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Design and Synthesis of Bis 1-Chloromethyl-5-hydroxy-1,2-dihydro-3*H*-benz[e]indole (*seco*-CBI)-Pyrrole Polyamide Conjugates

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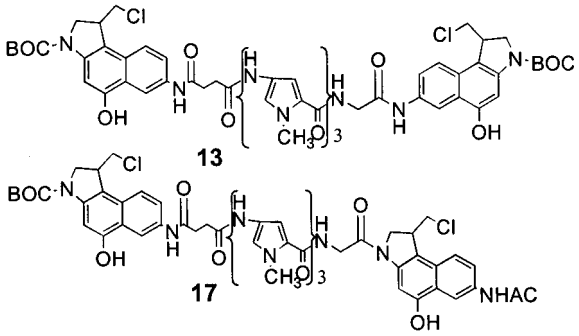
ABSTRACT



The design and synthesis of bis 1-chloromethyl-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI)-pyrrole polyamide conjugates (13, 17) as DNA minor groove binding agents are described.

Sequence-specific DNA alkylating agents have played an important role in molecular biology and human medicine in cancer chemotherapy. The cyclopropylindole class of antitumor antibiotics, exemplified by CC-1065 and duocarmycin A (Figure 1), is the parent member of a potent class of naturally occurring antitumor antibiotics that exert their biological properties through the sequence-selective alkylation of DNA.[1−10] Cellular investigations have shown that CC-1065 alkylates B-DNA reversibly with a high sequence selectivity at AT regions of the minor groove sites of 5′-d (A/GNTTA)-3′ and 5′-d (AAAAA)-3′, by the N-3 position of the 3′-adenine by the cyclopropylindole unit present in the molecule.[11−12]

Compared with other anticancer agents (+)-CC-1065 has a high bioactivity and is 400 times more potent than

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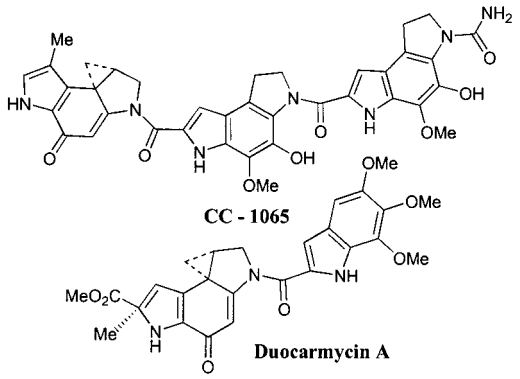


Figure 1.

doxorubicin, 80 times more potent than actinomycin D, and about twice as potent as maytansine against L1210 leukemia cells in vitro. Despite its high potency CC-1065 cannot be used in humans because it was found that it caused delayed death in the experimental animals. Because of the unique structure and properties of these natural products, many chemists were stimulated to synthesize derivatives and analogues of CC-1065 and duocarmycins with better antitumor selectivity and DNA-sequence-specific binding properties,[13] in an attempt to avoid the undesired side effects while retaining potency against tumor cells.[13] As a successful example of modification of 1,2,8,8a-tetrahydro-7-methyl cyclopropa[*c*]pyrrolo[3,2-*e*]indole-4-one (CPI), the DNA alkylating moiety of CC-1065, Boger first reported that the simplified moiety, 1,2,9,9a-tetrahydrocyclo-propa[*c*]benzo-[*e*]indole-4-one (CBI) and its analogues were more stable and more potent than the CPI counterparts.[14]

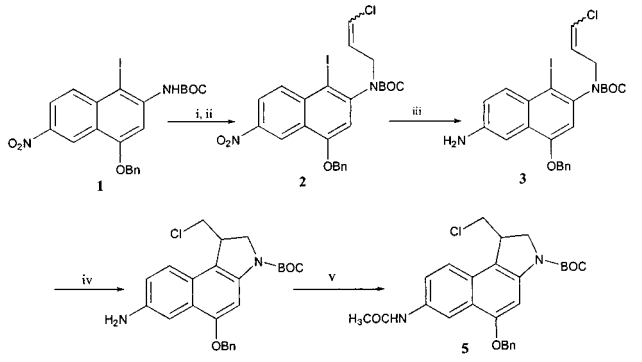
In addition, studies on netropsin, distamycin, and related compounds have led to the concept of polyamides as information reading agents.[15] A predominantly 4−5 AT base pair sequence is recognized by netropsin and distamycin in the minor groove of DNA. In our group attempts have been made to link CPI[16] and CBI[17] with polyamides, which are well-established DNA minor groove binders, to improve their pharmacological properties and potencies. We found that certain CPI-polyamide conjugates exhibit potency up to 10 000 times higher than that of CC-1065 against KB human cancer cells.[16] Studies also have shown that some synthetic compounds, which contain two CPI moieties linked from two positions by a flexible methylene chain of variable length, are significantly more potent than CC-1065 both in vitro and in vivo.[18]

