Side Chain Modifications in Lankacidin Group Antibiotics

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Novel N-acyl analogs of lankacidin may be prepared from 3-isocyanatolankone diformate [7,13-bis(formyloxy)-2-isocyanyano-1,4,10,19-tetramethyl-16-oxabicyclo[13.2.2]nonadeca-3,5,9,11-tetraene-17,18-dione]. Of seven such analogs evaluated in vitro only homolankacidin diformate showed significant activity. However, in a cell-free system two of the inactive analogs inhibited polypeptide synthesis as well as did lankacidin itself or erythromycin. Antibacterial activity, therefore, is a function of the ability of a congener to penetrate the bacterial cell membrane in addition to its intrinsic activity. Similarly, lankacidinol is as potent as lankacidin or erythromycin as an inhibitor of bacterial polypeptide synthesis in a cell-free system. This intrinsic activity is expressed as potent antibacterial activity against growing gram-positive cultures in O(2')-acyl derivatives with the proper lipophilicity.

An ideal antibiotic for treating common respiratory tract infections would be active against pathogenic strains of the following species, including those resistant to commercial agents such as ampicillin and erythromycin: Staphylococcus aureus, Streptococcus pyogenes, and Streptococcus pneumoniae (gram positive) and Haemophilus influenzae (gram negative). In addition, oral activity is required. Lankacidin, a 17-membered ring macroclide that has been known since 1960 (5, 7, 9, 11, 16, 19), possesses these properties to various degrees, and although not of commercial caliber itself, it affords an opportunity for semisynthesis with the aim of discovering a derivative worthy of clinical development. Earlier work with lankacidin involved its degradation, oxidation, and reduction products (6, 8), all showing greatly reduced activity. Some esters have been described (10), but at best these represent only pro-drug forms of the parent. Chemical progress in this area is difficult owing to the lability of lankacidin group antibiotics to even mildly acidic or basic conditions.

Lankacidinol (3), a reduction product of lankacidin, was first reported in 1969 (7) and is greatly inferior to lankacidin in both in vitro and in vivo evaluations. However, as will be shown in the present work, both lankacidin and lankacidinol are at least equal to erythromycin as inhibitors of polypeptide synthesis in a cell-free system. Hence, the therapeutic potential of 17-membered ring macroclides has not yet been realized.

Although we are not now able to report the discovery of a derivative for clinical study, the present work discloses some chemical procedures for modifying the side chain of lankacidin in an original way and describes derivatives of lankacidinol which are highly potent in vitro tests.

MATERIALS AND METHODS

General. All thin-layer and column chromatographies were performed with silica gel as the absorbent. Unless otherwise indicated methanol was the solvent used in UV absorption studies, chloroform was used for infrared (IR) studies, and chloroform-d for both 13C and 1H nuclear magnetic resonance (NMR) studies. Chemical shifts, δ (both 13C and 1H), are reported as parts per million downfield from tetramethylsilane internal standard. Melting points (mp) are uncorrected.

Criteria for purity. For lankacidin group antibiotics, thin-layer chromatography (TLC) serves well because of the sensitivity of the systems used; e.g., the syn- and anti-isomers of lankacidin oxime diformate are readily separable, as are the 2' epimers of lankacidinol. However, among lankacidinol derivatives, epimeric mixtures of O(2') acetates appear as single spots. Even though some of these esters are crystalline, sharp-melting materials, epimeric mixtures are assumed in all cases. A further criterion used here is the UV spectrum. The bis(1,3-butadiene)-like chromophore of lankacidin group compounds result in UV maxima at 223 to 229 nm with extinction coefficients of log ε 4.55 to 4.70. Degradation products usually result in strong new absorption peaks at longer wavelengths.

Therefore, samples not giving UV absorption intensities of this degree or showing absorption at longer wavelengths are easily recognized as being impure. The compounds described below were purified to single-spot materials (TLC) analysis with satisfactory UV spectra. In no case did impurities interfere with the assignment of the chemical structure by spectrographic methods.

