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SYNTHESIS OF LYSOPHOSPHATIDYLSERINE WITH 19:4 ACYL GROUP, AS A NOVEL SODIUM-POTASSIUM ATPASE INHIBITOR, IN RELATION TO DLIS-2, AN ENDOGENOUS DIGOXIN-LIKE SUBSTANCE

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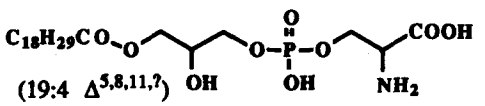
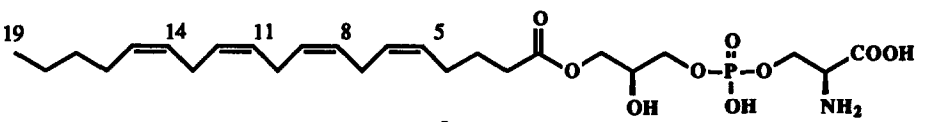
SUMMARY: A lysophosphatidylserine with 19:4 acyl group proposed as a candidate of DLIS-2, an endogenous digoxin-like substance was chemically synthesized. The synthetic compound showed a significant activity of Na^+,K^+-ATPase inhibition, which has been accepted as a major indicator activity for the essential hypertension.

It has been postulated that a participation of the circulating natriuretic factor, which inhibits Na^+,K^+-ATPase,[1] in the pathogenesis of essential hypertension is enhanced with sodium-loading. From this point of view, the endogenous digitalis-like factors have been searched in many laboratories.[2] As one of these factors, DLIS-2 (Digoxin-Like Immunoreactive Substance-2) (1) was isolated from human plasma of patients with essential hypertension,[3] and its structure was elucidated by the mass spectroscopic study by Kenny *et al.*, although the position of a double bond in a fatty acyl group and their geometric configurations were ambiguous.[4] The further structural study encountered with difficulty because of a small amount available from human source.



Proposed structure of DLIS-2 (1)



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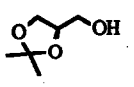
Thus, we attempted to establish the structure of this substance synthetically, especially the feature of unsaturation in the fatty acyl part, with guidance of the biological activities for synthetic DLIS analogs with various acyl groups. For this purpose the acyl group was introduced in the final step of this synthesis of the molecule in order to facilitate the substitution of fatty acyl groups.

In this study, we synthesized the DLIS analog 2 with 19:4 ($\Delta^{5,8,11,14}$) fatty acyl group corresponding to the structure proposed by Kenny *et al.* Isopropylideneglycerol 3 and benzylideneglycerol 4 were prepared by the usual methods.[5,6] The two serine derivatives, Fmoc-L-Ser(H)-OFm (5) and Boc-L-Ser(H)-OBu^t (6),[1] were

synthesized as follows: for preparation of **5**, Fmoc-L-Ser(Bu^t)-OH was esterified with 9-fluorenylmethanol and dicyclohexylcarbodiimide in the presence of 4-dimethylaminopyridine as a catalyst, and then the *t*-butyl group was removed with aqueous TFA. When this deprotection reaction was carried out under anhydrous conditions, the deprotected product was converted to its trifluoroacetate quantitatively. Another serine derivative **6** was prepared starting from Boc-L-Ser(Bzl)-OH according to the same concept. Formations of the phosphodiester linkages between either of *sn*-1,2-protected glycerols (**3**, **4**) and the serine derivatives (**5**, **6**) were performed at -20°C in THF by the phosphorodichloridite method[7] followed by oxidation with hydrogen peroxide according to the result of the preliminary investigation on the reaction procedure, especially for the order of phosphitylation and the reaction temperature. The compound **7** was adopted as a key intermediate of this synthesis, since **7** is superior to **8** in respect of a stability of the phosphotriester obtained. The phosphitylation reaction by the chloridite method for **5** carried out in the larger scale gave poor yield of the desired product **7**, but afforded the



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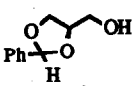

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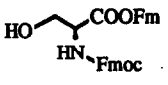

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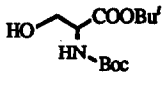

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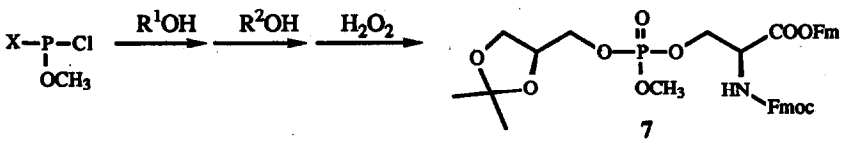

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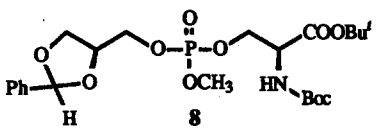
Table 1. Results of the Formation of Phosphate **7** from **3** and **5**.

X	R^1OH	R^2OH	Temperature	Yield(%)
Cl	3	5	-78	~0
Cl	3	5	-50	11
Cl	3	5	-18	22
Cl	3	5	4	16
Cl	3	5	18	5
Cl	5	3	-18	50
Cl	5	3	4	~0
	3	5	20	43
	5	3	20	77

* 1H-Tetrazole was used as the activator in the second stage of the reaction.



