Synthesis of DNA-Interactive Pyrrolo[2,1-*c*][1,4]benzodiazepines

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I. Introduction

In the area of molecular recognition there is growing interest in ring systems such as the pyrrolo[2,1-*c*][1,4]-benzodiazepines (PBDs) that can recognize and bind to specific sequences of DNA. Such compounds have potential as regulators of gene expression with possible application as therapeutic agents in the treatment of certain genetic disorders including some cancers.[1] They also have potential as highly selective anti-infective agents and as tools such as affinity-cleavage reagents for use in molecular biology.[1,2] The PBD ring system (1) (Scheme 1) is found in a group of naturally-occurring DNA-interactive antitumor antibiotics known as the "anthramycins". They are produced by various *Streptomyces* species; well-known members include anthramycin[3] (2) and tomaymycin[4] (6) (Scheme 2). Other antibiotics in the series include abbeymycin[5] (12), chicamycin A[6] (11), DC-81[7] (10), mazethramycin[8] (3), the neothramycins A and B[9] (9), prothracarcin[10] (7), sibanomicin (DC-102)[11] (8), sibiromycin[12] (5), and porothramycin B[13] (4). The biosynthesis of a number of PBDs has been studied by Hurley.[14]

The PBDs differ in the number, type, and position of substituents in both the aromatic A rings and pyrrolo C rings and in the nature of the C ring which is either fully saturated or unsaturated at either C2–C3 (endocyclic) or at C2 (exocyclic). All naturally-occurring compounds possess the (*S*)-configuration at C11a which provides the molecules with a right-handed twist when viewed from the C ring toward the A ring (Figure 1). This provides the appropriate 3-dimensional shape for a snug fit within the minor groove of DNA. Racemization at C11a can significantly reduce biological activity, and there is one example of a synthetic PBD with the (*R*)-configuration at C11a that is devoid of antitumor activity and DNA-binding affinity.[15a] The

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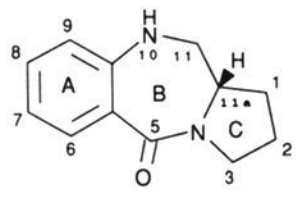
David E. Thurston was born on May 23, 1955, in London, England. He received his B.Sc. (Hons) in Pharmacy in 1976 from the University of Portsmouth (Hampshire, U.K.) specializing in medicinal chemistry and a Ph.D. in synthetic medicinal chemistry from the same institution in 1980 in collaboration with Glaxo Group Research Ltd. under the guidance of Drs. Colin G. Richards and Roger F. Newton. He then moved to the USA for postdoctoral studies with Professor Laurence H. Hurley, first at the University of Kentucky (1981) and then at the University of Texas at Austin (1982–1983) before joining the faculty in Austin as Assistant Professor (1983–1986). He returned to the U.K. in 1987 to take up a position in the School of Pharmacy and Biomedical Sciences at the University of Portsmouth where he is currently Reader and Head of Medicinal Chemistry. His research interests include the design, synthesis, and development of novel therapeutic agents and biochemical tools based on sequence-selective DNA-interactive ligands.

D. Subhas Bose was born on December 5, 1961, in Huzurabad, Andhra Pradesh, India. He received his M.Sc. in Chemistry from the Osmania University (India) in 1983 followed by a Ph.D. in synthetic chemistry from the University of Poona (India) under the guidance of Dr. A. V. Rama Rao (Director, Indian Institute of Chemical Technology, IICT, Hyderabad). He joined Dr. David E. Thurston at the University of Portsmouth in 1990 for postdoctoral studies and then returned to India in 1993 to take up a senior post in the Fine Chemicals Laboratory of the IICT, Hyderabad. His current research interests include the synthesis of DNA-interactive ligands.

Scheme 1



1

N10–C11 carbinolamine form (**13a**) (Scheme 3) may exist in the equivalent imine (**13b**) or carbinolamine methyl ether form (**13c**), depending upon the precise structure of the compound and the method of isolation or synthetic workup.[16] For example, the imine and methyl ether forms of DC-81 (**10**) may be interconverted by either dissolution of the imine in methanol or by several cycles of refluxing the methyl ether in $CHCl_3$ followed by evaporation of the solvent *in vacuo*.

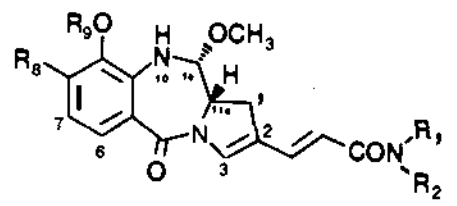
The mechanism of action of the PBDs derives from their ability to bind covalently within the minor groove, thus interfering with DNA function.[16a] After insertion in the minor groove, an aminal bond is formed through nucleophilic attack of the exocyclic N2 of a guanine at the electrophilic C11-position (Scheme 4 and Figure 2). The structure of the anthramycin–DNA adduct was initially studied by Hurley who utilized indirect techniques,[16a,17–21] but more recently, NMR,[22] fluorescence spectroscopy,[23] and molecular modeling[22c,e,f,23b,c,24] have been employed. Structure–activity relationship (SAR) predictions based upon CPK models have also been proposed by Thurston and Hurley.[16a,25] DNA footprinting studies have demonstrated that, in general, PBDs bind to DNA in a sequence-selective manner with a preference for 5′-Pu-G-Pu motifs (where Pu = either purine base and G = guanine).[15a,16a,26] Measurement of DNA-binding affinity of a series of PBDs using a sensitive and quantitative assay based on inhibition of linearization of plasmid pBR322 DNA by endonucleases (e.g. *BamH*1) has demonstrated a correlation between DNA-binding affinity and cytotoxicity in some cell lines.[16a,27] More recently, PBDs have been joined through their C8-positions to form potent irreversible DNA cross-linking agents with remarkable cross-linking efficiency and cytotoxicity.[28] NMR and modeling studies have shown that these PBD dimers span six or seven base pairs compared to three for the parent PBD units. A PBD has also been attached to EDTA to give a guanine-specific affinity-cleavage reagent.[29] Some general reviews of the PBDs have appeared.[16a,25,30]

II. General Remarks

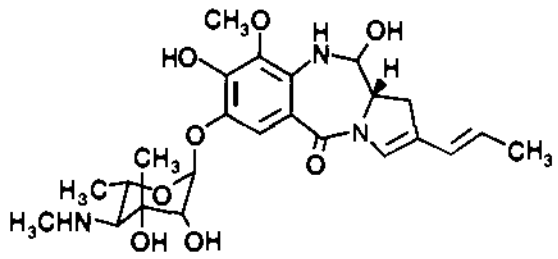
Although PBDs with either a secondary amine (e.g. **1**, Scheme 1, or **17**, Scheme 3) or amide functionality (e.g. **14**, Scheme 3) at N10–C11 are readily synthesized, the introduction of an imine or carbinolamine (or the equivalent) at this position is problematic due to the reactivity of these functional groups. In addition, the stability of a carbinolamine-containing PBD is related to the type and pattern of aromatic A-ring substituents and other structural features such as the degree of C-ring saturation.[16] For this reason, the carbinolamine moiety (or its equivalent) is usually generated during the final synthetic step under the mildest possible conditions. Furthermore, reaction conditions capable of causing racemization at the C11a-position must be avoided in order to maintain the correct 3-dimensional configuration to provide isohelicity with the minor groove of DNA.[15a] A number of partial reviews of the synthetic literature relating to the PBDs have appeared.[30c,d,f,g] The purpose of this review is to survey all known methods of PBD synthesis to date and to suggest other routes with potential use. This should allow the most appropriate strategy to be selected for a particular target molecule.

The first synthesis of a carbinolamine-containing PBD appeared in 1968 when Leimgruber reported the total synthesis of anthramycin.[31] This involved $NaBH_4$ or $LiAlH_4$ reduction of the corresponding pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (PBD-5,11-dione or "dilactam") of type **14** (Scheme 3). This method was

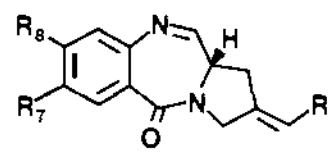
Scheme 2



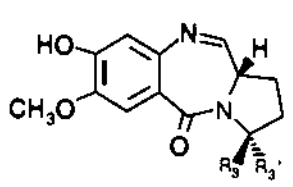
2 Anthramycin ($R_8 = CH_3$, $R_9 = R_1 = R_2 = H$)
3 Mazethramycin ($R_8 = R_1 = CH_3$, $R_9 = R_2 = H$)
4 Porothramycin B ($R_8 = H$, $R_9 = R_1 = R_2 = CH_3$)



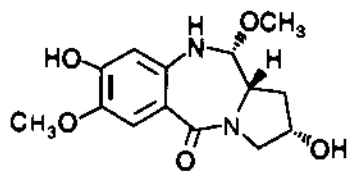
5 Sibiromycin



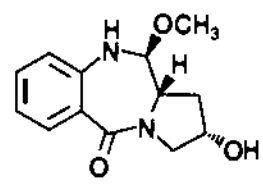
6 Tomaymycin ($R_7 = CH_3O$, $R_8 = HO$, $R = CH_3$)
7 Prothracarcin ($R_7 = R_8 = H$, $R = CH_3$)
8 Sibanomicin ($R_8 = H$, R_7 = sibirosamine pyranoside as in 5, R = Et)



9a Neothramycin A ($R_3 = H$, $R_{3'} = OH$)
9b Neothramycin B ($R_3 = OH$, $R_{3'} = H$)
10 DC-81 ($R_3 = R_{3'} = H$)



11 Chicamycin A



12 Abbeymycin

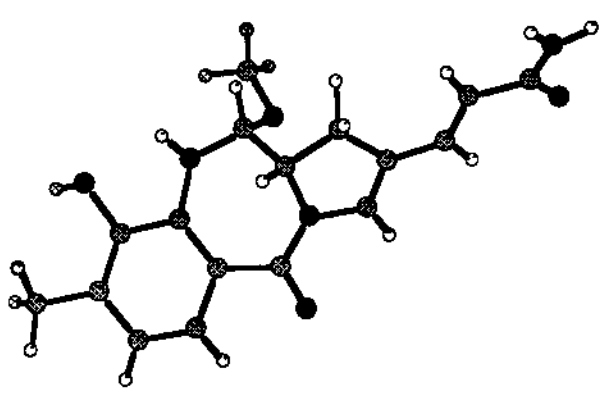


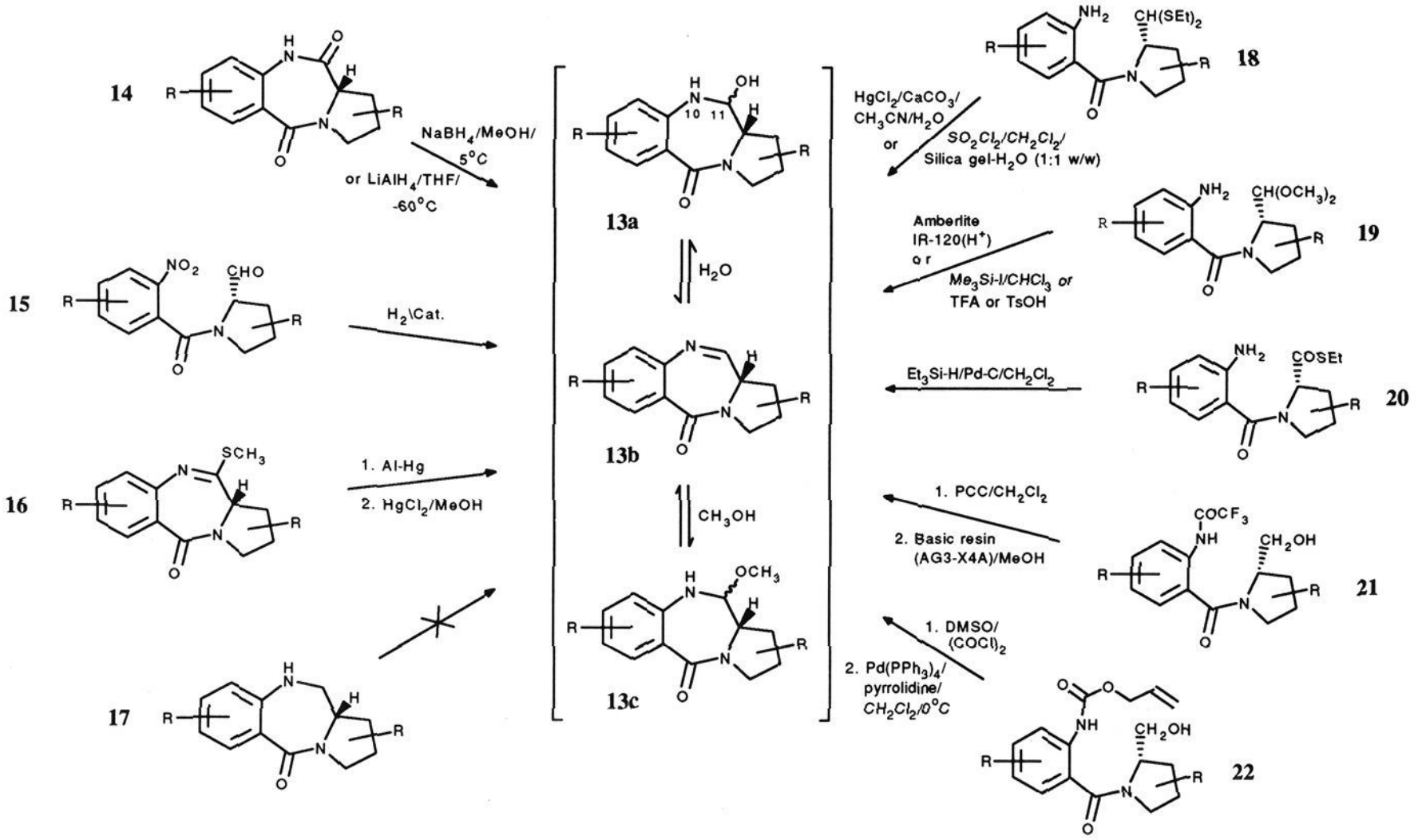
Figure 1. Structure of anthramycin methyl ether (2) based on X-ray crystallography data[3c] demonstrating the twist of the molecule that provides isohelicity with the minor groove of B-form DNA.

shown to have limitations with regard to A-ring substituent requirements;[32] however, it has been reported that the use of a MOM protecting group on the N10-position allows this approach to be used for the synthesis of PBDs such as neothramycin (**9**).[33] Lown and Joshua later reported[34] an alternative method involving reductive cyclization (H_2/catalyst) of a *N*-(2-nitrobenzoyl)pyrrolidine-2-carboxaldehyde of type **15**, a method which has also been applied to the total synthesis of tomaymycin[35] and the neothramycins A and B.[9b] This approach suffers from the limitation of producing varying amounts of N10–C11 secondary amine overreduction products of type **17**.[36] However, the mechanism of both of these reactions has been studied,[32,36] and their usefulness extended. In 1983, Kaneko reported an alternative approach, involving an aluminum-amalgam reduction of an imino thioether of type **16**, prepared in two steps from a PBD dilactam (**14**).[37] This method has been used to synthesize the natural products tomaymycin[37] and chicamycin.[38] A more recent development by Thurston and co-workers involves $HgCl_2$-mediated cyclization of *N*-(2-aminobenzoyl)pyrrolidine-2-carboxaldehyde diethyl thioacetals of type **18**,[39a] a route with many advantages that has been used to synthesize prothracarcin,[39a] DC-81,[40] both C8-[28] and C7-linked[41] PBD dimers, and some A-ring-modified PBD analogs (see Section VII.A). More recently, this approach has been modified by the introduction of sulfuryl chloride as the cyclization reagent rather than $HgCl_2/CaCO_3$ which provides an easier workup procedure and higher yields of products.[39b] Acid (TFA),[15a] $(CH_3)_3SiI$,[15b] or Amberlite IR-120[15c] catalyzed cyclization of amino dialkyl acetals of type **19** has also been used, but can lead to racemization at the C11a-position.[15a] Another method involving reduction of an amino ethyl thiol ester (**20**) with Et_3SiH/Pd–C was recently introduced by Fukuyama and co-workers and applied to the synthesis of the neothramycins.[42a] Cyclization of N-protected amino alcohols of type **21** or **22** has also been reported. Oxidation of the *N*-(trifluoroacetyl)-protected amino alcohol **21** affords a cyclized N-protected carbinolamine intermediate that can be deprotected by treatment with basic resin.[15a] A variation of this approach has been used by Fukuyama and co-workers[42b] to synthesize porothramycin B (**4**, Scheme 2). Swern oxidation of the *N*-allylcarbamate-protected amino alcohol **22** afforded a cyclized N-protected carbinolamine intermediate that could be deprotected with $Pd(Ph_3)_4$/pyrrolidine/CH_2Cl_2/0 °C.[42c] Finally, although a new and efficient route exists for the synthesis of N10–C11 secondary amines of type **17**,[43] there are presently no useful methods for converting the N10–C11 amine functionality to an imine or the equivalent. Each synthetic method outlined above will be reviewed in detail.

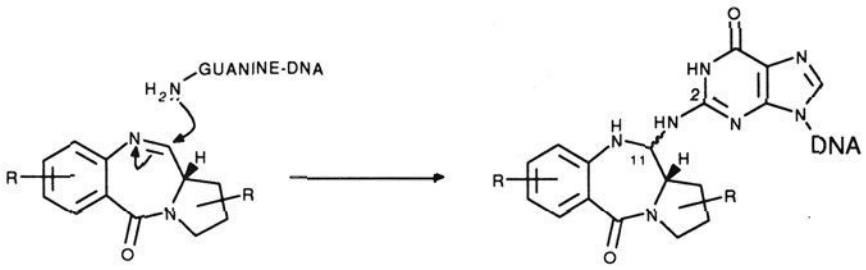
*III. Hydride Reduction of Pyrrolo[2,1-c][1,4]*benzodiazepine-5,11-diones (Dilactams)

This section describes the reduction of dilactams of type **14** (Scheme 3) to carbinolamines using hydride-donating reducing agents. It is divided into two subsections, the first describing the synthesis of dilactams and the second, their conversion into carbinolamines. It was the first reported method for the production of a carbinolamine-containing PBD and was used for the total synthesis of anthramycin (**2**, Scheme 2).[31] It suffers from limitations with respect to the type and pattern of A-ring substituents,[32] although a recent development by Mori[33] appears to have improved the scope. One potential advantage is that tritiated sodium boro[^{3}H]hydride could be used to produce radiolabeled PBD analogs useful for DNA-binding experiments.[32c]

Scheme 3



Scheme 4



A. Synthesis of Pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-diones

There are three major routes to pyrrolo[2,1-*c*][1,4]-benzodiazepine-5,11-diones. A fourth method is not used for preparative purposes:

1. Cyclization of N-(2-Nitrobenzoyl)pyrrolidine-2-carboxylic Acids or Esters

This involves cyclization of *N*-(2-nitrobenzoyl)pyrrolidine-2-carboxylic acids or esters, typically prepared by coupling the corresponding 2-nitrobenzoyl chlorides and prolines. For example, the *N*-(2-nitrobenzoyl)-pyrrolidine-2(*S*)-carboxylic acids of type **23a** (R_5 = H) cyclize during hydrogenation for up to 4 h (10% Pd–C/EtOH or MeOH or EtOAc/room temperature/H_2 atm) to afford dilactams of type **24a** in high yields (Scheme 5).[44a,b] Nitro esters such as **23b** ($R_1 = NO_2$, R_5 = Et), prepared by coupling the appropriate 2-nitrobenzoyl chloride and proline ethyl ester (Et_2O/$(Et)_3N$/0–5 °C), may also be hydrogenated to the corresponding amine (**23b**, $R_1 = NH_2$) which can then be cyclized by refluxing in toluene for 1 h[45a] or treatment with 1 N HCl.[45b] A similar two-step process (hydrogenation with Pd–C in EtOAc followed by refluxing in toluene) was used to prepare **24c** ($R_3 = R_7$ = HO; R_4 = CH_3O) from **23c** (R_3 = *p*-nitrobenzyloxy), an intermediate in the synthesis of chicamycins A and B (**11**, Scheme 2).[38] An attempt was also made to use this method for the preparation of the sibiromycin aglycon dilactam of type **24d**,[46] which is now known to lack unsaturation at C11a–C1 (**5**, Scheme 2).[12] However, upon catalytic hydrogenation of **23d** ($R_1 = NO_2$) using Lindlar's catalyst (Pd-$CaCO_3$/H_2 atm/toluene/room temperature/7 h), reduction of the propenyl side chain was faster than reduction of the nitro group. Chemical reduction of the nitro group was complicated by nucleophilic cleavage of the C5–amide bond, although triiron dodecacarbonyl ($Fe_3(CO)_{12}$/MeOH/PhH/1.7 h) gave a 30% yield of the required amine (**23d**; $R_1 = NH_2$) which was cyclized to **24d** (63% yield) upon refluxing

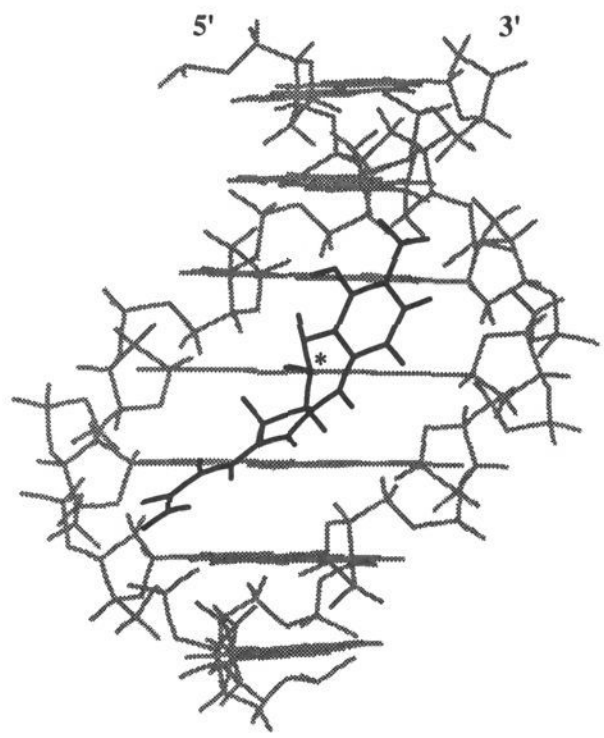
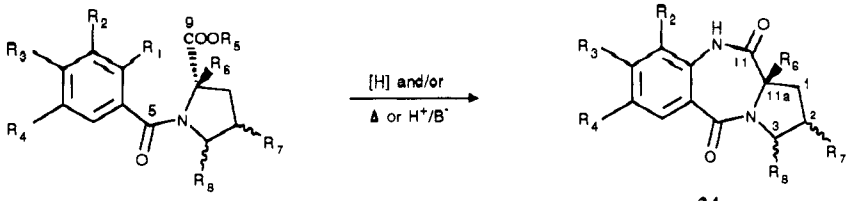


Figure 2. Model of anthramycin covalently linked [through a C11(*S*) bond] to the N2 of a guanine residue (marked with *) within the minor groove of B-form DNA (A ring oriented toward the 3′-end of the covalently-modified strand; model is not energy minimized).

Scheme 5



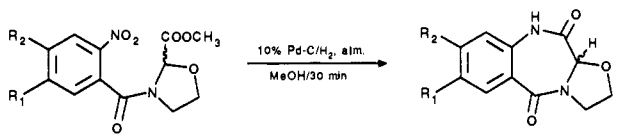
23 24

	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	C-Ring
a	NO_2	H or BnO	H, CH_3, HO, BzO, or BnO	H or CH_3O	H or Et	H	H or OH	H	-
b	NO_2 or NH_2	H or HO	H, HO CH_3 or EtO	H or CH_3O	CH_3 or Et	H or COOEt	H, OH, COOMe or COOEt, CH_2-CH=CH_2	H	C2=C3
c	NO_2 or NH_2	H or BnO	H, HO, CH_3 or p-NO_2BnO	H or CH_3O	Et	H, CH_2OH, CH_2-CH=CH_2	H or OH	H	-
d	NO_2 or NH_2	H or CH_3O	CH_3 or BnO	CH_3O	Et	-	CH_2=CH-CH_3 or H	H or OH	Pyrrole
e	NO_2	BnO	CH_3	H	Et	H	OH	H	-

in toluene for 40 min with a trace amount of *p*-toluenesulfonic acid. Chemical reduction was also utilized to prepare an early intermediate in the total synthesis of anthramycin (**2**).[31,47] Sodium dithionite ($Na_2S_2O_4$) in THF/H_2O at 40 °C was used to convert a nitro ester of type **23c** (R_1 = NO_2) into the corresponding amine (**23c**; R_1 = NH_2), which cyclized in 86% yield upon treatment with aqueous HCl. Confalone[48] used titanium trichloride (20% $TiCl_3$ in H_2O/MeOH) to reduce a nitro amide of type **23c** (R_1 = NO_2, R_6 = CH_2-CH=CH_2) to the amine (R_1 = NH_2) and then $NaOCH_3$ (25% in MeOH/18 h/room temperature) to effect ring closure (74% yield over two steps). Baraldi and coworkers have used an identical approach to synthesize dilactam analogs with heterocyclic A rings which is described in Section VII.A.

