Pergamon

Tetrahedron Letters 41 (2000) 9493–9498

TETRAHEDRON LETTERS

Synthesis and evaluation of a novel bleomycin A_2 analogue: continuing assessment of the linker domain†

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Received 28 August 2000; revised 18 September 2000; accepted 21 September 2000

Abstract

The synthesis and evaluation of a bithiazole ester analogue of deglycobleomycin A_2 that addresses the importance of the bithiazole–L-threonine amide for efficient DNA cleavage is described.

The bleomycins are a group of glycopeptide antibiotics isolated from the microorganism *Streptomyces verticillus* by Umezawa and co-workers.[1] Bleomycin A_2,[2–11] differing from other bleomycins only at the C-terminus, is the major component of the anticancer drug Blenoxane which has found clinical use in combination chemotherapy for the treatment of Hodgkin's lymphoma, carcinomas of the skin, head and neck, and testicular cancers.[12] Its properties are thought to arise from its ability to mediate the catalytic oxidative cleavage of duplex DNA,[13] and possibly RNA,[14,15] in the presence of a redox-active metal cation, oxygen, and a reductant.

Through extensive structural, physical, chemical, and biological studies on bleomycin A_2[3,16,17] and its derivatives,[18] four functional domains of the active molecule and their roles have been identified: the N-terminus domain, the C-terminus domain, the carbohydrate domain, and the linker domain. NMR solution structures of bleomycin–oligonucleotide complexes as well as point mutations in the structure of deglycobleomycin revealed that the substituents on the valerate and L-threonine subunits composing the linker domain induce bleomycin A_2 to adopt a single rigid compact conformation necessary for efficient DNA cleavage.[19] Among them, the L-threonine subunit substituent restricts the valerate–threonine amide to a single orientation, inducing a turn in the linker domain characteristic of the compact conformation observed in free and DNA-bound bleomycin A_2. In these prior studies, we also demonstrated that *N*-methylation of the valerate–threonine amide decreased DNA cleavage efficiency 10–15 fold and the DNA

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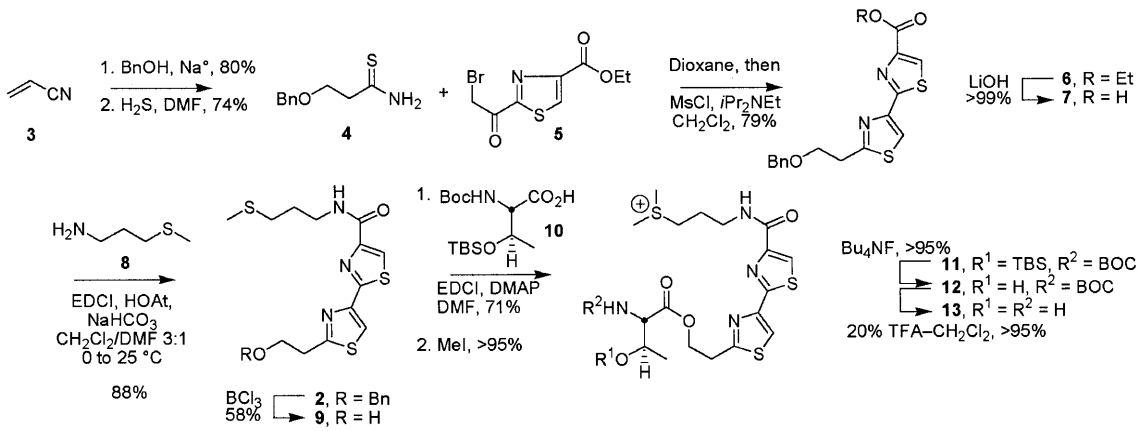
† Dedicated to Harry Wasserman on the occasion of his 80th birthday.

0040-4039/00/$ - see front matter
PII: S0040-4039(00)01633-6

cleavage sequence selectivity of bleomycin A_2 was nearly abolished.[20] Herein we describe the synthesis and evaluation of a bithiazole ester analogue replacing the adjacent L-threonine–bithiazole amide bond with a more flexible ester linkage (**1**) removing the hydrogen bond donor amide yet minimizing perturbation of the natural system.

