9,11-CYCLIC ACETAL DERIVATIVES OF (9*S*)-9-DIHYDROERYTHROMYCIN A

ERIC HUNT, DAVID J. C. KNOWLES, CHRISTINE SHILLINGFORD,
JENNIFER M. WILSON and ISKANDER I. ZOMAYA

Beecham Pharmaceuticals, Chemotherapeutic Research Centre,
Brockham Park, Betchworth, Surrey, RH3 7AJ, UK

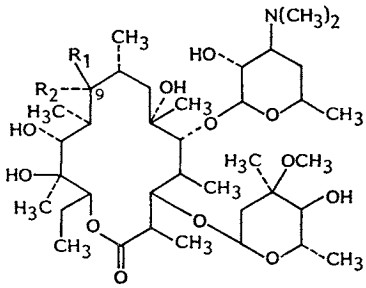
(Received for publication June 27, 1988)

A series of 9,11-cyclic acetal derivatives of (9*S*)-9-dihydroerythromycin A (**4**) have been prepared and their antibacterial activities compared to those of erythromycin A and 9-dihydroerythromycin A. Many of the cyclic acetal derivatives showed better antibacterial activity than their parent **4**. In particular, the acetaldehyde acetal (9-*O*,11-*O*-ethylidene-9-dihydroerythromycin A) (**8b**) showed good antibacterial activity in comparison with erythromycin A but was not sufficiently improved *in vivo* to warrant progression.

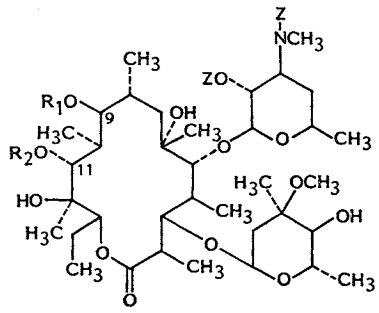
The nature of the substituent at position 9 in derivatives of erythromycin A (**1**) can have a marked effect on antibacterial activity. Thus erythromycin (**1**) and its 9-carbonyl condensation products, such as the 9-oxime **2**[1] and 9-hydrazone **3**[2], are all highly active against Gram-positive bacteria, whereas the reduced product, (9*S*)-9-dihydroerythromycin A (**4**)[3], has much weaker activity. In contrast to the alcohol **4**, the corresponding 9-amine, (9*S*)-erythromycylamine A (**5**)[4], is quite active. Also, those derivatives of **5** which retain a basic nitrogen at C-9 have good activity, whereas the 9-epimer of **5** and those derivatives of **5** which contain a non-basic nitrogen at C-9 have poor activity[4,5]. We now describe a series of 9,11-cyclic acetal derivatives of (9*S*)-9-dihydroerythromycin (**4**), some of which, in contrast to **4** itself, have quite good antibacterial activity.

The simplest representative of this type of acetal, the methylene acetal **8a**, was prepared by way of reaction of the bis-protected des-*N*-methyl-9-dihydroerythromycin **6** with chloroiodomethane and sodium hydride to give the methylene acetal **7**, followed by deprotection and reductive *N*-methylation[6] to give **8a**. Other acetals, as detailed in Table 1, were prepared by acid-catalysed acetalisation of either 9-dihydroerythromycin (**4**) or the *N*-protected form **6** with the appropriate aldehyde, or by an acid-catalysed transacetalisation using 9-dihydroerythromycin (**4**) and the dimethyl acetal of the aldehyde. All of the acetals in Table 1 were obtained as single diastereoisomers. Although the stereochemistry at the acetal carbon in these compounds is unknown, the correspondence of ^{13}C chemical shifts would imply that they all have the same stereochemistry at this centre. From thermodynamic considerations, the *R* configuration would be suggested (*i.e.* an equatorial configuration for the substituent on the methylenedioxy carbon of a chair-shaped 1,3-dioxane ring). The acetonide **9** was prepared by reaction of 9-dihydroerythromycin (**4**) and methyl isopropenyl ether in the presence of acid.

