Synthesis of 1-functionalized pyrenes from 1-lithiopyrene, and their application as fluorescent probes for the components of the *Ginkgo biloba L.* leaves extract

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Pyrene-1-carbaldehyde, pyrene-1-carboxylic acid, and 1-aminomethylpyrene were obtained from 1-lithiopyrene generated *in situ* from 1-bromopyrene. 1-Aminomethylpyrene was used for the fluorescent detection of terpene trilactones, components of the *Ginkgo biloba L.* leaves extract (ginkgolides and bilobalide).

Key words: pyrene derivatives, organolithium compounds, ginkgolides, trilactones, fluorescent detection.

Pyrene and its derivatives are of considerable interest for analytical and preparative organic chemistry due to their luminescent properties.[1,2] In particular, they are used as photoluminescence sensors for various analytes[3—5] and as photoluminescence labels (probes).[6—8] In this connection, a search for convenient methods for the preparation of pyrene derivatives with different reactive functional groups is an actual problem.

Initial introduction of carboxy or aldehyde group is of principal interest for the efficient functionalization of pyrene core. In particular, several methods for preparation of pyrene-1-carbaldehyde are described in the literature, such as a reaction of pyrene with the corresponding synthons in the presence of Lewis acids, for example, with dichloromethoxymethane in the presence of titanium tetrchloride,[9,10] with *N*-formyl-*N*-methylaniline[11,12] or DMF[13—15] and phosphorus oxychloride. There is described a synthesis of pyrene-1-carbaldehyde by oxidation of 1-methylpyrene using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[16] and by modification of 1-acetylpyrene.[17] Preparation of pyrene-1-carboxylic acid is possible through the oxidation of pyrene-1-carbaldehyde[18,19] or 1-acetylpyrene.[20,21] There are also syntheses which use $Et_3SiB(C_6F_5)_4$[22] and nitrile hydrolysis.[23] These methods are frequently limited to the availability of reagents and starting compounds.

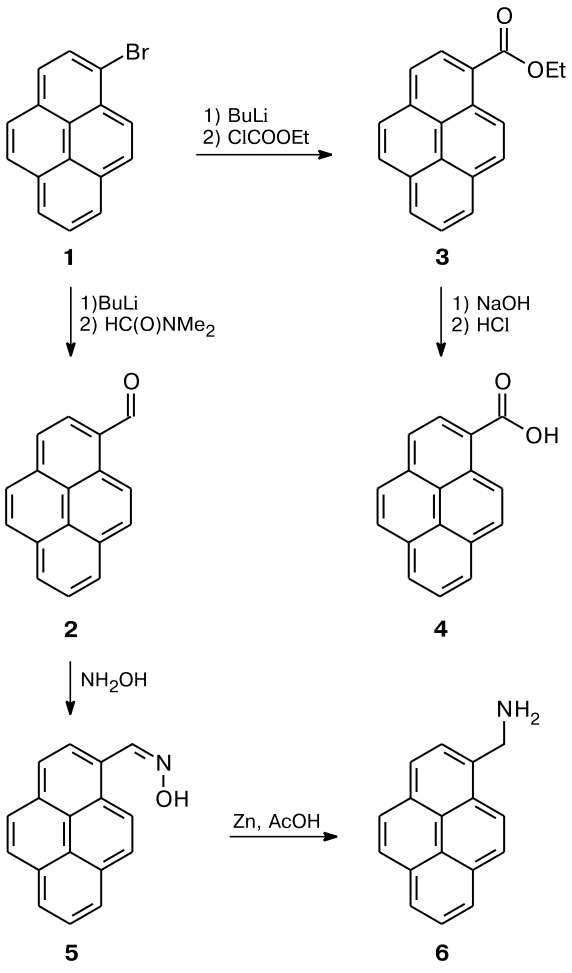
At the same time, little cases where pyrene is functionalized through lithiopyrenes generated *in situ* from bromopyrenes are known. In particular, 2-*tert*-butyl-6,8-diformylpyrene was synthesized based on the corresponding tribromopyrene (one bromine atom is lost in the course of the reaction)[24] by the reaction with Bu^tLi and then with DMF. Pyrene-1-carboxylic acid was produced by the reaction of 1-bromopyrene with phenyllithium and carbon dioxide,[25] whereas pyrene-1-carbaldehyde was obtained as a side product in the reaction of 1-bromopyrene with Bu^nLi and then with 2,5-dioctyldithieno[2,3-*b*:3′,2′-*d*]-thiophenene (on a very small scale).[26] Phenylaminocarbonylpyrene was obtained by the reaction of 1-lithiopyrene with phenyl isocyanate.[27] In turn, the starting 1-bromopyrene can be synthesized in high yield upon treatment of pyrene with *N*-bromosuccinimide.[28] To sum up, there are no literature data on the purposeful preparation of pyrene-1-carbaldehyde which uses organolithium intermediates, whereas synthetically poorly available reagents are suggested for the preparation of pyrene-1-carboxylic acid through 1-lithiopyrene.

