Orally Active Squalene Synthase Inhibitors: Bis((acyloxy)alkyl) Prodrugs of the α-Phosphonosulfonic Acid Moiety

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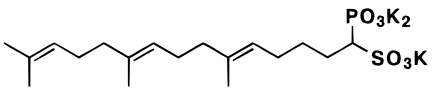
In the previous communication,[1] we reported that α-phosphonosulfonic acids (α-PSAs) are potent inhibitors of squalene synthase *in vitro*. A number of these inhibitors exhibited promising *in vivo* activity on intravenous dosing. However, potency upon oral dosing was poor (po-ED_{50} > 15 μmol/kg). The oral absorption of **1** in rats was low (3%), consistent with its high oral/iv ED_{50} ratio. We therefore sought to improve the oral absorption of prototype inhibitors **1** and **2** through a prodrug approach,[2] and our initial efforts in this direction are described herein.



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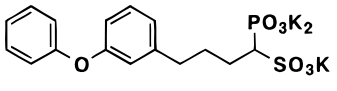
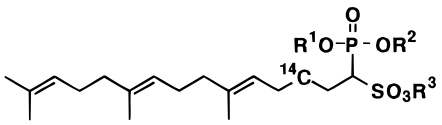


2

We surmised that the triacidic nature of the α-PSA inhibitors was the factor limiting oral absorption. For compounds with charged groups, masking the charge with a neutral, bioreversible prodrug ester can increase the membrane permeability and, hence, the absorption of the drug.[2] To determine how many acidic functions would have to be masked, we prepared a series of radiolabeled esters of **1** and determined the extent of oral absorption in bile-duct cannulated rats (Table 1).[3] Although both the parent triacid **1** and the monoester **3** were absorbed poorly (⩽6%), the diester **4** and the triester **5** exhibited good oral absorption (80% and 45%, respectively). Not surprisingly, the cyclohexyl sulfonate ester of **5** was found to be chemically unstable under physiological conditions.[4] In addition, triesters such as **5** suffered from poor aqueous solubility, whereas the diesters possessed sufficient solubility. We thus decided to focus our efforts on phosphonate diester prodrugs.

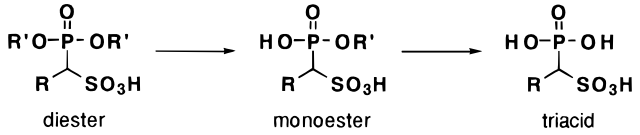
The discovery of an effective prodrug ester for oral administration has unique challenges. The prodrug must have appropriate lipophilicity and stability in order to permeate the intestinal wall. Once absorbed, the promoiety must be readily cleaved, either in the intestinal wall, upon reaching circulation, or at the site of action, to liberate the active parent drug. An effective prodrug must achieve a balance between two opposing tendencies: it must have sufficient stability to the esterases present in the intestinal lumen and mucosa, but must be susceptible to similar esterases in the intestinal wall, the plasma, or the liver. With the α-PSA inhibitors, this scenario is confounded by the need to mask two charges on the phosphonate group. Diester prodrugs must undergo two separate enzymatic conversions (Scheme 1). Since the monoesters (e.g. **3**, Table 1) are poorly absorbed, conversion of the diester to the monoester must occur postabsorption.

Table 1. Oral Absorption of Esters of [^{14}C]-**1** in Bile-Duct-Cannulated Rats

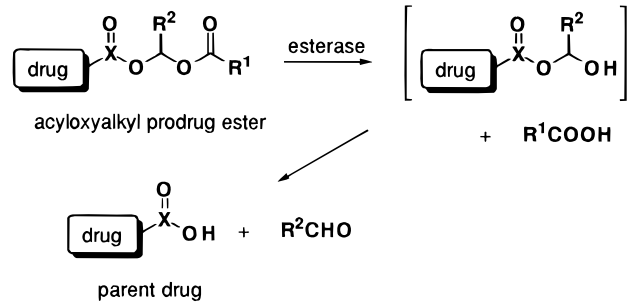


compd	R^1	R^2	R^3	% absorption
1	K	K	K	2.9
3	K	Et	K	6.0
4	Et	Et	K	79.9
5	Et	Et	*c*-C_6H_{11}	45.2

