Russian Journal of Bioorganic Chemistry, Vol. 28, No. 2, 2002, pp. 152–157. Translated from Bioorganicheskaya Khimiya, Vol. 28, No. 2, 2002, pp. 173–179.

Synthesis of New Photoaffine Probes on the Basis of Ganglioside GM1

E. V. Tsibizova,[1] E. L. Vodovozova, I. I. Mikhalyov, and Yul. G. Molotkovsky

Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, GSP-7 Moscow, 117997 Russia

Received June 13, 2001; in final form, July 02, 2001

Abstract—New photoaffine probes, photoreactive derivatives of ganglioside GM1 bearing a carbene-generating diazocyclopentadien-2-ylcarbonyl group at various distances from the carbohydrate moiety in their molecules were synthesized.

Key words: *diazocyclopentadien-2-ylcarbonyl label, gangliosides, lipid derivatives, photoaffinity labeling*

INTRODUCTION

Gangliosides, glycosphingolipids bearing one or several residues of sialic acid, are the obligatory components of eukaryotic plasmatic membranes.[2] Especially frequent in nervous tissue, they play an important role in intercellular interactions and in cell interactions with various exogenous factors [1]. Gangliosides are predominantly located on the outer side of the cell membrane bilayer where oligosaccharide determinants are exposed on the cell surface [1, 2]. It is known that they serve as receptors for various toxins [3–5], bacteria [6], and viruses [7, 8]; participate in the recognition processes by changing the expression of protein receptors [9, 10], modulate protein phosphorylation [11], are involved in the reception of interleukin-2 and -4 [12, 13], are specific markers of tumor cells [14, 15], and can suppress immunity [16, 17]. The polar (oligosaccharide) part of the ganglioside molecule plays the deciding role in these interactions; however, entire complexes of protein receptors in proximity to the apolar (ceramide) part of the molecule inside the cell membrane undergo conformational changes. Moreover, a direct contact of the ceramide residue with the recepted protein is also possible; this could result in the cross-linkage of a photolabeled ganglioside and the protein ligand.

Photolabeled gangliosides can be valuable tools in studying the membrane rearrangements that are caused by a ligand binding to a receptor; in these cases, the recepted protein directly binds to ganglioside or serves as a cofactor. It is important that PAL in the apolar part of the ganglioside molecule does not distort the structure of its oligosaccharide determinant and, therefore, allows the preparation of lipophilic probes for the study of various lipid–protein interactions inside the membranes.

Whereas phospholipid probes have long been used in membrane research, the use of ganglioside probes is rare. This is likely due to their difficult synthesis. As the total synthesis of labeled gangliosides is very laborious and unpractical, it is more appropriate to modify natural lipids at both their oligosaccharide (see, e.g., [18]) and ceramide moieties (see, e.g., [19–21]). The most effectual route for modification of the second type is the replacement of a fatty acid residue in a natural ganglioside molecule by a labeled acyl residue. This opportunity has appeared due to the development of the methods for the removal of acyl residue from the ceramide moiety of gangliosides by alkaline hydrolysis [22].

A number of groups (e.g., aryl azide, diazirine, and benzophenone) were recommended as photoreactive (photoaffinity, photoactivatable) labels, because they form highly reactive intermediates (nitrenes, carbenes, or biradicals) upon irradiation. However, none of them fully meets all the requirements imposed on such groups.

A relatively easy way for the synthesis of aryl azide derivatives is undoubtedly an important advantage for their practical use. Therefore, nitrene-generating phenyl azide groups are those most often used for PAL (see, e.g., [23]), and their photochemistry has been rather comprehensively studied. Phenyl azides can be photolyzed under rather mild conditions, which decrease the probability of damage to biomolecules ($\lambda > 300$ nm; though their absorption maxima are at $\lambda < 300$ nm). However, nitrenes resulting from their photolysis are low-reactive, which explains a low cross-linkage level of the probes to biomolecules.

Nitrenes generated from aryl azides have also been found [24] to undergo a rapid rearrangement to long-

[1] Please send correspondence to: phone: +7 (095) 330-6610; e-mail: parfin@mail.ru.

