Structural Modification of the Lincomycin Antibiotic

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In clinical practice the antibiotic lincomycin (Lincocin®) has been replaced by its chemically modified analogues 7-(*S*)-chloro-7-deoxylincomycin (clindamycin, Klimicin®, Dalacin®) and its 2-*O*-phosphate and palmitoyl ester derivatives. These new antibiotic analogues are active against Gram-positive and -negative anaerobic micro-organisms, possess high concentration in the tissues, and readily penetrate into the bones. These results suggest that further modification of the structure of the parent antibiotic, lincomycin, may be associated with additional, advantageous pharmacological effects.

Recently we have described our studies concerning the removal[1] of the acyl side-chain, its replacement[2] and the oxidation of the sulfur atom[3] of lincomycin. The antibacterial activity of one of the prepared compounds, 7-(*R*)-azido-7-deoxylincomycin (**2**) was found to be higher than that of lincomycin, but was lower than that of clindamycin[4].

The present paper reports on further chemical modification of compound **2**. For the transformation of the azido group by the Staudinger reaction[5] offers a promising possibility. Thus, **2** was first converted into the corresponding phosphinimine by treatment with triphenylphosphine, which further reacted with heterocyclic secondary amines (*N*-methyl-piperazine and morpholine) in the presence of carbon dioxide (Fig. 1) and resulted in the desired substituted C-7-ureido antibiotic derivatives (**3** and **4**). The spectral data supporting the proposed structures, and the physico-chemical properties of these compounds are shown in Tables 1 and 2.

Attempted reduction of the 7-(*R*)-azido compound (**2**) with sodium borohydride or sodium cyanoborohydride failed. At the same time, reaction of the peracetylated derivative (**5**) of **2** by the reaction route a.)→b.) (Fig. 1) resulted in 7-(*R*)-amino-7-deoxylincomycin (**6**) in good yield. The structure of **6** was unequivocally proved by the ^{1}H and ^{13}C-NMR spectroscopic data and the mass spectrometric fragmentation detailed in Fig. 2. Reductive alkylation of **6** with acetaldehyde in tetrahydrofuran furnished 7-(*R*)-diethylamino-7-deoxylincomycin (**7**). The presence of the diethylamino group in **7** was proved by the δ=1.15 ppm and 13.04 ppm chemical shift values in the ^{1}H and ^{13}C-NMR spectra, respectively, as well as by the *m/z*= 464 [M+H] peak in the FAB-MS spectrum.

The results of the biological studies showed that modification of the azido group at C-7 unfavourably modifies the antibacterial activity of the new antibiotic derivatives.

Of the substituted ureido derivatives only compound **3** possessed a moderate effect (in 6.2 μg/ml concentration) against *Bacteroidis fragilis* KB169 (ATCC 23745). However, similarly to **4**, this compound was inactive against the additional test organisms[3,4]. Development of the amino group at C-7 associated with a further decrease of the antibiotic effect: compound **6** was found to be active against *Staphylococcus aureus* KB210 (ATCC 6538p), *Bacteroidis fragilis* KB 169, and *Clostridium kainantoi* KB 133 (IFO 3353) only in 12.5 μg/ml concentration. However **4** has proved to be active against the two aformentioned strains only in concentrations of 25 and 50 μg/ml, respectively.

Experimental

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. ^{1}H (500 MHz) and ^{13}C-NMR spectra (125 MHz) were obtained with a Bruker DRX 500 spectrometer. Mass spectra were recorded with a VG-7035 instrument by the electron ionization [EI(+), 20 eV] and chemical ionization [CI, NH_3, 20 eV] techniques. FAB Mass spectra were obtained with a VG-70 HS spectrometer in a glycerol matrix (reference gas Xe). IR spectra were recorded in KBr pellets with a Perkin-Elmer 16 PC FT spectrophotometer. Specific optical rotations were measured with a Perkin-Elmer 141 MC polarimeter. Thin layer chromatography was carried out on Kieselgel 60 F_{254} (Merck) precoated plates, and column chromatography was performed on Kieselgel 60 (Merck, 0.063~0.2) adsorbent with the following eluent systems: (A) 9 : 1 : 0.02 $CHCl_3$-

Fig. 1. Chemical modification of 7-(*R*)-azido-7-deoxylincomycin.

