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Photolabile Carbonyl Protecting Group: A New Tool for Light-Controlled Release of Anticancer Agents

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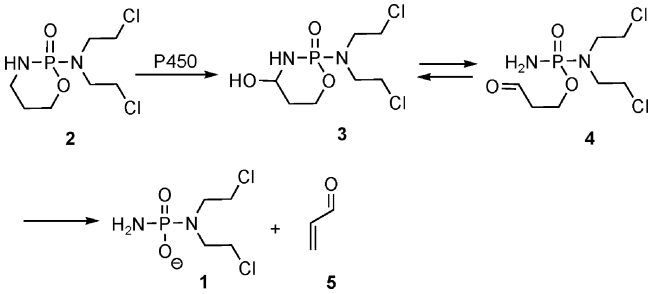
A new type of photolabile carbonyl-protecting group was utilized in releasing anticancer agents upon irradiation at 350 nm in an aqueous environment.

Introduction

Chemotherapy is an important therapeutic method in cancer treatment. Its major drawback is dose-limiting systemic toxicity of the therapeutic agents. To circumvent this limitation, highly cytotoxic anticancer drugs can be converted into less- or nontoxic prodrugs. These prodrugs can be selectively activated in the malignant tumor to achieve a high local concentration of anticancer agents for improved therapeutic outcomes without causing severe systemic toxicity.

One of the most studied anticancer agents for prodrug strategies is phosphamide mustard (PM, **1**), a cell-cycle-independent alkylating agent effective in killing a broad spectrum of cancer cells. Because it is chemically labile and cannot readily pass through cell membranes as a result of their anionic character at physiological pH, PM has to be used in prodrug form for clinical applications.[1] Among various PM prodrugs studied, cyclophosphamide (CP, **2**) is the most successful one and has been widely used in the clinic for decades.[2] However, in current regimens involving CP, only a fraction of the systemically administrated CP arrives at the liver, where it is oxidized by hepatic cytochrome P450 to 4-hydroxycyclophosphamide (**3**), which equilibrates with the ring-opened aldophosphamide (**4**; Scheme 1). Spontaneous β-elimination of **4** liberates cytotoxic PM (**1**) and acrolein (**5**). The anticancer activity of CP is mainly attributed to PM (**1**).[3]

The requirement for metabolic activation of CP limits its effectiveness and efficiency. Efforts have been dedicated to other PM prodrug strategies, one of which is to block the carbonyl group of aldophosphamide (**4**) to prevent generation of PM through β-elimination.[4] Upon appropriate activation in the tumor site, **4** can be regenerated to release PM (**1**).



Scheme 1. Activation pathway of cyclophosphamide (**2**).

We are interested in the activation of PM prodrugs with light. The precise tissue selectivity relies on physical placement of the light source in the vicinity of the tumor, or inside the tumor, rather than on insufficient differences in cellular parameters between tumor and normal tissues. This approach is similar to photodynamic therapy (PDT). However, different from the traditional PDT, it is oxygen-independent and thus more suitable for treating hypoxic solid tumors.[5]

Results and Discussion

As depicted in Scheme 2, the carbonyl group of **4** can be protected by a photolabile protecting group (PPG) to afford **6**, a photosensitive precursor of **4**. Upon irradiation, prodrug **6** can release aldophosphamide (**4**) with precise spatial and temporal control. A key issue of this strategy is to have a robust PPG with proper chemical and photochemical properties.

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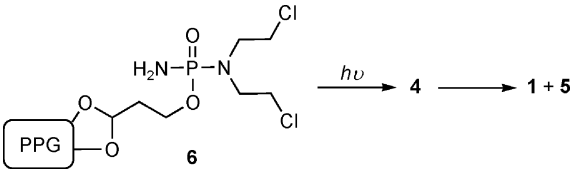
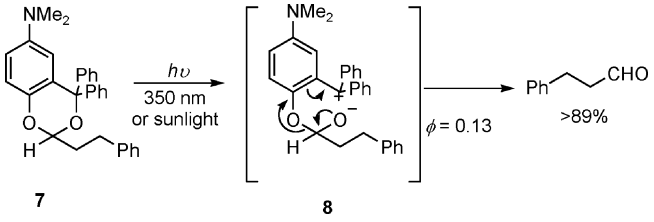


Scheme 2. Photosensitive precursor of aldophosphamide (**4**).

