THE SYNTHESIS OF β-CARBONHOMOLOGOUS INTERMEDIATE OF NORCARDICIN A. A GENERAL METHOD FOR SELECTIVE PREPARATION OF THE β-LACTAM RING IN THE PRESENCE OF FREE PHENOLIC HYDROXYL GROUPS

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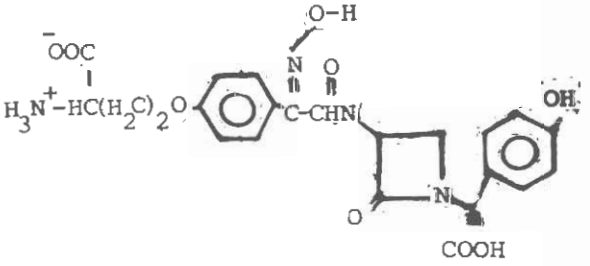
ABSTRACT

The synthesis of cis-N-[α -carbobenzyloxy- β-(p-hydroxy-phenyl)ethyl]-3-phthalimido-4-styryl-2-azetidinone is described. We have found that the electron-rich Schiff bases can afford the cis-β -lactam ring even in the presence of the free hydroxyl functions. The mechanisms of cis and trans- β -lactam ring formation are discussed. The discussions are consistent with the recent publications.

INTRODUCTION

Norcardicin A[1], the first monocyclic β -lactam antibiotic described, is remarkably active against Gram-negative organisms in vivo [2] although it displays but little activity in vitro[3,4]. It differs from all hitherto described β-lactam antibiotics, 1770-1780 cm^{-1}, in having a relatively unstrained (1725 cm^{-1}) β-lactam ring, and therefore being quite stable towards nucleophilic attack. It occured to us that the in vivo activation may well be linked to an oxidation of norcardicin A to the corresponding quinonemethine, in which the β -lactam frequency should be considerably augmented, thus leading to a chemically and therefore perhaps biologically reactive lactam. It should be noted that the epoxidation of the phenyl ring of the phenolic moiety of norcardicin A might as well be an alternative suggestion for the in vivo activation of the compound.



Norcardicin A

Because of the difficulties in preparing quinonemethines [5], the synthesis of the catechol derivatives of norcardicin A, in which an in vivo and/or in vitro oxidation to o-quinones may be more easily achieved and which may perhaps exist in part as the p-quinonemethine tautomer, was aleady reported [6]. This compound did not show any antibacterial activity. The synthesis of homocycloanalogues of norcardicin A and its biological evaluation [7] was the second goal of our research program in this area. However, the latter compound exhibited no antimicrobial activity. Because of lack of in vivo antibacterial activity of homocycloanalogues of norcardicin A, it occured to us that perhaps in these series of non-classical β-lactams, unlike the classical β-lactams such as penicillins or cephalosporins, the monocyclics are biologically active while the bicyclics display no antibacterial activity. Therefore, it was decided to prepare β-carbonhomologous of norcardicin A and study its biological activity.

Since the norcardicin A side-chain is relatively complex, the preparation of β-carbonhomologous derivatives of norcardicin A carrying a phthalimido side chain, as model compounds, is the subject of this paper. The experience gathered will be applid to the synthesis of β-carbonhomologous of norcardicin A with the proper side chain in the future.

EXPERIMENTAL

General: Reagent-grade solvents were distilled first and then stored over molecular sieves (type 4 Å). All starting materials were purchased from Fluka Chemical Company. Column Chromatography: Short columns of silica gel 60 Merck (230-400 mesh) were packed in glass columns (ϕ 2 or 3 cm) using 20-35 g of silica gel per g of curde mixture. TLC: Merck silica gel 60 F 254 anal. sheets. M.P. Buchi 510: uncorrected. IR Spectra: Beckman IR 8 spectrophotometer. ^{1}H-NMR spectra: Hitachi R-248 spectrometer.