Structure determination. All new compounds have been assigned structures consistent with their IR, UV, and 1H and 13C NMR spectra. Reference 1H and 13C NMR spectra are available in the literature (6, 8, 18). Only those features in the spectra most critical to the structural assignments of new compounds will be noted below.

(i) Lankacidin (1). Lankacidin (1) was supplied by W. D. Celmer and co-workers of the Antibiotic Screening Department, Central Research, Pfizer Inc., Groton, Conn.: mp 209 to 210°C dec. (ethyl acetate [EtOAc]) (6) 201 to 203°C dec.; IR (KBr) 3401 (NH), 1767, (CO-O), 1739 (C=O-C), 1724 (NCOCO), 1689 (NCOCO) cm⁻¹; UV max 227 nm (log ε 4.69); 1H NMR (DMSO-d₆) δ 2.39 (s, COOCO₂H), 8.04 (d, NHCO); 13C NMR (DMSO-d₆) δ 24.4 (q, COOCO₂H), 159.4 (s, NHCO), 170.0 (s, CO-O), 196.2 (s, NCOCO), 210.8 (s, C- CO-C). (ii) Lankacidin diformate (2). A solution of 4.59 g (0.01 mol) of lankacidin and 50 ml of pyridine was cooled to 0 to 5°C. With magnetic stirring, 10 ml of aceticformic anhydride was added cautiously. The reaction solution was allowed to warm to room temperature. After 1 h, the solution was poured into 600 ml of water to produce a colorless solid which was filtered and washed with water. The solid was taken up in 100 ml of CH₂Cl₂ and washed successively with 50 ml of water, 100 ml of water at pH 4 (adjusted with 1 N HCl), 50 ml of water, and 50 ml of saturated aqueous NaCl.
rapidly heated under reflux with 3 ml of ethanol for 1 h. The residue was stirred with 100 ml of Et₂O and evaporated. The residue was dried over anhydrous Na₂SO₄, filtered, and evaporated. The residue was stirred with 100 ml of Et₂O for 1 h. Insoluble material was filtered, and the ether was evaporated to furnish 4: yield of 460 mg (51%); IR 2247 (NCO), 1755, 1722 cm⁻¹; UV max 228 nm (log ε 4.59); ¹H NMR (lack of COOCO); ¹³C NMR (lack of NHCO=CH₂).

(v) 3-(3,5-Di-tert-butyl-4-hydroxybenzyl)oxy carbonylaminolankone diformate (5). A solution of 1.03 g (2.2 mol) of 4, 559 mg (2.37 mol) of 3,5-di-tert-butyl-4-hydroxybenzyl alcohol, 32 mg of iron(III) 2,4-pentandionate, and 12 ml of CH₂Cl₂ was allowed to stand at room temperature for 2 days. The reaction solution was directly chromatographed (EtOAc-hexane [1:1]) to furnish 5: yield of 722 mg (47%); IR 3610 (OH), 3425 (NH), 1754, 1727, 1715 (NHCO₂CH₂) cm⁻¹; UV max (unstable in MeOH); ¹H NMR δ 1.44, 2.45, 2.46, 3.99, 4.99 (s, CH₂CO₂CH₂); 7.16 (s, ArH).

(vi) 3-(Methoxycarbonylamino)lankone diformate (7, R = OCH₃). The isocyanate was prepared from 1.0 g of 3 in the manner described above and was dissolved in 45 ml of freshly prepared ethanol-free CHCl₃. After treatment with 0.2 ml of MeOH and 5 drops of dibutyltin dilaurate and standing at room temperature for 24 h, the solution was evaporated under reduced pressure. The residue was chromatographed (EtOAcl-hexane [1:1]) to furnish the title compound: yield of 306 mg (32% over two steps); IR 3472 (NH), 1773, 1751, 1738 (NHCO₂CH₂) cm⁻¹; UV max 227 nm (log ε 4.64); ¹H NMR δ 3.65, 3.66 (s, OCH₃).

