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RAPID 'SAR' VIA CLICK CHEMISTRY: AN ALKYNE-BEARING SPIRAMYCIN IS FUSED WITH DIVERSE AZIDES TO YIELD NEW TRIAZOLE-ANTIBACTERIAL CANDIDATES

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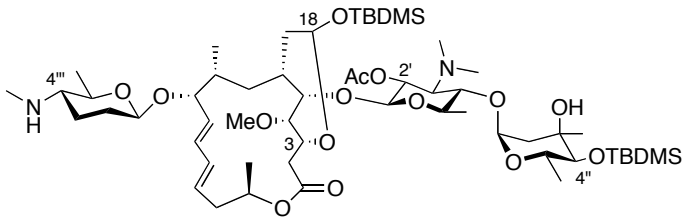
Abstract – The spiramycin analogue, 2'-acetyl-4'''-de-*N*-methyl-4''-*O*-*t*-butyldimethylsilylspiramycin I 3,18-(*O*-*t*-butyldimethylsilyl)acetal(**1**), was found to be an adequate antibacterial agent against MRSA strains. An acetylenic sidechain was attached to **1** producing the analog (**8**), with the core intact but displaying a tethered terminal alkyne, ready for reaction with 19 diverse azides. In the event, the copper-catalyzed Fokin-Huisgen triazole synthesis/coupling gave each of the nineteen new 4''-*O*-acyl triazole derivatives of **1** in good to nearly quantitative isolated yields.

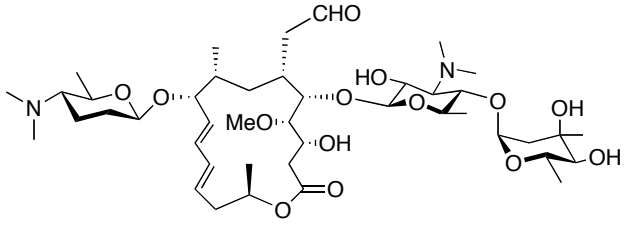
Macrolides such as spiramycin are an old and well-known family of oral antibiotics.[1] They are considered as the preferred therapeutic agents for treatment of upper and lower respiratory tract infections because of their safety and efficacy.[2] Spiramycin is a 16-membered macrolide antibiotic and is active against Gram-positive bacteria, mycoplasmas and toxoplasmas.[1] Spiramycin consists of four structural fragments; a 16-membered ring lactone, and three sugars (mycaminose, mycarose and forosamine).[3] Ōmura and co-workers have described the synthesis and antibacterial activity of a wide variety of spiramycin derivatives.[4] However, one of the most serious problem for antibiotics, including macrolides,

is that decades of clinical use have faced with the emergence of widespread bacterial resistance,[5] and as a result, new antibiotics must be developed. Therefore, various derivatives of spiramycin, synthesized at our institute, were retested against eight types of Gram-positive organism,[6] and one Gram-negative organism (**Figure 1**), including strains with resistance to macrolides. After screening our macrolide library, we found that 2'-acetyl-4'''-de-*N*-methyl-4''-*O*-*t*-butyldimethylsilylspiramycin I 3,18-(*O*-*t*-butyldimethylsilyl)acetal (**1**)[4c] showed moderate minimum inhibitory concentrations (MICs) against Gram-positive strains, including drug-resistance strains. For example, with MRSA N315 IR94[6] strain, derivative (**1**) had a MIC of 32 μg/mL, which indicated a marked improvement over the parent spiramycin I (**2**) (>256 μg/mL for MIC) (**Table 1**).

Figure 1



2'-acetyl-4'''-de-*N*-methyl-4''-*O*-*t*-butyldimethylsilyl-spiramycin I 3,18-(*O*-*t*-butyldimethylsilyl)acetal (**1**)



