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RAPID EXPLORATION OF NOVEL ANTHELMINTIC AGENTS FROM ALKYNE-BEARING AVERMECTIN DERIVATIVES VIA CLICK CHEMISTRY

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Abstract – We applied copper-catalyzed 1,4-disubstituted-1,2,3-triazole formation, a major connective reaction in "*Click Chemistry*", to synthesize novel avermectin derivatives. Among the synthetic triazole-containing avermectins, the 4-hydroxybutyltriazole derivative (**3a**) was found to demonstrate notable antinematodal activity against ivermectin-resistant *Trichinella spiralis in vitro*. Furthermore, a new compound (**3a**) also exhibited strong antinematode properties against ivermectin-resistant *Haemonchus contortus* in sheep following oral administration.

The avermectins, originally isolated from a culture broth of *Streptomyces avermitilis* (renamed *avermectinius*) in 1975, are a series of unique 16-membered macrolides that have been known to possess exceptionally potent anthelmintic, acaricidal and insecticidal activities with a novel mode of action.[1] In particular, avermectin B1a (Figure 1) is the most effective among the naturally-occurring avermectin analogs against insects and mites, and was commercialized for agricultural use in 1981.[2] Ivermectin, a derivative of avermectin with greater potency and lower toxicity, is composed of an imprecise 4:1 mixture of dihydroavermectin B1a with dihydroavermectin B1b. The main ingredient, 22,23-dihydroavermectin B1a (**IVM B1a**),[3] was found to target glutamategated chloride channels in parasites and insects, although understanding of the drugs actual mode of action remains incomplete.[1b,4] Ivermectin quickly proved to be the world's most effective and broad spectrum antiparasitic endectocidal drug ever developed. It was marketed as an anthelmintic for livestock and companion animals and quickly rose to be the world's Number 1 blockbuster drug in this respect. It can be administered by mouth, topically or via injection. As with most compounds, resistance arose fairly quickly after the compound's

introduction. In veterinary medicine, resistance to ivermectin is now widespread, although the mechanisms of resistance remain unresolved. Ivermectin was later found to be of unique use in controlling onchocerciasis, a neglected tropical disease of humans that had plagued the African continent for centuries. In 1987, ivermectin received approval for use in humans and was donated free of charge to help combat onchocerciasis. It has been widely recognized as a wonder drug, helping to control onchocerciasis and push the disease to the brink of elimination globally, while also being used in a worldwide program to eliminate another neglected tropical disease, lymphatic filariasis.[5] It has also been approved for treatment of human strongyloidiasis, scabies and head lice, and has shown promise against a wide variety of other human diseases and conditions. In an unprecedented development, despite over 30 years of continuous and intensive monotherapy with ivermectin, no resistance has been discovered in any of the helminthic human parasites that the drug is currently being used to target.

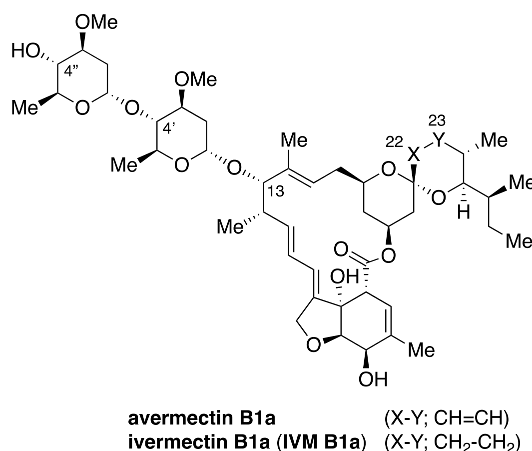


Figure 1. Structure of avermectin B1a and ivermectin B1a (**IVM B1a**)

