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Preparation and biological evaluation of novel leucomycin analogs derived from nitroso Diels–Alder reactions†

Baiyuan Yang,[a] Tina Zöllner,[b] Peter Gebhardt,[b] Ute Möllmann[b] and Marvin J. Miller*[a]

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A series of 10,13-disubstituted 16-membered macrolides was synthesized using nitroso Diels–Alder reactions of leucomycin A7. Despite the extensive constituent functionalities in leucomycin, the hetero cycloaddition reactions proceeded in a highly regio- and stereoselective fashion. Subsequent chemical modifications of the nitroso cycloadducts, including N–O bond reduction, were also conducted. Most leucomycin derivatives retained antibiotic profiles similar to leucomycin A7, and, in contrast to leucomycin itself, several exhibited moderate antiproliferative and cytotoxic activity.

Introduction

Antibiotic-resistant strains of pathogenic bacteria have become increasingly prevalent in the last decade.[1] Promising approaches to new drug scaffold discovery include mining underexplored microbial niches for natural products.[2] Natural products and their derivatives represent the most prolific source of molecular diversity in drug discovery.[3] Functional group transformation of natural products constitutes one of the main avenues for generating pharmacologically relevant compounds with altered and sometimes improved biological properties. Typical chemical derivatizations of natural products are often limited to standard modification of nucleophilic or electrophilic functional groups. Since many natural products contain multiple functional groups of the same or similar type, derivatization selectivity is often problematic. Hence, new methods for derivatization and semi-syntheses are still needed. Our effort in this area has involved nitroso Diels–Alder (NDA) reactions as efficient methods for derivatization and functionalization of diene-containing natural products.[4] Our early research has demonstrated that several complex diene-containing natural products, including leucomycin A7[5] (also known as turimycin H3) (Fig. 1), readily undergo nitroso cycloadditions, generating 1-amino-4-hydroxy-2-ene heterocycle scaffolds with high regio- and stereoselectivity.[4a] Our continued interest in natural product derivatization encouraged us to further explore the scope and limitation of this chemistry. In this regard, leucomycin A7, as a representative natural macrolide antibiotic containing a conjugated diene was chosen for further investigation of this method for modular enhancement of Nature's diversity (MEND).

Macrolide antibiotics have been widely used to treat bacterial infections for many years.[6] They inhibit protein biosyntheses by binding to bacterial ribosomal RNA (rRNA) to exhibit antibiotic activities.[7] As a large family of both natural and semisynthetic antibiotics, macrolides are classified according to the size of the lactone ring consisting of 12–16 atoms and to the number and type of carbohydrates attached.

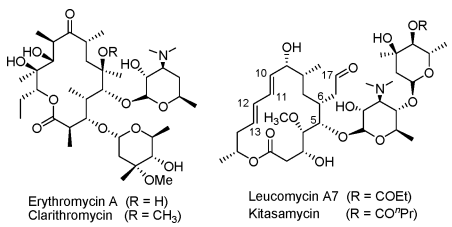

Fig. 1 Structures of representative 14- and 16-membered macrolides.

The erythromycin and leucomycin families (Fig. 1), as representatives of 14- and 16-membered macrolides, respectively, are the most commonly used macrolide antibiotics.[8] Unfortunately, despite their great success as therapeutic agents, bacteria are becoming increasingly resistant to these and other antibiotics. Thus, there is significant need for the rapid development of novel antibacterial agents.[2,9] Compared to extensive studies related to modification and derivatization of the 14-membered macrolide family, novel derivatization and semisyntheses of 16-membered macrolides, such as the leucomycins, have been only minimally explored.[10] Herein, we report full details of the syntheses and biological evaluation of 10,13-disubstituted 16-membered macrolides from nitroso Diels–Alder reactions of leucomycin A7 (**1**) and subsequent chemical modifications.

Results and discussion

Our previous studies demonstrated that, in contrast to reactions with nitrosobenzene **2**, or acylnitroso agents **3**, cycloaddition of leucomycin A7 with 6-methyl-2-nitrosopyridine **4a** afforded adduct **5a** as a single isomer in 90% yield at room temperature within 30 min (Table 1).[4a] Clearly, 6-methyl-2-nitrosopyridine **4a**, as a stabilized form of iminonitroso reagent, constitutes an ideal combination of reactivity and stability for NDA reactions with leucomycin A7, relative to benzenenitroso **2** and acylnitroso agent **3**, respectively.

A subsequent survey of solvent effects for the cycloaddition reaction of **1** with **4a** revealed that both THF and DCM were

[a]*Department of Chemistry and Biochemistry, University of Notre Dame, 251 Nieuwland ScienceHall, Notre Dame, Indiana, 46556, USA. E-mail: mmiller1@nd.edu; Fax: +1 574 631 6652; Tel: +1 574 631 7571*
[b]*Leibniz Institute for Natural Products Research and Infection Biology, Hans Knoell Institute, Beutenbergstrasse 11a, D-07745, Jena, Germany*
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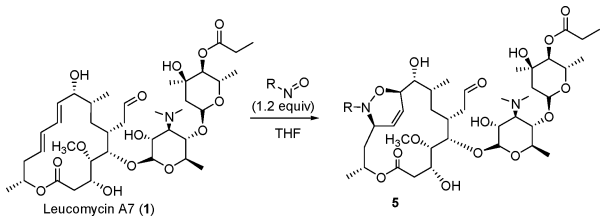
Table 1 Nitroso Diels–Alder reaction of leucomycin A7 with various nitroso agents



