4-1982

Analogues of Steffimycinone

David Wayne Elrod

ANALOGUES OF STEFFIMYCINONE

by

David Wayne Elrod

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Master of Arts
Department of Chemistry

Western Michigan University
Kalamazoo, Michigan
April 1982

ANALOGUES OF STEFFIMYCINONE

David Wayne Elrod, M.A.

Western Michigan University, 1982

Several analogues of Steffimycinone - an anthracycline antitumor antibiotic - have been prepared. Modifications were made at C-7 and C-10 of the non-aromatic A ring portion of the anthracycline nucleus. Compounds with nitrogen substituents were prepared. The synthesis of these compounds is discussed as well as their antitumor activity. The unsuccessful approaches to several other analogues are also discussed.

ACKNOWLEDGMENTS

I would like to express my appreciation to Dr. Paul F. Wiley of The Upjohn Company for his inspiration, guidance and encouragement. Without his patience and counsel this work would not have been possible. I am indebted to my advisor, Dr. Robert E. Harmon, for his guidance and patience throughout this project. I would also like to thank The Upjohn Company for providing educational assistance.

David Wayne Elrod

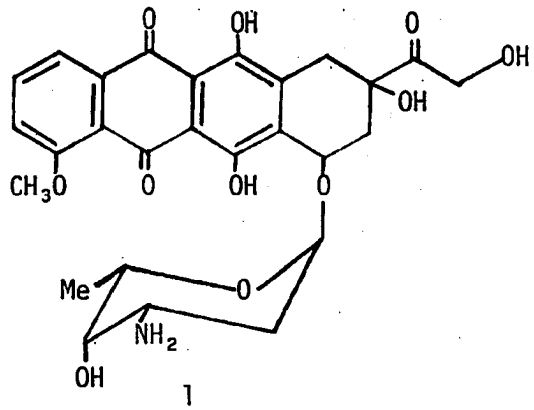
TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The anthracycline antibiotics are among the most important and most widely used drugs available for the treatment of cancer. The most prominent member of the anthracycline antibiotic family is doxorubicin (adriamycin) (1).



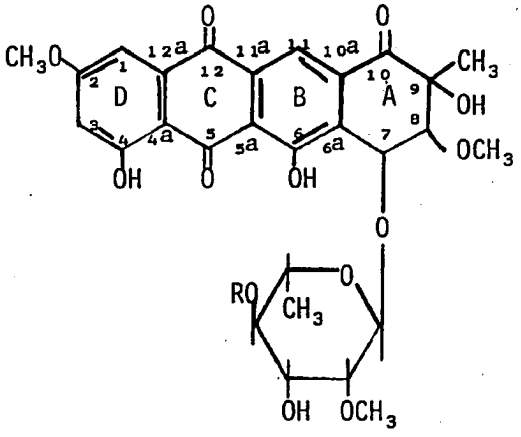
1

Doxorubicin has excellent activity against a wide variety of solid tumors and leukemias. In fact, doxorubicin has the broadest range of clinical usefulness of all the anticancer drugs currently in clinical use.[1] Its main limitation in cancer chemotherapy is the cumulative dose dependent cardiotoxicity which occurs when the total dose exceeds 550 mg/m². This cardiotoxicity leads to congestive heart failure in 30% of patients who exceed this dose[1] and is fatal in most of these cases. For this reason drug treatment must be suspended, even though the cancer is still responding to the doxorubicin. A considerable amount of effort has been devoted to pre-

paring other anthracyclines which would retain the anticancer activity of doxorubicin but not be as cardiotoxic. Several compounds have been prepared and are under evaluation by the National Cancer Institute. Included among these are AD-32, rubidazone, aclacinomycin A and 7-(R)-O-methylnogarol.[2] The development of improved anthracyclines by chemical modification has provided the impetus for this work.

BACKGROUND AND PURPOSE

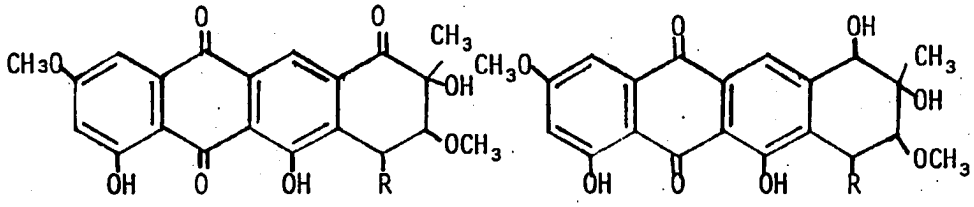
Steffimycin (2) and steffimycin B (3) are anthracycline antibiotics which are only very modestly active as antitumor agents. However, the steffimycins are members of a subgroup of three anthracyclines whose structures differ markedly from the other anthracyclines.[3]



2 R=H
3 R=CH_3

The steffimycins differ from doxorubicin by having a 2,4,6 substitution pattern on the anthracycline nucleus, compared to a 4,6,11 substitution for doxorubicin. Other differences consist of a methyl at C-9 instead of an oxygenated ethyl side chain, a methoxy group at C-8, a keto group at C-10 and a neutral sugar instead of an amino sugar at C-7. In view of these differences with other anthracyclines the steffimycins were thought to be worthwhile candidates for modification. Since the steffimycins showed such low antitumor activity the aglycone, steffimycinone (4) was chosen as the starting point.

Steffimycinone (4) has been prepared by acidic methanolysis of either steffimycin or steffimycin B.[4]



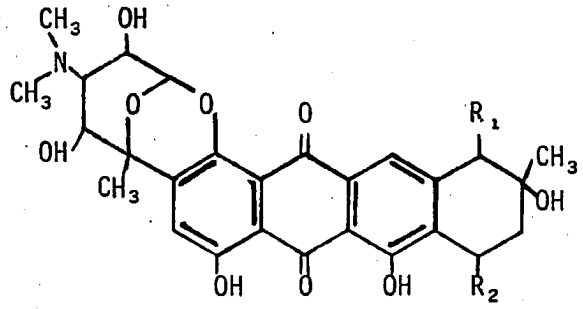
4	R=OH	6	R=OH
5	R=H	7	R=H

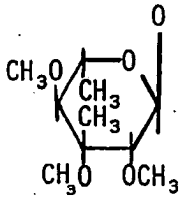
Several other steffimycinone derivatives were prepared during the structure determination of steffimycin. Catalytic reduction of 2 using 10% Pd/C in methanol gave 7-deoxysteffimycinone (5). The reduction of steffimycinone with 0.78 equivalents of sodium borohydride produced steffimycinol (6) while the use of 5 equivalents gave 7-deoxysteffimycinol (7). The ^{13}CNMR spectral assignments had been determined for compounds 2-7 in the course of the structure determination of steffimycin[4] and were confirmed during biosynthetic studies.[3] However, the stereochemistry of the A ring has not been established.[4]

The purpose of this research project was to prepare several derivatives of steffimycinone modified at C-7 and C-10. These positions were chosen for several reasons: 1) They are both in the non-aromatic A ring where they are more readily manipulated than in an aromatic ring. With the exception of modifications achieved by total synthesis, modifications in the anthraquinone portions of anthracyclines have been confined to demethylation of methoxyls, methylation and acetyl-

ation of hydroxyls and 5-imine formation.[2] 2) Both C-7 and C-10 have a functional group containing oxygen in different oxidation states -- a hydroxyl at C-7 and a ketone at C-10. Regioselectivity is assured by the different chemical behavior of the ketone and the quinone carbonyls.[5] In the case of reduction the reduced quinone readily reoxidizes in air.[5]

3) Modification of the anthracycline antibiotic nogalamycin (8) at C-7 and C-10 has produced a more active analogue, 7-(R)-O-methylnogarol (9).[6]





8 $R_1 = COOCH_3$ $R_2 =$

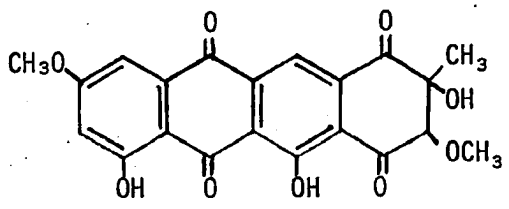
9 $R_1 = H$ $R_2 = OCH_3$

10 $R_1 = H$ $R_2 = NH_2$

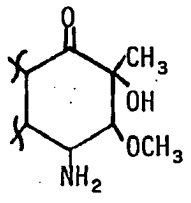
It was believed that an amino group would enhance the ability of the compound to intercalate between the strands of DNA. This intercalation of DNA is implicated in the mechanism of action of the anthracyclines.[1,2] The known active compounds doxorubicin and 7-(R)-O-methylnogarol both contain an amino group, although not directly connected to the A-ring. Several 7-amino analogues (10) of

7-(R)-O-methylnogarol have been prepared and showed antitumor activity.[6] Recently, Umezawa et al[7] reported on structure-activity relationships among 92 anthracyclines against L1210 leukemia in vitro. They concluded that an amino function either on the aglycone or on the sugar moiety was essential for in vitro activity. None of the compounds they tested had amino groups at either C-7 or C-10.