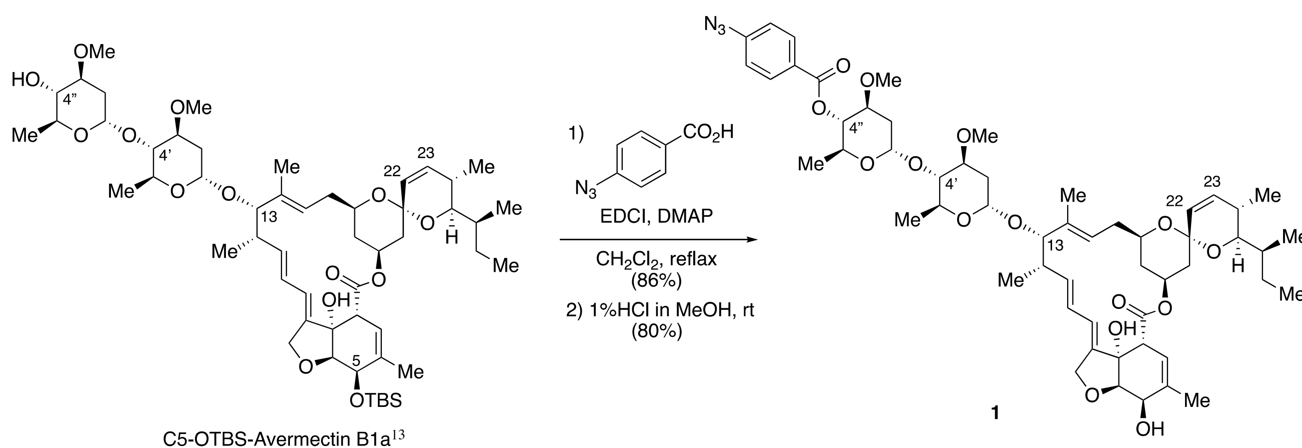
Since the development of ivermectin, various derivatives from the avermectins and differing ivermectin compounds have been synthesized to search for new anthelmintics showing a more potent and broader spectrum of activity.[6] We have a long-standing research program focusing on the search for new avermectin derivatives with the goal of finding compounds which will help to overcome the avermectin/ivermectin-resistance now seen widely in the treatment of livestock and pets – and to help delay the appearance of resistance in human medicine.

Our latest work has focused on antinematodal activity using assay on *Trichinella spiralis in vitro* and *Haemonchus contortus in vivo*. Trichinosis is a globally distributed human foodborne helminthic zoonosis caused by the *T. spiralis* nematodes.[7] *H. contortus* is a very common parasite, particularly in major livestock animals, and the most pathogenic nematode of ruminants.[8] The development of resistance to avermectins and/or ivermectin among these nematodes[9] stimulated interest in novel avermectin and

ivermectin derivatives as an alternative source of effective anthelmintic drugs. To find new drug candidates to help combat ivermectin-resistant nematodes, a rapid and highly-selective connection method would represent an important step potentially allowing access to many avermectin and/or ivermectin derivatives.

It is well known that 1,4-disubsutituted-1,2,3-triazole compounds are synthesized between a variety of azides and terminal acetylenes in the presence of copper(I) catalyst, a process which is utilized as a pivotal reaction in "*Click Chemistry*". The usefulness of this reaction was reported by Sharpless *et al.* in 2002.[10] In fact, 1,4-disubsutituted-1,2,3-triazole (*anti*-triazole) compounds are easy to prepare and suitable for establishing compound libraries in the search for a lead compound.[11] In this paper, we report the simple and quick preparation of avermectin and/or ivermectin derivatives via "*Click Chemistry*" from azide-bearing avermectin and ivermectin precursors with a variety of alkyne fragments to produce the *anti*-triazole derivatives. These were subsequently evaluated for antinematodal activity through screens using ivermectin-resistant nematodes.

