University of Huddersfield Repository

Hemming, Karl, Jamshaid, Faisal and Chambers, Christopher Steven

Intramolecular Azide to Alkene Cycloadditions for the Construction of Pyrrolobenzodiazepines and Azetidino-Benzodiazepines

Original Citation

Hemming, Karl, Jamshaid, Faisal and Chambers, Christopher Steven (2014) Intramolecular Azide to Alkene Cycloadditions for the Construction of Pyrrolobenzodiazepines and Azetidino-Benzodiazepines. Molecules, 19 (10). pp. 16737-16756. ISSN 1420-3049

This version is available at http://eprints.hud.ac.uk/id/eprint/24752/

The University Repository is a digital collection of the research output of the University, available on Open Access. Copyright and Moral Rights for the items on this site are retained by the individual author and/or other copyright owners. Users may access full items free of charge; copies of full text items generally can be reproduced, displayed or performed and given to third parties in any format or medium for personal research or study, educational or not-for-profit purposes without prior permission or charge, provided:

- The authors, title and full bibliographic details is credited in any copy;
- A hyperlink and/or URL is included for the original metadata page; and
- The content is not changed in any way.

For more information, including our policy and submission procedure, please contact the Repository Team at: E.mailbox@hud.ac.uk.

http://eprints.hud.ac.uk/
Intramolecular Azide to Alkene Cycloadditions for the Construction of Pyrrolobenzodiazepines and Azetidino-Benzodiazepines

Karl Hemming 1,*, Christopher S. Chambers 1,2,†, Faisal Jamshaid 1 and Paul A. O’Gorman 1,†

1 Department of Chemical Sciences, University of Huddersfield, Queensgate, Huddersfield, West Yorkshire HD1 3DH, UK
2 Laboratory of Biotransformation, Institute of Microbiology, Academy of Sciences of Czech Republic, Vídeňská 1083, Praha 4, 142 20, Czech Republic

† These authors contributed equally to this work.

* Author to whom correspondence should be addressed; E-Mail: k.hemming@hud.ac.uk; Tel.: +44-(0)-1484-472188; Fax: +44-(0)-1484-472182.

External Editor: Derek J. McPhee

Received: 31 July 2014; in revised form: 26 September 2014 / Accepted: 13 October 2014 / Published: 17 October 2014

Abstract: The coupling of proline- and azetidinone-substituted alkenes to 2-azidobenzoic and 2-azidobenzenesulfonic acid gives precursors that undergo intramolecular azide to alkene 1,3-dipolar cycloadditions to give imine-, triazole- or aziridine-containing pyrrolo[1,4]benzodiazepines (PBDs), pyrrolo[1,2,5]benzothiadiazepines (PBTDs), and azetidinob[1,4]benzodiazepines. The imines and aziridines are formed after loss of nitrogen from a triazoleine cycloadduct. The PBDs are a potent class of antitumour antibiotics.

Keywords: cycloaddition; 1,3-dipole; azide; pyrrolobenzodiazepine; azetidinone; antitumour; antibiotic; β-lactam

1. Introduction

The pyrrolobenzodiazepines (PBDs), of which the natural products abbeymycin (1), DC-81 (2) and fuligocandin B (3) (Figure 1) are typical examples, are a class of molecule that has attracted significant
interest due the antitumour and antibiotic activity of several members [1–5]. Synthetic hybrids [5–8] and
dimers [5,9–11] have also shown significant biological activity and members of the dimer class have
entered Phase II clinical development as antitumour compounds. PBDs with additional fused rings such
as the circumdatin (4) [12], bretazenil (5) [13] and the 1,2,3-triazolo-fused system 6 [14] are attractive
as potential antitumour compounds, neurological agents, and protease inhibitors, respectively. As a result
of this interest, the replacement of the PBD pyrrole ring with other rings has attracted attention [5,15]
and hence pyrido- [16], oxazolo- [17], thiazolo- [18] and imidazo- [19–21] analogues are all known. The
replacement of the PBD tertiary amide with a sulfonamide leads to the pyrrolobenzothiadiazepines
(PBTDs) which are synthetic analogues of the PBDs [22]. The PBTDs have received much less attention
than the PBDs, but it is known that PBTDs 7–9 are useful as non-nucleosidic reverse transcriptase
inhibitors [23], potent leukemia cell-line inhibitors [24] and Glut-1 transporter inhibitors with
chemotherapeutic potential [25], respectively.

Figure 1. Pyrrolobenzodiazepine (PBD) and pyrrolobenzothiadiazepine (PBTD) structures of interest.

We reported previously [26] that the 1,2,3-triazolo-fused PBDs and PBTDs 11 (Figure 2) are
available via intramolecular azide to alkyne 1,3-dipolar cycloaddition of 1-(azidoaryl)-2-alkynyl
pyrroles 10, and have recently shown [25] that the 1-(azidoaryl)-2-cyano pyrroles 12 give the
1,2,3,4-tetrazolo-fused PBD and PBTD systems 13.

Figure 2. Previous examples of PBDs and PBTDs synthesized by 1,3-dipolar cycloaddition.
In preliminary work involving alkenes, we demonstrated that 1-(azidoaryl)-2-alkenyl pyrroles 14 and 15 gave the aziridino-fused PBD 16 and PBTD 17 [27]. In this paper, we report the full details for the synthesis of compounds 16 and 17 and also discuss several new intramolecular cycloadditions involving the previously unreported 1-(azidoaryl)-2-alkenyl pyrroles 18 (Figure 3) along with the reactions of a second new series of compounds, the 1-(azidoaryl)-4-alkenyl-azetidin-2-ones 19.

Figure 3. Additional alkene based substrates studied in this paper.

2. Results and Discussion

2.1. Synthesis and Reactivity of the Pyrrolo-Based Systems

The largest and most researched class of PBDs, those represented by abbeymycin (1) and DC-81 (2), are able to interact with nucleophilic guanidine residues in the minor groove of DNA, a process that is reliant upon the presence of an electrophilic imine or carbinolamine based functional group [5]. It is of note that the (S)-chiral center that is present in the 3-dimensional structure of these PBDs gives the molecules a shape which enables them to twist into the DNA minor groove meaning that most syntheses are based upon derivatives of (S)-proline. It is known that intramolecular 1,3-dipolar cycloadditions of azides to alkenes can lead to either imines or aziridines via triazoline intermediates [28,29]. On this basis, we anticipated that we could access imine or aziridine containing PBDs using intramolecular 1,3-dipolar cycloadditions between azides and alkenes using alkenes derived from (S)-proline. Whilst we were interested in the possibility of producing an imine, we were more intrigued by the possibility of producing an aziridine due the known propensity [30] of aziridines to function as electrophiles and the potential biological activity that a novel system of this type might offer. We settled upon two approaches, as shown in Scheme 1.

In the first system, (S)-prolinol (20) was converted into the unstable alkene 24 using a modified literature protocol [31]. Thus, S-prolinol (20) was protected as the carbamate 21, oxidized to the aldehyde 22 and then converted to the N-protected alkene 23 by Wittig reaction. In-situ deprotection and coupling of the deprotected alkene 24 to 2-azidobenzoyl chloride or 2-azidobenzenesulfonyl chloride 27a/b gave the cycloaddition precursors 14 and 15 in 32% and 20% yield, respectively, from the carbamate 23. Heating compound 14 in chloroform gave a ~1:1 mixture of the imine 25 and the aziridine 16 (55% combined yield) whereas heating compound 15 in the same solvent gave the aziridine 17 as the only isolable product in 44% yield.

We assume these reactions proceed via intramolecular 1,3-dipolar cycloaddition to give an intermediate triazoline which then undergoes loss of two nitrogen atoms. It is of note that Broggini explored a similar process with halogenated aryl systems and obtained the triazolines 26 [32]. The presence of the aziridine ring in compounds 16 and 17 was clear in the NMR spectra from the extra CH₂ and CH groups and from the diagnostic lack of CH₂ geminal coupling in the aziridine CH₂.
Scheme 1. Synthesis of aziridinopyrrolobenzodiazepines and aziridinopyrrolobenzothiadiazepines.

Mass spectroscopy confirmed loss of two nitrogen atoms and precluded the triazolone. The relative stereochemical assignment in compounds 16 and 17 was made on the basis of the CH/CH coupling constant at 8–10 Hz, the lack of a CH to CH nOeSy correlation and the presence of aziridine CH to pyrrolidine CH correlation, and the unequivocal assignment of Broggin's system by X-ray crystallography [32]. Stereochemistry at the aziridine nitrogen was not determined. Although we used (S)-prolinol as the starting material (due to its ease of availability), we did not seek to confirm absolute stereochemical assignments, although it is of note that Sato and co-workers reported that the alkene 24 retains the (S)-configured centre [31].

In our second approach, also shown in Scheme 1, we coupled (S)-prolinol to 2-azidobenzyol chloride or 2-azidobenzenesulfonyl chloride 27a/b (80%–96%) and oxidized the alcohols 28 and 29 under Swern conditions to give the aldehydes 30 and 31 (72%–80%). Attempts to react the aldehydes 30 and 31 with (methylene)triphenylphosphorane were unsuccessful meaning that a more efficient route (compared to the synthesis and *in-situ* deprotection of 23) to the alkenes 14 and 15 was not possible. However, reactions of these aldehydes with (carbethoxymethylene)triphenylphosphorane in toluene were successful. In the case of reaction with aldehyde 30, the alkene 32 was isolated in 51% yield and then heated in chloroform, whereupon it converted into the aziridine 33 as a 1:1 mixture of diastereoisomers in 30% yield. In the case of aldehyde 31, the alkene 34 could not be isolated and the aziridine 35 was isolated as the only product in 34% yield from the aldehyde, this time as a single diastereoisomer, possibly due to the lower temperature at which reaction occurred. We assigned the aziridine-CH/pyrrolidine-CH stereochemistry on the same basis as that described above, but were unable to determine the stereochemistry at the CHCO₂Et chiral center. We assume that these products arise as a
result of azide to alkene cycloaddition and formation of an intermediate triazoline 39, which then undergoes loss of two nitrogen atoms. As discussed previously, this loss of nitrogen is known behavior in alkene to azide cycloaddition processes [28,29]. We also studied reactions involving (2-azido-4-benzyloxy-5-methoxy)benzoyl chloride 27c (X = CO, R¹ = OMe, R² = OBN) due to this having the substitution pattern present in the natural product DC-81 (2). This azide was available in six steps from commercially available 4-hydroxy-3-methoxybenzoic acid using a literature procedure [33,34]. Attempts to couple this acid chloride to the alkene 24 were unsuccessful and led to significant degradation of the azide staring material—possibly as a result of its intolerance to the extreme in-situ conditions under which the alkene 24 was generated [31]. However, coupling of the acid chloride 27c to prolinol was successful and subsequent oxidation gave the aldehyde 37. Attempted reaction of this aldehyde with (methylene)triphenylphosphorane was again unsuccessful. However, reaction with (carbethoxymethylene)triphenylphosphorane gave a new product. As was observed previously with aldehyde 31, the alkene 38 was not isolable. This time, however, the product was unexpectedly found to be the “reduced” pyrrolobenzodiazepine 40, formed as a single diastereoisomer in 21% yield from the aldehyde, with stereochemistry consistent with that discussed above (no CH to CH correlation by nOesy and a strong CH2CO2Et to pyrrolidine CH correlation). We assume that this product arises as a result of a free-radical based loss of nitrogen from the triazoline 39 followed by hydrogen abstraction from the toluene solvent rather than imine/aziridine formation as observed previously.