Prevously we reported the synthesis and biological evaluation of *seco*-CBI dimers against nine types of cancer cells. Certain examples showed significant activity against CCRT-CEM, HL-60 (TB), MOLT-4, leukemia, CNS cancer, melanoma, and prostate cancer cell lines with GI_{50} values <0.01 μm.[19]

To our knowledge no attempt has been made to synthesize bis 1-chloromethyl-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI)-pyrrole polyamide conjugates **13**, **17** (bis *seco*-CBI pyrrole polyamide dimers). To investigate the structure−activity relationship systematically, we have designed and synthesized bis *seco*-CBI pyrrole polyamide dimers, which contain two racemic CBI moieties linked from two different positions with pyrrole polyamide by a flexible methylene chain of variable length.

In our previous work the *seco*-CBI moiety was synthesized by using the following convenient route in good yield. Deprotonation of carbamate **1**,[17] using NaH, followed by alkylation of the resulting anion with 1,3-dichloropropene in the presence of phase transfer catalyst Bu_4NI gave an mixture of *Z* and *E* isomers of vinyl chloride **2**. Selective reduction of the nitro group of **2** using hydrazine provided amine **3**, the desired precursor for the intramolecular aryl radical cyclization on to a tethered vinyl chloride. A deoxygenated solution of **3** in dry benzene was heated at reflux for 15 h in the presence of 2 equiv of Bu_3SnH and a catalytic amount of AIBN to give the bifunctionalized *seco*-CBI prodrug **4**. To deactivate the amine group at the C7 position by reaction with acetyl chloride almost quantitatively to afford its acetyl derivative **5** (Scheme 1).

Scheme 1[a]



[a] (i) NaH; (ii) ClCH=CHCH$_2$Cl, Bu_4NI; (iii) hydrazine hydrate, $FeCl_3$, C; (iv) Bu_3SnH, AIBN; (v) CH_3COCl, DIEA.

Treatment of the *seco*-CBI **4** with 1.0 equiv succinic anhydride in the presence of triethylamine in dry THF at

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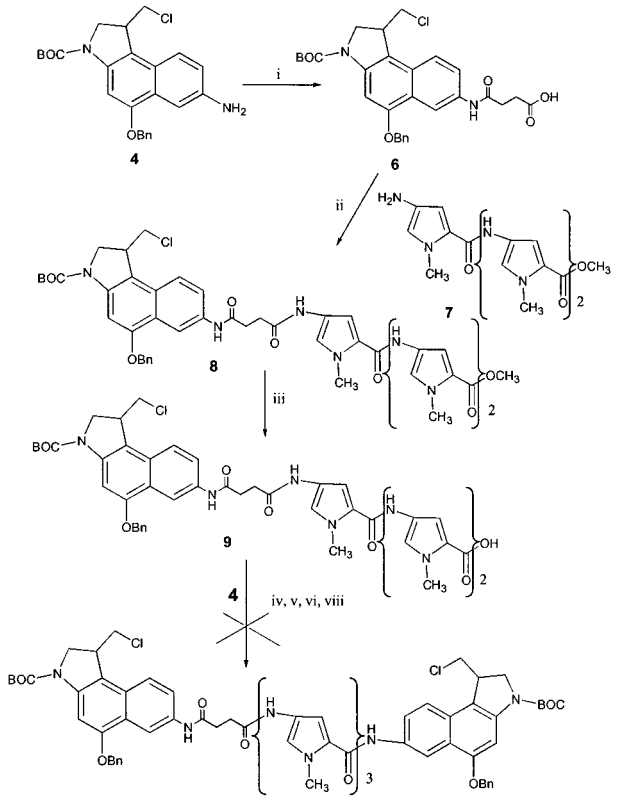
60 °C provided acid **6** in 80% yield. This acid **6** was then coupled with the amine moiety of pyrrole polyamide **7**,[20] using EDCI and HOBt as the coupling agents, in dry DMF at room temperature for about 12 h to afford the corresponding coupled *seco*-CBI polyamide methyl ester **8** in 80% yield, which upon hydrolysis with 1 N NaOH at room temperature produced the corresponding *seco*-CBI polyamide acid compound **9** in 70% yield. The corresponding amino compound was then prepared by hydrogenation of the corresponding nitro polyamide. This *seco*-CBI polyamide acid **9** was treated with the *seco*-CBI prodrug **4** under standard EDCI/HOBt coupling conditions and via its acid chloride route (Scheme 2). Unfortunately both reactions failed to produce the desired product because of the less reactive aromatic amino group of the *seco*-CBI **4**. In that case we needed to increase the reactivity of the amino group at the C7 position by introducing a more nucleophilic primary amine moiety in the *seco*-CBI **4** through a suitable linker.

