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Synthesis and DNA-Binding Affinity of *A*-C8/*C*-C2 Alkoxyamido-Linked Pyrrolo[2,1-*c*][1,4]benzodiazepine Dimers

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Abstract—The synthesis of new *A*-C8/*C*-C2 alkoxyamido-linked pyrrolo[2,1-*c*][1,4]-benzodiazepine dimers have been described in this report. These dimers exhibit significant DNA-binding ability with moderate anticancer activity.

DNA interstrand cross-linking agents that interact within the minor groove constitute an important class of antitumour drugs.[1] There has been considerable interest in the past few years in the design and synthesis of symmetrical cross-linking agents, particularly based on pyrrolobenzodiazepines (PBDs).[2] In the literature a number of PBD dimers have been designed and synthesized that have exhibited varying degrees of cytotoxicity and DNA cross-linking activity.[3] These PBD dimers have been joined through their different positions such as *A*-C7/*A*-C7′, *A*-C8/*A*-C8′ and *C*-C2/*C*-C2′, among these *A*-C8/*A*-C8- linked PBD dimers have shown promising cytotoxicity and efficient cross-linking property. Recently, Thurston and co-workers[4] have reported the first examples of ***A***-C8/***C***-C2 amido-linked PBD dimers with marginal cross-linking ability. In continuation of our efforts in the design and synthesis of PBD hybrids,[5] we have been interested in exploring anticancer potential and DNA-binding ability of *A*-C8/*C*-C2 alkoxyamido-linked PBD dimers. Further, we have also synthesized these PBD dimers with an amide functionality at N10-C11 of *C*-C2 component to unravel the aspect of non-covalent interactions in such dimers.

These PBD dimers have been synthesized by preparing the *A*-C8 acid component (**5**) and the *C*-C2 amino substituted component (**13**) individually and followed by the coupling of these two precursors. The C8 alkoxy acid has been synthesized in the following manner; the precursor (2*S*)-*N*-(4-hydroxy-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carboxaldehyde diethyl thioacetal **3** has been prepared by employing the literature method,[6] which upon etherification with methyl bromoalkanoates provides **4a–c** upon basic hydrolysis of these esters to give the desired intermediate acids **5a–c** (Scheme 1).

However, the *C*-C2 amino component has been prepared as described below: *trans*-4-hydroxy-L-proline methyl-ester hydrochloride has been coupled to the compound **6** to give the nitro ester **7**. The C2 hydroxy group is protected with TBDMS-Cl followed by reduction with DIBAL-H to produce the corresponding aldehyde, which is protected with EtSH/TMS-Cl. Surprisingly, in this reaction protection of aldehyde to diethyl thioacetal and deprotection of TBDMS takes place in the same step to afford the compound **10**. Mesylation upon C2 hydroxy group gives compound **11**. Azidation at C2 position proceeds through S_N2 reaction mechanism to afford compound **12**, followed by reduction to give compound **13**.

The key intermediates **14a–c** have been prepared by coupling of compound **5a–c** and compound **13**. Further, these upon reduction by $SnCl_2{\cdot}2H_2O$ in methanol and followed by deprotection of diaminothioacetal precursors **15a–c** using $HgCl_2/CaCO_3$ affords the PBD dimers **1a–c**[7] (Scheme 2).

The other key intermediates **20a–c** have been prepared by coupling compounds **5a–c** with compound **19**. Further, these upon reduction gives aminothioacetal precursors **21a–c** followed by deprotection to afford the desired PBD dimers **2a–c**[8] (Scheme 3).