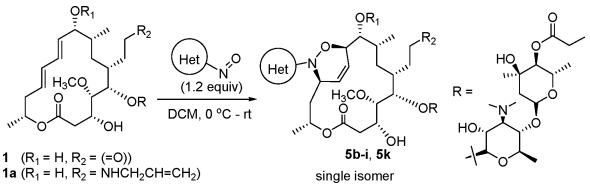
Entry	Nitroso		T/°C	Product (yield [%])
1	**2**		25 or 60	0
2	**3**[a]		60	**5** (<5)[b]
3	**4a**		25	**5a** (90)[c]

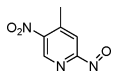
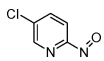
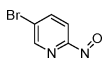
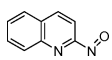
[a] Generated from thermal dissociation of the corresponding dimethylanthracene (DMA) adduct. [b] Based on LC/MS analysis. [c] Isolated yield.

suitable solvents for NDA reactions with leucomycin A7. On the other hand, in CH_3OH, the NDA reaction was found to be much slower, thus causing the decomposition of nitroso **4a** to be a major problem.[11] One possible explanation is that leucomycin A7 could form an acetal at the aldehyde position with CH_3OH, which consequently changes the conformation of the diene to slow down the NDA reaction. Two isomers were observed in DMSO for reasons that are not clear at this point.

To further extend the NDA chemistry, a series of 2-nitrosopyridine derivatives **4** was synthesized in a two-step sequence (*N*,*N*-dimethyl sulfilimine intermediate formation, followed by oxidation using *m*-CPBA).[12] It was noted that several nitroso agents were not very soluble in THF. Hence, considering the solvent effects, DCM was selected as the optimal solvent for extended cycloaddition reactions with **1**. The results are summarized in Table 2. Most of the nitroso agents generated, except for **4b** and **4c**, were stable and were used in pure form. The instability of nitroso agents **4b** and **4c** required their immediate use, so they were trapped *in situ* with **1**, giving cycloadducts **5b** and **5c** in low yields, respectively, but with high regio- and stereoselectivity (entries 1 and 2). The low yields might result from the labile nature of nitroso agents **5b** and **5c** as well as the instability of leucomycin A7 under oxidative conditions. Cycloaddition with 2-nitrosopyridine **4d** gave results similar to those previously obtained upon reaction with **4a** (entry 3). Halide substituents at the 5-position of the pyridine ring of the nitroso agents did not affect reactivity and selectivity. Thus, adducts **5e–h** were generated in good yields as single isomers (entries 4–7). Even when quinoline-based nitroso agent **4i** was used, a single-isomer product, **5i**, was obtained in 78% yield (entry 8). An alternative form of stabilized iminonitroso species, 5-methyl-3-nitrosoisoxazole **4j**, was also treated with **1**. Compared to the reactions with pyridinylnitroso agents **4e–i**, cycloaddition between **4j** and **1** occurred at a relatively slower rate, and gave a mixture of isomeric adducts in moderate yield (entry 9). Leucomycin A7 analog **1b**, synthesized from the reductive amination of the 17-

Table 2 Nitroso Diels–Alder reaction of leucomycins with various iminonitroso agents



Entry	Leucomycin	Nitroso		Adduct	Yield (%)[a]
1[b]	**1**	**4b**		**5b**	26
2[b]	**1**	**4c**		**5c**	13
3	**1**	**4d**		**5d**	91
4	**1**	**4e**		**5e**	90
5	**1**	**4f**		**5f**	88
6	**1**	**4g**		**5g**	86
7	**1**	**4h**		**5h**	89
8	**1**	**4i**		**5i**	78
9	**1**	**4j**		—	60[c]
10	**1a**	**4a**		**5k**	88

[a] Isolated yields. [b] *In situ* trapping required. [c] Multiple isomeric adducts were detected by ^{1}H NMR and LC/MS.

aldehyde of **1** with allylamine,[13] was also used in the reaction with nitroso agent **4a**. As a result, cycloadduct **5k** was obtained as a single isomer in 88% yield (entry 10). Since extensive 1D and 2D high resolution NMR studies have been established to conclusively determine the structure of cycloadduct **5a**, the stereochemistry of the other nitroso cycloadducts, **5a–i** and **5k**, were assigned by comparison of ^{1}H and ^{13}C NMR profiles with those of **5a**, which indicated that all the leucomycin A7 nitroso adducts obtained have the same regio- and stereochemistry. The excellent regio- and stereoselectivity of nitroso cycloadditions examined here have clearly shown that 2-nitrosopyridines **4** are very effective dienophiles with exquisite sensitivity to electronic and steric influences of complex diene-containing macrolides, which is probably derived from the unsymmetrical nature of the 2-nitrosopyridines studied.

Further diversification of leucomycin nitroso cycloadducts was carried out by N–O bond reduction with the intent to generate the corresponding macrolides with additional 1,4-amino alcohol substituents. Towards this end, adduct **5a** was chosen as a model

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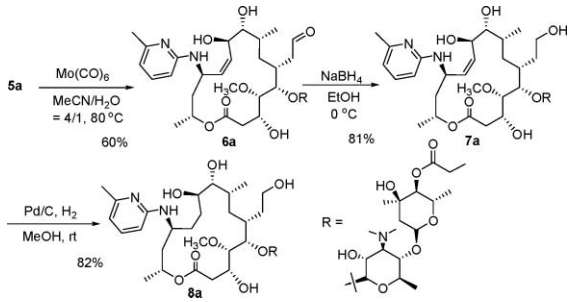
substrate. Reaction with $Mo(CO)_6$[14] at high temperature and in aqueous media successfully reduced the N–O bond of **5a** to give compound **6a** in 60% yield (Scheme 1). To further explore the structure–activity relationships of this novel family of macrolide antibiotics, 18-OH derivative **7a** was also obtained by $NaBH_4$-mediated reduction of the aldehyde of **6a**. Subsequent hydrogenation of **7a** reduced the remaining alkene to give compound **8a** in 82% isolated yield (Scheme 1).