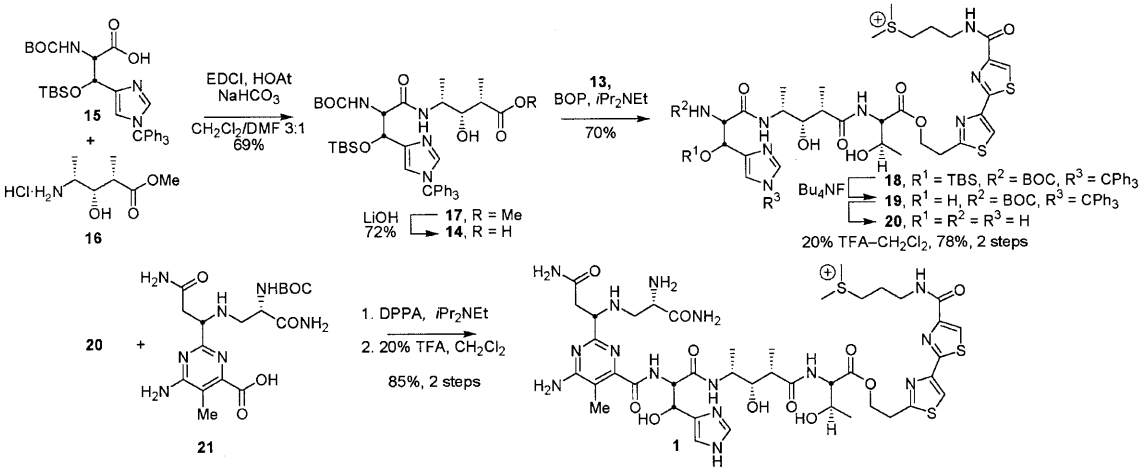
1. Synthesis

The first stage of the synthesis of **1** was the construction of the modified bithiazole subunit **2** (Scheme 1). Conjugate addition of benzyl alcohol to acrylonitrile (**3**, 1 equiv. BnOH, Na, 80°C, 80%) followed by conversion to the thioamide (1.2 equiv. Et_2NH, sat. with H_2S, DMF, 45°C, 74%) produced **4**. Its coupling with the α-bromo ketone **5**[18] to provide the bithiazole **6** was effected in two stages first by reaction in dioxane followed by elimination of water (2 equiv. MsCl, 2 equiv. iPr_2NEt, CH_2Cl_2, 79%). LiOH hydrolysis of the ethyl ester, followed by EDCI (1.5 equiv.) and HOAt (1.5 equiv.) mediated coupling of **7** (2 equiv. $NaHCO_3$, 3:1 CH_2Cl_2–DMF, 0–25°C, 88%) with **8** afforded the protected bithiazole subunit **2**. Benzyl deprotection (3 equiv. BCl_3, 0°C, 58%), coupling of the suitably protected L-threonine **10** with the free alcohol **9** (1.5 equiv. EDCI, 1.5 equiv. DMAP, DMF, 0°C, 71%), and formation of the sulfonium salt (1.1 equiv. MeI, MeOH, in the dark, >95%) afforded **11**. Stepwise deprotection of the OTBS and N-BOC protecting groups provided the modified tripeptide S sulfonium salt (**13**).



Scheme 1.

Dipeptide **14** was obtained from the hydrolysis (LiOH, 72%) of the coupling product between **15**[18] and **16**[18] (3 equiv. EDCI, 3 equiv. HOAt, 1.5 equiv. $NaHCO_3$, 3:1 CH_2Cl_2–DMF, 0°C 69%). Coupling of **13** with **14** utilizing BOP (3 equiv.) and Hunig's base (3 equiv.) yielded the pentapeptide **18** (DMF, 25°C, 70%, Scheme 2). Deprotection of the silyl ether (Bu_4NF) followed by simultaneous removal of the N-BOC and trityl protecting groups (20% TFA–CH_2Cl_2) set the stage for the final coupling step. In the presence of DPPA, N-BOC-pyrimidoblamic acid (**21**), prepared as previously described,[21] was reacted with **20** in high yield (1.1 equiv. DPPA, 2 equiv. iPr_2NEt, DMF, 0°C, 85%). Acid-catalyzed deprotection afforded the deglycobleomycin bithiazole ester analogue **1**.[22]



Scheme 2.