2.2. Synthesis and Reactivity of the Azetidino Based Systems

In order to obtain further examples of these cycloaddition processes, we next turned our attention to the use of 4-alkenyl-2-azetidinones (Scheme 2) as coupling partners for 2-azidobenzoyl chloride (27a). We chose azetidinone systems due to our long-standing interest in the chemistry and biological applications of the β-lactams and their derivatives [35,36], and also due to the lack of literature examples of azetidino analogues of the PB Ds. The 4-alkenyl-2-azetidinones 41 were synthesized in 58%–60% yield using a [2+2]-cycloaddition between a diene and chlorosulfonyl isocyanate followed by work-up to remove the N-sulfonyl group. Whilst the 4-methyl substituted β-lactam 41a coupled successfully (65% yield) to give the 1-(2'-azidobenzoyl)-2-azetidinone 42a, we were unable to obtain the corresponding demethyl system 42b from the β-lactam 41b. We thus converted the azetidinones 41a/b into the corresponding azetidinethiones 43a/b by reaction with Lawesson's reagent, reasoning that this may result in a nitrogen atom that was more nucleophilic, and in fact, the two azetidin-2-thiones 43a/b reacted successfully with 2-azidobenzoyl chloride (27a) to give the 1-(2'-azidobenzoyl)-2-azetidinthiones 44a/b in 84% and 71% yield, respectively. Each of the three azido alkenes 42a and 44a/b behaved differently when heated to reflux in boiling solvents. Compound 42a gave the imine 45 (38%) when heated in boiling chloroform for 72 h; compound 44a when heated in boiling chloroform also gave the corresponding imine 47, but this time formed the isolable triazoline 46 (61% yield) after 72 h and gave the imine in 32% yield after a further 7 days in boiling chloroform; compound 44b was stable in chloroform at reflux, but gave the aziridine 48 (48% yield) upon 24 h of heating in toluene at reflux. The aziridine ring in compound 48 was apparent from the CH2CHCHCH2 connectivity in the system, the distinctive lack of geminal proton coupling for the aziridine CH2 and the loss of two nitrogen atoms in the mass spectrum. The product was formed as a single diastereoisomer according to the 1H/13C-NMR
spectra. Stereochemistry at the aziridine nitrogen was not determined. The CH/CH stereochemistry was assigned by correlation to the systems described above on the basis of the similar 8.9 Hz coupling constant.

**Scheme 2.** Synthesis and reactivity of 1-(2'-azidobenzoyl)-4-alkenyl-1-azetidin-2-ones/2-thiones.

3. **Experimental Section**

3.1. **General Information**

Reactions were performed in oven-dried glassware under nitrogen dried through 4 Å molecular sieves and delivered through a gas manifold. Work-up procedures were carried out in air. Anhydrous grade solvents were freshly distilled using a continuous still under nitrogen. Chloroform was dried over 4 Å molecular sieves or distilled over phosphorus pentoxide (3% w/v). Dichloromethane, acetonitrile, ethyl acetate and toluene were distilled over calcium hydride (5% w/v) over 4–6 h. Diethyl ether and THF were pre-dried over sodium wire, and then distilled over sodium wire (1%–2% w/v) with benzophenone (0.2%–0.3% w/v) as an indicator. Other anhydrous solvents were purchased from Fisher Chemicals or Sigma-Aldrich. All reactions were monitored by TLC, which was carried out on 0.20 mm Macherey-Nagel Alugram® Sil G/UV254 silica gel-60 F254 precoated aluminium plates and visualisation was achieved using UV light or vanillin stain. Column chromatography was performed on Merck silica gel (0.063–0.200 mm, 60 Å). NMR spectra were recorded on a Bruker DPX-400 or on a Bruker Avance 500 instrument. IR spectra were recorded on a Nicolet 380 FT-IR instrument as a drop for oils and liquids or as neat powders for solids. Mass spectra were recorded on a Bruker Daltonics microTOF mass spectrometer operating at a positive ion mode under an electrospray ionization (ESI +) method. High resolution mass spectra were recorded on a Finnegar MAT 900 XTL instrument operated by the EPSRC National Mass Spectrometry service at the University of Swansea. 1-(2'-Azidobenzoyl)pyrrolidine-2-carbaldehyde [33,34], 2-azidobenzenesulfonic acid [37] and 2'-azido-4-(benzyloxy)-5-methoxybenzoic acid [33,34] were synthesized as described in the literature. The synthesis of N-2-ethenyl-1-ethoxycarbonylpyrrolidine (23) and its deprotection have been described previously [31], but full details are included below as we found some modification was necessary. Due to ease of availability, we started our synthesis with (S)-prolinol, but we did not seek to confirm the absolute stereochemical integrity of
subsequent materials. Full experimental procedures for the synthesis of compounds 16, 17 and 25 have not appeared previously, although we have reported this chemistry in preliminary form as part of an earlier study [27]. Similarly, we have described the synthesis and use of compounds 41 and 43 before as precursors in other chemistry [36], but have not before given detailed experimental procedures. Compounds 32–35, 38, 40, 42 and 44–48 are previously unreported.

3.2. Synthesis and Reactivity of the Pyrrolo-Based Systems

N-(Ethoxycarbonyl)-prolinol (21). To a stirring solution of S-prolinol (20, 1.00 g, 9.89 mmol) in 4 M NaOH (7 mL) was added ethyl chloroformate (1.13 mL, 1.29 g, 11.9 mmol) over 10 min at 0 °C. The reaction was stirred at 0 °C for 30 min followed by 30 min at ambient temperature. The reaction solution was neutralized with 2M HCl, the aqueous phase was separated and extracted with DCM (3 × 10 mL). The combined organic layers were dried (MgSO4), filtered and concentrated under reduced pressure to give the crude product 21 (1.64 g, 96%) as a yellow oil which was used directly in the next step. NMR: \( \delta_H \) (400 MHz, CDCl3): 1.27 (3H, t, \( J = 7.1 \), COCH2CH3), 1.57 (1H, m, CHH), 1.74–1.91 (2H, m, CHH + CHH), 1.99–2.08 (1H, m, CHH), 3.31–3.78 (1H, m, NCHH), 3.49–3.54 (1H, m, NCHH), 3.59–3.69 (2H, m, CH2OH), 3.97–4.03 (1H, m, CH2O), 4.14 (2H, q, \( J = 7.1 \), COCH2CH3), 4.61 (1H, dd, \( J = 7.6, 2.6 \), NCH/CH2); \( \delta_C \) (100 MHz, CDCl3): 14.7 (CH3), 24.1 (CH2), 28.6 (CH2), 47.3 (CH2), 60.6 (CH), 61.6 (CH2), 67.3 (CH2), 157.6 (q). IR: \( \nu_{\text{max}} \) (thin film cm\(^{-1}\)): 770 (s), 906 (m), 1046 (s), 1106 (s), 1333 (s), 1379 (s), 1414 (s), 1667 (s), 2876 (w), 2975 (w), 3400–3500 (br). LRMS (ESI+): Found 196.1 [M+Na]+, C8H15N4NaO3 requires 196.1.

N-(Ethoxycarbonyl)-prolinal (22). A 2 M solution of (COCl)2 in DCM (4.72 mL, 9.43 mmol) was diluted with dry DCM (12 mL) and cooled to −78 °C under N2. DMSO (1.34 mL, 1.47 g, 18.9 mmol) in DCM (5 mL) was added followed by the alcohol 21 (1.36 g, 7.86 mmol) in DCM (5 mL), over 15 min. The whole was maintained at −78 °C for 30 min before the addition of Et3N (5.48 mL, 3.98 g, 39.3 mmol) dropwise over 10 min. The whole was allowed to reach room temperature over an hour before being quenched with a mixture of Et2O (12.5 mL) and H2O (12.5 mL). The organic layer was separated and the aqueous phase was extracted with DCM (3 × 10 mL). The combined organics were dried (MgSO4), filtered, concentrated under reduced pressure and purified by silica chromatography (20 g) (EtOAc/Hex; 2:3) to yield the aldehyde 22 as a mixture of rotamers in the form of a yellow oil (1.19 g, 89%). NMR: \( \delta_H \) (400 MHz, CDCl3): 1.17 & 1.24 (3H, 2 × t, \( J = 7.1 & 7.1 \), COCH2CH3), 1.76–1.92 (2H, m, CH2), 1.93–2.09 (2H, m, CH2), 3.40–3.57 (2H, m, NCH2), 4.02–4.14 & 4.17–4.22 (3H, m, CO2CH2CH3 & NCHCHO), 9.48 & 9.56 (1H, 2 × d, \( J = 2.5, 1.7 \), CHO); \( \delta_C \) (100 MHz, CDCl3): 14.5/14.7 (CH3), 23.8/24.5 (CH2), 26.6/27.8 (CH2), 46.6/47.1 (CH2), 61.5 (2 × CH2), 64.8/65.1 (CH), 154.7/155.6 (q), 200.2/200.3 (CHO). IR: \( \nu_{\text{max}} \) (thin film cm\(^{-1}\)): 729 (s), 771 (s), 914 (m), 1021 (m), 1106 (s), 1172 (m), 1341 (s), 1380 (s), 1416 (s), 1466 (s), 1687 (s), 1733 (s), 2872 (m), 2980 (m). LRMS (ESI+): Found 194.1 [M+Na]+, CsH13NNaO3 requires 194.1.

N-2-Ethenyl-1-ethoxycarbonylpyrrolidine (23). n-BuLi in hexanes (1.6 M, 10.5 mL, 16.8 mmol) was added dropwise over 30 min to a stirring suspension of methyltriphenylphosphonium bromide (5.52 g, 15.4 mmol) in anhydrous THF (30 mL) at −78 °C under an inert atmosphere of nitrogen. The whole was allowed to reach −10 °C and kept at that temperature for 30 min before being cooled back to −78 °C. The
aldehyde 22 (1.20 g, 7.02 mmol) in anhydrous THF (5 mL) was added dropwise over 10 min. The whole was allowed to warm to −10 °C, and maintained at that temperature for 2 h and then the mixture was allowed to reach room temperature overnight. The mixture was quenched with saturated aqueous NH₄Cl (20 mL) and the aqueous layer was separated and extracted with EtOAc (3 × 10 mL). The combined organic layers were dried (MgSO₄), filtered, concentrated under reduced pressure and purified by silica chromatography (50 g) (EtOAc/Hex; 3:2) yielding an air sensitive product as a mixture of rotamers in the form of a pale orange oil (536 mg, 45%). NMR: δH (400 MHz, CDCl₃): 1.22–1.27 (3H, br, m, CH₃), 1.71 (1H, bs, CH₂CH₂CH₂), 1.81 (2H, br, m, NCHCH₂), 1.97 (1H, m, br, CH₂CHCHCH₂), 3.43 (2H, s, br, NCH₂), 4.13 (2H, br, m, OCH₂), 4.33 (1H, br, m, NCH), 5.04–5.12 (2H, br, m, CH=CCH₂), 5.72 (1H, br, m, CH=CH₂); δC (100 MHz, CDCl₃): 14.2/14.8 (CH₃), 22.6/23.4 (CH₂), 31.2/31.9 (CH₂), 46.3/46.5 (CH₂), 58.9/59.3 (CH), 60.4/60.8 (CH₂), 113.8/114.1 (CH₂), 138.2/138.5 (CH), 155.1/155.4 (q).

