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Synthesis of Neoglycolipids Based on D-Lactose

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Abstract—A synthesis was performed of amphiphilic D-lactose derivatives differing by the length and number of aliphatic chains. The compounds may be applied to the carbohydrate modification of phosphatidylcholine liposomes.

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Recently the research in the field of carbohydrate chemistry was aimed mainly on designing and preparation of artificial analogs of glycoconjugates containing natural structural and functional elements. Synthetic carbohydrate frameworks may possess properties similar to natural compounds, may simulate various biological processes or their stages, can be used in biochemical research involving isolation of lectins from natural sources, the study of their specificity, preparation of antibodies to carbohydrate determinants of desired structure, to serve as vectors of selective transport of biologically active substances into cells, and also be employed as sorbents in affine chromatography. Therewith the carbohydrate derivatives of low molecular weight can be obtained in preparative amounts in good yield by simpler procedures [1–4].

The goal of this study was the preparation of neoglycolipids **VII** and **XVI** that might be used in the composition of mixed liposomes for directional transport of pharmaceuticals and genetic material to desired target organs. The choice of the carbohydrate fragment in our study was due to the presence of a terminal galactosyl residue recognizable for membrane lectins of the cell surface specific thereto [5, 6].

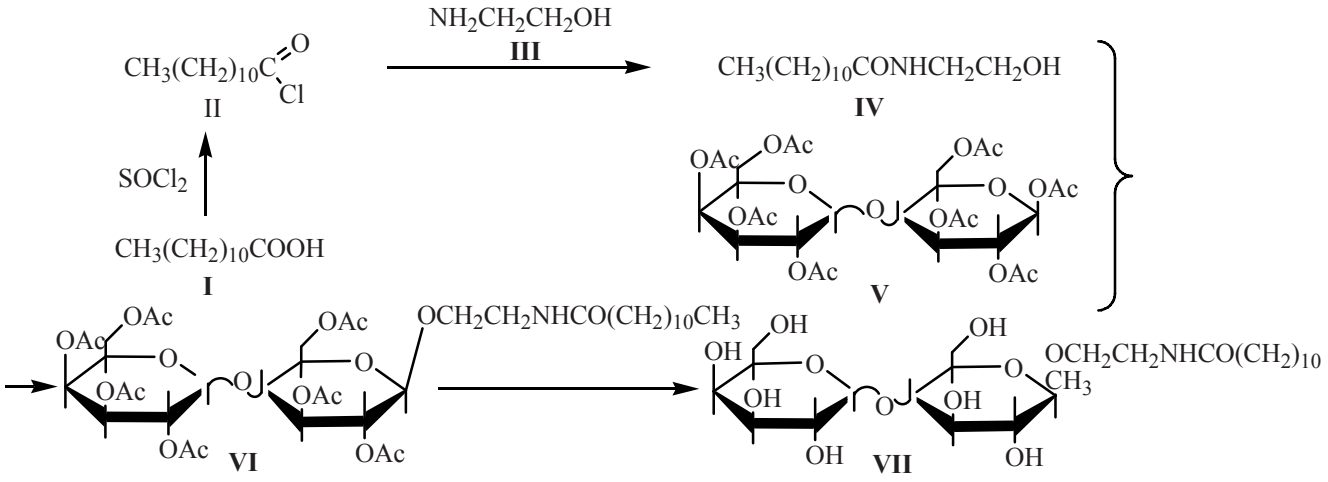
The hydrophobic component of synthetic amphiphiles is commonly composed of saturated or unsaturated aliphatic chains containing from 8 to 18 carbon atoms. The study of the effect of the chain length on the transfection efficiency in experiments both in vitro and in vivo revealed that the highest activity was inherent to compounds containing saturated hydrophobic chains 12 and 14 carbon atoms long, and to those containing oleic acid residues [7–10]. In the synthesis of neoglycolipids **VII** and **XVI** we used lauric acid (C_{12}) and tetradecanol (C_{14}).