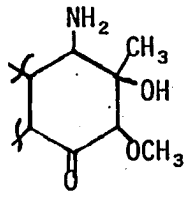
The following six compounds were the objectives of this project:



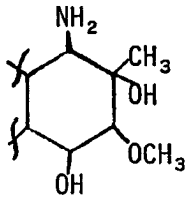
11



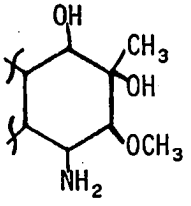
12



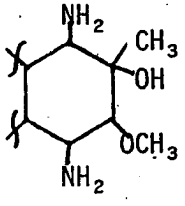
13



14



15



16

Compound 11 is the product of Jones oxidation of steffimycinone (4). Compound 12 is the 7-amino analogue of 4 and 13 is an isomer of 12 that has the keto and amino groups transposed. The pair of compounds 14 and 15 are analogous to the pair 12 and 13 but with the ketone reduced to a hydroxyl. The last compound, 16, has amino groups at both C-7 and C-10. There were two goals in this research problem:

1) To introduce an amino group into the A ring of steffimycinone and 2) To determine the structure-activity relationships (as measured by the *in vitro* activity against L1210 leukemia cells) between the position of the amino group and the position and oxidation level of the oxygen substituent in the A ring.

RESULTS AND DISCUSSION

The synthetic approaches to modification of steffimycinone at C-10 are summarized in figures 1 and 2.

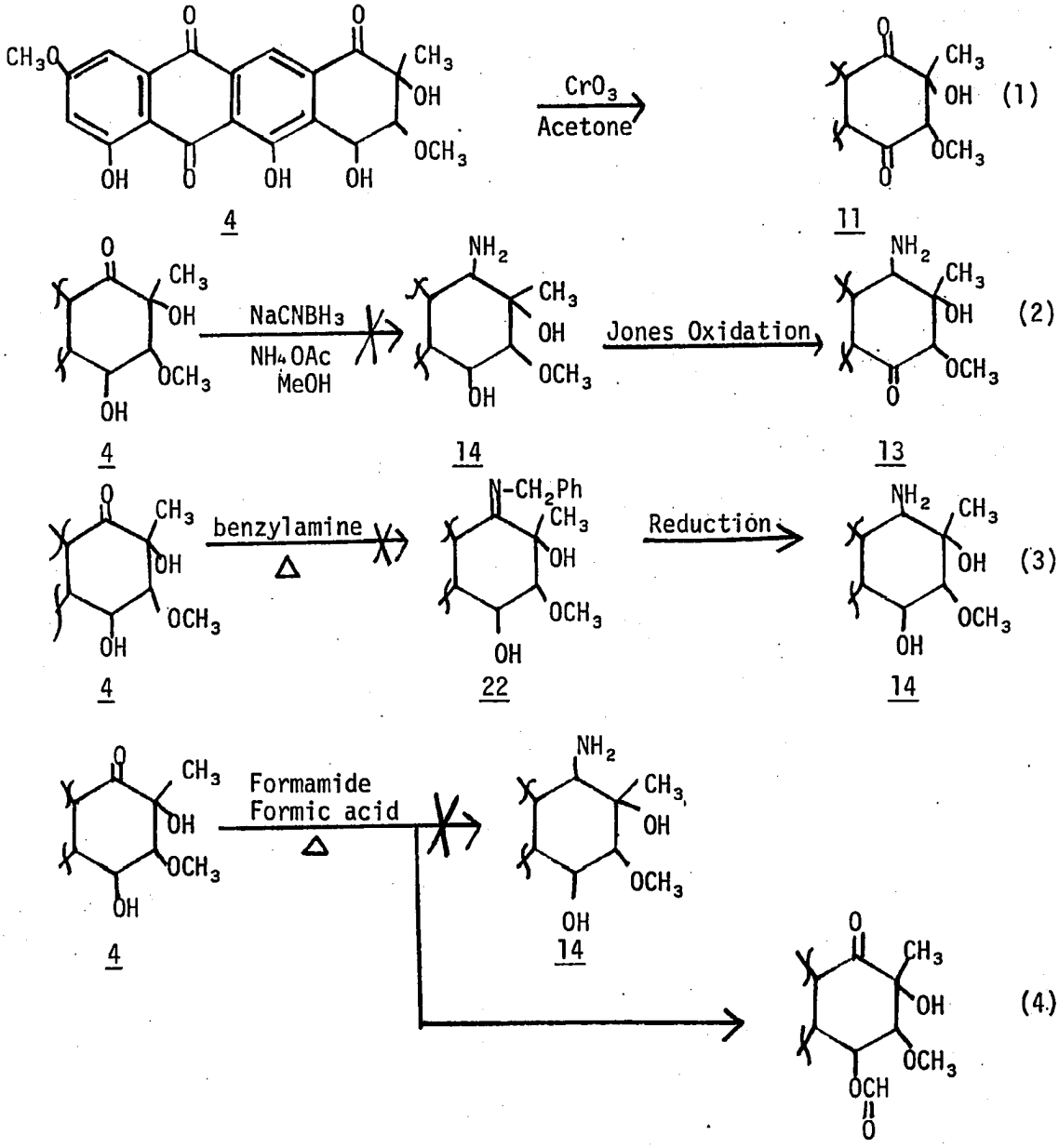


Figure 1. Synthetic Approaches

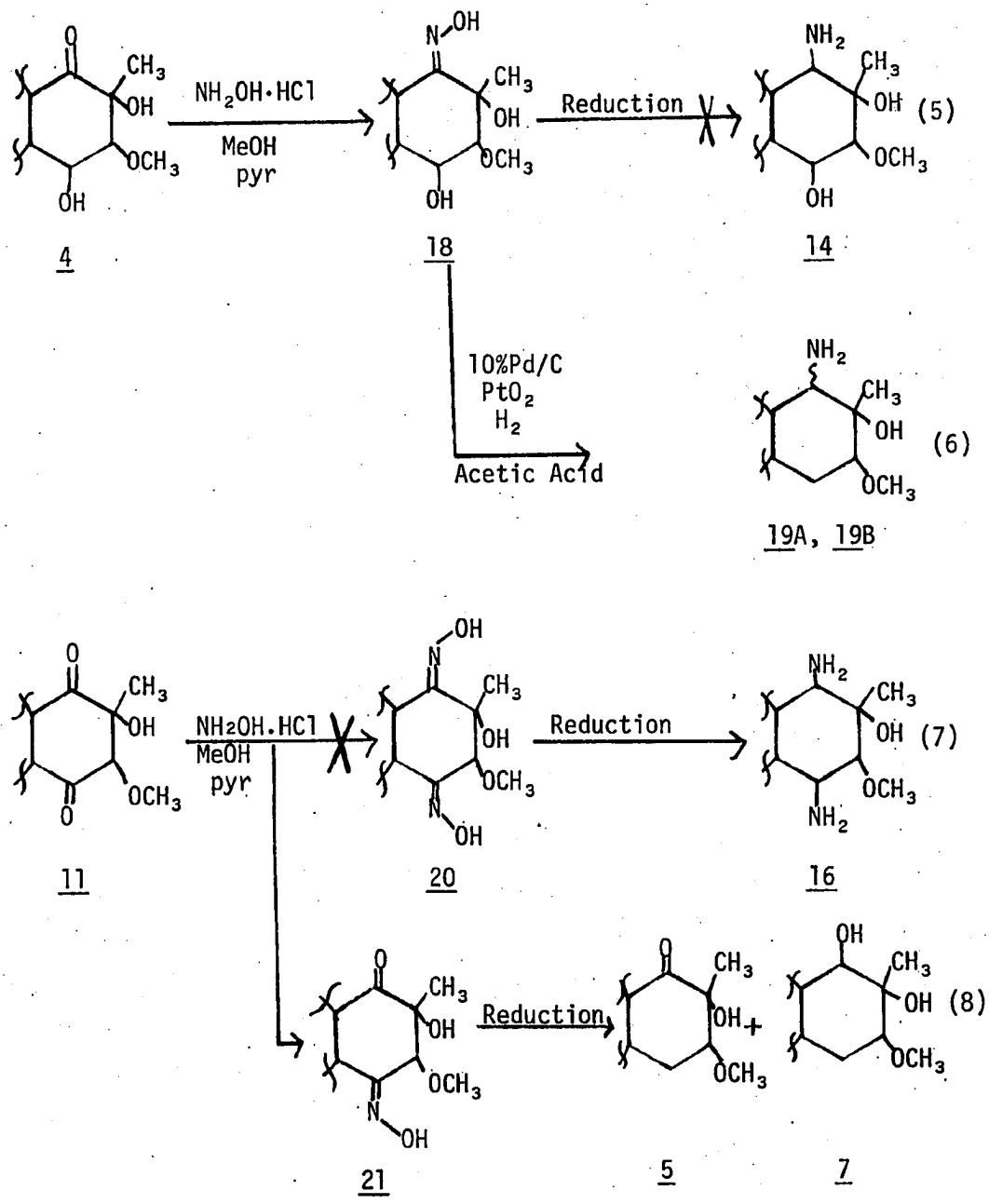


Figure 2. Oxime Approaches

Jones Oxidation

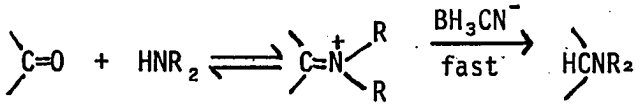
The first objective was the preparation of 7-keto-steffimycinone (11). The presence of a second ketone would change the conformation of the A ring and might be expected to modify the antitumor activity of 11 compared to steffimycinone.[2] Compound 11 could also serve as an intermediate in the preparation of the 7,10-diamino derivative (16), as shown in figure 1, equation (7). The 7-ketosteffimycinone (11) was readily prepared by Jones oxidation[8] of steffimycinone at 25°C in acetone. The product was recrystallized from acetone to give a 79% yield of 11. The ^{13}CNMR spectrum clearly showed two ketonic carbonyls at 196.4 ppm and 194.0 ppm. The molecular ion appeared at $^{m}/_{z}$ 412 in the mass spectrum, which is two mass units less than steffimycinone. The resonance for H-7, which occurred at δ5.24 in steffimycinone, is missing from the proton NMR spectrum of 11. This is what would be expected for oxidation of the hydroxyl at C-7 to a ketone.

