# Total Syntheses of (-)-Nocardicins A-G: A Biogenetic Approach

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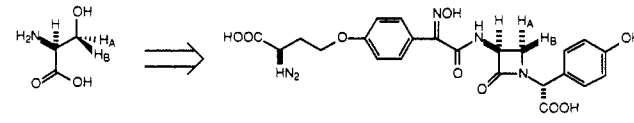
**Abstract:** The known nocardicins A-G have been synthesized, several for the first time, having geometric and stereoisomeric purities of a high order. The syntheses proceed through the central intermediacy of *tert*-butyl (-)-3-aminocardicinate (**14**), which has been prepared in a biogenetically patterned, modified Mitsunobu cyclodehydration reaction of a protected L-seryl-D-(*p*-hydroxyphenyl)glycine dipeptide **12**. An overall 54% yield from the precursor amino acids was achieved with complete stereochemical control at the labile C-5 position of **14**. Mixed anhydride coupling of **14** to *N*-(*tert*-butoxycarbonyl)-D-(*p*-hydroxyphenyl)glycine (**15**) and deprotection afforded nocardicin G (**7**), which was selectively converted to nocardicin F (**6**, anti-oxime 15:1) through the action of 30% hydrogen peroxide in the presence of a catalytic amount of sodium tungstate, or to nocardicin E (**5**, syn-oxime 20:1) in a transamination protocol and reaction with hydroxylamine. Methods were developed to prepare the aryl alkyl ether side-chain components **29-31** in high yield to enable the efficient synthesis of nocardicins A-D. Nocardicin A, for example, was prepared in an overall 22% yield from L-serine and D-(*p*-hydroxyphenyl)glycine.

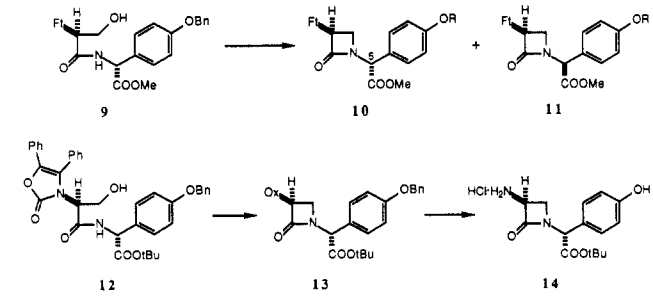
Nocardicin A (**1**) is the paradigmatic monocyclic β-lactam antibiotic. It is produced by the Actinomycete *Nocardia uniformis* ssp. *tsuyamanensis* together with six related but less abundant metabolites, nocardicins B-G (**2-7**, Chart I).[1,2] These structures may be viewed as *N*-acyl derivatives of (-)-3-aminonocardicinic acid (**8**, 3-ANA), differing by the presence or absence of a D-3-amino-3-carboxypropyl segment and by the nature of the substituent at C-2'.

Of the seven members of this antibiotic family, total syntheses of nocardicins A (**1**), B (**2**), and D (**4**) have been reported.[3-6] All published approaches have relied on reaction of the α-keto amide nocardicin D with hydroxylamine to give a separable mixture of isomeric oximes (**1** and **2**) favoring nocardicin A. Similarly, many syntheses of 3-ANA (**8**) and its protected versions have been reported, both in racemic[7,8] and optically active forms.[9-14] In this paper are described complete syntheses of all the known nocardicins in stereoisomerically pure form, several for the first time, and stereochemical ambiguities remaining in the original isolation and structure proof are resolved.[15] The availability of these materials has been essential to studies of the biosynthetic relationships among these metabolites[16,17] and to examination of the central issue of monocyclic β-lactam formation in vivo. Where comparisons can be made to earlier nocardicin syntheses, significant improvements have been achieved in stereocontrol and overall yield, in part through choice of starting materials and protecting groups, but more significantly in successfully developing methods (a) to control the labile C-5 stereocenter during β-lactam formation and through subsequent steps of the syntheses, (b) to selectively accomplish syn- or anti-oxime formation at C-2' and, (c) through a modified Mitsunobu coupling procedure, to obtain reproducibly high yields of aryl alkyl ethers. Finally, at a fundamental level, the parallel interplay of biosynthetic insights into this natural product series and the evolution of these syntheses will be illustrated such that (-)-nocardicin A could be prepared in 22% overall yield from serine and (*p*-hydroxyphenyl)glycine, its biosynthetic precursors.[18]

**Scheme I**



**Scheme II**



**(-)-3-Aminonocardicinic Acid.** The commonality of the 3-ANA structural unit led us, as it had earlier workers,[3-6] to the decision[19] that an efficient, convergent synthesis of the nocardicins could be attained best by condensing an appropriately protected de-

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(11) Townsend, C. A.; Nguyen, L. T. *J. Am. Chem. Soc.* **1981**, *103*, 4582-4583.

(12) Hatanaka, M.; Noguchi, N.; Ishimaru, T. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 1234-1237.

(13) Overman, L. E.; Osawa, T. *J. Am. Chem. Soc.* **1985**, *107*, 1698-1701.

(14) Hodgson, S. T.; Hollinshead, D. M.; Ley, S. V.; Low, C. M. R.; Williams, D. G. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2375-2381.

(15) A preliminary account of a portion of this work describing syntheses of (-)-nocardicins A and G has been published: Townsend, C. A.; Salituro, G. M.; Nguyen, L. T.; DiNovi, M. J. *Tetrahedron Lett.* **1986**, *27*, 3819-3822.

(16) Townsend, C. A.; Wilson, B. A. *J. Am. Chem. Soc.* **1988**, *110*, 3320-3321.

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(19) It is interesting to note that, while this decision was an obvious one from the point of view of synthetic organic chemistry, subsequently we were to discover that the biochemical solution with respect to the timing of this bond connection is quite different.[16]

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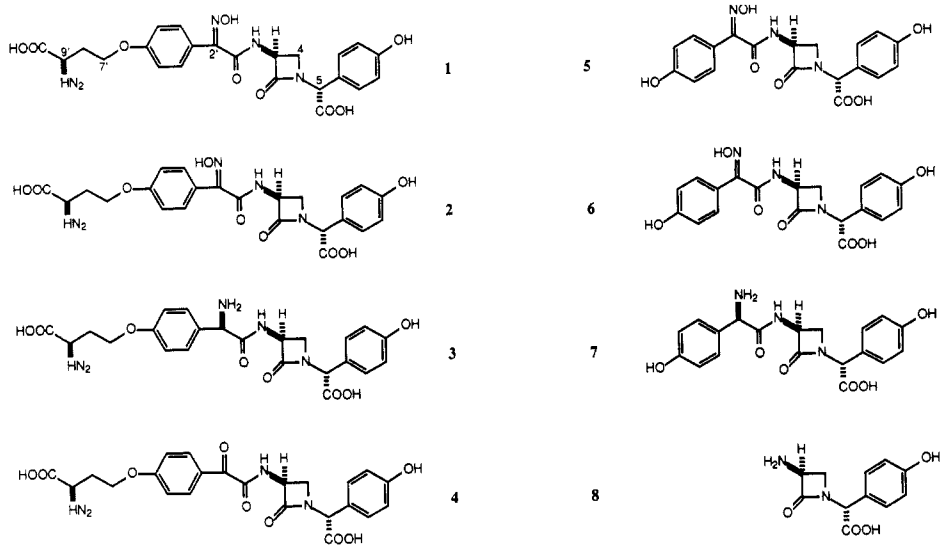
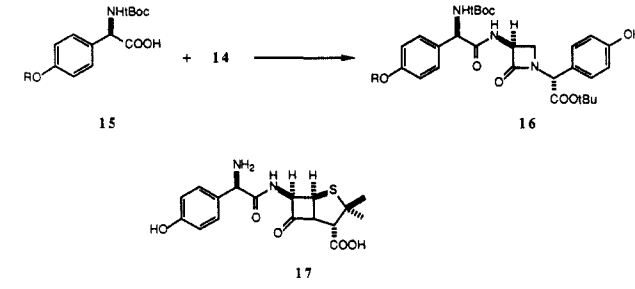
**Chart I**



rivative of (−)-3-aminonocardicinic acid (**8**) with various side-chain residues in a peptide bond forming reaction. The first experimental objective, therefore, was the preparation of 3-ANA in a form suitable for elaboration in this manner. Our approach was guided strongly by biogenetic considerations. Earlier experiments had demonstrated that the monocyclic β-lactam ring of nocardicin A is derived from L-serine.[18] Detailed examination of the key N–C-4 bond-forming process in vivo revealed that no change in oxidation state occurred at the seryl β-carbon and that clean stereochemical inversion was observed[20,21] at this center (Scheme I). Presuming the intermediacy of a peptide precursor, in keeping with what was then known about penicillin biosynthesis,[22] the simplest interpretation[20,21] of these observations was an intramolecular $S_N2$ displacement by amide nitrogen of the seryl hydroxyl, after activation of the latter in some way, possibly by phosphorylation. To explore the feasibility of such a proposal in a model system, the dipeptide **9** (Ft = phthalimido) was prepared and found to cyclize[23] in 15 min at room temperature to a 2:1 mixture of the monocyclic β-lactams **10** and **11** (R = $CH_2Ph$) under standard Mitsunobu conditions (triphenylphosphine/diethyl azodicarboxylate).[11] Removal of the benzyl groups by hydrogenolysis then allowed the biochemically correct L,D diastereomer **10** (R = H) to be isolated by fractional crystallization in 40–45% yield. While from the perspective of biomimicry this was a pleasing and mechanistically interesting result, from the point of view of preparative organic chemistry the failure to control the labile C-5 stereocenter was disappointing. That this difficulty had plagued previous nocardicin syntheses as well gave impetus to further investigation of the Mitsunobu reaction in the hope of finding a solution to this problem. As described more fully elsewhere,[21,24] these studies were ultimately successful in leading to the simple substitution of triethyl phosphite for triphenylphosphine to achieve cyclodehydrations of the model dipeptide **9** to >50:1 ratios of **10** to **11** (Scheme II). Unfortunately, but not unexpectedly, various demethylation[4,25] and dephthaloylation[4,26] protocols in our hands

**Scheme III**



neither afforded 3-aminonocardicinate (**8**) in synthetically useful amounts nor left uncompromised the carefully maintained integrity of the C-5 stereocenter.

The success of the Mitsunobu closure to form the β-lactam ring depended upon bidentate ligation of the seryl nitrogen to prevent competing aziridine formation in the cyclodehydration step. After surveying several potential nitrogen protecting groups,[27] an effective solution to the problems associated with deprotection of **10** (R = H) was provided by replacing the phthalimide with Sheehan's 4,5-diphenyl-4-oxazolin-2-one (Ox)[28] group and protecting the carboxyl as its *tert*-butyl ester. The known[28a] Ox-protected L-serine as its dicyclohexylammonium (DCA) salt and the *tert*-butyl ester of D-[*p*-(benzyloxy)phenyl]glycine,[4] isolated and purified as its *p*-toluenesulfonate salt, were coupled in the presence of dicyclohexylcarbodiimide/1-hydroxybenzotriazole hydrate (DCC/1-HBT)[29] to give **12** in 95% isolated yield. Cyclization of the dipeptide essentially as for **9** proceeded smoothly to afford the diastereomerically pure β-lactam **13** as a crystalline solid, mp 158–158.5 °C, in 84% yield after flash chromatography on silica gel. Differential deprotection of **13** was carried out by hydrogenation in the presence of 1 equiv of HCl to give *tert*-butyl (−)-3-aminonocardicinate hydrochloride (**14**) in 89% yield, $[\alpha]_D^{22}$ = −148.5°. The overall yield of **14** from L-serine was 54%.

(20) Townsend, C. A.; Brown, A. M. *J. Am. Chem. Soc.* **1982**, *104*, 1748–1750.
(21) Townsend, C. A.; Brown, A. M.; Nguyen, L. T. *J. Am. Chem. Soc.* **1983**, *105*, 919–927.
(22) Aberhart, J. D. *Tetrahedron* **1977**, *33*, 1545–1559. Queener, S. W.; Neuss, N. In *The Chemistry and Biology of β-Lactam Antibiotics*; Morin, R. B., Gorman, M., Eds.; Academic Press: New York, 1982; pp 1–81.
(23) This remarkably facile intramolecular cyclization process was not predictable a priori, indeed was unexpected, given the failure of even quite acidic benzamides to analogously react intermolecularly with 1-propanol: Wada, M.; Mitsunobu, O. *Tetrahedron Lett.* *13*, 1279–1282. Recognition of this fact led Miller to investigate related β-latam-forming reactions based on the more acidic seryl *O*-alkyl hydroxymates: Miller, M. J. *Acc. Chem. Res.* **1986**, *19*, 49–56.
(24) Townsend, C. A.; Nguyen, L. T. *Tetrahedron Lett.* **1982**, *23*, 4859–4862.
(25) Williams, D. R.; Sit, K.-Y. *J. Org. Chem.* **1982**, *47*, 2846–2851. Jung, M. E.; Lyster, M. A. *J. Org. Chem.* **1977**, *42*, 3761–3764. Felix A. M. *J. Org. Chem.* **1974**, *39*, 1427–1429.
(26) Kukolja, S.; Lammert, S. R. *J. Am. Chem. Soc.* **1975**, *97*, 5582–5583. Osby, J. O.; Martin, M. G.; Ganem, B. *Tetrahedron Lett.* **1984**, *25*, 2093–2096.
(27) Barany, G.; Merrifield, R. B. *J. Am. Chem. Soc.* **1977**, *99*, 7363–7365. Djuric, S.; Venit, J.; Magnus, P. *Tetrahedron Lett.* **1981**, *22*, 1787–1790. Matsumura, E.; Kobayashi, H.; Nishikawa, T.; Ariga, M.; Tohda, Y.; Kawashima, T. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 1961–1965.
(28) (a) Sheehan, J. C.; Guziec, F. S. *J. Am. Chem. Soc.* **1972**, *94*, 6561–6562. Sheehan, J. C.; Guzeic, F. S. *J. Org. Chem.* **1973**, *38*, 3034–3040. (b) Miller has also made extensive use of the Ox protecting group in β-lactam synthesis. See for example: Miller, M. J.; Mattingly, P. G. *Tetrahedron* **1983**, *39*, 2563–2570.
(29) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 2034–2040.

