: 828.5474 $[M+H]^+$, calcd. for $C_{45}H_{74}N_5O_9$: 828.5487.

20-Deoxy-20-[*N*-methyl-*N*-(1-phenyl-1*H*-1,2,3-triazol-4-yl) methylamino]-5-*O*-mycaminosyltylonolide (2c)

According to the general procedure of triazole formation, **1a** (100 mg, 0.154 mmol) and phenyl azide (22.0 mg, 0.184 mmol) were converted to **2c** (120 mg, 100%) as a pale yellow solid.

Mp: 109.1–113.9 °C; $[\alpha]\ ^{26}_D$ −30.3 (*c* 1.0, $CHCl_3$); IR (Diamond prism) cm^{-1}: 3352 (br), 2931, 1732, 1589, 1061, 756; ^{1}H NMR (500 MHz, CD_3OD) δ (p.p.m.): 8.67 (s, 1H), 7.95 (m, 2H), 7.60 (m, 2H), 7.50 (m, 1H), 7.21 (d, $J=15.5$ Hz, 1H), 6.48 (d, $J=15.5$ Hz, 1H), 5.76 (d, $J=10.3$ Hz, 1H), 4.84 (m, 1H), 4.20 (d, $J=7.5$ Hz, 1H), 3.87–3.84 (complex m, 2H), 3.65–3.50 (complex m, 4H), 3.34 (dd, $J=7.5$, 10.3 Hz, 1H), 3.18 (m, 1H), 3.11 (app t, $J=9.5$ Hz, 1H), 2.87–2.77 (complex m, 2H), 2.66 (m, 1H), 2.50 (s, 6H), 2.44 (m, 1H), 2.35 (m, 1H), 2.27 (m, 1H), 2.19 (s, 3H), 2.06 (d, $J=16.6$ Hz, 1H), 1.88 (m, 1H), 1.84 (s, 3H), 1.82–1.50 (complex m, 7H), 1.21 (d, $J=6.9$ Hz, 3H), 1.20 (d, $J=5.7$ Hz, 3H), 1.02 (d, $J=6.9$ Hz, 3H), 0.94 (t, $J=7.5$ Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ (p.p.m.): 206.5, 174.2, 149.6, 146.0, 144.8, 138.5, 136.3, 130.8 (2C), 130.0, 123.7, 122.1 (2C), 119.4, 105.6, 80.6, 76.2, 74.3, 72.6, 71.7, 71.6, 68.3, 62.5, 55.8, 52.8, 48.3, 46.6, 43.0, 42.4, 42.2 (2C), 40.4, 35.0, 34.3, 26.2, 26.1, 18.2, 17.9, 13.2, 10.1, 9.7; HRMS (ESI) *m/z*: 792.4521 [M+Na], calcd. for $C_{41}H_{63}N_5O_9$: 792.4524.

20-Deoxy-20-{*N*-methyl-*N*-[1-(3-pyridinyl)-1*H*-1,2,3-triazol-4-yl] methylamino}-5-*O*-mycaminosyltylonolide (2d)

According to the general procedure of triazole formation, **1a** (100 mg, 0.154 mmol) and 3-azidopyridine (25.0 mg, 0.208 mmol) were converted to **2d** (118 mg, 100%) as a pale yellow solid.

Mp: 116.0–119.2 °C; $[\alpha]\ ^{26}_D$ −138.6 (*c* 1.0, $CHCl_3$); IR (Diamond prism) cm^{-1}: 3402 (br), 2931, 1732, 1589, 1169, 1061, 752; ^{1}H NMR (500 MHz, CD_3OD) δ (p.p.m.): 9.21 (s, 1H), 8.79 (s, 1H), 8.68 (d, $J=4.6$ Hz, 1H), 8.44 (m, 1H), 7.69 (m, 1H), 7.18 (d, $J=15.5$ Hz, 1H), 6.48 (d, $J=15.5$ Hz, 1H), 5.74 (d, $J=10.3$ Hz, 1H), 4.81 (m, 1H), 4.23 (d, $J=7.5$ Hz, 1H), 3.88–3.84 (complex m, 2H), 3.66–3.50 (complex m, 4H), 3.35 (m, 1H), 3.22 (m, 1H), 3.13 (m, 1H), 2.87–2.79 (complex m, 2H), 2.65 (m, 1H), 2.50 (s, 6H), 2.43 (m, 1H), 2.38 (m, 1H), 2.29 (m, 1H), 2.18 (s, 3H), 2.07 (d, $J=16.6$ Hz, 1H), 1.88 (m, 1H), 1.84 (s, 3H), 1.82–1.52 (complex m, 7H), 1.22 (d, $J=6.9$ Hz, 3H), 1.21 (d, $J=7.5$ Hz, 3H), 1.02 (d, $J=6.9$ Hz, 3H), 0.94 (t, $J=7.2$ Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ (p.p.m.): 206.4, 174.4, 150.5, 149.6, 146.6, 144.8, 143.0, 136.3, 135.6, 130.5, 126.0, 123.9, 119.4, 105.6, 80.6, 76.3, 74.3, 72.6, 71.70, 71.65, 68.3, 62.5, 56.0, 52.7, 48.3, 46.6, 43.0, 42.5, 42.2 (2C), 40.4, 34.9, 34.2, 26.2, 26.1, 18.3, 17.9, 13.2, 10.1, 9.7; HRMS (ESI) *m/z*: 793.4473 $[M+Na]^+$, calcd. for $C_{40}H_{62}N_6O_9$: 793.4476.

20-Deoxy-20-{*N*-methyl-*N*-[1-(2-naphthyl)-1*H*-1,2,3-triazol-4-yl] methylamino}-5-*O*-mycaminosyltylonolide (2e)

According to the general procedure of triazole formation, **1a** (100 mg, 0.154 mmol) and 2-azidonaphthalene (34.5 mg, 0.204 mmol) were converted to **2e** (95.0 mg, 76%) as a pale yellow solid.

