1957

Synthesis of phosphatidyl peptides I. o-(distearoyl-l-a-glycerylphosphoryl)-l-serylglycylglycine / by Erich Baer, Jonas Maurukas, and Donald D. Clarke (From the Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada)

Erich Baer
University of Toronto. Banting Best Department of Medical Research

Jonas Maurukas
University of Toronto. Banting Best Department of Medical Research

Donald Dudley Clarke PhD
Fordham University, clarke@fordham.edu

Follow this and additional works at: https://fordham.bepress.com/chem_facultypubs
Part of the Biochemistry Commons

Recommended Citation
Baer, Erich; Maurukas, Jonas; and Clarke, Donald Dudley PhD, "Synthesis of phosphatidyl peptides I. o-(distearoyl-l-a-glycerylphosphoryl)-l-serylglycylglycine / by Erich Baer, Jonas Maurukas, and Donald D. Clarke (From the Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada)" (1957). Chemistry Faculty Publications. 41. https://fordham.bepress.com/chem_facultypubs/41

This Article is brought to you for free and open access by the Chemistry at DigitalResearch@Fordham. It has been accepted for inclusion in Chemistry Faculty Publications by an authorized administrator of DigitalResearch@Fordham. For more information, please contact considine@fordham.edu.
SYNTHESIS OF PHOSPHATIDYL PEPTIDES

I. O-(DISTEAROYL-L-\textalpha-GLYCERYLPHOSPHORYL)-L-SERYLGLYCEROLGLYCINE

BY ERICH BAER, JONAS MAURUKAS,* AND DONALD D. CLARKE†

(From the Banting and Best Department of Medical Research,
University of Toronto, Toronto, Canada)

(Received for publication, March 8, 1957)

During recent years reports (1–6) have appeared describing the isolation of naturally occurring phosphatides containing nitrogenous moieties considerably more complex than those of the classical phosphatides. It has been suggested that in some of these substances the basic moiety consists of a polypeptide. Although the reality of some of the phosphatidyl peptides has been questioned (7), it was felt that they were sufficiently interesting as a new class of phosphatides to attempt the synthesis of a simple representative by a procedure similar to those described in some of our earlier publications (8–13). A synthetic phosphatidyl peptide possessing a known structure should prove useful as a compound of reference in studies of the structure of natural lipopeptides and lipoproteins. The presence of serine (14–16) bound to phosphoric acid (2, 5, 17–23) in some of the naturally occurring phosphatides suggested that the nitrogenous moiety of the synthetic phosphatidyl peptide should contain at least 1 mole of serine and that the phosphatidyl group be linked in ester form to the alcoholic hydroxyl group of the hydroxyamino acid, as for instance in phosphatidylserine (21). Glycine was chosen as the second amino acid of the peptide, as it has been found to occur with serine in natural phosphatides (14–16) and does not introduce a new asymmetric center. The well known difficulties encountered in preparing peptides of even moderate chain length imposed certain restrictions on our initial attempts. Serylglycylglycine was selected for its simplicity as peptide moiety. A phosphatidylserylglycylglycine containing two identical saturated fatty acid substituents can occur theoretically in any of six stereoisomeric forms, i.e. four $\alpha$ and two $\beta$ forms. Recent investigations (8, 24, 25) seem to indicate that the glycerophosphoric acid moiety of natural phosphatides possesses the $\alpha$ structure and L configuration exclusively. Natural amino

*Sections of this paper form part of a thesis submitted by Jonas Maurukas to the Department of Pathological Chemistry of the University of Toronto in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

†Postdoctoral research fellow assisted by a grant from the National Research Council (Ottawa) to the senior author.
acids, as is well known, generally occur as L isomers. Hence, a phosphatidylserglycylglycine, if it were to occur in nature, most likely would be an α isomer with L configuration at both asymmetric centers. From a biological point of view, this isomer would be the most interesting one of the six and thus was selected for synthesis. As fatty acid substituents of the glyceride moiety of the phosphatidyl peptide, stearic acid was chosen, as it is one of the best known and most widely distributed acids of the saturated series.

