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Solid-Phase Synthesis of Bleomycin A_5 and Three Monosaccharide Analogues: Exploring the Role of the Carbohydrate Moiety in RNA Cleavage

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The bleomycins (BLMs), exemplified by BLM A_5 (**1**) (Figure 1), are anticancer antibiotics isolated from *Streptomyces verticillus*.[1] They effect single- and double-strand DNA cleavage,[2] as well as RNA cleavage[3] in the presence of a metal cofactor and oxygen.[4] Further, cleavage has been shown to be sequence- and shape-selective for both DNA and RNA.[2,3]

The role of the carbohydrate moiety is not well understood. It has been implicated in metal binding[5] and cellular uptake and likely participates in defining the nature of BLM binding to DNA, but has minimal effect on the sequence selectivity or potency of DNA cleavage.[6,7] While deglycoBLM (**2**) analogues have provided insights into the function of the amino acid constituents of BLM,[8] the sugar moiety has not been modified systematically. Boger et al. prepared BLM A_2 derivatives containing the monosaccharides α-D-mannose and 2-*O*-methyl-α-L-gulose.[9] These retained the DNA cleavage selectivity of BLM; however, only the 2-*O*-methyl-α-L-gulose BLM A_2 derivative cleaved DNA efficiently.

The accumulating evidence that RNA may be a plausible therapeutic locus for BLM has focused interest on the exploration of BLM analogues that target RNA specifically. While the carbohydrate moiety apprently has little influence on the sequence selectivity of DNA cleavage by BLM,[7] no study has defined the effect of the carbohydrate moiety on RNA cleavage. Presently, we describe the solid-phase synthesis of BLM A_5 and monosaccharide analogues of BLM A_5 containing α-D-mannose (**3**), α-L-gulose (**4**), and α-L-rhamnose (**5**). Also described is the ability of **3**−**5** to cleave DNA and RNA.

The solid-phase synthesis of BLM A_5 and the monosaccharide derivatives **3**−**5** was carried out in analogy with the synthesis of deglycoBLM.[10] The syntheses (Scheme 1) utilized Boc (**6**)- or NBS (2-nitrobenzenesulfonyl) (**7**)-protected spermidine resins. Addition of bithiazole intermediate (**8**)[10] to the free amine of **6** or **7** was accomplished utilizing *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) and Hunig's base in DMF over a 30-min period.[11] Fmoc group removal was accomplished using 20% piperidine in DMF. N^{α}-Fmoc-(*S*)-threonine (**9**) was then attached using HBTU, hydroxybenzotriazole (HOBt), and Hunig's base in DMF.[12] Following Fmoc deprotection, N^{α}-Fmoc-(2*S*,3*S*,4*R*)-4-amino-3-hydroxy-2-methylvalerate (**10**)[10] was conjugated to the resin in the same fashion.[12]

Fmoc- and trityl-protected histidine analogues **11**−**14** containing the native disaccharide and each of the three monosaccharide derivatives, respectively, were prepared using N^{α}-Fmoc-N^{im}-trityl-(*S*)-*erythro*-β-hydroxyhistidine.[13] Coupling of **11**−**14** to resin-bound tetrapeptide was accomplished using a combination of benzotriazol-1-yloxy-tris(dimethyamino)phosphonium hexafluorophosphate (BOP)

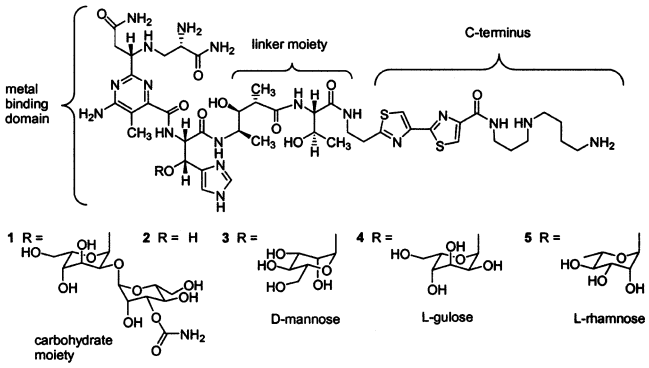
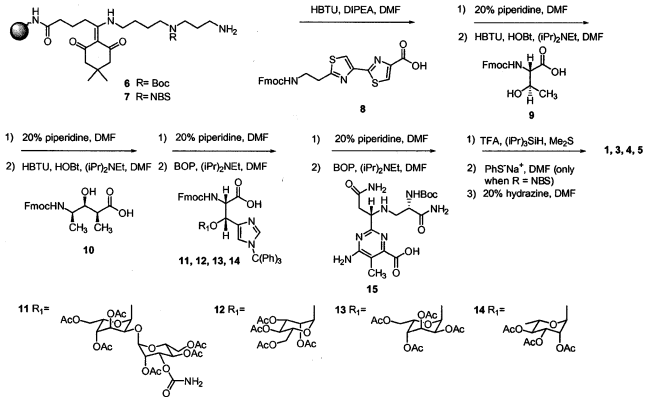