Attempts to reduce the time required for deprotection by using higher pressure (500 psi) led to epimerization at C-5. Various procedures for catalytic transfer hydrogenation were unsuccessful.[30]

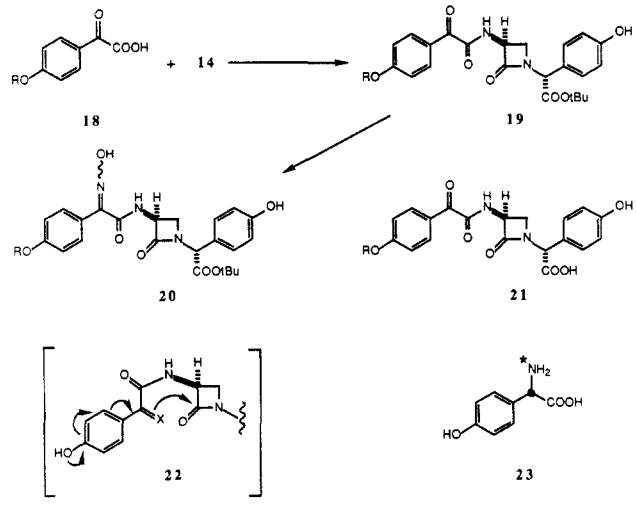
**Nocardicin G.** Nocardicin G (**7**) is the simplest structurally of the nocardicins and was chosen as the first test of the synthetic plan. Initially it was thought that protection of the terminal phenols would be desirable to enhance the yield of the envisioned peptide coupling reaction. Therefore, *N*-(*tert*-butoxycarbonyl)-D-[*p*-benzyloxy)phenyl]glycine (**15**, R = $CH_2Ph$) was prepared for reaction with *tert*-butyl 3-aminocardicinate (**14**), after silylation of the phenol (Scheme III). *N*-(Ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline (EEDQ),[31] which had been used in other nocardicin syntheses,[5,32] gave a disappointing 25% yield of the amide. A standard mixed anhydride procedure used successfully by Kamiya[4] in a related context gave a 10% yield. DCC/1-HBT gave a 15% yield and, as also seen in the above reactions, varying amounts of epimerization, presumably at C-5, were observed in the 400-MHz $^1H$ NMR spectra of **16** (R = $CH_2Ph$).

However, applying Naylor's method[33] to prepare 6-[D-$\alpha$-amino-(*p*-hydroxyphenyl)glycyl]penicillanic acid (**17**), where the free phenol of N-protected D-(*p*-hydroxyphenyl)glycine was reacted, **15** (R = H) and **14** were condensed to give a 34% yield of **16** with no epimerization detectable by high-field NMR spectroscopy. Systematic modification of the reaction conditions and use of 1.5 equiv of the mixed anhydride of **15** (R = H) afforded **16** (R = H) in a respectable 60–65% yield. Deprotection in trifluoroacetic acid (TFA)/anisole proceeded in 90% yield (55% from **14**) after crystallization from water and acetone. This material displayed physical and spectral properties in excellent agreement with those reported for the natural product.[2] The proposed[2] absolute configuration of the *N*-acyl side chain was, therefore, securely established as D. The stereoisomeric purity of the synthetic **7** was determined by HPLC analysis to be >99.95%.

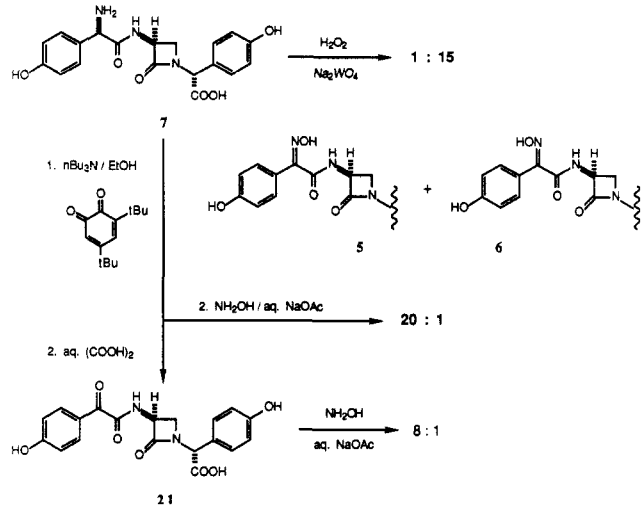
**Nocardicins E and F.** Nocardicins E/F and A/B are pairs of oxime isomers. No syntheses of the former have been reported heretofore, and all published approaches to nocardicin A have involved oximination of the corresponding $\alpha$-keto amide to give a mixture of configurational isomers favoring the syn-oxime of nocardicin A.[3-6] It was the failure of this approach in an opening attempt to prepare nocardicins E and F, as briefly described below, that led to further consideration of this problem and ultimately to an effective solution.

The initial objective, therefore, was to prepare the $\alpha$-keto amide **21** (R = H, nocardicin "H") for reaction with hydroxylamine to give **5** and **6**. (*p*-Hydroxybenzoyl)formate (**18**, R = H), commercially available as its sodium salt, was treated with ethyl chloroformate and 2,6-lutidine and reacted with *tert*-butyl 3-aminonocardicinate (**14**) to give **19** (R = H) in 64% yield. Unexpectedly, all attempts to remove the *tert*-butyl blocking group (TFA/anisole, 4 N HCl/dioxane, and several other methods[25,34]) resulted in complex reaction mixtures whose $^1H$ NMR spectra lacked the AMX spin system characteristic of the monocyclic $\beta$-lactam hydrogens. Reaction of **19** first with hydroxylamine to give **20** (75% yield) followed by deprotection similarly gave multiple products in which the $\beta$-lactam ring had been hydrolyzed.

**Scheme IV**




**Scheme V**




It is not clear why a deblocking protocol that worked well in the synthesis of nocardicin G (**7**) should fail completely in the analogous deprotections to nocardicins E (**5**) and F (**6**). Direct participation of the side-chain 2'-carbonyl or oxime in opening of the $\beta$-lactam ring would require a presumably unfavorable cis-amide, e.g., **22** (X = O, NOH; Scheme IV). Other means of activation toward hydrolysis of the four-membered ring could be imagined as well. Nonetheless, when the *p*-nitrobenzyl ether of **19** [R = *p*-$CH_2C_6H_4NO_2$ (PNB); chosen to reduce the electron-donating ability of the *p*-hydroxyl group] was treated with TFA/anisole, removal of the *tert*-butyl group could be readily carried out to give **21** (R = PNB). While this protection/deprotection scheme would have given a viable route to the desired $\alpha$-keto amide **21** (R = H) after hydrogenolysis, it added two steps to the synthesis and it still left unresolved the issue of selective syn- or anti-oxime formation.

The faltering promise of this synthetic plan brought our thoughts back to earlier observations about the biochemical formation of the oxime present in nocardicin A. Incorporation of 2-[$^{13}C$,$^{15}N$](*p*-hydroxyphenyl)glycine (**23**) into the antibiotic had demonstrated that the 2'-carbon/nitrogen bond is not broken in the course of this transformation, suggesting amine oxidation as the source of the oxime in vivo.[35] While numerous methods for the conversion of amines to carbonyl compounds have been developed,[36,37] very few are known for the direct oxidation of

(30) El Amin, B.; Anatharamaiah, G. M.; Roger, G. P.; Means, G. E. *J. Org. Chem.* **1979**, *44*, 3442–3444. Anwer, M. K.; Spatola, A. F. *Synthesis* **1980**, 929–932. Anatharamaiah, G. M.; Sivanandaiah, K. M. *J. Chem. Soc., Perkin Trans. 1* **1977**, 490–491. Jackson, A. E.; Johnstone, R. A. W. *Synthesis* **1976**, 685–587. Felix, A. M.; Heimer, E. P.; Lambros, T. J.; Tzougraki, C.; Meienhofer, J. *J. Org. Chem.* **1978**, *43*, 4194–4196.

(31) Belleau, B.; Malek, G. *J. Am. Chem. Soc.* **1968**, *90*, 1651–1653.

(32) Hakimelahi, G. M.; Just, G. *Can. J. Chem.* **1979**, *57*, 1932–1938. Hakimelahi, G. M.; Just, G. *Ibid.* **1979**, *57*, 1939–1944. Atherton, F. R.; Lambert, R. W. *Tetrahedron* **1983**, *39*, 2599–2608.

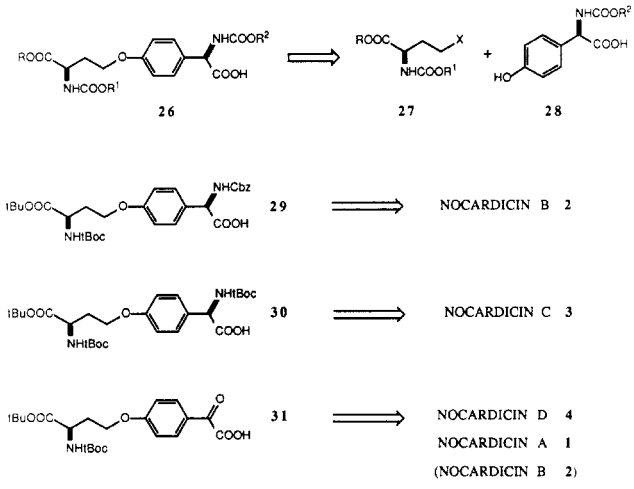
(33) Hakimelahi, G. M.; Just, G. *Can. J. Chem.* **1979**, *57*, 1932–1938. Hakimelahi, G. *M.*; Just, G. *Can. J. Chem.* **1979**, *57*, 1939–1944. Atherton, F. R.; Lambert, R. W. *Tetrahedron* **1983**, *3.*, 2599–2608. Long, A. A. W.; Nayler, J. H. C.; Smith, H.; Taylor, T.; Ward, N. *J. Chem. Soc.* **1971**, 1920–1922.

(34) Chandrasekaran, S.; Kluge, A. F.; Edwards, J. A. *J. Org. Chem.* **1977**, *42*, 3972–3974. Yamada, H.; Tobiki, H.; Tanno, H.; Suzuki, H.; Jimpo, K.; Ueda, S.; Nakagome, T. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 3333–3334.

(35) Townsend, C. A.; Salituro, G. M. *J. Chem. Soc., Chem. Commun.* **1984**, 1631–1632.

(36) Corey, E. J.; Achiwa, K. *J. Am. Chem. Soc.* **1969**, *91*, 1429–1432.

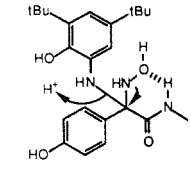
**Scheme VI**



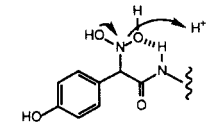

amines to oximes. The Kahr–Berther procedure,[38] however, using hydrogen peroxide in the presence of catalytic amounts of sodium tungstate, held promise to generate the oxime in conjugation with an electron-rich aromatic ring. Exposure of nocardicin G (**7**) to reaction conditions described for the conversion of benzylamine to benzaldehyde oxime (64% yield) resulted only in slow degradation to unidentifiable products. However, upon addition of 1 equiv of sodium bicarbonate to ensure deprotonation of the amine, the course of the reaction changed markedly where slow degradation was accompanied by faster formation of less polar oxime-containing products in a 15:1 ratio. Inter alia the chemical shift of the amide hydrogen is particularly diagnostic of the oxime configuration. Hydrogen bonding to the oxime oxygen in the syn geometry present in nocardicin E (**5**) and A (**1**) causes this hydrogen to appear at lower field ($\Delta\delta$ ca. 0.2 ppm) than in the corresponding anti configuration present in nocardicins F (**6**) and B (**2**).[1,2] On this basis the major isomer was shown to be nocardicin F (Scheme V). While unanticipated from what was undertaken as a biogenetically modeled route to the predominant syn-oxime in vivo, to observe anti selectivity was fortunate from the point of view of the developing syntheses. Under optimized conditions a 72% yield of nocardicin F (**6**) could be achieved in an oxidation of nocardicin G (**7**) with 20 equiv of 30% hydrogen peroxide and 8 mol % sodium tungstate.

Syn-selective oxime formation had been observed in earlier syntheses of nocardicin A (**1**) from nocardicin D (**4**) on reaction with hydroxylamine.[3-6] Treatment of the ethanol-soluble tributylammonium salt of nocardicin G (**7**) with Corey's transamination reagent, 3,5-di-*tert*-butyl-*o*-benzoquinone[36] followed by hydrolysis of the intermediate imine in aqueous oxalic acid, gave the corresponding $\alpha$-keto amide **21** (Scheme V). Reaction with hydroxylamine proceeded in good yield (89%) as expected to a mixture of nocardicins E (**5**) and F (**6**) in an 8:1 ratio typical of analogous transformations to nocardicins A and B.[3-6] It was known, however, from considerably earlier studies of Cordes and Jencks[39] that aniline catalyzes oxime formation. On this basis the imine intermediate itself, formed in the transamination reaction, was reacted directly with buffered aqueous hydroxylamine to give an equally good yield of oxime-containing products but now favoring the syn isomer, nocardicin E (**5**), by 20:1 (Scheme V).

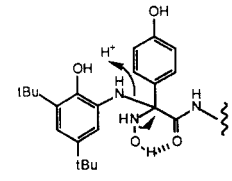
The configurational selectivity of the oxime-forming reactions deserves comment. Almost certainly the syn selectivity of the hydroxylamine additions stems from intramolecular hydrogen-bonding interactions to the adjacent amide carbonyl or NH, as illustrated in **24A** and **24B**. These effects may be amplified for



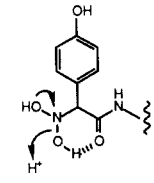

**24A**




**25A**




**24B**




**25B**

the imine of 2-hydroxy-3,5-di-*tert*-butylaniline owing to considerable steric compression between this and the *p*-hydroxyphenyl substituent in the obligatory tetrahedral intermediate **24A/B** leading to the high 20:1 ratio of syn product. In contrast, the mechanism of the tungstate-catalyzed amine oxidation is not known.[40] Presuming first oxidation to the corresponding hydroxylamine and then possibly to the *gem*-dihydroxylamine **25A/B**, intramolecular hydrogen-bonding interactions again may intervene to facilitate the elimination of water to give a transient nitroso compound. It may be argued on the basis of the principle of least motion and dipolar repulsions between the nitroso and the adjacent amide carbonyl, that anti-oxime formation may be favored in a rapid tautomerization to the conjugated oxime product. Alternatively, the *gem*-dihydroxylamine **25A/B** may dehydrate directly to the anti-oxime with the hydrogen-bonded hydroxyl being lost preferentially.