Modification of Steffimycinone at C-10

Reductive Amination with $NaCNBH_3$

Compound 14, 10-amino-10-deoxysteffimycinone, proved to be more difficult to prepare. Several approaches were attempted. The most obvious route was the reductive amination of steffimycinone using sodium cyanoborohydride. Sodium cyanoborohydride has been shown by Borch and coworkers[9] and others to be a versatile yet remarkably

selective reducing agent. It has been used to reduce numerous ketones to amines in the presence of ammonia. Some examples which share structural features with the present problem are: cyclohexanone is reduced to cyclohexylamine[9] in 61% yield; norbornanone to endo-norbornylamine in 63% yield[9], acetophenone to α-phenylethylamine in 80% yield[9] and 2-carboxyethylcyclohexanone (in the presence of dimethylamine) gave a (2:3) mixture of cis and trans 2-carboxyethyl cyclohexyl dimethylamine[9] in 53% combined yield. Portoghese and coworkers[10] reductively aminated the opoid antagonist naloxone at C-6 on the cyclohexanone ring to give both 6-amino isomers in 50% yield. The normal reaction conditions for reductive amination are 1 mole of ketone, 10 moles of ammonium acetate and 0.7 mole of sodium cyanoborohydride, stirred in absolute methanol for 24-48 hours at 25°C, with 3A molecular sieves to absorb water generated in the reaction. Steffimycinone was subjected to these conditions but gave only starting material. Ammonium formate and ammonium chloride were substituted for ammonium acetate but still no reaction was observed. No reaction occurred when the reaction mixture was refluxed 8 days either in methanol or in isopropanol. The mechanism proposed for this reaction is shown in equation (9).[9]



(9)

with the slow step the formation of the imine.

Hindered ketones such as isobutyrophenone can be reduced by cyanoborohydride to the alcohol but are not reductively aminated because the imine formation step is very difficult. Thus, if the imine does not form, then the compound cannot be reductively aminated using sodium cyanoborohydride. But, consequently, if the imine can be formed, then the second step, the reduction of the imine to the amine, should be rapid. To test this hypothesis the synthesis of the imine by a different route was attempted.[11,12]

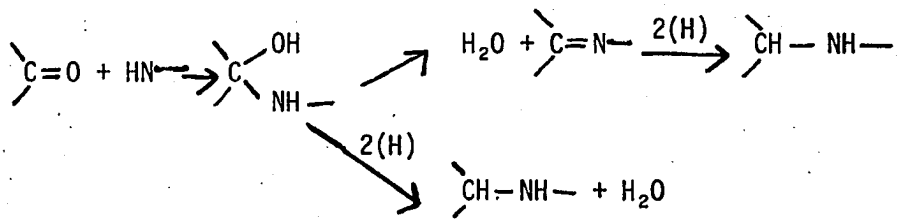
Imine Approach

Richer and Perelman have reported[13] the preparation of a number of cycloalkanone imines with benzylamine, which were isomerized to the corresponding benzaldehyde imine of cyclohexylamine using potassium tert-butoxide and then hydrolyzed to the cycloalkylamine and benzaldehyde. Steffimycinone was suspended in dry toluene and refluxed with one equivalent of benzylamine in a round-bottom flask fitted with a Dean-Stark water trap. No reaction was observed after 8 hours of reflux. The reaction was repeated adding 1 equivalent of para-toluenesulfonic acid and refluxing 16 hours. This time the steffimycinone was converted to a black tar which contained at least 8 components in nearly equal amounts. Several repetitions of this reaction gave the same result. Apparently the imine does not form at all in the absence of acid but there are numerous other competing reactions that occur in the presence of acid.

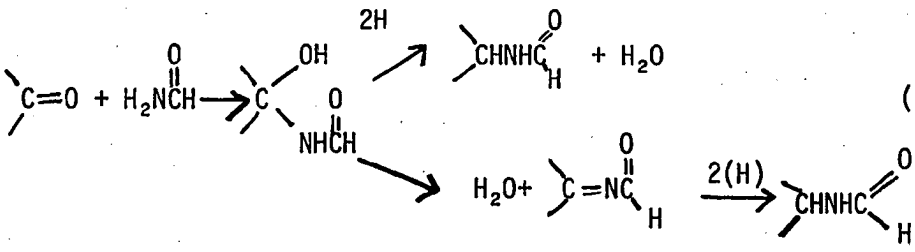
Leuckart Reaction

The Leuckart reaction has been used to reductively aminate

aldehydes and ketones including ketosteroids[14], acetophenone, and camphor.[15] The Leuckart reaction involves the reductive alkylation of ammonia, or primary or secondary amines, by aldehydes or ketones, where the distinguishing feature is the formic acid or formic acid derivative which serves as the reducing agent. An extensive review of the Leuckart reaction[16] suggests two possible mechanisms for the reaction which are shown in equations (10) and (11).



(10)



(11)

In equation (10) the ketone and amine form an addition product which may either be reduced directly to the amine product or which may lose water to give an imine which is reduced by the formic acid. In equation (11) the formamide reacts with the ketone to give rise to an addition product capable of reduction directly to the formyl derivative of the primary amine or of dehydration and then reduction to the formyl derivative of the amine. Either or both of these reactions may occur, depending upon experimental conditions. Sterically hindered alkyl-aryl ketones such as isobutyrophenone,

which was reported not to be reductively aminated by sodium cyanoborohydride[9], react successfully under Leuckart reaction conditions with yields from 50% to 85%.[16] Steffimycinone was treated with formamide and formic acid at 125° to 140°C. Approximately 30% of the steffimycinone was converted to a less polar yellow compound, which was isolated by chromotography on silica gel, eluting with methylene chloride-methanol (98:2). The bright yellow solid had mp. 210-212°C. A mass spectrum gave a molecular ion of 442, 28 mass units higher than steffimycinone, which corresponds to a formyl group. A ^{13}CNMR showed all of the carbons of steffimycinone at the appropriate resonances and one additional carbonyl carbon at 166.6 ppm -- which would be correct for a formyl carbonyl. The UV spectrum was nearly identical with that of steffimycinone suggesting that the formyl group was at C-7 where it did not affect the chromophore. When the formate (17) was treated with 1N sodium hydroxide at room temperature for 1 hour the only product obtained was steffimycinone (4). The Leuckart reaction was repeated on steffimycinone using ammonium formate and formic acid but the only products were a small yield of the formate (17) and starting material. Later it was found that steffimycinone was 95% converted to 17 by treatment with formic acid alone after 24 hours at room temperature. When formamide alone was used no reaction occurred. Thus steffimycinone could not be made to undergo the Leuckart reaction and the only reaction that occurred was esterification of the C-7 hydroxyl by the formic acid.

Oxime Approach

A different approach to the 10-amino compounds involves reduction of the oxime of the 10-ketone. The 10-oxime of steffimycinone (18) is readily prepared by refluxing steffimycinone in methanol and pyridine for 22 hours with an excess of hydroxylamine hydrochloride.[17] The oxime is obtained in 99% yield upon trituration with water, after evaporation of the reaction mixture. The IR spectrum of 18 was missing the band at 1710cm^{-1} which is due to the C-10 ketone in steffimycinone. The ^{13}CNMR spectrum showed the expected peaks for the oxime with the ketonic carbonyl at 198.5 ppm missing and a new peak at 152.0 ppm for the oxime C=N.

