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**SYNTHESIS LASALOCID ESTER WITH PENTADECAFLUORO-1-
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Abstract. One of the most important problems of contemporary agricultural chemistry is the search for new types of compounds showing biological activity and determination of the interactions of such compounds with metal ions in order to establish a correlation between the complex structure and its biological activity. Ionophores are hydrophobic molecules that have the ability to transport ions. Most of ionophores shows bactericidal effects and they are called ionophoretic antibiotics. One of the better-known carboxylic ionophore antibiotics is lasalocid. Lasalocid was prepared as a 1:1 complex of lasalocid with ethanol from the lasalocid sodium salt. A new ester of lasalocid with pentadecafluoro-1-octanol (LasF) was synthesized and its ability to complex formation with monovalent cations was studied. The reaction was carried out at room temperature

in the presence of N, N-dicyclohexylcarbodiimide (DCC), which is a catalyst in the esterification reaction of aromatic carboxylic acids. Scheme of the ester synthesis between lasalocid and 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-1-octanol is given. The purity of the obtained LasF ester was confirmed by thin layer liquid chromatography (TLC), high performance liquid chromatography (HPLC) and NMR spectra. Purification gave LasF ester 77 % yield (3.88 g). The calculations were carried out on the creation of complexes of LasF with Li⁺, Na⁺ and K⁺ cations by using semi-empirical calculations of the maximum surface packing density, Heat of Formation (HOF) and the geometric optimization were made using the WinMopac 2002 program.

Keywords: antibiotics, lasalocid, esterification, lasalocid ester, thin layer liquid chromatography, high performance liquid chromatography, quantum-mechanical calculations

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ЛАЗАЛОЦИДТІҢ ПЕНТАДЕКАФТОР-1-ОКТАНОЛМЕН ЭФИРІН (LasF) СИНТЕЗДЕУ ЖӘНЕ ОНЫҢ БІР ВАЛЕНТТІ КАТИОНДАРМЕН КОМПЛЕКСТЕРІН ЖАРТЫЛАЙ ЭМПИРИКАЛЫҚ ЗЕРТТЕУ

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Аннотация. Қазіргі ауылшаруашылық химиясының маңызды мәселелерінің бірі биологиялық белсенділік көрсететін қосылыстардың жаңа түрлерін іздестіру және күрделі құрылым мен оның биологиялық белсенділігі арасындағы

корреляцияны анықтау үшін осы қосылыстардың металл иондарымен әрекеттесуін анықтау болып табылады. Ионофорлар - иондарды тасымалдауға қабілетті гидрофобты молекулалар. Ионофорлардың көпшілігі бактерицидтік әсерге ие және ионтофоретикалық антибиотиктер деп аталады. Анағұрлым белгілі карбоксилді ионофор антибиотиктерінің бірі —лазалоцид. Лазалоцидті, оның натрийлі тұзынан этанолмен 1:1 қатынасында лазалоцид кешені түрінде алынды. Лазалоцидтің пентадекафтор-1-октанолмен (LasF) жаңа эфирі синтезделді және оның бір валентті катиондармен комплекс түзу қабілеті зерттелді. Реакция бөлме температурасында N,N—дициклогексил карбодиимидтің (DCC) қатысуымен жүргізілді, ол ароматты карбон қышқылдарының этерификация реакциясының катализаторы болып табылады. Лазалоцидтің 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-пентадекафтор-1-октанолмен күрделі эфирін синтездеу схемасы келтірілген. Алынған LasF эфирінің тазалығы жұқа қабат сұйық хроматографиясы (ЖҚСХ), тиімділігі жоғары сұйықтық хроматографиясы (ТЖСХ) және ЯМР спектрлері арқылы расталды. Тазарту нәтижесінде LasF эфирін 77 % (3,88 г) шығыммен алынды. Li⁺, Na⁺ және K⁺ катиондары бар LasF кешендерін құру бойынша есептеулер WinMорас 2002 бағдарламасының көмегімен беттік қаптаманың максималды тығыздығының, түзілу жылуының (HOF) және геометриялық оңтайландырудың жартылай эмпирикалық есептеулері арқылы жүргізілді.