Benzyl D,L-phenylalaninate (2a) and benzyl D,L-tyrozinate (2b). Both compounds were prepared in an identical manner The following is a representative procedure. Compound 1a (0.01 mol) was suspended in benzyl alcohol (120 ml). Thionyl chloride (0.02 mol) was added dropwise while stirring at -5°C within 1 h. The reaction mixture was refluxed for 5 h. The solution was then cooled and poured into ether (500 ml) to afford 2a.HCl as precipitate. Filtration gave D,L-phenylalaninate hydrochloride (95%). Compound 2a.HCl was suspended in ether and ammonia was bubbled into solution until saturation. After 15 min the resulting $NH_4^+Cl^-$ was filtered and the filtrate was evaporated to give

aminoester 2a(90%)as an oil.IR(neat): 3300-3450(NH_2),1745 (ester) .^{1}H-NMR ($CDCl_3$):2.79(m,2H,NH_2,exchanged with D_2O),3.45(t,J=6Hz,CH_2),4.39(br., 1 H,CH),7.10,7.22(2s,10 H,2 Ph),4.89 (br.s,2 H,CH_2O).

2b:Oil.IR(neat):3000-3500(NH_2, OH),1740(ester).^{1}H-NMR similar to that of 2a except for variation due to substitution.

4,4'-bis(t-butyldimethylsilyl-oxyphenyl)1,1'-methyl glycindiamide (3).Aminoester 2b (0.01 mol)was dissolved in DMF(70 ml).Imidazole(0.02 mol)was added.t-Butyldimethylsilyl chloride(0.015 mol)was added and the reaction mixture was stirred at 25°C for 38 h.The solution was partitioned between ether(150 ml)and water (150 ml).The organic layer was washed with water(5x100 ml),dried(Na_2SO_4)and evaporated to afford a residue.Chromatography on silica gel and elution with chloroform gave 3(85%),m.p.172-173 . IR(CH_2Cl_2):1680(amide),1110(ether) . ^{1}H-NMR($CDCl_3$):0.10(s,12H,4 CH_3), 0.95 (s,18 H,2t-Bu),2.99(d,J=6 Hz,4 H,2CH_2), 4.91(br.,2H,2NH),4.81(m, 2H,2CH) , 6.71(q,J_1=7 Hz,J_2=14 Hz,8H,2Ph).

Cis-N-(α-carbobenzyloxy-β-phenylethyl)-3-phthalimido-4-styryl-2-azetidinone(4a),cis-N-[α-carbobenzyloxy-β-(p-hydroxyphenyl)ethyl]-3 phthalimido-4-styryl -2-azetidinone (4b)cis-N-(phenyl)-3-phthalimido-4-phenyl-2-azetidinones 7a-d,trans-[N-(O-hydroxypyridyl)-3-phthalimido-4-(O-hydroxyphenyl)] -2- azetidinone (7e),trans-N-(O-hydroxypyridyl) -3-phthalimido-4-styryl-2-azetidinone (7f),O-substituted pyridyl amino-N-(O-substituted)benzylidenes 8a-c,and O-phthalimidoacetyloxypyridylamino -N-cinnamylidene(8d).All β-lactams 4a-b, 7a-d,and 7e-f were prepared in an identical manner and obtained in approximately 20-80% yield. Their spectra were similar except for variations due to substitutions.The following is a representative procedure:To a solution of 2a(0.01 mol)in 200 ml dry methylene chloride was added cinnamaldehyde (0.01 mol).The solution was brought to reflux and the methylene chloride distilled off slowly with the constant addition of dry methylene chloride so as to maintain the same volume of liquid in the reaction vessel.After the water of reaction was all removed(~5 h),the solution was cooled and $MgSO_4$ was added.After 1 h, it was filtered and evaporated to yield(100%)Schiff base 3a as an oil, which was used without purification for the next step.

To the freshly prepared Schiff base(4.15g,0.01 mol)in 200 ml dry CH_2Cl_2 was added at -10°C triethylamine(2.02 g,0.02 mol).A solution of phthalimidoacetyl chloride(1.2 g,0.01 mol)in 20 ml dry CH_2Cl_2 was added dropwise over 1 h.The solution was stirred for 2 h and then washed with water.The organic layer was dried

(Na_2SO_4), filtered, and evaporated to afford the curde product. Chromatography on silica gel and elution with $CHCl_3$ gave diastereoisomeric mixture of 4a (∿70%) as a foam. IR (CH_2Cl_2): 1765 (β-lactam), 1745 (ester), 1710-1720 (phthalimido). ^{1}H-NMR ($CDCl_3$): 3.39 (dd, J_1=6.5 Hz, J_2=7 Hz, 2 H, CH_2), 4.30-4.80 (m, 2 H, CH, and H-C(4)), 5.20 (s, 2H, CH_2O), 5.41 (d, J=5 Hz, H-C(3)), 6.10-6.80 (m, 2 H, CH=CH), 7.10-7.81 (m, 14 H, 3 Ph).

