Макрогетероциклы
http://macroheterocycles.isuct.ru

DOI: 10.6060/mhc151084s

Synthesis and Anti–Actinomycotic Activity of the Oligomycin A Thiocyanato Derivative Modified at 2–Oxypropyl Side Chain

Lyudmila N. Lysenkova,[a@1] Ivan A. Godovikov,[b] Alexander M. Korolev,[a] Valery N. Danilenko,[c] Olga B. Bekker,[c] Dilara A. Mavletova,[c] Aleksey A. Vatlin,[c] Andrey E. Shchekotikhin,[a,d@2] and Maria N. Preobrazhenskaya[a]

We dedicate this work to the memory of Professor Maria N. Preobrazhenskaya, the eminent scholar in heterocyclic and medicinal chemistry

[a]*G.F. Gause Institute of New Antibiotics, 119021 Moscow, Russian Federation*
[b]*A.N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, 119991 Moscow, Russian Federation*
[c]*N.I. Vavilov Institute of General Genetics, Russian Academy of Sciences, 119991 Moscow, Russian Federation*
[d]*D.I. Mendeleev University of Chemical Technology of Russia, 125047 Moscow, Russian Federation*
[@1]*Corresponding author E-mail: lyudmil-lys@yandex.ru*
[@2]*Corresponding author E-mail: shchekotikhin@mail.ru*

A novel way of chemical modification of the antibiotic oligomycin A at the 2-oxypropyl side chain was developed. Previously obtained 33-O-mesyl oligomycin A was used at the reaction with the potassium thiocyanate to produce (33S)-33-deoxy-33-thiocyanatooligomycin in 66 % yield. (33S)-33-Deoxy-33-thiocyanatooligomycin A has demonstrated a lower potency active against S. fradiae and S. albus than oligomycin A.

Keywords: (33*S*)-33-Deoxy-33-thiocyanatooligomycin, oligomycin A, macrolide antibiotic, anti-actinomycotic activity, ATP-synthase inhibitor.

Синтез и противоактиномикозная активность производного олигомицина А, модифицированного тиоцианатом по 2–гидроксипропильной цепи

Л. Н. Лысенкова,[a@1] И. А. Годовиков,[b] А. М. Королев,[a] В. Н. Даниленко,[c] О. Б. Беккер,[c] Д. А. Мавлетова,[c] А. А. Ватлин,[c] А. Е. Щекотихин,[a,d@2] М. Н. Преображенская[a]

Работа посвящается памяти выдающегося ученого в области гетероциклической и медицинской химии, профессора Марии Преображенской

[a]*Институт по изысканию новых антибиотиков им. Г.Ф. Гаузе, 119021 Москва, Россия*
[b]*Институт элементоорганических соединений им. А.Н. Несмеянова РАН, 119991 Москва, Россия*
[c]*Институт общей генетики им. Н.И. Вавилова РАН, 119991 Москва, Россия*
[d]*Российский химико-технологический университет им. Д.И. Менделеева, 125047 Москва, Россия*
[@1]*E-mail: lyudmil-lys@yandex.ru*
[@2]*E-mail: shchekotikhin@mail.ru*

Разработан новый способ химической модификации антибиотика олигомицина А в боковой цепи. Взаимодействием 33-О-мезилолигомицина с тиоцианатом калия синтезирован (33S)-33-дезокси-33-тиоцианатоолигомицин с выходом 66 %. (33S)-33-Дезокси-33-тиоцианатоолигомицин показал несколько меньшую активность в отношении S. fradiae и S. albus, чем исходный олигомицин А.

Ключевые слова: (33*S*)-33-Дезокси-33-тиоцианатоолигомицин, олигомицин А, макролидный антибиотик, противоактиномикозная активность, АТФ-синтаза.

