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A Methyl Glucuronate Prodrug of Phosphorodiamidic Mustard

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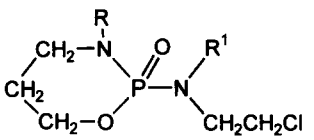
Abstract: 4-[Methyl (β-D-glucopyranosyl)uronate]benzyl N,N,N',N'-tetrakis(2-chloroethyl) phosphorodiamidate, **3**, was synthesized as a neutral prodrug of N,N,N',N'-tetrakis(2-chloroethyl) phosphorodiamidate mustard, **2c**. In the presence of carboxylate esterase and β-D-glucuronidase, **3** was converted to **2c** and 4-hydroxybenzyl alcohol, **7**.

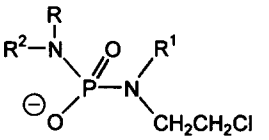
Cyclophosphamide, **1a**, and ifosfamide, **1b** (Fig. 1) are structurally-related anticancer agents that exhibit broad spectrum clinical efficacy.[1,2] These compounds are not active in their own right but are oxidatively biotransformed in vivo to unstable intermediates that are believed[3,4] to transport the ultimate active metabolites, phosphoroamidic mustard, **2a**, and phosphorodiamidic mustard, **2b** (Fig. 2), respectively, into cells.

Fig. 1



1a, R = H, $R^1 = CH_2CH_2Cl$
1b, $R = CH_2CH_2Cl$, R^1 = H

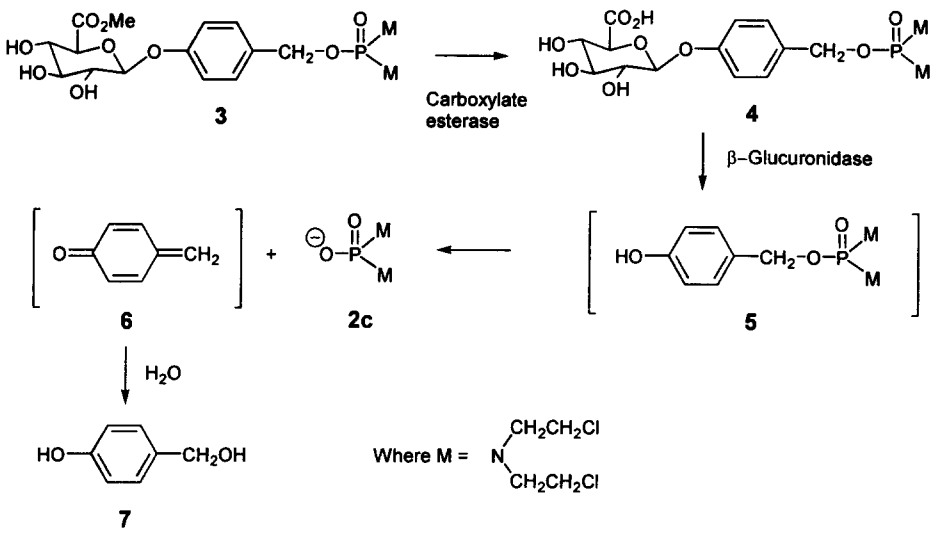
Fig. 2



2a, $R = R^2$ = H, $R^1 = CH_2CH_2Cl$
2b, $R = CH_2CH_2Cl$, $R^1 = R^2$ = H
2c, $R = R^1 = R^2 = CH_2CH_2Cl$

Despite their widespread clinical use, cyclophosphamide and ifosfamide give rise to toxic byproducts that limit their therapeutic efficacy. Both agents generate acrolein, a metabolite that has been implicated in kidney and bladder toxicity.[5] Ifosfamide gives rise to chloroacetaldehyde, which is believed to cause CNS toxicity.[6] These side effects cannot be overcome by directly administering the active metabolites, **2a** and **2b**, because such compounds are ionic at physiologic pH and penetrate poorly into cells.[7,8] To avoid toxicologic problems associated with the liberation of acrolein and chloroacetaldehyde, we sought an alternative prodrug approach to deliver phosphoramide mustards into cells. One such approach is shown in Scheme I. Here, the hybrid mustard, **2c**, a potent cytotoxic DNA-crosslinking agent [9], has been selected to investigate the strategy.