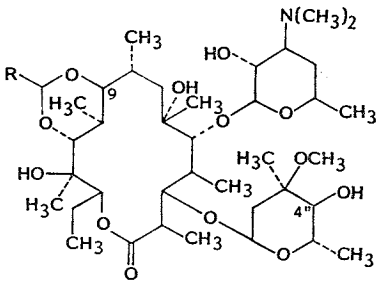
For comparison with the above acetals, three other compounds were prepared. The 9,11-cyclic carbonate **10** was obtained by reaction of 9-dihydroerythromycin (**4**) with carbonyl diimidazole. The cyclic ortho ester **8m** was prepared by treating **4** with trimethyl orthoformate in the presence of acid; in contrast to the acetals **8b**~**8l**, the orthoformate **8m** was obtained as an inseparable mixture of two diastereoisomers. The 9-ether **11** was prepared from the *N*-protected 9-dihydroerythromycin **6** by reaction with sodium hydride and 2-methoxyethoxymethyl chloride, followed by the usual deprotection



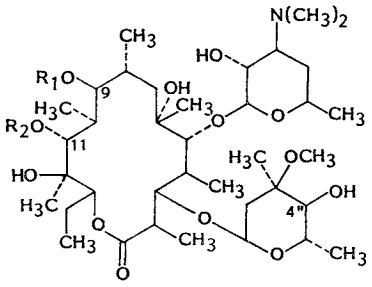
1 $R_1+R_2=O$
2 $R_1+R_2=NOH$
3 $R_1+R_2=NNH_2$
4 $R_1=OH$ $R_2=H$
5 $R_1=NH_2$ $R_2=H$



6 $R_1=R_2=H$ $Z=COOCH_2Ph$
7 $R_1+R_2=CH_2$ $Z=COOCH_2Ph$



8a R=H
8b~8l See Table 1
8m $R=OCH_3$



9 $R_1+R_2=C(CH_3)_2$
10 $R_1+R_2=C=O$
11 $R_1=CH_3OCH_2CH_2OCH_2$ $R_2=H$

Table 1. Preparation and physical properties of 9,11-cyclic acetals.

Compound	R	Method[a]	Yield (%)	MP (°C)	$[\alpha]_D$ (°)[b]	Molecular formula[c]	M^+
8b	CH_3	A	48	143~145	−31.2	$C_{39}H_{71}NO_{13}$	761.49
8c	Et	B	20	127~129	−36.0	$C_{40}H_{73}NO_{13}$	775.51
8d	*n*-Pr	B	16	(Gum)	−38.6	—	789.52
8e	$(CH_3)_2CH$	B	11	(Gum)	−35.5	—	789.52
8f	Ph	A	27	141~142	−67.1	$C_{44}H_{73}NO_{13}\cdot H_2O$	823.51
8g	2-Furanyl	A	40	115~119 (foam)	−45.8	$C_{42}H_{71}NO_{14}$	813.49
8h	$4\text{-}HOC_6H_4$	C	64	200~201	−64.8	$C_{44}H_{73}NO_{14}\cdot H_2O$	839.51
8i	$4\text{-}CH_3OC_6H_4$	C	56	142~144	−73.0	$C_{45}H_{75}NO_{14}\cdot H_2O$	853.52
8j	$4\text{-}(CH_3OCH_2CH_2O\text{-}CH_2O)C_6H_4$	C	63	123~125	−66.2	$C_{48}H_{81}NO_{16}$	927.55
8k	$3,4\text{-}(HO)_2C_6H_3$	C	77	165~167	—	$C_{44}H_{73}NO_{15}$	855.50
8l	3,4-Methylenedioxyphenyl	C	76	167~168.5	−67.6	$C_{45}H_{73}NO_{15}$	867.51

[a] See Experimental section. Method A: Aldehyde + **4**. Method B: Aldehyde + **6**. Method C: Dimethyl acetal + **4**.
[b] Determined for 1% solution in $CHCl_3$ at ambient temperature (20~25°C).
[c] Satisfactory microanalytical data were determined (except for **8d** and **8e**, which were gums).
—: Not measured.

Table 2. *In vitro* antibacterial activity for erythromycin (**1**) and derivatives of 9-dihydroerythromycin (**4**).

