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EVALUATION OF (2*S*,4*S*)/(2*R*,4*R*) AND (2*S*,4*R*)/(2*R*,4*S*) 6,6-*N*,*N*-DIMETHYL-2-METHYL-2-OXO-1,3-DIOXA-4-HEXADECYL-6-AZA-2-PHOSPHACYCLOOCTANE BROMIDE AS INHIBITORS FOR PROTEIN KINASE C, CARNITINE OCTANOYLTRANSFERASE, AND CARNITINE PALMITOYLTRANSFERASE

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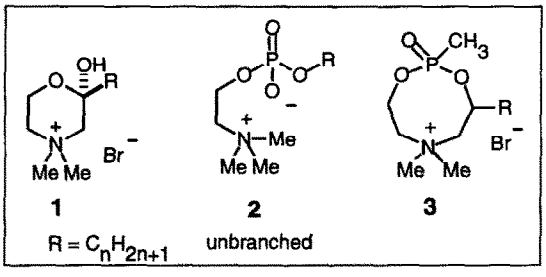
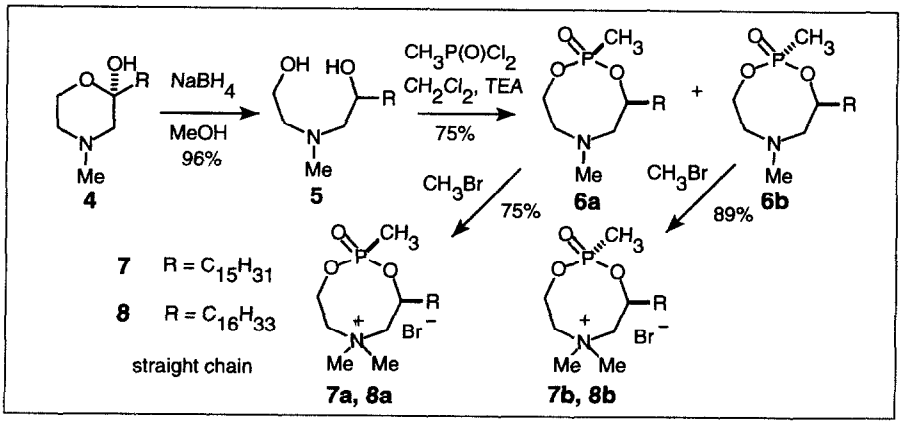
Abstract: (2*S*,4*S*)/(2*R*,4*R*) and (2*S*,4*R*)/(2*R*,4*S*) 6,6-*N*,*N*-dimethyl-2-methyl-2-oxo-1,3-dioxa-4-hexadecyl-6-aza-2-phosphacyclooctane bromide strongly inhibited protein kinase C and moderately inhibited carnitine octanoyltransferase and carnitine palmitoyltransferase.

Lipids that contain a quaternary ammonium group are present in biological membranes, facilitate fat metabolism, and regulate cellular functions, e.g., signal transduction.[1,2] Our hemicholinium, **1**, and related lipids[3] inhibit protein kinase C (PKC),[4] a key enzyme in signal transduction. The membrane component phosphatidylcholine,[5] and a single-chain analogue, hexadecyl phosphatidylcholine (miltofosine), **2**, also inhibit PKC.[6] A combination of the two structures might prove more effective. To test this idea, we have made an eight-membered phosphorous heterocycle,[7] **3**, with a quaternary ammonium ion and a alkyl side chain. By using a phosphonate in lieu of phosphate we can probe any



that arise from stereochemistry. Herein, we describe the syntheses, and inhibition constants of the two diastereomers of **3** for PKC and two enzymes that use quaternary ammonium lipids, carnitine palmitoyltransferase (CPT) and carnitine octanoyltransferase (COT).

Condensation of 1-bromo-2-octadecanone[4] with 2-(*N*-methylamino)ethanol produces **4**. Reduction of **4** gives the aminodiol **5**, which when treated with methyl phosphonic



dichloride in methylene chloride in the presence of triethylamine cyclizes to the diastereomers, **6a** and **6b**, which are separable by column chromatography. Quaternization of each isomer with CH_3Br produces **8a** and **8b**. Single crystal analysis of **7a**, (Figure 1) a lower homolog of **8a**, reveals that the methyl group on the phosphorus and the alkyl chain are cis to each other. By comparing the ^1H NMR spectra of **7a**, **8a**, and **8b**, we assign the structures of **8a** and **8b**.

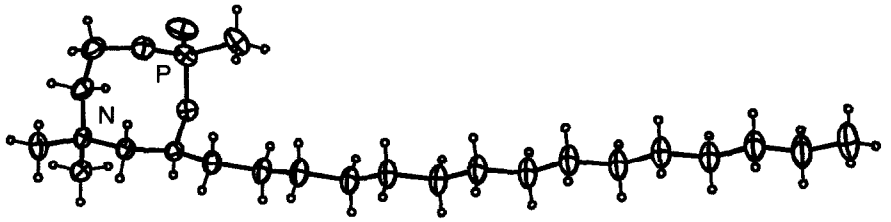


Figure 1. ORTEP drawing of **7a**. The bromide counter ion is not shown.