Introduction

Oligomycin A, an inhibitor of F_0F_1ATP synthases of bacteria and eukaryotes, disrupts bioenergetic metabolism.[1,2] The extremely high potency of oligomycin A makes this antibiotic an attractive scaffold for rational design of new chemotherapeutic agents. Oligomycins belong to the class of macrolide antibiotics which have highly functionalized molecules with keto- and hydroxyl groups, lactone and spiro moieties, and double bonds. The complexity of oligomycin and its lability in basic conditions[3] significantly impede drug modification and applicability. These challenges have prompted us to develop preparative ways to diversify the oligomycin scaffold. Additionally, novel semi-synthetic derivatives with a point modification of functional groups at oligomycin A (**1**) would be also valuable for SAR studies, validation of intracellular targets, and depicting the mechanism of F_0F_1ATP synthase inhibition.[4] Of interest is the development of modifications of the oligomycin A 2-oxypropyl side chain, as a hydroxyl group of this moiety plays a key role at the inhibition of proton translocation in F_0F_1ATP synthase.[5] Recently a fluorinated oligomycin A has been obtained selectively despite the presence of five hydroxyl groups indicating the special properties of 33-OH group.[6]

Experimental

*(33S)-33-Deoxy-33-thiocyanatooligomycin $C_{46}H_{73}NO_{10}S$ (**3**).* To a stirring solution of *33-O-mesyl-oligomycin A (**2**)*[7] (30 mg, 0.034 mmol) in hexamethylphosphoric triamide (HMPA) (3.5 ml) under argon flow, KSCN (67 mg, 0.7 mmol) was added. A flask with reaction mixture was put into the bath heated to 105 °C. The temperature was kept at 105–115 °C. An additional portion of KSCN (0.015 g, 0.15 mmol) was added after 1 h. The reaction mixture was stirred for 3 hrs; after the reaction was completed (TLC analyzed in hexane/acetone, 10:7), the resulting solution was cooled and quenched by adding water (10 ml). The aqueous