A variation of this type of cyclization was reported by De Martino.[49a] An amino diester of type **23b** (R_1 = NH_2, R_5 = Et, R_6 = COOEt), prepared by catalytic reduction (Pd–C/H_2) of the corresponding nitro diester (R_1 = NO_2), lost ethyl carbonate on heating to afford the dilactam **24b** (R_6 = H). A similar approach using a nitro ester (**23c**) with R_6 = CH_2OH afforded a dilactam (**24c**) with R_6 = CH_2OH. A new reagent combination, $FeSO_4$/NH_3 (Δ), EtOH/H_2O, 2 h), has also been used to cyclize diesters of type **23b** (R_5 = Et, R_6 = COOEt) to give dilactams of type **24b** (R_6 = COOEt).[49b] A similar approach *via* the diester but using an alternative reducing agent was used to prepare the C8-*O*-benzoyl-protected neothramycin dilactam.[50] Treatment of an ester of type **23d** (C11a–C1 saturated, C2=C3, R_3 = BnO, R_4 = CH_3O, R_5 = Et, R_7 = R_8 = H) with sodium dithionite ($Na_2S_2O_4$) in methanol at room temperature afforded the corresponding dilactam directly, which was hydrated to give the neothramycin dilactam **24d** (C ring = pyrrolidine, R_3 = BnO, R_4 = CH_3O, R_7 = H, R_8 = OH) upon treatment with aqueous formic acid. Pena and Stille[51] have prepared the dilactam **24e** by reductive cyclization of the nitro ester **23e** using

Scheme 6



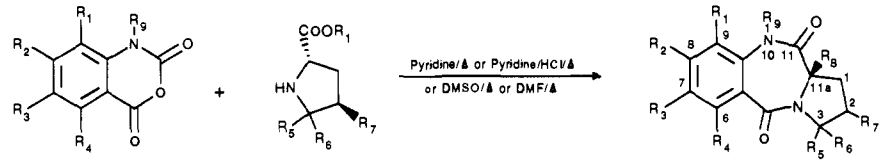
25 a R_1 = R_2 = H
b R_1 = CH_3O, R_2 = BnO

26 a R_1 = R_2 = H
b R_1 = CH_3O, R_2 = HO

$Na_2S_2O_4$. A number of dilactam analogs of neothramycin of type **24** (including R_4 = H or Cl, C2=C3, R_6 = COOEt and R_8 = OH) have also been prepared by the diester method[52] using $FeSO_4$ as the reducing agent. Thermal cyclization of amino esters of type **23** has appeared in the patent literature[53] and involves heating for 0.5–2 h in a solvent such as benzene, toluene, or xylene. A related method involving formation of an amino acid *in situ via* hydrolysis of a nitrile group has been reported by Artico[54] who demonstrated that an amino nitrile of type **23** (R_1 = NH_2, C5 = CH_2, C9 = C≡N) could be hydrolyzed under alkaline conditions to afford the corresponding dilactam. It is noteworthy that examples of the oxazolo[2,3-*c*][1,4]benzodiazepine-5,11-dione (OBD) ring system[55] (e.g. **26a,b**, Scheme 6) were obtained in one step by hydrogenation of the corresponding nitro methyl esters (**25a,b**) with 10% Pd–C/H_2 atm/MeOH for 30 min. In contrast to the PBD system, the rapid ring closure (i.e. 30 min at room temperature) probably reflects the electron-withdrawing effect of the C-ring oxygen. Reductive cyclization of nitro acids and esters of type **23** (R_5 = H or Et) in low yield has also been achieved in the presence of liver microsomes.[56]

Finally, the type of approaches described in this section have also been used to synthesize pyrrolo[2,1-*c*][1,4]benzothiazepine-5,11-diones and their equivalent N10–C11 secondary amines, which is discussed in Section VII.B.

Scheme 7



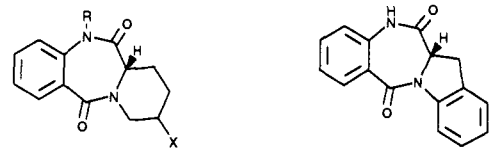
27 28 29

	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	C-Ring
a	H	H	H	H	H	H	-CH=CH-$COOCH_3$	H	H	C2=C3
b	H	H	H	H	H	H	-$COOCH_3$	H	H	C2=C3
c	H	H	F, H or NO_2	H	H	H	H or OH	H	H or CH_3	-
d	H	H	H	H	H or CH_3	H or CH_3	H	H or CH_3	H or CH_3	-
e	OCH_3	CH_3	H	H	H	H	OH or =O	H	H	-
f	H or CH_3	H	H or CH_3	H or CH_3	CH_3	CH_3	H	H	H	-
g	BnO	H	H	H	H	H	H	H	H	-
h	H	H	H	H	H	H	OH	H	H	-

2. Cyclocondensation of Isatoic Anhydrides with Substituted Prolines

An early description of this method[45b,57] involved a two-step synthesis of dilactams **29a** and **29b** (Scheme 7) *via* condensation of isatoic anhydride (**27a**) in pyridine with crude samples of the unstable pyrrolines **28a** or **28b**, formed by the copper oxide-promoted 1,6-addition of methyl isocyanoacetate to either methyl pentadienoate or methyl acrylate in benzene, the latter providing 35% yield. Reaction of isatoic anhydride and a proline derivative in DMSO or DMF at 100–110 °C for 1–20 h is a more popular method and has been used to produce dilactams of type **29** with substituents both in the aromatic A ring and in the R7- and R8-positions.[58a-g] This method has been used to prepare an intermediate (**29g**) used in the total synthesis of tilivalline,[58h] and also to prepare a previously published intermediate **29e** (R_7 = OH) used in a route to anthramycin. In this case, a novel, sequential directed ortho-metalation strategy was used to make a substituted anthranilic acid[58e] that was converted into an isatoic anhydride of type **27e** in high yield upon brief exposure (3 min) to oxalyl chloride in refluxing benzene. This was coupled to *trans*-4-hydroxy-L-proline (**28h**) in DMSO, to afford an intermediate of type **29e** (R_7 = OH). Pena and Stille also employed an isatoic anhydride to prepare **29h**, an intermediate used for the synthesis of anthramycin type molecules.[58f] The parent unsubstituted dilactam **29h** (R_7 = H) has also been prepared by this route,[37,58g] and 5-nitroisatoic anhydride (**27c**, R_3 = NO_2, R_9 = H) and L-proline methyl ester have been coupled to give the dilactam **29c** (R_3 = NO_2, R_7 = R_9 = H) using pyridine as solvent.[32c] Condensation of isatoic anhydride (**27a**) and *trans*-4-hydroxyproline (**28h**) to give dilactam **29h** was found to be more efficient using DMF[58i] as solvent rather than DMSO.[37,58a] After oxidation to the corresponding PBD-2,5,11-trione **29h** (R_7 = =O), heating in $POCl_3$ for 1.5 h at 80–90 °C gave the novel 5,10,11,11a-tetrahydro-2-chloro-1*H*-PBD-5,-11-dione (**29h**, R_7 = Cl, C2=C3).[58i] Dilactams prepared by this route have also been converted into their C11-thiolactam and C5,C11-dithiolactam equivalents.[58j,k] In one case, conversion of both these types of thiolactams to N10–C11 unsubstituted or substituted amidines was observed upon treatment with $HgCl_2$/THF/50 °C and either NH_3[58k] or R-NH_2,[58l] respectively. In addition, treatment of a C5,C11-dithiolactam with $HgCl_2/H_2O$ was shown to selectively convert it to the corresponding C5-thiolactam.[58k] Similarly, selective desulfurization of the C5-position of C5,C11-dithiolactams has recently been accomplished by an enzymatic process involving Baker's yeast (EtOH/buffer: pH 11.2/37 °C/2 days).[58m] The preparation of isatoic anhydrides has been reported by Schultz and co-workers[58g] who also synthesized methyl dilactams of type **29f** (R_1 or R_3 or R_4 = CH_3) by refluxing the appropriate isatoic anhydrides and proline with 1 equiv of pyridine hydrochloride in pyridine for 6 h (73–91% yields). The synthesis and reactivity of isatoic anhydrides has also been reviewed by Coppola.[59] Pyridinobenzodiazepines of type **30**,[33,57,60] pyrrolobenzodiazocines,[61] and the tetracyclic dilactam **31**[57,60] have also been reported (Scheme 8). Dilactams with fully saturated A rings have also been synthesized.[58g] More recently, the isatoic anhydride **33** (Scheme 9) was prepared by treatment of the anthranilic acid derivative **32** with *N,N'*-carbonyldiimidazole (CDI) (stirring for 1 h in DMF at 50 °C, followed by 3 days at room temperature; 67% yield). This was coupled to (*S*)-3,4-didehydroproline (dry DMSO/100 °C/3.5 h) to afford the novel 1,2-didehydrodilactam **34** in 57% yield.[15a]

Scheme 8

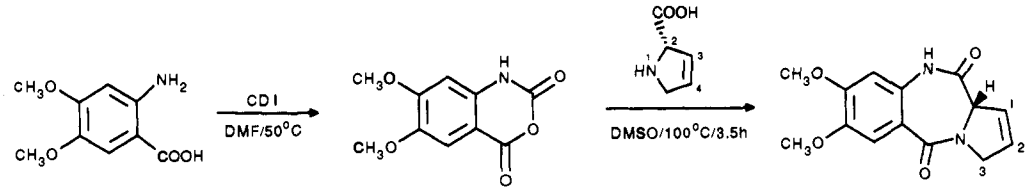


30 R = H or CH_3, X = H or OH **31**

3. Palladium-Catalyzed Carbonylation of Proline 2-Haloanilides

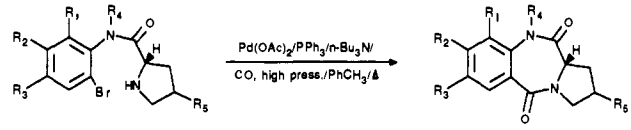
Ishikura and co-workers first reported[62] the use of palladium-catalyzed carbonylation to prepare dilactam **36a**, a precursor of a previously reported intermediate (**36g**) used in the synthesis of anthramycin.[31] The

Scheme 9



32 33 34

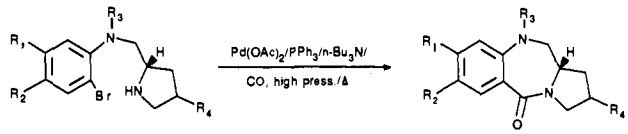
Scheme 10



35 36

	R_1	R_2	R_3	R_4	R_5
a	CH_3O	CH_3	Cl	CH_2OCH_3	OCH_2OCH_3
b	H	H	H	H or CH_3	H
c	H	HO	CH_3O	CH_2OCH_3	H
d	H	H	H	CH_2OCH_3	=CH-CH_3 (E)
e	H	TsO	CH_3O	CH_2OCH_3	=CH-CH_3 (E)
f	H	TsO	CH_3O	H	H
g	BnO	CH_3	H	H	=O

Scheme 11

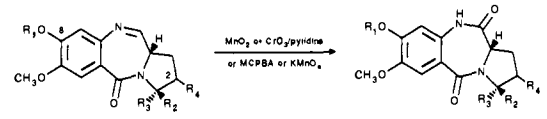


37 38

a R_3 = $COCH_3$, R_1 = R_2 = R_4 = H
b R_3 = CHO, R_1 = R_2 = R_4 = H
c R_3 = COPh, R_1 = R_2 = R_4 = H
d R_3 = SO_2CH_3, R_1 = R_2 = R_4 = H
e R_3 = TsO, R_2 = CH_3O, R_3 = COPh, R_4 = OAc
f R_1 = HO, R_2 = CH_3O, R_3 = H, R_4 = =CH-CH_3 (E)

o-bromoacetanilide (**35a**) was cyclized upon treatment with $Pd(OAc)_2$ (10 mol %), PPh_3 (1 molar equiv), and n-Bu_3N (1.5 molar equiv) in toluene under carbon monoxide (5 atm) at 110 °C for 48 h (27% yield after chromatography) (Scheme 10). Difficulties were encountered when attempting to insert carbon monoxide into the less substituted amides of type **35b**; either no reaction occurred or the products of intramolecular (imide) or intermolecular reaction formed.[43] For these less substituted cases, the dilactam could be produced in low yield if the amide was first reduced to an amine with $NaBH_4$/acetic acid, followed by substitution of the nitrogen. For example, the bromo amines **37a–d** (Scheme 11) gave the PBD N10–C11 secondary or tertiary amines **38a–d** in yields of 15–54% with two byproducts resulting from acyl group migration and/or quinoxaline formation.[63] Using this approach, the N-benzoylamine **37e** was converted into **38e** (54% yield) upon treatment with 4 atm of carbon monoxide at 100 °C in the presence of $Pd(OAc)_2$ (10 mol %), PPh_3, and n-Bu_3N for 40 h. This compound was converted into the non-electrophilic PBD natural product SEN-215 (**38f**).[63,64] Similar methodology has been used to prepare the appropriate skeletons for the synthesis of precursors of the neothramycins A and B (**36c**),[33] prothracarcin (**36d**),[65a] and tomaymycin (**36e**).[65b] A "one-pot" variation of this method was also reported[33] in which initial insertion of carbon monoxide occurs between a proline amino acid ester and an o-haloaniline (the Heck reaction), followed by spontaneous cyclization of the second lactam bond. This was found to take place if K_2CO_3 was utilized as base instead of n-Bu_3N. Using this method, the reaction of methyl 1-prolinates of type **28d** (R_5 = R_6 = H, Scheme 7) with o-iodoaniline and 2-bromo-4-methoxy-5-(tosyloxy)aniline gave dilactams **36b** (R_4 = H) and **36f** in 79% and 41% yield, respectively. Unfortunately, an effort to synthesize the neothramycins from methyl pyroglutamate and o-iodoaniline afforded a quinazoline instead. The yield of this type of one-pot reaction is particularly poor when 4-hydroxyproline derivatives replace proline as the amino acid component. The two-step procedure outlined in Scheme 10 had to be used for synthesis of the prothracarcin and tomaymycin dilactam precursors, **36d** and **36e**, respectively.

Scheme 12



39 40

a R_1 = CH_3, R_2 = R_3 = H, R_4 = =CH-CH_3 (E)
b R_1 = Bn, R_2 = H, R_3 = OH, R_4 = H
c R_1 = Bn, R_2 = OH, R_3 = R_4 = H
d R_1 = Ac, R_2 = R_3 = H, R_4 = OAc

a R_1 = CH_3, R_2 = R_3 = H, R_4 = =CH-CH_3 (E)
b R_1 = Bn, $R_2$$R_3$ = =O, R_4 = H
c R_1 = Ac, R_2 = R_3 = H, R_4 = OAc

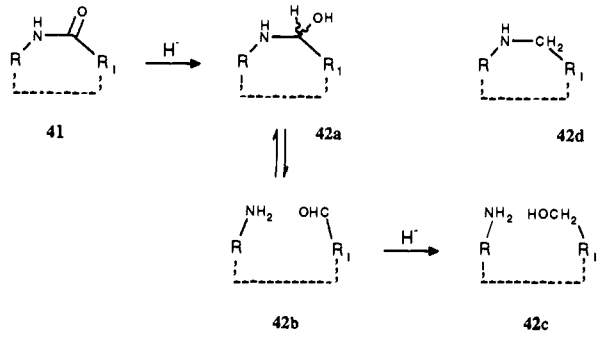
4. Oxidation of Pyrrolo[2,1-c][1,4]benzodiazepine N10–C11 Imines

The N10–C11 imine form of PBDs may be oxidized directly to the corresponding dilactams. For example, the imine form of C8-O-methyltomaymycin (**39a**) is oxidized to C8-O-methyl-11-oxotomaymycin (**40a**) by various oxidizing agents such as manganese dioxide, chromium trioxide–pyridine, and m-chloroperbenzoic acid (Scheme 12).[45a] The latter reagent in dichloromethane at −20 °C afforded the best result (20% yield). Similarly, a mixture of C8-O-benzylneothramycins A and B (**39b** and **39c**) were oxidized to the 3-oxodilactam (**40b**) with $KMnO_4$ in acetone at room temperature for 1 h.[9b] The imine form of 2,8-diacetylchicamycin (**39d**) was treated with m-chloroperbenzoic acid in $CHCl_3$ at −20 °C for 3 h to afford a 50% yield of the dilactam **40c**.[6]

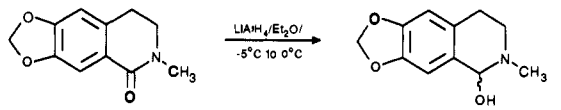
B. Reduction to the Carbinolamine Form

In general, the reduction of amides or lactams with hydride-donating reducing agents can lead to a number of different products depending upon the structure of the substrate, the type of reducing agent, and the reaction conditions (Scheme 13). For example, the carbonyl of amides and lactams (e.g. **41**) can be reduced with $LiAlH_4$ to afford the corresponding amines (**42d**), a reaction that is useful for the N-alkylation of amines *via* N-formyl derivatives,[66] and for the preparation of secondary amine containing heterocycles from lac-

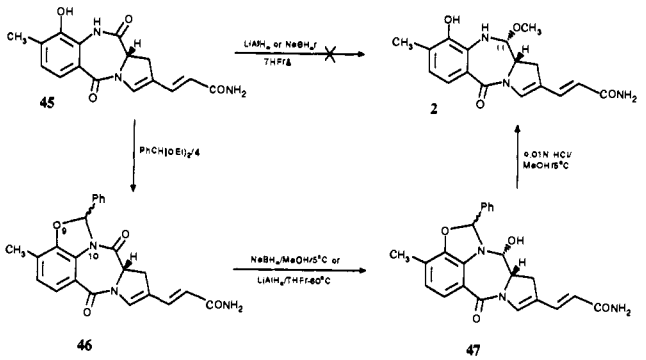
Scheme 13



Scheme 14



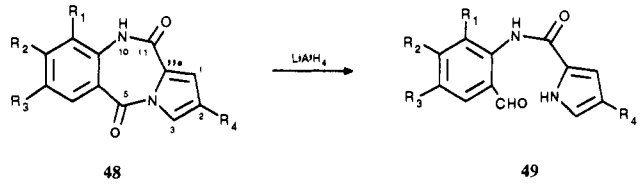
Scheme 15



tams.[67] Under mild conditions, aldehydes can sometimes be obtained *via* partial reduction to the carbinolamine (**42a**) which then dissociates to afford the corresponding amine and aldehyde (i.e. **42b**). This process is used for the preparation of aldehydes in high yield from both aliphatic and aromatic carboxylic acids by reduction of *N*-methylanilide derivatives with $LiAlH_4$. The aromatic component of the anilide is thought to furnish the required mesomeric resonance to prevent further reduction to amines or alcohols.[68a,b] Finally, in disubstituted amides and some other structures, the amide bond can be cleaved to afford the corresponding alcohols and amines (i.e. **42c**). The prerequisite for this reaction appears to be the presence of strongly electron-withdrawing substituents at the amide nitrogen which is therefore made electron deficient.[69] This encourages spontaneous dissociation of the carbinolamine intermediate (i.e. **42a** → **42b**), ensuring further reduction of the aldehyde (i.e. **42b** → **42c**).

One of the first reports of partial reduction of the carbonyl of a lactam to a stable carbinolamine was the final step of the synthesis of hydrastinine (**44**)[70] from the equivalent lactam (oxyhydrastinine, **43**) using $LiAlH_4$ in diethyl ether at low temperature (−5 °C to 0 °C) (Scheme 14). Leimgruber first reported partial reduction of a PBD dilactam to a N10–C11 carbinolamine as part of the total synthesis of anthramycin (Scheme 15).[31] However, it has now been established[32a–c] that the successful partial reduction of dilactams is dependent upon the type and pattern of A-ring substituents (e.g. electron-withdrawing groups facilitate reduction). Interestingly, dilactam forms of neothramycin, tomaymycin, and the sibiromycin aglycon (11-

Scheme 16

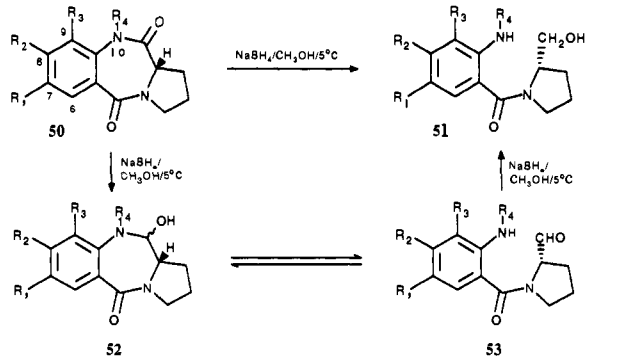


a $R_1 = R_3 = CH_3O$, $R_2 = CH_3$, $R_4 = -CH{=}CH{-}CH_3$
b $R_1 = R_2 = R_3 = R_4 = H$

oxosibiromycinone) have been known since 1979,[50] 1971,[45a] and 1981,[12,46] respectively; however, reduction to the parent carbinolamines has only recently been claimed by Mori and co-workers for the neothramycin[33,71] and tomaymycin[65a] dilactams, and the sibiromycin aglycon has not yet been successfully reduced.[46] Initial investigations[46] into the feasibility of reducing **48a** (Scheme 16) indicated that the C5-carbonyl attached to the pyrrole nitrogen is more reactive toward hydride ion than the other C11-lactam carbonyl. The model dilactam **48b** upon treatment with $LiAlH_4$ gave the aldehyde **49b**, presumably *via* opening of the C5 carbinolamine. Unfortunately, the structure of sibiromycin aglycon has now been revised and is known to contain a pyrroline (C2=C3 only) rather than a pyrrole C ring.[12] In the case of the total synthesis of anthramycin methyl ether (**2**, Scheme 15), the equivalent dilactam (**45**) is itself inert toward $LiAlH_4$ in refluxing THF. Leimgruber and co-workers incorporated the N10 and O9 atoms into a benzoxazoline ring system (i.e. **46**) by treatment with $PhCH(OEt)_2$, on the basis that *N*-methylanilides are converted to aldehydes in high yield by reduction with $LiAlH_4$.[68b] Dilactam **46** could then be successfully reduced to the carbinolamine **47** in 70% yield, using either $NaBH_4$ in methanol at 5 °C (preferred method) or $LiAlH_4$ in THF at −60 °C, followed by hydrolysis of the oxazoline ring (0.01 N HCl/MeOH/5 °C) to afford anthramycin methyl ether **2** in 70% overall yield.[31,47] Pena and Stille[51] have also prepared dilactam **46** *via* a different route involving the Pd-catalyzed coupling of a PBD vinyl triflate with an organostannane or an olefin (see Scheme 19) and successfully reduced it to the carbinolamine methyl ether **47** (C11-OMe) which could be converted to anthramycin (**2**) by hydrolysis. The three subsequently reported[45b,58e,62] "formal" total syntheses of anthramycin were thus based entirely on the unique partial reduction of the benzoxazoline dilactam of type **46**. Use of the benzoxazoline bridge was also reported by Takanabe and co-workers[53] who described the potential use of KBH_4 and $LiBH_4$ at −50 °C to effect reduction.