[2] Abbreviations: Dcp, diazocyclopentadien-2-ylcarbonyl; DcpOH, diazocyclopentadien-2-ylcarboxylic acid; lyso-GM1, lysoganglioside GM1; Np, *p*-nitrophenyl; PAL, photoaffinity labeling; Py, pyridine; and Tf_2O, trifluoromethanesulfonic anhydride.

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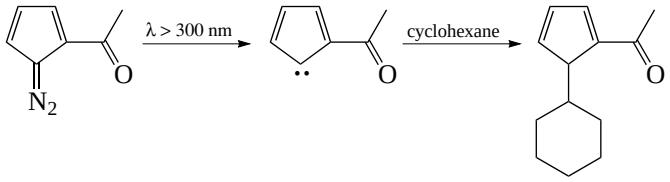


Scheme 1.

living dehydroazepines, which first and foremost react with nucleophiles, such as amino and thiol residues.

Photolysis of diazirine derivatives results in carbenes, which possess a higher reactivity than nitrenes [25]. Previously, a number of diazirine derivatives have been obtained, with 3-(trifluoromethyl)-3-phenyldiazirine group possessing the best characteristics [26]. The multistage synthesis of diazirine ring hinders the wide use of these compounds.

In recent years, benzophenone groups have often been used for PAL. The compounds containing them have several obvious advantages: they are more chemically stable than aryl azides and diazirines, are activated at 350–360 nm, and, after photoactivation, can react with nonactivated C–H bonds even in the presence of aqueous solvents [27]. However, this label is very bulky, and the generated biradical has a certain stereospecificity. Moreover, the benzophenone chromophore needs a durable irradiation for its photoactivation [28], which is undesirable in some cases.

To ensure the high sensitivity of a photoaffine probe, it should include radionuclides with high radiation energy (^{125}I, ^{131}I, ^{32}P, etc.). The lifetime of such compounds is very short; thus, it is reasonable to introduce the radioisotope into the cold probe immediately before use. To facilitate further analysis of the cross linkage products, the direct radiolabeling of the photoaffinity group is also desirable. Some functionalization methods for ^{125}I introduction were developed for a number of photoaffinity labels, namely, perfluorophenyl azide [29], 3-(trifluoromethyl)-3-phenyldiazirine, [30], and benzophenone groups [31]. However, this immediate introduction substantially complicates the probe synthesis.

Therefore, a search for new photoaffinity groups still remains a topical problem in membranology.

The carbene-generating Dcp group was first suggested for PAL by Nielsen *et al.* [32]. This labeling group is small in size, a substantial absorption in the near UV region (λ_{max} 314 nm, ε 15900 M^{-1} cm^{-1}) is characteristic of it, and it is readily photolyzed to give carbene. The carbene was shown to be highly reactive: it is effectively inserted into nonactivated C–H bonds [33] (Scheme 1).[3] Starting DcpOH can be easily obtained by three-stage synthesis from cyclopentadiene and is stable in the absence of UV light under various conditions: mild basic and acidic media (including peracetic acid) [34], heating (at ≥150°C), and oxidizers. We had previously synthesized the Dcp derivatives of ^{14}C/^{3}H-labeled phosphatidylcholine and sphingomyelin [33] and successfully used them for studying the membrane topography of cytochrome P-450 [35]. We further investigated some other characteristics of the Dcp group and found that it can be easily iodinated under oxidizing conditions, with its ability to generate a highly reactive carbene upon photoactivation being retained [36].

In this work, we report the synthesis of new photoaffine probes, ganglioside GM1 derivatives bearing Dcp group at various distances from the carbohydrate moiety of the molecule.