Scheme 1 Chemical modification of leucomycin adduct **5a**.

With the biological profile of parent leucomycin compound **1** known for comparison, the macrolide derivatives generated in this work, and ciprofloxacin as a control, were tested for their *in vitro* antibacterial activities against various strains of Gram-positive and Gram-negative bacteria as well as *Mycobacterium vaccae*, using agar diffusion assays (Table 3). In general, most leucomycin nitroso cycloadducts exhibited antibiotic profiles similar to leucomycin A7 (**1**) itself against most strains tested, although relatively decreased activities were observed. Unlike parent compound **1**, most derivatives except **5f** were inactive against *Pseudomonas aeruginosa* K799/61. While N–O bond-cleaved compound **6a** showed similar antibiotic activity to cycloadducts, both 18-OH derivatives, **7a** and **8a**, were found to be relatively inactive against selected bacteria, which indicated that the ethylaldehyde function at the C6 position of leucomycin might be essential for bioactivity. The importance of this ethylaldehyde substituent of several other 16-membered macrolide antibiotics in the inhibition of protein biosyntheses, including, for example, carbomycin A and spiramycin, was also reported by Steitz *et al*.[15]

Leucomycin analogs obtained were also subjected to antiproliferative and cytotoxicity assays. The results are summarized in Table 4. Interestingly, leucomycin cycloadducts derived from **1**, except **5c** and **5d**, showed moderate antiproliferative activity in L-929 (mouse fibroblasts) and K-562 (human leukaemia) cell lines, as well as moderate cytotoxic activity in HeLa (human cervix carcinoma), PC-3 (prostate cancer) and MCF-7 (breast cancer) cell lines. In contrast, leucomycin A7 (**1**) was not active at all in these assays (entries 1–9). Noteworthy here is the fact that the only difference among these cycloadducts is the substituent group in the pyridine ring, which changed the activity from none (**5c** and **5d**) to notable (**5a–b**, **5e–f**, **5h–i**). Particularly, adduct **5i** inhibited both PC-3 and MCF-7 cancer cells at the low micromolar level (entry 9). Cycloadducts **5k** derived from leucomycin analog **1a** had antiproliferative profiles similar to that of **5a** (entries 10 and 2). The N–O bond-reduced analogs **6a**, **7a** and **8a** were found to be relatively inactive against PC-3 and MCF-7 cells (entries 11–13), suggesting that the incorporated substituted rigid N–O heterocycle might be essential for antiproliferative and cytotoxic activity. Overall, these biological assays indicated that the nitroso heterocycle expanded the scope of the biological activity profile of its parent natural product.

Conclusions

We have demonstrated an efficient approach to 11,13-disubstituted leucomycin derivatives using nitroso Diels–Alder reactions and related chemistry for the development of new macrolide antibiotics. Most derivatives showed moderate antiproliferative and anticancer activity, with relatively lower antibacterial activity compared to that of the native macrolide.

Table 3 Antibacterial activity of leucomycin A7 and its analogs in the agar diffusion assay

	Growth inhibition zones in mm (9 mm well diameter)							
	Gram-positive bacteria				Gram-negative bacteria			
	B. subtilis	*S. aureus*	*S. aureus*	*E. faecalis*	*P. aeruginosa*		*E. coli*	*M. vaccae*
Compds[a]	ATCC 6633	SG 511	134/93 MRSA	1528 VRE	SG 137	K799/61	SG 458	IMET 10670
1	29	30	10	16.5	11	21.5	19	40
5a	26.5	27.5	0	18p	0	11P	0	30
5b	19/22P[b]	20	0	13P	0	0	0	13/19P
5c	17/20P	18	0	12P	0	0	0	14P
5d	28	29	19P	15	0	0	14p	32
5f	26	25	0	16	0	16P	13	27
5h	21/25p[c]	24.4	14P	13/17p	0	0	15.5	23/32p
5k	13/15p	14	0	10/15P	0	0	0	19/24P
6a	19/23	20	0	14p	0	0	16	29/34P
7a	0	13	0	0	0	0	0	15p
8a	0	0	0	0	0	0	0	14
cipro	28	17.5	0	16	24	17	20	18

[a] Exactly 50 μL of a 2.0 mM solution (DMSO–MeOH) of each compound was filled in 9 mm wells in agar media (standard I Nutrient Agar, Serva). Inhibition zones read after incubation at 37 °C for 24 h. Cipro (ciprofloxacin) was dissolved in H_2O to give a 5 μg mL^{-1} solution. [b] P, unclear inhibition zone/many colonies in the inhibition zone. [c] p, partially clear inhibition zone/colonies in the inhibition zone.

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Table 4 Antiproliferative activity and cytotoxicity of leucomycin derivatives

Entry	Compds	L-929 GI_{50}/μM	K-562 GI_{50}/μM	HeLa CC_{50}/μM	PC-3 IC_{50}/μM	MCF-7 IC_{50}/μM
1	**1**	>66	>66	>66	>50	>50
2	**5a**	31	34	44	20	14
3	**5b**	9.1	36.3	33.5	nd[a]	nd
4	**5c**	>100	>100	>100	nd	nd
5	**5d**	>100	>100	>100	>100	>100
6	**5e**	nd	nd	nd	30	18
7	**5f**	15.6	15	23.5	16	11
8	**5h**	30	20	40	10	6
9	**5i**	nd	nd	nd	8	6
10	**5k**	18	8.3	37	nd	nd
11	**6a**	>50	>50	>50	50	>50
12	**7a**	nd	nd	nd	40	>50
13	**8a**	nd	nd	nd	20	42

[a] Not determined.

