CHEMICAL REVIEWS

REVIEW

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

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1. INTRODUCTION

1.1. General Background

Discovered in the 1960s,[1] the pyrrolobenzodiazepines (PBDs) are an important class of sequence-selective DNA-interactive agents that bind covalently to guanine bases within the minor groove of DNA.[2] There are now two recognized subfamilies of pyrrolobenzodiazepines (Figure 1). The first is the PBD monomer subfamily that represents the agents originally discovered in cultures of *Streptomyces* species (e.g., anthramycin and tomaymycin) along with a wealth of more-recent synthetic analogues. A total of 13 distinct PBD monomer types based on their A- and C-ring substitution patterns have been reported since the original discovery of anthramycin, including the recently discovered Limazepines A−F from a *Micrococcus* species.[3] The PBD monomers are remarkable in possessing a 3-dimensional shape that allows them to fit perfectly within the minor groove of DNA, partly due to the longitudinal twist created by the chiral center at their C11a-position. Once located in a position of low energy in the groove (i.e., a preferred DNA sequence), largely dictated by substituents in the A- and C-rings, the electrophilic C11-position then alkylates the C2−NH_2 group of an adjacent guanine base, thus producing a robust covalent adduct capable of blocking biological processes such as transcription factor binding and RNA polymerase progression. The PBD monomers have both antibacterial properties and selective cytotoxicity toward tumor cells, and their production by

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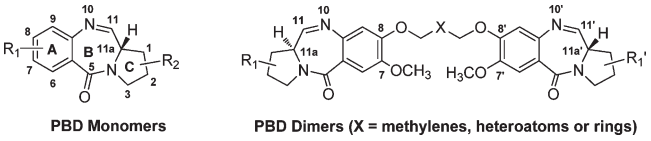
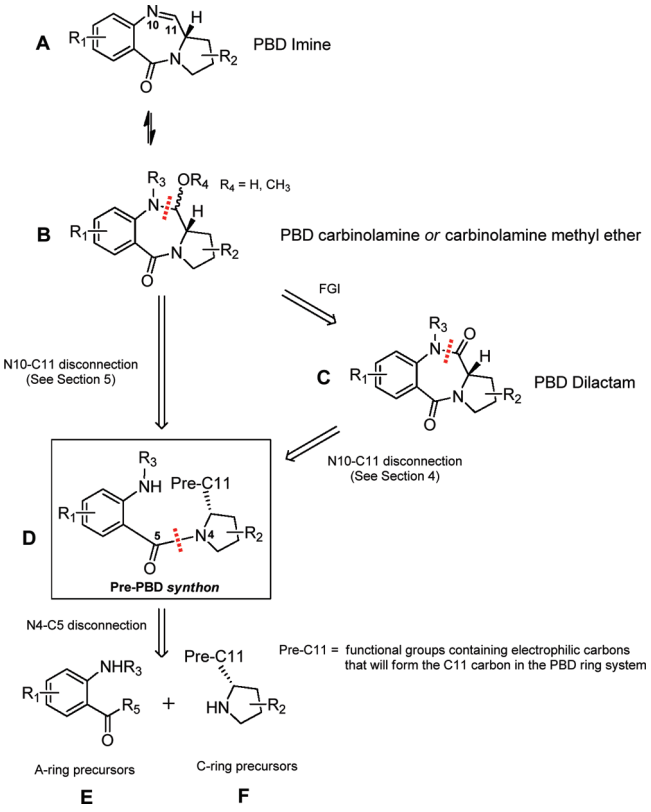


Figure 1. Structures of the PBD monomer and dimer subfamilies.

Scheme 1. Retrosynthetic Analysis of the Pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) Ring System[32]



Streptomyces and *Micrococcus* species has presumably evolved as a means of chemical attack or defense. In addition to these naturally occurring PBD monomers, a wide range of analogues have been produced synthetically over the last 50 years.

The second subfamily, the PBD dimers, is not naturally occurring, and the first C7-[4] and C8-linked[5] examples were designed to span greater lengths of DNA than the PBD monomers, to have enhanced sequence-selectivity, and to form DNA cross-links that might be more difficult for tumor cells to repair. It is now known that PBD dimers can form both interstrand and intrastrand cross-links, as well as monoadducts under certain conditions,[6] although the interstrand cross-linked adduct is still thought to be the most toxic in cells. One example of a C8/C8′-linked PBD dimer, SJG-136, has just completed Phase I clinical trials in the oncology area, where it has demonstrated sufficient therapeutic benefit to warrant progression to Phase II studies.[7,8] In addition, some PBD dimers have shown significant activity against MRSA strains of bacteria which has generated interest in the anti-infectives area.[9] Finally, both PBD monomers and dimers continue to be of interest as chemical probes to study DNA structure and function.[6,10]

Synthetic methodologies relating to the pyrrolobenzodiazepine (PBD) family were reviewed in this journal in 1994.[11] Since that time, a significant volume of new information has been published (i.e., >170 publications in the primary chemical literature along with >20 patents) including novel synthetic methodologies, improvements to previously reported routes, and reports of synthetic pathways to novel PBD structures. The intention of this review is to provide a comprehensive update of the synthetic chemistry literature relating to PBDs since the previous review.[11] Although a number of biological reviews have been published since 1994,[12,13] they have not focused on the synthetic chemistry aspects of PBDs.

As in the 1994 publication, this new review is organized around the crucial "B-ring cyclization" reaction that allows formation of the PBD skeleton (see Scheme 1). Thus, some of the newly reviewed work fits into similar sections to those used previously. However, some entirely novel chemical strategies reported in the intervening years have necessitated the addition of new sections. These generally involve the application of modern synthetic methodologies to PBD synthesis and include ring-closing metathesis (section 4.1.1), the reduction of azides to amines for the cyclization of methyl *N*-(2-azidobenzoyl) pyrrolidine-2-carboxylates (section 4.1.2), the bis(trifluoroacetoxy)iodobenzene (PIFA)-mediated cyclization of 1-benzoyl-*N*-methoxypyrrolidine-2-carboxamides (section 4.1.4), the intramolecular cyclization of aryl triflates (section 4.1.5), the synthesis of more-complex molecular structures that contain the PBD ring system (section 5.2), the use of the aza-Wittig reaction on *N*-(2-azidobenzoyl)pyrrolidine-2-carboxaldehydes (section 5.4), the oxidation of cyclic secondary amines (section 6), the use of solid-phase and parallel synthetic methodologies (section 7), the use of self-immolative protecting groups important for adapting PBDs as "warheads" for use in therapeutic targeting strategies (section 8.1), and, finally, chemical transformations relevant to improving the biological properties of PBDs (i.e., C11-modifications, section 8.2).

Two particular aspects of PBD synthesis have emerged since the 1994 review that are worth highlighting here. First, the robustness of the N10−C11 imine moiety to a variety of synthetic steps has become more apparent. Previously, the N10−C11 imine moiety was thought to be a highly chemically unstable functionality best introduced at the last step of a lengthy PBD synthesis. However, a number of recent studies have demonstrated that imine-containing PBDs are sufficiently robust to undergo a number of synthetic steps[14−16] including coupling to an antibody.[17] Second, PBD dilactams containing the oxidized N10−C11 amidic moiety have become more popular as robust chemical scaffolds for lengthy syntheses because they require less use of protecting groups, and there are now efficient methods for converting the N10−C11 amide functionality to the DNA-alkylating imine at the last step. Also, there is growing interest in the PBD dilactams themselves due to their ability to interact with DNA in a noncovalent manner.

1.2. Interconvertible N10−C11 Position of PBDs

When studying or planning a synthetic route to PBD structures, it is important to note that the molecules can exist in three interconvertible forms at their N10−C11 position: imine, carbinolamine, and carbinolamine methyl ether species (Figure 2).

A PBD produced synthetically, semisynthetically, or isolated from natural sources can exist as a mixture of two or even three of the above forms or can exist predominantly as just one.[2] Leimgruber and co-workers were the first to demonstrate this phenomenon through their work on anthramycin.[1] The

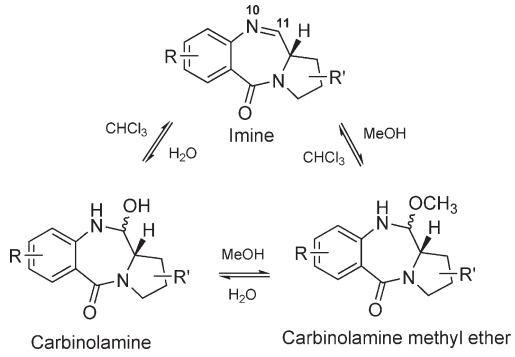


Figure 2. Three interconvertible forms of PBDs (imine, carbinolamine, and carbinolamine methyl ether) considered to be biologically equivalent.

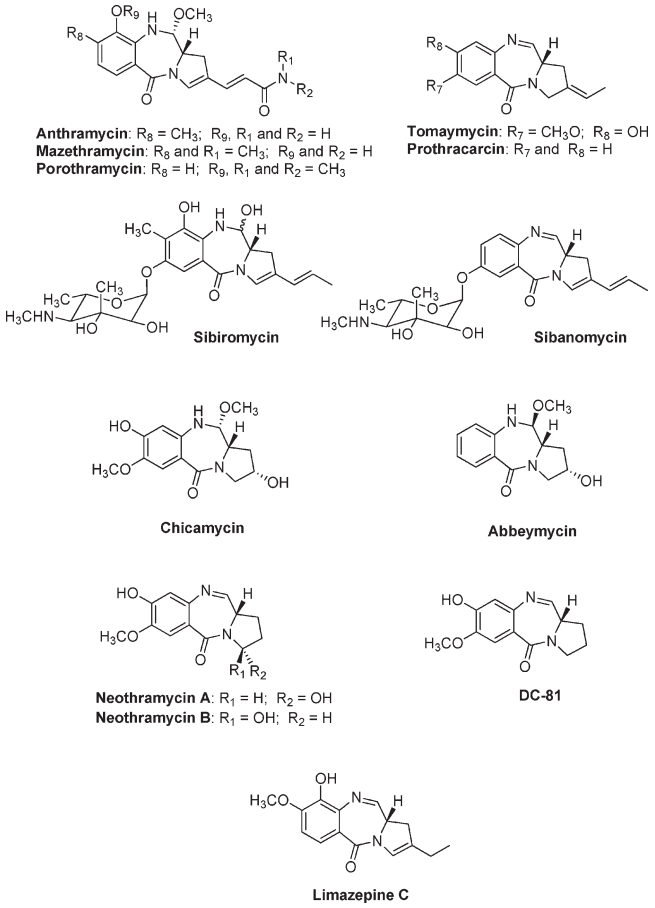


Figure 3. Structures of the naturally occurring PBDs. These are all produced by *Streptomyces* species with the exception of Limazepine C, which is produced by a *Micrococcus*.

preference for a particular N10−C11 form for an individual PBD molecule is subtle and appears to be influenced by a number of factors including the precise structure of the PBD (e.g., type of A- and C-ring substituents, degree of C-ring unsaturation and its conjugation to a C2-substituent), the method of synthesis and workup (or method of isolation if a natural product), and the solvents and conditions used for final purification steps. For example, anthramycin itself has been isolated from a fermentation broth in the carbinolamine form, crystallized from methanol as the C11−carbinolamine methyl ether, and converted to the imine form by gentle heating with acetonitrile. Therefore, although all three forms are chemically distinct and can be individually characterized by analytical techniques including HPLC, NMR and MS, they are generally considered to be completely equivalent and, together, to represent the parent PBD structure. It is also important to note that, for biophysical, biochemical, or biological experiments, PBDs are always dissolved in aqueous solutions, sometimes containing small amounts of water-miscible organic solvents such as methanol, ethanol, dimethylsulfoxide (DMSO), or dimethylformamide (DMF), depending on the solubility characteristics of the PBD being studied. In this aqueous environment, imine and carbinolamine methyl ether forms are usually converted to the carbinolamine form. Hence, such studies are generally considered to have been carried out with the single carbinolamine species irrespective of the structure of the PBD before dissolution. It follows that it is most likely the carbinolamine species that interacts with duplex DNA prior to the alkylation event, although dehydration to the imine form in the environment of the minor groove prior to nucleophilic attack of a guanine C2−NH_2 group cannot be ruled out. So far, no chemical or physical analytical techniques have been capable of elucidating the final steps of the alkylation event in detail.

1.3. Naturally Occurring PBDs

During the last 50 years, PBDs have been isolated from various species of *Streptomyces* and, most recently, from a *Micrococcus* (i.e., the Limazepines).[3] They span a range of structures distinguished from anthramycin principally by the position and type of substituents in the A- and C-rings, and the degree and position of points of unsaturation in the C-ring. To date, 13 naturally occurring PBDs have been reported with different A-ring substitution patterns and with the C-ring either fully saturated (e.g., neothramycin A and B), C2/C3-*endo*-unsaturated (e.g., anthramycin), or C2-*exo*-unsaturated (e.g., tomaymycin) (Figure 3). PBDs with either C2-*exo*- or C2-*endo*-unsaturation are, on average, more biologically potent than compounds with saturated C-rings. The recent isolation of the limazepines from a *Micrococcus* species[3] over 40 years since the discovery of anthramycin suggests that the PBD structure may be more ubiquitous and widely distributed than previously thought.

1.4. Mechanism of Action of PBDs

Many studies since the discovery of anthramycin have proved that the biological activity of PBDs is due to their ability to bind covalently through their N10−C11 functionality to a C2−NH_2 group of guanine within the minor groove of DNA (Figure 4A). The molecules have a longitudinal right-handed twist due to their chiral C11a position, which allows them to fit perfectly within the minor groove. Various biochemical and structural studies on PBD−DNA adducts have suggested that the molecules locate themselves with their N10-position pointing toward the floor of the minor groove (a prerequisite for covalent bond formation between the C11-position and the C2−NH_2 group of guanine) and with their C2- and C8-substituents following the floor and walls of the minor groove, thus enhancing DNA-binding affinity through hydrogen bond formation and other contacts such as electrostatic and van der Waals interactions. For example, anthramycin can form hydrogen bonds to DNA bases from its 9-OH A-ring substituent and the −NH_2 group at the end of its C-ring C2−acrylamide tail. Thus, anthramycin can stabilize duplex DNA to a greater extent than tomaymycin in which the A-ring −OH group is transposed to the C8-position, and the hydrogen-bonding ability of the C2-tail is eliminated (e.g., ΔT_m = 13.0 vs 2.6 °C for anthramycin vs tomaymycin,

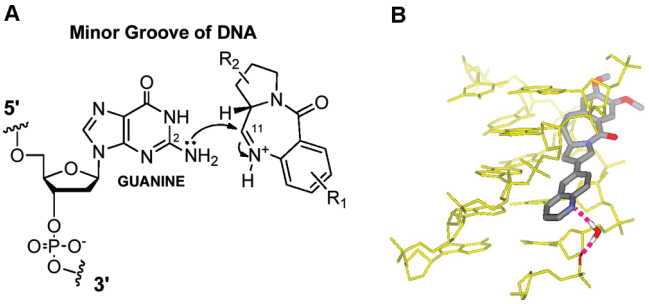


Figure 4. (A) Mechanism of the covalent binding of a PBD to the C2−NH2 group of a guanine within the DNA minor groove. (B) A molecular model[22] of a C2−quinolinyl PBD covalently bound to a guanine within the minor groove of DNA showing a H_2O-bridged H-bonding interaction between the nitrogen atom in the C2-quinolinyl substituent and backbone atoms of the opposite noncovalently modified DNA strand (see also Figs. 6 and 10 in ref 18 for a C2−naphthyl-substituted PBD).

respectively, in thermal denaturation experiments with calf thymus DNA).[18]

Furthermore, PBDs have a preference to position themselves within the minor groove with their A-ring orientated toward the 3′-end of the covalently modified strand with the C6-position of the A-ring pointing out of the groove (Figure 4B). Despite a lack of direct evidence for the precise mechanism of alkylation, it is speculated that the N10−C11 iminium species may form within the relatively hydrophobic environment of the minor groove through protonation of N10 prior to nucleophilic attack by the guanine C2−NH_2.

A further important feature of the PBDs is that they interact selectively with specific DNA sequences.[19,20] The results of numerous DNA-footprinting, X-ray crystallography, and high-field NMR experiments, associated with molecular modeling studies, suggest that the naturally occurring PBDs anthramycin and tomaymycin have a rank order of preference for 5′-Pu-G-Pu > Pu-G-Py > Py-G-Pu > Py-G-Py-3′ sequences. This preference has been suggested to arise from base-stacking characteristics and the inherently small helix twist at purine−purine sequences.[21] Also, noncovalent interactions unique to individual PBD structures and DNA sequences are assumed to further stabilize PBD−DNA adducts to different degrees.

The link between PBD−DNA adduct formation and selective antitumor activity for both PBD monomers[22,23] and dimers[24,25] is not entirely clear at present. Biochemical experiments such as "in vitro transcription stop" assays have demonstrated that PBDs can block transcription in a sequence-selective manner in the coding region of genes,[20,26] and other experiments (e.g., EMSA and ChIP assays) have shown that they can inhibit transcription factor binding.[27] Both of these properties could exert a selective effect in tumor cells where particular genes are being over-expressed compared to healthy cells. An alternative explanation is that tumor cells are often deficient in various DNA repair pathways, and so PBD−DNA adducts may be challenging for cancer cells to repair compared to healthy cells.[28,29] This scenario may be particularly relevant in the case of PBD dimers which can form DNA interstrand and intrastrand cross-links that might be more difficult to repair.

Finally, there is growing evidence that PBD dilactams, which contain a nonelectrophilic amidic functionality at the N10−C11 position (Figure 5), can also interact with DNA, albeit noncovalently. A study by Jones and co-workers[30] in 1990 originally

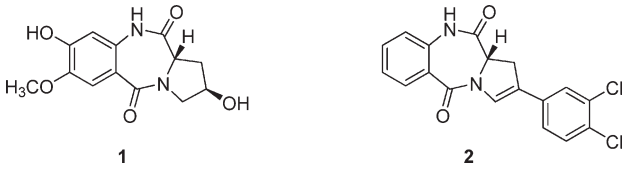


Figure 5. Structures of some PBD dilactams with amide functionalities at the N10−C11 position. Although nonelectrophilic at the C11-position, these molecules are still isohelical with the DNA minor groove and can interact noncovalently.[30,31]

demonstrated that the dilactam **1** can raise the melting temperature of calf thymus DNA by 3.3 °C, a phenomenon rationalized in terms of hydrogen-bonding interactions. However, Antonow and co-workers[31] recently prepared a number of C2−aryl PBD dilactams (e.g., **2**) with fewer hydrogen-bonding possibilities that can still increase the melting temperature of calf-thymus DNA by up to 2.4 °C, most likely demonstrating the contribution of hydrophobic interactions to overall binding. The fact that the covalently binding tomaymycin stabilizes DNA to a similar extent (i.e., $\Delta T_m = 2.6$ °C)[18] suggests that overall binding affinity is a subtle combination of covalent and noncovalent interactions.

2. GENERAL INFORMATION

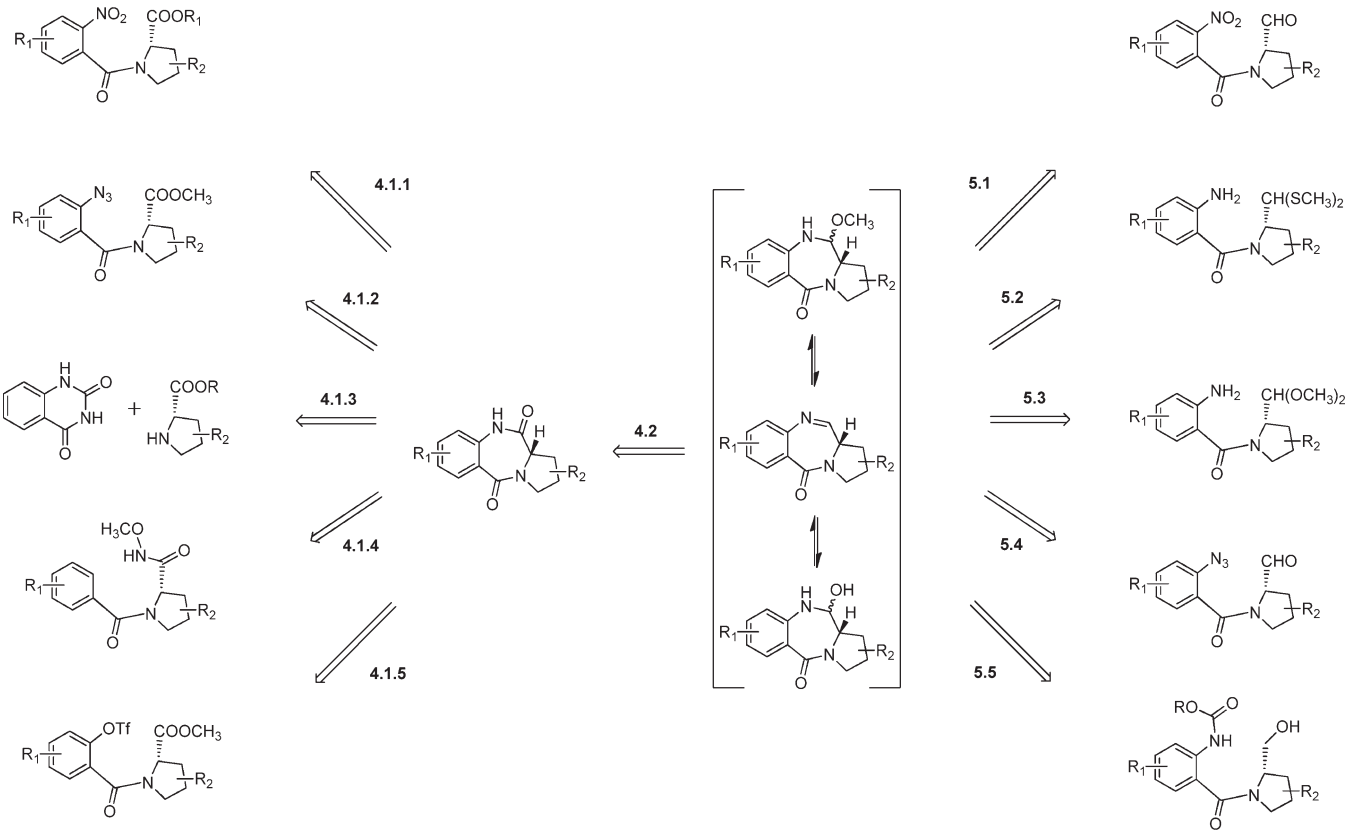
This review is organized according to the type of reactions used to produce the PBD ring system according to the "root" retrosynthetic analysis shown in Scheme 1. The two most investigated synthetic targets to emerge from this analysis are the PBD dilactams (section 4) and the PBD carbinolamines (section 5), both of which can lead to biologically active PBD N10−C11 imines. A full description of all synthetic routes is beyond the scope of this review; however, new approaches to the synthesis of key intermediates and for achieving important structural modifications are discussed and compared in relevant sections. An extensive amount of research has been carried out on the solid-phase synthesis of PBDs, and this is described in section 7. Synthetic modifications to the N10- and C11-positions of the PBD skeleton have recently emerged as a strategy to modulate biological activity, and these are described in section 8. A full discussion of biological results for individual PBD compounds or families is also beyond the scope of this review; however, significant biological features are mentioned in relevant sections. Other reviews are available that focus on the biological aspects of PBDs.[2,12,13]

3. RETROSYNTHETIC ANALYSIS OF THE PYRROLO[2,1-*C*][1,4]BENZODIAZEPINE RING SYSTEM

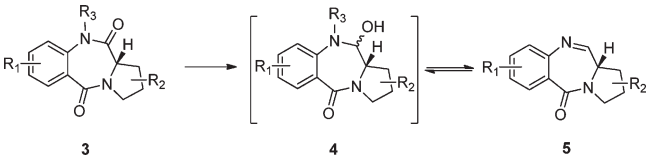
Since the discovery of anthramycin, the synthesis of PBD molecules has attracted many chemists because of the challenging combination of sensitive functional groups, chiral centers, substitution patterns, and potential use as lead molecules for anticancer and antibacterial drug discovery. Over the last 40 years, many synthetic methods have been published, with most approaches leaving formation of the N10−C11 electrophilic moiety to the end of the synthesis.

Retrosynthetic analysis of the PBD ring system (**A**, Scheme 1) reveals the A- and C-ring precursors (**E** and **F**) as likely starting points. The carbinolamine (**B**) or amide (i.e., dilactam) (**C**) forms of the PBD ring system are also candidates for disconnection. Functional group interconversions (FGIs) then lead to intermediates with electrophilic sp^2 pre-C11 groups[32] represented by the

Scheme 2. Retrosynthetic Analysis of the PBD Skeleton Showing the Most Commonly Encountered Pre-PBD *Synthons* in Published Synthetic Routes; The Numbers on the Double Arrows Relate to Relevant Sections in the Review in Which Individual Synthons are Described



Scheme 3. General Representation of the Hydride Reduction Approach to the Synthesis of PBD Imines from PBD Dilactams



pre-PBD *synthon* (**D**), which can in turn be disconnected to afford fragments **E** and **F**. A-ring building blocks for PBD synthesis are readily available with some exceptions depending on the type and position of substituents. Many A-ring building blocks are based on naturally occurring anthranilic acids, with the nucleophilic amine substituent often masked as nitro or azido groups prior to B-ring cyclization. Similarly, C-ring precursors can be obtained from L-proline fragments, many of which are derived from natural sources and provide the C11a(*S*)-stereochemistry required in the final PBD structure. The most common C-ring building blocks used to construct PBD skeletons are 2(*S*)-proline and 2(*S*),4(*R*)-4-hydroxy-2-proline methyl ester. These also provide the pre-C11 electrophilic group necessary for B-ring closure in the form of carboxylic acid, carboxylic acid methyl ester, or, after conversion, aldehyde functionalities. Pre-C11−aldehydes are normally protected as acetals or thioacetals that can be deprotected at a convenient stage of the synthetic route. Therefore, the pre-PBD *synthon* (**D**) occupies a significant and strategic position in the retrosynthetic analysis of the PBD skeleton and features prominently in many published synthetic routes. The most commonly used structures representing *synthon* **D** (Scheme 1) are summarized in Scheme 2. Each of the *forward* reactions corresponding to a key disconnection is discussed in the following sections and labeled accordingly.

4. HYDRIDE REDUCTION OF PYRROLO[2,1-*C*]-[1,4]BENZODIAZEPINE-5,11-DIONES (PBD DILACTAMS)

Leimgruber and co-workers elucidated the structure of anthramycin in 1965 and reported its first total synthesis three years later.[1] Their synthetic strategy was based on reduction of the N10−C11 amide functionality of a PBD dilactam intermediate of type **3** using lithium borohydride. This provided a N10−C11 carbinolamine of intermediate of type **4**, which eliminated water to provide the N10−C11 imine **5** (Scheme 3). Over 40 years have now passed, but this pioneering methodology for PBD synthesis still features regularly in the literature. The popularity of this approach is largely due to the efficiency of diazepine ring (B-ring) formation when PBD dilactams are obtained. The energy released in formation of the cyclic C═O bond is reflected in the high yields for these types of B-ring cyclizations. The PBD dilactam core can be obtained in only two steps involving initial coupling of the A-ring precursor to a proline derivative followed

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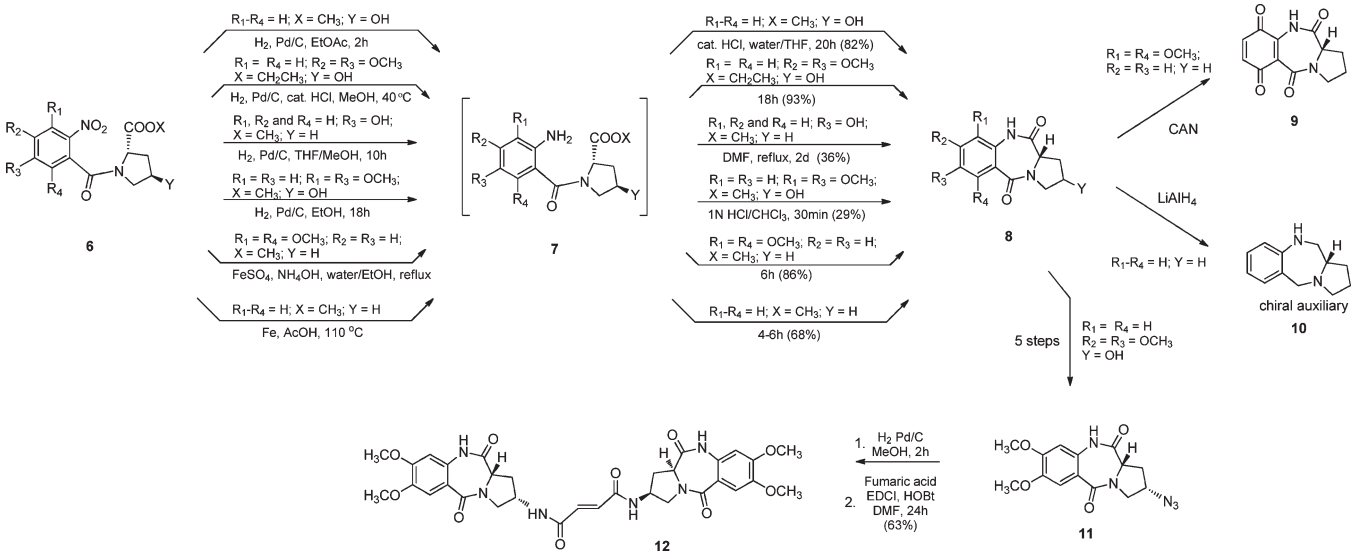
Scheme 4. Summary of Methodologies Used for the Formation of PBD Dilactams with Fully Saturated C-Rings via the Reductive Cyclization Approach



by B-ring closure. Less commonly, PBD dilactams can be constructed in just one step from isatoic anhydride and proline fragments (see section 4.1.3). PBD dilactams are robust chemical scaffolds that tolerate most synthetic processes and are amenable to a wide variety of further functionalization. The main limitation of this approach is that, depending on the methodology used, the N10−C11 hydride reduction step can lead to overreduction to give biologically inactive N10−C11 secondary amines rather than the electrophilic carbinolamine/imine species. Another potential problem is that PBD dilactams are susceptible to racemization at the C11a-position,[33−35] presumably due to the vicinal C11−carbonyl increasing the acidity of the C11a-proton. In this section, the different methods of synthesizing PBD dilactams and their subsequent conversion into N10−C11 carbinolamines/imines using hydride reducing agents are described. The section closes with an overview of methodologies for the reduction of PBD dilactams to DNA-alkylating PBD imines (section 4.2).

