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Phosphate Prodrugs of PD154075

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Abstract—In the preparation of phosphate prodrugs of PD154075, several strategies of linking a phosphate group to the indole moiety were studied. A novel linker, *p*-hydroxymethylbenzoyloxymethoxycarbonyl, was discovered to provide the phosphate prodrug of PD154075 (compound **9**) with significantly increased aqueous solubility, sufficient stability in aqueous solution and good bio-reconversion in vivo. © 2000 Elsevier Science Ltd. All rights reserved.

The need for more effective anti-emetic agents[1] has promoted further interests in identifying alternative mechanisms by which nausea and vomiting may be mediated. Recently, the tachykinin NK_1 receptor antagonists have demonstrated potent broad-spectrum anti-emetic activity and were effective against both acute and delayed emesis induced by chemotherapeutic agents in animal models.[2] In our investigation, PD154075[3] was a highly potent and selective NK_1 receptor antagonist. However, poor aqueous solubility of less than 1 μg/mL made the formulation of PD154075 problematic, especially for intravenous administration. One of the efforts that has been taken to overcome this problem was to prepare a prodrug of PD154075. The prodrug needs to have sufficient aqueous solubility (at least 5 mg/mL), stability in aqueous solution, and high reconversion in vivo.

The structure of PD154075 is characterized by an absence of functional groups which can be readily derivatized toward a prodrug. The only possible sites for the attachment of a prodrug moiety are the nitrogens of the amide bond, the carbamate bond and the indole moiety. In the past, extensive investigations[4] on the use of amide and carbamate groups for the preparation of prodrugs were taken due to the enormous interest[5] in improving bioavaibility of drug candidates, such as peptide and peptide mimetics. None of the existing prodrug approaches for amide and carbamate functionalities fulfilled our criteria for the prodrugs of PD154075. Although prodrugs for the indole group have been documented,[6] to the best of our knowledge there has been no report on the use of an indole group for the preparation of phosphate prodrugs. Here, we report our investigation on the phosphate prodrugs generated through an indole group.

Phosphate prodrugs as sodium salts are generally freely soluble in water and readily hydrolyzed in vivo by alkaline phosphatase, an enzyme widely distributed in a variety of tissues. As a nonspecific enzyme, alkaline phosphatase catalyzes the hydrolysis of phosphomonoesters, as well as phosphoramidates.[7] Phosphomonoesters are better substrates for alkaline phosphatase than phosphoramidates. Furthermore, phosphate prodrugs derived from phosphoramidates are scarce in the literature.[8] This raised the question of whether a phosphoryl group directly attached to the nitrogen of an indole group would be an enzymatic substrate. A double prodrug approach[4a] with the attachment of a phosphoryl group through an appropriate self-cleavable linker seemed to be more feasible.

Among the choices of self-cleavable linkers for a double prodrug approach, the hydroxymethyl group has been successfully used with a variety of heterocycles containing a NH group,[9] such as phenytoin, allopurinol and 5-fluorouracil. The self-cleavage rate of a hydroxymethyl group is dependent on the pK_a of the NH group to which it is attached. To achieve a cleavage half-life of

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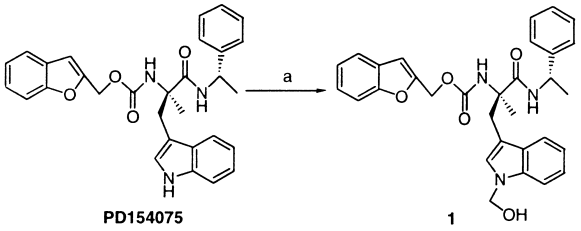
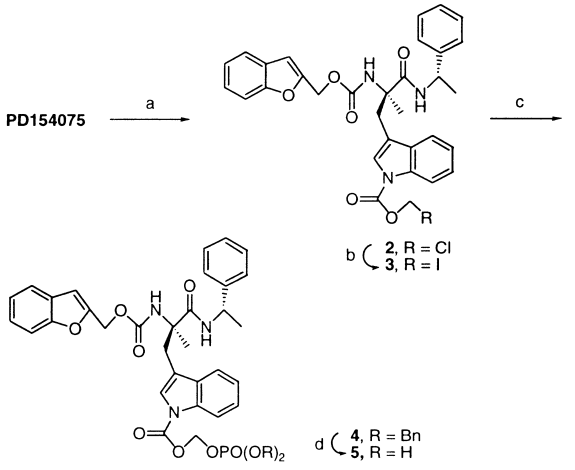
less than 1 h at pH 7.4 and 37 °C, a pK_a of less than 13.1 is required for the parent NH group.[9] The pK_a of the indole moiety of PD154075 was expected to be around 15,[10] which made the use of this approach questionable. This was proved by the preparation of the N^{in}–hydroxymethyl derivative of PD154075 (**1**).

