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Lincomycin. 14. An Improved Synthesis and Resolution of the Antimalarial Agent, 1′-Demethyl-4′-depropyl-4′(*R*)- and -(*S*)-pentylclindamycin Hydrochloride (U-24, 729A)[1]

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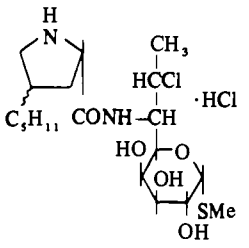
The synthesis of racemic 4-*n*-pentylproline hydrochloride (7) in high yield from α-*n*-pentylacrolein and diethyl acetamidomalonate is described. 4-*n*-Pentylproline is the key intermediate in a practical synthesis of the antimalarial and antibacterial agent, 1′-demethyl-4′-depropyl-4′(*R*)- and -(*S*)-*n*-pentylclindamycin hydrochloride (1) (U-24,279A).

1′-Demethyl-4′-depropyl-4′(*R*)- and -(*S*)-*n*-pentylclindamycin hydrochloride (U-24,729A) (1), a derived antibiotic related to lincomycin and clindamycin, was reported to possess broad-spectrum *in vitro* antibacterial activity and also significant *in vivo* activity.[2] The effectiveness of 1 as an antimalarial agent against *Plasmodium berghei* in the mouse was reported by Lewis[3] who also showed that 1 was not cross resistant with chloroquine or dimethyl diphenyl sulfone. Curative activity against blood inoculated *Plasmodium cynomolgi* in rhesus monkeys was also described.[4]



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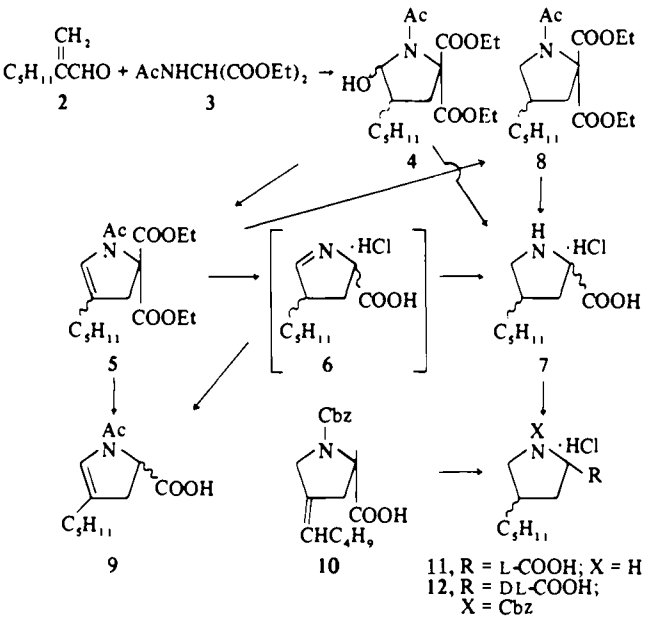
We have previously disclosed two closely related sequences for the synthesis of 4′-alkyl-1′-demethyl-4′-depropylclindamycin.[2b] In each case, the amino acid moiety was synthesized by a multistep process having as its key intermediate relatively expensive 1-carbobenzoxy-4-keto-L-proline. We now describe an efficient synthesis of racemic 4-*n*-pentylproline hydrochloride (7) and its facile conversion to 1′-demethyl-4′-depropyl-4′(*R*)- and -(*S*)-*n*-pentylclindamycin hydrochloride (1) in high yield.

The reaction sequence for the synthesis of racemic 4-*n*-pentylproline hydrochloride (7) outlined in Scheme I is similar to the synthesis of racemic 4-*n*-propylproline hydrochloride,[5] but possesses significant process improvements. α-*n*-Pentylacrolein (2), prepared by the method of Green and Hickinbottom,[6] was condensed with diethyl acetamidomalonate (3) to form pyrrolidine 4. While reduction of 4 with zinc–hydrochloric acid[7] gives 7 directly, higher yields of superior quality 7 were obtained as follows. Dehydration of 4 by anhydrous acid yielded 5 which in the presence of refluxing aqueous acid was hydrolyzed and decarboxylated to form acid 6. Catalytic reduction of 6 led to the isolation

Scheme I



of crystalline 4-*n*-pentylproline hydrochloride (7). The overall yield of 4-*n*-pentylproline hydrochloride (7) from diethyl acetamidomalonate, without isolation of intermediates, was consistently 65–70%.