At the same time, long ago there are known approaches to aromatic aldehydes and carboxylic acid esters by the reaction of aryllithium compounds (generated *in situ* upon treatment of bromo- or iodoarenes with alkyllithium derivatives), respectively, with DMF[29,30] and alkyl chloroformates.[31,32] However, these methods were not applied for pyrene derivatives. In the present work, we filled this gap in the synthetic organic chemistry.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 6, pp. 1312—1316, June, 2014.

1066-5285/14/6306-1312

Scheme 1



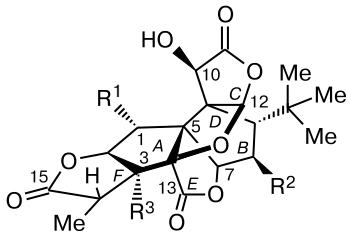
Reaction of 1-lithiopyrene (generated *in situ* from 1-bromopyrene **1**) with DMF in THF provides good yield of pyrene-1-carbaldehyde **2**. The structure of the product agrees with the ^{1}H and ^{13}C NMR spectroscopic and mass spectrometric data, as well as with elemental analysis. In particular, the ^{1}H NMR spectrum contains an indicative singlet for the aldehyde proton in the region δ 10.81.

The reaction of 1-lithiopyrene with ethyl chloroformate leads to ethyl pyrene-1-carboxylate **3**, which without isolation was subjected to the alkali hydrolysis in aqueous ethanol with the quantitative formation of pyrene-1-carboxylic acid **4**. The structure of **4** was confirmed by ^{1}H NMR spectroscopy and mass spectrometry. In particular, the ^{1}H NMR spectrum contains an indicative broad singlet for the proton of the carboxy group in the region δ 13.03.

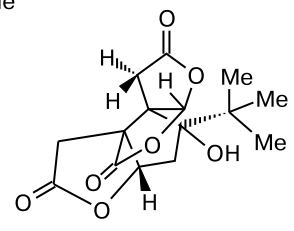
The reaction of aldehyde **2** with hydroxylamine in ethanol under argon at 50—55 °C leads to oxime **5** in good yield. Our studies showed that carrying out this reaction in air, as well as at elevated temperature leads to a considerable decrease in the yield of **5** because of resinification of the reaction mixture. The structure of the product agrees with the ^{1}H NMR spectroscopic and mass spectrometric data, as well as with elemental analysis. In particular, the ^{1}H NMR spectrum contains indicative singlets for the methine proton CH (δ 7.78) and the proton of the N—OH group (δ 9.14).

In order to synthesize the target 1-aminomethylpyrene **6**, we studied several approaches. For example, the application of $NaBH_4$ in ethanol successfully used for the reduction of oximes and imines[33] did not lead to compound **6** even upon prolonged reflux. A procedure described earlier[34] for the reduction of oxime **5** with zinc dust in acetic acid appeared to be the most successful. This procedure was modified in order to achieve the optimal yields of 1-aminomethylpyrene **6**: treatment of the filtered reaction mixture with ammonia led to the formation of a precipitate of pure product **6** as a hydroacetate, which was free of inorganic impurities. The structure of the product agrees with the ^{1}H NMR spectroscopic and mass spectrometric data, as well as with elemental analysis. In particular, the ^{1}H NMR spectrum contains an indicative singlet for the protons of the acetate anion in the region δ 1.86 and a signal for the protons of the methylene group (δ 4.51).