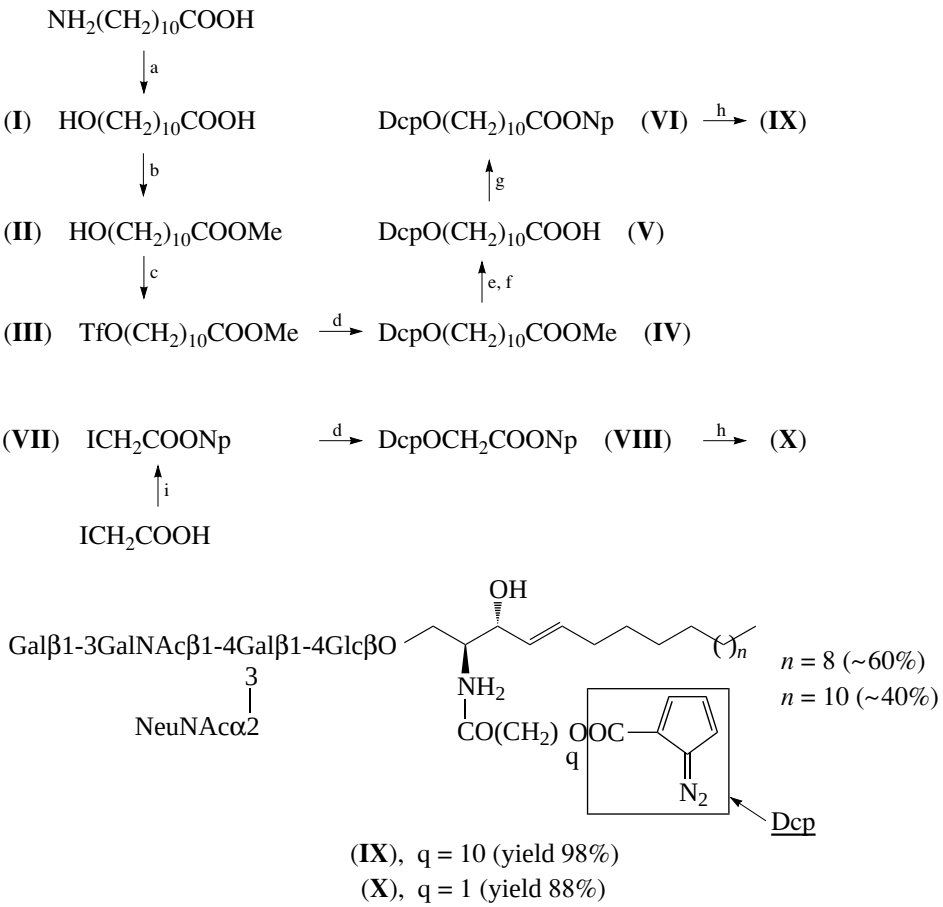
RESULTS AND DISCUSSION

*1. Long-Chain Dcp-GM1 probe (**IX**)*

When studying biological systems with photoaffinity probes, the presence of a labile bond between the photoaffinity label and the remainder of the probe molecule substantially facilitates the analysis of the cross-linkage products. Study of the lipid–protein interactions is primarily interesting, and, hence, ester bond, which, unlike peptide bond, is labile under alkaline conditions, is appropriate as such a labile bond. Therefore, we synthesized an aliphatic acid bearing Dcp residue on the terminal carbon atom (**V**) as a synthon for obtaining various lipid probes, which can be applied in examining the central region of lipid bilayer in the biological and model membranes (see Scheme 2).

11-Hydroxyundecanoic acid (**I**) was synthesized from the available 11-aminoundecanoic acid by deamination with nitrous acid at heating in water [37]. The resulting mixture of three products included (according to TLC) undecylenic acid (R_f 0.45, somewhat less than a half of the total amount), 10-hydroxyundecanoic acid (R_f 0.35, negligible amount), and the target product (**I**) (R_f 0.2, approximately a half of the total amount), isolated by chromatography on silica gel in 38% yield. Its ^{1}H NMR spectrum contained a triplet resonance characteristic of protons of a methylene group at the primary hydroxyl (see the Experimental section). Its IR spectrum also confirmed the structure of the resulting product (**I**).

[3] The structural study of the insertion product by ^{1}H NMR will be published elsewhere.



Scheme 2.

Reagents: a. HNO_2; b. MeOH/HCl; c. Tf_2O/Py; d. DcpOH/Et_3N; e. KOH, Pr^iOH/H_2O; f. HCl; g. CF_3COONp/Py; h. lyso-GM1/Et_3N; i. HONp, DCC.