Түйін сөздер: антибиотиктер, лазалоцид, этерификация, лазалоцид эфирі, жұқа қабатты сұйықтық хроматографиясы, тиімділігі жоғары сұйықтық хроматографиясы, кванттық механикалық есептеулер

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СИНТЕЗ ЭФИРА ЛАЗАЛОЦИДА С ПЕНТАДЕКАФТОР-1-ОКТАНОЛОМ (LasF) И ПОЛУЭМПИРИЧЕСКОЕ ИССЛЕДОВАНИЕ ЕГО КОМПЛЕКСОВ С ОДНОВАЛЕНТНЫМИ КАТИОНАМИ

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Аннотация. Одной из важнейших задач современной агрохимии является поиск новых типов соединений, проявляющих биологическую активность, и определение взаимодействия таких соединений с ионами металлов с целью установления корреляции между строением комплекса и его биологической активностью. Ионофоры представляют собой гидрофобные молекулы, способные переносить ионы. Большинство ионофоров проявляют бактерицидное действие и их называют ионофоретическими антибиотиками. Один из наиболее известных карбоновых ионофорных антибиотиков — это ласалоцид. Лазалоцид получили из натриевой соли лазалоцида с этанолом в соотношении 1:1 в виде комплекса лазалоцида. Синтезирован новый эфир лазалоцида с пентадекафтор-1-октанолом (LasF) и изучена его способность к комплексообразованию с одновалентными катионами. Реакцию проводили при комнатной температуре в присутствии N,N-дициклогексилкарбодиимида (ДЦК), который является катализатором реакции этерификации ароматических карбоновых кислот. Приведена схема синтеза сложного эфира лазалоцида с 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-пентадекафтор-1-октанолом. Чистота полученного эфира LasF подтверждена тонкослойной жидкостной хроматографией (ТСХ), высокоэффективной жидкостной хроматографией (ВЭЖХ) и спектрами ЯМР. Очистка дала сложный эфир LasF с выходом 77 % (3,88 г). Проведены расчеты по созданию комплексов LasF с катионами Li^+ , Na^+ и K^+ с использованием полуэмпирических расчетов максимальной поверхностной плотности упаковки, теплоты образования (HOF) и геометрической оптимизации по программе WinMорас 2002.

Ключевые слова: антибиотики, лазалоцид, этерификация, эфир лазалоцида, тонкослойная жидкостная хроматография, высокоэффективная жидкостная хроматография, квантово-механические расчеты

Introduction

Discovery of natural macrocyclic antibiotics, capable of complexation of ions and neutral molecules and permitting transport of alkali metal ions through semi-permeable membranes has directed much interest into the linear and cyclic systems containing heteroatoms with lone electron pair. The formation and activity of supramolecular species is determined by the intermolecular interactions in the systems with matching size, shape and stereoelectronic properties. The possibility of guesthost complexes formation is a result of an excellent complementarity of the receptor and substrate molecules. Determination of the nature of interactions of biologically active agents with the environment requires a comprehensive study by a number of methods.

Antibiotics-ionophores are widely used as compounds that used in the investigation of ion transport processes through membranes.

Ionophores are hydrophobic molecules that have the ability to transport ions.

Transport takes place from the hydrophilic layer to the hydrophobic layer. These compounds have also the ability to transport ions through the lipid cell membrane. Ionophores are acceptor molecules of the host, which operate on the basis of the type of guest — host, coordinating Ca^{2+} , Na^{+} or K^{+} . The formation of complexes of this type is possible through the specific construction of ionophores (Moore et al., 1964).

Most of ionophores shows bactericidal effects and they are called ionophoretic antibiotics (Pressman et al., 1967). Ionophores, such as monensin, lasalocid, salinomycin and narasin are antimicrobial compounds, which are used as an additive to animal feed (Schroeder et al., 2005). They increase growth by changing the bacterial flora in the intestines and stomach in most animal species. This results in improved metabolism, digestion and absorption of essential nutrients including carbohydrates, proteins, amino acids, minerals and vitamins, thanks to these animals need less nutrition suitable feed (Burgermeister et al., 1977). Ionophores transport ions across cell membranes, which compensates gradients, ion balance is disturbed, sodium potassium, which is an increase of osmotic pressure in the cell and the cell dies. However, not all bacteria are sensitive to ionophores, e.g., several species. *Prevotella ruminicola*, *Streptococcus bovis aminophilum Clostridium*, *Streptococcus ruminantium* and *Prevotella bryantii* has a pronounced resistance to ionophores (Schroeder et al., 2005).