Compound **1** was prepared with deprotonation of PD154075 by potassium bis(trimethylsilyl)amide followed by the addition of monomeric formaldehyde in THF at –78 °C (Scheme 1). Indeed, compound **1** was quite stable with less than 5% reconversion to PD154075 at pH 9 in 4 days, making this approach impractical.

Hydroxymethoxycarbonyl moiety was studied[11] as a self-cleavable linker for phosphate prodrugs and found to be especially useful for amino groups. It was expected that the use of the hydroxymethoxycarbonyl linker, as shown in compound **5**, could overcome the slow self-cleavage problem encountered with hydroxymethyl group. The preparation of compound **5** (Scheme 2) started with the treatment of the potassium salt of PD154075 with chloromethylchloroformate at −78 °C to give the N^{in}-chloromethoxycarbonyl derivative **2**. The chloro group was converted to an iodo group (**3**) under Finkelstein conditions. Coupling of compound **3** with the silver salt of dibenzylphosphate in toluene at reflux gave compound **4**, which was subsequently deprotected by transfer hydrogenation to give compound **5**.



Scheme 1. (a) (1) $KN(TMS)_2$, THF, −78 °C; (2) CH_2O; 70%.

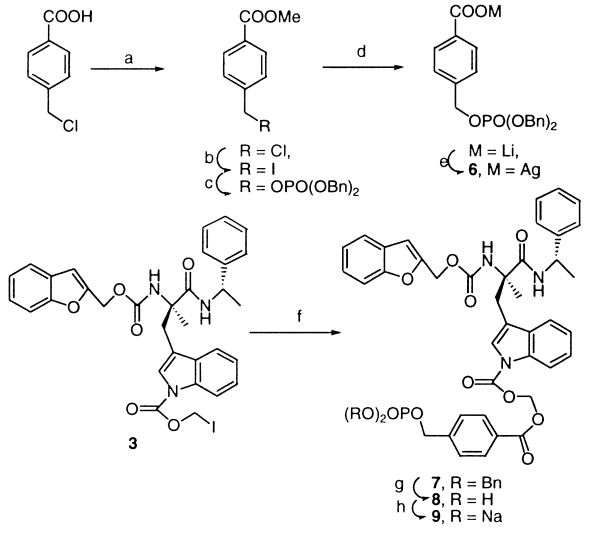


Scheme 2. (a) (1) $KN(TMS)_2$, THF, −78 °C; (2) chloromethyl chloroformate; 99%; (b) NaI, acetone, reflux, 97%; (c) $AgOPO(OBn)_2$, toluene, reflux, 68%; (d) 1,4-cyclohexadiene, 10% Pd/C, 42%.

All attempts to convert **5** to its corresponding sodium salt resulted in rapid decomposition to give back PD154075. The poor stability of **5** under basic conditions was likely due to the accelerated hydrolysis of the carbamate moiety of the linker through intramolecular catalysis of the anionic phosphate group. This was also proposed[11] by Safadi and co-workers for the poor stability of phosphoryloxymethoxycarbonate ester prodrugs. Block of the intramolecular catalysis would likely increase the stability of the prodrug. Bundgaard and coworkers reported[12] the use of *N*-substituted-(aminomethyl)benzoate esters as stabilized forms of amino acid esters. The incorporation of a benzene ring between the amino group and the ester group served to block the facile cleavage of amino acid esters through intramolecular nucleophilic catalysis, intramolecular general base catalysis or general-acid-specific-base catalysis. Using the same idea, we envisioned that incorporation of a toluoyl group between the phosphate and the hydroxymethoxycarbonyl group, as shown in compound **9**, would increase the stability.

The preparation of compound **9** (Scheme 3) started with generation of the silver salt of *p*-(dibenzylphosphoryloxymethyl)benzoate (**6**) from *p*-(chloromethyl)benzoic acid in 5 steps. Coupling of compound **3** and **6** in toluene at reflux gave the dibenzyl protected derivative **7**. Deprotection of **7** by transferhydrogenation gave compound **8** which, in sharp contrast to **5**, was subsequently converted to the disodium salt **9**.