Acylation of non-ultraviolet-absorbing 2-carboxy-4-*n*-pentyl-5-pyrrolidine hydrochloride (6) with acetic anhydride–pyridine[8] gave 45% yield of an acid, possessing ultraviolet absorption at 236 nm, whose nuclear magnetic resonance, infrared, and high-resolution mass spectral data indicate acid 9, previously prepared by saponification and decarboxylation of 5. On this basis the position of the unsaturation in intermediates 5 and 6 was assigned as shown.

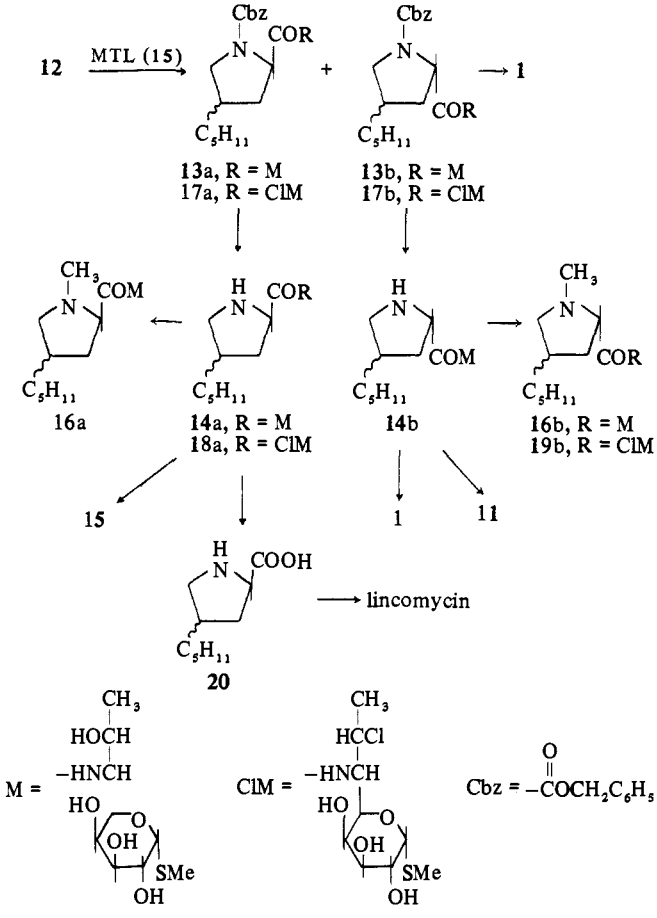
Variations in the order of the steps required to convert the initial condensation product 4 to 4-*n*-pentylproline (7) such as 5 → 8 → 7, seemed to offer no advantage over the original pathway.

cis- and *trans*-4-*n*-pentyl-L-proline hydrochloride (11) was prepared by concomitant hydrogenation–hydrogenolysis

of unsaturated acid of **10**[9] and used for tlc comparison with racemic amino acid **11** and also with *cis*- and *trans*-4-*n*-pentyl-L-proline obtained by degradation as described below.

Since optical resolution of **7** or its 1-carbobenzoxy derivative **12** was not initially rewarding, **12** was condensed with methyl thiolincosaminide (**15**)[10] to form epimeric amides **13a** and **13b**. The D isomer **13a** proved to be quite insoluble, precipitating from the reaction mixture, while the L isomer **13b** crystallized on concentration of the mother liquors. The desired L isomer (**13b**) of satisfactory optical purity for use as described in subsequent steps was obtained from the reaction mixture in over 40% yield (Scheme II).

Scheme II



In order to establish the identity of the coupled products, **13b** was decarbobenzoxylated to **14b**, which on acid hydrolysis afforded 4-pentyl-L-proline (**11**), identical with a sample prepared as described above. Chlorination of **14b** by the methods previously disclosed[2b] afforded **1**, identical in both its physical and microbiological properties with a known sample. Thus, racemic 4-*n*-pentylproline was satisfactorily converted to 1′-demethyl-4′-depropyl-4′-*n*-pentylclindamycin hydrochloride (**1**) (U-24,729A).

In our earlier synthesis of 1′-demethyl-4′-depropyl-4′-alkyllincomycins and clindamycins,[2b] we pointed out that these compounds were obtained as a mixture of isomers at C-4′. The ratio of trans to cis isomer was reported to be about 1 to 3 or 4 in favor of the cis isomer. This estimate was arrived at by N-methylation followed by chromatography. In order to establish the approximate isomer ratio at C-4′ in **14b**, this material was reductively methylated to form **16b**, identical with a known sample. Tlc and antibacterial assay indicated about the same isomer ratio in this material as in that prepared by the earlier synthesis.