The target 1-aminomethylpyrene **6** obtained as described above is a commonly used photoluminescence label for derivatization of biological objects.[35,36] In the present work, we supposed to use amine **6** for the photoluminescent detection of components in the *Ginkgo biloba L* leaves extract, *viz.*, terpene trilactones. To achieve this, we planned to carry out the reaction of amine **6** with terpene trilactones, one of two active groups of compounds, of the ginkgo biloba leaves extract (ginkgolides **7A—C,J,M** and bilobalide **8**). Nowadays, this extract is one of the best selling nootropic agents of plant origin.[37] A standardized leaves extract possesses antioxidant, antiinflammatory, and antiprolipherative activity.[38] Agents on its basis are used in the therapy of Alzheimer disease[39] and cognitive disorders.[40]



7A—C,J,M



8

Ginkgolide **7**	R^1	R^2	R^3
A	H	H	OH
B	OH	H	OH
C	OH	OH	OH
J	H	OH	OH
M	OH	OH	H

Terpene trilactones (TTL) are specific compounds for *Ginkgo biloba L.* There is description of four major ginkgolides (**A**, **B**, **C**, and **J**), minor ginkgolide **M**, and bilobalide **8**. The structure of the TTL molecule differs from most pharmaceutical compounds: they are sterically hindered, bent as a rosette compounds (cage molecules, Fig. 1, method mp2, the ChemOffice program), whose reactivity is low. Thus, these compound withstand reflux in a dilute solution of hydrogen peroxide.[41] This is the reason why quantitative detection of these compounds is very difficult problem, and development of convenient method for its solution is extremely actual.

When derivatization of TTL **8** with 1-aminomethylpyrene **6** was carried out upon prolonged heating in anhydrous *o*-xylene or dioxane in the presence of *p*-toluenesulfonic acid or cationite Dowex-50 in the H-form as a catalyst, no any signs of reaction between reactants were observed. When an aliquot of the reaction mixture (heated for 6 h) was injected directly into the mass spectrometer, the mass spectrum exhibited only peaks of molecular ions of the starting ginkgolides and bilobalide **8**. Assuming that water can be involved in this reaction, we carried out the derivatization in 1 *M* solution of hydrochloric acid. During prolonged heating of one mole of TTL **8** with 2.5 moles of 1-aminomethylpyrene **6** (calculated on the average molar weight), we took aliquots of the reaction mixture 5, 11, and 14 h after beginning of the heating (aliquots *1*, *2*, and *3*, respectively) and observed a growth of the peaks belonging to the derivatization products in aliquots *1* and *2*, with the content of the starting ginkgolides in the probes being stable enough. Analysis of aliquot *3* showed that the intensities of the peaks of derivatization products decreased against the presence of peaks of the starting TTL **8**. Based of the data obtained, we draw a conclusion that an equilibrium exists between the derivatization products and the starting reagents (the products of which were not found) in the course of the reaction of 1-aminomethylpyrene with TTL. A detailed analysis of the mass spectra of aliquots *1* and *2* can give a rough evaluation of the depth of the reaction of TTL with aminomethylpyrene (80%). Note the easiness of the reaction of ginkgolide **A** with 1-aminomethylpyrene as compared to the other ginkgo TTL, that is indicated by the higher intensity of the signals of derivatization products and the appearance of these signals at the beginning of the reaction already in the first aliquot.