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Experimental section

General comments

Commercial grade reagents and solvents were used without further purification. Flash chromatography was performed with silica gel 60 (230–400 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded at ambient temperature with the residual solvent peaks as internal standards. Infrared spectra were recorded by a FT-IR spectrometer and reported as cm^{-1}. Specific rotations were measured with a Rudolf Research Autopol III polarimeter at 589 nm and 25 °C. All melting points were recorded uncorrected. High-resolution mass spectra (HRMS) data were obtained as specified. (nr) not resolved, ^{1}H NMR signals are assigned based on HMQC- and HMBC-shifts.

General procedure for the synthesis of 5a and 5d–i. To a solution of leucomycin A7 (**1**) (50 mg, 0.066 mmol) in DCM (2 mL) was added 2-nitrosopyridine **4** (0.079 mmol, 1.2 eq.) in DCM (1 mL) slowly at 0 °C. The reaction mixture was gradually warmed to room temperature, and stirred for an additional 30 min until **1** was consumed. Then the solvent was removed under reduced pressure, and the crude product was purified using silica gel chromatography (DCM–MeOH, 20 : 1 to 12 : 1) to afford nitroso Diels–Alder adduct **5**.

Leucomycin adduct 5b. To a solution of *N*-(4-methyl-5-nitro-2-pyridyl)-dimethylsulfilimine (46.92 mg, 0.22 mmol) in 2 mL of DCM was added 64.9 mg of 3-chloro-benzoyl peroxide at 0 °C under inert gas atmosphere. After 1.5 h, 20 μL of dimethylsulfide was added and stirred for 10 min. Then, leucomycin A7 (**1**) (151 mg, 0.2 mmol) was added. After stirring for an additional 3 h, the reaction was allowed to stand overnight at 4 °C and after this, the solvent was removed under reduced pressure. The mixture was diluted with 15 mL of ethyl acetate, washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4, and then filtered. The organic solvent was removed under reduced pressure and the crude product was purified using silica gel chromatography ($CHCl_3$–MeOH, 98 : 2 with ammonia, 7 N) to afford **5b** as a yellow solid (48 mg, 26% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.74 (s, 1 H), 8.96 (s, 1 H), 6.88 (s, 1 H), 6.22 (ddd, J = 10.4, 4.4, 2.2 Hz, 1 H), 5.76 (d, J = 10.4 Hz, 1 H), 5.28 (m, 1H), 5.20–5.25 (m, 1 H), 5.02 (d, J = 3.6 Hz, 1 H), 4.76 (d, J = 6.9 Hz, 1 H), 4.57 (d, J = 10.2 Hz, 1 H), 4.42 (m, 1 H), 4.29 (d, J = 7.5 Hz, 1 H), 4.17-4.25 (m, 1 H), 3.90 (dd, J = 7.6, 3.6 Hz, 1 H), 3.79 (dd, J = 7.7, 1.5 Hz, 1 H), 3.44 (s, 3 H), 3.41 (dd, J = 10.6, 8.0 Hz, 1 H), 3.25 (m, nr), 3.23 (m, 1 H), 3.06 (dd, J = 7.7, 2.0 Hz, 1 H), 2.91 (dd, J = 18.2, 8.2 Hz, 1 H), 2.63 (nr), 2.63 (s, nr), 2.55 (nr), ~2.43 (m, nr), 2.43 (s, 6 H), 2.40 (nr), 2.25 (m, nr), 2.18 (nr), 1.97 (nr), 1.96 (d, J = 14.3 Hz, 1 H), 1.79 (dd, J = 14.3, 3.9 Hz, 1 H), 1.60 (m, nr), 1.48–1.55 (m, nr), 1.33 (d, J = 6.5 Hz, 1H), 1.19 (d, J = 5.8 Hz, 3 H), 1.14 (t, J = 7.6, 7.6 Hz, 3 H), 1.13 (nr), ~1.09 (nr), 0.95 (nr); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.9, 174.4, 171.8, 159.6, 147.7, 146.1, 138.4, 127.3, 127.3, 108.2, 104.2, 96.9, 84.4, 80.1, 77.1, 75.6, 73.8, 73.0, 72.4, 71.4, 69.3, 68.8, 68.3, 67.4, 63.4, 61.3, 53.7, 45.8, 41.9, 41.7, 40.4, 38.0, 37.5, 32.8, 32.3, 27.6, 25.2, 21.7, 19.9, 18.9, 17.7, 16.7, 9.3; HRMS (ESI) $[M + H]^+$ calcd for $C_{44}H_{67}N_4O_{17}$ 923.4501, found 923.4515.

Leucomycin adduct 5c. Yield: 13%; yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.73 (s, 1 H), 8.40 (d, J = 4.6 Hz, 2 H), 6.67 (t, J = 4.8 Hz, 1 H), 6.18 (ddd, J = 10.4, 4.5, 2.1 Hz, 1 H), 5.76 (d, J = 10.3 Hz, 1 H), 5.20–5.30 (m, 1 H), 5.09–5.15 (m, 1 H), 5.02 (d, J = 2.7 Hz, 1 H), 4.85–4.90 (m, 1 H), 4.56 (nr), 4.43 (nr), 4.12–4.19 (m, 1 H), 4.22 (d, J = 7.4 Hz, 1 H), 3.90–3.97 (m, 1 H), 3.74 (d, J = 7.0 Hz, 1 H), 3.46 (s, 3 H), 3.45 (nr), 3.25 (nr), 3.20 (nr), 3.19 (nr), 2.87 (dd, J = 17.9, 8.5 Hz, 1 H), 2.72 (dd, J = 15.0, 7.6 Hz, 1 H), 2.58 (dd, J = 15.1, 4.9 Hz, 1 H), 2.49 (nr), 2.43 (s, 6 H), 2.40 (nr), 2.34 (nr), 2.18 (nr), 1.97 (nr), 1.92 (nr), 1.56–1.65 (nr), 1.82 (nr), 1.78 (nr), 1.28 (d, J = 6.3 Hz, 3 H), 1.19 (d, J = 5.8 Hz, 3 H), 1.16 (n.r), 1.10 (d, J = 6.9 Hz, 3 H), 1.07 (nr), 0.82–0.91 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.9, 174.4, 171.6, 162.2, 158.2, 128.3, 126.8, 112.8, 104.9, 96.8, 83.1, 80.9, 77.2, 75.4, 74.6, 73.0, 71.7, 71.6, 69.3, 68.8, 68.4, 67.0, 63.4, 61.2, 54.8, 46.0, 42.0, 41.6, 40.4, 38.3, 38.0, 34.1, 32.6, 27.6, 25.2, 20.8, 19.0, 17.8, 16.9, 9.3; HRMS (ESI) $[M + H]^+$ calcd for $C_{42}H_{67}N_4O_{15}$ 867.4603, found 867.4577.