(vii) 3-Formamidolankone diformate (7, R = H). A solution of the isocyanate (690 mg, 1.46 mmol) in 25 ml of ethanol-free CHCl₃ was treated with 0.1 ml of formic acid and 0.3 ml of acetoformic anhydride and allowed to stand at room temperature for 5 h. The solution was then washed successively with water, saturated aqueous NaHCO₃, water, and saturated aqueous NaCl. The organic phase was dried over anhydrous Na₂SO₄, filtered, and evaporated to furnish a yellow solid which was chromatographed (EtOAcl-hexane [1:1]). A colorless solid (7, R = H) was obtained: yield of 230 mg (33%); IR 3472 (NH), 1779, 1755-1745, 1712 (NHCOH) cm⁻¹; UV max 228 nm (log ε 4.58); ¹H NMR δ 7.98, 8.02, 8.18 (s, HCON).

(viii) 3-(1,1-Dioxobutylamino)lankone diformate (7, R = COCH₃H₃; homolankacidin diformate). In a manner similar to the preparation described above, the title com-

**TABLE 1.** MICs of lankacidin and its diacate and diacetate in comparison with MICs of erythromycin and ampicillin against strains of *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae,* and *Haemophilus influenzae*

| Strain                        | MIC (µg/ml)²    |
|-------------------------------|----------------|
|                               | Lankacidin     | Diformate | Diacetate | Erythromycin | Ampicillin |
| *Staphylococcus aureus*       | 1.56           | 6.25      | 50        | 0.10         | ≤0.10      |
| 01A005                        | 0.78           | 3.12      | 25        | 0.20         | ≤0.10      |
| 01A002                        | 0.78           | 1.56      | 25        | >25          | 1.56       |
| 01A110                        | 0.78           | 0.78      | 12.5      | >25          | 3.12       |
| 01A110                        | 0.78           | 3.12      | 25        | 6.25         | 25         |
| *Streptococcus pyogenes*²     |                |           |           |              |            |
| 02C040                        | 0.20           | 1.56      | 12.5      | >50          | ≤0.10      |
| 02C203                        | 0.39           | 0.78      | 12.5      | 0.05         | ≤0.025     |
| *Streptococcus pneumoniae*²   | 0.39           | ND        | ND        | 6.25         | 0.78       |
| 021017                        | 0.39           | 12.5      | ND        | 6.25         | 0.78       |
| *Haemophilus influenzae*      |                |           |           |              |            |
| 54A010                        | 1.56           | ND        | ND        | 3.12         | 50         |
| 54A051                        | 0.39           | 12.5      | ND        | 6.25         | 0.78       |

¹ ND, Not determined.
² A high level of inoculum was used in this study; overnight cultures were diluted only 10-fold.
pound was obtained from 613 mg (1.3 mmol) of 4 and 119 mg (1.2 mmol) of 2-oxobutyric acid: yield of 50 mg (8%); IR 3450 (NH), 1780, 1745, 1708 (NHCO) cm⁻¹; UV max 228 nm (log ε 4.64); ¹H NMR δ 1.08 (t, COOCH₂CH₃), 2.90 (q, COOCH₂CH₃).

(ix) 3-Acetamidolankone diformate (7, R = CH₃). The carbamate 5 was prepared in situ from a solution of 1.03 g (2.2 mmol) of the isocyanate 4, 568 mg (2.4 mmol) of 3,5-di-tert-butyl-4-hydroxybenzyl alcohol, and 0.1 ml of dibutyltin dilaurate in 40 ml of CH₂Cl₂. After 3 days at room temperature when the isocyanate was no longer evident (TLC or IR), the solution was treated successively with 0.2 ml of acetyl chloride, 0.6 ml of AC₂O, and 0.8 ml of diisopropylethylamine. After 2 days at room temperature, the volatile components were evaporated under reduced pressure, and the residue was chromatographed (EtOAc) to furnish a light-yellow solid (7, R = CH₃): yield of 350 mg (33%). Crystallization from Et₂O afforded pure material: mp 163 to 164°C; IR 3484 (NH), 1770, 1733, 1695 (NHCO) cm⁻¹; UV max 229 nm (log ε 4.60); ¹H NMR δ 2.00 (s, NHCOCH₃).