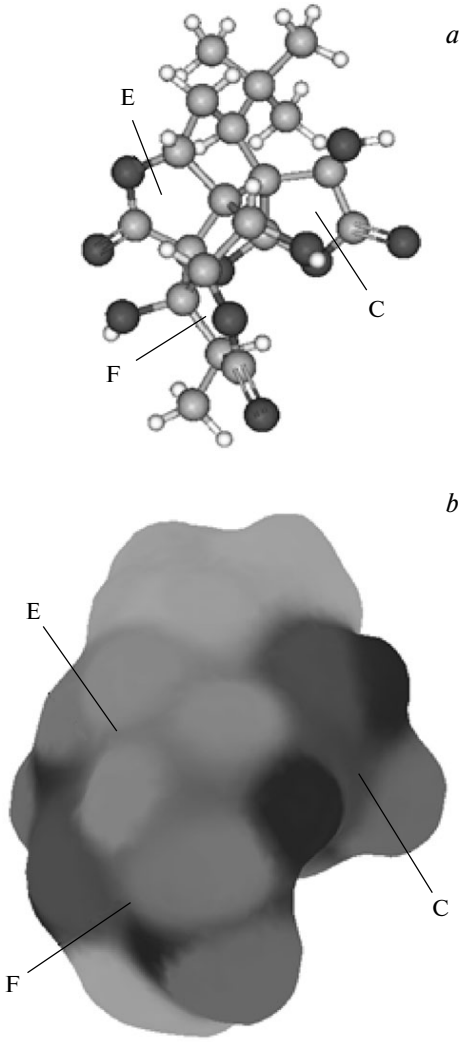
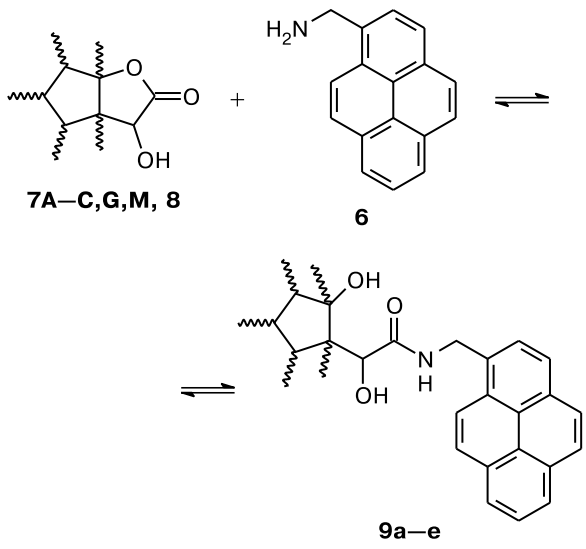


Fig. 1. The structure of ginkgolide **B** (*a*) and the model of the charge distribution over the molecule (*b*). The letters indicate the corresponding rings. Ring F is under the image, ring C is in the center to the right.

To sum up, by now our efforts to reach acceptable depth of the derivatization reaction of pyrene-containing fluorescent label **6** for the quantitative determination of TTL failed. Such a behavior of TTL can be explained by the steric hindrance of both the lactone ring of TTL and 1-aminomethylpyrene, as well as by the equilibrium hydrolysis of the reaction products leading to the starting compounds. Attempted shift of the equilibrium towards of derivatization products also failed. Despite that TTL contains three lactone rings, no products of the addition of several 1-aminomethylpyrene molecules to one molecule of TTL were observed (Scheme 2, the structure of TTL is given in the simplified form). When a blank experiment with TTL **7** and **8** in aqueous hydrochloric acid was carried out without aminomethylpyrene **6**, no hydrolysis products of TTL were detected, no $[M+H_2O+H]^+$ peak were observed. Apparently, the equilibrium during hydrolysis of TTL is strongly shifted to the side of the starting compounds and the hydrolysis products are present only in the quasistationary concentration and are detected only by subsequent reaction with aminomethylpyrene as an amide.

The known from the literature[42] higher reactivity of ring C in all the TTL explains the observed data by the formation of amides **9** exactly at this lactone. Due to the pyrene moiety incorporated in compounds **9**, this derivatization method can be used for the quantitative detection

Scheme 2



of trace amounts of TTL ginkgo biloba by HPLC with the fluorescence detector. The detection procedure is now under development.