N-(2'-Azidobenzoyl)-2-ethenylpyrrolidine (14). To a vigorously stirred suspension of finely ground KOH (2.31 g, 41.2 mmol) in ethylene glycol (7.2 mL) was added hydrazine hydrate (0.25 mL, 7.92 mmol) and 2-ethenyl-1-ethoxycarbonylpyrrolidine (23) (0.2706 g, 1.58 mmol) and the whole was heated to reflux (195 °C) for 4 h. The reaction was allowed to reach ambient temperature before being diluted with a mixture of Et₂O (4 mL) and H₂O (4 mL). The thick syrup was extracted with Et₂O (3 × 5 mL) and dried with finely ground NaOH. Et₃N (0.33 mL, 2.37 mmol) was added to the ethereal solution containing the amine at 0 °C under an inert atmosphere and was stirred for 10 min before the freshly prepared acid chloride 27a (0.48 g, 2.30 mmol) in Et₂O (5 mL) was added dropwise over 10 min and the whole was allowed to reach ambient temperature overnight. The reaction was diluted with water (20 mL), the ethereal layer was separated and the aqueous phase was extracted with ether (3 × 10 mL). The combined organics were dried (MgSO₄), filtered, concentrated under reduced pressure and purified by silica chromatography (21 g) (EtOAc/Hex; 2:3) to yield the product as a mixture of rotamers (0.1230 g, 32%) in the form of a yellow oil. NMR: δH (400 MHz, CDCl₃): 1.73–2.15 (4H, m, 2 × CH₂), 3.17–3.23 & 3.30–3.36 (1H, m, CCH₂), 3.64–3.71 & 3.30–3.36 (1H, m, CCH₂), 4.11–4.15 & 4.84–4.87 (1H, br, m, CH₂CH₂), 4.70 & 5.35 (1H, 2 × d, J = 16.9, 17.1, CH=CH₂), 4.89 & 5.20 (1H, 2 × d, J = 10.3 & 10.4, CH₂CH₂), 4.89 & 5.20 (1H, 2 × d, J = 10.3 & 10.4, CH₂CH₂), 5.56 & 5.90 (1H, 2 × ddd, J = 6.2, 10.4, 16.9 & 4.8, 10.4, 17.1, CH₂CH₂) 7.10–7.24 (3H, m, ArH), 7.32–7.45 (1H, m, ArH); δC (100 MHz, CDCl₃): 22.1/23.6 (CH₂), 30.9/32.3 (CH₂), 45.9/48.2 (CH₂), 58.4/61.1 (CH₂), 114.5/114.9 (CH₂), 118.4/118.5 (CH), 124.6/125.2 (CH), 127.9/128.3 (CH), 129.6/130.1 (q), 130.2/130.3 (CH), 136.1 (q), 136.9/137.6 (CH), 166.8/167.4 (q). IR: νmax (thin film cm⁻¹): 750 (s), 932 (s), 1083 (m), 1149 (m), 1292 (s), 1415 (s), 1449 (s), 1598 (s), 1631 (s), 2128 (s), 2878 (m), 2973 (m), 3078 (w). LRMS (ESI+): Found 265.1 [M+Na]⁺, 507.2 [2M+Na]⁺. HRMS (ESI+): Found 265.1064 [M+Na]⁺, C₁₃H₁₄N₄NaO requires 265.1060.

Pyrrolobenzodiazepine (25) and aziridinopyrrolobenzodiazepine (16). The alkene 14 (78.0 mg, 0.32 mmol) was dissolved in CHCl₃ (10 mL) and heated at reflux under an inert atmosphere of nitrogen for 16 h whilst being monitored by TLC (EtOAc/Hex; 3:2). The reaction mixture was concentrated under reduced pressure and purified by silica chromatography (20 g) (EtOAc/Hex; 3:2–4:1) to yield two inseparable close-running spots on TLC which were found to be the aziridine 16 and the methyl imine 25 in a 1:1 ratio (estimated by ¹H-NMR, 43 mg, 55%). NMR: δH (400 MHz, CDCl₃): 1.96–2.06 (2H, m, CH₂), 2.00
N-(2'-Azidobenzenesulfonyl)-2-ethenylpyrrolidine (15). To a stirring suspension of KOH (4.280 g, 76.5 mmol) in ethylene glycol (15 mL), hydrazine hydrate (0.46 mL, 471 mg, 14.7 mmol) was added under nitrogen followed by the ester protected alkene 23 (497 mg, 2.94 mmol), and the whole was heated at reflux (195 °C) for 4.5 h. The reaction mixture was cooled to ambient temperature and was diluted with Et2O (9 mL) and water H2O (9 mL). The organic layer was separated and the aqueous layer was extracted with Et2O (3 × 5 mL) and dried over NaOH. Et3N (0.62 mL, 4.41 mmol) was added to the ethereal solution containing the amine at 0 °C and the mixture was stirred for 10 min under an inert atmosphere of nitrogen before addition of freshly prepared 2-azidobenzenesulfonyl chloride 27b [which was prepared by heating at reflux 2-azidobenzenesulfonic acid (900 mg, 4.41 mmol) with 2M oxalyl chloride in DCM (4.4 mL, 8.82 mmol) which was concentrated under reduced pressure and suspended in Et2O (10 mL)]. The whole was allowed to reach room temperature overnight. The reaction was diluted with water (30 mL), the ethereal layer was separated and the aqueous layer was extracted with Et2O (3 × 10 mL). The combined organics were dried (MgSO4), filtered, concentrated under reduced pressure and purified by silica chromatography (20 g) (EtOAc/Hex; 1:4) to yield the product as a yellow oil (163 mg, 20%). NMR: δH (400 MHz, CDCl3): 1.30–1.40 (1H, m, CH2), 1.83–1.92 (2H, m, CH2), 2.07–2.10 (1H, m, CH/H), 2.92–2.99 (1H, m, CH/H), 3.54–3.57 (1H, m, CH/H), 3.93–3.98 (1H, m, NCH), 4.37–4.40 (1H, m, CH=CH2), 4.54 (1H, dd, J = 12.6, 17.8, CH=CH/H), 5.30–5.37 (1H, m, CH=CH2), 7.16 (1H, dd, J = 8.0, 8.0, ArH), 7.45 (1H, dd, J = 8.4, 8.4, ArH), 7.83 (1H, d, J = 8.4, ArH), 7.91 (1H, d, J = 8.0, ArH). IR: υmax (thin film cm⁻¹): 926 (m), 1026 (s), 1099 (m), 1157 (m), 1182 (s), 1296 (m), 1330 (m), 1445 (m), 1471 (s), 1518 (m), 1592 (s), 2137 (s), 2871 (m), 2977 (m). LRMS (ESI+): Found 215.1 [M+H]+, 237.1 [M+Na]+, 451.2 [2M+Na]+, 665.3 [3M+Na]+. HRMS (ESI+): Found 215.1179 [M+H]+, C13H15N2O requires 215.1179.

Aziridinopyrrolobenzothiadiazepine (17). The N-(2'-azidobenzenesulfonyl)-2-ethenylpyrrolidine (15) (125 mg, 0.449 mmol) was dissolved in toluene (10 mL) and heated to reflux for 18 h. The reaction was allowed to reach room temperature before the solvent was removed and the crude was purified by silica chromatography (25 g) (EtOAc/Hex;2:3) to yield the aziridine product 17 as a tan colored oil (50 mg, 44%). NMR: δH (500 MHz, CDCl3): 1.81–2.07 (4H, m, 2 × CH2), 2.91–2.95 (1H, m, SO2NCH/H), 3.32 (1H, dd, J = 9.9, 11.7, ArNCH/H), 3.50 (1H, m, SO2NCH/H), 3.57 (1H, dd, J = 3.9, 11.7, ArNCH/H), 3.89
(1H, ddd, J = 2.9, 2.9, 7.9, SO₂NCH), 4.84–4.87 (1H, m, ArNCH), 6.70 (1H, d, J = 8.1, ArH), 6.84 (1H, dd, J = 7.8, 7.8, ArH), 7.20 (1H, ddd, J = 7.8, 7.8, 1.3, ArH), 7.70 (1H, dd, J = 8.1, 1.3, ArH); δC (125 MHz, CDCl₃): 24.5 (CH₂), 25.8 (CH₂), 43.4 (CH₂), 49.2 (CH₂), 59.3 (CH), 62.9 (CH), 118.6 (CH), 119.9 (CH), 125.7 (q), 129.2 (CH), 132.7 (CH), 144.6 (CH). IR: νmax (thin film cm⁻¹): 759 (s), 1009 (m), 1037 (s), 1081 (m), 1181 (m), 1252 (s), 1274 (m), 1333 (s), 1482 (s), 1520 (m), 1591 (s), 2926 (m), 2970 (w), 3066 (m), 3362 (w).