Compound **VII** was prepared along Scheme 1. Amidoalcohol **IV** was obtained from ethanolamine (**III**) and lauroyl chloride (**II**) in the presence of triethylamine. The subsequent reaction of 2-hydroxyethyldodecylamide (**IV**) with D-lactose octaacetate (**V**) was carried out in dichloromethane for 12 h at room temperature and was catalyzed by boron trifluoride etherate [11]. On removing the protective groups by treating with 0.1M sodium methylate solution in methanol we obtained (2-dodecylamido)ethyl-β-D-lactoside (**VII**) in 94% yield.

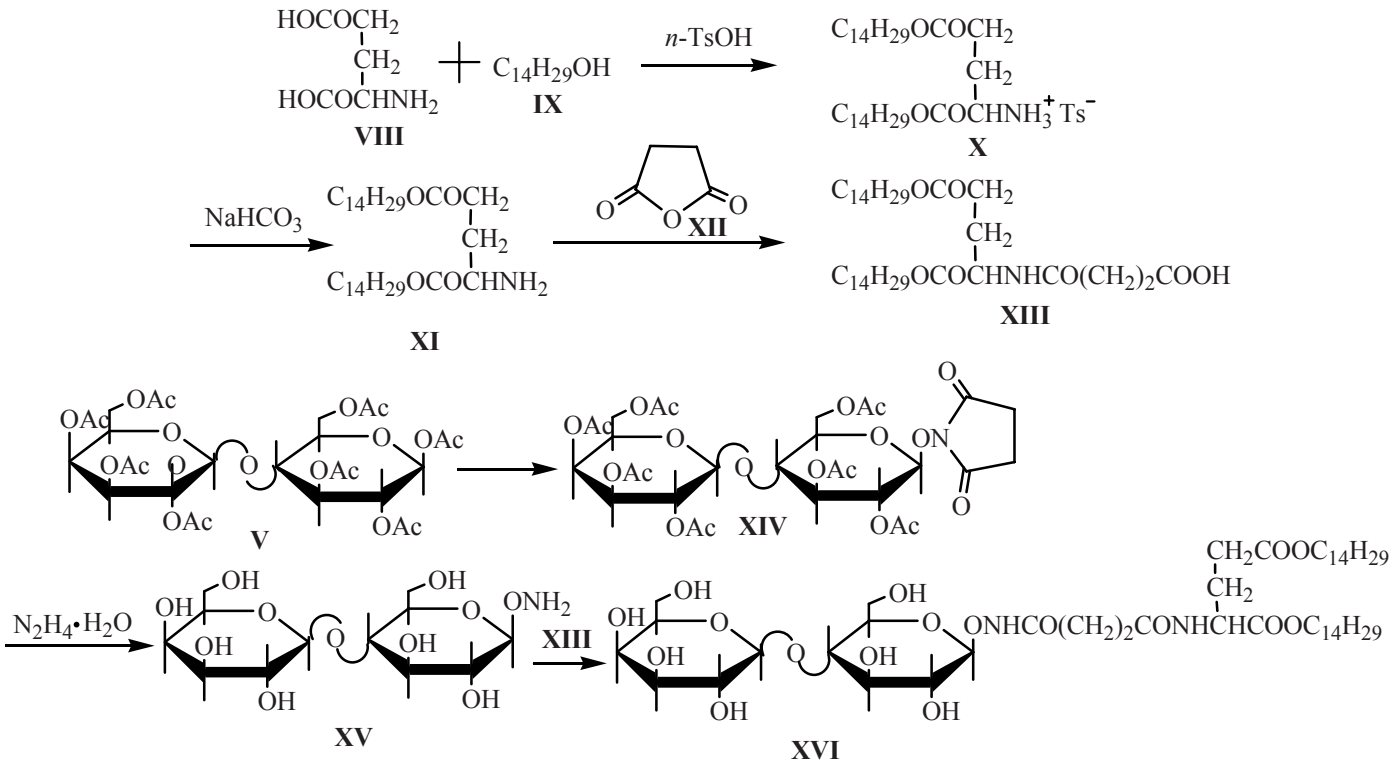
The structure of compounds **VI** and **VII** were confirmed by ^{1}H, IR, and mass spectra. In the ^{1}H NMR spectrum of compound **VI** signals appeared from the protons of acetyl and aliphatic groups, ethylene fragment, and carbohydrate skeleton. The signal of anomeric proton H^1 was observed as a doublet with the chemical shift 4.51–4.53 ppm ($J_{1,2}$ 7.4 Hz) indicating the β-configuration of the anomeric center. No signal of the α-anomer was seen in the ^{1}H NMR spectrum of compound **VI**. In the IR spectrum of glycoside **VII** the absorption bands of hydroxy groups and of amide moiety were observed. The mass spectrum of target compound **VII** contained the molecular ion peak of *m/z* 589.2 (I_{rel} 4.2%).