(-)-Nocardicins E (**5**) and F (**6**) are widely separated on reverse-phase chromatography. The isomeric oximes for cell-free and enzymatic studies could be readily obtained by preparative HPLC in >99.95% purity. Alternatively, pure nocardicin E was obtained by chromatography on LH-20 eluting with ethanol and crystallization from ethanol/isopropyl ether.

**Nocardicins B and C.** Syntheses of the more complex 3-amino-3-carboxypropyl ether containing nocardicins A–D require the preparation of suitably protected acyl side-chain fragments for coupling to *tert*-butyl 3-aminonocardicinate (**14**). Initially it was envisioned that a common aryl alkyl ether **26** (Scheme VI, top) could be obtained by reaction of a D-homoserine derivative **27** (X = OH or halogen) with protected D-(*p*-hydroxyphenyl)-glycine **28**. Specific choice of the protecting groups R = $R^1$ = *tert*-butyl and $R^2$ = $CH_2Ph$, i.e., compound **29**, would permit differential deprotection of the C-2′ amine by hydrogenolysis, after peptide coupling with *tert*-butyl 3-aminonocardicinate (**14**). Selective manipulation of the 2′-amino function using the chemistry developed above then, in principle, would allow preferential formation of the 2′-keto, or the 2′-syn- or anti-oxime. However, a further and important consideration was the necessity to achieve maximum efficiency for the sake of planned radiochemical and stable-isotope syntheses of these compounds for biochemical study. Notwithstanding, a brief series of attempts was made to convert nocardicin C directly into nocardicins A, B, and D using the methods developed for the conversion of nocardicin G to E and F. Selective reaction of the benzylic 2′-amine versus the 9′-amine could not be achieved. Therefore, to minimize the number of individual steps to nocardicins A–D, side-chain fragment **29** was to be used only in the preparation of nocardicin B (**2**) by the sodium tungstate/hydrogen peroxide method. Nocardicin C (**3**) was to be generated in a single deprotection step after coupling

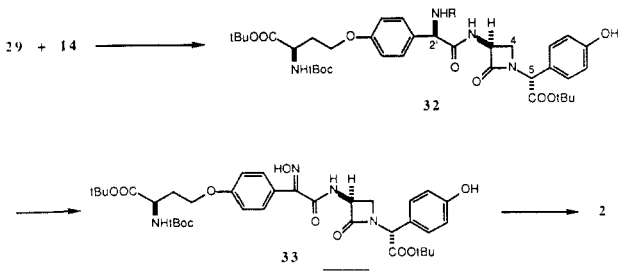
(37) Buckley, T. F.; Rapoport, H. *J. Am. Chem. Soc.* **1982**, *104*, 4446–4450. Ohta, S.; Okamoto, M. *Synthesis* **1982**, 756–758. Hoffman, R. V. *J. Am. Chem. Soc.* **1976**, *98*, 6702–6704. Rawalay, S. S.; Shechter, H. *J. Org. Chem.* **1967**, *32*, 3129–3131. Audette, R. J.; Quail, F. W.; Smith, P. J. *Tetrahedron Lett.* **1971**, *12*, 279–282. Czarny, M. R. *J. Chem. Soc., Chem. Commun.* **1976**, 81. Hoffman, R. V.; Kumar, A. *J. Org. Chem.* **1984**, *49*, 4011–4014. Lee, G. A.; Freedman, H. H. *Tetrahedron Lett.* **1976**, *17*, 1641–1644.

(38) Kahr, K.; Berther, C. *Chem. Ber.* **1960**, *93*, 132–136.

(39) Cordes, E. H.; Jencks, W. P. *J. Am. Chem. Soc.* **1962**, *84*, 826–831.

(40) For an excellent comprehensive overview of inorganic oxygen transfer chemistry see: Holm, R. H. *Chem. Rev.* **1987**, *87*, 1401–1449.

**Scheme VII**



to **30**, and both nocardicins D (**4**) and A (**1**) were to be prepared from **31** (Scheme VI).

*tert*-Butyl *N*-(*tert*-butoxycarbonyl)-L-homoserinate has been synthesized in five steps from L-aspartic acid.[41] The corresponding D antipode **27** (R = R$^1$ = *t*-Bu, X = OH) was prepared with minor alterations of the published procedure to afford the protected amino acid whose specific optical rotation, $[\alpha]_D = +38.3°$ (*c* = 1, EtOH), compared favorably with that reported for its enantiomer, $[\alpha]_D = -37.5°$ (*c* = 1, EtOH).[41] Application then of the method of Hooz and Gilani[42] gave a greater than 90% yield of the corresponding bromide **27** (R = R$^1$ = *t*-Bu, X = Br).

*N*-(Carbobenzyloxy)-D-(*p*-hydroxyphenyl)glycine (**28**, R$^2$ = $CH_2Ph$) was treated with 2 equiv of potassium hydride in DMF and reacted with bromide **27** (R = R$^1$ = *t*-Bu, X = Br) to give an 85% yield of aryl alkyl ether **29** without detectable epimerization at C-2′ as revealed in later steps (vide infra). The presence of the free carboxylate throughout this reaction, as hoped, preserved the sensitive $\alpha$-center and returned this function directly without need for intervening protection/deprotection steps for DCC/1-HBT coupling to *tert*-butyl 3-aminonocardicinate (**14**, Scheme VII). The fully protected nocardicin C derivative **32** (R = $COOCH_2Ph$) was obtained in 88% yield. Hydrogenolysis in the presence of 1.2 equiv of HCl gave the corresponding amine hydrochloride **32** (R = H·HCl). A methanol solution of the latter was reacted with successive additions of 30% hydrogen peroxide in the presence of sodium tungstate and sodium bicarbonate. The anti-oxime **33** was formed as the predominant product together with small amounts of the syn isomer. Flash chromatography on silica gel afforded pure **33** in 58% yield, which was deprotected in TFA/2-mercaptoethanol[43] to give nocardicin B (**2**) in 47% yield after recrystallization from water at pH 2.5. The purified product exhibited spectral and physical properties in accord with those reported for the natural product.[1]

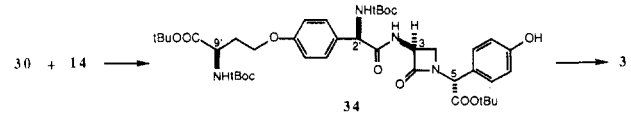
The success and operational efficiency of this sequence is to be contrasted with preliminary experiments in which *N*-(*tert*-butoxycarbonyl)-D-(*p*-hydroxyphenyl)glycine (**28**, R$^2$ = *t*-Bu) was further protected as its benzyl ester. Attempted aryl alkyl ether synthesis using this doubly protected amino acid with **27** (Scheme VI, top) under normal and modified (vide infra) Mitsunobu (with **27**, X = OH) or Williamson (with **27**, X = Br, I) conditions gave substantial epimerization at the base-labile (*p*-hydroxyphenyl)-glycine $\alpha$-center. Approximately 3:2 (D:L) mixtures were commonly encountered at C-2′, as revealed in the subsequent debenzylation/peptide coupling steps to generate **32** (R = COO-*t*-Bu). The presence of C-2′ epimer was readily apparent in the $^1$H NMR spectrum of **32** by the observation of overlapping resonances for H-2′, H-3, and H-4$\alpha$, and most diagnostically useful, the well-separated doublet of doublets corresponding to H-4$\beta$, appearing at ~2.8 ppm for the desired (2′*R*) stereoisomer, was quite distinct from its (2′*S*) epimer, which appeared at ~2.9 ppm. Similarly, the sharp singlet corresponding to H-5 at ~5.3 ppm gave rise to a new signal for this diastereomer at ~0.01 ppm upfield. By way of comparison, the position of the H-5 resonance has also been diagnostic of the sterochemical integrity at C-5. Epimerization here, however, results in a substantially larger

(41) Ramsamy, K.; Olsen, R. K.; Emergy, T. *Synthesis* **1982**, 42–43, and references cited therein.

(42) Hooz, J.; Gilani, S. S. H. *Can. J. Chem.* **1968**, *46*, 86–87.

(43) Lundt, B. F.; Johansen, N. L.; Volund, A.; Markussen, J. *Int. J. Pept. Protein Res.* **1978**, *12*, 258–268.

**Scheme VIII**



upfield shift to H-5 (ca. 0.1 ppm) and marked movements of the H-3, H-4$\alpha$, and H-4$\beta$ resonances[21,24] that are unambiguously distinct from those of a C-2′ epimer. The homoseryl and seryl $\alpha$-centers C-9′ and C-3 respectively, are by contrast far more resistant to racemization/epimerization and presented minimal problems of stereocontrol under the conditions of these syntheses.

In parallel with the synthesis of nocardicin B (**2**), *N*-(*tert*-butoxycarbonyl)-D-(*p*-hydroxyphenyl)glycine (**28**, R$^2$ = *t*-Bu) was condensed with **27** (R = R$^1$ = *t*-Bu, X = Br) in the presence of 2 equiv of potassium hydride to give the acyl side-chain component **30**. Amide bond formation as before (DCC/1-HBT, 83%) gave **34** (Scheme VIII) diastereomerically homogeneous at C-2′ and C-5. After flash chromatography on silica gel, this material was deprotected in TFA/anisole to give nocardicin C (**3**): mp 233–236 °C; $[\alpha]_D = -161°$ (*c* = 0.5, $H_2O$).

A discrepancy exists in the literature with respect to the specific optical rotation of nocardicin C. In the original isolation of the natural product by workers at Fujisawa[2] a notably lower value was reported, $[\alpha]_D = -95°$ (*c* = 0.6, $H_2O$), which has not been revised in subsequent reviews of this work.[44] In the interim a Takeda group has described the isolation and characterization of the formadicins,[45] which appear to be metabolically elaborated forms of nocardicin C. In the course of their structure proof, a stereochemical correlation was made by reduction of nocardicin A to an epimeric mixture of 2′-amines (Scheme IX) which, when separated, gave optical rotations of $[\alpha]_D = -162°$ and $-53.7°$ (*c* = 0.5, $H_2O$). The diastereomer having the greater rotation was separately converted to nocardicin G (**7**), securing, therefore, the C-2′ configuration as D (vide supra). Similarly, the stereoisomer having the lower rotation was converted to epinocardicin G (**36**).[16] Therefore, supported by both the total synthesis above and semisynthesis from nocardicin A,[45] nocardicin C (**3**, DDLD configuration; Scheme IX) has a specific optical rotation of $-161/-162°$, while epinocardicin C (**35**, DLLD configuration) has a rotation of $-53.7°$.[46] It would seem that the lower rotation reported by the Fujisawa group for nocardicin C is either a clerical or typographical error.

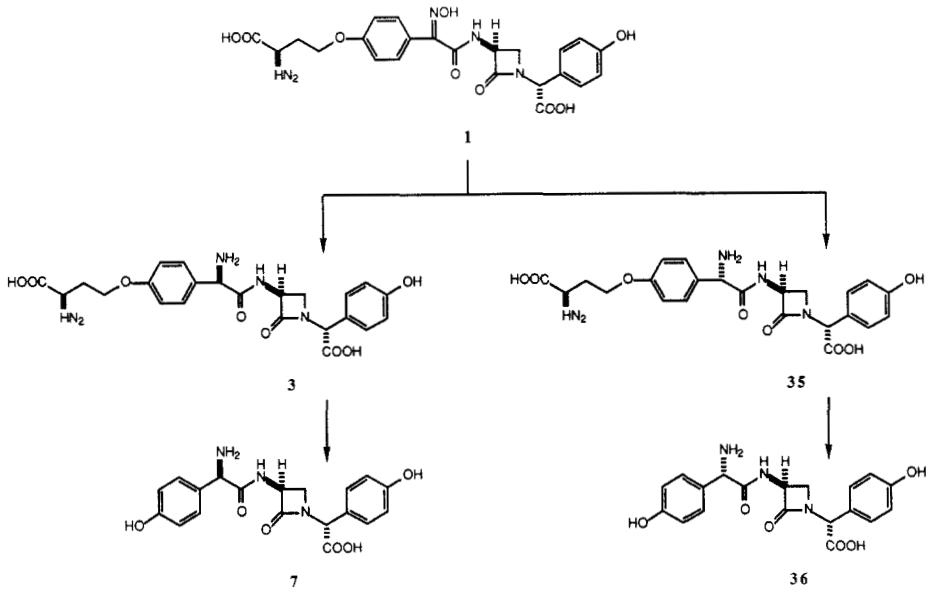
**Nocardicins A and D.** Reaction of (*p*-hydroxybenzoyl)formate sodium salt with dimethyl sulfate in DMSO gave the highly crystalline methyl ester **37**. Condensation of **37** with protected D-homoserine **27** (Scheme X) under standard Mitsunobu conditions[5,6] gave complex product mixtures of which the desired aryl alkyl ether **31** (R = Me, Scheme X) constituted less than 40%. Examination of the other products formed revealed that the symmetrical alkyl alkyl ether of *tert*-butyl *N*-(*tert*-butoxycarbonyl)homoserine accounted for 30–35% of the mass balance, reflecting a common problem in our experience in aryl alkyl ether forming reactions of this kind. Bearing in mind the probable kinetic order of the Mitsunobu reaction, to suppress symmetrical alkyl alkyl ether formation, the concentrations of the homoserine derivative **27** and the coupling reagent were kept low by the slow, simultaneous addition of **27** and diethyl azodicarboxylate by syringe pump. Such an addition carried out over 2 h resulted in a much cleaner reaction from which **31** (R = Me, Scheme X)

(44) See, for example: Kamiya, T.; Aoki, H.; Mine, Y. In *Chemistry and Biology of β-Lactam Antibiotics*; Mortin, R. B., Gorman, M., Eds.; Academic Press: New York, 1982; Vol. 2, pp 165–225.

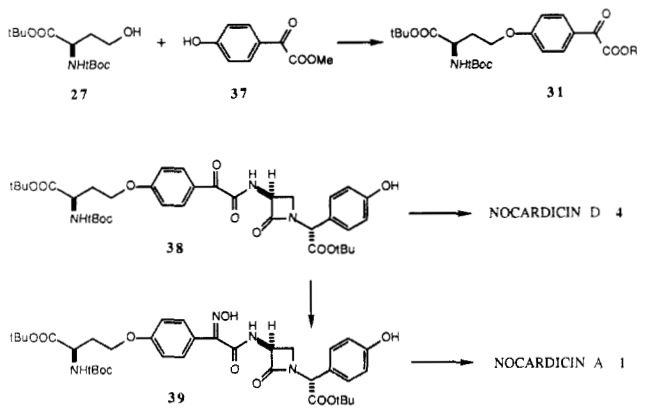
(45) Hida, T.; Tsubotani, S.; Katayama, N.; Okazaki, H.; Harada, S. *J. Antibiot.* **1985**, *38*, 1128–1140.