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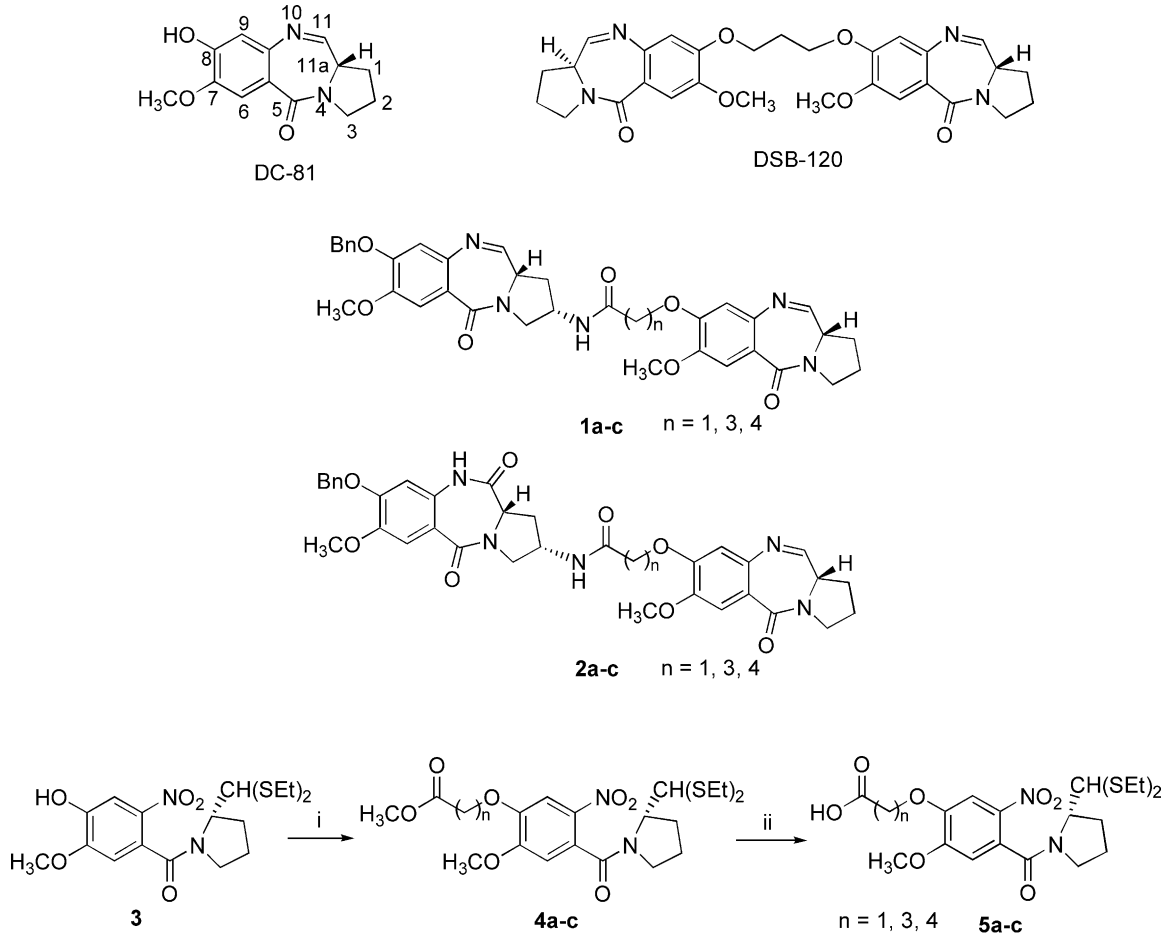
