acid analogs were dissolved in sterile water and added aseptically to the previously autoclaved assay tubes. In all assays the amount of growth was determined photometrically at 625 mμ with a Bausch and Lomb Spectronic 20 spectrophotometer, in terms of absorbance readings of the turbid culture medium against a blank of uninoculated medium set a zero absorbance. For *E. coli* the data in Table I are recorded as absorbance readings which are related to the milligrams of dry cells calculated from a standard curve of milligrams of dry cells per milliliter *vs.* absorbance readings.

Lincomycin. VI. 4′-Alkyl Analogs of Lincomycin. Relationship between Structure and Antibacterial Activity[1]

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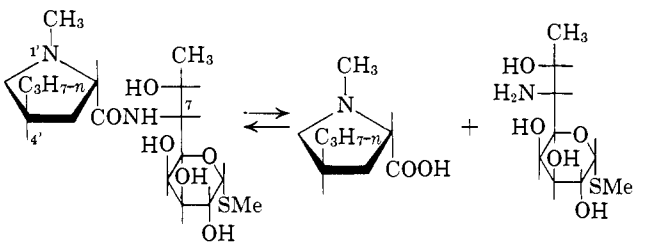
The partial synthesis of a series of 4′-alkyl analogs of lincomycin and 1′-demethyl-1′-ethyllincomycin is reported. The *in vitro* antibacterial activity of some of these compounds was three to four times that of lincomycin. Replacement of the 7-hydroxyl group of these compounds by chlorine further enhanced the antibacterial activity. Relationships are drawn between structure and *in vitro* and *in vivo* antibacterial activity.

Lincomycin, a water-soluble antibiotic,[2] is orally effective in man for the treatment of diseases caused by gram-positive organisms.[3] The elucidation of the structure of lincomycin (**1**) showed that it was not chemically related to any of the major antibiotics.[4]

Lincomycin may be cleaved into an amino acid fragment, *trans*-1-methyl-4-*n*-propyl-L-proline (**2**) and an amino sugar, methyl thiolincosaminide (**3**). These fragments may be recombined to yield lincomycin by employing one of the standard methods for amide formation.[5] The unique chemical stability of lincomycin

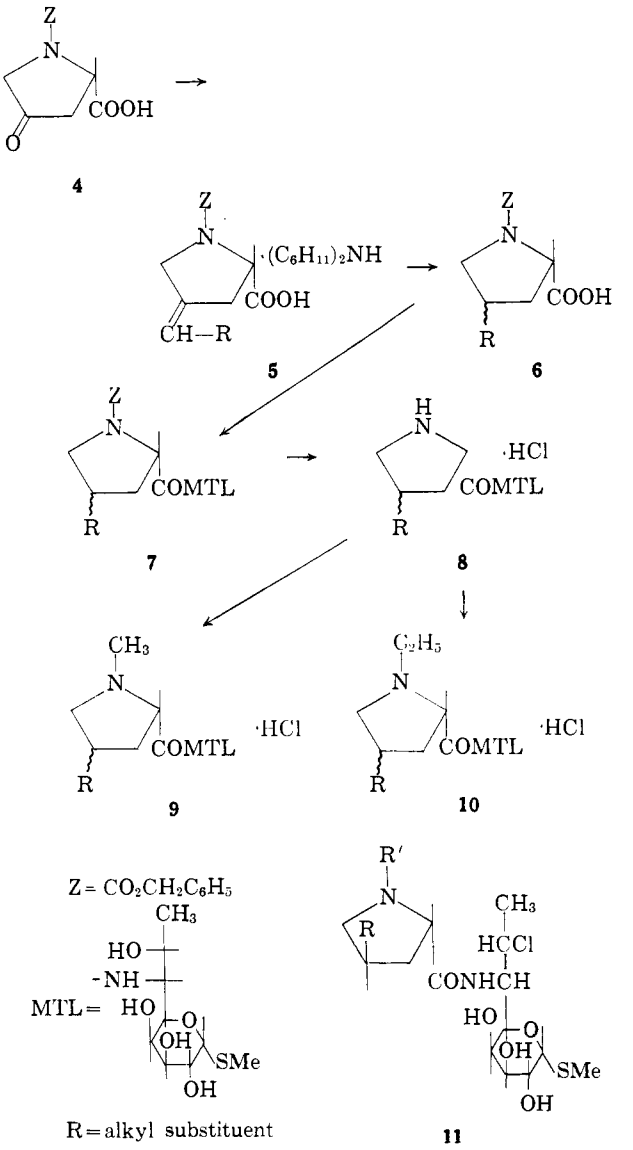


1 2 3

and the cleavage–recombination sequence established the antibiotic as a highly desirable substrate in which to study the effect of chemical modification on antibacterial activity. The synthesis and antibacterial properties of lincomycin analogs having various alkyl groups at N-1′ and C-4′, and in some cases having the 7-hydroxyl replaced by chlorine, are now described.