Compound	MIC (μg/ml)				
	S.a.	*S.p.*	*S.f.*	*H.i.*	*B.c.*
1	0.25	<0.015	0.5	1	0.13
4	2	0.13	2	4	0.5
8a	0.5	0.25	2	16	1
8b	0.13	0.03	1	2	0.06
8c	0.25	0.03	2	2	0.13
8d	1	0.06	4	4	0.5
8e	0.5	0.03	2	4	0.5
8f	0.5	0.03	2	4	0.5
8g	1	0.03	4	4	0.5
8h	0.5	0.06	1	4	0.25
8i	0.5	0.03	2	2	0.25
8j	2	0.13	4	4	1
8k	0.5	0.13	—	8	0.5
8l	1	0.13	4	4	1
8m	1	<0.015	2	2	0.5
9	0.5	0.06	1	4	0.13
10	0.5	0.03	2	1	0.25
11	32	0.5	32	64	4

Medium: Blood Agar Base +5% lysed horse blood. Inoculum: $10^5 \sim 10^6$ cfu. Incubation: 18 hours at 37°C.

Organisms: *S.a.*, *Staphylococcus aureus* Oxford; *S.p.*, *Streptococcus pneumoniae* 1761; *S.f.*, *Streptococcus faecalis* I; *H.i.*, *Haemophilus influenzae* Wy 21; *B.c.*, *Branhamella catarrhalis* 1502.

and reductive *N*-methylation.

The antibacterial activities of the new derivatives are shown in Table 2. The best of the 9,11-cyclic acetals was the ethylidene derivative **8b**, which had good activity when compared with erythromycin (**1**) and was significantly more active than the parent 9-dihydroerythromycin (**4**). The other 9,11-cyclic derivatives in Table 2 were generally less active than the ethylidene derivative **8b**, although many of them were more active than 9-dihydroerythromycin (**4**), especially against *Staphylococcus aureus* and *Streptococcus pneumoniae*. In contrast to the cyclic compounds, the non-cyclic 9-ether **11** had very poor activity, being even less active than the parent 9-alcohol **4**.

The ethylidene derivative **8b** was also tested against experimental infections (*S. aureus* and *S. pneumoniae*) in mice, where it proved to be marginally more active than erythromycin. This improvement over erythromycin was insufficient for the compound to be progressed further.

Experimental

MP's were determined using a Kofler hot-stage apparatus. Specific rotations were measured for solutions in $CHCl_3$. Electron impact mass spectra (EI-MS) were obtained on a VG ZAB IF mass spectrometer operated at 8 kV using 70 eV electrons and a source temperature of 200°C. The structures of all compounds were confirmed by ^1H and ^{13}C NMR spectra for solutions in $CDCl_3$.

Solutions were dried with anhydrous sodium sulfate and solvents were evaporated using a rotary evaporator with bath temperature below 30°C. Except where stated otherwise, Merck Silica gel 60 was used for column chromatography. For chromatography on silanised silica gel, Merck Silica gel 60H silanised was used.

(9*S*)-2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-9-dihydroerythromycin A (**6**)

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethylerythromycin A[6] (2 g) in 1,2-dimethoxyethane

(30 ml) was stirred at −15°C while sodium borohydride (150 mg) was added in small portions during 1 hour. The mixture was stirred for a further 1.5 hours during which the temperature was allowed to rise to 0°C. Acetic acid (2 ml) was added and the mixture was stirred for 5 minutes. The mixture was diluted with ethyl acetate (150 ml) and was washed with water, satd $NaHCO_3$, and water. The solution was dried, the solvent was removed, and the residue was crystallised from Et_2O - hexane to give the title compound as colourless crystals (1.72 g): MP 115~118°C; $[\alpha]_D^{20}$ −68.2° (*c* 0.49).

Anal Calcd for $C_{52}H_{79}NO_{17}$: C 63.07, H 8.04, N 1.41.
Found: C 63.13, H 7.88, N 1.49.

