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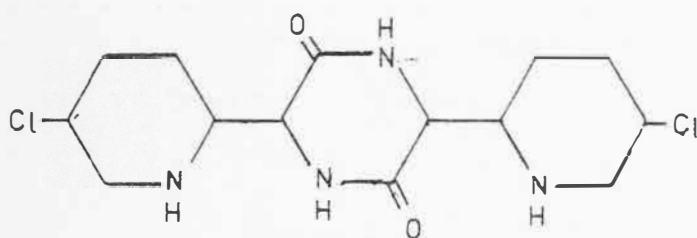


Senior Thesis

Studies Toward the Total Synthesis of Antibiotic 593A

Robert B. Wardle

In 1970 an article was published in the Journal of Antibiotics in which the isolation of a substance from the south African soil micro-organism Streptomyces griseolus designated 593A (NSC-135758) was reported¹. It was reported to inhibit the growth of human tumors in the chick embryo. Arison and Beck² in 1973 proposed the structure to be 3,6-bis-(5-chloro-2-piperidyl)-2,5-piperizinedione based on spectral data.



593A

fig. 1

In the ensuing years a number of further studies were made on the activity of 593A. It was found to be active against a number of solid tumors, several variants of leukemia, and in selectively inhibiting DNA synthesis^{3a,b}. A possible mode of operation was suggested^{3b} in which the Antibiotic would form a bis-aziridine derivative which acts as an alkylating agent.

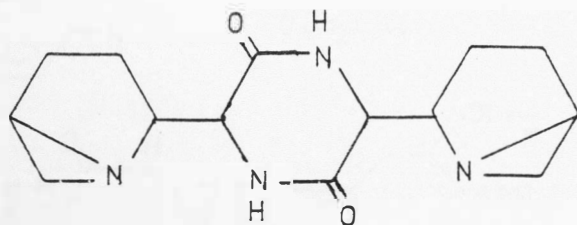
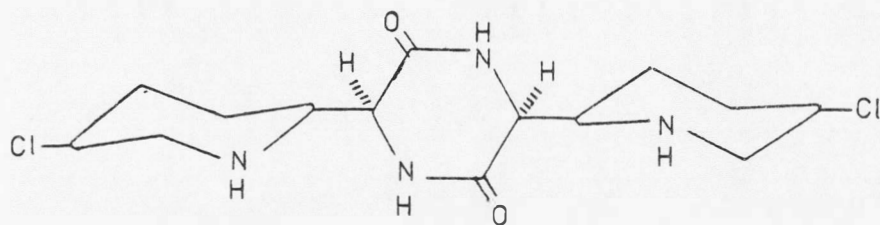


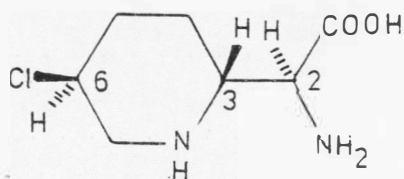
fig. 2

The proposed structure was confirmed and the configuration of the chiral centers was established by Petit and coworkers⁴ in 1976 by converting 593A·hydrochloride to the sulfate salt which has a crystal structure

suitable for X-ray crystallographic analysis. The molecule is a dimer of the novel amino acid Streptolutine (also shown below).



593A



Streptolutine, 6S, 3R, 2S

fig. 3

Recently Fukuyama and coworkers⁵ at Rice University reported a d,l synthesis of 593A. The key step in the synthesis was the dimerization of a substituted β -lactam to form the diketopiperazine ring as shown below.