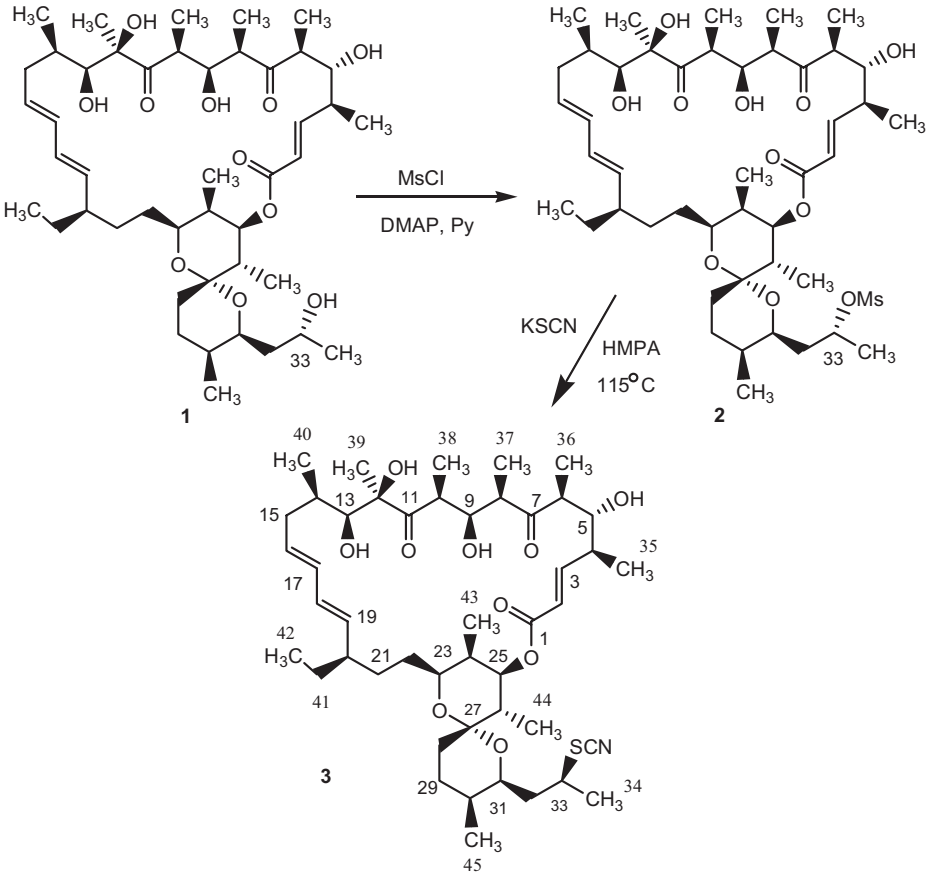

Figure 1. Synthesis of (33*S*)-33-deoxy-33-thiocyanatooligomycin A **3**.

Table 1. ^{1}H and ^{13}C NMR spectra of compounds **2** and **3** with comparison to oligomycin A **1** in $CDCl_3$.