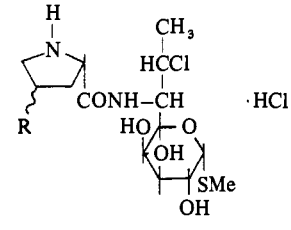
The identity of the D isomer **13a** was established in a similar manner to that for **13b**. Hydrogenolysis of **13a** afforded **14a** which on acid degradation gave 4-*n*-pentyl-D-proline (**20**) whose optical rotation was equal, but opposite in sign to that of **11**. Methylation of **14a** afforded **16a** as a mixture of isomers at C-4′ which was essentially inactive *vs. Sarcina lutea* as was also **14a**. The **16a** isomers were not separated from **16b** by tlc and therefore were not purified further.

Since it was uneconomical to discard that half of the resolving agent, methyl thiolincosaminide **15**, which was coupled with the D-amino acid, a method to recover the amino sugar was required. Hydrazinolysis[10] of **14a** gave in high yield methyl thiolincosaminide (**15**) whose identity was established by the usual physical measurements as well as by conversion to lincomycin.

Similarly 1-carbobenzoxy-4-*n*-pentylproline (**12**) was condensed with methyl 7(*S*)-chloro-7-deoxythiolincosaminide[2b] to form a mixture of **17a** and **17b**. This mixture was readily separated by fractional crystallization. Hydrogenolysis of **17b** afforded **1**, while **17a** gave inactive **18a**. While this process was somewhat more convenient than the method first described, the inability to satisfactorily cleave clindamycins with hydrazine hydrate led to its disfavor.

We have previously reported that in a series of 1′-demethyl-

Table I. Antibacterial Assay of 1′-Demethyl-4′-depropyl-4′(*R*)- and -(*S*)-pentylclindamycin Hydrochlorides (**1**)



Compound	Standard curve assay *vs. Sarcina lutea*[a] ATCC 9341	Serial dilution minimal inhibitory concentration[b] μg/ml: *Staphylococcus aureus* OSU 284	*Staph. aureus* UC 552	*Streptococcus faecalis* UC 3235	*Escherichia coli* ATCC 28	*Proteus vulgaris* ATCC 8247	*Salmonella schottmuelleri* ATTCC 9149	Mouse protection assay[c] *vs. Staphylococcus aureus* Dose, mg/kg: Subcutaneous	Oral
Lincomycin	1	0.4	0.8	12.5	400	800	4000		
R = *trans*-C_5H_{11}	13.7	0.05	0.025	0.2	12.5	50	25	1.5	5.7
R = *cis*-C_5H_{11}	10	0.025	0.025	0.1	6.4	50	25	2.1	6.6

[a] Ref 11. [b] Determinations made in brain heart infusion medium (Difco). Inocula consisted of about 10^5 organisms/ml of medium. Twofold dilutions of the antibiotic were used in each sensitivity determination. End points were read at 20 hr and are expressed as minimal inhibitory concentration of compound in μg/ml. [c] Ref 12.

Table II. Antimalarial Assay of 1'-Demethyl-4'-depropyl-4'(*R*)- and -(*S*)-pentylclindamycin Hydrochlorides in *P. berghei* Infected Mice[a]

Compound	MED, mg/kg[b]		CD_{50}, mg/kg[c]		CD_{100}, mg/kg[d]	
	Subcutaneous	Oral	Subcutaneous	Oral	Subcutaneous	Oral
(*R*) Epimer	0.75		6.0		>12	
(*S*) Epimer	0.62	1.56	7.0	14	>20	50
(*R*)-(*S*) Mixture	0.312	1.25	5.0	15	20	>20

[a]Data generously supplied by C. Lewis of these laboratories. [b]Dosage at which median survival time (ST_{50}) was increased significantly (p = 0.05) over ST_{50} of untreated controls. [c]CD_{50} is median protective dose (95% limits). [d]Dosage that protected 100% of animals; no parasites could be demonstrated by staining or subinfecting normal mice.

4'-depropyl-4'-alkyllincomycins and clindamycins the trans isomers [4'(*R*)-alkyl] were about twice as potent as the cis isomers [4'(*S*)-alkyl] in antibacterial assays.[2b] Therefore, we were interested to determine whether this structure-activity relationship was valid in the 1'-demethylclindamycin series.

An examination by tlc of the intermediates in the synthesis of 1'-demethyl-4'-depropyl-4'-*n*-pentylclindamycin hydrochloride (**1**) revealed that partial separation of the 4' epimers could likely be achieved by chromatography of the carbobenzoxy derivative **13b**. Moreover, vpc analysis of the trifluoroacetate derivatives of **13b** offered a quantitative method for estimation of each isomer. When chromatographed over silica gel in a succession of five columns, 5% of the trans and 43% of the cis isomer were separated. Each isomer showed greater than 90% purity when assayed by vpc analysis of its trifluoroacetate derivative. Using the methods described above each epimer was subjected to hydrogenolysis and chlorination to yield the trans and cis isomer of **1**, respectively. Again vpc assay of the trifluoroacetate derivative showed greater then 90% purity.