Ionophores, due to its antibiotic properties are effective as drugs against coccidiosis. This is a parasitic disease-causing diarrhea, weight loss and sometimes death due to inflammation of the small intestine. Intensive livestock promotes the conditions in which the parasite *Coccidia* can achieve such a high level that there causing clinical symptoms in animals. Consequently, the animals must be continuously dosed ionophores. This prevents the formation and spread of disease in the herd (Burgermeister et al., 1977).

Ionophores have been approved in mid-1970 as feed additives for livestock, and since then their use has become routine for feeding ruminants (Hilgenfeld et al., 1982).

Thereby, studying methods for obtaining a new class of ionophores is one of the most important problems of modern agricultural chemistry. There appears the importance of searching for new types of compounds demonstrating biological activity and determining the interactions of these compounds with metallic ions in order to establish the connection between complex structures and their biological activity.

One of the better-known carboxylic ionophore antibiotics is lasalocid. Despite the many desirable properties, characterized by high toxicity. Receiving lasalocid acid derivatives, can lead to a reduction of its harmfulness, and thus increase its use (Pankiewicz et al., 2006; 2001; 2007; 2005).

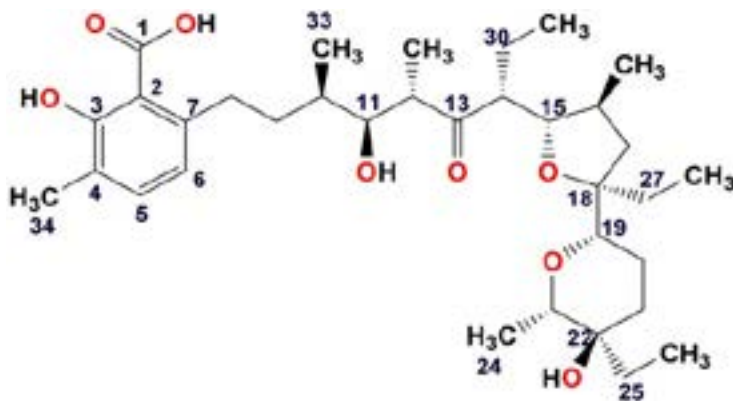


Fig. 1. Lasalocid acid

Lasalocid acid (Fig. 1) for the first time was isolated from *Streptomyces lasaliensis* in 1951 by Berger et al. (Berger et al., 1951). From among all homologues of lasalocid acid, lasalocid A, the main product of biosynthesis, shows the greatest antibacterial activity.

In previous studies, we studied the synthesis of a new lasalocid ester of cinnamyl acid and the results of a spectroscopic and semi-empirical study of its ability to form complexes with certain monovalent cations (Tukibayeva et al., 2016).

The aim of this work is investigation of lasalocid ester with pentadecafluoro-1-octanol (LasF) synthesis and its complexes with monovalent cations by spectroscopic, semiempirical methods.

Novelty of obtained results:

– lasalocid ester with 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-1-octanol (LASF) was synthesized for the first time and the purity of the ester obtained was confirmed by thin-layer liquid chromatography, high-performance liquid chromatography, NMR and IR Fourier spectra.

Methods and materials

Preparation of Lasalocid acid ester with 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-1-octanol. The ester synthesis was as follows: 3.06 g (5.17×10^{-3} mol) of lasalocid acid were weighed into a 250 ml round bottom flask and dissolved in 100 ml of methylene chloride. The flask and the stirrer were placed on a magnetic stirrer. Then 5 % excess 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-1-octanol (2.13 g, 5.33×10^{-3} mol). After complete substrate mixing, a sample was taken and applied to a TLC plate. In a further step, 10 % excess N, N-dicyclohexylcarbodiimide (1.15 g, 5.58×10^{-3} mol) was dissolved and stirring in the solution. After approximately 30 minutes, all the substrates were dissolved and the sample was taken and applied to a TLC plate. A 500 mL beaker was used as a chromatographic chamber to which a mobile phase was poured in a suitable ratio (acetone: methylene chloride) and a plate was placed on which the samples were previously applied. After a few attempts, a suitable development phase of 1: 5 was chosen, acetone: methylene chloride.