The reduction of the oxime proved exceedingly difficult. A wide variety of reducing agents was tried to reduce the oxime and the results were as follows: 1) Sodium in refluxing ethanol[18] gave a very polar acid insoluble purple material, 2) Sodium in refluxing isopropanol also gave a very polar acid insoluble purple material[19] 3) Magnesium in refluxing methanol[20] -- the reaction mixture changed from the yellow of steffimycinone 10-oxime to a dark purple but no reaction could be seen by TLC, 4) No reaction was seen when sodium cyanoborohydride in methanol was used.[9] 5) Sodium borohydride in refluxing isopropanol[21] gave acid insoluble 10-dihydrosteffimycinone (6) (Steffimycinol) and starting material. 6) Trifluoroacetic anhydride in 1,2-dimethoxyethane[22] gave only tar. 7) Lithium aluminum hydride[23] formed an insoluble purple precipitate and a colorless ether solution when added to the steffi-

mycinone 10-oxime solution. When this purple ppt. was treated with EtOAc to destroy the lithium aluminum hydride, the starting material was regenerated. Evidently the LAH complexes with the oxime, and this complex does not react even after 24 hours of reflux. Other anthracyclines have been reported to chelate metals and this must be what occurs here.[2] 8) Formic acid and formamide (Leuckart reaction conditions) gave 6 yellow acid insoluble components.[16] 9) Hydrogenation over 10% Pd/C[21] in either acetic acid, acetic acid plus acetic anhydride, or ethanol with acetylchloride gave only mixtures of 6 to 8 acid insoluble components. 10) Hydrogenation over PtO_2[21] in either acetic acid, acetic acid plus acetic anhydride or ethanol plus acetylchloride gave only mixtures of 6 to 8 acid insoluble components. 11) Hydrogenation of the oxime using both PtO_2 and 10% Pd/C in glacial acetic acid as solvent gave two acid soluble products. These two acid soluble products were purified by partitioning the reaction mixture between water and methylene chloride. The desired products stayed in the acidic aqueous phase, which was neutralized and extracted with methylene chloride to obtain the free amino compounds. A combination of column chromatography and preparative thick layer chromatography were used to separate the two compounds 19A and 19B in 8.7% and 7.2% yields respectively. The mass spectra for both compounds gave a molecular ion at $^{m}/_{z}$ 399, which was 16 mass units less than expected. This molecular weight corresponds to a molecular formula $C_{21}H_{21}O_7N$. The ^{13}CNMR spectra of both 19A and 19B show quite clearly that the oxime

has been reduced. The oxime carbon peak at 152 ppm is missing. In its place is a new peak at 55.8 ppm for 19A and 56.2 ppm for 19B, which would be correct for a carbon attached to an amino group. The other striking difference between 19A and 19B and the oxime is that C-7 has shifted from 67.7 to 26.0 (26.2 for 19B). This is exactly the same as the chemical shift of C-7 in 7-deoxysteffimycinone(5). The other two carbons in ring A, C-8 and C-9 have shifted about 5 ppm upfield compared to the resonances for those carbons in steffimycinone. The rest of the ^{13}CNMR spectra looks nearly identical to steffimycinone. Thus, the structure of the two reduction products of steffimycinone 10-oxime (18) are the epimeric 7-deoxy-10-amino-10-deoxysteffimycinones 19A and 19B. The ^{1}HNMR spectra are consistent with these structures. The H-7 proton has shifted from δ5.24 in steffimycinone to δ3.58 (3.60) in 19A (19B) and integrates for two hydrogens. The C-7 protons appear at δ3.33 in 7-deoxysteffimycinol (7). A new singlet, corresponding to the proton at C-10, appears at δ4.10 in 19A and δ3.72 in 19B. The hydrogenolysis of the hydroxyl group at C-7 was unexpected but is not too surprising, considering that hydrogenation of steffimycinone (4) with Pd/C gave 7-deoxysteffimycinone (5).[4]

The next compound whose synthesis was attempted was 16, the 7,10-diamino, 7,10-dideoxy derivative. The approach used was to prepare the 7,10-bisoxime of steffimycinone by treating the 7-ketosteffimycinone (11) with hydroxylamine hydrochloride at reflux in methanol and pyridine.[17] However, the product, which was isolated

in 57% yield after purification by preparative TLC, was not the expected 7,10-bis oxime (20) but instead the 7-oxime (21). The ^{13}CNMR showed quite clearly that the product had three carbonyls, the C-10 ketone at 197.8 ppm, and two quinone carbonyls, at 187.9 and 180.1 ppm and only one oxime carbon, at 151.7. The rest of the spectrum was consistent with this structure (21). The IR spectrum still showed a strong band at 1710 cm^{-1}, which in steffimycinone is due to the ketonic carbonyl at C-10. The analytical data also support this structure. The mono oxime was calculated to contain 3.27% nitrogen and 3.23% was found. Also, a molecular ion ion was found at m/z 427. Reduction of the 7-oxime with sodium cyanoborohydride in methanol gave 6 acid insoluble products. When the 7-oxime was reduced under conditions identical with those used to prepare 19A and 19B (PtO_2, 10%Pd/C, acetic acid, H_2), shown in equation (8), no amino products were obtained. Instead, four non-polar acid insoluble products were formed, two of which were identified by TLC as the 7-deoxy compounds 5 and 7. It is possible that the 7-oxime is reduced to the 7-amine but under the reaction conditions hydrogenolysis occurs to give the 7-deoxy compounds. Hydrogenolysis of the 7-substituent appears to be facile, as shown by the formation of 19.

In summary, the introduction of a nitrogen atom at C-10 of the A ring of steffimycinone was achieved. Compounds 19A and 19B, isomeric 7-deoxy-10-amino-10-deoxysteffimycinones, were prepared in 8.7% and 7.2% respectively. They were prepared by hydrogenation of steffimycinone 10-oxime (18) with PtO_2 and 10% Pd/C as catalysts

and acetic acid as the solvent. Six different hydrogenation conditions were tried, using either 10% Pd/C or PtO_2, but none were successful. Also, seven other reducing agents were tried, including sodium in ethanol (or 2-propanol), lithium aluminum hydride, magnesium in methanol, sodium cyanoborohydride, sodium borohydride, trifluoroacetic anhydride, and Leuckart reaction conditions, but all failed to give any amino product. The other unsuccessful approaches to a 10-amino steffimycinone were 1) reductive amination with sodium cyanoborohydride, 2) the Leuckart reaction, and 3) the formation and reduction of the imine of the ketone at C-10. These three approaches required the formation of an imine of the C-10 ketone. This ketone fails to form an imine, probably due to the fact that it is an aromatic ketone as well as being sterically hindered[12] by the adjacent methyl and hydroxyl at C-9. Attempts to prepare the 7,10-diamino compound (16) were also unsuccessful (see equations (7) and (8)). A byproduct (17) of the Leuckart reaction was the formylation of the C-7 hydroxyl by the formic acid present in that reaction.

Modification of Steffimycinone at C-7

The approach used to introduce an amino group into the 7 position of steffimycinone differed substantially from the approaches tried at C-10. There is a hydroxyl group at C-7 and a keto group at C-10. Instead of a reductive amination as was attempted at C-10, the C-7 hydroxyl lends itself to a nucleophilic substitution. If the hydroxyl were converted to a better leaving group, such as tosylate or mesylate then it should be possible to displace

the leaving group with a nitrogen nucleophile, such as ammonia, to give the amino derivative directly. Alternatively, the tosylate could be displaced by azide to give the 7-azido compound which could be reduced to the amine. This approach is illustrated in figure 3.

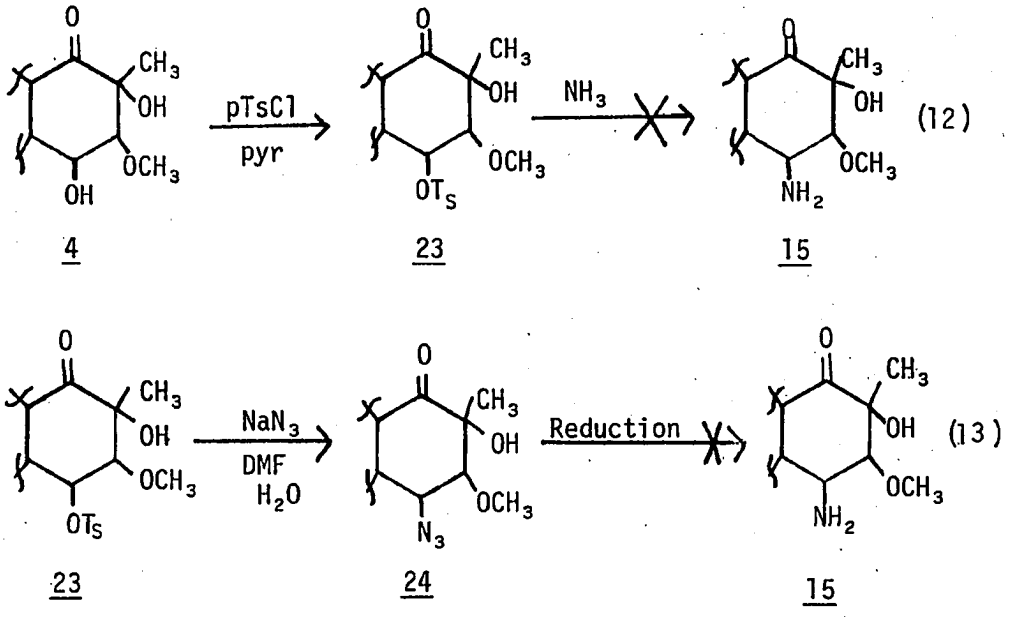


Figure 3. Modification at C-7

Regioselectivity at C-7 should be achieved since the hydroxyl at C-7 is both secondary and benzylic. The other hydroxyls at 2,4 and 9 are either phenolic or tertiary. The reduction of the 7-oxime (21) is shown in equation (8) and is discussed in the previous section.

Tosylate Preparation

The tosylate (23) was prepared by treating steffimycinone with 4.4 equivalents of p-toluenesulfonyl chloride in pyridine at room

temperature for 22 to 48 hours. This procedure is a modification of the procedure of Marvel[24] in that four times as much tosyl chloride was required to get complete reaction. Also, a longer reaction time and a slightly higher temperature were required. The tosylate was isolated by pouring the reaction mixture into methylene chloride and extracting with 1N HCl to remove the pyridine. The tosylate was normally used for subsequent reaction without further purification. However, for analytical purposes 23 was purified by preparative TLC. The mass spectrum gave a molecular ion at 568, which corresponds to a monotosylate, $C_{28}H_{24}O_{11}S$. Microanalysis found 5.63% sulfur, with 5.64% calculated on the basis of the molecular formula. The UV spectrum is very similar to that for steffimycinone, suggesting that the tosyl group is at C-7. The tosyl group carbons can be seen in the ^{13}CNMR spectrum where the tosyl methyl appears at 21.1 ppm, the carbon bearing the sulfonyl group at 146.0 ppm, the 4' carbon at 142.7, the 2' and 6' carbons at 130.1 and the 3' and 5' carbons at 128.4 ppm. The rest of the steffimycinone carbons appear at the appropriate resonances for this structure. The tosyl group protons are also clearly evident in the ^{1}HNMR spectrum of 23.