4.1. Synthesis of Pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-diones (PBD Dilactams)

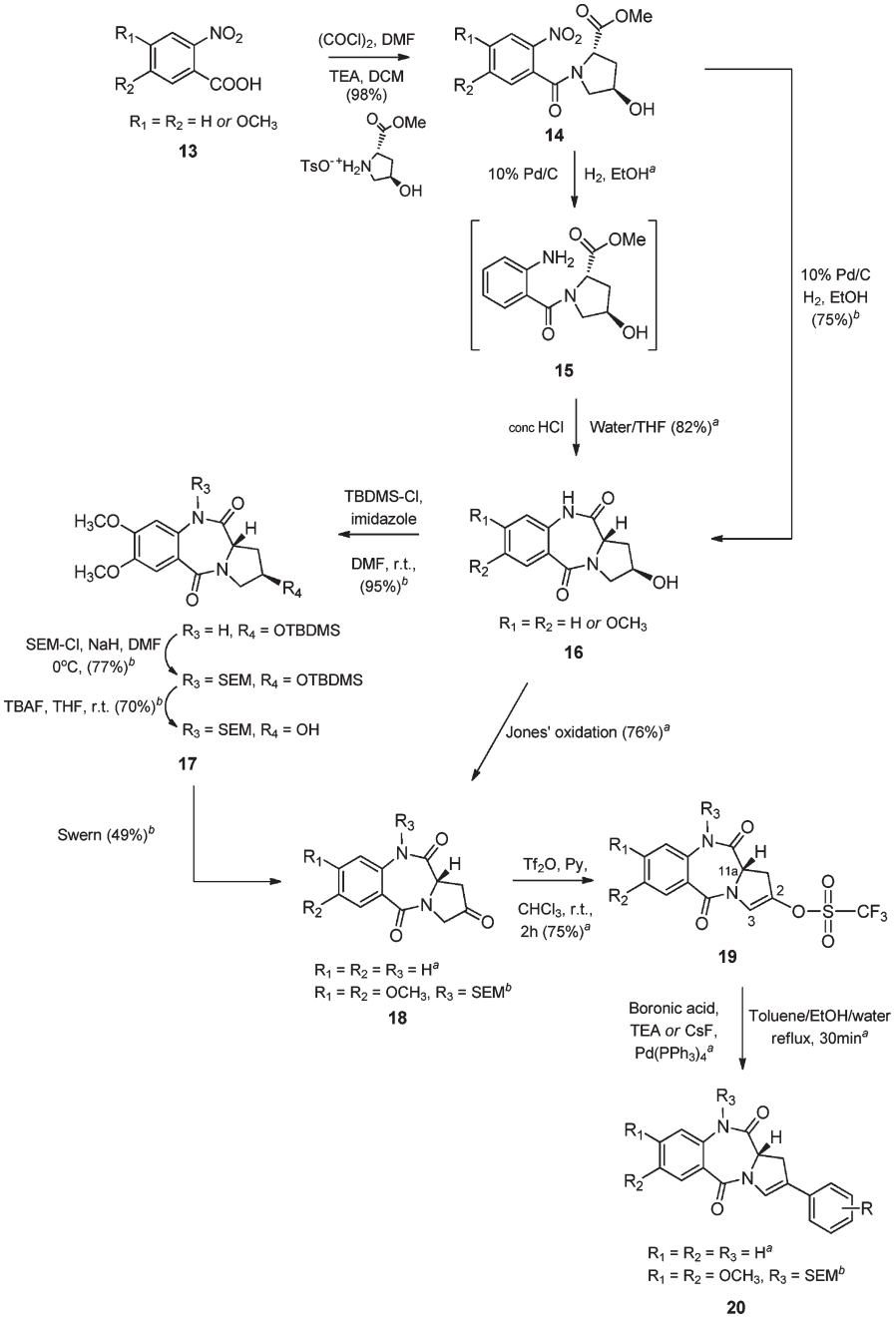
PBD dilactams can be obtained through seven different routes, and the sections below (4.1.1−4.1.5) describe five methods in which standard B-ring cyclization can be achieved. A novel B-ring cyclization method[36] employing pentapeptides containing aspartic acid has also been reported using solid-supported reagents (see section 7). It is noteworthy that some PBD dilactams have biological activity and have been targeted as final products themselves.[31,33,37−39]

4.1.1. Cyclization of Methyl and Ethyl *N*-(2-Nitrobenzoyl)pyrrolidine-2-carboxylates. Methyl and ethyl *N*-(2-nitrobenzoyl)pyrrolidine-2-carboxylates are readily obtained by condensing 2-nitrobenzoic acid derivatives (i.e., A-ring precursors) with pyrrolidine-derived building blocks (i.e., C-ring precursors) (Scheme 4). The nitro group in the benzene ring is the key functionality, undergoing reduction to a nucleophilic anilinic amine which then reacts with the electrophilic pre-C11-carbon from the pyrrolidine ring, thus leading to a 7-*exotrig* B-ring closure. Occasionally, this energetically favorable cyclization can be achieved in situ during the nitro-reduction in a single step.[40,41] However, in most cases, catalytic amounts of HCl and/or refluxing is required to achieve cyclization.

Since the last review of PBD dilactam-forming methodologies,[11] a single-step reductive cyclization has been accomplished in 86% yield by refluxing a nitro ester of type **6** with iron(II) sulfate and ammonium hydroxide in ethanol/water (1:1) for 6 h to provide an intermediate of type **8** suitable for the synthesis of pyrrolo[1,4]benzodiazepinequinones (**9**) after demethylation using ceric ammonium nitrate (CAN) (Scheme 4).[42] Elemental iron in glacial acetic acid at 110 °C has also been employed for larger-scale (e.g., 12.5 g of starting material) reduction of the nitro group.[43,44] The overall reaction time, including B-ring cyclization, ranged from 4 to 6 h, and the product was obtained after recrystallization in good yield (68%). In this case, the resulting PBD dilactam (**8**) was treated with $LiAlH_4$, and the overreduced PBD (**10**) used as a chiral auxiliary for enantioselective Morita−Baylis−Hillman reactions.[45]

Catalytic hydrogenation in the presence of palladium on charcoal has also been used extensively for the reduction of nitro groups within *N*-(2-nitrobenzoyl)pyrrolidine derivatives. This technique produces the PBD ring system in varying yields (29−93%) and is suitable for larger-scale preparations (e.g., 33 g of starting material) in which the PBD dilactam products can often be purified by recrystallization.[46] The nitro-reduction can proceed relatively rapidly (e.g., 2 h),[31] but the subsequent acid-catalyzed cyclization step may take up to 20 h in THF/water 1:10. The cyclization time can be shortened to approximately 30 min by using a 1:1 mixture of 1 N HCl/$CHCl_3$, although the yield reported in this case was much lower (i.e., 29%).[46] Alternatively, catalytic amounts of concentrated HCl can be added to the hydrogenation vessel at the beginning of the reduction step, thus shortening the overall reduction/cyclization time to ∼18 h.[47] This strategy has been used by Al-Said for synthesis of the symmetrical C2/C2′-coupled PBD dilactam dimer **12**, which has the two PBD units joined through a fumarate linker.[47] A thermal cyclization process has also appeared in the literature,[48] involving reflux of the hydrogenation product in DMF for 2 days to

Scheme 5. Synthesis of C2−Aryl C2/C3-*endo*-Unsaturated PBD Dilactams via the Reductive Cyclization Route



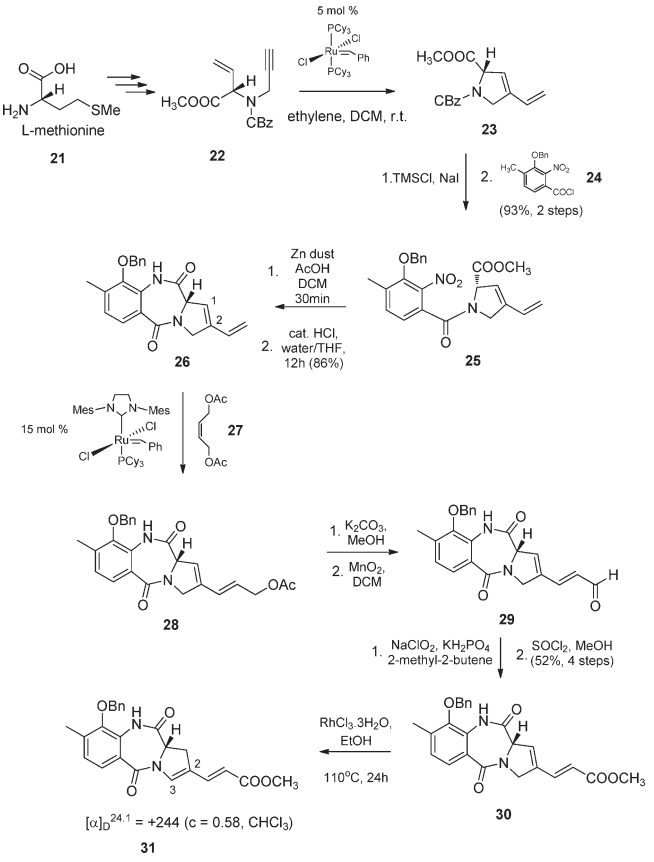
[a] Reaction conditions for $R_1 = R_2 = R_3 = H$, according to Antonow and co-workers.[31] [b] Reaction conditions for $R_1 = R_2 = OCH_3$, R_3 = SEM, according to Cooper and co-workers.[40]

complete cyclization. However, the reported yield of only 36% highlights the advantage of acid-catalyzed cyclization.

Catalytic hydrogenation has also been employed for the synthesis of a novel library of C2−aryl PBD dilactams (Scheme 5).[31,40] The enol−triflate building block **19** was used as a substrate for palladium-catalyzed C−C bond formation at the C2-position, and was treated with several different boronic acids under Suzuki coupling conditions. However, the C11a chiral center racemized during the cross-coupling step, which utilized a relatively strong base (i.e., Na_2CO_3). One possible explanation for this is the proximity of the amidic C11−carbonyl to the C11a-proton that presumably increases its acidity. Racemization was avoided by using milder bases such as tetraethylammonium (TEA) or cesium fluoride (CsF).[31] DNA thermal denaturation studies showed that the additional C2-aryl functionalities in compounds of type **20** ($R_1 = R_2 = R_3 = H$) enhanced interaction with DNA compared to C2-unsubstituted PBD dilactams.[31] This was attributed to hydrophobic interactions resulting from close contact of the C2−aryl ring with DNA bases in the minor groove, a hypothesis later confirmed through NMR studies with C2-naphthyl PBD imines.[18]

A disadvantage of employing Pd-catalyzed hydrogenation for B-ring cyclization is that building blocks containing unsaturation

Scheme 6. Use of Ruthenium-Catalyzed Metathesis for the Synthesis of C2/C3-*endo*-Unsaturated PBDs, and $RhCl_3 \cdot 3H_2O$ for the Isomerization of C1/C2-*endo*-Unsaturation to the C2/C3 Position



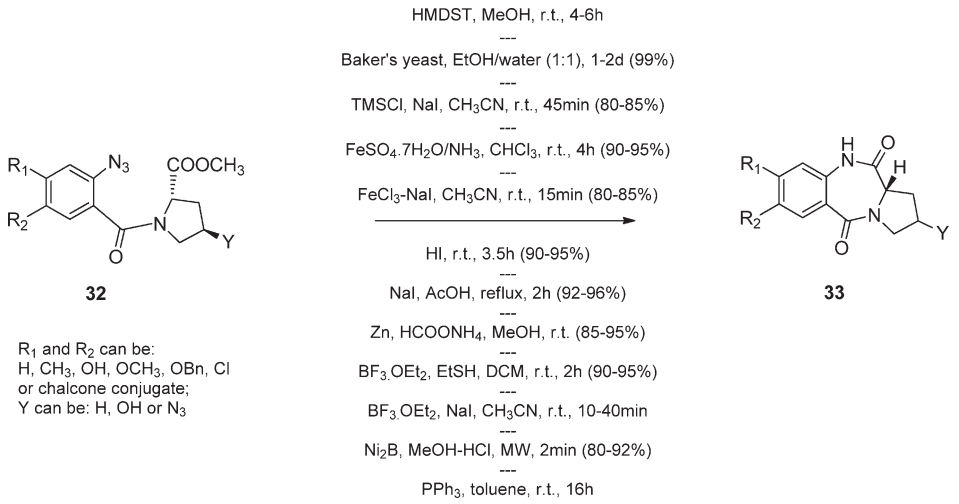
(e.g., C-ring building blocks with pre-C2/C3-*endo*-unsaturation) are reduced. In these cases, chemoselective synthetic methods can be used. For example, the building block **25**, an equivalent of the pre-PBD *synthon* **D** shown in Scheme 1, was elegantly prepared from **22** by Kitamura and co-workers using a ring-closing enyne metathesis (Scheme 6).[49] This intermediate was itself prepared in a 5-step synthesis from L-methionine (**21**). Intermediate **25** was subjected to selective nitro-reduction with zinc dust in dichloromethane (DCM) for 30 min in the presence of acetic acid, which effected cyclization to **26** without affecting either the *endo*- or *exo*-unsaturation associated with the pyrrolidine ring. As in most of the examples of hydrogenation discussed above, the anilinic amine required treatment with catalytic HCl over a period of 12 h in THF/water (1:1) for cyclization to be achieved in good yield (i.e., 86%). The cyclization product **26** underwent cross-metathesis with the diacetate **27**, thus extending the C2-position. However, the subsequent intermediates in this route (i.e., **28**, **29**, and **30**) contained undesired C1/C2-*endo*-unsaturation. To overcome this problem, isomerization of the C1/C2 double bond to the C2/C3 position was carried out with $RhCl_3 \cdot 3H_2O$.[49] The resulting isomerized product **31** was reported to have intact C11a(*S*)-stereochemistry ($[\alpha]_D^{24} = +244$; $c = 0.58CHCl_3$), thus confirming the utility of $RhCl_3 \cdot 3H_2O$ as a reagent to isomerize a C1/C2 double bond within the C-ring of a PBD. This discovery could represent a general opportunity to salvage C2/C3-*endo*-unsaturated PBDs (preferred due to their higher DNA-binding affinity and chemical stability)[50] from synthetic routes that inadvertently produce C1/C2-*endo*-unsaturated kinetic products (e.g., formation of C1/C2-*endo* enol−triflates in the presence of strong bases,[50] Scheme 42, section 5.5).

4.1.2. Cyclization of Methyl *N*-(2-Azidobenzoyl)-pyrrolidine-2-carboxylates. Successful methodologies for the reductive cyclization of methyl *N*-(2-azidobenzoyl)-pyrrolidine-2-carboxylates to produce PBDs have been pioneered by Kamal and co-workers (Scheme 7).[51] Apart from several synthetic methods based on reagents typically used to reduce azides to amines, a quantitative biocatalytic B-ring cyclization procedure has also been reported.[52] From a practical standpoint, compared to the direct reduction of *N*-(2-nitrobenzoyl)pyrrolidine derivatives (e.g., **6**), one disadvantage is the additional step required to produce the azido intermediate (i.e., **32**). This is typically achieved by treating *N*-(2-nitrobenzoyl)pyrrolidine derivatives with sodium azide (NaN_3) in hexamethylphosphoramide (HMPA) at room temperature.[51] A further disadvantage is that this preliminary step and the subsequent reductive cyclization reactions often require the chromatographic purification of products. However, it is a useful alternative reaction sequence when palladium-catalyzed hydrogenations either will not work or cannot be undertaken for reasons relating to chemoselectivity.[53]

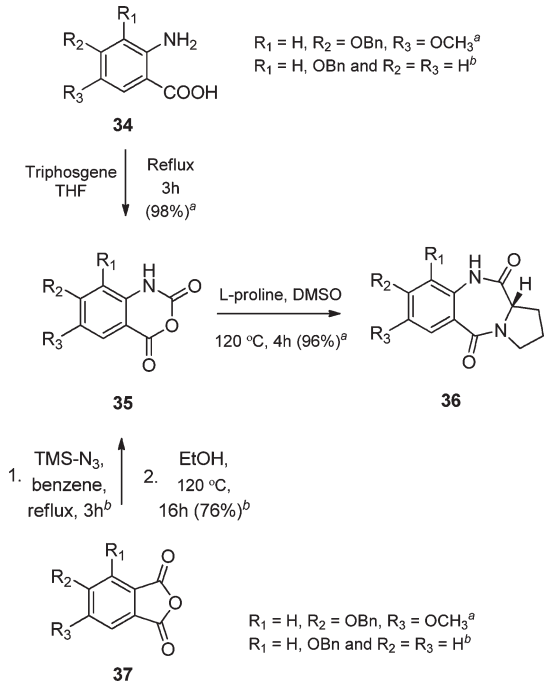
In contrast to the nitro reduction strategy (section 4.1.1), azide reductive cyclizations occur without the need for a second acid-catalyzed step, which is one potential advantage. Hexamethyldisilathiane (HMDST) in methanol was the first reducing agent to be employed to form a PBD skeleton from azidobenzoyl building blocks.[51] This method has also been employed for the synthesis of C2-azido-substituted PBDs, as HMDST selectively reduces aryl but not alkylazido groups.[54] Another silicon-based reagent (trimethylsilyl iodide, TMSI), generated in situ from the reaction of trimethylsilyl chloride (TMSCl) with sodium iodide in acetonitrile, has been shown to reduce arylazido groups en route to both PBD dilactam monomers and dimers.[55] Similarly, ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) in 25% aqueous ammonia solution has been used to reduce arylazides to produce dilactams in excellent yields (90−95%),[56] and ferric chloride ($FeCl_3$) with sodium iodide has delivered comparable yields for the synthesis of similar PBD dilactams but with significantly shorter reaction times (15 min).[57] Zinc and ammonium formate ($HCOONH_4$) in methanol can convert nitro and azido arenes to *N*-arylformamides and have been successfully used to synthesize PBDs.[58] An aqueous solution of hydriodic acid (HI) has also been used to produce excellent yields of PBD dilactams via arylazide reduction.[53] A nonaqueous variant of this method was developed using sodium iodide in acetic acid under reflux,[59] and this in situ formation of HI afforded similar yields to direct use of the reagent. Recently, boron trifluoride diethyl etherate ($BF_3 \cdot OEt_2$) has been used in conjunction with ethanethiol (EtSH)[60] or sodium iodide[61] as a mild an efficient method for the reductive cyclization of arylazides of type **32** to afford PBD dilactams (e.g., **33**). Microwave radiation (MW) has also been used in conjunction with nickel boride (Ni_2B) in acidic methanolic media to effect cyclization in good yields.[62] A solid-support-based method has also been reported in which triphenyl phosphine (PPh_3) is used to reduce an arylazide tethered to a chalcone derivative to a PBD−chalcone conjugate (see sections 5.4 and 7).[63]

4.1.3. Cyclocondensation of Isatoic Anhydrides with Substituted Prolines. This method involves the direct reaction

Scheme 7. Summary of Methodologies for the Synthesis of PBD Dilactams via the Reductive Cyclization of Aryl Azides



Scheme 8. Isatoic Anhydride Route for PBD Dilactam Synthesis

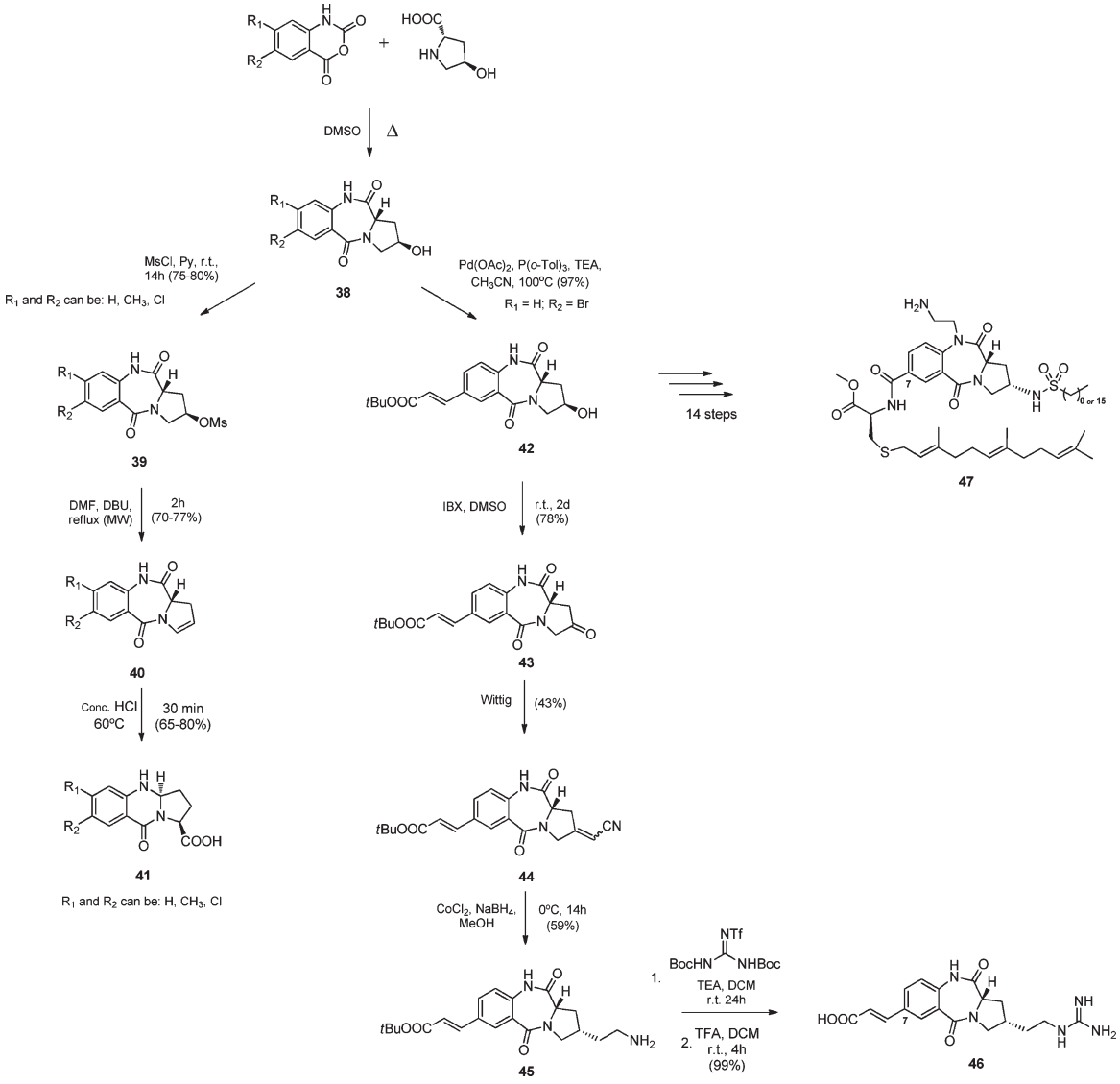


[a] Reaction conditions for R_1 = H, R_2 = OBn, and R_3 = OCH_3 according to Wang and co-workers.[65,66] [b] Reaction conditions for R_1, R_2, and R_3 = H according to Nagasaka and Koseki.[70]

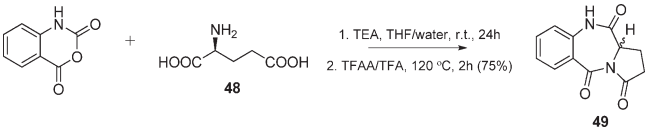
of an anthranilic acid *N*-carboxylic anhydride (isatoic anhydride) with an L-proline derivative. A typical condensation protocol involves simply heating the two components together in DMSO for 4 h at 100−120 °C (Scheme 8).[64−66] Refluxing the components in DMF for 15 min has also been reported but results in lower yields (63%).[67] The heating of finely ground isatoic anhydrides with L-proline to 150 °C for 18 h in the absence of solvent has also been reported by the pharmaceutical industry as a more efficient method for PBD dilactam preparation,[68] and comparable results have been reported using microwave radiation for only 3 min.[69] Isatoic anhydrides (**35**) are commercially available but can also be prepared in excellent yields by refluxing the corresponding anthranilic acid derivative (**34**) with triphosgene in tetrahydrofuran (THF).[65,66] Alternatively, isatoic anhydrides can be synthesized via a modified Curtius rearrangement by treating phthalic anhydrides (**37**) with trimethylsilylazide (TMS-N_3), as exemplified in the synthesis of tilivalline by Nagasaka and Koseki.[70] The main advantages of using isatoic anhydrides as A-ring precursors are the simplicity of the coupling procedure, the high yields obtained, the limited byproduct formation, the straightforward workup and ease of purification, all of which allow for large-scale preparations of PBD dilactams.

This versatile condensation is not limited to the use of L-proline, and *trans*-4-hydroxy-L-proline and L-glutamic acid have also been used for the synthesis of PBD-based chemical scaffolds. For example, Jolivet-Fouchet and co-workers have shown that *trans*-4-hydroxy-L-proline reacts with isatoic anhydride in DMSO under microwave radiation to give PBD dilactam **38** in just 30 min (Scheme 9).[71] Subsequent treatment with mesyl choride afforded **39**, and base (DBU) was then used to introduce C2/C3-*endo*-unsaturation through a bimolecular elimination. The resulting enamide **40** was treated with hydrochloric acid at 60 °C to induce rearrangement into the pyrrolo[2,1-*b*]quinazoline ring system (**41**) in good yield. A similar condensation was reported by Giannis and co-workers as an initial step toward the synthesis of **46**,[33] a potential integrin antagonist (i.e., **46**),[33] and acyl protein thioesterase (APT1) inhibitors of type **47** (Scheme 9).[37,38] Both syntheses involved a palladium-catalyzed Heck coupling with *tert*-butyl acrylate at the C7-position. In the case of **46**, oxidation of the C2−hydroxyl was effected with 1-hydroxy-(1*H*)-1,2-benziodoxol-3-one 1-oxide (IBX) to provide the C2−ketone **43**. C−C bond formation was then achieved at the C2-position via Horner−Wadsworth−Emmons olefination to afford **44** (see Table 3 for similar C2-olefinations). This was followed by simultaneous reduction of the nitrile group and C2-*exo*-unsaturation with sodium borohydride ($NaBH_4$) and 2 equiv of cobalt chloride ($CoCl_2$) to provide the primary amine **45**. Finally, the guanidine terminus was installed at the end of the C2-side chain by reaction with *N*,*N*′-bis-*tert*-butoxycarbonyl-*N*″-trifluoromethanesulfonyl guanidine followed by removal

Scheme 9. Cyclocondensation of Isatoic Anhydrides and 4-Hydroxyproline for the Synthesis of PBD Dilactams Analogues[33,37,38,71]



Scheme 10. Reaction of Isatoic Anhydride with Acyclic Amines[35]



of the protecting groups with trifluoroacetic acid (TFA) to afford **46** (Scheme 9).

Acyclic amines such as L-glutamic acid have also been used by Roos and Dastlik in synthetic studies toward the alkaloid (+)-ifforestine (Scheme 10).[35] In this case, the condensation was carried out with isatoic anhydride, triethylamine (TEA), and L-glutamic acid in THF/water at room temperature for 24 h, followed by 2 h treatment with trifluoroacetic anhydride (TFAA) and trifluoroacetic acid (TFA) at 120 °C. Although a good yield (75%) was reported, **49** was obtained as a racemic mixture.

Li and co-workers have also used isatoic anhydrides in the initial stage of the synthesis of pyrroloimidazobenzodiazepines of type **51** in a search for selective ligands of $GABA_A$/Bz receptors (Scheme 11).[72,73] Once formed, a PBD dilactam (**50**) was treated with diethyl phosphorochloridate ($(EtO)_2POCl$) and lithium diisopropylamide (LDA),[72] sodium hydride (NaH),[73] or potassium *t*-butoxide (*t*-BuO^-K^+),[74] followed by addition of isocyanoacetates pretreated with LDA to incorporate the N10−C11 imidazole ring.

Similarly, Breslin and co-workers have incorporated an external imidazole-2-thione moiety at the N10−C11 position of a PBD ring as part of a structure−activity relationship study of HIV-1 replication inhibitors (Scheme 12).[75] Their synthesis started from 5-chloroisatoic anhydride that condensed with *trans*-hydroxy-L-proline to give **52**. This was followed by Jones' oxidation of the C2-hydroxy group (**53a**) and then A-ring

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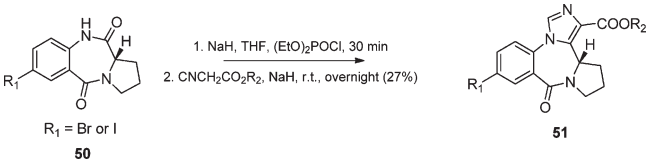
Scheme 11. Synthesis of Pyrroloimidazobenzodiazepines[73]

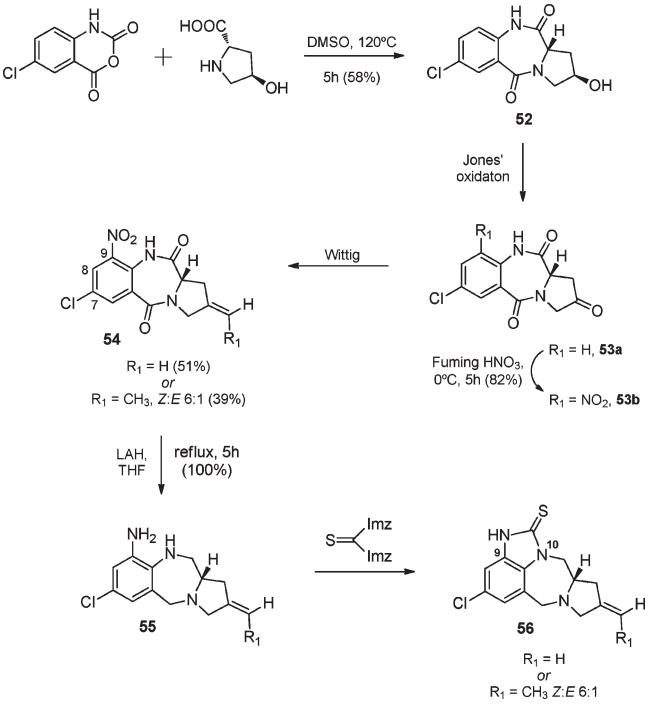


nitration with fuming nitric acid to afford **53b** in good yield (82%), a key intermediate for Wittig olefination to give **54**. The C9−NO_2 and the dilactam ring were then simultaneously reduced with lithium aluminum hydride (LAH) to provide the two adjacent C9- and N10-nucleophilic nitrogens of **55**. This intermediate reacted readily with 1,1′-thiocarbonyldiimidazole to provide structure **56** containing the additional N10−C11 imidazole-2-thione ring.

Another use of the isatoic anhydride approach is in the enantioselective C11a-methylation of PBDs developed by MacQuarrie-Hunter and Carlier (Scheme 13).[76] This elegant C11a-alkylation process is based on the "memory of chirality" inherent in PBD ring systems of type **58** when these molecules are exposed to strong bases (i.e., LDA).[76,77] The concept of "memory of chirality" as a strategy for asymmetric synthesis has been reviewed recently by the same group.[78] It initially appears to be a counterintuitive approach, as deprotonation at the C11a-position would be expected to destroy the sp^3-hybridized chiral center of the starting material. However, in the case of **58**, chiral high-performance liquid chromatography (HPLC) analysis of the products (**59**) showed that the chiral C11a−C11 enolate formed under the basic conditions must racemize slowly on the time scale of the alkylation reaction, thus allowing sufficient time for the kinetic enolate of **58** to attack the electrophile (i.e., halogenated alkyl) with the original stereochemistry maintained. Furthermore, the researchers were able to optimize the method to maximize chemical yield while still maintaining enantioselectivity.[76] It was found that a cryogenic temperature of −100 °C and a 1 min exposure to LDA before adding the electrophile (e.g., methyl triflate (MeOTf)) afforded the desired C11a−Me PBD in 68% yield and with 99.5% ee (enantiomeric excess). Temperature and time appeared to be critical for the success of the reaction, as a temperature of −78 °C instead of −100 °C led to complete racemization within 5 min of reaction. An in situ protocol was simultaneously reported in which the electrophile was included in the initial reaction mixture.[76] In this case, the sterically hindered potassium hexamethyldisilazane (KHMDS) was used as base to prevent reaction between the base and the electrophile. It is possible that this approach could be used to design future generations of "racemization-resistant" PBDs for biological studies. For example, the C11a-racemization reported by Antonow and co-workers[31] could be circumvented by the presence of a quaternary C11a carbon (e.g., (*S*)-C11a−Me), although the extent to which an alkyl group at the C11a-position of a PBD may inhibit DNA interaction and covalent binding remains to be investigated.

4.1.4. Cyclization of 1-Benzoyl-*N*-methoxypyrrolidine-2-carboxamides. Recently, Correa and co-workers reported a new method for forming the PBD ring system based on the hypervalent iodine reagent PIFA [phenyliodine(III) bis-(trifluoroacetate)] (Scheme 14).[79] The route started with benzoylation of L-proline methyl ester using standard peptide coupling conditions to afford **61**. Subsequent saponification with

Scheme 12. Synthesis of C9−N10-Bridged PBDs from Isatoic Anhydrides[75]



LiOH to give **62** followed by a second amide coupling with methoxyamine provided the *N*-alkoxyamide **63**, the substrate required for the novel B-ring cyclization. The next step is based on an intramolecular electrophilic aromatic substitution promoted by the *N*-acylnitrenium species of type **64** obtained from treatment of **63** with PIFA. For this reason, the nucleophilic character of the phenyl group (A-ring precursor) is crucial, and cyclization only occurs provided the pre-C7 and pre-C8 substituents are electron-donating groups. The B-ring closure was best achieved in DCM at room temperature and provided satisfactory yields of dilactam **65** ranging from 57 to 70%. Subsequent treatment with molybdenum hexacarbonyl ($Mo(CO)_6$) removed the N10-methoxy group in good yield (73−85%). Intermediate **66** was then converted to the N10−C11 secondary amine **67** upon treatment with sodium borohydride. This could be converted into the N10−C11 imine species (i.e., C8−benzyl DC-81) by oxidation with *N*-methylmorpholine *N*-oxide (NMO)/tetrapropylammonium perruthenate (TPAP)[79] (see section 6). Overall, although this synthetic approach does not produce very high yields in the crucial B-ring cyclization step, it has the advantage compared to other methods of allowing diversification of A-ring structure such as the inclusion of heterocyclic rings (e.g., **68** and **69**).