Synthesis of 1-[2'-Azido-4-(benzyloxy)-5-methoxybenzoyl]prolinal (37)

Step 1: Synthesis of 1-[2'-Azido-4-(benzyloxy)-5-methoxybenzoyl]prolinal

2'-Azido-4-(benzyloxy)-5-methoxybenzoic acid (236 mg, 0.789 mmol) was dissolved in dry toluene (5 mL) and SOCl₂ (1.5 mL) was added. The solution was placed in a preheated oil bath at 85 °C for 3 h. The reaction was cooled to ambient temperature, concentrated in vacuo, dissolved in fresh DCM (3 × 10 mL) and concentrated in vacuo, to remove the excess SOCl₂. K₂CO₃ (218 mg, 1.58 mmol) in water (2 mL) was added in one portion to a stirring solution of S-prolinol (0.162 mL, 168 mg, 1.66 mmol) in DCM (2 mL) and the whole was stirred for 10 min. The acid chloride in DCM (5 mL) was added drop-wise and the whole was stirred at ambient temperature overnight before the organic layer was separated. The aqueous phase was extracted with DCM (3 × 10 mL) and the combined organic layers were dried (MgSO₄), filtered, concentrated and purified by silica chromatography (20 mg) (EtOAc/Hex; 3:1) to yield the product as an orange oil (262 mg, 87%). NMR: δH (400 MHz, CDCl₃): 1.56–1.84 (3H, m, CH₂ + CH₃), 2.06–2.13 (1H, m, CH₂), 3.20–3.30 (2H, m, CH₂), 3.62–3.67 (1H, m, CH₂O⁻), 3.73–3.77 (1H, m, CH₂OH), 3.80 (3H, s, OMe), 4.23–4.29 (1H, m, CH₂O⁻), 5.10 (2H, s, OCH₂), 5.10 (2H, s, OCH₂), 6.59 (1H, s, ArH), 6.76 (1H, s, ArH), 7.24–7.38 (5H, m, ArH); δC (100 MHz, CDCl₃): 24.5 (CH₂), 28.5 (CH₂), 49.6 (CH₂), 56.3 (CH₃), 61.2 (CH), 66.6 (CH₂), 71.2 (CH₂), 104.2 (CH), 110.7 (CH), 121.4 (q), 127.3 (CH), 128.2 (CH), 128.7 (CH), 135.9 (q), 147.2 (q), 149.8 (q), 168.8 (q). IR: νmax (thin film cm⁻¹): 742 (s), 1004 (m), 1048 (m), 1077 (m), 1178 (s), 1214 (s), 1242 (s), 1433 (s), 1511 (s), 1603 (s), 1684 (s), 2109 (s), 2882 (w), 2937 (w), 3145–3593 (br). LRMS (ESI⁺): Found 383.2 [M+H]⁺, 765.3 [2M+H]⁺, 787.3 [2M+Na]⁺. HRMS (ESI⁺): Found 383.1708 [M+H]⁺, C₂₀H₂₂N₄O₄ + H⁺ requires 383.1714.

Step 2: Oxidation to 1-[2'-azido-4-(benzyloxy)-5-methoxybenzoyl]prolinal (37)

DMSO (0.14 mL, 154 mg, 1.97 mmol) in DCM (1 mL) and the alcohol from the previous step (250 mg, 0.654 mmol) in DCM (2 mL) were added dropwise, respectively, over 15 min, to a solution of 2 M oxalyl chloride (0.39 mL, 0.785 mmol) at −78 °C in DCM (1 mL). After 10 min, Et₃N (0.24 mL, 174 mg, 1.72 mmol) was added dropwise to the mixture at −78 °C. The whole was allowed to reach room temperature over two h before being quenched with a mixture of Et₂O (10 mL) and H₂O (10 mL). The organic layer was separated and the aqueous layer was extracted with DCM (3 × 10 mL). The combined organics were dried (MgSO₄), filtered, concentrated and purified by silica chromatography (20 g) (EtOAc/Hex; 3:1) to give the product as a mixture of rotamers in the form of an orange oil (161 mg, 65%). NMR: δH (400 MHz, CDCl₃): 1.79–1.89 (2H, m, CH₂), 1.97–2.04 (1H, m,
Molecules 2014, 19 16747

CHH), 2.07–2.20 (1H, m, CH/H), 3.28–3.34 & 3.35–3.41 (2H, m, NCH2), 3.76 & 3.81 (3H, 2 × s, OCH3), 4.12–4.17 & 4.52–4.57 (1H, m, NC/HCHO), 5.08 & 5.12 (2H, 2 × s, OCH2), 6.53 & 6.61 (1H, 2 × d, J = 1.4 & 1.8, CHO); δC (100 MHz, CDCl3): 24.9/26.4 (CH2), 46.8/48.6 (CH2), 56.3/56.4 (CH3), 60.4 (CH2), 64.8/66.5 (CH), 71.3/71.3 (CH2), 104.2/104.4 (CH), 111.2/111.5 (CH), 120.7/120.8 (q), 127.4 (CH), 128.3 (CH), 128.8 (CH), 135.9/136.0 (q), 147.3/147.3 (q), 150.0/150.1 (q), 167.2/167.4 (q), 198.0/199.4 (CH).

IR: υmax (thin film cm−1): 1245 (s), 1430 (s), 1454 (s), 1512 (s), 1604 (s), 1622 (s), 1731 (m), 2110 (s), 2942 (m). LRMS (ESI+): Found 381.2 [M+H]+, 403.1 [M+Na]+, 783.3 [2M+Na]+. HRMS (ESI+): Found 403.1375 [M+Na]+, C20H20N4O4 + Na+ requires 403.1377.

(1-(2’-Azidobenzenesulfonyl)pyrrolidin-2-yl)methanol (29). 2-Azidobenzenesulfonic acid (2.95 g, 14.8 mmol) was heated at reflux in a 2M solution of (COCl)2 in dichloromethane (14.8 mL, 29.6 mmol) with a drop of DMF under an inert atmosphere of nitrogen for 5 h. The reaction was allowed to reach room temperature before the crude acid chloride was concentrated in vacuo and washed with dichloromethane (3 × 10 mL). K2CO3 (3.30 g, 23.7 mmol) in water (10 mL) was added in one portion to a stirring solution of L-prolinol (0.60 g, 5.9 mmol) in dichloromethane (15 mL). The crude sulfonyl chloride in dichloromethane (10 mL) was added slowly and the whole was stirred for 18 h at room temperature. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried (MgSO4), filtered, and concentrated to give the product as a pure orange oil (1.61 g, 96% from prolinol). NMR: δH (400 MHz, CDCl3): 1.67–1.78 (1H, m, CH/H), 1.79–1.99 (3H, m, CH2/H + CH2), 2.79 (1H, bs, CH2OH), 3.38 (1H, dt, J = 6.3, 10.2, NCH/H), 3.49–3.56 (1H, m, NCH/H), 3.62 (1H, dd, J = 5.6, 11.5, CH/CHOH), 3.70 (1H, dd, J = 4.2, 11.5, CH/CHOH), 4.02–4.08 (1H, m, CH2/HOH), 7.27 (1H, ddd, J = 1.0, 7.8, 7.8, ArH), 7.32 (1H, dd, J = 1.0, 8.0, ArH), 7.62 (1H, dd, J = 1.5, 7.8, 7.8, ArH), 8.02 (1H, dd, J = 1.5, 8.0, ArH); δC (100 MHz, CDCl3): 24.7 (CH2), 29.0 (CH2), 49.5 (CH2), 61.8 (CH), 65.5 (CH2), 119.9 (CH), 124.8 (CH), 129.0 (q), 132.6 (CH), 134.2 (CH), 138.2 (q). IR: υmax (thin film cm−1): 819, 870, 900, 928, 992, 1043, 1069, 1122, 1146, 1155, 1199, 1264, 1287, 1323, 1439, 1471, 1574, 1583, 1660, 2120, 2876, 2953, 3172–3693 (br). LRMS (ESI+): Found 305.1 [M+Na]+, 587.1 [2M+Na]+. HRMS (ESI+): Found 305.0676 [M+Na]+, C11H14N4O3S + Na+ requires 305.0679.

(1-(2’-Azidobenzenesulfonyl)pyrrolidin-2-yl)carbaldehyde (31). A 2M solution of oxalyl chloride in dichloromethane (1.88 mL, 3.75 mmol) was diluted with dichloromethane (10 mL) and cooled to −78 °C under nitrogen. DMSO (0.53 mL, 0.59 g, 7.5 mmol) in dichloromethane (10 mL) and the alcohol (0.80 g, 2.83 mmol) in dichloromethane (5 mL) were each added over 10 min. The whole was maintained at −78 °C for 30 min before dropwise addition of Et3N (2.18 mL, 1.58 g, 15.6 mmol) after which the whole was allowed to reach room temperature. The reaction was quenched with a mixture of Et2O (10 mL) and H2O (10 mL). The organic layer was separated and the aqueous phase was extracted with dichloromethane (3 × 10 mL). The combined organic layers were dried (MgSO4), filtered, concentrated and purified on silica (20 g) (EtOAc/Hex; 2:3) to yield the product as a white solid (0.57 g, 72%) which rapidly decomposed to an orange oil and was used immediately in subsequent reactions. NMR: δH (400 MHz, CDCl3): 1.83–1.95 (2H, m, CH2), 1.98–2.09 (1H, m, CH/H), 2.15–2.23 (1H, m, CH/H), 3.40 (1H, dt, J = 9.7, 7.2, NCH/H), 3.57 (1H, ddd, J = 5.5, 6.8, 9.7, CH/H), 4.47 (1H, ddd, J = 1.9, 4.5, 8.5,
CHCHO), 7.28 (1H, ddd, J = 7.8, 7.8, 1.0, ArH), 7.34 (1H, dd, J = 8.0, 1.0, ArH), 7.64 (1H, ddd, J = 7.8, 7.8, 1.6, ArH), 8.03 (1H, dd, J = 8.0, 1.6, ArH), 9.72 (1H, d, J = 1.6, CHO); δC (100 MHz, CDCl3): 25.0 (CH2), 27.7 (CH2), 48.8 (CH2), 67.1 (CH), 119.9 (CH), 124.9 (CH), 128.9 (q), 132.5 (CH), 134.4 (CH), 138.2 (q), 200.5 (CH). IR: νmax (thin film cm⁻¹): 820, 865, 999, 1080, 1122, 1156, 1199, 1265, 1287, 1332, 1439, 1471, 1574, 1583, 1603, 1730, 2122, 2953. HRMS (ESI+): compound degraded.

N-(2'-Azidobenzoyl)-2-(carbethoxy-1''-ethenyl)-pyrrolidine (32). The aldehyde 30 [33,34] (273 mg, 1.12 mmol) was dissolved in toluene (10 mL). (Carbethoxymethylene) triphenylphosphorane (390 mg, 1.12 mmol) was added in one portion and the whole was stirred at room temperature under an inert atmosphere of nitrogen for 12 h before being concentrated in vacuo and purified by silica chromatography (40 g) (EtOAc/Hex; 3:2) to yield the product 32 in the form of a yellow oil as a mixture of rotamers (178 mg, 51%). NMR: δH (400 MHz, CDCl3): 1.29 & 1.31 (3H, t, J = 7.1, 2 × COCH2C6H5), 1.80–1.99 & 2.01–2.43 (4H, m, CH2), 3.21–3.28 & 3.78–3.86 (1H, m, CHH), 3.34–3.40 & 3.69–3.75 (1H, m, CHH), 4.13 & 4.21 (2H, q, J = 7.1 & 7.1, COC2H2CH3), 4.24–4.30 & 4.96–5.00 (1H, m, NCCH2), 5.46 & 6.15 (1H, dd & d, J = 15.6, 1.1 & 15.6, CHC2HCO2Et), 6.59 & 6.94 (1H, dd & dd, J = 15.6, 4.6 & 15.6, 4.9, CHCHCO2Et), 7.12 (1H, dd, J = 7.5, 7.5, ArH), 7.14 (1H, d, J = 7.3, ArH), 7.19–7.25 (1H, m, ArH), 7.40 & 7.45 (2H, 2 × m, ArH); δC (100 MHz, CDCl3): 14.2/14.3 (CH3), 22.3/23.8 (CH2), 30.5/32.1 (CH2), 46.1/48.2 (CH2), 57.2 (CH), 60.4/60.6 (CH2), 118.5 (CH), 121.2/121.3 (CH), 124.9/125.2 (CH), 127.9 (CH), 129.1/129.5 (q), 130.6 (CH), 133.7/136.2 (q), 146.5 (CH), 165.8/166.5 (q), 167.1/167.4 (q). IR: νmax (thin film cm⁻¹): 753 (s), 1043 (m), 1093 (m), 1180 (s), 1301 (s), 1369 (m), 1414 (s), 1451 (s), 1475 (m), 1633 (s), 1716 (s), 2130 (s), 2238 (w), 2880 (m), 2979 (m). LRMS (ESI+): Found 315.1 [M+H]+, 337.1 [M+Na]+, 651.3 [2M+Na]+. HRMS (ESI+): Found 315.1451 [M+H]+, C16H18N4O3 + H+ requires 315.1452.