To investigate the generality of this procedure, Thurston and co-workers[32a,b] prepared a number of dilactams with different substituents both in the aromatic A ring and at the N10–position (**50a–f**) (Scheme 17). Using similar conditions to those reported by Leimgruber[31,47] ($NaBH_4$/methanol/5 °C), no reduction was found to occur if N10 was unsubstituted (**50a** or **50b**). This was attributed to initial reaction of the hydride reducing agent with the amidic N10 proton (and/or C9-hydroxyl in the case of **50b**) followed by precipitation of an insoluble non-reducible complex. Compounds with increasing steric (alkyl) bulk at the N10-position (e.g. **50c**) afforded mixtures of starting materials and the corresponding ring-opened amino alcohols of type **51c**. N-Benzylated derivatives **50d** and

Scheme 17

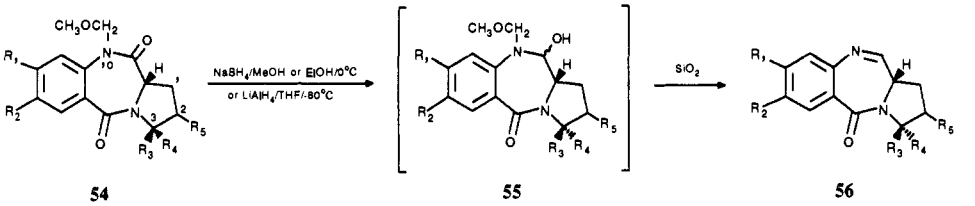


	R_1	R_2	R_3	R_4
a	H	H	H	H
b	H	CH_3	OH	H
c	H	H	H	CH_3, Et, Pr, $CH(CH_3)_2$, $(CH_2)_3CH_3$, Bn
d	H	H	OCH_3	Bn
e	H	CH_3	OBn	Bn
f	H	CH_3	·····O$CHPh$·····	
g	NO_2	H	H	H
h	NH_2 or NEt_2	H	H	H
i	$N^+Et_2CH_3$	H	H	H
j	$NHCOCH_3$	H	H	H

50e with electron-donating groups in the aromatic ring failed to reduce at all. This was attributed to the relatively higher electron density on the N10-nitrogen due to reduced resonance into the ring, which would presumably facilitate interaction of the nitrogen lone pair with the adjacent carbonyl, hence decreasing its electrophilicity toward hydride. Incorporating the N10-nitrogen and O9-oxygen substituents into a benzoxazoline ring system as in **50f** allowed smooth conversion to the benzoxazoline carbinolamine **52f**, which could then be hydrolyzed (0.01 N HCl/MeOH/5 °C) to the methyl ether form (C11-OMe) of the carbinolamine **52b** in high yield. A proposal has been advanced by Thurston and co-workers[32a,b] to rationalize the overreduction of dilactams of type **50**. It has been suggested that dissociation of the initial carbinolamine complexes (**52**) to amino aldehydes of type **53** occurs, which are then further reduced to the amino alcohols (**51**). Since dissociation of the carbinolamine to the amino aldehyde is the critical step that commits the reaction to proceed to overreduction, two factors were considered that might favor this process. First, transient protonation of the N10-nitrogen by methanol, when the nitrogen has sufficient basicity, might facilitate dissociation to the amino aldehyde. Second, dissociation could result in a negatively charged N10-nitrogen which should be favored if resonance stabilization can occur through the aromatic ring. Although there appear to be many examples in related systems where stabilization of a nitrogen anion by ring substituents favors overreduction, in the PBD dilactam system it would seem that nitrogen protonation is more important than resonance extension from the nitrogen. Hence, inclusion of N10 into a benzoxazoline ring (i.e. as in **46** and **50f**) lowers the electron density on the nitrogen (base weakening) *via* inductive transmission through the benzal carbon, therefore reducing the resonance effect of the C9 oxygen. The nitrogen is less easily protonated, reducing the possibility of dissociation to the amino aldehyde. This concept was later supported by the work of Suggs[32c] who prepared the 7-nitro dilactam **50g** which reduced smoothly (3-fold molar excess of $NaBH_4$ with a saturated solution of **50g** in methanol, followed by precipitation with H_2O) to the carbinolamine **52g** in 68% yield, with only trace amounts of overreduction products formed. The nitro carbinolamine **52g** is very stable (mp 215 °C dec), providing additional evidence that electron-withdrawing groups in the aromatic ring of a PBD enhances stability of the N10–C11 carbinolamine form, thus reducing biological activity.[15a] The amides **50h** failed to reduce, possibly due to an enhanced electron density at N10,[32a,b] and the quaternary salt **50i** was inert, possibly due to deprotonation of the amide (due to an increased acidity), as demonstrated by UV spectroscopy. The acetamide derivative **50j** reduced slowly with a large excess of $NaBH_4$ to the acyclic amino alcohol **51j**. All of these results appear to support the proposed mechanism of reduction.[32a,b]

More recently, Mori reported[33] that introduction of a N10-methoxymethyl (MOM) substituent allows selective $NaBH_4$ reduction (MeOH/0 °C) of a PDB dilactam to a N10–C11 carbinolamine, presumably due to the MOM group playing a similar role to the O9–N10 benzoxazoline system (e.g. **46** or **50f**) in reducing the basicity of the N10-nitrogen. Reduction of **54a** (Scheme 18) with $NaBH_4$ (methanol/0 °C) followed by silica gel chromatography was reported to afford the imine **56a** in high yield (80%), presumably *via* the carbinolamine intermediate **55a**. Conversion of the neothramycin dilactam models **54b** and **54c** to the imines **56b** (33%) and **56c** (36%) was also reported, although some unchanged starting materials always appeared to remain. In a later publication,[65a] a solution of the prothracarcin precursor **54d** in ethanol was treated with a 10-fold excess of $NaBH_4$ for 3 h at 0 °C. Extraction into benzene, slurrying with silica gel, and then column chromatography on silica gel (*n*-hexane/acetone) afforded prothracarcin (**56d**) in high yield (89%). The corresponding *Z*-isomer (**56e**) was prepared from **54e** in 84% yield by a similar process. The equivalent N10-MOM precursor of tomaymycin (**54f**) reduced only slowly with $NaBH_4$ at 0 °C. However, reduction with $LiAlH_4$ (1 equiv/THF/−60 °C) followed by treatment with silica gel in EtOAc and purification by column chromatography (silica gel) was reported to give pretomaymycin (**56f**) in 92% yield. Similarly, **54g** was treated with $LiAlH_4$ in THF for 30 min at −60 °C, followed by the addition of $Na_2SO_4 \cdot 10H_2O$ with stirring for several hours. Extraction into CH_2Cl_2, followed by treatment with silica gel in methanol and finally column chromatography (silica gel), afforded the corresponding *Z*-isomer of tomaymycin (**56g**) in 75% yield. Imines **56f** and **56g** were also reduced with $NaCNBH_3$ in acidic methanol to afford the naturally-occurring PBD N10–C11 secondary amine, SEN-215, and the equivalent *Z*-isomer.[65b] Mori also reported[71] that the C3-*O*-methylneothramycin precursors **54h** and **54i** could be converted to the C3-*O*-methylneothramycins (**56h** and **56i**) upon reduction with $LiAlH_4$ in THF, followed by treatment with silica gel. These compounds could then be converted into neothramycins A and B (**9a**, **9b**, Scheme 2) by mild hydrolysis with 0.01 M HCl in dioxan at 0 °C for 20 min. Intending to use this methodology, Pena and Stille[51,58f] prepared a model intermediate (**59a**, Scheme 19) for the total synthesis of anthramycin (**2**). Dilactam **57a** was obtained from condensation of isatoic anhydride with hydroxyproline, followed by oxidation. It was then treated with pyridine and triflic anhydride to afford the triflate ester **58a**. Pd-catalyzed coupling of the triflate ester with an organostannane or an olefin (Heck-type reaction) gave **59a**, a novel formation of

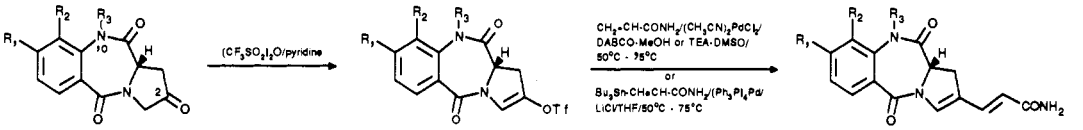
Scheme 18



54 55 56

	R_1	R_2	R_3	R_4	R_5
a	H	H	H	H	H
b	H	H	OCH_3	H	H
c	H	H	H	OCH_3	H
d	H	H	H	H	=CH-CH_3 (E)
e	H	H	H	H	=CH-CH_3 (Z)
f	HO	CH_3O	H	H	=CH-CH_3 (E)
g	HO	CH_3O	H	H	=CH-CH_3 (Z)
h	HO	CH_3O	H	OCH_3	H
i	HO	CH_3O	OCH_3	H	H

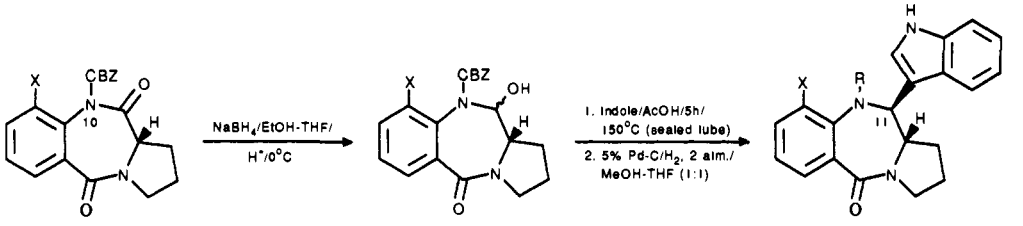
Scheme 19



57 58 59

a $R_1 = R_2 = H$, $R_3 = CH_2OCH_3$
b $R_1 = CH_3$, R_2-R_3 = -O-CH(Ph)-

Scheme 20



60a X = H
60b X = OBn

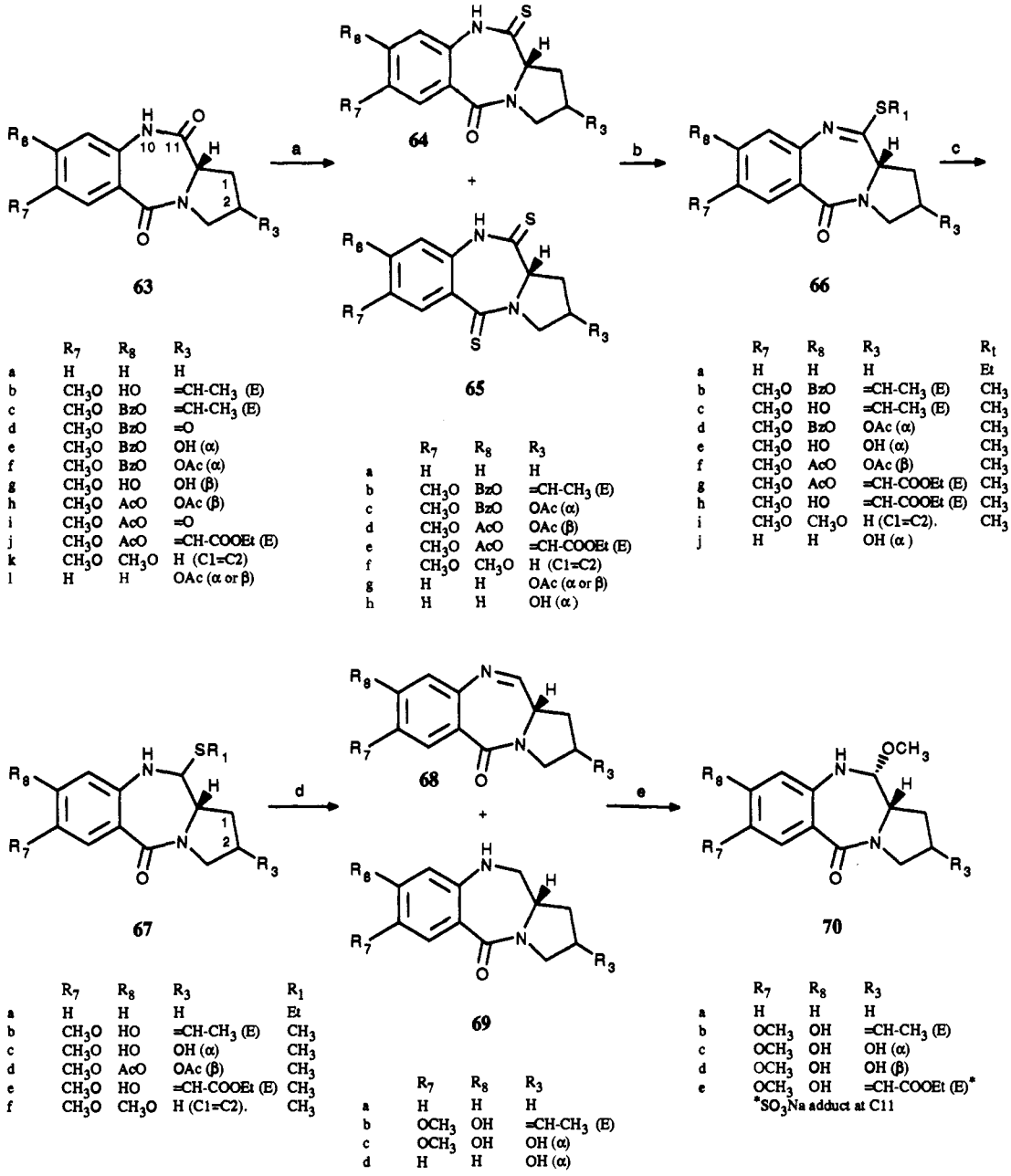
61a X = H
61b X = OBn

62a X = H, R = CBZ
62b X = OBn, R = CBZ
62c X = R = H
62d X = OH, R = H

the anthramycin side chain. Unfortunately, reduction of this N10-MOM-protected dilactam to an anthramycin analog was not reported. Interestingly, using the original methodology of Leimgruber and co-workers,[31] the O9,N10-benzal-protected anthramycin precursor (**59b**) prepared in a similar way (**57b** → **58b** → **59b**; Scheme 19) was successfully reduced with $NaBH_4$/MeOH and then hydrolyzed (HCl/MeOH) to remove the benzal protecting group and afford anthramycin in high yield. Nagasaka and co-workers[58h] have used a related method involving a N10-carboxybenzyl (CBZ) group to synthesize tilivalline (**62d**, Scheme 20). Controlled reduction of **60a** and **60b** with $NaBH_4$ (EtOH–THF/H^+/0 °C) afforded the carbinolamines **61a** and **61b** (69–76% yield) which were condensed with indole to produce **62a** and **62b** in 50–93% yield. The stereoselectivity at C11 was ascribed to attack of the nucleophile from the less-hindered face of the iminium ion intermediate. Hydrogenation with 5% Pd–C (H_2, 2 atm/MeOH–THF, 1:1) removed the CBZ protecting groups to provide **62c** and **62d**, respectively.

In conclusion, the partial reduction of PBD dilactams to carbinolamines appears to have great potential because of the many efficient methods available for the synthesis of dilactams in high yield. In addition, all of the known methods of dilactam synthesis maintain the required (*S*)-stereochemistry at C11a, and racemization does not appear to occur during hydride reduction. Until the recent reports by Mori and co-workers[33,65,71] of the use of a N10-MOM-protecting group, the use of dilactams seemed to be limited either to compounds with a 9-hydroxyl substituent that could be included in a benzoxazoline ring system, or to compounds with electron-withdrawing groups in the aromatic A ring. However, Mori's method has not yet been successfully applied by other workers. For example, synthesis of the N10-MOM-protected precursor of anthramycin (**59a**, Scheme 19) has been achieved by Pena and Stille,[51,58f] but its successful reduction has not yet been reported. Instead, the O9,N10-benzal protecting group of Leimgruber[31] (i.e. **59b**) was utilized.[51] Similarly, variously substituted N10-MOM-protected dilactams have failed to reduce to carbinolamine-containing PBDs in other laboratories.[39a,65c] The extensive use of silica gel in this method may also be a concern, as some N10–C11 carbinolamine-containing PBDs are unstable in the presence of silica gel.[36,39a] However, due to the ease of preparation and stability

Scheme 21[a]



	R_7	R_8	R_3
a	H	H	H
b	CH_3O	HO	=CH-CH_3 (E)
c	CH_3O	BzO	=CH-CH_3 (E)
d	CH_3O	BzO	=O
e	CH_3O	BzO	OH (α)
f	CH_3O	BzO	OAc (α)
g	CH_3O	HO	OH (β)
h	CH_3O	AcO	OAc (β)
i	CH_3O	AcO	=O
j	CH_3O	AcO	=CH-COOEt (E)
k	CH_3O	CH_3O	H (C1=C2)
l	H	H	OAc (α or β)

	R_7	R_8	R_3
a	H	H	H
b	CH_3O	BzO	=CH-CH_3 (E)
c	CH_3O	BzO	OAc (α)
d	CH_3O	AcO	OAc (β)
e	CH_3O	AcO	=CH-COOEt (E)
f	CH_3O	CH_3O	H (C1=C2)
g	H	H	OAc (α or β)
h	H	H	OH (α)

	R_7	R_8	R_3	R_1
a	H	H	H	Et
b	CH_3O	BzO	=CH-CH_3 (E)	CH_3
c	CH_3O	HO	=CH-CH_3 (E)	CH_3
d	CH_3O	BzO	OAc (α)	CH_3
e	CH_3O	HO	OH (α)	CH_3
f	CH_3O	AcO	OAc (β)	CH_3
g	CH_3O	AcO	=CH-COOEt (E)	CH_3
h	CH_3O	HO	=CH-COOEt (E)	CH_3
i	CH_3O	CH_3O	H (C1=C2).	CH_3
j	H	H	OH (α)	

	R_7	R_8	R_3	R_1
a	H	H	H	Et
b	CH_3O	HO	=CH-CH_3 (E)	CH_3
c	CH_3O	HO	OH (α)	CH_3
d	CH_3O	AcO	OAc (β)	CH_3
e	CH_3O	HO	=CH-COOEt (E)	CH_3
f	CH_3O	CH_3O	H (C1=C2).	CH_3

	R_7	R_8	R_3
a	H	H	H
b	OCH_3	OH	=CH-CH_3 (E)
c	OCH_3	OH	OH (α)
d	H	H	OH (α)

	R_7	R_8	R_3
a	H	H	H
b	OCH_3	OH	=CH-CH_3 (E)
c	OCH_3	OH	OH (α)
d	OCH_3	OH	OH (β)
e	OCH_3	OH	=CH-COOEt (E)*

*SO_3Na adduct at C11

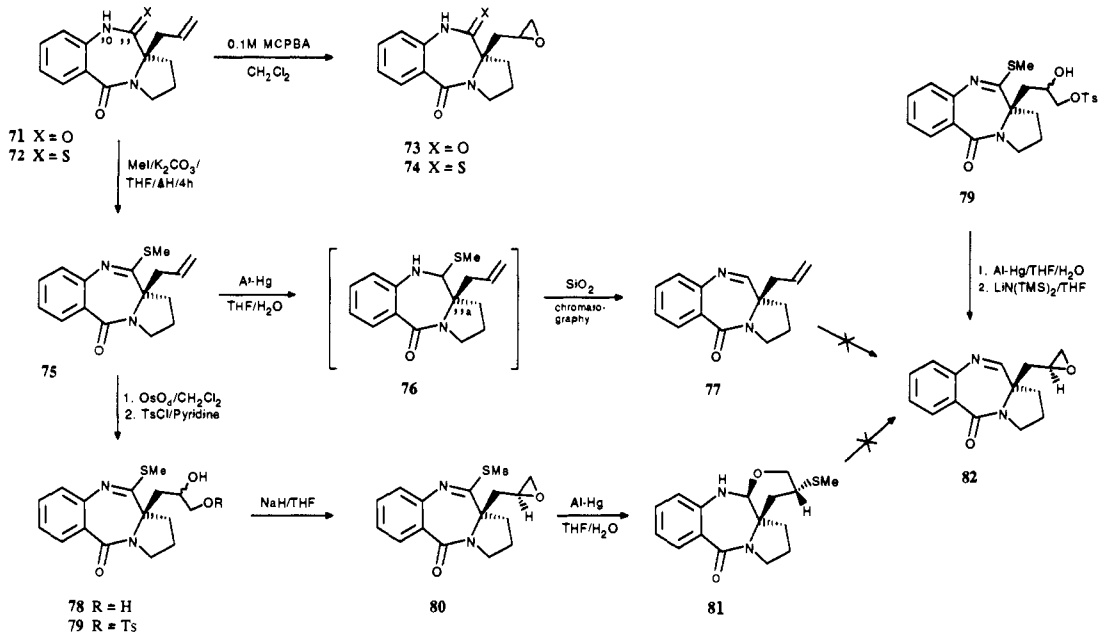
[a] (a) P_2S_5/benzene/80 °C or P_2S_5/$NaHCO_3$/CH_3CN/Δ/15 min or (p-$CH_3OC_6H_4PS_2)_2$/PhH/80 °C; (b) Et_3OBF_4/CH_2Cl_2/$KHCO_3$ or CH_3I/K_2CO_3/THF and/or DMF; (c) Al-Hg/aq. THF and/or KH_2PO_4/0–5 °C/14 h, d: 0.1N methanolic $HgCl_2$/0 °C and/or SiO_2 chromatography/5 °C, e: CH_3OH.

of PBD dilactams, the use of N10-protecting groups to facilitate and direct hydride reduction deserves further study.

IV. Reduction of Pyrrolo[2,1-c][1,4]benzodiazepine N10–C11 Imino Thioethers

This method, which is based on the well-known reduction of thiazolines (a form of imino thioether) to thiazolidines[72] using an aluminum amalgam was introduced in 1983 by Kaneko and co-workers[37] as a general and mild method for the reduction of PBD dilactams to the carbinolamine oxidation level. Treatment of the parent dilactam **63a** with P_2S_5 (PhH/80 °C/2.5 h) afforded the N10–C11 mono thiolactam **64a** which was alkylated with triethyloxonium tetrafluoroborate [Et_3OBF_4/CH_2Cl_2/$KHCO_3$] to give the imino thioether **66a** in 91% yield (Scheme 21). Reduction of **66a** with an excess of freshly prepared Al–Hg amalgam in aqueous THF at 0–5 °C for 14 h[72] afforded the thiocarbinolamine **67a** in 37% yield which crystallized upon workup. Silica gel chromatography converted **67a** to the imine **68a**, although a final yield was not reported. A minor byproduct, the N10–C11 secondary amine **69a**, was also produced. The same approach, but using different reagents, was applied[37] to the conversion of naturally-occurring oxotomaymycin[45a] (**63b**) to tomaymycin methyl ether (**70b**). **63b** was first benzoylated at the phenolic C8-OH to give **63c** (NaH/PhCOCl/DMF/–20 °C → room temperature), which was then treated with freshly prepared Lawesson's reagent[73] [(p-$CH_3OC_6H_4PS_2)_2$/PhH/80 °C/1.5 h] to afford the N10–C11 thioamide **64b**. Treatment with CH_3I/K_2CO_3/THF/12 h afforded the imino thioether **66b**. The benzoyl protecting group was then removed by hydrolysis (K_2CO_3/MeOH/0 °C) without affecting the

Scheme 22



imino thioether functionality to afford **66c**. Treatment with Al–Hg amalgam (prepared from 7 equiv of aluminum foil) at 0–5 °C in aqueous THF for 22 h afforded the initial reaction product **67b**, which was treated with 0.1 N methanolic $HgCl_2$ solution at 0 °C and then chromatographed on SiO_2 at 5 °C to afford 38% of the imine form of tomaymycin (pretomaymycin, **68b**) and 8% of the overreduction product **69b**. The overall yield for the six-step process was 29%. When **68b** was dissolved in methanol and allowed to stand in the freezer overnight, conversion to tomaymycin methyl ether **70b** was quantitative.

A similar methodology was used to synthesize the naturally-occurring pyrrolobenzodiazepine, chicamycin A[38a] (**70c**). Stereoselective reduction of the ketone **63d** with $NaBH_4$/EtOH afforded the alcohol **63e** (62%). Acetylation [$(Ac)_2O$/pyridine] afforded **63f**, which was treated with Lawesson's reagent (PhH/80 °C/14 h) to afford the thioamide **64c** in 52% yield (over two steps). Alkylation with CH_3I (K_2CO_3/THF/14 h) afforded **66d** which was hydrolyzed under mild conditions (K_2CO_3/MeOH/0 °C) to give the imino thioether **66e** in quantitative yield. Treatment with an aluminum amalgam (Al–Hg/aqueous THF/0–5 °C/18 h) afforded the thiocarbinolamine **67c** which was treated with 0.1 N methanolic $HgCl_2$ at 0 °C followed by chromatography on silica gel at 5 °C to afford chicamycin A (**70c**, 49%) along with the overreduction product **69c** (18%) (overall yield for chicamycin A: 10%).

Similar methodology was used to synthesize a number of bicyclic and tricyclic analogs of anthramycin for SAR studies.[38b] The chicamycin A C2-epimer (**70d**) was synthesized from the 3-hydroxy dilactam **63g**. This involved initial acetylation of both hydroxyl functionalities [$(Ac)_2O$/pyridine] to give **63h** (76%) followed by treatment with Lawesson's reagent (**64d**, 86%), CH_3I/K_2CO_3 (**66f**, 100%), and Al–Hg (**67d**, 58%) before hydrolysis with K_2CO_3/MeOH to afford the 2(*R*)-hydroxy epimer **70d** in 37% yield.

In a related synthesis, the ketone (**63i**) was reacted with the Wittig reagent $Ph_3P{=}CHCOOEt$ to afford the C2-alkylidene dilactam (**63j**, 63% yield) which was treated with Lawesson's reagent (**64e**, 79%) and then CH_3I/K_2CO_3 to afford the imino thioether **66g**. This was hydrolyzed with K_2CO_3/MeOH (**66h**) (94% for the last two steps), reduced with Al–Hg (**67e**), and then treated with aqueous $NaHSO_3$ solution to afford the bisulfite addition product **70e** (24%) directly. The carbinolamine methyl ether of **70e** was too unstable to isolate. The C1–C2 unsaturated thiocarbinolamine **67f** has also been synthesized by this method. The dilactam **63k** afforded the thiolactam **64f** in 81% yield upon reaction with $P_2S_5/NaHCO_3/CH_3CN$ (reflux for 15 min). Treatment with CH_3I/K_2CO_3/THF/DMF/room temperature/4 h afforded the imino thioether **66l** (76%), which was reduced to **67f** with Al–Hg amalgam (0 °C/KH_2PO_4/THF/35 h; 49% yield). **67f** was found to be labile and subject to facile loss of methanethiol.[15a] Baraldi and co-workers have also used this cyclization method to synthesize PBD analogs with heterocyclic A rings (see Section VII.A).