The method for preparation of 4′-alkyl analogs of lincomycin was a modification of the previously described partial synthesis of lincomycin.[6] The synthetic scheme is outlined in Chart I.

CHART I



R = alkyl substituent

1-Carbobenz-

(1) A portion of this work was reported earlier. Abstracts of Papers, Vth Interscience Conference on Antimicrobial Agents and Chemotherapy and IVth International Congress of Chemotherapy, Washington, D. C., Oct 17–21, 1965, p 17.

(2) (a) D. J. Mason, A. Dietz, and C. DeBoer in "Antimicrobial Agents and Chemotherapy—1962," J. C. Sylvester, Ed., American Society for Microbiology, Ann Arbor, Mich., 1963, p 554; (b) R. R. Herr and M. E. Bergy, *ibid.*, p 560; (c) C. Lewis, H. W. Clapp, and J. E. Grady, *ibid.*, p 570. Lincocin® is lincomycin hydrochloride.

(3) (a) W. J. Holloway, R. H. Kahlbaugh, and E. G. Scott in "Antimicrobial Agents and Chemotherapy—1963," J. C. Sylvester, Ed., American Society for Microbiology, Ann Arbor, Mich., 1964, p 200; (b) J. Harnecker, J. Contreras, B. Gilabert, and V. Ubilla, *ibid.*, p 204; (c) E. W. Walters, M. J. Romansky, and A. C. Johnson, *ibid.*, p 210; (d) J. C. Trakas and H. E. Lind, *ibid.*, p 216.

(4) H. Hoeksema, B. Bannister, R. D. Birkenmeyer, F. Kagan, B. J. Magerlein, F. A. MacKellar, W. Schroeder, G. Slomp, and R. R. Herr, *J. Am. Chem. Soc.*, **86**, 4223 (1964).

(5) W. Schroeder, B. Bannister, and H. Hoeksema, *ibid.*, in press.

(6) B. J. Magerlein, R. D. Birkenmeyer, R. R. Herr, and F. Kagan, *ibid.*, in press.

oxy-4-keto-L-proline (**1**) was treated with the appropriate Wittig reagents[7] in dimethyl sulfoxide solution to form 1-carbobenzoxy-4-alkylidene-L-prolines. The acids usually failed to crystallize, but formed crystalline dicyclohexylamine salts (**5**). The free acids were regenerated from the salts and hydrogenated over a platinum–Dowex 1 catalyst[8] to afford oily saturated acids **6**. The acids **6** were obtained as a mixture of isomers at C-4. Separation of this mixture by thin layer chromatography was unsuccessful, the entire mixture being used in the subsequent step. The acids **6** were condensed with methyl thiolincosaminide (**3**) by the mixed anhydride procedure affording amides **7** in high yield. The carbobenzoxy group of **7** was readily removed by hydrogenolysis to yield methyl N-(4′-*n*-alkyl-L-prolyl)-α-thiolincosaminides isolated as their crystalline hydrochlorides **8**. Both **7** and **8** were isolated as *cis–trans* mixtures at C-4′ since experience showed that separation of isomers was more readily accomplished after introduction of an alkyl substituent on the amino acid nitrogen. Reductive methylation of **8**[9] followed by careful column chromatography over silica gel led to the isolation of the *trans* and *cis* isomers which were characterized as their crystalline hydrochlorides (**9**). The assignment of the configuration as *trans* or *cis* was made on the basis of the relative mobility of the isomers on thin layer chromatography. Lincomycin (*trans*) has a greater R_f on tlc than does its *cis* isomer.[8] Therefore, the *trans* configuration was assigned to that isomer of the pair showing the greater mobility on tlc. These derived antibiotics[10] were isolated as variable hydrates depending on the conditions of drying. Most analytical data for these compounds was obtained using samples dried under high vacuum at ambient temperature. The elemental analyses were corrected for the water found to be present. The experimental section contains the details for the preparation of *trans* and *cis* methyl N-(1′-methyl-4′-*n*-butyl-L-prolyl)-α-thiolincosaminide (**9**, R = C_1H_9) and *trans* and *cis* methyl N-(1-ethyl-4-*n*-butyl-L-prolyl)-α-thiolincosaminide (**10**, R = *n*-C_4H_9). Data for the other 4′-alkyl analogs of lincomycin and their intermediates are found in Tables I–V.

The remarkable potentiating effect on the antibacterial activity of lincomycin observed when the 7-hydroxyl group is replaced by chlorine[11] caused us to introduce chlorine into a number of derived lincomycins. The 7-deoxy-7(S)-chloro analogs (**11**) which were prepared and obtained analytically pure are tabulated in Table VI. Due to the limited supply of substrate available, analytical data for several chloro analogs were not obtained, though in each case only a single spot was detected on tlc.