Methyl 11-hydroxyundecanoate (**II**) was obtained by the esterification of (**I**) with a methanolic HCl in 53% yield. Its treatment with Tf_2O in the presence of pyridine in chloroform at –10°C led to methyl 11-(trifluoromethanesulfonyloxy)undecanoate (**III**) (yield 86%), which was further used without additional purification.

The reaction of triflate (**III**) with DcpOH in anhydrous acetone in the presence of a small excess of triethylamine gave methyl 11-(Dcp-oxy)undecanoate (**IV**) in 50% yield. The structure of (**IV**) was confirmed by mass spectrometry and ^{1}H NMR spectroscopy.

Methyl ester (**IV**) was hydrolyzed with KOH in aqueous isopropanol at room temperature. The rate of alkaline hydrolysis of the ester bond formed by DcpOH is substantially lower than that of the aliphatic ester. The reaction was carried out for 20 h until the appearance of traces of free DcpOH (TLC). The successive chromatography on silica gel and the reversed phase yielded 40% of 11-(Dcp-oxy)undecanoic acid (**V**), which was then transformed into its *p*-nitrophenyl ester (**VI**) with *p*-nitrophenyltrifluoroacetate in pyridine.

The long-chain ganglioside probe (**IX**) was synthesized by the reaction of lyso-GM1 with *p*-nitrophenyl ester (**VI**) in DMSO in the presence of triethylamine and isolated by gel filtration in yield (98%), determined from its mass and UV-absorption of the Dcp label.

*2. Short-Chain Dcp-GM1 probe (**X**)*

To obtain probe (**X**) bearing the label in the close proximity to the ganglioside polar head, we somewhat changed the synthetic scheme.

We failed to obtain *p*-nitrophenyl iodoacetate (**VII**) in the same way as ester (**VI**) using *p*-nitrophenyl trifluoroacetate, because the reagent had almost the same chromatographic mobility as the target product. Ester (**VII**) was obtained by the esterification of iodoacetic acid with *p*-nitrophenol in the presence of DCC. The structure of the resulting compound was confirmed by ^{1}H NMR and elemental analysis.

DcpOH was treated with (**VII**) in anhydrous acetone in the presence of triethylamine. The reaction product, Dcp-oxyacetic acid *p*-nitrophenyl ester (**VIII**), was isolated by column chromatography on silica gel in

84% yield, and its structure was confirmed by mass spectrometry and ^{1}H NMR.

Photoreactive ganglioside (**X**) bearing the Dcp-oxyacetyl residue was synthesized, like (**IX**), from lyso-GM1 and Dcp-oxyacetic acid *p*-nitrophenyl ester (**VIII**). Its yield (88%) was somewhat less than that of (**IX**), which is obviously due to the steric hindrances during acylation of the amino group of sphingosine residue.

High yields of probes (**IX**) and (**X**) can be explained by the advantages of our approach, namely, the use of DMSO as a solvent for the reaction of lysoganglioside with active esters (**VI**) and (**VIII**) and the use of gel filtration instead of absorption and reversed phase chromatography for the purification of micromolar amounts of target products.

EXPERIMENTAL

11-Aminoundecanoic acid, trifluoromethanesulfonic anhydride, trifluoroacetic anhydride, and triethylamine were from Fluka (Switzerland); DMSO and anhydrous pyridine from Merck (Germany); and iodoacetic and acetic acids (reagent grade) were of domestic production. DCC (Serva, Germany) was used as 1 M solution in tetrachloromethane. To remove water, acetone was distilled over K_2CO_3 and chloroform, over P_2O_5. Other reagents and solvents (Reakhim, Russia) were purified by standard procedures. *p*-Nitrophenyl trifluoroacetate was obtained from *p*-nitrophenol and trifluoroacetic anhydride [38]. DcpOH was synthesized as described in [34]. Lysoganglioside was obtained from the natural ganglioside by hydrolysis [21].