Some limitations of this approach were uncovered by Confalone and co-workers[48] (Scheme 22) when it was used to synthesize an unusual epoxide-containing anthramycin analog **82**. The dilactam **71**, prepared from a 2-allylproline methyl ester intermediate, was initially converted to the epoxide **73** (0.1 M *m*-chloroperbenzoic acid/CH_2Cl_2; 81% yield), but this failed to form the thiolactam **74** under a variety of conditions. However, Lawesson's reagent in glyme converted **71** to the thiolactam **72**, which was successfully alkylated with CH_3I/K_2CO_3/THF/Δ/4 h to give the imino thioether **75** in 84% yield. Reduction with Al–Hg amalgam (H_2O/THF) followed by chromatography on silica gel afforded the imine **77** directly (31%), presumably through loss of methyl mercaptan from the thiocarbinolamine **76** due to steric crowding at the C11a-position. Attempts to oxidize **77** to the epoxide **82** failed due to the reactivity of the N10–C11 imine functionality, which was preferentially oxidized. However, **75** was successfully oxidized with OsO_4/CH_2Cl_2 to give the diastereomeric alcohols **78** (1:1, 89%), which were readily tosylated (TsCl/pyridine) at the primary alcohol position (**79**) (75% total yield). Although treatment of one isolated diastereomeric tosylate with NaH/THF afforded the epoxide **80**, reduction with Al–Hg amalgam (THF/H_2O)

afforded, instead of **82**, the rearrangement product **81**, resulting from initial intramolecular alkylation of the dihydro thioether sulfur by the epoxide. However, initial reduction of the tosylate (**79**) with Al–Hg amalgam afforded a rearrangement product similar to **81** that was converted to the imino epoxide **82** in the presence of base ($LiN(TMS)_2$/THF) in 48% yield.

The two major disadvantages of this approach were highlighted by Thurston and co-workers in an attempt to synthesize abbeymycin.[58j] Reaction of the precursors **63l** with Lawesson's reagent afforded a mixture of mono- (**64g**) and dithiolated (**65g**) products necessitating chromatographic separation. At higher temperatures dithiolation was found to be almost quantitative. After hydrolysis (**64h**) and alkylation of the monothiolated (C3-α) compound to give **66j**, reduction with Al–Hg amalgam afforded the overreduced N10–C11 secondary amine **69d** (25%) as the only isolable product. Interestingly, the dithiolactam **65g** (C3-α), after hydrolysis (**65h**), alkylation (**66j**, C5=S), and reduction, gave C5-thioabbeymycin (**68d**; C5=S) in 28% yield.

In conclusion, as with the hydride reduction approach (see Section III.B), this route appears to be very attractive because of the ease of preparation and stability of PBD dilactams. The reaction conditions are mild (neutral and low temperature) and compatible with many functional groups. In addition, due to the stability of dilactams, the stereochemistry at the C11a-position is usually maintained. Furthermore, it has been demonstrated that both acetyl and benzoyl C8-protecting groups can be removed from the molecule at the imino thioether stage,[37,38] thus extending the scope of the methodology. An additional advantage is that double bonds remain intact throughout the reaction sequence. As with other methods, the form of the final product (e.g. N10–C11 imine or carbinolamine) is dependent upon the isolation procedure. For example, if a methanol-containing solvent mixture is used for the silica gel chromatography during the last step, a carbinolamine methyl ether is generally obtained, whereas if non-methanol-containing solvent mixtures and/or short silica gel contact times are used, an imine usually results.

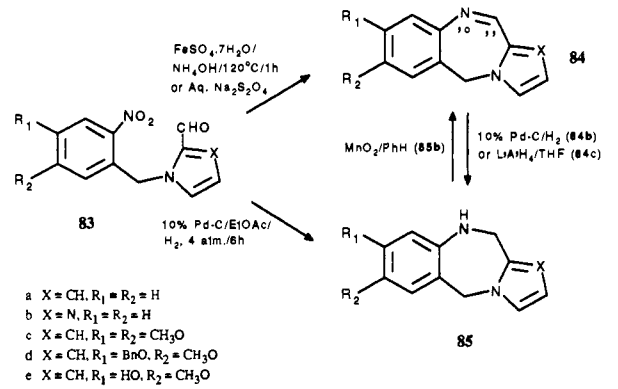
Potential disadvantages of this approach include C5,-C11-dithiolation at the first step, or overreduction at the last step,[58j] both of which necessitate chromatographic separation and reduce the overall yield. One attempt to synthesize abbeymycin by this route failed completely, and only the N10–C11 secondary amine overreduction product could be isolated.[58j] A further disadvantage is that Al–Hg amalgam[72] and Lawesson's reagent[73] must both be freshly prepared for optimum yields, which can be a time-consuming process.

V. Cyclization of Substituted N-Benzoylpyrrolidine Precursors

A. Reductive Cyclization of N-(2-Nitrobenzoyl)-pyrrolidine-2-carboxaldehydes

This method involves the controlled reductive cyclization of *N*-(2-nitrobenzoyl)pyrrolidine-2-carboxaldehydes of type **15** (Scheme 3) *via* catalytic hydrogenation or chemical reduction. It was first reported in relation to the preparation of 5-deoxypyrrolo[2,1-*c*]-[1,4]benzodiazepine analogs of type **84** or **85** (Scheme 23), both unsaturated in the pyrrolo C ring.[54] However,

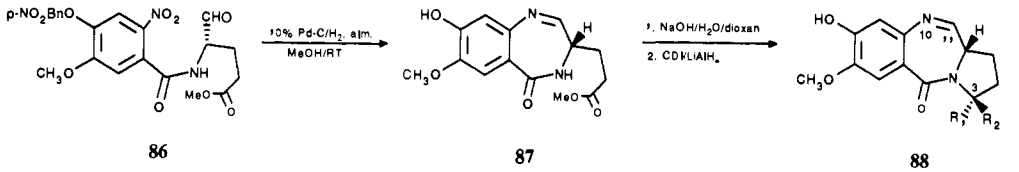
Scheme 23



catalytic hydrogenation of the nitro aldehyde **83a** over Pd–C catalyst afforded only the 10,11-dihydro derivative **85a** (i.e. the secondary amine) in low yield instead of **84a**. Miyamoto and co-workers reported the first total synthesis of an N10–C11 imine-containing PBD (neothramycins A and B) *via* reductive cyclization[9b] (Scheme 24). A methanolic solution of nitro aldehyde **86** was subjected to catalytic hydrogenation over 10% Pd–C at room temperature for 30 min (H_2 atm) to afford the imine **87** in 45% yield. Hydrolysis ($NaOH/H_2O$/dioxan) and reduction (*N,N'*-carbonyldiimidazole/$LiAlH_4$) of the ester group afforded a mixture of the neothramycins A and B (**88a**/**88b**) in low yield (3–10% from **86**). It is likely that sensitivity of the N10–C11 imine to hydrolysis accounted for the poor yield. Further hydrogenation of the 3-*O*-butyl ether (**88c**) (10% Pd–C/dioxan/room temperature/1.5 h/2.1 K g cm^{-2}) afforded the secondary amine, C3-*O*-butyl N10,-C11-dihydroneothramycin A (**88d**). This technique was also used to construct the parent unsaturated PBD imine[34] **90a** (Scheme 25). Catalytic hydrogenation of a methanolic solution of the nitro aldehyde **89a** over 5% Pd–$BaCO_3$ for 17 h (H_2 atm/room temperature) afforded the imine **90a** in low yield (8.5%) after chromatography on silica gel (benzene–methanol, 19:1). Further reduction of **90a** with non-poisoned catalyst (5% Pd–C/H_2 atm/MeOH/24 h) afforded the secondary amine **91a** in almost quantitative yield. This compound could also be prepared by direct reduction of the nitro aldehyde (**89a**) using non-poisoned catalyst (5% Pd–C/H_2 atm/MeOH/24 h) in 60% yield.

The total synthesis of both (*E*)- and (*Z*)-tomaymycins was also accomplished using the reductive cyclization technique[35] (Scheme 25). It was initially found that the nitro aldehyde **89b** upon hydrogenation over 10% Pd–C (THF/3 h/H_2 atm) afforded the imine **90c** in 90% yield which was unstable under acid or base conditions and, not surprisingly, could not be oxidized at the C2-position by a number of methods. Concluding that incorporation of the C2-ethylidene moiety must take place prior to cyclization of the B ring, the isomeric ethylidene-substituted nitro aldehydes **89d** were prepared and successfully cyclized to the imine forms (**90e**) upon reductive cyclization with 5% Pd–$BaSO_4$/THF/room temperature/3 h/H_2 atm (*E*:*Z*, 70:60). The corresponding N10–C11 carbinolamine methyl ether forms (**90e**: N10–C11 = $NHCH(OCH_3)$) were obtained, if methanol was used as solvent for the reduction process instead of THF. The same technique has been used to prepare some tomaymycin analogs[74] (Scheme 26). For example, **92a–g** all cyclized after hydrogenation with 5% Pd–$BaSO_4$ in MeOH or EtOAc/MeOH (1.5–1.8 wt

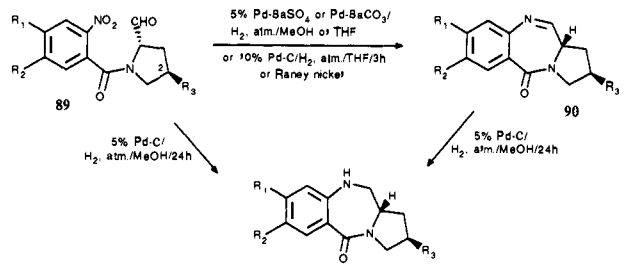
Scheme 24



86 87 88

a R_1 = OH, R_2 = H
b R_1 = H, R_2 = OH
c R_1 = OBu, R_2 = H
d R_1 = OBu, R_2 = H, N10-C11 = -NH-CH_2-

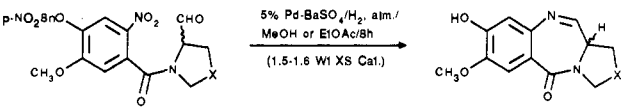
Scheme 25



89 90 91

a $R_1 = R_2 = R_3 = H$
b R_1 = p-NO_2-BnO, $R_2 = CH_3O$, R_3 = OH
c R_1 = HO, $R_2 = CH_3O$, R_3 = OH
d R_1 = BnO or p-NO_2-BnO, $R_2 = CH_3O$, R_3 = =CH-CH_3 (E or Z)
e R_1 = HO, $R_2 = CH_3O$, R_3 = =CH-CH_3 (E or Z)

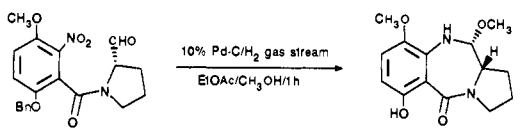
Scheme 26



92 93

X
a -CH_2-
b -CH_2CH_2-
c -S-
d C=N-OCH_3
e CH-CN
f C=O
g CH-O.CO-$(CH_2)_{14}$-CH_3

Scheme 27

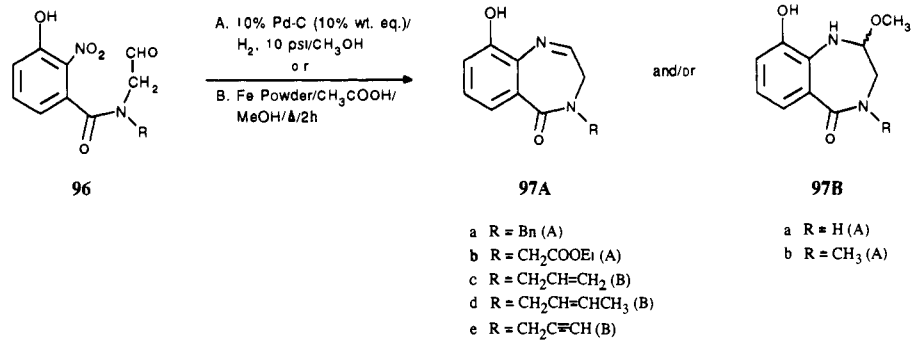


94 95

excess/H_2 atm) for 8 h to afford the imines **93a–g** in 18–89% yield. Nitro aldehyde **94** was similarly converted[74] to the methyl ether **95** (75% yield) using non-poisoned 10% Pd–C catalyst in EtOAc/MeOH and hydrogen as a gas stream for 1 h (Scheme 27). Kaneko and co-workers[38b] have reported the synthesis of PBD-related benzodiazepine analogs in either their imine (**97Aa–e**) or methyl ether forms (**97Ba,b**) (Scheme 28) in approximately 30% yield *via* either hydrogenation of methanolic solutions of the corresponding nitro aldehydes (**96**) at 10 psi over 10% Pd–C, or by chemical reduction with Fe powder/CH_3COOH/MeOH/Δ. Mohr and co-workers[75] described the isolation and synthesis of tilivalline **99a** (C11 = β), a novel PBD from *Klebsiella pneumoniae*; this was the first report of a PBD from a bacterium rather than a lower fungi (Scheme 29). The nitro ketone **98a** was hydrogenated in ethanol over 10% Pd–C (H_2 atm/room temperature/4 h) to afford **99a** in 98% yield as an epimeric mixture at C11 (C11α:β, 92:8). Similarly, **98b** was hydrogenated to afford **99b** in 96% yield (C11α:β, 52:48; 8%). Working toward a total synthesis of the neothramycins A and B, Langlois and co-workers[76a] reported that the model nitro aldehydes **100a** and **100b** (Scheme 30) (prepared *via* a novel route involving formation of the pyrrolidine ring through intramolecular cyclization of an amido epoxide species) could be reductively cyclized to **101a** and **101b** in 62% and 75% yields, respectively, using the conditions of Tozuka and co-workers[74] (5% Pd–$BaSO_4$/H_2 atm/MeOH or EtOAc–MeOH). The same authors also reported the use of Raney nickel for this type of cyclization;[76b] the model nitro aldehydes **100a** and **100b** and the precursors of neothramycins A and B (**100c**) were reduced with Raney nickel to afford the imines **101a** (67%) and **101b** (78%) and the neothramycins A and B, respectively (**101d** and **101e**; 40%) (Scheme 30). This approach with Raney nickel was also used to cyclize the unsubstituted nitro aldehyde **89a** (Scheme 25) to the imine **90a** in 75% yield.[76c] The same workers[76c] also attempted to cyclize the sulfonamide analog **100f** (Scheme 30) with an excess of Raney nickel (as a slurry in water) in a mixture of ethyl acetate and methanol at room temperature. Interestingly, the amino aldehyde intermediate **100g** was isolated. This intermediate was slowly transformed (1.5 h) into the methyl or ethyl carbinolamine alkyl ethers (**101h**, R = Me or Et) upon standing at room temperature in methanol- or ethanol-containing dichloromethane, respectively, in the presence of trifluoroacetic acid. This reluctance to cyclize (i.e. the unusual stability of the amino aldehyde intermediate, **100g**) contrasts with the PBD system (i.e. C5 = C=O) where N10–C11 imines form spontaneously from the corresponding amino aldehydes, no examples of which have been isolated. This may be due to the electron-withdrawing ability of a sulfonyl *vs* a C5-carbonyl group, which may reduce the nucleophilicity of the aromatic amine. Using an optically pure alcohol, (*S*)-ethyl lactate, instead of MeOH or EtOH, only one diastereomer of structure **101h** was detected by ^{1}H-NMR, indicating that no epimerization at the asymmetric C11a-position had occurred during the reaction.

Thurston and Langley[36] have carried out a study of the mechanism of the reductive cyclization of a series of *N*-(2-nitrobenzoyl)pyrrolidine-2-carboxaldehydes of type **102** (Scheme 31). Numerous attempts to repeat the reductive cyclization of **102a**, as reported by Lown[34] failed, although the previously unknown N10-hydroxy-carbinolamine methyl ether (**106**, $R_1 = CH_3$), or after longer reduction times, the secondary amine **110a** was isolated. This result was intriguing, as it was inconsistent with, for example, the work of Tozuka[35] and

Scheme 28



96

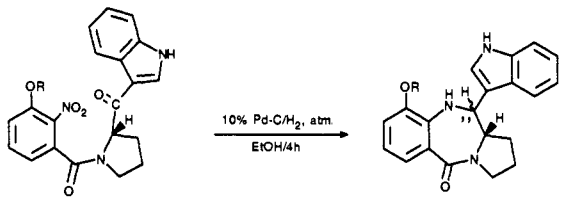
97A

a R = Bn (A)
b R = CH_2COOEt (A)
c R = $CH_2CH{=}CH_2$ (B)
d R = $CH_2CH{=}CHCH_3$ (B)
e R = $CH_2C{\equiv}CH$ (B)

97B

a R = H (A)
b R = CH_3 (A)

Scheme 29



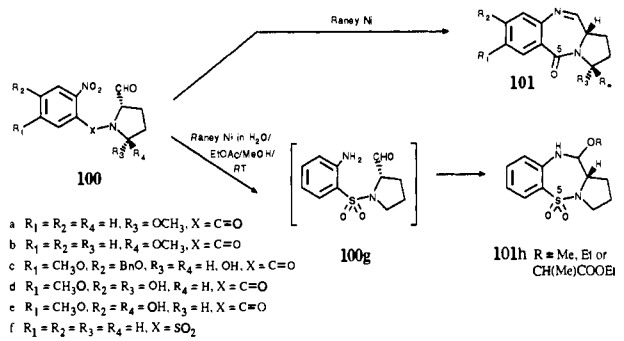
98

a R = Bn
b R = CH_3

99

a R = H
b R = CH_3

Scheme 30



100

a $R_1 = R_2 = R_4 = H$, $R_3 = OCH_3$, X = C=O
b $R_1 = R_2 = R_3 = H$, $R_4 = OCH_3$, X = C=O
c $R_1 = CH_3O$, $R_2 = BnO$, $R_3 = R_4 = H$, OH, X = C=O
d $R_1 = CH_3O$, $R_2 = R_3 = OH$, $R_4 = H$, X = C=O
e $R_1 = CH_3O$, $R_2 = R_4 = OH$, $R_3 = H$, X = C=O
f $R_1 = R_2 = R_3 = R_4 = H$, $X = SO_2$

101

100g

101h R = Me, Et or CH(Me)COOEt

Miyamoto[9b] who prepared tomaymycin methyl ether (**90e**, *E*-form, Scheme 25) and a number of related analogs, and **87** (Scheme 24), an intermediate in the synthesis of neothramycin, by reductive cyclization of the corresponding nitro aldehydes **89d** or **86** using 5% Pd–$BaSO_4$ or 10% Pd–C, respectively.

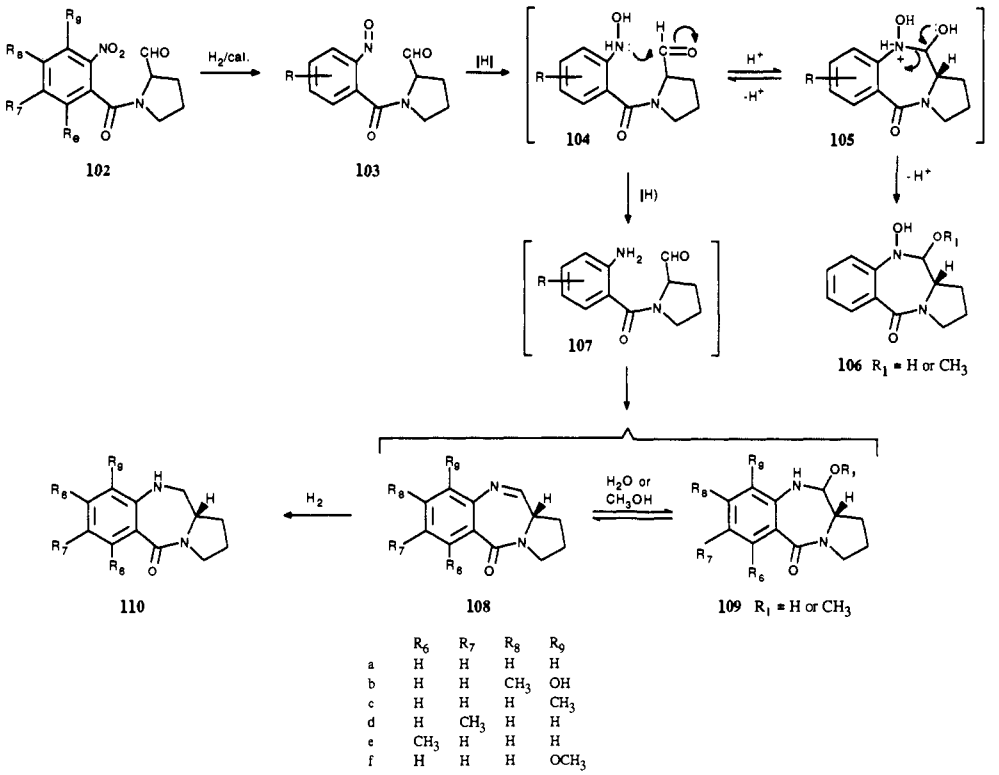
The formation of imines in a number of the examples above[9b,35,38b] is surprising as, with non-poisoned catalyst (and particularly at higher pressures), rapid formation of the corresponding secondary amine would be anticipated. Studies by both Lown[34] and Thurston[36] indicate that reduction of a number of nitro aldehydes under similar conditions always affords the corresponding secondary amines of type **110** in nearly quantitative yield. In addition, Artico and co-workers[54] have reported that catalytic hydrogenation of **83a** (Scheme 23) with Pd–C (strength not reported) affords the secondary amine **85a**, and similar treatment of 1-(2-nitrobenzyl)imidazole-2-carboxaldehyde (**83b**) (10% Pd–C/EtOAc/50 °C/H_2, 4 atm/6 h) has been shown[77] to afford the corresponding secondary amine **85b** (92% yield). Similarly, hydrogenolysis of **83d** in EtOAc for 2 h affords the secondary amine **85e** in 70% yield.[78b]

An HPLC time-course investigation[36] of the reduction of **102a** with 5% Pd–$BaSO_4$ revealed a sequence of intermediates that included the secondary amine **110a**, the carbinolamine (**109a**, R_1 = H) and its methyl ether (**109a**, $R_1 = CH_3$), the *N*-hydroxycarbinolamine **106** (R_1 = H) and its methyl ether **106** ($R_1 = CH_3$), and possibly a nitroso compound **103** (R = H). It was determined that **104** is a key intermediate which, once formed, can either be further reduced to the amino aldehyde **107** (R = H), leading to the carbinolamine or related species **109** or **108**, or cyclize to give the *N*-hydroxy intermediate **105**. Thurston suggested[36] a mechanism which implied that factors leading to an increase in electron density (e.g. base-strengthening) on the N10-nitrogen (such as electron-donating substituents in the aromatic ring) would favor production of the secondary amine **110** by causing the equilibrium between **104** and **105** to lie in favor of **104**. In five cases with electron-donating groups (**102b–f**), only species of type **108**/**109** and **110** were observed by RP MP-HPLC, with a notable absence of *N*-hydroxy compounds of type **106**, thereby supporting the concept that nitrogen protonation in a species of type **105** may direct the course of reaction.

Investigations to optimize yield[36] established that the type and amount of catalyst, the volume of solvent, and reaction time are all critical. Catalyst activity appeared to vary from batch to batch, and storage under anhydrous conditions was important for maintaining reduction efficiency. The optimum amount of catalyst and solvent volume were found to be 0.35 g and 100 mL, respectively, per 1 mmol of nitro aldehyde starting material. The reaction time was found to be critical, with longer reaction times leading to higher yields of the corresponding secondary amines. In most cases, the carbinolamines and secondary amine products could be isolated by simple filtration and evaporation of solvent at 40 °C, followed by purification using MP-HPLC with CH_3CN/H_2O (1:1) as mobile phase. On evaporation of the appropriate fractions, the pure product in each case was obtained essentially in the carbinolamine form, presumably due to the aqueous solvent system.