4.1.5. Cyclization of *N*-(2-Trifluoromethylsulfonyloxybenzoyl)pyrrolidine-2-carboxylic Esters. The range of aromatic building blocks that can be used as A-ring precursors for the synthesis of PBDs has been extended by an approach employing salicylic acid derivatives as starting materials (Scheme 15).[34] This method is based on the intramolecular cyclization of aryl triflates prepared from L-proline methyl ester (**70**) or (*S*)-prolinol (**72**) C-ring precursors. The pre-N10-B-ring nitrogen is inserted via an external NH_3-based reagent that displaces the aryl triflate. Thus, treatment of **70** with ammonium

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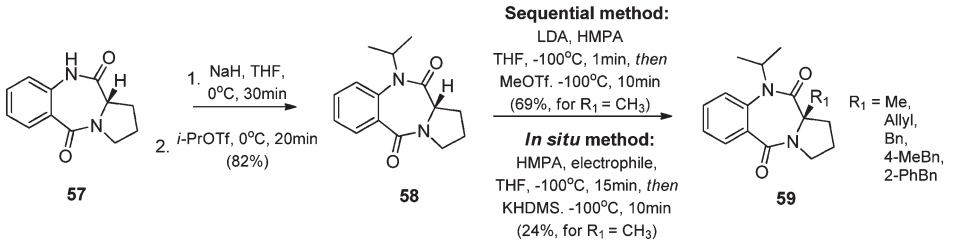
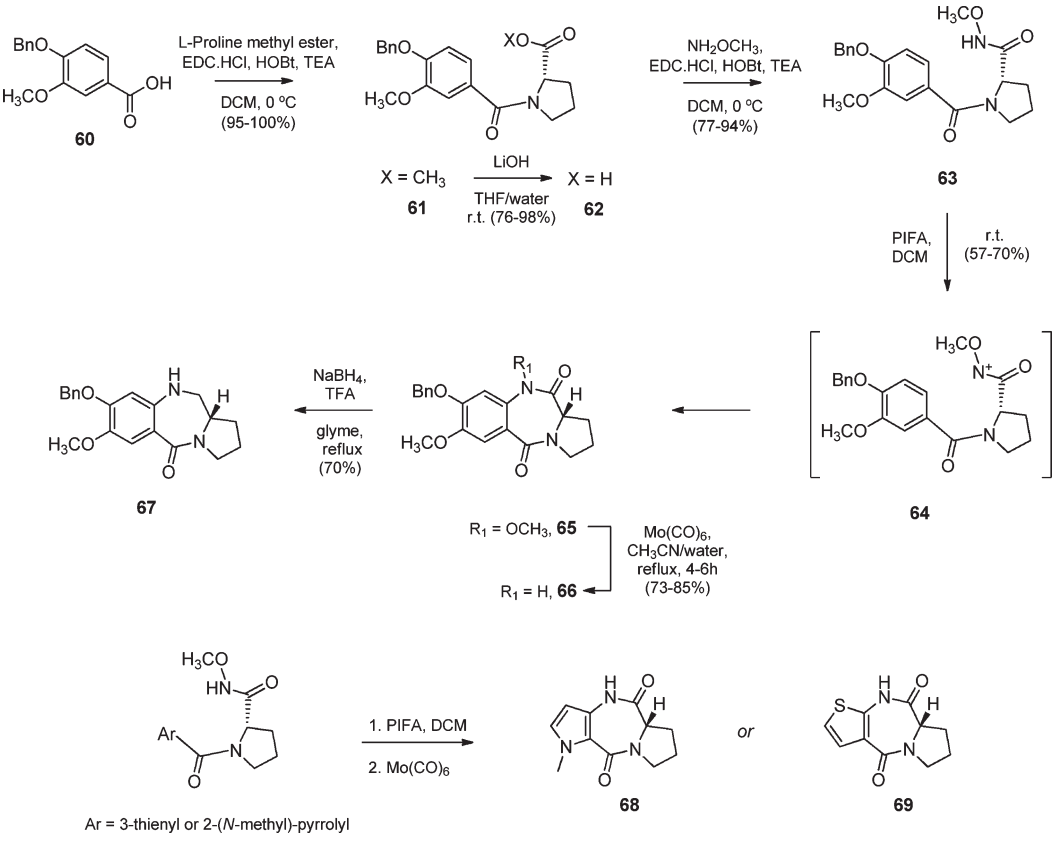
Scheme 13. Enantioselective C11a-alkylation of PBDs through the "Memory of Chirality" Approach[76]



Scheme 14. Synthesis of PBDs through the Cyclization of 1-Benzoyl-*N*-methoxypyrrolidine-2-carboxamides[79]

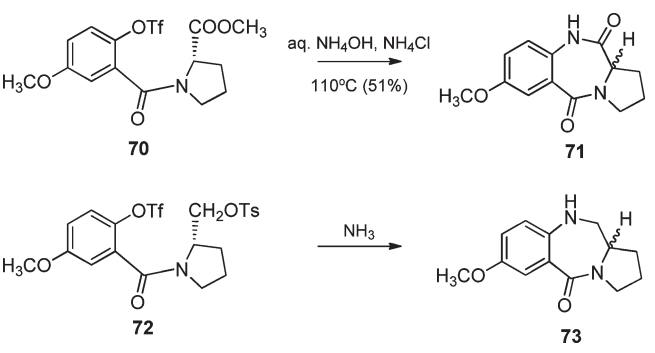


hydroxide and ammonium chloride affords the PBD dilactam **71**, although some racemization at the C11a position occurred. Similarly, compound **72** was treated with aqueous ammonia at 110 °C to afford the secondary amine **73** in 51% yield.[80] It is noteworthy that an aryl iodide, synthetically equivalent to **70**, has recently been used for aryl amination followed by B-ring cyclization, thus generating N10-substituted PBD dilactams in 78% yield and good optical purity (i.e., 93% ee).[226]

4.2. Reduction of PBD Dilactams to PBD N10−C11 Carbinolamines/Imines

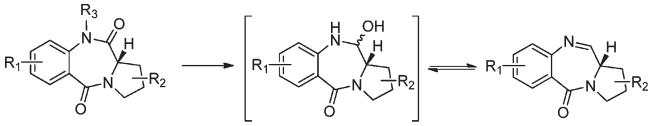
The ease of preparation and stability of PBD dilactams justify the popularity of this chemical scaffold as a basis for PBD synthesis. However, dilactams are of only modest interest for their DNA-interactive properties because of the absence of the

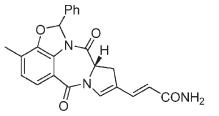
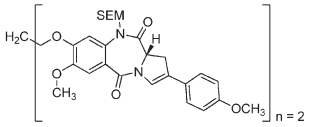
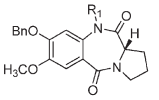
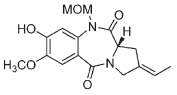
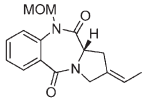
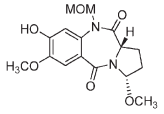
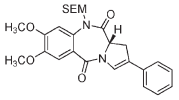
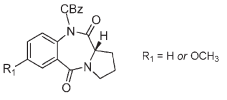
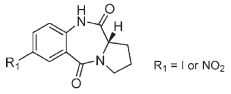
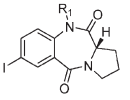
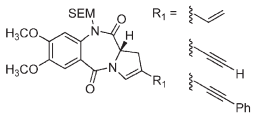
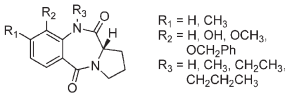
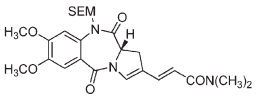
Scheme 15. Synthesis of PBDs via Cyclization of *N*-(2-Trifluoromethylsulfonyloxy)benzoyl)pyrrolidine-2-carboxylic Esters[34,192]



dx.doi.org/10.1021/cr100120f |Chem. Rev. 2011, 111, 2815–2864

Table 1. Effect of A- and C-Ring Substituents and N10-Protective Groups on the Conversion of PBD Dilactams to PBD Imines/Carbinolamines Using Different Hydride Reagents[a]



PBD Dilactam Structure (74)	Hydride	Yield (Carbinolamine/Imine) (75)	Reference (76)
	$NaBH_4$	100%	(Peña and Stille, 1989)[82] (Leimgruber *et al.*, 1968)[1]
	$LiBH_4$	94%	(Howard *et al.*, 2009)[81]
	$LiBH_4$ $NaBH_4$	95% (R_1 = MOM) Over-reduced (R_1 = H)	(Hu *et al*, 2001)[65] (Correa *et al.*, 2005)[79]
	$LiAlH_4$	92%	(Mori *et al.*, 1986)[83]
	$NaBH_4$	89%	(Mori *et al.*, 1986)[83]
	$LiAlH_4$	85%	(Mori *et al.*, 1986)[84]
	$NaBH_4$	74%	(Cooper *et al.*, 2002)[40]
	$NaBH_4$	69% (R_1 = H) 47% (R_1 = OCH_3)	(Nagasaka and Koseki, 1998)[70] (Kraus and Liu)[34]
	$NaBH_4$ $LiAlH_4$	68% (R_1 = NO_2) Over-reduced (R_1 = I)	(Suggs *et al.*, 1985)[85] (Katsifis *et al*, 1998)[67]
	$LiAlH_4$ $NaBH_4$	47% (R_1 = MOM) Over-reduced (R_1 = MEM)	(Katsifis *et al*, 1998)[67]
	$NaBH_4$	ca. 25%	(Tiberghien *et al.*, 2004)[41]
	$NaBH_4$	Over-reduced to B-ring-opened amino alcohol	(Thurston *et al.*, 1984)[86]
	$NaBH_4$	No reaction	(Chen *et el.*, 2004)[87]

[a] References prior to 1994 (i.e., refs 82−86) are included for comparison only and are not discussed in the text.

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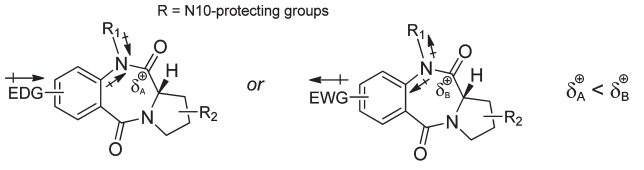
N10−C11 imine/carbinolamine functionality. Therefore, efficient methods to reduce the N10−C11 amide group of PBD dilactams to DNA-alkylating imine or carbinolamine moieties are highly sought after. Until the most recent reports,[15,81] reduction has traditionally been achieved at the end of synthetic pathways using covalent hydrides, and these methodologies have been reviewed by Thurston and Bose[11] and Katsifis et al.[67] This regioselective reduction of the C11−carbonyl requires careful strategic planning around the final synthetic steps as its efficiency is dependent upon a number of factors such as A-ring substituents, N10-protecting groups, the C-ring substitution pattern, and the source of hydride (summarized in Table 1). The two most common problems are that, first, some PBD dilactams are simply refractory to hydride reducing agents, and second, the reaction may proceed further than intended to produce overreduced N10−C11 secondary amines or B-ring-opened amino aldehydes.[86] Since the N10−nitrogen is directly attached to the aromatic A-ring, it is thought that substituents in the aromatic ring affect the electron density of this nitrogen, which in turn may affect the electrophilicity of the C11−carbonyl and its reactivity toward hydride. Electron-donating groups (EDGs) in the A-ring appear to reduce the electrophilicity of the C11−carbonyl toward hydride by feeding electrons through, whereas electron-withdrawing groups (EWGs) reduce the interaction of the N10 lone pair with the adjacent C11−carbonyl, thus facilitating nucleophilic attack by hydrides (Scheme 16). Thurston and Bose have suggested that overreduction to B-ring opened amino alcohols may be facilitated when carbinolamine forms dissociate to B-ring opened amino aldehyde forms, a process which may be influenced by the basicity of the N10−nitrogen.[11] In this context, inclusion of an appropriate N10-protecting group (R group, Scheme 16) may assist dilactam reduction by lowering the electron density on the nitrogen (base weakening), thereby increasing the electrophilicity of C11−carbonyl. An N10-protecting group can be easily installed by treating a PBD dilactam with a kinetic base (e.g., NaH), followed by addition of the electrophile (e.g., MOM−Cl), all of which maintains the C11a-*S* stereochemistry of the PBD.[67] A representative protocol for hydride reduction involves excess sodium borohydride ($NaBH_4$) in ethanol/THF at room temperature.[46] Finally, it should be noted that the equilibrium between N10−C11 carbinolamine and imine forms can be shifted toward the latter by treatment with silica gel before final chromatographic purification.

Scheme 16. Schematic Representation of Electronic Factors Influencing the Hydride Reduction of PBD Dilactams to PBD N10−C11 Carbinolamines/Imines



A combination of electronic factors based on substituents in the A-ring and N10-protecting groups can affect the electrophilicity of the C11-carbon (i.e., $\delta^{\oplus}$) and, consequently, the susceptibility of PBD dilactams to hydride reduction.

5. CYCLIZATION OF SUBSTITUTED *N*-BENZOYLPYRROLIDINE PRECURSORS

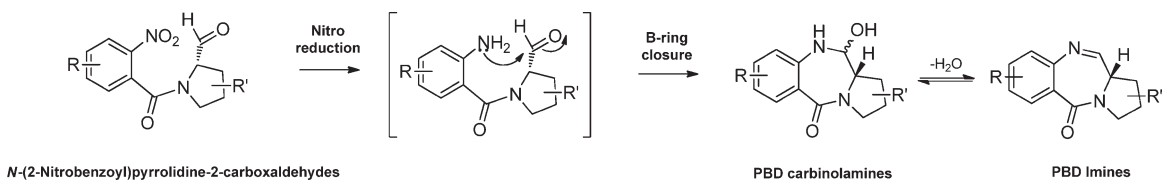
This section describes synthetic methodologies used for B-ring cyclization in which the electrophilic group at the pre-C11-position of the pre-PBD *synthon* (section 3) is an aldehyde, a protected aldehyde (e.g., dialkyl acetal or thioacetal), or a hydroxyl group that can be oxidized back to an aldehyde at the cyclization step. Mechanistically, this cyclization procedure differs from the formation of PBD dilactams in that a leaving group (e.g., alkoxy or hydroxy) is not involved. In this case, the newly formed N10−C11 carbinolamine bond results from intramolecular nucleophilic attack of an aromatic amine on the pre-C11−aldehyde or its equivalent (Scheme 17). The initially formed hemiaminal intermediate (i.e., the carbinolamine) can then form the electrophilic N10−C11 imine by loss of water. This type of cyclization has traditionally been carried out at the end of a synthetic pathway due to the chemical sensitivity of the N10−C11 carbinolamine or imine functionality. However, recent studies[14,17] have shown that the PBD imine can withstand a number of reaction conditions (e.g., Schemes 20 and 39). Although some of the intermediates used in this approach can be fragile, with appropriate planning the route can be useful in avoiding problems that arise with the PBD dilactam approach (i.e., difficulties with reduction of dilactams to imine/carbinolamine final products). Finally, it should be noted that the carbinolamine functionality, once formed, can be protected as, for example, a carbinolamine silyl ether to allow further modifications to the A- or C-rings.

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

In this method, the aldehyde group at the pre-C11-position is unprotected and cyclization results from reduction of the A-ring nitro group (Scheme 17). Therefore, this route has similarities with the cyclization of nitrobenzoylpyrrolidine-2-carboxylic esters to give dilactams as described in section 4.1.1. Reductive cyclization was first reported using catalytic hydrogenation in the presence of palladium on charcoal.[88] However, Thurston and Langley[89] subsequently used this approach but reported the formation of overreduced N10−C11−secondary amine PBDs as final products. This problem has been previously reviewed by Thurston and Bose.[11]

Langlois and co-workers have used the reductive cyclization approach for the synthesis of (+)-porothramycin and anthramycin[90] analogues **84** and **85** (Scheme 18).[91] They obtained the PBD ring system by selective reduction of the nitro aldehydes of type **83** with Raney-Ni catalyst. Although the

Scheme 17. Mechanism for the Reductive Cyclization of *N*-(2-Nitrobenzoyl)pyrrolidine-2-carboxaldehydes to PBD Carbinolamines or Imines



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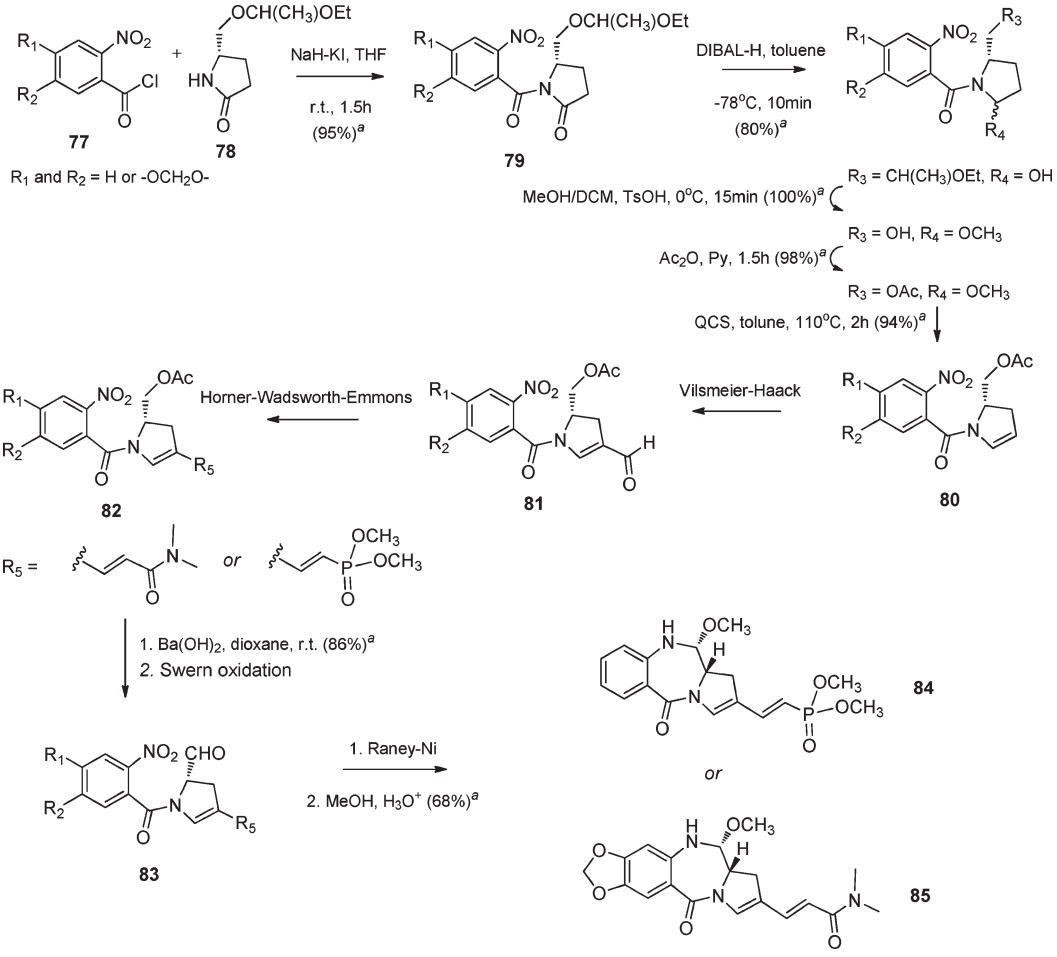
Scheme 18. Synthesis of Anthramycin and (+)-Porothramycin Analogues via Reductive Cyclization of *N*-(2-Nitrobenzoyl)pyrrolidine-2-carboxaldehydes[91]



[a] Reaction conditions for $R_1 = R_2 = H$ according to Rojas-Rousseau and Langlois.[91]

reported yields for this cyclization were not high (i.e., 36 and 68%, respectively), this methodology allowed diversification at the C2-position, which was not possible using the thioacetal approach (section 5.2).[92] They utilized the ethoxy−methyl protected pyrrolidone **78** as a C-ring precursor, which was obtained from commercially available L-pyroglutamic acid methyl ester. The pre-C3−carbonyl of **79** was then reduced with diisobutylaluminum hydride (DIBAL), thus avoiding pyrrolidine ring-opening to afford a diastereoisomeric mixture of secondary alcohols. After successive deprotection/reprotection of the acetal group, the enamide **80** was obtained via elimination of the pre-C3−methoxy group catalyzed by quinolinium camphorsulfonate (QCS). Next, a Vilsmeier−Haack formylation produced the pre-C2−aldehydes of type **81**, which were further functionalized at the pre-C2-position with phosphorus-based olefinating reagents using the Horner−Wadsworth−Emmons reaction to afford intermediates of type **82**. Deprotection of the pre-C11a acetate followed by Swern oxidation afforded the nitro aldehyde intemediates of type **83**, which were then converted to the final products **84** and **85** using Raney-Ni (see also Table 3, section 5.5 for a comparison of approaches used for the olefination of pre-C2−ketone intermediates.

A variant of this approach was developed by Kamal and coworkers using *N*,*N*-dimethylhydrazine with ferric chloride hexadrydrate ($FeCl_3 \cdot 6H_2O$) as reagents for a one-pot synthesis of the PBD N10−C11 carbinolamine **88** (Scheme 19).[93] To investigate the mechanism of this cyclization process, the reactions were carried out in two successive steps to establish that intermediate **87** was involved in B-ring formation. This method avoided the problem of racemization at the C11a−carbon, which had been observed in previous attempts to reductively cyclize *N*-(2-nitrobenzoyl)pyrrolidine-2-carboxaldehydes using iron−acetic acid.[94] Furthermore, for nitroaldehydes of type **86**, this represents an alternative to palladium-catalyzed hydrogenation, which can lead to overreduced biologically inactive N10−C11 secondary amines.[95]

Another method for reductive cyclization has been reported that uses sodium dithionite (sodium hydrosulfite, $Na_2S_2O_4$, 5−8 equiv) in THF/water at room temperature (Scheme 20).[17] Using this method during the synthesis of PBD "warheads" to attach to antibodies, it was demonstrated that treatment of **93** with $Na_2S_2O_4$ gave initially the C11−hydrogen sulfite intermediate **94** that could be directly observed by electrospray mass spectrometry (ES-MS).[17] The imine product **95** was then obtained in 85% yield by treatment with acetyl chloride (AcCl) in MeOH. Using various strategies, this methodology was applied to the synthesis of several tomaymycin derivatives including C8/C8′-linked PBD dimers. In another case, B-ring formation was

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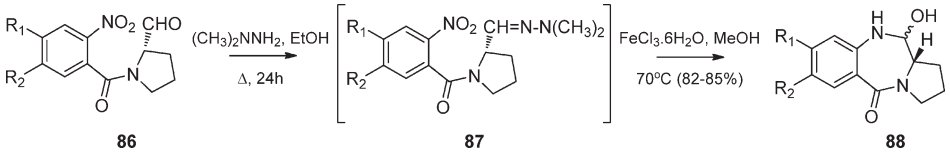
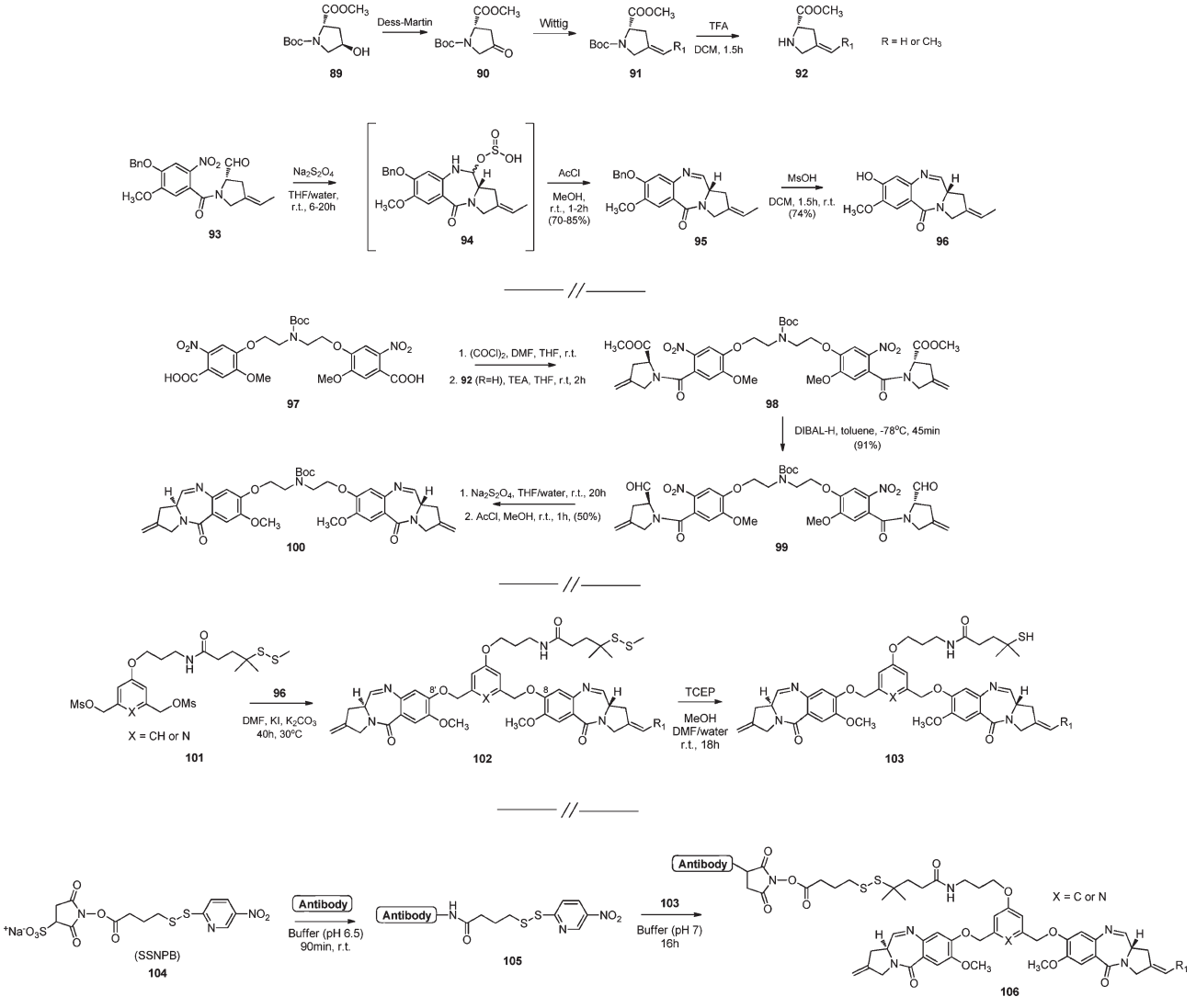
Scheme 19. Alternative Reductive Cyclization Approach Using *N,N*-Dimethylhydrazine and Ferric Chloride ($FeCl_3 \cdot 6H_2O$) Hexahydrate[93]



Scheme 20. Use of Sodium Dithionite ($Na_2S_2O_4$) for Reductive Cyclization and Its Application to the Synthesis of PBD "Warheads" for Use in Antibody Drug Conjugates (ADCs)[17]



achieved in a linear fashion starting from the symmetrical dimer intermediate **97**. Other PBD dimers were synthesized directly from the PBD imine monomer **96**, with the final steps of the synthetic route using the novel linker **101**.[17,96] This particular synthetic route was significant in that it demonstrated that PBD imine structures can be used intact as versatile scaffolds, thus avoiding the necessity of additional steps such as N10−C11 protection/deprotection or hydride reduction (i.e., for PBD dilactams) at the end of a synthetic pathway. The C8/C8′ linkers (e.g., **101**) used to tether two molecules of the PBD **96** in these studies provided a mercapto group that allowed a disulfide exchange reaction to take place with a 5-nitro-2-pyridyldithiobutanoate group[97] attached to an antibody (i.e., **105**) to provide tumor-selective immunoconjugates of type **106** that had both in vitro and in vivo activity.[17]

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

This approach is one of the most widely used for PBD synthesis and, in particular, for the synthesis of PBD conjugates.

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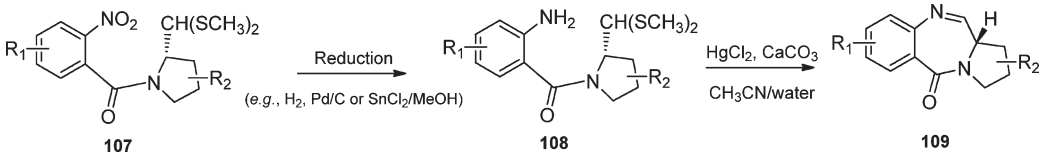
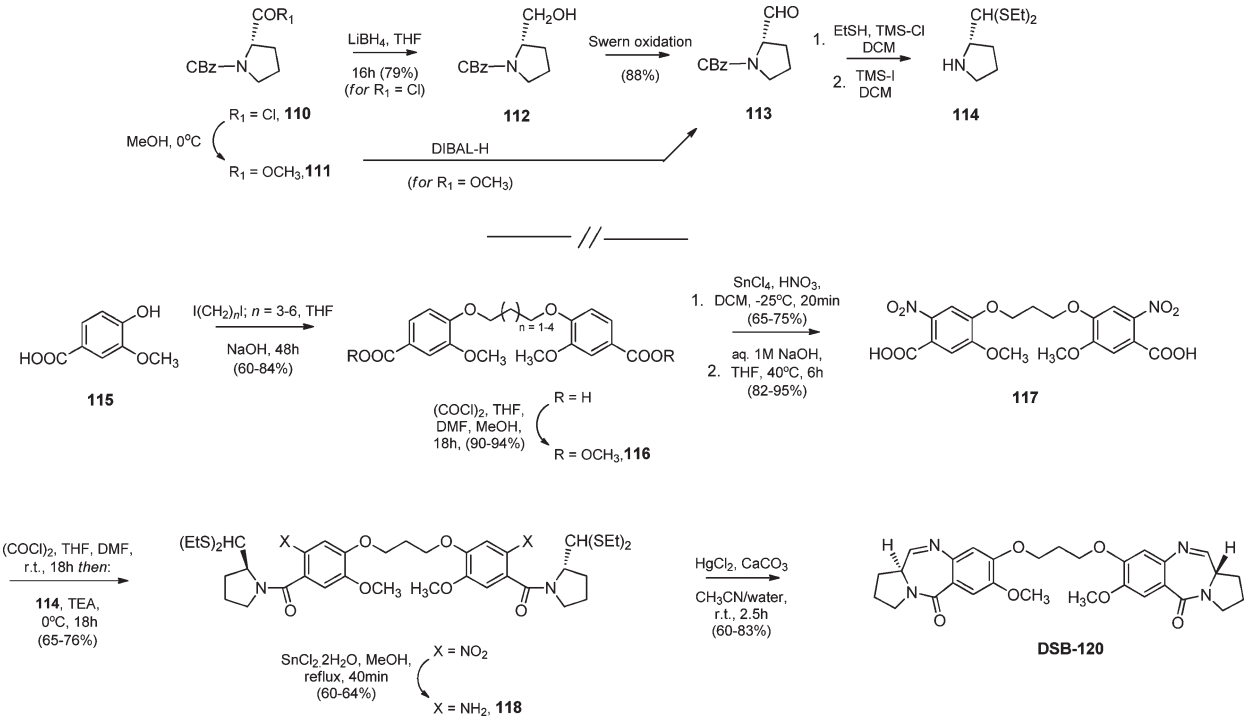
Scheme 21. Cyclization of *N*-(2-Aminobenzoyl)pyrrolidine-2-carboxaldehyde Diethyl Thioacetals to Provide PBD Imines



Scheme 22. Synthesis of the PBD Dimer DSB-120 Using the Amino Thioacetal Approach[101]



It is based on the concept of protecting a pre-C11-position aldehyde group as a diethyl thioacetal functionality. Protection of the aldehyde can either be carried out after A- to C-ring coupling (i.e., to give *N*-(2-nitrobenzoyl)pyrrolidine-2-carboxaldehydes of type **107**, Scheme 21), or a C-ring building block already carrying a diethyl thioacetal group can be coupled to an A-ring in a convergent manner. Apart from the versatility of introducing the thioacetal group either pre- or post-A/C-ring coupling, other advantages include compatibility with a wide range of reaction conditions and no reported racemization at the C11a-position of the final PBD structures. In particular, the robustness of the thioacetal protecting group allows many significant modifications to be carried out to nitro thioacetal scaffolds of type **107**. Mercury(II) chloride ($HgCl_2$) has been the reagent of choice for removing the thioacetal group and effecting cyclization, although the formation of mercuric salts can make the isolation of products difficult and can adversely affect yields.[98] This problem, along with the stench of ethyl mercaptan released during the protection/deprotection steps, and the fact that $HgCl_2$ is toxic and dangerous for the environment are all disadvantages of this method. However, more recently, $FeCl_3 \cdot 6H_2O$[99] or bismuth triflate[100] have been successfully used for the deprotection of thioacetals to overcome some of these problems.