We have assayed **8a** and **8b** as inhibitors for PKC. (Table I) The inhibition studies are done with recombinant PKC, which is partially activated with tetradecyl phorbol acetate.[8]

Diastereomer **8a** inhibits twice as well as **8b**. Compounds **8a** and **8b** behave like the hemicholinium lipids and probably, sphingolipids.[9] In comparison, phosphatidylcholine inhibits PKC that is activated only by calcium and phosphatidylserine. When phorbol 12,13-dibutyrate is added, phosphatidylcholine does not inhibit; it activates.[10]

Table I. Inhibitory Potencies, IC_{50} (μM), of **8a** and **8b** on Protein Kinase C, Carnitine Octanoyltransferase, and Carnitine Palmitoyltransferase

Compound	PKC	COT	CPT-II
8a	4.8*	475 ± 25	128 ± 12
8b	9.9*	912 ± 88	305 ± 5

Mean values ± SD for two or more determinations except * which are single determinations.

Because palmitoylcarnitine inhibits PKC, we have assayed **8a** and **8b** as inhibitors of COT[11] and CPT-II.[12,13,14] (Table I) These compounds moderately inhibit CPT-II and COT. Compared to hemicholinium inhibition of COT (IC_{50}, 1300-4000 μM) and CPT-II (IC_{50}, 32-235 μM),[15] **8a** and **8b** are better for COT and worse for CPT-II. Hemipalmitoylcarnitinium (**HPC**), a carboxymethyl derivative of **1**, potently inhibits purified CPT-II[14] and strongly inhibits both CPT-I[16] in intact mitochondria and purified COT.[14] Adding a carboxymethyl group to the morpholinium ring substantially improves inhibition.

In conclusion, these data encourage us to prepare the individual enantiomers to probe enantioselectivity in PKC inhibition. The factor of two in IC_{50}'s for all three assays may indicate a common structural feature in the recognition sites on the enzymes or just be coincidence. The improved inhibition for COT when comparing **3** and **1** suggests that a carboxymethyl analog of **3** might be a potent inhibitor of COT. We are continuing this project with these efforts in mind.

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References and Notes

1. Hilggard, P.; Klenner, T.; Stekar, J.; Unger, C. *Cancer Chemother. Pharmacol.* **1993**, *32*, 90.

2. Endo, K; Igarashi, Y.; Nisar, M.; Zhou, Q.; Hakomori, S-i. *Cancer Res.* **1991**, *51*, 1613.

3. Gandour, R. D.; Kumaravel, G. U. S. Patent No. 5,196,418.

4. Kumaravel, G.; Ashendel, C. L.; Gandour, R. D. *J. Med. Chem.*, **1993**, *36*, 177.

5. Kaibuchi, K; Takai, Y.; Nishizuka, Y. *J. Biol. Chem.*, **1981**, *256*, 7146.

6. Geilen, C. C.; Haase, R.; Buchner, K.; Wieder, T.; Hucho, F.; Reutter, W. *Eur. J. Cancer*, **1991**, *27*, 1650.

7. Godovikov, N. N.; Vikhreva, L. A.; Kabachnik, M. I. *Zh. Obshch. Khim.*, **1975**, *45*, 728.

8. Compounds were assayed in duplicate for the inhibition of PKC using the published method[4] with slight modifications. Instead of rat brain PKC, we used a mixture (1:1) of recombinant PKC (from mouse) alpha and beta-2 (prepared by expression in insect cells and partially purified). The compounds were assayed in MeOH and the concentration ranged from 160-0.016 μg/ml (5 values).

9. Hannun, Y. A.; Bell, R. M. *Science*, **1989**, *243*, 500.

10. Nakadate, T.; Blumberg, P. M. *Cancer Res.*, **1987**, *47*, 6537.

11. The enzyme was purified from bovine liver by the published method. Ramsay, R. R.; Derrick, J. P.; Friend, A. S.; Tubbs, P. K. *Biochem. J.* **1987**, *244*, 271.

12. The enzyme was purified from bovine liver by the published method. Clarke, P. R. H.; Bieber, L. L. *J. Biol. Chem.* **1981**, *256*, 9861.

13. COT and CPT-II were assayed as described previously.[14]

14. Nic a' Bháird, N.; Kumaravel, G.; Gandour, R. D.; Krueger, M. J.; Ramsay, R. R. *Biochem. J.* **1993**, *294*, 645.

15. Kumaravel, G; Nic a' Bháird, N.; Ramsay, R. R.; Gandour, R. D. (Unpublished results)

16. Gandour, R. D.; Leung, O.-t.; Greway, A. T.; Ramsay, R. R.; Nic a' Bháird, N., Fronczek, F. R., Bellard, B. M.; Kumaravel, G. *J. Med. Chem.* **1993**, *36*, 237.

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