Column chromatography was carried out on Silica gel 60 (40–63 μm; Merck, Germany) and Silica gel L (40–100 and 100–160 μm; Chemapol, Czech Republic). Silica gel RP-18 (25–40 μm; Merck, Germany) was used for the reversed phase chromatography. Gel filtration was carried out on Sephadex LH-20 (Pharmacia, Sweden). For TLC, the silica gel 60 F_{254} and RP-18 F_{254} (Merck, Germany) precoated plates were used; solvent systems were: (A) 50 : 10 : 1 toluene–ethyl acetate–acetic acid, (B) 9 : 1 chloroform–methanol, (C) 7 : 3 methanol–water, (D) 9 : 1 methanol–water, (E) 1 : 1 methanol–water, (F) 6 : 4 methanol–water, (G) 1 : 1 chloroform–methanol, and (H) 2 : 3 : 1 isopropanol–ethyl acetate–water. Substance spots were visualized by (a) 5% phosphomolybdic acid in ethanol, (b) water, (c) UV irradiation, (d) ammonia vapor, (e) 0.2% ninhydrin in ethanol, and (f) resorcinol. Solvents were evaporated in a vacuum at temperatures below 40°C. After purification, the reaction products were dried at 20 Pa. Melting points were determined on a Kofler apparatus and were uncorrected.

All manipulations with Dcp derivatives were carried out under yellow light.

UV spectra were registered on an Ultrospec II 4050 (LKB, Sweden) spectrophotometer in ethanol. Mass spectra were taken on a MSBKh (Sumy, Ukraine) plasma desorption time-of-flight mass spectrometer (ionization with the products of ^{252}Cf fission at an accelerating voltage of +15 eV) or on a Finnigan MAT 9005 (ESI) mass spectrometer. IR spectra were recorded on a Specord 75IR (Carl Zeiss, Germany) instrument. ^{1}H NMR spectra were registered on a Bruker WM-500 (United States) spectrometer.

11-Hydroxyundecanoic acid (I). A solution of $NaNO_2$ (483 mg, 7 mmol) in water (2 ml) was added to a solution of 11-aminoundecanoic acid (1 g, 5 mmol) in a 4 : 1 water–acetic acid mixture (5 ml). The reaction mixture was heated for 1 h in a boiling water bath (100°C), kept overnight at room temperature, acidified with 1 N HCl to pH 3.0, and extracted with chloroform (3 × 5 ml). The extract was filtered though cotton wool, evaporated, dried, and chromatographed on Silica gel L (100–160 μm). Elution with an 0 ⟶ 10% gradient of 10 : 1 isopropanol—acetic acid in chloroform gave 382 g (38%) of (**I**) as a white solid, R_f 0.2 (A; a and b). The product (white needles from water) had: mp 64–65°C (lit. [39]: mp 65–66°C); IR (ν, cm^{-1}): 3620 (OH), 2933 and 2860 (CH_2), 1700 (C=O), and 1226 and 1200 (OH–COOH); ^{1}H NMR ($CDCl_3$, δ, ppm): 1.29 (12 H, m, $(CH_2)_6$), 1.55 (2 H, m, $C\underline{H}_2CH_2COOH$); 1.64 (2 H, m, $HOCH_2C\underline{H}_2$), 2.35 (2 H, t, CH_2COOH), and 3.64 (2 H, t, $HOCH_2$).

Methyl 11-hydroxyundecanoate (II). Acetyl chloride (5 ml) was added dropwise to methanol (50 ml) at vigorous stirring, and the resulting mixture was immediately added to acid (**I**) (200 mg, 1 mmol). The reaction mixture was gently stirred for 3.5 h at 40°C and evaporated. The residue was chromatographed on Silica gel L (40–100 μm); elution with system B gave 115 mg (53%) of ester (**II**) as a colorless oil; R_f 0.45 (A; a).

Methyl 11-(trifluoromethanesulfonyloxy)undecanoate (III). Ester (**II**) (115 mg, 0.53 mmol) was dissolved in anhydrous chloroform (6 ml) containing anhydrous Py (70 μl, 0.87 mmol) and the resulting solution was added dropwise to a solution of Tf_2O (144 μl, 0.87 mmol) in anhydrous chloroform (6 ml) under stirring and cooling in an ice–salt bath (–10°C). The mixture was then stirred for 2 h in the stream of argon at 0°C and then extracted with water (3 × 3 ml). The organic phase was filtered through cotton wool, concentrated, and dried to give 159 mg (86%) of ester (**III**) as a chromatographically pure colorless oil; R_f 0.8 (A; a).