Glycopolyaminoacidic derivative of D-lactose **XVI** was prepared along Scheme 2. The initial hydrophobic fragment of the amphiphile **XI** was obtained by esterification of L-glutamic acid (**VIII**) with tetradecanol (**IX**) in the presence of *p*-toluenesulfonic acid followed

Scheme 1.



Scheme 2.



by the treatment of the arising salt **X** with 5% solution of sodium hydrogen carbonate [12]. The obtained chromatographically pure compound **XI** without further purification was treated with succinic anhydride in order to react it further with D-lactose derivative.

The structure of compound **XIII** possessing a free carboxy group was confirmed by ^{1}H and IR spectra. The ^{1}H NMR spectrum contained the proton signals from the aliphatic chains, L-glutamic acid residue, and also the signals of the methylene groups of the succinic acid residue as a multiplet at 3.59–3.70 ppm. In the IR spectrum of compound **XIII** absorption bands were observed belonging to the hydroxy and ester groups, and also to two amide bands.

β-Aminolactoside **XV** [13] was obtained by treating the D-lactose octaacetate (**V**) with *N*-hydroxysuccinimide

under acid catalysis followed by reacting the obtained compound **XIV** with hydrazine hydrate.

The target N-substituted amino-β-D-lactoside **XVI** was obtained by treating compound **XV** with the diester of L-glutamic acid **XIII** in DMF in the presence of dicyclohexylcarbodiimide and 4-(dimethylamino)pyridine in 50% yield [14]. The IR spectrum of compound **XVI** contained absorption bands of hydroxy and ester groups, and also bands amide **I** and **II**. The mass spectrum contained the molecular ion peak of *m/z* 976.5 (I_{rel} 2%).

The synthesized neoglycolipids **VII** and **XVI** were applied to the preparation of mixed phosphatidylcholine dispersions at the ratio phosphatidylcholine–glycoside 95:5. The modified liposomes are considerably more stable than the control phosphatidylcholine liposomes. At the storage of water dispersions of mixed liposomes for 24 h at room temperature no precipitation of aggregates was observed.

Hence we performed a synthesis of two amphiphiles based on D-lactose that could be used for preparation of carbohydrate-containing transport system and for further biochemical investigations.

EXPERIMENTAL

^{1}H NMR spectra were registered on a pulse spectrometer Bruker WM-200 at operating frequency 200 MHz from solutions in deuterochloroform. IR spectra were recorded on a spectrophotometer Shimadzu IR-435 from thin films or mulls in mineral oil. Electron-impact mass spectra were measured on a Finnigan Polaris Q instrument, ionizing chamber temperature 150°C, ionizing electrons energy 70 eV. The melting points were determined on a Boëtius heating block.

The thin-layer chromatography was performed on Sorbfil plates (Krasnodar, Russia) using the following eluent systems: chloroform–methanol, 3:1 (A), chloroform–methanol, 9:1 (B), chloroform–methanol, 12:1 (C), chloroform–ethanol, 5:1 (D), toluene–acetonitrile, 3:1 (E), toluene–ethyl acetate, 3:1 (F), toluene–ethyl acetate, 5:1 (G), acetone–petroleum ether, 1:5 (H), acetone–petroleum ether, 3:1 (I), ethyl acetate–petroleum ether, 3:1 (J), acetone–methanol, 1:1 (K).

The preparative TLC was carried out on silica gel L5/40 μm (Czechia), the column chromatography, on silica gel L40/100 μ (Czechia).

The spot visualizing at TLC was performed in iodine vapor or by heating over the flame of a spirit-lamp. The substances containing free amino groups were developed with a 5% ninhydrin solution followed by heating at 50–80°C. The substances containing *N*-hydroxysuccinimide group were visualized by a specific developer for N hydroxysuccinimides [15].

