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A NOVEL CATALYST FOR O-ACYLATION IN LIPID CHEMISTRY

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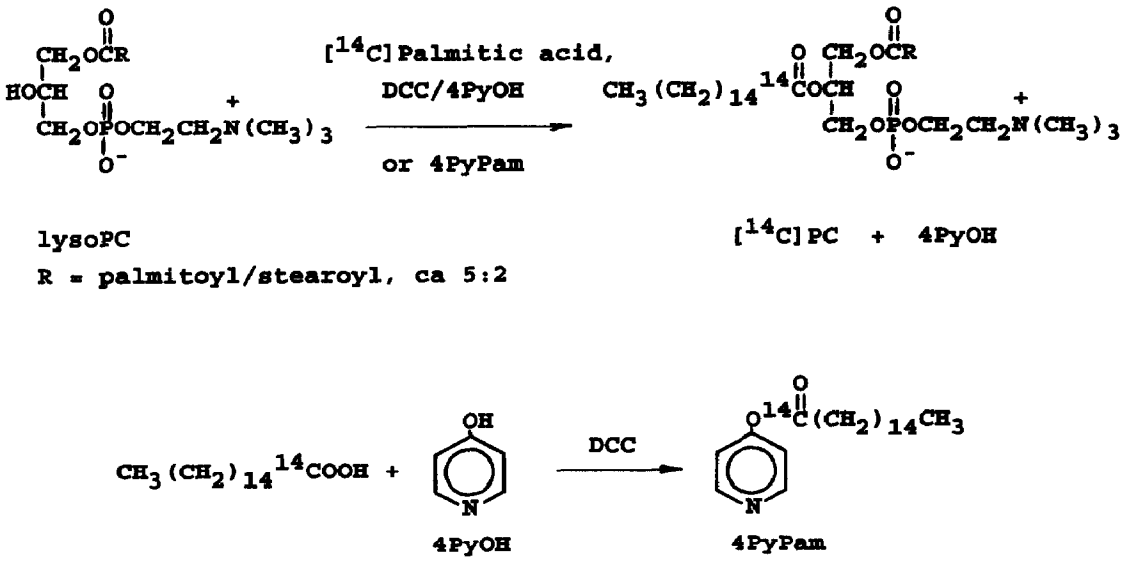
Abstract: *A new route for acylation of lysophosphatidylcholine (lysoPC) by condensation it with fatty acid/dicyclohexylcarbodiimide (DCC) under catalysis of 4-hydroxypyridine (4PyOH) is described; as suggested O-acylation proceeds via formation of the activated 4PyOH-ester. This one-pot method does not need fatty acid excess, it is especially suitable for the small-scale syntheses of phosphatidylcholines (PC) with precious or labile (e.g. photoaffine) fatty acid residues.*

The most routine procedure for the synthesis of common phospholipids (phospatidylethanolamine, phosphatidylinositol etc) modified in fatty acid residue at 2-position (or in both residues) of the glycerol backbone is the enzymatic transesterification of the corresponding **PC**. The latter in turn may be obtained by acylation of **lysoPC** (or glycerophosphocholine) with a modified fatty acid. This step is complicated by low 2-OH group reactivity; a wide variety of fatty acid derivatives were proposed as acylating agents: acylchlorides[1], anhydrides[2], imidazolides[3,4], trifluoroacetic anhydrides[5], 2-thiopyridyl esters[6] etc. All these methods are not quite perfect because of side product formation[1], need of elevated temperature[1,3] or strong base[4] action, of the fatty acid derivative excess, and low yields[1,5].

The most convenient method for the **lysoPC** (or glycero-3-phosphocholine and relative compounds) acylation was proposed by Gupta et al.[7] who used for this purpose moderate amounts of fatty acid anhydrides with 4-dimethylaminopyridine (**DMAP**) as a catalyst. Reaction proceeds under mild conditions and gives as a rule good yields; a number of improvements of the method has been published, among them the use of 4-pyrrolidinopyridine (**PPy**) as a catalyst[8,9] and of ultrasound treatment for the reaction accelerating[10] should be noted. But some excess of fatty acid anhydride is needed even here; a detailed kinetic study[11] has shown that the best results of **lysoPC** acylation with 1.5 equivalent of anhydride (5-min reaction time, 90% yield) were achieved by application of 200-fold **PPy** excess. The procedure requires strict anhydrous conditions which are difficult to achieve in small-scale

synthesis. Besides, acylation with anhydrides is resulted even theoretically in loss of a half of original fatty acid, which can be re-cycled not in each case. A one-pot version of the synthesis was developed[12] where **lysoPC**, fatty acid, **DCC** and a catalyst were reacted in one step; with moderate excesses of fatty acid this method ensured acceptable (20-50%), but not always reproducible yields of end product. To reduce N-acylurea formation in the last procedure it was suggested to apply *p*-toluenesulfonic acid[13]. However, when dealing with the synthesis of labile photoaffine PC the presence of such acidic catalyst is undesirable since it causes the photoaffine group damage (data for 2-diazocyclopentadienecarbonyl group, unpublished results).