Methyl 11-(Dcp-oxy)undecanoate (IV). Ester (**III**) (159 mg, 0.46 mmol) in anhydrous acetone (2.6 ml) was added to a solution of DcpOH (20 mg, 0.15 mmol) and triethylamine (80 μl, 0.58 mmol) in anhydrous acetone (1 ml). The reaction mixture was stirred for 40 min at room temperature and evaporated. The residue was chromatographed on a Silica gel 60 column eluted with a 0 ⟶ 7% gradient of ethyl acetate in heptane to give 25 mg (50%) of (**IV**) as a chromatographically pure yellowish oil; R_f 0.63 (A; a and c); MS (^{252}Cf), *m*/*z*: 334 $[M]^+$; UV [λ_{max}, nm (ε)]: 215

(12 400) and 314 (15 900); ^{1}H NMR ($CDCl_3$, δ, ppm): 1.29 (12 H, m, $(CH_2)_6$), 1.38 (2 H, m), 1.61 (2 H, m), 1.70 (2 H, t, *J* 6.72 Hz, C$\underline{H}_2$COO), 3.66 (3 H, s, CH_3), 4.24 (2 H, t, DcpOC$\underline{H}_2$), 6.07 (1 H, dd, $J_{4'5'}$ 4.60 Hz and $J_{4'3'}$ 3.20 Hz, H4'), 6.82 (1 H, dd, $J_{3'4'}$ 3.18 Hz and $J_{3'5'}$ 1.96 Hz, H3'), and 7.02 (1 H, dd, $J_{5'4'}$ 4.64 Hz and $J_{5'3'}$ 1.96 Hz, H5').

11-(Dcp-oxy)undecanoic acid (V). A solution of ester (**V**) (25 mg, 75 μmol) in isopropanol (16 ml) and 5% KOH (0.4 ml) was kept until the appearance of traces of DcpOH (TLC monitoring; system A; a and c). The reaction was stopped by neutralization with 1 N HCl up to pH 7.0, evaporated, and the dry residue was suspended in water (1 ml), acidified up to pH 2.0–3.0, and extracted with chloroform (3 × 0.5 ml). The extract was washed with a small amount of water and filtered through cotton wool. The filtrate was evaporated and chromatographed on a Silica gel 60 column eluted with a 0 ⟶ 5% gradient of 10 : 1 isopropanol–acetic acid in chloroform. Fractions containing (**V**) and traces of DcpOH were combined and chromatographed on a reversed phase column successively eluted with systems C and D to yield 8.2 mg (34%) of acid (**V**) as a yellowish amorphous substance; R_f 0.44 (A; a and c); its UV spectrum was similar to that of ester (**IV**).

***p*-Nitrophenyl 11-(Dcp-oxy)undecanoate (VI).** *p*-Nitrophenyl trifluoroacetate (5.8 mg, 0.024 mmol) in anhydrous Py (200 μl) was kept with acid (**V**) (4 mg, 12 μmol) at room temperature for 5 h under gentle stirring. The reaction mixture was evaporated and chromatographed on a Silica gel 60 column eluted with a 0 ⟶ 2% gradient of ethyl acetate in toluene to give 2.7 mg (50%) of (**VI**) as a yellow oil; R_f 0.78 (A; a, c, and d); MS (^{252}Cf), *m/z*: 441.6 $[M]^+$. A solution of ester (**VI**) in DMSO (1 μmol/50 μl) was prepared.

