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Solid-Phase Synthesis of Bleomycin Group Antibiotics. Elaboration of Deglycobleomycin A_5

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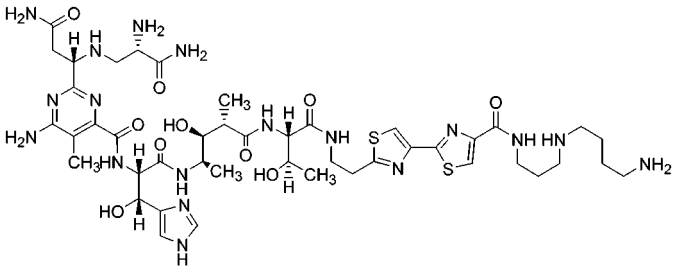
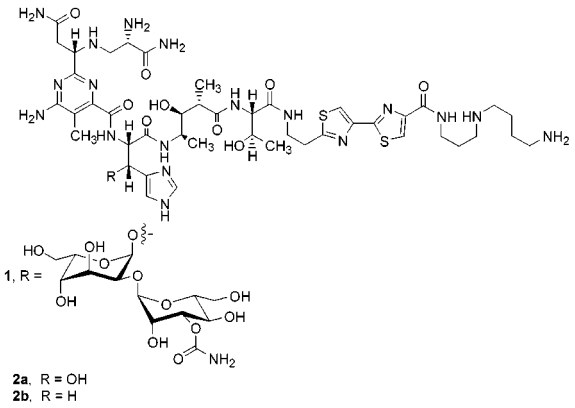
ABSTRACT



The solid-phase syntheses of two deglycobleomycin A_5 analogues were achieved using a commercially available polystyrene resin containing triphenylmethyl-linked spermidine. The final products were deblocked and released from the resin, analyzed, and purified by C_{18} reversed phase HPLC and characterized by high-field ^1H NMR spectroscopy and mass spectrometry. The purified products relaxed supercoiled plasmid DNA in a concentration-dependent fashion and to the same extent as authentic material derived from natural BLM A_5.

The bleomycins (BLMs), exemplified by bleomycin A_5 (**1**) in Figure 1, are naturally occurring, polypeptide-derived antitumor antibiotics[1] that are used extensively in the clinic for the treatment of several cancers.[2] In the presence of certain metal ions and dioxygen, bleomycin effects the sequence selective, oxidative degradation of DNA[1,3] and RNA;[1,4] the effects on one or both of these targets are likely responsible for the antitumor activity of this class of compounds. Deglycobleomycin, lacking the carbohydrate moiety, exhibits DNA cleavage properties very similar to those of bleomycin itself at the levels of potency, sequence selectivity, and actual chemistry of DNA degradation.[5]



2a, R = OH
2b, R = H

Figure 1. Structures of BLM A_5 (**1**) and deglycoBLM A_5 analogues **2a** and **2b**.

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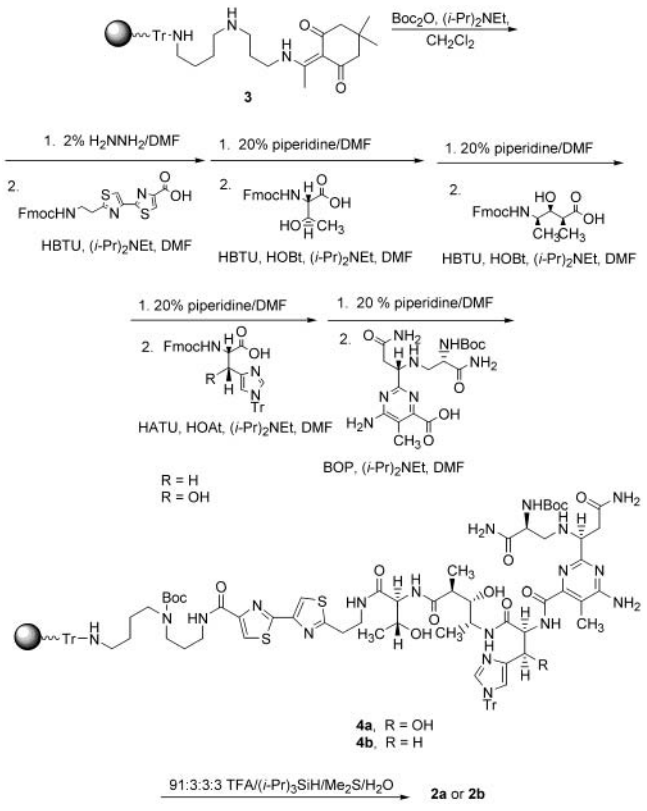
In the past two decades, numerous analogues of bleomycin and deglycobleomycin have been synthesized;[6−9] these studies have contributed importantly to our understanding of the way in which BLM binds to and degrades its nucleic acid substrates, as well as the roles of individual structural elements. However, with the exception of our discovery that the carbohydrate moiety of bleomycin is not essential for DNA cleavage,[5] none of the analogues reported to date can be argued to have improved properties, or a more readily synthetically accessible structure, that might lead to the development of improved antitumor agents.

