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SYNTHESIS AND ANTITUMOR EVALUATION OF PACLITAXEL PHOSPHONOOXYMETHYL ETHERS: A NOVEL CLASS OF WATER SOLUBLE PACLITAXEL PRO-DRUGS

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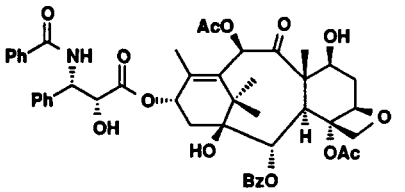
Abstract: *The synthesis, pharmacokinetic properties, and antitumor evaluation of novel paclitaxel phosphonooxymethyl ether derivatives 8-11 and salts thereof is described. These compounds exhibit improved water solubility as compared to paclitaxel (1) and upon incubation with plasma and alkaline phosphatase they readily release parent drug. The in vivo antitumor evaluation of compounds 8-11 established them as suitable pro-drugs of paclitaxel.*

At present, paclitaxel (Taxol®), **1**≠ is a well established anticancer drug for the treatment of ovarian and breast carcinomas,[1] yet clinical trials still continue in an effort to optimize and expand its therapeutic profile. Despite impressive efficacy of paclitaxel against solid tumors, its clinical formulation containing Cremophore EL and ethanol has presented excipient related problems of hypersensitivity reactions in patients.



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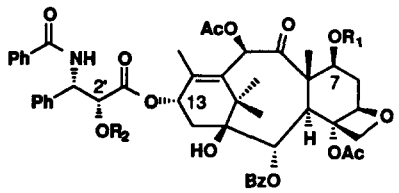
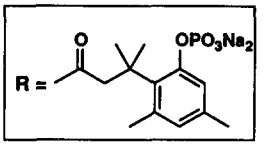
The severity of these reactions is minimized by administering extensive premedication and extending the duration of infusion. In order to supplant this current iv formulation major chemistry efforts are in progress in academia and industry aimed at identifying derivatives/pro-drugs of paclitaxel that exhibit increased solubility in aqueous media.[2] Accordingly, in a series of recent publications we have reported a pro-drug approach utilizing enzyme cleavable paclitaxel phosphates.[3, 4] While our earliest candidates namely, paclitaxel phosphates **2**, **3** and **4** fulfilled the criteria of solubility in water, exceeding 10 mg/mL, they were stable in vitro in the presence of plasma and alkaline phosphatases and were devoid of antitumor efficacy in vivo.[3] We speculated that the phosphate moieties directly attached to the taxane core as in **2**, **3**, and **4** were not accessible for the enzymatic cleavage due to steric crowding. Subsequently, paclitaxel double pro-drugs **5** and **6** were synthesized and evaluated in our laboratories.[4] In this series, the phosphate moiety is attached to the paclitaxel nucleus through a so-called 'self-immolating' linker; enzymatic cleavage of the phosphate

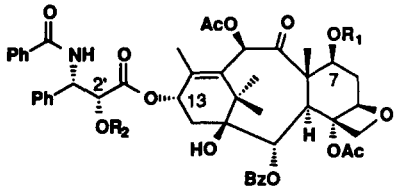
bond triggered spontaneous δ-lactonization of the o-hydroxyphenylpropionate with concomitant release of parent paclitaxel. Importantly, the desired water solubility of ca. 5 mg/mL was achieved with this class of pro-drugs and despite their strong binding to plasma proteins in vitro, they were found to have in vivo antitumor activity in murine tumor models.



Entry	R_1	R_2
(2):	PO_3Na_2	H
(3):	H	PO_3Na_2
(4):	PO_3Na_2	PO_3Na_2
(5):	H	R
(6):	R	H
(7):	H	$C(O)OCH_2OPO_3H_2$