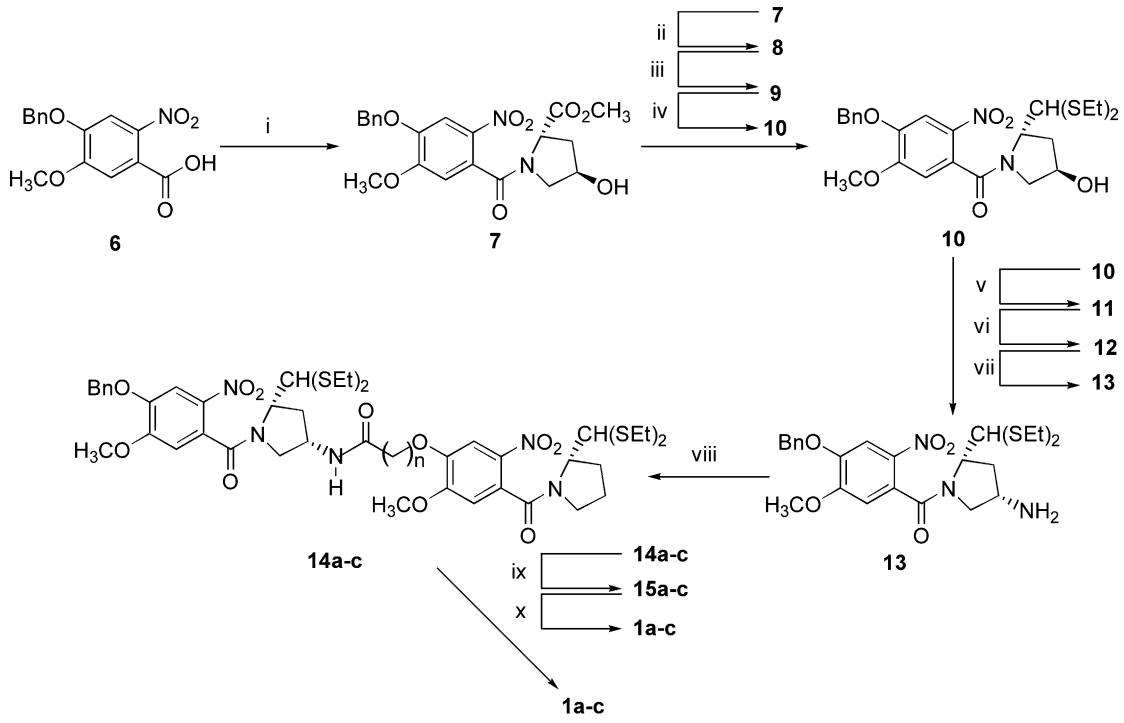
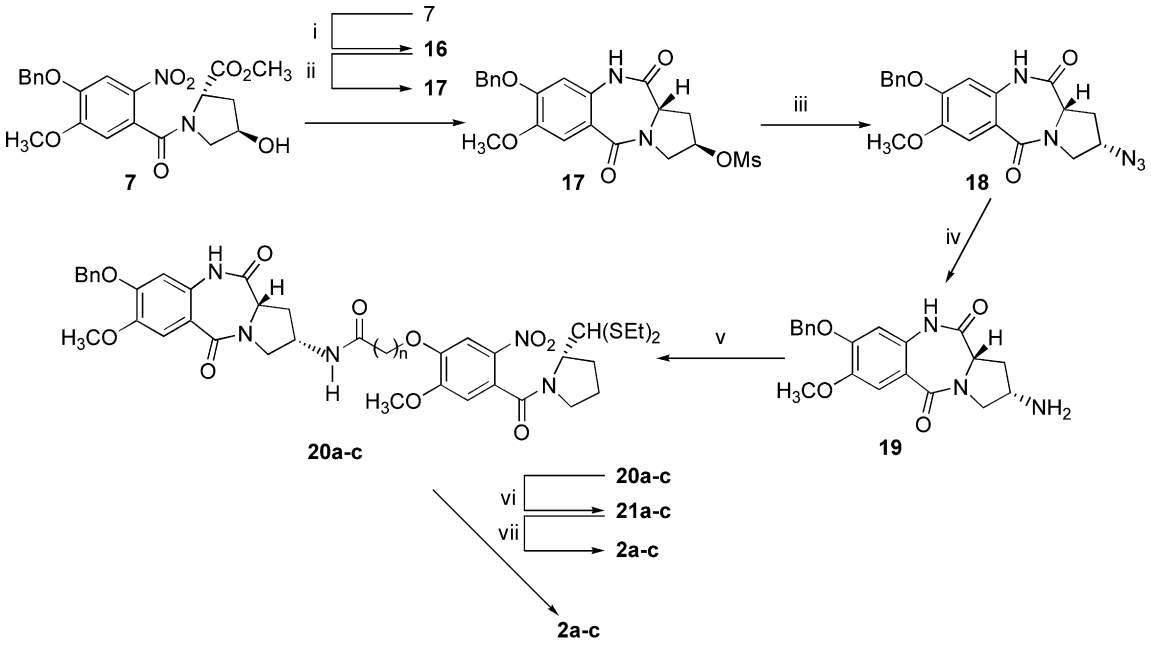