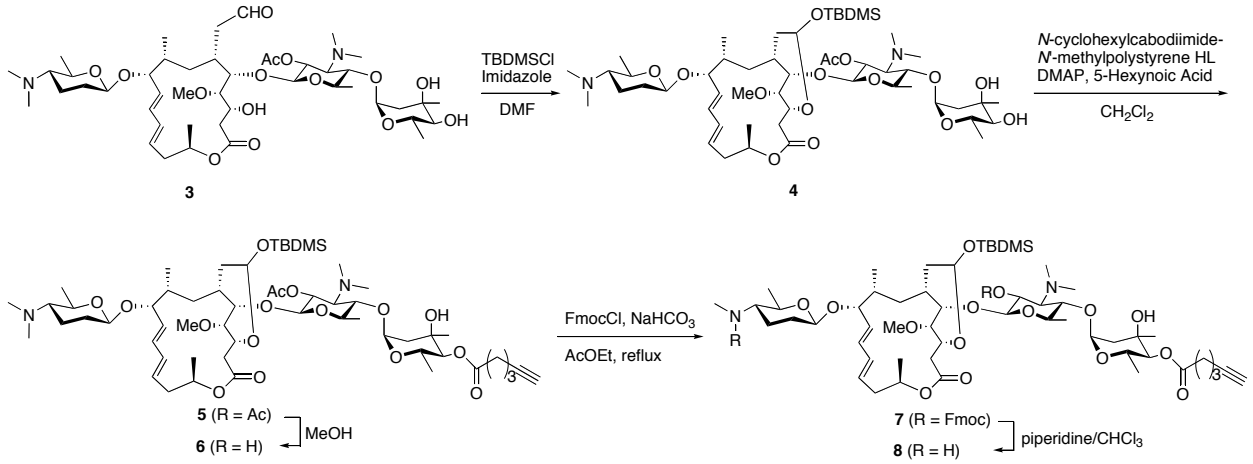
spiramycin I (**2; SPM I**)

Since the discovery of a good lead spiramycin analogue (**1**) for resistant bacteria strains, our efforts have turned to the preparation of new analogues in this series, and the utilization of the "click chemistry"[7] provides an important approach for simple and rapid evaluation of functional activity. The concept of click chemistry is designating powerful and selective reactions for an efficient synthesis of interesting compounds and combinatorial libraries through heteroatom links, when the Huisgen 1,3-dipoler cycloaddition of azides and alkynes is regarded as the reliable connection method. The advantages of click chemistry in biological studies have recently been demonstrated in several applications.[7b,7e,8] Herein, we focus on 4"-modification of **1** with a variety of acyl functions instead of the TBDMS group via click chemistry, and we report a quick and simple route for the preparation of 4"-acyl derivatives from one common alkyne precursor with nineteen kinds of azide blocks and their anti-bacterial activities.

Our basic synthetic approach envisioned selective 3,18-*O*-silylacetalization, 4"'-*N*-protective demethylation, 4"-*O*-alkyne-acylation and total deprotection, thus allowing us to explore modification at 4" by the click process. 2'-Acetylspiramycin I (**3**)[4a] was used as a starting material. The reaction of **3** with TBDMSCl in the presence of imidazole provided 3,18-*O*-TBDMS acetal (**4**)[9] in 80% yield. The condensation of **4** with 5-hexynoic acid using *N*-cyclohexylcabodiimide *N'*-methylpolystyrene HL (solid-supported cabodiimide)[10] in the presence of DMAP resulted in 4"-*O*-acyl product (**5**)[9] in 83% yield. After deacetylation (100%) of **5**[9] in MeOH, protective demethylation at 4"'-*N* with FmocCl[4c] furnished

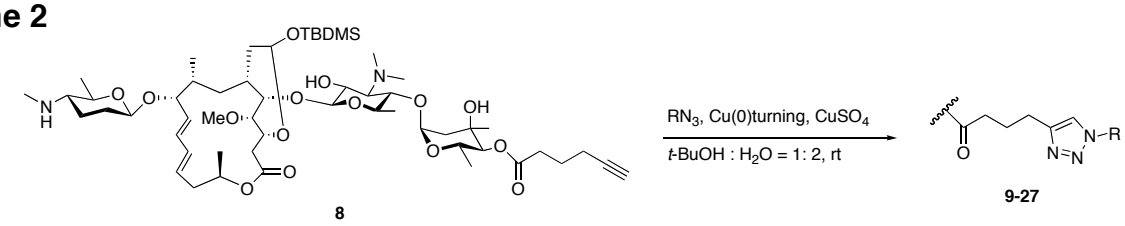
bis Fmoc (**7**)[9] in 83% yield. Finally, deprotection of the bis-Fmoc groups with piperidine afforded **8**[9] in 54% yield, and this was used as the common precursor for click process derivatization (**Scheme 1**).

