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SYNTHESIS AND PLA_2-INHIBITORY PROPERTIES OF 2(*R*)-ACETAMIDO-ALKYLPHOSPHOMETHANOLS WITH A VARIABLE AGGREGATE ANCHOR

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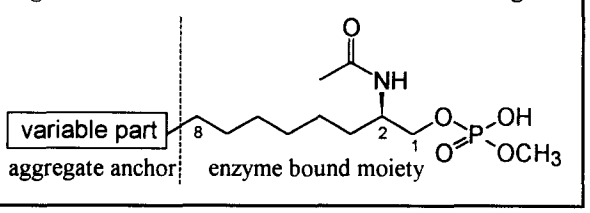
Abstract: Acylamino inhibitors of 14 kDa-PLA_2 were synthesized which differ in the moiety that is not bound into the enzyme's active site but immersed in the lipid aggregate when a ternary inhibitory complex is formed. Our results indicate that this part of the inhibitors does not significantly influence inhibitory properties as long the amphiphilic character is retained. So, inhibitory and biophysical properties should be variable independently.

Phospholipases A_2 (PLA_2) catalyze the hydrolysis of *sn*-2 fatty acids from membrane glycerophospholipids. Increased activity of human non-pancreatic secretory PLA_2 (hnps PLA_2) has been demonstrated in several inflammatory and non-inflammatory diseases such as rheumatoid arthritis, septic shock, ARDS and neoplastic diseases.[1-5] Inhibition of hnps PLA_2 may therefore be a promising therapeutic strategy. Acylaminophospholipids are a class of potent substrate analogous inhibitors of hnps PLA_2 and other 14 kDa-PLA_2.[6-10] They form ternary inhibitory complexes consisting of the enzyme, an inhibitor molecule and the substrate aggregate.[11] It is known from X-ray studies that both the polar headgroup and the amide moiety of acylaminophospholipids are bound into the active site of 14 kDa-PLA_2.[12-14] In contrast, the apolar part of long chain acylaminophospholipids is not completely bound into the enzyme, but is partly immersed in the substrate aggregate when a ternary complex is formed.[15,16] From the inhibitory properties of acylaminophospholipids and related inhibitors investigated by us[17] and others [9,10] we concluded that in ternary complexes the apolar parts of 2-acylamino-alkylphospholipids are immersed in the lipid aggregate approximately from the nineth carbon of the alkyl chain up as shown in fig. 1. This is in line with an X-ray structure of hnps PLA_2 complexed with an acetamido-alkylphospholipid inhibitor published by Oh.[13] To investigate the influence of the moiety of acylaminophospholipids immersed in the lipid aggregate (in the following referred to as aggregate anchor) on PLA_2 inhibition we synthesized a series of 2(*R*)-acetamido-alkylphosphomethanols with identical enzyme bound part but with aggregate anchors differing in both size and polarity.

Figure 1. General structure of the inhibitors investigated



Chemistry[18]

The central steps to synthesize the chiral inhibitors **11b-g** (fig. 3) were Wittig reactions of the respective