Leucomycin adduct 5e. Yield: 90%; yellow solid; $[\alpha]_D^{20} = -84.5$ (c = 1.0, $CHCl_3$); mp 128–130 °C; IR (neat) 3465, 3020, 2954, 1723, 1579, 1521, 1424, 1215, 1046, 929, 773, 669 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.79 (s, 1H), 8.11 (d, J = 2.8 Hz, 1 H), 7.40 (m, 1 H), 7.09 (dd, J = 8.9, 3.5 Hz, 1 H), 6.23 (ddd, J = 10.4, 4.6, 2.3 Hz, 1 H), 5.77 (d, J = 10.5 Hz, 1 H), 5.28 (m, 1 H), 5.15

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(m, 1 H), 5.08 (m, 1 H), 4.62 (d, J = 10.2 Hz, 1 H), 4.47 (m, 1 H), 4.34 (d, J = 7.5 Hz, 1 H), 4.28 (m, 1 H), 3.92 (dd, J = 7.7, 4.3 Hz, 1 H), 3.86 (dd, J = 7.7, 2.0 Hz, 1 H), 3.50 (s, 3 H), 3.48 (m, 1 H), 3.30 (m, 1 H), 3.26 (t, J = 9.4 Hz, 1 H), 3.19 (dd, J = 7.6, 2.4 Hz, 1 H), 2.95 (dd, J = 18.8, 7.8 Hz, 1 H), 2.67–2.58 (m, 1 H), 2.51–2.40 (m, 10 H), 2.32 (m, 1 H), 2.14 (m, 1 H), 2.05–1.92 (m, 2 H), 1.84 (dd, J = 14.4, 4.2 Hz, 1 H), 1.77 (m, 1 H), 1.62 (m, 1 H), 1.32 (d, J = 6.6 Hz, 3 H), 1.23 (d, J = 5.9 Hz, 3 H), 1.18 (t, J = 7.6 Hz, 3 H), 1.13 (d, J = 6.1 Hz, 3 H), 1.12 (s, 3 H), 1.08 (d, J = 6.9 Hz, 3 H), 0.89 (m, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.4, 174.7, 172.4, 155.9, 154.5 (d, J = 156.7 Hz), 135.5 (d, J = 25.1 Hz), 128.1, 127.1, 125.8 (d, J = 20.0 Hz), 109.9, 104.6, 97.1, 84.6, 80.8, 77.5, 77.4, 75.7, 73.2, 71.8, 69.6, 69.0, 68.6, 67.9, 63.6, 61.6, 53.7, 46.1, 42.4, 41.9, 40.6, 38.4, 37.9, 33.2, 33.1, 31.9, 29.2, 27.8, 25.5, 20.4, 19.2, 18.1, 17.2, 9.6; HRMS (ESI) $[M + H]^+$ calcd for $C_{43}H_{67}FN_3O_{15}$ 884.4551, found 884.4565.

Leucomycin adduct 5g. Yield: 86%; yellow solid; $[\alpha]_D^{20} = -115.2$ (c = 2.2, $CHCl_3$); mp 134–136 °C; IR (neat) 3485, 3019, 2934, 1723, 1585, 1520, 1424, 1215, 1165, 1059, 1015, 929, 767, 669 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.78 (s, 1H), 8.26 (d, J = 2.4 Hz, 1 H), 7.69 (dd, J = 8.8, 2.4 Hz, 1 H), 7.00 (d, J = 8.8 Hz, 1 H), 6.23 (ddd, J = 10.6, 4.4, 2.4 Hz, 1 H), 5.75 (d, J = 10.3 Hz, 1 H), 5.28 (m, 1 H), 5.16 (m, 1 H), 5.07 (m, 1 H), 4.66 (m, 1 H), 4.61 (d, J = 10.3 Hz, 1 H), 4.416 (m, 1 H), 4.33 (d, J = 7.6 Hz, 1 H), 4.26 (m, 1 H), 3.92 (dd, J = 7.9, 3.5 Hz, 1 H), 3.84 (dd, J = 7.6, 1.4 Hz, 1 H), 3.48 (s, 3H), 3.47 (m, 1 H), 3.29 (m, 1 H), 3.26 (t, J = 8.2 Hz,1 H), 3.14 (dd, J = 7.6, 2.0 Hz, 1 H), 3.00 (br s, 1 H), 2.94 (dd, J = 18.5, 8.2 Hz, 1 H), 2.66–2.58 (m, 2 H), 2.51–2.40 (m, 10 H), 2.29 (m, 1 H), 2.14 (m, 1 H), 2.02–1.81 (m, 3 H), 1.72 (m, 1 H), 1.61 (m, 1H), 1.32 (d, J = 6.5 Hz, 3 H), 1.22 (d, J = 5.9 Hz, 3 H), 1.17 (t, J = 7.6 Hz, 3 H), 1.12 (d, J = 6.5 Hz, 3 H), 1.11 (s, 3 H), 1.08 (d, J = 7.0 Hz, 3 H), 0.96 (m, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.3, 174.6, 172.2, 157.3, 149.1, 140.6, 127.8, 127.3, 110.6, 110.2, 104.5, 97.0, 84.5, 80.6, 77.3, 75.7, 73.7, 73.1, 71.7, 69.5, 68.9, 68.5, 67.7, 63.6, 62.0, 61.5, 53.5, 45.6, 42.0, 41.8, 40.5, 38.3, 37.8, 32.8, 31.8, 27.8, 25.5, 20.3, 19.2, 18.0, 17.0, 9.5; HRMS (ESI) $[M + H]^+$ calcd for $C_{43}H_{67}BrN_3O_{15}$ 944.3750, found 944.3728.