DSB-120, the first C8/C8′-linked PBD dimer to be reported, was synthesized by Thurston and co-workers using this methodology in a convergent approach based on the dimeric intermediate **118** derived from the thioacetal **114** (Scheme 22).[101] The C-ring precursor **114** was readily obtained from the N-protected L-proline acyl chloride derivative **110** in four steps. These studies also highlighted the advantage of using the acyl chloride intermediate **110** rather than the methyl ester **111** as a precursor to aldehyde **113**. In the case of methyl ester **111**, the aldehyde functionality can be directly produced via DIBAL−H reduction, although this method often provides only moderate yields due to incomplete reaction and/or the production of overreduced alcohol (i.e., **112**). However, the acyl chloride **110** can be reduced to the corresponding alcohol (**112**) in high yield (79%) and then converted to the aldehyde via Swern oxidation, a strategy that outperforms the more direct DIBAL−H reductive approach in large-scale preparations in terms of both yield and purity. The aldehyde **113** was then converted to the *N*-unprotected pyrrolidine thioacetal intermediate **114** in two steps. Next, the dimer core **117** was constructed by tethering two units of vanillic acid (**115**) using diiodoalkanes of various length in the presence of potassium carbonate.[102] Subsequent coupling of the propyl-linked dimer core (**117**) to **114** afforded **118**, the synthetic

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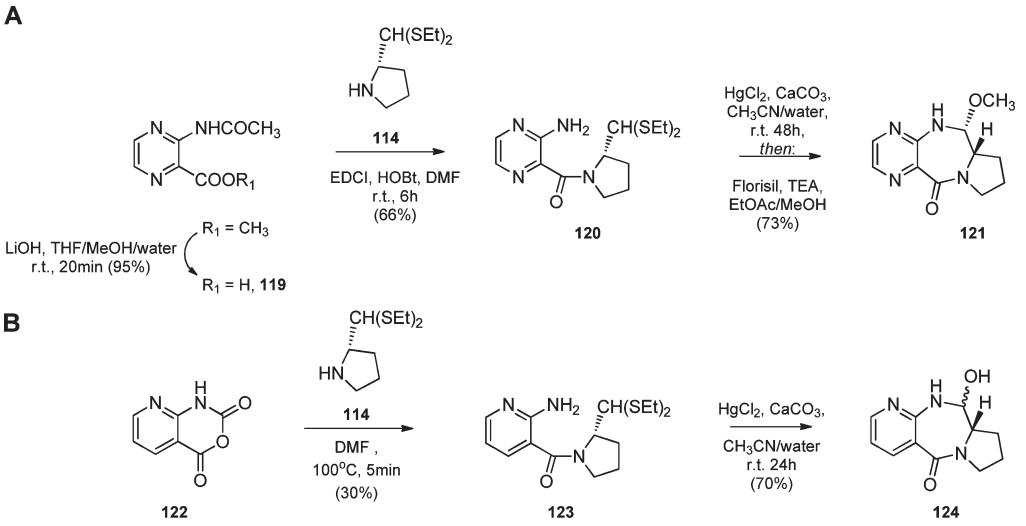
Scheme 23. Use of the Thioacetal Approach to Synthesize A-Ring-Modified PBDs[103]



equivalent of the pre-PBD *synthon* described in section 3. Reduction of the previously installed nitro groups with tin chloride dihydrate ($SnCl_2 \cdot 2H_2O$) afforded the bis-amino thioacetal **118**, which spontaneously cyclized to the bis-imine DSB-120 upon treatment with $HgCl_2$, maintaining the "*S*" stereochemistry at the C11a/C11a′ positions (Scheme 22).

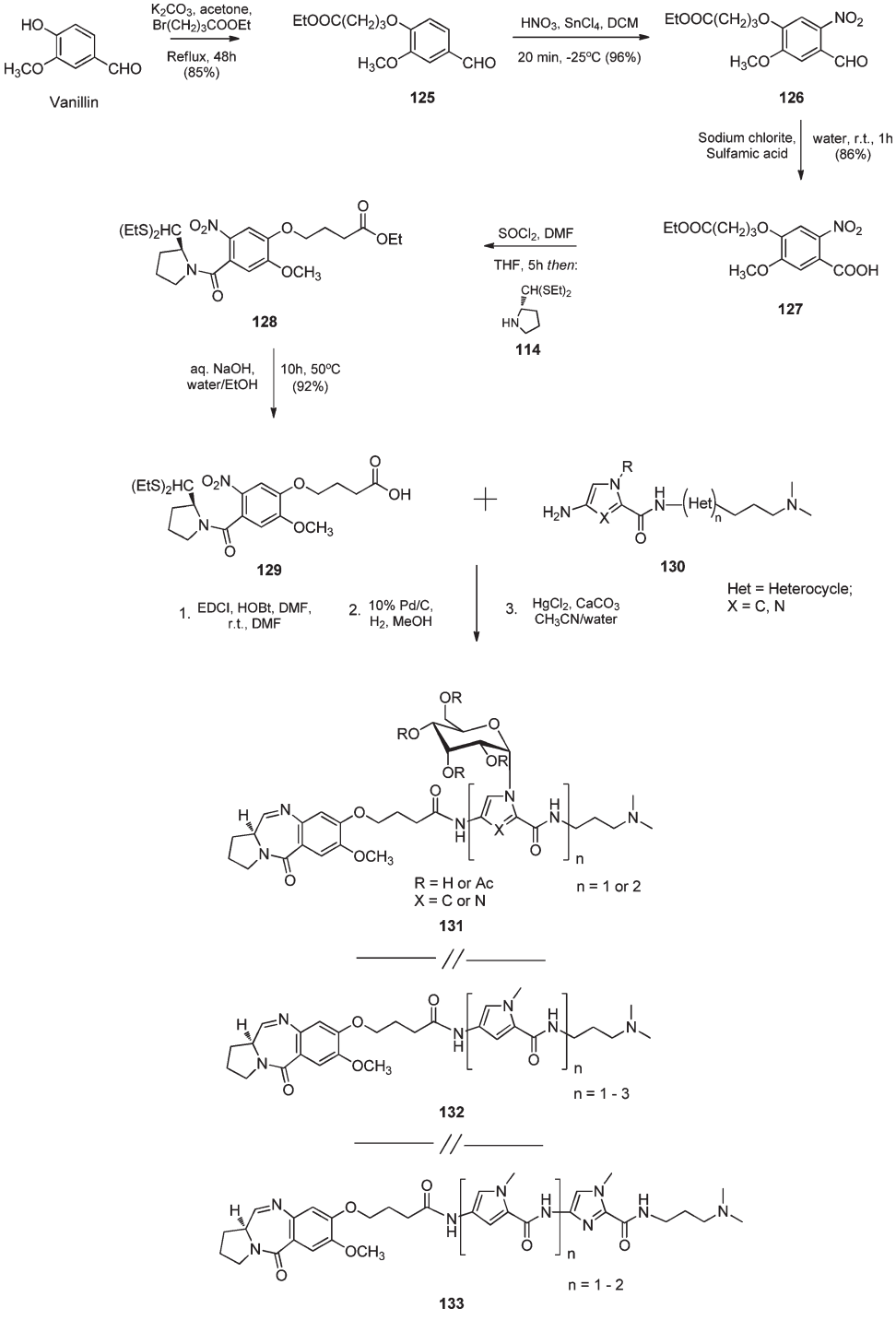
The thioacetal C-ring building block **114** has also been used to synthesize several A-ring-modified analogues of DC-81.[103] For example, Scheme 23A shows a convergent synthetic route involving the coupling of **114** to the A-ring precursor methyl 3-(acetylamino)-2-pyrazinecarboxylate (**119**) to give the amino thioacetal intermediate **120**. Treatment with $HgCl_2/CaCO_3$ afforded the carbinolamine methyl ether **121**, which was obtained exclusively as the 11*R*,11a*S* diastereomer after purification by flash chromatography using TEA-treated Florisil and EtOAc/MeOH as solvent. A similar approach was used to prepare a pyridine analogue (**124**) as shown in Scheme 23B starting from **114** and the pyridinic isatoic anhydride **122** (see also section 4.1.3). Other aza-heterocyles have been coupled to **114** according to the route shown in Scheme 23 to produce a variety of A-ring-modified PBDs.[103] Cytotoxicity and DNA-binding studies showed that none of these changes to the PBD A-ring significantly improved biological activity compared to DC-81.[103]

There have been numerous synthetic studies linking the PBD ring system to various combinations of pyrrole and imidazole heterocycles in order to extend their base-pair span within the DNA minor groove and to potentially enhance their recognition of specific base-pair sequences. DNA-alkylating (e.g., (+)-cyclopropapyrroloindole (CPI))[104] and intercalating (e.g., naphthalimide)[105] moieties have also been linked to PBDs to afford a variety of hybrid molecules. This interest in combining the PBD scaffold with other types of DNA minor-groove binding agents has been driven by the potential use of such molecules to target specific gene sequences for therapeutic purposes.[106] Such agents could be used to selectively bind to known promoter or response elements in the genome to inhibit the transcription of genes involved in a range of pathologies, including cancer. For example, one such PBD conjugate (GWL-78, **316**, n = 2, Scheme 45, section 5.5) has been shown to bind to inverted CAAT-box sequences, thus inhibiting interaction of the transcription factor NF-Y.[27]

Lown and co-workers have reported synthetic studies on a range of C8-linked conjugates with the PBD joined to pyrrole,[107] glycosylated pyrrole,[108] imidazole,[109,110] and imidazole/pyrrole[111] moieties with the objective of improving both water solubility and DNA sequence selectivity[112] (Scheme 24). Using a convergent approach, the pyrrole/imidazole units were attached to the nitro thioacetal pre-PBD building block **129**. This equivalent of the pre-PBD *synthon* (see Scheme 1) features frequently in the syntheses of many reported C8-linked PBD hybrids. It is prepared by coupling the C-ring thioacetal **114** to intermediate **127**, which is obtained from vanillin in three steps, followed by ester hydrolysis. Attachment of intermediate **129** to the heterocyclic units of type **130** utilized typical amide coupling conditions (e.g., EDCI and HOBt). Reduction of the nitro group followed by B-ring closure with H_2/Pd and $HgCl_2$, respectively, generated conjugates of type **131**−**133**. The cytotoxicity of these compounds was found to be influenced by the number and position of the pyrrole and imidazole rings. For the glycosylated PBD conjugates (**131**), differential activity in the NCI 60 cell line screen was observed, depending on whether the sugar moiety was protected or not.

The same DNA-targeting strategy was used by Lown and co-workers to synthesize C2-linked PBD−polypyrrole conjugates[113] and C2/C2′-linked PBD dimers (Scheme 25).[111,114] In these cases, the pre-C11−aldehyde was protected after A- and C-ring coupling to give intermediate **134**. This was followed by DMSO-mediated oxidation of the pro-C2−hydroxy group using acetic anhydride as activating agent to furnish the pro-C2−ketone **135** in 55% yield. This was used in a Wittig olefination reaction to give the C2-*exo*-acryl ester **136** exclusively as the *E*-isomer in 69% yield (see Table 3 for similar C2-olefination reactions). The next step involved strong base under refluxing conditions to saponify **136**. The resulting carboxylic acid (**137**) was successfully dimerized in the presence of amide-coupling reagents and 0.5 equiv of appropriate diamines to give **139** (n = 1−3). In addition, the C2-group was extended by coupling a β-alanine methyl ester to **137** followed by hydrolysis with 1 N

Scheme 24. Use of the Thioacetal Approach to Synthesize C8−Heterocyclic PBD Conjugates[112]



sodium hydroxide to afford the carboxylic acid **140**, which was then coupled to polypyrrole fragments of type **141**. These final intermediates were subsequently reduced and subjected to $HgCl_2$-mediated B-ring cyclization to afford the C2-linked PBD−polypyrrole conjugates of type **142**. In general, the C8-linked PBD−polypyrrole conjugates (**142**) had a higher cytotoxic potency than the C2/C2′-linked dimers (**139**) that are known to be inferior to C8/C8′-linked PBD dimers in terms of DNA interstrand cross-linking ability, binding affinity and cytotoxicity.[111]

Polyaminoalkyl moieties are known to reversibly interact with DNA and have been conjugated to PBDs in an attempt to modify their DNA-binding affinity and sequence-recognition properties. For example, Matsumoto and co-workers[115,116] joined the polyaminoalkanes of type **143** to the pre-C7−fluoro nitro thioacetal intermediate **144** through a nucleophilic aromatic substitution reaction (Scheme 26). In this case, the *para*-nitro group most likely assisted displacement of the fluorine due to its electron-withdrawing properties. After reduction of the nitro group of intermediates **145** with stannous dichloride, B-ring closure to

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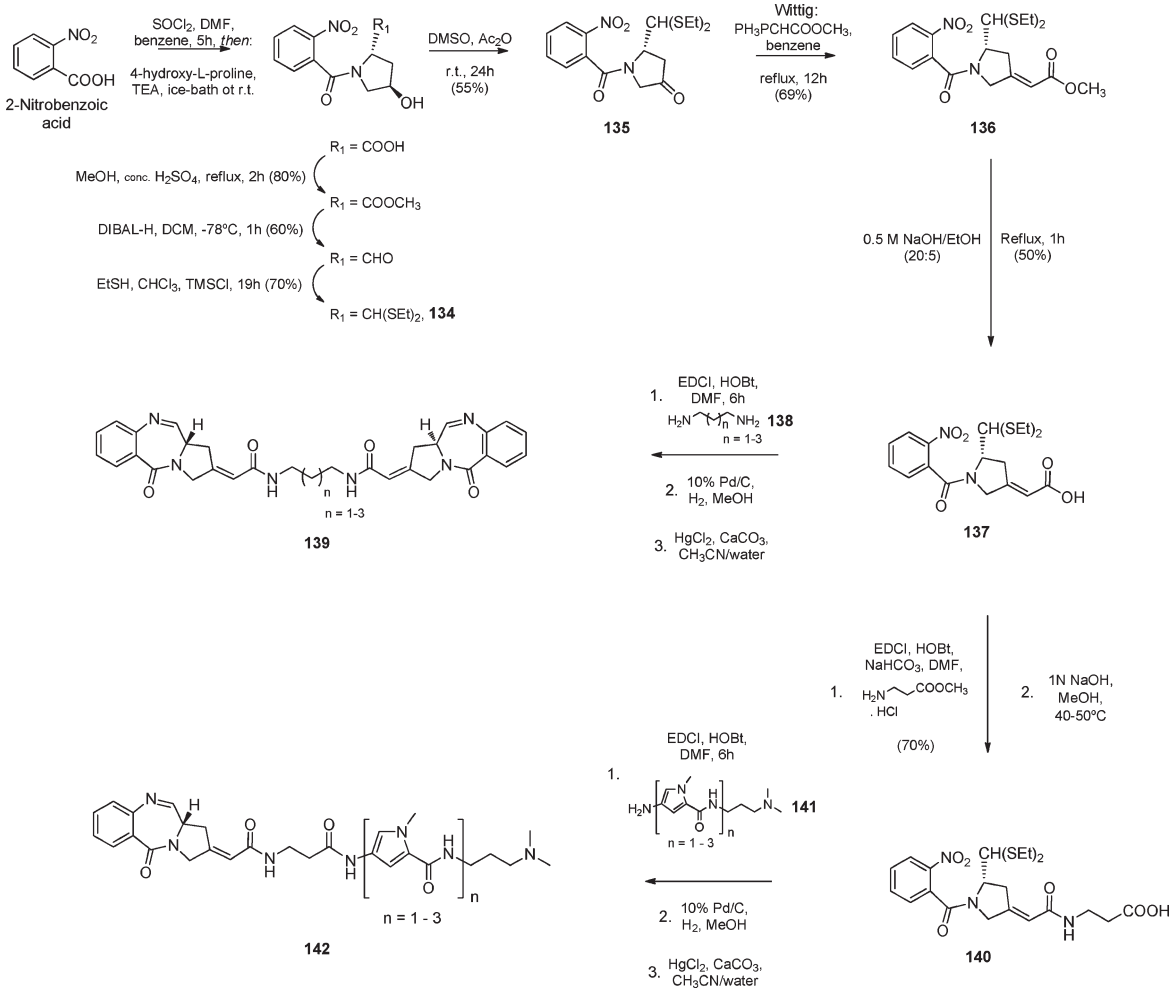
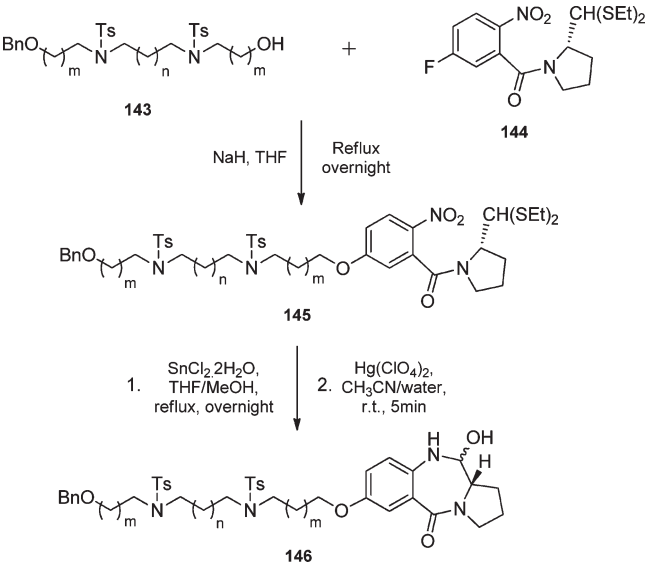
Scheme 25. Synthesis of C2/C2′-Linked PBD Dimers and C2-Linked PBD Polypyrrole Conjugates[111,114]



provide the PBD ring system (**146**) was achieved with mercury perchlorate [$Hg(ClO_4)_2$]. This dithioacetal deprotecting agent afforded carbinolamines of type **146** in yields of no higher than 46% and required a centrifugation step to separate the mercury salts from the organic material. Thus, $Hg(ClO_4)_2$ does not compare favorably with the more commonly used $HgCl_2$ as a cyclization reagent, and in addition is even more toxic and dangerous to the environment. Presumably the tosyl groups in the C7−polyaminoalkyl chain had to be removed prior to biological evaluation, although no details of this or any biological data were reported.

In a further attempt to improve the DNA-sequence targeting properties of PBDs, Zhilina and co-workers synthesized a C8-linked PBD−oligonucleotide conjugate.[117] The triplex-forming oligonucleotide (TFO) component (**149**) contained 23 nucleotides and was designed to target a polypurine tract in the major groove of DNA by forming a triple-helix adduct through Hoogsteen H-bonding. Furthermore, the PBD component and linker (**148**) were designed to reach through to the minor groove where formation of a covalent adduct could occur, thus potentially stabilizing the triple helix. The PBD intermediate **148** was synthesized from **147** according to the strategy shown in Scheme 27, and containing a displaceable *p*-nitrophenyl ester group at the end of a C8−butyric side chain. Palladium-catalyzed

Scheme 26. Synthesis of C7-Linked PBD−Polyaminoalkyl Conjugates[115,116]



removal of the N10−Alloc group (see section 5.5) gave the PBD imine **148**, which was coupled to the TFO unit **149** that

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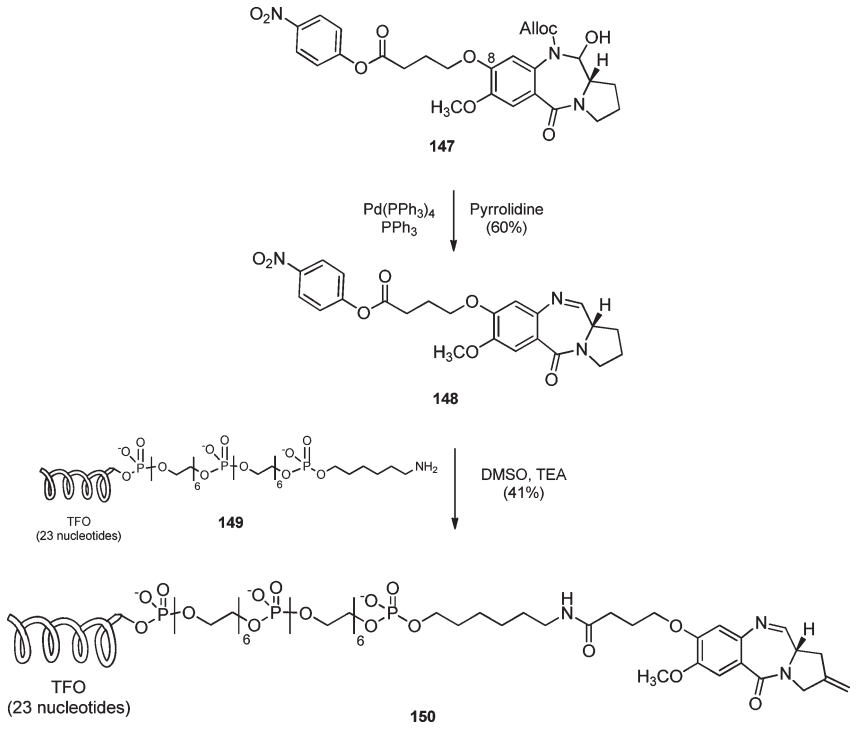
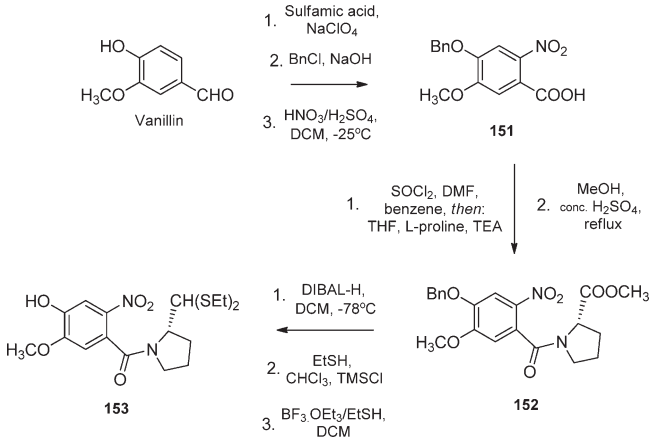
Scheme 27. Synthesis of a C8-Linked PBD−Oligonucleotide Conjugate Potentially Capable of Interacting in Both the Minor and Major Grooves of DNA[117]



consisted of the oligonucleotide linked to a hexa(ethylene glycol) triphosphate alkylamine chain suitable for amide coupling to the labile ester of **148**. Final HPLC purification and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis revealed the presence of all components of the PBD−TFO conjugate **150**, which was isolated in 41% yield.

Kamal and co-workers have combined PBDs with several therapeutically relevant scaffolds in an effort to improve biological activity. These include naphthalimide,[105,118,119,144] methanesulphonate,[120] chrysene,[121] pyrene,[122,123] acridone/acridine,[124] benzimidazole,[125,126] anthraquinone,[127,128] flavone,[129] fluoroaryl pyrimidine,[130] fluoroquinolone,[131] azepane,[132] quinazolinone,[133] 6-chloropurine,[134] 1,2,4-benzothiadiazine,[135] 1,2,3-triazole,[136] triazolo[1,2,4]benzothiadiazine,[137] benzothiazole/benzoxazole,[138] anthranilamide,[139] G1 dendrimer,[140] anilino-substituted pyrimidine,[220] phenanthrylphenol,[221] 3,5-diaryl isoxazoline/isoxazole,[222] cinnamido,[223] and 2,5-diaryloxadiazole[224] moieties, all linked through the C8-position of DC-81. Most of these hybrids have been synthesized from the pre-C8−hydroxy intermediate **153**, obtained in eight steps from vanillin (Scheme 28). The typical synthetic approach involved coupling the second therapeutically relevant moiety to the pre-C8-position of **153**, followed by nitro reduction and final $HgCl_2$-mediated B-ring cyclization. The methodologies used to attach the additional moieties to the C8-position can be divided into four subgroups as shown in Table 2A−D. The first subgroup (2A) involves a pre-PBD unit of type **154** containing a C8-side chain with a carboxylic acid terminus. This was coupled to amine-containing ancillary moieties, and the products were reduced and cyclized to give C8-linked PBD hybrids of type **155** and **156**. The

Scheme 28. Synthesis of the *N*-(2-Nitrobenzoyl)pyrrolidine-2-carboxaldehyde Diethyl Thioacetal 153, a Key Building Block for the Construction of DC-81-based C8-Linked PBD Hybrids[105]

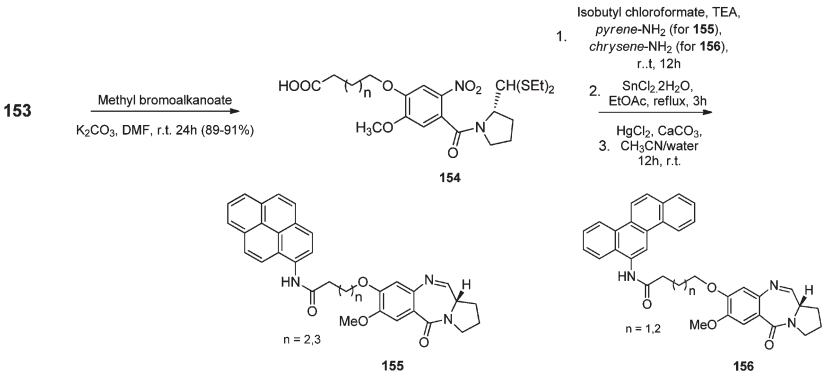


second subgroup (2B) is the reverse of this, with the synthesis based on a pre-PBD unit of type **157** containing a C8-side chain with an amino terminus. This was coupled to carboxylic acid-containing ancillary moieties to provide intermediates that were reduced and cyclized to give PBD conjugates **158**−**160**. In the third subgroup (2C), a haloalkyl moiety was initially linked to the ancillary unit that was then attached to the pre-C8-position of **153** in the presence of base to effect an S_N2 reaction. The resulting intermediate **161** was then converted to conjugates of type **162**−**168** using standard methodology. In the final

dx.doi.org/10.1021/cr100120f |*Chem. Rev.* 2011, 111, 2815–2864

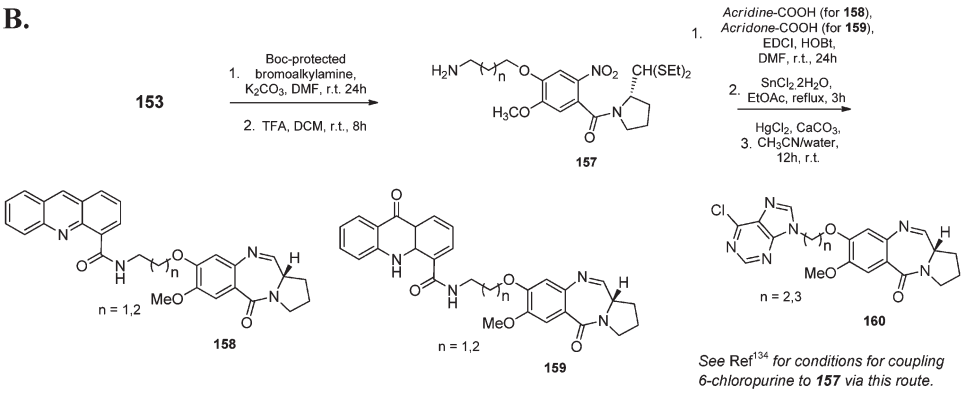
Table 2. PBD C8-Hybrid Molecules Synthesized by Kamal and Co-workers

A.



Ref[123] Ref[121]

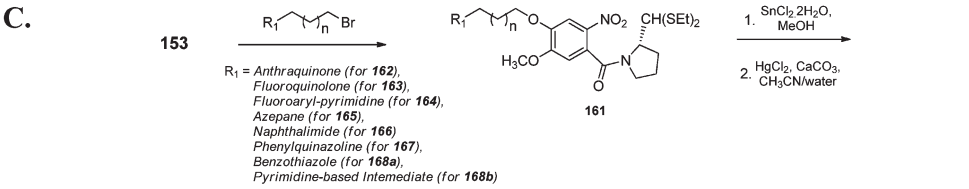
B.

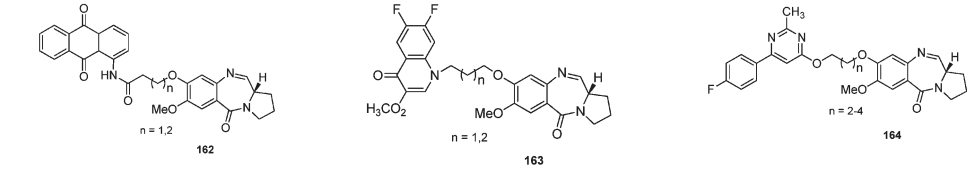


See Ref[134] *for conditions for coupling 6-chloropurine to* ***157*** *via this route.*

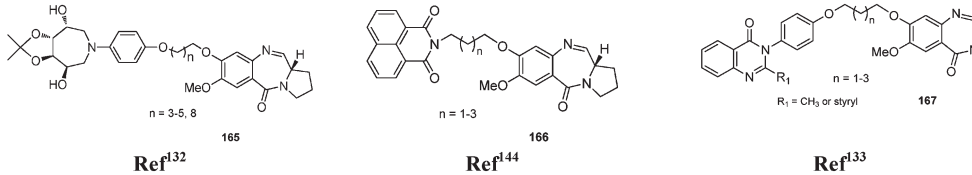
Ref[124] Ref[124] Ref[134]

C.

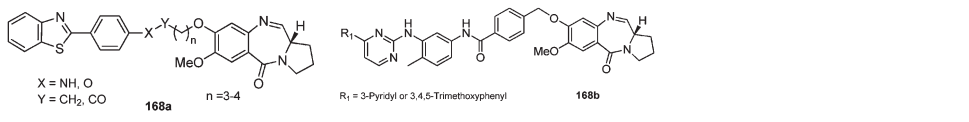




Ref[127,128] Ref[131] Ref[130]



Ref[132] Ref[144] Ref[133]

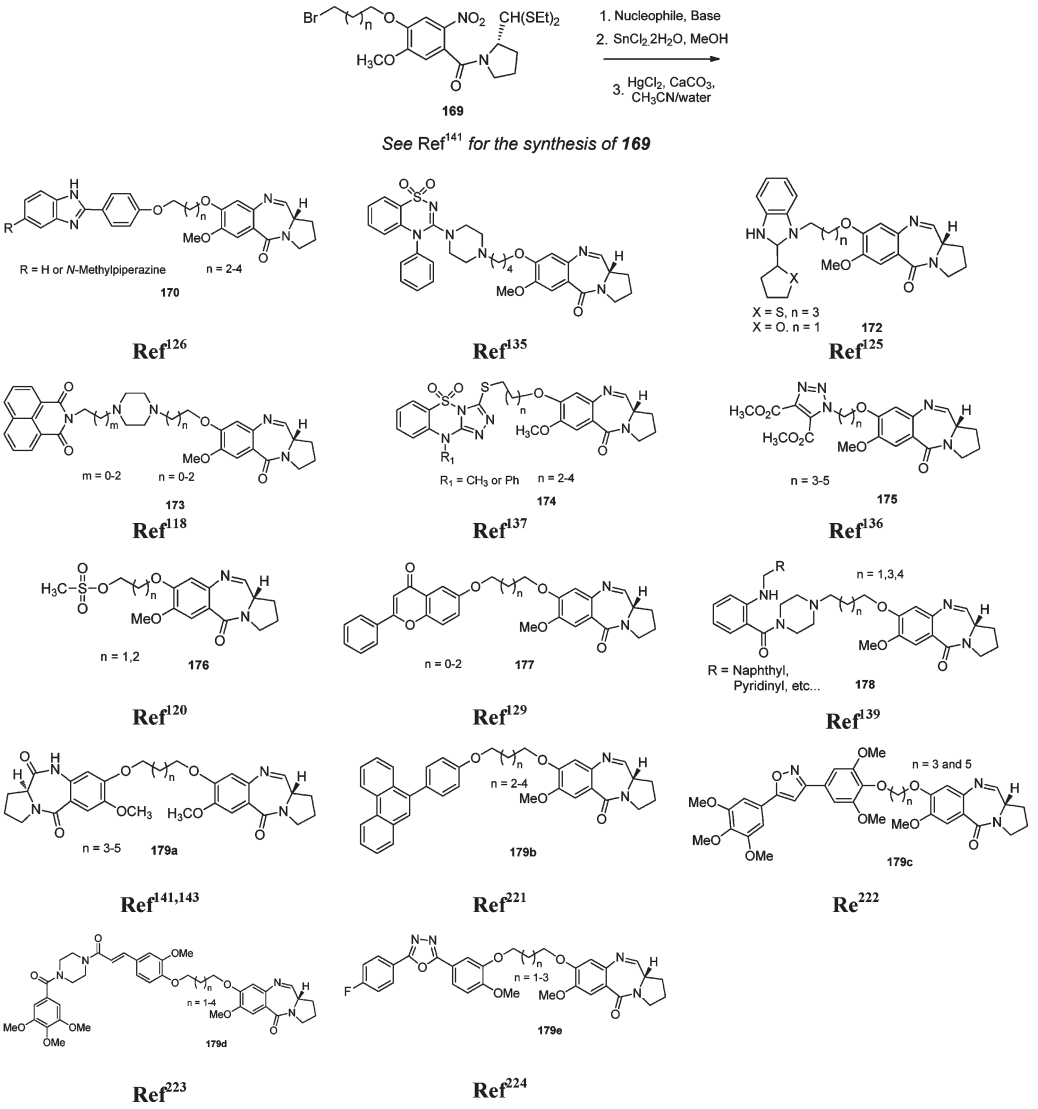


Ref[138] Ref[220]

dx.doi.org/10.1021/cr100120f |*Chem. Rev.* 2011, 111, 2815–2864

Table 2. Continued

D.