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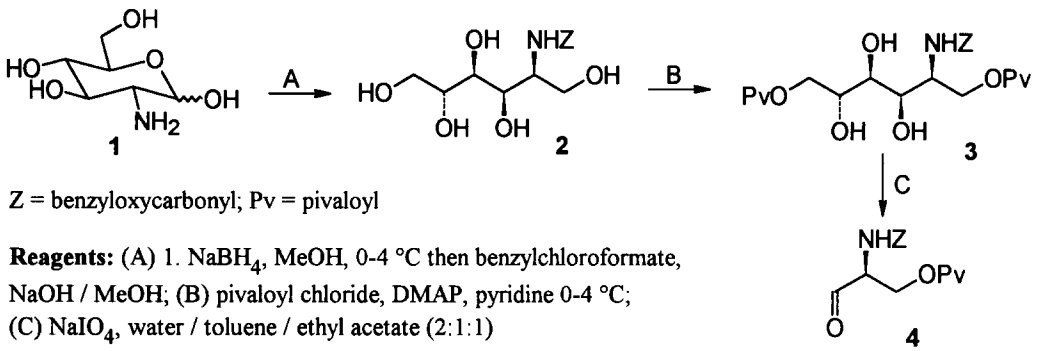
triphenylphosphonium bromides **6a-c**[19] with *N*-Z-*O*-Pv-(*S*)-serinal (**4**), which was prepared from glucosamine (**1**) (fig. 2). Reduction of the anomeric center of **1** with sodium borohydride and subsequent Z-protection of the amino group afforded **2**. After column filtration to remove borates, the primary hydroxyl groups were selectively pivaloylated to afford **3** as a powder in 45 % yield with respect to **1**. In order to avoid racemization, the aldehyde **4** was prepared from **3** by periodate oxidation only immediately before the following Wittig reaction and reacted as crude product without previous chromatographic purification.

Due to the presence of equimolar amounts of pivaloyloxyacetaldehyde in the crude **4**, two equivalents of the respective triphenylphosphonium bromides **6a-c** had to be applied in the Wittig reactions (fig. 3). Deprotection of the Witttig products **7a-c** was accomplished by acidic cleavage without affecting the benzyl ether in **7a**. The oxazaphospholane intermediates[20,21], generated by reacting **8a-c** with methyl dichlorophosphate, were opened with 6 N HCl to afford the zwitterionic phosphomethanols **9a-c**. Acetylation (to give **10a-c**) and hydrogenolysis afforded the inhibitors **11b-d**. The esters **11e-f** were prepared from the alcohol **11d** using the respective acid chlorides.[22]

Enantiomeric purity was checked by ^{19}F NMR spectroscopy. For this purpose, **8c** was hydrogenated to give 2(*R*)-amino-1-octadecanol which was quantitatively reacted with excess mosher's acid chloride[23] (ee ≥ 98 %).

Figure 2. Synthesis of *N*-Z-*O*-Pv-(*S*)-serinal (**4**)