2. Evaluation

The ability of the Fe(II) complex of **1** to cleave duplex DNA was conducted first through examination of single-strand and double-strand cleavage of supercoiled ΦX174 DNA (Form I) to produce relaxed (Form II) and linear (Form III) DNA, respectively. The Fe(II) complex of **1** was found to cleave the ΦX174 DNA with a 2 fold decrease in efficiency as compared to bleomycin A_2 (Fig. 1) which is the identical reduction in cleavage efficiency shown by deglycobleomycin A_2.

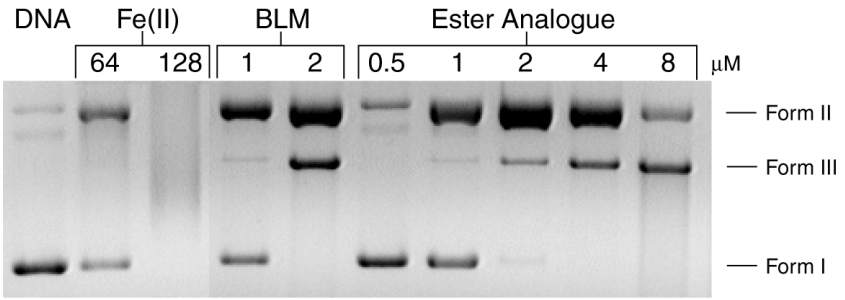


Figure 1. Agarose gel illustrating the cleavage reactions of supercoiled ΦX174 DNA by Fe(II)-agents at 25°C for 1 h in buffer solutions containing 2-mercaptoethanol[23]

The sequence selectivity of DNA cleavage was examined with duplex w794 DNA[24] by monitoring strand cleavage of singly [^{32}P]-5′-end labeled duplex DNA after exposure to the Fe(III) complexes of the agents following activation with H_2O_2.[25,26] This protocol is much more sensitive to the distinctions in the relative efficiency of DNA cleavage than the supercoiled DNA cleavage assays. Consequently, bleomycin and its analogues exhibit cleavage over a much broader range of concentrations. As seen in Fig. 2, the bithiazole ester analogue performed with the same trends observed in the supercoiled DNA cleavage assay, namely a minor 2–5 fold decrease in cleavage efficiency relative to bleomycin analogous to the 2–5 reduction observed with deglycobleomycin. By contrast, *N*-methylation of the adjacent valerate–threonine amide

provided an analogue that cleaved w794 DNA only at a concentration of 128 μM[20] versus the 1–2 μM observed with **1**. Notably, the DNA cleavage selectivity is not compromised and **1** displayed the identical preference toward cleavage of 5′-GC and 5′-GT sites.

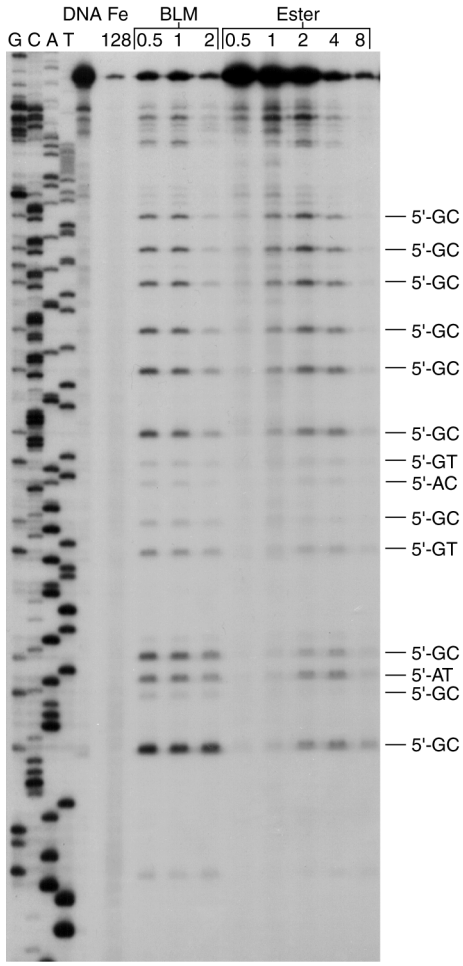
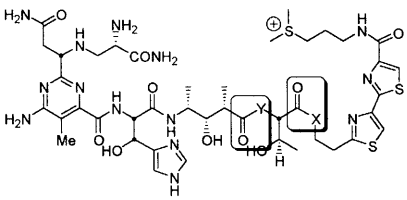