The *in vitro* and *in vivo* antibacterial assays of these epimers are given in Table I. In contrast to the greater activity of the trans isomers of the 1'-alkyl series, in the 1'-demethyl series no significant difference of potency was noted in either the *in vitro* or *in vivo* antibacterial assays. Similarly no significant difference in potency for the two isomers was noted when assayed *vs. P. berghei* in the mouse (Table II).

A simple and efficient synthesis of racemic 4-*n*-pentylproline hydrochloride followed by a high yield condensation-resolution sequence forms the basis of an improved synthesis of 1'-demethyl-4'-depropyl-4'(*R*)- and -(*S*)-*n*-pentylclindamycin hydrochloride (U-24,729A). A method for the separation of the 4'(*R*) and 4'(*S*) epimers and a comparison of their antibacterial potency was described.

Experimental Section†

α-*n*-Pentylacrolein (2). *n*-Heptanal (587 g) was converted to 593 g of α-*n*-pentylacrolein, bp 157–159°, $n^{25}D$ 1.4458, by the procedure of Green and Hickinbottom.[6] *Anal.* ($C_8H_{14}O$) C, H.

1-Acetyl-2,2-dicarboethoxy-4-*n*-pentyl-4-pyrroline (5). α-*n*-Pentylacrolein (**2**) (55.6 g, 0.44 mole) was added to a solution of 86.8 g (0.4 mole) of diethyl acetamidomalonate (**3**) in 800 ml of benzene followed by 2 ml of 25% NaOMe in MeOH. After standing at ambient temperature for 18 hr, the solvent was distilled *in vacuo*. Tlc (C_6H_{12}–Me_2CO, 2:1) showed the absence of **3** and the appearance of 1-acetyl-2,2-dicarboethoxy-5-hydroxy-4-pentylpyrrolidine (**4**) as a slower moving spot (R_f, 0.5) which was not absorbed in the uv.

†Melting points were taken in a Thomas-Hoover Uni-Melt apparatus and are corrected for stem exposure. Thin-layer chromatography (tlc) was carried out on microslides coated with Brinkman silica gel GF_{254}. Column chromatography employed silica gel, 0.05–0.20 mm, for chromatography, Brinkman Instruments, Inc., Westbury, L.I., N. Y. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements are within ±0.4% of the theoretical values. Absorption bands of spectra (ir, nmr) were as expected for all compounds.

Crude **4** was dissolved in 450 ml of abs EtOH and HCl bubbled in for 5 min. After 1.5 hr, the slightly cloudy solution was filtered and evaporated to dryness. Tlc (C_6H_{12}–Me_2CO, 2:1) showed the absence of **4** and the appearance of a strongly uv-absorbing spot moving slightly faster than **4**. This material was usually used in the following step without purification.

A sample of **5** was purified by chromatography using C_6H_{12}–Me_2CO (2:1) for elution. The major fraction selected on the basis of tlc analysis gave the following data: λ_{max} 236 nm (ε 9300) (Et_2O). *Anal.* ($C_{17}H_{27}NO_5$) C, H, N.

4-*n*-Pentylproline Hydrochloride (7). Method A. To the crude product from the previous step was added 460 ml of 6 *N* HCl and the resulting mixture heated at reflux for 2.5 hr. The cooled solution was extracted with ether to remove a small amount of deeply colored oil. The aqueous solution was evaporated under vacuum to give crude 4-*n*-pentyl-1-pyrroline-5-carboxylic acid hydrochloride (**6**). Ultraviolet absorption showed only end absorption with a weak shoulder at 255 nm. Crude **6** was dissolved in 1.2 l. of MeOH and hydrogenated for 18 hr over 50 g of 10% Pd/C. The catalyst was removed by filtration and the solvent evaporated. The residue was triturated with Me_2CO and filtered. The yield of **7**, mp 194–199° (sinter 187°), was 60.1 g (68% overall yield based on **3**). Recrystallization (aqueous MeCN) afforded an analytical sample, mp 201–204° (sinter 193°). *Anal.* ($C_{10}H_{20}ClNO_2$) C, H, N, M^+.

Method B. Crude **5** was hydrogenated over Pd/C and hydrolyzed with 6 *N* HCl as described above to yield **7**, mp 191–194° (sinter 176°), in significantly lower yield than in method A.