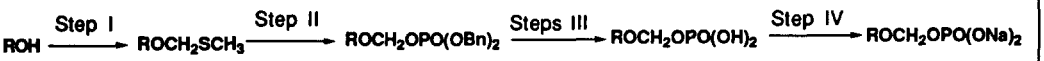
As a further extension of the 'self immolating' linker prodrug technology we also synthesized 2'-phosphonooxymethyl carbonate **7** by adapting the chemistry devised by Folkmann and Lund.[5] Unfortunately during purification of the free acid (**7**) and subsequent preparation of its water soluble salts rapid decomposition ensued; this precluded its use as a suiatble pro-drug of paclitaxel.[6] To address the stability issue, we decided to replace the carbonate moiety in **7** by a methylene acetal as a 'self-immolating' linker. This strategy led us to design a series of paclitaxel phosphonooxymethyl ether derivatives (**8-11**) as prototypical synthetic targets. Herein, we wish to report the synthesis and preliminary in vivo antitumor evaluation of these novel paclitaxel pro-drugs.



Entry	R_1	R_2
(8):	$CH_2OPO(OH)_2$	H
(9):	H	$CH_2OPO(OH)_2$
(10):	$CH_2OPO(OH)_2$	$CH_2OPO(OH)_2$
(11):	$CH_2OPO(OH)_2$	$CO_2C_2H_5$

A general four step procedure (Scheme 1) starting from paclitaxel (ROH, R=C-7 and/or C-2') was developed towards the synthesis of phosphonooxymethyl ethers of paclitaxel:

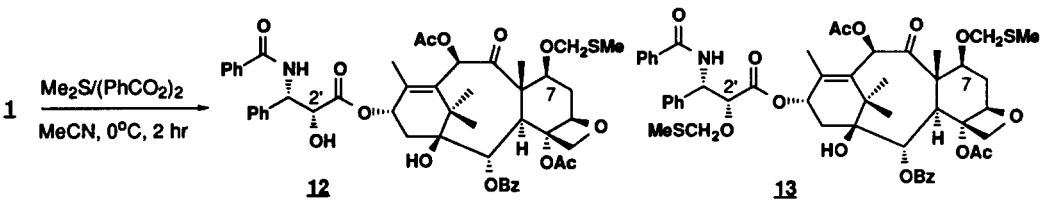
Scheme 1

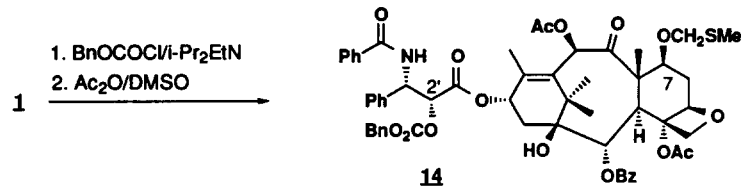


Step I: $Me_2S/(PhCO_2)_2$ (method a), or $DMSO/Ac_2O$ (method b); Step II: $HOPO(OBn)_2$/NIS, THF/mol. sieves; Step III: H_2 /10% Pd - C, AcOEt; Step IV: 1 - 2 equiv. $NaHCO_3$.

The key intermediates in this process namely the 2'- or/and 7-methylthiomethyl ethers (MTM-ethers), can be synthesized by several known procedures.[7-11] We selected two of them which under neutral conditions yielded MTM ethers of paclitaxel in satisfactory yield. Thus, a direct methylthiomethylation of paclitaxel (**1**)

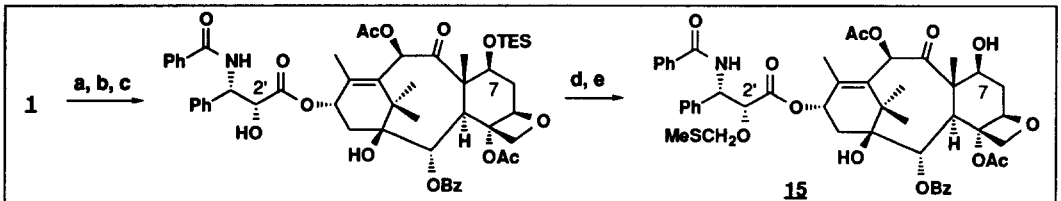
with dimethyl sulfide/benzoyl peroxide in acetonitrile[7] (method a, Scheme1) afforded primarily the 7-MTM ether **12**; the hydroxyl group at the C-7 position, being more nucleophilic than the one at the C-2' position reacts almost exclusively (95% yield). In this reaction the by-products 2',7-di-MTM ether **13** and unreacted paclitaxel were discernible (TLC) in minute quantities.