In conclusion, we suggested approaches to the efficient functionalization of pyrene at position 1 with a possibility of introduction of a wide range of functional group, as well as showed a principal possibility of application of 1-aminomethylpyrene obtained in this work with the purpose of fluorescent detection of TTL ginkgo biloba.

Experimental

^{1}H NMR spectra were recorded on a Bruker Avance-400 spectrometer (400 MHz), using $SiMe_4$ as an internal standard. Melting points were measured on a Boetius heating stage. Mass spectra (electrospray or atmospheric pressure chemical ionization) (APCI) were recorded on a Bruker Daltonics series MicrOTOF-Q II instrument (Bremen, Germany). 1-Bromopyrene **1** was synthesized according to the known procedure.[28]

Pyrene-1-carbaldehyde (2). Dry 1-bromopyrene (500 mg, 1.78 mmol) was dissolved in anhydrous THF (20 mL). This solution was placed into a Schlenk flask and cooled to −78 °C under argon. A solution of BuLi in hexane (2.5 mol L^{-1}, 0.75 mL) was added to the solution, a mixture obtained was stirred at −78 °C for 5 min, followed by addition of anhydrous DMF (0.15 mL, 1.96 mmol) and stirring at room temperature for 12 h. Then water (30 mL) was added and the product was extracted with ethyl acetate (3×15 mL). The extract was dried with anhydrous sodium sulfate. The solvents were evaporated at reduced pressure. The product was purified by recrystallization (toluene). The yield was 250 mg (61%), m.p. 124—126 °C. ^{1}H NMR ($CDCl_3$), δ: 8.12 (d, 1 H, J = 8.0 Hz); 8.22—8.41 (m, 6 H); 8.47 (d, 1 H, J = 8.0 Hz); 9.45 (d, 1 H, H(2), J = 9.2 Hz); 10.81 (s, 1 H, CHO). MS (APCI), m/z (%): 231.08 $[M + H]^+$ (100).

Pyrene-1-carboxylic acid (4). Dry 1-bromopyrene (500 mg, 1.78 mmol) was dissolved in anhydrous THF (20 mL). This solution was placed into a Schlenk flask and cooled to −78 °C under argon. A solution of BuLi in hexane (2.5 mol L^{-1}, 0.75 mL) was added to the solution, a mixture obtained was stirred at −78 °C for 5 min, followed by addition of ethyl chloroformate (0.18 mL, 1.87 mmol) and stirring for 12 h at room temperature. Then, the reaction mixture was washed with aqueous NH_4Cl. The intermediate ethyl ester (**3**) was extracted with ethyl acetate (3×15 mL). The extract was dried with anhydrous sodium sulfate. The solvents were evaporated at reduced pressure. Potassium hydroxide (1.5 g, 26.7 mmol) in water (3 mL) was added to the residue ethanolic solution (50 mL) and the mixture obtained was stirred with reflux for 10 min, during which several portions of water were added until the last of them began to cause disappearance of opalescence. The solvents were evaporated at reduced pressure. Water (100 mL) and hydrochloric acid (10% aqueous, to pH = 3) were sequentially added to the residue. A precipitate formed was filtered off, washed with water, and dried. The yield was 350 mg (81%), m.p. > 250 °C. Found (%): C, 82.84; H, 4.01; $C_{17}H_{10}O_2$. Calculated (%): C, 82.91; H, 4.09. ^{1}H NMR (DMSO-d_6), δ: 8.09 (dd, 1 H, H(7), J = 7.6 Hz, J = 7.6 Hz); 8.17 (d, 1 H, J = 8.8 Hz); 8.20—8.42 (m, 5 H); 8.64 (d, 1 H, J = 8.0 Hz); 9.31 (d, 1 H, H(2), J = 9.2 Hz); 13.03 (br.s, 1 H, COOH). MS (ESI), m/z (%): 245.06 $[M - H]^-$ (100).