7a: m.p. 194-195°C. IR (CH_2Cl_2): 1765 (β-lactam), 1710 (phthalimido). ^{1}H-NMR ($CDCl_3$): 5.35 (d, J=5 Hz, H-C(4)), 5.60 (d, J=5 Hz, H-C(3)), 7.11-7.95 (m, 14 H, 3 Ph).

7f: m.p. 205-206°C. IR (CH_2Cl_2): 3300-3350 (OH), 1780 (β-lactam), 1705 (phthalimido). ^{1}H-NMR ($CDCl_3$): 5.40 (dd, J_1=2 Hz, J_2=3 Hz H-C(4)), 5.63 (d, J=2 Hz, H-C(3)), 6.75 (m, 2 [illegible] =CH), 7.10-8.35 (m, 13 H, 2 Ph and py.OH).

8a: Oil. IR (CH_2Cl_2): 1640 (CH=N), 1750 (ester), 1710 (amide). ^{1}H-NMR ($CDCl_3$): 4.30 (s, 2 H, CH_2), 7.00-8.20 (m, 13 H, Ph, PhOH, py. and CH=N).

trans-N-(pyrimidyl)-3-phthalimido-4-styryl-2-azetidinone (13a) and trans-N-(pyrimidyl)-3-phthalimido 4-carbomethoxy-2-azetidinone (13b). Compounds 13a-b were prepared (∿30%) in the same manner which was described for the preparation of 4a except that amine 10 was refluxed first with HMDS and the resulting silylated product 12 was converted to the corresponding Schiff bases (by refluxing in benzene and subsequently treated according to conditions described) for 4a). Their spectra were similar which will be described for 13a only.

13a: Foam. IR (CH_2Cl_2): 1780 (β-lactam), 1710 (phthalimido), ^{1}H-NMR (DMSO-d6): 4.70 (br.d, J=2 Hz, H-C(4)), 5.61 (d, J=2 Hz, H-C(3)), 7.20 (m, 2 H, CH=CH), 7.90-9.10 (m, 12 H, 2 Ph and pyrim.).