Mp: 113.3–117.6 °C; $[\alpha]\ ^{25}_D$ −16.2 (*c* 1.0, $CHCl_3$); IR (Diamond prism) cm^{-1}: 3450 (br), 2935, 1732, 1589, 1169, 1053, 748; ^{1}H NMR (500 MHz, CD_3OD) δ (p.p.m.): 8.77 (s, 1H), 8.40 (s, 1H), 8.11–8.06 (complex m, 2H), 8.02–7.97 (complex m, 2H), 7.61–7.57 (complex m, 2H), 7.16 (d, $J=15.5$ Hz, 1H), 6.46 (d, $J=15.5$ Hz, 1H), 5.52 (d, $J=10.9$ Hz, 1H), 4.62 (m, 1H), 4.20 (d, $J=7.5$ Hz, 1H), 3.90 (d, $J=13.8$ Hz, 1H), 3.85 (d, $J=9.8$ Hz, 1H), 3.57 (d, $J=10.3$ Hz, 1H), 3.51 (d, $J=13.8$ Hz, 1H), 3.38 (dd, $J=3.4$, 10.9 Hz, 1H), 3.34 (dd, $J=7.5$, 10.3 Hz, 1H), 3.22–3.09 (complex m, 3H), 2.82 (m, 1H), 2.75 (m, 1H), 2.66 (m, 1H), 2.48 (s, 6H), 2.43 (m, 1H), 2.34 (m, 1H), 2.28 (m, 1H), 2.23 (s, 3H), 2.04 (d, $J=17.2$ Hz, 1H), 1.84–1.67 (complex m, 5H), 1.79 (s, 3H), 1.57–1.47 (complex m, 3H), 1.22 (d, $J=6.3$ Hz, 3H), 1.20 (d, $J=6.9$ Hz, 3H), 1.02 (d, $J=6.9$ Hz, 3H), 0.87 (t, $J=7.2$ Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ (p.p.m.): 206.5, 174.2, 149.6, 146.2, 144.8, 136.4, 135.9, 134.6, 134.5, 131.0, 129.6, 129.0, 128.5, 128.2, 123.9, 120.6, 120.4, 119.4, 105.7, 80.6, 76.1, 74.3, 72.6, 71.71, 71.65, 68.4, 62.3, 55.8, 52.8, 48.3, 46.6, 43.1, 42.6, 42.2 (2C), 40.4, 35.0, 34.2, 26.2, 26.1, 18.2, 17.9, 13.2, 10.0, 9.7; HRMS (ESI) *m/z*: 820.4852 $[M+H]^+$, calcd. for $C_{45}H_{66}N_5O_9$: 820.4861.

20-Deoxy-20-{*N*-methyl-*N*-[1-(3-quinolyl)-1*H*-1,2,3-triazol-4-yl] methylamino}-5-*O*-mycaminosyltylonolide (2f)

According to the general procedure of triazole formation, **1a** (1.00 g, 1.54 mmol) and 3-azidoquinoline (392 mg, 2.30 mmol) were converted to **2 f** (1.21 g, 96%) as a pale yellow solid.

Mp: 118.0–119.0 °C; $[\alpha]\ ^{31}_D$ −114.1 (*c* 1.0, $CHCl_3$); IR (Diamond prism) cm^{-1}: 3421 (br), 2935, 1728, 1589, 1173, 1049, 756; ^{1}H NMR (500 MHz, CD_3OD) δ (p.p.m.): 9.49 (d, $J=2.3$ Hz, 1H), 8.91 (d, $J=2.3$ Hz, 1H), 8.89 (s, 1H), 8.18 (d, $J=8.6$ Hz, 1H), 8.14 (d, $J=8.0$ Hz, 1H), 7.90 (m, 1H), 7.75 (m, 1H), 7.15 (d, $J=15.5$ Hz, 1H), 6.48 (d, $J=15.5$ Hz, 1H), 5.51 (d, $J=10.3$ Hz, 1H), 4.51 (m, 1H), 4.23 (d, $J=7.5$ Hz, 1H), 3.93 (d, $J=13.8$ Hz, 1H), 3.85 (m, 1H), 3.59 (d, $J=10.3$ Hz, 1H), 3.52 (d, $J=13.8$ Hz, 1H), 3.42 (dd, $J=4.0$, 11.5 Hz, 1H), 3.35 (dd, $J=7.5$, 10.3 Hz, 1H), 3.24–3.20 (complex m, 2H), 3.13 (app t, $J=9.5$ Hz, 1H), 2.87 (m, 1H), 2.77 (m, 1H), 2.66 (m, 1H), 2.50 (s, 6H), 2.45–2.29 (complex m, 3H), 2.23 (s, 3H), 2.05 (d, $J=16.6$ Hz, 1H), 1.88–1.78 (complex m, 3H), 1.81 (s, 3H), 1.73–1.66 (complex m, 2H), 1.58–1.45 (complex m, 3H), 1.24 (d, $J=5.7$ Hz, 3H), 1.22 (d, $J=6.9$ Hz, 3H), 1.03 (d, $J=6.3$ Hz, 3H), 0.84 (t, $J=7.2$ Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ (p.p.m.): 206.5, 174.3, 149.6, 148.4, 146.8, 145.2, 144.9, 136.5, 132.2, 132.0, 129.9, 129.7, 129.5, 129.1, 129.0, 124.3, 119.4, 105.7, 80.6, 76.1, 74.3, 72.6, 71.74, 71.67, 68.4, 62.4, 56.0 52.7, 48.4, 46.7, 43.1, 42.7, 42.2 (2C), 40.4, 35.0, 34.1, 26.2, 26.1, 18.3, 17.9, 13.2, 9.9, 9.7; HRMS (ESI^+) *m/z*:821.4812 $[M+H]^+$, calcd for $C_{44}H_{65}N_6O_9$: 821.4813.

20-Deoxy-20-{*N*-methyl-*N*-[1-(6-quinolyl)-1*H*-1,2,3-triazol-4-yl] methylamino}-5-*O*-mycaminosyltylonolide (2g)

According to the general procedure of triazole formation, **1a** (100 mg, 0.154 mmol) and 6-azidoquinoline (39.1 mg, 0.230 mmol) were converted to **2 g** (115 mg, 91%) as a pale yellow solid.