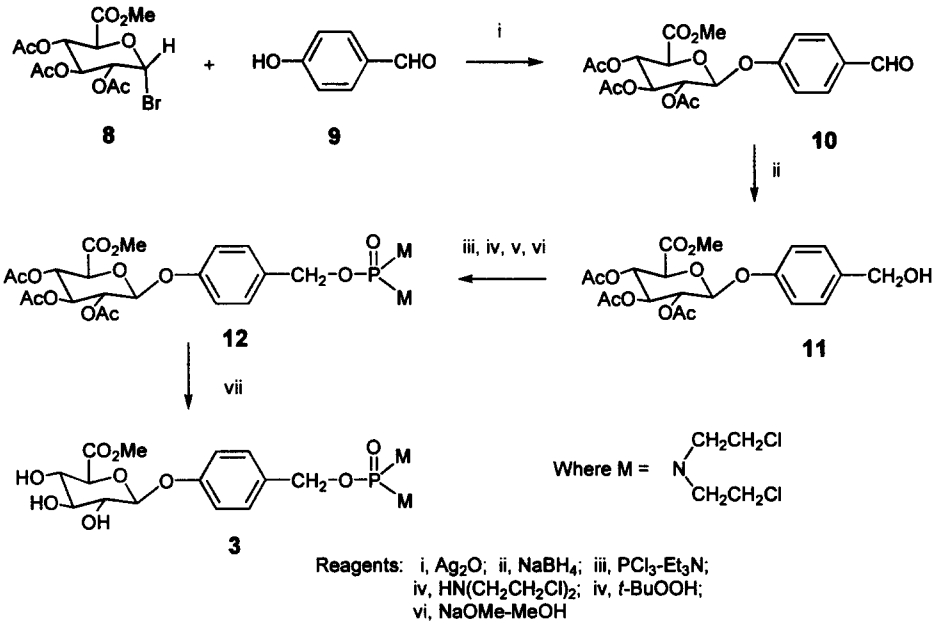
The prodrug **3** was anticipated to be fairly stable in aqueous media at neutral pH. When administered *in vivo*, however, the methyl glucuronate should be hydrolyzed to the free glucuronide, **4**, by carboxylate esterases,[10]



Scheme I

enzymes that are ubiquitous in tissue. Once formed, **4** should be further cleaved to the phenolic intermediate, **5**, by tissue β-glucuronidases. Interestingly, β-glucuronidases have been reported to be elevated in human breast tumors.[11] **5** should be inherently unstable and spontaneously dissociate to tetrakis(2-chloroethyl) amine phosphorodiamidate, **2c**, and the quinone methide, **6**. The latter is expected to rapidly hydrate to 4-hydroxy benzyl alcohol, **7**. Although the toxicologic properties of **6** and **7** are not well established, neither compound (unlike acrolein) has been reported to induce acute or chronic organ damage in mammals.

3 was synthesized as shown in Scheme II. A solution of *p*-hydroxybenzaldehyde, **9**, (2.0 g, 16.0 mmol) in CH_3CN (20 mL) was added with stirring to a mixture of methyl (tri-O-acetyl-α-D-glucopyranosyl bromide)uronate, **8**[12] (6.0 g, 15.0 mmol), and dry, freshly-prepared Ag_2O (7.4 g, 30 mmol) in CH_3CN (110 mL). After 6 h at ambient temperature, the Ag salt was filtered, and the product remaining after solvent evaporation was purified by chromatography over silica (200-400 mesh, Merck Inc.) using hexane-EtOAc as mobile phase. It was crystallized from hexane-EtOAc to afford **10** as a white solid (4 g, 60%), mp 170-171 °C. Treatment of **10** (2.3 g, 5.25 mmol) with $NaBH_4$ (0.42 g, 10.95 mmol) in a mixture of dry $CHCl_3$ (30 mL) and dry $(CH_3)_2CHOH$ (7 mL) gave the benzyl alcohol **11** as a white solid (1.7 g, 75%), mp 132-133 °C. **11** (1.16 g, 2.63 mmol) was added to a stirred solution of PCl_3 (0.25 mL, 2.9 mmol) and Et_3N (1 equivalent) maintained at 0 °C under a N_2 atmosphere. After 20 min, bis(2-chloroethyl) amine hydrochloride (1.05 g, 5.79 mmol) was added followed by Et_3N (2.35 mL, 18.4 mmol) and stirring was continued for 75 min at 0 °C and then for 30 min at ambient temperature. The reaction mixture was cooled to -20 °C and a solution of 5.2 M *tert*-butyl hydroperoxide solution in decane (0.5 mL, 2.63 mmol) was added. After 1.5 h, the solvent was removed and the product was extracted



Scheme II

with EtOAc. It was purified by chromatography on silica, using hexane-EtOAc as eluent, to yield **12**[13] as a gummy liquid (0.9 g, 44%). **12** (0.9 g, 1.17 mmol) was deacetylated with NaOMe (63 mg, 1.17 mmol) in anhydrous MeOH (40 mL) to give **3**[14] (0.68 g, 90%) as a thick gum. The compound resisted all attempts at crystallization, but was judged to be pure by chromatographic (HPLC; TLC on silica) and NMR criteria.