In order to conveniently append various fragments to avermectin and/or ivermectin scaffolds, we envisaged the triazole via a spacer, which is connected at the oleandrose C4-position of avermectin. Generally, because acetylene reagents are more readily available than azide compounds, azide-bearing avermectin B1a or **IVM B1a** were the good starting points for synthesizing a range of derivatives. As the spacer, we simply selected *p*-azidobenzoic acid. It could be expected that the conjugated acyl moiety would be more stable against enzymatic or non-enzymatic hydrolysis than the alkylacyl functions. Our basic synthetic approach envisioned the introduction of *p*-azidobenzoate to the C4"-OH of avermectin B1a (**1**),[12] which was prepared from the known C5-OTBS avermectin B1a derivative[13] by the condensation of *p*-azidobenzoic acid using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) in the presence of DMAP and, subsequently, the deprotection of the TBS group under acid hydrolysis (Scheme 1).



Scheme 1. Preparation of azide-bearing avermectin B1a (**1**)

Table 1. Preparation of 1,4-disubstituted-1,2,3-triazole derivatives and antinematodal activity against ivermectin-resistant *Trichinella spiralis*

1 —(≡—R, $[Cu(MeCN)_4]PF_6$ / TBTA, Cu(0) turning, MeOH, rt (30 ~ 100%)[a])→ compounds **2** ~ **16**

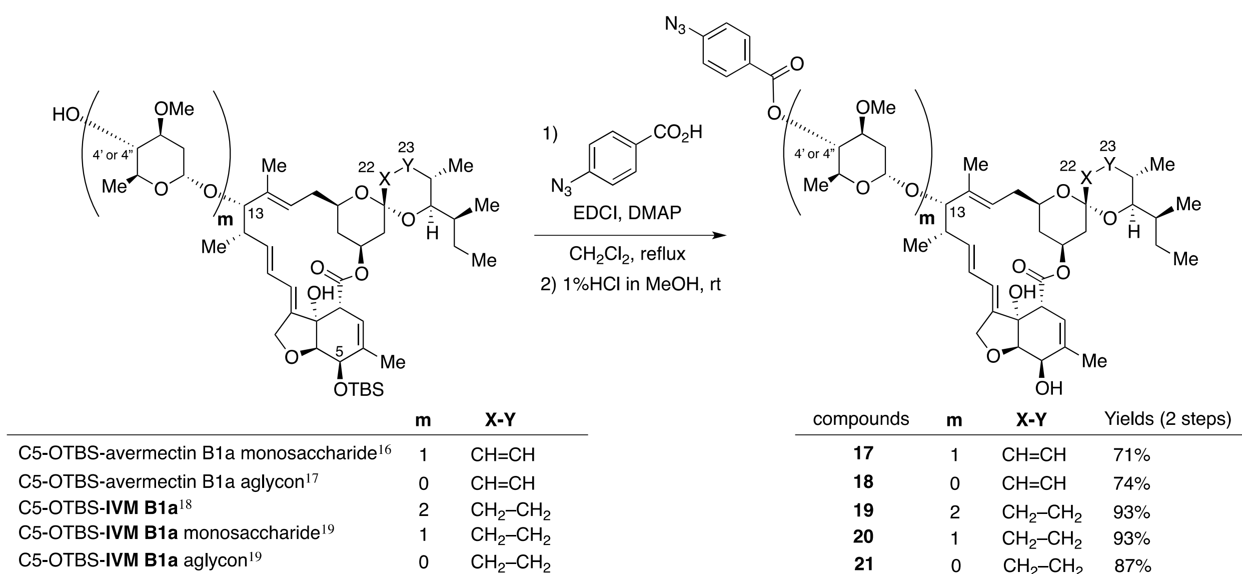
Compounds	R	Yields[b]	*Trichinella spiralis* nematode larval mortality / motility assay[c]		
			100 ppm	10 ppm	1 ppm
2	Me	54%	+	-	-
3a	HO	83%	+++	++	+
4	Cl	30%	++	+	-
5	Me, O, O	80%	-	-	-
6	OH	73%	-	-	-
7		96%	+	+	-
8		87%	-	-	-
9	O, O	quant.	+	-	-
10	H_2N	90%	+	-	-
11	NO_2	86%	+	-	-
12	Me	85%	-	-	-
13	MeO	quant.	-	-	-
14	N	90%	+	-	-
15	N, N, Me	34%	+	-	-
16	S	82%	-	-	-
IVM B1a			+	-	-