Leucomycin adduct 5i. Yield: 78%; yellow solid; $[\alpha]_D^{20} = -117.8$ (c = 1.7, $CHCl_3$); mp 140–142 °C; IR (neat) 3453, 3020, 2934, 1723, 1571, 1521, 1476, 1425, 1215, 1047, 929, 774, 669 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ δ 9.78 (s, 1H), 8.06 (d, J = 9.0 Hz, 1 H), 7.76 (d, J = 8.4 Hz, 1 H), 7.69 (d, J = 8.0 Hz, 1 H), 7.60 (m, 1 H), 7.37–7.33 (m, 2 H), 6.28 (ddd, J = 10.6, 4.4, 2.2 Hz, 1 H), 5.76 (d, J = 10.4 Hz, 1 H), 5.54 (m, 1 H), 5.31 (m, 1 H), 5.05 (m, 1 H), 4.68 (m, 1 H), 4.60 (d, J = 10.2 Hz, 1 H), 4.43 (m, 1 H), 4.30 (m, 2 H), 4.02 (dd, J = 7.8, 3.0 Hz, 1 H), 3.85 (dd, J = 7.8, 1.4 Hz, 1 H), 3.47 (s, 3 H), 3.41 (m, 1 H), 3.26 (m, 1 H), 3.2 (m, 1 H), 3.14 (d, J = 7.8 Hz, 1 H), 3.04 (br s, 1 H), 2.92 (dd, J = 18.1, 9.0 Hz, 1 H), 2.67 (d, J = 6.2 Hz, 3 H), 2.40-2.32 (m, 12 H), 2.19 (m, 1 H), 2.00–1.94 (m, 3 H), 1.81 (dd, J = 14.4, 3.8 Hz, 1 H), 1.77 (m, 1 H), 1.72 (m, 1 H), 1.36 (d, J = 6.8 Hz, 3 H), 1.19 (d, J = 7.6 Hz, 3 H), 1.17 (J = 7.6 Hz, 3 H), 1.11 (s, 3 H), 1.10 (d, J = 5.8 Hz, 3 H), 0.89 (m, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.4, 174.7, 172.3, 157.2, 147.5, 138.6, 130.1, 127.8, 127.7, 127.6, 127.5, 124.3, 123.9, 110.1, 104.4, 97.0, 84.5, 80.6, 77.4, 75.6, 74.1, 73.0, 73.5, 71.2, 69.6, 69.5, 68.8, 68.6, 67.8, 63.8, 63.4, 61.6, 54.0, 53.7, 46.1, 42.1, 41.7, 40.4, 38.0, 37.9, 32.6, 22.7, 20.2, 19.1, 17.9, 17.1, 9.5; HRMS (ESI) $[M + Na]^+$ calcd for C_{47} $H_{69}N_3NaO_{15}$ 938.4621, found 938.4616.

Leucomycin adduct 5l. Yield: 88%; yellow solid; $[\alpha]_D^{20} = -132.7$ (c = 1.0, MeOH); IR (neat) 3418, 2934, 2878, 1728, 1589, 1449, 1376, 1271, 1165, 1119, 1056, 1014, 911, 841, 784 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.45 (dd, J = 8.2, 7.6 Hz, 1 H), 6.85 (d, J = 8.2 Hz, 1 H), 6.57 (d, J = 7.6 Hz, 1 H), 6.20 (ddd, J = 10.4, 4.3, 2.2 Hz, 1H), 5.70 (dt, J = 10.4, 1.5 Hz, 1 H), 5.25 (m, 1 H), 5.24 (m, 1 H), 5.03 (d, J = 3.4 Hz, 1 H), 4.65 (m, 1 H), 4.58 (d, J = 10.2 Hz, 1 H), 4.44 (m, 1 H), 4.32 (d, J = 7.5 Hz, 1 H), 4.22 (m, 1 H), 3.90 (d, J = 8.8 Hz, 1 H), 3.87 (dd, J = 7.7, 3.3 Hz, 1 H), 3.54 (s, 3 H), 3.42 (dd, J = 7.5, 5.9 Hz, 1 H), 3.26 (m, 1 H)), 3.21 (m, 1H), 3.13 (dd, J = 8.6, 0.8 Hz, 1 H); 2.74–2.60 (m, 2H), 2.65–2.48 (m, 2H), 2.44 (s, 6H), 2.42 (m, 1H), 2.41 (q, J = 7.7 Hz, 2 H), 2.06 (ddd, J = 15.7, 7.5, 3.0 Hz, 1 H), 1.98 (d, J = 14.1 Hz, 1 H), 1.88 (m, 1H), 1.81 (m, 1H), 1.80 (dd, J = 14.3, 3.9 Hz, 1 H), 1.70 (m, 1 H), 1.69 (m, 1 H), 1.70–1.52 (m, 2H), 1.32 (d, J = 6.6 Hz, 3 H), 1.21 (d, J = 5.7 Hz, 3 H), 1.15 (t, J = 7.6 Hz, 3 H), 1.11 (d, J = 3.5 Hz, 3 H), 1.10 (s, 3H), 1.09 (d, J = 5.7 Hz, 3 H), 0.95 (br-m, 1H; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.4, 172.0, 157.9, 157.2, 136.4, 129.7, 127.7, 104.9, 96.8, 84.9, 79.9, 77.2, 75.5, 74.3, 73.0, 71.8, 69.3, 69.2, 68.8, 68.7, 67.4, 63.4, 61.3, 53.9, 46.7, 42.0, 41.7, 40.3, 38.0, 37.7, 34.6, 33.0, 29.9, 27.6, 25.3, 20.0, 19.0, 17.8, 17.0, 9.3; HRMS (ESI) $[M + H]^+$ calcd for $C_{47}H_{77}N_4O_{14}$ 921.5431 found 921.5400.

