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Efficient synthesis of 3-*O*-thia-cPA and preliminary analysis of its biological activity toward autotaxin

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ABSTRACT

The efficient synthesis of 3-*O*-thia-cPAs (**4a–d**), sulfur analogues of cyclic phosphatidic acid (cPA), has been achieved. The key step of the synthesis is an intramolecular Arbuzov reaction to construct the cyclic thiophosphate moiety. The present synthetic route enables the synthesis of **4a–d** in only four steps from the commercially available glycidol. Preliminary biological experiments showed that **4a–d** exhibited a similar inhibitory effect on autotaxin (ATX) as original cPA.

PHYLPA **1**, which was discovered in 1992 by Murakami-Murofushi et al. from myxoamoebae of a true slime mold, *Physarum polycephalum*, is the first example of a naturally occurring cyclic phosphatidic acid (cPA) (Fig. 1).[1] Later, cPAs were found in mammalian organs such as human sera and porcine brain.[2a,b] cPA is regarded as a cyclic derivative of lysophosphatidic acid (LPA), which elicits a number of physiological effects such as cell proliferation, invasion and metastasis of cancer cells. Previously, we demonstrated that cPAs **2a–d** induce biological effects that are contrary to those elicited by LPA. These observations suggest that cell proliferation, invasion and metastasis of cancer cells are controlled by LPA and cPA. We focused on the synthesis of enzymatically and chemically stable derivatives of cPA-oriented molecules in order to develop novel agents of medicinal importance. The concentration of LPA is controlled through product inhibition of autotaxin (ATX, nucleotide pyrophosphate/phosphodiesterase-2) by LPA.[3] Thus, it seemed likely that cPA could interact with ATX resulting in the inhibition of cancer cell invasion and metastasis. In this context, we previously prepared carba analogues of cPA, 2-*O*-ccPA **3a–d** and 3-*O*-ccPA **3′a–d**. These carba analogues were found to be potent inhibitors of ATX activity and to exhibit more potent activities than cPAs **2a–d** in the suppression of cancer cell invasion and metastasis.[4a,b] We were also interested in the preparation and biological activity of the sulfur analogue of cPA, 3-*O*-thia-cPA **4** (Fig. 1). In the field of nucleic acid, it is well known that the replacement of a phosphate oxygen atom with a sulfur atom results in an increase in stability to nuclease-mediated and base-catalyzed hydrolysis.[5] We report here the preparation of **4** and its preliminary biological activity toward ATX.

In order to synthesize several analogues of 3-*O*-thia-cPAs, a concise approach toward the target molecules was required. For this purpose, 3-*O*-thia-cyclicphosphate III was selected as a common intermediate, and we planned to construct the cyclic thiophosphate moiety via the intramolecular Arbuzov reaction of the phosphate II, which could readily be prepared from hydroxythiol I (Scheme 1).

We first investigated a synthetic route in a racemic form. Thus, the synthesis of 3-*O*-thia-cPAs commenced with glycidyl ether **5**, which is derived from (±)-glycidol (Scheme 2). The epoxide **5** was converted to the disulfide **8**, the precursor of the intramolecular Arbuzov reaction, in three steps: (1) epoxide opening with thioacetic acid, (2) methanolysis, and (3) sulfenylation with 2,4-dinitrobenzenesulfenyl chloride. When disulfide **8** was treated with $PCl(OMe)_2$ the initially formed phosphite **9** was found to undergo a simultaneous intramolecular Arbuzov reaction to give the desired cyclic thiophosphate **10**.[6] The chemical structure of **10** was confirmed by means of NMR (^1H NMR, ^{31}P NMR and HH-COSY) and mass spectrometry.