Mp: 128.7–130.8 °C; $[\alpha]^{25}_D$ −38.5 (*c* 1.0, $CHCl_3$); IR (Diamond prism) cm^{-1}: 3383 (br), 2934, 1728, 1589, 1057, 752; ^{1}H NMR (500 MHz, CD_3OD) δ (p.p.m.): 8.93 (dd, $J=1.2$, 4.0 Hz, 1H), 8.83 (s, 1H), 8.52 (s, 1H), 8.51 (d, $J=8.0$ Hz, 1H), 8.40 (m, 1H), 8.25 (d, $J=9.2$ Hz, 1H), 7.64 (dd, $J=4.0$, 8.0 Hz, 1H), 7.18 (d, $J=14.9$ Hz, 1H), 6.47 (d, $J=14.9$ Hz, 1H), 5.64 (d, $J=10.3$ Hz, 1H), 4.69 (m, 1H), 4.21 (d, $J=7.5$ Hz, 1H), 3.90 (d, $J=13.8$ Hz, 1H), 3.85 (d, $J=9.7$ Hz, 1H), 3.58 (d, $J=10.3$ Hz, 1H), 3.54 (d, $J=13.8$ Hz, 1H), 3.47 (dd, $J=3.4$, 10.9 Hz, 1H), 3.35 (dd, $J=8.0$, 10.9 Hz, 1H), 3.33 (m, 1H), 3.20 (m, 1H), 3.12 (app t, $J=9.5$ Hz, 1H), 2.87–2.75 (complex m, 2H), 2.66 (m, 1H), 2.50 (s, 6H), 2.44 (m, 1H), 2.36 (m, 1H), 2.29 (m, 1H), 2.21 (s, 3H), 2.05 (d, $J=17.2$ Hz, 1H), 1.81 (s, 3H), 1.84–1.76 (complex m, 4H), 1.69 (m, 1H), 1.57–1.51 (complex m, 3H), 1.22 (d, $J=5.7$ Hz, 3H), 1.21 (d, $J=5.7$ Hz, 3H), 1.03 (d, $J=6.9$ Hz, 3H), 0.88 (t, $J=7.5$ Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ (p.p.m.): 206.5, 174.3, 152.4, 149.6, 148.2, 146.5, 144.7, 138.8, 136.47, 136.44, 131.3, 130.0, 124.6, 123.9, 123.8, 120.3, 119.5, 105.7, 80.6, 76.1, 74.3, 72.5, 71.7 (2C), 68.4, 62.4, 55.8, 52.9, 48.2, 46.6, 43.1, 42.5, 42.2 (2C), 40.5, 35.0, 34.2, 26.2, 26.0, 18.2, 17.9, 13.2, 10.0, 9.7; HRMS (ESI^+) *m/z*: 821.4815 $[M+H]^+$, calcd. for $C_{44}H_{65}N_6O_9$: 821.4813.

20-Deoxy-20-{*N*-methyl-*N*-[1-(5-quinolyl)-1*H*-1,2,3-triazol-4-yl] methylamino}-5-*O*-mycaminosyltylonolide (2h)

According to the general procedure of triazole formation, **1a** (100 mg, 0.154 mmol) and 5-azidoquinoline (39.1 mg, 0.230 mmol) were converted to **2 h** (113 mg, 90%) as a pale yellow solid.

Mp: 124.1–128.8 °C; [α] $^{27}_{D}$ −109.4 (*c* 0.5, $CHCl_3$); IR (Diamond prism) cm^{-1}: 3380 (br), 2935, 1732, 1589, 1169, 1057, 752; ^{1}H NMR (500 MHz, CD_3OD) δ (p.p.m.): 8.99 (m, 1H), 8.68 (s, 1H), 8.29 (d, *J*=8.6 Hz, 1H), 8.25 (d, *J*=8.6 Hz, 1H), 8.01 (app t, *J*=8.0 Hz, 1H), 7.93 (d, *J*=7.5 Hz, 1H), 7.65 (dd, *J*=4.6, 8.6 Hz, 1H), 7.06 (d, *J*=15.5 Hz, 1H), 6.42 (d, *J*=15.5 Hz, 1H), 5.13 (d, *J*=10.9 Hz, 1H), 4.26 (d, *J*=7.5 Hz, 1H), 4.05 (m, 1H), 3.95 (d, *J*=13.8 Hz, 1H), 3.78 (d, *J*=9.2 Hz, 1H), 3.57 (d, *J*=10.3 Hz, 1H), 3.52 (d, *J*=14.3 Hz, 1H), 3.49 (dd, *J*=4.0, 11.5 Hz, 1H), 3.36 (dd, *J*=7.5, 10.3 Hz, 1H), 3.30–3.24 (complex m, 2H), 3.14 (app t, *J*=9.5 Hz, 1H), 2.86 (m, 1H), 2.72–2.62 (complex m, 2H), 2.51 (s, 6H), 2.37–2.26 (complex m, 3H), 2.25 (s, 3H), 1.95 (d, *J* =17.2 Hz, 1H), 1.90–1.76 (complex m, 3H), 1.74 (s, 3H), 1.68–1.63 (complex m, 2H), 1.58–1.52 (complex m, 2H), 1.44 (m, 1H), 1.26 (d, *J*=6.3 Hz, 3H), 1.20 (d, *J*=6.3 Hz, 3H), 1.00 (d, *J*=6.3 Hz, 3H), 0.76 (t, *J*=7.2 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ (p.p.m.): 206.4, 173.8, 152.6, 149.6, 149.0, 145.8, 145.1, 136.2, 135.2, 133.9, 131.7, 130.4, 128.2, 125.8, 125.6, 124.1, 119.1, 105.7, 80.7, 76.1, 74.3, 72.6, 71.74, 71.69, 68.3, 62.3, 56.1, 52.4, 48.4, 46.7, 43.0, 42.8, 42.2 (2C), 40.1, 35.0, 34.0, 26.2, 25.9, 18.3, 17.9, 13.1, 10.0, 9.6; HRMS (ESI) *m/z*: 821.4797 $[M+H]^+$, calcd. for $C_{44}H_{65}N_6O_9$: 821.4813.