(x) 3-Propionamidolankone diformate (7, R = CH₃; 2'-deoxolankacidin diformate). In a manner similar to that described above, the title compound was obtained from a solution of 2.47 g (5.2 mmol) of 4, propionyl chloride, and propionic anhydride: yield of 756 mg (35%); IR 3484 (NH), 1770, 1739-1730, 1689 (NHCO) cm⁻¹; UV max 228 nm (log ε 4.68); ¹H NMR δ 1.14 (t, COCH₂CH₃), 2.22 (q, COCH₂CH₃).

(xi) 3-Acetamidolankone (8, R = CH₃). A solution of 500 mg (1.0 mmol) of 7, R = CH₃, 5 ml of tetrahydrofuran (THF), 5 ml of MeOH, 2.5 ml of water, and 1.5 ml of 1 N K₂CO₃ was allowed to stand at room temperature for 45 min. A few drops of 6 N HCl were added to bring the solution to...
pH 4. The solution was then evaporated under reduced pressure to produce an amber gum. Water was added, and the organic matter was extracted with EtOAc. After further aqueous washes, drying, and filtering, the organic phase was evaporated, and the residue was chromatographed (EtOAc-MeOH [50:1]). The title compound was obtained: yield of 35 mg (8%); IR (KBr) 3415 (NH), 1757, 1724, 1672 (NHCO) cm⁻¹; UV max 227 nm (log ε 4.66); ¹H NMR (DMSO-δ6) δ 1.88 (s, 3H, NHCOCH₃) and 3.10 (br, 2H, NHCOCH₂CH₃).

(xii) 3-Propionamidolankone (8, R = CH₃CH₂; 2-deoxolankacidin). In a manner similar to that described above, the title compound was obtained. M. 560 mg of 7, R = CH₃CH₂; yield of 114 mg (23%); IR 3410 (NH), 1754, 1712, 1672 (NHCO) cm⁻¹; UV max 227 nm (log ε 4.66); ¹H NMR (DMSO-δ6) δ 1.00 (t, COCH₂CH₃), 2.3 (NCOCH₂H₂ hidden among other absorptions).

(xiii) Lankacidinoldiformate (9). A magnetically stirred solution of 1.0 g (1.9 mmol) of 2, 30 ml of MeOH, and 30 ml of THF was cooled to 5°C and treated with 18 mg (0.47 mmol) of NaBH₄. After 30 min, the solution was evacuated under reduced pressure. The residue was taken up in CH₂Cl₂ and washed with water and saturated aqueous NaCl. The organic phase was dried over anhydrous Na₂SO₄, filtered, and evaporated to afford 0.6 g of pale-yellow foam. This material was chromatographed (eluant, EtOAc) to furnish the less polar epimer of lankacidinoldiformate (9A; 140 mg, 14% yield) and the more polar epimer (9B; 204 mg, 20% yield). There was also an intermediate fraction containing both epimers (10%).

(xiv) Lankacidinol (10). In a manner similar to that for preparing 9, 1 was reduced by NaBH₄ to afford 10 as a mixture of less polar and more polar epimers. To visualize these epimers on TLC it was necessary to elute the plate three times with EtOAc. To obtain the pure epimers it was expedient to hydrolyze the pure epimeric diformate derivatives 9A and 9B.

(xv) 10A. A solution of 180 mg of 9A, 5 ml of MeOH, 5 ml of THF, 1 ml of water, and 15 drops of 1 M K₂CO₃ was allowed to stand at room temperature for 15 min. The solution was acidified with 3 drops of 6 N HCl and evaporated under reduced pressure to furnish solids in an aqueous suspension. The mixture was filtered, washed thoroughly with water, and allowed to dry. The pure less polar epimer of lankacidinol (10A) was obtained: yield of 110 mg (69%); mp 184 to 185°C (mp 178 to 179°C [1]).