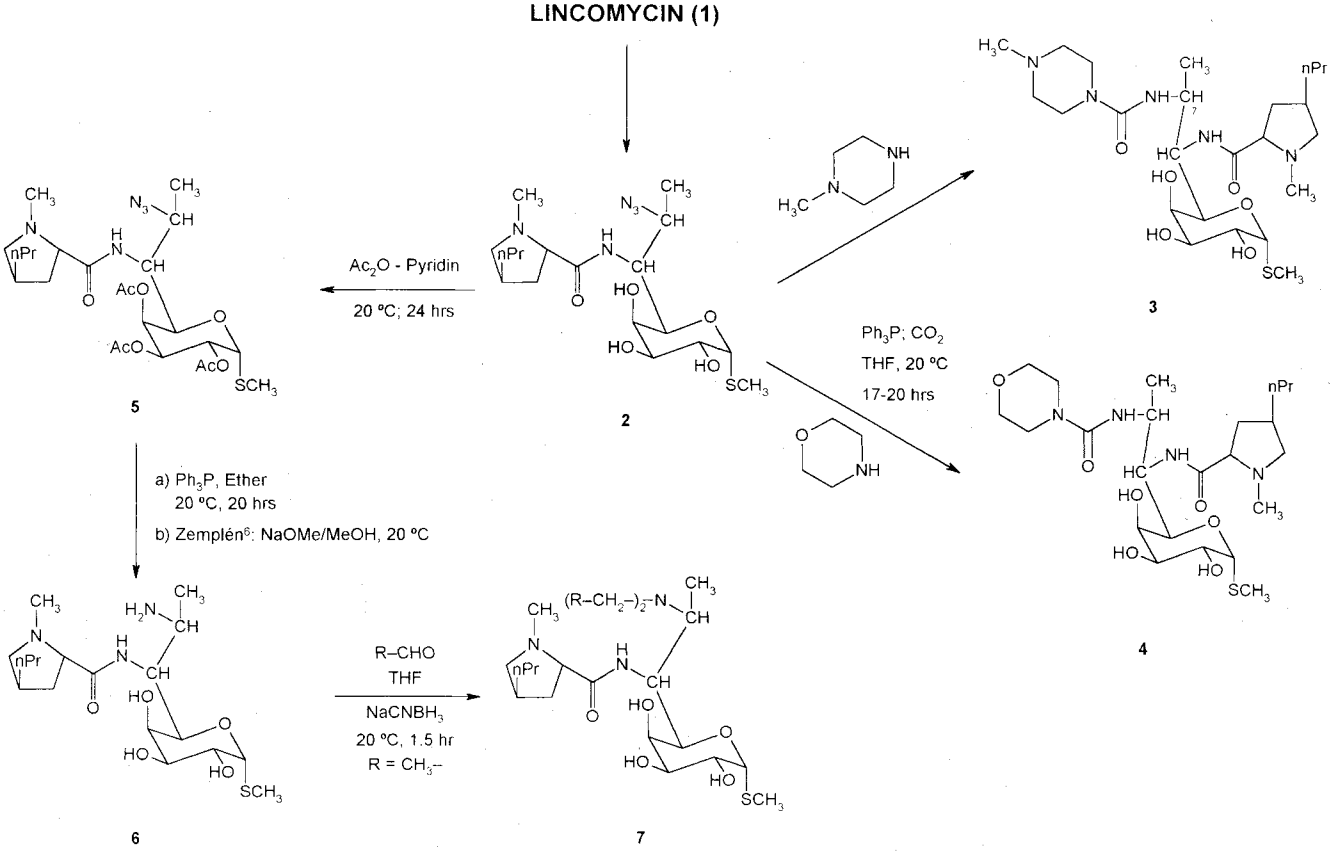


Table 1. Physico-chemical properties and IR spectroscopic data of the new lincomycin derivatives.