Lauroyl chloride (II). A mixture of 1 g (5 mmol) of lauric acid (**I**), 1 ml (14 mmol) of thionyl chloride, and 1 drop of DMF was heated at 88°C on an oil bath for 2 h. Excess $SOCl_2$ was distilled off in a vacuum (10 mm Hg) at the bath temperature 25°C [16]. Yield 1.1 g (99%). Compound **II** was used in further stage of the synthesis without additional purification.

***N*-(2-Hydroxyethyl)dodecylamide (IV)**. A solution of 0.22 ml (5.5 mmol) of ethanolamine and 0.61 g (6 mmol) of triethylamine in 10 ml of anhydrous dichloromethane was cooled to –10°C, 1.1 g (5 mmol) of compound **II** was added, and the mixture was kept for 30 min at room temperature. The separated precipitate was filtered off and washed with dichloromethane, and the solvent was removed in a vacuum. The reaction product was purified by column chromatography (eluent system G). Yield 0.6 g (50%), colorless crystals, R_f 0.41 (E), mp 78–79°C (CH_2Cl_2). IR spectrum (mull in mineral oil), cm^{-1}: 3300 (NH), 3250 (OH), 1640 (C=O), 1540 (NH), 1465, 1460, 1380, 710 (CH), 1050 (C–N).

***N*-{2-[4-O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-β-D-glycopyranosido]ethyl}dodecylamide (VI)**. To solution of 0.76 g (1.1 mmol) octaacetyl-β-D-lactose (**V**) in 10 ml of anhydrous dichloromethane was added 0.16 ml (1.2 mmol) of boron trifluoride etherate. After 15 min into the reaction mixture was added 0.3 g (1.2 mmol) of compound **IV**, and the mixture was left standing for 12 h at room temperature. On completion of the reaction the mixture was neutralized with 25% ammonia solution till pH 7, washed with water (3×100 ml), the organic layer was dried with anhydrous sodium sulfate, the solvent was distilled off in a vacuum, and the reaction product was purified by column chromatography (eluent system H). Yield 0.3 g (30%), colorless oily substance, R_f 0.48 (eluent system I). IR spectrum (thin film), cm^{-1}: 3300 (NH), 1750 (C=O), 1640 (C=O), 1530 (NH), 2845, 1460, 1375, 710 (CH), 1050 (C–N), 1210 (C–O), 1160–1035 (4 bands, carbohydrate skeleton). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.86–0.91 t (3H, CH_3), 1.26 s (16H, CH_2), 1.98 s, 2.06 s, 2.07 s, 2.13 s, 2.17 s, 2.18 s, 2.19 s (21H, $COCH_3$), 1.60–1.64 t (2H, $COCH_2CH_2$), 2.67 m (2H, $COCH_2CH_2$), 3.75–3.82 t (2H, NCH_2), 3.86–3.91 t (2H, OCH_2), 4.12–4.19 m (4H, H^5, $H^{5'}$, H^6), 4.47–4.50 t (2H,

$H^{1'}$, $J_{1,2}$ 8.0 Hz), 4.51–4.53 t (2H, H^1, $J_{1,2}$ 7.4 Hz), 4.46–4.53 m (2H, $H^{6'}$), 4.85–4.99 m (2H, H^2, $H^{2'}$), 5.10–5.22 m (2H, H^3, $H^{4'}$), 5.36–5.39 m (2H, $H^{3'}$, H^4), 5.89–5.92 t (1H, NH).

***N*-{2-[4-O-(β-D-Galactopyranosyl)-β-D-glycopyranosido]ethyl}dodecylamide (VII).** To a solution of 0.3 g of compound **VI** in 3 ml of methanol was added at room temperature while stirring 0.75 ml of freshly obtained 0.1 M solution of sodium methylate in methanol till pH 8. After 20 min the solution was treated with ion-exchange resin KU-2 till pH 7, the resin was filtered off, the solvent was distilled off in a vacuum. Yield 0.2 g (94%), R_f 0.45 (eluent system A), mp 146–147°C (MeOH). IR spectrum (mull in mineral oil), cm^{-1}: 3280 (NH), 3250 (OH), 1630 (C=O), 1560 (NH), 2890, 1324, 700 (CH), 1210–1060 (4 bands, carbohydrate skeleton). Mass spectrum, *m/z* (I_{rel}, %): 589.2 (4.2) $[M]^+$, 399.2 (18.9), 280.1 (19.9), 279.1 (100), 206.1 (25), 191.2 (70), 163.2 (23.8), 147.1 (39.8), 111.1 (44.5), 85.1 (57.9), 71.0 (68), 57.1 (89.1).