We next attempted a deprotection of the PMB group of **10**. We investigated a range of conditions, such as oxidative cleavage using DDQ or CAN (Table 1, entries 1–3), Lewis acid-mediated

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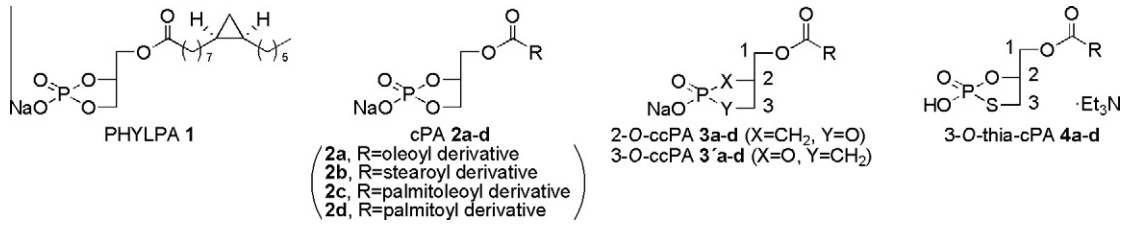
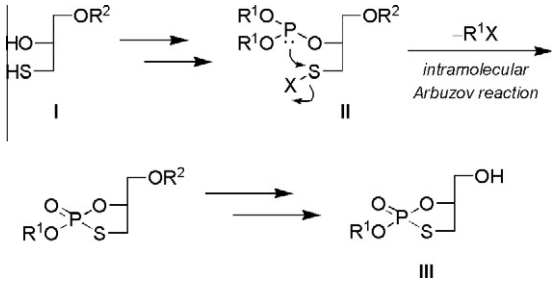
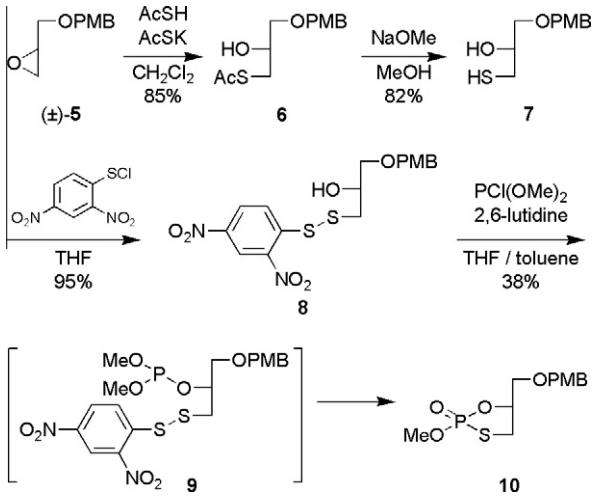


Figure 1. Structure of PHYLPA **1** and its analogues **2**–**4**.



Scheme 1. Construction of cyclic thiophosphate moiety.



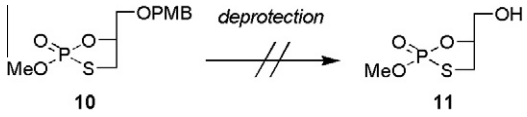
Scheme 2. Synthesis of cyclic thiophosphate **10**.

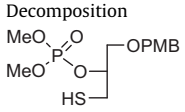
deprotection (entries 4–7) and hydrogenolysis (entry 9), but were unable to obtain the desired compound **11**. In each case, the cyclic thiophosphate moiety decomposed resulting in the formation of a complex mixture of products.