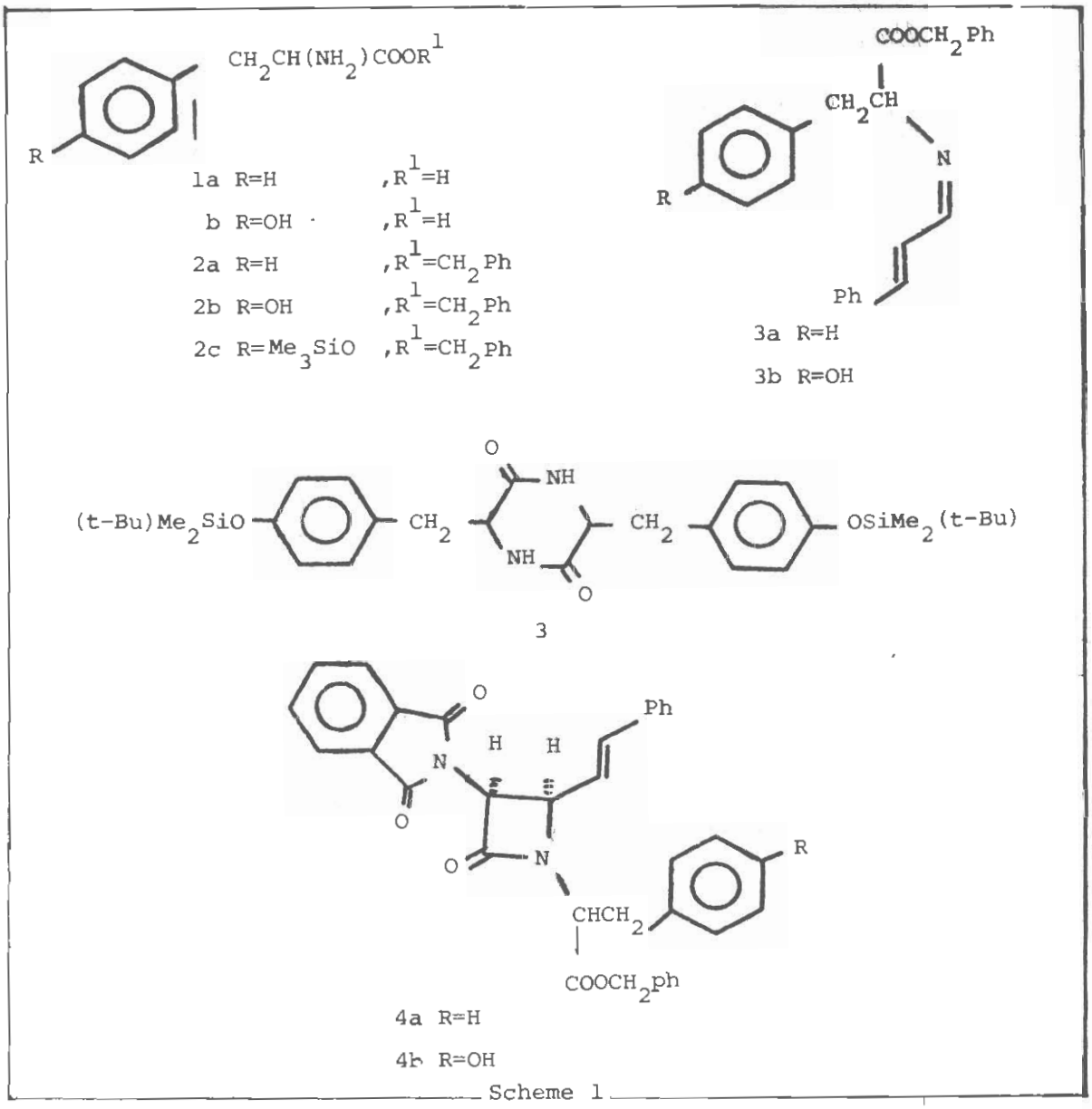
Dibenzyl-2-{[cis-2-Oxo-3-phthalimido-4-(O-hydroxyphenyl)-1-azetidinyl]}malonate (15). Aminoester 14 (1 mmol) and aldehyde 6b (1.1 mmol) was mixed together. After 10 min. CH_2Cl_2 (50 ml) and $MgSO_4$ (10 g) were added and filtered. To the filtrate were added NEt_3 (2.2 mmol) and phthalimidoacetyl chloride (1.1 mmol). After 1 h, the solution was evaporated and the residue was purified on silica gel using $CHCl_3$ as eluent. Compound 15 (50%) was obtained as a foam. IR (CH_2Cl_2): 3300 (OH), 1770 (β-lactam), 1745 (ester), 1710 (phthalimido). ^{1}H-NMR ($CDCl_3$): 4.45 (d, J=5 1H, H-C(4)), 4.81 (s, 4 H, 2 CH_2), 5.11 (s, 1 H, CH), 5.26 (d, J=5 Hz, H-C(3)), 7.10-8.01 (m, 15 H, 2 Ph and Ph OH).

RESULTS AND DISCUSSION

Phenylalanine (1a) and tyrosine (1b) were transformed to their respective benzyl esters 2a-b (∿90%). Silylation of the hydroxyl function in 2b with tertbutyldimethylsilyl chloride in the presence of imidazole in DMF did not afford the correspon-

ding silyl derivative 2c but instead gave the cyclic amide 3 (~85%). Therefore, it was decided to examine the ability of our methods in selective preparation of the β-lactam ring in the presence of the free hydroxyl functions.

Benzyl D,L-phenylalaninate (2a) and Benzyl D,L-tyrosinate (2b) were converted to their cinnamylidine Schiff bases 3a-b. Reactions with phthalimidoacetyl chloride [7-10] using the methods described by Doyle et al. [11] and by ourselves [12,13], gave the corresponding β-lactams 4a-b as a mixture of epimers at the carboxy bearing carbon (Scheme 1). All the β-lactams obtained by this method were cis-fused [14], as could be determined by ^{1}H-NMR (J=5 Hz) of all derivatives in which the relevant protons did not overlap with other signals.



Scheme 1

Since we established a simple method for the synthesis of β-crabon-homologous intermediate of norcardicin A, involving the use of the acyl chloride and triethylamine in the presence of the phenolic hydroxyl group, it was decided to examine the generality and mildness of the method in the selective preparation of the β-lactam ring using substrates possessing free hydroxyl functions.