20-Deoxy-20-{*N*-methyl-*N*-[1-(5-isoquinolyl)-1*H*-1,2,3-triazol-4-yl] methylamino}-5-*O*-mycaminosyltylonolide (2i)

According to the general procedure of triazole formation, **1a** (100 mg, 0.154 mmol) and 5-azidoisoquinoline (39.1 mg, 0.230 mmol) were converted to **2i** (89.2 mg, 71%) as a pale yellow solid.

Mp: 125.2–126.4 °C; [α] $^{27}_{D}$ −99.6 (*c* 1.0, $CHCl_3$); IR (Diamond prism) cm^{-1}: 3380 (br), 2935, 1732, 1589, 1169, 1057, 752; ^{1}H NMR (500 MHz, CD_3OD) δ (p.p.m.): 9.43 (s, 1H), 8.70 (s, 1H), 8.56 (d, *J*=6.3 Hz, 1H), 8.38 (d, *J*=8.6 Hz, 1H), 8.11 (d, *J*=6.9 Hz, 1H), 7.93 (app t, *J*=7.7 Hz, 1H), 7.73 (d, *J*=5.7 Hz, 1H), 7.07 (d, *J*=15.5 Hz, 1H), 6.43 (d, *J*=14.9 Hz, 1H), 5.22 (d, *J*=10.3 Hz, 1H), 4.25 (d, *J*=7.5 Hz, 1H), 4.16 (m, 1H), 3.94 (dd, *J*=13.2 Hz, 1H), 3.79 (d, *J*=9.2 Hz, 1H), 3.57 (d, *J*=10.3 Hz, 1H), 3.55 (d, *J*=14.3 Hz, 1H), 3.49 (dd, *J*=4.0, 10.9 Hz, 1H), 3.36 (dd, *J*=7.5, 10.3 Hz, 1H), 3.33 (m, 1H), 3.26 (m, 1H), 3.14 (app t, *J*=9.5 Hz, 1H), 2.86 (m, 1H), 2.73–2.64 (complex m, 2H), 2.51 (s, 6H), 2.41–2.28 (complex m, 3H), 2.25 (s, 3H), 1.96 (d, *J* =16.6 Hz, 1H), 1.90–1.43 (complex m, 8H), 1.75 (s, 3H), 1.26 (d, *J*=6.3 Hz, 3H), 1.19 (d, *J*=6.9 Hz, 3H), 0.99 (d, *J*=6.9 Hz, 3H), 0.77 (t, *J*=7.2 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ (p.p.m.): 206.4, 173.9, 153.7, 149.6, 145.8, 145.0, 144.8, 136.2, 134.3, 132.7, 131.4, 130.5, 129.9, 128.6, 128.1, 119.2, 117.6, 105.7, 80.7, 76.1, 74.3, 72.6, 71.72, 71.67, 68.3, 62.3, 56.0, 52.5, 48.3, 46.7, 43.0, 42.7, 42.2 (2C), 40.1, 35.0, 34.1, 26.2, 25.9, 18.3, 17.9, 13.1, 10.0, 9.6; HRMS (ESI^+) *m/z*: 821.4813 $[M+H]^+$, calcd. for $C_{44}H_{65}N_6O_9$: 821.4813.

20-Deoxy-20-{*N*-methyl-*N*-[1-(1-naphthyl)-1*H*-1,2,3-triazol-4-yl] methylamino}-5-*O*-mycaminosyltylonolide (2j)

According to the general procedure of triazole formation, **1a** (100 mg, 0.154 mmol) and 1-azidonaphthalene (39.1 mg, 0.230 mmol) were converted to **2j** (110 mg, 87%) as a pale yellow solid.

Mp: 119.2–121.7 °C; [α] $^{27}_{D}$ −93.9 (*c* 1.0, $CHCl_3$); IR (Diamond prism) cm^{-1}: 3398 (br), 2931, 1732, 1173, 1053, 771; ^{1}H NMR (500 MHz, CD_3OD) δ (p.p.m.): 8.61 (s, 1H), 8.14 (d, *J*=8.0 Hz, 1H), 8.06 (d, *J*=7.5 Hz, 1H), 7.75 (d, *J*=7.5 Hz, 1H), 7.70 (m, 1H), 7.65–7.60 (complex m, 3H), 7.09 (d, *J*=15.5 Hz, 1H), 6.42 (d, *J*=14.9 Hz, 1H), 5.08 (d, *J*=9.7 Hz, 1H), 4.25 (d, *J*=7.5 Hz, 1H), 4.08 (m, 1H), 3.96 (d, *J*=13.8 Hz, 1H), 3.80 (d, *J*=9.2 Hz, 1H), 3.58 (d, *J*=10.3 Hz, 1H), 3.53 (d, *J*=13.8 Hz, 1H), 3.45 (dd, *J*=4.0, 10.9 Hz, 1H), 3.36 (dd, *J*=7.6, 10.3 Hz, 1H), 3.29–3.22 (complex m, 2H), 3.14 (app t, *J*=9.5 Hz, 1H), 2.86 (m, 1H), 2.71–2.63 (complex m, 2H), 2.51 (s, 6H), 2.41–2.27 (complex m, 3H), 2.25 (s, 3H), 1.95 (d, *J* =17.2 Hz, 1H), 1.90–1.77 (complex m, 3H), 1.74 (s, 3H), 1.43 (m, 1H), 1.26 (d, *J*=5.7 Hz, 3H), 1.20 (d, *J*=6.3 Hz, 3H), 0.99 (d, *J*=6.9 Hz, 3H), 0.76 (t, *J*=7.2 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ (p.p.m.): 206.4, 173.7, 149.7, 145.4, 145.2, 136.2, 135.6, 135.3, 131.6, 130.1, 129.4, 129.0, 128.4, 128.3, 126.3, 125.4, 123.7, 119.1, 105.7, 80.7, 76.1, 74.3, 72.6, 71.74, 71.68, 68.3, 62.4, 56.0, 52.5, 48.4, 46.7, 43.0, 42.8, 42.2 (2C), 40.1, 35.0, 34.1, 26.2, 26.0, 18.3, 17.9, 13.1, 10.0, 9.6; HRMS (ESI) *m/z*: 820.4840 $[M+H]^+$, calcd. for $C_{45}H_{66}N_5O_9$: 820.4861.

