MODIFIED BLEOMYCINS. SYNTHESIS OF NEW BLEOMYCINS VIA REACTION OF DEMETHYL-BLM A2 WITH BROMOACETYL DERIVATIVES

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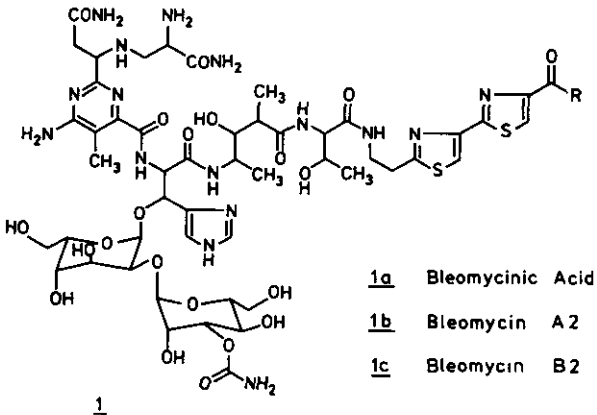
Abstract - Reaction of demethyl-BLM A2 with bromoacetyl derivatives leads to the corresponding sulfonium salts which constitute a new class of biologically active bleomycins.

The glycopeptide antibiotics known as bleomycins (1) discovered by Umezawa[1] have received a great deal of attention in view of their antitumour properties. The various bleomycins possess a common acyl structural moiety corresponding to bleomycinic acid (1a), and differ from one another in the nature of the terminal amine which is linked via an amide bond to this acid. A mixture of bleomycins (BLM) containing A2 (1b, 70%) and B2 (1c, 20%) is currently employed in the clinic in the treatment of squamous cell carcinoma, Hodgkin's disease, lymphomas and testis tumors[2]. Administration of bleomycins (A2 + B2) in large dosage is, however, limited due to the side effect of pulmonary toxicity. The development of modified bleomycins with lower pulmonary toxicity and increased therapeutic effect on human cancer is, consequently, of great importance.

Umezawa and coworkers have studied the pulmonary toxicity of modified bleomycins and, based upon "lung fibrosis index" data, have shown that this side effect is influenced by structural changes in the terminal amine part of the molecule[3]. These results suggest the need for developing simple methods for modification of the side chain which would allow convenient access to series of structurally related bleomycins. In this communication we describe one such method and its application to the synthesis of several new bleomycins, together with preliminary



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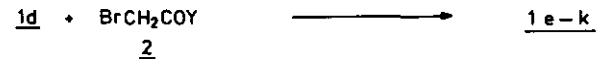
			R
1a	Bleomycinic	Acid	OH
1b	Bleomycin	A 2	$NH(CH_2)_3-\overset{+}{S}(CH_3)_2$
1c	Bleomycin	B 2	$NH(CH_2)_4-NHC(=NH)NH_2$
1d	Demethyl	A 2	$NH(CH_2)_3-S-CH_3$

Scheme



1d + $BrCH_2COY$ (2) ⟶ 1e–k

Y		R	$X^- = 1/2\ SO_4^{2-}$	δ CH_3
		1b $NH(CH_2)_3\overset{+}{S}(CH_3)CH_3$	X^-	2.94
		1d $NH(CH_2)_3SCH_3$		2.11
2a	OH	1e $NH(CH_2)_3\overset{+}{S}(CH_3)CH_2COO^-$		2.92
2b	OEt	1f $NH(CH_2)_3\overset{+}{S}(CH_3)CH_2COOEt$	X^-	3.04
2c	NH_2	1g $NH(CH_2)_3\overset{+}{S}(CH_3)CH_2CONH_2$	X^-	3.00
2d	N⟨ ⟩	1h $NH(CH_2)_3\overset{+}{S}(CH_3)CH_2CON$⟨ ⟩	X^-	3.00
2e	N⟨ ⟩O	1i $NH(CH_2)_3\overset{+}{S}(CH_3)CH_2CON$⟨ ⟩O	X^-	2.99
2f	NH⟨	1j $NH(CH_2)_3\overset{+}{S}(CH_3)CH_2CONH$⟨	X^-	3.00
2g	NH⟨▷	1k $NH(CH_2)_3\overset{+}{S}(CH_3)CH_2CONH$⟨▷	X^-	3.00

data on their biological activity.