Scheme 1



Scheme 2



In contrast to carboxylate esters, simple diesters of phosphonates are not generally bioconverted to the corresponding free acid.[5] For example, diethyl phosphonate **4** showed no *in vivo* activity upon either iv or po dosing.[6] An alternative strategy is the use of an (acyloxy)alkyl ester, where esterase hydrolysis at the acyloxy group results in the eventual release of the parent drug (Scheme 2).[7,8] This strategy has found wide use with carboxylate drugs and is precedented for phosphinic monoacids with the ACE inhibitor fosinopril.[9] The use of (acyloxy)alkyl esters as prodrugs for phosphorus diacids has received limited attention.[5,10]

As a surrogate endpoint for oral absorption, prodrugs were evaluated in fasted rats for their ability to inhibit cholesterol biosynthesis from acetate.[11,12] In addition, the prodrugs were tested for susceptibility to rat intestinal esterases utilizing a mucosal preparation.[13,14] The results for bis((acyloxy)alkyl) prodrugs of **1** are shown in Table 2. A 4-fold improvement in oral potency over the triacid was observed for **8** and **9**. These two prodrugs exhibited poor iv potency and similar suscep-

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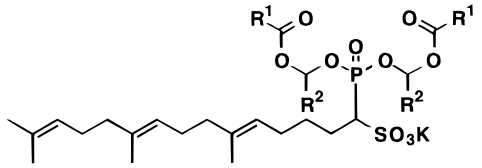
Table 2. Inhibition of Cholesterol Biosynthesis in Rats by Prodrugs of **1**



compd	R^1	R^2	rat model ED_{50} (μmol/kg) iv [SEM]	rat model ED_{50} (μmol/kg) po [SEM]	relative esterase susceptibility[a]
1	parent tri-K^+ salt		0.25 [0.10]	16.5 [1.9]	
6	*n*-hexyl	H	0.18 [0.08]	14.0 [2.9]	6.5
7	*i*-Pr	H	1.7[b]	18.8 [3.0]	8.1
8	*c*-$C_6H_{11}CH_2$	H	−35% at 4	4.1 [1.9]	0.49
9	*t*-Bu	H	−40% at 22	3.9 [1.1]	1
10	Ph	H	1.5[b]	15.0 [4.1]	1.5
11	Et	Me	7.0[b]	11.6 [4.3]	1.7
12	Et	*i*-Pr	nd[c]	−43% at 28	1.1
13	*t*-Bu	Me	nd	−35% at 28	0.20
14	Ph	Me	nd	−33% at 28	0.21

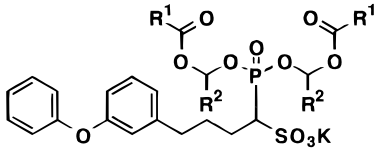
[a] Initial hydrolysis rate by rat intestine mucosal esterases relative to **9**; see ref 13 for further details. [b] Data based upon one determination. [c] nd = not determined.

tibility toward the mucosal esterases. The poor iv potency of **8** and **9** suggests that they were not readily cleaved by plasma or hepatic esterases and were probably converted to inhibitor **1** in the intestinal wall during absorption. Esters **6** and **7** were equipotent to the parent triacid on oral dosing. The higher rate of enzymatic hydrolysis *in vitro* and good potency upon iv dosing suggests that these esters are being converted to **1** in the intestinal lumen prior to absorption. Conversely, prodrugs **13** and **14** exhibited relatively low mucosal esterase susceptibility as well as poor oral activity, suggesting that these inhibitors are converted to **1** too slowly to express good potency. These results indicate that there is a delicate balance between esterase susceptibility and oral efficacy. If conversion is too rapid, preabsorptive hydrolysis occurs in the intestinal lumen. If conversion is too slow, the active substance is not revealed before the prodrug suffers other fates (e.g., excretion or alternate metabolism). In the case of α-PSA **1**, the ((cyclohexylmethyl)carboxy)-methyl (**8**) and (pivaloyloxy)methyl (**9**) esters strike the optimal balance among the derivatives evaluated in this study.