Position	Type	Oligomycin A (**1**)		33-*O*-Mesyloligomycin A (**2**)		33-Deoxy-33-thiocyanato-oligomycin A (**3**)	
		δ_C, ppm	δ_H, ppm, mult (*J* in Hz)	δ_C, ppm	δ_H, ppm, mult (*J* in Hz)	δ_C, ppm	δ_H, ppm, mult (*J* in Hz)
1	O=CO	165.0	–	165.0	–	165.1	–
2	CH	122.6	5.80, dd (15.6, 0.7)	122.6	5.80, dd (15.7, 0.7)	122.7	5.86, d (15.3)
3	CH	148.3	6.62, dd (15.6, 10.1)	148.4	6.62, dd (15.6, 10.0)	148.6	6.67, dd (15.3, 10.8)
4	CH	40.1	2.36, tq (10.0, 6.6)	40.0	2.37, tq (10.0, 6.5)	40.2	2.42, m
5	CH	72.9	3.75, dd (10.1, 1.3)	72.9	3.76, dd (10.1, 1.2)	73.0	3.82, d (9.8)
6	CH	46.4	2.70, dq (1.3, 7.4)	46.5	2.71, qd (7.3, 1.3)	46.7	2.74, m
7	C=O	220.2[a)]	–	220.2[a)]	–	220.4	–
8	CH	41.9[b)]	3.59, dq (8.6, 6.9)	41.8[b)]	3.58, dq (8.4, 6.9)	41.7	3.35, m
9	CH	72.6	3.94, dd (8.6, 3.1)	72.5	3.94, dd (8.4, 3.0)	72.3	3.98, m
10	CH	45.6[b)]	2.74, dq (3.0, 7.1)	45.6[b)]	2.75, qd (7.0, 3.0)	45.7	2.8 m
11	C=O	219.9[a)]	–	219.8[a)]	–	220.0	–
12	C-O	82.9	–	82.9	–	83.1	–
13	CH	72.2	3.89, d (1.9)	72.2	3.92, d (1.8)	68.5	3.84, m
14	CH	33.4	1.88, m	33.4	1.85, m	45.9	1.92, m
15	CH_2	38.3	2.17, bd; 1.98dt	38.4	2.18, m; 1.97, m	38.38	1.28, m; 1.46, m
16	CH	129.3	5.42, ddd (14.8, 10.5, 4.1)	129.3	5.44, ddd (14.7, 10.6, 3.9)	129.8	5.49, t (11.0)
17	CH	132.3	6.00, ddd (14.7, 10.4, 1.4)	132.3	6.01, ddd (14.6, 10.3, 1.6)	130.6	5.98, m
18	CH	130.2	5.90, dd (14.9, 10.5)	130.3	5.92, dd (14.7, 10.4)	132.4	6.05, m
19	CH	137.7	5.21, dd (14.8, 9.6)	137.6	5.24, dd (14.7, 9.6)	137.3	5.27, dd (15.3, 9.9)
20	CH	45.9	1.85, m	45.7	1.85, m	45.74	1.92, m
21	CH_2	31.4	1.52, m; 1.35, m	31.4	1.40, m	38.5	1.94, m; 2.17 m
22	CH_2	30.9	1.59, ddd	30.4	1.59, m; 1.07, m	30.7	1.18–1.23, m
23	CH	68.9	3.78, ddd (9.8, 2.7, 2.4)	69.1	3.71, ddd (10.0, 3.7, 2.1)	41.97	3.63, m
24	CH	35.7	2.11, ddq (5.0, 2.2, 6.9)	35.6	2.11, ddq (5.0, 2.0, 7.0)	35.8	2.16, m
25	CH	76.1	4.91, dd (11.4, 5.0)	75.8	4.93, dd (11.4, 4.9)	75.9	4.99, dd (12.0, 4.3)
26	CH	37.6	1.78, dq (11.4, 6.6)	37.6	1.78, dq (11.4, 6.6)	37.7	1.83, m
27	OCO	99.1	–	99.2	–	99.5	–
28	CH_2	25.9	1.90, m; 1.23,m	25.8	1.88, m; 1.22, m	21.8	1.46, m; 1.28 m
29	CH_2	26.4	2.07, m; 1.38, m	26.1	2.08, m; 1.48, m	26.23	1.96, m; 2.21, m
30	CH	30.4	1.54, m	29.6	1.60, m	33.6	1.99, m
31	CH	67.1	3.96, dt (10.3, 2.5)	67.9	3.83, ddd (8.2, 5.0, 2.5)	69.6	3.70, m
32	CH_2	42.4	1.55, m; 1.25m	40.6	1.83, m; 1.73, m	40.9	1.80, m; 1.98, m
33	CH	64.6	4.00, ddq (9.2, 3.1, 6.2)	78.2	4.87, ddq	41.8	3.35, m
34	CH_3	24.6	1.21, d (6.2)	22.0	1.48, d (6.2)	22.5	1.62, d (6.6)
35	CH_3	17.8	1.16, d (6.6)	17.8	1.16, d (6.5)	18.0	1.22, d (6.0)
36	CH_3	8.2	1.05, d (7.3)	8.2	1.05, d (7.3)	8.4	1.10, d (7.3)
37	CH_3	14.0	1.09, d (6.9)	13.9	1.08, d (6.9)	14.2	1.13, d (6.1)
38	CH_3	9.2	1.01, d (7.0)	9.3	1.02, d (7.0)	9.4	1.06, d (6.6)
39	CH_3	20.9	1.11, s	20.9	1.12, s	21.0	1.16, s
40	CH_3	14.4	0.98, d (6.6)	14.4	0.98, d (6.6)	11.1	0.94, d (6.9)
41	CH_2	28.4	1.35, m; 1.25, m	28.2	1.35, m; 1.25, m	28.5	1.39, m; 1.29, m
42	CH_3	12.0	0.80, t (7.4)	12.0	0.81, t (7.4)	12.1	0.86, m
43	CH_3	6.0	0.82, d (6.9)	5.9	0.82, d (6.9)	6.0	0.87, d (6.0)
44	CH_3	11.7	0.95, d (6.6)	11.7	0.94, d (6.6)	12.1	1.00, d (6.0)
45	CH_3	11.1	0.88, d (6.9)	11.0	0.91, d (7.0)	14.5	1.03, d (6.4)
46	S-C	–	–	39.1	2.99s	111.3	–

[a), b)] Assignment of reverse signals of the same compound, labeled the same letters.