20-Deoxy-20-{*N*-benzyl-*N*-[1-(3-quinolyl)-1*H*-1,2,3-triazol-4-yl] methylamino}-5-*O*-mycaminosyltylonolide (2k)

According to the general procedure of triazole formation, **1b** (250 mg, 0.344 mmol) and 3-azidoquinoline (58.5 mg, 0.413 mmol) were converted to **2k** (310.5 mg, 100%) as a pale yellow solid.

Mp: 118.6–120.2 °C; [α]$^{25}_{D}$ −124.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD) δ (p.p.m.): 9.45 (d, *J*=1.7 Hz, 1H), 8.92 (s, 1H), 8.84 (s, 1H), 8.12 (d, *J*=8.6 Hz, 1H), 8.08 (d, *J*=8.0 Hz, 1H), 7.83 (m, 1H), 7.70 (m, 1H), 7.42 (m, 2H), 7.38 (m, 2H), 7.26 (m, 1H), 7.09 (d, *J*=15.5 Hz, 1H), 6.46 (d, *J*=15.5 Hz, 1H), 5.73 (d, *J*=10.9 Hz, 1H), 4.78 (m, 1H), 4.00–3.92 (complex m, 4H), 3.57–3.40 (complex m, 4H), 3.26 (dd, *J*=7.5, 10.3 Hz, 1H), 3.17 (d, *J*=12.6 Hz, 1H), 3.02 (app t, *J*=9.5 Hz, 1H), 2.94 (m, 1H), 2.84–2.78 (complex m, 2H), 2.66 (m, 1H), 2.53 (dd, *J*=10.3, 17.2 Hz, 1H), 2.44 (s, 6H), 2.21–2.14 (complex m, 2H), 2.07 (d, *J*=16.6 Hz, 1H), 1.93 (m, 1H), 1.82 (s, 3H), 1.81–1.45 (complex m, 7H), 1.19 (d, *J*=6.3 Hz, 3H), 1.03 (d, *J*=5.7 Hz, 3H), 1.03 (d, *J*=5.7 Hz, 3H), 0.90 (t, *J*=7.5 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ (p.p.m.): 206.6, 174.6, 149.5, 148.3, 147.7, 144.6, 144.5, 139.6, 136.6, 132.1, 131.9, 130.8 (2C), 129.8, 129.6, 129.5 (2C), 129.4, 128.9, 128.3, 128.2, 124.2, 119.6, 105.6, 80.4, 76.3, 74.2, 72.5, 71.6 (2C), 68.6, 62.5, 59.8, 52.3, 50.4, 48.3, 46.5, 42.8, 42.1 (2C), 40.5, 34.9, 34.1, 26.2 (2C), 18.1, 17.9, 13.3, 10.0, 9.8; HRMS (ESI^+) *m/z*: 897.5111 $[M+H]^+$, calcd. for $C_{50}H_{69}N_6O_9$: 897.5126.

20-Deoxy-20-{*N*-methyl-*N*-[1-(3-quinolyl)-1*H*-1,2,3-triazol-4-yl] methylamino}-5-*O*-mycaminosyltylonolide (2l)

According to the general procedure of triazole formation, **1c** (100.0 mg, 0.157 mmol) with 3-azidoquinoline (40.1 mg, 0.236 mmol) was converted to **2l** (97.9 mg, 77%) as a colorless solid.

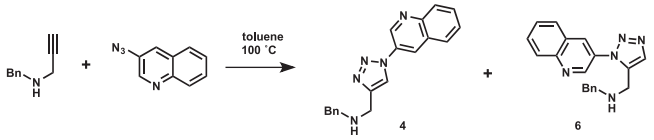
Mp: 126.5–128.0 °C; [α]$^{26}_{D}$ −111.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD) δ (p.p.m.): 9.48 (d, *J*=2.9 Hz, 1H), 8.89 (d, *J*=2.3 Hz, 1H), 8.82 (s, 1H), 8.16 (d, *J*=8.6 Hz, 1H), 8.13 (d, *J*=8.6 Hz, 1H), 7.88 (dt, *J*=1.2, 8.0 Hz, 1H), 7.74 (dt, *J*=1.2, 8.0 Hz, 1H), 7.23 (d, *J*=14.9 Hz, 1H), 6.48 (d, *J*=15.5 Hz, 1H), 5.70 (d, *J*=10.3 Hz, 1H), 4.63 (app t, *J*=8.6 Hz, 1H), 4.26 (d, *J*=7.5 Hz, 1H), 4.00 (d, *J*=14.3 Hz, 1H), 3.86 (d, *J*=13.8 Hz, 1H), 3.80 (d, *J*=10.3 Hz, 1H), 3.65 (d, *J*=10.3 Hz, 1H), 3.51 (dd, *J*=3.4, 11.2 Hz, 1H), 3.41–3.22 (complex m, 3H), 3.14 (t, *J*=9.2, 9.7 Hz, 1H), 2.92 (m, 1H), 2.83–2.73 (complex m, 2H), 2.66 (m, 1H), 2.51 (s, 6H), 2.45–2.38 (complex m, 2H), 2.04 (d, *J* =17.2 Hz, 1H), 1.89–1.66 (complex m, 5H), 1.82 (s, 3H), 1.60–1.45 (complex m, 3H), 1.24 (d, *J*=6.3 Hz, 3H), 1.22 (d, *J*=6.9 Hz, 3H), 1.03 (d, *J*=6.9 Hz, 3H), 0.84 (t, *J*=7.5 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ (p.p.m.): 206.6, 174.6, 149.7, 148.4, 148.3, 144.7 (2C), 136.6, 132.2, 131.9, 129.8, 129.6, 129.4, 128.9, 128.3, 123.2, 119.5, 105.7, 80.6, 76.2, 74.3, 72.6, 71.7 (2C), 68.3, 62.5, 48.3, 47.1, 46.5, 44.5, 42.8, 42.2 (2C), 40.4, 34.7, 34.1, 27.8, 26.1, 18.2, 17.9, 13.2, 10.0, 9.7; HRMS (ESI) *m/z*:829.4478 $[M+Na]^+$, calcd for $C_{43}H_{62}N_6O_9Na$: 829.4476.