Method C. Treatment of 13.7 g of **4** with HCl and Zn afforded 1.4 g of recrystallized **7**, mp 197–200° (sinter 183°).

1-Acetyl-4-*n*-pentyl-4-pyrroline-2-carboxylic Acid (9). Method A. A mixture of 5 g of crude **6**, 10 ml of Ac_2O, and 10 ml of C_5H_5N was heated on a steam bath for 1 hr. An acidic fraction of 3.75 g was isolated. Chromatography of this fraction over silica gel gave 1.1 g (18.1%) of **9**: $\lambda_{max}^{Et_2O}$ 241 nm (ε 5400); ($C_{12}H_{19}NO_3$) M^+ 225 (mol wt 225).

Method B. A solution of 6 g of **5** in 26 ml of 1 *N* NaOH was heated at reflux for 30 min. The solution was acidified with HCl and heated to the bp where evolution of CO_2 was noted. Extraction with CH_2Cl_2 afforded 2.6 g (62.5%) of light colored oil which was not separated on tlc (MeOH–NH_4OH, 19:1) from **9** prepared by method A.

4-*n*-Pentyl-L-proline (11). 1-Carbobenzoxy-4-pentylidene-L-proline (**10**) (10 g) was hydrogenated over Pd/Dowex as previously described.[9] Hydrogenolysis over 10% Pd/C afforded 2.65 g of crude product, mp 201–218° dec, which on recrystallization gave 1.96 g (33.4%) of **11**, mp 218–225° dec, $[\alpha]D$ −21° (10 *N* HCl). *Anal.* ($C_{10}H_{19}NO_2$) C, H, N.

***N*-Carbobenzoxy-4-pentylproline (12).** Carbobenzoxy chloride (26 ml) and 415 ml of 4 *N* NaOH were simultaneously added to a solution of 27.5 g of 4-*n*-pentylproline hydrochloride (**7**) in 450 ml of H_2O and 62.5 ml of 4 *N* NaOH over a 30-min period. The usual work-up yielded 37.2 g (94%) of **12** as an oil. A portion was dried under high vacuum. *Anal.* ($C_{18}H_{25}NO_4$) C, H, mol wt. Dicyclohexylamine (0.59 ml) was added to 1.06 g of **12** in 5 ml of Et_2O. The crystals which formed rapidly were collected, washed with Et_2O, and dried. The yield of salt, mp 118–120°, was 1.0 g (62.1%). A portion was recrystallized from MeCN–H_2O. It melted at 116–120°. *Anal.* ($C_{30}H_{48}N_2O_4$) C, H, N.

1'-Carbobenzoxy-1'-demethyl-4'-depropyl-4'(*S*)- and -(*R*)-*n*-pentyllincomycin (13b) and 1'-Carbobenzoxy-1'-demethyl-4'-depropyl-4'(*S*)- and -(*R*)-*n*-pentyl-D-lincomycin (13a). To a solution of 6.16 g of *N*-carbobenzoxy-4-*n*-pentylproline (**12**) and 2.71 ml of Et_3N in 100 ml of MeCN at 0° was added 2.65 ml of isobutyl chloroformate. After stirring for 10 min, a solution of 4.88 g of methyl thiolincosaminide (**15**) in 59 ml of H_2O was added. The reaction mixture was maintained 1 hr at 5° and 4 hr at ambient temperature. The crystals which precipitated were collected, washed with Me_2CO, and dried. The solution was filtered to yield 3.8 g (35.6%) of

13a: mp 206–209°; [α]D +177° (MeOH). *Anal.* ($C_{27}H_{42}N_2O_8S$) C, H, N, S.

The mother liquors from the above filtration were concentrated under vacuum to about 90 ml and again filtered. Crude **13b**, mp 181–184°, [α]D +105° (MeOH), weighing 2.4 g (22.7%) was thus obtained. Recrystallization from MeOH afforded 13b: mp 182–186°; [α]D +116° (MeOH). *Anal.* ($C_{27}H_{42}N_2O_8S$) C, H, N, S.

The mother liquors from above were concentrated and the residue stirred with CH_2Cl_2. Filtration afforded 1.58 g (14.7%) of additional **13b**: mp 182–186°; [α]D +115° (MeOH). Work-up of the CH_2Cl_2 extract gave 0.57 g (5.3%) of poor quality 13b: mp 158–164°; [α]D +96° (MeOH).