[a] Brief reaction condition: Each alkyne compound (2.0 eq.) was reacted with the azide avermectin derivative (**1**) (1.0 eq.) in the presence of $Cu[(MeCN)_4]PF_6$ (0.01 eq.), tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) (0.02 eq.) and copper turning (a small piece) in MeOH at rt under stirring. After reactions completion, the mixtures were diluted with sat. aq. NH_4Cl solution and extracted with AcOEt. The organic layer was dried over Na_2SO_4 and concentrated. Preparative TLC purification ($CHCl_3$:acetone) afforded the corresponding triazole products in 30~100% yields; [b] Isolation yields; [c] Efficacy in larval assay: +++ means that all larvae dead, ++ means that more than 50% larvae dead in comparison with untreated control, + means that fewer larvae live in comparison with untreated control, - means that larval mortality is equal to untreated control.

A variety of 1,2,3-*anti*-triazole avermectin derivatives were synthesized, as expected, in moderate to excellent yields, in the presence of catalytic amounts of [Cu(MeCN)$_4$]PF$_6$ with Cu(0) turning and tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) in MeOH. Exceptionally, **4** was generated in very low yield with many unidentified by-products, due to the instability of the haloalkyl moiety in MeOH solution. In case of the preparation of **15**, although the reaction very clearly proceeded, the product was obtained in low yield as the polarity of the **15** and the reagent obstructed the isolation process (Table 1).

In vitro antinematodal activity (against ivermectin-resistant *Trichinella spiralis*) of triazoles (**2-16**) and **IVM B1a** are also summarized in Table 1. The method used for *in vitro* screening assay for *Trichinella spiralis* was that reported by Jenkins and Carrington.[14] Biological evaluation indicated that, among the various triazole compounds tested, both **3a**[15] and **4** showed stronger antinematodal activity than that of **IVM B1a**. In particular, **3a** showed the most potent antinematodal activity. In most cases, the derivatives with simple alkyls, including cyclic forms (**2** and **7**), benzoyloxyalkyl (**9**) and aromatic functions (**10**, **11**, **14** and **15**) on the triazole ring, showed the moderate activity similar to that of **IVM B1a**. The other derivatives (**5**, **6**, **8**, **12**, **13** and **16**) were less potent than **IVM B1a**.

Our interest then turned to focus on making triazole derivatives with different lengths of hydroxyalkyl groups, based on avermectin B1a, avermectin B1a-desaccharide derivatives, **IVM B1a** and **IVM B1a**-desaccharide derivatives, to provide better understanding of Structure-Activity-Relationships (SARs). Consequently, we prepared the C4", C4' or C13-*O*-(*p*-azidobenzoyl) derivatives from the corresponding C5-OTBS-avermectin and ivermectin intermediates,[16-19] respectively, to synthesize further hydroxyalkyl-triazole derivatives (Scheme 2).



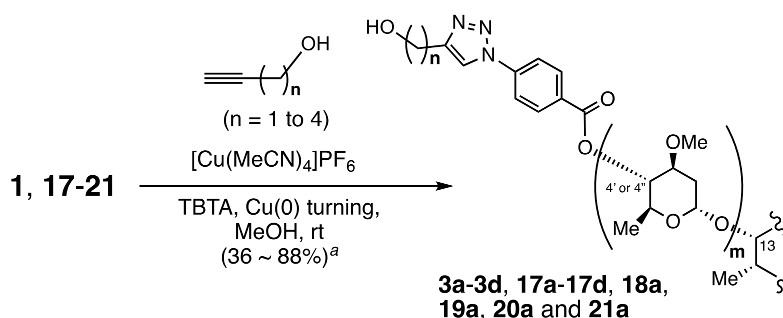
	m	X-Y
C5-OTBS-avermectin B1a monosaccharide[16]	1	CH=CH
C5-OTBS-avermectin B1a aglycon[17]	0	CH=CH
C5-OTBS-**IVM B1a**[18]	2	CH_2–CH_2
C5-OTBS-**IVM B1a** monosaccharide[19]	1	CH_2–CH_2
C5-OTBS-**IVM B1a** aglycon[19]	0	CH_2–CH_2

compounds	m	X-Y	Yields (2 steps)
17	1	CH=CH	71%
18	0	CH=CH	74%
19	2	CH_2–CH_2	93%
20	1	CH_2–CH_2	93%
21	0	CH_2–CH_2	87%