The synthesis of the O-(distearoyl-L-α-glycerylphosphoryl)-L-serglycylglycine follows closely our procedure for the synthesis of phosphatidylserine (12) with the exception that the N-carbobenzyox-L-serine benzyl ester is replaced by N-carbobenzyox-L-serglycylglycine benzyl ester. At first the substituted tripeptide was prepared by a procedure requiring ten steps (26). Subsequently, a somewhat simpler procedure for its synthesis was developed involving only seven steps (26). More recently an even greater simplification in the preparation of the tripeptide derivative was achieved (Reaction Scheme I) by coupling N-carbobenzyox-L-serine (I) with glycylglycine benzyl ester (II), by using the carbodiimide procedure of Sheehan, Goodman, and Hess (27). This procedure which requires only three steps is described in the experimental part of this paper. Removal of the protective groups of the N-carbobenzyox-L-serglycylglycine benzyl ester by hydrogenolysis with palladium as catalyst gave
L-serylglycylglycine possessing a specific rotation that was identical with that reported for L-serylglycylglycine prepared by two other methods (26). Thus it seems safe to assume that by the present, as well as by the two earlier, methods L-serylglycylglycine and its precursor are obtained in an optically pure state.

The synthesis of the phosphatidyl tripeptide (IX) was carried out as follows (see Reaction Scheme II). The D-α,β-distearin (V) was phosphorylated with phenylphosphoryl dichloride in the presence of quinoline, giving rise to the formation of distearoyl-L-α-glycerylphenylphosphoryl chloride (VI) and the by-product, bis(distearoyl-L-α-glyceryl)phenylphosphate (VII). Owing to the formation of by-product VII, some of the phenylphosphoryl dichloride remains unchanged. In the second phase of the phosphorylation, this material occasions an appreciable loss of the valuable tripeptide derivative via two reactions, one leading to the formation of phenylphosphoryl bis(N-carbobenzoxy-L-serylglycylglycine benzyl ester) and the other presumably to a breakdown of the tripeptide. To prevent these losses, attempts were made to free the distearoyl-L-α-glycerylphenylphosphoryl chloride (VI) of phenylphosphoryl dichloride, prior to the esterification of compound VI with the tripeptide derivative (III). These attempts were abandoned, however, when it was found that it is difficult to obtain products of compound VI with constant chlorine content. Hence, without isolating the distearoyl-L-α-glycerylphenylphosphoryl chloride (VI), it was esterified with N-carbobenzoxy-L-serylglycylglycine benzyl ester (III) in the presence of an organic base. When pyridine was used, the reaction mixture became deep red. By changing to 2,6-lutidine, which Atherton, Howard, and Todd (28) found to be an efficient base for phosphorylations, the formation of colored by-products was greatly reduced, and a better yield of distearoyl-L-α-glycerylphenylphosphoryl N-carbobenzoxy-L-serylglycylglycine benzyl ester (VIII) was obtained. Attempts to isolate compound VIII by treating the rather complex mixture of phosphorylation products with a number of selective solvents gave a product which was enriched in compound VIII but resisted further separation by the action of solvents alone. To obtain a purer compound recourse was had to a chromatographic separation of the enriched material on a column of silicic acid. By using as eluents a mixture of low boiling petroleum ether and diethyl ether (4:1, v/v), followed by diethyl ether, two fractions were obtained of which the first consisted mainly of bis(distearoyl-L-α-glyceryl)phenylphosphate (VII) and the second of fairly pure distearoyl-L-α-glycerylphenylphosphoryl N-carbobenzoxy-L-serylglycylglycine benzyl ester (VIII). A check of the nitrogen balance, however, revealed that appreciable quantities of nitrogenous material had been retained by the column. This material could be eluted
with methanol. It contained a petroleum ether-soluble compound which after further purification gave nitrogen and phosphorus values in agreement with those required by theory for phenylphosphoryl bis(N-carbobenzyloxy-L-serylglycylglycine benzyl ester).