***p*-Nitrophenyl iodoacetate (VII).** A solution of *p*-nitrophenol (46 mg, 0.36 mmol) and DCC (80 mg, 0.4 mmol) in anhydrous chloroform (3 ml) was cooled in the ice bath to 0°C and added to a solution of iodoacetic acid (56 mg, 0.3 mmol) in anhydrous chloroform (3 ml) under stirring. The mixture was kept for 1 h at 0°C and overnight at room temperature, filtered through cotton wool, and evaporated. The dry residue containing (**VII**) and dicyclohexylurea was treated with toluene and filtered. The filtrate was chromatographed on a Silica gel 60 column eluted with toluene containing 0.5% acetic acid to give 51 mg (55%) of chromatographically pure (**VII**); R_f 0.8 (A; c and d). The product was crystallized from ethanol to give white monoclinic crystals; mp 80°C; ^{1}H NMR ($CDCl_3$, δ, ppm): 3.95 (2H, s, IC$\underline{H}_2$CO), 7.33 (2 H, d, $J_{2'3'} = J_{6'5'}$ 9.17 Hz, 2'-H and 6'-H), and 8.30 (2 H, d, $J_{3'2'} = J_{5'6'}$ = 9.17 Hz, H3' and H5'). Found, %: C, 31.24; H, 2.00; N, 4.42; I, 41.00. $C_8H_6INO_4$. Calculated, %: C, 31.29; H, 1.97; N, 4.56; I, 41.33.

***p*-Nitrophenyl Dcp-oxyacetate (VIII).** A solution of DcpOH (13.8 mg, 0.1 mmol) and triethylamine (28 μl, 200 μmol) in anhydrous acetone (1 ml) was stirred for 2–3 min, and ester (**VII**) (31.5 mg, 0.1 mmol) in anhydrous acetone (1 ml) was added. The solution was stirred overnight and evaporated. The residue was chromatographed on a reversed phase column successively eluted with systems E and F to yield 25 mg (80%) of chromatographically pure (**VIII**); R_f 0.67 (A; c and d); MS (^{252}Cf), *m/z*: 315.7 $[M]^+$; ^{1}H NMR ($CDCl_3$, δ, ppm): 5.04 (2 H, s, OC$\underline{H}_2$COO), 6.14 (1 H, dd, $J_{4'5'}$ 4.58 Hz, $J_{4'3'}$ 3.30 Hz, H4'), 6.97 (1 H, dd, $J_{3'4'}$ 3.10 Hz, $J_{3'5'}$ 2.14 Hz, H3'), 7.12 (1 H, dd, $J_{5'4'}$ 4.58 Hz, $J_{5'3'}$ 1.98 Hz, H5'), 7.36 (2 H, d, $J_{2'3'} = J_{6'5'}$ = 9.15 Hz, H2' and H6'), and 8.30 (2 H, d, $J_{3'2'} = J_{5'6'}$ = 9.15 Hz, H3' and H5'). A solution of ester (**VIII**) in DMSO (0.38 μmol/50 μl) was prepared.

11-(Dcp-oxy)undecanoyl GM1 (IX). Triethylamine (1 μl, 7 μmol) and a solution of (**VI**) (530 μg, 1.2 μmol) in DMSO (50 μl) were added to lyso-GM1 (1 mg, ~1 μmol) in DMSO (50 μl). The reaction mixture was kept for 16 h at room temperature, treated with a drop of water, diluted with system F (0.1 ml), and applied onto a Sephadex LH-20 column (0.5 × 20 cm) equilibrated with system F. Elution with system E resulted in (**IX**); yield 98% (from UV absorption); R_f 0.25, $R_{f\ start}$ 0.07 (E; a, c, e, and f); ^{1}H NMR (CD_3OD, δ, ppm): 1.08 (3 H, t, *J* 7.10 Hz, CH_3), 1.45–1.55 (~36 H, m), 1.91 (2 H, m, C$\underline{H}_2$CH$_2$CO); 2.11 (1 H, dd ~ t, *J* 12.10 Hz, H3$_a$ of Neu5Acα), 2.20 and 2.18 (2 × 3 H, 2 s, 2 NAc), 2.36 (2 H, t, *J* 7.52 Hz, C$\underline{H}_2$CO), 2.92 (1 H, dd, ^2J 12.20 Hz, ^3J 4.42 Hz, H3$_e$ of Neu5Acα), 4.05 (1 H, m, H2), 4.63, 4.55, and 4.49 (3 × 1 H, 3 dd, *J* 7.61, 8.03, and 7.81 Hz, 3 H1 of hexapyranoses β), 5.64 (1 H, dd, $J_{4'5'}$ 15.21, $J_{4'3'}$ 7.78 Hz, H4 of (*E*)C=C), 5.87 (1 H, dt, $J_{5'4'}$ 15.34, $J_{5'6'}$ 7.72 Hz, H5 of (*E*)C=C), 6.26 (1 H, br. dd, $J_{4'5'}$ 4.37 and $J_{4'3'}$ 3.46 Hz, H4'), 6.97 (1 H, br. t (dd), $J_{3'4'}$ 3.40 and $J_{3'5'}$ 1.80 Hz, H3'), and 7.35 (1 H, br. dd, $J_{5'4'}$ 4.58 and $J_{5'3'}$ 1.83 Hz, H5'); MS (ESI), *m/z*: 1582.875 $[M_1 + H]^+$, 1604.869 $[M_1 + Na]^+$, 1620.837 $[M_1 + K]^+$ {calculated for $C_{72}H_{119}N_5O_{33}$ $[M_1]^+$ = 1581.7885 and $[M_1 + H]^+$ = 1582.7963}; 1610.912 $[M_2 + H]^+$, 1632.893 $[M_2 + Na]^+$, 1648.866 $[M_2 + K]^+$ {calculated for $C_{74}H_{123}N_5O_{33}$ $[M_2]^+ = [M_1]^+ + 2CH_2$ = 1609.8198, $[M_2 + H]^+$ = 1610.8276); UV spectrum is similar to that of ester (**VI**).