N-methyl-*N*-[1-(3-quinolinyl)-1*H*-1,2,3-triazol-4-yl]methylamine (3)

To a solution of 3-azidoquinoline (492 mg, 2.89 mmol) in *t*-BuOH/H_2O (29 ml) was added *N*-methylpropargylamine (136.3 μl, 2.82 mmol), $CuSO_4$•$5H_2O$ (72.3 mg, 0.289 mmol) and sodium L-ascorbate (279.3 mg, 1.41 mmol). After being stirred for 15 min, to the reaction mixture was added saturated aqueous Rochelle salt (20 ml). The mixture was extracted with $CHCl_3$ (100 ml), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH=30/1) to give **3** (629 mg, 91%) as a yellow solid.

Mp; 127.9–131.4 °C; IR (Diamond prism) cm^{-1}: 3290, 3120, 3070, 810, 748; ^{1}H NMR (500 MHz, CD_3OD) δ (p.p.m.): 9.39 (d, *J*=2.9 Hz, 1H), 8.77 (d, *J*=2.9 Hz, 1H), 8.63 (s, 1H), 8.11 (d, *J*=8.6 Hz, 1H), 8.06 (d, *J*=8.0 Hz, 1H), 7.85 (m, 1H), 7.71 (m, 1H), 3.95 (s, 2H), 2.48 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ (p.p.m.): 148.4, 148.0, 144.2, 132.1, 132.0, 129.7, 129.61, 129.55, 129.0, 127.9, 122.9, 46.5, 35.6; HRMS (FAB) *m/z*: found *m/z*: 240.1244 $[M+H]^+$, calcd. for $C_{13}H_{14}N_5$: 240.1249.

N-Benzyl-*N*-[1-(3-quinolyl)-1*H*-1,2,3-triazol-4-yl]methylamine (4) and *N*-Benzyl-*N*-[1-(3-quinolyl)-1*H*-1,2,3-triazol-5-yl]methylamine (6)



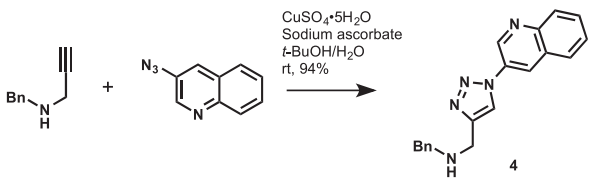
3-Azidoquinoline (132 mg, 0.773 mmol) and *N*-benzylpropargylamine (102 mg, 0.702 mmol) were dissolved in toluene (3.5 ml), and the mixture was stirred at 100 °C. After stirring for 35 h, the reaction mixture was concentrated to give the crude product. The residue was purified by flash column chromatography on silica gel (Hexane/EtOAc = 2/1 to 1/2) to give **4** (71.6 mg, 32%) as a brown solid and **6** (43.0 mg, 19%) as a brown liquid.

4; Mp; 126.6–129.0 °C; IR (KBr) cm^{-1}: 3332, 3151, 2796, 1496, 1427, 1350, 1215, 1045, 741, 702; ^{1}H NMR (500 MHz, $CDCl_3$) δ (p.p.m.) 9.30 (s, 1H), 8.47 (s, 1H), 8.16 (d, *J* = 8.6 Hz, 1H), 8.07 (s, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.78 (m, 1H), 7.64 (m, 1H), 7.37–7.24 (complex m, 5H), 4.05 (s, 2H), 3.90 (s, 2H), 2.05 (br s, 1H); ^{13}C NMR (125 MHz, ($CDCl_3$) δ (p.p.m.) 148.3, 147.7, 143.1, 139.8, 130.5, 129.7, 128.6, 128.4 (2C), 128.3, 128.2, 127.4, 126.1, 120.2, 52.5, 44.2; HRMS (ESI) *m/z*: 338.1377 $[M+Na]^+$, calcd. for $C_{19}H_{17}N_5Na$: 338.1382.

6; IR (KBr) cm^{-1}: 3306, 2931, 1670, 1238, 1041, 748; ^{1}H NMR (500 MHz, $CDCl_3$) δ (p.p.m.) 9.23 (d, *J* = 2.3 Hz, 1H), 8.56 (d, *J* = 2.3 Hz, 1H), 8.22 (d, *J* = 8.6 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.85 (m, 1H), 7.82 (s, 1H), 7.68 (m, 1H), 7.28–7.21 (complex m, 5H), 3.92 (s, 2H), 3.82 (s, 2H), 1.76 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (p.p.m.) 148.0, 146.5, 138.9, 136.7, 134.5, 131.1, 131.0, 130.2, 129.7, 128.7 (2C), 128.5, 128.2 (2C), 128.1, 127.5, 53.3, 41.4; HRMS (FAB) *m/z*: 316.1557 $[M+H]^+$, calcd. for $C_{19}H_{18}N_5$: 316.1562.

To confirm whether compounds were 1,4- or 1,5-triazole compounds, we carried out the following reaction, as a copper-catalyzed triazole reaction exclusively gives a 1,4-triazole product.

N-Benzyl-*N*-[1-(3-quinolyl)-1*H*-1,2,3-triazol-4-yl]methylamine (4)



To a solution of *N*-benzylpropargylamine (3.34 g, 23.0 mmol) and 3-azidoquinoline (3.92 g, 23.0 mmol) in *t*-BuOH/H_2O = 2/1 (23 ml) were added $CuSO_4 \cdot 5H_2O$ (57.4 mg, 0.230 mmol) and sodium L-ascorbate (2.28 g, 11.5 mmol). The reaction mixture was stirred at room temperature. After stirring for 40 min, to the reaction mixture was added saturated aqueous $NaHCO_3$ (20 ml) and the resulting mixture was extracted with $CHCl_3$ (30 ml × 2). The combined organic layers were dried over Na_2SO_4 and concentrated at reduced pressure. The residue was recrystallized from $CHCl_3$/hexane at −78 °C to give product **4** (6.84 g, 94%) as a pale yellow solid.