The concurrent removal of the phenyl-, benzyl-, and carbobenzoxy groups
of compound VIII by catalytic hydrogenolysis in glacial acetic acid in the presence of a mixture of platinum and palladium catalysts gave \(O\)-(distearoyl-L-\(\alpha\)-glycerylphosphoryl)-L-serylglycylglycine (IX). The phosphatidyl tripeptide, a white solid melting with decomposition from 181-182°, was obtained in over-all yields ranging from 10 to 12 per cent. This product is soluble in warm chloroform, 95 per cent ethanol, or glacial acetic acid, but insoluble in ether, petroleum ether, or acetone. In its optical behavior it resembles the lecithins and cephalins, rather than phosphatidylserine, in that its specific rotation (\(+7.6^\circ\)) is closer to that of the corresponding lecithin (\(+6.1^\circ\)) or cephalin (\(+6.0^\circ\)) than to that of phosphatidylserine (\([\alpha]_{D}^{20} -18^\circ \neq [\alpha]_{D}^{40} -6.0^\circ\)). Moreover, the optical activity of the phosphatidyl tripeptide fails to show the marked temperature effect exhibited by phosphatidylserine (12).

Possession of the phosphatidyl tripeptide permitted us to carry out a few experiments, which, we hoped, would yield some information regarding the action of alcohol on lipoproteins and lipopeptides. The failure of the usual fat solvents (ether, petroleum ether, benzene, or chloroform) to extract phosphatides quantitatively from plant and animal tissues is well known. The remarkable efficiency of ethanol and other alcohols for this purpose seemed to indicate some influence more complex than mere solvent action. It has been suggested that the alcohols cause dissociation of certain linkages in the lipoprotein complexes, some of which are so weak that they can be ruptured by ether when an outer barrier (presumably water) has been removed, while others seem to require the more drastic treatment with boiling alcohol. However, it has never been established satisfactorily whether or not the action of alcohol, which is known to release phosphatides from lipoproteins, could cause the rupture of peptide bonds in lipoproteins or lipopeptides. To test this rather unlikely possibility, a 2 per cent solution of the phosphatidyl tripeptide in 95 per cent ethanol was kept at 80° for 30 minutes. The almost complete recovery (96 per cent) of unchanged phosphatidyl tripeptide, on cooling to room temperature, indicates that under these conditions none of the bonds has been ruptured. Thus it seems unlikely that the treatment of organs or tissues with hot alcohol to extract the phosphatides involves rupture of peptide bonds. The conclusion seems warranted that the alcohol-soluble phosphatides of tissue lipoproteins represent material that is not bound to protein by covalent bonds.

With regard to firmly bound (chemically bound?) phosphatides of some natural lipoproteins (such as lipovitellin) and lipopeptides, much of the present uncertainty as to their structure can be attributed to the lack of methods which would allow one to separate these moieties from protein
without causing structural changes in the phosphatide moiety. 2 years ago (29) we reported that cephalins and phosphatidylserines, but not lecithins, when treated with diazomethane, are cleaved at the ester bond linking the phosphatidic acid to the nitrogenous moiety. This cleavage (diazometholysis) which proceeds without structural changes in the phosphatidyl moiety gives in a good yield the optically pure dimethyl ester of the phosphatidic acid, whose structure and configuration can be established readily by comparison with phosphatidic acid esters of known constitution. Subsequently, the reaction was used successfully in the elucidation of the structure of the glycerophosphoric acid moiety of phosphatidylserine (29) that had been isolated from ox brain by the method of Folch. We have now found that diazomethane reacts also with the phosphatidyl tripeptide, giving in a good yield (90 per cent) the dimethyl ester of L-α-distearoylglycerophosphoric acid. This would suggest that the "diazo-metholysis" of natural lipopeptides and lipoproteins may provide a method of setting free "chemically bound" phosphatidyl moieties in a form in which their structure and configuration can readily be determined.

EXPERIMENTAL

\textit{N-Carbobenzoxy-L-serilylglycylglycine Benzyl Ester (III)}—To a solution of 3.6 gm. of glycylglycine benzyl ester hydrochloride (26) and 3 ml. of triethylamine in 30 ml. of dry and ethanol-free chloroform were added 3.3 gm. of \textit{N}-carbobenzoxy-L-serine (12) dissolved in 30 ml. of freshly distilled acetonitrile and 3.0 gm. of dicyclohexyl carbodiimide in 9 ml. of dry chloroform. After standing overnight at room temperature, 75 ml. of dichloromethane were added, and the mixture was filtered with suction. To separate the tripeptide derivative and dicyclohexylurea, the solid material was suspended in 75 ml. of boiling 1,2-dimethoxyethane, and the warm mixture was separated by centrifugation. The precipitate was extracted once more in the same manner with 75 ml. of boiling dimethoxyethane. To the combined extracts were added 300 ml. of petroleum ether (b.p. 30–60°), and the precipitate was recrystallized from 120 ml. of dimethoxyethane. The yield of fairly pure \textit{N}-carbobenzoxy-L-serilylglycylglycine benzyl ester, melting from 148–151°, was 3.6 gm. (56.4 per cent of theory). This material is quite satisfactory for further use. For analysis, 0.36 gm. of the material was treated with two 6 ml. portions of boiling ethyl acetate and washed with low boiling petroleum ether. The benzyl ester which was recovered in a yield of 75 per cent melted from 150–152°; reported melting point 151–153° (26). Mixed melting point with authentic material gave no depression.