Scheme 2. Preparation of azide-bearing avermectin and **IVM B1a** derivatives (**17-21**)

All azide-bearing derivatives (**17-21**) could be synthesized by adapting to the preparation method of **1** in good yields. Furthermore, triazole formations of **1** and **17-21** with different lengths of alkyl alcohols (n=1~4; shown in Table 2) were conducted under the standard copper-catalyzed *anti*-triazole formation to obtain the corresponding products, as the focused derivatives related to **3a**, in moderate to good yields, respectively.

Table 2. Preparation of focused derivatives related to **3a** and antinematodal activity against ivermectin-resistant *Trichinella spiralis*



Compounds	X-Y	m	n	Yields[b]	*Trichinella spiralis* nematode larval mortality / motility assay[c]		
					100 ppm	10 ppm	1 ppm
3a	CH = CH	2	4	83%	+++	++	+
3b	CH = CH	2	3	88%	+	-	-
3c	CH = CH	2	2	79%	+	+	-
3d	CH = CH	2	1	75%	+	+	-
17a	CH = CH	1	4	77%	-	-	-
17b	CH = CH	1	3	52%	-	-	-
17c	CH = CH	1	2	63%	+	-	-
17d	CH = CH	1	1	55%	+	-	-
18a	CH = CH	0	4	77%	-	-	-
19a	CH_2 - CH_2	2	4	80%	-	-	-
20a	CH_2 - CH_2	1	4	36%	+	-	-
21a	CH_2 - CH_2	0	4	57%	+	-	-
IVM B1a	CH_2 - CH_2	2	–	–	+	-	-

[a] Accrording to the procedure in Table 1; [b] Isolation yields; [c] Efficacy in larval assay: **+++** means that all larvae dead, **++** means that more than 50% larvae dead in comparison with untreated control, **+** means that fewer larvae live in comparison with untreated control, **-** means that larval mortality is equal to untreated control.

In vitro antinematodal activity of hydroxyalkyltriazole derivatives (**3a-d**, **17a-d**, **18a-21a**) are summarized in Table 2. Biological evaluation indicated that the triazoles with the avermectin B1a scaffold (**3a-3d**) had better efficacy than the other types of derivatives (**17a-d** and **18a-21a**), suggesting that the scaffold of this type of derivative (**3a-3d**) might be important. The observation of the activity between **3a-d**, **17a-d** and **18a** also indicated that the disaccharide moiety of avermectin B1a might be significant. Conversely, ivermectin derivatives (**19a-21a** and **IVM B1a**) did not show the correlation with SARs of the avermectin-type derivatives with respect to activity against nematode. The lengths of the hydroxy alkyl chain moiety also showed no correlation among these derivatives. As a result, no

derivatives with more potent activity than **3a** were obtained. Consequently, we concluded that the antinematodal activity of 4-hydroxybutyl and avermectin B1a conjunct via an *anti*-triazolylbenzoyl tether produced the greatest antinematode impact.

