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IMPROVED ASYMMETRIC SYNTHESIS OF (−)-3-AMINONOCARDICINIC ACID AND FURTHER OBSERVATIONS OF THE MITSUNOBU REACTION FOR β-LACTAM FORMATION IN SERYL PEPTIDES

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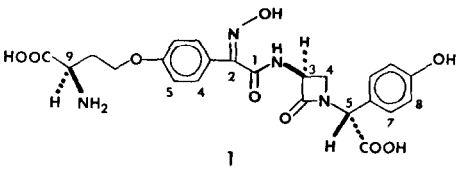
Abstract *Protected seryl peptide 2 was treated under a variety of conditions with triphenylphosphine or triethylphosphite and diethyl azodicarboxylate to give mechanistic insight into the β-lactam forming reaction and an improved route to (−)-3-aminonocardicinic acid (7)*

Mitsunobu, early in his series of papers exploring the scope of intermolecular dehydration reactions caused by the action of triphenylphosphine and diethyl azodicarboxylate (DEAD)[2] examined the coupling of benzamide (pk_a 13-14) and p-nitrobenzamide with n-propanol No N-alkylation was observed in either case, a finding which was attributed to the low acidity of amide hydrogens [3] This conclusion was of concern when peptide 2 (Ft=phthalimido) was to be synthesized with the intention of cyclizing it intramolecularly as a potential biogenetic model for monocyclic β-lactam formation in the antibiotic nocardicin A (1) [4] Biosynthetic



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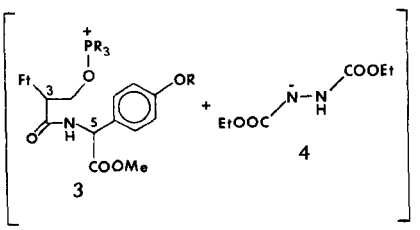
studies had revealed *inter alia* that the three carbons of the β-lactam ring of 1 were derived from L-serine without change in the oxidation state at the β-carbon[5] and later that four-membered ring formation occurred with clean stereochemical inversion at this center [6] In fact, as reported previously,[4] treatment of protected peptide 2 with 2 5 equivalents of PPh_3/DEAD in dry THF remarkably proceeded within 15 min at room temperature to afford a 2 1 mixture of the diastereomers 5a and 6a Debenzylation and fractional crystallization readily afforded optically pure 5b (i e epimerization had occurred only at C-5 in the cyclization[7,8]) The latter, by published procedures,[8] could be fully deprotected to (−)-3-aminonocardicinic acid (7), the structural element common to all members of the nocardicin family In this Letter further observations are made on the use of the Mitsunobu reagent in this cyclization and



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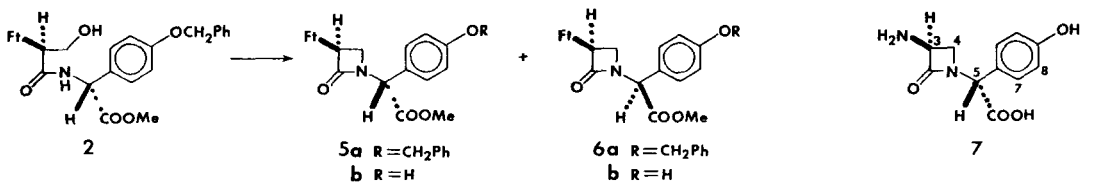


2 5a R=CH_2Ph b R=H 6a R=CH_2Ph b R=H 7

modified conditions are reported which afford specific transformation of peptide 2 to β-lactam product 5 having diastereomeric purity of >50 1

First, the intramolecular dehydration of 2 was examined as a function of varying amounts of PPh_3/DEAD under otherwise constant conditions for 15 minutes The results of these experiments are summarized in Table 1 Contrary to our first impressions published in an earlier communication,[4] when the distribution of products was examined after silica gel chromatography by FT-NMR, trace to modest amounts of dehydroalanylpeptide 8[9] were detectable (7% under the originally reported conditions, 2 5 equivalents) With the exception of 1 0 equivalent of Mitsunobu reagent, all reactions went to completion as judged by the disappearance of 2 by analytical TLC (silica, ethyl acetate hexanes 3 2) Interestingly, the ratio of 5 6 was essentially unchanged at 2 1 but sharply increasing amounts of dehydropeptide 8 were observed as the amount of PPh_3/DEAD was decreased