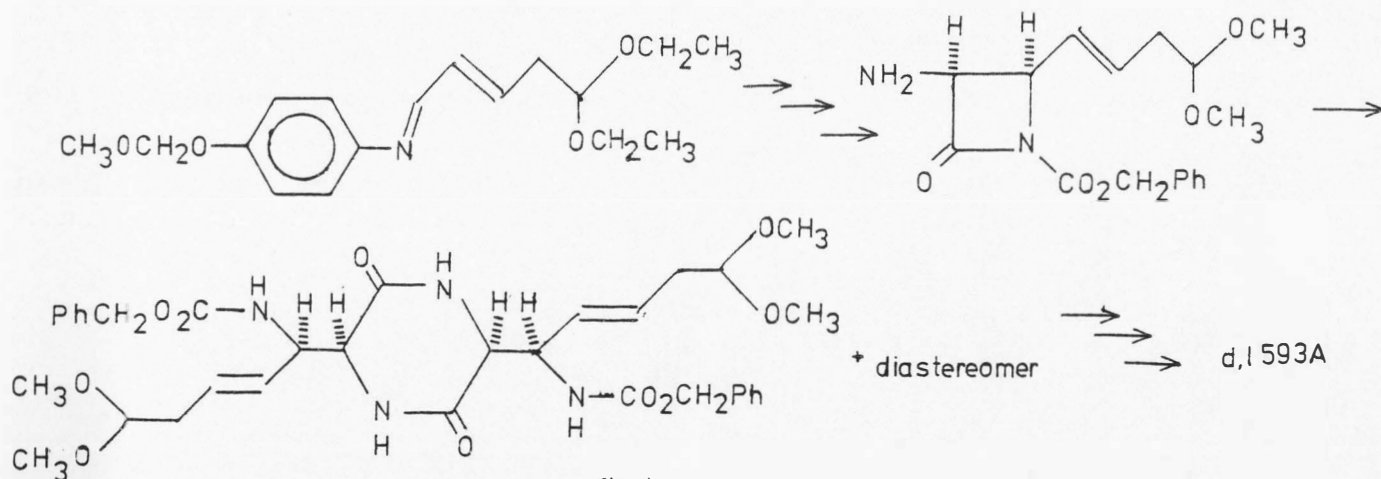
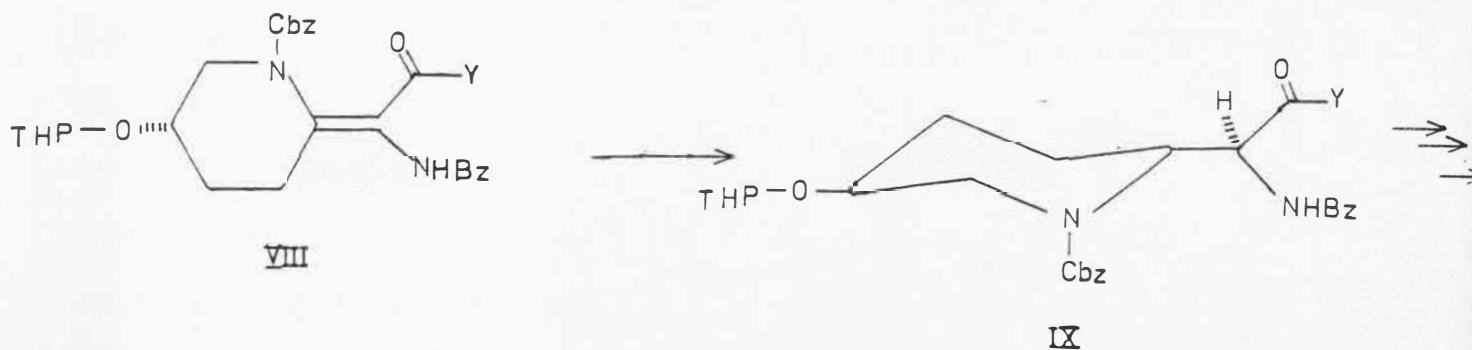
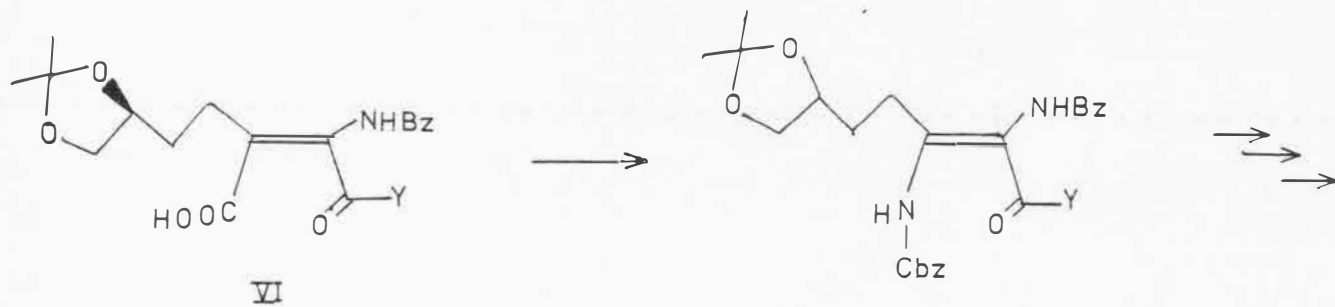
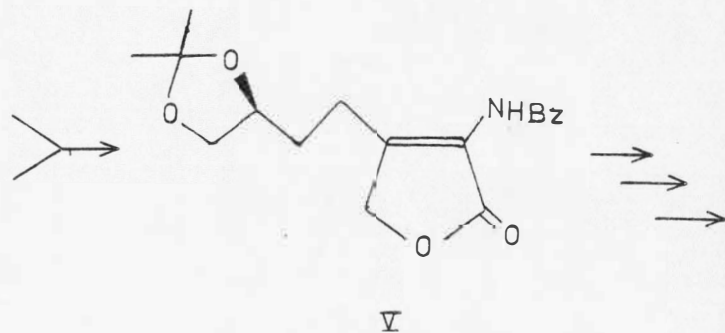
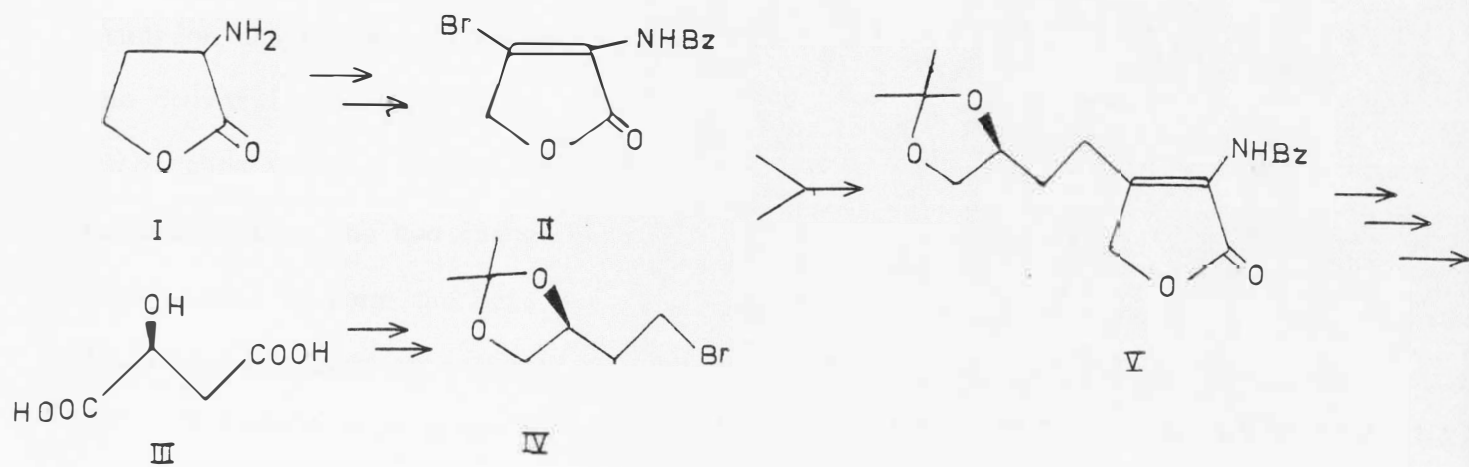


fig. 4

As mentioned this was a d,l synthesis of the antibiotic and since for medicinal or biological evaluation a stereospecific synthesis is

necessary, the synthetic route undertaken in our lab is to be stereo-specific. The general route, as funded by NIH, is as below.