Pyrene-1-carbaldehyde oxime (5). Pyrene-1-carbaldehyde (250 mg, 1.09 mmol) was dissolved in ethanol (60 mL). A solution of hydroxylamine hydrochloride (360 mg, 5.45 mmol) and sodium acetate (450 mg, 5.48 mmol) in water (5 mL) was added to the solution. A mixture obtained was stirred at 55 °C under argon for 5 h. Ethanol was evaporated at reduced pressure. The residue was purified by column chromatography (silica gel, eluent chloroform, R_f = 0.7). The yield was 180 mg (67%), m.p. 191—193 °C. Found (%): C, 83.06; H, 4.44; N, 5.53. $C_{17}H_{11}NO$. Calculated (%): C, 83.25; H, 4.52; N 5.71. ^{1}H NMR ($CDCl_3$), δ: 7.78 (s, 1 H, OH); 8.00—8.08 (m, 2 H); 8.11 (d, 1 H, J = 8.8 Hz), 8.16 (m, 2 H); 8.22 (m, 2 H); 8.37 (d, 1 H, J = 7.9 Hz); 8.58 (d, 1 H, H(2), H(2), J = 9.2 Hz); 9.14 (s, 1 H, ArC<u>H</u>=NOH). MS (ESI), m/z (%): 246.09 $[M+H]^+$ (100).

1-Aminomethylpyrene hydroacetate (6·AcOH). Pyrene-1-carbaldehyde oxime (1 g, 4.08 mmol) was dissolved in glacial acetic acid (30 mL). After addition of zinc dust (3.8 g), the mixture obtained was stirred for 15 h at room temperature. A resulting solution was filtered, aqueous ammonia was added to the filtrate to pH = 9. A precipitate formed was filtered off and dried *in vacuo*. The yield was 40%, m.p. > 250 °C. Found (%): C, 78.15; H, 4.89; N, 4.52. $C_{17}H_{14}N\cdot(CH_3COO-)$. Calculated (%): C, 78.33; H, 5.08; N, 4.81. ^{1}H NMR (DMSO-d_6), δ: 1.86 (s, 3 H, Ac); 4.51 (s, 2 H, CH_2); 7.97—8.22 (m, 8 H); 8.39 (d, 1 H, H(2), J = 9.2 Hz). MS (ESI), m/z (%): 215.08 $(M+H-NH_3)^+$ (100).

Concentration of TTL from the *Ginkgo biloba L.* extract was performed according to the procedure described in the literature,[41] excluding purification by column chromatography. The content of TTL was 40%.

Functionalization of TTL with 1-aminomethylpyrene in *o*-xylene. The TTL concentrate (68 mg) was dehydrated in *o*-xylene (10 mL) using a Dean—Stark trap. 1-Aminomethylpyrene (20 mg) and the catalyst (Dowex-50 resin, 200 mg) were added to the mixture, which was refluxed for 6 h.

Functionalization of TTL with 1-aminomethylpyrene in 10% aqueous hydrochloric acid. A mixture of the TTL concentrate (68 mg), 1-aminomethylpyrene (35 mg), and 1 *M* hydrochloric acid (33 mL) was refluxed. Aliquots were taken 5, 11, and 14 h

after beginning the reflux. The maximal intensity of peaks of the TTL derivative was observed in the aliquot *2* (reflux for 11 h). MS, $[M + H]^+$, m/z (I_{rel} (%)): **9a**, 640.26 (21000), calculated for $C_{37}H_{37}NO_9$ 640.25; **9b,d**, 656.25 (3880), calculated for $C_{37}H_{37}NO_{10}$ 656.25; **9c**, 672.25 (875), calculated for $C_{37}H_{37}NO_{11}$ 672.24; **9e**, 558.22 (960), calculated for $C_{32}H_{31}NO_8$ 558.21.

This work was financially supported by the Ministry of Education and Science of the Russian Federation (State Contract 14.A18.21.0817), the Council on Grants at the President of the Russian Federation (Program of State Support for Leading Scientific Schools and Young Scientists of the Russian Federation, Grant MK-1511.2013.3), as well as by the Government of the Russian Federation (Program 211, Agreement No. 02.A03.21.0006).

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Received February 17, 2014;
in revised form May 15, 2014