Aziridinopyrrolobenzodiazepine (33). The carbethoxy alkene 32 (178 mg, 0.567 mmol) was heated at reflux under nitrogen in CHCl3 (10 mL) for 48 h before being concentrated and purified by silica chromatography (20 g) (EtOAc/Hex; 3:2) to yield the product 33 as a ~1:1 mixture of two diastereoisomers in the form of a yellow oil (47 mg, 30%). NMR: δH (400 MHz, CDCl3) [mixture of isomers]: 1.23 (3H, t, J = 7.1, COCH2CH3, isomer “a”), 1.29 (3H, t, J = 7.1, COCH2CH3, isomer “b”), 1.74–1.80 (2H, m, CH2), 1.81–2.27 (4H, m, 2 × CH2), 2.77 (1H, d, J = 2.6, CHCO2Et, isomer “a”), 2.87 (1H, d, J = 2.7, CHCO2Et, isomer “b”); 3.08 (1H, dd, J = 9.6, 2.6, ArNH, isomer “a”), 3.37 (1H, dd, J = 8.7, 2.7, ArNCH, isomer “b”), 3.57 (1H, dd, J = 7.3, 7.3, 12.0, CHH), 3.66–3.73 (4H, m, COCH2CH3, isomer “a”), 4.20 (2H, quartet, J = 7.1, COCH2CH3, isomer “a”), 4.23 (2H, quartet, J = 7.1, COCH2CH3, isomer “b”), 4.38 (1H, bd, J = 9.6, CONCH), 6.69 (1H, d, J = 7.4, ArH), 6.94 (1H, dd, J = 7.5, 7.5, 0.9, ArH), 7.01 (1H, ddd, J = 7.5, 7.5, 1.0, ArH), 7.07 (1H, d, J = 8.0, ArH), 7.19 (1H, ddd, J = 7.6, 7.6, 1.6, ArH), 7.28 (1H, ddd, J = 7.7, 7.8, 1.6, ArH), 7.70 (2H, dd, J = 7.8, 1.8, ArH); δC (100 MHz, CDCl3): 14.2 (CH3), 14.5 (CH3), 23.1 (CH2), 25.6 (CH2), 29.5 (2 × CH2), 42.6 (CH), 46.4 (CH2), 47.0 (CH), 50.4 (CH), 56.6 (CH), 57.2 (CH), 60.1 (CH), 61.4 (CH2), 62.0 (CH2), 68.0 (CH2), 121.3 (CH), 122.0 (CH), 123.1 (CH), 123.3 (CH), 125.3 (q), 126.6 (q), 130.7 (CH), 131.0 (CH), 132.0 (CH), 132.2 (CH), 142.9 (q), 148.1 (q), 166.0 (q), 166.3 (q), 167.9 (q), 168.8 (q). IR: νmax (thin film cm⁻¹): 752 (m), 1024 (s), 1178 (s), 1217 (s), 1260 (s), 1372 (m), 1454 (m), 1503 (m), 1602 (s), 1622 (s), 1725 (s), 2871 (m), 2926 (m), 2977 (m). LRMS (ESI+): Found. 309.1 [M+Na]+. HRMS (ESI+): Found 309.1206 [M+Na]+, C16H18N4O3 + Na+ requires 309.1210.
**Aziridinopyrrolobenzothiadiazepine** (35). The aldehyde 31 (286 mg, 1.13 mmol) was dissolved in toluene (10 mL) and (carbethoxymethylene)triphenylphosphorane (500 mg, 1.43 mmol) was added in one portion and the whole was stirred at room temperature for 18 h. The reaction mixture was concentrated and purified by silica chromatography (40 g) (EtOAc/Hex; 3:2) to yield, as a single isomer, the aziridine 35 as a yellow oil (123 mg, 34%). NMR: δH (500 MHz, CDCl3): 1.41 (3H, t, J = 7.1, COCH2C6H5), 1.75–1.80 (1H, m, CHH), 1.87–2.01 (1H, m, CHH), 2.09–2.16 (1H, m, CHH), 2.27–2.35 (1H, m, CHH), 3.19 (1H, dd, J = 5.0, 9.6, 9.6, SO2NC6H5), 3.67–3.73 (2H, m, SO2NCH2CH3), 4.94 (1H, d, J = 9.8, ArNCH), 7.43 (1H, dd, J = 7.7, 7.7, 1.1, ArH), 7.55 (1H, dd, J = 7.7, 7.7, 1.5, ArH), 8.04 (1H, dd, J = 8.0, 1.5, ArH); δC (125 MHz, CDCl3): 14.1 (CH3), 22.7 (CH2), 28.9 (CH2), 46.4 (CH2), 60.7 (CH), 61.8 (CH), 63.0 (CH2), 85.4 (CH), 123.8 (CH), 126.6 (CH), 128.5 (CH), 131.6 (q), 133.6 (CH), 138.4 (q), 167.3 (q). IR: υmax (thin film cm⁻¹): 1035 (m), 1066 (m), 1092 (s), 1135 (m), 1167 (s), 1205 (m), 1247 (m), 1271 (m), 1344 (s), 1469 (s), 1503 (m), 1589 (m), 1738 (s), 2981 (m). LRMS (ESI+): Found 345.1 [M+Na]+, 723.2 [2M+Na]+. HRMS (ESI+): Found 345.0875 [M+Na]+, C15H18N2O4S + Na+ requires 345.0879.

**3-Benzylx-4-methoxy-11-ethyl-ethanoyl-[1,4]-pyrrolo[2,1-c] benzodiazepin-5-one** (40). The aldehyde 37 (198 mg, 0.58 mmol) was dissolved in toluene (10 mL) and (carbethoxymethylene)triphenylphosphorane (200 mg, 0.58 mmol) was added in one portion and the whole was stirred at room temperature for 18 h. The reaction was concentrated in vacuo and purified by silica chromatography (30 g) (EtOAc/Hex; 4:1) to yield the product 40 as a yellow oil (52 mg, 21%). NMR: δH (500 MHz, CDCl3): 1.32 (3H, t, J = 7.2, CO2CH2C6H5), 1.74–1.80 (1H, m, CHH), 1.93–2.19 (3H, m, CHH + CH2), 2.31–2.24 (2H, m, CH2CO2Et), 3.43–3.47 (1H, m, NHC6H5), 3.62–3.66 (1H, m, NHC6H5CH), 3.68–3.73 (1H, m, NCH3), 3.76–3.80 (1H, m, NCH3), 3.89 (3H, s, OMe), 4.06 (2H, q, J = 7.2, CO2C6H5CH3), 5.13 (1H, d, J = 12.3, PhCH2OH), 5.18 (1H, d, J = 12.3, PhCH2OH), 6.34 (1H, s, ArH), 7.31–7.50 (7H, m, 6 × ArH + NH); δC (125 MHz, CDCl3): 14.2 (CH3), 23.2 (CH2), 29.9 (CH2), 37.3 (CH2), 46.9 (CH2), 56.3 (CH3), 60.1 (CH), 61.0 (CH2), 62.7 (CH), 70.9 (CH2), 108.1 (CH), 113.1 (CH), 120.0 (q), 127.4 (CH), 128.0 (CH), 128.4 (CH), 136.5 (q), 137.8 (q), 145.0 (q), 150.9 (q), 168.3 (q), 171.9 (q). IR: υmax (thin film cm⁻¹): 723 (m), 1025 (s), 1119 (s), 1178 (s), 1218 (s), 1260 (s), 1373 (m), 1432 (s), 1453 (m), 1503 (m), 1602 (s), 1623 (m), 1726 (m), 2860 (m), 2924 (s), 2953 (m). LRMS (ESI+): Found 447.2 [M+Na]+. HRMS (ESI+): Found 447.1895 [M+Na]+ requires C24H28N2O5 + Na+ requires 447.1890.

### 3.3. Synthesis and Reactivity of the Azetidino-Based Systems

**4-Methyl-4-ethenyl-1-azetidin-2-one** (41a). To a stirred solution of isoprene (2.33 g, 3.43 mL, 34.41 mmol) in dry diethyl ether (15 mL) at −78 °C was added a solution of chlorosulfonyl isocyanate (4.88 g, 3.01 mL, 34.01 mmol) in dry ether (10 mL) dropwise over one hour. The reaction mixture was allowed to warm to −10 °C dropwise over one hour. The reaction mixture was allowed to warm to −10 °C and then the reaction flask was transferred to an ice-salt bath and stirred for 30 min. The cooled solution was added dropwise to a vigorously stirred solution of water (50 mL), sodium carbonate (9.00 g), sodium sulfite (6.01 g) and ice (30 g) over 10 min. The mixture was stirred at −10 °C for 1 h and then allowed to warm to room temperature and extracted with diethyl ether (6 × 20 mL). The combined organic extracts were dried (MgSO4) and the solvent removed under reduced pressure to give the product as a pale yellow
oil (2.20 g, 58%). NMR: δH (400 MHz, CDCl3): 1.50 (3H, s, Me), 2.79 (2H, s, CH2), 5.10 (1H, dd, J = 10.6, 0.7, CH=CHH), 5.22 (1H, dd, J = 17.2, 0.7, CH=CHH), 6.02 (1H, dd, J = 10.6, 17.2, CH=CH2), 6.83 (1H, br, NH); δC (100 MHz, CDCl3): 24.8 (CH2), 50.7 (CH3), 54.5 (q), 113.8 (CH), 141.1 (CH2), 167.6 (q). IR: υmax (thin film cm⁻¹): 923 (m), 1153 (m), 1186 (m), 1226 (m), 1274 (w), 1304 (m), 1372 (m), 1412 (m), 1643 (m), 1720 (s), 2970 (w), 3235 (m). LRMS (ESI+): Found 134.1 [M+Na]+, 291.2 [2M+3Na]+.