Compound	Yield (%)	m.p. (°C)	$[\alpha]_D^{20}$	MS (*m/z*)	IR (KBr) cm^{-1}
3	76.4	119~120	+93.9° (*c* 0.17, $CHCl_3$)	533[a]	3338, 2922, 2872, 2794, 2358, 1652, 1532, 1456, 1384, 1338, 1292, 1264, 1242, 1140, 1092, 1058, 1002, 902, 856, 694
4	71	101~103	+91.5° (*c* 0.15, $CHCl_3$)	591[b]	3350, 2958, 2922, 2854, 2784, 2362, 1652, 1530, 1456, 1398, 1304, 1274, 1258, 1160, 1094, 1072, 1000, 902, 694
6	80.7	91~93	+122.5° (*c* 0.18, $CHCl_3$)	435[c] $[M+NH_4]^+$	3336, 2956, 2920, 2870, 2786, 2348, 1658, 1566, 1454, 1438, 1402, 1330, 1210, 1096, 1056, 922, 906, 870, 694
7	42	67~69	+114.7° (*c* 0.19, $CHCl_3$)	464[a]	3350, 2964, 2922, 2872, 1652, 1520, 1456, 1386, 1306, 1206, 1186, 1090, 1066, 900, 694

FAB: [a] $(M+H)^+$, [b] $(M+2H)$; CI: [c] $(M+NH_4)^+$.

Table 2. Assignment of the signals of the ^{1}H and ^{13}C-NMR spectra of the new synthetic lincomycin analogues.

Compound	^{1}H-NMR (500 MHz, D_2O) δ ppm	Position	^{13}C-NMR (125 MHz, D_2O) δ ppm
3	5.38 (1H, d, H-1, $J_{1,2}$=5.9 Hz)	C-1	88.95
	4.45 (1H, dd, H-6, $J_{6,7}$=4.1 Hz)	C-3	71.08
	4.21 (1H, d, H-5, $J_{5,6}$=9.8 Hz)	C-5	69.38
	4.16 (1H, dq, H-7, $J_{7,Me}$=7.0 Hz)	C-4	69.01
	4.13 (1H, dd, H-2, $J_{2,3}$=10.1 Hz)	C-2	68.21
	3.89 (1H, d, H-4, $J_{4,5}\approx 0$)	C-5′	62.57
	3.61 (1H, dd, H-3, $J_{3,4}$=3.2 Hz)	2×NCH_2	53.82
	3.40 (2H, bs, NCH_2)	C-6	51.37
	3.33 (2H, bs, NCH_2)	C-7	46.91
	3.21 =1H, dd, H-5′a, $J_{5'a,5'b}$=9.3 Hz)	N-Me″	44.88
	3.12 (1H, dd, H-2′, $J_{2',3'a}$=6.1 Hz, $J_{2',3'b}$=9.7 Hz)	2×NCH_2	43.52
		NMe′	40.79
	2.45 (4H, bs, 2×NCH_2)	C-4′	37.36
	2.36 (3H, s, NMe′)	C-3′	36.94
	2.27 (3H, s, NMe″)	CH_2 (Pr, 4′)	35.94
	2.23 (1H, m, H-4′)	CH_2 (Pr)	21.07
	2.18 (3H, s, SMe)	Me (Pr)	13.71
	2.113 (1H, t, 5′b)	Me (C-7)	13.55
	1.99 (1H, m, H-3′a)	S-Me	13.38
	1.88 (1H, m, H-3′b)		
	1.35 (2H, m, CH_2 (Pr))		
	1.30 (2H, m, CH_2 (Pr))		
	1.14 (3H, d, Me (C-7))		
	0.88 (3H, t, Me (Pr))		
4	5.32 (1H, d, H-1, $J_{1,2}$=5.8 Hz)	C-1	88.94
	4.40 (1H, dd, H-6, $J_{6,7}$=4.0 Hz)	C-3	71.07
	4.15 (1H, d, H-5, $J_{5,6}$=9.9 Hz)	C-5	69.35
	4.11 (1H, dq, H-7, $J_{7,Me}$=6.9 Hz)	C-2′	69.11
	4.07 (1H, dd, H-2, $J_{2,3}$=10.4 Hz)	C-4	68.97
	3.83 (1H, d, H-4, $J_{4,5}\approx 0$)	C-2	68.20
	3.65 (4H, m, 2×OCH_2)	2×OCH_2	66.52
	3.55 (1H, dd, H-3, $J_{3,4}$=3.2 Hz)	C-5′	62.62
	3.36~3.24 (4H, m, 2×NCH_2)	C-6	51.22
	3.12 (1H, dd, H-5′a, $J_{5'a,4'}$=6.7 Hz, $J_{5'a,5'b}$=9 Hz)	C-7	46.96
	3.02 (1H, dd, H-2′, $J_{2',3a'}$=6.1 Hz, $J_{2',3'b}$=9.5 Hz)	2×NCH_2	43.96
	2.28 (3H, s, 5′-NCH_3)	NCH_3 (5′)	40.69
	2.15 (1H, m, H-4′)	C-4′	37.24
	2.12 (3H, s, S-Me)	C-3′	36.96
	2.04 (1H, t, H-5′b, $J_{5'a,5'b}$=9.5 Hz)	CH_2 (Pr)	36.08
	1.90 (1H, m, H-3′a)	CH_2 (Pr)	21.12
	1.79 (1H, m, H-3′b)	Me (Pr)	13.65
	1.34~1.20 (4H, m, 2×CH_2(Pr))	S-Me	13.46
	1.08 (3H, d, Me-C-7)	7-Me	13.39
	0.82 (3H, t, Me (Pr))		