20-Deoxy-20-{*N*-benzyl-*N*-[1-(3-quinolyl)-1*H*-1,2,3-triazol-4-yl] methylamino}-desmycosin (5)

According to the general procedure for reductive amination, desmycosin (3.67 g, 4.76 mmol) and **4** (1.50 g, 4.76 mmol) were converted to **5** (4.60 g, 90%) as a pale yellow solid.

Mp: 111.7–113.6°C; [α] $^{25}_D$ −52.3 (*c* 1.0, $CHCl_3$); IR (KBr) cm^{-1}: 3429 (br), 2935, 1728, 1589, 1165, 1057, 748; ^{1}H NMR (500 MHz, CD_3OD) δ (p.p.m.): 9.50 (d, *J* = 2.9 Hz, 1H), 8.96 (s, 1H), 8.91 (d, *J* = 2.3 Hz, 1H), 8.17 (d, *J* = 8.6 Hz, 1H), 8.12 (d, *J* = 8.0 Hz, 1H), 7.89 (m, 1H), 7.75 (app t, *J* = 7.7 Hz, 1H), 7.45–7.44 (complex m, 2H), 7.39 (app t, *J* = 7.5 Hz, 2H), 7.28 (app t, *J* = 7.5 Hz, 1H), 7.04 (d, *J* = 15.5 Hz, 1H), 6.48 (d, *J* = 15.5 Hz, 1H), 5.71 (d, *J* = 10.3 Hz, 1H), 4.84 (m, 1H), 4.50 (d, *J* = 8.0 Hz, 1H), 4.03–3.94 (complex m, 4H), 3.81 (dd, *J* = 4.0, 9.7 Hz, 1H), 3.73 (t, *J* = 2.9 Hz, 1H), 3.65 (m, 1H), 3.57 (s, 3H), 3.53 (d, *J* = 10.3 Hz, 1H), 3.44 (d, *J* = 14.3 Hz, 1H), 3.40 (s, 3H), 3.34 (m, 1H), 3.26 (dd, *J* = 7.5, 10.3 Hz, 1H), 3.19 (d, *J* = 13.2 Hz, 1H), 3.14 (dd, *J* = 2.9, 9.7 Hz, 1H), 3.04–2.90 (complex m, 4H), 2.83 (m, 1H), 2.67 (m, 1H), 2.55 (dd, *J* = 10.3, 17.2 Hz, 1H), 2.45 (s, 6H), 2.22–2.13 (complex m, 2H), 2.09 (d, *J* = 17.2 Hz, 1H), 1.95 (m, 1H), 1.89–1.44 (complex m, 7H), 1.82 (s, 3H), 1.22–1.20 (complex m, 6H), 1.03 (app d, *J* = 6.3 Hz, 6H), 0.93 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ (p.p.m.): 206.5, 174.6, 149.4, 148.3, 147.7, 144.7, 144.3, 139.6, 136.3, 132.1, 131.9, 130.8 (2C), 129.8, 129.7, 129.5 (3C), 128.9, 128.2 (2C), 124.1, 119.7, 105.6, 102.3, 82.8, 81.6, 80.3, 76.2, 74.6, 74.2, 72.5, 71.6 (2C), 71.0, 70.1, 68.6, 62.2, 59.8, 59.6, 52.3, 50.4, 46.5, 46.1, 42.8, 42.1 (2C), 40.5, 34.8, 34.1, 26.2, 26.1, 18.14, 18.10, 17.9, 13.3, 10.1, 9.9; HRMS (ESI) *m/z*: 1071.6022 $[M+H]^+$, calcd. for $C_{58}H_{83}N_6O_{13}$: 1071.6018.

20-Deoxy-20-{*N*-benzyl-*N*-[1-(3-quinolyl)-1*H*-1,2,3-triazol-5-yl] methylamino}-5-*O*-mycaminosyltylonolide (7)

According to the general procedure of reductive amination, OMT (73.9 mg, 0.124 mmol) and **6** (43.0 mg, 0.136 mmol) were converted to **7** (107 mg, 96%) as a pale yellow solid.