Antibacterial Activity.—Preliminary *in vitro* and *in vivo* antibacterial assays of the lincomycin analogs are found in Table VII. The activities of the analogs are expressed relative to lincomycin taken as unity. The *in vitro* assay was the standard curve agar diffusion assay for lincomycin using the test organism *S. lutea*.[12] The *in vivo* antibacterial activity was measured in the mouse protection assay against *Staphylococcus aureus*.[13] The compounds were administered orally.

The standard curve assays for the 4′-alkyl analogs of lincomycin indicate that the *trans* isomers show a significant increase in activity over lincomycin, a maximum of almost four times the antibacterial activity of lincomycin being realized before the cut-off point is reached at C-6. A similar effect was found for the *cis* isomers, which are consistently about one-half as active as the *trans* isomers.

The increased lipophilicity of these compounds due to the increased size of the 4′-alkyl substituent likely permits increased penetration of the molecule to the site of action.

A similar situation is found in the N-ethyl series. Again the *in vitro* assays increase with lengthening of the 4′-substituent, a maximum being reached at C-5.

The *in vivo* assays also indicate enhanced antibacterial activity for the 4′-substituted lincomycin analogs, though the increase is not as noteworthy as found in the *in vitro* assays.

The antibacterial spectrum was not changed as the homologous series was ascended. However, the analogs containing an N-ethyl substituent showed significantly greater activity against gram-negative organisms.

While the initial members of the N-demethyllincomycin series (**8**) possessed only slight antibacterial activity, activity gradually increased with increased lipid solubility. The N-demethyllincomycins were characterized by a several fold greater activity against streptococci organisms than would be predicted from their standard curve assays.

The replacement of the 7-hydroxy group in 4′-substituted analogs of lincomycin by chlorine consistently raised the antibacterial activity, both *in vitro* and *in vivo*.

From the foregoing, we note that the described variations in the lincomycin molecule at C-4′, N-1′, and C-7 lead to derived antibiotics possessing greater antibacterial activity than lincomycin. Moreover, in the case of the 7-deoxy-7(S)-chloro-4′-alkyl analogs, the potentiating affects of the 7(S)-chloro group and the 4′-alkyl are roughly additive.

Experimental Section

Melting points were determined on a Thomas–Hoover capillary melting point apparatus using a thermometer calibrated for stem exposure. Optical rotations were measured in the solvent noted (*c* ~1). Thin layer chromatograms (tlc) were run on 2.5 × 7.5 cm microslides coated with Brinkmann silica gel GF_{254} using a solvent mixture of ethyl acetate–acetone–water (8:5:1). Detection was effected by spraying with Lemieux Reagent.[13]

1-Carbobenzoxy-4-*n*-butylidene-L-proline Dicyclohexylamine Salt (5, R = C_3H_7).—Sodium hydride (19 g of 53% suspension in mineral oil) was added to 350 ml of dimethyl sulfoxide (DMSO) and the mixture was stirred and heated at 70 ± 3° until the hydride had reacted. The solution was cooled to 32° and 162 g of *n*-butyltriphenylphosphonium bromide was added. The resulting reaction mixture was stirred for 1 hr. A solution of 26 g

(7) M. Bethell, G. W. Kenner, and R. C. Sheppard, *Nature*, **194**, 864 (1962).

(8) (a) F. J. McQuillin, W. O. Ord, and P. L. Simpson, *J. Chem. Soc.*, 5996 (1963). (b) See ref 6 for a discussion on the use of this catalyst.

(9) Thin layer chromatography of the crude alkylation mixture usually indicated an isomeric ratio of about 1–4 in favor of the unnatural *cis* isomer.

(10) V. Bryson in "Survey of Biological Progress," Vol. IV, B. Glass, Ed., Academic Press Inc., New York, N. Y., 1962, p 374, suggests the term "derived antibiotics" for biosynthetics related to antibiotics.

(11) R. D. Birkenmeyer, ref 1.

(12) L. J. Hauka, D. J. Mason, M. R. Burch, and R. W. Treick, ref 2a, p 565.

(13) R. U. Lemieux and H. F. Bauer, *Anal. Chem.*, **26**, 920 (1954).