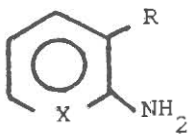
Compounds 5a-c were reacted to the respective aldehydes 6a-c to afford the corresponding Schiff bases, which upon separate reactions with phthalimidoacetyl chloride and triethylamine in methylene chloride at -5°C gave β-lactams 7a-d((∿ 70-80%), scheme 2). It thus seems that relatively electron-rich anilines (as measured by their PK_a (∿ 4.63) [15]) give high yields of cis-β-lactams 7a-d even when the corresponding Schiff bases possess free hydroxyl functions. However, at PK_a ranges below 2.4 (i.e. 5c) one obtains either a mixture of β-lactam 7e-f (∿5%) and esters 8a-d (75%) or only ester 8d. It is of interest to note that the amines with no free hydroxyl group at PK_a ranges of 2.4-4.6 give consistently cis-β-lactams [16,17] while those with the PK_a ranges below 2.4 afford either a mixture of cis/trans isomers or only trans isomer[16]. Therefore we became interested to examine the β-lactam formation with amines having PK_a∿1. Since the preparation of β-lactams possessing a pyrimidine ring is not yet described and this type of compounds might as well have interesting biological activity and toxicity, 2-aminopyrimidine (10) was chosen as the starting material. Compound 10 was prepared from 2-chloropyrimidine (9). Nucleophilic replacement of the chlorine atom in 9 with ammonia, using the standard literature procedure[18], gave, only, 40% of the desired compound 10. However, the above reaction in NH_4OH at 25°C afforded 10 in nearly quantitative yield (table 1).

Table 1- Aminolysis of 2-Chloropyrimidine after 72 h.

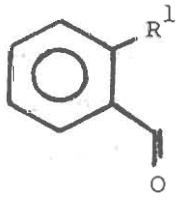
Compound	Conditions	Product (yield)	Compound (yield)
9	$MeOH/NH_3$ (100°) Pressure bottle	10 (40%)	11 (40%)
11	〃	—	11 (100%)
9	t-BuOH (dry, 100°) Pressure bottle	10 (50%)	9 (50%)
9	t-BuOH (wet, 100°) Pressure bottle	10 (70%)	9 (30%)
9	NH_4OH (25°)	10 (100%)	—

This clearly indicates that the nucleophilic displacement of the chlorine function in pyrimidines is faster in a more polar solvent.

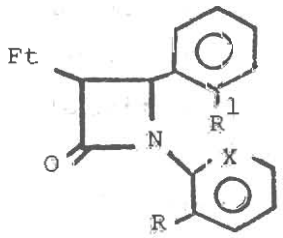
Compound 10 could not be dissolved in solvents,such as benzene or methylene cholride,which are suitable for the Schiff base formation. Therefore it was decided to prepare its silyl derivative 12.Reaction of 2-aminopyrimidine(10)with hexamethyldisilazane(HMDS)in the presence of ammonium sulfate afforded 12 (100%). Compound 12 was transformed to its Schiff bases,which upon reactions,in situe,with phthalimidoacetyl chloride and triethylamine in methylene chloride gave the corresponding trans β-