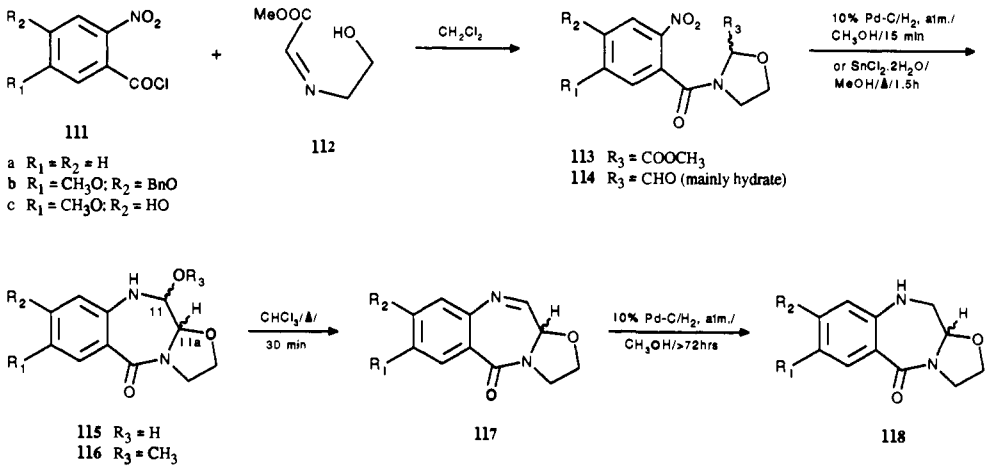
Jones[15c,55] has also used the reductive cyclization procedure to form the oxazolo[2,3-c][1,4]benzodiazepine (OBD) ring system of type **115** (Scheme 32). Methyl glyoxalate was condensed with ethanolamine (CH_2Cl_2/room temperature/molecular sieves) to give the imino alcohol **112**, which was treated with the substituted 2-nitrobenzoyl chlorides **111a,b** to afford nitro esters of type **113a,b**. Reduction with DIBAL-H afforded the corresponding aldehydes of type **114**, which, unlike pyrrolidine-2-aldehydes, existed mainly in the hydrated form owing to the electron-withdrawing effect of the C-ring oxygen. Reductive cyclization of the aldehydes using either 10% Pd–C/H_2 atm/MeOH/15 min/room temperature or $SnCl_2{\cdot}2H_2O$/MeOH/Δ/90 °C gave the OBD carbinolamines **115a–c** in high yield (approxi-

Scheme 31



	R_6	R_7	R_8	R_9
a	H	H	H	H
b	H	H	CH_3	OH
c	H	H	H	CH_3
d	H	CH_3	H	H
e	CH_3	H	H	H
f	H	H	H	OCH_3

Scheme 32



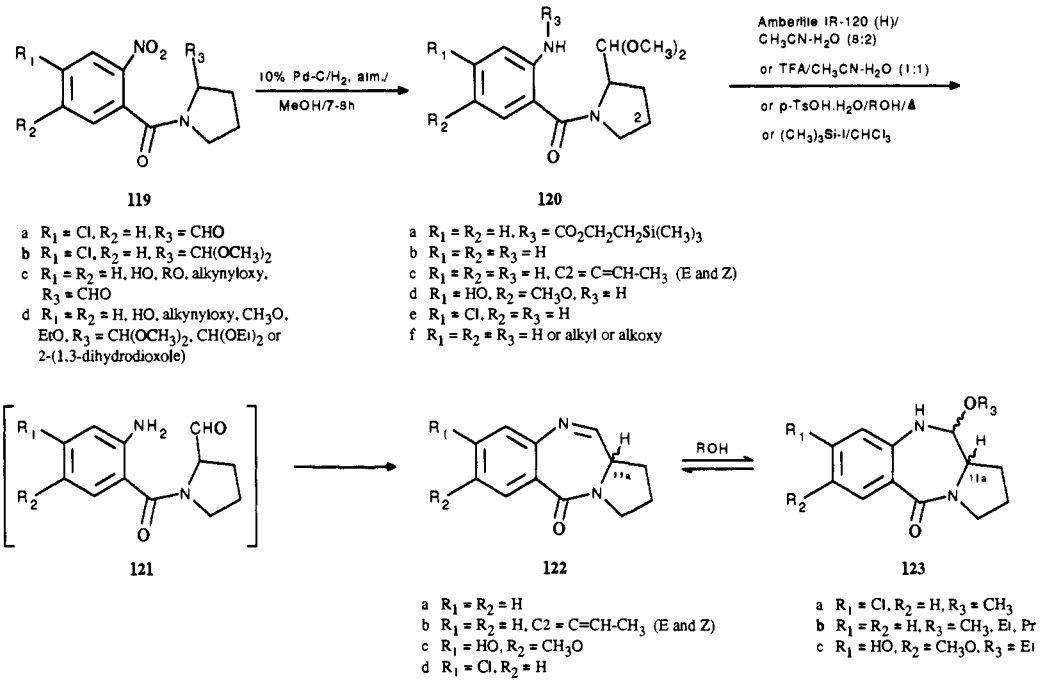
mately 80%). In contrast to the PBDs, methyl ether formation (**115a–c** → **116a–c**) (MeOH/Δ/2 h) occurred stereospecifically to give the (11*R*,11a*S*) and (11*S*,11a*R*) forms which appeared as one species by NMR spectroscopy, suggesting a mixture of enantiomers with the H11 and H11a protons in the cis configuration. Refluxing the carbinolamines in $CHCl_3$ for 30 min afforded the corresponding OBD imines **117a–c**. Greater stability of the OBD carbinolamine/imine forms compared to their PBD equivalents is suggested by their resistance to overreduction (*via* imine intermediates) to the corresponding N10–C11 secondary amines (e.g. **118**). Unlike PBDs, the OBD carbinolamines can withstand prolonged hydrogenation; >72 h hydrogenation at room temperature is required for reduction to the secondary amine forms.

Chemical reducing agents have also been used to effect reductive cyclization. Stefancich has reported[77] (Scheme 23) that reduction of **83b** with $FeSO_4 \cdot 7H_2O$/NH_4OH (120 °C/1 h) afforded the imine **84b** in 84% yield, whereas catalytic hydrogenation (*vide supra*) afforded the secondary amine **85b** in 92% yield. Compounds **84b** and **85b** were shown to be interconvertible by either reduction with 10% Pd–C (95% yield) or oxidation with manganese dioxide in dry benzene (69%).

Iron powder and acetic acid (Δ/2 h) has been used to cyclize nitro aldehydes of type **96c–e** to imines **97Ac–e** in approximately 30% yield[38b] (Scheme 28). Aqueous sodium dithionite ($Na_2S_2O_4$) has also been used to reduce the nitro aldehydes **83c** and **83d** to the imine forms **84c** (47%)[78a] and **84d**,[78b] respectively (Scheme 23). Interestingly, the imine **84c** has also been reduced in 83% yield to the amine **85c** by treatment with $LiAlH_4$/THF.[78a] Jones and co-workers[15c,55] also used $SnCl_2 \cdot 2H_2O$/MeOH/Δ/90 min to effect reductive cyclization of the OBD nitro aldehydes of type **114a–c** (Scheme 32).

On the basis of the above, reductive cyclization is not, in general, a preferred method for preparing carbinolamine-containing PBDs due to low yields, the formation of byproducts (i.e. N10–C11 secondary

Scheme 33



119

a R_1 = Cl, R_2 = H, R_3 = CHO
b R_1 = Cl, R_2 = H, R_3 = $CH(OCH_3)_2$
c R_1 = R_2 = H, HO, RO, alkynyloxy, R_3 = CHO
d R_1 = R_2 = H, HO, alkynyloxy, CH_3O, EtO, R_3 = $CH(OCH_3)_2$, $CH(OEt)_2$ or 2-(1,3-dihydrodioxole)

120

a R_1 = R_2 = H, R_3 = $CO_2CH_2CH_2Si(CH_3)_3$
b R_1 = R_2 = R_3 = H
c R_1 = R_2 = R_3 = H, C2 = C=CH-CH_3 (E and Z)
d R_1 = HO, R_2 = CH_3O, R_3 = H
e R_1 = Cl, R_2 = R_3 = H
f R_1 = R_2 = R_3 = H or alkyl or alkoxy

121

122

a R_1 = R_2 = H
b R_1 = R_2 = H, C2 = C=CH-CH_3 (E and Z)
c R_1 = HO, R_2 = CH_3O
d R_1 = Cl, R_2 = H

123

a R_1 = Cl, R_2 = H, R_3 = CH_3
b R_1 = R_2 = H, R_3 = CH_3, Et, Pr
c R_1 = HO, R_2 = CH_3O, R_3 = Et

amines and/or N10-hydroxy compounds), and the possibility of reduction of other double bonds in the molecule. However, the introduction of Raney nickel as a cyclization catalyst by Langlois and Andriamialisoa[76] appears to be promising (e.g. 75% reported yield for **89a** → **90a** in Scheme 25), apparently overcoming the problems of sequential reduction of the newly generated N10–C11 imine to the secondary amine and/or the incomplete reduction of the aromatic nitro group prior to the cyclization step leading to N10-hydroxy compounds.[36] The scope and general applicability of the use of Raney nickel therefore deserves further study.

B. Cyclization of *N*-(2-Aminobenzoyl)pyrrolidine-2-carboxaldehyde Dialkyl Acetals

This route involves the deprotection of an amino acetal of type **120** to form an amino aldehyde intermediate of type 121 that can spontaneously cyclize to a PBD imine (**122**) or carbinolamine (**123**), depending upon the workup conditions (Scheme 33). Both nonacidic [$(CH_3)_3SiI/CHCl_3$] and acidic [TFA, *p*-TsOH·H_2O or Amberlite Resin IR-120 (H)] reagents have been used to effect cyclization. However, one of the major problems associated with this method is that deprotection of the acetal can lead to racemization at the C11a-position of the product, particularly under acidic conditions.

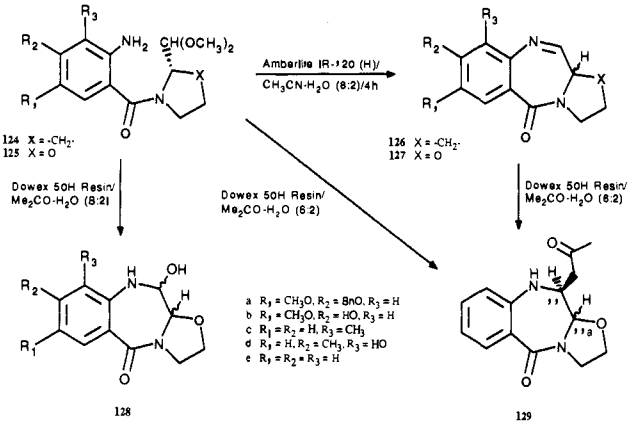
Mazzocchi and Schuda first reported[79] a synthesis of the parent PBD imine **122a** in 51% yield *via* deprotection of the amino acetal **120b** using $(CH_3)_3SiI$ in $CHCl_3$. The *N*-TMS-protected acetal precursor (**120a**) was produced *via* a lengthy nine-step ring-opening ring-closing procedure, and deprotection with tetrabutylammonium fluoride (CH_3CN/55 °C) afforded the amino acetal **120b** in 60% yield. However, the possibility of racemization at the C11a-position of the product was not addressed, and optical rotation measurements not reported. Similarly, a mixture of prothracarcin A and B precursors[15b] (e.g. **120c**) led to a mixture of prothracarcins A and B (**122b**) in low yield. DC-81 (**122c**) was also prepared from the precursor **120d** in 35% yield.

A different cyclization reagent has been used by other workers[15a] to prepare the 8-chloro analog **123a** and its imine form **122d**. The precursor nitro acetal **119b** was readily prepared (68% yield) by treatment of the corresponding nitro aldehyde **119a** with 2,2-dimethoxypropane/MeOH/CH_3SO_3H (stirring at room temperature for 2 h). Hydrogenation at atmospheric pressure over 10% Pd–C catalyst in MeOH for 7–8 h gave the amino acetal **120e** in 30% yield. The key deprotection step involved dissolving **120e** in CH_3CN/H_2O (1:1) followed by the addition of TFA with stirring at room temperature. Reaction was complete after 3 h as judged by HPLC [32% yield; precipitated as a mixture of the carbinolamine (**123a**) and imine (**122d**) forms]. Optical activities of the products were not reported, although it is likely that some racemization at C11a would occur under the acidic reaction conditions.

Karyone and co-workers[80] have also reported the preparation of carbinolamine-containing PBDs of type **123b** and **123c**, through treatment of solutions of the amino methyl acetals of type **120f** (and also the equivalent ethyl acetals) in refluxing EtOH, MeOH, or PrOH with *p*-TsOH·H_2O for approximately 5 min. The corresponding nitro acetals **119d** were prepared by treatment of the aldehydes of type **119c** with the appropriate alcohols (MeOH, EtOH, or $HOCH_2CH_2OH$) in the presence of catalysts such as BF_3·Et_2O (24 h/room temperature), followed by chromatography on silica gel.

A different reagent, Amberlite IR-120 (H) resin in CH_3CN–H_2O (8:2), was used by Jones and co-workers[15c,40a,55] to effect cyclization of amino acetals (Scheme 34). Amino acetals **124a–d** were prepared by treatment of the corresponding nitro aldehydes with Amberlite IR-120 (H) or Dowex 50X resin and $(CH_3O)_3CH/CH_2Cl_2$/16 h/room temperature followed by reduction with 10% Pd–C/H_2 atm/MeOH (for **124b–d**) or $SnCl_2$·$2H_2O$/

Scheme 34



MeOH/Δ (for **124a**). Stirring with Amberlite IR-120 (H) resin in CH_3CN–H_2O (8:2) for approximately 4 h afforded the imines **126a–d** in low yield (14–47%). An advantage of this method is the simple workup procedure which involves removal of the resin by filtration followed by evaporation of the solvent *in vacuo*. The compounds were typically obtained as yellow gums consisting of a mixture of the imine and carbinolamine forms. These could be further purified by column chromatography on silica gel to afford predominantly the imine forms.[15c]

Shioiri and co-workers[81] have utilized this general method for a stereoselective synthesis of tilivalline (**133a**), a PBD metabolite from a *Klebsiella pneumoniae* sp. (Scheme 35). The amino acetal intermediates **132** were obtained in 84% yield by condensation of anthranilates of type **130a–c** with L-prolinal dimethyl acetal (**131**) using diethyl phosphorocyanidate [DEPC, $(EtO)_2P(O)CN$], Et_3N, and 4-Å molecular sieves (THF, 0 °C for 1 h, then room temperature for 40 min). In a one-pot process, intermediates **132** were treated sequentially with TMS-Cl/NaI/pyridine in CH_3CN (–20 °C, 0.5 h, argon), indole (2 equiv, room temperature, 0.5 h), and $ZnCl_2$ (room temperature overnight, then 55 °C for 3 h). The products **133a–c** were formed through a Mannich-type condensation of the acetal with indole in the presence of TMS-Cl/NaI. The reaction was completely stereoselective (in contrast to the synthesis of Mohr and Budzikiewicz[75]) with the nucleophile (indole) approaching from the less hindered β-face.

Jones and co-workers[15c,55] have also used this cyclization process to prepare oxazolobenzodiazepines (OBDs). The amino acetals of type **125a,b** (Scheme 34) were treated with Dowex 50H resin in acetone–H_2O (8:2) to give the corresponding carbinolamines (**128a,b**). In the case of **125e**, a mixture of the carbinolamine (**128e**) and the unusual acetone addition product (the C11-oxopropyl adduct, **129**) was formed. In contrast to the OBD carbinolamines of type **128**, the coupling between the H11 and H11a protons in the ^{1}H-NMR spectrum indicated a trans arrangement, suggesting that the C11-oxopropyl adduct exists as a mixture of (11*S*,-11a*S*) and (11*R*,11a*R*) enantiomers. Quantitative conversion of the OBD imine **127e** (obtained by refluxing **128e** in $CHCl_3$ for 30 min) to the acetone adduct (**129**) could also be achieved under the same reaction conditions. This stereospecific reaction of a carbon nucleophile at the C11-position is unusual in the PBD ring system and may indicate a more reactive imine form.

Although this general method uses simple chemistry and provides high yields, the potential loss of stereochemistry at C11a could be problematic. For example, Thurston and co-workers have carried out a direct comparison of the optical purity and biological activity of samples of DC-81 (e.g. **122c**, Scheme 33, or **126b**, Scheme 34) obtained from different synthetic routes.[40a] A sample prepared by acid-catalyzed Amberlite IR-120 (H) cyclization was racemic and had a lower cytotoxicity (IC_{50} in L1210: 5.3 μM) compared to a sample prepared by the amino thioacetal route (see Section V.C) which had an $[\alpha]^{23}_D$ +371 (c = 0.68, $CHCl_3$) and an IC_{50} (L1210) of 0.38 μM. Although racemization of the sample of DC-81 prepared by acid-catalyzed cyclization could have occurred during either workup or storage, this result suggests that other routes such as the thioacetal/$HgCl_2$ process should be utilized if the optical purity and biological activity of final products is crucial.

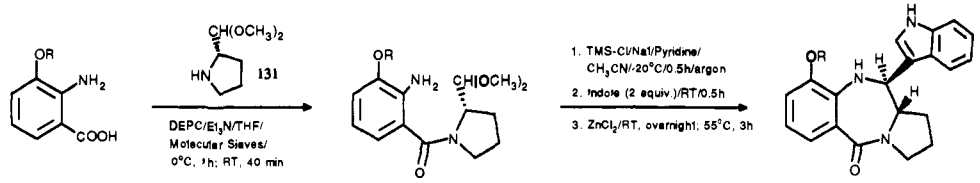
C. Cyclization of *N*-(2-Aminobenzoyl)pyrrolidine-2-carboxaldehyde Diethyl Thioacetals

This method was specifically developed to overcome the limitations of the previously described techniques.[39a] A method was sought that was generally applicable and not dependent on the type or pattern of ring substituents, that involved non-hydrogenolytic conditions to preserve points of unsaturation in the molecule, that maintained stereochemical integrity at C11a of the product, and that was adaptable to a convergent synthesis. The essential requirements of such a sequence are reduction of a nitro to an amino functionality under non-hydrogenolytic conditions and careful formation, protection, and deprotection of an aldehyde in a mild and nonracemizing environment. Cyclization of *N*-(2-aminobenzoyl)pyrrolidine-2-carboxaldehyde diethyl thioacetals was found to meet all of these criteria.

Nitro aldehydes of type **135**, prepared *via* DIBAL-H reduction[82] of the corresponding nitro esters of type **134**, were converted in almost quantitative yield to diethyl thioacetals[83] (**136**) by stirring at room temperature with ethanethiol and trimethylsilyl chloride ($CHCl_3$/16–48 h) (Scheme 36). These stable intermediates could be efficiently reduced by refluxing for 1–2 h with stannous chloride dihydrate in MeOH[84] to afford the corresponding amino diethyl thioacetals of type **137** in nearly quantitative yield. Efficient deprotection with concomitant cyclization to the carbinolamine (**138**) or imine (**139**) equivalents was then effected by treatment at room temperature with $HgCl_2$ and $CaCO_3$ in CH_3CN/H_2O for 12–48 h.[85] The products were usually isolated in the imine form **139** because of the workup procedure which involved extraction into chloroform.[3a,35,36,40a]

The procedure was initially tested on three model nitro aldehydes **135a–c** which afforded, over three steps, the cyclized imines **139a–c** in overall yields of 53%, 72%, and 68%, respectively. In each case, apart from a trace amount of starting material, the final products were clean and consisted of single components asvisualized by TLC. Significantly, cyclization took place with both substituted and unsubstituted aromatic A rings, whereas other synthetic methods may typically fail in one or the other case.[32,36] Furthermore, it was conclusively demonstrated that the stereochemistry of

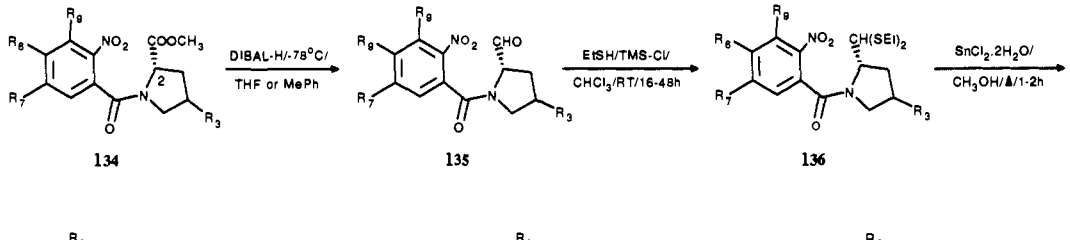
Scheme 35



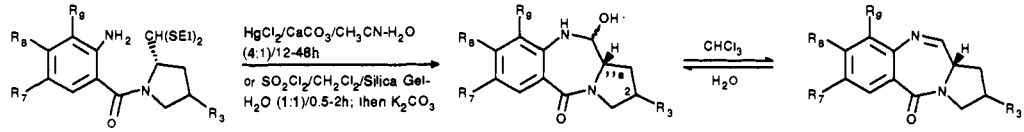
130 132 133

a R = H
b R = CH_3
c R = Bn

Scheme 36



134 135 136



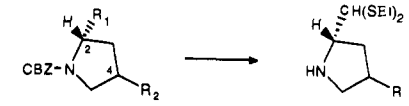
137 138 139

a $R_7 = R_8 = R_9 = R_3 = H$
b $R_7 = R_8 = R_3 = H$, $R_9 = CH_3$
c $R_7 = CH_3$, $R_8 = R_9 = R_3 = H$
d $R_7 = R_8 = R_9 = H$, $R_3 = =CH\text{-}CH_3$ (E)
e $R_7 = R_8 = R_9 = H$, $R_3 = =CH\text{-}CH_3$ (Z)
f $R_7 = CH_3O$, $R_8 = BnO$, $R_3 = R_9 = H$
g $R_7 = CH_3O$, $R_8 = HO$, $R_3 = R_9 = H$
h $R_7 = CH_3O$, $R_8 = BnO$, $R_3 = R_9 = H$, C11a(R)

the aldehyde-bearing carbon is preserved during diethyl thioacetal formation and subsequent deprotection.[39a] Optically active unsubstituted nitro aldehyde **135a** of $[\alpha]^{25}_D$ −146.9 (c = 0.049) was converted into the nitro diethyl thioacetal (**136a**) and then back to the nitro aldehyde by treatment with $HgCl_2$ and $CaCO_3$ in CH_3CN/H_2O. The resulting product had an almost identical optical rotation ($[\alpha]^{25}_D$ −146.1) to starting material **135a**, indicating that the overall process preserves C11a stereochemistry in the product. As a demonstration of the preservation of unsaturation, the total synthesis of the naturally occurring PBD prothracarcin[10] (**139d**) was undertaken. Both optically active prothracarcin (**139d**) (28% overall yield) and its unnatural Z-isomer (**139e**) were synthesized from the appropriate C2-substituted nitro aldehydes **135d,e**. This allowed confirmation of the E-configuration of the C2-ethylidene side chain of naturally-occurring prothracarcin.

Of further interest is the potential of this route to be adapted for a convergent approach to analog synthesis. For example, (2S)-pyrrolidine-2-carboxaldehyde diethyl thioacetal (**143**) was prepared in bulk in an overall yield of 56%, *via* deprotection (TMS-I/CH_2Cl_2/argon/room temperature/30 min) of the N-CBZ derivative (**142**), itself derived from intermediates **140** and **141** (Scheme 37).[39a] A more efficient deprotection method for CBZ-protected pyrrolidines of type **142** utilizing $BF_3{\cdot}OEt_2$/EtSH has also been reported.[86] After coupling of **143** to a 2-nitrobenzoyl derivative, an efficient conversion to an imine-containing product may then be achieved in two high-yielding steps. For example, **143** was coupled to 2-nitrobenzoyl chloride to afford **136a** in

Scheme 37



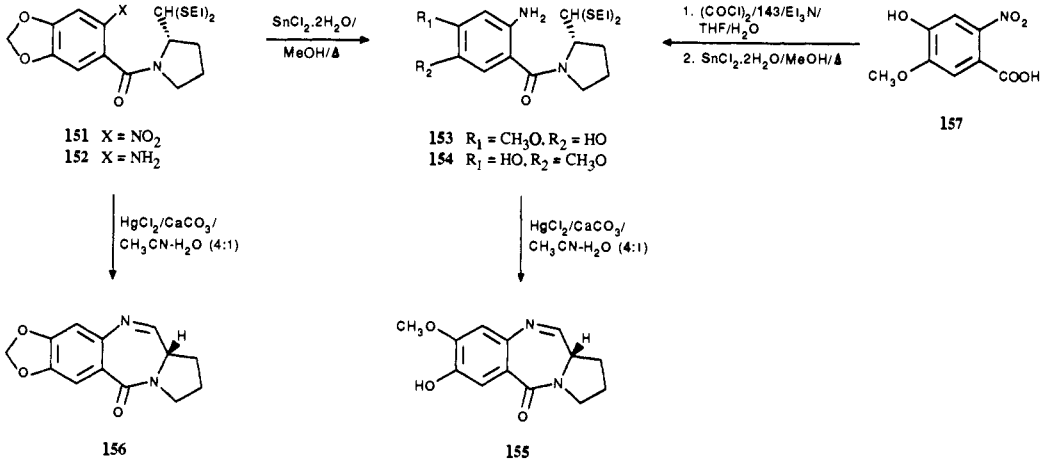
140 $R_1 = COOCH_3$, $R_2 = H$
141 $R_1 = CHO$, $R_2 = H$
142 $R_1 = CH(SEt)_2$, $R_2 = H$
144 $R_1 = COOH$, $R_2 = OH$
145 $R_1 = COOH$, $R_2 = =O$
146 $R_1 = COOH$, $R_2 = =CH\text{-}CH_3$*
147 $R_1 = COOCH_3$, $R_2 = =CH\text{-}CH_3$(E)
148 $R_1 = CHO$, $R_2 = =CH\text{-}CH_3$(E)
149 $R_1 = CH(SEt)_2$, $R_2 = =CH\text{-}CH_3$(E)
*E,Z mixture

143 R = H
150 $R = =CH\text{-}CH_3$(E)

92% yield. This product had a similar optical activity to that of **136a** produced *via* the linear synthesis described above (Scheme 36). Continuation of the synthesis through **137a** afforded the final product **139a** in 69% overall yield from **143**. Similarly, (2S)-(*E*)-4-ethylidenepyrrolidine-2-carboxaldehyde diethyl thioacetal (**150**)[87] has been produced in six steps (**144** → **150**) starting from CBZ-protected (2S,4R)-hydroxyproline (**144**) (Scheme 37). This is the C-ring precursor of both prothracarcin and tomaymycin and has been used in convergent total syntheses of both prothracarcin[39a] and a tomaymycin analog.[87] As both have the E-configuration at C2, separation of E- and Z-isomers after the Wittig reaction (**145** → **146**) was necessary, although the E-isomer was formed in higher yield.