\textit{d-α,β-Distearin}—The diglyceride (30) was prepared from d-mannitol via 1,2,5,6-diacetone d-mannitol (31), d-acetone glycerol (31), d-acetone
glycerol α-benzyl ether (30, 32), L-α-benzyl glycerol ether (30, 32), D-α,β-
distearin α-benzyl ether (30, 32), with the exception that the diacetone
D-mannitol was recrystallized from a mixture (1:1, v/v) of benzene and
petroleum ether (b.p. 60–80°), and that the preparation of n-acetone gly-
cerol α-benzyl ether and L-α'-benzyl glycerol ether was carried out by the
modified procedures of Howe and Malkin (32).

O-(Distearoyl-L-α-glycerylphenylphosphoryl) N-Carbobenzoxy-L-serglycyl-
glycine Benzyl Ester; Phosphorylation—In a 200 ml. round bottomed,
3-necked flask equipped with an oil-sealed stirrer, calcium chloride tube,
and dropping funnel were placed 1.68 gm. (8.0 mmoles) of freshly frac-
tionated phenylphosphoryl dichloride, 1.24 gm. of anhydrous quinoline
(9.6 mmoles), and 25 ml. of anhydrous and ethanol-free chloroform. To
the vigorously stirred phosphorylating mixture, which was kept at 25°,
was added dropwise over a period of 2½ hours a solution of 5.0 gm. (8.0
mmoles) of D-α,β-distearin in 50 ml. of chloroform. 1 hour after com-
pleting the addition of distearin, the flask was immersed in a water bath
at 45°, a solution of 3.55 gm. (8.0 mmoles) of N-carbobenzoxy-L-serglycyl-
glycine benzyl ester in 45 ml. of warm lutidine was added rapidly with
stirring, and the mixture was kept for 2 hours at 45°. At the end of this
period, the slightly colored solution was concentrated under reduced pres-
sure, and the residue was freed as thoroughly as possible of quinoline and
lutidine in a high vacuum (0.1 mm. of Hg) at a bath temperature not ex-
ceeding 50°.

Isolation of Compound VIII—The residue was extracted with 50 ml.
of boiling petroleum ether (b.p. 30–60°), and the mixture was separated
by centrifugation. The extraction was repeated four times with 20 ml.
portions of fresh petroleum ether, and the combined petroleum ether ex-
tracts were brought to dryness under reduced pressure. The solid residue
was dissolved in 75 ml. of chloroform, and the solution was washed as
rapidly as possible with 20 ml. portions of ice-cold 1.5 N sulfuric acid until
the acid was free of lutidine and quinoline, as indicated by a negative test
with ammonium reineckate, and with distilled water until the wash water
remained neutral. After drying the chloroform solution with anhydrous
sodium sulfate, the solvent was removed under reduced pressure, and the

1 Commercial 2,6-lutidine was freed of picolines by the method of Cathcart and
Reynolds (33). The purified material was dried by boiling under reflux over barium
oxide and was fractionally distilled by using a Vigreux column.