Based on the *in vitro* results, we subsequently carried out the more advanced biological evaluation of antinematodal activity using the best compound (**3a**) in the ivermectin-resistant *Haemonchus contortus* infection model in sheep. Commercial sheep (hoggets milk teeth age) of 22-23 kg in average weight were used for testing. In the group receiving commercial ivermectin (n=12), sheep were treated via subcutaneous injection of a commercial ivermectin product, dosage 0.2 mg per kg bodyweight. In the group receiving compound **3a** (n=12), sheep were treated orally through a drenching gun with an aqueous suspension containing 0.25% (v/w) of **3a**; dose volume 0.08 mL suspension per kg bodyweight corresponding to 0.2 mg of **3a** per kg bodyweight. Treatments of **3a** and ivermectin were performed on day 0 of the trial. Fecal sampling took place on Day-1 and Day-14 post-treatment. The method used for parasite egg count was that reported by Robert and O'Sullivan[20] and comparison was made with that found in a group of untreated control animals (n=10). The anthelmintic efficacy against each nematode was calculated using the geometric mean of the nematode number in each experimental treated group compared to the geometric mean of the control group, as per the following formula: Efficacy (%) = [(control-treated) control^{-1}] x 100. As a result, *Haemonchus contortus* infected sheep under commercial ivermectin treatment had an average geometric mean parasite egg count value of 2640.8. Whereas, with the **3a** dose, the average geometric mean value was 1536.7. The comparable value in the control group was 2753.3. Therefore, the efficacy of treatment in the commercial ivermectin group was a lowly 4.1%. Compared with the efficacy of commercial ivermectin, efficacy in the group receiving **3a** through oral administration was 44.2%, meaning that **3a** exhibited stronger antinematodal activity against ivermectin-resistant species *in vivo* than commercial ivermectin given via subcutaneous administration.

In conclusion, we applied a copper(I) catalyzed *anti*-triazole formation to synthesize a variety of avermectin and ivermectin derivatives. Among them, compound **3a** exhibited antinematodal activity against ivermectin-resistant *Trichinella spiralis* nematodes *in vitro* and *Haemonchus contortus* nematodes in sheep when given via oral administration. We believe that the results provide a useful insight for future drug development to help suppress or overcome ivermectin-resistant parasites, with respect to avermectins derivatives as anthelmintic agents, for both human and animal health.

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12. C4”-*O*-*p*-Azidobenzoate avermectin B1a (**1**): Colorless amorphous material. $[\alpha]_D^{29}$ +79.8 (*c* 0.1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.07 (ddd, *J*= 2.4, 4.4, 8.8 Hz, 2H), 7.08 (ddd, *J*= 2.4, 4.4,

8.8 Hz, 2H), 5.89-5.73 (m, 4H), 5.56 (dd, *J*= 2.5, 9.9 Hz, 1H), 5.42-5.37 (m, 3H), 5.00 (dd, *J*= 3.8, 10.1 Hz, 1H), 4.92 (t, *J*= 9.3 Hz, 1H), 4.79 (d, *J*= 3.1 Hz, 1H), 4.71 (dd, *J*= 2.3, 3.8 Hz, 1H), 4.66 (dd, *J*= 2.3, 14.4 Hz, 1H), 4.29 (d, *J*= 5.9 Hz, 1H), 4.12-3.95 (m, 4H), 3.88 (m, 1H), 3.75 (m, 1H), 3.65 (m, 1H), 3.47 (t, *J*= 10.0 Hz, 1H), 3.46 (s, 3H), 3.34 (s, 3H), 3.30 (ds, *J*= 4.1, 6.3 Hz, 1H), 3.26 (t, *J*= 9.0 Hz, 1H), 2.54 (bt, 1H), 2.40-2.22 (m, 5H), 2.02 (ddd, *J*= 1.1, 4.5, 7.5 Hz, 1H), 1.87 (s, 3H), 1.80-1.44 (m, 8H), 1.50 (s, 3H), 1.29 (d, *J*= 6.2 Hz, 3H), 1.24-1.27 (m, 1H), 1.19 (t, *J*= 6.8 Hz, 3H), 1.18 (d, *J*= 6.2 Hz, 3H), 0.93 (t, *J*= 7.4 Hz, 3H), 0.92-0.83 (m, 7H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 173.8, 165.1, 144.9, 139.7, 138.0 (Cx2), 136.3, 135.2, 131.6 (Cx2), 127.7, 126.6 (Cx2), 124.8, 120.4, 118.9, 118.3, 118.0, 98.6, 95.8, 95.1, 82.1, 81.0, 80.4, 79.2, 79.1, 77.6, 75.9, 74.9, 68.5, 68.4 (Cx2), 67.7, 67.2, 66.6, 57.2, 56.6, 45.7, 40.5, 39.8, 36.7, 35.2 (Cx2), 34.5, 34.3, 30.6, 27.5, 20.3, 20.0, 18.4, 17.5, 16.4, 15.1, 13.0, 12.1; IR (KBr) ν (cm^{-1}) 3018, 2967, 2932, 2361, 2339, 2122, 1716, 1604, 1272, 1174, 1119, 1050, 544, 503, 489, 458, 422, 412; ESI-MS: *m/z* calcd for $C_{55}H_{75}N_3O_{15}Na$, $[M+Na]^+$ 1040.5096. Found: *m/z* 1040.5106.