Streptolutine $\xrightarrow{\quad}$ 593 A

GENERAL SYNTHETIC ROUTE

fig. 5

The synthesis up to the formation of compound V was completed by William J. Hennen, a doctoral student. He also completed a partial study on the hydrogenation of the double bond which must occur during the conversion of VIII to IX. The first step which was not properly researched was the conversion of the δ -lactone V to the Carbobenzyl-oxyamine VII. The two changes involved here would be the opening of the lactone to form the acid VI and then a Curtius Rearrangement or analogous reaction to convert the acid VI to the Carbobenzyl-oxyamine VII. The final step which had not been researched was the conversion of VII to the cyclic compound VIII. This would involve opening the acetonide, selective protection, and ring closure.

Part 1

The Curtius rearrangement is an example of a rearrangement of an alkyl or aryl group to an electron deficient Nitrogen atom to form an isocyanate intermediate product. A number of other reactions, some of the oldest known in organic chemistry, proceed by this same mechanism. Some examples are: the Hofmann degradation, the Lossen reaction, and the Schmidt reaction⁶. These reactions are of great general synthetic utility because of the large number of different final products that can be obtained by treating the isocyanate intermediate with various reagents. Figure 6 shows the general mechanism and a number of the more common trappings:

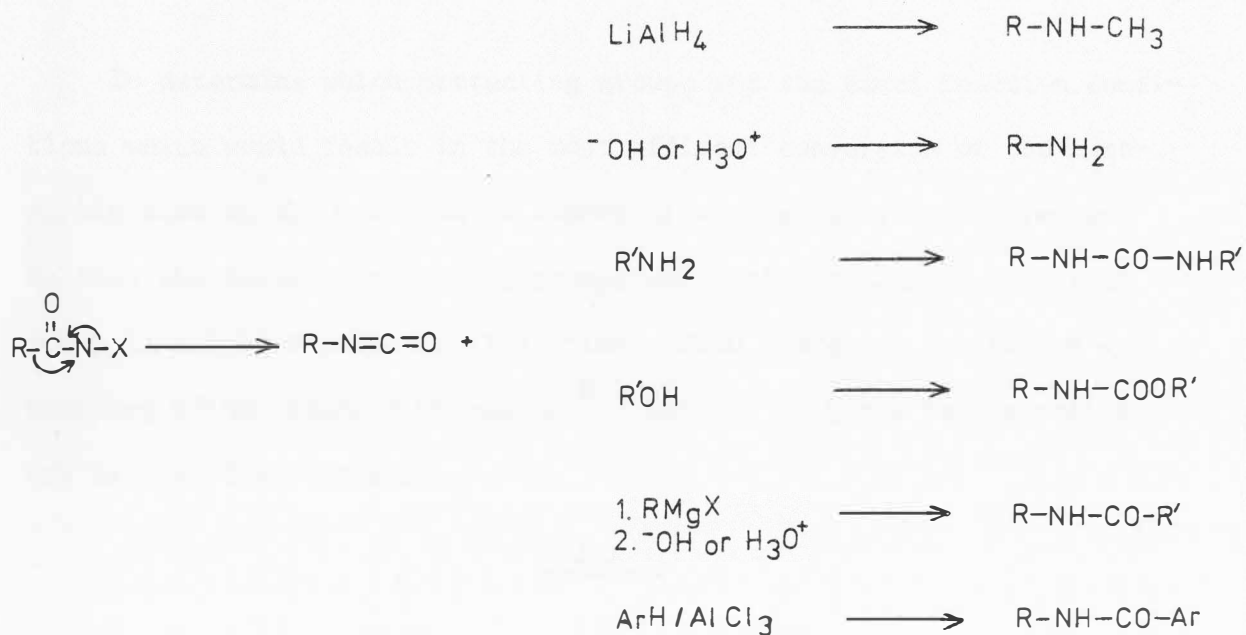


fig. 6

In the particular system involved, a carboxylic acid must be converted to a carbobenzyloxy protected amine--a urethane. Because of the other functionalities in the molecule, particularly mild conditions are needed to effect the conversion. A very suitable version of the Curtius Rearrangement was one published by Yamada and coworkers⁷ which utilizes triethylamine, diphenylphosphoryl azide, hot benzene, and a benzylalcohol trap. The mechanism of the reaction is given here:

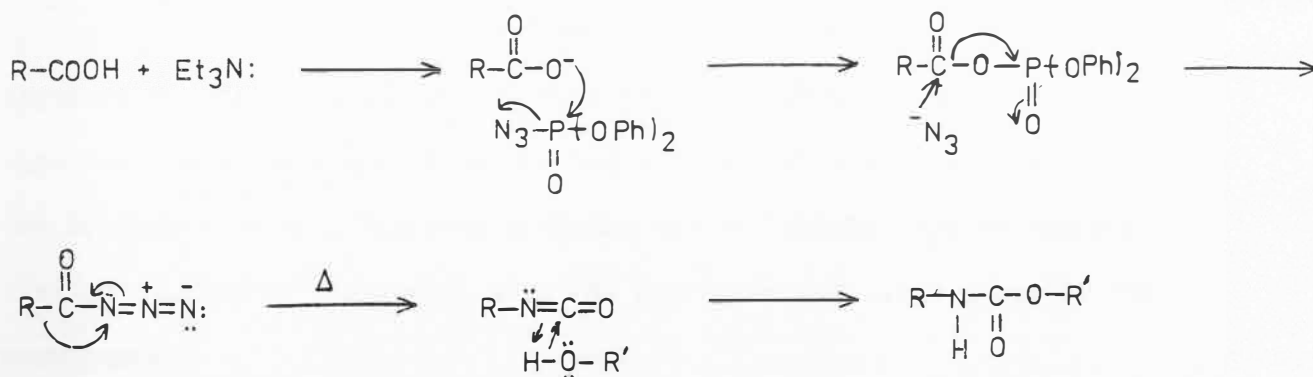
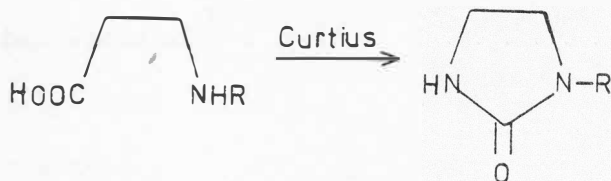