TABLE I

1-CARBOBENZOXY-4-ALKYLIDENE-L-PROLINE DICYCLOHEXYLAMINE SALTS (5)

R	Yield, %	Mp, °C	$[\alpha]_D$ ($CHCl_3$), deg	Formula	C, % Calcd	C, % Found	H, % Calcd	H, % Found	N, % Calcd	N, % Found
n-C_3H_7	46.8	142–144	−4	$C_{29}H_{44}N_2O_4$	71.86	71.69	9.15	9.30	5.78	5.74
n-C_4H_9	49.5	124–128	−6	$C_{30}H_{46}N_2O_4$	72.25	72.38	9.30	9.52	5.62	5.97
n-C_5H_{11}	38.0	109–111	−7	$C_{31}H_{48}N_2O_4$	72.62	72.70	9.44	9.43	5.46	5.71
n-C_7H_{15}	43.4	113–118	−11	$C_{33}H_{52}N_2O_4$	73.29	73.32	9.69	10.06	5.18	5.28
n-$C_{17}H_{35}$	51.0	106–110	−7	$C_{43}H_{72}N_2O_4$	75.83	75.85	10.66	10.85	4.11	4.05

TABLE II

METHYL N-(1′-CARBOBENZOXY-4′-n-ALKYL-L-PROLYL)THIOLINCOSAMINIDES (7)

R	Yield, %	Mp, °C	$[\alpha]_D$ (CH_3OH), deg	Formula	C, % Calcd	C, % Found	H, % Calcd	H, % Found	N, % Calcd	N, % Found	S, % Calcd	S, % Found
n-C_4H_9	72.9	197.5–200	+110	$C_{26}H_{40}N_2O_8S$	57.75	57.58	7.46	7.16	5.18	5.50	5.93	6.01
n-C_5H_{11}	81.1	191–193	+108	$C_{27}H_{42}N_2O_8S$	58.46	58.32	7.63	7.52	5.05	4.95		
n-C_6H_{13}	91.0	176–180	+103	$C_{28}H_{44}N_2O_8S$	59.13	59.16	7.80	7.46	4.93	5.09	5.64	5.96
n-C_8H_{17}	89.2	181–202	+99	$C_{30}H_{48}N_2O_8S$	60.38	60.35	8.11	8.08	4.70	4.73		
n-$C_{18}H_{37}$	49.9	137–141	+72	$C_{40}H_{68}N_2O_8S$	65.18	65.23	9.30	9.46	3.80	3.90	4.35	4.18

TABLE III

METHYL N-(4′-n-ALKYL-L-PROLYL)THIOLINCOSAMINIDE HYDROCHLORIDES (8)

R	Yield, %	Mp, °C	$[\alpha]_D$ (H_2O), deg	Formula	C, % Calcd	C, % Found	H, % Calcd	H, % Found	N, % Calcd	N, % Found	S, % Calcd	S, % Found	H_2O, % Found
H	70.6	273–280		$C_{14}H_{26}N_2O_6S\cdot HCl$[b]	43.46	43.33	7.04	6.72	7.24	6.98			
n-C_4H_9	73.0	197–199 dec	+150	$C_{18}H_{34}N_2O_6S\cdot HCl$[a]	48.80	48.58	7.96	8.19	6.32	6.04	7.24	7.36	5.54
n-C_5H_{11}	69.7	212–214 dec	+141	$C_{19}H_{36}N_2O_6S\cdot HCl$[a]	49.93	50.22	8.16	7.96	6.13	6.09	7.02	7.18	5.43
n-C_6H_{13}	98.0	210–212 dec	+134	$C_{20}H_{38}N_2O_6S\cdot HCl$[b]	50.99	50.81	8.35	8.75	5.95	5.80	6.81	6.65	
n-C_8H_{17}	93.1	181–200 dec	+128	$C_{22}H_{42}N_2O_6S\cdot HCl$[b]	52.94	52.62	8.68	8.36	5.61	5.61	6.43	6.36	
n-$C_{18}H_{37}$	54.0	165–175 dec	+127	$C_{32}H_{62}N_2O_6S\cdot HCl$[b]	60.11	60.35	9.93	10.03	4.38	4.60	5.02	4.98	

[a] Dried at 26° under vacuum, analyses corrected for water content. [b] Dried at 80° under vacuum.

TABLE IV

METHYL N-(1′-METHYL-4′-n-ALKYL-L-PROLYL)THIOLINCOSAMINIDE HYDROCHLORIDES (9)

R	Yield, %	Mp, °C	$[\alpha]_D$ (H_2O), deg	Formula[a]	C, % Calcd	C, % Found	H, % Calcd	H, % Found	N, % Calcd	N, % Found	S, % Calcd	S, % Found	H_2O, % Found
H	77	262–264	+160	$C_{15}H_{28}N_2O_6S\cdot HCl$	44.93	44.77	7.29	6.99	6.99	7.00			0.25
n-C_4H_9 *trans*	30[b]	161–168 dec	+138	$C_{19}H_{36}N_2O_6S\cdot HCl$	49.93	50.32[a]	8.16	7.98[a]	6.13	6.20	7.02	6.67	4.07
cis	30[b]	194–198 dec	+132			49.82		8.20		6.05		6.65	4.34
n-C_5H_{11} *trans*	21	188–191 dec	+133	$C_{20}H_{38}N_2O_6S\cdot HCl$	50.99	50.66	8.35	8.60	5.95	5.96			2.78
cis	44					50.95		8.27		5.78			4.32
n-C_6H_{13} *trans*	17	115–117 dec	+125	$C_{21}H_{40}N_2O_6S\cdot HCl$	51.99	51.67[a]	8.52	8.75[a]	5.78	5.71	6.61	6.46	5.62
cis	13	141–144 dec	+121			51.82[a]		8.80[a]		5.72		6.35	3.79
n-C_8H_{17} *trans*		110–114 dec	...	$C_{23}H_{44}N_2O_6S\cdot HCl$		...		...	5.46	5.40	6.25	6.29	5.74
cis		118–122 dec	+123		53.83	53.64	8.84	9.01		5.37		5.99	5.42

[a] Sample dried at 100° under vacuum. [b] Yield taken from a scale up of the experiment described.