Ditetradecyl L-glutamate (XI). A mixture of 2.3 g (15.6 mmol) of L-glutamic acid (**VIII**), 10 g (46.9 mmol) of tetradecanol (**IX**), and 4.15 g (15.6 mmol) finely dispersed in a mortar *p*-toluenesulfonic acid was heated on an oil bath at 110°C for 1.5 h. The completion of the reaction was determined by the disappearance of the L-glutamic acid; then the reaction mixture was cooled to room temperature and recrystallized from acetone. The precipitate was filtered off, dissolved in 100 ml of chloroform, washed with 5% solution of sodium hydrogen carbonate (5×35 ml), with water till pH 7, and dried with sodium sulfate. The solvent was removed in a vacuum. Yield 10 g (96%), colorless amorphous substance, R_f 0.53 (eluent system E). IR spectrum (mull in mineral oil), cm^{-1}: 3450 (NH), 2926, 2904, 1450, 1375, 700 (CH), 1736 (C=O), 1172 (C–N), 1145, 1028 (C–O).

The obtained chromatographically pure compound **XI** without further purification was used in the next reaction stage.

3-[1,3-Di(tetradecyloxycarbonyl)propylcarbamoyl]propanoic acid (XIII). To a solution of 1.53 g (2.8 mmol) of ester **XI** in 20 ml of toluene was added 0.29 g (2.8 mmol) of succinic anhydride **XII**, and the mixture was stirred for 12 h at room temperature. Toluene was distilled off in a vacuum, the residue was dissolved in 50 ml of chloroform, washed with 0.1 N HCl, with water till pH 7, and dried. The solvent was removed in a vacuum. Yield 1.6 g (91%), colorless crystals, R_f 0.4 (eluent system C), mp 33–34°C ($CH_3C_6H_5$). IR spectrum (mull in mineral oil), cm^{-1}: 3390 (OH), 3290 (NH), 2960, 1465,1375, 715 (CH), 1730, 1720 (C=O), 1640 (C=O, NH), 1389 (C–N). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 0.83–0.92 t (6H, CH_3), 1.15–1.40 s (44H, CH_2), 1.50–1.70 m (4H, β-CH_2CH_2OCO), 2.35–2.42 m (2H, β-CH_2), 2.62–2.77 m (2H, δ-CH_2), 3.59–3.70 m (4H, CH_2CO), 4.03–4.08 t (2H, α-CH_2OCO), 4.10–4.15 t (2H, α-CH_2OCO), 4.57–4.64 m (1H, CH).

***N*-[4-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glycopyranosido]succinimide (XIV).** To a solution of 5 g (7.4 mmol) of octaacetyl-β-D-lactose (**V**) in 22 ml of anhydrous dichloromethane was added 1 ml (8.1 mmol) of boron trifluoride etherate. After 15 min the reaction mixture was treated with 0.93 g (8.1 mmol) of *N*-hydroxysuccinimide and left standing for 12 h at room temperature. On completion of the reaction the mixture was neutralized with 25% ammonia solution till pH 7 and washed with water (5×100 ml). The organic layer was dried, the solvent was distilled off in a vacuum, the residue was subjected to column chromatography (eluent system J). Yield 2.4 g (43%), colorless crystals, R_f 0.42 (eluent system B), mp 90–91°C (EtOH) (92.5–93.7°C [17]). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 1.90 s, 1.99 s, 2.00 s, 2.02 s, 2.04 s, 2.06 s, 2.09 s (21H, $COCH_3$), 2.73 s (4H, CH_2CH_2), 3.72–3.78 m (1H, H^5), 3.86–3.91 m (1H, $H^{5'}$), 4.05–4.23 m (4H, H^6, $H^{6'}$), 4.41–4.45 d.d (1H, H^4), 4.53–4.56 d (1H, $H^{1'}$, $J_{1,2}$ 7.9 Hz), 4.94–4.98 d.d (1H, $H^{3'}$), 5.06–5.10 d (1H, H^1, $J_{1,2}$ 6.3 Hz), 5.14–5.22 m (2H, H^2, $H^{2'}$, H^3), 5.33–5.35 d.d (1H, $H^{4'}$). Found, %: C 48.97; H 5.43; N 2.09. $C_{30}H_{39}NO_{20}$. Calculated, %: C 49.12; H 5.36; N 1.91.