Compound 6a. To a solution of cycloadduct **5a** (24 mg, 0.027 mmol) in MeCN–H_2O (4 : 1, 3 mL) was added $Mo(CO)_6$ (8.6 mg, 0.033 mmol). The mixture was quickly heated to reflux (80 °C), and monitored by TLC analysis. After **5a** was consumed (12 h), the reaction mixture was cooled to room temperature and diluted with MeOH–DCM (1 : 1, 3 mL), then filtered through a pad of Celite. The solvent was removed under reduced pressure and the crude product was purified using silica gel chromatography (CH_2Cl_2–MeOH 12 : 1) to afford compound **6a** as a white solid in 60% yield. $[\alpha]_D^{20} = -55.3$ (c = 1.2, $CHCl_3$); mp 128–130 °C; IR (neat) 3397, 2928, 2875, 1733, 1603, 1465, 1165, 1084, 1052, 911, 780 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.18 (s, 1 H), 7.27 (t, J = 7.8 Hz, 1 H), 6.43 (d, J = 7.4 Hz, 1 H), 6.22 (d, J = 8.4 Hz, 1 H), 5.80 (dd, J = 10.4, 8.8 Hz, 1 H), 5.60 (m, 1 H), 5.27 (m, 1 H), 5.24 (d, J = 9.6 Hz, 1 H), 5.04 (d, J = 3.4 Hz, 1 H), 4.84 (m, 1 H), 4.81 (m, 1 H), 4.61 (d, J = 10.4 Hz, 1 H), 4.44 (m, 1 H), 4.41 (m, 1 H), 4.38 (d, J = 7.6 Hz, 1 H), 3.88 (dd, J = 8.0, 2.0 Hz, 1 H), 3.53 (s, 3 H), 3.50 (m, 1 H), 3.46 (m, 1 H), 3.39 (dd, J = 8.0, 2.3 Hz, 1 H), 3.25 (m, 1 H), 3.23 (m, 1 H), 2.73 (m, 1 H), 2.68 (m, 1 H), 2.65 (m, 1 H), 2.50 (s, 6 H), 2.47–2.37 (m, 4 H), 2.33 (s, 3 H), 2.05–1.72 (m, 6 H), 1.32 (d, J = 6.2 Hz, 3 H), 1.25 (m, 1H), 1.17 (t, J = 7.6 Hz, 3 H), 1.15 (d, J = 6.2 Hz, 3 H), 1.12 (d, J = 6.2 Hz, 3 H), 1.10 (s, 3 H), 1.09 (d, J = 6.8 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 203.2, 174.6, 173.0, 171.2, 156.4, 138.7, 137.3, 131.4, 113.4, 106.7, 104.2, 97.0, 85.6, 79.8, 77.3, 75.8, 73.1, 71.6, 69.6, 69.3, 69.0, 68.8, 63.6, 63.1, 61.6, 52.2, 44.1, 43.3, 42.1, 41.8, 40.8, 32.2, 30.2, 29.9, 28.5, 27.7, 25.4, 23.6, 22.6, 21.4, 20.9, 19.0, 17.9, 9.5; HRMS (FAB) $[M + H]^+$ calcd for $C_{44}H_{72}N_3O_{15}$ 882.4963 found 882.4961.

Compound 7a. To a solution of compound **6a** (23.3 mg, 0.03 mmol) in 3 mL of anhydrous ethanol at 0 °C was slowly added $NaBH_4$ (4.0 mg, 0.1 mmol) under argon. The reaction mixture was