TABLE V

METHYL N-(1′-ETHYL-4′-n-ALKYL-L-PROLYL)THIOLINCOSAMINIDE HYDROCHLORIDES (10)

R	Yield, %	Mp, °C	$[\alpha]_D$ (H_2O), deg	Formula	C, % Calcd	C, % Found	H, % Calcd	H, % Found	N, % Calcd	N, % Found	S, % Calcd	S, % Found	H_2O, % Found
H	24	210–213	+161	$C_{16}H_{30}N_2O_6S\cdot HCl$	46.13	46.32	7.53	7.18	6.75	6.67			
n-C_4H_9 *trans*	16	148–151	+134	$C_{20}H_{38}N_2O_6S\cdot HCl$	50.99	51.33	8.35	8.68	5.95	5.81	6.81	6.57	4.08
cis	14	134–138	+130			50.94		8.54		5.98		6.50	3.30
n-C_5H_{11} *trans*	7	130–138	+128	$C_{21}H_{40}N_2O_6S\cdot HCl$	51.99	51.65	8.52	8.61	5.78	...	...	...	5.40
cis	13	150–157	+121			51.51		8.64		6.03	...	...	0.89
n-C_6H_{13} *trans*	8	125–128	+128	$C_{22}H_{42}N_2O_6S\cdot HCl$	52.94	53.28	8.68	8.90	5.61	5.61	6.43	6.31	5.88
cis	4	93–106											
n-C_8H_{17} *trans*	11	78–90	+118	$C_{24}H_{46}N_2O_6S$	58.74	58.70	9.45	9.68	5.71	5.78	6.54	6.57	4.54
cis	*a*		+91	$C_{24}H_{46}N_2O_6S\cdot HCl$	54.68	54.30	8.99	8.91	5.32	5.22	6.08	5.40	1.76
n-$C_{18}H_{37}$ *trans*		135–153		$C_{34}H_{66}N_2O_6S\cdot HCl$	61.18	61.63	10.12	10.39					

[a] Noncrystalline.

TABLE VI

METHYL N-(1′-ALKYL-4′-*n*-ALKYL-L-PROLYL)-7-DEOXY-7(*S*)-CHLOROTHIOLINCOSAMINIDES (**11**)[a]

R	R′	Yield, %	Formula	C, % Calcd	C, % Found	H, % Calcd	H, % Found	N, % Calcd	N, % Found	S, % Calcd	S, % Found	Cl, % Calcd	Cl, % Found	H$_2$O, % Found	$[\alpha]$D (H$_2$O), deg
C_2H_5 *trans*	CH_3	21	$C_{17}H_{32}Cl_2N_2O_5S$	45.63	45.25	7.21	6.62	6.26	5.95	7.18	7.10	15.85	15.45	7.90	+149
C_3H_9 *cis*	CH_3	45	$C_{18}H_{36}Cl_2N_2O_5S$	Tlc only[b]											
trans	CH_3	39	$C_{18}H_{36}Cl_2N_2O_5S$	Tlc only[b]											
C_6H_{11} *cis*	CH_3	12	$C_{20}H_{38}Cl_2N_2O_5S$	Tlc only[b]											
trans	CH_3	22	$C_{20}H_{38}Cl_2N_2O_5S$	47.33	46.73	7.94	8.00	5.52	5.34	6.32	6.26	13.97	13.91	3.84	+125
C_3H_7 *trans*	C_2H_5	22	$C_{19}H_{36}Cl_2N_2O_5S$	47.99	48.20	7.63	7.81	5.89	6.07	6.74	6.48	14.91	15.05	4.57	+133
C_4H_9 *cis*	C_2H_5	11	$C_{20}H_{38}Cl_2N_2O_5S$	49.07	48.91	7.82	7.91	5.72	6.14	6.55	5.97	14.49	14.80	5.37	+106
trans	C_2H_5	19	$C_{20}H_{38}Cl_2N_2O_5S$	49.07	48.38	7.82	8.03	5.72	5.56	6.55	6.43	14.49	15.06	3.02	
C_5H_{11} *cis*	C_2H_5	5	$C_{21}H_{40}Cl_2N_2O_5S$	Tlc only[b]											
trans	C_2H_5	28	$C_{21}H_{40}Cl_2N_2O_5S$	Tlc only[b]											
C_6H_{13} *trans*	C_2H_5	24	$C_{22}H_{42}Cl_2N_2O_5S$	51.05	50.85	8.18	7.26	5.41	5.47	6.201	6.79	13.70	13.43	3.38	+124

[a] These compounds were highly solvated and melted from 125–155°. [b] One spot on tlc using $CHCl_3$–MeOH (5:1).