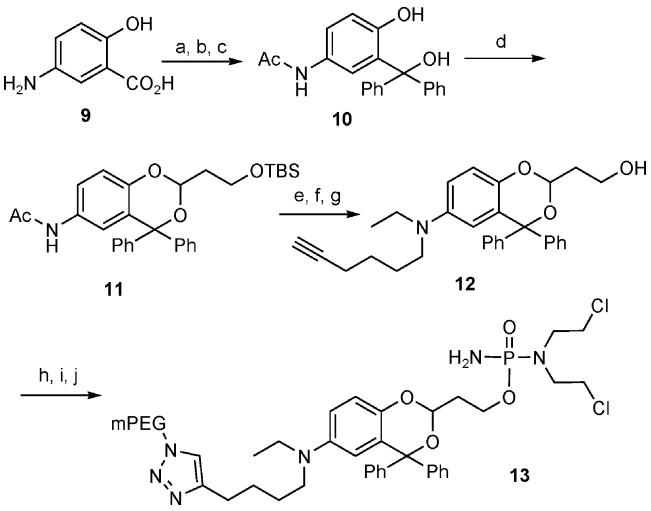
We recently developed a series of novel PPGs for carbonyl groups.[6] These new PPGs have demonstrated distinct advantages over existing ones. For example, the new PPG in acetal **7** ($\varepsilon_{327nm} = 3.38 \times 10^3$ M^{-1}cm^{-1}, $\Phi = 0.13$) can be readily removed with 350 nm irradiation or sunlight to release 3-phenylpropanal in high yields, presumably via intermediate **8** (Scheme 3).[6c] Importantly, the PPG can undergo flexible structural adjustment for prodrug applications without compromising their photochemical reactivity.



Scheme 3. Photolabile carbonyl protecting group.

To use the new PPG, proper modification of its structure is required to tune pharmacological properties of the prodrug. Thus, synthesis of the photoactivated prodrug commenced from commercially available 5-aminosalicylic acid (**9**) (Scheme 4). Esterification and acetylation followed by reduction of the ester moiety with an excess amount of phenyllithium provided salicyl alcohol **10** (82% yield, three steps). Formation of acetal **11** from **10** and 3-[(TBS)oxy]-propanal under neutral conditions without using any other chemical reagents was achieved in 68% yield.[7] Subsequent reduction of the amide, alkylation of the nitrogen atom, and removal of the TBS group provided photosensitive acetal **12** (57% yield, three steps). Acetal **12** was equipped with the *N*-hex-5-yn-1-yl handle for linking a hydrophilic (or a tumor targeting) unit at a later stage of the synthesis to adjust the pharmacological properties of the prodrug. Addition of **12** to known *N*,*N*-bis(2-chloroethyl)phosphoric acid dichloride under basic conditions followed by treatment with ammonia led to the phosphorus diamide as an inseparable diastereomeric mixture. To demonstrate the facility of adjusting the properties of the prodrugs, a PEG unit (1 K Dalton) was conveniently linked through click chemistry to provide water-soluble model prodrug **13** (56% yield, three steps).[8]

Prodrug **13** is stable under laboratory lighting, but sensitive to sunlight and UV irradiation. Prodrug **13** (16 mM in unbuffered D_2O) was irradiated with 350 nm light in a Rayonet photochemical reactor at room temperature. ^{31}P NMR spectra were acquired after 10 min and 20 min irradiation (Figure 1).[9] The signals at δ = 20.65 and 20.70 ppm were assigned to aldehyde **4** and its hydrate **14**. The presence of **4** was also confirmed by the ^{1}H NMR signal at δ = 9.6 ppm. The ^{31}P NMR shifts at δ = 12.40 and 12.54 ppm were ascribed to **3**-*cis* and **3**-*trans*, respectively. Borch and co-workers demonstrated that a pre-equilibrium existed among **4**, **14**, and **3** prior to rate-limiting expulsion of **1**



Scheme 4. Synthesis of light-activated anticancer prodrug **13**. Reagents and conditions: (a) MeOH, H_2SO_4 (cat.), reflux, 96%; (b) AcCl, Et_3N, CH_2Cl_2, 93%; (c) PhLi, THF, –78 °C, 92%; (d) 3-[(TBS)oxy]propanal, *p*-xylene, 140 °C, 68%; (e) LAH, THF, reflux, 65%; (f) $HCC(CH_2)_4OTs$, K_2CO_3, KI, Bu_4NCl, MeCN, reflux, 92%; (g) TBAF, THF, 95%; (h) *t*BuOK, $Cl_2P(O)N(CH_2CH_2Cl)_2$, THF, 72%; (i) NH_3, dioxane, 85%; (j) mPEG-N_3, Cu powder, MeCN/H_2O (10:1), 92%.