Due to a large excess (typically 4 equiv.) of benzoyl peroxide and dimethyl sulfide (typically 8-10 equiv.) required to optimize this process, considerable quantities of DMSO, benzoic anhydride, benzoic acid, and MTM-benzoate are generated necessitating purification by chromatography. By doubling the ratio of the reagents, the 2',7-di-MTM ether of paclitaxel (**13**) becomes the major product, however, the reaction in this case is not as efficient as for the mono methylthiomethylation protocol. Consequently, an extensive chromatographic purification is necessary, resulting in lowering the yield of this step to ca. 53%. Alternatively, a direct methylthiomethylation of paclitaxel at the C-7 position with Ac_2O/DMSO[8,12] (method b, Scheme 1) was attempted but this led to decomposition products only. However, prior acylation at the 2' position with benzyl chloroformate in the presence of di i-propylethyl amine in methylene chloride (93% yield) followed by treatment with Ac_2O/DMSO afforded intermediate **14** in 94% yield.

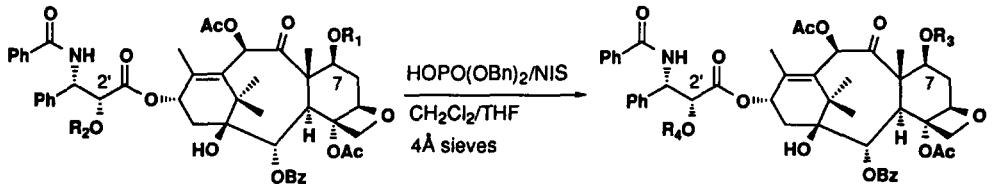
Scheme 2



We next focused our attention towards the synthesis of the C-2'-MTM ether derivative **15**. The sequence leading to **15** (Scheme 2) required following protection/deprotection steps: (a) protection of the 2' position with benzyl carbonate; (b) protection of the 7 position with triethyl silyl ether; (c) deprotection of the 2'-OH by catalytic hydrogenation; and (e) deprotection of the 7-OH by mild hydrolysis in acidic conditions after

introduction of MTM group at the 2' position. It is noteworthy that in both methylthiomethylation procedures described above we did not discern any epimerization at C-7, and that the tertiary hydroxyl group at C-1, as well as the C-13 side chain, remained unaffected.

Conversion of MTM ethers **12**, **13**, **14**, and **15** to dibenzylphosphonooxymethyl ethers (di-BnPM ethers) **16**, **17**, **18**, and **19**, respectively, was achieved in N-iodosuccinimide (NIS) mediated esterification with dibenzylphosphate in methylene chloride / tetrahydrofuran (step II, Scheme 1) in the presence of 4Å molecular sieves with 80-90% yield.[13]



R_1= CH_2SMe, R_2 = H, **12**
R_1 = R_2 = CH_2SMe, **13**
R_1 = CH_2SMe, R_2 = CO_2Bn, **14**
R_1 = H, R_2= CH_2SMe, **15**

R_3 = $CH_2OP(O)(OBn)_2$, R_4 = H, **16**
R_3 = R_4 = $CH_2OP(O)(OBn)_2$, **17**
R_3 = $CH_2OP(O)(OBn)_2$, R_4 = CO_2Bn, **18**
R_3 = $CH_2OP(O)(OBn)_2$, R_4= H, **19**

Compounds **16**, **17**, **18**, and **19** were found to be labile and each decomposed gradually during chromatographic purification. A fast work up of the crude products by flash chromatography was developed in order to minimize their decomposition. Subsequently, hydrogenation (Step III, Scheme 1), using 10% palladium on activated carbon in ethyl acetate at 60 PSI, 2-3 hrs, RT, led to the more stable phosphates **8**, **9** and **10**. These were converted (Step IV, Scheme 1) to their various salts such as sodium, triethylamine, triethanolamine, etc. and required purification by reverse phase column chromatography in acetonitrile:water (20-30%) followed by precipitation with ethyl acetate/hexane.