TABLE VII

ANTIBACTERIAL ACTIVITIES OF LINCOMYCIN ANALOGS

No.	R	Std curve assay *vs. S. btor*[a]	Mouse protection assay *vs. S. btor*[b] (Oral)
9	H	0.1025	
	n-C_2H_5 *trans*[c]	0.3	0.58
	n-C_3H_7 *trans*	1.0	1.00
	n-C_4H_9 *trans*	2.1	1.20
	n-C_5H_{11} *trans*	3.4	1.35
	n-C_6H_{13} *trans*	3.6	
	n-C_7H_{15} *trans*[d]	1.5	0.51
	n-C_8H_{17} *trans*	1.0	0.30
	n-C_3H_7 *cis*	0.5	
	n-C_4H_9 *cis*	1.3	
	n-C_5H_{11} *cis*	1.8	
	n-C_6H_{13} *cis*	2.1	
	n-C_7H_{15} *cis*	0.9	
	n-C_8H_{17} *cis*	0.6	
10	H	0.02	
	n-C_3H_7 *trans*	1.0	1.00
	n-C_4H_9 *trans*	1.2	1.33
	n-C_5H_{10} *trans*	3.0	0.89
	n-C_6H_{13} *trans*	2.0	0.55
	n-C_8H_{17} *trans*	0.4	0.12
	n-C_3H_7 *cis*	0.5	
	n-C_4H_9 *cis*	0.7	
	n-C_5H_{10} *cis*	1.3	
	n-C_6H_{13} *cis*	1.2	
	n-C_8H_{17} *cis*	0.4	
8	H	0.1025	
	n-C_3H_7 (*cis–trans*)	0.05	
	n-C_4H_9 (*cis–trans*)	0.01	
	n-C_5H_{11} (*cis–trans*)	0.15	
	n-C_6H_{13} (*cis–trans*)	0.33	
	n-C_7H_{15} (*cis–trans*)[d]	0.47	
	n-C_8H_{17} (*cis–trans*)	0.60	
	n-$C_{18}H_{37}$ (*cis–trans*)	<0.01	
	R R′		
11	C_2H_5 CH_3 *trans*	1.4	0.58
	n-C_3H_7 CH_3 *trans*	3.7	1.84
	n-C_4H_9 CH_3 *trans*	3.6	1.40
	n-C_5H_{10} CH_3 *trans*	5.0	1.88
	n-C_3H_7 C_2H_5 *trans*	2.0	1.40
	n-C_4H_9 C_2H_5 *trans*	2.4	1.50
	n-C_5H_{11} C_2H_5 *trans*	4.1	
	n-C_6H_{13} C_2H_5 *trans*	1.0	0.60

[a] Lincomycin = 1. [b] Lincomycin = 1. [c] The authors are indebted to Dr. A. Argoudelis for the use of these data. [d] The authors are indebted to Dr. B. Bannister for the use of these data.

of 1-carbobenzoxy-4-keto-L-proline (**4**)[11] in 100 ml of DMSO was added, and the reaction mixture was heated at 70° for 4 hr. The reaction mixture was cooled to 25° and 1 l. of 2.5% $KHCO_3$ solution was added. This mixture was extracted twice with 700-ml portions of ether which in turn were back extracted with 2.5% $KHCO_3$ solution. The combined aqueous extract was acidified (HCl) and extracted with four 500-ml portions of ether. The combined ether extract was washed successively with 250 ml of water, three 250-ml portions of saturated $NaHSO_3$ solution, and 250 ml of water. After drying (Na_2SO_4), the solvent was removed under vacuum leaving an oily residue of 24 g. This oil was dissolved in 31 ml of acetonitrile and 18 ml of dicyclohexylamine. The crystals, which precipitated on refrigeration, were collected, washed with acetonitrile, and dried *in vacuo*. The yield of crystalline salt, mp 136–140°, was 21 g (46.8%).

Similar material after two recrystallizations from acetonitrile melted at 142–144° and gave $[\alpha]$D −4° ($CHCl_3$).

Anal. Calcd for $C_{29}H_{44}N_2O_7$: C, 71.86; H, 9.15; N, 5.78. Found: C, 71.69; H, 9.30; N, 5.74.