Displacement of Tosyl Group with Amines

The direct displacement of the tosyl group by an amine[23] (equation 11) was unsuccessful. When 23 was treated with ammonia in tetrahydrofuran two very polar purple products were obtained which were insoluble in acid. When the tosylate was treated with either diethylamine or 2-methoxyethylamine the tosylate was converted to two methylene chloride soluble, nonpolar purple products which were

insoluble in 1N HCl. The same result was obtained when steffimycin (2) was used instead of the tosylate. Apparently the amine reacts with the quinone carbonyls to give imine products, analogous to the preparation of 5-iminodaunorubicin by treatment of daunorubicin with methanolic ammonia.[2]

Tosylate-Azide Approach

Since the tosylate-amine reaction failed, the alternative reaction, the displacement of the tosylate with azide was tried.[23,25,26] As shown in equation (13) the tosylate was heated with sodium azide in dimethylformamide and water. The tosylate was converted to two more polar red products which were fairly unstable. The purification of the azide products was very difficult due to their instability and pure compounds were never obtained. Preparative TLC of 100 mg of crude azide gave 40 mg of sample that was no cleaner than the starting material. The azide (24) appeared to decompose on the TLC plate and also did not elute from the silica gel. The identity of the product was demonstrated by the IR spectrum which showed a very strong N≡N band at 2150 cm^{-1} along with the other bands expected for steffimycinone. The formation of two azides was somewhat surprising, considering the well-known stereospecificity of nucleophilic substitutions on tosylates.[25] However, closer inspection of the tosylate (23) reveals that it is benzylic as well as secondary. Since a carbonium ion at a secondary benzylic position should be quite well stabilized[27] the stereospecificity expected in the displacement by azide would be lost. Racemization

could occur with the azide attacking the carbonium ion at C-7 from either side, giving rise to two epimeric azides.

All attempts to reduce the azide were unsuccessful. The usual method for reduction of azides is with lithium aluminum hydride.[23,25] When the azide (24) was refluxed with LAH in ether several more polar red products were formed, all of which were acid insoluble. Catalytic reduction[28,29] of 24 with PtO_2 in ethanol produced nine acid insoluble products while reduction with 10% Pd/C gave six acid insoluble components. Sodium borohydride in refluxing isopropanol gave four polar products, all of which lacked an amino group. It has been reported[23,30,31,32] that azides are reduced to amines by triphenylphosphine according to the following reaction:

$$RN_3 + PPh_3 \xrightarrow{-N_2} RN{=}PPh_3 \xrightarrow{H_2O} RNH_2 + Ph_3PO \qquad (14)$$

When the azide (24) was refluxed in benzene with triphenylphosphine for 16 hours, then treated with a 1:1 mixture of acetic acid and 48% hydrobromic acid the major product obtained was steffimycinone (4). Traces of two other components were also observed but these also were acid insoluble.

A related method that used the tosylate as the starting material was the Gabriel Synthesis of amines.[33] This reaction is normally employed to prepare primary amines, but in a few cases secondary amines have been made this way.[33] The Gabriel Synthesis involves heating a halide or tosylate with potassium phthalimide in dimethylformamide to form an N-alkyl phthalimide. Then the N-alkyl phthalimide is converted into the corresponding amine by hydrolysis or hydrazinolysis. When steffimycinone 7-tosylate was heated with

potassium phthalimide in DMF a black tar was obtained from which no products could be isolated.

Bell[34] has shown that reduction of a carbonyl ortho or para to a phenolic hydroxyl proceeds to the methylene derivative but stops at the carbinol stage when the carbonyl is meta to the phenolic hydroxyl. He also found that a benzylic hydroxyl was reduced to a methylene group if it was ortho or para to a phenolic hydroxyl. These observations would explain the difficulties in obtaining a 7-amino derivative of steffimycinone. The phenolic hydroxyl at C-6 of steffimycinone is oriented ortho to the C-7 position, rendering any substituent at that position particularly susceptible to hydrogenolysis. According to Norman[35], a benzylic group, when attached to OH, OR, OCOR, NR_2, SR or halogen, readily undergoes hydrogenolysis to the methylene group during reduction. The effect of the ortho phenolic hydroxyl noted by Bell would serve to make the C-7 position of steffimycinone even more prone to hydrogenolysis. A reexamination of the reduction of the 10-oxime (18) and the 7-oxime (21) provides evidence of this propensity towards hydrogenolysis. Reduction of the 10-oxime (equation (6)) gave an amino group at C-10, as would be expected[34] for a substituent meta to the hydroxyl at C-6. Concomitantly the C-7 hydroxyl ortho to the C-6 hydroxyl was removed. When the 7-oxime was reduced (equation (8)) considerable hydrogenolysis occurred at C-7 to give mainly the 7-deoxy compounds 5 and 7. Thus, it seems unlikely that a 7-amino derivative of steffimycinone can be prepared by a method that involves the reduction at C-7 of an intermediate. Perhaps an approach employing some type of substitution reaction at C-7

would be more likely to succeed.

Antitumor Activity

The second part of this project was the comparison of the antitumor activities of the compounds prepared, to determine the relationship of structure to activity. Since the preparation of all of the original target compounds was not achieved the structure-activity relationships could not be determined. However, the antitumor activities of the compounds which were prepared could be determined and compared. The standard *in vitro* assay for antitumor activity is the L1210 mouse leukemia assay. In this assay, the cytotoxicity against L1210 cells *in vitro* is determined using a tube-dilution technique reported by Li *et al*[36] and values are expressed as nanomolar concentrations necessary to inhibit the growth of the cells by 50% and 90% with smaller numbers indicating greater activity. The results of the L1210 *in vitro* assay are shown in table 1.

Table 1.

Activity of Steffimycin Analogues Against L1210 Mouse Leukemia In Vitro

No.	Compound	ID_{50}[a]	ID_{90}
2	Steffimycin	3.0	7.3
4	Steffimycinone	1.8	4.3
11	7-Ketosteffimycinone	1.0	3.2
17	7-Formylsteffimycinone	2.3	5.2
18	Steffimycinone 10-oxime	5.6	12.6
19A	7-Deoxy-10-amino--10-deoxysteffimycinone A	2.4	5.5
19B	7-Deoxy-10-amino-10-deoxysteffimycinone B	1.6	3.8
21	Steffimycinone 7-oxime	2.1	4.7
23	7-Tosylsteffimycinone	1.4	3.2

[a]Activities against L1210 cells are expressed as the nM concentration necessary to inhibit cell growth by 50% and 90%.

As can be seen from this table the ID_{50}'s range from 1.0 to 5.6 nM with the ID_{50} for steffimycinone at 1.8, which is about in the middle of the range. None of the other compounds differs significantly in activity from steffimycinone. A five fold difference in ID_{50} is probably the minimum difference that is significant. Due to the disappointingly low *in vitro* activities none of the compounds warranted testing *in vivo*.

EXPERIMENTAL

General

Instrumentation

Melting points were determined on a Culatti Melting Point Apparatus and are uncorrected. Proton NMR spectra were determined on either a Varian A-60D 60MHz or a Varian EM 390 90 MHz spectrometer. Chemical shift values are reported on the δ scale from tetramethylsilane. Carbon-13 NMR spectra were determined on either a Varian CFT-20 or Varian FT 80A Fourier Transform NMR Spectrometer. Chemical shift values are reported on the δ scale (ppm) downfield from tetramethylsilane. Infrared spectra were recorded on either a Perkin Elmer 735 or Perkin Elmer 299B IR spectrophotometer. Ultraviolet spectra were obtained on a Cary 15 spectrophotometer. Mass spectra and C, H, N, S analyses were provided by the PAC Department of The Upjohn Company. The L1210 *in vitro* leukemia assay data were generously provided by S.L. Kuentzel of The Upjohn Company. Steffimycinone was prepared according to the literature.[4]