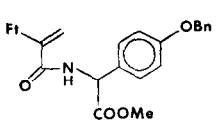
Table 1 Proportion of dehydropeptide 8 relative to β-lactams 5a and 6a on reaction of dipeptide 2 (50 mM) with PPh_3/DEAD in dry THF for 15 min at room temperature

equivalents PPh_3/DEAD	extent of reaction	8 in total product mixt [a]	ratio[a] 5a 6a
1 0	90-95%	23%	2 1 1
1 5	100%	16%	1 9 1
1 8	100%	12%	2 0 1
2 5	100%	7%	2 0,1
4 0	100%	3%	1 8 1
5 0	100%	<2%	2 0 1

[a]Determined by integration of ^{1}H-NMR spectra (80MHz, $CDCl_3$)

The following further observations were made In the 1 0 equivalent reaction cited in Table 1 a small amount of unreacted dipeptide was recovered by preparative TLC, crystallized once and found to have melting point and ^{1}H-NMR spectrum identical to that of optically pure starting material 2 [4] In a separate reaction using 2 5 equivalents of Mitsunobu reagent and quenching after 15 min with deuterium oxide, ^{1}H-NMR analysis showed no detectable deuterium incorporation into 5 or 6 Lastly, pure 5a, obtained as described below, was treated with 2 5 equivalents of PPh_3/DEAD in THF for 15 min The ^{1}H-NMR spectrum of the isolated β lactam product(s) showed that the 2 1 ratio of 5 6 observed for the reactions in Table 1 had been reestablished, a result also obtained, albeit more slowly, in $CDCl_3$ in the presence of a small amount of triethylamine

In sum these findings suggest that if deprotonation occurs at C-3 in 2, elimination rather than epimerization is the result as 5b was obtained optically pure [4] Hence, epimerization must take place only at C-5 but not in the peptide itself prior to cyclization The 2 1 ratio of 5 6 in all reactions including exposure of pure 5a to the Mitsunobu conditions or triethylamine implies that this is the thermodynamic mixture at C-5, an equilibrium which is rapidly achieved in the β-lactam-forming reaction itself and not on workup as shown by the absence of deuterium incorporation upon quenching



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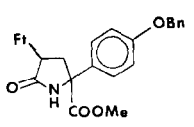

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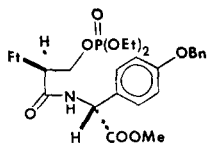

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Replacing triphenylphosphine with triethylphosphite, the course of the cyclization was significantly altered First, in a series of reactions with 2 5 equivalents of $P(OEt)_3$/DEAD under conditions of temperature and concentration employed above, the rate of closure was markedly slower requiring about 45 min to go to completion but the diastereomeric ratio of products was considerably more favorable with regard to the desired 5 accompanied by 9% or less of a by-product which we believe to be γ-lactam 9[10] but no detectable dehydropeptide 8 As shown in Table 2, continued exposure to excess $P(OEt)_3$/DEAD very gradually reduced the ratio of 5 6 from 6 8 1 to 6 5 1 and finally 3 1 1 after 161 hr Assuming the approach to the 2 1 equilibrium mixture would be a first-order process,[11] it is clear that epimerization of the β-lactam products alone would not account for the extent of epimerization observed after 1 hr at C-5 However, bearing in mind that peptide 2 was recovered without loss of optical purity, the implication of these kinetic considerations is that a relatively substantial degree of epimerization must occur in an intermediate(s) which is effectively not formed reversibly from 2 but which preceeds closure to β-lactam

Table 2 Product composition of β-lactams 5 and 6 on reaction of dipeptide 2 (50 mM) with $P(OEt)_3$/DEAD in dry THF at room temperature for the times shown

equivalents $P(OEt)_3$/DEAD	reaction time (hr)	extent of reaction	ratio[a] 5a 6a
2 5	1	100%	6 8 1
2 5	16	100%	6 5.1
2 5	161	100%	3 1 1
1 0	4	90-95%	>50 1
1 0	15	90-95%	~50 1[b]

[a]Determined by integration of the ^{1}H-NMR spectrum (80MHz, $CDCl_3$) [b]See text