Table 2. (Continued)

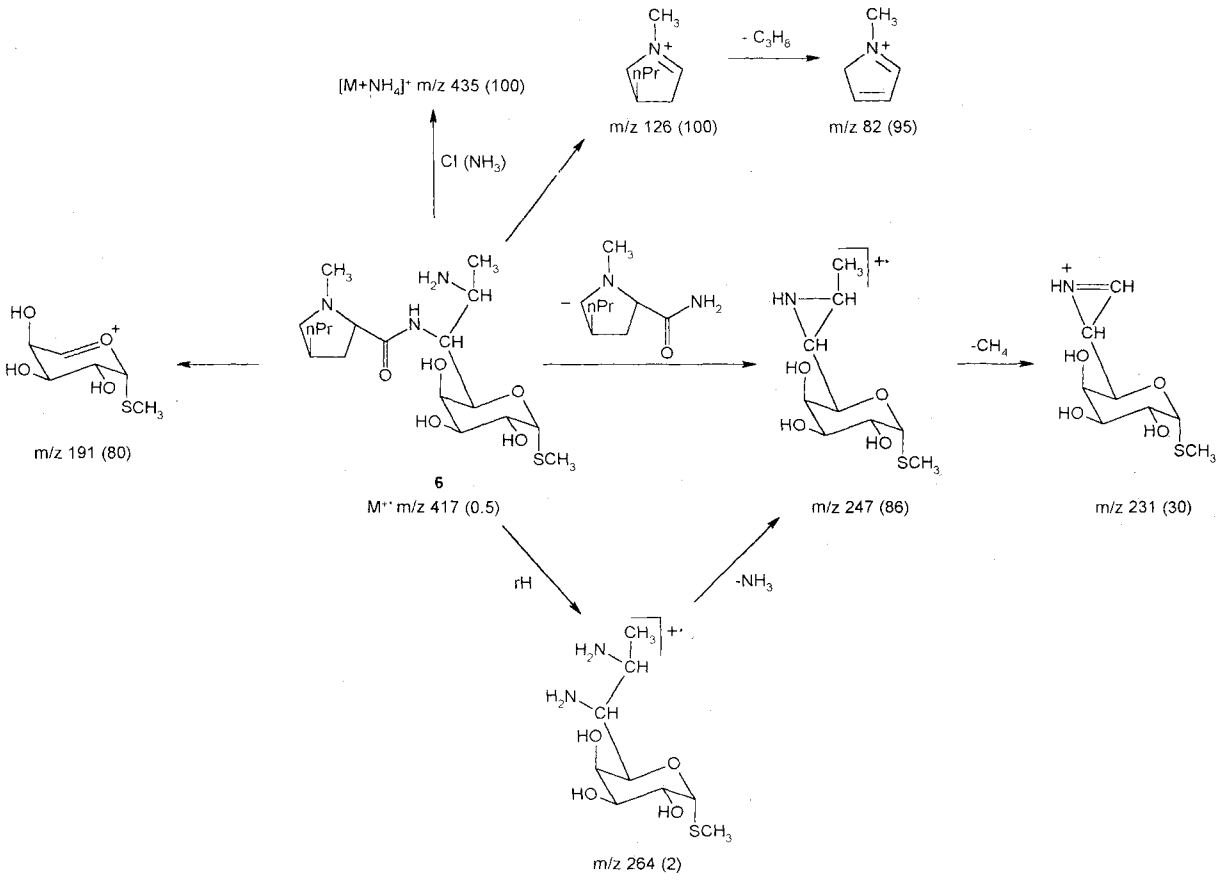
Compound	^{1}H-NMR (500 MHz, D_2O) δ ppm	Position	^{13}C-NMR (125 MHz, D_2O) δ ppm
6	5.40 (1H, d, H-1, $J_{1,2}$=5.8 Hz)	C-1	88.68
	4.41 (1H, dd, H-6, $J_{6,7}$=4.2 Hz)	C-3	70.81
	4.26 (1H, d, H-5, $J_{5,6}$=10.2 Hz)	C-5	70.06
	4.13 (1H, dd, H-2, $J_{2,3}$=10.4 Hz)	C-2′	68.83
	3.88 (1H, d, H-4, $J_{4,5}\approx$0 Hz)	C-4	68.70
	3.64 (1H, dd, H-3, $J_{3,4}$=3.0 Hz)	C-2	68.05
	3.56 (1H, m, H-7, $J_{7,Me}$=6.4 Hz)	C-5′	62.55
	3.22 (1H, dd, H-5′a, $J_{5'a,4'}$=6 Hz, $J_{5'a,5'b}$=8.3 Hz)	C-6	51.53
	3.18 (1H, dd, H-2′, $J_{2',3'a}$=6.2 Hz, $J_{2'a,3'b}$=9.9 Hz)	C-7	47.72
	2.38 (3H, s, NMe)	N-Me	41.05
	2.20 (1H, m, H-4′)	C-4′	37.34
	2.16 (3H, s, S-Me)	C-3′	36.84
	2.14 (1H, t, H-5′b, $J_{5'a,5'b}$=10.0 Hz)	CH_2 (Pr)	35.90
	2.03 (1H, m, H-3′a)	CH_2 (Pr)	21.12
	1.88 (1H, m, H-3′b)	7-Me	14.17
	1.4~1.25 (4H, m, 2×CH_2 (Pr))	Me (Pr)	13.72
	1.18 (3H, d, 7-Me)	S-Me	13.33