Since its introduction about 40 years[10] ago, solid-phase chemistry has been an invaluable tool for the synthesis of peptides. Following attachment of the carboxylate moiety of the C-terminal amino acid to an insoluble polymer matrix, peptides can be synthesized in high yields and good purity by the sequential coupling of N-blocked amino acids and deblocking of the newly attached amino acid constituent. Despite the obvious structural complexity of bleomycin, and potential sensitivity of its functional groups to the protocols employed for solid-phase peptide synthesis, we report herein (i) the successful synthesis of two deglycobleomycin analogues (**2a** and **2b**) and (ii) the ability of these analogues to effect DNA relaxation in a concentration-dependent fashion.

As outlined in Scheme 1, a commercially available polystrene resin (37−73 μm), containing triphenylmethyl spermidine **3**, was Boc protected using di-*tert*-butyl dicarbonate and Hunig's base in CH_2Cl_2.[11−13] Treatment of the protected resin with 2% hydrazine in DMF liberated the 9-amino group for coupling with the bithiazole moiety of bleomycin.[15] The Fmoc-protected bithiazole was activated with HBTU[16] and Hunig's base in DMF; this solution was added to the free amino resin and the mixture was shaken under N_2 at 25 °C for 30 min. After the resin was washed with DMF and CH_2Cl_2, a small sample was utilized for qualitative verification of coupling by colorimetric assays using the Kaiser[17] and bromophenol blue[18] tests for amines.

Scheme 1. Synthesis of DeglycoBLM A_5 Analogues **2a** and **2b**



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(12) A portion of the Boc-protected resin was treated with 5% TFA in CH_2Cl_2; mass spectral analysis of the cleaved product gave the expected $(M + H)^+$ ion at 410.2. Portions of each of the coupled intermediates were removed from the resin analogously, and their molecular weights were verified by mass spectrometry.

(13) Although syntheses of BLMs and deglycoBLMs are well documented,[5−9] none of the previous synthetic strategies was compatible with the solid-phase linkers available. It was, therefore, decided to utilize the Fmoc[14] protecting group for the internal fragments and the acid-labile triphenylmethyl linker.

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The coupling was quantified by Fmoc cleavage and indicated a loading of 0.35 mmol/g of resin, which represented a coupling efficiency of 65%.[19] Successive treatments of the resin with 20% piperidine in DMF, followed by Fmoc-threonine, and then in the same fashion for attachment of Fmoc-methylvaleric acid, resulted in attachment of these two amino acids in yields of >99% (0.34 mmol/g) and 95% (0.30 mmol/g), respectively.

The resin-bound oligopeptide was deblocked with piperidine in DMF, and portions were treated either with Fmoc-β-hydroxyhistidine or Fmoc-histidine using the highly reactive couping reagent HATU[20] since the use of HBTU afforded lower yields. Fmoc-β-hydroxyhistidine was attached to the oligopeptide in 90% yield (0.24 mmol/g), while attachment of Fmoc-histidine proceeded in 92% yield (0.25 mmol/g).

Boc pyrimidoblamic acid[21] was coupled to the resin using BOP[22] and Hunig's base in the absence of light for 16 h. After the resin was washed with DMF and methylene chloride, the protected resins **4a** and **4b** were treated with 91:3:3:3 TFA−triisopropylsilane−H_2O−Me_2S for 4 h, and the supernatant was concentrated and treated with diethyl ether; then the residue was dissolved in water and lyophilized. The crude product was purified by C_{18} reversed phase HPLC. The fractions containing the desired product were collected and lyophilized. The resulting colorless solids were analyzed by ESI-MS and exhibited molecular ions at *m*/*z* 1073.5 $(M + H)^+$ and 1057.5 $(M + H)^+$ for **2a** and **2b**, respectively. The overall yields[23] for these products were 55% for **2b** and 35% for **2a**.

DeglycoBLM A_5 was also characterized by its ability to relax Form I DNA in the presence of Fe^{2+}. Concentration-dependent relaxation of supercoiled pSP64 plasmid DNA is shown in Figure 2 for synthetic deglycoBLM A_5 **2a** at 3 and 5 μM concentrations. This synthetic BLM analogue had the same potency as an authentic sample of **2a** derived from BLM A_5 by treatment with HF.[24]

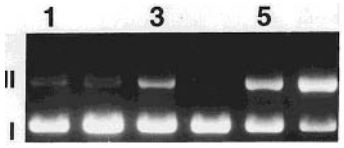

Figure 2. Relaxation of pSP64 Form I DNA by synthetic Fe(II)·deglycoBLM A_5: lane 1, DNA + 3 μM dgBLM A_5; lane 2, DNA + 5 μM dgBLM A_5; lane 3, DNA + 3 μM Fe^{2+}; lane 4, DNA alone; lane 5, DNA + 3 μM Fe^{2+} + 3 μM dgBLM A_5; lane 6, DNA + 3 μM Fe^{2+} + 5 μM dgBLM A_5.

The successful preparation of deglycoBLMs **2a** and **2b** by stepwise solid-phase synthesis establishes the feasibility of this approach for the evaluation of analogues of BLM. While the complexity of the amino acid building blocks results in product formation in yields less than those accessible for simple peptides, individual bleomycins can nonetheless be prepared conveniently in reasonable yields and less than 48 h starting from the protected amino acids. This strategy should greatly facilitate the synthesis of much larger numbers of BLM analogues from which it may be possible to select species having specific improved properties.

Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) Fmoc cleavage was determined by UV measurement of the dibenzylfulvene−piperidine adduct formed upon treatment of the resin with piperidine. The optical density of 5540 M^{-1} at 290 nm and 7300 M^{-1} at 300 nm was used to calculate the loading from a known weight of dry resin.

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