Reactions

7-Ketosteffimycinone (11). Steffimycinone (4) (1.03g, 2.5 mmol) was dissolved in the 250 mL dry acetone (Burdick and Jackson) and 0.65 mL Jones Reagent[37] (a 5% excess) was added. The yellow acetone solution turned lighter yellow when the Jones reagent was added. The reaction mixture was stirred at room temperature for 21 h. TLC (SiO_2;$CHCl_3$-MeOH 9:1) showed the reaction to be about 75% complete. Then 0.35 ml Jones reagent was added and stirring was continued at room temperature for an additional 17 h (38 h total). The reaction mixture was a cloudy brown solution with a purple-brown precipitate. The reaction mixture was poured into 250 mL water, concentrated under reduced pressure to remove the acetone, and then extracted with 3x150 ml CH_2Cl_2. The combined CH_2Cl_2 extracts were dried over $MgSO_4$, filtered and evaporated in vacuo to a brown residue which was recrystallized from acetone to give brown solids, 810 mg, 79% yield. mp 177-182°C; Rf. 0.29 (SiO_2, $CHCl_3$-CH_3OH 9:1); UV (EtOH) λ_{max} 203 nm (ε 30,580), 233 (ε 25,250), 252 sh (ε 20,830) 280 sh (ε 13,640) 450 (ε 7,960), IR (Nujol) 3570, 3500, 3400, 3075, 2740, 2600, 1710, 1675, 1625, 1600, 1560, 1420, 1360, 1330, 1280, 1235, 1200, 1180, 1110, 1070, 1015, 970, 960, 935, 880, 860, 840, 820, 800, 780, 750, 730, 710, 680 cm^{-1}; ^{1}HNMR ($CDCl_3$ + CD_3OD) δ 1.35 (S, 3H, CH_3C), 2.10 (S, 1H, H-8), 3.40 (s, 3H, CH_3O), 3.90 (S, 3H, CH_3O), 6.62 (1H, H-3), 7.30 (1H, H-1), 8.32 (S, 1H, H-11); ^{13}CNMR ($CDCl_3$ + CD_3OD) δ 196.4 (C-10, C=O), 194.0 (C-7, C=O), 189.9 (C-5, C=O), 180.5 (C-12, C=O), 167.8 (C-2), 166.1 (C-4), 164.0 (C-6), 139.3 (C-10a), 137.5 (C-11a), 135.8 (C-12a), 134.6 (C-6a), 117.4 (C-5a), 109.9 (C-11),

109.4 (C-4a), 107.4 (C-1), 107.1 (C-3), 92.0 (C-8), 87.3 (C-9), 59.4 (CH_3O, C-8), 56.6 (CH_3O, C-2), 21.4 (CH_3, C-9); mass spectrum $^{m}/_{z}$ 412 (M^+).

Anal. Calcd for $C_{21}H_{16}O_9$: C, 61.19; H, 3.91; Found: C, 60.33; H, 4.10.

7-O-formylsteffimycinone (17). A solution of 200 mg (0.48 mmol) steffimycinone (4) in 10 mL 97% formic acid (Eastman) was stirred at room temperature for 46 h. The reaction mixture was poured into 70 mL water and extracted with 3 x 50 mL CH_2Cl_2. The combined CH_2Cl_2 extracts were extracted with 3 x 50 mL water and then the CH_2Cl_2 solution was evaporated in vacuo to give 224 mg of crude yellow product. The 224 mg of product was coated on 2 g silica gel and chromatographed on 20 g silica gel using CH_2Cl_2-CH_3OH (98:2), collecting 5 mL fractions. Fractions 8-24 were pooled and evaporated in vacuo to give 152 mg of yellow product which still contained some steffimycinone. A second chromatography was carried out, first coating the sample on 4 g silica gel and then chromatographing over 40 g silica gel and collecting 5 ml fractions. The column was eluted first with 1500 mL CH_2Cl_2, then with 300 mL CH_2Cl_2-CH_3OH (99.5:0.5), then with CH_2Cl_2-CH_3OH (99:1). A 300 mL forerun was discarded then fractions 29-34 were combined and evaporated under reduced pressure, weight 45 mg (21% yield): mp 210-212°C; Rf 0.72 (SiO_2, $CHCl_3$-CH_3OH 9:1); UV (EtOH) λ_{max} 210 nm (ε 38,400), 232 (ε 36,730), 255 sh (ε 26,770), 275 sh (ε 24,780), 437 (ε 16,810); IR (Nujol) 3625, 3500, 1720, 1710, 1680, 1620, 1600, 1560, 1410, 1380, 1325, 1300, 1260, 1240, 1210, 1170, 1150, 1120, 1050, 1025, 980, 930, 900, 870, 840, 830, 790, 770, 760,

730 cm^{-1}; ^{1}HNMR ($CDCl_3$) δ1.48 (s, 3H, CH_3C), 3.62 (s,3H, CH_3O), 3.77 (d, 1H, J=2.5Hz, H-8), 3.96 (s, 3H, CH_3O), 6.65 (d, 1H, H-7), 6.75 (d, 1H, J=2.5 Hz, H-3), 7.48 (d, 1H, J=2.5 Hz, H-1), 8.26 (s, 1H, H-11), 8.42 (s, 1H, HCOO); ^{13}CNMR ($DMSO_{d_6}$) 197.7 (C-10, C=O), 189.3 (C-5, C=O), 180.1 (C-12, C=O), 166.6 (formyl C=O), 164.6 (C-2), 161.4 (C-4), 160.5 (C-6), 136.1 (C-10a), 134.6 (C-11a), 133.5 (C-12a), 130.0 (C-6a), 118.0 (C-5a), 115.3 (C-11), 110.4 (C-4a), 108.1 (C-1), 106.7 (C-3), 84.2 (C-8), 76.1 (C-9), 64.3 (C-7), 59.8 (CH_3O, C-8), 56.5 (CH_3O, C-2), 22.3 (CH_3C, C-9); mass spectrum $^{m}/_{z}$ 442 (M+).

Anal. Calcd for $C_{22}H_{18}O_{10}$: C, 59.73, H, 4.10; Found: C, 59.95; H, 4.78.

Steffimycinone 10-Oxime (18). A mixture of 1.0 g (2.4 mmol) steffimycinone (4), 470 mg (6.8 mmol) hydroxylamine hydrochloride, 50 mL dry CH_3OH and 25 mL dry pyridine was stirred and heated under reflux for 22 h. The solvent was removed by evaporation under reduced pressure to give a yellow-brown residue. The residue was triturated with 100 mL water and the solid was collected by filtration and air dried to give brown solids, 1.03 g, (99% yield) mp 195°C dec., Rf 0.43 (SiO_2, $CHCl_3$-CH_3OH 9:1); UV (EtOH) λ_{max} 208 nm (ε34,330), 230 (ε31,970), 284 (ε19,640), 436 (ε11,220); IR (Nujol) 3200, 1670, 1620, 1600, 1560, 1310, 1280, 1250, 1210, 1160, 1120, 1100, 1040, 970, 920, 850, 660 and 624 cm^{-1}; ^{1}HNMR ($DMSO_{d_6}$) δ1.48 (s, 3H, CH_3C), 3.35 (s, 1H, H-8), 3.60 (s, 3H, CH_3O), 3.82 (s, 3H, CH_3O) 4.97 (1H, H-7), 6.65 (1H, H-3), 7.05 (1H, H-1), 8.57 (1H, H-11); ^{13}CNMR 189.8 (C-5, C=O), 180.6 (C-12, C=O), 166.2 (C-2), 164.5 (C-4), 160.3 (C-6) 152.0 (C-10, C=NOH), 135.1 (C-10a), 134.7 (C-11a),

133.9 (C-12a), 130.8 (C-6a), 120.7 (C-5a), 114.8 (C-11), 109.8 (C-4a), 107.8 (C-1), 106.3 (C-3), 88.8 (C-8), 74.5 (C-9), 67.7 (C-7), 60.0 (CH_3O, C-8), 56.2 (CH_3O, C-2), 22.4 (CH_3, C-9); mass spectrum: M^+ not obtained, only decomposition.

Anal. Calcd for $C_{21}H_{19}O_9N$: C, 58.73; H, 4.46; N, 3.26; Found: C, 56.15; H, 4.63, N, 4.00.

7-deoxy-10-amino-10-deoxysteffimycinones 19A and 19B. Steffimycinone 10-oxime (18), 1.0 g (2.3 mmol), was dissolved in 200 mL glacial acetic acid and added 250 mg PtO_2 and 250 mg of 10% Pd/C. The mixture was shaken under hydrogen at an initial pressure of 40 psi in a Parr Hydrogenation Apparatus for 5.25 hours. The catalyst was removed by filtration and the filtrate was poured into 500 mL water, the pH was adjusted to pH 7.6 with 50% NaOH and extracted with 7x300 mL CH_2Cl_2. The CH_2Cl_2 extracts were combined and evaporated under reduced pressure. The residue was redissolved in 500 mL CH_2Cl_2, extracted with 500 mL water, which was discarded, and then was extracted with 3 x 500 mL 1N HCl. A red gummy precipitate formed during the extraction with HCl which was collected by filtration, 361 mg. The combined HCl extracts were adjusted to pH 7.6 with 50% NaOH, and extracted with 5x300 mL CH_2Cl_2. The combined CH_2Cl_2 extracts were evaporated in vacuo to give 137 mg of crude yellow residue. The 137 mg of residue was purified by preparative TLC on 2 Analtech 1000μSi GF 20x20 cm TLC plates, using $CHCl_3$-CH_3OH 9:1 for elution. Two overlapped bands Rf 0.29 to 0.38 were scraped off the TLC plate together, extracted from the silica gel with