fig. 7

To determine which protecting groups and the exact reaction conditions which would result in the most efficient conversion of the carboxylic acid to the urethane, a number of analogous systems were used to test the various protecting groups and configurations. The first group is simple N-protected β -alanine. Each protected derivative was prepared using standard procedures⁸. Results of these test reactions are tabulated in table 1:

TABLE 1



<u>Reactant</u>		<u>Product</u>	<u>Yield</u>
<u>1</u>	R= Benzoyl	<u>10</u>	74%
<u>2</u>	R= Carbobenzyloxy	<u>11</u>	55%
<u>3</u>	R= t-Butyloxycarbonyl	<u>12</u>	27%

These results were unexpected as up to that time we had no reason to know of the internal trap to form the N-protected-2-imidizolidones. Apparently, the rearrangement to form the isocyanate proceeded as was expected, then the trapping occurs internally and intermolecularly. The mechanism of this internal trapping can be hypothesized to operate similar to that of the workup with the protected amine, as shown on the next page.

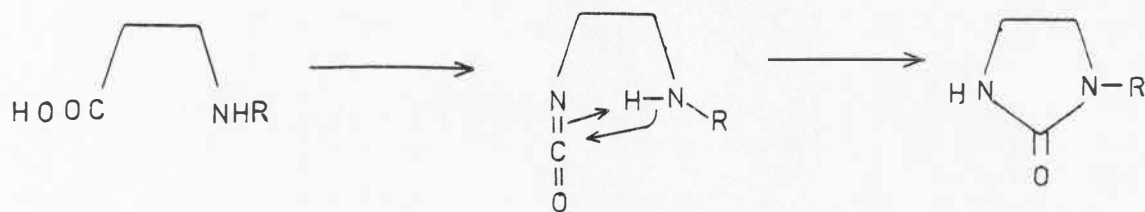
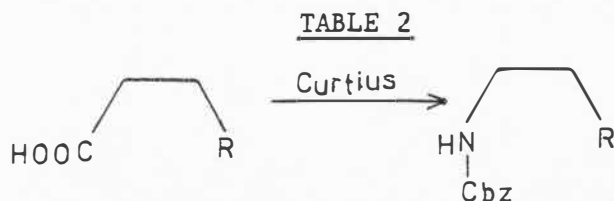


fig. 8

It should also be noted that the bulkier the protecting group, the lower the yield of 2-imidizolidone. In the case of the *t*-Boc- β -alanine, the expected product was recovered in roughly 20% yield, which was lower than that of the 2-imidizolidone, but significant.

An article by Okumura and coworkers⁹ at Kyoto University, which was first found after this work had been done, showed that this internal trapping had been observed previously on some very similar systems.

A series of acids were made lacking hydrogen on another nitrogen in the molecule. The starting materials were made in one step from β -alanine (4) and from succinic anhydride (5 and 6) using standard procedures¹⁰.

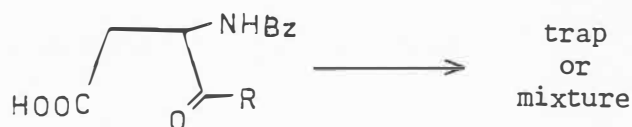


<u>Reactant</u>		<u>Product</u>	<u>Yield</u>
<u>4</u>	R= N-Phthaloyl	<u>13</u>	68%
<u>5</u>	R= CONMe ₂	<u>14</u>	60%
<u>6</u>	R= CO ₂ Me	<u>15</u>	-

With these groups attached, the rearrangement took place as desired in reasonably good yields and with at most very minor side products.

A number of more highly functionalized compounds were made to more closely parallel the actual system. These compounds were prepared by literature methods (7)¹¹ and by reacting lactones whose preparation had been worked out by William Hennen with an amine and then oxidizing the resulting alcohol to the acid (8 and 9). A summary of the results of reacting these compounds under the conditions of the Curtius Rearrangement is given in table 3.

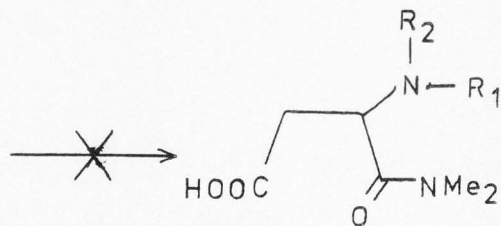
TABLE 3



<u>Reactant</u>		<u>Product</u>	<u>Yield</u>
<u>7</u>	R= OMe	<u>16</u>	60%
<u>8</u>	R= NHMe	complex mixture	
<u>9</u>	$\nabla^{2,3}$ R= NMe ₂	complex mixture	

In the case of 7 a clear yield of ring closure compound was isolated. Compounds 8 and 9 yielded mixtures of large numbers of products, none of which corresponded with the desired products in significant yields.