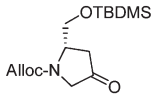
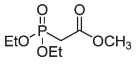
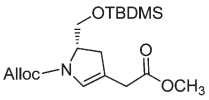
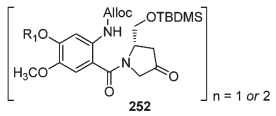
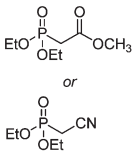
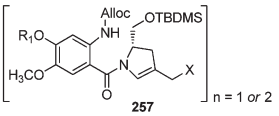
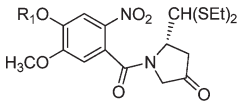
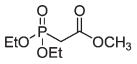
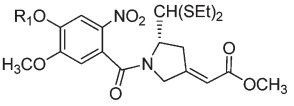
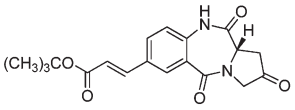
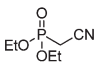
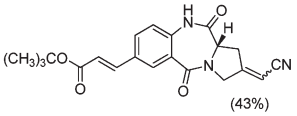
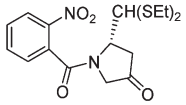
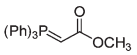
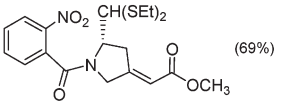
subgroup (2D), intermediates of type **169** were prepared from vanillic acid methyl ester in four steps.[141] The bromide at the terminus of the side chain was then substituted with nucleophilic ancillary moieties in the presence of base to provide intermediates that could be reduced and cyclized to give hybrids of type **170**−**179a**−**e**.

In general, this approach of hybrid formation by Kamal and co-workers appears to improve cytotoxicity and DNA-binding affinity of PBDs to some extent. For example, the PBD−benzimidazole hybrids of type **170** were particularly cytotoxic in the melanoma panel of the NCI 60 cell-line screen and also stabilized duplex DNA to a significant level as determined by thermal denaturation studies (ΔT_m = 22.6 °C after 18 h incubation at 37 °C). More recently, DNA adducts of a *N*-methylpiperazinyl-substituted analogue of **170** have been investigated by NMR, confirming that interaction occurs in the minor groove.[142] Thioacetal intermediates of type **169** have also been used for the synthesis of mixed N10−C11 imine−amide C8/C8′ PBD dimers (e.g., **179**) using a C8−hydroxy PBD dilactam as the ancillary moiety.[141,143] These dilactam/imine dimers are unable to cross-link double-stranded DNA but can still form monoadducts, with **179a**(n = 5) providing significant DNA-duplex stabilization.[143]

C8-linked PBD hybrids containing enediyne (e.g., **181**)[15] and indole (e.g., **183**)[16] moieties have been reported by Wang and co-workers (Scheme 29). The interesting feature of these syntheses is that, in both cases, the ancillary moiety was added to a fully formed DC-81 molecule containing an electrophilic N10−C11 imine functionality. The DC-81 building block was synthesized from a PBD dilactam precursor using the isatoic anhydride approach (see section 4.1.3).[65] Conjugation of the ancillary moieties was then achieved by substitution reactions involving the C8−OH of DC-81 and alkyl bromide functionalities in the enediyne and indole building blocks. From a practical

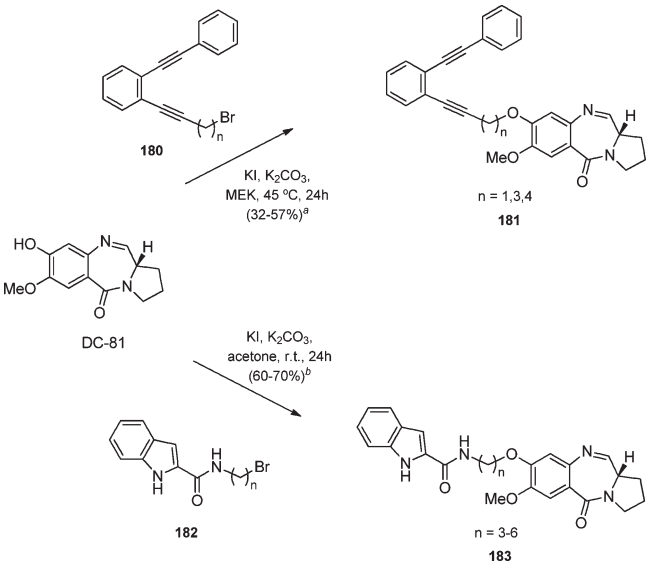
dx.doi.org/10.1021/cr100120f |*Chem. Rev.* 2011, 111, 2815–2864

Table 3. Comparison of Different Methods of C2-Olefination Used to Produce C2-*exo*- and C2/C3-*endo*-Unsaturated PBDs

Reference	Substrate	Ylid Precursor	Base/ Equivalents	Solvent/ Temperature	Time	Product (Yield)
Thurston and Howard[171]			NaH (3.5 Equiv.)[a]	THF 0°C (ice-acetone)[a]	16h[a]	 (63%)
Gregson *et al*[168,171]	 **252** n = 1 *or* 2; n = 1, R_1 = Bn, n = 1, R_1 = CH_3, n = 2, R_1 = $(-CH_2-)_3$		NaH (2.5 Equiv.)	THF Room Temperature	16h	 **257** n = 1 *or* 2; n = 1, R_1 = Bn, X = CO_2CH_3 or CN (80 and 63%); n = 1, R_1 = CH_3, X = CO_2CH_3 (73%); n = 2, R_1 = $(-CH_2-)_3$, X = CO_2CH_3 (79%)
Kamal *et al*[145]	 R_1 = CH_3 or Bn		NaH (2.0 Equiv.)	THF 0°C	2h	 R_1 = CH_3 or Bn (83 and 80%)
Addicks *et al*[33]			BuLi (2.2 Equiv.)	THF -50°C	12h[b]	 (43%)
Reddy *et al*[111]			---	Benzene reflux	12h	 (69%)

[a] Initial 16-h treatment at room temperature with 2.1 equiv of NaH gave mainly the C2-*exo*-product. The reaction was then transferred to another flask and exposed to an additional 1.4 equiv of NaH for a further 40 min at 0 °C to afford the *endo*-product. [b] The reaction (initially at −50 °C) was allowed to warm to room temperature over 3 h; stirring was then continued for 12 h at room temperature.

Scheme 29. Synthesis of C8-Linked PBD Hybrids Through Addition of the Ancillary Unit to a Fully-Formed DC-81 Molecule[15,16] *a,b*

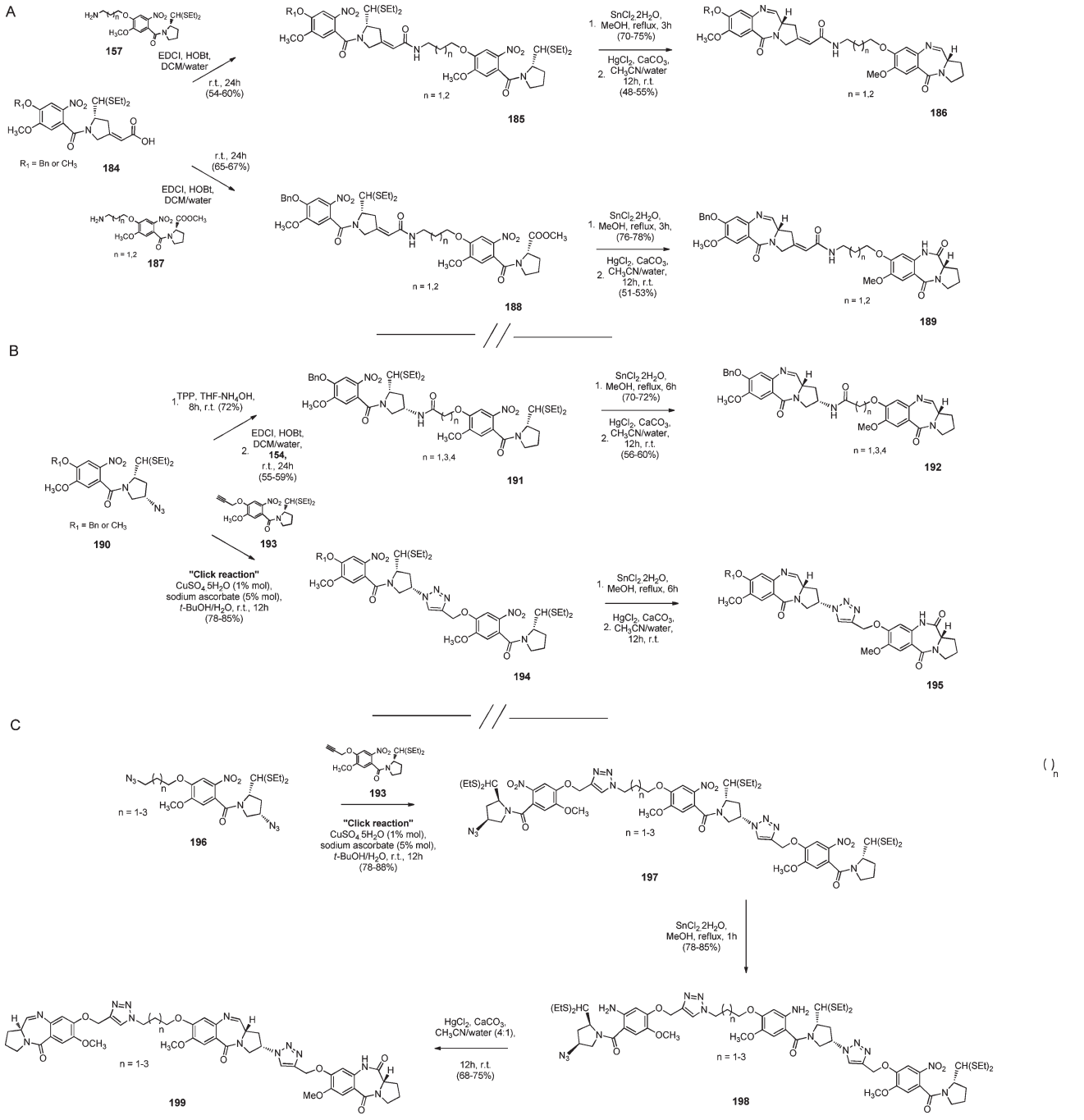


[a] Reaction conditions according to Hu and co-workers.[15] [b] Reaction conditions according to Wang and co-workers.[16]

standpoint, these syntheses demonstrated that the N10−C11 imine functionality of a PBD can withstand conjugation conditions based on potassium iodide and potassium carbonate in acetone or methyl ethyl ketone (MEK). The apparent robustness of the N10−C11 imine functionality under these conditions suggests that other PBD scaffolds may not require N10−C11 protection during a multistep synthesis. This could provide altenative synthetic strategies to the currently accepted approach involving N10−C11 protection through to the end of a synthetic pathway.

Kamal and co-workers have reported the synthesis of C2/C8-linked PBD dimers via the thioacetal approach using an intermediate of type **184** (Scheme 30).[145] This intermediate contains a C2-*exo*-acrylic acid moiety and was obtained using an approach similar to that described in Scheme 25. The free C2-carboxylic acid group of **184** enabled coupling with C8−alkylamine intermediates of type **157** (Table 2) and **187** to form C2/C8-linked intermediates (**185** and **188**) which, after reduction and B-ring cyclization, afforded the C2/C8 cross-linking and monoalkylating asymmetrical PBD dimers **186** and **189**, respectively. Similarly, compounds of type **134** (Scheme 25) have been converted through mesylation, substitution with sodium azide and inversion of C2-configuration, into C2-azido derivatives such as **190** (Scheme 30B). The C2-azido functionality can then be selectively reduced with triphenyl phosphine (TPP) in the presence of ammonium hydroxide in THF to afford the C2-amine. This can be coupled to **154** using standard coupling conditions followed by further elaboration to provide C8/C2-linked PBD dimers of type **192**.[146] Alternatively, dimerization can be conducted via dipolar cycloaddition with the C8−alkyne **193** (Scheme 30B).[147] In this example of "click" chemistry, a 1,2,3-triazole ring is produced that connects the two PBD units together (i.e., **194**), ultimately leading to C8/C8′−triazole-linked monoalkylating PBD dimers of type **195**. This "click" synthetic protocol has also been used to generate bis-1,2,3-triazolo-bridged PBD trimers of type **199** containing three imine functionalities in a single molecule.[225]

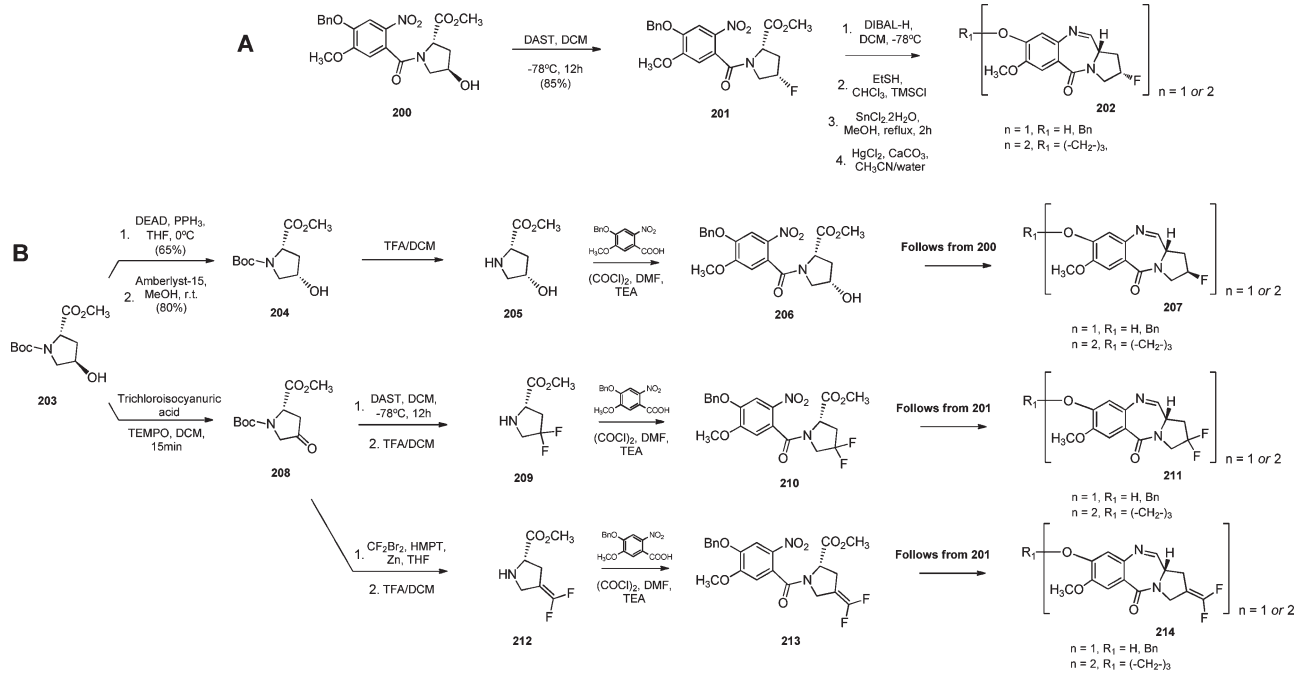
Scheme 30. Synthesis of C2/C8-Linked PBD Dimers[145−147]



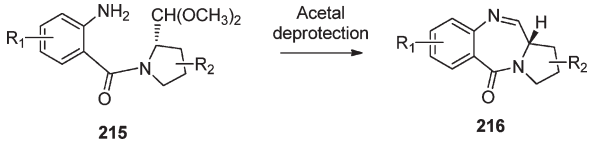
Finally, fluorine has been inserted at the C2-position of PBD monomers and dimers using the thioacetal approach. This has been achieved using the fluorinating agent diethylaminosulfur trifluoride (DAST) on pre-C2−hydroxy nitro thioacetal intermediates of type **200**, and C2−hydroxy (**204**) or C2−keto (**208**) proline derivatives.[148−150] The pre-C2(*S*)-fluoro intermediate **201** was obtained with inversion of configuration at the pre-C2-position by treating the pre-C2−hydroxy intermediate **200** with DAST (Scheme 31A). In addition, O'Neil and coworkers[151] have devised a strategy for obtaining C2(*R*)−fluoro isomers via an intramolecular Mitsunobu reaction on the pre-C2−hydroxy C-ring building block **203**. A lactone ring is initially formed using diethyl azodicarboxylate/triphenylphosphine (DEAD/PPh_3), and then opened with Amberlyst-15 to regain the hydroxyl group (**204**) on the same face as the ester group (Scheme 31B).[151,152] Alternatively, DAST has been used to prepare C2−difluoro-substituted PBD monomers and dimers (Scheme 31B). In this case, fluorination was carried out on the pyrrolidin-4-one C-ring precursor **208** prior to coupling to a 2-nitrobenzoic acid derivative. Finally, fluorinated analogues of SJG-136 (i.e., **214**, $n = 2$) have been synthesized from **208** using zinc carbenoids generated from dibromodifluoromethane (CF_2Br_2) in the presence of hexamethylphosphoric triamide (HMPT) and zinc.[150] This method converts the pre-C2−ketone

dx.doi.org/10.1021/cr100120f |*Chem. Rev.* 2011, 111, 2815–2864

Scheme 31. Synthesis of Monomeric and Dimeric PBDs with Fluorine Substituents at the C2-Position



Scheme 32. General Representation of the Cyclization of *N*-(2-Aminobenzoyl)pyrrolidine-2-carboxaldehyde Dimethyl Acetals to PBD Imines



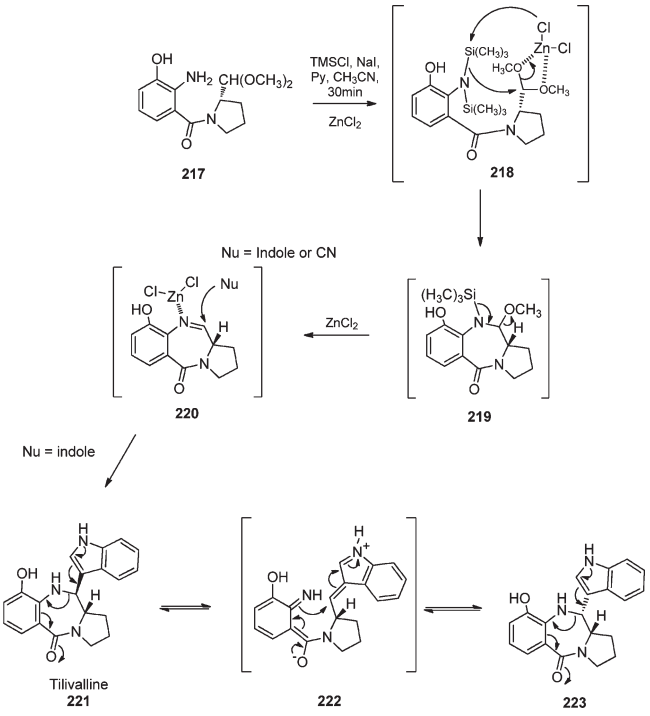
group into the difluorinated C2-*exo*-unsaturated functionality appearing in the C-ring of the final products.

5.3. Cyclization of *N*-(2-Aminobenzoyl)pyrrolidine-2-carboxaldehyde Dimethyl Acetals

In this method, the synthetic equivalent of the pro-PBD *synthon* (**D**, Scheme 1, section 3) has an electrophilic pre-C11−carbonyl group protected as a dimethyl acetal (e.g., **215**, Scheme 32). Upon deprotection under mild acidic conditions, the released aldehyde readily reacts with the A-ring amine to effect cyclization. Thurston and Bose[11] suggested that this approach may lead to racemization of the PBD C11a-position and carried out optical purity and cytotoxicity measurements on various batches of DC-81 synthesized through different cyclization approaches. It was established that DC-81 derived from an amino dimethyl acetal of type **215** was less biologically active than a sample synthesized by the amino thioacetal route (section 5.2).[11] Wilson and co-workers[153] synthesized a C8-epoxide-functionalized PBD using this method, and also highlighted the problem of C11a racemization (see Scheme 48, section 5.5).

Matsumoto and co-workers have also used the dimethyl acetal approach for the stereoselective synthesis of tilivalline (**221**), a naturally occurring PBD isolated from *Klebsiella pneumoniae* var. *oxytoca* (Scheme 33).[154,155] Chlorotrimethylsilane and zinc

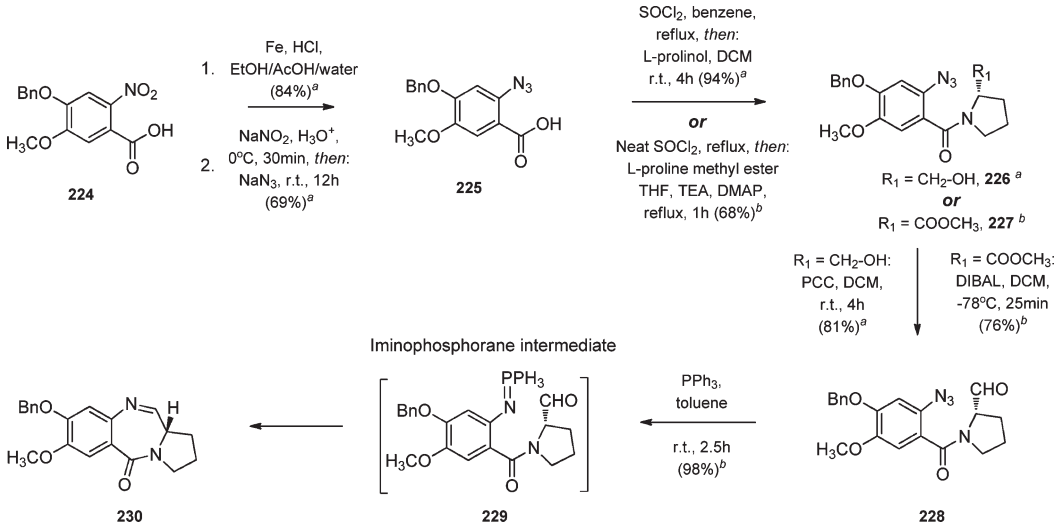
Scheme 33. Stereoselective Synthesis of Tilivalline Using the *N*-(2-Aminobenzoyl)pyrrolidine-2-carboxaldehyde Dimethyl Acetal Approach[154,157]



chloride ($ZnCl_2$) were used to promote cyclization of the dimethyl acetal intermediate **217** in a stereoselective manner. A mechanism for this reaction was proposed by Aoyama and Shioiri[156] who suggested formation of a complex between $ZnCl_2$

dx.doi.org/10.1021/cr100120f |*Chem. Rev.* 2011, 111, 2815–2864

Scheme 34. Synthesis of PBD Imines via Cyclization of *N*-(2-Azidobenzoyl)pyrrolidine-2-carboxaldehydes



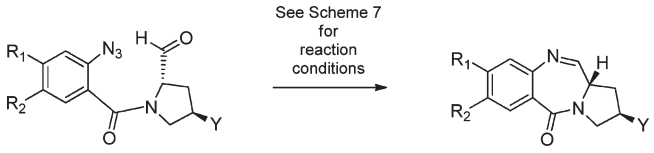
[a] Reaction conditions according to Molina and co-workers.[160] [b] Reaction conditions according to Eguchi and co-workers.[159]

and the dimethyl acetal group (i.e., **218**, Scheme 33). The PBD imine (**220**) was then subjected to a Mannich reaction with indole at −15 °C. In this step the $ZnCl_2$ acted as a Lewis acid, coordinating with the N10−nitrogen and increasing electrophilicity at the C11-position of **220**. This allowed stereoselective nucleophilic attack by indole to provide the Mannich base **221** known as Tilivalline. Interestingly, Matsumoto and co-workers observed that Tilivalline epimerized to **223** in the presence of $ZnCl_2$ if produced at higher temperature (55 °C) and a longer reaction time (24 h). Computational analysis[154] suggested that this C11-epimerization most likely occurred via the tautomeric intermediate **222**. This hypothesis was supported by a higher level of epimerization when cyanide was used as a nucleophile rather than indole, probably due to its smaller size.[157] Interestingly, the C11(*R*)-cyano derivative was found to be ∼100-fold more cytotoxic than the C11(*S*)−cyano epimer.[158]

5.4. Cyclization of *N*-(2-Azidobenzoyl)pyrrolidine-2-carboxaldehydes

In 1995 two different groups, Eguchi and co-workers[159] and Molina and co-workers,[160] independently described a new method for PBD synthesis involving consecutive Staudinger/intramolecular aza-Wittig reactions of *N*-(2-azidobenzoyl)-pyrrolidine-2-carboxaldehydes (Scheme 34). Both groups used this approach to synthesize C8−OBn-protected DC-81 (**230**, Scheme 34) with only minor differences in the reagents used. The azido group was first installed on the nitro-substituted A-ring precursor **224** via nitroreduction, diazotization, and azidation reactions (see section 4.1.2). Subsequent coupling to L-prolinol or proline methyl ester followed by oxidation or reduction steps, respectively, led to the common intermediate **228** containing a pre-C11a aldehyde group. The key synthetic step involved treatment of **228** with triphenylphosphine that led to in situ formation of the iminophosphorane **229** (Staudinger reaction). This underwent a spontaneous intramolecular aza-Wittig reaction at room temperature to afford the PBD ring system (**230**) in excellent yield. O'Neil and co-workers also explored the use of PPh_3 for the synthesis of PBDs via the aza-Wittig reaction.[161] However, they reported difficulties in separating the byproduct

Scheme 35. Synthesis of C2-Substituted PBD Imines via Cyclization of *N*-(2-Azidobenzoyl)pyrrolidine-2-carboxaldehydes



triphenylphosphine oxide (Ph_3PO) from the PBD by column chromatography, finding that purification was best achieved after using 0.5 equiv of 1,2-bis(diphenylphosphino)ethane (DPPE) rather than Ph_3PO.[162]

Kamal and co-workers have investigated the use of immobilized reagents for this approach, synthesizing DC-81 as an example (Scheme 34). Polymer-supported (PS) reagents (including PS-PPh_3) were employed throughout the synthetic pathway, with all intermediates purified by simple filtration and evaporation. This provided not only DC-81 but also an array of PBD imine analogues.[163]

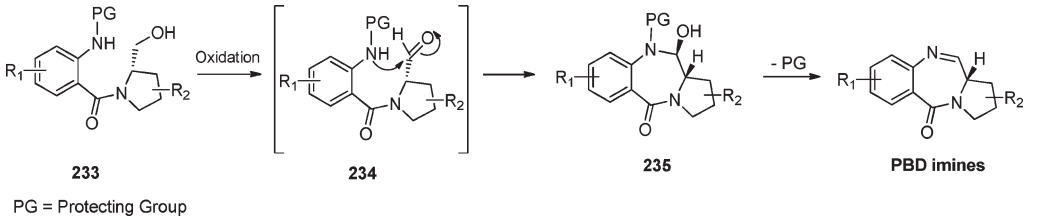
Kamal and co-workers have also successfully utilized *N*-(2-azidobenzoyl)pyrrolidine-2-carboxaldehydes of type **231** for the synthesis of PBD imines (Scheme 35).[51,58,60] Their methodology involved reduction of the azide group to an amine and is described in detail in section 4.1.2.

5.5. Oxidative Cyclization of *N*-(2-(Protected)aminobenzoyl)pyrrolidine-2-methanol Precursors

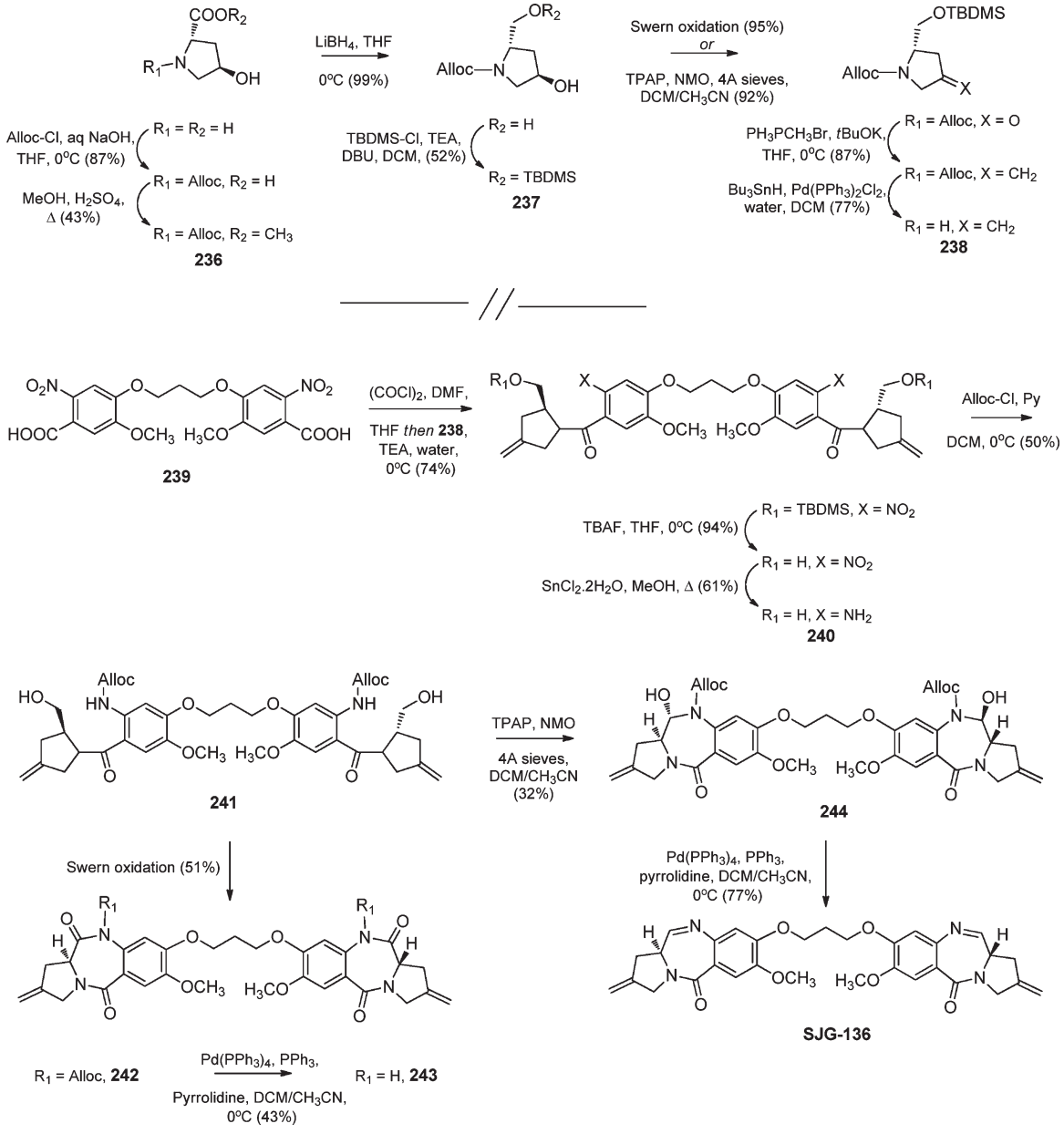
This method of cyclization initially produces the PBD ring system with a N10-protected N10−C11 carbinolamine moiety (Scheme 36). The N10-protecting group then has to be removed to generate the free PBD N10−C11 imine. Originally introduced by Fukuyama and co-workers[164] as part of their pioneering work on the palladium-catalyzed synthesis of aldehydes from

dx.doi.org/10.1021/cr100120f |*Chem. Rev.* 2011, 111, 2815–2864

Scheme 36. Oxidative Cyclization of *N*-(2-(Protected)aminobenzoyl)pyrrolidine-2-methanol Intermediates to N10-Protected PBD Carbinolamines



Scheme 37. Synthesis of the PBD Dimer SJG-136 Using the N10-Protection Approach[165]



thioesters, this was the first PBD synthesis to introduce the concept of using N10-protecting groups (i.e., N10-Alloc in their original synthesis). This was an important development that led to extensive investigations of the use of N10−carbamate protecting groups in PBD syntheses. Therefore, this method, which is now very popular for PBD synthesis, is frequently referred to as

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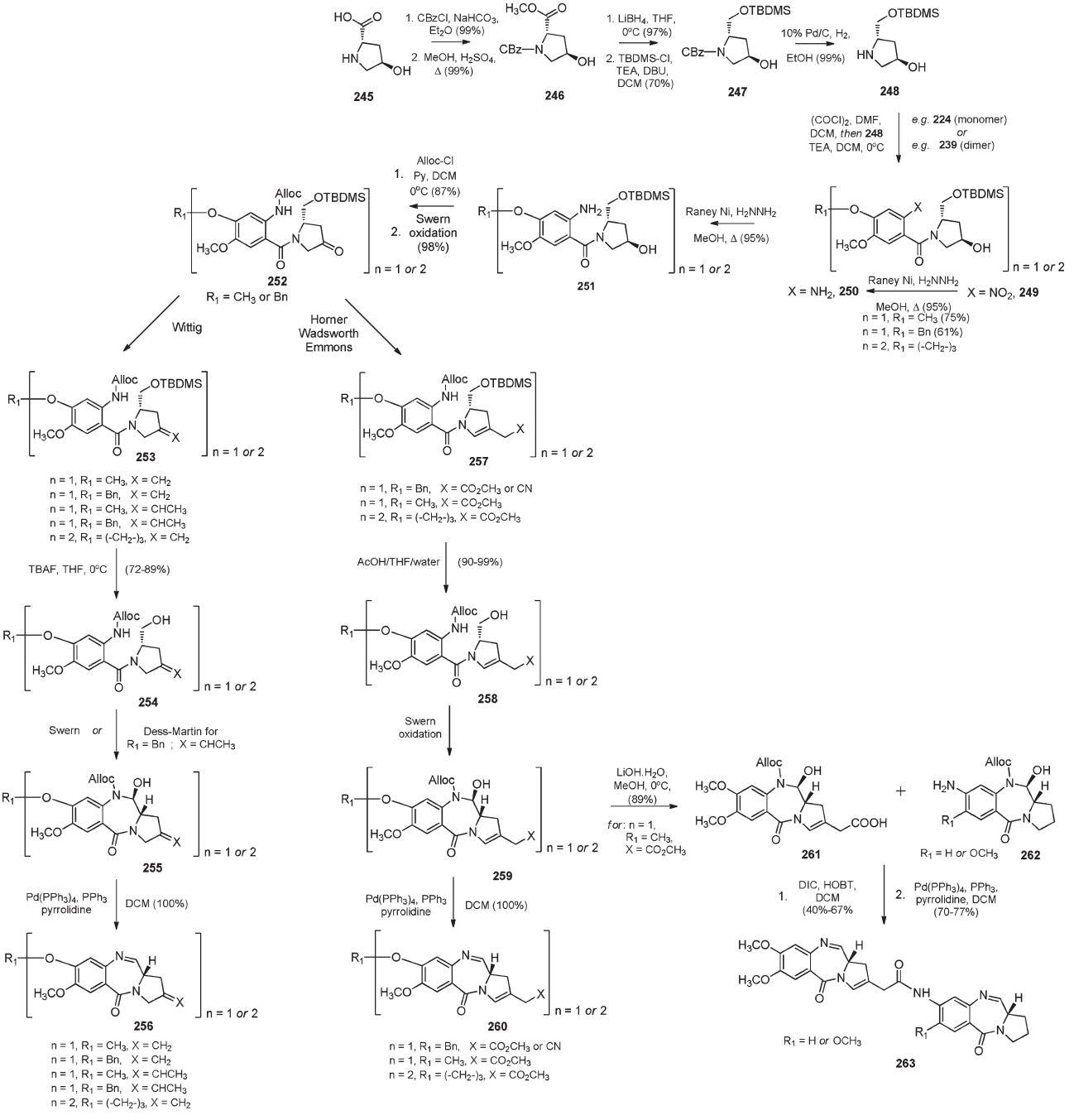
Scheme 38. Synthesis of Monomeric and Dimeric PBD Imines Using the N10-Protecting Approach[166,168−170]



the "Fukuyama approach" in the chemical literature. Generally, the nitrogen of a N10−carbamate should not be sufficiently nucleophilic to react with the adjacent pre-C2 aldehyde directly. However, in cases where this does occur, a favorable 7-*exotrig*-cyclization affords the cyclized N10-protected C11−hydroxy PBD system (e.g., **235**, Scheme 36). More significantly, the N10−carbamate protecting group prevents imine formation through elimination, thus allowing a range of A- and C-ring modifications to be carried out to the PBD scaffold without any degradation of the N10−C11 imine moiety. The N10-protecting group can then be removed at the last step of the synthetic route to release the N10−C11 imine.