Similar results were obtained with prodrugs of the metabolically stable diphenyl ether α-PSA **2**.[1] The bis-((pivaloyloxy)methyl) derivative **15** was found to be the most potent analog on oral dosing, exhibiting a 6-fold enhancement over the parent triacid **2** (Table 3). Prodrug **15** was also evaluated as a cholesterol-lowering agent in hamsters.[15] At an oral dose of 31 μmol/kg for 5 days, **15** lowered plasma cholesterol levels by 22%, whereas 300 μmol/kg of the parent triacid **2** was required to achieve the same effect. Therefore, a 10-fold increase in oral potency was achieved with this prodrug ester in the hamster model. Consistent with these results, the systemic bioavailability of **2** following an oral dose of **15** in rats was 17%.[16,17] The hepatic oral bioavailability[18] of **2** following an oral dose of **15** in bile-duct-cannulated rats was 28%, indicating significant first pass extraction.

We postulated that prodrugs of **1** and **2** are being absorbed primarily as the intact diesters, not as the corresponding monoesters, on the basis of the data shown in Table 1. This hypothesis is supported by the

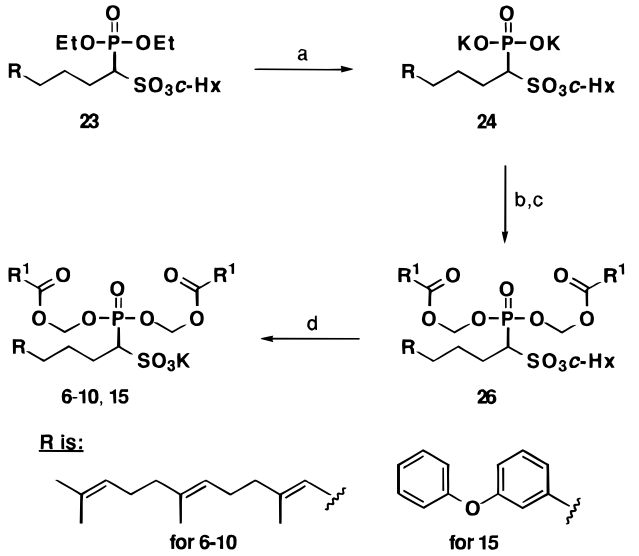
Table 3. Inhibition of Cholesterol Biosynthesis in Rats by Prodrugs of **2**



compd	R^1	R^2	rat model ED_{50} (μmol/kg) iv [SEM]	rat model ED_{50} (μmol/kg) po [SEM]	relative esterase susceptibility[a]
2	parent tri-K^+ salt		0.20 [0.05]	19.5 [5.2]	
15	*t*-Bu	H	1.7 [0.39]	3.5 [1.3]	15
16	Et	Me	nd[c]	12.6[b]	17
17	Et	Et	nd	10.9[b]	nd
18	Et	*i*-Pr	nd	7.9[b]	nd
19	*i*-Pr	Me	nd	10.1[b]	nd
20	*i*-Pr	*i*-Pr	nd	−45% at 28	nd
21	*t*-Bu	Me	nd	12.8 [2.0]	0.45

[a] Initial hydrolysis rate by rat intestine mucosal esterases relative to **9**; see ref 13 for further details. [b] Data based upon one determination. [c] nd = not determined.

Scheme 3[a]

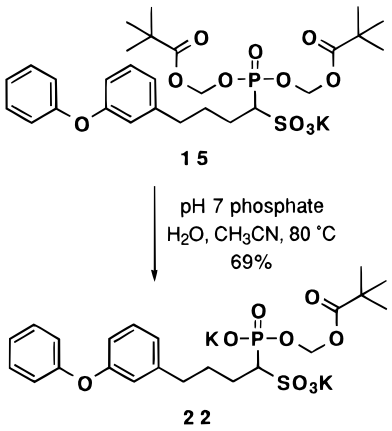


[a] (a) TMSBr, allyl-TMS, CH_2Cl_2, then KOH/H_2O; (b) $AgNO_3$, H_2O; (c) $R^1CO_2CH_2I$ (**25**), toluene, 0 °C (55−70% from **23**); (d) KOAc, CF_3CH_2OH, H_2O, 40 °C (72−97%).