Column preparation. The dry and clean column was placed vertically on a tripod. At the outlet of the column, a beaker was needed to collect the eluent. The column was then washed several times with methylene chloride. 0.5 cm of methylene chloride at the bottom of the column was left. In a subsequent step in a suitable size beaker, a silica gel slurry in methylene chloride was prepared so that it contained no air bubbles. Then the slurry in the beaker was stirred and decisive, but it was not poured very quickly into the walls of the column to minimize the formation of air bubbles. After filling the column with silica gel, the tap of the column was gently opened and the Pasteur glass pipette was washed over the column walls of the silica gel residue that was deposited during the slurry pouring.

After complete separation of the liquid and solid parts, the solvent was poured until the eluent was about 1 cm above the surface of the carrier. In the meantime, it was considered that the level of liquid did not fall below the surface of silica gel, ie there was no “drying out of the column”.

Results and discussion

The previously obtained lasalocid acid was used for the synthesis of lasalocid esters. The reaction was carried out at room temperature in the presence of N, N-dicyclohexylcarbodiimide (DCC), which is a catalyst in the esterification reaction of aromatic carboxylic acids. DCC binds with water, and the resulting dicyclohexylurea (DCU) breaks down as a white precipitate from the reaction (Fig.2.) (Kabyzbekova et al. 2017).

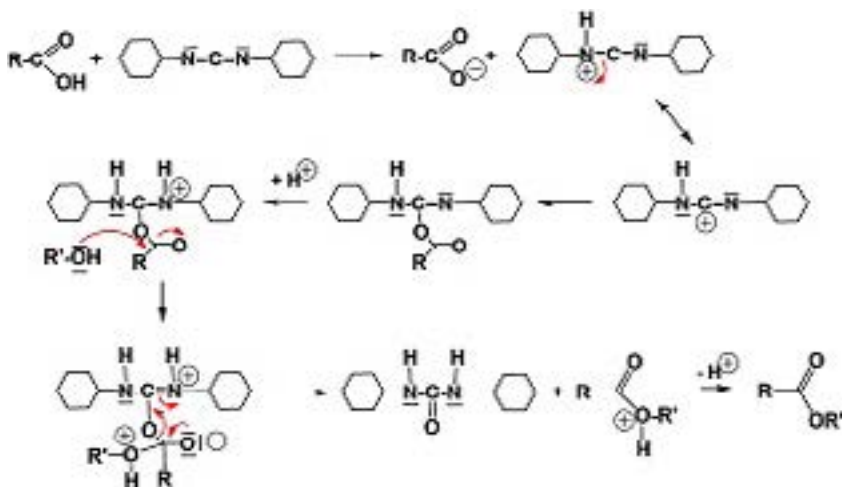


Fig. 2. Exemplary esterification mechanism with DCC

Prior to each esterification reaction using greater amounts of lasalocid acid and alcohol, pilot reactions were carried out on smaller amounts of reagents to optimize optimum reaction conditions and increase process efficiency.

Lasalocid acid ester with 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-1-octanol (Fig.3.):



Fig. 3. Scheme of the ester synthesis between lasalocid ester and 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-1-octanol

After the plate was expanded, the reaction was not complete, the unreacted lasalocid acid was visible in the mixture, so the reaction was continued for 2 hours by TLC thin layer chromatography. N, N-dicyclohexylurea (DCU) white precipitate was precipitated in the solution.