Gregson and co-workers[165] have used this approach to synthesize SJG-136, a C2/C2′-*exo*-unsaturated C8/C8′-linked PBD dimer currently in clinical trials[7,8] (Scheme 37). The Alloc-protected intermediate **241** was obtained through a convergent route employing the C-ring building block **238** (synthesized from L-4-hydroxyproline over seven steps) and the A-ring dimer core **239**. The oxidative cyclization step occurred in a stereospecific manner in the presence of tetrapropylammonium perruthenate (TPAP) and *N*-methylmorpholine *N*-oxide (NMO) to give the bis-carbinolamine **244** in good yield. However, an attempt to use Swern conditions led to overoxidation to the PBD tetralactam **243**. This type of

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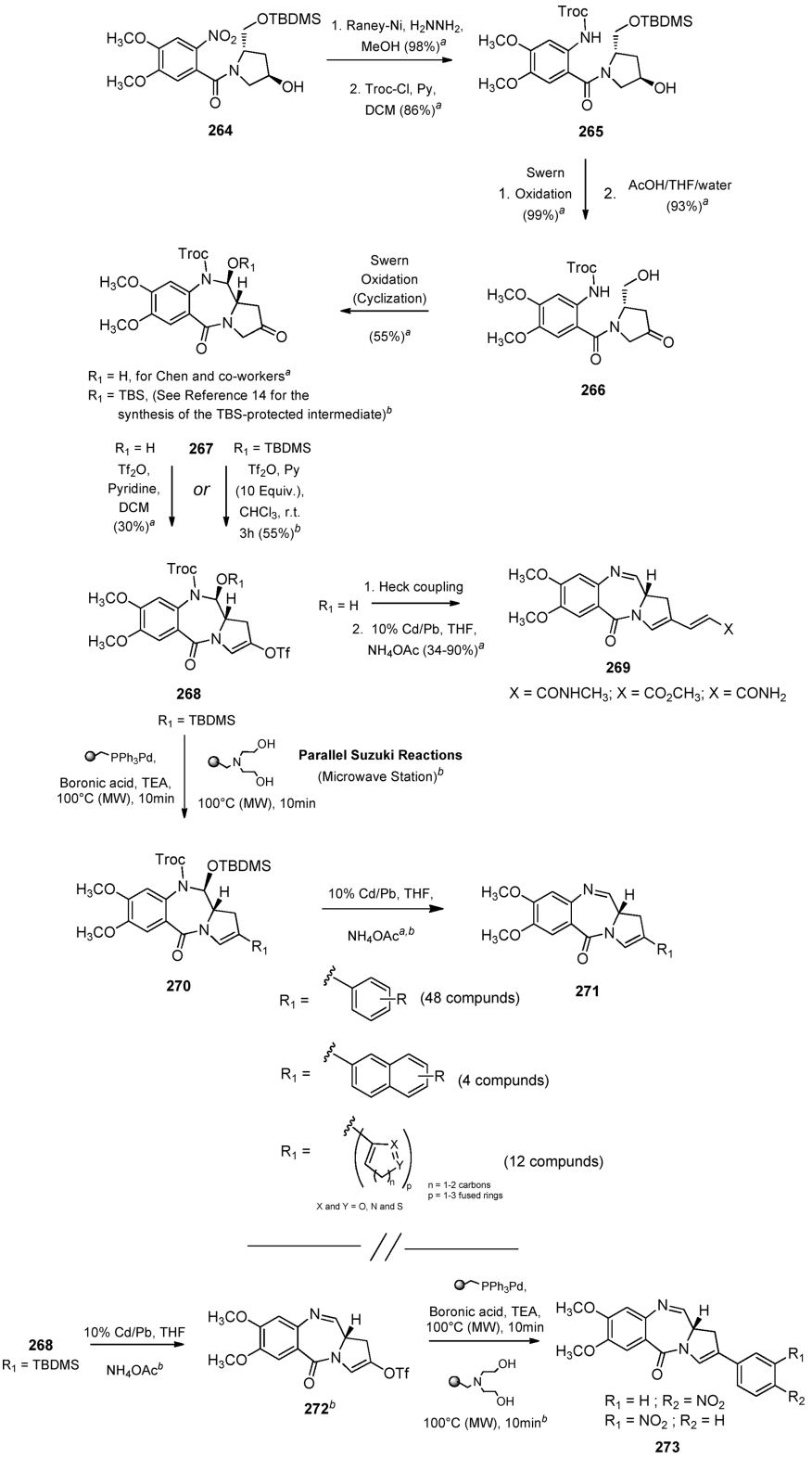
Scheme 39. Synthesis of C2-Modified PBD Imines Using Palladium-Catalyzed Coupling



[a] Reaction conditions according to Chen and co-workers.[87] [b] Reaction conditions according to Antonow and co-workers.[14,22]

overoxidation is a potential drawback for this oxidative ring closure (Scheme 37).[166,167]

Gregson and co-workers have also used this approach to synthesize some tomaymycin analogues to evaluate the influence of C2-*exo*-unsaturation on DNA-binding affinity and cytotoxicity (Scheme 38).[168,171] Compounds of type **256** were obtained via Wittig olefination of ketone **252** prior to B-ring closure, and these exhibited enhanced cytotoxicity and DNA-binding affinity

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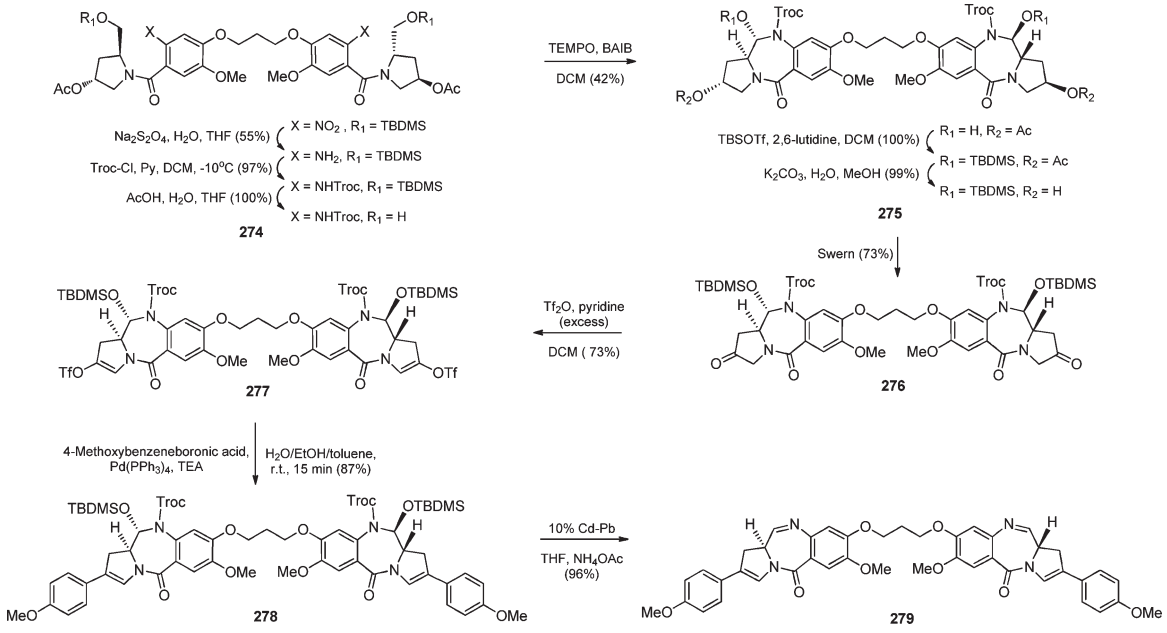
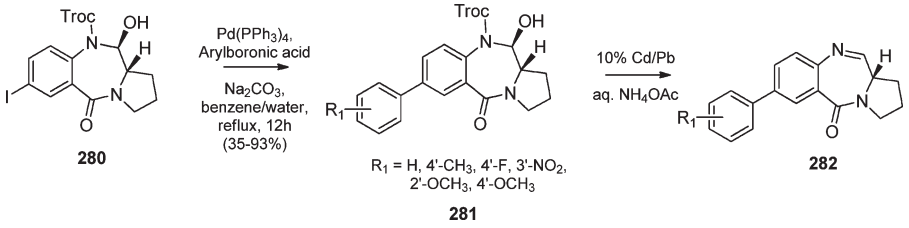
Scheme 40. Synthesis of C2/C2′−*Bis*-Aryl C8/C8′-Linked PBD Dimers[173,174]



Scheme 41. Synthesis of C7−Aryl PBD Imines[176]



compared to their saturated C-ring counterparts. The Horner−Wadsworth−Emmons reaction was also used to produce C2/C3-*endo*-unsaturated PBDs of this type. For example, the C2−ketone **252** was treated with different phosphonates in the presence of NaH.[169] Spontaneous migration of the initially formed *exo*-double bond into the C-ring occurred to provide monomeric and dimeric pre-PBD synthons of type **257** containing C2/C3-*endo* unsaturated C-rings. This methodology is comparable to the similar C2-olefination reactions shown in Table 3. The intermediates of type **257** were used to provide both monomeric[166,168] and dimeric[167,169] PBD imines[170] (i.e., **260**), all of which, like the C2-*exo*-unsaturated analogues (**256**), had enhanced DNA-binding affinity and cytotoxicity compared to their fully saturated C-ring analogues. The same authors also reported a synthesis of the *A*-C8/*C*-C2-linked asymmetrical PBD dimer **263**[170] obtained from the Alloc-protected building blocks **261** and **262**, derived from the same oxidative cyclization approach and joined using conventional amide coupling conditions.[166] Most importantly, Gregson and co-workers reported a five-step synthesis of the C-ring building block **248** from *trans*-4-hydroxy-L-proline.[166] This is a key intermediate for convergent PBD syntheses, and is highly versatile due to its chemical stability and the presence of a pre-C2−hydroxyl group, which can be elaborated into a C2-side chain at a later stage in the synthesis.

Chen and co-workers demonstrated application of the Heck reaction to PBD intermediates of type **268** to install C2−acrylyl substituents via the C2/C3-*endo*-enol triflate (Scheme 39).[87] The precursor (i.e., **264**) of the intermediate **266** required for the oxidative cyclization step was obtained by coupling the appropriate *o*-nitrobenzoic acid with the C-ring building block **248** using oxalyl chloride in the presence of a catalytic amount of DMF. The C-ring *endo*-unsaturation was generated by allowing the C2−ketone **267** to react with triflic anhydride and pyridine to give the thermodynamic product **268** in 30% yield, a useful starting material for palladium-catalyzed coupling reactions. It is noteworthy that, due to the use of Pd(0) catalyst in the coupling reaction, the *N*-trichloroethoxycarbonyl (Troc) group was employed as an alternative to the N10−Alloc protecting group. The Troc group can be efficiently removed with 10% Cd/Pb couple that can be easily prepared by stirring cadmium dust and lead oxide in 50% aqueous AcOH.[172] Although this deprotection reaction proceeds under mild conditions, the 10% Cd/Pb, which has to be used in excess, is very toxic and dangerous for the environment. Furthermore, it can remain in small quantities in final products and would be a concern in batches intended for clinical use. Interestingly, **269** was shown to have an average cytotoxic potency higher than anthramycin, demonstrating that the 9-OH group of the latter is not essential for enhanced activity.

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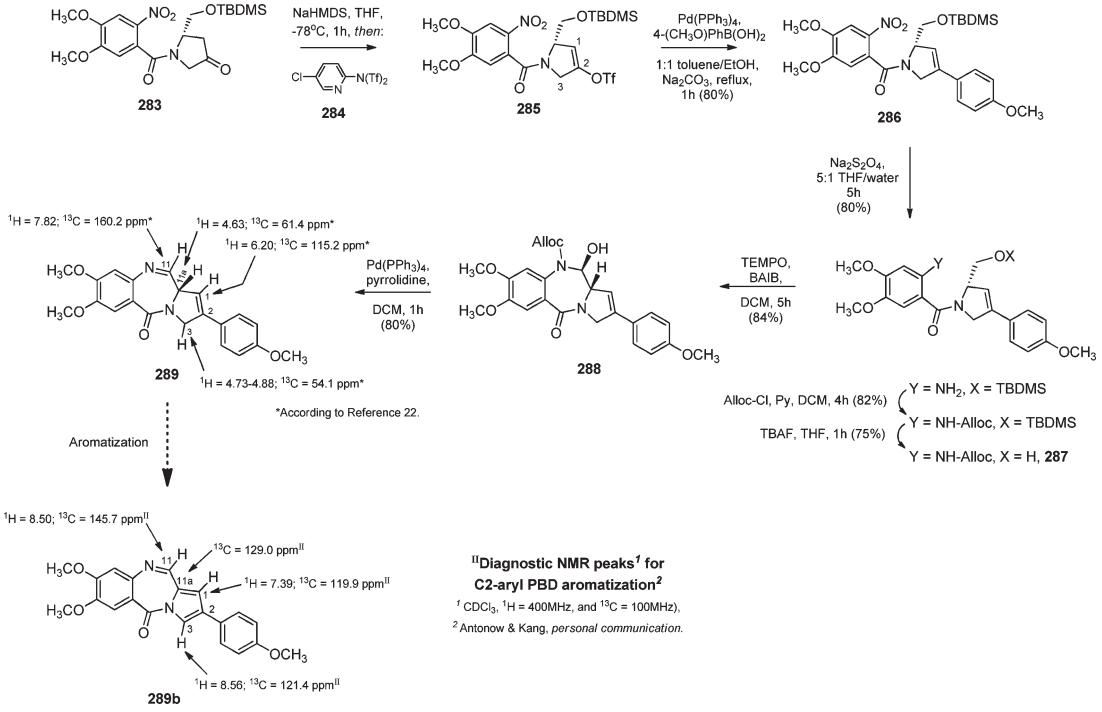
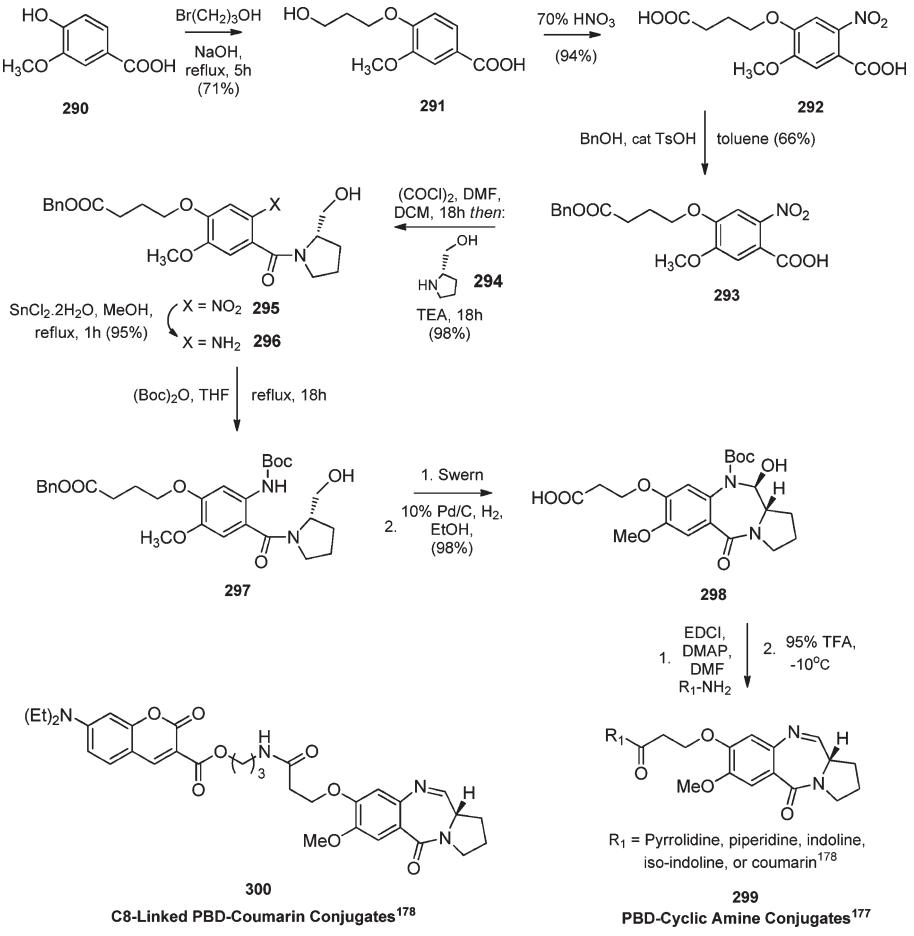
Scheme 42. Synthesis of C1/C2-*endo*-Unsaturated C2−Aryl PBD Monomers[50]



Scheme 43. Synthesis of C8-Linked PBD−Cyclic Amine Conjugates[177178]



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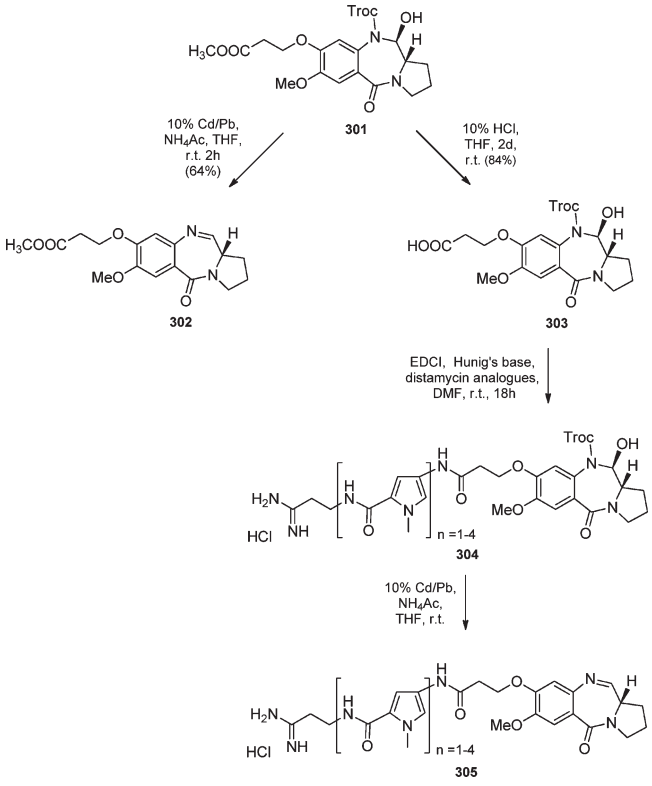
Scheme 44. Synthesis of C8-Linked PBD−Polyamide Conjugates Using the Method of Baraldi and Co-workers[179]



A similar synthetic route was employed by Antonow and co-workers to incorporate aryl groups at the C2-position of PBDs via Suzuki coupling of an enol triflate of type **268** (Scheme 39).[14,22] In this case, the oxidative cyclization approach allowed preparation of **267** on a large scale (i.e., 60 g), and the synthetic route was based on a parallel approach for the Suzuki coupling using PS−PPh_3Pd (catalyst) and polystyrene-bound diethanolamine (PS-DEAM) (scavenger) under microwave radiation. The use of scavengers, immobilized catalyst, and phase-separator cartridges allowed simultaneous workup of multiple reactions and the rapid synthesis of a library of 66 N10−C11 imine-containing C2−aryl-substituted PBDs with stereochemical integrity at their C11a-positions. Compounds with nitro substituents in the C2−aryl ring had to be synthesized by a different procedure because the 10% Cd/Pb couple step would reduce them to amines. Additionally, intermediate **268** was subjected to Troc-deprotection conditions to afford the N10−C11 imine-containing enol triflate PBD **272**. This was found to be a suitable substrate for Suzuki coupling and was used to produce PBDs of type **273**. The Suzuki coupling methodology has also been applied to the synthesis of C2/C2′−aryl C8/C8′-linked PBD dimers (Scheme 40).[173,174]

Chen[173] and Howard and Gregson[174] have synthesized **274**, and closure of the PBD ring system was achieved using 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and bis(acetoxyiodo)-benzene (BAIB) as oxidizing agents leading ultimately to the C2/C2′−ketone **276** (Scheme 40). Use of a large excess (i.e., 22 equiv) of triflic anhydride and pyridine allowed formation of the *bis*-enol−triflate PBD dimer **277** in 73% yield. Next, Suzuki coupling was achieved in excellent yield using TEA as base at room temperature for just 15 min. Final Troc deprotection under mild conditions (10% Cd/Pb) furnished **279**, the first example of a C2−aryl PBD dimer that was shown to have potent antitumor activity.[175]

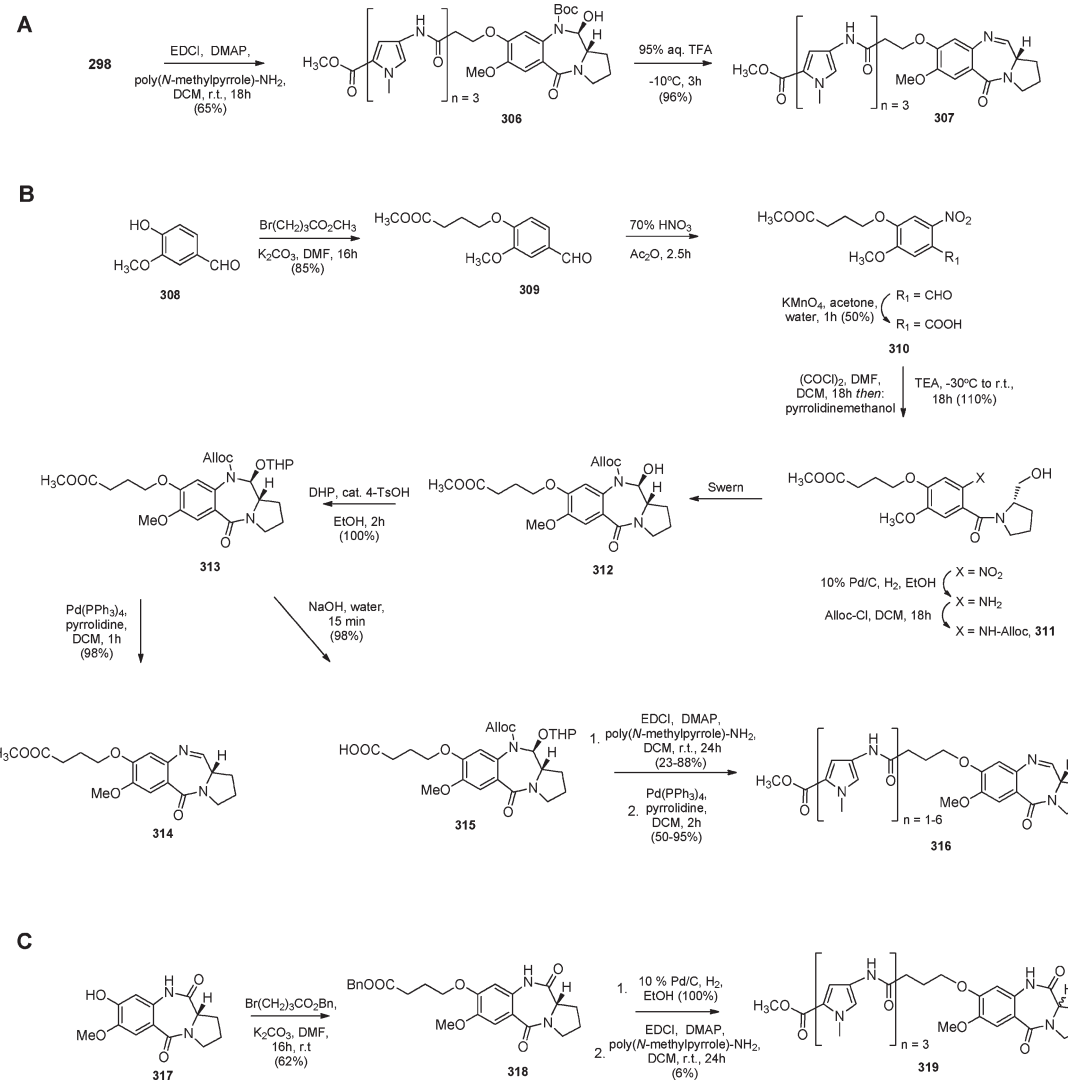
Guiotto and co-workers[176] have attached aryl groups to the C7-position of PBDs using Suzuki coupling to investigate whether substituents at this position affect the electronic characteristics of the N10−C11 imine with regard to its electrophilicity and ability to interact with DNA (Scheme 41). The 7-iodo N10−Troc-protected compound **280** was subjected to palladium-catalyzed Suzuki coupling involving six different phenylboronic acids in the presence of Na_2CO_3 in refluxing benzene to afford intermediates of type **281**. The yields for this coupling reaction ranged from 35% to 93%, and the Troc group was subsequently removed with 10% Cd/Pb to afford the corresponding C7−aryl PBD imines of type **282**.[176]

The C1/C2-*endo*-unsaturated C2−aryl PBD monomer **289** has been synthesized by Kang and co-workers using a different route involving kinetic enolization of the ketone **283** prior to B-ring closure (Scheme 42).[50] Using a sterically hindered base (i.e., NaHMDS) and 5-chloro-2-(*N*,*N*-[trifluoromethanesulfonyl]amino)pyridine (**284**) as triflating agent, the kinetic C1/C2-*endo*-enol triflate **285** was formed almost exclusively in 50% yield. This was subjected to Suzuki coupling with 4-methoxybenzene boronic acid in the presence of a relatively strong base (Na_2CO_3) under reflux to afford **286** in 80% yield. Preservation of the C1/C2-*endo*-unsaturation was surprising since the pK_a of the C3-proton is presumably low due to the vicinity of the amidic nitrogen. Furthermore, the C1/C2-*endo*-unsaturation was sufficiently robust to withstand all subsequent steps including B-ring closure using TEMPO/BAIB to give **288** in 85% yield. It is noteworthy that the final C2−aryl product **289** obtained by palladium-mediated Alloc deprotection was relatively unstable, with a tendency to aromatize to the biologically inactive fully unsaturated C-ring form with double bonds at both the C11a−1 and C2−3 positions.[50]

The aqueous solubility of a drug candidate is usually a prime concern in drug discovery. Hence, for PBD antitumor agents, numerous methods to improve water solubility have been investigated. For example, Masterson and co-workers have attached hydrophilic moieties such as cyclic amines to the C8-position of PBDs using the Boc-protected intermediate **298** and standard amide bond-forming conditions (Scheme 43).[177] Use of the Boc protecting group allowed large-scale (e.g., 60 g) preparation of the versatile PBD building block **298** in seven steps from the readily available **290** using simple procedures with minimal chromatographic purification. More recently, Wells and co-workers used the same building block to synthesize C8-linked PBD−coumarin conjugates.[178] In particular, the fluorescent 7-diethylaminocoumarin **300** prepared by this route allowed investigation of the cellular penetration of PBDs.

The oxidative cyclization approach combined with N10-protecting groups has also been extensively used to synthesize sequence-selective DNA-interactive agents for gene-targeting purposes (see also section 5.2). For example, Baraldi and co-workers have synthesized the N10−Troc-protected intermediate **301** via oxidative B-ring cyclization (Scheme 44).[179] In this case, MeOH and Troc−Cl were used to protect the C8−carboxylic acid and anilinic N10−amine, respectively. After hydrolysis of the methyl ester under acidic conditions to afford **303**, amidino poly(*N*-methylpyrrole) moieties resembling the natural product distamycin were joined to give conjugates of type

Scheme 45. Synthesis of C8-Linked PBD−Polyamide Conjugates Using the Method of Wells and Co-workers[20]



304. Troc deprotection with 10% Cd/Pb and ammonium acetate in THF afforded a series of PBD−distamycin hybrids as stable HCl salts (**305**). They also produced the parent PBD analogue **302** for comparative purposes in biological experiments. It is noteworthy that Baraldi and co-workers have also successfully utilized the "thioacetal approach" (see section 5.2) to synthesize N10−Alloc-protected analogues for use in the route shown in Scheme 44.[180,181] However, the final N10-deprotection of a N10−Alloc-protected analogue of **304** (n = 3) in the presence of $Pd(PPh_3)_4$ led to a very poor yield of the PBD imine product.