Scheme 1. (i) Methyl bromoalkanoate, K_2CO_3, DMF, rt, 24 h, 90%; (ii) 1 N LiOH, THF–MeOH–H_2O, 12 h, rt, 78%.



Scheme 2. (i) $SOCl_2$, C_6H_6, *trans*-4-hydroxyprolinemethylesterhydrochloride, H_2O, 2 h, rt, 75%; (ii) TBDMSCl, imidazole, CH_2Cl_2, 8 h, rt, 80%; (iii) DIBAL-H, CH_2Cl_2, 1 h, −78 °C, 70%; (iv) EtSH, TMSCl, CH_2Cl_2, 8 h, rt, 62%; (v) TEA, MsCl, CH_2Cl_2, 5 h, 0 °C, 78%; (vi) NaN_3, DMF, 60 °C, 16 h, 76%; (vii) TPP, THF–NH_4OH, 8 h, rt, 72%; (viii) HOBt, EDCl, compound **5**, CH_2Cl_2–H_2O, 8 h, rt, 55–59%; (ix) $SnCl_2$–$2H_2O$, MeOH, 6 h, reflux, 70–72%; (x) $HgCl_2$, $CaCO_3$, MeCN–H_2O, 12 h, rt, 56–60%.



Scheme 3. (i) TEA, MsCl, CH_2Cl_2, 5 h, 0 °C, 78%; (ii) $SnCl_2$–$2H_2O$, MeOH, 2 h, reflux, 68%; (iii) NaN_3, DMF, 16 h, 60 °C, 76%; (iv) TPP, THF–H_2O, 8 h, rt, 72%; (v) HOBt, EDCl, compound **5**, CH_2Cl_2–H_2O, 8 h, rt, 63–65%; (vi) $SnCl_2$–$2H_2O$, MeOH, 2 h, reflux, 68–73%; (vii) $HgCl_2$, $CaCO_3$, CH_3CN–H_2O, 12 h, 51–55%.

Compounds **1a–c** and **2a–b** have been evaluated for the primary anticancer activity in the standard three-cell line panel comprising of the MCF7 (breast), NCI-H460 (lung) and SF-268 (CNS), and none of these compounds showed significant cytotoxicity. The DNA binding ability for these novel *A*-C8/*C*-C2 alkoxyamido-linked PBD dimers has been examined by thermal denaturation studies using calf thymus (CT) DNA. Melting studies show (Table 1) that these compounds stabilize the thermal helix → coil or melting stabilization (ΔT_m) for the CT–DNA duplex at pH 7.0, incubated at 37 °C, where PBD/DNA molar ratio is 1:5. The compounds **1a–c** exhibited a large value of ΔT_m particularly after 18 h incubation in comparison to DC-81 and for compounds **2a–c** these values are insignificant.

It is observed from this preliminary data that imine-imine PBD dimers (**1a–c**) have interesting profile of DNA binding ability. On the contrary, the imine-amide dimers (**2a–c**) have not exhibited any significant DNA binding ability. Unlike for the previously reported PBD-dimers a correlation between the DNA-binding affinity and cytotoxictiy could not be derived in this class of head to tail PBD dimers. Therefore, preparation of structurally modified analogues of such dimers particularly by incorporating *exo*- and *endo*-unsaturation at C2 and variation of linker length could probably address the factors responsible for the insignificant cytotoxicity of such efficient DNA-binding PBD dimers. The detailed cross-linking ability, anticancer activity and molecular modelling studies for these compounds is in progress and will be published in due course.

Table 1. Thermal denaturation data for *A*-C8/*C*-C2 alkoxyamido-linked PBD dimers with CT–DNA

PBD dimers	[PBD]/[DNA] molar ratio[b]	ΔT_m (°C)[a] after incubation at 37 °C for	
		0 h	18 h
1a	1:5	0.9	4.7
1b	1:5	3.7	9.3
1c	1:5	6.1	10.9
DC-81	1:5	0.3	0.7
DSB-120	1:5	10.2	15.1

[a]For CT–DNA alone at pH 7.00±0.01, T_m = 69.2 °C±0.01 (mean value from 10 separate determinations), all ΔT_m values are ±0.1–0.2 °C.
[b]For a 1:5 molar ratio of [PBD]/[DNA], where CT–DNA concentration = 100 μM and ligand concentration = 20 μM in aqueous sodium phosphate buffer [10 mM sodium phosphate + 1 mM EDTA, pH 7.00±0.01].

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7. Selected data for compound **1c**: ^{1}H NMR ($CDCl_3$) δ 1.20–2.30 (m, 4H), 1.82–2.70 (m, 10H), 3.75–3.85 (m, 3H), 3.90 (s, 3H), 3.95 (s, 3H), 4.20 (t, 2H), 4.50–4.60 (m, 2H), 5.20 (s, 2H), 6.62 (NH, exchangeable), 6.80 (s, 1H), 6.85 (s, 1H), 7.30–7.45 (m, 7H), 7.65 (d, 1H, $J = 4.2$ Hz), 7.70 (d, 1H, $J = 5.07$ Hz); MS (FAB) 680 $[M + H]^{+\cdot}$.

8. Selected data for compound **2c**: ^{1}H NMR ($CDCl_3$) δ 1.62–1.80 (m, 4H), 1.90–2.70 (m, 10H), 3.45–3.75 (m, 4H), 3.80 (s, 3H), 3.92 (s, 3H), 3.98–4.20 (m, 2H), 4.42–4.55 (m, 1H), 5.15 (s, 2H), 6.55 (s, 1H), 6.80 (s, 1H), 6.80 (s, 1H), 7.00 (m, 1H), 7.24–7.42 (m, 7H), 7.70 (d, 1H, $J = 4.6$ Hz), 9.15 (s, 1H); MS (FAB) 696 $[M + H]^{+\cdot}$.