After a further TLC analysis, no presence in the lasalocid acid solution was found, indicating the complete reactivity of the substrates and the end of the reaction. Therefore, then a white DCU precipitate was filtered off on an ordinary funnel. The clear solution of methylene chloride was evaporated on a vacuum evaporator until yellow oil was obtained. In the next step, the oil was re-dissolved in methylene chloride. Then the DCU precipitates again on the surface of the solution. Once again, the amount of white precipitate was filtered off and the clear solution evaporated to a thick yellow oil. This action was repeated twice more. A diaphragm pump was used to get rid of the solvent residue. As a result, 5.21 g of lasalocid acid ester were obtained from 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-1-octanol (LasF). Then, to remove any impurities visible on the TLC plates, the ester was purified by column chromatography. Purification gave LasF ester 77 % yield (3.88 g). Potentially it is possible that 1.33 g were contaminants, probably the remains of alcohol and DCU. Making another TLC plate after cleaning, confirmed a higher purity of the ester obtained.

A pilot reaction was performed prior to the main ester synthesis reaction. The synthesis process was analogous to the one described above. 0.117 g (3.0×10^{-4} mol) of lasalocid acid was used in the reaction, which was dissolved in 50 ml of methylene chloride in a 100 ml round bottom flask. Then 5 % excess 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-1-octanol (0.126 g, 3.15×10^{-4} mol) and 10 % excess DCC (0.068g, 3.3×10^{-4} mol). Synthesis was also controlled by thin-layer TLC liquid chromatography. As a result, 0.344 g of the ester was obtained before purification, and purification of 0.205 g of ester in 60 % yield.

By comparing the yields of the two syntheses, it can be concluded that the pilot reaction has potentially contributed to increasing the efficiency of the main reaction by selecting more optimal reaction conditions and reducing product losses.

The purity of the obtained LasF ester was confirmed by thin layer liquid chromatography (TLC), high performance liquid chromatography (HPLC) and NMR spectra.

Ester purification using column chromatography. To purge visible impurities, DCU residue and alcohol in the obtained esters, purification was carried out on a chromatography column.

Ester introduction to the column. After the column was prepared, the lasalocid acid ester was dissolved in a small amount of methylene chloride. Then very carefully using a Pasteur glass pipette a solution of methylene chloride ester in silica gel was added. When the level of the introduced solution was equal to the surface of the silica gel, methylene chloride was added dropwise. Eluent was introduced very slowly, so as not to “agitate” the silica gel layer. When the test substance was completely absorbed in the top of the column, a column of about 100 cm³ of methylene chloride was charged and the separation of the constituents of the ester solution in methylene chloride was started.

The flowing mobile phase was collected in 50 cm³ conical flasks. During the separation, constant flow of the eluent was maintained by the silica gel, primarily by maintaining a constant level of mobile phase over the column filling. When the flasks were filled to 25 cc, TLC thin-layer liquid chromatography was tested on a regular basis. Due to the high dilution, each sample was applied several times (about 10 times) to one place of the TLC plate, preventing enlargement and spotting. Then one of the spots was checked under the UV lamp.

Column separation. The column separation for each of the esters looked quite different due to the different polarity of the alcohols used for synthesis and visible impurities.

In the case of lasalocid acid ester 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-1-octanol, the separation on the chromatographic column was easier and lasted much shorter than the 2-pyridinepropanol ester. During the separation of the solution from LasF, methylene chloride itself was used as the first mobile phase. Then, after collecting 15 samples, the eluent polarity was increased by adding acetone and methylene chloride in a ratio of 0.5:10 as the mobile phase. In the next step, 20 fractions were collected and the phase polarity was increased, increasing the amount of acetone (1:10 ratio). This increase in polarity caused a wash out of the column of impurities that were examined by developing them on the plates. TLC plates were again found lacking ester in previous samples, so that the eluent polarity was further increased by adding to the mobile phase acetone ratio (2:10). This additive of acetone has washed away the pure lasalocid acid ester. The purity of the ester was confirmed by TLC thin layer chromatography and HPLC liquid chromatography. In the next step, the fractions containing the pure ester were evaporated by evaporator, then the solvent residues were removed by means of a diaphragm pump. As a result, a purified lasalocid acid derivative was prepared from 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-1-octanol (Fig.4.). The split time is 8 hours.