[4-O-(β-D-Galactopyranosyl)-β-D-glycopyranosyl]oxyamine (XV). To a solution of 100 mg (0.1 mmol) of compound **XIV** in 15 ml of ethanol was added 1.65 ml (0.95 mmol) of hydrazine hydrate. The mixture was left standing at room temperature for 24 h. The reaction progress was monitored by TLC (eluent system K). On completion of the reaction the excess hydrazine hydrate and the solvent were removed in a vacuum. The reaction product was purified by recrystallization from ethanol. Yield 0.03 g (33%), R_f 0.6 (eluent system K), mp 138–139°C (EtOH). IR spectrum (mull in mineral oil), cm^{-1}: 3340 (OH), 3320 (NH), 2920 (CH), 1638 (NH), 1435,1356 (CH), 1212 (C–O), 1114–1035 (C–O, 4 bands, carbohydrate skeleton). ^1H NMR spectrum (D_2O), δ, ppm: 3.41 d.d (1H, H^2), 3.53–3.69 m (3H, $H^{2'}$, H^3, $H^{3'}$), 3.76–3.81 m (1H, H^6, $H^{6'}$), 3.88 d (1H, $H^{4'}$), 4.07–4.18 m (2H,

H^5, $H^{5'}$), 4.24 m (1H, H^4), 4.34 d (1H, H^1, $J_{1,2}$ 8.0 Hz), 4.92 d (1H, $H^{1'}$, $J_{1,2}$ 7.5 Hz).

Bistetradecyl *N*-{3-[*N*'-4-O-(β-D-galactopyranosyl)-β-D-glycopyranosyloxy]carbamoyl}propionyl)-glutamate (XVI). To a solution of 0.21 g (0.32 mmol) of acid **XIII** and 0.1 g (0.49 mmol) of dicyclohexylcarbodiimide in 10 ml of DMF was added 0.12 g (0.32 mmol) of 1-O-amino-β-D-lactoside (**XV**) and 0.01 g (0.08 mmol) of 4-(dimethylamino)pyridine. The reaction mixture was stirred for 24 h. On completion of the reaction the separated precipitate was filtered of, the solvent was distilled off in a vacuum, the reaction product was subjected to column chromatography (eluent system B). Yield 0.16 g (51%), colorless crystals, R_f 0.50 (eluent system D), mp 155–156°C (MeOH). IR spectrum (mull in mineral oil), cm^{-1}: 3350 (OH), 3314 (NH), 1731 (C=O), 1650 (C=O), 1572 (NH), 2902, 2870, 1370, 700 (CH), 1163–1040 (4 bands, carbohydrate skeleton). Mass spectrum, *m/z* (I_{rel}, %): 976.5 (1) $[M]^+$, 531.3 (39), 530.2 (100), 515.2 (59.5), 232.2 (45), 219.2 (56.1), 189.1 (100), 179.1 (23), 149.1 (51.9), 147.1 (81.8), 111.0 (55), 97.0 (58.8), 95.1 (55.2), 81.0 (44.2), 57.1 (68).

Preparation of liposomes. A solution of 40 mg of phosphatidylcholine and 2 mg of compound **VII** or **XVI** in 3 ml of a mixture chloroform–methanol, 2:1, was slowly evaporated on a rotary evaporator. The residue was dried in a vacuum for 2 h at 20°C. Then 3 ml of distilled water was added, and at 60°C the mixture was shaken for 15 min.

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