Direct evidence for such an intermediate was encountered when the cyclization of 2 was carried out with 1 0 equivalent of $P(OEt)_3$/DEAD While monitoring progress of the reaction by TLC, a new, weak lower R_f spot was observed which grew fainter as peptide disappeared over time Rapid removal of the solvent from a 100 mg scale reaction and preparative TLC of the residue yielded a small sample of the transient intermediate ^{1}H-NMR analysis at 300MHz of this material gave the spectrum of a single diastereomer quite similar to that of protected dipeptide 2 However the ABX spin system for the C-3 and C-4 hydrogens of the seryl residue was shifted to lower field and the AB portion (C-4 methylene) was split into 14 lines rather than the expected maximum of eight Extraction of the coupling constants was easily carried out to disclose interaction with a fourth spin (J=7 9Hz) Signals for ethoxy ligands were visible upfield and led to structure 10 for the isolated species which we propose is derived from the true intermediate 3 (R´=OEt) by Arbuzov or Perkow-like reaction on exposure to silica gel This structural assignment was further substantiated by examination of the $^{13}C\{^1H\}$-NMR spectrum which contained doublets for the ethylphosphate methyl (δ16 0, $^3J_{P-C}$=6 4Hz) and methylene (δ63 5, $^2J_{P-C}$=5 9Hz), with C-4 appearing at δ64 1($^2J_{P-C}$=6 3Hz) Treatment of dipeptide 2 with 0 9 equivalent of sodium hydride and diethyl chlorophosphate (THF, -23° → 25°) afforded a sample of 10 whose ^{1}H-NMR spectrum[12] and R_f on TLC were identical to those obtained for the isolated intermediate above This reaction also produced ca 10% of the diastereomeric phosphate triester presumably arising from epimerization at C-5

Of notable practical value, when the reaction of 2 in the presence of 1 0 equivalent

was allowed to run until the disappearance of starting material had ceased (Table 2), ^{1}H-NMR analysis of the product mixture showed 5a as the sole β-lactam product (to the limits of detection), <3% of byproduct 9 and no dehydropeptide 8. Debenzylation and crystallization from ethanol gave optically pure 5b identical with material obtained earlier [4] The obtention of β-lactam as a single diastereomer was consistent with the isolation of 10 as one diastereomer, but we suggest that in the presence of excess cyclizing reagent that epimerization takes place at C-5 in 3 (R´=OEt) prior to ultimate closure to β-lactam That anion formation occurs at C-5 in 3 more readily than in 5/6 perhaps can be accounted for in the fact that a zwitterion is formed in the former and that some small amount of cyclization to 9, the observed byproduct, does take place

In conclusion, the cyclization of 2 to β-lactam product(s) under Mitsunobu conditions appears to take place in the expected fashion[2] via an intermediate as 3 (R´=OEt or Ph), although the selectivity of the transformation is remarkable given the comparative acidities of H-3 and H-5 The comparatively slower rate of reaction with triethyl phosphite may derive from its lower nucleophilicity and hence a lesser tendency to form the initial DEAD adduct This factor may further account for the significantly slower approach to equilibrium of the β-lactam products 5 and 6 on exposure to excess phosphite as opposed to phosphine reagent Of importance too may be the relative leaving group abilities of the positively charged phosphorus species generated in 3, i e $-OP^+(OEt)_3$ is better and β-lactam closure may compete more effectively with C-5 epimerization for available Mitsunobu base 4. Returning to Mitsunobu's original observation that intermolecular reaction of amide and primary alcohol failed,[3] the success of this intramolecular cyclization, apparently in a nonaberrant manner as evidenced above, suggests a unique mechanistic situation which will be explored further elsewhere

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9 An authentic sample of 8 was prepared by mesylation of 2 (MsCl, Et_3N, CH_2Cl_2) and elimination (Et_3N, CH_2Cl_2), ^{1}H-NMR (80MHz, $CDCl_3$) δ3 74 (s, 3H), 5 04 (s, 2H), 5 58 (d, J=6 7, 1H, H-5), 5 85 (d, J=1 4, 1H, vinyl), 6 24 (d, J=1 4, 1H, vinyl), 6 95 (d, J=8 9, 2H), 7 32 (d, J=8 9, 2H), 7 38 (brs, 5H), 7 82 (sym m, 4H, Ft)
10 This assignment is based on the appearance of a diagnostic broad triplet at δ5 95 (J~5Hz) corresponding to H-3 and a multiplet at δ1 9-2 1 for the C-4 methylene hydrogens
11 A A Frost and R G Pearson, "Kinetics and Mechanism," 2nd edn , Wiley, New York (1952)
12 (300MHz, $CDCl_3$) δ1 25 (br q, J ~ 6 5, 6H), 3 69 (s, 3H), 4 02 (br quintet, J ~ 7 5, 4H), 4 65-4 81 (14 lines centered at 4 71, ABX-P, J_{AX}=6 1, J_{BX}=8 2, J_{AB}=11 2, $^3J_{P-H}$=7 9, 2H, H-4), 5 04 (s, 2H), 5 17 (ABX as dxd, J=6 1, 8 2 1H, H-3), 5 49 (d, J=7 0, 1H, H=5), 6 94 (AA´XX´, J_{app}=8 5, 2H, H-8), 7 27 (AA´XX´, J_{app}=8 5, 2H, H-7), 7 3-7 45 (br m, 5H), 7 75-7 77 (4 lines 2H, Ft), 7 88-7 91 (4 lines, 2H, Ft)

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