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15. Triazole compound **3a**: Colorless amorphous material. $[\alpha]_D^{29}$ +70.42 (*c* 0.1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J*= 9.0 Hz, 2H), 7.85 (d, *J*= 9.0 Hz, 2H), 5.88 (m, 1H), 5.81-5.71 (m, 3H), 5.56 (dd, *J*= 2.6, 9.9 Hz, 1H), 5.45-5.37 (m, 3H), 5.01 (dd, *J*= 4.3, 10.3 Hz, 1H), 4.96 (t, *J*= 9.4 Hz, 1H), 4.80 (d, *J*= 3.4 Hz, 1H), 4.72 (dd, *J*= 2.5, 14.6 Hz, 1H), 4.67 (dd, *J*= 2.3, 14.5 Hz, 1H), 4.30 (t, *J*= 6.8 Hz, 1H), 4.06-3.63 (m, 8H), 3.48 (m, 1H), 3.47 (s, 3H), 3.37 (s, 3H), 3.31 (dd, *J*= 2.4, 4.5 Hz, 1H), 3.28 (t, *J*= 9.1 Hz, 1H), 2.86 (t, *J*= 7.4 Hz, 2H), 2.55 (m, 1H), 2.42 (dd, *J*= 4.9, 13.4 Hz, 1H), 2.32-2.24 (m, 4H), 2.02 (dd, *J*= 3.6, 4.9 Hz, 1H), 1.90-1.45 (m, 14H), 1.88 (s, 3H), 1.51 (s, 3H), 1.31 (d, *J*= 6.2 Hz, 3H), 1.21 (d, *J*= 6.2 Hz, 3H), 1.20 (d, *J*= 6.9 Hz, 3H), 0.97-0.85 (m, 7H), 0.92 (d, *J*= 7.2 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 173.8, 164.7, 149.2, 140.3, 139.7, 138.0 (Cx2), 136.3, 135.1, 131.5, 129.9 (Cx2), 127.7, 124.8, 120.4, 120.0 (Cx2), 119.7, 118.7, 118.4, 118.0, 98.6, 95.8, 95.0, 82.1, 81.1, 80.4, 79.2, 79.1, 77.4, 75.8, 74.9, 68.5, 68.3, 67.7, 67.2, 66.6, 62.5, 57.1, 56.6, 45.7, 40.5, 39.8, 36.6, 35.2, 35.2, 34.5, 34.3, 32.1, 30.6, 27.5, 25.5, 25.3, 20.2, 20.0, 18.4, 17.5, 16.4, 15.1, 13.0, 12.0; IR (KBr) ν (cm^{-1}) 3450, 2965, 2934, 2873, 2359, 2343, 2334, 1724, 1609, 1519, 1377, 1342, 1308, 1270, 1160, 1119, 858, 534, 510, 484, 474, 423, 410; ESI-MS: *m/z* calcd for $C_{61}H_{85}N_3O_{16}Na$ $[M+Na]^+$ 1138.5828. Found: *m/z* 1138.5836.
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18. C5-OTBS-**IVM B1a** was obtained from selective silylation (TBSCl, imidazole, DMF) from the

commercially available ivermctin B1a by according to the procedure of reference 14.

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