Attempts were also made to form two other compounds for testing, but were unsuccessful (see diagram on the next page).



17 R_1 and $\text{R}_2 = \text{Phthaloyl}$

18 $\text{R}_1 = \text{Benzoyl}$ $\text{R}_2 = \text{H}$

The study showed that to effect the transformation of compound VI to compound VII protecting groups must be utilized which block all possibilities of internal trappings. The most effective groups may be the methyl ester or the phthaloyl. Clearly problems do exist in effecting the transformation as previously planned and the general approach must be modified to overcome these difficulties.

Part 2

In the cyclization study the conversion would effect only a portion of compound VII as shown here:

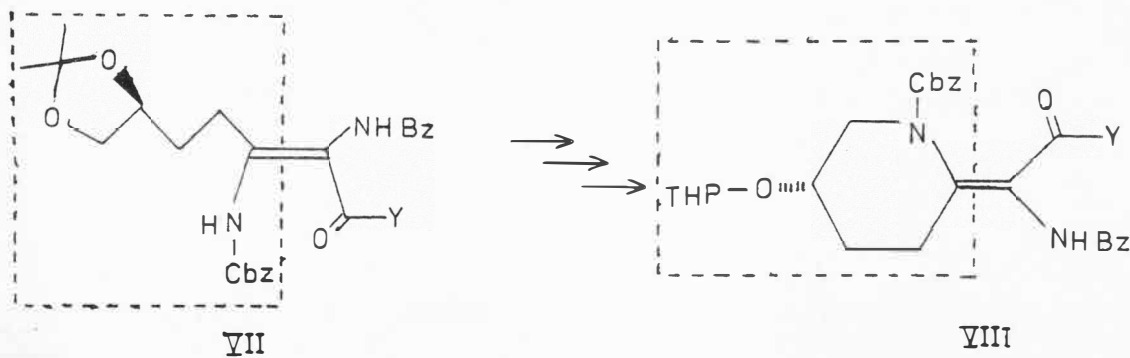


fig. 10

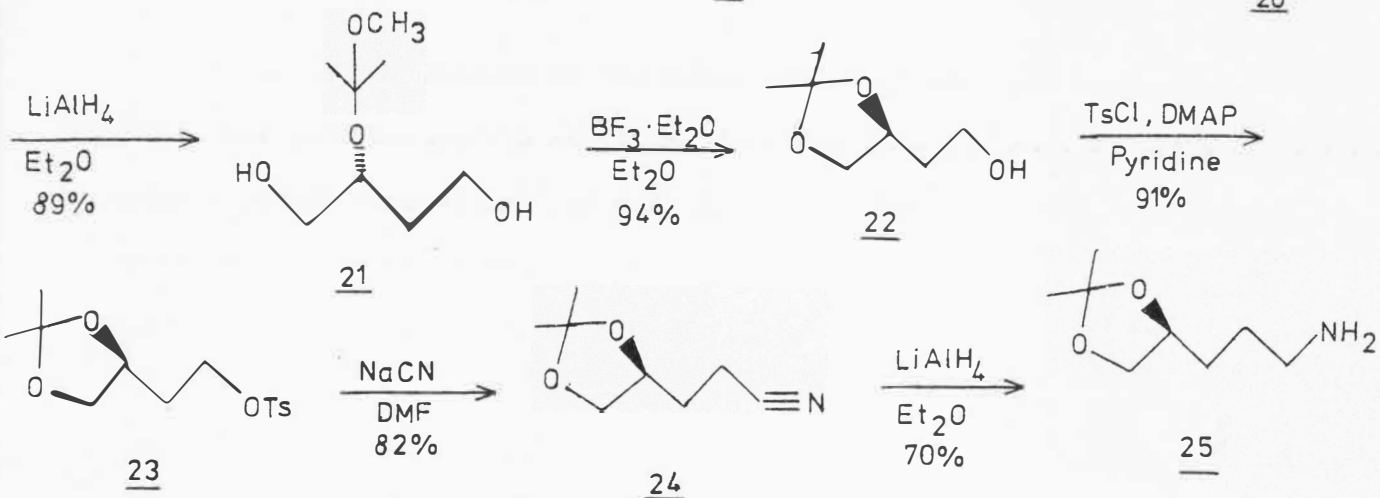
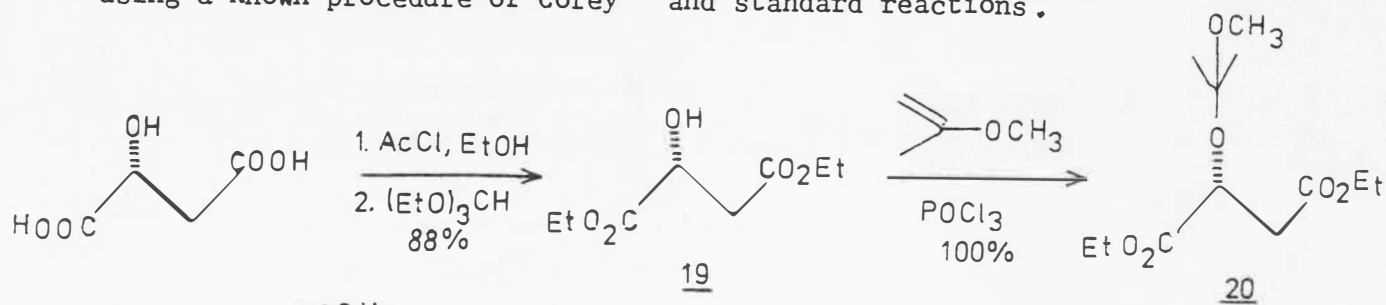
Therefore, a molecule was designed that would have the same important functionalities as VII, but be much simpler to construct:



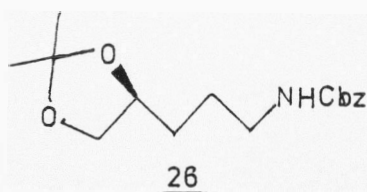
26

fig. 11

This compound (26) was prepared from L-(-)-malic acid as shown below, using a known procedure of Corey¹² and standard reactions.