Figure 1. Structures of bleomycin A_5 (**1**), deglycobleomycin A_5 (**2**), and BLM A_5 analogues containing α-D-mannose (**3**), α-L-gulose (**4**), and α-L-rhamnose (**5**).

Scheme 1



and Hunig's base in DMF at 0 °C over a 12-h period.[12] Coupling of the Boc pyrimidoblamic acid moiety (**15**)[14] to the resin-bound pentapeptide was effected using BOP reagent and Hunig's base in DMF at 0 °C for 12 h. The resin-bound, fully functionalized BLM A_5 and each of the monosaccharide analogues was then deprotected[15] and cleaved from the resin, affording BLM A_5 (**1**) and BLM derivatives **3**, **4**, and **5**.[12]

The DNA cleavage efficiency, selectivity, and the ratio of double-strand to single-strand DNA cleavage was determined for each of the monosaccharide analogues. The α-L-gulosyl BLM A_5 analogue **4** effected single- and double-strand DNA cleavage to nearly the same extent as BLM A_5 itself (not shown). The α-D-mannosyl analogue of BLM A_5 (**3**) and the α-L-rhamnosyl analogue (**5**) of BLM A_5 had diminished DNA cleavage efficiencies comparable to that of deglycoBLM A_5. Monosacharide analogues **3**−**5** each had the same sequence selectivity of DNA cleavage as BLM A_5. These results agree with those of Boger et al.[8,9] and support the

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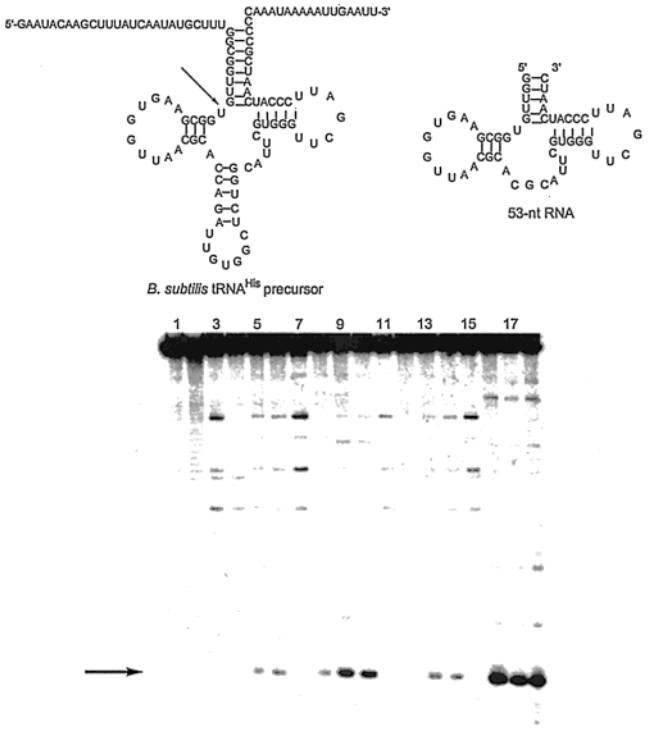