Wells and co-workers have utilized the intermediate **298** to synthesize the C8-linked PBD−poly(*N*-methylpyrrole) conjugate **307** (Scheme 45A).[20,182] This conjugate is structurally different from compounds of type **305** in that it has a terminal ester rather than an amidino terminus, which was shown to be associated with improved nuclear uptake compared to other PBD−polyamide conjugates.[20] However, modeling studies suggested that an additional methylene in the linker between the poly(*N*-methylpyrrole) chain and the C8−oxygen of the PBD should improve the DNA-binding affinity of hybrids of this type. For this reason, a new PBD building block **312** containing a 4-carbon C8-linker was designed and synthesized using the Fukuyama approach with a N10−Alloc protecting group (Scheme 45B). However, C11a-racemization occurred during the saponification step required to provide the free C8-acid. This was thought to arise from B-ring-opening followed by enolization and subsequent ring closure under the basic conditions, a process that should be preventable by protecting the C11−OH group. Several protecting groups were investigated for this purpose,[183] and tetrahydropyranyl (THP) was found to be the most effective at preventing C11a-racemization during the ester hydrolysis step. Therefore, the C11−*O*-THP PBD building block **315**[20] was prepared from **312** and coupled to six *N*-methyl pyrrole oligomers of varying lengths using standard peptide coupling reagents. Final treatment with $Pd(PPh_3)_4$ and pyrrolidine resulted in simultaneous removal of the N10−Alloc and C11−*O*-THP protecting groups to give the PBD imine conjugates of type **316** in 50−95% yields. Interestingly, the PBD dilactams of type **319**, prepared for use as controls in biological experiments, also racemized during synthesis (Scheme 45C). It is possible that the C8-alkylation step involving treatment of the dilactam (**317**) with potassium carbonate (K_2CO_3) was responsible, as Antonow

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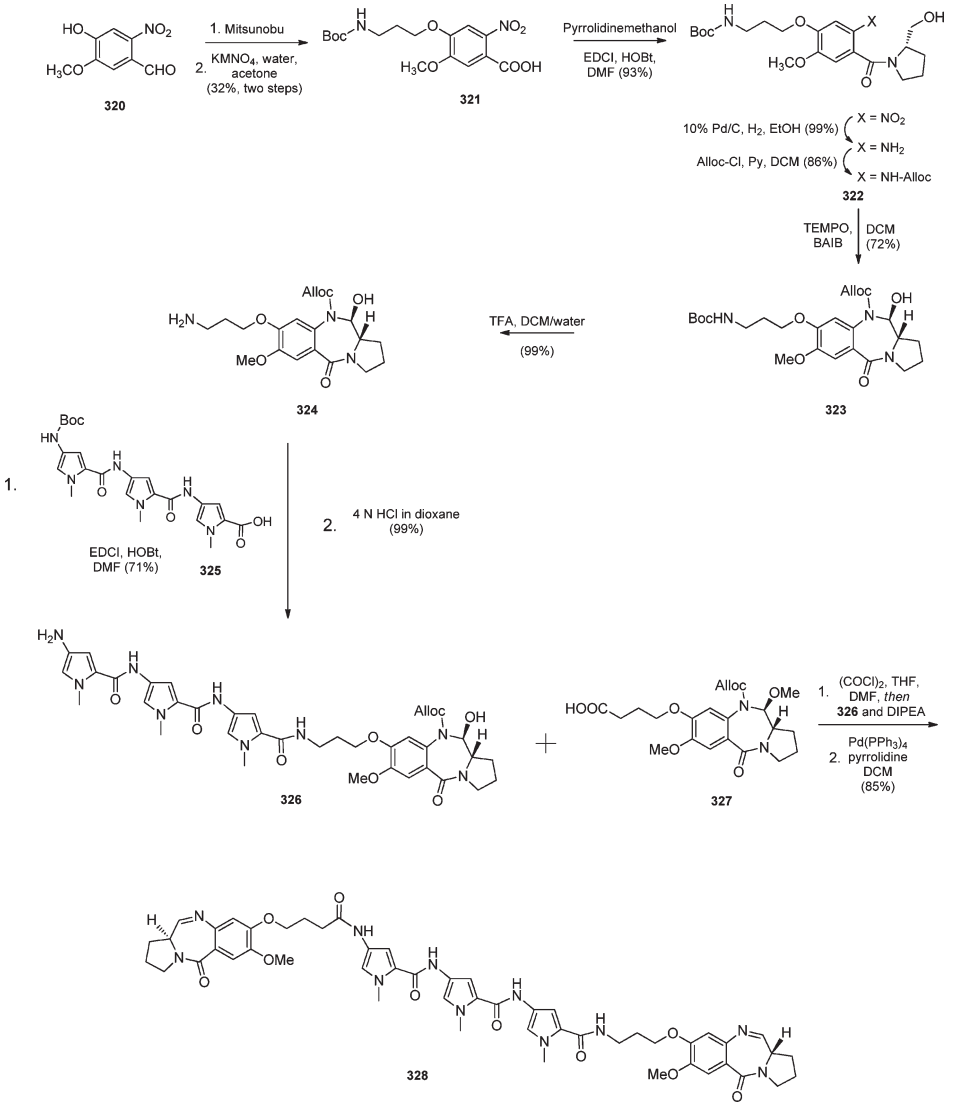
Scheme 46. Synthesis of the Asymmetric C8/C8′−Tripyrrole-Linked PBD Dimer 328[185]



and co-workers[31] have reported the racemization of C2−aryl PBD dilactams due to the use of Na_2CO_3 during a palladium-catalyzed step (see Scheme 5, section 4.1.1).

More recently, Tiberghien and co-workers applied the oxidative cyclization approach to the synthesis of an asymmetric C8/C8′−tripyrrole-linked PBD dimer (**328**, Scheme 46).[184,185] Their synthesis utilized the Alloc-protected PBD capping unit **324**, which contained a C8-side chain with an amino terminus. This C8-moiety was added to the A-ring fragment **320** via a Mitsunobu reaction, and **324** was synthesized from **322** using TEMPO/BAIB as the oxidizing agents for B-ring cyclization. Building block **324** was then coupled to the tripyrrole **325** to afford the C8-conjugated PBD **326**. This was coupled to a N10-Alloc−C11−methoxy-protected PBD capping unit (**327**) containing a free carboxylic acid at the C8-position. Final N10/N10′−Alloc deprotection provided the novel sequence-selective PBD dimer **328** in good yield (85%). Despite its high molecular weight (Mol. Wt. 984.07), this compound exhibited submicromolar cytotoxicity and was shown to bind with high affinity to an interstrand cross-linking site spanning 11 DNA base pairs.[185]

In 2001, Zhou and co-workers described the synthesis of an adenine−guanine cross-linking agent **322** containing both PBD and (+)-cyclopropanepyrroloindole (CPI) pharmacophores (Scheme 47A).[104] The reaction sequence started with the advanced PBD intermediate **329**, which had its pre-C11-position protected as a thioacetal (see section 5.2) and its pre-N10-position protected as a Fmoc carbamate in an adaption of the Fukuyama approach.[164] Cyclization and deprotection of the C8−carboxylic acid group afforded the intermediate **330**, which was coupled to the CPI unit **331** using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI). Final Fmoc deprotection with tetrabutylammonium fluoride (TBAF) provided **332**, which contained both imine and cyclopropane alkylating moieties and was shown to be capable of alkylating both guanine and adenine DNA bases in a cross-linking assay. In 2003, Tercel and co-workers reported the synthesis of some related PBD−*seco*-cyclopropa[*c*]benz[*e*]indol-4-one (*seco*-CBI) conjugates of type **326** with mixed-base cross-linking properties (Scheme 47B).[186] They first synthesized a number of N10−Alloc-protected intermediates of type **334** containing C8−alkyl

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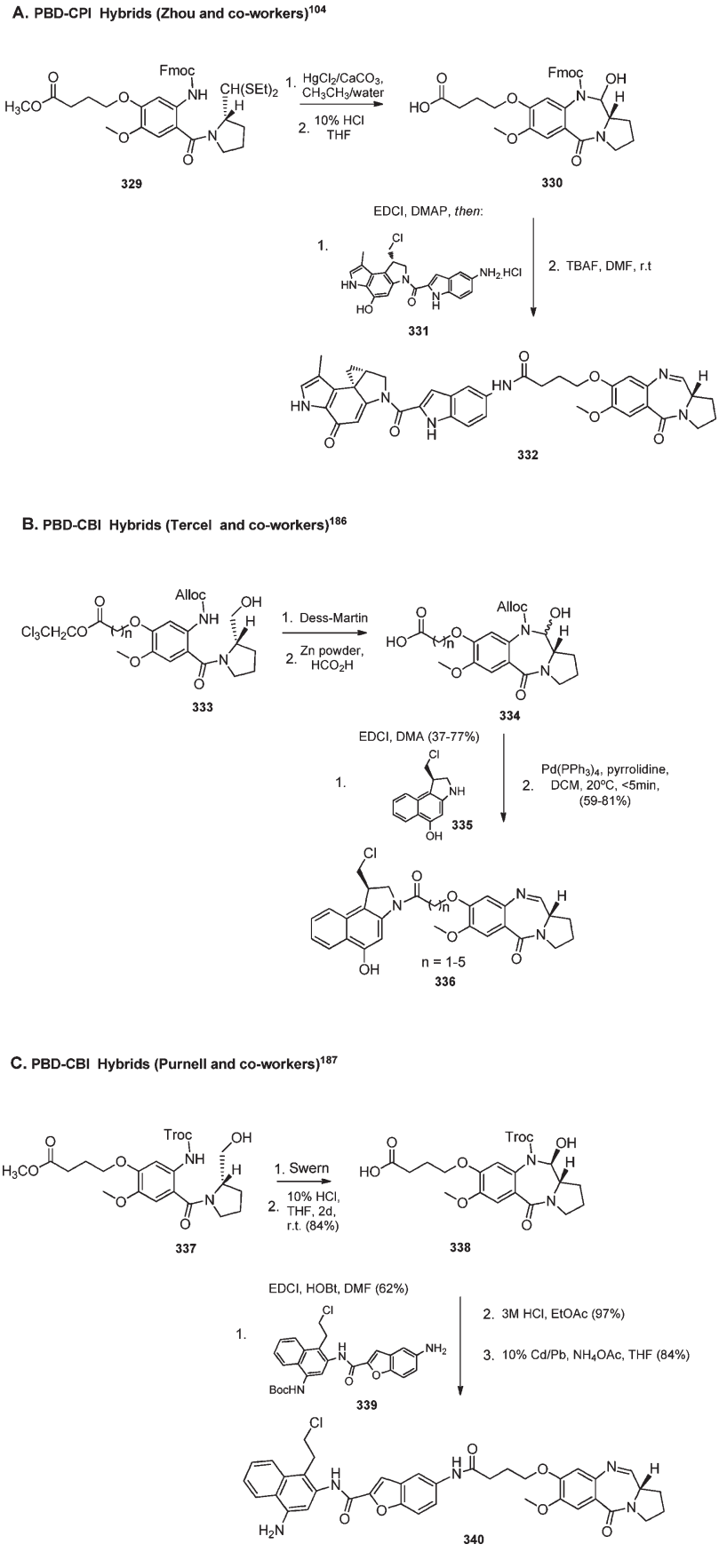
Scheme 47. Synthesis of C8-Linked PBD−CPI and PBD−CBI DNA Cross-Linking Hybrid Molecules



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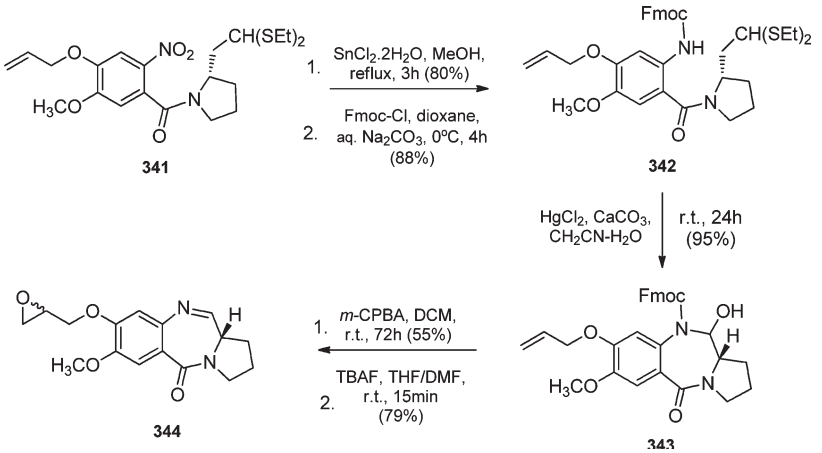
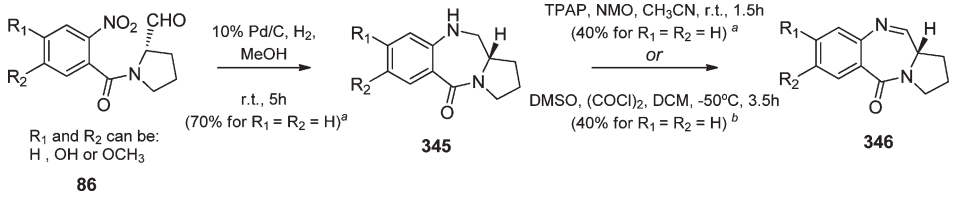
Scheme 48. Synthesis of C8−Epoxide-Containing PBD Conjugate 151[153]

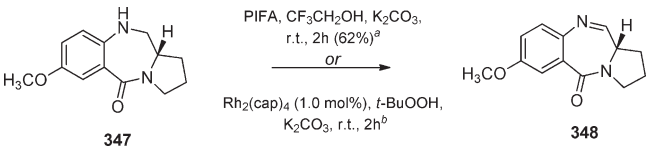


Scheme 49. Synthesis of N10−C11 PBD Imines Through the Oxidation of N10−C11 PBD Secondary Amines According to the Method of Kamal and Co-workers[98,190]



[a] Reaction conditions according to Kamal and co-workers.[190] [b] Reaction conditions according to Kamal and co-workers.[98] Also see ref 191 for related polymer-supported methodologies.

Scheme 50. Synthesis of N10−C11 PBD Imines Through the Oxidation of N10−C11 PBD Secondary Amines According to the Method of Kraus and Melekhov[192] **and Choi and Doyle**[193]



[a] Reaction conditions according to Kraus and Melekhov.[192] [b] Reaction conditions according to Choi and Doyle.[193]

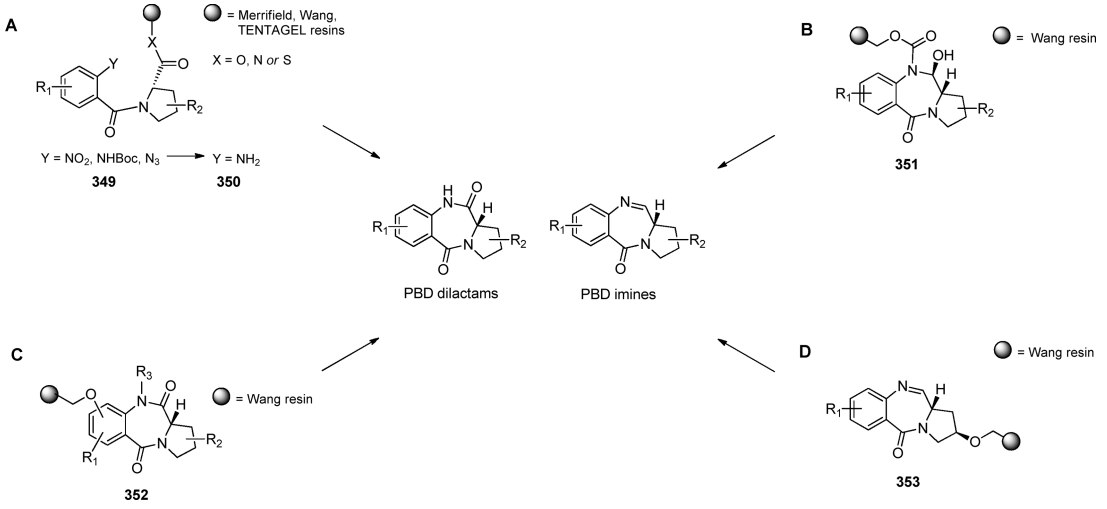
side chains of varying lengths starting from the precursor **333** and using a B-ring oxidative cyclization procedure employing the Dess-Martin reagent followed by Troc removal using Zn powder. These intermediates were coupled to the Boc-protected CBI building block **335** to form a set of DNA cross-linking agents of type **336** containing central linkers of varying length. Interestingly, a DNA thermal cleavage assay using a 512 base-pair DNA fragment demonstrated that the *seco*-CBI portion of the molecule controlled the sequence selectivity as alkylation at guanine sites was not observed. More recently, using a similar approach, Purnell and co-workers[187] synthesized the bisalkylating PBD−(*seco*-CBI) conjugate **340** from the N10−Troc-protected PBD intermediate **338** (containing a C8-side chain homologous to **301** as shown in Scheme 44) and the *seco*-CBI intermediate **339** (Scheme 47C). Compound **340** was found to covalently react with adenine−N3 positions at AT-rich sequences within the minor groove and produce DNA interstrand cross-links.[187]

To investigate the possibility of attaching PBDs to solid supports for the high-throughput screening of DNA fragments, Hardy and co-workers tethered the Fmoc-protected intermediate **330** (Scheme 47A) via its C8−carboxylic acid to different peptide sequences attached to a solid support.[188] This allowed "on-bead" visualization of rhodamine-labeled double-stranded DNA sequences that had interacted specifically with a particular immobilized PBD.

A C8−epoxide-containing PBD conjugate **344** has been synthesized by Wilson and co-workers from the pre-C8−allyl precursor **341** (Scheme 48) using the thioacetal approach to effect PBD B-ring formation and addition of a Fmoc-group at the N10-position (see section 5.2) to give intermediate **343**.[153] This N10-protecting group was included to prevent oxidation of the N10−C11 imine moiety to an amide with *m*-CPBA during the epoxidation step. Selective epoxidation of the pre-C8-side chain of intermediate **341** was attempted prior to B-ring cyclization. However, this led to oxidation of the pre-C11-thioacetal group to sulfoxide and sulfone species in addition to epoxide formation. In contrast, treating the cyclized N10−Fmoc-protected intermediate **343** with *m*-CPBA followed by Fmoc deprotection with tetrabutylammonium fluoride (TBAF) afforded the C8−epoxide-containing PBD **344** in good yield. This bifunctional compound was shown to form interstrand DNA cross-links in vitro.[153]

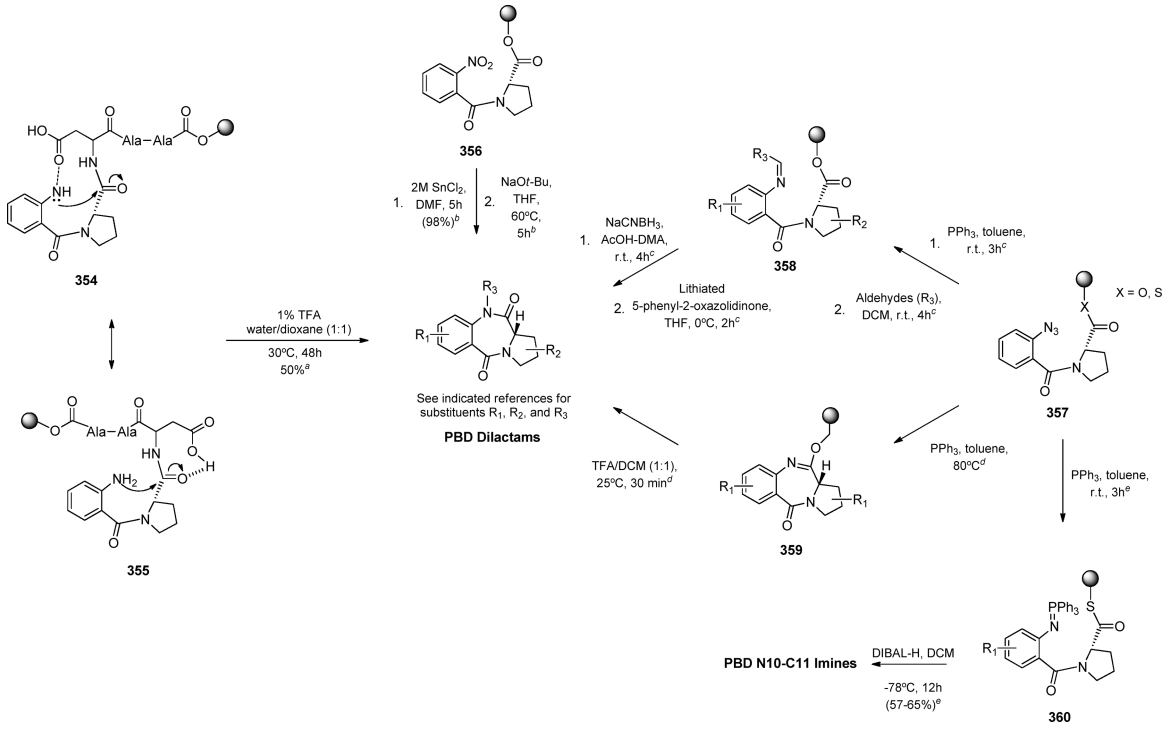
dx.doi.org/10.1021/cr100120f |*Chem. Rev.* 2011, 111, 2815–2864

Scheme 51. Four Possible Sites for Attachment of PBDs to Solid-Phase Resins[a]



[a] A, Pre-C11-ester, -thioester or -amide linkages; B, N10-carbamate linkages; C, A-ring ether linkages; D, C2-ether linkages.

Scheme 52. Solid-Phase Synthesis of PBD Monomers via Pre-C11-Linkage to the Resin[a−e]



[a] Reaction conditions according to Moroder and co-workers.[36] [b] Reaction conditions according to Mayer and co-workers.[195] [c] Reaction conditions according to Kamal and co-workers.[39] [d] Reaction conditions according to Ohlmeyer and co-workers.[197] [e] Reaction conditions according to Kamal and co-workers.[196]

6. OXIDATION OF CYCLIC N10−C11 SECONDARY AMINES

This approach involves selective oxidation of the N10−C11 secondary amine functionality in the diazepine ring of biologically inactive PBD structures of type **345** (Scheme 49). These cyclic amine precursors can be readily obtained via reductive cyclization of *N*-(2-nitrobenzoyl)pyrrolidine-2-carboxaldehydes of type **86** (see section 5.1) using catalytic hydrogenation over palladium on charcoal. However, this hydrogenation step is incompatible with either *endo*- or *exo*-unsaturation in the C-ring of the PBD, and only PBD imines with fully saturated C-rings have been synthesized by this method. Cyclic secondary amines of type **345** have also been obtained by hydride reduction of N10−C11 PBD dilactams (see section 4.2), although this has not yet been reported as a means to obtain PBD secondary amines for subsequent oxidation to N10−C11 PBD imines.

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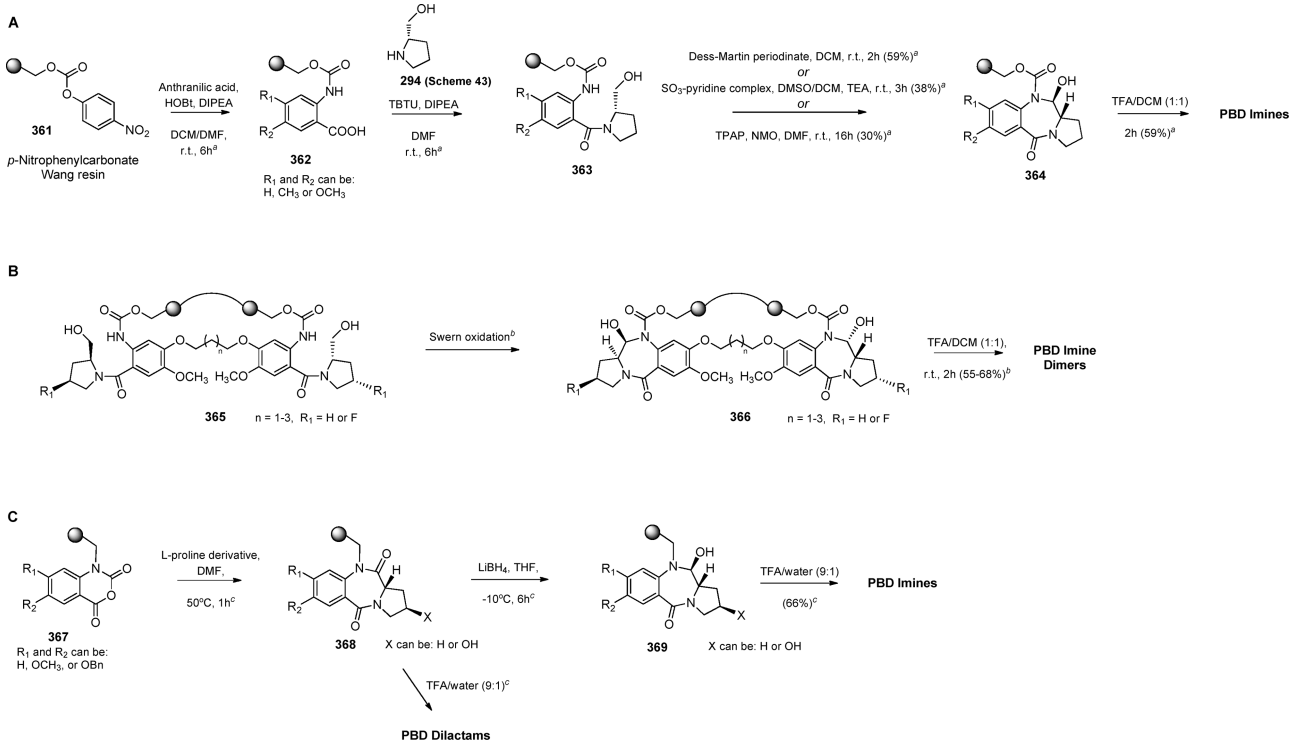
Scheme 53. Solid-Phase Synthesis of PBD Monomers and Dimers Using Pre-N10-Linkages to the Resin [a−c]



[a] Final yield after cleavage from solid support. Reaction conditions for $R_1 = R_2 = OCH_3$ according to Berry and co-workers.[198] [b] Reaction conditions for R_1 = F according to Kamal and co-workers.[199] [c] Reaction conditions for $R_1 = R_2$ = H according to Kamal and co-workers.[200]

Kamal and Rao have reported that PBD N10−C11 secondary amines can be converted to biologically active N10−C11 imine-containing PBDs via Swern oxidation, although yields are low (40%) (Scheme 49).[189] In a follow-up study, Kamal and co-workers improved the yield by using catalytic amounts of tetra-*n*-propylammonium perruthenate (TPAP) and *N*-methylmorpholine *N*-oxide (NMO).[190] More recently, the same researchers reported a variant of this reaction involving polymer-supported sulfoxide (PSS) for the Swern oxidation and polymer-supported perruthenate (PSP) for the TPAP oxidation with the isolated yields for these "on-bead" oxidations ranging from 50−75%.[191] It is noteworthy that oxidation with catalytic amounts of PSP in conjunction with NMO provided higher yields than PSS with oxalyl chloride. A solution-phase NMO/TPAP oxidation of N10−C11 PBD secondary amines has also been used by Correa and co-workers for the synthesis of DC-81.[79]

Finally, Kraus and Melekhov[192] have demonstrated that the PBD N10−C11 secondary amine **347** can be oxidized to the equivalent N10−C11 PBD imine (**348**) using bis(trifluoroacetoxy)iodobenzene (PIFA) in 2,2,2-trifluoroethanol in the presence of potassium carbonate (Scheme 50).[192] Although the reaction appeared to be very sensitive to aqueous workup, direct flash chromatography of the crude product afforded **348** in 62% yield. The same substrate (**347**) was used by Choi and Doyle to produce **348** in 80% yield using catalytic amounts of dirhodium caprolactamate $[Rh_2(cap)_4]$ in the presence of *tert*-butyl hydroperoxide (*t*-BuOOH).[193]

7. SOLID-PHASE AND PARALLEL SYNTHESES OF PYRROLO[2,1-*C*][1,4]BENZODIAZEPINES

In the 1990s, combinatorial chemistry approaches were widely used in medicinal chemistry projects in the pharmaceutical industry to explore molecular diversity. Typically, large chemical libraries were synthesized and then evaluated using a variety of high-throughput screening (HTS) methodologies. Today, combinatorial chemistry is still a powerful tool in drug discovery, but the emphasis has shifted to the synthesis of small- to medium-size libraries in which each member is pure and well-characterized. These focused libraries are often produced by rounds of parallel synthesis in which solid-phase chemistry allows facile purification and automation. In the field of PBD chemistry, several synthetic approaches have been developed on solid-support using a range of different resins, with the Wang resin being the most popular choice. Most of these approaches have been based on existing solution-phase methodologies already described in previous sections, and have been recently reviewed by Kamal and co-workers.[194] Essentially, there are four positions on the PBD scaffold that can be linked to resin as C11-esters, thioesters, or amides (Scheme 51A), N10-carbamates (Scheme 51B), A-ring ethers (Scheme 51C), or C2-ethers (Scheme 51D). The position of the PBD scaffold attached to the resin is of fundamental importance as it can directly affect the structure of the final products (e.g., whether the N10−C11 position is in the imine or amide form). Scheme 51 summarizes the solid-supported approaches reported in the literature to date.