CbzCl, MgO
H₂O
93%



26

fig. 12

The final steps to form the cyclic analog of VIII, 30, were effected using known reactions and conditions¹³.

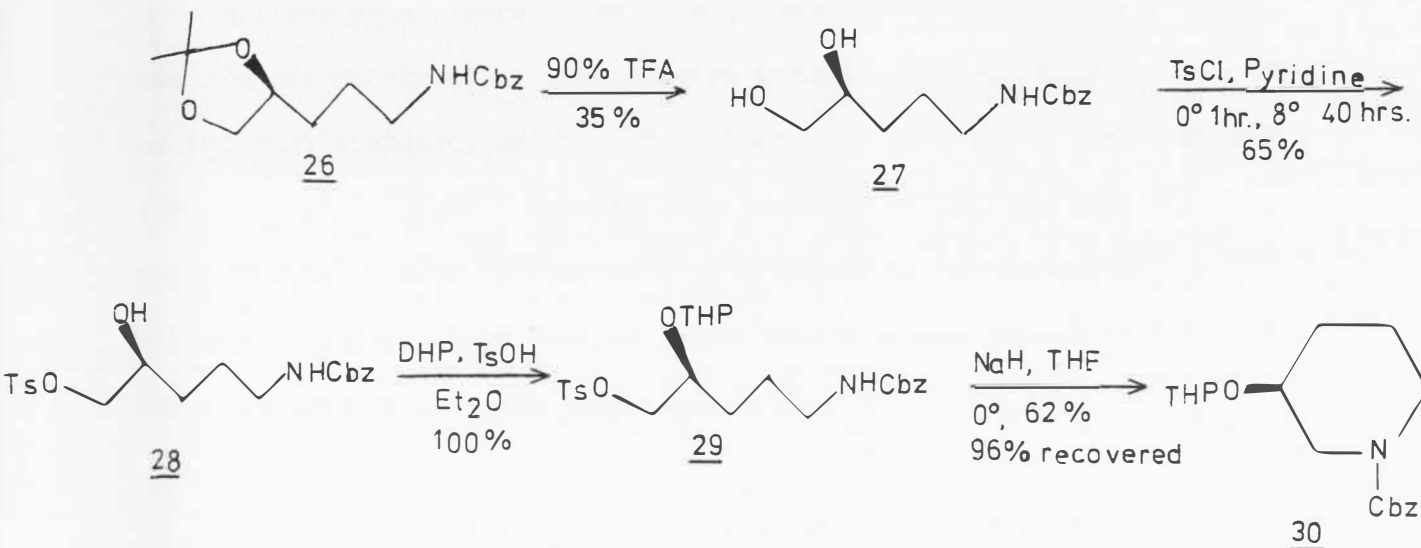


fig. 13

To verify the success of the formation of 30 and to check out the steps that will be used in the final deprotection, 30 was deprotected under standard conditions¹⁴ to form 32, (S)-3-piperidinol. This is a known compound with standard spectral data already accumulated, which made for easy verification of our synthesis¹⁵.

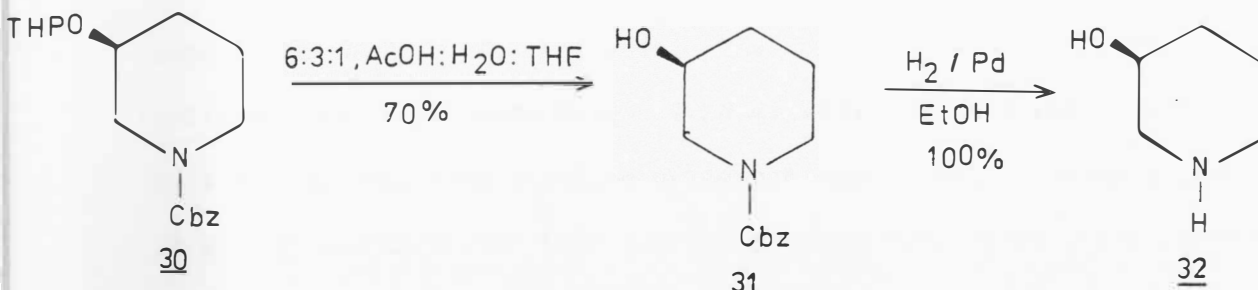


fig. 14

This analog study worked quite well without any major problems, showing that this pathway is a synthetically useful method of preparing the ring functionality.

EXPERIMENTAL SECTION

General Comments:

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. NMR spectra were determined in CDCl_3 with TMS as internal standard, unless noted otherwise, by using a Varian EM-360 spectrometer. Infrared spectra were recorded on Perkin Elmer 710B spectrometer. Mass Spectra were recorded on an LKB Bromma 2091 GC/MS. Elemental analysis were done at M-H-W Laboratories. Specific rotations were measured on a Perkin Elmer Polarimeter 241.

Compounds 1 through 7 were synthesized according to the literature procedures previously cited and exhibited melting points and spectral data that coincided with that previously reported.

8 was prepared in two steps from α -benzamido- γ -butyrolactone. 2.05 g (10 mmol) of α -benzamido- γ -butyrolactone was dissolved in 50 ml of redistilled CH_2Cl_2 . Methylamine was generated from a 50% solution in water by adding it dropwise to NaOH pellets. The methylamine was then bubbled through the solution until starting material was completely consumed, according to TLC analysis. The solution was evaporated and the solid recrystallized from EtOH. 2.23 g; 94%; M.P. 154-155. This alcohol was then oxidized using one equivalent of Jones reagent to give 8 in 92% yield; EtOH recrystallization after extraction using base and then acid; M.P. 176-177.