(46) In other work in this laboratory, isonocardicin C (LDLD-configuration) has been prepared (J. J. Hangeland and C. A. Townsend, unpublished). As nocardicin C was isolated from washed mycelia rather than from the fermentation broth, on the basis of recent cell-free results,[17] it might be expected that nocardicin C is actually a mixture of nocardicin C and isonocardicin C. However, given the specific rotations of the pure diastereomers (and mixtures), this proposal cannot account for the lower rotation reported for nocardicin C by the Fujisawa group.[2]

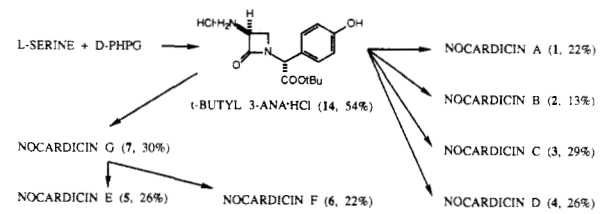
**Scheme IX**



**Scheme X**



**Scheme XI**



could be isolated by flash chromatography on silica gel in 84% yield. Saponification in methanol containing 1.1 equiv of sodium hydroxide readily afforded the free acid **33** (R = H, Scheme X).

Coupling of the $\alpha$-keto acid **31** (R = H, Scheme X) and *tert*-butyl 3-aminonocardicinate (**14**) was best accomplished by using the mixed anhydride method successfully applied earlier in the preparation of nocardicin G (**7**) to afford a 78% yield of **38**, after chromatography on silica gel. Deprotection in TFA/anisole at 0 °C or dilute TFA/anisole in methylene chloride at room temperature gave poor yields of nocardicin D (**4**). However, treatment of **38** at room temperature with TFA/anisole for 6 min followed by rapid removal of the trifluoroacetic acid in vacuo and trituration of the crude product with ether gave a more favorable outcome. Recrystallization of the crude product from water at pH 3 gave nocardicin D (**4**) in 62% yield having physical and spectral properties identical with those reported for the natural product[2] and for material obtained by treatment of nocardicin A with sodium bisulfite.[1]

The preparation of nocardicin A (**1**) directly from nocardicin D (**4**) has been reported in several previous syntheses.[3,4,6] Similarly, oxime formation has been carried out with protected forms of nocardicin D[5] and analogues.[32] In the interest of minimizing exposure of the $\beta$-lactam and the sensitive C-5 position to aqueous base, we elected to treat the protected form of nocardicin D **38** with hydroxylamine in absolute ethanol. A slow but clean transformation was observed to give two principal products in unequal amounts. Careful $^1$H NMR analysis in DMSO-$d_6$ solution showed no extraneous resonances for H-5, H-4$\alpha$, and H-4$\beta$, indicating that the C-2′ and C-5 stereocenters were unaffected. Homonuclear decoupling of H-3 of the major and minor components unambiguously established the identity of the side-chain NH, which for the major component appeared 0.26 ppm downfield from the corresponding signal in the minor isomer. These data suggested formation of the syn- and anti-oxime isomers as the major and minor products, respectively, in the absence of detectable epimerization (vide supra). These protected nocardicin A and B derivatives were isolated in 79% and 10% yields, respectively, by preparative thin-layer chromatography. The minor component was shown to be identical to **33** prepared above (Scheme VII), and treatment of its syn isomer **39** with TFA/mercaptoethanol[43] for 7 min afforded nocardicin A (**1**) in 67% yield, after recrystallization from water at pH 2.5. Subsequently it was found that the mixture of oxime isomer could be deprotected without recourse to separation to give a comparable yield of nocardicin A, after crystallization from water, having spectral properties in complete accord with those of the natural product; $[\alpha]_D = -148°$ ($c$ = 1, 1% $NaHCO_3$) [lit.[1] $[\alpha]_D = -146°$ ($c$ = 1, 1% $NaHCO_3$)].[47]

**Conclusion.** All of the known natural nocardicins A–G have been synthesized, several for the first time, having stereoisomeric purities of a high order. The precursor amino acids in vivo, serine and (*p*-hydroxyphenyl)glycine (PHPG), taken here in their L and D forms, respectively, have been coupled to give a protected seryl-PHPG dipeptide and cyclized in a biomimetic sense by use of a modified Mitsunobu procedure to provide, after deprotection of the phenol and N-terminus, *tert*-butyl (−)-3-aminonocardicinate (**14**) in an efficient 54% overall yield from the starting amino acids. As summarized in Scheme XI, this central intermediate has been joined in peptide bond forming reactions with appropriate side-chain fragments to give the individual nocardicins in the indicated *overall* yields from serine and PHPG. The high overall efficiencies of these syntheses and the maintenance of stereochemical control may be attributed to several developments. Amine oxidation catalyzed by sodium tungstate in the presence of excess hydrogen peroxide permitted selective formation of the anti-oxime char-

(47) It is amusing to note that natural nocardicin A isolated from fermentation broths when analyzed for its stereoisomeric purity contains trace amounts of isonocardicin A.[17]

acteristic of nocardicins F and B, directly from nocardicin G and from a partially protected form of nocardicin C, respectively. Transamination of nocardicin G and reaction with hydroxylamine in turn gave the syn-oxime of nocardicin E selectively, and analogously, addition to a protected form of nocardicin D gave nocardicin A. In the preparation of nocardicins B and C, the dianion of N-protected PHPG was reacted directly with **27** (R = $R^1$ = *t*-Bu, X = Br) to provide the aryl alkyl ethers **29** and **30** (Scheme VI) without detectable epimerization at the benzylic center destined to become C-2′ in the final products. Similarly, in the synthesis of nocardicins A and D, the Mitsunobu coupling of methyl (*p*-hydroxybenzyl)formate (**37**) and the slow addition of *tert*-butyl *N*-(*tert*-butoxycarbonyl)-D-homoserine (**27**, Scheme X) using a syringe pump overcame competing alkyl alkyl ether formation to provide a generally applicable method for the preparation of aryl alkyl ethers in high yield. The availability of these cometabolites of nocardicin A from total synthesis in stereoisomerically pure form(s) has been absolutely essential to investigation of the intermediate and late stages of nocardicin biosynthesis. Initial findings from these studies have been published recently in preliminary form,[16,17] and further results will be reported in due course.

## Experimental Section

**D-*tert*-Butyl-[*p*-(benzyloxy)phenyl]glycine Toluenesulfonate.** In an oven-dried 500-mL Parr pressure bottle D-[*p*-(benzyloxy)phenyl]glycine[48] (10.0 g, 49.4 mmol) was suspended in 100 mL of reagent-grade dioxane and cooled to 0 °C. Concentrated sulfuric acid (10 mL) was added, and the bottle was cooled for 15 min. Isobutylene (100 mL of liquid) was condensed from the gas into a flame-dried 125-mL graduated Erlenmeyer flask cooled to −78 °C and added to the pressure bottle. The bottle was stoppered and shaken for 6 h on a Parr apparatus. The pressure was released and the contents of the flask were quickly poured into an ice-cold mixture of 400 mL of 1 N NaOH and 500 mL of ether. After separation, the aqueous layer was extracted three times with 200-mL portions of ether. The combined ether extracts were washed with 300 mL of brine and dried over anhydrous magnesium sulfate. Filtration and evaporation in vacuo afforded a viscous oil, which was dissolved in 75 mL of anhydrous ether. Addition of an anhydrous magnesium sulfate dried ethereal solution of toluenesulfonic acid monohydrate (9.4 g, 49.4 mmol) precipitated the product (16.25 g, 68%) as a white powder: mp 176–180 °C dec; $[\alpha]_D^{22}$ = −53.8° (*c* = 0.95, MeOH); $^1$H NMR (DMSO-$d_6$) δ 8.59 (3 H, Br s, $^+NH_3$), 7.05–7.48 (13 H, m, Ar), 5.13 (2 H, s, $PhCH_2$), 4.98 (1 H, s, H-2), 2.08 (3 H, s, Ar $CH_3$), 1.38 (9 H, s, *tert*-butyl); IR (KBr) 3200–2500, 1720, 1580, 1475, 1360, 1250-1000 cm$^{-1}$. Anal. ($C_{26}H_{31}NO_6S$) C, H, N.

***tert*-Butyl *N*-(4,6-Diphenyl-4-oxazolin-2-onyl)(Ox)-L-seryl-D-[*p*-(benzyloxy)phenyl]glycinate (12).** A flame-dried 2-L round-bottomed flask fitted with a nitrogen inlet and a magnetic stirring bar was charged with L-Ox-serine dicyclohexylammonium salt[28a] (37.00 g, 75.0 mmol) and D-*tert*-butyl[*p*-benzyloxy)phenyl]glycine toluenesulfonate (36.41 g, 75.0 mmol). Addition of 50 mL of DMF and 700 mL of methylene chloride produced a solution that was cooled to 0 °C in an ice bath. 1-Hydroxybenzotriazole monohydrate (12.63 g, 82.5 mmol) (predried on an abderhalden heated by refluxing ethanol over phosphorus pentoxide) and freshly distilled dicyclohexylcarbodiimide (17.02 g, 82.5 mmol) were added, and the reaction was stirred for 2.5 h at 0 °C and 1.5 h at room temperature. The suspension was cooled to 4 °C in a refrigerator and filtered to remove the precipitated dicyclohexylurea. The filtrate was evaporated in vacuo and the residue redissolved in 300 mL of ethyl acetate. The organic solution was washed once with 200 mL of water, three times with 200-mL portions of 10% bicarbonate, two times with 200-mL portions of 1 N HCl, once with 200 mL of water, and once with 200 mL of brine and dried over anhydrous magnesium sulfate. Filtration and evaporation in vacuo provided a light yellow oil, which was crystallized from 200 mL of ethyl acetate and 150 mL of hexane to give the product (39.84 g, 86%) as white crystals. A second crop was obtained from the mother liquors by evaporation in vacuo and purification of the residue by flash chromatography (200 g of silica gel, 2:1 petroleum ether/ethyl acetate), providing peptide (4.46 g, 9%) identical with the first crop: total yield 44.3 g, 95%; mp 153.5–155 °C; $[\alpha]_D^{25}$ = −92.2° (*c* = 1.0, $CHCl_3$); $^1$H NMR ($CDCl_3$) δ 6.8–7.6 (19 H, m and $AB_q$, *J* = 6.8 Hz, Ar), 5.35 (1 H, d, *J* = 6.8 Hz, PHPG H-2), 5.04 (2 H, s, $PhCH_2$), 4.21 and 4.12 (3 H, m, Ser H-2 and H-3), 1.38 (9 H, s, *tert*-butyl); IR ($CHCl_3$) 3380, 3015, 2950, 1745, 1640, 1500, 1360, 1225, 1150 cm$^{-1}$. Anal. ($C_{37}H_{36}N_2O_7$) C, H, N.

**(−)-3-*N*-(4,5-Diphenyl-4-oxazolin-2-onyl)Aminonocardicinic Acid *tert*-Butyl Ester (13).** An oven-dried 2-L round-bottomed flask equipped with a nitrogen inlet, a rubber septum, and a magnetic stirring bar was charged with protected dipeptide **12** (44.3 g, 71.37 mmol) and 1.25 L of THF. To the solution were added triethyl phosphite (13.46 mL, 78.51 mmol) and diethyl azodicarboxylate (12.36 mL, 78.51 mmol), and the reaction was stirred under nitrogen for 4.5 h. The THF was evaporated in vacuo, and the residue was triturated with ethyl acetate/hexane to precipitate diethyl hydrazinedicarboxylate, which was removed by filtration. The filtrate was evaporated in vacuo and purified by flash chromatography (600 g of silica gel, 5:1 petroleum ether/ethyl acetate). Crystallization of the resulting white powder from ethyl acetate/hexanes provided the desired β-lactam (36.29 g, 84%) as white powder: mp 158–158.5 °C; $[\alpha]_D^{25}$ = −152° (*c* = 0.1, $CHCl_3$); $^1$H NMR ($CDCl_3$) δ 7.41 (10 H, cm, Ar), 7.18 (5 H, m, Ar), 6.98 (2 H, AB q, H-8), 5.43 (1 H, s, H-5), 5.08 (2 H, s, $PhCH_2$), 4.88 (1 H, dd, *J* = 3.0, 5.8 Hz, H-3), 3.75 (1 H, t, *J* = 5.8 Hz, H-4α), 3.42 (1 H, dd, *J* = 3.0, 5.8 Hz, H-4β), 1.42 (9 H, s, *tert*-butyl); IR ($CHCl_3$) 3000, 2980, 1755, 1610, 1510, 1385, 1370, 1240, 1150 cm$^{-1}$. Anal. ($C_{37}H_{34}N_2O_6$) C, H, N.

**(−)-*tert*-Butyl 3-Aminonocardicinate Hydrochloride (14).** A 500-mL Parr pressure bottle was charged with β-lactam **13** (2.00 g, 3.32 mmol), 50 mL of reagent-grade ethyl acetate, 20 mL of reagent-grade methanol, and 20 mL of glacial acetic acid. The resulting solution was deoxygenated by sparging with argon for 5 min. HCl (3.5 mL of 1 N) was added followed by 10% palladium on carbon (0.75 g, Aldrich). Hydrogenolysis was carried out at 40 psi for 44 h on a Parr apparatus. The catalyst was removed by filtration through Celite and the filtrate evaporated in vacuo with azeotropic removal of acetic acid by adding 100-mL portions of cyclohexane followed by concentration four times. The solid white residue was dried under vacuum and triturated with 25-mL portions of ether three times and twice with 25 mL of pentane. The resulting solid was dried in vacuo to provide the product (967 mg, 89%) as a white powder. Alternatively, the residue from evaporation may be purified by flash chromatography (50 g of silica gel, 49:49:2 ethyl acetate/methylene chloride/methanol) to afford the product in similar yield: mp 128 °C dec; $[\alpha]_D^{22}$ = −148.5° (*c* = 0.12, MeOH); $^1$H NMR (DMSO-$d_6$) δ 9.75 (1 H, br s, OH), 9.0 (3 H, br s, $NH_3$), 6.98 (4 H, AB q, *J* = 8.8 Hz, Ar), 5.31 (1 H, s, H-5), 4.52 (1 H, cm, H-3), 3.64 (1 H, t, *J* = 5.9 Hz, H-4α), 3.07 (1 H, dd, *J* = 2.6, 5.9 Hz, H-4β), 1.41 (9 H, s, *tert*-butyl); IR (KBr) 3500–2400, 1750, 1720, 1580, 1245, 1150, 1100 cm$^{-1}$. Anal. Calcd for $C_{15}H_{21}N_2O_4Cl{\cdot}1.5H_2O$: C, 50.63; H, 6.76; N, 7.87. Found: C, 50.87; H, 6.36; N, 7.83.