The key intermediate **143** has also been coupled to 4-(benzyloxy)-5-methoxy-2-nitrobenzoic acid to afford **136f** (91% yield) which was reduced (**137f**, 75%) and cyclized to afford the imine **139f** (68%) (Scheme 36). Debenzylation by transfer catalysis afforded the natural product DC-81 (**139g**) in 89% yield.[40b] In all convergent

Scheme 38



151 X = NO_2
152 X = NH_2

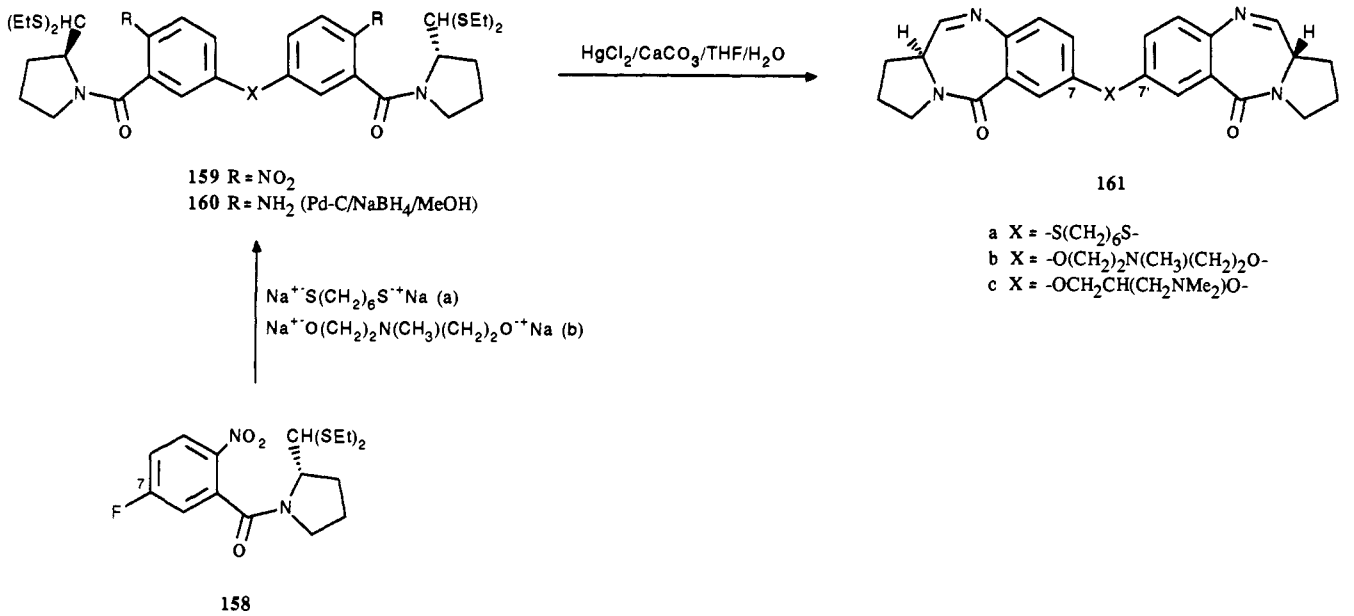
153 R_1 = CH_3O, R_2 = HO
154 R_1 = HO, R_2 = CH_3O

157

156

155

Scheme 39



159 R = NO_2
160 R = NH_2 (Pd-C/$NaBH_4$/MeOH)

161

a X = -$S(CH_2)_6S$-
b X = -$O(CH_2)_2N(CH_3)(CH_2)_2O$-
c X = -$OCH_2CH(CH_2NMe_2)O$-

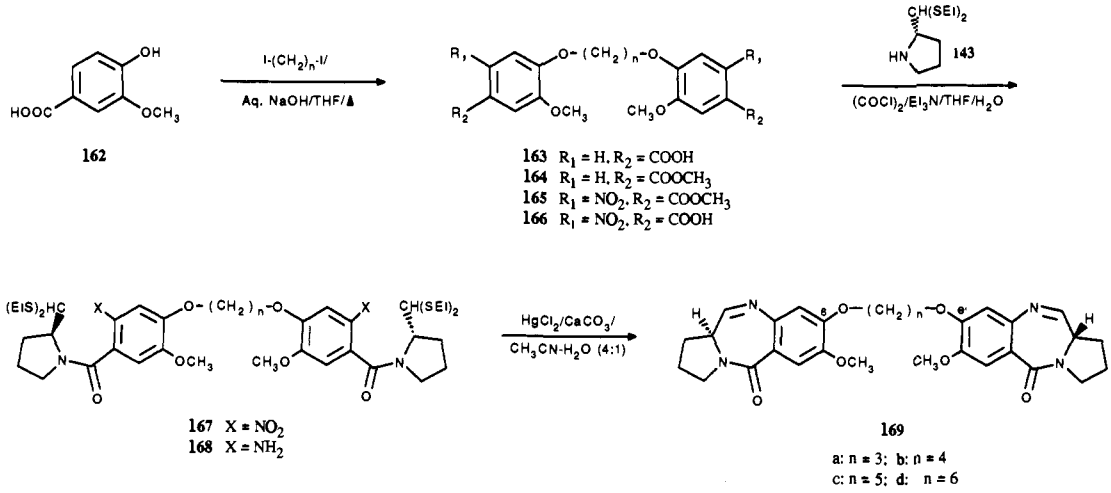
158

syntheses of this type, the phenolic C8-hydroxyl is usually protected with a group such as benzyl or benzoate. However, it has recently been shown[40a] that 6-nitrovanillic acid (**157**) can be coupled directly with **143** followed by reduction with $SnCl_2 \cdot 2H_2O$ to afford **154** (Scheme 38). The C11a-enantiomeric form of **139f** (Scheme 36) has also been synthesized[15a] by this route. D-Proline was coupled to 4-(benzyloxy)-5-methoxy-2-nitrobenzoic acid (89%) and converted to the C11a-(*R*)-imine **139h** in 80% yield. Recently, this route was also used to synthesize iso-DC-81 (**155**),[27b,88] and a novel dioxolo[4,5-*h*]pyrrolo[2,1-*c*]benzodiazepine (**156**)[89] (Scheme 38). The intermediate **151** was prepared from 2-nitropiperonylic acid and **143**. Attempted reduction of the aromatic nitro group with $SnCl_2 \cdot 2H_2O$ gave the ring-opened product **153** instead of the expected amino thioacetal **152**.[89] This represents the first example of a regiospecific tin-catalyzed cleavage of this type and provided the opportunity to synthesize *iso*-DC-81 (**155**), a new type of C7-hydroxy-C8-methoxy-substituted PBD, isomeric with the DC-81, tomaymycin, and neothramycin A-ring substitution pattern. Interestingly, catalytic hydrogenation of **151** afforded the corresponding amine (**152**) which was successfully cyclized to afford **156**, the first example of a dioxolo-[4,5-*h*]pyrrolobenzodiazepine.[89]

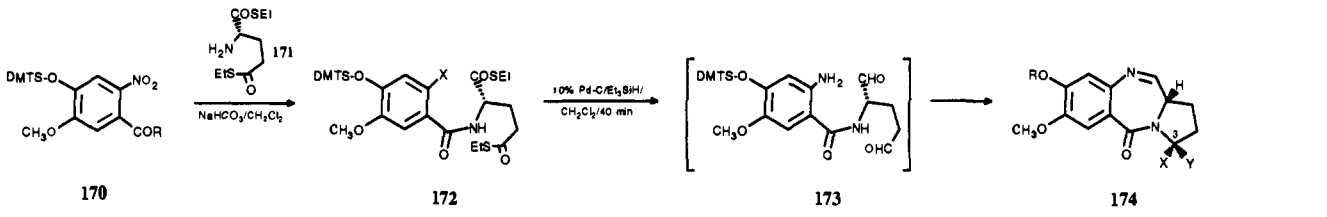
The same methodology has been used by Suggs and co-workers[23d,41] to prepare C7-linked PBD dimers of type **161a,b** (Scheme 39) from precursor amino thioacetals (**160a,b**) using $HgCl_2/CaCO_3/THF/H_2O$. These dimers were shown to have DNA cross-linking activity. The amino thioacetals **160a,b** were prepared from the corresponding nitro thioacetals (**159a,b**) *via* reduction with Pd–C/$NaBH_4$. Interestingly, the nitro thioacetal of type **159** with a $NH(CH_2)_3N(CH_3)(CH_2)_3NH$ linker failed to give a final product of type **161** under a variety of conditions, and only decomposition products resulted. The nitro thioacetal precursors (**159**) were prepared by nucleophilic displacement of a C7-fluorine substituent in the monomer nitro thioacetal fragment **158** with sodium salts of the linker fragments. C7-linked DC-81 dimers such as **161c** with one or more chiral centers in the linker have also been reported.[41c]

Thurston and co-workers have recently synthesized C8-linked DC-81 dimers (Scheme 40).[28,87] These dimers of type **169** are extremely cytotoxic (e.g. IC_{50} = 0.0005 μM in ADJ/PC6 cells) and are approximately 350- and 50-fold more efficient than melphalan and cisplatin, respectively, as irreversible DNA interstrand cross-linking agents. Molecular modeling and NMR studies have shown that, at least for **169a**, the molecule is completely isohelical with the minor groove of B-DNA,

Scheme 40



Scheme 41



bonding to guanines on opposite strands. In contrast to the synthetic approach adopted by Suggs for the C7-linked dimers, this route involved the joining of two units of vanillic acid (**162**) through their C8-phenolic hydroxyls with α,ω-diiodoalkanes of varying length to afford dimer acids of type **163**. However, all attempts to obtain nitro acids of type **166** by direct nitration of **163a–d** failed using a variety of reaction conditions, due to the insoluble nature of the dimer acids. Following conversion to the corresponding methyl esters **164a–d**, nitration with $SnCl_4/HNO_3$ proceeded smoothly to afford the nitro esters **165a–d** in high yield. Mild hydrolysis of the esters with aqueous NaOH at room temperature for 6 h afforded the nitro acids **166a–d** in quantitative yield. These could be coupled to **143** to afford the bis(amides) **167a–d** in approximately 65% yield, which were subsequently reduced to the amino thioacetals **168a–d**. Cyclization with $HgCl_2/CaCO_3$ in CH_3CN/H_2O afforded the target C8-linked dimers **169a–d** in good yields. Baraldi and co-workers have also used this $HgCl_2$-mediated cyclization method to synthesize PBD analogs with heterocyclic A rings (see Section VII.A).

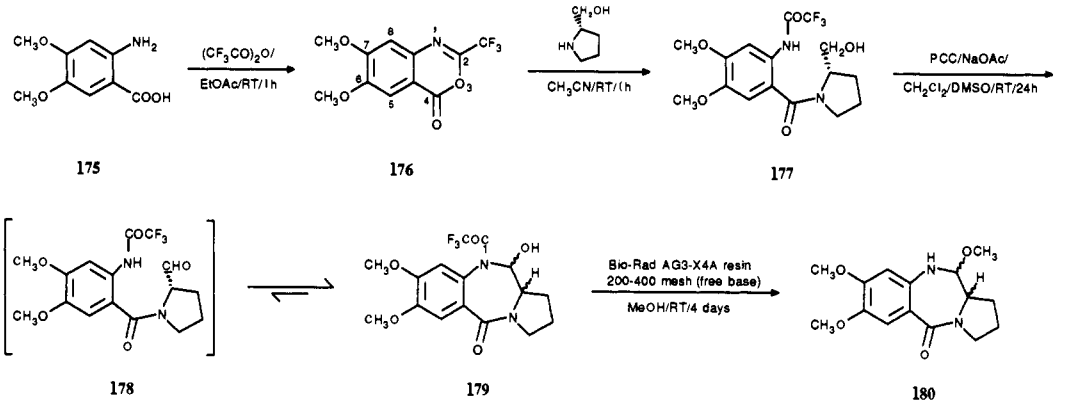
More recently, Courtney and Thurston[39b] have reported the use of sulfuryl chloride to effect the cyclization of amino thioacetals. A solution of SO_2Cl_2 in CH_2Cl_2 was added dropwise to a stirred mixture of **137a** or **137f** and wet silica gel (silica gel/H_2O, 1:1 by weight) in CH_2Cl_2 (Scheme 36). After the mixture was stirred for 0.5–2 h until TLC indicated that reaction was complete, K_2CO_3 was added and stirring continued for a further 0.5 h. The reaction mixture was then filtered and the filtrate evaporated *in vacuo* to afford the crude imine products **139a** and **139f** in almost quantitative yield. A further advantage over the use of $HgCl_2/CaCO_3$ is that no Hg-containing byproducts are produced, thus allowing a simple and rapid workup procedure. For example, even after one purification by column chromatography, 20% and 60% yields of **139a** and **139f**, respectively, were obtained at a purity level suitable for biochemical and pharmacological evaluation.

In conclusion, the cyclization of amino thioacetals has significant advantages over other routes. In particular, (a) it is insensitive to substituent types and patterns in the A ring, (b) stereochemistry is preserved at the aldehyde-bearing carbon, (c) unsaturation is preserved in the final product, (d) yields are reasonable, and (e) it is adaptable to a convergent approach. Considerable experience with this method of cyclization has now been gained in a number of independent laboratories, and its application to the synthesis of PBD monomers and dimers, and analogs with heterocyclic A rings (see Section VII.A) attests to its versatility and scope. The recent introduction of sulfuryl chloride as the cyclization reagent appears to be advantageous in terms of higher yields and reduced formation of byproducts, although further work is required to establish its full potential.

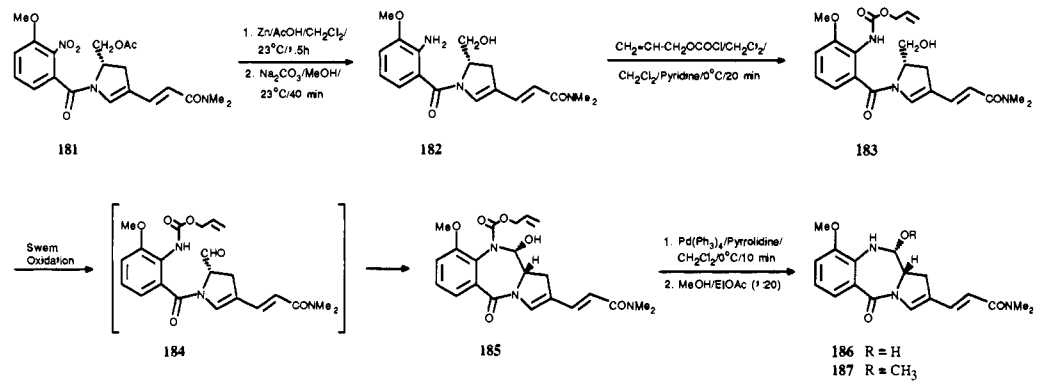
D. Cyclization of Ethyl *N*-(2-Aminobenzoyl)-pyrrolidine-2-thiocarboxylates

Fukuyama[42a] has reported the only known example of the synthesis of an imine form of a PBD (i.e. neothramycin A) *via* the production of an aldehyde functionality in the presence of an A-ring amine (Scheme 41). This was made possible through a new method of aldehyde synthesis involving the reduction of an ethyl thioester (derived from a carboxylic acid) with triethylsilane and a catalytic amount of Pd–C catalyst. The dimethylthexylsilyl (DMTS)-protected

Scheme 42



Scheme 43



A-ring fragment of neothramycin A (**170a**) was prepared from vanillin in five steps in 70% overall yield. This was converted to the acid chloride **170b** using $(COCl)_2$/PhH/60 °C and coupled ($NaHCO_3/CH_2Cl_2$) to the unstable amino dithioester **171** (derived in one step from *N*-Boc-L-glutamic acid) in 73% yield. The resulting amide **172a** was then reduced to the aromatic amine **172b** using activated Zn/acetic acid/ether/23 °C in 80% yield. The critical double cyclization of **172b** was performed by treatment with 10% Pd–C (15 mol %) and Et_3SiH (5 equiv) in CH_2Cl_2 (40 min). The unstable neothramycin silyl ethers formed (**174a**), presumably through the bisaldehyde intermediate **173**, were isolated as an epimeric mixture of the more stable C3-*O*-methyl ethers (**174b**) by treatment with camphorsulfonic acid/methanol (66% yield from **172b**). Finally, deprotection of the more predominant DMTS ether was achieved with *n*-$Bu_4N^+F^-$/AcOH/MeOH to give the C3-α-epimer **174c** in 65% yield. Although this method appears to be a promising and high-yielding route to the PBDs, there is presently only one reported example of its use, and so its general applicability is unknown.

E. Cyclization of a *N*-(2-((Trifluoroacetyl)amino)-benzoyl)pyrrolidine-2-methanol

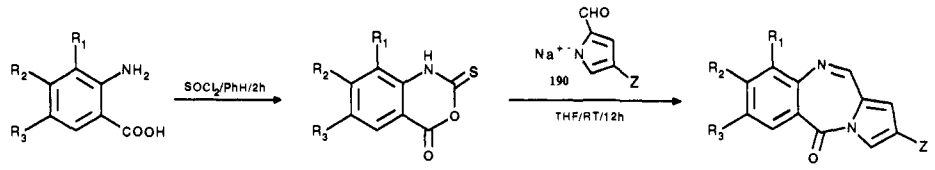
This method involves oxidation and deprotection of an *N*-(trifluoroacetyl)-protected amino alcohol of type **177**.[15a] The intermediates are prepared by a reaction involving acylation of a pyrrolidine fragment (e.g. pyrrolidinemethanol) with a benzoxazin-4-one of type **176**. Treatment of a solution of the disubstituted anthranilic acid **175** in ethyl acetate with trifluoroacetic anhydride for 1 h at room temperature afforded 6,7-dimethoxy-2-(trifluoromethyl)-4*H*-1,3-benzoxazin-4-one (**176**) in 96% yield (Scheme 42). Reaction of **176** with (*S*)-2-pyrrolidinemethanol in CH_3CN for 1 h at room temperature afforded the N10-trifluoroacetyl alcohol **177** in almost quantitative yield, which was oxidized with pyridinium chlorochromate (CH_2Cl_2/DMSO/NaOAc, stirring overnight at room temperature) to afford the cyclized N10-(trifluoroacetyl)-protected carbinolamine **179** in 60% yield, presumably *via* the N10-(trifluoroacetyl)-protected amino aldehyde intermediate **178**. A suspension of **179** and Bio-Rad AG3-X4A resin (200–400 mesh, free base form) in methanol was stirred at room temperature for 4 days to afford the corresponding carbinolamine methyl ether (**180**) in 21% yield after recrystallization from EtOAc/cyclohexane.

This would appear to be a useful but relatively low-yielding synthetic procedure for producing imine- or carbinolamine-containing PBDs. However, there is only one reported example of its use, and so its general applicability is unknown. In addition, optical rotations were not reported, and so it is unclear whether C11a-stereochemistry is maintained.

F. Cyclization of a *N*-(2-((Allyloxy)carboxamido)benzoyl)pyrrolidine-2-methanol

Fukuyama and co-workers[42b] have recently reported a cyclization technique based on the oxidation and deprotection of an *N*-(allyloxycarbonyl)-protected amino alcohol of type **183** (Scheme 43). Starting from the nitro acetate **181**, reduction ($Zn/CH_3COOH/CH_2Cl_2$/23 °C/1.5 h) and hydrolysis (saturated Na_2CO_3/MeOH/23 °C/40 min) afforded the amino alcohol **182**. N-Carbamoylation with allyl chloroformate/pyridine (CH_2Cl_2/0 °C/20 min) gave the N-protected amino alcohol **183** (64% yield in three steps from **181**).

Scheme 44



188 189 191

a $R_2 = CH_3$, $R_1 = R_3 = H$, $Z = H$
b $R_1 = R_2 = R_3 = H$, $Z = H$
c $R_2 = CH_3$, $R_1 = R_3 = CH_3O$ (Z = CH=CH-CH_3, trans)
d $R_2 = CH_3$, $R_1 = R_3 = HO$ (Z = CH=CH-CH_3, trans)
e $R_2 = R_3 = CH_3O$, $R_1 = H$, $Z = H$

Spontaneous cyclization to form a single isomer of N-protected porothramycin A (**185**) occurred upon oxidation under Swern conditions (72% yield), presumably *via* the N-protected amino aldehyde intermediate **184**. Removal of the allylurethane protecting group was carried out according to the procedure of Deziel[42c] using Pd(0) [$Pd(PPh_3)_4$/pyrrolidine/CH_2Cl_2/0 °C/10 min] to provide unstable non-crystalline porothramycin A (**186**) in 67% yield after rapid purification by flash chromatography. Crystallization from MeOH–EtOAc (1:20) provided pure porothramycin B (**187**), which was identical to the natural product based on NMR, MS, and optical rotation data. In summary, this synthetic route appears to have potential in terms of yield and optical activity of the final product. Further work is required to establish its general applicability.

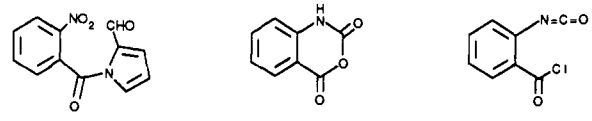
VI. Synthesis of C11a–C1, C2–C3 Unsaturated PBDs

Until the structure of sibiromycin was corrected,[12] the fully unsaturated pyrrolobenzodiazepine system of type **191** was of interest because it was thought to constitute the parent ring system of anhydrosibiromycinone (**191d**) (Scheme 44). Consequently, a number of synthetic approaches have been explored. The N10–C11 imine functionality of this system is exceptionally stable in comparison to analogs with either a partially (C2–C3 unsaturated) or fully saturated C ring. The carbinolamine or carbinolamine methyl ether forms of the fully unsaturated systems have never been observed, presumably due to the stability derived from extended conjugation of the N10–C11 imine through to the C ring. Similarly, the N10–C11 imine of unsaturated systems of this type is inert to attack by nucleophiles. The four known approaches to the fully unsaturated system are outlined below.

A. Cyclocondensation of Sulfinamide Anhydrides with Substituted Prolines

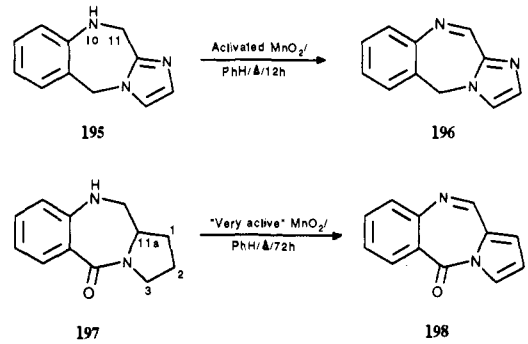
An approach to the initially published (but incorrect) anhydrosibiromycinone structure was made by Parker and co-workers.[90] Working first on a model system, the sulfinamide anhydride **189a** (Scheme 44) was prepared by refluxing the corresponding anthranilic acid **188a** with $SOCl_2$ in benzene for 2 h. Reaction with the sodium salt of pyrrole-2-carboxaldehyde (**190a**) by stirring in THF at room temperature for 12 h afforded the imine **191a** as bright yellow crystals in high yield (91%). Interestingly, attempts to prepare **191b** by reductive cyclization of the nitro aldehyde **192** (Scheme 45) or by condensation of pyrrole-2-carboxaldehyde or its sodium salt (**190a**) with isatoic anhydride **193** or the isocyanate **194** failed (Scheme 45).

Scheme 45



192 193 194

Scheme 46



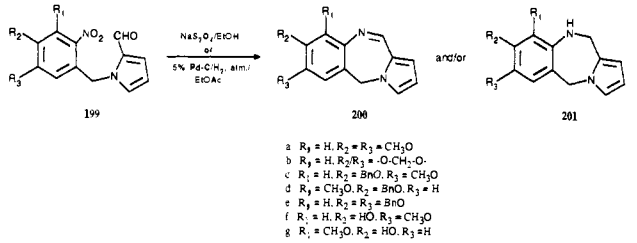
195 196 197 198

Similar methodology was used to convert the anthranilic acid **188c** to sulfinamide anhydride **189c**, which was coupled to the pyrrole aldehyde **190c** (prepared in three steps from the known pyrrole-2-carboxylic ethyl thioester), to afford the dimethylanhydrosibiromycinone (**191c**) in 67% yield. Demethylation with BBr_3/CH_2Cl_2 at 0 °C afforded **191d** (originally thought to be anhydrosibiromycinone) in 65% yield. A similar route has been used[15a] to prepare the 7,8-dimethoxy analog **191e**. Treatment of dimethoxyanthranilic acid **188e** with $SOCl_2$ (toluene/100 °C/2 h) afforded the sulfinamide anhydride (**189e**) which was coupled with **190a** (in anhydrous Et_2O) to afford the imine **191e** in 31% yield.

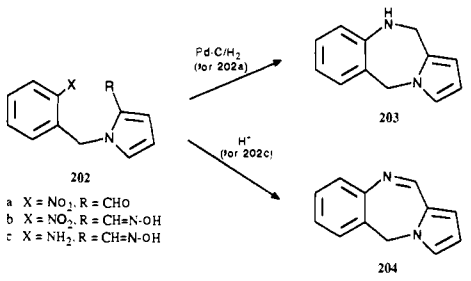
B. Oxidation of PBD N10–C11 Secondary Amines with Manganese Dioxide

In the related 5*H*-imidazo[2,1-*c*][1,4]benzodiazepine ring system (e.g. **196**, Scheme 46), the N10–C11 secondary amine **195** can be oxidized to the imine form (**196**) in 69% yield upon treatment with activated manganese dioxide[77] (dry PhH, stirring, Δ/12 h). On the basis of these results, recent work in this laboratory[15c] has demonstrated that the fully saturated PBD amine **197** can be oxidized to the fully unsaturated derivative (**198**) in 77% yield upon treatment with activated (very active) manganese dioxide (PhH/Δ/72 h). Unfortunately, at the present time, there appears

Scheme 47



Scheme 48



to be no means to control the selectivity to achieve oxidation at only the N10–C11 position. Attempts to oxidize the OBD secondary amine (e.g. **118**, Scheme 32) failed completely.[15c]

C. Reductive Cyclization of Nitro Aldehydes with Sodium Dithionite

Scalzo and co-workers[78a,b] demonstrated that reductive cyclization of the (*o*-nitrobenzyl)pyrrole aldehydes (**199a–e**) with sodium dithionite in alcoholic solution afforded the fully unsaturated 5-deoxy PBD analogs **200a–e** in yields of 47–85% (Scheme 47). However, in the case of nitro aldehydes **199c** and **199d**, catalytic hydrogenation (5% Pd–C/H_2 atm/EtOAc) afforded the secondary amines **201f** and **201g** both in yields of 70%. Treatment of the nitro aldehydes **199c–e** with alcoholic sodium dithionite in the presence of ammonium hydroxide afforded the secondary amines **201c–e** in yields of 75%, 70%, and 75%, respectively.