9 was similarly prepared in two steps, but from $2,3$ - α -benzamido- γ -butyrolactone. Dimethyl amine was evolved as was the methylamine and bubbled through a solution of 0.15g (0.74 mmol) in 25 ml dry THF until

all starting material was consumed. The solvent was evaporated and the product purified by chromatography (EtOAc). 0.06g, 33%; NMR (CDCl_3): δ 3.2 (d on m, 9H), 5.8 (m, 1H), 7.6-8.3 (m, 6H). A considerable amount of Michael addition product was also isolated. This was oxidized to the acid 9 by using one equivalent of Jones reagent and reacting for 3/4 hr. Quenched with isopropanol, dissolved in 5% NaHCO_3 , extracted neutral compounds with EtOAc, acidified with 1 N HCl, and extracted the product with EtOAc. Obtained product in 60% yield. NMR (DMSO): δ 3.2 (d, 6H), 5.8 (m, 1H), 7.3-8.0 (m, 6H).

General Conditions of the Curtius Rearrangement:

Approximately 1 mmol of compound was accurately weighed out and placed in a small flask with 2ml benzene. Also added was 1 eq. of triethylamine and 2 eq. diphenylphosphorylazide along with 1ml benzene with each. The reaction was allowed to heat up to a moderate reflux of the benzene solvent in an oil bath. Added 0.23 ml benzylalcohol. The reaction was run overnight at reflux. Unless a precipitate had formed, the solvent was evaporated and the products separated by chromatography.

10 was recovered in 74% by preparative TLC in EtOAc. M.P. 101-104.

11 was recovered in 55% yield. All spectral data is obtainable from Willial Hennen.

12 was recovered in 27% yield by preparative TLC in 9:1, CHCl_3 : EtOH. NMR (CDCl_3): δ 1.8 (s, 9H), 3.5-4.1 (dt, 4H), 6.8 (s, 1H).

13 was recovered in 68% yield. Product had precipitated out during the reaction and was recrystallized from hexanes after half sat. NaHCO_3 and 0.5 N HCl washes of benzene solution. M.P.: 161-62; NMR (CDCl_3): δ 3.6-4.0 (m, 4H), 5.2 (s, 2H), 7.4 (s above d, 7H), 7.9 (d, 2H).

14 was recovered in 60% yields by preparative TLC in EtOAc. NMR (CDCl_3): δ 2.6 (t, 2H), 3.0 (s, 6H), 3.5 (t, 2H), 5.2 (s, 2H), 7.4 (s, 5H).

Yields and spectral data on 15 can be obtained from William Hennen.

16 was recovered in 60% yields by preparative TLC in EtOAc. NMR (CDCl_3): δ 3.6 (m, 2H), 3.9 (s, 3H), 5.2 (q, 1H), 6.2 (broad s, 1H), 7.4-7.9 (m, 5H).

Curtius reaction on 8 afforded a mixture of internal traps to both nitrogens with available hydrogen and a number of unidentifiable products.

Curtius reaction on 9 afforded a number of products, none of which exhibited the N-dimethyl peaks in NMR.

Attempted synthesis of 17 was unseccessful because the conversion of N-phthaloyl- α -amino- γ -butyrolactone, which was prepared by standard procedures of phthalation, to the corresponding N-dimethyl ring opened compound yielded an untractable mixture of products.

The synthesis of 18 was unsuccessful because no conditions were found to oxidize the N-dimethyl ring opened compound to the acid 18. The ring opening was done as for compound 9, but upon evaporation of

the solvent a solid formed which proved to be the ring opened compound. It was recrystallized from Abs. EtOH to give 73% yield; M.P. 124-125°. Both the Jones oxidation and a basic KMnO_4 oxidation were attempted.

Compounds 19 through 22 were synthesized according to the Corey procedure previously mentioned and exhibited spectral data as in that procedure.

23 was prepared by cooling 9.04g (1 eq.) of TsCl , 0.06g DMAP (0.01 eq.), and 4.75ml pyridine to 0° C. Added quickly 6.92g 22. Stirred 1 hr. at 0° and 40 hrs. at 8° C. The solution turned slightly pink. Stirred $\frac{1}{2}$ hr. at room temperature then poured onto 90ml ice-water. Washed water 3x with Et_2O (50ml). Washed combined Et_2O with 10ml 5% NaHCO_3 2x, 10ml half-saturated CuSO_4 3x (CuSO_4 solution became dark blue, Et_2O clear), 5ml sat. CuSO_4 , 5ml H_2O , 5ml CuSO_4 , 10ml H_2O 2x, and 10ml sat. NaCl . Dried Et_2O with K_2CO_3 , filtered, evaporated solvent, and had 11.74g product. NMR (CDCl_3): δ 1.4 (d, 6H), 2.0 (t, 2H), 2.6 (s, 3H), 3.5-4.4 (m, 5H), 7.4-7.9 (dd, 4H).

24 was prepared by dissolving 17.79g 23 in 170ml DMF, adding 4.36g NaCN (1.5 eq.), and heating at 85° for 3.5 hrs.. Cooled flask to r.t., evaporated largest portion of DMF and dissolved residue in CH_2Cl_2 . Washed CH_2Cl_2 with 80ml 1N $\text{Na}_2\text{S}_2\text{O}_3$ 2x, 80ml sat. NaCl 2x, and dried over K_2CO_3 (a slight orange color persists in the solution). Filter, evaporate, and kugelrohr distill product at 72-75°, 0.9 torr. NMR (CDCl_3): δ 1.4 (d, 6H), 2.0 (t, 2H), 2.6 (t, 2H), 3.7 (m 1H), 4.2 (m, 2H).