Elaborating the last procedure for the small-scale acylation of **lysoPC** with [^{14}C]palmitic acid, we found that the use of unpurified **PPy** (Fluka) containing up to 12% of admixtures as a catalyst gives higher yields of the product ([^{14}C]**PC**) in comparison with the use of chromatographically pure **PPy**. Our further studies revealed that this phenomenon was brought about by a more strong catalyst presenting in crude **PPy**, which appeared to be **4PyOH** (MS, *m/z*: 95 (M^+); ^{1}H-NMR in D_6-DMSO, δ, ppm: 6.01 (broadened, 1H, OH), 6.46 (dd, 2H, 3-H and 5-H), 7.97 (dd, 2H, 2-H and 6-H);



Scheme 1

lit.[14]: 5.10, 6.22, 7.74)), the original substance for **PPy** synthesis. Yields of [^{14}C]**PC** in runs catalysed with **PPy** or **4PyOH** are presented in Table 1. The data show that the **4PyOH** use instead of **PPy** leads to the considerable rise of [^{14}C]**PC** yield. The nitrogen atom in the aromatic ring of **PPy** is much more nucleophilic than **4PyOH**'s one because of the substancial electron donation of pyrrolidine ring. So high

catalytic activity of **4PyOH** cannot be explained by its basic properties exclusively. The only possible supposition on the mechanism of catalysis is the formation of intermediate ester - 4-pyridyl[^{14}C]palmitate (**4PyPam**) - which subsequently acylates secondary OH group in **lysoPC** molecule (Scheme 1).

Indirectly such reaction scheme is confirmed by the following. When taken in equivalent amounts **4PyOH** doesn't catalyze the acylation significantly. It seems to be due to the reduction of the intermediate ester formation speed (and accordingly the rise of N-acylurea by-product formation). By the other hand moderate excess of **4PyOH** (2.5 eq) in the presence of the same quantity of more strong base, **PPy**, leads to the significant increase of the acylation rate. It may be connected with the additional base-catalysed **lysoPC** acylation by intermediate ester. In the case of 5-fold excess of **4PyOH** the latter may serve both as an alcohol to provide sufficiently rapid formation of intermediate ester and a base which catalyzes the transesterification step. The evidence of the intermediate ester formation in **lysoPC** acylation with [^{14}C]palmitic acid in the presence of **4PyOH** was obtained by direct synthesis of **4PyPam** from **4PyOH** and palmitic acid in the presence of **DCC** (yield 86%; FAB-MS: 334 = 333[M] + 1[H^+]). The substance with identical chromatographic properties was detected in the reaction mixture within **lysoPC** acylation process (TLC data).

Table 1. Yields[a] of [^{14}C]**PC** on the Acylation of **lysoPC** with [^{14}C]Palmitic Acid and **DCC**[b].

Catalyst	Reaction time (hours)		
	2	17	24
PPy (5 eq)	12	30	32
4PyOH (5 eq)	66	72	73
PPy (2.5 eq) + 4PyOH (2.5 eq)	59	73	75
4PyOH (1 eq)	3	3.5	3.5

a. Yield of [^{14}C]PC was estimated as a ratio (in %) of corresponding spot radioactivity to the total radioactivity of all zones, from sample origin to solvent front on TLC silica plate (system: chloroform/methanol/water, 65:25:4, by vol., visualization by phosphomolybdic acid).

b. LysoPC (trifluoroacetate salt, 2 μmol) thoroughly dried in high vacuum overnight, [^{14}C]palmitic acid (2 μmol, 100 μCi) and appropriate amounts of catalyst in 0.3 ml of dry chloroform were treated with DCC (3 μmol as 20% solution in CCl_4), the mixture was stirred in argon atmosphere.

An unusual O-acylation with activated ester prompted us to examine the possibility of a similar reaction with activated esters of 1-hydroxybenzotriazole, N-hydroxysuccinimide and 2-pyridinol which are widely used for N-acylation. None of three substances catalyzed the acylation of **lysoPC**, alone or in the presence of triethylamine.

Thus we have a unique example of base-catalyzed O-acylation by intermediate activated ester, which may be of a good practical value, especially for syntheses of esters with the precious or labile fatty acids residues. In our hands, acylation of 2 μmol **lysoPC** with equimolar quantity of [^{14}C]palmitic acid gave after gel filtration and reversed phase chromatography 58% yield of [14C]**PC**; analogous synthesis with **lysoPC** and photoaffine 12-(2-diazocyclopentadienecarbonylamino)-[^{14}C]dodecanoic acid led to the corresponding photoaffine ^{14}C-labeled **PC** with 54% yield.

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