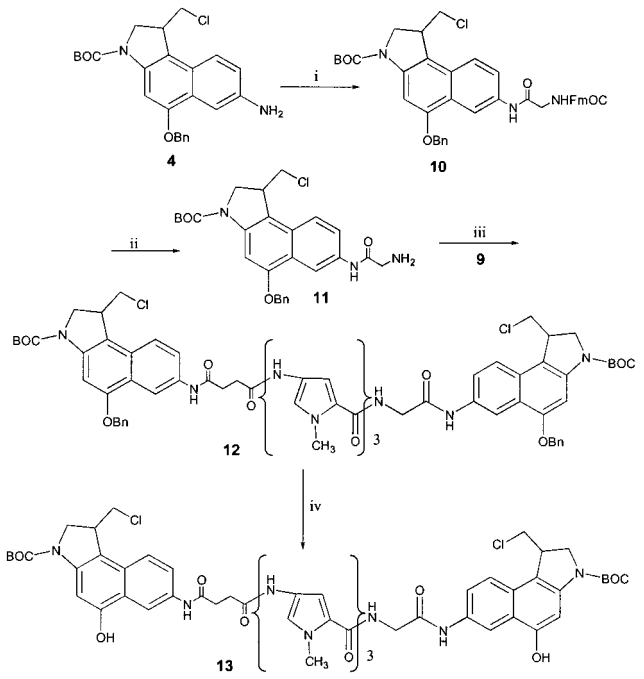
Scheme 2[a]



[a] (i) Succinic anhydride, THF, rt; (ii) EDCI, HOBt, DMF, rt; (iii) 1 N NaOH, THF/MeOH (1:1), rt; (iv) EDCI, HOBt, DMF, rt; (v) DCC, HOBt, DMF, rt; (vi) EDCI, DMF, rt; (viii) (1) TBDMS, imidazole, rt, (2) oxalyl chloride/DCM 0 °C, (3) Et_3N, THF, rt.

Condensation of the *seco*-CBI **4** in the presence of EDCI and HOBt in dry DMF at room temperature with 1.0 equiv *N*-Fmoc glycine gave compound **10** in 70% yield. Detachment of the Fmoc group from **10** with TBAF in dry THF, followed by coupling with 1.0 equiv of *seco*-CBI polyamide acid **9** using EDCI and HOBt as the coupling agents in dry DMF at room temperature for about 12 h afforded the corresponding coupled bis *seco*-CBI polyamide **12** in 70% yield. Hydrogenolysis of the bis *seco*-CBI polyamide **12** in THF with 4.0 equiv of ammonium formate in the presence of Pd−C for about 2 h to remove the benzyl ether almost quantitatively provided the final C7−C7 bis *seco*-CBI pyrrole polyamide dimer **13** [21] in 80−90% yield (Scheme 3).

Scheme 3[a]



[a] (i) *N*-FmOC glycine, EDCI, HOBt, DMF, rt; (ii) TBAF, THF, rt; (iii) **9**, EDCI, HOBt, DMF, rt; (iv) $HCOONH_4$, Pd/C, THF, rt.

Acid-mediated deprotection of the Boc group from **5** followed by coupling with 1.0 equiv of *N*-Boc glycine under standard EDCI/HOBt coupling conditions in dry DMF