(xvi) 10B. In a similar manner to that described above there was obtained from 126 mg of 9B 38 mg (34%) of the more polar epimer of lankacidinol (10B); mp 139 to 141°C (mp 169 to 171°C [1]).

(xvii) O(2')-acyl lankacidinol diformates (11) from acid anhydrides. A solution of 2.0 g of 9 in 20 ml of pyridine was treated with 2 molar equivalents of the acid anhydride and

FIG. 2. Preparation of N-acyl derivatives of 3-aminolankone diformate (6) via 3-[(3,5-di-tert-butyl-4-hydroxybenzyl)oxycarbonylamino]lankone diformate (5).
allowed to stand at room temperature for 24 h. The solution was poured into 100 ml of water, and the organic matter was extracted from the aqueous suspension with CH₂Cl₂. The organic phase was washed successively with 2 N HCl, water, and saturated aqueous NaCl. It was then dried over anhydrous Na₂SO₄, filtered and evaporated to nearly pure 11. Chromatography (EtOAc-hexane) afforded an epimeric mixture of 11 (yield of 50 to 65%).

(xviii) O(2')-acyl lankacidin diformates (11) from acid chlorides. A solution of 1.5 g of 9 in 15 ml of pyridine and 15 ml of CH₂Cl₂ was cooled to 5°C and treated with 2 to 4 ml of the acid chloride. After 2 to 3 h, the reaction solution was poured into 100 ml of water, 2 N HCl was added to bring the mixture to pH 4, and the mixture was then extracted with CH₂Cl₂. After washing, drying, filtering, evaporating, and column chromatography, 11 as an epimeric mixture was obtained (yield of ca. 50%).

(xix) O(2')-acyl lankacidinols (12). A solution of 1.0 g of 11, 10 ml of THF, 10 ml of MeOH, 5 ml of water, and 2 ml of 1 N K₂CO₃ was allowed to stand at room temperature for 20 min. The solution changed from pH 10.8 to 9.8 during this time, was adjusted to pH 4.5 by the addition of 6 N HCl, and was concentrated under reduced pressure to furnish an aqueous suspension of organic solids. More water was added, and the mixture was extracted with CH₂Cl₂. The organic phase was dried over anhydrous Na₂SO₄, filtered, and evaporated. The residue was chromatographed to afford an epimeric mixture of 12 (yield of 42 to 82%).

MICs were determined by standard agar plate dilution techniques (15).

Studies on the ability of macrolide antibiotics to inhibit cell-free MS2 viral mRNA-directed polypeptide synthesis were conducted as described by English and co-workers (2). Cell extract (S 30) was prepared from Escherichia coli MRE 600 RNase T as described elsewhere (14). A 1H-amino acid mixture was used as a source of radioactivity.

RESULTS AND DISCUSSION

In general, there are severe limitations to chemically modifying lankacidin because of its sensitivity to even mildly acidic or basic conditions. Outside of the range pH 3 to 12, the compound decomposes rapidly; at pH 4 or 11 it can be manipulated at room temperature for up to 20 min without significant degradation; within the range pH 5 to 8 the compound is stable for many hours at room temperature.

Formyl satisfactorily protects the hydroxy groups. Not only is 2 easily prepared in good yield, but at later stages in the semisynthesis sequence the formyl groups can be removed under mild chemical conditions. In contrast, acetate esters, although easily formed, cannot be hydrolyzed chemically under conditions compatible with the stability of the lankacidin system. The earlier literature reports that such esters can be hydrolyzed by enzymatic methods (4, 13), but that is much less convenient. The diformate esters of lankacidin group compounds may also be tested in vitro directly with only the loss of one or two levels in the dilution sequence; diacetate esters are generally much less active (Table 1 and reference 10).