CH_2Cl_2-CH_3OH(9:1), filtered, and evaporated in vacuo to give 70 mg. The 70 mg of residue was further purified by preparative TLC on an Analtech 1000μ SiGF 20 x 20 cm TLC plate, eluting with $CHCl_3$-Acetone-CH_3OH-conc. NH_4OH (71:23:4.5:1.5). Two bands were obtained 19B, R_f 0.75, 20 mg (2.1%) and 19A, R_f 0.50 31 mg (3.3% yield). The 361 mg of red gummy residue was purified by HPLC over 55 g silica gel, using $CHCl_3$-CH_3OH-NH_4OH (97:3:0.1) for elution and collecting 5 mL fractions. Fractions #48-80 were pooled and evaporated in vacuo to give 19A, 50 mg (5.4% yield) and fractions #100 to 200 were pooled and evaporated to dryness to give 19B , 47 mg (5.0%). Total yield of 19A, 81 mg (8.7%) mp 159-164°C, R_f 0.49 (SiO_2, $CHCl_3$-CH_3OH 9:1) UV (EtOH) λ_{max} 224 nm (ε31,470), 245 sh (ε15,540), 264 sh (ε20,120), 270 (ε23,310), 289 sh (ε14,340, 432 (ε12,970); IR (Nujol) 3375, 1675, 1630, 1600, 1565, 1420, 1310, 1250, 1210, 1165, 1100, 1030, 960, 920, 860, 760, and 730 cm^{-1}; ^{1}HNMR ($CDCl_3$) δ1.07 (s, 3H, CH_3C), 3.10 (1H, H8), 3.43 (s, 3H, CH_3O), 3.58 (m, 2H, H-7), 3.88 (s, 3H, CH_3O), 4.10 (s, 1H, H-10), 6.53 (d, 1H, J=3Hz, H-3), 7.17 (1H, H-1), 7.91 (s, 1H, H-11); ^{13}CNMR ($CDCl_3$ + CD_3OD) δ191.5 (C-5, C=O), 181.5 (C-12, C=O), 166.9 (C-2), 165.2 (C-4), 160.3 (C-6), 147.2 (C-10a), 135.3 (C-11a), 131.3 (C-12a), 129.9 (C-6a), 118.1 (C-5a), 108.5 (C-11), 108.5 (C-4a), 106.7 (C-1), 106.7 (C-3), 81.2 (C-8), 73.3 (C-9), 57.6 (CH_3O, C-8), 56.2 (CH_3O, C-2), 55.8 (C-10, C-NH_2), 26.0 (C-7), 18.4 (CH_3, C-9); mass spectrum $^m/_z$ 399 (M^+).

Anal. Calcd for $C_{21}H_{21}O_7N$: C, 63.15; H, 5.30; N, 3.51. Found: C, 61.85; H, 5.47, N, 3.14.

Total yield of 19B, 67 mg (7.2%) mp 168-174°C, R_f 0.43 (SiO_2, $CHCl_3$-CH_2OH9:1); UV (EtOH) λ_{max} 224 nm (ε31,870), 246 sh (ε15,940), 264 sh (ε20,120), 270 (ε21,315), 290 sh (ε14,340), 434 (ε12,770); IR (Nujol) 3400, 1670, 1620, 1600, 1560, 1410, 1305, 1275, 1250, 1205, 1155, 1100, 1090, 1030, 1000, 960, 950, 925, 865, 840, 770, 750, and 720 cm^{-1}; ^{1}HNMR ($CDCl_3$) δ1.23 (s, 3H, CH_3C), 3.20 (m, 1H, H-8), 3.47 (s, 3H, CH_3O), 3.60 (m, 2H, H-7), 3.72 (s, 1H, H-10), 3.89 (s, 3H, CH_3O), 6.60 (d, 1H, H-3), 7.20 (d, 1H, H-1), 7.76 (s, 1H, H-11); ^{13}CNMR ($CDCl_3$ + CD_3OD) δ190.5 (C-5, C=O), 181.4 (C-12, C=O), 167.0 (C-2), 165.3 (C-4), 160.8 (C-6), 146.3 (C-10a), 135.2 (C-11a), 131.4 (C-12a), 129.4 (C-6a), 121.6 (C-5a), 113.8 (C-11), 108.9 (C-4a), 106.6 (C-1), 106.6 (C-3), 82.4 (C-8), 70.3 (C-9), 58.5 (CH_3O, C-8), 57.9 (CH_3O, C-2), 56.2 (C-10, C-NH_2), 26.2 (C-7, CH_2), 23.8 (CH_3, C-9); mass spectrum $^m/_z$ 399 (M^+).

Anal. Calcd for $C_{21}H_{21}O_7N$: C, 63.15; H, 5.30; N, 3.51. Found: C, 59.36; H, 5.30; N, 2.97.

Steffimycinone 7-Oxime (21). A mixture of 110 mg (0.27 mmol) 7-ketosteffimycinone (11), 108 mg (1.6 mmol) hydroxylamine hydrochloride, 12 mL dry CH_3OH and 5 mL dry pyridine were stirred under reflux for 21 hours. The solvent was removed by evaporation under reduced pressure to give a brown residue. The residue was triturated with 50 ml water and the brown solids collected by filtration to give 114 mg of crude product. This product was purified by preparative TLC on an Analtech 1000μ SiGF 20 x 20 cm TLC plate, eluted with

$CHCl_3$-CH_3OH(9:1). The purple band at R_f 0.41 was scraped off, extracted from the silica gel with $CHCl_3$-CH_3OH(9:1), filtered and evaporated in vacuo to give orange solids 68 mg (57% yield) mp 182°C dec. R_f 0.32, (SiO_2, $CHCl_3$-CH_3OH 9:1); UV (EtOH) λ_{max} 252 nm (ϵ 32,300), 285 (ϵ 21,240), 445 (ϵ 13,720). IR (Nujol) 3300, 1710, 1680, 1630, 1600, 1315, 1290, 1240, 1205, 1165, 1100, 1025, 980, 870, 835, 770 cm^{-1}; ^{1}HNMR ($DMSO_{d_6}$) δ 1.25 (s, 3H, CH_3C), 3.28 (s, 3H, CH_3O), 3.83 (s, 3H, CH_3O), 6.63 (1H, H-3), 6.96 (1H, H-1), 8.05 (s, 1H, H-11); ^{13}CNMR ($DMSO_{d_6}$) 197.8 (C-10, C=O), 187.9 (C-5, C=O), 180.1 (C-12, C=O), 165.9 (C-2), 164.6 (C-4), 160.4 (C-6), 151.7 (C-7, C=NOH), 134.8 (C-10a), 134.0 (C-11a), 133.0 (C-12a), 125.0 (c-6a), 120.1 (C-5a), 115.7 (C-11), 110.4 (C-4a), 107.1 (C-1), 106.7 (C-3), 77.8 (C-8), 76.9 (C-9), 57.9 (CH_3O, C-8), 56.3 (CH_3O, C-2), 21.9 (CH_3C, C-9); mass spectrum m/z 427 (M^+).

Anal. Calcd for $C_{21}H_{17}O_9N_1$: C, 59.01; H, 4.01; N, 3.27; Found: C, 56.58; H, 4.70; N, 3.23.

7-O-p-Toluenesulfonylsteffimycinone (23). Steffimycinone (4) (1.2 g, 2.9 mmol) was dissolved in 60 mL pyridine, cooled to 0°C in an ice bath and then 2.4 g (12.6 mmol) p-toluenesulfonyl chloride was added. The ice bath was removed, the reaction mixture was allowed to warm to room temperature and was stirred at room temperature for 22 hours. The reaction mixture was poured into 1000 mL of 1N HCl, which was extracted with 4 x 200 mL CH_2Cl_2. The combined CH_2Cl_2 extracts were dried over sodium sulfate, filtered, and evaporated in vacuo to give 2.21 g bright yellow solids. A 100 mg portion of the tosylate was purified by preparative TLC on an Analtech

1000 μ SiGF 20 x 20 cm TLC plate, and eluted with $CHCl_3$-CH_3OH (97:3). The major band, Rf0.21, was scraped off, extracted with CH_2Cl_2-CH_3OH (9:1), filtered, and evaporated in vacuo to give 38 mg, 51% yield mp 225°C dec; Rf 0.66 (SiO_2, $CHCl_3$-CH_3OH 9:1); UV (EtOH) λ_{max} 218 nm (30,110), 236 sh (ε 23,300), 256 (ε26,560), 275 sh (ε20,740), 415 (ε 5400); IR (Nujol) 3500, 1705, 1680, 1640, 1600, 1355, 1330, 1305, 1250, 1190, 1180, 1150, 1100, 1085, 1045, 1010, 975, 960, 925, 895, 870, 820, 800, 760, 740, 720, and 660 cm^{-1}; ^{1}HNMR ($CDCl_3$) δ1.56 (s, 3H, CH_3C), 2.45 (s, 3H, CH_3, tosyl), 3.46 (s, 3H, CH_3O), 3.80 (d, 1H; J=3Hz, H-8), 3.90 (s, 3H, CH_3O), 5.50 (m, 1H, H-7), 6.60 (d, 1H, J=3Hz, H-3), 7.30 (d, 1H, J-3Hz, H-1), 7.35 (d, 2H, J=9Hz, H-3',5'), 7.88 (d, 2H, J=9Hz, H-2',6'), 8.72 (S, 1H, H-11); ^{13}CNMR (DMSO-d_6) δ 198.6 (C-10, C=0), 184.3 (C-5, C=0), 179.9 (C-12, C=0), 165.8 (C-2), 164.3 (C-4), 147.1 (C-6), 146.0 (C-1'), 142.7 (C-4'), 135.3 (C-10a), 134.2 (C-11a), 132.7 (C-12a), 132.0 (C-6a), 130.1 (C-2' and 6') 128.4 (C-3' and 5'), 123.2 (C-5a), 111.0 (C-11), 106.8 (C-4a, C-1 and C-3), 87.3 (C-8), 76.1 (C-9), 59.4 (CH_3O, C-8), 56.4 (CH_3O, C-2), 23.2 (CH_3, C-9), 21.2 (CH_3, tosyl); mass spectrum $^m/_z$ 568 (M^+).