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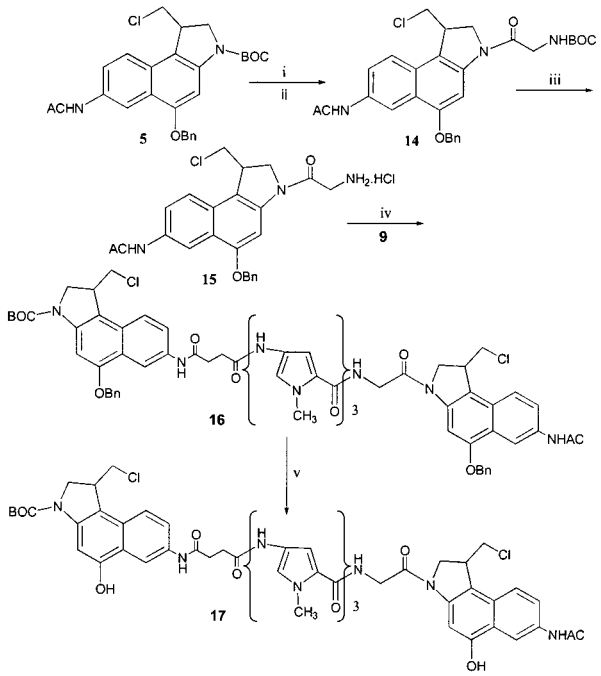
(21) Spectral data for compound **13**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.52 (s, 18H, 2 × Boc-H), 2.56−2.70 (m, 4H, 2 × CH_2CO−), 3.81 (s, 3H, −NCH_3), 3.82 (s, 3H, −NCH_3), 3.85 (s, 3H, −NCH_3), 3.90−4.10 (m, 12H, Cl, 2-H, 2 × CH_2Cl, 2 × CH_2N, NHC**H**$_2$), 6.88 (d, 1H, J = 1.8 Hz, Py-H), 6.96 (d, 1H, J = 1.8 Hz, Py-H), 7.05 (d, 1H, J = 1.8 Hz, Py-H), 7.18 (d, 1H, J = 1.8 Hz, Py-H), 7.24 (d, 1H, J = 1.8 Hz, Py-H), 7.26 (d, 1H, J = 1.8 Hz, Py-H), 7.59−7.75 (m, 6H, 2 × C4−H, C7−H, C8−H), 8.32 (m, 1H, N**H**CH_2), 8.40 (s, 2H, 2 × C6−H), 9.89 (s, 1H), 9.91 (s, 1H), 9.95 (s, 1H), 10.09 (s, 1H), 10.12 (s, 1H), 10.30 (s, 1H), 10.32 (s, 1H). ES-MS *m/z* calcd for $C_{60}H_{65}N_{11}O_{12}Cl_2Na$ 1224.40, found 1224.40 ($M + Na^+$).

Scheme 4[a]



[a] (i) 4 M HCl in dioxane, rt, 2 h; (ii) *N*-Boc glycine, EDCI, HOBt, $NaHCO_3$, DMF, rt; (iii) 4 M HCl in dioxane, rt, 2 h; (iv) **9**, EDCI, HOBt, $NaHCO_3$, DMF, rt; (v) $HCOONH_4$, Pd/C, THF, rt.

afforded compound **14** in good yield. Detachment of the Boc group from **14**, followed by coupling with 1.0 equiv of *seco*-CBI polyamide acid **9** using EDCI and HOBt as the coupling agents in dry DMF produced benzyl-protected C7−N3 bis *seco*-CBI pyrrole polyamide dimer **16** in 60−70% yield. Treatment of **16** with ammonium formate in the presence of Pd−C for about 2 h provided the final C7−N3 bis *seco*-CBI pyrrole polyamide dimer **17**[22] in 80% yield (Scheme 4).

In summary, we have described the first synthesis of the bis 1-chloromethyl-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (*seco*-CBI)-pyrrole polyamide conjugates **13** and **17** (bis *seco*-CBI pyrrole polyamide dimers). Results on the DNA sequence preferences and biological evaluation will be reported in due course.

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(22) Spectral data for compound **17**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.52 (s, 9H, Boc-H), 2.04 (s, 3H, CH_3CON), 2.58−2.70 (m, 4H, 2 × CH_2CO−), 3.81 (s, 3H, −NCH_3), 3.82 (s, 3H, −NCH_3), 3.85 (s, 3H, −NCH_3), 3.90−4.40 (m, 12H, Cl, 2-H, 2 × CH_2Cl, 2 × CH_2N, NHC**H**$_2$), 6.88 (d, 1H, J = 1.5 Hz, Py-H), 6.96 (d, 1H, J = 1.5 Hz, Py-H), 7.05 (d, 1H, J = 1.5 Hz, Py-H), 7.16 (d, 1H, J = 1.5 Hz, Py-H), 7.23 (d, 1H, J = 1.5 Hz, Py-H), 7.26 (d, 1H, J = 1.5 Hz, Py-H), 7.60−7.79 (m, 5H, 2 × C8, C9−H, C4−H), 7.90 (d, 1H, C4−H), 8.20−8.30 (m, 1H, N**H**CH_2), 8.35−8.42 (m, 2H, 2 × C6−H), 9.91 (s, 1H), 9.94 (s, 1H), 10.03 (s, 1H), 10.05 (s, 1H), 10.07 (s, 1H), 10.14 (s, 1H), 10.32 (s, 1H). HR-ESMS *m/z* calcd for $C_{57}H_{59}N_{11}O_{11}Cl_2Na$ 1166.367, found 1166.368 ($M + Na^+$).