Scheme 1

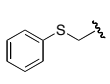
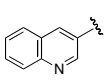


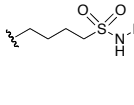
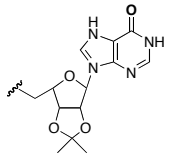
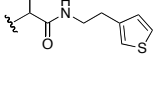
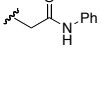
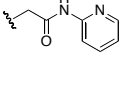
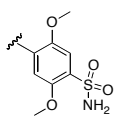
Nineteen azide compounds[7,8], representing a diverse range of hydrophobic and hydrophilic groups, were subjected to the copper-catalyzed Fokin-Huisgen cycloaddition process[7c] with spiramycin alkyne (**8**) under copper-catalyzed conditions.[11] Each of these azide molecules was linked to **8** to give nineteen triazole candidates (**9**-**27**)[9] in good to excellent isolated yields (50-98%) (**Scheme 2**). [Such reliability has become expected of this copper-catalyzed Fokin-Huisgen process,[7c] and even seen alongside just the next best click reaction competitors, at least for the moment, it sits clear and away at the top.]

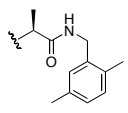
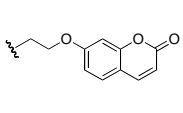
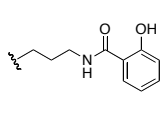
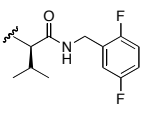
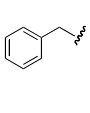
Scheme 2



8 **9-27**

	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
R								
times	17 h	13 h	15 h	15 h	11 h	14 h	17 h	15 h
yields	78%	82%	70%	71%	62%	50%	56%	79%

	(17)	(18)	(19)	(20)	(21)	(22)
R						
times	20 h	24 h	15 h	17 h	24 h	27 h
yields	80%	89%	88%	74%	96%	57%

	(23)	(24)	(25)	(26)	(27)
R					
times	24 h	27 h	19 h	14 h	14 h
yields	72%	77%	98%	54%	89%

The efficiency of the present click chemistry process allowed the preparation of each triazole candidates in sufficient purity for *in vitro* antibacterial testing against eight Gram-positive strains and one Gram-negative strain using standard serial-dilution techniques (**Table 1**).

Table 1. Antibacterial Activity of Spiramycin I and Analogues

	MIC (μg/mL)								
	S.aureus								*E. coli*
Compound No.	FDA209P[a]	Smith[a]	MRSA N315 IR94[b]	MRSA N315 IR94 HR-1[b]	MRSA 70[b]	MRSA 92-1191[b]	ISP447[c]	ISP217[d]	NIHJ JC-2[e]
1	32	32	32	32	32	64	64	32	>64
2 (SPM)	2	4	>256	>256	>256	–	8	>256	>256
8	16	16	16	16	16	32	32	16	>128
9	8	8	8	8	8	16	16	8	>128
10	8	8	8	8	8	16	16	8	>128
11	8	16	16	16	16	32	32	8	>128
12	16	32	32	16	32	32	64	16	>128
13	16	16	16	16	16	64	64	16	>128
14	64	128	64	64	64	128	>128	64	>128
15	16	32	32	16	32	64	64	16	>128
16	32	128	64	64	–	128	128	64	>128
17	16	64	16	16	32	64	64	16	>128
18	32	128	32	32	32	>128	64	32	>128
19	16	32	16	16	16	32	32	16	>128
20	8	16	16	16	16	16	32	16	>128
21	16	32	16	16	16	32	32	16	>128
22	16	32	16	16	32	32	32	16	>128
23	8	16	16	16	8	32	16	16	>128
24	8	32	16	16	16	64	32	16	>128
25	8	16	16	16	16	16	16	8	>128
26	8	32	16	16	16	32	32	16	>128
27	8	32	16	16	16	32	32	16	>128

[a] *S. aureus* FDA209P and Smith: susceptible strains. [b] *S. aureus* MRSA N315 IR94, MRSA N315 IR94 HR-1, MRSA 70, and MRSA 92-1191: MRSA strains isolated from clinical patients. [c] *S. aureus* ISP447: inducibly-resistant strain. [d] *S. aureus* ISP217: constitutively-resistant strain. [e] *E. coli* NIHJ JC-2: susceptible Gram-negative strain.