solution was extracted with EtOAc (2×20 ml). The combined organic layer was carefully washed with water (5×20 ml), brine (20 ml), dried over Na_2SO_4 and evaporated. The residue was purified twice by column chromatography on silica gel in hexane:acetone (10:7) and then $CHCl_3$:MeOH (10:0.5) After evaporation of the solvent a colorless amorphous powder was obtained. Yield: 0.019 g (66 %). R_f=0.58 (hexane:acetone, 10:7); R_t = 17.11, 96.4 %. Mass spectrum (ESI) *m/z* (%): 854.4861 (100) [$(M+Na)^+$]. IR (film) ν cm^{-1}: 3447 m, 2969 s, 2933 s, 2875 s, 2152 w, 1713 s, 1651 w, 1457 s, 1384 m, 1333 m, 1273 m, 1223 m, 1173 s, 1090 m, 984 s, 894 s (Figure S11). UV-Vis (CH_3OH) λ nm (lgε): 260 (4.4), 280 (4.2) (Figure S10). $[\alpha]_D^{20}$ -41.6° (c 0.576, methanol). M.p.: 103–104 °C.

*Evaluation of the antibacterial activity of compounds **1** and **3***. The antibacterial activity of oligomycin A (**1**) and its derivative (**3**) was determined as the diameter of the growth inhibition halo of *S. fradiae* ATCC-19609 cells around paper discs impregnated with tested compounds.[8] The agar MG medium (0.7 % agar), p*H* = 7.5, was mixed with *S. fradiae* spore suspension (10^7 spores per dish) and plated on Petri dishes with agar MG medium (2 % agar). Dishes were overlaid with paper discs containing different concentrations of tested compounds. The growth inhibition halo was measured after incubation for 24 hrs at 28 °C. The compound's concentrations showed the smallest halo diameter were compared.

The details of measurements are given in *Supplementary Information*.

Results and Discussion

Previously the selective transformation of 33-hydroxyl group of oligomycin A side chain **1** into 33-mesyloxy group was observed.[7] The reaction of **1** with methanesulfonyl chloride in pyridine in the presence of DMAP has resulted to 33-*O*-mesylate of oligomycin **2** with a good yield. It is known that the mesyloxy moiety is an excellent leaving group that can be replaced with wide range of nucleophiles. Therefore, further studies toward the transformation of **2** were pursued. In this study, we have described the modification of **2** into (33*S*)-33-deoxy-33-thiocyanatooligomycin A **3** and compared their antibiotic activity.

After extensive studies, we have found the reaction conditions for successful transformation of **2** to **3** (Figure 1). Treatment of **2** with an excess of KSCN in hexamethylphosphoric triamide (HMPA) at 105–115 °C for 3–4 hrs has given **3** in good yield (66 %). To avoid side reactions, a flask with reaction mixture was put into the bath heated to 105 °C. We were unable to accomplish this reaction in other solvents such as *N,N*-dimethylformamide, *N,N*-dimethylacetamide, or *N*-methylpyrrolidone.

The structure of **3** was confirmed by ^1H and ^{13}C NMR studies, high-resolution mass spectrometry (HRMS ESI), and IR spectroscopy. The assigned stereochemistry of **3** was based on the general features of S_N2-type reactions, which led to Walden inversion of substituents at 33-C. The strong characteristic band at 2153 cm^{-1} for the SCN group was observed in the IR spectrum of **3**.

In ^1H and ^{13}C NMR spectra of **3** the sizeable low field shifts of 33-H signal ($\Delta\delta_H \sim 3.5$ ppm) and 33-C ($\Delta\delta_C \sim 37$ ppm) were noted in comparison with corresponding signals of **2**. Additionally, the quaternary signal at 111.26 ppm in ^{13}C NMR spectra and JMOD-HQ experiment have proved the structure of (33*S*)-33-deoxy-33-thiocyanatooligomycin A (**3**). The NMR spectra (Table 1) were elucidated using 2D $^1H^1H$-COSY, $^1H^1H$-NOESY and 2D inverse hetero $^1H^{13}C$-HMQC and $^1H^{13}C$-HMBC correlations (Figures S1-S9).