	0.88 (3H, t, Me (Pr))		
7	7.79 (1H, d, NH, $J_{NH,6}$=9.7 Hz)	C=O	174.62
	5.36 (1H, d, H-1, $J_{1,2}$=5.5 Hz)	C-1	89.61
	4.28 (1H, dt, H-6, $J_{6,7}$=9.5 Hz)	C-5	75.39
	4.19 (1H, dd, H-2, $J_{2,3}$=9.6 Hz)	C-3	72.55
	4.06 (1H, d, H-5, $J_{5,6}$=2.5 Hz)	C-2	69.81
	3.90 (1H, d, H-4, $J_{4,5}\approx$0 Hz)	C-4	69.13
	3.51 (1 H, dd, H-3, $J_{3,4}$=3.2 Hz)	C-2′	69.07
	3.42 (1H, m, H-7, $J_{7,7\text{-Me}}$=6.9 Hz)	C-5′	63.52
	3.14 (1H, m, H-5′a)	C-7	52.68
	3.02 (1H, dd, H-2′a, $J_{2',3a}$=4.2 Hz, $J_{2',3'b}$=11 Hz)	C-6	51.93
	2.80 (2H, m, CH_2 (Et))	2×CH_2 (Et)	44.52
	2.41 (2H, m, CH_2 (Et))	N-Me	42.64
	2.38 (3H, s, N-Me)	C-4′	38.41
	2.21 (3H, s, S-Me)	C-3′	38.15
	2.1~2.04 (2H, m, H-4′, H-5′b)	CH_2 (Pr)	36.08
	1.86 (1H, m, H-3′b)	CH_2 (Pr)	21.93
	1.37~1.25 (4H, m, 2×CH_2 (Pr))	S-Me	14.69
	1.15 (6H, t, 2×CH_3 (Et))	Me (Pr)	14.58
	0.99 (3H, d, 7-Me)	2×CH_3 (Et)	13.04
	0.91 (3H, t, Me (Pr))	7-Me	9.77