Anal. Calcd for $C_{28}H_{24}O_{11}S$: C, 59.15; H, 4.25; S, 5.64; Found: C, 59.41, H, 4.29, S, 5.63.

7-Azido-7-deoxysteffimycinone (24). 7-p-Tosylsteffimycinone (23) was dissolved in 100 mL dimethylformamide, added 50 mL water and then 600 mg (9.2 mmol) sodium azide was added. The reaction mixture was

heated to 80°C for 21 hours. After cooling to room temperature the reaction mixture was poured into 900 mL saturated sodium chloride solution and extracted with 3 x 350 mL ethyl ether. The combined ether extracts were washed with 300 mL saturated sodium chloride solution. The ether solution was evaporated in vacuo to give 550 mg of a red residue, which contained three components -- steffimycinone and the 2 more polar epimeric azido compounds. A 100 mg sample of this red residue was chromatographed on an Analtech 100 20 x 20 cm silica gel GF Prep TLC plate, eluting with Skellysolve B-acetone (7:3). The major band, at R_f 0.02 was scraped off, extracted from the silica gel with chloroform-methanol (9:1) and evaporated in vacuo to give 40 mg of red residue that had the same three components as the reaction mixture. R_f 0.58 and 0.54 (SiO_2, $CHCl_3$-CH_3OH 9:1); IR (Nujol) 3450, 3325, 2150, 2100, 1710, 1675, 1620, 1580, 1420, 1320, 1250, 1195, 1175, 1110, 1045, 1020, 965, 930, 850, 820, 750, 730, 690, and 660 cm^{-1}.

References

(1) Pratt, W.B.; Ruddon, R.W. "The Anticancer Drugs"; Oxford University Press: New York, 1979; p 155-170.

(2) Arcamone, F. "Doxorubicin: Anticancer Antibiotics"; Academic Press: New York, 1981.

(3) Wiley, P.F.; Elrod, D.W.; Marshall, V.P. Biosynthesis of the Anthracycline Antibiotics Nogalomycin and Steffimycin B. J. Org. Chem. 1978, 43, 3457-61.

(4) Kelly, R.C.; Schletter, I.; Koert, J.M.; MacKellar, F.A.; Wiley, P.F. Structures of Steffimycin and Steffimycin B. J. Org. Chem. 1977, 42, 3591-96.

(5) Arcamone, F. In "Anticancer Agents Based on Natural Product Models", Cassady, J.M.; Douros, J.D., Ed.; Academic Press: New York, 1980; Chapter 1.

(6) Wiley, P.F.; Elrod, D.W.; Houser, D.J.; Richard, F.A. Structure-Activity Relationships in Nogalamycin Analogs. J. Med. Chem., in press.

(7) Matsuzawa, Y.; Oki, T.; Takeuchi, T.; Umezawa, H. Structure-Activity Relationships of Anthracyclines Relative to Cytotoxicity and Effects on Macromolecular Synthesis in L1210 Leukemia Cells. J. Antibiot. 1981, 34, 1596-1607.

(8) Bowers, A.; Halsall, T.G.; Jones, E.R.H.; Lemin, A.J. The Chemistry of the Triterpenes and Related Compounds. Part XVIII. Elucidation of the Structure of Polyporenic Acid C. J. Chem. Soc. 1953, 2548-60.

(9) Borch, R.F.; Bernstein, M.D.; Durst, H.D. The Cyanohydridoborate Anion as a Selective Reducing Agent. J. Am. Chem. Soc. 1971, 93, 2897-2904.

(10) Jiang, J.B.; Hanson, R.N.; Portoghese, P.S.; Takemori, A.E. Stereochemical Studies on Medicinal Agents. 23. Synthesis and Biological Evaluation of 6-Amino Derivatives of Naloxone and Naltrexone. J. Med. Chem. 1977, 20, 1100-1102.

(11) Pirkle, W.H.; Hauske, J.R. Design of Chiral Derivatizing Agents for the Chromatographic Resolution of Optical Isomers. Asymmetric Synthesis of Some Chiral Fluoroalkylated Amines. J. Org. Chem. 1977, 42, 2436-39.

(12) Layer, R.W. The Chemistry of Imines. Chem. Rev. 1967, 63, 489-510.

(13) Richer, J.; Perelman, D. Equilibration d'Imines Cyclaniques. Can. J. Chem. 1970, 48, 570-78.

(14) Panouse, J.J.; Schmitt, J.; Cornu, P.J.; Hallot, A.; Pluchet, H.; Camoy, P. Recherches sur les Amino-Stéroides. VIII. Monoamines en C3 et Diamines en C3 et en C20 Préparées au Moyen de la Reaction de Leuckart. Bull. Soc. Chim. Fr. 1963, 1753-60; Chem. Abstr. 1964, 60, 583c.

(15) Ingersoll, A.W.; Brown, J.H.; Kim, C.K.; Beauchamp, W.D.; Jennings, G. Extensions of the Leuckart Synthesis of Amines. J. Am. Chem. Soc. 1936, 58, 1808-11.

(16) Moore, M.L. In "Organic Reactions", Adams, R., Ed.; John Wiley and Sons: New York, 1949; Vol. 5, Chapter 7, p 301-330.

(17) Kotera, K.; Kitahonoki, K. In "Organic Synthesis": Baumgarten, H.E., Ed; John Wiley and Sons: New York, 1973; Coll. Vol. 5, p 83-87.

(18) Shoppee, C.W.; Evans, D.E.; Richards, H.C.; Summers, G.H.R. Steroids and Walden Inversion. Part XXX. The Epimeric Coprostan-3-ylamines and Cholest-4-en-3-ylamines. J. Chem. Soc. 1956, 1649-55.

(19) Shoppee, C.W.; Cremlyn, R.J.W.; Evans, D.E.; Summers, G.H.R. Steroids and Walden Inversion. Part XXXVIII. The Deamination of Epimeric Cholestan-2-,-4-, and -7-ylamines. J. Chem. Soc. 1957, 4364-69.

(20) Sugden, J.K. Reduction of Oximes. Chem. and Ind. 1969, 260.

(21) House, H.O. "Modern Synthetic Reactions", 2nd ed.; W.A. Benjamin: Menlo Park, Ca., 1972; Chapters 1 and 2.

(22) Emmons, W.D. The Synthesis of Nitrosoalkane Dimers. J. Am. Chem. Soc. 1957, 79, 6522-24.

(23) Buehler, C.A.; Pearson, D.C. "Survey of Organic Syntheses"; Wiley-Interscience: New York, 1970; Vol 1, p 423-50.

(24) Sekera, V.C.; Marvel, C.S. Higher Alkyl Sulfonates. J. Am. Chem. Soc. 1933, 55, 345-9.

(25) Bose, A.K.; Kistner, J.F.; Farber, L. A Convenient Synthesis of Axial Amines. J. Org. Chem. 1962, 27, 2425-27.

(26) Montgomery, R.; Wiggins, L.F. The Anhydrides of Polyhydric Alcohols. Part V. 2,5-Diamino-1,4-3,6-Dianhydro Mannitol and Sorbitol and their Sulphanilamide Derivatives. J. Chem. Soc. 1946, 393-6.

(27) Morrison, R.T.; Boyd, R.N. "Organic Chemistry", 2nd ed.; Allyn and Bacon: Boston, 1966; p 466-91.

(28) Rylander, P.N. "Catalytic Hydrogenation Over Platinum Metals"; Academic Press: New York, 1967, p 494-6.

(29) Augustine, R.L. "Catalytic Hydrogenation"; Marcel Dekker: New York, 1965, p 95-6.

(30) Horner, L.; Hoffman, H. In "Newer Methods of Preparative Organic Syntheses"; Foerst, W., Ed.; Academic Press: New York, 1963, Vol. 2, p 163-212.

(31) Horner, L.; Gross, A. Die Verwendung der Phosphin-imine zur Einführung primärer Aminogruppen. Justus Liebigs Ann. Chem. 1955, 591, 117-134.

(32) Hellman, H.; Lingens, F.; Folz, E. Synthesen der αα'-Diamino-glutarsäure. Chem. Ber. 1956, 89, 2433-36.

(33) Gibson, M.S.; Bradshaw, R.W. The Gabriel Synthesis of Primary Amines. Angew. Chem. Int. Ed. Engl. 1968, 7, 919-30.

(34) Bell, K.H. Reductive Removal by Sodium Borohydride of Side-Chain Oxygen from Phenolic Ketones. Aust. J. Chem. 1969, 22, 601-5.

(35) Norman, R.O.C. "Principles of Organic Synthesis", 2nd ed.; Chapman and Hall: London, 1978; p 625-27.

(36) Li, L.H.; Kuentzel, S.L.; Murch, L.L.; Pschigoda, L.M.; Krueger, W.C. Comparative Biological and Biochemical Effects of Nogalamycin and Its Analogs on L1210 Leukemia. Cancer Res. 1979, 39, 4816-22.

(37) Fieser, L.F.; Fieser, M. "Reagents for Organic Synthesis", John Wiley and Sons: New York, 1967, p 142.