One of the more attractive approaches to side chain modification would be to remove the pyruvoyl group to produce the free amine, 3-amino-lankonane diformate, (Fig. 1, number 6). Such an intermediate could then be acylated with a variety of agents to produce a broad range of novel derivatives. Our initial attempts to remove pyruvoyl by classical methods demonstrated the extreme resistance of the amide group to electrophilic agents. As a definitive experiment, lankacidin diacetate in CH₂Cl₂ was treated with triethylxonium tetrafluoroborate. The solution changed from colorless to light purple (12 min) to dark purple (32 min). Samples were taken at 2, 12, and 32 min and evaluated by IR spectroscopy. Although most of the absorption bands changed during this obvious decomposition, the bands at 3390 and 1680 cm⁻¹, those associated with the amide function, remained unaltered in position and intensity. Clearly, imidate ester formation did not take place.

These results discouraged further attempts to directly break the N-CO bond and caused us to consider an indirect method. Swiss workers (17) reported removing the side chain of nocardicin A by means of a second order Beckmann rearrangement. Although the conditions employed there are much too severe for lankacidin, we followed a similar course with success. Figure 1 outlines the methods used to prepare 4. This isocyanate is highly hindered and is relatively sluggish in reactions typical of members of its class. Amines reacted with the isocyanate to produce ureas, but these products proved to be unstable, particularly in aqueous or

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**TABLE 2. MICs of lankacidin derivatives**

| Structure | R       | MICs (µg/ml) against *Staphylococcus aureus* |
|-----------|---------|--------------------------------------------|
|           |         | 01A005 (S)       | 01A400 (R)²  |
| 1         |         | 1.56            | 0.78         |
| 2         |         | 6.25            | 3.12         |
| 3         |         | 100             | 50           |
| 4         |         | >50             | >50          |
| 5         | OCH₃    | 100             | 50           |
| 6         | H       | >50             | 12.5         |
| 7         | COCH₂CH₃| 6.25            | 12.5         |
| 8         | CH₃     | >200            | >200         |
| 9         | CH₂CH₃  | >50             | >50          |
| 10        | CH₂CH₃  | >50             | >50          |
| Erythromycin |       | 0.2             | 6.25         |
| Ampicillin |         | ≤0.1            | 25           |

a. S, Susceptible to erythromycin; R, resistant to erythromycin.

b. 01A400 is an inducible erythromycin-resistant strain; it also harbors a β-lactamase and is tetracycline resistant.

**TABLE 3. Concentration of lankacidin derivatives giving 90% inhibition of viral mRNA-directed polypeptide synthesis in a cell-free system**

| Structure | R       | Compound                              | Conc giving 90% inhibition (µmol) |
|-----------|---------|---------------------------------------|----------------------------------|
| 1         |         | Lankacidin                            | 0.35                             |
| 2         |         | Lankacidin O(8,14) diacetate           | >10                              |
| 3         |         | Lankacidin O(14) diacetate             | >10                              |
| 4         |         | Lankacidin O(8) diacetate              | 0.4                              |
| 5         |         | 3-Isocyanatolankone diformate          | >10                              |
| 6         |         | Homolankacidin diformate               | >10                              |
| 7         |         | Lankacidinol                         | 0.10                             |
| 8         |         | 3-Acetamidolankone diformate           | 0.4                              |
| 9         |         | 2-Deoxolankacidin                    | 0.8                              |
| 10        |         | Lankacidinol mixed epimers           | 0.5                              |
| 10A       |         | Lankacidinol (less polar epimer)      | 0.5                              |
| 10B       |         | Lankacidinol (more polar epimer)      | 0.5                              |
| 12 (CH₃)   |         | Lankacidinol, O(2')-octanoyl     | 0.7                              |
| 12        | (CH₃)   | Erythromycin                          | 0.9                              |

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methanolic solution. Alcohols reacted more slowly to produce carbamates which are more stable than the ureas but nevertheless also decomposed in polar solvents.

It has long been known that isocyanates react directly with carboxylic acids to produce amides. Babusiaux and co-workers (1) published a definitive study of this reaction, and their ideas are incorporated in the latter half of Fig. 1 to illustrate how two compounds in the present work were prepared. Formic acid and the isocyanate 4 reacted within 5 h to produce the formamide 7, \( R = H \) in a 33% yield.