Mp: 108.8–111.3 °C; [α] $^{26}_D$ +4.4 (*c* 1.0, $CHCl_3$); IR (Diamond prism) cm^{-1}: 3394 (br), 2927, 1716, 1589, 1169, 1057, 748; ^{1}H NMR (500 MHz, CD_3OD) δ (p.p.m.): 9.11 (d, *J* = 2.3 Hz, 1H), 8.66 (d, *J* = 1.7 Hz, 1H), 8.20 (d, *J* = 8.6 Hz, 1H), 8.10 (d, *J* = 8.0 Hz, 1H), 8.04 (s, 1H), 7.95 (m, 1H), 7.78 (m, 1H), 7.08–7.03 (complex m, 6H), 6.42 (d, *J* = 15.5 Hz, 1H), 5.91 (d, *J* = 10.3 Hz, 1H), 5.01 (dt, *J* = 2.3, 9.2 Hz, 1H), 4.16 (d, *J* = 7.5 Hz, 1H), 3.89 (d, *J* = 14.9 Hz, 1H), 3.79 (d, *J* = 9.2 Hz, 1H), 3.70–3.55 (complex m, 5H), 3.41 (d, *J* = 13.2 Hz, 1H), 3.29 (m, 1H), 3.08–3.01 (complex m, 2H), 2.87 (m, 1H), 2.63–2.32 (complex m, 6H), 2.51 (s, 6H), 2.07 (dd, *J* = 1.5, 17.2 Hz, 1H), 1.91 (m, 1H), 1.85 (s, 3H), 1.74–1.60 (complex m, 4H), 1.52–1.28 (complex m, 2H), 1.14 (d, *J* = 6.9 Hz, 3H), 1.05–0–99 (complex m, 6H), 0.97 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ (p.p.m.): 206.3, 174.8, 149.3, 148.7, 148.1, 144.1, 139.2, 138.6, 136.6, 135.5, 134.1, 132.5, 131.4, 130.10, 130.06 (2C), 129.6, 129.3, 129.2 (2C), 128.7, 128.0, 119.7, 105.2, 80.5, 76.4, 74.2, 72.5, 71.7 (2C), 68.6, 62.7, 59.7, 52.8, 48.2, 46.8, 46.5, 42.5, 42.2 (2C), 40.8, 36.4, 34.5, 26.4, 26.3, 18.4, 17.8, 13.3, 10.1, 9.6; HRMS (ESI) *m/z*: 897.5128 $[M+H]^+$, calcd. for $C_{50}H_{69}N_6O_9$: 897.5126.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Ōmura, S. in: *Macrolide Antibiotics. Chemistry, Biology, and Practice* 2nd edn (ed. Ōmura, S.) (Academic Press, San Diego, 2002).
2 Hamill, R. L., Haney, M. E. Jr, Stamper, M. & Wiley, P. F. Tylosin, a new antibiotic. II. Isolation, properties, and preparation of desmycosin, a microbiologically active degradation product. *Antibiot. Chemother.* 328–334 (1961).
3 Ōmura, S. Microbial metabolites: 45 years of wandering, wondering and discovering. *Tetrahedron* **67**, 6420–6459 (2011).
4 Debono, M. *et al.* Synthesis and antimicrobial evaluation of 20-deoxo-20-(3,5-dimethylpiperidin-l-yl)desmycosin (tilmicosin, EL-870) and related cyclic amino derivatives. *J. Antibiot.* **42**, 1253–1267 (1989).
5 Miyake, T., Takita, M., Hamada, M., Takeuchi, T. & Umezawa, S. (Zaidan Hojin Biseibutsu Kagaku Kenkyu Kai). Macrolide antibiotics and treatment of pasteurellosis. WO2001016148A1 (2001).
6 Kumar, N. *et al.* Mastitis effects on reproductive performance in dairy cattle: a review Trop. *Anim. Health Prod.* **49**, 663–673 (2017).
7 Gruet, P., Maincent, P., Berthelot, X. & Kaltsatos, V. Bovine mastitis and intramammary drug delivery: review and perspectives. *Adv. Drug Deliv. Rev.* **50**, 245–259 (2001).
8 Marshall, S. A. *et al.* Proposed MIC quality control guidelines for National Committee for Clinical Laboratory Standards susceptibility tests using seven veterinary antimicrobial agents: ceftiofur, enrofloxacin, florfenicol, penicillin G-novobiocin, pirlimycin, premafloxacin, and spectinomycin. *J. Clin. Microbiol.* **34**, 2027–2029 (1996).
9 Phan, L. T. *et al.* 23-O-substituted 5-O-mycaminosyltylonide derivatives. WO2003089446A2 (Enanta Pharmaceuticals, Inc.) (2003).
10 Ma, Z. *et al.* Novel erythromycin derivatives with aryl groups tethered to the C-6 position are potent protein synthesis inhibitors and active against multidrug-resistant respiratory pathogens. *J. Med. Chem.* **44**, 4137–4156 (2001).
11 Tornøe, C. W., Christensen, C. & Meldal, M. Peptidotriazoles on solid phase: [1,2,3]-triazoles by regiospecific copper(I)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides. *J. Org. Chem.* **67**, 3057–3064 (2002).

12 Rostovtsev, V. V., Green, L. G., Fokin, V. V. & Sharpless, K. B. A stepwise Huisgen cycloaddition process: copper(I)-catalyzed regioselective ligation of azides and terminal alkynes. *Angew. Chem. Int. Ed.* **41**, 2596–2599 (2002).
13 Hirose, T. *et al*. Rapid 'SAR' *via* click chemistry: an alkyne-bearing spiramycin is fused with diverse azides to yield new triazole-antibacterial candidates. *Heterocycles* **69**, 55–61 (2006).
14 Sugawara, A. *et al*. Design and synthesis via click chemistry of 8,9-anhydroerythromycin A 6,9-hemiketal analogues with anti-MRSA and -VRE activity. *Bioorg. Med. Chem. Lett.* **17**, 6340–6344 (2007).
15 Galli, U. *et al*. Identification of a sirtuin 3 inhibitor that displays selectivity over sirtuin 1 and 2. *Eur. J. Med. Chem.* **55**, 58–66 (2012).
16 Lima-Neto, R. G. *et al*. Synthesis of 1,2,3-triazole derivatives and *in vitro* antifungal evaluation on *Candida* strains. *Molecules* **17**, 5882–5892 (2012).
17 Sashida, H., Fujii, A. & Tsuchiya, T. Studies on diazepines. XXIX. Syntheses of 3*H*- and 5*H*-1, 4-benzodiazepines from 3-azidoquinolines. *Chem. Pharm. Bull.* **35**, 4110–4116 (1987).
18 Ahammed, S., Saha, A. & Ranu, B. C. Hydrogenation of azides over copper nanoparticle surface using ammonium formate in Water. *J. Org. Chem.* **76**, 7235–7239 (2011).
19 Pokhodylo, N. T., Matiichuk, V. S. & Obushak, N. D. Methyl 3-cyclopropyl-3-oxopropanoate in the synthesis of heterocycles having a cyclopropyl substituent. *Russ. J. Org. Chem.* **46**, 894–897 (2010).
20 Hollywood, F. *et al*. Photolysis of quinolyl and isoquinolyl azides in primary and secondary aliphatic amines: synthesis of bicyclic azepines, diazepines, and quinolyl- and isoquinolyl-diamines. *J. Chem. Soc. Perkin Trans.* **1**, 421–429 (1982).
21 Mamidyala, S. K. & Cooper, M. A. Probing the reactivity of *o*-phthalaldehydic acid/ methyl ester: synthesis of N-isoindolinones and 3-arylaminophthalides. *Chem. Commun.* **49**, 8407–8409 (2013).
22 National Committee for Clinical Laboratory Standards Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved standard M31-A. NCCLS, Wayne, PA (1999).

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