The parent spiramycim (**2**) was inactive against clinically isolated MRSA strains (MICs >256 μg/mL), but the primary derivative (**1**) was effective against *staphylococcus* strains including MRSA strains (MICs 32~64 μg/mL). To date, adamantyl-triazole and thiophenylmethyl-triazole derivatives (**9** and **10**)

have shown promising antibacterial properties, with activity 4-fold greater than the lead derivative (**1**). Further in vitro and in vivo studies on these spiramycin analogues are in progress.

In conclusion, we have described a highly efficient approach to the preparation of the spiramycin analogues through the use of click chemistry. In fact, synthesis of any spiramycin analogues had been required 1) selective protection; 2) selective functionalisation; and 3) total deprotection for each derivatives with complicated handling. However the present protocol requires only one alkyne, which is expanded to a variety of triazole candidates with simple azide libraries. We believe that this method would be widely applied to preparation of natural product analogues in order to simply and rapidly identify high-affinity inhibitors.

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9. Dry DMF, ethyl Acetate and CH_2Cl_2 were purchased from Kanto Chemical Co. Precoated silica gel plates with a fluorescent indicator (Merck 60 F254) were used for analytical and preparative thin layer chromatography. Flash column chromatography was carried out with Merk silica gel 60 (Art. 1.09385). ^{1}H and ^{13}C NMR spectra were measured on JEOL JNM-EX270 (270 MHz). All infrared spectra were measured on a Horiba FT-210 spectrometer. High- and low-resolution mass spectra were measured on a JEOL JMS-DX300 and JEOL JMS-AX505 HA spectrometer. Elemental analysis data were measured on a Yanaco CHN CORDER MT-5. The data for common alkyne precursor (**8**) is included as an example: white amorphous powder, $[\alpha]_D^{28}$ –5.4° ($CHCl_3$, *c* 1.00); IR (KBr) ν cm^{-1} : 3489 (br), 3309 (br), 2937 (m), 1738 (m), 1633 (w), 1458 (w), 1375 (w), 1254 (m), 1161 (m), 1115 (m), 1068 (s), 1018 (m), 845 (m) cm^{-1}; ^{1}H-NMR (270 MHz, CD_3OD) δ (ppm) : 6.16 (2H, m), 5.94 (1H, m), 5.62 (1H, m), 5.15 (1H, m), 4.68 (2H, m), 4.59 (1H, d, *J* = 7.6 Hz), 4.46 (2H, m), 4.36 (1H, dd, *J* = 7.6 Hz), 4.26 (1H, m), 4.15 (1H, m), 3.65 (1H, dd, *J* = 7.6, 2.7 Hz), 3.44 (3H, s), 3.40-3.25 (3H, m), 2.59-2.25 (20H, m), 2.11 (2H, m), 2.00-1.71 (10H, m), 1.68-1.40 (8H, m), 1.31-1.29 (3H, d, *J* = 5.9 Hz), 1.27 (1H, m), 1.26-1.23 (3H, d, *J* = 7.8 Hz), 1.14-1.12 (3H, d, *J* = 5.9 Hz), 1.08 (s, 3H), 1.02-0.99 (3H, d, *J* = 6.8 Hz), 0.89 (9H, s), 0.55 (1H, m), 0.09 (6H, s); ^{13}C-NMR (67.5 MHz, CD_3OD) δ (ppm) : 174.7, 172.3, 141.1, 136.5, 128.5, 128.4, 105.5, 103.0, 100.5, 98.0, 87.9, 85.3, 83.2, 79.8, 78.9, 77.1, 76.8, 74.4, 72.2, 71.9, 71.2, 70.8, 70.7, 64.7, 62.2, 59.1, 43.1, 42.9, 42.8(x2), 41.1, 40.8, 39.3, 35.7, 34.7, 33.9, 33.8, 32.1, 32.0, 30.3, 28.7, 26.8, 26.7, 26.1, 25.2, 22.0, 20.8, 20.0, 19.5, 19.2, 18.8, 18.7, 18.6(x3), -3.4, -4.5; HR-MS (FAB) (matrix; m-NBA) *m/z* 1037.6356 [$(M+H)^+$; calcd for $C_{54}H_{92}N_2O_{15}Si$: 1037.6345]; *Anal.* Calcd for $C_{54}H_{91}N_2O_{15}Si\bullet H_2O$: C, 61.45; H, 8.98; N, 2.65. Found: C, 61.48, H, 8.84, N, 2.87.