1'-Demethyl-4'-depropyl-4'(*S*)- and -(*R*)-*n*-pentyllincomycin Hydrochloride (14b). Hydrogenolysis of 2.4 g of 13b in MeOH over 0.6 g of 10% Pd/C in the usual manner gave 1.61 g (80.4%) of **14b**, mp 210–215° dec, whose ir curve was identical with that of material previously described.[9]

1'-Demethyl-4'-depropyl-4'(*S*)- and -(*R*)-*n*-pentyl-D-lincomycin Hydrochloride (14a). Hydrogenolysis of 3.8 g of **13a** as described above afforded 2.41 g of **14a**: mp 236–238° dec. *Anal.* ($C_{19}H_{37}ClN_2O_6S$) C, H, N, S.

Hydrazinolysis of 14a. A solution of 3 g of **14a** in 25 ml of hydrazine hydrate was heated at reflux for 3 days. The hydrazine was distilled under vacuum; the crystalline residue was triturated with CH_2Cl_2, and filtered to give 19 g of crude **15**. Recrystallization (H_2O) gave 12.9 g of methyl thiolincosaminide **(15)**, mp 224–229°, whose infrared spectrum was identical with that of a known sample. When coupled with 4-*n*-propylhygric acid[5] **15** gave lincomycin hydrochloride, identical in physical and biological properties with known lincomycin.

1'-Demethyl-4'-depropyl-4'-*n*-pentylclindamycin Hydrochloride, U-24,729A (1). Method A. Chlorination of 6.98 g of **14b** as previously described[2b] gave 1.30 g (42.9%) of **1**: mp 207–211° (sp 190°); [α]D 142° (H_2O). *Anal.* ($C_{19}H_{36}Cl_2N_2O_5S$) C, H, N, S, Cl. This material gave identical spectral and antibacterial data when run in parallel with known **1**.[2]

Method B. Hydrogenolysis of 4.2 g of **17b** afforded 1.95 g of hydrochloride **1**: mp 222–226°. This material was identical by infrared, mass spectrum, and microbiological assay with authentic **1**.

4'-Depropyl-4'(*S*)- and -(*R*)-*n*-pentyllincomycin Hydrochloride (16b). Reductive methylation of 100 mg of **14b** afforded **16b** which was identical by tlc ($CHCl_3$–MeOH, 4:1; EtOAc–Me_2CO–H_2O, 8:5:1) with known **16b** showing about the same ratio of isomers. Bioautograph *vs. S. lutea* indicated the same level of activity as that of known **16b**.

4'-Depropyl-4'(*S*)- and -(*R*)-*n*-pentyl-D-lincomycin Hydrochloride (16a). Reductive methylation of 100 mg of **14a** gave material not separated from **16b** by tlc, but essentially inactive *vs. S. lutea*.

1'-Carbobenzoxy-1'-demethyl-4'-depropyl-4'(*R*)- and -(*S*)-*n*-pentylclindamycin (17b). As described above, 13.3 g of *N*-carbobenzoxy-4-*n*-pentylproline **(12)** was condensed with 10.1 g of methyl 7-deoxy-7(*S*)-chlorothiolincosaminide.[2b] The precipitate was collected and dried yielding 3.1 g of **17b**: mp 199–201° dec. Concentration of the mother liquors gave 10 g of a sticky solid which gave an additional 3.8 g of **17b**: mp 189–190° dec. A third crop of 400 mg of **17b**, mp 204–206°, precipitated on standing. Recrystallization of first crop crystals from Me_2CO gave **17b**: mp 208–210° dec; [α]D +132° (MeOH). *Anal.* ($C_{27}H_{41}ClN_2O_8S$) C, H, N.

The D isomer **(17a)** remaining in the mother liquors was not purified but converted to **18a** as described below.

1'-Demethyl-4'-depropyl-4'(*R*)- and -(*S*)-*n*-pentyl-D-clindamycin (18a). Hydrogenolysis of 10.2 g of crude **17a** found in the mother liquors after crystallization of **17b** gave after chromatography over silica gel and conversion to the hydrochloride 1.9 g of **18a**: mp 165–168°. Recrystallization from aqueous Me_2CO raised the mp to 171–173° dec. *Anal.* ($C_{19}H_{36}Cl_2N_2O_5S$) C, H, N, S, Cl.

4'-Depropyl-4'(*R*)- and -(*S*)-*n*-pentylclindamycin (19b). Reductive alkylation of **16b** prepared from 17b gave **19b**, mp 175–179°, which on tlc (EtOAc–Me_2CO–H_2O, 8:5:1; $CHCl_3$–MeOH, 4:1) showed two spots not separated from authentic 4'-depropyl-4'(*R*)- and 4'-depropyl-4'(*S*)-*n*-pentylclindamycin **(1)**[2b] when detected by H_2SO_4 or by bioautograph.