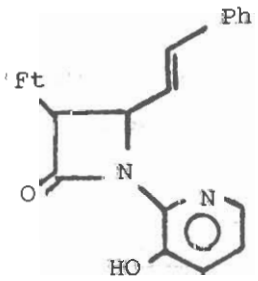
5a R=H ,X=CH
b R=OH,X=CH
c R=OH,X=N



6a R^1=H
b R^1=OH
c=cinnamaldehyde

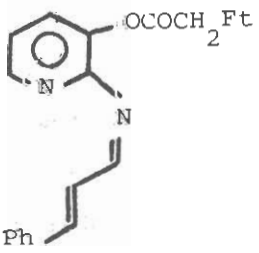


7a R=H ,R^1=H ,X=CH
b R=OH,R^1=H ,X=CH
c R=H ,R^1=OH,X=CH
d R=OH,R^1=OH,X=CH
e R=OH,R^1=OH,X=N

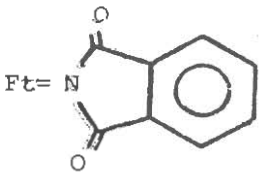


7f

8a R=$OCOCH_2Ft$,R^1=OH
8b R=OH ,R^1=$OCOCH_2Ft$
8c R=$OCOCH_2Ft$,R^1=$OCOCH_2Ft$



8d



Scheme 2

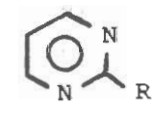
lactams 13a-b(∿30%,Scheme 3). It seems to us that the change of stereoche - mistry reflects a change in the mecha- nism of cycloaddition,where electron- rich Schiff bases give cis-β-lactam by the mechanism proposed by Doyle et al[9]and supported by Sullivan et al[19].They proposed that formation of an immonium ion,followed by cycli- zation, in which the cis-stereo - chemistry is ensured by electrostatic interaction of the carbanion adja - cent to the phthalimido group and charged cinnamylidene group.With a lowering of the nucleophilicity of the Schiff base nitrogen one could expect the rate of this reaction to slow down.A competing reaction path, involving perhaps phthalimidoketene, may then give trans β-lactam.Luck and Kagan[20,21]have shown that with a pure aldoketene,only trans-β-lac- tamas are formed.

The selective β-lactam ring formation in the presence of the phenolic hydroxyl group can also be due to the nucleophilicity of the Schiff bases.Electron rich Schiff bases give the β-lactam ring in a much faster rate than the ester formation of the phenolic hydroxyl functin. With lowering of the nucleophilicity of the Schiff base nitrogen,the rate of the β-lactam ring formation has to be much slower than the rate of the ester formation.

These facts made us possible to prepare a β-carbonhomologous inter - mediate of norcardicin A.It has to be mentioned that the preparation of β- lactam rings in the presence of a free alcoholic hydroxyl function,even with electron rich Schiff bases,failed and resulted in the formation of an ester function.

It should be noted that the reaction of 14 with 6b and the subs- equent treatment of the resulting Schiff base with phthalimidoacetyl chloride and triethylamine gave 15 in good yield.

Attempts for the conversion of 15 ⟶ 16 is in progress.The results will be reported in future.

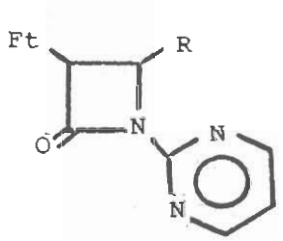


9 R=Cl

10 R=NH_2

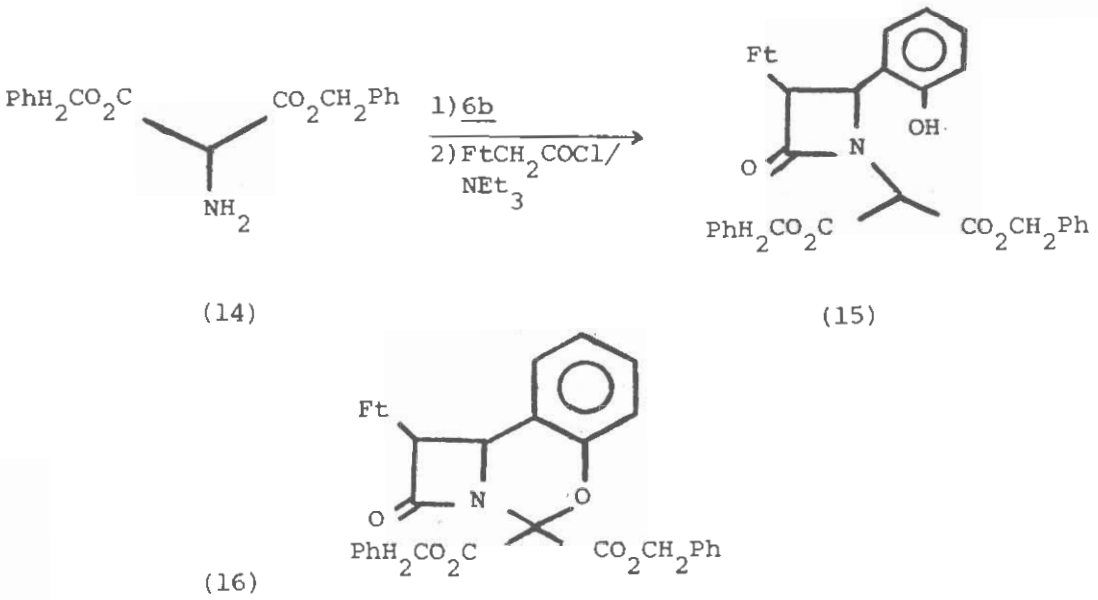
11 R=OMe

12 R=$NHSiMe_3$



13a R=Styryl

b R=COOMe



(14) (15)

(16)

Scheme 3

Acknowledgement

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