***N*-(*tert*-Butoxycarbonyl)-D-(*p*-hydroxyphenyl)glycine (15, R = H).** A 250-mL round-bottomed flask equipped with a magnetic stirring bar was charged with D-(*p*-hydroxyphenyl)glycine (3.00 g, 18.0 mmol), 18 mL of 1 N NaOH, 18 mL of water, and 18 mL of reagent-grade dioxane. To the vigorously stirred solution was added a solution of di-*tert*-butyl dicarbonate (4.29 g, 25.0 mmol) in 18 mL of dioxane. After 4 h at room temperature the dioxane was evaporated in vacuo and the aqueous solution washed once with a 25-mL portion of ether. The aqueous solution was adjusted to pH 2–3 with solid $KHSO_4$ and extracted three times with 25-mL portions of ethyl acetate. The organic extracts were combined, washed once with 30 mL of brine, and dried over anhydrous magnesium sulfate. Filtration and evaporation in vacuo provided a clear oil, which was crystallized from ethyl acetate to yield the product (4.42 g, 97%) as a white solid: mp 199 °C (dec starting at 160 °C) [lit.[28b] mp 200 °C (dec starting at 137 °C)]; $[\alpha]_D^{24}$ = −130° (*c* = 1.08 EtOH); $^1$H NMR (DMSO-$d_6$) δ 9.43 (1 H, s, OH), 7.39 (1 H, br d, *J* = 8.3 Hz, NH), 7.16 (2 H, d, *J* = 8.5 Ar), 6.69 (2 H, d, *J* = 8.5 Hz, Ar), 4.94 (1 H, br d, *J* = 8.3 Hz, H-2), 1.37 (9 H, s, *tert*-butyl).

**Protected Nocardicin G (16, R = H).** In an oven-dried, three-necked 100-mL round-bottomed flask equipped with a nitrogen inlet, a rubber septum, and a magnetic stirring bar D-*N*-*t*-Boc-PHPG (**15**, R = H) (1.804 g, 6.75 mmol) was dissolved in 15 mL of freshly distilled acetone and cooled to −20 °C in a dry ice/carbon tetrachloride bath. After 15 min 2,6-lutidine (715 μL, 6.14 mmol) was added and 5 min later a solution of iso-butyl chloroformate (796 μL, 6.14 mmol) in 5 mL of acetone was added dropwise over 5 min. The reaction was stirred at −20 °C for 20 min then transferred to a 0 °C ice bath and stirred for an additional 30 min.

An oven-dried, 20-mL pear-shaped flask equipped with a nitrogen inlet, a rubber septum, and a magnetic stirring bar was charged with 3-ANA derivative **14** (1.480 g, 4.50 mmol) and 15 mL of DMF and cooled to 0 °C in an ice bath. To the solution was added 2,6-lutidine (524 μL, 4.50 mmol) and stirring was continued for 5 min. The resulting solution was added, by syringe, to the suspension of mixed anhydride with the aid of two 2-mL acetone rinses. The reaction was stirred at 0 °C for 30 min, then allowed to come to room temperature, and stirred for an

(48) Kamiya, T.; Hashimoto, M.; Nakaguchi, O.; Oku, T. *Tetrahedron* **1979**, *35*, 323–328.

additional 2.0 h. The mixture was diluted with 100 mL of water, 50 mL of brine, and 5 mL of 1 N HCl and extracted four times with 75-mL portions of ethyl acetate. The combined extracts were washed twice with 150-mL portions of water, twice with 100-mL portions of 0.5 N HCl, once with 100 mL of water, and twice with 75 mL portions of brine and dried over anhydrous magnesium sulfate. Filtration and evaporation in vacuo provided a yellow foam, which was purified by flash chromatography (100 g of silica gel, 4:1 methylene chloride/acetone), to yield the product **16** (1.508 g, 61%) as a white powder. Analytically pure material may be obtained by crystallization from chloroform/petroleum ether: mp 223–225 °C dec; $[\alpha]^{23}_D = -179°$ ($c$ = 0.50, EtOH); $^1$H NMR (acetone-$d_6$) $\delta$ 8.35 (1 H, br s, OH), 8.11 (1 H, br s, OH), 7.66 (1 H, br d, $J$ = 7.3 Hz, C-3 NH), 7.15–6.8 (8 H, 2 AB q, Ar), 6.00 (1 H, br d, $J$ = 6.9 Hz, C-2′ NH), 5.34 (1 H, s, H-5), 5.12 (2 H, br m, H-3 and H-2′), 3.88 (1 H, t, $J$ = 5.3 Hz, H-4$\alpha$), 3.00 (1 H, dd, $J$ = 5.5, 2.5 Hz, H-4$\beta$), 1.46 (9 H, s, *tert*-butyl), 1.40 (9 H, s, *tert*-butyl); IR (KBr) 3350, 3180, 2960, 2920, 1745, 1720, 1685, 1635, 1600, 1470, 1355, 1220, 1150 cm$^{-1}$. Anal. ($C_{28}H_{35}N_3O_8$) C, H, N.

**Nocardicin G (7).** A flame-dried 50-mL round-bottomed flask equipped with a nitrogen inlet and a magnetic stirring bar was charged with protected nocardicin G (**16**, R = H) (948 mg, 1.75 mmol) and 3.5 mL of anisole and cooled to 0 °C in an ice bath. Trifluoroacetic acid (35 mL) was cooled to 0 °C and added to the mixture, which was then stirred for 15 min at 0 °C and for 30 min at room temperature. The trifluoroacetic acid was evaporated in vacuo and the residue dried under high vacuum for 30 min. The resulting viscous oil was triturated four times with 10-mL portions of ether and three times with 10-mL portions of pentane to provide a light yellow powder, which was dried in vacuo. The powder was dissolved in 4 mL of water containing 4 drops of 1 N HCl and filtered to remove insoluble impurities. The filtrate was adjusted to pH 4.5 with 4 N NaOH and crystallization was caused by addition of 4 mL of acetone. After 3 h in a freezer, the mixture was filtered and dried to a white powder: 605 mg, 90%; mp 225–227 °C dec (lit.[2] mp 227 °C dec); $[\alpha]^{25}_D = -198°$ ($c$ = 1.0, 1% $NaHCO_3$) [lit.[2] $[\alpha]^{25}_D = -205°$ ($c$ = 1.0, 1% $NaHCO_3$)]; $^1$H NMR ($D_2O$) $\delta$ 7.31 (2 H, d, $J$ = 8.9 Hz, Ar), 7.22 (2 H, d, $J$ = 8.4 Hz, Ar), 6.94 (2 H, d, $J$ = 8.4 Hz, Ar), 6.89 (2 H, d, $J$ = 8.9 Hz, Ar), 5.25 (1 H, s, H-5), 5.05 (1 H, s, H-2′), 4.88 (1 H, dd, $J$ = 5.0, 2.2 Hz, H-3), 3.75 (1 H, $t$, $J$ = 5.6 Hz, H-4$\alpha$), 3.03 (1 H, dd, $J$ = 5.6, 2.6 Hz, H-4$\beta$).

Stereoisomeric purity (>99.95%) was monitored by HPLC with a LiChrosorb RP-18 (5 $\mu$m) analytical column (250 mm) eluting with 0.01 M $(NH_4)_2CO_3$ adjusted to pH 5.5 with acetic acid (0.7 mL/min). The retention time of nocardicin G was 15.2 min, epinocardicin G 11.3 min.[16]

**Nocardicin E (5).** In a flame-dried 25-mL round-bottomed flask equipped with a nitrogen inlet and a magnetic stirring bar, nocardicin G (**7**) (154 mg, 0.40 mmol) was added to a briskly stirred solution of 3,5-di-*tert*-butyl-*o*-benzoquinone (86 mg, 0.39 mmol) and tri-*n*-butylamine (95 $\mu$L, 0.40 mmol) in 4 mL of absolute ethanol. A red solution resulted after 5 min, and the stirring was continued at room temperature for 1.5 h. Hydroxylamine hydrochloride (278 mg, 4.0 mmol) and 4 mL of pH 4.5 0.1 M sodium acetate buffer were added, and stirring was continued for 3 h at room temperature. The reaction was diluted with 100 mL of 0.1 M sodium bicarbonate and washed four times with 40-mL portions of ether. The aqueous solution was acidified to pH 2 with 6 N HCl, and approximately 10 g of sodium chloride was added. The solution was extracted with 50-mL portions of ethyl acetate eight times. The combined ethyl acetate extracts were washed twice with 50-mL portions of 0.5 N HCl and once with 50 mL of brine and dried over anhydrous sodium sulfate. Filtration and evaporation in vacuo provided a red solid, which was triturated three times with 5-mL portions of diisopropyl ether, once with 5 mL of methylene chloride, and twice with 5-mL portions of pentane, yielding the product (137 mg, 88%) as a 20:1 mixture of **5**/**6**: mp 224–225 °C dec (lit.[2] mp 228–231 °C dec; $[\alpha]^{24}_D = -194°$ ($c$ = 1.0 $H_2O$) [lit.[2] $[\alpha]^{24}_D = -192°$ ($c$ = 1, $H_2O$)]; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.17 (1 H, s, OH), 9.81 (1 H, s, OH), 9.59 (1 H, s, OH), 9.08 (1 H, d, $J$ = 7.9 Hz, NH), 7.31 (2 H, d, $J$ = 8.2 Hz, Ar), 7.15 (2 H, d, $J$ = 8.5 Hz, Ar), 6.75 (4 H, d, $J$ = 8.5 Hz, Ar), 5.33 (1 H, s, H-5), 4.96 (1 H, m, H-3), 3.77 (1 H, t, $J$ = 5.4 Hz, H-4$\alpha$), 3.07 (1 H, dd, $J$ = 4.8, 2.4 Hz, H-4$\beta$).

Nocardicins E and F were readily separable (to purities of >99.95%) by reverse-phase HPLC. On an analytical scale this was accomplished with a LiChrosorb RP-18 column (250 mm, 5 $\mu$m) eluting with 0.1% TFA/10% acetonitrile (0.7 mL/min). The retention times of nocardicins E and F were 13.2 and 22.5 min, respectively. Alternatively, purification to >99% could be achieved by chromatography on LH-20. For example, the reaction above run on 1 mmol of nocardicin G after workup was applied to an LH-20 column (1.25 × 50 cm) and eluted with ethanol. The $FeCl_3$-positive fractions were combined, the solvent was removed in vacuo, and the rsidue was crystallized from ethanol/isopropyl ether to give nocardicin E in 62% yield.[17]

**Nocardicin F (6).** A 10-mL round-bottomed flask equipped with a magnetic stirring bar was charged with sodium bicarbonate (21.0 mg, 0.25 mmol), sodium tungstate dihydrate (6.6 mg, 0.02 mmol), nocardicin G (7) (88.8 mg, 0.23 mmol), 0.5 mL of distilled water, and 0.5 mL of methanol. To the gently stirred suspension was added 30% hydrogen peroxide (440 $\mu$L, 5.0 mmol), and the resulting yellow solution was stirred at room temperature for 6 h. The solution was adjusted to pH 2 by the addition of 2 drops of 6 N HCl and evaporated to dryness in vacuo. The residue was suspended in 3 mL of ethanol, sonicated, and filtered through Celite to remove inorganic salts. The filtrate was evaporated to a tan residue, which was dissolved in 0.5 mL of absolute ethanol and precipitated by the addition of dichloroethane to yield the product (66 mg, 72%) as a 15:1 mixture of **6**/**5**: mp 199–202 °C dec (lit.[2] mp 230–231 °C dec; $[\alpha]^{24}_D = -179°$ ($c$ = 1, $H_2O$) [lit.[2] $[\alpha]^{24}_D = -181°$ ($c$ = 1, $H_2O$)]; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.66 (1 H, s, OH), 9.76 (1 H, s, OH), 9.57 (1 H, s, OH), 8.84 (1 H, d, $J$ = 8.6 Hz, NH), 7.37 (2 H, d, $J$ = 8.9 Hz, Ar), 7.16 (2 H, d, $J$ = 8.6 Hz, Ar), 6.75 (2 H, d, $J$ = 8.5 Hz, Ar), 6.75 (2 H, d, $J$ = 8.9 Hz, Ar), 5.30 (1 H, s, H-5), 4.92 (1 H, m, H-3), 3.69 (1 H, t, $J$ = 5.1 Hz, H-4$\alpha$), 3.12 (1 H, dd, $J$ = 5.0, 2.4 Hz, H-4$\beta$).

For further purification, see nocardicin E (**6**) above.

***tert*-Butyl D-*N*-(*tert*-Butoxycarbonyl)homoserinate (27, R = R$^1$ = *t*-Bu, X = OH).** In a flame-dried 100-mL round-bottomed flask equipped with a nitrogen inlet, a rubber septum, and a magnetic stirring bar were dissolved $\alpha$-*tert*-butyl *N*-(*tert*-butoxycarbonyl)-D-aspartate[49] (3.468 g, 12 mmol) and triethylamine (1.67 mL, 12 mmol) in 12 mL of THF, and the solution was cooled to −5 °C in an ice/salt bath. A solution of ethyl chloroformate (1.205 mL, 12.6 mmol) in 12 mL of THF was added dropwise over 10 min to the reaction. The resulting suspension was stirred at room temperature for 30 min and then filtered into a 50-mL addition funnel with the aid of 10 mL of THF. The filtrate was added dropwise over 30 min to an ice-cold solution of sodium borohydride (946 mg, 25 mmol) in 6 mL of water. The mixture was stirred at room temperature for 4 h and then acidified to pH 2 with 1 N HCl. The THF layer separated and was collected. The aqueous layer was extracted twice with 30-mL portions of ethyl acetate. The combined organic phases were washed twice with 50-mL portions of 1 M $NaHCO_3$ and once with 50 mL of brine and dried over anhydrous magnesium sulfate. Filtration and evaporation of the solvents provided a light yellow oil, which was purified by flash chromatography (150 g of silica gel, 7:3 hexane/acetone) to afford the product (2.51 g, 76%) as a clear, highly viscous oil: $[\alpha]^{25}_D = +38.3°$ ($c$ = 1.0, EtOH) [lit.[41] $[\alpha]^{25}_D$ (for L isomer) = −37.5° ($c$ = 1, EtOH)]; $^1$H NMR ($CDCl_3$) $\delta$ 5.34 (1 H, br d, $J$ = 6.6 Hz, NH), 4.39 (1 H, br m, H-2), 3.6–3.8 (2 H, cm, H-4), 2.15 (2 H, cm, H-3), 1.48 (9 H, s, *tert*-butyl), 1.46 (9 H, s, *tert*-butyl).