(9*S*)-2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-9-*O*,11-*O*-methylene-9-dihydroerythromycin A (7)

(9*S*)-2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-9-dihydroerythromycin A (**6**) (800 mg) in dry DMF (10 ml) was treated with K_2CO_3 powder (500 mg), 15-crown-5-ether (1,4,7,10,13-pentaoxacyclopentadecane) (2 drops), and chloroiodomethane (1 ml). The mixture was stirred at 0°C while sodium hydride (50% dispersion in oil; 100 mg) was added in one portion. The mixture was stirred at 0°C for 30 minutes and was then allowed to warm to room temperature during 10 minutes. The mixture was diluted with ethyl acetate (100 ml) and was washed with Na_2SO_3 soln (30 ml) and water (3×40 ml). The solution was dried, the solvent was removed, and the residue was chromatographed using EtOAc - hexane to give the title compound as a white solid. Crystallisation from Et_2O - hexane gave **7** as colourless crystals (520 mg): MP 118~119°C; $[\alpha]_D^{21}$ −74.3° (*c* 1.35).

Anal Calcd for $C_{53}H_{79}NO_{17}$: C 63.52, H 7.95, N 1.40.
Found: C 63.58, H 8.07, N 1.34.

(9*S*)-9-*O*,11-*O*-Methylene-9-dihydroerythromycin A (**8a**)

(9*S*)-2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-9-*O*,11-*O*-methylene-9-dihydroerythromycin A (**7**) (500 mg) in EtOH (20 ml) and acetate buffer (pH 4.8, 0.73 M, 2 ml) was shaken with 10% palladium - carbon (150 mg) under hydrogen (1 atmosphere) for 30 minutes. 37% formaldehyde (2 ml) was added and hydrogenation was continued for 1.5 hours. The catalyst was removed by filtration and was washed with EtOH and water. The EtOH was removed from the filtrate under reduced pressure, and the aqueous solution was diluted with water, basified (pH 12) using K_2CO_3, and extracted with ethyl acetate (3×40 ml). The combined extracts were washed with water and dried. The solvent was removed to yield a white foam (380 mg). Crystallisation from CH_2Cl_2 - hexane gave the title compound as colourless crystals (340 mg): MP 140~142°C; $[\alpha]_D^{25}$ −63.3° (*c* 1.0); EI-MS *m/z* 747 (M, found 747.4761; calcd for $C_{38}H_{69}NO_{13}$ 747.4752).

Anal Calcd for $C_{38}H_{69}NO_{13}$: C 61.02, H 9.30, N 1.87.
Found: C 60.64, H 9.07, N 1.76.

(9*S*)-9-*O*,11-*O*-Ethylidene-9-dihydroerythromycin A (**8b**)

(9*S*)-9-Dihydroerythromycin A (1.0 g) and pyridinium *p*-toluenesulfonate (360 mg) in acetaldehyde (5 ml) were treated with anhydrous copper (II) sulfate (1.0 g) and the mixture was stirred for 10 days. The mixture was diluted with ethyl acetate (50 ml) and washed with 10% K_2CO_3 soln (30 ml) and water (3×30 ml). The solution was dried, the solvent was removed, and the residue was chromatographed on silanised silica gel using MeOH - phosphate buffer (0.067 M, pH 7.0) (3:2) to give the title compound as colourless crystals (500 mg), from CH_2Cl_2 - hexane: MP 143~145°C; $[\alpha]_D^{20}$ −31.2° (*c* 1.0).

Anal Calcd for $C_{39}H_{71}NO_{13}$: C 61.47, H 9.39, N 1.84.
Found: C 61.19, H 9.38, N 1.76.

Compounds **8f** and **8g** were prepared similarly.

(9*S*)-9-*O*,11-*O*-*n*-Propylidene-9-dihydroerythromycin A (**8c**)

(9*S*)-2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-9-dihydroerythromycin A (**6**) (500 mg) and pyridinium *p*-toluenesulfonate (70 mg) were dissolved in a mixture of 1,2-dimethoxyethane (5 ml) and propionaldehyde (5 ml). Anhydrous calcium sulfate (1.0 g) was added and the mixture was stirred for 10 days. The mixture was diluted with ethyl acetate (50 ml) and filtered. The filtrate was

washed with water (2×30 ml) and dried. The solvent was removed and the residue was chromatographed using EtOAc - hexane to give (9*S*)-2′-*O*,3′-*N*-bis(benzyloxycarbonyl)-*N*-demethyl-9-*O*,11-*O*-*n*-propylidene-9-dihydroerythromycin A as a colourless gum (105 mg).