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11. Each azide compound (1.2 eq.) was reacted with the spiramycin alkyne (**8**) (1.0 eq.) in the presence of $CuSO_4$ (0.1 eq.) and copper turning (5 mg) at room temperature. The solvent mixture was in the ratio of 1:2-*t*-BuOH:H_2O. Excess azide compound was used to drive all alkyne react to product. The reaction were done in small vials with stirring and allowed to occur for 11-27 hrs. The reactions were monitored by TLC plate($CHCl_3$:MeOH:NH_4OH-10:1:0.1) and LC/MS (Waters 2695 and ZQ) with MassLynx 4.0. After reactions completion, the reaction mixtures were extracted with $CHCl_3$(x3), the combined organic layers were washed with sat. NaCl aq. solution (x1), dried over Na_2SO_4 and concentration. Then flash chromatography ($CHCl_3$:MeOH:NH_4OH-50:1:0.1) afforded triazole products in 50-98% yields. The data for benzyltriazole derivative (**27**) in included as an example: white amorphous powder, $[\alpha]_D^{28}$ –5.3° ($CHCl_3$, *c* 1.00); IR (KBr) ν cm^{-1} : 3456 (br), 2937 (m), 1735 (m), 1629 (w), 1456 (w), 1371 (w), 1254 (m), 1159 (m), 1126 (m), 1066 (s), 1018 (m), 845 (m) cm^{-1}; ^{1}H-NMR (600 MHz, CD_3OD) δ (ppm) : 7.79 (1H, s), 7.42-7.34 (5H, m), 6.26 (1H, dd, *J* = 10.2, 5.0 Hz), 6.16 (1H, dd, *J* = 10.5, 4.7 Hz), 6.00 (1H, dd, *J* = 9.9, 6.1 Hz), 5.68 (1H, dq, *J* = 7.7, 7.4 Hz), 5.60 (2H, s), 5.19 (1H, d, *J* = 3.3 Hz), 4.77 (1H, m), 4.73 (1H, d, *J* = 7.2 Hz), 4.62 (1H, d, *J* = 10.2 Hz,), 4.48 (2H, m), 4.40 (1H, d, *J* = 7.4 Hz), 4.30 (1H, t, *J* = 7.2 Hz), 4.21 (1H, m), 3.69 (1H, dd, *J* = 7.4, 2.8 Hz), 3.49 (3H, s), 3.41 (1H, m), 3.33 (2H, m), 2.78 (2H, t, *J* = 7.4 Hz), 2.63-2.35 (16H, m), 2.57 (6H, s), 2.17 (2H, m), 2.06-1.91 (5H, m), 1.81 (1H, m), 1.69 (1H, d, *J* = 13.8 Hz), 1.60 (1H, m), 1.50 (1H, m), 1.36-1.34 (8H, dd, *J* = 4.7, 1.1 Hz), 1.28-1.27 (3H, d, *J* = 6.1 Hz), 1.16-1.15 (3H, d, *J* = 6.1 Hz), 1.11 (3H, s), 1.06-1.05 (3H, d, *J* = 7.2 Hz), 0.94 (9H, s), 0.60 (1H, t, *J* = 12.4 Hz), 0.13 (6H, s); ^{13}C-NMR (67.5 MHz, $CDCl_3$) δ (ppm) : 174.5, 172.0, 148.6, 140.8, 136.9, 136.3, 130.0(x2), 129.5, 129.0(x2),128.2, 128.1, 123.4, 105.2, 102.7, 100.2, 97.7, 87.5, 85.0, 82.9, 78.6, 76.8, 76.5, 74.1, 72.0, 71.9, 71.6, 70.9, 70.5, 64.4, 61.9, 58.8, 54.8, 42.7(x2), 42.6, 42.4, 40.8, 40.5, 39.0, 35.4, 34.4, 34.1,33.6, 31.8, 28.3, 26.3(x3), 25.8, 25.7, 25.6, 21.6, 20.5, 19.6, 19.2, 18.9, 18.3, -3.7, -4.8; HR-MS (FAB) (matrix; m-NBA) *m/z* 1170.6984 [$(M+H)^+$; calcd for $C_{61}H_{99}N_5O_{15}Si$: 1170.6985] ; *Anal*. Calcd for $C_{61}H_{98}N_5O_{15}Si\bullet 4/5H_2O$: C, 61.83; H, 8.56; N, 5.91. Found: C, 61.96, H, 8.84, N, 5.95.