Acid Degradation of 14b. A solution of 2.0 g of **14b** in 50 ml of 1 *N* HCl was heated at reflux for 20 hr. The dark solution was evaporated to dryness. The residue was dissolved in MeOH and again evaporated. This residue was dissolved in 20 ml of MeOH, and 40 ml of anhydrous Et_2O was added. The solution was filtered and concentrated to give 1.30 g of residue. The crude product was dissolved in 25 ml of H_2O and passed over 18 ml of Dowex-50 (H^+). The column was washed with H_2O to neutrality. Elution with 1 *N* NH_4OH followed by evaporation of the fractions showing presence of **11** gave 0.92 g of **11**. Chromatography over silica gel using NH_4OH–MeOH (5:95) for elution gave a major fraction of 477 mg which was recrystallized from water using Darco G-60 to decolorize. There was thus obtained 120 mg of **11**: mp 210–222° dec. This material was recrystallized from H_2O to give **11**: mp 227–230°; [α]D −22° (10 *N* HCl), not separated by tlc from previously prepared material.

Acid Degradation of 18a. Acid degradation of 1.9 g of **18a** as described above led to the isolation of 90 mg of 4-*n*-pentyl-D-proline **20**: mp 203–217°; [α]D +20° (10 *N* HCl).

Chromatographic Separation of 1'-Carbobenzoxy-1'-demethyl-4'-depropyl-4 (*R*)- and -(*S*)-pentyllincomycin. A solution of 26 g of **13b** in 250 ml of a solvent mixture of EtAc–Me_2CO–H_2O (8:5:1) was passed over a column of 4 kg of silica gel. The column was eluted with the same solvent mixture, collecting 200-ml fractions. After evaporation of the solvent, each fraction was assayed as follows. A sample of 5–10 mg was dissolved in 0.5 ml of chloroform, and 0.2 ml of *N*-trifluoroacetylimidazole was added. After standing for a few minutes, 2 μl of this solution was injected into a Hewlett-Packard gas chromatograph Model 402, fitted with a 6-ft column packed with 1% OV-17 maintained at 270° and using a carrier gas flow of 25 cm³/min. Fractions showing greater than 90% of one isomer were combined. The fractions showing mixtures were rechromatographed. There was thus obtained 1.22 g (4.7%) of trans isomer of 90–92% purity and 11.1 g (42.7%) of cis isomer of about 95% purity.

1'-Demethyl-4'-depropyl-4'(*R*)-pentyllincomycin. The 1.22 g of trans isomer was dissolved in 50 ml of MeOH and hydrogenolyzed over 420 mg of 10% Pd/C for 17 hr. As the reaction was not complete, additional catalyst (250 mg) was added and hydrogenolysis continued for 24 hr. The catalyst was removed by filtration, and the solvent evaporated to yield 0.98 g purified but used in the next step.

1'-Demethyl-4'-depropyl-4'(*S*)-pentyllincomycin. In the manner described above, 2.5 g of the cis isomer was hydrogenolyzed in 40 ml of MeOH containing 0.9 g of 10% Pd/C. Evaporation of the solvent after removal of the catalyst by filtration afforded 1.4 g of crystalline product. This material was dissolved in acetone and acidified with HCl to give, after filtration, 1.25 g of HCl salt.

1'-Demethyl-4'-depropyl-4'(*R*)-pentylclindamycin Hydrochloride. A mixture of 0.98 g of trans isomer, 2.36 g of triphenylphosphine, 30 ml of MeCN, and 10 ml of CCl_4 was stirred at ambient temperature for 18 hr. Methanol (2 ml) was added and the solution concentrated *in vacuo*. The residue was partitioned between EtOAc and H_2O. The aqueous solution was made basic and extracted with CH_2Cl_2. The CH_2Cl_2 extract was dried and concentrated. The residue was dissolved in MeOH (25 ml) and heated at reflux for 1 hr. After evaporation of the solvent, the residue was partitioned between EtOAc and 0.1 *N* HCl. The acid fraction was again made alkaline and the product obtained by extraction. Conversion to the HCl salt failed to give crystals so that the crude **6** was recovered and chromatographed over 100 g of silica gel taking 5-ml fractions. After a forerun of about 125 ml, fractions 57–83 were combined to give a residue of 0.265 g of product. This glassy solid was dissolved in 15 ml of Me_2CO, and 3 drops of 6 *N* HCl was added. The crystals which formed on cooling weighed 165 mg and melted at 157–160° dec. Vpc assay indicated about 90% trans isomer. A second crop of crystals, mp 211–221°, weighed 34 mg and assayed for 90% trans isomer. Both fractions moved as expected on tlc. The low mp of the initial fraction was likely due to solvation as its vpc and tlc data were as expected.