1-Carbobenzoxy-4-*n*-butyl-L-proline (*cis* **and** *trans*) (**6**, **R** = *n*-C_4H_9).—1-Carbobenzoxy-4-*n*-butylidene-L-proline (10 g) was shaken with ether and excess 5% KOH until no solid remained. The aqueous layer was separated. This solution was acidified with HCl and repeatedly extracted with ether. The ethereal extract was dried (Na_2SO_4) and concentrated *in vacuo*. The oily residue weighed 6.3 g (95%). This acid was dissolved in 200 ml of methanol and shaken for 17 hr over 2.1 g of Pt–Dowex 1 catalyst[8a] under 2.8 kg/cm^2 of H_2. The catalyst was removed by filtration. The filtrate on evaporation yielded 6.3 g of oily product which was used without purification in the next step.

Methyl N-(1′-Carbenzoxy-4′-*n*-butyl-L-prolyl)thiolincosaminide (*cis* **and** *trans*) (**7**, **R** = *n*-C_4H_9).—The acid from above (6.3 g) was dissolved in 175 ml of acetonitrile and cooled to 0°. To this solution was added 3.46 ml of triethylamine followed by 3.34 ml of isobutyl chloroformate. After stirring at 0 ± 3° for 15 min there was added a solution of 6.2 g of methyl thiolincosaminide dissolved in 85 ml of water. The reaction mixture was stirred in the cooling bath for 0.5 hr and at 25° for 1 hr. The mixture was filtered to yield, after drying, 4.57 g (37.7%) of **7** (R = *n*-C_4H_9). The mother liquors were concentrated under vacuum and an additional 4.25 g (35.2%) of product was recovered. Recrystallization from acetonitrile afforded **7** (R = *n*-C_4H_9), mp 194–196°. The analytical sample prepared by recrystallization from the same solvent melted at 197.5–200° and showed $[\alpha]$D +140° (CH_3OH).

Anal. Calcd for $C_{26}H_{39}N_2O_6S$: C, 57.75; H, 7.46; N, 5.18; S, 5.93. Found: C, 57.58; H, 7.16; N, 5.50; S, 6.01.

Methyl N-(4′-*n*-Butyl-L-prolyl)thiolincosaminide Hydrochloride (*cis* **and** *trans*) (**8**, **R** = *n*-C_4H_9).—A quantity of 7.8 g of **7** (R = *n*-C_4H_9) was dissolved in 200 ml of methanol and shaken under 2.8 kg/cm^2 of H_2 over 2 g of 10% Pd-C for 17 hr. The catalyst was removed by filtration and the filtrate was evaporated under vacuum. The residue was dissolved in a mixture of 20 ml of acetone and 20 ml of water. The solution was acidified by the

[11] A. A. Patchett and R. Wickop, *J. Am. Chem. Soc.*, **79**, 185 (1957).

addition of 6 *N* HCl and diluted with a fourfold volume of acetone. The crystals were collected by filtration and dried. They melted at 188–194° dec and weighed 4.7 g (73.0%). The analytical sample obtained by recrystallization from the same solvent melted at 197–199° and showed $[\alpha]D$ +150° (H_2O).

Anal. Calcd for $C_{18}H_{34}N_2O_6S \cdot HCl$: C, 48.80; H, 7.96; N, 6.32; S, 7.24. Found (corrected for 5.54% water): C, 48.58; H, 8.19; N, 6.04; S, 7.36.

Methyl N-(1′-Methyl-4′-*n*-butyl-L-prolyl)thiolincosaminide Hydrochloride (*cis* and *trans*) (9, R = *n*-C_4H_9).—A mixture of 2.0 g of **8** (R = *n*-C_4H_9), 2.0 ml of 37% formalin, 150 ml of methanol, and 500 mg of 10% Pd–C was shaken under 2.45 kg/cm² of H_2 for 3.5 hr. The catalyst was removed by filtration. Evaporation of the methanol yielded a partially crystalline residue which when assayed by tlc consisted chiefly of the *cis* and *trans* isomers of **9** (R = *n*-C_4H_9) in a ratio of about 3:1.

Separation of Isomers.—The above product was dissolved in a mixture of methanol–methylene chloride and 1.5 ml of triethylamine was added. To this solution was added 7 g of silica gel and the solvent was distilled under vacuum. This solid was sifted on top of a chromatographic column of 200 g of silica gel packed with a solvent mixture consisting of ethyl acetate, acetone, and water in a ratio of 8:5:1. The column was developed by eluting with the same solvent and 20-ml fractions were collected. Tlc of each fraction showed that fractions 31–38, 310 mg, were essentially pure *trans* isomer and 49–74, 326 mg, were essentially pure *cis* isomer. Fractions 39–48 consisted of a mixture of isomers which could be further separated by repeated chromatography. Each isomer was dissolved in a few drops of dilute HCl and the hydrochloride precipitated by addition of acetone. In this manner there was obtained 50 mg of *trans* **9** (R = C_4H_9), mp 135–137° dec, and about 150 mg of *cis* isomer, mp 105°, softening with further melting at 175–185° dec.