Recently, masked lactones have received attention as self-cleavable linkers for phosphate prodrugs.[13] With the occurrence of rapid lactone formation upon enzymatic cleavage of the phosphate group, the masked lactone approach provided solutions to overcome slow enzymatic cleavage encountered by phosphate prodrugs linked to hindered hydroxy groups, as well as an amino group. However, no example has been reported on the



Scheme 3. (a) CH_2N_2, ether, 75%; (b) NaI, acetone, reflux, 97%; (c) $AgOPO(OBn)_2$, toluene, reflux, 92%; (d) LiOH, THF, MeOH, H_2O; (e) $AgNO_3$, H_2O, 66%; (f) **6**, toluene, reflux, 75%; (g) 1,4-cyclohexadiene, 10% Pd/C, ethanol, 65%; (h) NaOH, H_2O, 20%.

use of this approach on aromatic heterocycles. Two masked lactone linkers were selected to test this approach on the nitrogen of the indole. The masked tetramethyl-substituted dihydrocoumarin,[13a–d] as shown in compound **16**, has been successfully used in a variety of prodrugs and has demonstrated the ability to undergo rapid lactonization due to the 'trimethyl lock' effect. The masked phthalide,[13c] as shown in compound **21**, was used successfully in the phosphate prodrug of taxol. The efficiency of the lactonization (effective molarity) of the corresponding hydroxyacid to form 4,4,5,7-tetramethyl-3,4-dihydrocoumarin (**10**) was reported[14] to be six orders of magnitude higher than that of the corresponding hydroxyacid to form phthalide (**11**) (Fig. 1).

The preparation of compound **16** (Scheme 4) started with the preparation of compound **12** by the procedure of Nicolaou and co-workers.[13b] The coupling of the potassium salt of PD154075 with a mixed anhydride of **12** (compound **13**) at −78 °C give compound **14**. Deprotection of the benzyl groups by transfer hydrogenation followed by the treatment with NaOH gave the disodium salt **16**.

The preparation of compound **21** was completed in a sequential manner (Scheme 5). The coupling of the potassium salt of PD154075 with *o*-chloromethyl benzoyl chloride[15] at −78 °C gave the corresponding chloro derivative (**17**). Iodo-chloro exchange under Finkelstein conditions gave the corresponding iodo derivative (**18**). Coupling of **18** with the silver salt of dibenzyl phosphate in toluene at reflux gave compound **19**. Deprotection of the benzyl groups, followed by the treatment of NaOH gave the disodium salt **21**.

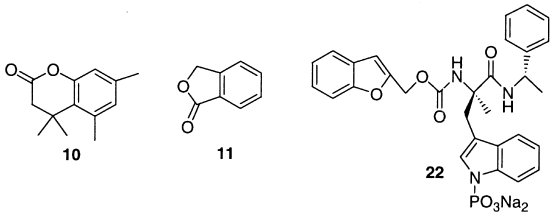
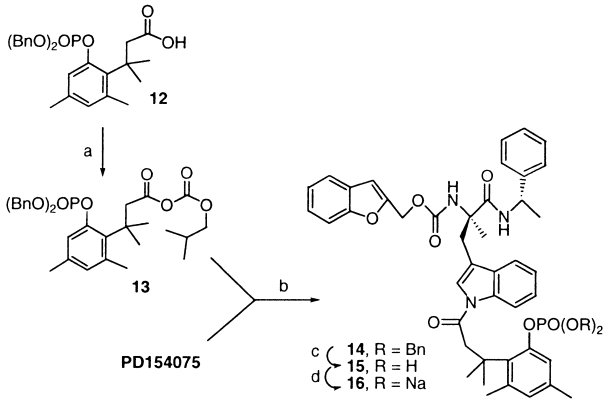


Figure 1.