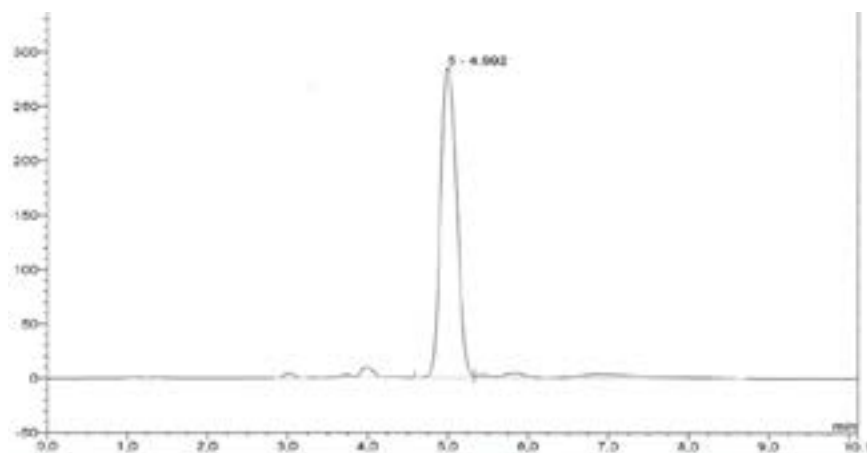


Fig. 4. Chromatogram of lasalocid ester with pentadecafluoro-1-octanol (LasF) after purification

PM6 semiempirical calculation. Theoretical calculations were carried out on the creation of complexes of LasF with Li^+ , Na^+ and K^+ cations (Kabylbekova, 2017). Table 1 shows the heat of formation (HOF) LasF complexes with cations of lithium, sodium and potassium.

Table 1. Heat of formation (HOF, kJ/mol) of LasF and its complexes with Li^+ , Na^+ , K^+ cations calculated by PM6 method

Compound	HOF (kJ/mol)	ΔHOF
LasF	-4651.20	-
LasF: Li^+ (complexed)	-4389.19	-
LasF Li^+ (uncomplexed)	-4036.06	353.13
LasF: Na^+ (complexed)	-4524.12	-
LasF Na^+ (uncomplexed)	-4106.12	418.00
LasF: K^+ (complexed)	-4428.89	-
LasF K^+ (uncomplexed)	-4194.83	234.06
	$\Delta\text{HOF} = \text{HOF}_{(\text{complexed})} - \text{HOF}_{(\text{uncomplexed})}$	

Calculations were made using semi-empirical calculations of the maximum surface packing density, Heat of Formation (HOF) and the geometric optimization were made using the WinMopac 2002 program (Fig.5.).

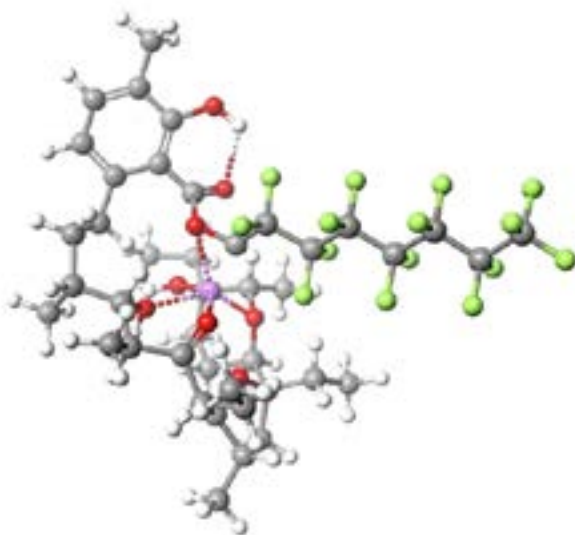


Fig. 5. Calculated structure of LasF with Li^+ cation

Conclusion

The synthesis of lasalocid acid ester with 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-1-octanol were researched for the first time. The reaction was carried out at room temperature in the presence of N, N-dicyclohexyl carbodiimide (DCC), which is a catalyst in the esterification reaction of aromatic carboxylic acids. In order to remove any impurities visible on the TLC plates, the ester was purified by column chromatography. Purification gave LasF ester 77 % yield (3.88 g). The purity of the obtained LasF ester was confirmed by thin layer liquid chromatography (TLC), high performance liquid chromatography (HPLC) and NMR spectra. The calculations were carried out on the creation of complexes of LasF with Li^+ , Na^+ and K^+ cations by using PM6 method.

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