The *trans* isomer was recrystallized from the same solvent and then melted at 161–168° dec.

Anal. Calcd for $C_{19}H_{36}N_2O_6S \cdot HCl$: C, 49.93; H, 8.16; N, 6.13; S, 7.02. Found: C, 50.32; H, 7.98; N, 6.20; S, 6.67. N and S analysis corrected for 4.07% H_2O; C and H sample dried at 100°.

Similarly, recrystallization of the *cis* isomer gave a product melting at 194–198° dec.

Anal. Found (corrected for 4.34% water): C, 49.82; H, 8.20; N, 6.05; S, 6.65.

Methyl N-(1′-Ethyl-4′-*n*-butyl-L-prolyl)thiolincosaminide Hydrochloride (*cis* and *trans*) (10, R = *n*-C_4H_9).—A mixture of 2.0 g of **8** (R = *n*-C_4H_9), 1.5 ml of acetaldehyde, and 750mg of 10% Pd-C in 150 ml of methanol was shaken under 2.45 kg/cm² of H_2 for 5.5 hr. The catalyst was removed by filtration to give a residue consisting chiefly of *cis* and *trans* **10**, R = *n*-C_4H_9.

Separation of Isomers.—As described above, the mixture of isomers (2 g) was chromatographed over 200 g of silica gel, using for elution a solvent system of ethyl acetate–acetone–water (8:5:1). Fractions 33–42 appeared by tlc to be pure *trans* isomer and were combined. Fractions 47–64 were essentially pure *cis* isomer and were also combined. Fractions 43–48 were a mixture of isomers which could be purified by rechromatography. Each isomer was dissolved in a few drops of dilute HCl and the crystalline hydrochloride precipitated on dilution with a large volume of acetone.

The *trans* isomer fraction of 415 mg gave 340 mg (15.9%) of crystalline *trans* product, mp 144–151°. Recrystallization from dilute acetone raised the melting point to 148–151°.

Anal. Calcd for $C_{20}H_{38}N_2O_6S \cdot HCl$: C, 50.99; H, 8.35; N, 5.95; S, 6.81. Found (corrected for 4.08% water): C, 51.33; H, 8.68; N, 5.81; S, 6.57.

The *cis* isomer fraction of 645 mg afforded 300 mg (14.1%) of crystalline hydrochloride, mp 135–139°. Recrystallization from dilute acetone gave crystals, mp 134–138°.

Anal. Found (corrected for 3.30% water): C, 50.94; H, 8.54; N, 5.98; S, 6.50.

Methyl N-(1′-Ethyl-4′-*n*-hexyl-L-prolyl)-7-deoxy-7(S)-chlorothiolincosaminide (11, R = *n*-C_6H_{11}).—Thionyl chloride (1.4 ml) was added to a stirred suspension of methyl N-(1′-ethyl-4′-*n*-hexyl-L-prolyl)thiolincosaminide (1.4 g) in 28 ml of CCl_4. The reaction mixture was heated at reflux for 2 hr and evaporated to dryness under vacuum. Chloroform (100 ml) was added and then removed under vacuum. The $CHCl_3$ addition and evaporation were repeated two more times to ensure complete removal of residual $SOCl_2$. The solid residue was dissolved in 5 ml of ethanol, cooled in an ice bath, and the pH was adjusted to 10 by the addition of 1 *N* NaOH. Water (200 ml) was added and the basic solution was extracted well with $CHCl_3$. The extracts were dried and evaporated to give 1.0 g of crude product. This material was purified by chromatography over silica gel using MeOH–$CHCl_3$ (1:6) for elution. The fractions containing the desired material as determined by tlc were combined and evaporated. The residue was converted to its hydrochloride salt as described above.

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The 6-Deoxytetracyclines. IX. Imidomethylation

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Reaction of 6-demethyl-6-deoxytetracycline with N-hydroxymethylphthalimide gives 7-phthalimidomethyl-6-demethyl-6-deoxytetracycline. Reaction of other N-methylolimides is also described. 7-Citraconimidomethyl-6-demethyl-6-deoxytetracycline has an *in vitro* biological activity 12 times that of tetracycline.

The reaction of an aromatic compound with an N-hydroxymethylamide or an N-hydroxymethylimide to form an amidomethyl- or imidomethyl-substituted product has beeen known for many years as the Tscherniac–Einhorn reaction.[1] This reaction has recently been reviewed excellently by Zaugg and Martin[2a] as well as by other workers;[2b,c] however, the reaction does not seem to have enjoyed very wide synthetic import. No example of a natural product having been subjected to this reaction is reported by these reviewers, although it is clear that the general scope that it encompasses is broad.

Nitration[3] and halogenation[4] of 6-deoxytetracyclines

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