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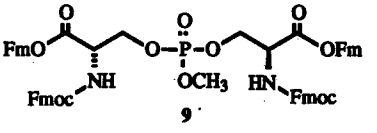

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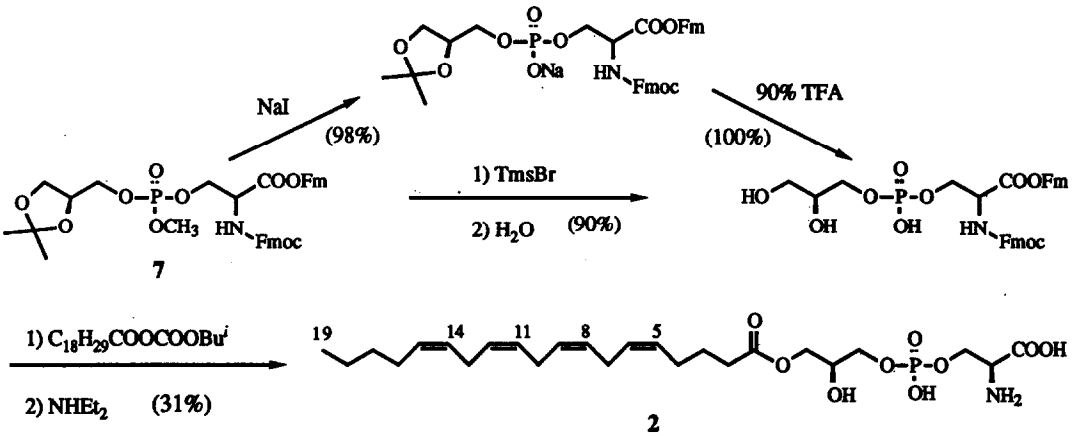
compound **9** as a major product, since there seems to be not enough difference in the reactivities between the two stages of the phosphitylation. Then we tried the monochloromonoamidite method[8] which gave the satisfactory result for achievement of the stepwise phosphitylation, even if the larger-scale preparation was applied. The result of the reactions was summarized in Table 1.



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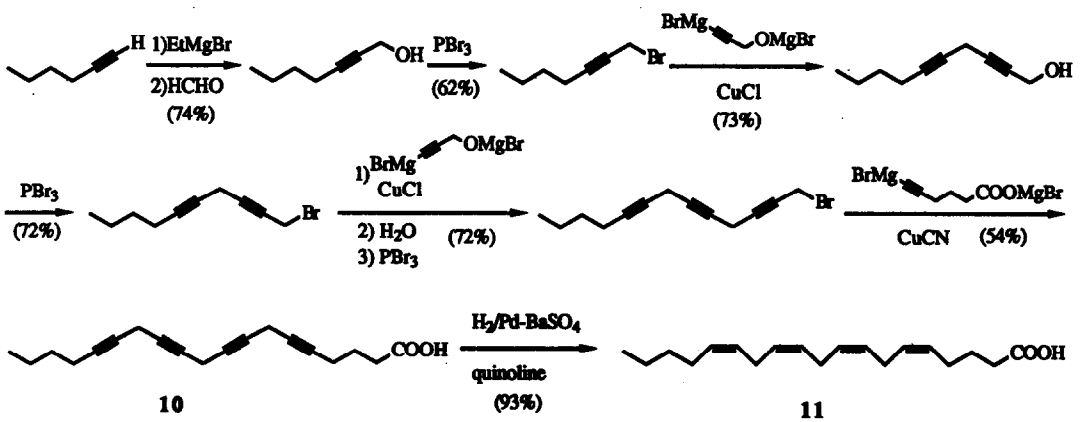
The methyl group of phosphate **7** was removed with sodium iodide in 2-butanone and then the isopropylidene group was deprotected with aqueous trifluoroacetic acid to avoid a cyclization of the phosphate which occurred in case of the reverse order of these deprotection procedures. Although the one-step deprotection with bromotrimethylsilane[9] was possible, the stepwise procedure was preferable in respect of the ease of purification.



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Nonadecatetraynoic acid (**10**) was synthesized according to the acetylide coupling method used in the synthesis of *cis*-docosatetraenoic acid by Sprecher.[10] The tetrayne fatty acid **10** thus obtained was hydrogenated in the presence of Pd-$BaSO_4$/quinoline catalyst to give all-*cis* nonadecatetraenoic ($\Delta^{5,8,11,14}$) acid (**11**). The structure of **11** was assigned by ^{1}H- and ^{13}C-NMR in comparison with arachidonic acid. The monoacylation of the glycerol moiety at 1-position with the acid **11** was performed by the mixed anhydride method. The structure of the product was assigned in ^{1}H-NMR. The removal of Fmoc and Fm groups was subsequently achieved by treatment with diethylamine in DMF.[11] This reaction procedure seems to be applicable for another unsaturated fatty acyl group, since no disturbance may be arisen from the unsaturated bond in series of the reactions.

The lysophosphatidylserine **2** thus synthesized inhibited the Na^+,K^+-ATPase from dog kidney (Sigma A-0142) with IC_{50} of approximately 10^{-5} M.[12] This result strongly suggested that the synthetic compound **2** can not be denied as a possible structure of DLIS-2 as far as the ATPase inhibition is concerned. We are now engaging in study on the structure-inhibitory activity relationship of DLIS analogs, whose results will be soon reported in detail elsewhere.

REFERENCES AND NOTES

1. Abbreviations: ATPase, adenosine 5'-triphosphatase; Boc, *t*-butoxycarbonyl; Fm, 9-fluorenylmethyl; Fmoc, 9-fluorenylmethoxycarbonyl; IC_{50}, the concentration required for 50% inhibition of enzyme.
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12. The inhibitory activity of this lysophosphatidylserine is approximately one tenth of that of ouabain, a hypertensive steroid, but comparable to that of SPAI-1, an endogenous Na^+,K^+-ATPase inhibitory peptide, recently characterized by Eisai group (K. Araki, J. Kuroki, O. Ito, M. Kuwada, and S. Tachibana, *Biochem. Biophys. Res. Commun.*, **164**, 496 (1989)).

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