1'-Demethyl-4'-depropyl-4'(*S*)-pentylclindamycin Hydrochloride. Chlorination of 1.2 g of the cis isomer in the manner described afforded, after chromatography and conversion to the HCl salt, 0.51 g of crystals, mp 196–198° dec. Vpc assay indicated greater than 88% purity.

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Synthesis of Some Histidine Analogs and Their Effect on the Growth of a Histidine-Requiring Mutant of *Leuconostoc mesenteroides*†

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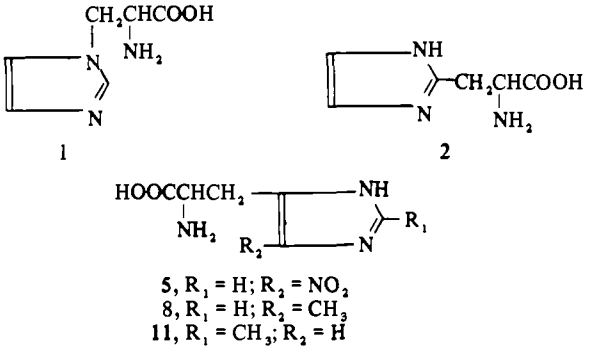
A number of histidine analogs and isomers have been synthesized and examined for biological activity by their ability to support the growth of a histidine-requiring bacterial mutant. None of the compounds investigated were capable of replacing histidine in the system selected even when present in large concentrations. 2-Isohistidine, although incapable of supporting growth of the organism, was shown to stimulate the growth produced by limiting amounts of the natural amino acid. No antimetabolic action of the analogs was detected.

Despite the importance of histidine, relatively little is known of the structural essentials for biological function. Studies in which the alanyl side chain of histidine has been modified, as in α-methylhistidine[1] and α-hydrazinoimidazolepropionic acid,[2] or in which the imidazole ring has been replaced, as in 2-pyridylalanine,[3] 2-thiazolealanine,[3] and 1,2,4-triazolealanine,[4] have been reported. The biological action of histidine where substituents have been added to the imidazole ring or where the position of the alanyl side chain has been modified has received scant attention, although disturbances in the structure of alkaline phosphatase from *Escherichia coli* grown on media containing 2-methylhistidine[5] have been described. The action of histidine decarboxylase on a range of derivatives and analogs of histidine has also been reported.[6,7]

The present study is an attempt to determine the importance of various sites on the imidazole nucleus of histidine in influencing subsequent biological activity. The structural modifications selected were (1) transfer of the alanyl side chain from the 4(5) position to the 1 and 2 positions of the ring and (2) substitution of a methyl or nitro group on the imidazole ring. Biological activity was evaluated by an examination of the growth of a histidine-requiring mutant of *Leuconostoc mesenteroides* in a histidine assay medium[8] supplemented with either the analog alone or with the analog and limiting amounts of L-histidine.

Chemistry. The synthesis of α-amino-β-(1-imidazolyl)-propionic acid (1-isohistidine) by Michael condensation of methyl 2-acetamidoacrylate with imidazole and hydrolysis of the product has been briefly described[9] but the free amino acid was not isolated. An essentially identical method was initially employed in the present study and extended to obtain both the hydrochloride and the free base in crystalline form. A more convenient synthesis was subsequently developed by condensing diethyl α-acetamido-α-dimethylaminomethylmalonate methiodide[10] with sodium imidazole in liquid NH_3. Attempts to use EtOH as solvent were not successful though some 1-isohistidine was detected by tlc.



The remaining analogs in the present series were prepared by a standard route in which the corresponding hydroxymethyl derivative was converted to the chloromethyl compound, condensed with sodium diethyl acetamidomalonate, and hydrolyzed. 4-Nitro-5-chloromethylimidazole for the preparation of 4-nitrohistidine was prepared by direct nitration of 5-chloromethylimidazole hydrochloride and isolated by precipitation on dilution with ice water. The synthesis of 2-methylhistidine[6,11] by the general route required 2-methyl-4-hydroxymethylimidazole prepared in the present investigation by LAH reduction of ester obtained from 2-methylimidazole-4,5-dicarboxylic acid.[12] This approach minimized the risk of contamination of the final product with traces of histidine as is possible in alternative methods.[11]

The amino acids were used for biological study as racemates though some investigation of their resolution was undertaken. In exploratory experiments the standard enzymatic procedure utilizing the *N*-acetyl derivative[13] was extremely slow and in some cases the enzyme appeared to be subject to end-product inhibition. It did not appear to be practicable to effect resolution by this route.

Metabolic Activity. The metabolic activity of the com-

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