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kept stirring at 0 °C for 2 h. TLC analysis indicated the complete consumption of **6a**. The reaction was quenched by slowly adding of 1 mL of H_2O. Ethanol was removed under reduced pressure. The resultant mixture was diluted with DCM–H_2O (3 mL : 2 mL), then separated. The aqueous layer was washed with 3 × 3 mL of DCM. The combined organic solvent was washed with brine and dried over Na_2SO_4. The solvent was filtered, then removed under reduced pressure to afford **7a** as a white solid in 81% yield. $[\alpha]_D^{20}$ = –42.8 (c = 1.1, $CHCl_3$); mp 137–139 °C; IR (neat) 3453, 3019, 2977, 1605, 1520, 1476, 1424, 1216, 1046, 929, 849, 761, 669 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.31 (t, J = 7.6 Hz, 1 H), 6.46 (d, J = 7.4 Hz, 1 H), 6.29 (d, J = 8.4 Hz, 1 H), 5.77 (dd, J = 10.4, 8.8 Hz, 1 H), 5.27 (t, J = 9.8 Hz, 1 H), 5.19 (m, 1 H), 5.06 (d, J = 3.6 Hz, 1 H), 4.82 (m, 1 H), 4.76 (m, 1 H), 4.61 (d, J = 10.2 Hz, 1 H), 4.58 (m, 1 H), 4.48–4.41 (m, 2 H), 4.41 (m, 1 H), 3.98 (d, J = 7.8 Hz, 1 H), 3.56 (s, 3 H), 3.49–3.47 (m, 2 H), 3.41 (dd, J = 8.0, 2.0 Hz, 1 H), 3.32–3.25 (m, 2 H), 2.71 (m, 1 H), 2.63 (dd, J = 14.3, 4.2 Hz, 1 H), 2.51 (s, 6 H), 2.49–2.41 (m, 3 H), 2.35 (s, 3 H), 2.19–2.11 (m, 3 H), 1.99 (d, J = 14.2 Hz, 1 H), 1.85 (d, J = 3.8 Hz, 1 H), 1.82 (d, J = 3.6 Hz, 1 H), 1.80–1.73 (m, 2 H), 1.68–1.62 (m, 2 H), 1.33 (d, J = 6.2 Hz, 3 H), 1.21 (d, J = 5.8 Hz, 3 H), 1.18 (t, J = 7.6 Hz, 3 H), 1.13 (d, J = 6.8 Hz, 3 H), 1.12 (s, 3 H), d, J = 6.2 Hz, 3 H), 1.09 (d, J = 6.8 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.7, 171.2, 157.2, 157.0, 138.6, 137.1, 131.7, 113.5, 107.5, 105.1, 97.1, 85.0, 80.9, 77.7, 77.4, 75.8, 73.3, 71.9, 69.6, 69.1, 68.9, 63.6, 63.2, 61.4, 59.8, 52.5, 43.5, 42.2, 41.8, 40.6, 34.9, 32.8, 31.8, 29.9, 29.3, 27.8, 25.4, 23.7, 22.8, 20.9, 19.1, 17.9, 14.3, 9.5; HRMS (FAB) $[M + H]^+$ calcd for $C_{44}H_{74}N_3O_{15}$ 884.5120, found 884.5108.

Compound 8a. To a solution of compound **7a** (36.3 mg, 0.041 mmol) in 2 ml MeOH was added Pd/C (7.2 mg, 10% wt) in one portion. The mixture was stirred under 1 atm H_2 at room temperature for 24 h, then filtered through a pad of Celite and concentrated under reduced pressure. The crude product was purified using silica gel chromatography (CH_2Cl_2–MeOH 12 : 1) to afford compound **8a** as a white solid in 82% yield. $[\alpha]_D^{20}$ = –51.6 (c = 0.7, $CHCl_3$); mp 125–127 °C; IR (neat) 3456, 3020, 2975, 1516, 1424, 1215, 1047, 929, 755, 669 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.31 (t, J = 7.8 Hz, 1 H), 6.40 (d, J = 7.2 Hz, 1 H), 6.28 (d, J = 8.4 Hz, 1 H), 5.15 (br s, 1 H), 5.07 (d, J = 3.8 Hz, 1 H), 4.62 (d, J = 10.2 Hz, 1 H), 4.47 (m, 1 H), 4.40 (d, J = 7.6 Hz, 1 H), 4.27 (br s, 1 H), 3.97 (d, J = 9.0 Hz, 1 H), 3.83 (m, 1 H), 3.70 (m, 2 H), 3.63 (m, 1 H), 3.59 (s, 3 H), 3.57–3.46 (m, 2 H), 3.39 (d, J = 7.8 Hz, 1 H), 3.35 (m, 1 H), 3.28 (t, J = 9.8 Hz, 1 H), 3.10 (dd, J = 14.7, 7.4 Hz, 2 H), 2.74 (dd, J = 14.2, 8.6 Hz, 1 H), 2.64 (dd, J = 14.4, 5.2 Hz, 1 H), 2.51 (s, 6 H), 2.48–2.40 (m, 3 H), 2.36 (s, 3 H), 2.22 (m, 1 H), 2.00 (d, J = 14.0 Hz, 1 H), 1.94 (br m, 1 H), 1.84 (dd, J = 14.4, 4.0 Hz, 1 H), 1.79–1.58 (m, 3 H), 1.39 (t, J = 7.4 Hz, 3 H), 1.30 (d, J = 6.2 Hz, 3 H), 1.26 (d, J = 6.0 Hz, 3 H), 1.18 (t, J = 7.6 Hz, 3 H), 1.13 (d, J = 6.2 Hz, 3 H), 1.12 (s, 3 H), 1.05 (d, J = 6.8 Hz, 3 H), 0.90–0.84 (m, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 172.7, 171.5, 157.5, 157.3, 138.5, 112.0, 106.2, 97.1, 79.6, 77.3, 77.1, 76.9, 75.8, 75.6, 73.6, 73.3, 71.9, 71.8, 69.6, 69.3, 68.8, 63.6, 62.0, 61.4, 60.6, 54.3, 43.6, 42.3, 41.9, 34.9, 31.8, 29.9, 27.8, 25.5, 25.4, 24.9, 23.9, 22.9, 20.9, 20.9, 19.1, 18.0, 14.3, 9.5; HRMS (FAB) $[M + H]^+$ calcd for $C_{44}H_{76}N_3O_{15}$ 886.5276, found 886.5302.

***In vitro* cytotoxic assays.** MCF-7 cells and PC-3 cells are both adherent cell lines for the assay. Cells were added to a 96 well microplate, and incubated at 37 °C and 5% CO_2 for 24 h to allow the cells to adhere. They were then treated with 20 μM concentration of each sample. After 72 h incubation with sample compound, the media was removed from the cells and the cells were fixed with a solution of glutaraldehyde. Cells were stained with crystal violet, washed and air dried. The stain was eluted with solution of Triton-X-100. Optical density was measured at 595 nm. Compounds that had over 50% inhibition at 20 μM were then assayed at 8 different concentrations to estimate an IC_{50}.

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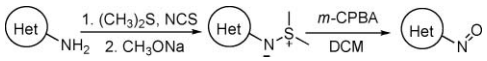

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