***tert*-Butyl D-2-[(*tert*-Butoxycarbonyl)amino]-4-bromobutyrate (27, R = R$^1$ = *t*-Bu, X = Br).** In a flame-dried 50-mL round-bottomed flask equipped with a nitrogen inlet, a rubber septum, and a magnetic stirring bar were dissolved *tert*-butyl *N*-(*tert*-butoxycarbonyl)-D-homoserinate (485 mg, 1.76 mmol) and carbon tetrabromide (1.17 g, 3.52 mmol) in 15 mL of freshly distilled methylene chloride, and the solution was cooled to 0 °C. To the stirred colorless solution was added a solution of triphenylphosphine (924 mg, 3.52 mmol) in 5 mL of freshly distilled methylene chloride (which had been prepared in dry glassware) dropwise over 5 min. The resulting solution was stirred at room temperature for 2 h. The solvent was evaporated in vacuo to provide a yellow residue. Trituration with ether/petroleum ether precipitated triphenylphosphine oxide, which was removed by filtration. The filtrate was evaporated in vacuo to a yellow oil, which was purified by flash chromatography (20 g of silica gel, 8:1 petroleum ether/ether) to provide the product (542 mg, 91%) as a colorless oil: $[\alpha]^{23}_D = -10.1°$ ($c$ = 1.05, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 5.10 (1 H, br d, $J$ = 7.0 Hz, NH), 4.28 (1 H, cm, H-2), 3.42 (2 H, cm, H-4), 2.35 and 2.15 (2 H, 2 cm, H-3), 1.48 (9 H, s, *tert*-butyl), 1.44 (9 H, s, *tert*-butyl); IR ($CHCl_3$) 3410, 2890, 1705, 1695, 1350, 1135. Anal. ($C_{13}H_{24}NO_4Br$) C, H, N.

**D-*N*-(Benzyloxycarbonyl)-2-[4-[D-3-[(*tert*-butoxycarbonyl)amino]-3-(*tert*-butoxycarbonyl)propoxy]phenyl]glycine (29).** In an oven-dried, three-necked 25-mL round-bottomed flask equipped with a nitrogen inlet, a rubber septum, and a magnetic stirring bar was suspended 35% potassium hydride (121 mg, 1.06 mmol) in 2.5 mL of dry DMF. To the suspension was added *N*-(benzyloxycarbonyl)-D-(*p*-hydroxyphenyl)-glycine (**28**, R = $CH_2Ph$) (152.5 mg, 0.508 mmol). The mixture bubbled for 5 min and was stirred for an additional 5 min at room temperature. To the resulting yellow solution was added a solution of *tert*-butyl D-2-[(*tert*-butoxycarbonyl)amino]-4-bromobutyrate (**27**, R = R$^1$ = *t*-Bu, X = Br) (143 mg, 0.423 mmol) in 1.5 mL of dry DMF dropwise over 10 min. The resulting solution was stirred at room temperature for 3 h. The reaction was diluted with 50 mL of ethyl acetate and 30 mL of 0.1

(49) Yang, C. C.; Merrifield, R. B. *J. Org. Chem.* **1976**, *41*, 1032–1040.

N HCl. The layers were separated and the organic phase was washed with three 30-mL portions of water, one 30-mL portion of 0.5 N HCl, and one 30-mL portion of brine. The ethyl acetate solution was dried over anhydrous magnesium sulfate, filtered, and evaporated in vacuo to a yellow oil. The oil was purified by flash chromatography (15 g of silica gel, 3:1 hexane/acetone containing 2.5% methanol and 0.5% acetic acid) to provide the product (202 mg, 85%) as a white foam: mp 99–102 °,C; $[\alpha]^{25}_D = -95.5°$ ($c$ = 1.1, $CHCl_3$); $^1H$ NMR (DMSO-$d_6$) $\delta$ 7.96 (1 H, d, $J$ = 7.9 Hz, NH), 7.34 (5 H, cm, $CH_2Ph$), 7.30 (2 H, d, $J$ = 8.4 Hz, Ar), 7.23 (1 H, d, $J$ = 7.9 Hz, NH), 6.87 (2 H, d, $J$ = 8.4 Hz, Ar), 5.04 (3 H, m, $CH_2Ph$ and ArCH), 3.99 (3 H, m, homoserine H-2 and H-4), 1.96 and 2.06 (2 H, 2 cm, homoserine H-3); IR ($CHCl_3$) 3410, 3320, 2910, 2860, 1755, 1745, 1705, 1680, 1605, 1450, 1355, 1145 $cm^{-1}$. Anal. ($C_{29}H_{38}N_2O_9$) C, H, N.

**Protected Nocardicin C (32, R = $COOCH_2Ph$).** An oven-dried 25-mL round-bottomed flask equipped with a nitrogen inlet and a magnetic stirring bar was charged with **29** (122 mg, 0.218 mmol), **14** (72 mg, 0.218 mmol), and 2.0 mL of THF and cooled to 0 °C. To the resulting solution were added 2,6-lutidine (28 $\mu$L, 0.240 mmol), 1-hydroxybenzotriazole (37 mg, 0.240 mmol), and dicyclohexylcarbodiimide (49.5 mg, 0.240 mmol). The reaction was stirred at 0 °C for 30 min and at room temperature for 1.5 h. The suspension was filtered to remove precipitated dicyclohexylurea, and the filtrate was diluted with 25 mL of ethyl acetate. The organic solution was washed once with 15 mL of water, twice with 15-mL portions of 0.5 N HCl, once again with 15 mL of water, and once with 15 mL of brine. The organic layer was dried over anhydrous magnesium sulfate, filtered, and evaporated. The residue was purified by flash chromatography (10 g of silica gel, 5:2 hexanes/acetone) to provide the product (161 mg, 88%) as a white foam: mp 89–92 °C; $[\alpha]^{23}_D = -86.3°$ ($c$ = 0.59, $CHCl_3$); $^1H$ NMR (DMSO-$d_6$) $\delta$ 9.61 (1 H, s, OH), 8.86 (1 H, d, $J$ = 8.2 Hz, C-3 NH), 7.89 (1 H, d, $J$ = 8.2 Hz, C-2′ NH), 7.32 (6 H, m, Ph and C-9′ NH), 7.25 (2 H, d, $J$ = 8.5 Hz, Ar), 7.11 (2 H, d, $J$ = 8.1 Hz, Ar), 6.82 (2 H, d, $J$ = 8.5 Hz, Ar), 6.76 (2 H, d, $J$ = 8.1 Hz, Ar), 5.27 (1 H, s, H-5), 5.10 (1 H, d, $J$ = 8.3 Hz, H-2), 5.00 (2 H, br s, $CH_2Ph$), 4.85 (1 H, br m, H-3), 3.95 (3 H, m, H-7′ and H-9′), 3.65 (1 H, t, $J$ = 5.4 Hz, H-4$\alpha$), 2.82 (1 H, br, dd, H-4$\beta$), 2.04 and 1.94 (2 H, 2 cm, H-8′), 1.37 (9 H, s, *tert*-butyl), 1.36 (9 H, s, *tert*-butyl), 1.35 (9 H, s, *tert*-butyl); IR ($CHCl_3$) 3400, 2910, 2880, 1750, 1730, 1710, 1675, 1605, 1595, 1355, 1145 $cm^{-1}$. Anal. ($C_{44}H_{56}N_4O_{12}$) C, H, N.

**Di-*tert*-butyl 9′-*N*-(*tert*-Butoxycarbonyl)nocardicin C (32, R = H).** In a 200-mL Parr pressure bottle was dissolved **32** (R = $CH_2Ph$) (222 mg, 0.266 mmol) in 13 mL of absolute ethanol. Nitrogen was bubbled through the solution for 5 min to remove dissolved oxygen, and 75 mg of 10% palladium on carbon was added followed by 0.32 mL of 1.0 N HCl. The suspension was placed on a Parr Hydrogenator, and the reaction was shaken under 40 psi of $H_2$ for 3 h, filtered through Celite, and evaporated in vacuo to a clear residue. The residue was dissolved in 2 mL of chloroform, and product was precipitated as a white powder by the addition of 4 mL of petroleum ether. The product (191 mg, 98%) was collected by filtration: mp 210–240 °C dec; $[\alpha]^{23}_D = -98.5°$ ($c$ = 0.39, $CHCl_3$); $^1H$ NMR (DMSO-$d_6$) $\delta$ 9.63 (1 H, s, OH), 9.05 (1 H, d, $J$ = 8.1 Hz, C-3 NH), 8.56 (3 H, dt, $J$ = 3, 4.9 Hz, $NH_3$), 7.33 (2 H, d, $J$ = 8.9 Hz, Ar), 7.24 (1 H, d, $J$ = 7.9 Hz, NH), 7.11 (2 H, d, $J$ = 8.2 Hz, Ar), 6.96 (2 H, d, $J$ = 8.9 Hz, Ar), 6.75 (2 H, d, $J$ = 8.2 Hz, Ar), 5.29 (1 H, s, H-5), 4.88 (1 H, m, H-2′), 4.80 (1 H, br m, H-3), 4.00 (3 H, m, H-7′ and H-9′), 3.68 (1 H, t, $J$ = 5.4 Hz, H-4$\alpha$), 2.87 (1 H, br dd, $J$ = 3.9, 5.4 Hz, H-4$\beta$), 2.04 and 1.94 (2 H, 2 cm, H-8′) 1.37 (9 H, s, *tert*-butyl), 1.37 (9 H, s, *tert*-butyl), 1.36 (9 H, s, *tert*-butyl); IR ($CHCl_3$) 3400, 3200, 2900, 2860, 1745, 1730, 1720, 1695, 1605, 1355, 1145 $cm^{-1}$. Anal. Calcd for $C_{36}H_{51}N_4O_{10}Cl$: C, 58.50; H, 6.99; N, 7.62. Found: C, 60.08; H, 7.14; N, 7.5.

**Protected Nocardicin B (33).** To a solution of nocardicin C derivative **32** (R = H) (147 mg, 0.20 mmol) in 1.5 mL of absolute methanol in a 10-mL round-bottomed flask equipped with a magnetic stirring bar and a nitrogen inlet was added a solution of sodium bicarbonate (16.8 mg, 0.20 mmol) and sodium tungstate dihydrate (5.3 mg, 0.016 mmol) in 0.5 mL of distilled water. To the resulting gently stirred solution was added 30% hydrogen peroxide (176 $\mu$L, 2.0 mmol). Aliquots of 176 $\mu$L of 30% hydrogen peroxide were added every hour for 5 h. After 6 h the reaction mixture was transferred to a separatory funnel and diluted with 75 mL of ethyl acetate and 40 mL of water. The layers were separated, and the ethyl acetate solution was washed twice with 40-mL portions of water, twice with 40-mL portions of 0.5 N HCl, twice with 40-mL portions of 0.5 N NaOAc, and once with 40 mL of brine. The organic solution was dried over anhydrous sodium sulfate, filtered, and evaporated to a yellow oil. Purification by flash chromatography (6 g of silica gel, methylene chloride with 2.5% methanol) provided the product (82 mg, 58%) as a white powder: mp 167–169 °C dec; $[\alpha]^{23}_D = -87.5°$ ($c$ = 0.36, $CHCl_3$); $^1H$ NMR (DMSO-$d_6$) $\delta$ 9.59 (1 H, s, OH), 8.87 (1 H, d, $J$ = 8.5 Hz, C-3 NH), 7.44 (2 H, d, $J$ = 8.8 Hz, Ar), 7.25 (1 H, d, $J$ = 8.0 Hz, NH), 7.13 (2 H, d, $J$ = 8.5 Hz, Ar), 6.92 (2 H, d, $J$ = 8.8 Hz, Ar), 6.76 (2 H, d, $J$ = 8.5 Hz, Ar), 5.26 (1 H, s, H-5), 4.93 (1 H, m, H-3), 4.01 (3 H, m, H-7′ and H-9′), 3.67 (1 H, t, $J$ = 5.4 Hz, H-4$\alpha$) 3.11 (1 H, dd, $J$ = 2.6, 4.8 Hz, H-4$\beta$), 2.03 and 1.95 (2 H, 2 cm, H-8′), 1.40 (9 H, s, *tert*-butyl), 1.38 (9 H, s, *tert*-butyl), 1.36 (9 H, s, *tert*-butyl); IR ($CHCl_3$) 3390, 3300, 2900, 1745, 1730, 1720, 1695, 1675, 1600, 1355, 1145 $cm^{-1}$. Anal. ($C_{36}H_{48}N_4O_{11}$) C, H, N.

**Nocardicin B (2).** A flame-dried 25-mL round-bottomed flask equipped with a magnetic stirring bar and a nitrogen inlet was charged with protected nocardicin B (**33**) (36.7 mg, 0.051 mmol), 50 $\mu$L of 2-mercaptoethanol, and 0.50 mL of trifluoroacetic acid. The resulting yellow solution was stirred at room temperature for 6 min. Anhydrous ether, approximately 5 mL, and cyclohexane, approximately 10 mL, were added to precipitate the product. The solvents were removed under high vacuum. The brown residue was triturated with three 5-mL portions of anhydrous ether and dried to a tan powder, nocardicin B trifluoroacetate salt. Nocardicin B was purified by dissolving the salt in 1 mL of water and filtering through a cotton plug to remove insoluble impurities. The filtrate was adjusted to pH 2.5 with 1 N NaOH to precpitate the product (12 mg, 47%) as an off-white powder: mp 259–263 °C dec (lit.[1] mp 262–264 °C dec; $[\alpha]^{23}_D = -176°$ ($c$ = 0.5, $H_2O$) [lit.[1] $[\alpha]_D = -162°$ ($c$ = 1, $H_2O$)] for the sodium salt; $^1H$ NMR ($D_2O$ + NaOD) $\delta$ 7.18 (2 H, d, $J$ = 8.2 Hz, Ar), 7.05 (2 H, d, $J$ = 7.2 Hz, Ar), 7.00 (2 H, d, $J$ = 8.2 Hz, Ar), 6.59 (2 H, d, $J$ = 7.8 Hz, Ar), 5.17 (1 H, s, H-5), 4.98 (1 H, m, H-3), 4.16 (2 H, m, H-7′), 3.77 (1 H, t, $J$ = 6 Hz, H-9′), 3.43 (1 H, t, $J$ = 5 Hz, H-4$\alpha$), 3.21 (1 H, dd, $J$ = 4.8, 1.7 Hz, H-4$\beta$), 2.14 and 1.95 (1 H, 2 cm, H-8′).