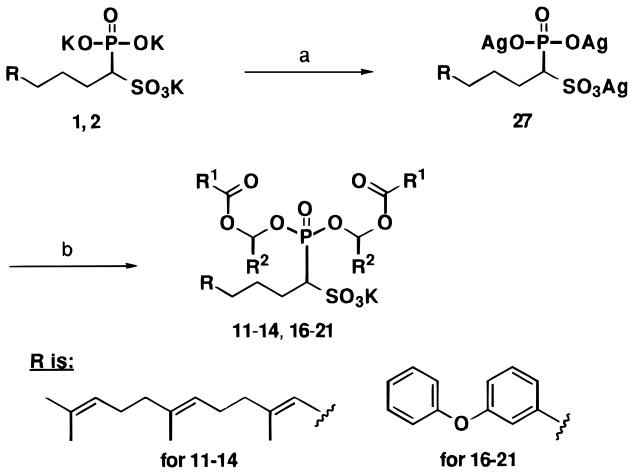
observation that mono((pivaloyloxy)methyl) ester **22** exhibited oral activity in rats (po ED_{50} = 13 μmol/kg) weaker than the corresponding diester **15**. The bioavailability of **2** on dosing **15** to rats is probably limited by the hydrolysis of the prodrug to the monoester and triacid in the intestinal lumen.[19]

The synthesis of bis((acyloxy)methyl) prodrugs (R^2 = H) of **1** and **2** commenced with the selective deesterification of triester **23**[1] with TMSBr[20] (Scheme 3). The resulting potassium salt **24** was converted to the corresponding silver salt and alkylated with iodide **25**[21] to provide the protected prodrug **26**, *via* a procedure similar to that reported by Farquhar.[10f] Removal of the cyclohexyl sulfonate protecting group required very mild reaction conditions, in order to spare the sensitive acyloxymethyl functionality. Solvolysis of **26** in aqueous trifluoroethanol buffered with potassium acetate proved to be an extremely gentle and effective method for deesterification of the cyclohexyl sulfonate. Using this procedure, prodrugs **6**−**10** and **15** were obtained as water soluble potassium salts in pure form directly from

Scheme 4



Scheme 5[a]



[a] (a) $AgNO_3$, H_2O (100%); (b) (i) $R^1CO_2CH(R^2)Cl$ (**28**), 2,4,6-collidine, CH_2Cl_2, (ii) aqueous $KHCO_3$ workup (34−68%).

the reaction mixture. Monoester **22** was prepared by selective hydrolysis of **15** at pH 7 (Scheme 4).

Preparation of the bis((acyloxy)alkyl) prodrugs (R^2 = alkyl) could also be accomplished via the method described in Scheme 3; however, in the final solvolytic deprotection step, formation of the desired product was accompanied by further hydrolysis of one of the prodrug ester side chains, resulting in a mixture of bis- and mono((acyloxy)alkyl) products.[22] This problem was circumvented by performing the esterification with the sulfonate unprotected. The tripotassium salt **1** or **2** was converted to the corresponding trisilver salt **27** as an isolable solid (Scheme 5). Alkylation with the appropriately-substituted chloride **28**[23] followed by an aqueous $KHCO_3$ workup provided the potassium salts of prodrugs **11**−**14** and **16**−**21** directly.

In summary, the systematic application of a prodrug strategy was applied to achieve a large improvement in the oral potency of two highly charged parent drugs. These efforts resulted in α-PSA squalene synthase inhibitors which are potent oral cholesterol lowering agents in animal models. The synthesis and biological evaluation of α-PSAs in optically pure form will be the subject of future publications.

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(3) Four groups of three bile-duct-cannulated rats each were given single 15 μmol/kg oral doses of [^{14}C]-**1** or one of the esters in Table 1. Bile, urine, and feces were collected from each animal for 12 h. Animals were sacrificed at 12 h after dosing. Radioactivity in all samples, including the carcass, was determined by liquid scintillation counting. The absorption was estimated by the sum of radioactivity recovered in bile, urine, and carcass (minus the gastrointestinal tract).

(4) Incubation of triester **5** with boiled plasma and boiled homogenates of liver and intestines from rats (4 h, 37 °C) all resulted in the extensive conversion of triester **5** to the diester **4**, presumably via a solvolytic mechanism.