The above product was converted into the title compound using the process described for the preparation of compound **8a**. The title compound was obtained as colourless crystals (70 mg): MP 127~129°C; $[\alpha]_D^{20}$ −36.0° (*c* 1.0).

Anal Calcd for $C_{40}H_{73}NO_{13}$: C 61.91, H 9.48, N 1.80.
Found: C 62.01, H 9.59, N 1.60.

Compounds **8d** and **8e** were prepared similarly.

(9*S*)-9-*O*,11-*O*-(3,4-Methylenedioxyphenyl)methylene-9-dihydroerythromycin A (**8l**)

(9*S*)-9-Dihydroerythromycin A (200 mg) in dry, ethanol - free $CHCl_3$ (2 ml) treated with piperonaldehyde dimethyl acetal (500 mg) and pyridinium *p*-toluenesulfonate (80 mg). The solution was kept for 7 days, and was then diluted with ethyl acetate and washed with K_2CO_3 soln and water. The solution was dried, the solvent was removed, and the residue was chromatographed using 35% ammonia - MeOH - $CHCl_3$ (1:9:90) to give a white foam. The foam was then chromatographed on silanised silica gel using MeOH - phosphate buffer (0.067 M, pH 7.0) (3:2) to give the title compound as colourless prisms (180 mg), from EtOAc - hexane: MP 167~168.5°C; $[\alpha]_D^{20}$ −67.6° (*c* 1.0).

Anal Calcd for $C_{45}H_{73}NO_{15}$: C 62.26, H 8.47, N 1.61.
Found: C 62.33, H 8.44, N 1.54.

Compounds **8h**, **8i**, **8j** and **8k** were prepared similarly.

(9*S*)-9-*O*,11-*O*-Methoxymethylene-9-dihydroerythromycin A (**8m**)

(9*S*)-9-Dihydroerythromycin A (200 mg) in dry, ethanol - free $CHCl_3$ (2 ml) was treated with trimethyl orthoformate (1 ml) and pyridinium *p*-toluenesulfonate (70 mg) and the mixture was stirred for 6 days. The mixture was diluted with ethyl acetate and washed with 10% K_2CO_3 soln and water. The solution was dried and the solvent was removed to yield a colourless gum (230 mg). The gum was dissolved in MeOH (8 ml) and acetic acid (0.1 ml) was added†. The solution was kept for 24 hours and was then diluted with ethyl acetate and washed with $NaHCO_3$ soln and water. The solution was dried, the solvent was removed, and the residue was chromatographed on silanised silica gel using MeOH - phosphate buffer (0.067 M, pH 7.0) (3:2) to give the title compound as a white foam (140 mg): $[\alpha]_D^{21}$ −56.6° (*c* 1.0); EI-MS *m/z* 777 (M, found 777.4839; calcd for $C_{39}H_{71}NO_{14}$ 777.4858). ^{13}C NMR showed that the product was a 1:1 mixture of methoxymethylene diastereoisomers.

(9*S*)-9-*O*,11-*O*-Isopropylidene-9-dihydroerythromycin A (**9**)

(9*S*)-9-Dihydroerythromycin A (500 mg) and 2-methoxypropene (0.7 ml) were dissolved in dry, EtOH - free $CHCl_3$ (10 ml). Pyridinium chloride (120 mg) was added and the mixture was stirred for 16 hours. The solution was washed with 10% K_2CO_3 soln and then dried. The solvent was removed and the residue was dissolved in a mixture of acetone (10 ml) and water (10 ml). The solution was brought to pH 3.5 using 1 M HCl, and was kept for 2 hours††. The solution was basified (pH 11) using K_2CO_3 and was extracted with ethyl acetate. The extract was dried, the solvent was removed, and the residue was chromatographed on silanised silica gel using MeOH - phosphate buffer (0.067 M, pH 7.0) (3:2) to give the title compound as a white foam (320 mg): MP 101~109°C; $[\alpha]_D^{25}$ −30.4° (*c* 1.0); EI-MS *m/z* 775 (M, found 775.5075; calcd for $C_{40}H_{73}NO_{13}$ 775.5086).

Anal Calcd for $C_{40}H_{73}NO_{13}$: C 61.91, H 9.48, N 1.80.
Found: C 61.46, H 9.34, N 1.62.