Figure 2. Cleavage of a 53-nucleotide RNA related to *B. subtilis* $tRNA^{His}$ precursor by Fe(II)·BLM derivatives. Lane 1, RNA alone; lane 2, 100 μM Fe^{2+}; lane 3, 20 μM **3**; lane 4, 1 μM **3** + 1 μM Fe^{2+}; lane 5, 10 μM **3** + 10 μM Fe^{2+}; lane 6, 20 μM **3** + 100 μM Fe^{2+}; lane 7, 20 μM **4**; lane 8, 1 μM **4** + 1 μM Fe^{2+}; lane 9, 10 μM **4** + 10 μM Fe^{2+}; lane 10, 20 μM **4** + 100 μM Fe^{2+}; lane 11, 20 μM **5**; lane 12, 1 μM **5** + 1 μM Fe^{2+}; lane 13, 10 μM **5** + 10 μM Fe^{2+}; lane 14, 20 μM **5** + 100 μM Fe^{2+}; lane 15, 20 μM **1**; lane 16, 1 μM **1** + 1 μM Fe^{2+}; lane 17, 10 μM **1** + 10 μM Fe^{2+}; lane 18, 20 μM **1** + 100 μM Fe^{2+}.

suggestion that the nature of the first carbohydrate moiety is important to BLM function.

The cleavage of a 53-nt RNA[16] by each of the monosaccharide analogues (Figure 2) showed that each retained a selectivity pattern for hydrolytic (lanes 3, 7, 11, and 15) and oxidative RNA cleavage (lanes 4−6, 8−10, 12−14, and 16−18) most similar to that of BLM A_5 itself. Again, only the α-L-gulosyl analogue (**4**) exhibited a relatively high level of oxidative RNA cleavage. Quantification of the major oxidative cleavage site (arrow) revealed that **4** cleaved the RNA substrate 47% as efficiently as BLM A_5, while **3** and **5** effected 16 and 19% cleavage, respectively. Interestingly, the hydrolytic RNA cleavage efficiency of analogue **4** was superior to that of BLM A_5.

The role of the carbohydrate moiety during DNA and RNA cleavage has been the subject of much speculation. It has been postulated that the carbohydrate moiety forms part of a protective pocket for the reactive metal−oxygen intermediates that are directly responsible for both DNA and RNA strand scission.[17] It is clear from the present experiments that the carbohydrate moiety of BLM plays a significant role in defining DNA and RNA cleavage competence. Further, it is apparent that the sugar can be of critical importance to the efficiency of oxidative DNA and RNA cleavage.

This work establishes the utility of our solid-phase synthesis strategy for the facile production of BLM analogues containing one or more sugar moieties. The elaboration of BLM libraries that include modified carbohydrate moieties is now technically feasible.

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Supporting Information Available: Experimental procedures and spectral data for BLM and its synthetic intermediates as well as experimental details of the cleavage of the 53-nt 5′-^{32}P end-labeled RNA (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Quantification of coupling efficiency employed Fmoc analysis involving piperidine treatment of a known weight of dry resin and subsequent spectrophotometric measurement of the resulting dibenzylfulvene-piperidine adduct.[10,12] The resin-bound dipeptide derived from **6** (used for the synthesis of **1**) was produced in 43% yield from the initial resin; the resin-bound dipeptide derived from **7** (employed for the syntheses of **3**−**5**) was obtained in yields >75%.

(12) Threonine was attached in >85% yield, as judged by Fmoc analysis. The valerate was attached in >90% yield. Yields for the coupling of each of the monosaccharide-containing histidine intermediates and the native disaccharide were consistently >85%. Boc pyrimidoblamic acid coupling to the pentapeptide containing **11** gave **1** in 15% overall yield following deprotection and removal from the resin. The respective yields for **3**, **4**, and **5** were 22, 29, and 17%.

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(15) Removal of the NBS group from resin **7** and its coupling products required repeated exposure to the sodium salt of thiophenol. This treatment removed the carbamoyl group from the disaccharide of **1**, prompting the use of resin-bound Boc-protected spermidine **6** for the synthesis of **1**. Deprotection of both Boc and trityl groups from resin-bound **1** was accomplished by treatment with TFA, isopropylsilane, and dimethyl sulfide. Following deprotection, fully functionalized BLM A_5 (**1**) was removed from the resin using 20% hydrazine in DMF, which also effected disaccharide moiety deacetylation. The monosaccharide derivatives, lacking the carbamoyl group, were prepared using the NBS-protected spermidine resin **7**.

(16) This RNA, identical in structure with the core of *B. subtilis* $tRNA^{His}$ precursor transcript,[3a] has been shown to undergo oxidative cleavage predominantly at a single position[13] that is presumably analogous to U_{35} in *B. subtilis* $tRNA^{His}$ precursor transcript.

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