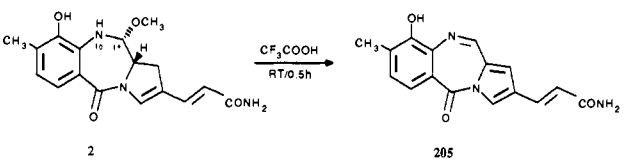
D. Hydrolysis of *N*-(2-Aminobenzyl)pyrrole-2-carbaldoximes

Artico and co-workers[54] noted that catalytic hydrogenation of the nitro aldehyde **202a** (over Pd–C) afforded only the N10–C11 secondary amine **203** (Scheme 48). However, conversion to the oxime (**202b**), followed by reduction (PtO_2/H_2), afforded the amino carbaldoxime (**202c**) which could be hydrolyzed in acid medium to afford the 5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine **204**. The stability of **204** in acid conditions is most likely due to the extended conjugation of the system, and this method is unlikely to be of use for PBDs with saturated C rings.

E. Miscellaneous

Treatment of anthramycin methyl ether **2** with TFA (room temperature/30 min) afforded the fully unsaturated derivative **205** in 50% yield[91] (Scheme 49). A mechanism involving initial elimination of methanol from the N10–C11 position followed by dehydrogenation of the pyrrolo C ring *via* a TFA-catalyzed disproportionation reaction was postulated to explain this result.

Scheme 49



In conclusion, the N10–C11 imine form of PBDs with fully unsaturated C rings is unreactive toward nucleophiles due to the extended conjugation of the system and is, therefore, of little interest from the biological standpoint. However, it can react with hydride ion; for example, treatment of the imines **200a** and **200c** (Scheme 47) with $LiAlH_4$ in THF affords the secondary amines **201a** and **201c** in yields of 83% and 95%, respectively.[78]

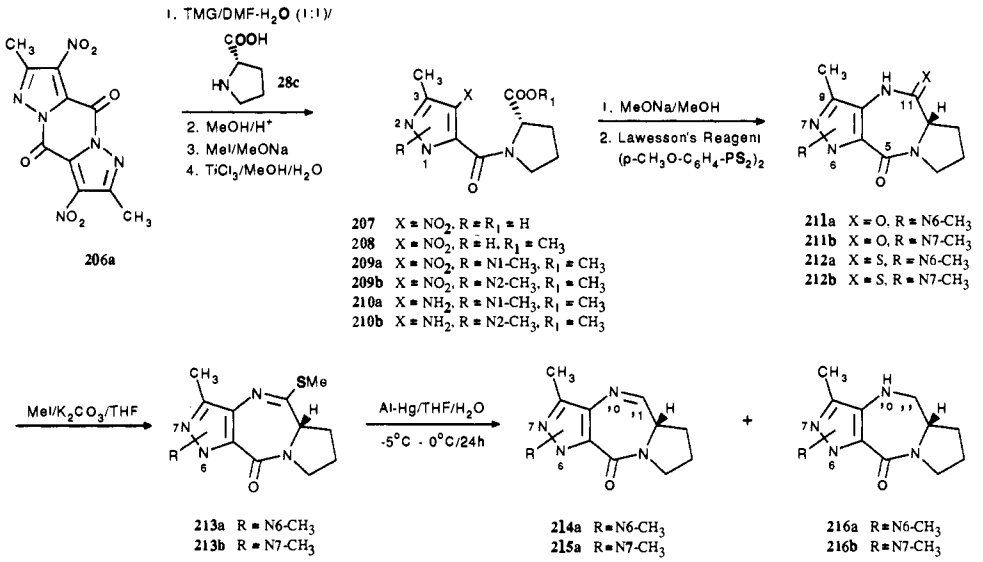
VII. Synthesis of Ring-Modified PBDs

A. A-Ring Modification

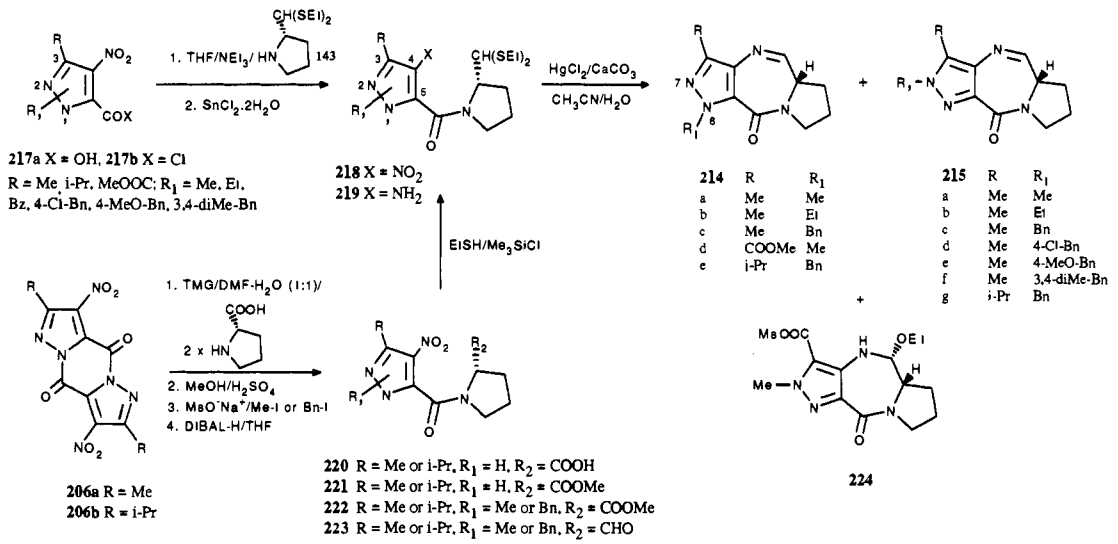
A variety of A-ring heterosubstituted PBD analogs have been reported by Baraldi and co-workers.[92–94] A new class of electrophilic pyrazolo[4,3-*e*]pyrrolo[1,2-*a*][1,4]diazepinones of type **214** and **215** (Scheme 50) with 1,3-dimethyl- and 1,2-dimethyl-substituted pyrazole A rings have been synthesized[92] *via* the imino thioether route of Kaneko and co-workers.[37] The pyrazole A ring was initially coupled to L-proline (**28c**) in good yield *via* a novel acylation reaction using the readily available diketopiperazine (**206a**) in a 1:1 mixture of DMF–H_2O in the presence of 1,1,3,3-tetramethylguanidine (TMG). The resulting nitro acid (**207**) was converted to the ester (**208**) by treatment with MeOH/H^+ and was then methylated with CH_3I/NaOMe/MeOH to afford a 1:1 mixture of the corresponding (N1)-1,3-dimethyl (**209a**) and (N2)-2,3-dimethyl (**209b**) derivatives. After chromatographic separation, **209a** and **209b** were reduced with aqueous $TiCl_3$/MeOH/H_2O to afford the amines **210a** and **210b** which were cyclized *in situ* with NaOMe/MeOH to afford the corresponding dilactams **211a** and **211b**. Treatment with Lawesson's reagent [(*p*-$CH_3OC_6H_4$-$PS_2)_2$] gave the thioamides **212a** and **212b** which were methylated with CH_3I/K_2CO_3/THF to afford the imino thioethers **213a** and **213b** in good yield. Treatment with Al–Hg amalgam in aqueous THF at 0–5 °C for 24 h afforded a mixture of products which, after purification, led to the target imines **214a** (N6-Me) and **215a** (N7-Me) in 35% and 42% yields, respectively, along with the corresponding overreduction products **216a** (15%) and **216b** (12%). Interestingly, **214a** and **215a** were found to exist preferentially in their imine forms based on ^1H- and ^{13}C-NMR studies. Their ability to interact with DNA has not been established; however, they are comparable in cytotoxicity to L-PAM in L1210 cells but between 100- and 1000-fold less cytotoxic than tomaymycin.

A range of N6- and N7-alkyl substituted derivatives (**214a–e** and **215a–g**) have also been prepared by Leoni[93] using the convergent amino thioacetal route of Thurston and co-workers[39a] (Scheme 51). 4-Nitropyrazole-3-carboxylic acids of type **217a** were converted to their corresponding acid chlorides (**217b**) with $(COCl)_2$ and then coupled to (2*S*)-pyrrolidine-2-carboxaldehyde diethyl thioacetal (**143**)[39a] to afford the nitro thioacetals

Scheme 50



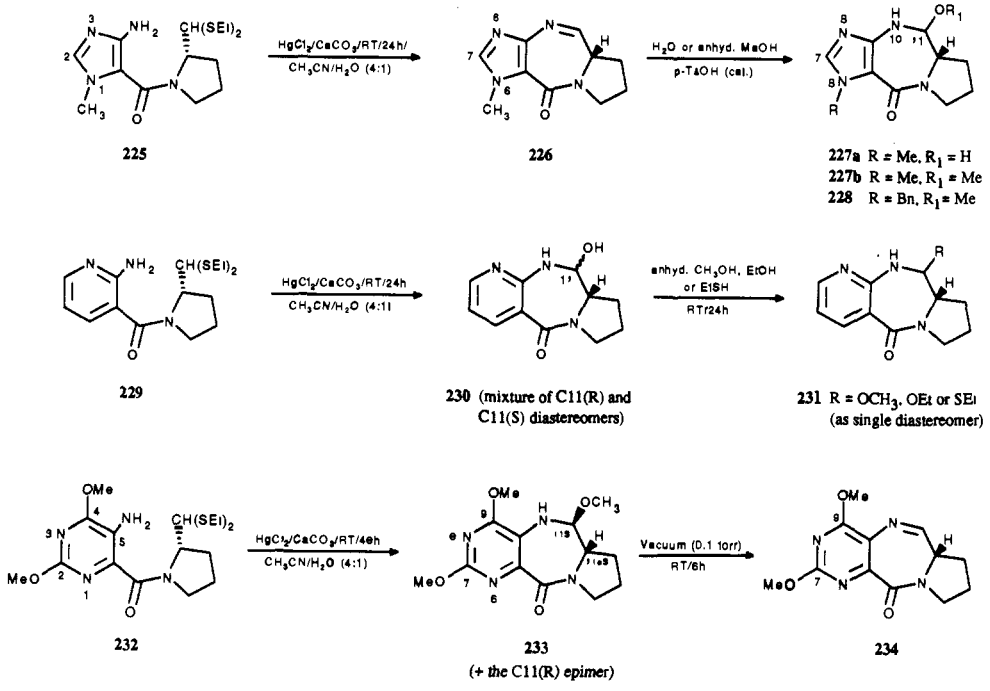
Scheme 51



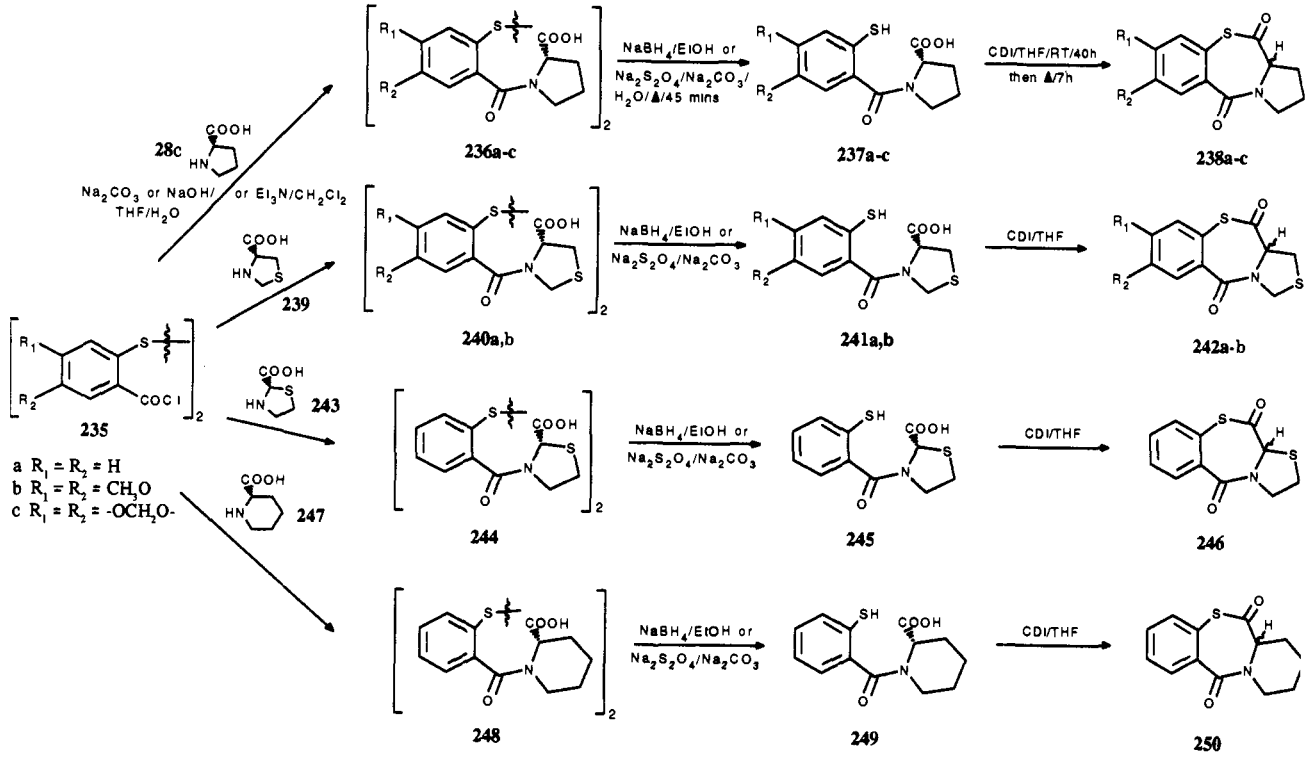
of type **218** which were reduced with $SnCl_2 \cdot 2H_2O$ to give the corresponding amino thioacetals (**219**). Treatment with $HgCl_2/CaCO_3$ in CH_3CN/H_2O afforded the N6- and N7-substituted N10–C11 imine series **214a–e** and **215a–f**, respectively, and **224** which was isolated in a stable form as the C11(*R*)-carbinolamine ethyl ether. A linear version of this route involved reaction of the Me- and i-Pr-substituted diketopiperazines of type **206a** or **b** with 2 mol of L-proline to afford amides of type **220**. These were treated with $MeOH/H_2SO_4$ to effect esterification (**221**), and then alkylated with MeI or $PhCH_2I$ in the presence of MeONa to provide N1- and N2-substituted compounds of type **222**.[93] After reduction to the corresponding aldehydes (**223**) using DIBAL-H/THF, treatment with $EtSH/Me_3SiCl$ afforded the nitro thioacetals of type **218** which were reduced and cyclized as before. Analogs **214a**, **215a**, and **215g** were synthesized by this route. The most cytotoxic compounds were **215d** and **215e** which were up to 24-fold more potent than melphalan but 60–200-fold less cytotoxic than tomaymycin in a L1210 cell line.

Baraldi and co-workers have also reported[94] the related imidazolo[5,4-*e*]pyrrolo[1,2-*a*][1,4]diazepinone ring system of type **226**, prepared by $HgCl_2/CaCO_3$ cyclization of the corresponding amino thioacetal **225** (Scheme 52). Treatment with H_2O or anhydrous MeOH using *p*-toluenesulfonic acid as catalyst gave the corresponding addition products of type **227a** or **227b**. Additional alkyl analogs were described, such as the N6-benzyl C11-*O*-methyl ether **228**, and other compounds alkylated at N8 instead of N6 that also formed addition products. Similarly, cyclization of the pyridine amino thioacetal intermediate **229** afforded the pyrido[2,3-*e*]pyrrolo[1,2-*a*][1,4]diazepinone ring system of type **230**. This compound was isolated in the carbinolamine form as a mixture of C11(*R*)- and C11(*S*)-diastereomers. Treatment of **230** with anhydrous methanol, ethanol, or ethanethiol for 24 h at room temperature gave the corresponding addition products of type **231** as single diastereomeric species. Finally, cyclization of the pyrimidine amino thioether **232** gave a mixture of the two C11-diastereomers of (11a*S*)-7,9,-11-trimethoxy-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo-[2,1-*c*]pyrimido[5,4-*e*][1,4]diazepin-5-one (**233**). The pure C11(*S*)-isomer was isolated by medium-pressure HPLC using C_{18} and $H_2O/MeOH$ (8:2) as mobile phase. Interestingly, this analog preferred to exist as the C11-methyl ether, and the relatively unstable imine form (**234**) could only be prepared after standing in a vacuum

Scheme 52



Scheme 53



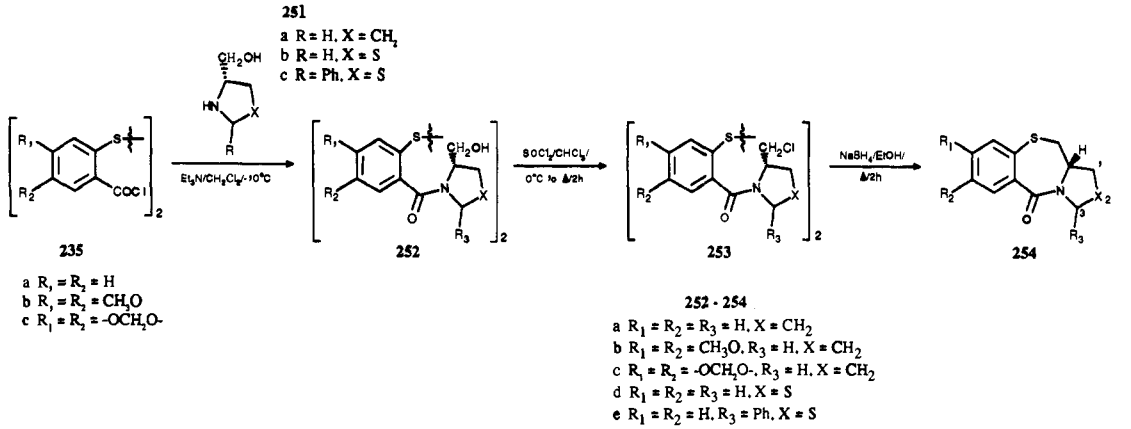
at 0.1 Torr for 6 h at room temperature. Other ring systems with 1,2- and 1,4-diazine and variously coupled thiophene-type A rings were also described.[94]

One conclusion from these studies is that analogs with six-membered heterocyclic A rings prefer to exist in the N10–C11 carbinolamine or carbinolamine methyl ether forms, whereas the five-membered A-ring compounds appear to be stable in their equivalent imine forms.

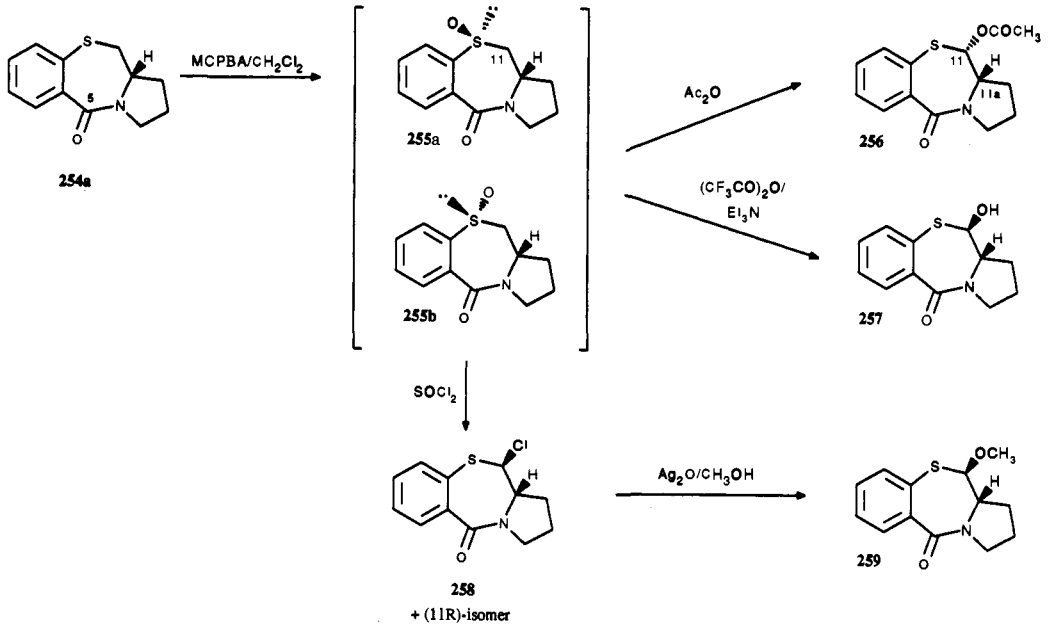
B. B-Ring Modification

Nacci and co-workers have reported[95–100] the synthesis of pyrrolo[2,1-c][1,4]benzothiazepine compounds as sulfur-containing B-ring-modified analogs of the PBDs (Schemes 53–55). For example, coupling of bis(2-(chlorocarbonyl)phenyl) disulfides of type **235** with the cyclic amino acids **28c**, **239**, **243**, and **247** under Schotten–Baumann conditions (Na_2CO_3/THF/H_2O or NaOH/H_2O or Et_3N/CH_2Cl_2) afforded the (*S*)-bis[2-[[2-(hydroxycarbonyl)-1-pyrrolidinyl]carbonyl]phenyl] disulfides of type **236**, **240**, **244**, and **248**, respectively (Schemes 53). These were reduced with $NaBH_4$/EtOH or sodium dithionite/Na_2CO_3/Δ/H_2O to afford the corresponding monomeric thiols of type **237**, **241**, **245**, and **249** in high yields. The final cyclizations were carried out with CDI/THF (room temperature for 40

Scheme 54



Scheme 55



h, then Δ for 7 h), leading to pyrrolo[2,1-*c*][1,4]-benzothiazepine-5,10-diones (**238a–c**), thiazolo[4,3-*c*]-[1,4]benzothiazepines (**242a,b**), the thiazolo[2,3-*c*][1,4]-benzothiazepine (**246**), and the pyrido[2,1-*c*]-[1,4]benzothiazepine (**250**).[95-97] Dilactams of type **238**, **242**, **246**, and **250** were reported to be optically inactive due to racemization at the C11a-position, and the use of other condensing reagents (i.e. DCC/copper(II) chloride or DCC/1-hydroxybenzotriazole) did not prevent racemization. The thiol acid **237a** was also prepared by less preferred routes involving four or five steps from *o*-(methylthio)benzoic acid and L-proline *tert*-butyl ester, or *o*-(methylthio)benzoyl chloride and L-proline (**28c**), respectively.[96]

Similarly, the acid chlorides **235a–c** were reacted (Et_3N/CH_2Cl_2) with amino alcohols **251a–c** to provide the dimeric alcohols **252a–e** which were converted into the dichloro derivatives **253a–e** by treatment with thionyl chloride in $CHCl_3$ (0 °C → Δ/2 h) (Scheme 54). Cyclization to give the 5-oxo-2,3,11,11a-tetrahydro-1*H*,5*H*-pyrrolo[2,1-*c*][1,4]benzothiazepines (**254a–c**) and the thiazolo[4,3-*c*][1,4]benzothiazepines (**254d,e**) was achieved by treatment with $NaBH_4$ in refluxing EtOH. These compounds were produced as single stereoisomers, although configuration at C3 of **254e** was not assigned. Interestingly, reduction at room temperature for 30 min cleaved the disulfide bond instead of effecting cyclization. The disulfides of type **253** were also prepared by alternative routes involving the direct and quantitative condensation of the acid chlorides of type **235** with (*S*)-2-(chloromethyl)pyrrolidine hydrochloride (Et_3N/CH_2Cl_2/−10 °C) or, in the case of **253a**, treatment of the monomeric derivative of **252a** with $SOCl_2$/PhH/Δ/90 min.[96,97] Compound **254a** could also be made by treatment of (*S*)-*N*-(2-mercaptobenzoyl)-2-(chloromethyl)pyrrolidine, the monomeric thiol derivative of **253a**, with sodium metal/EtOH.[96]

5-Oxo-2,3,11,11a-tetrahydro-1*H*,5*H*-pyrrolo[2,1-*c*]-[1,4]benzodiazepine (**254a**) was used to prepare 11-substituted 5-oxo-2,3,11,11a-tetrahydro-1*H*,5*H*-pyrrolo-[2,1-*c*][1,4]benzothiazepines of type **256–259** (Scheme 55). Oxidation of **254a** with *m*-chloroperbenzoic acid gave the sulfoxides **255a/b** (51% and 35%, respectively) in a 1.5:1 ratio, which were separated by flash chromatography on silica gel. Their precise stereochemistry was elucidated by ^{1}H-NMR and molecular modeling studies which suggested that the α-proton at C11 is deshielded in the case of the equatorial sulfoxide, whereas for the axial, both C11 protons are equally affected by the SO group. The less and more polar sulfoxides were thus identified as the (10*S*,11a*S*)-2,3,-11,11a-tetrahydro-1*H*,5*H*-pyrrolo[2,1-*c*][1,4]-

benzothiazepin-5-one 10-oxide (**255a**, equatorial) and the (10*R*,11a*S*)-isomer **255b** (axial). Both sulfoxides (**255a/b**) were subjected to a Pummerer rearrangement in order to functionalize the 11-position. In contrast to a previous literature observation, treatment of either **255a** or **255b** with acetic anhydride afforded (11*S*,11a*S*)-11-acetoxy-2,3,11,11a-tetrahydro-1*H*,5*H*-pyrrolo[2,1-*c*]-[1,4]benzothiazepin-5-one (**256**) as a single isomer. Similarly, reaction with trifluoroacetic anhydride/triethylamine afforded (11*R*,11a*S*)-11-hydroxy-2,3,11,-11a-tetrahydro-1*H*,5*H*-pyrrolo[2,1-*c*][1,4]-benzothiazepin-5-one (**257**) as a single isomer. Conversely, reaction with thionyl chloride afforded a diastereomeric mixture of 11-chloro derivatives, from which only one isomer, the (11*S*,11a*S*)-11-chloro-2,3,-11,11a-tetrahydro-1*H*,5*H*-pyrrolo[2,1-*c*][1,4]benzothiazepine-5-one (**258**), was isolated by column chromatography in 80% yield. Attempts to isolate the other diastereomer by HPLC or fractional crystallization were unsuccessful. Treatment of **258** with silver oxide (Ag_2O/MeOH) led to the methoxy derivative **259** with retention of configuration at C11. The absolute configuration of **258** was unambiguously assigned by X-ray crystallography and, based on this, the configurations of **256**, **257** and **259** could be assigned by ^{1}H-NMR. In general, the coupling constant values for protons at C11 and C11a of compounds of this type are 9.5–10.2 or 1.0–3.0 Hz depending on a relative *trans* or *cis* configuration, respectively. A degree of cytotoxicity was observed for compounds **238a**, **238b**, **242b**, **254a**, **254b**, and **254e** in L1210 cells (20–30% growth inhibition at 100 μg/mL), whereas compounds **242a**, **246**, and **250** were significantly more active (75–94% inhibition). C5-Deoxy derivatives of compounds of type **254** and **255** with fully unsaturated C rings and with or without 11-oxo substituents have also been synthesized.[98-100]

A number of C5-deoxy PBD analogs have also been reported, and some examples are given elsewhere in this review (e.g. see Schemes 23, 46, 47, and 48). Similarly, C5-sulfoxide PBD analogs have been reported[76b] and are described earlier in this review (see Scheme 30).