**TABLE 4. MICs of O(2')-acyl lankacidinol O, O(8,14) diformates (11) against Streptococcus pyogenes and Staphylococcus aureus strains**

| Structure | R         | Streptococcus 02C203 | Staphylococcus aureus* |
|-----------|-----------|----------------------|------------------------|
|           |           | 01A005 (S) | 01A052 (S) | 01A400 (R) |
| 9         |           | 3.12       | 50         | 50         | 50         |
| 11        | \((CH_2)_3-H\) | 1.56       | 50         | 25         | 25         |
| 11        | \((CH_3)_3-H\) | 1.56       | 25         | 12.5       | 25         |
| 11        | \((CH_2)_3-H\) | 0.20       | 3.12       | 3.12       | 3.12       |
| 11        | \((CH_2)_5-H\) | 0.20       | 6.25       | 3.12       | 12.5       |
| 11        | \((CH_2)_6-H\) | 0.10       | 6.25       | 1.56       | 12.5       |
| 11        | \((CH_2)_7-H\) | >50        | >50        | >50        | >50        |
| 2         |           | 0.78       | 6.25       | 3.12       | 12.5       |

Erythromycin

Ampicillin

| MICs against | 01A005 (S) | 01A052 (S) | 01A400 (R) |
|--------------|------------|------------|------------|
| Erythromycin | ≤0.025     | ≤0.10      | ≤0.10      |
| Ampicillin   | ≤0.025     | ≤0.20      | ≤0.025     |

* All isolates are erythromycin susceptible except 01A400, which is an inducible erythromycin-resistant strain that also harbors a β-lactamase and is tetracycline resistant.

* S, Susceptible to erythromycin; R, resistant to erythromycin.
reaction with 2-oxobutyric acid to produce 7. \( R = \text{COCH}_2\text{CH}_3 \) in an 8% yield took 3 days. Under these conditions, acetic and propionic acids worked even more poorly.

To improve the acylation results a novel scheme was used to produce 3-aminolankide diformate (6) in situ under mild conditions. Advantage was taken of the fact that carbamates can be formed cleanly and relatively quickly when iron or tin salts are used as catalysts. Further, a method was needed to release the amine from the carbamate under mild conditions; the one eventually used (Fig. 2) was modelled after the work of Kemp and Hoyng (12). Starting with the isocyanate, the amides are prepared in a three-step, one-pot reaction. The 3-acyaminolankide diformates (7) were hydrolyzed in aqueous THF-MeOH starting at pH 10.8 and dropping naturally to pH 9.8 over a 45-min period to prepare 3-acyaminolankides. The low yields were a result of sacrificing some product to ensure complete removal of the formyl groups.

Table 2 summarizes some in vitro test results for the new compounds prepared. MICs against \textit{Staphylococcus aureus} 01A005 (an erythromycin-susceptible organism) and \textit{Staphylococcus aureus} 01A400 (an erythromycin-resistant organism) are typical for a wide variety of gram-positive pathogenic isolates. With the exception of 7. \( R = \text{COCH}_2\text{CH}_3 \) the MIC data suggest that structural modifications of lankacidin greatly reduce potency. This may be due to the inability of the derivatives to penetrate the bacterial cell membrane rather than the loss of intrinsic activity per se. Studies of antibacterial action in a cell-free system should shed some light on this question. 8, \( R = \text{CH}_3 \) and 8, \( R = \text{CH}_2\text{CH}_3 \) inhibited viral mRNA-directed polypeptide synthesis in a cell-free system as well as did lankacidin itself or erythromycin (Table 3). The data also show that analogs with the C-14 hydroxy group protected do not express intrinsic activity. Apparently, during MIC incubations, the formyl group is hydrolyzed much better than is acetyl.