(Dcp-oxy)acetyl GM1 (X) was synthesized as described for (**IX**) from lyso-GM1 (~1 μmol) and (**VIII**) (380 μg, 1.2 μmol); yield 88% (from UV absorption); R_f 0.15 (E; a, c, e, and f); ^{1}H NMR (CD_3OD, δ, ppm): 1.09 (3 H, t, *J* 7.10 Hz, CH_3), 1.45–1.57 (~22 H, m), 2.20 (2 × 3 H, br. s, 2 NAc), 2.85 (2 H, s, COC$\underline{H}_2$ODcp), 2.92 (1 H, dd, ^2J 12.20 and ^3J 4.40 Hz, H3$_e$ of Neu5Acα), 4.03 (1 H, m, H2), 4.63, 4.59, and 4.50 (3 × 1 H, 3 dd, *J* 7.60, 8.02, and 8.02 Hz, 3 H1 of hexapyranoses β), 5.65 (1 H, dd, $J_{4'5'}$ 15.20 and $J_{4'3'}$ 7.72 Hz, H4 of (*E*)C=C), 5.89 (1 H, dt, $J_{5'4'}$ 15.32,

$J_{5'6'}$ 7.70 Hz, H5 of (*E*)C=C), 6.29 (1 H, br. t (dd), $J_{4'5'}$ 4.30 and $J_{4'3'}$ 3.30 Hz, H4'), 7.09 (1 H, br. t, H3'), and 7.40 (1 H, br. dd, $J_{5'4'}$ 4.60 and $J_{5'3'}$ 1.80 Hz, H5'); MS (ESI), *m/z*: 1456.740 $[M_1 + H]^+$, 1478.714 $[M_1 + Na]^+$, 1494.701 $[M_1 + K]^+$ {calculated for $C_{63}H_{101}N_5O_{33}$ $[M_1]^+$ = 1455.6477 and $[M_1 + H]^+$ = 1456.6555}, 1484.723 $[M_2 + H]^+$, 1506.740 $[M_2 + Na]^+$, 1522.722 $[M_2 + K]^+$ {calculated for $C_{65}H_{105}N_5O_{33}$ $[M_2]^+ = [M_1]^+ + 2CH_2$ = 1483.6790 and $[M_2 + H]^+$ = 1484.6868}. UV spectrum is similar to that of ester (**VI**).

ACKNOWLEDGMENTS

We are grateful to Dr. A.B. Tuzikov (Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences) for assistance in interpretation of ^{1}H NMR spectra and to Dr. Bo Ek (The Swedish University of Agricultural Sciences, Uppsala) for registration of high-resolution mass spectra.

^{1}H NMR spectra were registered with the partial support of the Ministry of Science of the Russian Federation, project no. 96-03-08, and the Russian Foundation for Basic Research, project no. 00-04-55024.

The work was partly supported by the Russian Foundation for Basic Research, project no. 00-04-48922.

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