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APPLICATION OF THE "TRIMETHYL LOCK"[1] TO GANCICLOVIR, A PRO-PRODRUG WITH INCREASED ORAL BIOAVAILABILITY[2]

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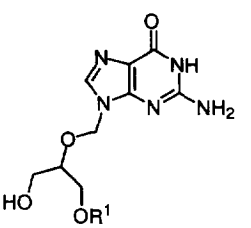
Abstract: The synthesis and oral bioavailability of two potential prodrugs of Ganciclovir (**1**) based on the "trimethyl lock" is described. The mono-3-(2'-Acetoxy-4'6'-dimethylphenyl)-3,3-dimethylpropanoic ester (**2**) showed a four-fold increase in oral bioavailability over the parent drug in rats. Copyright © 1996 Elsevier Science Ltd

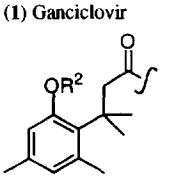
The approach of chemically modifying drugs to improve pharmacokinetic properties has been successfully applied to a variety of molecules and their advantages in overcoming unfavorable chemical properties reviewed.[4-6] The pro-prodrug (double prodrug) is an extension of this concept where, after absorption, an enzymatic step first liberates a prodrug that is then converted to the parent drug by a secondary mechanism.[7]

In this letter we wish to report the application of the "trimethyl lock" as a pro-prodrug strategy for Ganciclovir[8] (**1**) resulting in the synthesis of the mono-3-(2'-acetoxy-4'6'-dimethylphenyl)-3,3-dimethyl propanoic ester (**2**). This derivative demonstrated four times the oral bioavailability of the parent drug in rats.

Ganciclovir is the current drug of choice for treatment and maintenance therapy of CMV (cytomegalovirus) retinitis, however, its oral bioavailability in man is such that patients are often required to take in excess of 1.0 g of the oral formulation (Cytovene®) daily, as an alternative to iv therapy. Thus, a prodrug with increased bioavailability is of definite clinical utility.



R^1 = H (**1**) Ganciclovir

R^1 = 

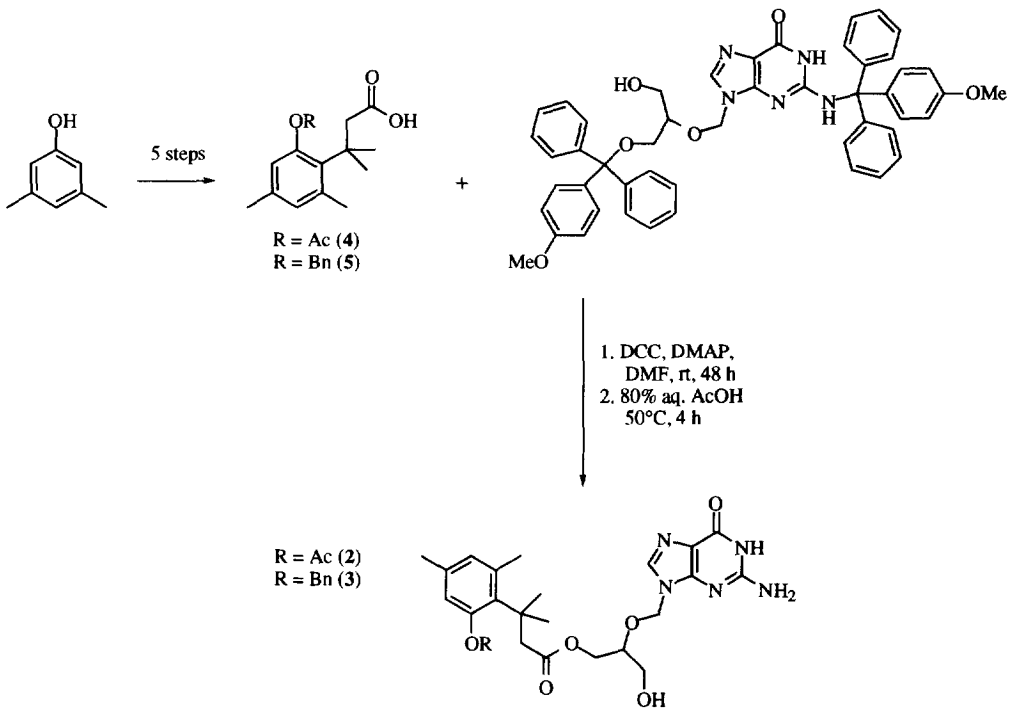
R^2 = Ac (**2**)
R^2 = Bn (**3**)