Since BLM A2, the major component of clinical bleomycin, is a sulfonium salt and since, according to the currently accepted mechanism of action of bleomycin, the positively charged sulfur atom is involved in an electrostatic interaction with DNA[4], it was felt that the modified side chain ought to retain this specific feature of the antibiotic. The simplest manner in which this could be achieved was visualized via the replacement of one of the methyl groups of the sulfonium moiety, by an alkyl residue bearing functionalized substituents. In view of the presence of a large number of sensitive structural elements in the molecule of bleomycin, we recognized that the reactions which were to be employed for the proposed chemical conversions had to be highly selective in their operation.

As starting material for our studies we employed clinically outdated Blenoxane^R, provided by Bristol-Myers[5]. Bleomycin A2 was isolated from Blenoxane^R by chromatography[6] and subsequently demethylated to demethyl A2 (1d), employing essentially the procedures described in the literature[7]. Reaction of 1d with a variety of electrophiles was investigated, in order to develop a specific reaction at the sulfur site. The results of this study led to the conclusion that electrophiles of type $BrCH_2COY$ (2, Scheme) were most effective in causing the desired transformation in a practical, useful reaction. Thus, when 1d was allowed to react with 2a-g[8,9] (pH 1.60, RT 24 h), the sulfonium salts ("modified bleomycins" 1e-k) were obtained in fair yields (~ 60%, Scheme). Details of the chromatographic separation and isolation of the products will be presented elsewhere. Structure elucidation of 1e-k required detailed NMR spectra of the "parent" molecule 1b. Utilizing high resolution (500 MHz) ^1H and ^{13}C NMR in conjunction with two dimensional Fourier transform techniques an unambiguous and complete interpretation of the ^1H and ^{13}C NMR spectra of BLM A2 (1h) has been made[10]. The most significant change in the ^1H NMR spectra accompanying the conversion of 1d to 1e-k is the downfield displacement of the signal of the S-methyl group. The chemical shifts of the sulfonium methyls in 1e-k are presented in the Scheme. The chemical shifts of the S-methyl in 1b and 1d are provided for comparison. Mass measurements of the modified bleomycins by Fast Atom Bombardment (FAB) method are in progress.

Biological Results: The effect of bleomycin analogues 1f-k on the survival of B16 mouse melanoma cells *in vitro* has been studied. These results and the comparative data for bleomycin are presented in the Table.

Table

A comparative study of the effect of Bleomycin and analogues 1f-k on the survival of B16 melanoma cells in vitro.

Compound	Dose µg/ml	Percent survival
Bleomycin[a]	10	41 ± 2
	100	29 ± 2
1f	10	43 ± 2
	100	27 ± 1
1g	10	38 ± 2
	100	21 ± 2
1h	10	35 ± 3
	100	26 ± 5
1i	10	51 ± 2
	100	28 ± 2
1j	10	41 ± 3
	100	23 ± 3
1k	10	32 ± 3
	100	23 ± 3

[a] Bleomycin Lundbeck, obtained from H. Lundbeck and Co A/S Copenhagen.

B16 mouse melanoma cells from log phase cultures were suspended in fresh medium [Dulbecco's modified minimum essential eagle medium with 10% new born calf serum (Gibco, Europe)] at $5x10^4$ cells/ml. For the experiments, "6 Multiwell" plates were employed, with two ml of the cell suspension in each well. After 24 hour the test agents were incubated with the B16 melanoma cells during 21 hours. After treatment, the cells were washed twice and fresh medium added, 2 ml/well. 48 Hours after the start of the incubation the cells were trypsinized and counted on a Sysmex microcell-counter. The number of cells surviving a particular drug treatment could be estimated by comparing counts of untreated cells with those of treated cells. Viability was estimated by Trypsan blue exclusion. Two doses were tested: 10 µg/ml and 100 µg/ml. Percent survival was calculated according to the formula T/C x 100. T = treated cells, C = untreated control. The number is the mean of three samples.

The procedure for modification of the side chain of bleomycin, described in this report, can be applied to a large range of α-halogenated amides and peptides, including those incorporating structural moieties which might be expected to show specific interaction with the DNA bases. Work in this direction is currently in progress.

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5. We are indebted to Dr J. Vida (Bristol-Myers) for providing us generous quantities of clinically outdated Blenoxane.
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7. A. Fujii, ibid., p. 343. The product was isolated as the sulphate salt.
8. The details of the syntheses of electrophiles 2d-g will be reported elsewhere.
9. A referee has drawn our attention to U.S. Patent 4,339,426 (C.A., 98, 5358m, 1983), in which reactions of 1d with 1-(p-bromoacetamidophenyl)-EDTA and 1-(p-bromoacetamidobenzyl)-EDTA are described.[5]
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