MeOH - NH_4OH; (B) 10 : 0.2 $CHCl_3$ - MeOH; (C) 6 : 4 : 0.02 $CHCl_3$ - MeOH - NH_4OH; (D) 9 : 1 $CHCl_3$ - MeOH; and (E) 7 : 3 $CHCl_3$ - MeOH. Evaporations were carried out under diminished pressure (bath temperature below 40°C). Each of the synthesized compounds possessed appropriate microanalytical data.

7-(*N*-Methylpiperazinyl-ureido)-7-deoxylincomycin (**3**)

Compound **2** (0.2 mmol) and *N*-methylpiperazine (0.2 mmol) were dissolved in dry tetrahydrofuran (2 ml) and the solution was saturated with carbon dioxide. A solution of triphenylphosphine (0.2 mmol) in dry tetrahydrofuran (2 ml) was added dropwise to the reaction mixture, which was then stirred at room temperature for 20 hours. Following evaporation, the residue was submitted to

Fig. 2. EI(+) (20 eV) Mass spectroscopic fragmentation pattern of compound **6**.



column chromatography (A) to obtain 76.4% of pure **3**.

7-(Morpholinoyl-ureido)-7-deoxylincomycin (**4**)

The reaction of **2** with morpholine was carried out (with 17 hours reaction time) as described above for the preparation of **3** to furnish 71% of pure **4**.

7-Azido-7-deoxy-peracetyllincomycin (**5**)

To an ice-cold solution of **2** (0.15 mmol) in dry pyridine (1 ml) acetic anhydride (1 ml) was added dropwise, the reaction mixture was allowed to stand at room temperature for 24 hours, and poured onto ice-water. The mixture was extracted with chloroform (2×5 ml), the organic layer was washed with dilute acetic acid, water, and saturated aq. $NaHCO_3$, and then dried over Na_2SO_4. The residue obtained after evaporation of the solvent was purified by means of column chromatography (B) to afford 71.6% of the pure product **5**, m.p. 54~55°C, $[\alpha]_D^{20}=93.8°$ (*c* 0.25, $CHCl_3$).

7-(*R*)-Amino-7-deoxylincomycin (**6**)

A solution of **5** (0.55 mmol) and triphenylphosphine (0.55 mmol) in dry ether (3 ml) was kept at room temperature for 20 hours. The solvent was distilled off under diminished pressure, and the residue was triturated with hexane to obtain 94.3% of a crystalline residue, m.p. 103~106°C. This was *O*-decetylated under the Zemplen conditions,[6] and the residue, obtained after usual work-up, was purified by column chromatography (gradient elution D→E) to furnish pure crystalline **6** after trituration with ether.

7-Diethylamino-7-deoxylincomycin (**7**)

To a mixture of **6** (0.1 mmol) and acetaldehyde (0.15 mmol) in dry tetrahydrofuran sodium cyanoborohydride (0.11 mmol) was added and the mixture was strirred at room temperature for 1.5 hours. It was then concentrated under diminished pressure and the residue was purified by means of column chromatography (D) to obtain the pure title compound **7**.

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