Actinobacteria of the *Streptomyces* genus, a cause of actinomycosis, has been found to be more sensitive to **1** than other bacteria.[9] The *Streptomyces fradiae* strain highly sensitive to **1 (**MIC <0.001 nmol/ml, <0.0005 nmol/disc) has been validated by us as a useful test system for screening the derivatives of **1**.[8] To estimate the activity of the novel semi-synthetic derivative **3**, we have tested its potency against *S. fradiae* in comparison with **1.** The screening has shown that although **3** has strongly inhibited the growth of the test culture at subnanomolar concentrations (Table 2), it was noticeably less potent than **1**. This result has correlated with crystallographic data for a drug-enzyme complex that showed an important role of the hydroxyl group in the propanol side chain of oligomycin A **1** for binding to subunit *c* of the F_0F_1ATP synthase.[4]

Conclusions

We have developed a method for a point modification of the complex molecule of oligomycin A at the position 33 of the side chain. Although (33*S*)-33-deoxy-33-thiocyanatooligomycin A **3** obtained by this method was less potent against *S. fradiae* and *S. albus* than oligomycin A, **3** can be useful for investigation of the action mechanism of oligomycins and functioning of ATP synthase in eukaryotic or microbial cells. Further development of the routes to transform 33-*O*-mesyl-oligomycin A (**2**) potentially useful for diversification of oligomycin A is in progress.

Acknowledgements. This study was supported by grants of the Russian Science Foundation (agreement № 15-15-00141). The authors thank A. N. Maliutina (Gause Institute of New Antibiotics) for IR, UV and HPLS analysis.

Table 2. Comparative *in vitro* activity of oligomycin A and (33*S*)-33-thiocyanate derivative against *S. fradiae* ATCC-19609 and *S. albus* ATCC-21132.

Compound		Oligomycin A (**1**)		33-Deoxy-33-thiocyanatooligomycin (**3**)	
Concentration, nmol/disc[a)]		0.001	0.01	0.01	0.1
Halo diameter, mm	*S. fradiae* ATCC-19609	10.0±0.3 [b)]	19.0±0.5	9.0±0.5	12.0±0.5
	S. albus ATCC-21132	–	10.5±0.5	–	7.5±0.5

[a)]disc diameter 7 mm;
[b)]± S.D. of three independent measurements.

References

1. Antoniel M., Giorgio V., Fogolari F., Glick G.D., Bernardi P., Lippe G. *Int. J. Mol. Sci.* **2014**, *15(5)*, 7513–7536.
2. Pagliarani A., Nesci S., Ventrella V. *Mitochondrion* **2013**, *13*, 312–319.
3. Lysenkova L.N., Turchin K.F., Korolev A.M., Danilenko V.N., Bekker O.B., Dezhenkova L.G., Shtil A.A., Preobrazhenskaya M.N. *J. Antibiot*. **2014**, *67*, 153–158.
4. Symersky J., Osowski D., Walters D.E., Mueller D.M. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 13961–13965.
5. Salomon A.R., Voehringer D.W., Herzenberg L.A., Khosla C. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 14766–14771.
6. Sladojevich F., Arlow S., Tang P., Ritter T. *J. Am. Chem. Soc.* **2013**, *135*, 2470–2473.
7. Lysenkova L.N., Turchin K.F., Korolev A.M., Dezhenkova L.G., Bekker O.B., Shtil A.A., Danilenko V.N., Preobrazhenskaya M.N. *Bioorg. Med. Chem.* **2013**, *21*, 2918–2924.
8. Alekseeva M.G., Elizarov S.M., Bekker O.B., Lubimova I.K., Danilenko V.N. *Biochem. (Moscow) Suppl. A Membr. Cell. Biol.* **2009**, *3*, 16–23.
9. Hensel M., Achmus H., Deckers-Hebestreit G., Altendorf K. *Biochem. Biophys. Acta* **1996**, *1274*, 101–108.

Received 16.10.2015
Accepted 14.11.2015