This observation of potent intrinsic activity in lankacidin derivatives that otherwise would be considered inactive raised the question as to whether lankacidinol was truly inactive at the site of action (the ribosome) or whether lankacidinol was merely another case of an intrinsically active antibiotic not being able to penetrate the bacterial cell membrane. Experiments demonstrate that lankacidinol is a highly potent inhibitor of cell-free polypeptide synthesis, and it makes no difference whether the more polar or less polar epimer is evaluated (Table 3). Thus, a new possibility for a clinically effective member of the lankacidin group antibiotics emerged. Because lankacidinol is more hydrophilic than its parent, lankacidin, it seemed probable that derivatives of lankacidinol with greater lipophilic character would exhibit the desired in vitro activity, that is, show significant MICs. Simple esters of lankacidinol should meet this requirement. However, esterification of the C-14 hydroxyl group results in the loss of both in vitro and intrinsic activity among lankacidin derivatives (10 and this work). Esterification of the C-8 hydroxyl group is compatible with activity (10 and this work), but selective esterification at C-8 is limited in scope and requires enzymatic methods (4, 13) or tedious chromatographic separation of isomers from partially esterified compounds.

Until now a convenient method to prepare esters at the 2' position selectively has not been available. A straightforward procedure is outlined in Fig. 3. Following this approach, we prepared \( O'2' \)-acyl derivatives of 11 and 12 and evaluated them in vitro as antibacterial agents (Tables 4 and 5). As in the lankacidin series, the diesters could be tested directly in vitro with the expectation of detecting activity, although with some loss of potency. It is evident from the data in Table 5 that optimum potency was achieved with 12 [\( R = (\text{CH}_2)_7\text{H} \)]. This derivative was not only equal to lankacidin in activity against \textit{Staphylococcus aureus} strains but was dramatically superior in potency against \textit{Streptococcus pyogenes}.

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**TABLE 5. MICs of O(2')-acyl lankacidinols (12) against \textit{Streptococcus pyogenes} and \textit{Staphylococcus aureus} strains**

| Structure | R          | \textit{MICs} against (\( \mu \text{g/ml} \)) | \textit{Streptococcus pyogenes} | \textit{Staphylococcus aureus} |
|-----------|------------|-------------------------------------------|--------------------------------|-------------------------------|
|           |            | \textit{MICs} against (\( \mu \text{g/ml} \)) | 02C203                         | 01A005 (S)                    | 01A052 (S)                    | 01A400 (R)                    |
| 10        |            | 3.12 | >50 | >50 | >50 |
| 12        | (\( \text{CH}_2 \))_3\text{H} | 0.78 | 25 | 6.25 | 25 |
| 12        | (\( \text{CH}_2 \))_4\text{H} | 0.78 | 12.5 | 3.12 | 12.5 |
| 12        | (\( \text{CH}_2 \))_5\text{H} | 0.10 | 3.12 | 3.12 | 1.56 |
| 12        | (\( \text{CH}_2 \))_6\text{H} | 0.39 | 50 | 1.56 | 1.56 |
| 12        | (\( \text{CH}_2 \))_7\text{H} | 0.006 | 1.56 | 0.20 | 0.39 |
| 12        | (\( \text{CH}_2 \))_8\text{H} | 0.025 | 3.12 | 0.39 | 1.56 |
| 12        | (\( \text{CH}_2 \))_9\text{H} | 0.05 | 3.12 | 0.78 | 0.78 |
| 12        | (\( \text{CH}_2 \))_{10}\text{H} | 0.39 | 6.25 | 1.56 | 1.56 |
| 12        | (\( \text{CH}_2 \))_{11}\text{H} | 0.20 | >50 | >50 | >50 |
| 1         |            | 0.39 | 1.56 | 0.78 | 0.78 |
| Erythromycin |    | \( \leq 0.025 \) | 0.20 | 0.39 | 6.25 |
| Ampicillin    |    | \( \leq 0.025 \) | \( \leq 0.10 \) | 0.39 | 25 |

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a All isolates are erythromycin susceptible except 01A400, which is an inducible erythromycin-resistant strain that also harbors a β-lactamase and is tetracycline resistant.

\( ^{a} \) S, Susceptible to erythromycin; R, resistant to erythromycin.
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