4-Methyl-4-ethenyl-1-azetidin-2-thione (43a). To the azetidin-2-one (0.55 g, 4.97 mmol) in dry THF (15 mL) was added Lawesson’s reagent (1.00 g, 2.48 mmol) and the whole was stirred at room temperature for an hour before being heated to reflux for two h. The reaction was cooled to ambient temperature before being concentrated under reduced pressure and purified by silica chromatography (75 g) (EtOAc/petroleum ether; 1:3) to give the product as a yellow oil (0.35 g, 56%). The reaction was higher yielding (up to 70%) on a larger scale (2 g of lactam), but less convenient to purify (stench). NMR: δH (400 MHz, CDCl3): 1.60 (3H, s, Me), 2.98 (2H, s, CSCH2), 5.21 (1H, d, J = 10.6, CH=CHH), 5.28 (1H, d, J = 17.2, CH=CH2), 6.05 (1H, dd, J = 10.6, 17.2 CH=CH2), 8.78 (1H, bs, NH); δC (100 MHz, CDCl3): 23.8 (CH3), 54.6 (CH2), 63.7 (q), 115.1 (CH2), 139.0 (CH), 202.2 (q). IR: υmax (thin film cm⁻¹): 924 (s), 989 (m), 1016 (m), 1081 (s), 1217 (m), 1288 (m), 1374 (m), 1404 (s), 1466 (s), 2971 (w), 3147 (m).

4-Ethenyl-2-azetidinone (41b). To 1,3-butadiene (10 mL) condensed into anhydrous ether (40 mL) at −10 °C, was added a solution of chlorosulfonyl isocyanate (3.0 mL, 34.5 mmol) in anhydrous ether (10 mL) over one hour, under an atmosphere of nitrogen. The temperature was maintained at −10 °C for a further period of 3 h and warmed slowly to room temperature overnight, to produce a clear, yellow solution. The solution was added to an ice cold mixture of water (70 mL), ice (30 g), NaHCO3 (9.0 g) and Na2SO3 (6.0 g) and stirred for one hour at −10 °C. The reaction was allowed to warm to room temperature before being extracted with ether (6 × 20 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure to yield 4-ethenyl-2-azetidinone (2.01 g, 60% yield) as a clear yellow oil. NMR: δH (400 MHz, CDCl3): 6.49 (1H, s, N−H), 5.92 (1H, ddd, J = 17.2, 10.2, 3.2, =CH), 5.32 (1H, d, J = 17.2, =CH2), 5.20 (1H, d, J = 10.2, =CH2), 4.13 (1H, m, NCH), 3.25 (1H, m, ring-CH2), 2.70 (1H, d, J = 17.2 ring-CH2); δC (100 MHz, CDCl3): 167.82 (C=O), 137.43 (CH), 116.93 (CH2), 49.40 (CH2), 48.93 (CH). IR: υmax (thin film cm⁻¹): 3274.6 (m, broad), 1755.4 (s), 1482.2 (m), 1413.8 (m), 1380.1 (m), 1237.1 (m).

4-Ethenylazetidin-2-thione (43b). To the lactam (1.00 g, 10.3 mmol) dissolved in anhydrous THF (15 mL) was added Lawesson’s reagent (2.08 g, 5.15 mmol) in one portion, with stirring. The reaction was stirred at ambient temperature, under an inert atmosphere of dry nitrogen for one hour, before being heated at reflux for an additional hour. The reaction was monitored for completion by tlc and was subsequently cooled to room temperature, to give a crude product as a clear orange liquid. The sample was concentrated by rotary evaporation and purified using silica column chromatography (eluent: petroleum ether-ethyl acetate 4:1), to yield 4-ethenyl-azetidin-2-thione (0.67 g, 58%) as a clear, yellow oil. NMR: δH (400 MHz, CDCl3): 6.88 (1H, s, N−H), 5.96 (1H, ddd, J = 17.1, 10.3, 3.0, =CH), 5.35 (1H, d, J = 17.1, =CH2), 5.26 (1H, d, J = 10.3, =CH2), 4.62 (1H, m, NCH), 3.30 (1H, m, ring-CH2), 2.84 (1H, m, ring-CH2); δC (100 MHz, CDCl3): 203.65 (C=S), 135.14 (CH), 118.34 (CH2), 58.01 (CH), 48.34 (CH2). IR: υmax (thin film cm⁻¹): 3174.1 (m, broad), 1482.6 (s), 1426.0 (m), 1407.2 (w), 1237.1 (m).
1-(2′-Azidobenzoyl)-4-methyl-4-vinylazetidin-2-one (42a). 2-Azidobenzoic acid (508 mg, 3.02 mmol) was heated to reflux in SOCl₂ (4 mL) for 3 h under nitrogen. The excess SOCl₂ was removed in vacuo and the crude acid chloride was dissolved in DCM (3 × 5 mL) which was removed in vacuo to yield the crude 2-azidobenzoylchloride. The β-lactam 41 (457 mg, 4.12 mmol) in DCM (25 mL) and DMAP (100 mg) were chilled to −10 °C. The crude acid chloride in DCM (5 mL) was added dropwise to the solution over 10 min. The whole was maintained at −10 °C for 30 min before the addition of Et₃N (0.89 mL, 646 mg, 6.40 mmol) and the whole was allowed to reach room temperature overnight. The reaction was concentrated under reduced pressure and purified by silica chromatography (70 g) (EtOAc/Hex; 1:4) to give the product 42a as a mixture of rotamers in the form of a dark yellow oil (498 mg, 65%). NMR: δH (400 MHz, CDCl₃) rotamer 1: 1.87 (3H, s, Me), 2.98 (1H, d, J = 16.2, COCH₂), 3.08 (1H, d, J = 16.2, COCH), 5.35 (1H, d, J = 10.7, CH=CH), 5.45 (1H, d, J = 17.3, CHH=CH₂), 6.24 (1H, dd, J = 10.7, 17.3, CH=CH₂), 7.22 (1H, dd, J = 7.6, 7.6, ArH), 7.23 (1H, d, J = 8.3, ArH), 7.42 (1H, dd, J = 7.6, 1.4, ArH), 7.52 (1H, ddd, J = 8.3, 8.3, 1.4, ArH).

δC (100 MHz, CDCl₃) rotamer 1: 21.7 (CH₃), 49.2 (CH₂), 58.9 (q), 114.9 (CH₂), 117.5 (CH), 123.7 (CH), 125.8 (q), 128.1 (CH), 131.2 (CH), 137.1 (q), 137.2 (CH), 162.4 (q), 162.5 (q); δC (100 MHz, CDCl₃) rotamer 2: 27.6 (CH₃), 39.5 (CH₂), 58.6 (q), 114.3 (CH₂), 119.9 (CH), 123.5 (q), 124.7 (CH), 130.8 (CH), 132.1 (CH), 138.9 (q), 139.9 (CH), 151.5 (q), 165.3 (q). IR: υmax (thin film cm⁻¹): 1084 (m), 1216 (s), 1329 (s), 1391 (s), 1451 (m), 1478 (m), 1519 (s), 1604 (m), 1656 (m), 1682 (m), 1801 (s), 2131 (s), 2853 (m), 2925 (m). LRMS (ESI+): 279.1 [M+Na]+, 535.2 [2M+Na]+. C₁₃H₁₂N₄NaO₂ requires 279.1.

8,9-Dimethylazetidino[2,1-a][1,4]benzodiazepin-2,11-dione (45). The azetidinone 42a (250 mg, 0.97 mmol) was heated to reflux in CHCl₃ (10 mL) under an atmosphere of dry nitrogen and monitored by NMR every 24 h. After 72 h, the reaction mixture was concentrated under reduced pressure and purified by silica chromatography (22 g) using graduated elution (EtOAc/Hex; 1:4–3:1) to give the imine 45 as a yellow oil (84 mg, 38%). NMR: δH (500 MHz, CDCl₃): 2.00 (3H, s, Me), 2.49 (1H, s, Me), 3.42 (1H, d, J = 16.0, COCHH), 3.77 (1H, d, J = 16.0, COCHH), 7.52 (1H, ddd, J = 7.7, 7.7, 1.0, ArH), 7.70 (1H, dd, J = 8.0, 1.0, ArH), 7.78 (1H, ddd, J = 7.7, 7.7, 1.6, ArH), 8.22 (1H, ddd, J = 8.0, 1.6, ArH); δC (125MHz, CDCl₃): 20.1 (CH₃), 26.2 (CH₃), 43.6 (CH₂), 73.3 (q), 123.7 (q), 126.5 (CH), 126.7 (CH), 127.4 (CH), 134.3 (CH), 149.7 (q), 155.2 (q), 158.1(q), 204.2 (q). IR: υmax (thin film cm⁻¹): 804 (m), 1216 (s), 1329 (s), 1391 (s), 1451 (m), 1478 (m), 1519 (s), 1604 (m), 1656 (m), 1682 (m), 1801 (s), 2131 (s), 2853 (m), 2925 (m). LRMS (ESI+): 279.1 [M+Na]+, 535.2 [2M+Na]+. C₁₃H₁₂N₄NaO₂ requires 279.1. HRMS (ESI+): 279.0862 [M+Na]+, C₁₃H₁₂N₄NaO₂ requires 279.0852.

1-(2′-Azidobenzoyl)-4-methyl-4-vinyl-1-azetidin-2-thione (44a). 2-Azidobenzoic acid (214 mg, 1.3 mmol) was heated to reflux under nitrogen in SOCl₂ (4 mL) for 4 h. The excess thionyl chloride was removed in vacuo and the product was dissolved in DCM (3 × 5 mL) and concentrated in vacuo to give the crude acid chloride. The thiolactam (250 mg, 1.97 mmol) in DCM (25 mL) and DMAP (100 mg) was chilled to −10 °C. The crude acid chloride in DCM (5 mL) was added dropwise over 10 min and the whole was
maintained at −10 °C for 30 min before dropwise addition of Et$_3$N (0.41 mL, 299 mg, 2.95 mmol). The whole was allowed to reach room temperature overnight and the reaction was concentrated and purified by silica chromatography (22 g) (EtOAc/Hex; 1:4) to give the product 44a as an orange oil (296 mg, 84%). NMR: $\delta_H$ (400 MHz, CDCl$_3$): 1.92 (3H, s, Me), 2.93 (1H, d, $J = 16.9$, CSC/H), 3.04 (1H, d, $J = 10.8$, C/H=CH), 5.38 (1H, d, $J = 17.3$, CHH=CH), 6.31 (1H, dd, $J = 17.3$, 10.8 CHH=CH), 7.20 (1H, dd, $J = 8.2$, 0.8, ArH), 7.24 (1H, ddd, $J = 7.6$, 7.6, 0.8, ArH), 7.36 (1H, dd, $J = 7.6$, 1.5, ArH), 7.54 (1H, ddd, $J = 8.2$, 8.2, 1.5, ArH); $\delta_C$ (100 MHz, CDCl$_3$): 22.3 (CH$_3$), 54.5 (CH$_2$), 67.5 (q), 116.4 (CH$_2$), 118.5 (CH), 125.1 (CH), 126.6 (q), 128.9 (CH), 132.1 (CH), 137.7 (CH), 138.3 (q), 164.2 (q), 201.7 (q). IR: $\upsilon_{\text{max}}$ (thin film cm$^{-1}$): 909 (s), 931 (m), 1215 (s), 1322 (s), 1358 (m), 1448 (w), 1478 (m), 1674 (s), 2131 (s). LRMS (ESI+): Found 295.1 [M+Na]$^+$, 567.1 [2M+Na]$^+$. HRMS (ESI+): Found 295.0623 [M+Na]$^+$, C$_{13}$H$_{12}$N$_4$NaOS requires 295.0624.