Figure 2. Cleavage of double stranded DNA by Fe(III)-agents (SV40 DNA fragment, 144 base pairs, nucleotide no. 5238–138, clone w794) in phosphate–KCl buffer containing H_2O_2[27]

Analysis of the linker domain through single point changes in the structure of deglycobleomycin A_2, such as the one detailed herein, have revealed many crucial elements necessary



Agent	ΦX174 Efficiency	ds:ss	Selectivity
bleomycin A2	2 – 5	1 : 6	5'-GC, 5'-GT > 5'-GA
deglycobleomycin A2 X = NH, Y = NH	1	1 : 12	5'-GC, 5'-GT > 5'-GA
1, X = O, Y = NH	0.9	nd	5'-GC, 5'-GT > 5'-GA
X = NH, Y = NMe	0.08	1 : 58	weak 5'-GC

Figure 3.

for efficient DNA cleavage.[19] With the synthesis of the bithiazole ester analogue **1**, we have shown that the replacement of the threonine–bithiazole amide with a depsipeptide ester linkage does not effect the properties (Fig. 3) and does not alter the linker domain's ability to adopt the decisive compact DNA bound conformation. The absence of a substantial change in cleavage efficiency and sequence selectivity indicates that neither the conformational or H-bond properties of the threonine–bithiazole amide nitrogen are critical.

Acknowledgements

We gratefully acknowledge the financial support of the National Institutes of Health (CA42056, D.L.B.) and the Skaggs Institute for Chemical Biology.

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22. All intermediates and final compounds were characterized by IR, NMR, and HRMALDI FTMS. Spectroscopic data for **1**: $R_f = 0.08$ (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]_D^{25}$ −20.7 (*c* 0.29, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.86 (s, 1H), 8.22 (s, 2H), 7.57 (s, 1H), 5.42 (d, $J = 4.4$ Hz, 1H), 4.78 (d, $J = 9.2$ Hz, 1H), 4.68 (dd, $J = 1.9$, 4.7 Hz, 1H), 4.60 (d, $J = 6.1$ Hz, 1H), 4.8 (d, $J = 6.1$ Hz, 1H), 4.46 (d, $J = 1.6$ Hz, 1H), 4.51 (m, 1H), 3.70 (m, 2H), 3.60 (t, $J = 9.6$ Hz, 2H), 3.46 (t, $J = 3.0$ Hz, 2H), 3.40 (t, $J = 3.6$ Hz,

2H), 3.20 (m, 2H), 3.02 (t, $J=5.8$ Hz, 2H), 2.95 (s, 6H), 2.63 (m, 1H), 2.27 (s, 3H), 2.16 (t, $J=3.6$ Hz, 2H), 1.22 (d, $J=3.4$ Hz, 3H), 1.21 (d, $J=3.4$ Hz, 3H), 1.15 (d, $J=3.3$ Hz, 3H); IR (neat) ν_{max} 3346, 2997, 1646, 1551, 1426, 1259, 1065 cm^{-1}; HRMALDI FTMS (DHB) m/z 1048.3869 (M^+, $C_{42}H_{62}N_{15}O_{11}S_3^+$ requires 1048.3915).

23. After electrophoresis on a 1% agarose gel, the gel was stained with 0.1 μg/mL ethidium bromide and visualized on a UV transilluminator. The image has been inverted for clarity.
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27. The DNA cleavage reactions were run for 30 min at 37°C and electrophoresis was run on an 8% denaturing PAGE and visualized by autoradiography.