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NOTE

Synthesis and properties of a novel brominated oligomycin A derivative

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Oligomycins belong to the class of highly functionalized macrolide antibiotics that contain the hydroxyl groups, the lactone and spiro moieties, as well as the double bonds. The mechanism of cytocidal activity of oligomycins involves the inhibition of oxidative phosphorylation by preventing ATP synthesis. Design of new compounds on oligomycin A scaffold is of interest as ATP synthase inhibitors are drug candidates for the treatment of bacterial infections and cancer.[1] Recently, we reported the examples of modification at the C_7 carbonyl group and the $C_2{=}C_3$ double bond of the antibiotic.[2] In search of selective methods to modify oligomycin A (**1**), we carried out bromination of (**1**) with NBS in DMF at neutral pH; this procedure yielded a mono-bromo derivative (**2**) in 45% yield (Figure 1). NMR studies showed the presence of a single bond C_{16}–C_{17} (instead of the double bond in (**1**) and a tetrahydropyrane cycle due to the formation of $C_{13}H$–O–$C_{17}H$ bond confirmed by a low field shift of C_{13} ($\Delta \sim 10$ p.p.m.) in comparison with ^{13}C NMR spectrum of (**1**) (81.73 and 72.15 p.p.m. for C_{13} in (**1**) and (**2**), respectively) (Table 1).[2] The tetrahydropyrane ring contains Br in position C_{16}. The spin coupling constants in the tetrahydropyrane ring correlate with 'chair' conformation and correspond to the following orientation of hydrogen atoms: $C_{13}H_{eq}$, $C_{14}H_{eq}$, $C_{16}H_{ax}$ and $C_{17}H_{ax}$. On the basis of these coupling constant values and taking in mind that oligomycin A moiety has 13*S* and 14*R* configuration,[3] we can ascribe 13*S*, 14*R*, 16*R* and 17*S* stereochemistry to bromooligomycin (**2**). This structure represents a new skeleton in this type of antibiotics.

The NMR spectra were elucidated using homo ^1H, ^1H (2D COSY) and hetero ^1H, ^{13}C (2D HETCOR) correlation as well as ^1H and ^{13}C 1D spectra of (**1**) and its derivatives and were registered using unity +400 (Varian, Palo Alto, CA, USA) spectrometer at 400.0 MHz for ^1H nuclei, and at 100.6 MHz for ^{13}C nuclei.

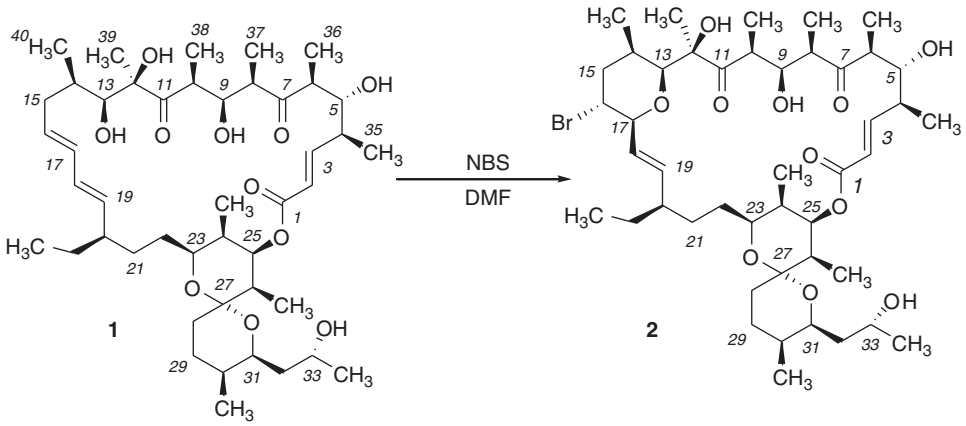

Figure 1 Synthesis of the bromo derivative of **1** (compound **2**).

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Table 1 The ^{1}H and ^{13}C NMR spectra of 1 and 2