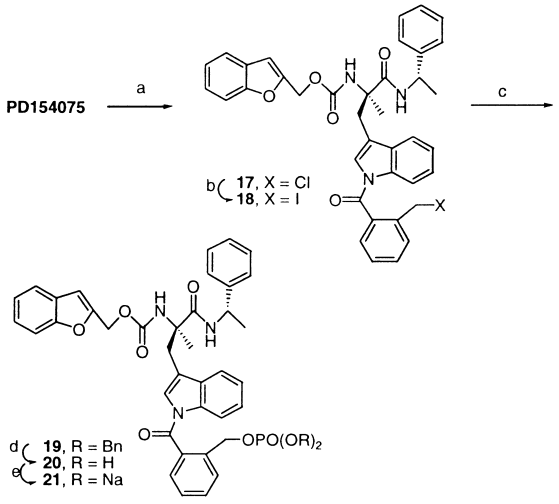


Scheme 4. (a) Isobutyl chloroformate, NMM, CH_2Cl_2, −10 °C; (b) (1) $KN(TMS)_2$, THF, −78 °C, 2) **13**; 87%; (c) 1,4-cyclohexandiene, 10% Pd/C, EtOH; (d) 0.1 M NaOH, H_2O, 50% two steps.

The derivative with a phosphoryl group directly attached to the nitrogen of the indole moiety (compound **22**, Fig. 1) was prepared by coupling the potassium salt of PD154075 with dibenzyl phosphoryl chloridate followed by debenzylation and generation of the sodium salt.

All of the disodium salts of phosphate derivatives of PD154075 (compound **9**, **16**, **21**, and **22**) were found to have substantially increased aqueous solubility between 31 and 76 mg/mL (Table 1). The ability of compound **9**, **16**, **21**, and **22** to act as prodrugs was evaluated in vivo in male Wistar rats after intravenous administration. The percentage of bio-reconversion of the four compounds to PD154075 is listed in Table 1.

Compound **22**, with a phosphoryl group directly attached to the nitrogen of the indole moiety, was converted to PD154075 by only 11.7%. This finding was not unexpected due to the generally poor ability of alkaline phosphatase to hydrolyze phosphoramidates. The bio-reconversions of compound **16** and **21** to PD154075 were only 9.35 and 19.9%, respectively, contrary to the reportedly[13b–d] successful use of these two



Scheme 5. (a) (1) $KN(TMS)_2$, THF, −78 °C; (2) *o*-chloromethylbenzoyl chloride; 99%; (b) NaI, acetone, reflux, quantitative; (c) $AgOPO(OBn)_2$, toluene, reflux, 83%; (d) 1,4-cyclohexandiene, 10% Pd/C, EtOH; (e) 0.1 M NaOH, H_2O, 67%, two steps.

Table 1. Conversion of prodrugs to PD154075 in male Wistar rats and solubility

Compounds	Dose[a] equiv to PD154075 (mg/mL)	f[b] (%)	Solubility[c] (mg/mL)
PD154075			< 0.001
9	5.3	59.5±21.3	58
16	5.0	9.35± 4.12	31
21	4.6	19.9± 1.07	55
22	5.7	11.7± 0.84	76

[a]Each rat received about 5 mg/kg equivalent of PD154075 as an intravenous bolus ($n = 3$ each). The dosing solutions of the prodrugs were prepared as 10 mg/mL in 5% dextrose in water.
[b]Percentage of prodrug reconversion to PD154075 from plasma.
[c]Solubility in water.

masked lactone linkers for phosphate prodrugs. The even lower bio-reconversion rate of compound **16** over compound **21** implied that the poor enzymatic cleavage of the phosphate groups, rather than the rate of the subsequent lactonization of the linkers, was more likely to be responsible for the low reconversion. This limited enzymatic access to the phosphate groups of compound **16** and **21** could be due to a hindered environment imposed by the indole ring or possibly due to tight binding of the compounds to plasma protein.[13d] Compound **9**, with *p*-hydroxymethylbenzoyloxymethoxycarbonyl as the linker, was reconverted back to PD154075 in 59.5% in vivo. This high reconversion of **9** to PD154075 could attribute to its longer and more flexible linker which provides easier access for alkaline phosphatase.

In conclusion, in the preparation of aqueous soluble prodrugs of PD154075, a novel phosphate prodrug approach for an indole group has been developed. By using *p*-hydroxymethylbenzoyloxymethoxycarbonyl as the linker, compound **9** was found to be a good prodrug candidate for PD154075 with significantly increased aqueous solubility, sufficient stability in aqueous solution and extensive bio-reconversion in vivo. With the increasing challenges in drug delivery of complex therapeutic agents, this approach provides an important new prodrug strategy for drug candidates containing an indole moiety.

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