25 was prepared by slurring 2.05g LiAlH_4 (1 eq.) in 85ml Abs. Et_2O , and adding 7.89g 24 dissolved in 30ml Abs. Et_2O at a rate so as to maintain a moderate reflux. Continue assisted reflux for 5hrs. The reaction was quenched by cooling the vessel to 0° and adding very slowly 2ml H_2O , 2ml 15% NaOH, and 2ml H_2O . LAH salts were filtered off and reslurried 2x in CH_2Cl_2 . Combined CH_2Cl_2 and Et_2O were dried over K_2CO_3 and then filtered and evaporated. Kugelrohr distillation of product at $72-75^\circ$, 0.65 torr. 5.72g ptd. (70%). NMR (CDCl_3): δ 1.4 (d, 6H), 2.0 (t, 2H), 2.6 (t, 2H), 3.7 (m, 1H), 4.2 (m, 2H).

26 was prepared by washing 5.72g 25 into a flask containing 2.87g MgO (1 eq.) with 200ml sat. MgO sol'n., cooling to 0° , and adding dropwise quickly 7.2ml CbzCl (1 eq.). Stirred at r.t. overnight. Filter, wash solids with 70ml CH_2Cl_2 3x, separate water and wash with 80ml CH_2Cl_2 2x. Dried combined CH_2Cl_2 with K_2CO_3 , filter, and evaporate the solvent. Chromatographic purification in 9:1 Hex.:Ace.. Recovered 9.85g product (93%). NMR (CDCl_3): δ 1.4 (d, 6H), 1.6 (m, 4H), 3.3 (m, 2H), 3.6 (m, 1H), 4.1 (m, 2H), 4.7 (d, 1H), 5.2 (s, 2H), 7.5 (s, 5H).

27 was prepared by adding 4.2ml 90% TFA to 0.5g 26 and stirring for 5 min.. Evaporated TFA on high vacuum, dissolved oil in CHCl_3 and added K_2CO_3 to neutralize any excess TFA. Filtered over a celite pad and evaporated solvent. Crystallized with Et_2O (0.23g, M.P. $61-62^\circ$). Recrystallization with EtOAc . Yield: 0.15g (35%); M.P. $68-70^\circ$. Elemental Analysis done (William Hennen has actual data).

28 was prepared by dissolving 0.10g 27 in 6.7ml pyridine, cooling to 0° , and adding 0.074g TsCl (exactly 1 eq.). Stirred 1 hr. at 0° ,

then 40 hrs. at 8°. Poured reaction mixture onto 10ml 6N HCl, extracted two times with CH₂Cl₂, acidifying after the first wash, and then washed the combined CH₂Cl₂ washes with 3N HCl. Dried sol'n. over K₂CO₃, filtered, evaporated, and had 0.10g pdt (62%). NMR (CDCl₃): δ 1.5 (m, 6H), 2.4 (s, 3H), 2.9-3.4 (m, 3H), 3.9 (broad s, 2H), 5.1 (s, 2H), 7.4 (s, 7H), 7.9 (d, 2H).

29 was prepared by dissolving 0.65g 28 in 3.5ml Et₂O, adding 0.29ml of dihydropyran, and a catalytic amount of TsOH. Stirred at r.t. with aluminium foil wrapped around the flask overnight. TLC shows one spot. Washed Et₂O with 3ml H₂O. Dried Et₂O with MgSO₄, filtered, and evaporated the solvent. Yield: 0.78g (100%). NMR (CDCl₃): δ 1.3-1.7 (m, 10H), 2.4 (s, 3H), 3.1-4.2 (m, 8H), 4.6-4.7 (m, 1H), 5.1 (s, 2H), 7.4 (s, 7H), 7.9 (d, 2H), specific rotation: -3.9°.

30 was prepared by placing 0.05g 50% NaH in oil dispersion in a flask, under N₂, washing with hexanes, and adding 15ml dry THF to slurry. To this was added, at 0°, 0.25g 29 in 5ml THF slowly. Ran 1 hr. at 0°, then overnight at r.t.. Solution has a slight yellow tint. Added 0.1ml H₂O to quench. No noticeable reaction took place. THF was evaporated and the resulting oil dissolved in CH₂Cl₂. The CH₂Cl₂ solution was washed with 5% NaHCO₃, which was then washed with CH₂Cl₂. The combined CH₂Cl₂ washings were washed with sat. NaCl solution, dried over K₂CO₃, and evaporated. The product was purified by preparatory TLC 7:3 hexanes:acetone. Yield: 0.10g (62%) plus 0.09g starting material, therefore, 96% recovered yield. NMR (CDCl₃): δ 1.5-1.9 (m, 10H), 3.3-4.3 (m, 7H), 4.7-4.9 (m, 1H), 5.1 (s, 2H), 7.4 (s, 5H), specific rotation: +4.23°.

31 was prepared from 30 by dissolving 50mg of 30 in 2ml THF, then adding a solution of 9ml acetic acid and 4.5ml H₂O. The reaction was stirred at r.t. for 1 hr., then all reactants were evaporated under reduced pressure. The residue was dissolved in EtOAc and washed with a NaCl sat. solution. The EtOAc was dried with MgSO₄, filtered, and evaporated. By TLC analysis only one product existed which was more polar than the starting material. Yield: 25mg (70%); IR (neat): 3350 cm⁻¹, 3050 cm⁻¹, 2950 cm⁻¹, 1700 cm⁻¹, 1530 cm⁻¹.

32 was prepared by catalytic Hydrogenation of 31. 30mg of 31 was dissolved in 10ml EtOH and a trace (abt. 3mg) of Pd on charcoal was added. The solution was placed on the Parr Hydrogenator at 50 p.s.i. and reacted overnight. The solution was filtered on a celite pad and roto-evaporated. Yield: 13mg (100%); MS m/e: 44 (base peak), 56, 57, 70, 100, 101, 102.

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