In 1996, Moroder and co-workers reported the first synthesis of PBDs on solid support as part of a combinatorial campaign to generate benzodiazepine diversomers.[36] They explored the general tendency of N^{α}-(2-aminobenzoyl)peptides to undergo acid-catalyzed cyclization to 1,4-benzodiazepine-2,5-one structures including PBDs (Scheme 52). This elegant solid-supported approach involved the use of Merrifield resin loaded with a tripeptide containing an aspartic acid terminus (**354**,

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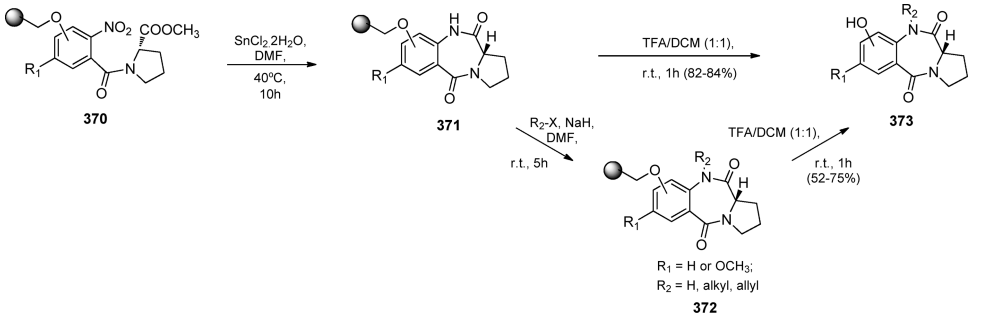
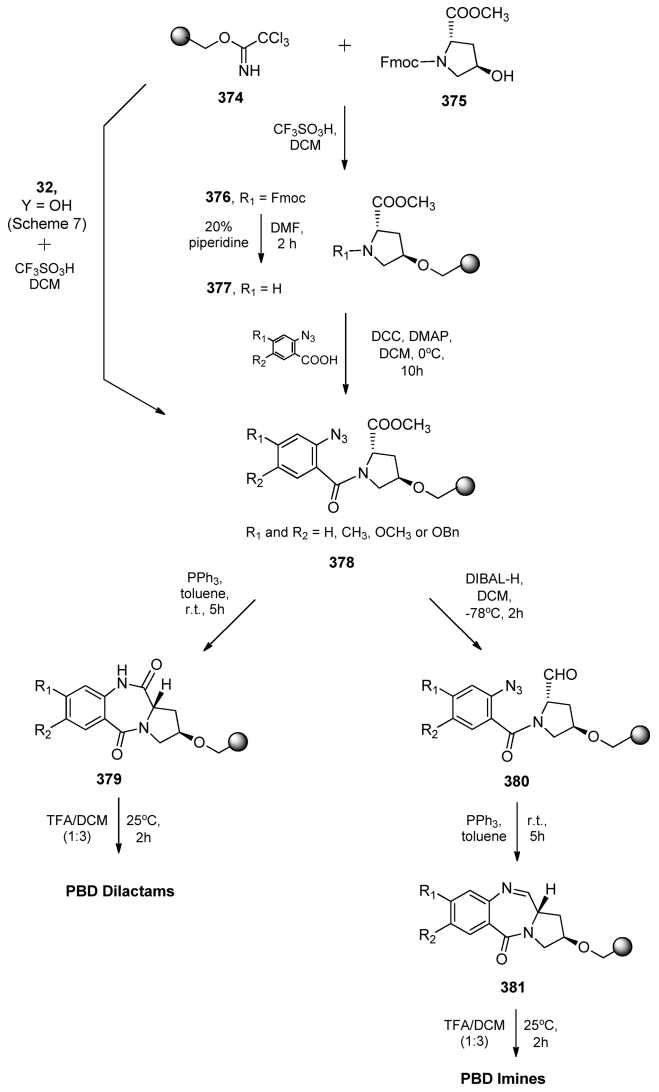
Scheme 54. Solid-Phase Synthesis of PBD Monomers Using A-Ring-Linkages to the Resin[201]



Scheme 55. Solid-Phase Synthesis of PBD Monomers Using C2-Linkages to the Resin[202]



Scheme 52). In this case, the carboxyl side chain of the aspartic acid residue appeared to be involved either in intramolecular protonation of the pre-C11 proline carbonyl group (**355**) or in hydrogen bonding to the pre-N10 amino group of the anthranilic acid residue (**354**), both leading to cyclization and release of a PBD dilactam from the resin. Subsequent washing of the resin followed by treatment with 1% TFA in dioxane/water (1:1) afforded PBD dilactams in 50% yield and with a high degree of purity. From a synthetic standpoint, this approach to the PBD ring system has also been investigated in solution phase,[36] although the integrity of the C11a-stereochemistry in the final PBD dilactam products was not reported. In the same year, Mayer and co-workers showed that B-ring cyclization could also be achieved on solid support in the presence of base.[195] They sequentially coupled Wang resin to proline and then *o*-nitrobenzoic acid to give **356** (Scheme 52). The nitro group then was reduced on-bead using tin chloride in DMF, followed by cyclization and release of the PBD dilactam from the resin by heating in THF in the presence of sodium *tert*-butoxide. Although the reported yields (79%) and purity of the crude PBD dilactam products were satisfactory, no information was provided on the integrity of the C11a-stereochemistry. A combinatorial method has also been reported by Kamal and co-workers using immobilized *N*-(2-azidobenzoyl)pyrrolidine-2-carboxylic esters (see section 4.1.2) to generate diversification at three sites on the PBD scaffold (i.e., A-ring, N10, and C2).[39] They used Staudinger and aza-Wittig reactions on the solid-supported intermediate **357** at room temperature to generate chemical diversity at the pre-N10-position and to generate immobilized *N*-(2-(benzylideneamino)benzoyl)pyrrolidine-2-carboxylic esters (e.g., **358**, Scheme 52). Further chemoselective reduction of the imino group with sodium cyanoborohydride ($NaCNBH_3$) provided the secondary amine, which cyclized and then released from the resin upon treatment with lithiated oxazolidinone as base to afford free PBD dilactams. In all, 2 different types of prolines, 11 azidobenzoic acids, and 9 aldehydes were utilized to produce 126 compounds, thus highlighting the combinatorial advantage of this approach. The aza-Wittig reaction had been previously used on the immobilized iminophosphorane **360** by the same researchers.[196] The thioester linkage to the resin was then reduced to the pre-C11 aldehyde with DIBAL−H in DCM, which afforded PBD imines through cleavage from the resin. Interestingly, Ohlmeyer has shown that PBD carbimide resins of type **359** are obtained directly from intermediates of type **357** (X = O) when the Staudinger reaction is conducted at 80 °C.[197]

In 2000, Berry and co-workers reported the solid-phase synthesis of PBD imines with saturated C-rings through a process involving attachment of the pre-N10 position to the resin (i.e., Schemes 51B and 53A).[198] They used a *p*-nitrophenylcarbonate-functionalized Wang resin (i.e., **361**) to synthesize four

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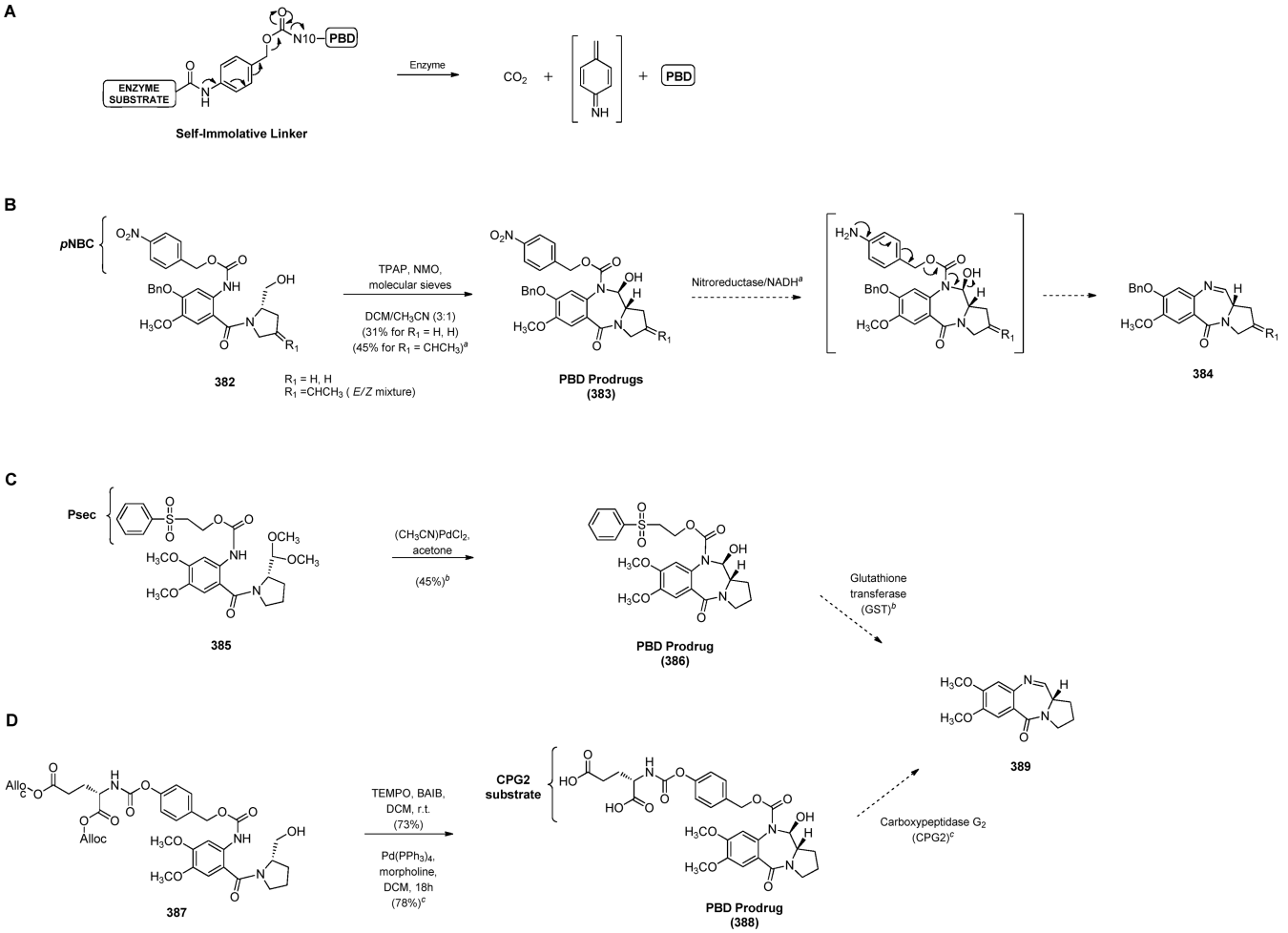
Scheme 56. Use of Therapeutically Cleavable N10−Carbamate Protecting Groups to Produce PBD Prodrugs (Dashed Arrows Represent the In Vivo Deprotection Step)



[a] Reaction conditions according to Sagnou and co-workers.[205] [b] Reaction conditions according to Berry and co-workers.[207] [c] Reaction conditions according to Masterson and co-workers.[208]

A-ring-modified analogues of DC-81. The key intermediates (e.g., **363**) were built-up on the polymeric matrix through a pre-N10−carbamate linkage via sequential reaction with the anthranilic (A-ring) and prolinol (C-ring) components. Oxidative cyclization (i.e., the Fukuyama's approach, section 5.5) was then carried out using three different reaction conditions to provide the pro-N10-protected PBD ring system **364**, which could be released from the resin in the N10−C11 imine form upon treatment with TFA in DCM (Scheme 53A). This oxidative cyclization approach has also been investigated by Kamal and co-workers for the solid-phase synthesis of PBD dimers via their N10/N10′-positions using the same functionalized Wang resin (Scheme 53B).[199] Interestingly, the PBD−dimer core (i.e., **365**) cross-linked to two carbamate sites on the resin, potentially due to the low percentage (1%) of cross-linking divinyl benzene (DVB) in the polymeric matrix allowing more flexibility and site-to-site interactions within the resin. The oxidative cyclization was achieved through Swern oxidation, and the products of type **366** were cleaved from the resin with TFA in DCM. To date, this is the only reported example of the solid-phase synthesis of PBD dimers.

The "isatoic anhydride approach" (see section 4.1.3) has also been successfully transferred to solid support by Kamal and co-workers to produce both PBD dilactams and PBD imines (Scheme 53C).[200] First, variously substituted isatoic anhydrides were coupled to chloromethyl Wang resin through a S_N2 reaction using sodium hydride as base to give solid-supported intermediates of type **367**. Subsequent condensation with proline derivatives in DMF proceeded at 50 °C to give the tethered PBD dilactams of type **368**, which could then be cleaved from the resin with TFA/water (9:1). Alternatively, the immobilized PBD dilactams (**368**) could be reduced with $LiBH_4$ to the N10−C11 carbinolamine intermediates (**369**) before cleavage with TFA/water, to afford the corresponding N10−C11 PBD imines. This entire synthetic sequence was achieved in only four steps with overall yields ranging from 60 to 66%. It is noteworthy that reduction with $LiBH_4$ did not release the PBD from the resin as might be expected based on experiments with MOM- and SEM−N10-protecting groups. Crucially, the C11a-stereocenter appeared to remain intact throughout the synthetic pathway according to optical rotation measurements reported for representative final products.

Kamal and co-workers have also shown that PBD dilactams can be constructed on a solid support by attaching 2-nitro-substituted 3- or 4-hydroxymethylbenzoates to Wang resin

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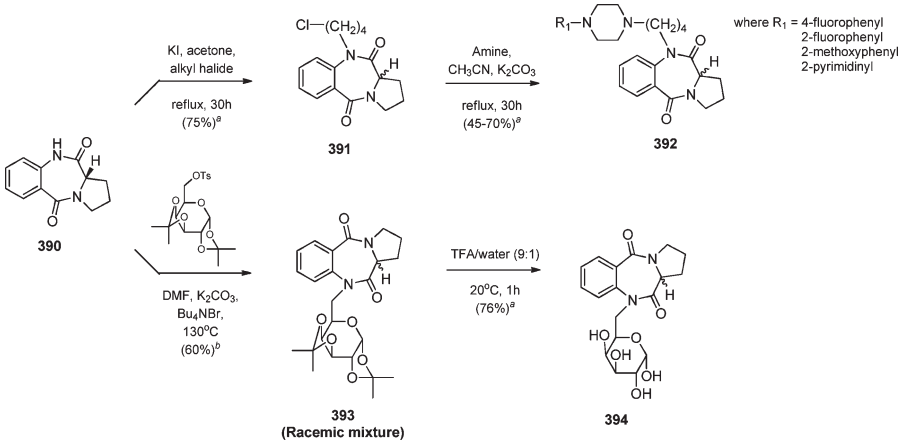
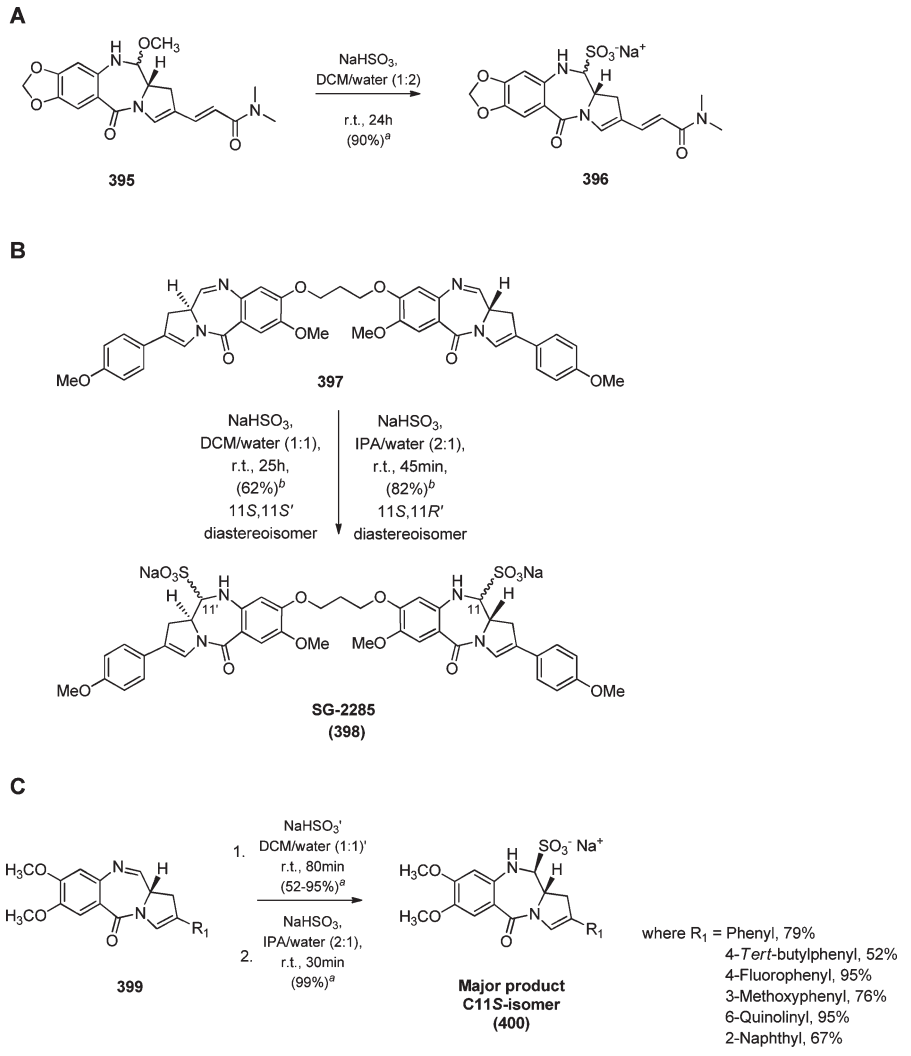
Scheme 57. Incorporation of N10−Alkyl Protecting Groups into PBD Dilactams



[a] Reaction conditions according to Kossakowski and co-workers.[211] [b] Reaction conditions according to Bouhlal and co-workers.[213]

Scheme 58. Synthesis of C11−(Sodium Bisulfite)-Containing PBD Adducts



[a] Reaction conditions according to Langlois and co-workers.[90] [b] Reaction conditions according to Howard and co-workers.[81] [c] Reaction conditions according to Antonow and co-workers.[22]

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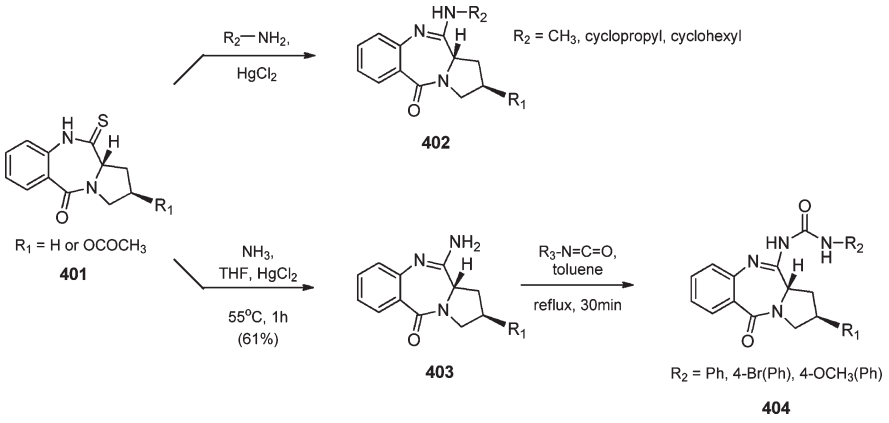
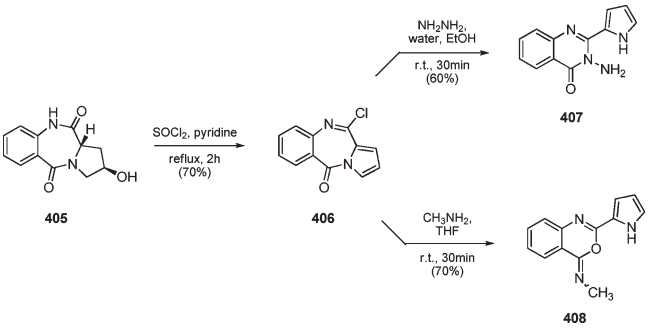
Scheme 59. Synthesis of C11−Amidine-Containing PBDs[218]



through their A-rings via Mitsunobu reaction followed by condensation with a proline methyl ester fragment (i.e., Schemes 51C and 54).[201] The PBD ring system was then generated on-bead (i.e., **371**) by nitro reduction with tin chloride according to the methodologies described in section 4.1.1. The C11a-stereochemistry remained unaffected throughout this synthetic route as confirmed by optical rotation measurements of products released from **371** by treatment with TFA (i.e., **373**). Alkyl or allyl groups were also introduced to the N10-position prior to cleavage (i.e., **372**). To date, this is the only reported example[201] of linking PBD-precursors to a solid support via their A-rings.

The option of using the C2-position of a PBD as an attachment point for a solid support (i.e., Scheme 51D) has been investigated by Kamal and co-workers for the solid-phase synthesis of PBD imines and dilactams.[202] Their route was based on the nitro/azide reductive approaches described in sections 4.1.1 and 4.1.2. For example, they utilized a Wang resin that had been pretreated with trichloroacetonitrile (Cl_3CCN) to contain trichloroacetamidate groups in its polymeric matrix (**374**, Scheme 55). This functionality was easily displaced with the pre-C2−hydroxy group of Fmoc-protected 4-hydroxyproline methyl ester (**375**) in the presence of trifluoromethanesulphonic acid (CF_3SO_3H) to form the tethered N−Fmoc-protected pre-C-ring building block **376**. Subsequent N-deprotection to give **377** followed by coupling to variously substituted 2-azidobenzoic acids afforded intermediates of type **378**. These could be converted to resin-bound PBD dilactams (**379**) via azide reduction with triphenylphosphine, and then released from the resin by treatment with TFA/DCM. Alternatively, the resin-bound intermediates of type **378** could be reduced to aldehyde-containing intermediates (**380**) with DIBAL−H, which formed N10−C11 PBD imines on-bead (i.e., **381**) after treatment with PPh_3/toluene. These could be released from the resin upon treatment with TFA to give free PBD imines. It is noteworthy that the PBD dilactams produced by this route were sufficiently pure that final purification by chromatography was not required. In two other cases, the authors reported the use of indium[203] or aluminum[204] in conjunction with ammonium chloride (NH_4Cl) as efficient alternatives to tin chloride for nitro/azide reduction of PBD intermediates on solid support. Similarly, nickel boride (Ni_2B) in acidic methanolic media in conjunction with microwave radiation was recently reported[62] for the tandem azide reductive

Scheme 60. Synthesis of C11−Chloro-Containing PBDs and Their Reaction with Amines[219]



cyclization/PBD resin cleavage of constructs of type **378** to afford PBD dilactams.[62,203,204] In these cases, intermediates of type **378** were obtained by coupling pre-C2-hydroxy functionalized methyl *N*-(2-azidobenzoyl)pyrrolidine-2-carboxylates (i.e., Y = OH in **32**, Scheme 7) directly to **374**.

8. SYNTHESIS OF N10- AND C11-SUBSTITUTED PYRROLO[2,1-C][1,4]BENZODIAZEPINES

8.1. N10-Modification

In section 5.5, the advantages of using pre-N10- or N10-protecting groups to facilitate PBD synthesis through the oxidative cyclization approach were described. However, N10-protecting groups have also been incorporated into the PBD ring system with the intention of regulating biological activity (i.e., Scheme 56A). Therefore, N10-protection with therapeutically cleavable moieties is discussed separately in this section.

N10−(*p*-Nitrobenzyl)carbamate (*p*NBC)-protected PBDs such as **383** (Scheme 56B) have been synthesized as potential prodrugs by Sagnou and co-workers via oxidative cyclization of intermediates of type **382** using TPAP and NMO.[205,206] The N10 group of prodrugs such as **383** blocks the ability of the N10−C11 carbinolamine/imine moiety to alkylate DNA. However, these biologically inert compounds can regain their electrophilic imine group through enzymatic cleavage of the protecting group (e.g., with nitroreductase). The electronic properties of the protecting group are changed when the aromatic nitro moiety is reduced to an amine, making it labile and prone to degradation

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(i.e., deprotection). A 1,6-elimination driven by the formation of CO_2 (i.e., HCO_3^-) releases the free biologically active PBD molecule **384** (Figure 56B). More importantly, this deprotection process can be site-targeted by using antibodies or viral vectors to deliver the enzyme to certain cancer cell types (e.g., the Antibody-Directed Enzyme Prodrug Therapy (ADEPT) or Gene-Directed Enzyme Prodrug Therapy (GDEPT) approaches, respectively). A similar concept prompted Berry and co-workers to use a 2-phenylsulphonylethylcarbonyl (Psec) group at the N10-position (**386**, Scheme 56C).[206,207] The Psec group possesses acidic protons at the α-position to the sulphonyl group, and molecular modeling studies had suggested that these protons might be removable by the action of the enzyme glutathione transferase (GST), thus leading to fragmentation of the Psec group and subsequent release of active N10−C11 PBD imines (**389**). More recently, Masterson and co-workers have utilized L-glutamic acid as a protecting group (i.e., **388**, Scheme 56D) to make PBD prodrugs that are substrates for the enzyme carboxypeptidase G2 (CPG2).[208] Prodrugs of this type may be used for ADEPT therapies,[209] and this approach has also been used to make PBD dimer prodrugs.[208,210]

Protecting groups more robust than carbamates have been installed at the N10-position of the PBD skeleton. For example, N10-alkylation can be performed on the PBD dilactam scaffold (**390**, Scheme 57) by refluxing with an acetone solution of 1-bromo-4-chlorobutane in the presence of potassium iodide, thus creating an opportunity for further elaboration of the newly formed N10-(4-chlorobutyl) chain (**391**).[211] However, it is noteworthy that the C11a-stereochemistry of the final compounds of type **392** was not reported.[211] Sugar moieties have also been incorporated at the N10-position of PBDs by Bouhlal and co-workers through the condensation of protected glycosydic building blocks in DMF at 130 °C in the presence of base (i.e., K_2CO_3).[212,213] However, using protected D-galactopyranose as an example, it was found that the reaction conditions caused racemization at the C11a-position of the products according to ^{13}C NMR and X-ray analyses of **393**. It is worth noting that these reaction conditions resemble the ones used by Antonow and co-workers[31] (see section 4.1.1) for the synthesis of C2−aryl PBD dilactams, which also led to C11a-racemization when Na_2CO_3 was used, whereas no epimerization was observed with weak bases such as triethylamine.

8.2. C11-Modification

The C11-position of the PBD skeleton is the electrophilic center that interacts covalently with guanines in the minor groove of DNA. Thus, there has been a tendency to avoid synthetic modifications at this position, especially those involving carbon−carbon bond formation that are difficult to reverse. However, the C11−bisulfite functionality has emerged as a convenient modification due to its reversibility and ability to enhance water solubility (Scheme 58).[214,215] Thus, synthetic methodology relating to C11−bisulfite preparation and biological data relating to bisulfite adducts have appeared in both the chemical and patent literature.[216,217] For example, Langlois and co-workers have reported a synthesis of the anthramycin-related compound **396** (Scheme 58A) that contains a C11−bisulfite functionality,[90] and more recently, Howard and co-workers synthesized the C2−aryl C11−bisulfite PBD dimer SG-2285 (**398**; Scheme 58B).[81,175,216,217]

The synthesis of C11−bisulfite adducts is simple, involving reaction of the N10−C11 imine or carbinolamine methyl ether forms of a PBD with aqueous sodium bisulphite in either biphasic (e.g., DCM/water) or monophasic (e.g., IPA/water) solvent systems. The reaction normally proceeds smoothly at room temperature. In the case of the biphasic system, unreacted N10−C11 imine remains in the DCM layer, and the hydrophilic C11−bisulfite product is obtained as a solid after lyophilization of the aqueous phase. However, although excess sodium bisulfite is also found in the aqueous phase and can complicate the isolation and purification process, in general the ease of purification for the biphasic system ensures good yields. For the monophasic procedure, almost quantitative yields can be obtained, although an accurately measured 1:1 stoichiometry of reagents is required. Furthermore, although easy to synthesize, characterization of the stereochemistry of the newly formed C11−SO3$^-$ bond can be challenging and time-consuming, especially for PBD dimers. Interestingly, for the synthesis of **398**, Howard and co-workers reported that different C11/C11′-diastereoisomers could be obtained depending upon the solvent system used for sodium bisulfite addition.[81] Recently, Antonow and co-workers carried out a NMR study of the reaction of sodium bisulfite with C2−aryl PBD monomers of type **399** to elucidate the stereochemistry of the C11−SO_3^- bond formed (**400**, Scheme 58C).[22] The study showed that for these monomeric C2−aryl PBDs the major species formed in biphasic solvent systems (i.e., DCM/water) after 80 min was in the C11(*S*)-configuration. Time-resolved NMR experiments showed that the imines are first converted to the "ring-opened" form upon exposure to sodium bisulfite in DCM/water. However, after a short time (i.e., 5 min), the (*S*)-C11−$SO_3^-Na^+$ PBD is the major species and can be isolated in good yield after lyophilization. In the same study, MS-MALDI experiments demonstrated that C11(*S*)−bisulfite PBD monomers react covalently with DNA in the same way as PBD imines, thus providing evidence that C11−bisulfite adducts act as true "prodrugs".[22]

Another example of C11-modication has been reported by Foloppe and co-workers who synthesized C11−amidine-containing PBDs (Scheme 59).[218] They treated the thiolactam **401**, obtained by reaction between compounds of type **390** (see Scheme 57) and Lawesson's reagent, with primary amines and ammonia in the presence of mercuric chloride to afford adducts of type **402** and **403**, respectively. In the case of **403**, it was subsequently reacted with different isocyanates to afford substituted amidines of type **404**. These products were evaluated for DNA-binding affinity but were shown to interact only weakly,[218] presumably due to the lack of ability to bind covalently.

Finally, Fabis and co-workers have reported that the fully aromatized C11−chloro PBD **406** can be obtained in 70% yield from the dilactam **405** by treatment with thionyl chloride and pyridine (Scheme 60).[219] The reactivity of **406** was investigated as this type of scaffold could be used to synthesize other types of biologically relevant heterocycles. Treatment of **406** with amines gave rearrangement products including the quinazoline **407** and the benzoxazine **408**.

9. CONCLUSIONS AND PERSPECTIVES

There is growing interest in the synthesis of PBDs as evident from the increasing number of research articles published and patents filed and granted since the last review in this journal 15 years ago.[11] These publications fall broadly into three areas. One of these relates to the chemical synthesis of PBDs and their use in

mechanistic studies. A good example of this is the work by Carlier and co-workers[77] on the novel concept of the memory of chirality (section 4.1.3). The second area relates to synthetic medicinal chemistry studies aimed at understanding the structure−activity relationship of PBD monomers and dimers with the objective of enhancing DNA-binding affinity and sequence-selectivity, cytotoxicity, and in vivo antitumor activity. The third area relates to the synthesis of PBD-based "targeted therapies" such as enzymatically released prodrugs and PBD−antibody conjugates.

Since the previous review,[11] there have been notable improvements in synthetic methodologies available for making PBD molecules. The new range of pre-N10-protecting groups reported for use within the oxidative cylization method originally described by Fukuyama and co-workers are a notable example (section 5.5). There has also been an increasing interest in the synthesis and chemistry of PBD dilactams, partly due to their ability to interact with DNA noncovalently. The small number of relatively robust synthetic steps required to prepare PBD dilactams and the numerous methods now available to convert them to N10−C11 imine-containing PBDs are notable features. Interestingly, although the application of solid-phase methodologies to the preparation of PBD libraries initially gained prominence during the past decade, it appears to be declining now, possibly due to the introduction of a number of novel alternative solution-phase technologies such as solid-supported reagents, scavengers, and "catch−release" cartridges.

A large number of publications and patents during the past 15 years have focused on enhancing the biological activity of PBDs through the addition or modification of substituents in the A- and/or C-rings, the conjugation of other DNA-interactive moieties to the PBD skeleton, or joining two PBD units together to afford PBD dimers that derive their biological potency from the formation of DNA cross-links. The conjugative approach has led to a large number of reported syntheses of "PBD hybrids" consisting of a single PBD unit joined, usually through the C8-position, to other DNA-interactive moieties such an intercalative, alkylating, or minor-groove binding agents. Significantly, the "dimerization" approach has led to one PBD dimer, SJG-136, that has successfully completed Phase I clinical trials and has progressed to Phase II. This has prompted the publication of a number of novel synthetic routes to PBD dimers. These are generally longer than synthetic pathways used for PBD monomers, but the key synthetic steps for formation of the PBD skeleton are usually comparable.

An exciting development in the oncology area is the move toward so-called "targeted" agents such as enzyme-releasable prodrugs and antibody−drug conjugates. In both cases, the PBDs have been recognized as highly potent low-molecular-weight "warheads" that are relatively easy to synthesize, and that can be joined to enzymatically-sensitive protecting groups or antibodies. At present, molecules such as the enediynes or maytansinoids are popular for this purpose but suffer from significant drawbacks including high molecular weight and limited availability from natural sources. In the case of enzyme-releasing strategies, a number of synthetic routes have now been published to prepare N10-protected PBD prodrugs that, while biologically inactive themselves due to masking of their electrophilic N10−C11 moiety, cleave to form potent DNA-binding cytotoxic species upon exposure to relevant enzymes produced at the tumor site in certain therapies (e.g., nitroreductase-based gene therapy, or ADEPT). Synthetic pathways have also been published demonstrating that potent PBDs "warheads" can be coupled to therapeutic antibodies and then targeted to tumor cells. It is likely that the use of PBDs for tumor targeting will continue to increase in the future.

A significant effort has also been made to increase the base-pair span and sequence-selectivity of PBD molecules so that they might be used as gene-targeting agents in biological experiments and possibly as therapeutic agents. In this context, they have the potential to selectively downregulate genes in tumor or bacterial cells, perhaps leading to novel oncology or anti-infective agents. Toward this goal, experimental PBD-based agents have been reported that can selectively inhibit transcription factor binding (e.g., NF-Y),[27] or block transcription.[20]

Finally, as PBD-based anticancer agents progress toward licensing and clinical use, there is likely to be an increasing number of publications and patents relating to commercial scale-up. Furthermore, an ever-growing concern over the environment means that there is likely to be an emphasis on the increased use of nontoxic reagents for PBD synthesis while minimizing harmful chemical and solvent waste. This shift from laboratory scale to commercial production is already evident from the sharp increase in the number of patent applications relating to discoveries in the chemistry and biology of PBDs.

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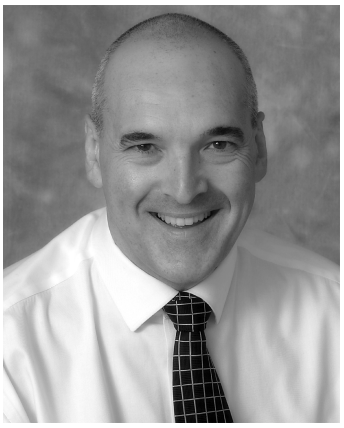
BIOGRAPHIES

Dyeison Antonow graduated as a pharmacist in 2003 from the Universidade Federal do Rio Grande do Sul (UFRGS) after completing his clinical training in radiopharmeuticals and nuclear medicine at Santa Rita Hospital (ISCMPA) in Porto Alegre, Brazil. He then moved to the University of London (U.K.) to complete his Ph.D. in organic chemistry on the synthesis of DNA-interactive agents and NMR-based structural investigations into drug−DNA adducts. In 2007, he took up a position as a Cancer Research UK Research Fellow working on the discovery of novel inhibitors of protein−protein interactions for cancer chemotherapy, particularly inhibitors of the HIF-1 and STAT3 signaling pathways. Dr. Antonow has also worked as a consultant for the biotech company Spirogen Ltd. on the development of novel DNA-targeted anticancer drugs. He is currently interested

in fragment-based drug discovery using NMR and target-guided synthesis.



David Thurston is Professor of Anticancer Drug Discovery at the School of Pharmacy, University of London. He has a first degree in pharmacy and a Ph.D. in synthetic medicinal chemistry, and has worked at two schools of pharmacy in the U.S.A. (University of Texas at Austin and Kentucky) as well as the Portsmouth, Nottingham, and London Schools in the U.K. He has supervised numerous Ph.D. and postdoctoral researchers throughout his career and has published extensively in the primary chemical literature. He is also author of the textbook *Chemistry and Pharmacology of Anticancer Drugs* and is co-inventor on a number of patents. His laboratory discovered SJG-136, a first-in-class sequence-selective DNA minor-groove cross-linking agent based on the pyrrolobenzodiazepine scaffold. SJG-136 has recently completed Phase I clinical trials in the U.S.A. (with the NCI) and the U.K. (with Cancer Research UK) and has progressed to Phase II studies. In 2000, Professor Thurston cofounded the oncology biotech company Spirogen Ltd., which is partly based on technologies relating to the pyrrolobenzodiazepines. Although still working on DNA-interactive agents, his academic laboratory has recently broadened to work on the discovery of novel protein−protein interaction inhibitors relating to the HIF and STAT3 signaling pathways.

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NOTE ADDED IN PROOF

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