12-Methyl-1,2,3-triazolino[1,5-a]azetidino[1,4-c][1,4]benzodiazepin-2-on-14-thione (46). The 1-(2'-'azidobenzoyl)-azetidin-2-thione 44a (295 mg, 1.08 mmol) was heated at reflux in CHCl$_3$ (10 mL) under nitrogen for 36 h before being concentrated under reduced pressure and purified by silica chromatography (22 g) (EtOAc/Hex; 1:1) to give the triazolino product 46 as a yellow oil (180 mg, 61%). NMR: $\delta_H$ (500 MHz, CDCl$_3$): 1.22 (3H, s, Me), 2.89 (1H, d, $J = 16.8$, SCC/H), 2.94 (1H, d, $J = 16.8$, SCCH), 4.27 (1H, dd, $J = 6.1$, 12.2, CH$_3$C/HN), 4.37 (1H, dd, $J = 6.1$, 17.7, N$_3$CH), 4.71 (1H, dd, $J = 12.2$, 17.7, N$_3$CH), 7.14 (1H, ddd, $J = 7.1$, 7.1, 1.0, ArH), 7.52 (1H, ddd, $J = 7.2$, 7.2, 1.6, ArH), 8.04 (1H, dd, $J = 8.4$, 1.0, ArH), 8.2 (1H, dd, $J = 8.4$, 1.6, ArH); $\delta_C$ (125 MHz, CDCl$_3$): 16.8 (CH$_3$), 50.5 (CH$_2$), 59.6 (CH), 65.0 (q), 70.4 (CH$_2$), 115.9 (q), 118.6 (CH), 123.4 (CH), 134.2 (CH), 134.5 (CH), 138.3 (q), 161.8 (q), 198.1 (q). IR: $\upsilon_{\text{max}}$ (thin film cm$^{-1}$): 745 (s), 1103 (m), 1162 (m), 1211 (m), 1251 (m), 1322 (s), 1461 (s), 1720 (m), 2921 (w), 3000 (w). LRMS (ESI+): Found 295.1 [M+Na]$^+$, 567.1 [2M+Na]$^+$. HRMS (ESI+): Found 295.0622 [M+Na]$^+$, C$_{13}$H$_{12}$N$_4$NaOS requires 295.0624.

11-Thioxo-8,9-dimethyl-azetidino[2,1-c][1,4]benzodiazepin-2-one (47). A sample of the triazolo compound 46 from above (87 mg, 0.32 mmol) was heated at reflux in CHCl$_3$ (10 mL) under nitrogen for a week before being concentrated under reduced pressure and purified by silica chromatography (15 g) (EtOAc/Hex; 3:2) to give the methyl imine product as a yellow oil (25 mg, 32%). NMR: $\delta_H$ (400 MHz, CDCl$_3$): 1.98 (3H, s, Me), 2.47 (3H, s, Me), 3.40 (1H, d, $J = 16.0$, CSC/H), 3.77 (1H, d, $J = 16.0$, CSCHH), 7.50 (1H, dd, $J = 6.1$, 7.7, 1.0, ArH), 7.68 (1H, dd, $J = 8.1$, 1.5, ArH); $\delta_C$ (100 MHz, CDCl$_3$): 20.1 (CH$_3$), 26.3 (CH$_3$), 43.6 (CH$_2$), 73.3 (q), 126.6 (CH), 126.7 (CH), 127.3 (CH), 134.3 (CH), 149.6 (q), 155.2 (q), 158.0 (q), 204.4 (q). IR: $\upsilon_{\text{max}}$ (thin film cm$^{-1}$): 647 (s), 770 (s), 1103 (m), 1115 (m), 1132 (m), 1300 (m), 1323 (s), 1346 (s), 1460 (s), 1606 (s), 1651 (s), 1676 (s), 2928 (w), 2975 (w). LRMS (ESI+): Found 267.1 [M+Na]$^+$, C$_{13}$H$_{12}$N$_2$NaOS requires 267.0563.

1-(2'-Azidobenzoyl)-4-ethenyl-1-azetidin-2-thione (44b). To a solution of 4-ethenyl-1-azetidin-2-thione (43b, 0.16 g, 1.42 mmol) and dimethylaminopyridine (0.1 g, 0.82 mmol) in anhydrous dichloromethane (20 mL), was added, with stirring and under an atmosphere of dry nitrogen, 2-azidobenzoylchloride (0.28 g, 1.56 mmol) in anhydrous dichloromethane (10 mL) [prepared as described previously], dropwise over 20 min at −10 °C. The reaction was stirred for 30 min before triethylamine (0.29 mL, 2.83 mmol)
was added dropwise over 10 min at −10 °C. The reaction mixture was warmed to room temperature and left to stir at ambient temperature for 24 h, before being concentrated by rotary evaporation under reduced pressure and purified by flash silica column chromatography (eluent: petroleum ether:ethyl acetate, 4:1) to yield 1-(o-azidobenzoyl)-4-ethenyl-1-azetidin-2-thione (0.26 g, 71%) as a yellow solid, melting point: 79–82 °C. NMR: δH (500 MHz, CDCl3): 2.84 (1H, dd, J = 17.1, 3.1, CH/H), 3.24 (1H, dd, J = 17.1, 5.9, CH/H), 5.15 (1H, m, CH), 5.38 (1H, d, J = 10.4, CHH=CH), 5.49 (1H, d, J = 17.2, CHH=CH), 6.09 (1H, ddd, J = 17.2, 10.4, 7.0, =CH), 7.21 (2H, m, 2 × ArH), 7.39 (1H, ddd, J = 7.6, 1.2, ArH), 7.53 (1H, ddd, J = 7.8, 7.8, 1.5, ArH); δC (125 MHz, CDCl3): 47.10 (CH2), 59.42 (CH), 118.52 (CH), 119.46 (CH2), 124.97 (CH), 126.06 (q), 129.32 (CH), 132.40 (CH), 133.59 (CH), 133.59 (CH), 163.97 (C=O), 201.89 (C=S). IR: νmax (thin film cm−1): 2927.9 (s), 2853.4 (s), 2128.3 (s), 1687.0 (s), 1462.9 (m), 1375.5 (m). HRMS (ESI+): calc. for C12H10N4OS + H+ = 259.0648, measured = 259.0648.

(Aziridino[1,2-a]azetidino[2,1-c][1,4]benzodiazipine-7-one-9-thione (48). A solution of 1-(2'-azidobenzoyl)-4-ethenyl-1-azetidin-2-thione (44b, 0.074 g, 2.87 mmol) dissolved in anhydrous toluene (6 mL) was heated at reflux under an atmosphere of dry nitrogen for 24 h at which point point TLC confirmed that the reaction had gone to completion. The sample was cooled to room temperature, concentrated under reduced pressure, and purified by silica column chromatography (eluent: petroleum ether:ethyl acetate, 1:2) to yield the title compound 48 (0.032 g, 48% yield) as a yellow oil. NMR: δH (500 MHz, CDCl3): 2.26 (1H, d, J = 3.5, NCH/H), 2.81 (1H, d, J = 4.4, NC/H), 3.10 (1H, dd, J = 16.8, 2.8, CSCH/H), 3.17 (1H, ddd, J = 8.9, 4.4, 3.5, ArNCH), 3.31 (1H, dd, J = 16.8, 5.7, CSCH/H), 4.20 (1H, ddd, J = 8.9, 5.7, 2.8, CONCH), 7.10 (1H, ddd, J = 7.6, 7.6, 0.8, ArH), 7.17 (1H, d, J = 8.1, ArH), 7.45 (1H, ddd, J = 7.7, 7.7, 1.6, ArH), 7.80 (1H, dd, J = 8.1, 1.6, ArH); δC (125 MHz, CDCl3): 35.13 (CH2), 42.00 (CH), 45.14 (CH2), 58.86 (CH), 122.90 (CH), 123.47 (CH), 132.20 (CH), 133.76 (CH), 150.04 (q), 163.89 (C=O), 199.55 (C=S). IR: νmax (thin film cm−1): 2873.3 (w), 1698.2 (s), 1653.5 (s), 1601.9 (m), 1483.4 (m), 1355.8 (m), 1345.6 (m), 1195.4 (m). HRMS (ESI+): calc. for C12H10N2OS + H+ = 231.0587, measured = 231.0588.

4. Conclusions

Intramolecular 1,3-dipolar cycloadditions between an azide and an alkene in 1-(2'-azidoaryl)-2-alkenyl-proline based systems led to aziridino-fused pyrrolobenzodiazipines and pyrrolobenzothiadiazepines (4 examples), or to pyrrolobenzodiazipines with a methyl substituted imine (1 example) or a pyrrolobenzodiazipine with a carbethoxymethylene substituted amine (1 example). 1-(2'-Azidoaryl)-4-alkenyl-azetidin-2-ones reacted to give the corresponding aziridino-fused azetidinobenzodiazipine (1 example), triazolino-fused azetidinobenzodiazipine (1 example) or azetidinobenzodiazipine with a methyl substituted imine (2 examples). These reactivity patterns are unpredictable but can all be rationalized by the formation of a common triazoline intermediate. We are currently further exploring the chemistry of the 4-ethenyl-1-azetidin-2-one derivatives 41–44 and are also investigating the antitumour and antibiotic potential of the PBD, PBTM and azetidinobenzodiazipines reported in this paper.

Acknowledgments

We thank the EPSRC for DTA funding to C.S.C., and the University of Huddersfield for a PhD bursary to P.A.O’G. We thank Neil McLay, University of Huddersfield, for NMR and mass
spectroscopic support. We are grateful to the EPSRC National Mass Spectrometry Service, University of Wales, Swansea for HRMS.

**Author Contributions**

Hemming designed the project and is the principal and corresponding author and wrote the text. Chambers, O’Gorman and Jamshaid conducted the practical chemistry shown in Schemes 1 and 2 and provided the experimental procedures and characterization data.

**Conflicts of Interest**

The authors declare no conflict of interest.