2 The petroleum ether-insoluble material, on dissolving in chloroform and washing
with cold dilute sulfuric acid, gave a material which, after recrystallization from
acetone, contained nitrogen and phosphorus in a ratio of approximately 2:1. This
ratio suggests that it is mainly a phosphatidyl dipeptide. It is possible that the
terminal amino acid has been removed by the action of phenylphosphoryl dichloride.
A similar observation has been reported by Pascu and Wilson (34).
residue was freed thoroughly of chloroform in a high vacuum at a bath temperature of 35–40°. The solid material, weighing 7 gm., was dissolved in 100 ml. of hot acetone, and the solution was allowed to attain room temperature (25°) gradually. The precipitate, which had formed on standing, was centrifuged off and washed with two 20 ml. portions of acetone. The mother liquor and washings were combined, and the acetone was distilled off under reduced pressure. The residue was taken up in 50 ml. of hot petroleum ether, and the solution, after being allowed to stand overnight at room temperature, was freed by centrifugation of a slight precipitate. The clear solution, containing approximately 4 gm. of material, was placed on a chromatographic column which had been prepared by pouring a slurry of 50 gm. of silicic acid3 in petroleum ether (b.p. 35–60°) into a glass tube of 45 cm. height and 2.8 cm. diameter while tapping the side of the tube firmly with a rubber mallet (35). The column was washed successively with 200 ml. of low boiling (b.p. 35–60°) petroleum ether (Eluate 1), 300 ml. of a mixture of 4 volumes of low boiling petroleum ether and 1 volume of diethyl ether (Eluate 2), and 300 ml. of diethyl ether (Eluate 3); the eluates were collected separately. On removal of the solvents under reduced pressure (bath temperature 30–35°), Eluate 1 left only traces of a solid residue, while Eluate 2 gave 1.2 gm. of solid material which consisted largely of bis(distearoyl-L-α-glyceryl)phenylphosphate (calculated, P 2.24, N 0.0; found, P 2.20, N 0.3), and Eluate 3 gave 1.8 gm. (18.7 per cent of theory) of fairly pure distearoyl-L-α-glycerylphenylphosphoryl N-carbobenzoxy-L-serylglycylglycine benzyl ester.

\[
\text{C}_{67}\text{H}_{110}\text{O}_{14}\text{N}_2\text{P} (1206.5). \text{Calculated.} \quad \text{N 3.48, P 2.57} \\
\text{Found.}^4 \quad " 3.40, " 2.50 \\
\quad " 3.59, " 2.44
\]

O-(Distearoyl-L-α-glycerylphosphoryl)-L-serylglycylglycine (IX)—A solution of 1.8 gm. of distearoyl-L-α-glycerylphenylphosphoryl N-carbobenzoxy-L-serylglycylglycine benzyl ester (VIII) in 50 ml. of lukewarm glacial acetic acid, together with 0.5 gm. of platinum oxide5 and 0.5 gm. of palladium black (37), was placed in an all-glass hydrogenation vessel of 250 ml. capacity, and the mixture was vigorously shaken in an atmosphere of pure hydrogen6 at an initial pressure of about 50 cm. of water until the absorption of hydrogen ceased. After displacing the hydrogen with nitrogen, the mixture was transferred to a centrifuge tube, the precipitate was

3 The silicic acid (Merck reagent grade) was sifted to remove particles finer than 74 μ (United States sieve No. 200).

4 Analyses of several independent preparations.

5 The catalyst was prepared as described (36), except that the sodium nitrate was replaced by an equimolecular amount of potassium nitrate.

6 Electrolytically produced hydrogen was used.
brought into solution by warming the mixture to 65°, and the catalyst was removed by a brief centrifugation of the warm mixture. The mixture of catalysts was extracted with two 10 ml. portions of warm glacial acetic acid. The clear supernatant solutions were combined and kept at +6° for 1 hour. The precipitate was collected with suction on a Büchner funnel, washed with a few ml. of cold glacial acetic acid, followed by ether, and dried in vacuo over sodium hydroxide pellets. This material was trituated with 10 ml. of chloroform, filtered with suction, and washed on the filter with four 5 ml. portions of chloroform. The dried material, weighing 0.77 gm. (57 per cent of theory, over-all yield 10.6 per cent) and melting from 180–181°, was dissolved in 15 ml. of warm chloroform. To the solution were added dropwise and with swirling 10 ml. of acetone, and the mixture, while still warm, was centrifuged briefly. The clear supernatant solution was decanted and kept at 6° for several hours. The phosphatidyl tripeptide was centrifuged and resuspended in acetone, and the mixture was separated by centrifugation. After repeating the treatment with acetone once more, the substance was dried in vacuo at room temperature; recovery 85 per cent, m.p. 181–182° (decomposition), [α]D +7.6° (±0.2°) in ethanol-free chloroform (c, 5). The rotation does not change measurably within the temperature range 15–40°. The phosphatidyl tripeptide is insoluble in ether, petroleum ether, or acetone, slightly soluble in glacial acetic acid at room temperature (20–25°), and readily soluble in warm (60°) glacial acetic acid, 95 per cent ethanol, or chloroform.