Stability Studies of 3.

Compound **3** was degraded with a half life of 282 min when incubated at a concentration of 10^{-4} M in 0.05 M phosphate buffer, pH 7.4, at 37°C. Under similar conditions in the presence of carboxylate esterase (2 units), the half-life of **3** was reduced to 21 min. In both cases, HPLC analysis of the reaction mixture[15] indicated a single product with a retention time of 3.0 min. When **3** was incubated simultaneously with carboxylate esterase (2 units) and β-glucuronidase (20 units), the peak at 3.0 min was again formed, but was gradually replaced with a new peak with a retention time (1.84 min) identical to that of an authentic sample of 4-hydroxybenzyl alcohol, **7**. These results suggest that the product formed in the presence of esterase is the free glucuronide, **4**, and that this product is cleaved by β-glucuronidase to the hydroxybenzyl intermediate, **5**. Once generated, **5** is expected to spontaneously disproportionate to 4-hydroxybenzyl alcohol, **7**, and the chemically reactive mustard, **2c**. Since **2c** does not contain a strong chromophore, it was not evident in the reaction mixture using a U.V. detector. Further analysis of this same reaction mixture was conducted by LC/MS. Three product peaks were now evident. One showed a four-chlorine molecular ion cluster with M = 627, consistent with the free glucuronide, **4**. Another

showed a prominent peak at M = 107 consistent with the hydroxytropylium ion formed by rearrangement of the 4-hydroxybenzyl cation ion derived from **7**. The third peak, not evident when the mixture was analyzed using a UV detector, showed a four-chlorine molecular ion cluster with M = 345 characteristic of the free mustard, **2c**.[16] These findings strongly support the rationale invoked in the design of prodrug **3**. Biological studies of this compound are in progress and will be reported in the future.

Acknowledgment

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13. TLC, Silica; R_f = 0.44 (EtOAc-hexane, 3:2). ^{1}H NMR ($CDCl_3$): δ 7.32 (d, 2 H, ArH), 7.0 (d, 2 H, ArH), 5.28-5.37 (m, 3 H), 5.15 (d, 1 H, anomeric proton, J= 6.9 Hz), 5.00 (d, 2 H, CH_2OP, J = 8.05 Hz), 4.16-4.21 (m, 1 H), 3.73 (s, 3 H, CO_2Me), 3.55-3.65 (m, 8 H, $(NCH_2)_4$), 3.31-3.43 (m, 8 H, $(CH_2Cl)_4$), 2.07 (s, 3 H, OCOMe), 2.05 (brs, 6H, 2 x OCOMe). Found: C, 44.15; H, 5.28; N, 3.42. $C_{28}H_{39}O_{12}N_2PCl_4$ requires C, 43.97; H, 5.12; N, 3.65.
14. TLC, Silica; R_f= 0.57 ($CHCl_3$-MeOH, 9:1). ^{1}H NMR ($CDCl_3$): δ 7.30 (d, 2 , ArH), 7.02 (d, 2 H, ArH), 5.05-4.85 (m, 3 H, CH_2OP and anomeric proton), 4.02 (d, 1 H), 3.90-3.65 (m, 9 H), 3.63-3.50 (m, 8 H, $(NCH_2)_4$), 3.45-3.20 (m, 8 H, $(CH_2Cl)_4$). Found: C, 41.14; H, 5.57; N, 4.01. $C_{22}H_{33}O_9N_2PCl_4.CH_3OH$ requires C, 40.97; H, 5.53; N, 4.15.
15. A solution of **1** (10^{-4} M) in 0.05 M phosphate buffer, pH 7.4 (2 mL) was incubated at 37 °C either in the absence or the presence of the enzymes. At different time intervals, 100 μL aliquots were removed and analyzed directly for parent drug and metabolites by HPLC on a C-18 reverse phase column (Phenomenex, 150 x 3.90 mm). The mobile phase was acetonitrile-0.05 M phosphate buffer, pH 7.0 (35:65) at a flow rate of 1.0 mL/min. Eluted compounds were monitored with a variable-wavelength UV detector set at 260 nm with 0.01 AUFS sensitivity (Water 484 model), and quantitated electronically as a function of time using a NEC Pinwriter P6200 integrator. The retention times of the parent drug **1** and 4-hydroxybenzyl alcohol, **6**, were 10.2 min and 1.8 min, respectively.
16. An authentic sample of the free mustard, **2c**, was prepared by catalytic hydrogenation (Pd/H_2) of benzyl N,N,N',N'-tetrakis(2-chloroethyl) phosphorodiamidate, and characterized by NMR and LC/MS analysis.

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