In addition to compounds **8**, **9**, and **10** we synthesized paclitaxel 2'-ethylcarbonate-7-PM ether **11** following the above procedure, replacing the benzylcarbonate group at the 2' position with ethyl carbonate by acylating paclitaxel with commercially available ethyl chloroformate. Our reasoning for the preparation of **11** was based on earlier SAR studies on 2'-acyl and 2'-carbonates of paclitaxel showing that these simple derivatives are metabolized in mice to paclitaxel exhibiting antitumor activity comparable to that of paclitaxel. Paclitaxel 2'-ethyl carbonate (**20**) was superior to the other carbonates tested.[14,15]

The sodium salts of **8**-**11** were found to be ca. 10^3 times more soluble than paclitaxel and these solutions were stable at 37 °C, pH 7.4 with T_{90} > 20 hrs. When treated with bovine intestinal alkaline phosphatase in vitro compounds **8**, **9**, and **10** readily generated paclitaxel, while compound **11** was converted to the 2'-ethyl carbonate **20**. Both **8** and **11** reacted at a comparable rate when incubated with purified bovine intestinal alkaline phosphatase to form **1** and **20**, respectively. Upon incubation in fresh mouse plasma (37 °C), both compounds generated **1**, suggesting that the conversion of **20** to **1** would be a facile reaction, in vivo.

After iv administration of **8** (30 mmol/kg) to tumor-bearing mice, paclitaxel (**1**) was detectable in plasma, liver and tumor within 15 min. of dosing, indicating rapid pro-drug conversion, in vivo. After iv

administration of **11** (30 mmol/kg) to tumor-bearing mice, it also was eliminated rapidly from plasma (T_{50} = 5 min). In plasma, liver and tumor, both **1** and **20** were detectable within 15 min of dosing, with concentrations of **20** > **1** until about 4-6 hrs after dosing, when paclitaxel concentrations became predominant.[16]

As expected, compounds **8-11** were devoid of in vitro cytotoxicity. In vivo studies[17] using the ip M109 tumor model have been conducted with compounds **9** and **11** and the results are tabulated below:

	Compound		Paclitaxel Parallel Data	
	Dose [mg/kg/inj]	Max. [%] T/C	Dose [mg/kg/inj]	Max. [%] T/C
9	200	144	60	237
11	64	188	44	175

Based on the activity (increase in life span) criteria[17] of T/C ≥ 125 in this tumor model both prodrugs **11** and **9** are considered efficacious. Compound **11** was comparable in efficacy to paclitaxel, whereas **9** produced a modestly active maximum T/C of 144% whereas paclitaxel yielded a maximum T/C of 237%. The optimal doses of **11** and paclitaxel were similar, 40-64 mg/kg/inj, but the optimal dose of **9** was greater (≥200 mg/kg/inj).Subsequent secondary evaluation of **8-11** against subcutaneously implanted M109 tumor and drug administration by the intravenous route (in non-Cremophore aqueous vehicle) have been successful and these results will be published seperately in full. Thus, in summary, the phosphonooxymethyl ethers of paclitaxel reported herein provide novel paclitaxel pro-drugs endowed with adequate water solubility and in vivo efficacy against the M109 murine tumor model. They represent a simple chemical alternative to the previously disclosed pro-drugs from this laboratory.

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References and Notes:

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≠ Taxol® is a registered trademark of Bristol-Myers Squibb Company.

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16. Compounds **8** and **11** were administered iv to mice bearing sc-implanted M109 tumors. At intervals after dosing, plasma, liver and tumor samples were obtained. Concentrations of paclitaxel (**1**), **8**, **11**, and **20** were determined in these samples using a specific HPLC-UV (227 nm) assay following solid-phase extraction. Additional details will be published in a separate paper.
17. Compounds were administered ip on days 5 and 8 post-tumor implant; paclitaxel was evaluated concomitantly in each experiment. All compounds were evaluated at several dose levels in a vehicle consisting of 10% ethanol/10% Cremophor/80% water (or 0.9% NaCl for paclitaxel). For additional details related to this antitumor test see: Rose, W. C. *Cancer Treat. Repts.* **1981**, *65*, 299.

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