The most common prodrugs are usually esters of drugs that already contain carboxyl or hydroxyl moieties since many enzymes are capable of hydrolyzing simple esters. However, there are cases where enzymatic hydrolysis is slow, resulting in reduced biological activity, for example with Naproxen[9] and some penicillins;[10] or, where hydrolysis is fast, occurring before absorption, resulting in no significant increase in biological activity over the parent drug. A pro-prodrug approach provides a viable alternative in either case.

The "trimethyl lock", a prodrug initially developed for amines,[11] utilizes an intramolecular lactonization step to liberate the parent molecule. It derives its name from the substitution of the methyl groups at the 4' and 3

positions within the molecule; these are positioned such that, once the lock is triggered, intramolecular cyclization occurs at a rate many orders of magnitude faster than in the unsubstituted case.[1]

Two mono-ester derivatives of Ganciclovir were synthesized; first, where the hydroxyl trigger to the "trimethyl lock" was protected by an esterase labile acetate group (**2**); second, where the acetate was replaced with an enzymatically more stable benzyl group (**3**). The synthetic route is outlined below.



3,5-Trimethyl phenol was converted in five steps into 3-(2'-acetoxy-4'6'-dimethylphenyl)-3,3-dimethylpropanoic acid[1] (**4**) and 3-(2'-benzyloxy-4'6'-dimethylphenyl)-3,3-dimethylpropanoic acid[11] (**5**), using a modification of the literature procedures.[12] Coupling of these with N^2-4-methoxyphenyl diphenylmethyl-9-[(3-hydroxy-1-(4-methoxyphenyldiphenylmethoxy)-2-propoxy)-methyl] guanine, available from Ganciclovir by treatment with 2.5 equivalents of 4-methoxyphenyldiphenylmethyl chloride in DMF in the presence of triethylamine and DMAP,[13] was achieved under standard conditions. Removal of the protecting groups was accomplished using 80% aqueous acetic acid at 50 °C yielding the desired products in reasonable yields.[14]

Determination of the oral bioavailability[15] of Ganciclovir and both ester derivatives in rats (see table) indicated the acetate derivative (**2**) to be 15.6 % bioavailable. This represents a greater than four fold increase over the value obtained for Ganciclovir, 3.6%. Statistical analysis of the areas under the curves were computed using bootstrap resampling techniques,[16] the standard deviations and 99 % confidence intervals are indicated (see table).

compound	area under curve	standard deviation	99 % confidence intervals	oral bioavailability[15]
Ganciclovir (**1**)	209.7	59.8	55.7-363.7	3.6 %
(**2**)	893.7	168.9	458.6, 1328.8	15.6 %
(**3**)	-	-	-	0 %

No significant plasma levels of Ganciclovir were observed for the benzyl derivative (**3**). This indicates that, in this molecule, hydrolysis of the ester linkage is not occurring prior to absorption, where a bioavailability similar to that measured for Ganciclovir would be expected; or, that absorption is occurring and hydrolysis taking place without activation of the trigger, where a bioavailability of greater than zero would be observed. Extrapolation of these conclusions to the acetate derivative (**2**) indicate that its increased oral bioavailability over Ganciclovir results from absorption followed by esterase activated hydrolysis of the acetate trigger, this facilitates the rapid intramolecular cyclization outlined before[1] liberating the parent drug, a clear demonstration of the pro-prodrug principle in action. Studies aimed at increasing the oral bioavailability further by derivatization of Ganciclovir and experiments to determine the bioavailability of these compounds in other species are underway and will be reported elsewhere.[17]