	1					*2*				
No		δ_C		δ_H	$J_{H,H}$		δ_C		δ_H	$J_{H,H}$
1	165.02	O–CO				165.13	O–CO		—	
2	122.61	CH	sp^2	5.80dd	15.6, 0.7	122.57	CH	sp^2	5.87dd	15.7, 0,6
3	148.29	CH	sp^2	6.62dd	15.6, 10.1	148.83	CH	sp^2	6.61dd	15.7, 9.8
4	40.06	CH	sp^3	2.36tq	10.0, 6.6	39.21	CH	sp^3	2.40ddq	10.1, 9.8, 6.4
5	72.88	CH	sp^3	3.75dd	10.1, 1.3	72.78	CH	sp^3	3.74d	10.1
6	46.38	CH	sp^3	2.70dq	1.3, 7.4	49.00	CH	sp^3	2.49dq	1.2, 7.4
7	220.16[a]	CO	—			220.79[a]	CO	—		
8	41.88[b]	CH	sp^3	3.59dq	8.6, 6.9	40.30[b]	CH	sp^3	3.14dq	2.7, 7.1
9	72.57	CH	sp^3	3.94dd	8.6, 3.1	73.44	CH	sp^3	3.82dd	7.9, 2.7
10	45.63[b]	CH	sp^3	2.74dq	3.0, 7.1	46.40[b]	CH	sp^3	2.86dq	7.9, 6.7
11	219.93[a]	CO		—		219.31[a]	CO		—	
12	82.91	C_q–O	sp^3	—		81.98	C_q	sp^3	—	
13	72.15	CH	sp^3	3.89d	1.9	81.73	CH	sp^3	4.05d	2.2
14	33.41	CH	sp^3	1.88m		31.67	CH	sp^3	2.02dddq	4.4, 2.8, 2.2, 7.1
15	38.33	CH_2	sp^3	2.17bd; 1.98dt		43.68	CH_2	sp^3	2.29ddd, 2.22ddd[c]	
16	129.30	CH	sp^2	5.42ddd	14.8, 10.5, 4.1	46.40	CH	sp^3	3.97ddd	11.5, 10.5, 4.6
17	132.30	CH	sp^2	6.00ddd	14.7, 10.4, 1.4	79.91	CH	sp^3	3.92ddt	10.5, 4.6, 0.9
18	130.19	CH	sp^2	5.90dd	14.9, 10.5	126.02	CH	sp^2	5.49ddd	16.1, 4.6, 0.9
19	137.67	CH	sp^2	5.21dd	14.8, 9.6	138.27	CH	sp^2	5.62ddd	16.1, 6.8, 0.9
20	45.94	CH	sp^3	1.85m		41.81	CH	sp^3	2.04m	
21	31.38	CH_2	sp^3	1.52m; 1.35m		28.61	CH_2	sp^3	1.22m, 1.51m	
22	30.90	CH_2	sp^3	1.59ddd; ~1.02m		26.70	CH_2	sp^3	1.18m, 1.47m	
23	68.95	CH	sp^3	3.78ddd	9.8, 2.7, 2.4	68.40	CH	sp^3	3.77ddd	7.7, 6.0, 2.1
24	35.74	CH	sp^3	2.11ddq	5.0, 2.2, 6.9	34.88	CH	sp^3	1.90m	
25	76.10	CH	sp^3	4.91dd	11.4, 5.0	74.99	CH	sp^3	5.11dd	11.4, 5.0
26	37.61	CH	sp^3	1.78dq	11.4, 6.6	38.03	CH	sp^3	1.75dq	11.4, 6.7
27	99.11	O–C–O	sp^3	—		99.27	C_q	sp^3	—	
28	25.88	CH_2	sp^3	1.90m; 1.23m		25.68	CH_2	sp^3	1.19m, 1.89m	
39	26.41	CH_2	sp^3	2.07m; 1.38m		25.50	CH_2	sp^3	1.47m, 2.09m	
30	30.38	CH	sp^3	1.54m		30.38	CH	sp^3	1.53m	
31	67.14	CH	sp^3	3.96dt	10.3, 2.5	67.07	CH	sp^3	3.97m	
32	42.46	CH_2	sp^3	1.55m, 1.25m		42.29	CH_2	sp^3	1.60m, 1.22m	
33	64.57	CH	sp^3	4.00ddq	9.2, 3.1, 6.2	64.67	CH	sp^3	4.00m	
34	24.66	CH_3	sp^3	1.213d	6.2	24.70	CH_3	sp^3	1.216d	6.2
35	17.84	CH_3	sp^3	1.159d	6.6	16.74	CH_3	sp^3	1.188d	6.4
36	8.21	CH_3	sp^3	1.047d	7.3	7.91	CH_3	sp^3	1.175d	7.4
37	13.99	CH_3	sp^3	1.085d	6.9	9.83	CH_3	sp^3	0.949d	7.1
38	9.16	CH_3	sp^3	1.011d	7.0	13.33	CH_3	sp^3	1.098d	6.7
39	20.92	CH_3	sp^3	1.110s		20.08	CH_3	sp^3	1.072s	
40	14.39	CH_3	sp^3	0.977d	6.6	13.48	CH_3	sp^3	1.095d	7.1
41	28.42	CH_2	sp^3	~1.35m, 1.25m		24.70	CH_2	sp^3	1.34m, 1.40m	
42	11.98	CH_3	sp^3	0.799t	7.4	11.90	CH_3	sp^3	0.844t	7.3
43	5.97	CH_3	sp^3	0.822d	6.9	5.83	CH_3	sp^3	0.808d	6.9
44	11.67	CH_3	sp^3	0.950d	6.6	11.48	CH_3	sp^3	0.922d	6.7
45	11.13	CH_3	sp^3	0.884d	6.9	11.06	CH_3	sp^3	0.878d	7.0

[a]Reverse assignments of signals are possible.
[b]5-OH: δ 3.07, ^{3}J=2.6 Hz; 13-OH: δ 2.12, ^{3}J=8.3 Hz.
[c]15-CH_2: H_{eq}: δ 2.29ddd, J 13.2, 4.6, 2.8 Hz; H_{ax}: δ 2.22ddd, J 13.2, 11.5, 4.4 Hz.