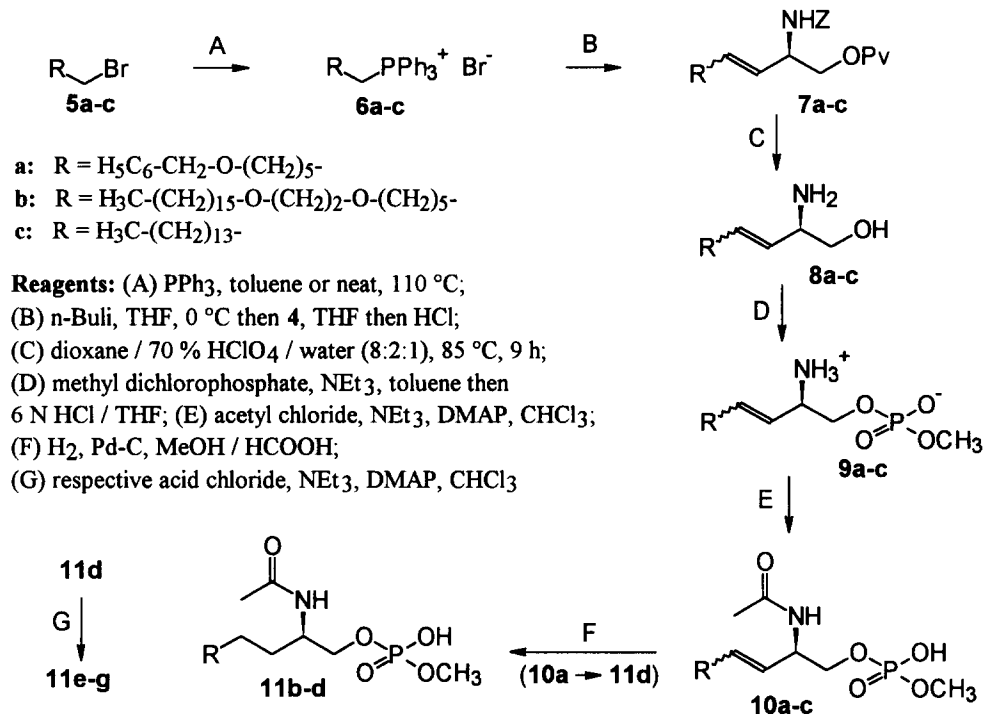
Z = benzyloxycarbonyl; Pv = pivaloyl

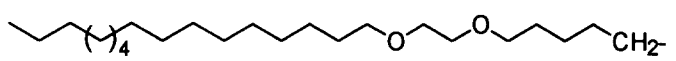
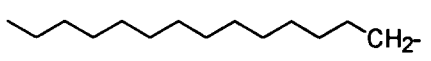
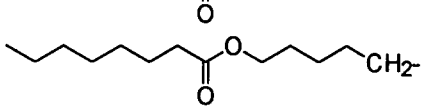
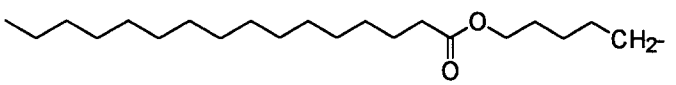
Reagents: (A) 1. $NaBH_4$, MeOH, 0-4 °C then benzylchloroformate, NaOH / MeOH; (B) pivaloyl chloride, DMAP, pyridine 0-4 °C; (C) $NaIO_4$, water / toluene / ethyl acetate (2:1:1)

Discussion

The inhibitors **11b-g** listed in fig. 3 have identical enzyme bound parts, but different aggregate anchors (compare fig.1). The non-amphiphilic compound **11d** is a poor inhibitor, whereas the inhibitory properties[24,25] of all other (amphiphilic) compounds are comparable with those of other potent PLA_2 inhibitors.[6-10,26] This difference is likely due to the accumulation of amphiphilic but not hydrophilic inhibitors in the substrate aggregate, i.e. in the immediate vicinity of the active enzyme. Within the amphiphilic inhibitors (**11a-c,e-f**) both the overall size of the aggregate anchor and the insertion of ester or ether groups into it only marginally affect inhibition. These results imply that biophysical parameters of acylaminophospholipids such as critical micelle concentration (CMC) and hydrophilic-lipophilic balance (HLB) values can be varied by modifying the aggregate anchor without relevantly affecting the inhibitory properties as long as the overall amphiphilic character is

Figure 3. Synthesis and PLA_2-inhibitory properties[24] of the 2-acetamido-alkylphosphomethanols **11b-g**



Structure of R	No	IC_{50} (μM)[25]	$X_i(50)$[25]
	**11b**	1.7	0.00029
	**11c**	3.4	0.00057
	11d	350	0.058
	11e	2.3	0.00039
	**11f**	3.1	0.00052
	**11g**	5.0	0.00084

retained. As biophysical properties influence pharmacokinetics, it should be possible to combine enhanced inhibitory power (which can be achieved by modifying the enzyme bound moiety of the inhtitors as demonstrated by others[14,26]) with an adaptation of the parmacokinetic behavior to different pharmacologic needs by varying size and polarity of the inhibitors' aggregate anchors.

References and Notes

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18. All compounds reported gave spectral data in accord with assigned structures.
19. The educts **5a** and **5b** were prepared according to standard procedures by alkylation of benzylic alcohol or ethylene glycol, respectively.
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Kley, J. T.; Unger, C.; Massing, U. *Biochim. Biophys. Acta* **1998**, *1392*, 193
25. IC_{50} values of amphiphilic PLA_2 inhibitors (**11b,c,e-g**) depend on the substrate concentration applied in the assay. To allow comparison of our data with results from others $X_i(50)$ values are given in addition to the IC_{50} values. X_i = [inhibitor] / ([substrate] + [inhibitor])
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