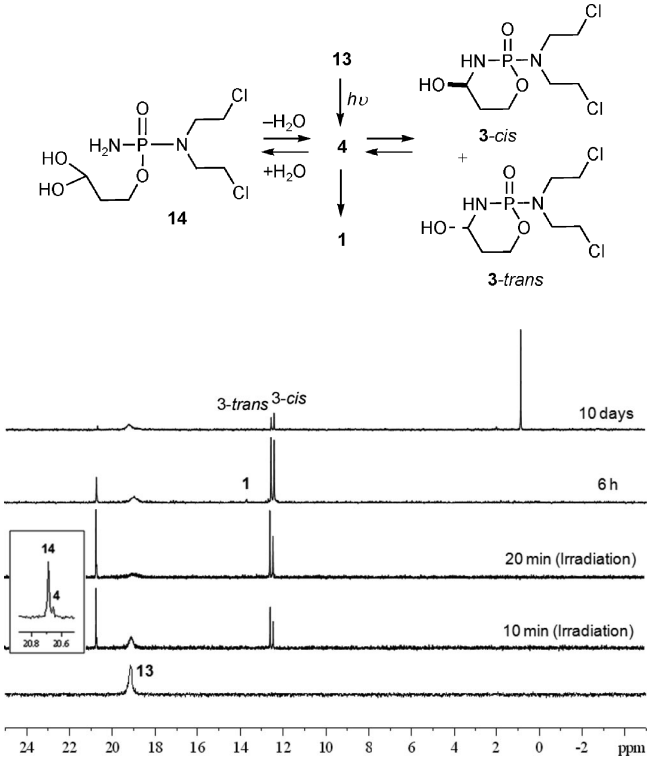


Figure 1. ^{31}P NMR spectra recorded as a function of time.

from **4** in buffer solutions. In their pseudoequilibrium mixtures obtained with different approaches, aldophosphamide (**4**) was the minor component.[10] Our results from unbuffered D_2O are in agreement with the early observations. PM (**1**), the β-elimination product of **4**, only appeared as a weak peak (δ = 13.68 ppm) in the spectrum taken after 6 h. Nevertheless, the signal of the inorganic phosphate (δ = 0.90 ppm),[11] the hydrolysis product from **1** via the phosphoramidic diacid intermediate, appeared after 6 h and steadily grew at the expense of the other peaks.[12]

The NMR study shows that the fate of photochemically generated **4** is consistent with that of aldophosphamide obtained through other approaches,[10] proving that compound **13** is a useful precursor to **4**. In addition, cytotoxic acrolein and the postulated *O*-quinone methide intermediate from fragmentation of **13** will be produced at the activation site to enhance cancer cell death.[2b,13] Although acrolein is known as an alkylating agent and to be highly toxic to cancer cell lines, it does not contribute to the anticancer activity of **2**; instead, it causes hemorrhagic cystitis.[14] With the new approach, locally produced acrolein should have a much reduced side effect on other organs. The pharmacological properties of prodrug **13** are currently under investigation.

Conclusions

A novel photoactivated phosphamide mustard prodrug equipped with a new phototrigger was designed and synthesized. The prodrug effectively released aldophosphamide upon irradiation at 350 nm, but it is stable under laboratory lighting. The PPG moiety has remarkable dark stability and was carried through a multistep synthesis. It was readily modified for the prodrug application and can be potentially useful in releasing other biologically important substances in aqueous environments.

Experimental Section

***N*-{2-[2-(TBS)oxyethyl]-4,4-diphenyl-4*H*–1,3-benzodioxin-6-yl}acetamide (11):** A stirring mixture of **10** (900 mg, 2.70 mmol) and 3-(*tert*-butyldimethylsilyloxy)-1-propanal (762 mg, 4.05 mmol) in *p*-xylene (4.0 mL) was heated to 140 °C in a sealed tube under an argon atmosphere. After 1.5 h, the reaction was allowed to attain room temperature and the solvent was removed under vacuum. The crude products were purified by silica-gel column chromatography (petroleum ether/ethyl acetate, 2:1; R_f = 0.3) to produce **11** (1.41 g, 89%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.48–7.42 (br. s, 1 H), 7.37 (dd, J = 8.9, 2.6 Hz, 1 H), 7.35–7.27 (m, 5 H), 7.25–7.15 (m, 5 H), 6.84 (d, J = 9.0 Hz, 1 H), 6.82 (d, J = 2.7 Hz, 1 H), 5.13 (t, J = 5.4 Hz, 1 H), 3.82–3.71 (m, 2 H), 2.06 (q, J = 5.9 Hz, 2 H), 1.94 (s, 3 H), 0.74 (s, 9 H), –0.06 (s, 6 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 200.4, 168.3, 149.1, 145.9, 143.7, 130.4, 129.0, 128.1, 128.0, 127.8, 127.5, 125.6, 121.6, 121.3, 117.3, 93.1, 84.1, 58.0, 37.9, 25.7, 24.1, 18.0, –5.46, –5.51 ppm. IR (neat): $\tilde{\nu}$ = 3019, 2957, 2930, 1678, 1497, 1393 cm^{-1}. HRMS (ESI): calcd. for $C_{26}H_{28}NO_4Si$ [M – C_4H_9]$^+$ 446.1788; found 446.1794.

Irradiation of 13: Prodrug **13** (14.5 mg, 8.6 mol) in D_2O (0.55 mL) in a Pyrex NMR tube was irradiated in a RPR-200 Rayonet photoreactor equipped with 16 of 350 nm lamps for 10 and 20 min. The progress of the photoreaction was monitored with NMR spectroscopy.

Supporting Information (see footnote on the first page of this article): Experimental details, spectroscopic data, ^{1}H and ^{13}C NMR spectra.

Acknowledgments

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[11] The chemical shifts of the intermediates are not identical to the values reported by others,[10,12] owing to the sensitivity of

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