**References**

1. Hochlowski, J.E.; Andres, W.W.; Theriault, R.J.; Jackson, M.; McAlpine, J.B. Abbeymycin, a new anthramycin-type antibiotic produced by a streptomycete. *J. Antibiot.* **1987**, *40*, 145–148.
2. Wang, T.; Lui, A.S.; Cloudsdale, I.S. A novel route to pyrrolo[2,1-c][1,4]benzodiazepin-5-ones. Formal total synthesis of (±)-DC-81. *Org. Lett.* **1999**, *1*, 1835–1837.
3. Pettersson, B.; Hasimbegovic, V.; Bergman, J. One-pot Eschenmoser episulfide contractions in DMSO: Applications to the synthesis of fuligocandins A and B and a number of vinylogous amides. *J. Org. Chem.* **2011**, *76*, 1554–1561.
4. Gerratana, B. Biosynthesis, synthesis and biological applications of pyrrolobenzodiazepines. *Med. Res. Rev.* **2012**, *32*, 254–293.
5. Antonow, D.; Thurston, D.E. Synthesis of DNA-interactive pyrrolo[2,1-c][1,4]benzodiazepines (PBDs). *Chem. Rev.* **2011**, *111*, 2815–2864.
6. Brucoli, F.; Hawkins, R.M.; James, C.H.; Jackson, P.J.M.; Wells, G.; Jenkins, T.C.; Ellis, T.; Kotecha, M.; Hochhauser, D.; Hartley, J.A.; *et al*. An extended pyrrolobenzodiazepine-polyamide conjugate with selectivity for a DNA sequence containing the ICB2 transcription factor binding site. *J. Med. Chem.* **2013**, *56*, 6339–6351.
7. Kamal, A.; Ramakrishna, G.; Janaki Ramaiah, M.; Viswanath, A.; Subba Rao, A.V.; Bagul, C.; Mukhopadhyay, D.; Pushpavalli, S.N.C.V.L.; Pal-Bhadra, M. Design, synthesis and biological evaluation of imidazo[1,5-a]pyridine–PBD conjugates as potential DNA-directed alkylating agents. *Med. Chem. Commun.* **2013**, *4*, 697–703.
8. Rahman, K.M.; Jackson, P.J.M.; James, C.H.; Piku Basu, B.; Hartley, J.A.; de la Fuente, M.; Schatzlein, A.; Robson, M.; Barbara Pedley, R.; Pepper, C.; *et al*. GC-targeted C8-linked pyrrolobenzodiazepine-biaryl conjugates with femtomolar *in vitro* cytotoxicity and *in vivo* antitumor activity in mouse models. *J. Med. Chem.* **2013**, *56*, 2911–2935.
9. Hartley, J.A.; Hamaguchi, A.; Suggitt, M.; Gregson, S.J.; Thurston, D.E.; Howard, P.W. DNA interstrand cross-linking and *in vivo* antitumor activity of the extended pyrrolo[2,1-c][1,4]benzodiazepine dimer SG2057. *Investig. New Drugs* **2012**, *30*, 950–958.
10. Gregson, S.J.; Howard, P.W.; Hartley, J.A.; Brooks, N.A.; Adams, L.J.; Jenkins, T.C.; Kelland, L.R.; Thurston, D.E. Design, synthesis, and evaluation of a novel pyrrolobenzodiazepine DNA-interactive agent with highly efficient cross-linking ability and potent cytotoxicity. J. Med. Chem. 2001, 44, 737–748.
11. Rahman, K.M.; James, C.H.; Thurston, D.E. Effect of base sequence on the DNA cross-linking properties of pyrrolobenzodiazepine (PBD) dimers. Nucleic Acids Res. 2011, 39, 5800–5812.
12. Witt, A.; Bergman, J. Total syntheses of the benzodiazepine alkaloids curcumdatin F and curcumdatin C. J. Org. Chem. 2001, 66, 2784–2788.
13. López-Romero, B.; Evrard, G.; Durant, F.; Sevrin, M.; George, P. Molecular structure and stereoelectronic properties of sarmazenil—A weak inverse agonist at the omega modulatory sites (benzodiazepine receptors): Comparison to bretazenil and flumazenil. Bioorg. Med. Chem. 1998, 6, 1745–1757.
14. Mohapatra, D.K.; Maity, P.K.; Shabab, M.; Khan, M.I. Click chemistry based rapid one-pot synthesis and evaluation for protease inhibition of new tetracyclic fused benzodiazepine derivatives. Bioorg. Med. Chem. Lett. 2009, 19, 5241–5245.
15. Thurston, D.E.; Bose, D.S. Synthesis of DNA-interactive pyrrolo[2.1-c][1,4]benzodiazepines. Chem. Rev. 1994, 94, 433–465.
16. Markandeya, N.; Shankaraiah, N.; Reddy, C.S.; Santos, L.S.; Kamal, A. Asymmetric syntheses of piperidino-benzodiazepines through ‘cation-pool’ host/guest supramolecular approach and their DNA-binding studies. Tetrahedron Asymmetry 2010, 21, 2625–2630.
17. Thurston, D.E.; Jones, G.B.; Davis, M.E. Synthesis and reactivity of a novel oxazolo[2,3-c][1,4]benzodiazepine ring system with DNA recognition potential: A new class of anthramycins. J. Chem. Soc. Chem. Commun. 1990, 874–876.
18. Tozuka, Z.; Yazawa, H.; Murata, M.; Takaya, T. Studies on tomaymycin. III. Synthesis and antitumor activity of tomaymycin analogs. J. Antibiot. 1983, 36, 1699–1708.
19. Stefancich, G.; Artico, M.; Massa, S.; Corelli, F. Research on nitrogen heterocyclic compounds; XIII. Synthesis of 5H-imidazo[2,1-c][1,4]benzodiazepine, a novel tricyclic ring system. Synthesis 1981, 13, 321–322.
20. Mitra, S.; Darira, H.; Chattopadhyay, P. Efficient synthesis of imidazole-fused benzodiazepines using palladium-catalyzed intramolecular C–N bond formation reaction. Synthesis 2013, 45, 85–92.
21. Basolo, L.; Beccalli, E.M.; Borsini, E.; Broggini, G.; Khansaa, M.; Rigamonti, M. Access to a novel class of tetracyclic 1,4-benzodiazepin-5-ones starting from α-amino acids by Pd-catalysed amination/1,3-dipolar cycloaddition as the key steps. Eur. J. Org. Chem. 2010, 2010, 1694–1703.
22. Hemming, K.; Loukou, C. Synthetic approaches to 1,2,5-benzothiadiazepine 1,1-dioxides—Sulfonamide analogues of the 1,4-benzodiazepines. J. Chem. Res. 2005, 2005, 1–12.
23. Artico, M.; Silvestri, R.; Pagnozzi, E.; Stefancich, G.; Massa, S.; Loi, A.G.; Putzolu, M.; Corrias, S.; Spiga, M.G.; La Colla, P. 5H-Pyrrolo[1,2-b][1,2,5]benzothiadiazepines (PBTDs): A novel class of non-nucleoside reverse transcriptase inhibitors. Bioorg. Med. Chem. 1996, 4, 837–850.
24. Di Stefano, C.; Marfe, G.; Trawinska, M.M.; Sinibaldi-Salimei, P.; Silvestri, R.; Amadori, S.; Abruzzese, E. Pyrrolo[1,2-b][1,2,5]benzothiadiazepines (PBTDs) exert their anti-proliferative activity by interfering with Akt-mTOR signaling and bax: Bcl-2 ratio modulation in cells from chronic myeloid leukemic patients. Cancer Sci. 2010, 101, 991–1000.
25. Hemming, K.; Chambers, C.S.; Hamasharif, M.S.; João, H.; Khan, M.N.; Patel, N.; Airley, R.; Day, S. Azide based routes to tetrazolo and oxadiazolo derivatives of pyrrolobenzodiazepines and pyrrolobenzothiadiazepines. *Tetrahedron* **2014**, *70*, 7306–7317.

26. Chambers, C.S.; Patel, N.; Hemming, K. Intramolecular 1,3-dipolar cycloaddition as a route to triazolobenzodiazepines and pyrrolobenzodiazepines. *Tetrahedron Lett.* **2010**, *51*, 4859–4861.

27. Patel, N.; Chambers, C.S.; Hemming, K. Synthesis of benzothiadiazines, benzothiadiazepines, and benzothiadiazocines from intramolecular azide reactions and iodocyclizations. *Synlett* **2009**, 3043–3047.

28. Broggini, G.; Garanti, L.; Molteni, G.; Pilati, T. Stereoselective intramolecular cycloadditions of homochiral N-alkenoyl aryl azides. *Tetrahedron Asymmetry* **2001**, *12*, 1201–1206.

29. Beccalli, E.; Broggini, G.; Paladino, G.; Pilati, T.; Pontremoli, G. Diastereoselective synthesis of enantiopure (αR)-2-methyl-4-(α-phenylethyl)-1,2,3,4-tetrahydro-benzo[e][1,4]diazepine-5-ones. *Tetrahedron Asymmetry* **2004**, *15*, 687–692.

30. Bass, P.D.; Gubler, D.A.; Judd, T.C.; Williams, R.M. Mitomycinoid alkaloids: Mechanism of action, biosynthesis, total syntheses, and synthetic approaches. *Chem. Rev.* **2013**, *113*, 6816–6863.

31. Sato, T.; Tsujimoto, K.; Matsubayashi, K.-I.; Ishibashi, H.; Ikeda, M. Carbamoylmethyl radical cyclization: Formal synthesis of (−)-trachelanthamidine. *Chem. Pharm. Bull.* **1992**, *40*, 2308–2312.

32. Broggini, G.; de Marchi, I.; Paladino, G.; Pilati, T.; Terraneo, A. Effective synthesis of enantiopure [1,2,3]triazolo[1,5-a]- and pyrazolo[1,5-a]-pyrrolo[2,1-c][1,4]benzodiazepines by diastereoselective intramolecular azide and nitrilimine cycloadditions. *Synthesis* **2005**, 2246–2252.

33. Molina, P.; Díaz, I.; Tárraga, A. Synthesis of pyrrolo[2,1-c][1,4]benzodiazepines via intramolecular aza-Wittig reaction. Synthesis of the antibiotic DC-81. *Tetrahedron* **1995**, *51*, 5617–5630.

34. Eguchi, S.; Yamashita, K.; Matsushita, Y.; Kakehi, A. Facile synthesis of 1,4-benzodiazepin-5-one derivatives via intramolecular aza-Wittig reaction. Application to an efficient synthesis of O-benzyl DC-81. *J. Org. Chem.* **1995**, *60*, 4006–4012.

35. Tsang, W.Y.; Ahmed, N.; Hemming, K.; Page, M.I. An activated sulfonylating agent that undergoes general base-catalysed hydrolysis by amines in preference to aminolysis. *J. Org. Chem.* **2008**, *73*, 4504–4512.

36. Hemming, K.; O’Gorman, P.A.; Page, M.I. The synthesis of azabicyclo[4.2.1]nonenes by the addition of a cyclopropenone to 4-vinyl substituted 1-azetines—Isomers of the homotropane nucleus. *Tetrahedron Lett.* **2006**, *47*, 425–428.

37. Blackburn, C.; Achab, A.; Elder, A.; Ghosh, S.; Guo, J.; Harriman, G.; Jones, M. Synthesis of 3-amino-1,2,4-benzothiadiazine 1,1-dioxides via a tandem aza-Wittig/heterocumulene annulation. *J. Org. Chem.* **2005**, *70*, 10206–10209.

Sample Availability: Samples of the compounds **11** and **13** (X = SO₂, R¹ = R² = H) are available from the corresponding author.

© 2014 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/4.0/).