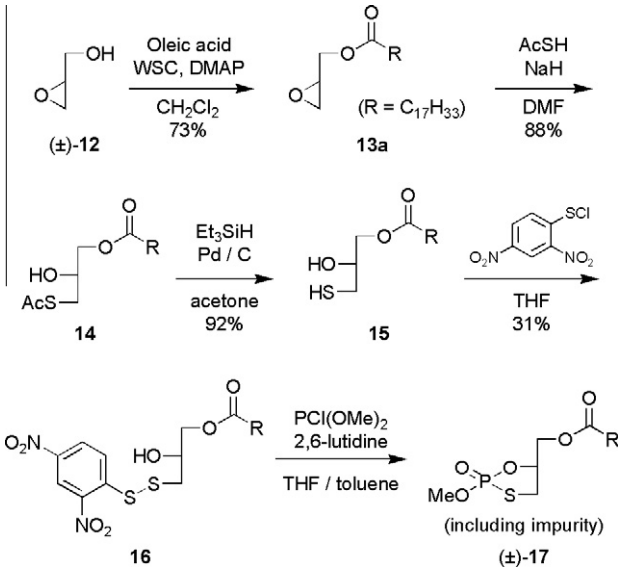
As an alternative approach, we examined the construction of the cyclic thiophosphate with as a 1-*O*-acyl derivative (Scheme 3). At first, racemic glycidol **12** was esterified with oleic acid using WSC and DMAP. Epoxide **13a** was cleaved with sodium thiolacetate, and the thiol ester was reductively cleaved with Et_3SiH and Pd/C to give thiol **15**.[7] Thiol **15** was sulfenylated, and the resulting disulfide **16** was treated with $PCl(OMe)_2$ in a similar manner to the case of PMB ether **8**. Although the desired cyclic thiophosphate **17** was detected in the crude mixture, we were unable to isolate **17** in a pure form due to the presence of several polar by-products that proved to be inseparable. We reasoned that the relatively high nucleophilicity of the thiolate anion might cause undesirable side reactions. Therefore, we investigated using an alternative leaving group.

Next, we examined the thiocyanide group. Thiocyanide could be obtained from the epoxide **13a** in one step by the reaction with

Table 1
Deprotection of PMB group



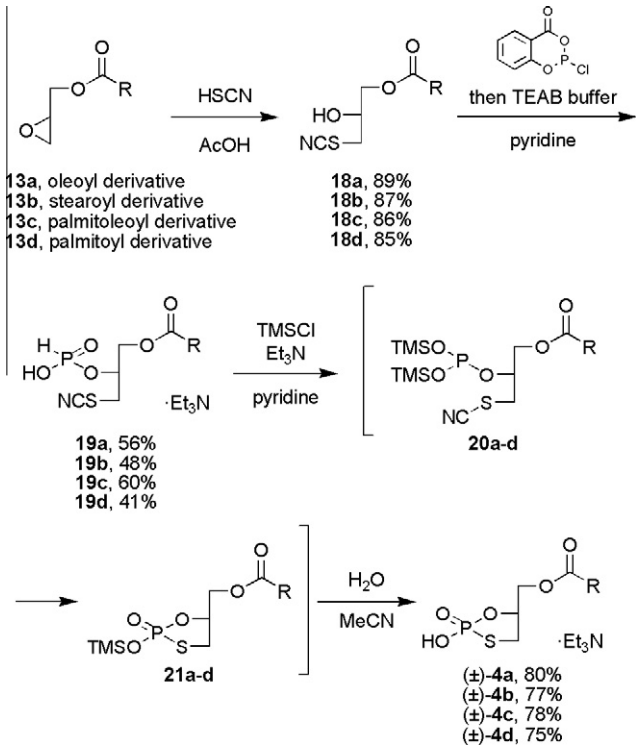
Entry	Reagent	Solvent	Temp.	Result
1	DDQ	CH_2Cl_2/H_2O	rt	Decomposition
2	DDQ	CH_2Cl_2/buffer	rt	Decomposition
3	CAN	$MeCN/H_2O$	rt	Decomposition
4	BBr_3	CH_2Cl_2	−78 °C	Decomposition
5	BCl_3	CH_2Cl_2	−78 °C	Decomposition
6	$MgBr_2$, Me_2S	CH_2Cl_2	−78 °C	Decomposition
7	TFA, anisole	CH_2Cl_2	rt	Decomposition
8	H_2, Pd/C	MeOH	rt	



Scheme 3. Synthesis of cyclic thiophosphate **17**.

thiocyanate. The cyanide group was then anticipated to act as a leaving group in the Arbuzov reaction.[8] We intended to employ the trimethylsilyl phosphite **20** as an Arbuzov reaction precursor in order to obtain the free 3-*O*-thia-cPA directly. Preparation of the Arbuzov reaction using the thiocyanate derivative is summarized in Scheme 4.