(5) In two cases, diphenyl esters of phosphonates have been used successfully as prodrugs to improve oral absorption: (a) Serafinowska, H. T.; Ashton, R. J.; Bailey, S.; Harnden, M. R.; Jackson, S. J.; Sutton, D. Synthesis and *in Vivo* Evaluation of Prodrugs of 9-[2-(Phosphonomethoxy)ethoxy]adenine. *J. Med. Chem.* **1995**, *38*, 1372−1379. (b) De Lombaert, S.; Erion, M. D.; Tan, J.; Blanchard, L.; El-Chehabi, L.; Ghai, R. D.; Sakane, Y.; Berry, C.; Trapani, A. J. *N*-Phosphonomethyl Dipeptides and Their Phosphonate Prodrugs, a New Generation of Neutral Endopeptidase (NEP, EC 3.4.24.11) Inhibitors. *J. Med. Chem.* **1994**, *37*, 498−511.

(6) After an oral dose of [^{14}C]-**4** or [^{14}C]-**5** to rats, no [^{14}C]-**1** was detected in bile and urine. The profiles of radioactivity in bile and urine after dosing with [^{14}C]-**5** were identical to those of [^{14}C]-**4**, but different from those of [^{14}C]-**1** after an intravenous dose. These results suggest that the phosphonate esters were not bioconverted to the active acid in rats.

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(13) Esterase susceptibility was determined from the initial rate of aldehyde release upon incubation of the prodrug with homogenized rat small intestinal mucosa. Prodrug (200 μM) was incubated with esterase preparation in 20 mM Tris, pH 7.0 at 37 °C in a 1 mL volume. Aliquots (150 μL) were removed at various times and quenched by addition to 120 μL of 0.5 M sodium acetate, pH 3.8. Released aldehydes were detected by a modification of the method of Sawicki *et al.*[14] Briefly, 30 μL of a 0.5% (w/v) solution of methylbenzothiazolinehydrazone was added to the quenched reaction mixture, the mixture vortexed and incubated at room temperature for 30 min. To this was added 150 μL of 0.25% (w/v) ferric chloride, and the mixture was vortexed and incubated for a further 10 min. Finally, the mixture was diluted with 450 μL of water and allowed to stand at room temperature for 60 min before measuring the absorbance at 625 nm. The corresponding aldehyde was used for a standard curve for quantitation. The esterase susceptibility of **9** was 3.7 pmol of aldehyde released/min per unit esterase, where one unit of esterase is defined as the amount of enzyme which will hydrolyze 1 nmol of 4-nitrophenylacetate/min at 25 °C and pH 7.0 at a substrate concentration of 0.7 mM. No attempt was made to differentiate between the rates of hydrolysis of the diester and the subsequently formed monoester. For prodrugs **6**, **8**, **9**, and **15**, >1 equiv of aldehyde was released during the assay period (3 h), indicating that the second ester was cleaved. In addition, the complete enzymatic hydrolyses of **9** to **1** and **15** to **2** have been demonstrated in the cytosolic fractions of rat liver and intestinal homogenates. In the latter experiments, the formation of **1** and **2** was assayed by an HPLC method with UV detection.

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(15) The hamster cholesterol-lowering model was executed as described in ref 12.

(16) The systemic bioavailability of **2** in rats was calculated on the basis of the ratios of areas under the plasma concentration of **2** vs time curve after an intravenous dose of **2** and an oral dose of **15**. The concentration of **2** was measured by an HPLC method with UV detection.

(17) The systemic bioavailability of the (*S*)-enantiomer of **2** upon oral dosing of the tripotassium salt was <3%.

(18) The hepatic bioavailability of **2** was calculated on the basis of the ratios of the amounts of **2** excreted in bile after an intravenous dose of **2** and an oral dose of **15**. The concentration of **2** was measured by an HPLC−UV assay.

(19) We cannot rule out the possibility that the absorption of monoester **22** is making a contribution to the bioavailability of **2** on dosing the diester **15**.

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(21) Iodides **25** were prepared according to ref 10e, and references cited therein.

(22) A number of deprotection conditions were attempted in order to prevent formation of the monoester byproduct. These included nucleophilic displacement with iodide, thiophenoxide, or azide and elimination with DBU; however, in all cases, the undesired monoester was a major contaminant of the reaction.

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