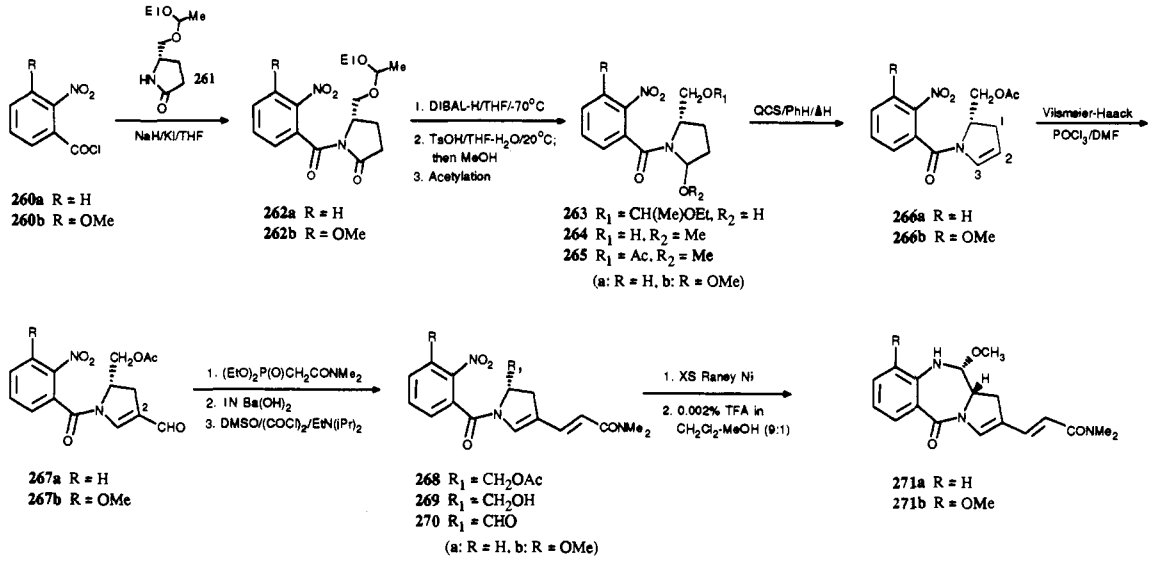
C. C-Ring Modification

A number of general modifications have been made to the C ring of the PBD system, and some examples are included throughout this review. For example, Section VI (Schemes 44, 46, 47, 48, and 49) describes the formation of molecules with C11a–C1 and/or C2–C3 unsaturation (see also Schemes 16, 23, and 58). Synthesis of a C1–C2 unsaturated PBD (**34**) has also been described (Scheme 9). Analogs with nitrogen (Schemes 23 and 46), oxygen (Schemes 32 and 34), and sulfur (Schemes 26, 53 and 54) in the C ring (with or without unsaturation) have also been described. In addition, compounds with pyridino (e.g. Schemes 8, 26, and 53) or indolo (e.g. Scheme 8) C rings have been described, and benzodiazepine analogs have been prepared devoid of the C ring (e.g. Scheme 28).

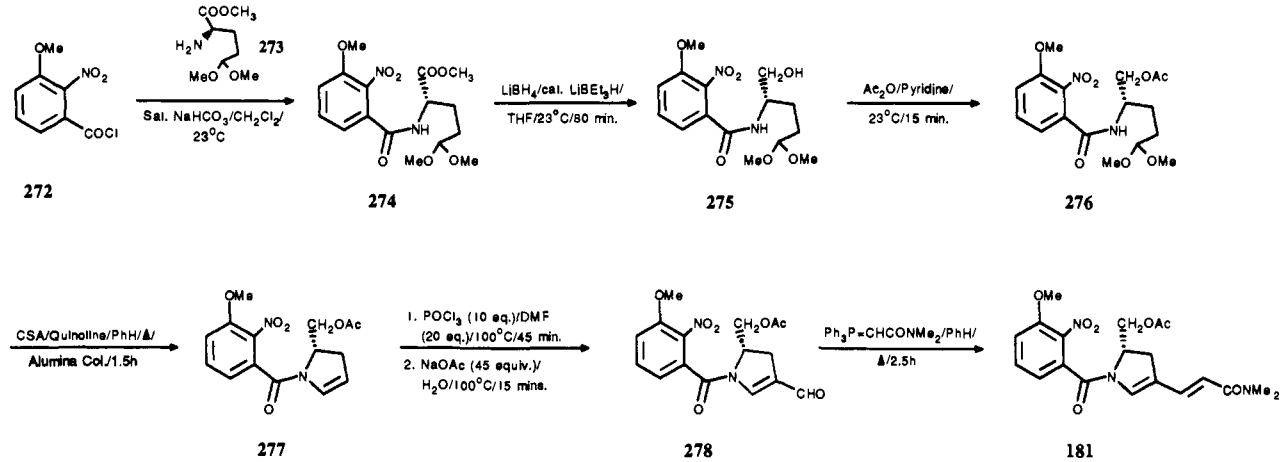
A number of attempts have also been made to synthesize analogs with substituents at the C2-position. This is important, as the cytotoxicity and DNA-binding ability of the PBDs appears to be greatly enhanced[16a] if there is either exocyclic unsaturation at C2 (e.g. tomaymycin, Scheme 2) or an unsaturated side chain in conjugation with a C2–C3 double bond (e.g. anthramycin, mazethramycin, porothramycin, or sibiromycin; Scheme 2). In addition to the difficulty of forming the N10–C11 imine or carbinolamine moiety in the PBD B ring, the incorporation of unsaturated C2 substituents into the C ring such as those found in anthramycin and sibiromycin is challenging. Two novel approaches to this problem have been developed by Pena and Stille[58f] and, independently, by Langlois and co-workers[101,102] and Fukuyama and co-workers.[42b] Stille and co-workers have demonstrated[51,58f] that enol triflate ethers of type **58** (Scheme 19), prepared from the corresponding ketones (**57**) by treatment with triflic anhydride in pyridine, may be coupled to either olefins or vinylstannanes *via* a palladium-catalyzed Heck-type coupling reaction to provide products of type **59**. This method was used to prepare **59b** for a total synthesis of anthramycin, where the C2-acrylamide side chain was attached in three steps[51] compared to six steps in the original Leimgruber synthesis.[31] The triflate **58b** was converted to **59b** in 50% yield by treatment with acrylamide and bis(acetonitrile)palladium(II) chloride [$(CH_3CN)_2PdCl_2$] using either 1,4-diazabicyclo[2.2.2]-octane (DABCO) or TEA as base in either methanol or DMSO, respectively, at 50–75 °C. The same reaction was accomplished in lower yield (22%) using the vinylstannane $Bu_3SnCH{=}CHCONH_2$ and tetrakis-(triphenylphosphine)palladium(0) [$Pd(PPh_3)_4$] as catalyst with LiCl (THF/50–75 °C). The use of reagents such as acrylamide, ethyl acrylate, or $Bu_3SnCH{=}CH_2$ offers many possibilities to synthesize anthramycin-type analogs with C2 substituents of varying structure.

Similarly, Langlois and co-workers[101] first reported that a C2–C3 didehydropyrrolidine (enamide) derivative of type **266a** (Scheme 56), prepared in high yield (90%) by elimination of a C5-alkoxy group from **265a** (using quinolinium camphosulfonate, 15 mol %/$PhCH_3$/90 °C/0.5 h) can provide an almost quantitative yield of the α,β-unsaturated aldehyde **267a** upon treatment with DMF–$POCl_3$ for 2.5 h. This was the first example of a Vilsmeier–Haack reaction of an intermediate of type **266** and demonstrated the potential to transform aldehydes of type **267** into a variety of PBD analogs with interesting C2 side chains *via* Wittig-type reactions. Based on this methodology, Langlois and co-workers later reported the total syntheses of 9-desmethoxyporothramycin B (**271a**) and porothramycin B (**271b**) (Scheme 56).[102] The imides of type **262** were prepared in high yield (e.g. 95% for **262b**) from treatment of the anion derived from (5*S*)-5-((1-ethoxyethoxy)methyl)-2-pyrrolidone (**261**) (NaH/THF/KI) with the 2-nitrobenzoyl chlorides (**260a** or **260b**). Partially regioselective reduction was achieved by addition of a hexane solution of DIBAL-H to a THF solution of the imides to afford carbinolamides of type **263** in good yield (e.g. 68% for **263b**). These were converted to the nitro alcohols of type **264** in high yield by treatment with TsOH/THF–H_2O/20 °C followed by MeOH. Quantitative acetylation then gave the acetates of type **265**. Elimination to form the enamides (**266**) was achieved in >85% yield by heating a toluene solution of **265a/b** in the presence of quinolinium camphorsulfonate (15 mol % QCS/90 °C/0.5 h for **265a**). The formyl group was introduced at C2 through a Vilsmeier–Haack reaction (e.g. $POCl_3$/DMF/2.5 h for **266a**), affording the key enamido aldehyde intermediates of type **267** in >85% yield. The *N*,*N*-dimethylacrylamide

Scheme 56



Scheme 57



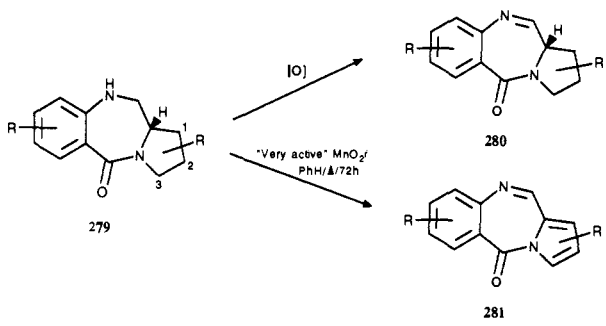
C2 side chain was added through a Wittig–Horner reaction with the suitably functionalized diethyl phosphonate, $(EtO)_2P(O)CH_2CONMe_2$. The products (**268**) were directly saponified with 1 N $Ba(OH)_2$ to provide the alcohols **269a** (97% from **267a**) and **269b** (78% from **267b**; non-optimized yield). Swern oxidation of these primary alcohols required the use of diisopropylethylamine as base to avoid partial racemization of the aldehyde products (**270a**, 96% and **270b**, 93%). Cyclization was achieved with an excess of Raney nickel at room temperature followed by treatment with a very dilute (0.002%) solution of trifluoroacetic acid in CH_2Cl_2–MeOH (9:1) to provide 9-desmethoxyporothramycin B (**271a**) and porothramycin B (**271b**) in 65% and 45% (non-optimized) yields, respectively.

Fukuyama and co-workers[42b] have also taken advantage of this approach to synthesize (+)-porothramycin B. The enamide **277** (Scheme 57), a suitable substrate for the Vilsmeier–Haack reaction, was obtained by a novel route involving cyclization of the C ring after coupling to the A-ring fragment. The route started from amino ester **273**, which was synthesized in seven steps from L-glutamic acid. It was first acylated with 3-methoxy-2-nitrobenzoyl chloride (**272**) in a two-phase reaction (saturated $NaHCO_3/CH_2Cl_2$/23 °C) to afford the amide **274** in 95% yield. Selective reduction of the ester functionality with lithium borohydride in the presence of a catalytic quantity of $LiBEt_3H$ (THF/23 °C/80 min) gave the primary alcohol **275**, which was isolated as the corresponding acetate **276** after treatment with acetic anhydride in pyridine (23 °C, 15 min). Using conditions similar to those reported by Langlois and co-workers,[101,102] **276** was subjected to a facile cyclization–elimination reaction by treatment with quinolinium camphorsulfonate (QCS) formed *in situ* from camphorsulfonic acid (CSA) and quinoline (PhH/23 °C/reflux through an alumina column/1.5 h) to afford the electron-rich enamide **277** in 79% yield (from **274**). Conversion of **277** to the aldehyde **278** was effected by the conventional Vilsmeier–Haack reaction conditions ($POCl_3$ [10 equiv]/DMF [20 equiv]/100 °C/45 min), followed by treatment with NaOAc ([45 equiv]/H_2O/100 °C/15 min). After acylation of a minor quantity of partially deacetylated alcohol produced during the reaction, the aldehyde **278** was converted to the conjugated amide **181** in 74% yield (from **277**) by treatment with the stabilized ylide, $Ph_3P{=}CHCONMe_2$ (PhH/Δ/2.5 h). This was used in the total synthesis of (+)-porothramycins A and B (**186** and **187**) as described in Section V.F (Scheme 43).

VIII. Approaches with Potential Application

This section describes methods that have been used to generate imines or carbinolamines in other types of heterocyclic ring systems and that might be applicable

Scheme 58



to the PBDs. Both synthetic and enzymic approaches are considered.

A. Synthetic Approaches

1. Oxidation of Secondary Amines

One approach which has not been extensively investigated is the oxidation of secondary amines of type **279** (Scheme 58) to N10–C11 imines or carbinolamines (e.g. **280**). This approach is attractive, as PBD secondary amines are readily prepared in high yield by a number of different methods including the reductive cyclization of *N*-(2-nitrobenzoyl)pyrrolidine-2-carboxaldehydes (see Section V.A) and the more recently developed palladium-catalyzed carbonylation reaction of Mori and co-workers (see Section III A.3). Recent investigations in this laboratory have demonstrated[15c] that "very active" MnO_2 (PhH/Δ/72 h) is capable of oxidizing the unsubstituted amine **279** (R = H) to the fully unsaturated PBD **281**. Studies are currently underway with ring systems substituted at the C1- or C2-position (e.g. the OBD **118**, Scheme 32), to explore whether this approach is capable of producing unsaturation selectively at N10–C11.

Other oxidizing agents may also prove useful. For example, Murahashi and co-workers[103] have reported the ruthenium-catalyzed [$RuCl_2(PPh_3)_3$] oxidation of various tetrahydroisoquinolines (e.g. **282a**) with *tert*-butyl hydroperoxide in benzene at room temperature (2 h) to the corresponding imines (e.g. **283a**) in good yield (typically >65%) (Scheme 59). It is possible that similar reagents may be capable of oxidizing PBD secondary amines of type **279** to imines. Ruthenium tetroxide (RuO_4) has also been used to oxidize the α-carbon in a number of heterocyclic systems containing secondary or tertiary amines and may be of use, particularly under controlled conditions, so that overoxidation to the corresponding dilactam does not occur.[104] The di-*tert*-butyliminoxyl radical, t-$Bu_2C{=}NO^{\bullet}$, is also a potentially useful reagent. It has been used by Cornejo and co-workers[105] to oxidize a series of primary and secondary amines in pentane or hexane under very mild conditions (room temperature/4 h) and in good yield (>68%).

2. Dehydration of PBD N10-Hydroxylamines

Murahashi and co-workers[106] have shown that hydroxylamines such as the tetrahydroisoquinoline hydroxylamines **282b** or **282c**, prepared by treatment of the appropriate amines with H_2O_2, may be converted into the corresponding imines (**283b** or **283c**) in high yield (>89%) *via* a dehydration reaction involving anhydrous $TiCl_3$ in dry THF at room temperature (Scheme 59). The application of this method to the formation of imine or carbinolamine-containing PBDs may be feasible.

3. Elimination of N10-Bromo Derivatives

Corey and co-workers[107] have shown that 2-spiro-substituted piperidine derivatives can be N-brominated with *N*-bromosuccinimide in THF at low temperature and then converted to the corresponding imines by elimination of HBr using t-AmO$^-$K$^+$/THF/−40 °C. It is possible that an N10-brominated PBD of type **284** could be prepared from **279** and made to eliminate HBr (**284** → **280**) under similar conditions (Scheme 59).

B. Enzymic Approaches

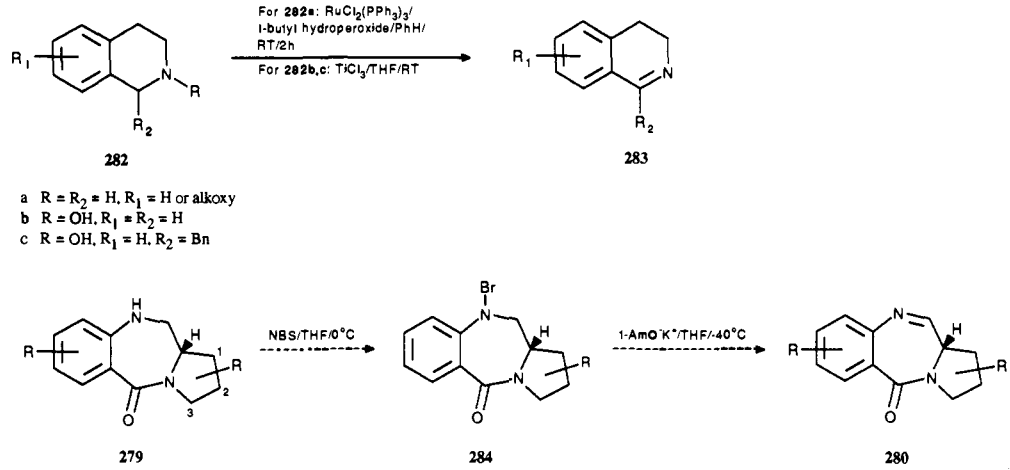
A number of enzymic systems are now known that are capable of oxidizing substituted amines. This type of biooxidation is related to the well-known metabolic process of N-demethylation. It is feasible that secondary amines of type **279** (Scheme 59) could be converted to imines or carbinolamines *via* this process. However, the C3-position may also be vulnerable to hydroxylation.

A second possibility is bioreduction of a dilactam of type **285** to a carbinolamine (**286**) or the imine equivalent (Scheme 60). There is a precedent for this type of conversion, as oxotomaymycin is found in the culture broth of *Streptomyces achromogenes* along with tomaymycin. Although it has not been proved experimentally, it is possible that the microorganism is capable of reducing oxotomaymycin to tomaymycin.[14b] A detailed study of this may reveal an enzyme (or enzymes) capable of the general reduction of PBD dilactams. It is also feasible that the oxidation and reduction reactions described above could be carried out electrochemically.

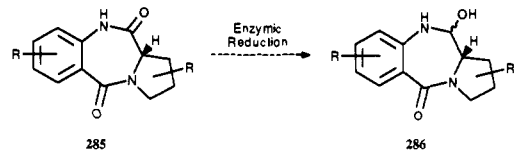
IX. Conclusions

The most serious problem encountered with the synthesis of carbinolamine-containing PBDs is the lability of the N10–C11 carbinolamine moiety (or its imine equivalent) which, with few exceptions, is incorporated at the last step of the synthesis. This strategy is adopted in most of the approaches reviewed above. Other problems include the potential loss of stereochemistry at C11a due to racemization, the loss of double bonds in either the pyrrole C ring or a C2 side chain under reductive conditions, and a dependency on a particular type and pattern of A-ring substituents. Two of the synthetic routes reviewed above have overcome these problems to varying degrees and have been the most widely applied by a number of independent laboratories. The synthesis developed by Kaneko and co-workers (see Section IV) involving the reduction of PBD N10–C11 imino thioethers starts from dilactams with fixed C11a(*S*) stereochemistry. Furthermore, only one step employs reducing conditions (Al–Hg amalgam/MeOH) that are normally too mild to cause reduction of isolated C=C bonds. This route has proven useful for a number of different types and patterns of A-ring substituents and can afford reasonable yields of products over three steps from the corresponding dilactams. It has been used by different groups to synthesize both PBD natural products and a number of related analogs. However, there is a

Scheme 59



Scheme 60



problem with C5/C10 selectivity in the initial thiolation step, and the final product is nearly always contaminated with the N10–C11 secondary amine resulting from overreduction of the imine by excess Al–Hg amalgam.

The approach developed by Langley and Thurston (see Section V.C) involving the cyclization of *N*-(2-aminobenzoyl)pyrrolidine-2-carboxaldehyde diethyl thioacetals is also versatile and efficient and provides reasonable yields of products. The aldehyde is protected as the diethyl thioacetal and then deprotected ($HgCl_2/CaCO_3$) under mild and nonracemizing conditions. Reduction of the *N*-(2-nitrobenzoyl) precursor is accomplished with stannous chloride, thus sparing double bonds in the molecule. Although the final PBDs are free of contamination with N10–C11 secondary amine overreduction products, excess mercuric salts have to be removed by chromatography. This approach has been used successfully by a number of independent laboratories for the synthesis of naturally occurring PBDs, numerous related analogs including those with heterocyclic A rings, and both C7- and C8-linked PBD dimers.

Many of the innovative, more recently introduced, approaches for cyclization of the PBD B ring appear to be promising and deserve further study to establish their scope and general applicability. In particular, the use of Raney nickel by Langlois and co-workers (see Section V.A) to cyclize *N*-(2-nitrobenzoyl)pyrrolidine-2-carboxaldehydes is of interest as it provides significant improvements over other methods of reductive cyclization.

It should also be remembered that the overall stability of the N10–C11 carbinolamine moiety (or imine) of a specific compound prepared by any of the methods described above is dependant upon the type and pattern of A- and C-ring substituents, and the degree of unsaturation in the C ring. The precise form of a final product (e.g. N10–C11 imine, carbinolamine, or carbinolamine alkyl ether) can also be influenced by the final workup conditions, as the three forms are interconvertible. However, chromatographic purification of final products is normally required whichever synthetic method is adopted.

Finally, based on current knowledge of the SAR of PBDs, the recent advances made by Pena and Stille, Langlois and co-workers and Fukuyama and co-workers in attaching anthramycin-type C2 side chains to the C-ring (see Section VII.C) are likely to play a significant future role in producing new types of PBD analogs with enhanced DNA-binding affinity, sequence-selectivity, and biological activity.

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Abbreviations Used: Δ, heating to reflux (unless other temperature given); Bn, benzyl ($PhCH_2$); Bz, benzoyl (PhCO); CBZ, carboxybenzyl; CDI, *N*,*N*′-carbonyldiimidazole; DABCO, diazabicyclo[2.2.2]octane; DCC, dicyclohexylcarbodiimide; DEPC, diethyl phosphorocyanidate; DIBAL-H, diisobutylaluminum hydride; DMTS, dimethylthexylsilyl; L-PAM, phenylalanine mustard; MCPBA, *m*-chloroperbenzoic acid; MOM, methoxymethyl; NBS, *N*-bromosuccinimide; PBD, pyrrolo[2,1-*c*][1,4]benzodiazepine; PCC, pyridinium chlorochromate; PTSA or *p*-TsOH, *p*-toluenesulfonic acid; Pu, purine; Py, pyrimidine; QCS, quinolinium camphosulfonate; TFA, trifluoroacetic acid; TMG, 1,1,3,3-tetramethylguanidine; TEA, triethylamine; TsCl, *p*-toluenesulfonyl chloride.

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