Treatment of the epoxide **13a** with HSCN in acetic acid afforded the thiocyanate **18a** in 89% yield. Epoxide opening was found to



Scheme 4. Synthesis of 3-*O*-thia-cPA **4a**.

proceed smoothly under acidic conditions. Then, the resulting **18a** was reacted with salicylchlorophosphite, followed by hydrolysis with TEAB buffer to give monoalkylphosphite **19a**.[9] *H*-Phosphonate moiety of **19a** was confirmed by the characteristic large P–H coupling constant (631 Hz) in ^{1}H NMR spectroscopy. We were delighted to obtain the desired cyclic thiophosphate **4a** in 80% yield as a triethylammonium salt by the treatment of **19a** with trimethylsilyl chloride and triethylamine in pyridine, followed by hydrolysis in acetonitrile. The reaction proceeded with the initial formation of bistrimethylsilyl phosphite **20a**, and the latter, in turn, underwent a simultaneous intramolecular Arbuzov reaction. The cyanide group was found to act as an excellent leaving group in the present Arbuzov reaction. The chemical structure of oleoyl ester **4a** was confirmed by means of NMR (^{1}H NMR, ^{31}P NMR and HH-COSY) and mass spectrometry.

We were thus able to establish an efficient route to 3-*O*-thia-cPA starting from commercially available glycidol.[10] In a similar way, stearoyl derivative **4b**, palmitoleoyl derivative **4c** and palmitoyl derivative **4d** were synthesized from corresponding glycidyl esters **13b–d** in four steps with an overall yield of 32%, 40% and 26%, respectively.

These 3-*O*-thia-cPAs **4a–d** were subjected to an ATX inhibition assay as previously described.[11] The catalytic activity of ATX in conditioned medium (CM) from MDA-MB-231 cells was significantly inhibited by 3-*O*-thia-cPAs in a dose-dependent manner. Moreover, variation in the acyl chain of these derivatives did not significantly affect the degree of ATX inhibition (Fig. 2). 2-*O*-ccPA **3c** was reported to be the most potent ATX inhibitor among naturally occurring cPA and chemically synthesized carba-analogues of cPA.[4a,12] Oleoyl derivatives of cPA **2a**, 3-*O*-thia-cPAs **4a–d** and palmitoleoyl derivative of 2-*O*-ccPA **3c** at 10 μM were found to give 25%, 55% and 66% inhibition, respectively. So the order of ATX inhibitory potency by cPA analogues was as follows: 2-*O*-ccPA **3c** > 3-*O*-thia-cPAs **4a–d** > cPA **2a**.

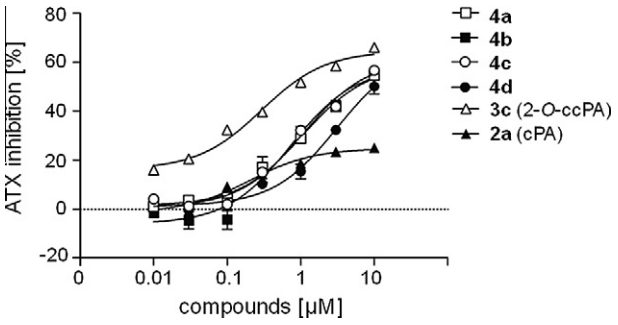


Figure 2. Dose–response relationship of ATX inhibition by 3-*O*-thia-cPAs **4a–d**, 2-*O*-ccPA **3c** and cPA **2a**.

In summary, we have established an efficient route to synthesize the sulfur-analogues of cPA, 3-*O*-thia-cPAs **4a–d**, from glycidol. The key feature of the present synthesis is an intramolecular Arbuzov reaction to construct the cyclic thiophosphate moiety. Preliminary biological experiments showed that 3-*O*-thia-cPAs exhibit a similar inhibitory effect on ATX as original cPA. The synthesis of enantiopure 3-*O*-thia-cPA and detailed evaluation of its biological activities is now underway.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedures, characterization data; ^{1}H and ^{13}C NMR spectra for new compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.05.083.

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