C₄₅H₆₀O₁₂N₃P (906.2). Calculated. C 60.96, H 9.78, N 4.68, P 3.42

Found. 61.02, 9.66, 4.78, 3.40

61.28, 9.87, 3.49

A solution of 50 mg. of the phosphatidyl tripeptide in 2.5 ml. of warm 95 per cent alcohol was kept in a bath at 80° (±1°) for 30 minutes. After standing for 2 hours at room temperature (24°), the precipitate was centrifuged and dried in vacuo over calcium chloride. The recovered material weighed 48 mg. (96 per cent of theory). Its melting point and phosphorus content (calculated, P 3.42; found, P 3.43) agreed with those of unchanged phosphatidylserglycylglycine.

Diazometholysis of Phosphatidyl Tripeptide—The diazometholysis of distearoyl-L-α-glycerylphosphoryl-L-σerylglycylglycine (150 mg.) and the isolation and purification of the phosphatidic acid ester were carried out as described for phosphatidylserine (29), except that a mixture of chloroform and ethanol (10:1, v/v) was used as solvent for the phosphatidyl
tripeptide and that the chromatographic purification of the cleavage product was carried out in ether solution. The distearoyl-L-α-glycerophosphoric acid dimethyl ester, which was obtained in a yield of 89 per cent of theory, on recrystallization from methanol melted from 50–51°. Authentic distearoyl-L-α-glycerophosphoric acid dimethyl ester melts at 52–53° (29); mixed melting point 51–52°.

L-Serylglycylglycine—The removal of the protective groups of N-carbobenzoxy-L-serylglucylglycine benzyl ester by catalytic hydrogenolysis and the purification of the tripeptide by reprecipitation from water with alcohol were carried out as described by us in an earlier publication (26). The tripeptide, which was obtained in a yield of 80 per cent of the theory, possessed the specific rotation \( \alpha \)D\n\( \pm 31.8° \) (1 N HCl; c, 5.6) and molecular rotation \( \alpha \)D\n\( \pm 69.7° \); reported for serylglycylglycine (26) \( \alpha \)D\n\( \pm 32.5° \) (1 N HCl; c, 5.4), \( M_D \)\n\( \pm 71.4° \). Both rotations are higher than those reported for L-serylglucose (38), i.e. \( \alpha \)D\n\( \pm 30.2° \) (1 N HCl), \( M_D \)\n\( \pm 48.9° \). On suspecting that this anomaly was caused by the acid medium, the optical activity of both peptides was determined in distilled water. In this solvent, the specific rotation of L-serylglucose, \( \alpha \)D\n\( \pm 34.0° \), not only is lower than that of L-serylglucose, \( \alpha \)D\n\( \pm 45.5° \) (c, 1.8), but also, in accordence with theoretical considerations, the molecular rotations of both peptides are now practically identical, +745° and +738°, respectively.

**SUMMARY**

The synthesis of a phosphatidyl tripeptide has been accomplished. It was prepared by (a) phosphorylation of D-α,β-distearin with phenylphosphoryl dichloride and pyridine, (b) esterification of the resulting distearoyl-L-α-glycerylphenylphosphoryl chloride with N-carbobenzoxy-L-serylglycylglycine benzyl ester in the presence of lutidine, and (c) removal of the protective groups by catalytic hydrogenolysis. The O-(distearoyl-L-α-glycerylphosphoryl)-L-serylglucylglycine was obtained in an over-all yield of 12 per cent.

The synthesis of N-carbobenzoxy-L-serylglucylglycine benzyl ester, for which two procedures have been reported, has been simplified considerably by condensing N-carbobenzoxy-L-serine with glycylglycine benzyl ester by means of \( N,N' \)-dicyclohexylcarbodiimide.

The phosphatidyl tripeptide is cleaved by diazomethane with the formation of the dimethyl ester of its phosphatidic acid moiety. This suggests that diazomethane may prove to be a valuable analytical tool in the elucidation of the structure of natural lipopeptides and lipoproteins.

The authors acknowledge with gratitude the support of their work by a Research Grant from the National Research Council, Division of Medicine, Ottawa, Canada.
BIBLIOGRAPHY