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References and Notes:

1. 3-(2'-Hydroxy-4'6'-dimethylphenyl)-3,3-dimethylpropanoic acid derivatives; see Amsberry, K. L.; Borchardt, R. T. *J. Org. Chem.* **1990**, *55*, 5867, and references cited therein.
2. Contribitution No. 939 from Chemical Research and Development.
3. Present address: Roche Bioscience, 3401 Hillview Avenue, Palo Alto, CA 94304.
4. Bundgaard, H. *Drugs Future* **1991**, *16*, 443.
5. *Design of Prodrugs*; Bundgaard, H., Ed.; Elsevier: Amsterdam 1985.
6. Stella, V. J.; Charman, W. N. A.; Naringrekar, V. H. *Drugs* **1985**, *29*, 455.
7. Bundgaard, H. *Adv. Drug Deliv. Rev.* **1989**, *3*, 39.
8. 9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine, also known as DHPG; Martin, J. C.; Dvorak, C. A.; Smee, D. F.; Matthews, T. R.; Verheyden, J. P. H. *J. Med. Chem.* **1983**, *26*, 759.
9. Harrison, I. T.; Lewis, B.; Nelson, P.; Rooks, W.; Roszkowski, A.; Tomolonis, A.; Fried, J. *J. Med. Chem.* **1970**, *13*, 203.
10. Ferres, H. *Drugs Today* **1983**, *19*, 499.
11. Amsberry, K. L.; Gerstenberger, A. E.; Borchardt, R. T. *Pharm. Res.* **1991**, *8*, 455.

12. In our hands conversion of the 3-(2'-acetoxy-4'6'-dimethylphenyl)-3,3-dimethyl propanol into the corresponding acid was best achieved by initial oxidation under Swern conditions followed by a second oxidation with sodium chlorite in the presence of 2-methyl-2-butene. Oxidation of 3-(2'-benzyloxy-4'6'-dimethylphenyl)-3,3-dimethyl propanol into the corresponding acid was accomplished is one step using potassium dichromate.
13. Martin, J. C.; Tippie, M. A.; McGee, D. P. C.; Verheyden, J. P. H. *J. Pharm. Sci.* **1987**, *76*, 180.
14. All new compounds gave spectral and analytical data consistent with their assigned structures.
15. To measure oral bioavailability, the plasma levels of Ganciclovir were determined in male rats at ten time points (15 min to 24 h) after a single oral dose (po) of either Ganciclovir or the pro-prodrug of Ganciclovir, respectively. A colony of 40 rats were dosed initially, 4 rats were removed at each time point and the bioavailability determined. For Ganciclovir the oral dose was 10 mg/kg, for the pro-prodrug esters the dose in each case was equimolar to 10 mg/kg of Ganciclovir. The dose vehicle for the oral dose consisted of 0.5% carboxymethyl cellulose, 0.4% polysorbate alcohol, 0.9% benzyl alcohol and 0.9% NaCl with the pH adjusted to 3.5 with HCl (rest water). The plasma levels of Ganciclovir obtained after oral administration of Ganciclovir or the pro-prodrug of Ganciclovir were compared with the plasma levels obtained after an iv administration of an equimolar amount of Ganciclovir (10 mg/kg) utilizing the area under the curve of the plasma concentration vs. time plot over a 24 h period following dosing. For iv administration the sodium salt of Ganciclovir was formulated in normal saline solution. Aliquots of plasma were analyzed by HPLC where Ganciclovir and an internal standard were detected by UV absorbency at 254 nm.
16. At each time point, one concentration was drawn at random form the observed values and an area under the curve calculated, this procedure was repeated 1000 times.
17. Dillon, M. P.; Cai, H.; Maag, H. manuscript in preparation.

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