**D-*N*-(*tert*-Butoxycarbonyl)-2-[4-[D-3-[(*tert*-butoxycarbonyl)amino]-3-(*tert*-butoxycarbonyl)propoxy]phenyl]glycine (30).** In an oven-dried, three-necked 25-mL round-bottomed flask equipped with a nitrogen inlet, a rubber septum, and a magnetic stirring bar was suspended 35% potassium hydride (Aldrich) (162 mg, 1.41 mmol) in 3 mL of dry DMF. To the suspension was added D-*N*-(*tert*-butoxycarbonyl)-(*p*-hydroxyphenyl)glycine (**15**, $R$ = H, or **28**, R = *t*-Bu) (181 mg, 0.678 mmol). The mixture bubbled for 5 min and stirred at room temperature for an additional 5 min. To the resulting yellow mixture was added a solution of *tert*-butyl D-2-*tert*-butoxycarbonylamino-4-bromobutyrate (**27**, R = $R^1$ = *t*-Bu, X = Br) (191 mg, 0.565 mmol) in 2 mL of dry DMF dropwise over 10 min. The resulting solution was stirred at room temperature for 3 h and then diluted with 75 mL of ethyl acetate and 50 mL of 0.1 N HCl. The layers were separated, and the organic phase was washed three times with 50-mL portions of water, once with 50 mL of 0.5 N HCl, and once with brine. The ethyl acetate solution was dried over anhydrous magnesium sulfate, filtered, and evaporated in vacuo to a yellow oil. The oil was purified by flash chromatography (20 g of silica, methylene chloride, 5% methanol, 0.5% acetic acid) to provide the product (217 mg, 73%) as a white foam: mp 73–74.5 °C; $[\alpha]^{24}_D = -90.0°$ ($c$ = 1.05, $CHCl_3$); $^1H$ NMR (DMSO-$d_6$) $\delta$ 7.47 (1 H, d, $J$ = 8.6 Hz, NH), 7.27 (2 H, d, $J$ = 8.5 Hz, Ar), 7.24 (1 H, d, $J$ = 8.0 Hz, NH), 6.86 (2 H, d, $J$ = 8.5 Hz, Ar), 5.00 (1 H, d, $J$ = 8.6 Hz, C*H*Ar), 3.98 (3 H, cm, $CH_2O$ and CH), 2.05 and 1.96 (2 H, 2 cm, $CH_2$), 1.48 (9 H, s, *tert*-butyl), 1.44 (9 H, s, *tert*-butyl), 1.40 (9 H, s, *tert*-butyl); IR ($CHCl_3$) 3450, 3400–2400, 1750, 1700, 1650, 1350, 850 $cm^{-1}$. Anal. ($C_{26}H_{40}N_2O_9$) C, H, N.

**Protected Nocardicin C (34).** An oven-dried 25-mL round-bottomed flask fitted with a nitrogen inlet, a rubber septum, and a magnetic stirring bar was charged with **30** (180 mg, 0.343 mmol), *tert*-butyl 3-aminonocardicinate hydrochloride (**14**) (113 mg, 0.343 mmol), 1-hydroxybenzotriazole (58 mg, 0.377 mmol), and 5 mL of freshly distilled THF, and the solution was cooled to 0 °C. To this mixture were added 2,6-lutidine (40 $\mu$L, 0.343 mmol) and dicyclohexylcarbodiimide (78 mg, 0.377 mol). The mixture was stirred for 45 min at 0 °C and for 1.5 h at room temperature. The reaction was cooled to 0 °C and then filtered to remove the precipitated dicyclohexylurea. The filtrate was evaporated in vacuo, and the residue was purified by flash chromatography (15 g of silica gel, 3:1 methylene chloride/ethyl acetate) to provide the product (227 mg, 83%) as a white foam: mp 74.5–76 °C; $[\alpha]^{23}_D = -78.8°$ ($c$ = 0.50, $CHCl_3$); $^1H$ NMR (DMSO)-$d_6$ $\delta$ 9.60 (1 H, br, s, OH), 8.80 (1 H, br d, $J$ = 8.7 Hz, NH), 7.23 (1 H, br d, $J$ = 8.8 Hz, NH), 7.23 (2 H, d, $J$ = 8.4 Hz, Ar), 7.05 (2 H, d, $J$ = 8.4, Ar), 6.82 (2 H, d, $J$ = 8.4 Hz, Ar), 6.76 (2 H, d, $J$ = 8.4 Hz, Ar), 5.27 (1 H, s, H-5), 5.03 (1 H, d, $J$ = 8.6 Hz, H-2′), 4.85 (1 H, br m, H-3), 3.97 (3 H, cm, H-7′ and H-9′), 3.64 (1 H, t, $J$ = 5.0 Hz, H-4$\alpha$), 2.83 (1 H, br dd, $J$ = 5.0, 2.2 Hz, H-4$\beta$), 2.02 and 1.95 (2 H, 2 cm, H-8′), 1.48–1.38 (36 H, 4 s, *tert*-butyl); IR ($CHCl_3$) 3400, 2890, 1760, 1750, 1650, 1600, 1350, 1140, 950 $cm^{-1}$. Anal. ($C_{41}H_{58}N_4O_{12}$) C, H, N.

**Nocardicin C (3).** In a flame-dried 25-mL round-bottomed flask equipped with a nitrogen inlet and a magnetic stirring bar was dissolved protected nocardicin C (**34**) (184 mg, 0.231 mmol) in 0.5 mL of anisole,

and the solution cooled to 0 °C. Trifluoroacetic acid, 5.0 mL, was cooled to 0 °C and then added to the flask. The resulting yellow solution was stirred at 0 °C for 15 min and at room temperature for 20 min. The trifluoroacetic acid was evaporated in vacuo, and the tan residue was dried under high vacuum for 10 min. The dried residue was triturated three times with 5-mL portions of ether to provide a light tan power. The powder was dissolved in 2 mL of water and adjusted to pH 3 with 1 N NaOH. Insoluble impurities were removed by filtration through a cotton plug. The filtrate was diluted with 10 mL of acetone, causing precipitation of the product. After being cooled in a freezer for 3 h, the mixture was filtered and dried to a white powder (51 mg, 45%). A second crop was obtained by concentrating the filtrate to approximately 1 mL and adding 8 mL of acetone (22 mg, 20%): total yield 73 mg, 65%; mp 233–236 °C dec (lit.[2] mp 225–230 °C dec); $[\alpha]^{24}_D = -161.3°$ ($c$ = 0.50, $H_2O$) [lit.[45] $[\alpha]^{25}_D = -162°$ ($c$ = 0.50, $H_2O$); $^1H$ NMR ($D_2O$) $\delta$ 7.29 (2 H, d, $J$ = 8.2 Hz, Ar), 7.05 (2 H, d, $J$ = 8.2 Hz, Ar), 6.99 (2 H, d, $J$ = 8.2 Hz, Ar), 6.59 (2 H, d, $J$ = 8.2 Hz, Ar), 5.17 (1 H, s, H-5), 4.46 (1 H, s, H-2′), 4.13 (2 H, t, $J$ = 6.0 Hz, H-7′), 3.72 (1 H, t, $J$ = 5.2 Hz, H-9′), 3.43 (1 H, m, H-4$\alpha$), 3.07 (1 H, m, H-4$\beta$), 2.12 and 1.98 (2 H, 2 cm, H-8′).

**Methyl (*p*-Hydroxybenzoyl)formate (37).** In a flame-dried 25-mL round-bottomed flask equipped with a nitrogen inlet and a magnetic stirring bar was suspended sodium (*p*-hydroxybenzoyl)formate (0.941 g, 5.0 mmol) in 5 mL of DMSO. Dimethyl sulfate (0.521 mL, 5.5 mmol) was added and the mixture slowly became a yellow solution, which was stirred for 18 h. The reaction was diluted with 30 mL of water and 50 mL of ether. The layers were separated, and the aqueous phase was extracted three times with 30-mL portions of ether. The combined ether extracts were washed twice with 50 mL portions of water, once with 50 mL of 0.1 M sodium bicarbonate, twice with 50-mL portions of water, and once with 50 mL of brine. The organic solution was dried over anhydrous sodium sulfate, filtered, and evaporated to a yellow solid. Crystallization from benzene provided the product (794 mg, 88%) as white needles: mp 64.5–65.5 °C; $^1H$ NMR ($CDCl_3$) $\delta$ 7.98 (2 H, d, $J$ = 8.8 Hz, Ar), 6.93 (2 H, d, $J$ = 8.8 Hz, Ar), 6.10 (1 H, br s, OH) 3.97 (3 H, s, $CH_3$); IR ($CHCl_3$) 3550, 3300–2900, 1725, 1665, 1580, 1310, 1280, 1150, 995 cm$^{-1}$. Anal. ($C_9H_8O_4$) C, H.

**Methyl [4-[D-3-[(*tert*-Butoxycarbonyl)amino]-3-(*tert*-butoxycarbonyl)propoxy]benzoyl]formate (31, R = Me).** An oven-dried, three-necked 50-mL round-bottomed flask equipped with a nitrogen inlet, a rubber septum, and a magnetic stirring bar was charged with methyl (*p*-hydroxybenzoyl)formate (**37**, 722 mg, 4.01 mmol), triphenylphosphine (1.201 g, 4.58 mmol), and 10 mL of freshly distilled THF. Solutions of *tert*-butyl D-*N*-(*tert*-butoxycarbonyl)homoserinate (**27**, R = R$^1$ = *t*-Bu, X = OH) (1.051 g, 3.82 mmol) in 5 mL of THF and diethyl azodicarboxylate (759 $\mu$L, 4.58 mmol) in 5 mL of THF were prepared separately in oven-dried glassware and added to the reaction mixture by syringe pump over 2 h. The reaction was allowed to stir for an additional 30 min and then evaporated in vacuo to a yellow oil. The product was obtained by flash chromatography (150 g of silica gel, 3:1 hexane/ethyl acetate) giving a clear oil: 1.235 g, 74%; $[\alpha]^{23}_D = -9.5°$ ($c$ = 1.18, $CHCl_3$); $^1H$ NMR ($CHCl_3$) $\delta$ 8.00 (2 H, d, $J$ = 8.8 Hz, Ar), 6.96 (2 H, d, $J$ = 8.8 Hz, Ar), 5.20 (1 H, br d, $J$ = 4.5 Hz, NH), 4.37 (1 H, br m, CH), 4.14 (2 H, cm, $CH_2O$), 3.97 (3 H, s, $CH_3$), 2.27 (2 H, cm, $CH_2$), 1.48 (9 H, s, *tert*-butyl) 1.43 (9 H, s, *tert*-butyl); IR ($CHCl_3$) 3420, 2985, 2910, 1735, 1700, 1675, 1595, 1365, 1150, 1000. Anal. ($C_{22}H_{31}NO_8$), C, H, N.

**[4-[D-3-[(*tert*-Butoxycarbonyl)amino]-3-(*tert*-butoxycarbonyl)propoxy]benzoyl]formic Acid (31, R = H).** In a 50-mL round-bottomed flask equipped with a plastic cap and a magnetic stirring bar was dissolved **31** (R = Me) (1.235 g, 2.82 mmol) in 25 mL of methanol and the solution was cooled to 0 °C. After 10 min, 3.1 mL of 1 N sodium hydroxide was added and the solution was stirred at 0 °C for 15 min. Eight drops of 1 N HCl were added, and the solution was evaporated in vacuo. The yellow residue was dissolved in 40 mL of ethyl acetate and washed twice with 30-mL portions of 1 N HCl, twice with 30-mL portions of water, and once with 30 mL of brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo to a white sticky foam (1.04 g, 88%). This unstable material was sufficiently pure for use in the next reaction: $[\alpha]^{23}_D = -18.7°$ ($c$ = 1.10, MeOH). $^1H$ NMR ($CHCl_3$) $\delta$ 8.45 (2 H, br d, $J$ = 8.4 Hz, Ar), 6.97 (2 H, d, $J$ = 8.4 Hz, Ar), 5.22 (1 H, br d, $J$ = 7 Hz, NH), 4.38 (1 H, br m, CH), 4.17 (2 H, dt, $J$ = 2.2, 6.2 Hz, $CH_2O$), 2.28 (2 H, cm, $CH_2$), 1.48 (9 H, s, *tert*-butyl), 1.44 (9 H, s, *tert*-butyl); IR ($CHCl_3$) 3405–3200, 2905, 2860, 1715, 1700, 1630, 1585, 1355, 1145.

**Di-*tert*-Butyl *N*-(*tert*-Butoxycarbonyl)nocardicin D (38).** In an oven-dried, three-necked 50-mL round-bottomed flask equipped with a nitrogen inlet, a rubber septum, and a magnetic stirring bar was dissolved keto acid **31** (R = H) (608 mg, 1.44 mmol) in 7.5 mL of freshly distilled methylene chloride and the solution was cooled to −20 °C. 2,6-Lutidine (168 $\mu$L, 1.44 mmol) was added followed by isobutyl chloroformate (187 $\mu$L, 1.44 mmol) 5 min later. The resulting solution was stirred at −20 °C for 45 min.