(9*S*)-9-Dihydroerythromycin A Cyclic 9,11-Carbonate (**10**)

(9*S*)-9-Dihydroerythromycin A (370 mg) in dry THF (3 ml) was treated with K_2CO_3 powder (300 mg) and carbonyl diimidazole (100 mg) and the mixture was stirred for 22 hours, then diluted with ethyl acetate and washed twice with water. The solution was dried, the solvent was removed, and the

† This acid solvolysis was necessary to remove the dimethoxymethyl moiety from the 4″-hydroxyl.
†† This acid hydrolysis was necessary to remove the (1-methoxy-1-methyl)ethyl moiety from the 4″-hydroxyl.

residue was chromatographed on silanised silica gel using MeOH - phosphate buffer (0.067 M, pH 7.0) (3: 2) to give the title compound as a white solid. Crystallisation from CH_2Cl_2 - hexane gave colourless needles (200 mg): MP 154~155.5°C; $[\alpha]_D^{21}$ −79.2° (*c* 1.0); EI-MS *m/z* 761 (M, found 761.4576; calcd for $C_{38}H_{67}NO_{14}$ 761.4565).

Anal Calcd for $C_{38}H_{67}NO_{14}$: C 59.90, H 8.86, N 1.84.
Found: C 59.91, H 9.00, N 1.89.

(9*S*)-9-*O*-(2-Methoxyethoxymethyl)-9-dihydroerythromycin A (**11**)

(9*S*)-2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-9-dihydroerythromycin A (**6**) (200 mg) in dry 1,2-dimethoxyethane (2 ml) was treated with sodium hydride (50% dispersion in oil; 10 mg) and the mixture was stirred for 5 minutes. 2-Methoxyethoxymethyl chloride (40 mg) was added and the mixture was stirred for 40 minutes. The mixture was diluted with ethyl acetate and washed with water, $NaHCO_3$ soln, and water. The solution was dried, the solvent was removed, and the residue was chromatographed using EtOAc - hexane to give (9*S*)-2′-*O*,3′-*N*-bis(benzyloxycarbonyl)-*N*-demethyl-9-*O*-(2-methoxyethoxymethyl)-9-dihydroerythromycin A as a colourless gum (140 mg): $[\alpha]_D^{22}$ −44.4° (*c* 1.0).

The above compound was converted into the title compound using the process described for the preparation of compound **8a**. The title compound **11** was obtained as a colourless gum (90 mg): $[\alpha]_D^{21}$ −31.9° (*c* 1.0); EI-MS *m/z* 823 (M, found 823.5285; calcd for $C_{41}H_{77}NO_{15}$ 823.5297).

Acknowledgements

We thank Dr. J. H. C. NAYLER for his interest and encouragmeent.

References

1) MASSEY, E. H.; B. KITCHELL, L. D. MARTIN, K. GERZON & H. W. MURPHY: Erythromycylamine. Tetrahedron Lett. 1970: 157~160, 1970
2) SIGAL, M. V., Jr.; P. F. WILEY, K. GERZON, E. H. FLYNN, U. C. QUARCK & O. WEAVER: Erythromycin. VI. Degradation studies. J. Am. Chem. Soc. 78: 388~395, 1956
3) GLABSKI, T.; H. BOJARSKA-DAHLIG & W. SLAVINSKI: Erythromycin derivatives. Part VIII. 9-Dihydroerythromycin A cyclic 11,12-carbonate. Rocz. Chem. 50: 1281~1284, 1976
4) RYDEN, R.; G. H. TIMMS, D. M. PRIME & E. WILDSMITH: *N*-Substituted derivatives of erythromycylamine. J. Med. Chem. 16: 1059~1060, 1973
5) MASSEY, E. H.; B. S. KITCHELL, L. D. MARTIN & K. GERZON: Antibacterial activity of 9(*S*)-erythromycylamine — aldehyde condensation products. J. Med. Chem. 17: 105~107, 1974
6) FLYNN, E. H.; H. W. MURPHY & R. E. MCMAHON: Erythromycin. II. Des-*N*-methylerythromycin and N-methyl-C^{14}-erythromycin. J. Am. Chem. Soc. 77: 3104~3106, 1955