The structure of (**2**) is membranous; a membranous structure has been demonstrated also for the product of the interaction of (**1**) with hydroxylamine.[2] It is likely that the formation of such structures is typical for this 26-membered macrolide as well as for 14-membered antibacterial macrolides (for example, erythromycin and its derivatives).[4]

Bromo-oligomycin A (**2**) was low cytotoxic against HCT116 human colon cancer cell line and K562 human leukemia cell line (IC_{50} 16.0 ± 2.2 and 9.0 ± 1.4 μM, respectively), whereas the parental compound (**1**) was significantly more potent (IC_{50} 3.3 ± 1.0 and 3.0 ± 1.0 μM, respectively) as determined by MTT-test after a 72 h incubation.[5] Furthermore, (**2**) was weakly active against *Streptomyces fradiae* strain that is extremely sensitive to (**1**) (250 nM per disk and 0.001 nM per disk, respectively).

A lowered potency of (**2**) for actinobacterial cells correlated with a decreased activity against filamentous fungi (moulds) and yeast.

Compound (**1**) was highly active against *Aspergillus niger* ATCC 16404 and *Fusarium oxysporum* VKM F-140 strains (MICs 0.125 and 1 μg ml^{-1}, respectively) and moderately active against *Candida albicans* ATCC 14053 and *Cryptococcus humicolus* ATCC 9949 (MICs 4 and 2 μg ml^{-1}, respectively, after a 24-h exposure). Compound (**2**) revealed the activity only against *C. humicolus* (MIC 2 μg ml^{-1}), whereas for other studied fungi and yeast strains (**2**) was practically inert (MIC >16 μg ml^{-1}). Determination of MICs was carried out according to NCCLS, Standards M27-A and M38-A.[6,7]

Low biological activities of compound (**2**) may be explained by distorted conformation of oligomycin A derivative in comparison with the starting antibiotic due to the formation of the webbed structure and also by the absence of free 13-OH group that participates at the interaction with oligomycin A target in cells. It was supposed that in oligomycins the hydroxyl groups could function as donors or acceptors of hydrogen bonds.[8]

EXPERIMENTAL PROCEDURE

Oligomycin A ((**1**); purity 95 %) was obtained in the Research Center for Biotechnology of Antibiotics BIOAN, Moscow using the *Streptomyces avermitilis NIC B62* strain (production of (**1**) $\sim$1 g l^{-1}). Fermentation was performed for 8 days at 28 °C in liquid medium. Isolation and purification involved the extraction with acetone-hexane mixture followed by crystallization.

Synthesis of bromo-oligomycin A (2).

To a solution of (**1**) (1.0 g, 1.3 mmol) in dry DMF (10 ml) N-Br-succinimide (1-bromopyrrolidine-2,5-dione) (0.25 g, 1.41 mmol) was added, the reaction mixture was stirred at room temperature for 2 h and analyzed by TLC in $CHCl_3$-MeOH (10 : 0.5). The mixture was diluted with water; the reaction product was extracted with toluene, washed and dried over Na_2SO_4. Then the reaction product was purified by column chromatography on silica gel 60 (Merck) in $CHCl_3$-MeOH (50 : 0.5) to give 0.45 g (45%) of (**2**) as colorless amorphous powder. MW calcd. for $C_{45}H_{73}BrO_{11}$ 868.4336. Found in ESI-mass spectrum (m/z) 869.4445 $(M + H)^+$, 871.4424 $(M + H)^+$, 891.4274 $(M + Na)^+$, 893.4265 $(M + Na)^+$ (Bruker Daltonics GmbH, Bremen, Germany). UV-spectrum (λ_{max} nm, MeOH), (ε): 209 (14 000) (UV/VIS double beam spectrometer, UNICO, Dayton, NJ, USA); IR V_{max}, cm^{-1} (film) 3417, 2967, 2934, 2877, 1698, 1644, 1456, 1365, 1277, 1224, 1172, 1092, 1046, 982, 881 and 763 (Nicolet_iS10 Fourier transform IR spectrometer, Nicolet, Madison, WI, USA); $[\alpha]_D^{20}$ −83.3 (c 0.23, MeOH) (AA55 Polarimeter, Optical Activity Ltd (Cambridge, UK)); R_t 14.01 (Shimadzu LC10 vp instrument, Kyoto, Japan, MeCN-0.01 M H_3PO_4 mixture, pH 2.6. The percentage of MeCN increased from 20 to 22% within 15 min, then to 90% within 15 min at a flow rate of 1 ml min^{-1}).

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