A flame-dried 25-mL pear-shaped flask equipped with a nitrogen inlet, a rubber septum, and a magnetic stirring bar was charged with *tert*-butyl 3-aminocardicinate hydrochloride (**14**) (473.5 mg, 1.44 mol) and 5 mL of dry DMF and cooled to −10 °C. To this solution was added 2,6-lutidine (168 $\mu$L, 1.44 mmol), and stirring was continued for 5 min. The resulting solution was added dropwise by syringe to the solution of the mixed anhydride. The transfer was completed by two rinses with 1.5 mL of dry DMF. The reaction was stirred at −20 °C for 1 h. The mixture was diluted with 75 mL of ethyl acetate and 60 mL of water. The layers were separated, and the aqueous phase was extracted three times with 50 mL of ethyl acetate. The combined organic extracts were washed twice with 60-mL portions of 0.5 N HCl, three times with 60-mL portions of water, and once with 75 mL of brine. The organic layer was dried over sodium sulfate, filtered, and evaporated to a yellow foam. The crude product was purified by flash chromatography (50 g of silica gel, 4:1 methylene chloride/ethyl acetate) to afford **38** as a white foam: 778 mg, 78%; mp 188–191 °C, $[\alpha]^{21}_D = -101.6°$ ($c$ = 1.05, MeOH); $^1H$ NMR ($CHCl_3$) $\delta$ 8.33 (2 H, dd, $J$ = 1.3, 9.0 Hz, Ar), 7.60 (1 H, br d, $J$ = 7.2 Hz, C-3 NH), 7.19 (2 H, d, $J$ = 8.4 Hz, Ar), 6.90 (2 H, d, $J$ = 8.4 Hz, Ar), 6.87 (2 H, dd, $J$ = 1.0, 9.0 Hz, Ar), 5.49 (1 H, s, H-5), 5.22 (1 H, br d, $J$ = 7.7 Hz, C-9′ NH), 5.11 (1 H, cm, H-3), 4.37 (1 H, br m, H-9′), 4.13 (2 H, cm, H-7′), 4.00 (1 H, t, $J$ = 5.5 Hz, H-4$\alpha$), 3.12 (1 H, dd, $J$ = 2.4, 5.8 Hz, H-4$\beta$), 2.05 and 1.90 (2 H, 2 cm, H-8′) 1.47 (9 H, s, *tert*-butyl), 1.46 (9 H, s, *tert*-butyl), 1.43 (9 H, s, *tert*-butyl); IR ($CHCl_3$) 3560, 3400, 3300, 2905, 2865, 1765, 1750, 1715, 1700, 1650, 1585, 950 cm$^{-1}$. Anal. Calcd for $C_{36}H_{47}N_3O_{11}$: C, 61.97; H, 6.79; N, 6.02. Found: C, 62.53; H, 7.44; N, 5.48.

**Nocardicin D (4).** In a flame-dried 25-mL round-bottomed flask equipped with a nitrogen inlet and a magnetic stirring bar was mixed protected nocardicin D **38** (175 mg, 0.25 mmol) with 150 $\mu$L of 2-mercaptoethanol and 3.0 mL of trifluoroacetic acid. The resulting solution was stirred at room temperature for 6 min and then 4 mL of anhydrous ether and 10 mL of cyclohexene were added to precipitate the product. The solvents were removed by evaporation under high vacuum. The brown residue was triturated with three 10-mL portions of anhydrous ether and dried to a tan powder, crude nocardicin D trifluoroacetate. The powder was dissolved in 3 mL of water and filtered through a cotton plug to remove insoluble impurities. The filtrate was adjusted to pH 3 with 1.0 N NaOH to precipitate the product (75 mg, 62%) as an off-white powder: mp 231–235 °C dec (lit.[2] mp 230–235 °C dec); $[\alpha]^{23}_D = -186°$ ($c$ = 1.0, 1% aqueous $NaHCO_3$) [lit.[1] $[\alpha]_D = -186°$ ($c$ = 1.0, 1% aqueous $NaHCO_3$)]; $^1H$ NMR ($D_2O$, NaOD) $\delta$ 7.90 (2 H, d, $J$ = 7.4 Hz, Ar), 7.07 (4 H, 2 overlapping d, $J$ = 7, 8.5 Hz, Ar), 6.61 (2 H, d, $J$ = 8.5 Hz, Ar), 5.19 (1 H, s, H-5), 5.10 (1 H, br dd, $J$ = 1.5, 4 Hz, H-3), 4.23 (2 H, t, $J$ = 5.8 Hz, H-7′), 3.84 (1 H, t, $J$ = 5.6 Hz, H-9′), 3.45 (1 H, t, $J$ = 6 Hz, H-4$\alpha$), 2.97 (1 H, dd, $J$ = 2, 6 Hz, H-4$\beta$), 2.16 and 2.01 (2 H, 2 m, H-8′).

**Di-*tert*-butyl *N*-(*tert*-Butoxycarbonyl)nocardicin A (39).** A flame-dried 10-mL round-bottomed flask equipped with a nitrogen inlet and a magnetic stirring bar was charged with protected nocardicin D (**38**) (104.7 mg, 0.15 mmol) and 1.5 mL of absolute ethanol and cooled to 0 °C. To the resulting solution was added hydroxylamine hydrochloride (52.1 mg, 0.75 mmol) followed by 2,6-lutidine (35 $\mu$L, 0.30 mmol). The reaction was stirred at room temperature for 11 h. The solvent was evaporated in vacuo, and the residue was suspended in 25 mL of ethyl acetate. The suspension was washed with 20 mL of water, twice with 20-mL portions of 0.5 N HCl, twice with 20-mL portions of water, and once with 20 mL of brine. The organic solution was dried over anhydrous sodium sulfate filtered, and evaporated to a clear glass. The residue was purified by preparative TLC (2.0-mm plate, 10% methanol in methylene chloride, developed three times) and eluted from the plate with acetonitrile. The products were identified as **33** (11 mg, 10%) and **39** (84 mg, 79%), the higher $R_f$ and lower $R_f$ bands, respectively. **39**: mp 138.5–141 °C dec; $[\alpha]^{24}_D = -90.1°$ ($c$ = 0.64, MeOH); $^1H$ NMR (DMSO-$d_6$) $\delta$ 9.59 (1 H, s, OH) 9.13 (1 H, d, $J$ = 8.3 Hz, C-3 NH), 7.44 (2 H, d, $J$ = 8.9 Hz, Ar), 7.24 (1 H, d, $J$ = 8.0 Hz, NH), 7.13 (2 H, d, $J$ = 8.6 Hz, Ar), 6.93 (2 H, d, $J$ = 8.9 Hz, Ar) 6.76 (2 H, d, $J$ = 8.6 Hz, Ar), 5.30 (1 H, s, H-5), 4.79 (1 H, m, H-3), 4.01 (3 H, m, H-7′ and H-9′), 3.76 (1 H, t, $J$ = 5.4 Hz, H-4$\alpha$), 3.05 (1 H, dd, $J$ = 2.3, 5.4 Hz, H-4$\beta$), 2.17 and 2.09 (2 H, 2 cm, H-8′), 1.41 (9 H, s, *tert*-butyl), 1.37 (9 H, s, *tert*-butyl), 1.36 (9 H, s, *tert*-butyl); IR ($CHCl_3$) 3560, 3400, 3310, 2910, 2860, 1750, 1730, 1680, 1600, 1355, 1350, 1145, 945 cm$^{-1}$. Anal. ($C_{36}H_{48}N_4O_{11}$) C, H, N.

**Nocardicin A (1).** A flame-dried 25-mL round-bottomed flask equipped with a nitrogen inlet and a magnetic stirring bar was charged with protected nocardicin A (**39**) (47 mg, 0.066 mmol), 100 $\mu$L of 2-mercaptoethanol, and 2.0 mL of trifluoroacetic acid. The resulting so-

lution was stirred at room temperature for 7 min and then 4 mL of anhydrous ether and 10 mL of cyclohexane were added to precipitate the product. The solvents were removed by evaporation under high vacuum. The tan residue was triturated with three 10-mL portions of anhydrous ether and dried to an off-white powder. The powder was dissolved in 4 mL of water and filtered through a cotton plug to remove insoluble impurities. The filtrate was adjusted to pH 2.5, causing precipitation of the product as a white powder: 22 mg, 67%; mp 211-214 °C dec (lit.[1] mp 214-216 °C dec); $[\alpha]^{23}_D = -148°$ $c = 1$, 1% aqueous $NaHCO_3$) [lit.[1] $[\alpha]_D = -146°$ ($c = 1.0$, 1% aqueous $NaHCO_3$); $^1H$ NMR ($D_2O$ + NaOD) $\delta$ 7.36 (2 H, d, $J = 8.9$ Hz, Ar), 7.06 (2 H, d, $J = 8.6$ Hz, Ar), 6.99 (2 H, d, $J = 8.6$ Hz, Ar), 6.60 (2 H, d, $J = 8.9$ Hz, Ar), 5.20 (1 H, s, H-5), 5.03 (1 H, dd, $J = 5$, 1.8 Hz, H-3), 4.16 (2 H, t, $J = 6.5$ Hz, H-7'), 3.82 (1 H, t, $J = 5.5$ Hz, H-9'), 3.44 (1 H, t, $J = 5.5$ Hz, H-4$\alpha$), 3.30 (1 H, dd, $J = 2$, 4 Hz, H-4$\beta$), 2.14 and 1.99 (2 H, 2 cm, H-8').

**Acknowledgment.** We are pleased to acknowledge and thank L. T. Nguyen, W. E. Stites, and Dr. M. J. DiNovi for preliminary studies that provided the foundation for the syntheses described above and A. McE. Reeve and J. J. Hangeland for collecting some of the analytical data. We are grateful to the National Institutes of Health (Grant AI 14937), the Alfred P. Sloan Foundation, and the Camille and Henry Dreyfus Foundation for financial support of this research. Major analytical instrumentation used in this work was acquired with funds from the NIH and NSF (NMR, Grants RR 01934 and PCM 83-03176; MS, Grant RR 02318).

**Registry No. 1**, 39391-39-4; **2**, 60134-71-6; **2** trifluoroacetate, 123004-78-4; **3**, 59511-12-5; **4**, 61425-17-0; **4** trifluoroacetate, 123004-81-9; **5**, 63555-59-9; **6**, 63598-46-9; **7**, 65309-11-7; **12**, 111216-46-7; **13**, 110207-46-0; **14**, 123004-73-9; **15** (R = H), 27460-85-1; **16** (R = H), 110207-48-2; **27** (R = R' = *t*-Bu; X = OH), 110207-49-3; **27** (R = R' = *t*-Bu; X = Br), 123004-74-0; **28** (R = $CH_2Ph$), 26787-75-7; **29**, 123004-75-1; **30**, 123004-79-5; **31** (R = Me), 110207-50-6; **31** (R = H), 110207-53-9; **32** (R = $CH_2Ph$), 123004-76-2; **32**·HCl (R = H), 123004-77-3; **33**, 110269-45-9; **34**, 123004-80-8; **37**, 15206-55-0; **38**, 110207-51-7; **39**, 110207-52-8; D-[*p*-(benzyloxy)phenyl]glycine, 69489-40-3; D-[*p*-(benzyloxy)phenyl]glycine *tert*-butyl ester toluenesulfonate salt, 123004-72-8; L-Ox-serine dicyclohexylammonium salt, 48201-16-7; D-(*p*-hydroxyphenyl)glycine, 22818-40-2; $\alpha$-*tert*-butyl *N*-(*tert*-butoxycarbonyl)-D-aspartate, 77004-75-2; sodium (*p*-hydroxybenzoyl)formate, 54537-30-3.

# Asymmetric Alkylations of a Phenylalanylglycinate Equivalent. Novel Route to Dipeptides Bearing $\alpha$-Alkyl-$\alpha$-amino Acid Residues

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**Abstract:** Asymmetric single and double alkylations of chiral $\beta$-lactam acetate **1**, which is a chiral glycinate as well as a phenylalanylglycinate equivalent, are studied. First, the sequential asymmetric double alkylation of **1a** is performed to give the corresponding doubly alkylated $\beta$-lactam esters **3** (>99% de) in high yields, which can readily be converted to the corresponding dipeptides (**4**) via dissolving metal reduction in good yield. The salient advantage of this method is that a quaternary chiral center of desired configuration can be created just by changing the order of the addition of two alkyl halides used ($R^1 \neq R^2$). Remarkable effect of temperature on stereoselectivity is observed in the asymmetric single alkylation of **1a**, e.g., in the lithium enolate formation and alkylation of **1a** with allyl bromide; the observed stereoselectivities ($R/S$) are 7.6/1 at −95 °C, >50/1 at −78 °C, 7.9/1 at −50 °C, and 4.4/1 at −30 °C. Similar dependence of stereoselectivity on the reaction temperature is also observed for the reactions with methyl iodide and benzyl bromide. When ethyl bromoacetate was used as an electrophile, the reaction gave the highest stereoselectivity at −97 °C (>50/1) rather than at −78 °C, and the stereoselectivity decreased along with the increase of temperature. A rationale for the observed effect of temperature on stereoselectivity is proposed. The single alkylation products can be readily converted to the corresponding dipeptides through dissolving metal reduction and then to amino acids by hydrolysis; hence this asymmetric single alkylation serves as a new and effective method for the synthesis of enantiomerically pure non-protein amino acids and their dipeptides. Finally, the sequential asymmetric triple alkylation of **1a** with methyl iodide, allyl bromide, and methyl iodide is successfully achieved to give **5a-1** with virtually complete stereoselectivity. Deprotection of the *tert*-butyl ester of **5a-1** followed by the cleavage of $\beta$-lactam ring as well as the removal of *N*-protection with $Li/NH_3/THF/t$-BuOH at −78 °C gave enantiomerically pure ($S$)-$\alpha$-methylphenylalanyl-($R$)-$\alpha$-allylalanine (**6a-1**) after purification on an ion-exchange column.

The significance of non-protein amino acids has recently been recognized in connection with design and synthesis of enzyme inhibitors as potential pharmaceutical drugs and also for the study of enzymatic reaction mechanisms.[2-5] In particular, $\alpha$-alkyl-$\alpha$-amino acids have been attracting medicinal and biochemical interests, i.e., (a) those amino acids which are known to be powerful enzyme inhibitors for e.g., dopa,[2] ornithine,[3] glutamate,[3] *S*-adenosylmethionine (SAM) decarboxylases,[4] and aspartate aminotransferase[5] and (b) those amino acids which act as conformational modifiers for physiologically active peptides.[6] $\alpha$-Alkyl-$\alpha$-amino acids also provide a challenging synthetic problem for chemists since the $\alpha$-alkyl-$\alpha$-amino acids have chiral quaternary carbons, and thus conventional enzymatic optical resolution technology cannot be applied effectively, viz., no racemization can take place at the chiral $\alpha$-carbons, and thus D isomers cannot be recycled to the optical resolution process.[7] Therefore, the asymmetric synthesis of optically pure $\alpha$-alkyl-$\alpha$-amino acids is

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