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# Facile synthesis of mutagen X (MX): 3-chloro-4-(dichloromethyl)-5-hydroxy-5*H*-furan-2-one

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ABSTRACT

3-Chloro-4-(dichloromethyl)-5-hydroxy-5*H*-furan-2-one (mutagen X, MX) was synthesized in six steps from commercially-available and inexpensive starting materials (27% overall yield). This synthesis enables the preparation of MX analogs and does not require the use of chlorine gas, as do previously reported methods.

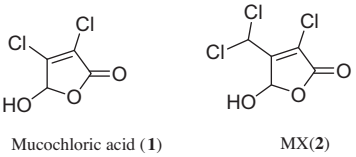


## Introduction

Halogenated organic substances containing vinyl chloride and chlorinated hydroxyfuranone functionalities such as mucochloric acid (**1**) and mutagen X (**2**, MX) are contaminants in chlorinated water and industrial chemical waste (Fig. 1).[1] Compounds **1** and **2** were discovered in the late 1970s, and shown to be mutagenic in the Ames assay.[2] MX was isolated initially from chlorine-bleached pulp mill effluents in 1979.[3] One fraction among the different concentrates showed consistent mutagenicity in *Salmonella typhimurium* strain TA100. Later the mutagenic compound was identified as MX.[4] These halogenated compounds were also isolated from chlorine-disinfected or treated drinking water.[5] They are formed by the reaction of $Cl_2$ with humic acids derived from microorganisms present in soil and water.[6] MX was shown to be present in detectable limits in these drinking water sources and at levels as high as 310 ng/L.[4] Though the concentration of MX in drinking water is typically 100- to 1000-fold lower than other common chlorinated by-products of concern such as trihalomethanes, it is believed that MX is more mutagenic. Smeds et al. analyzed drinking water samples from 35 locations and reported that MX accounted for up to 67% of the overall mutagenicity (*S. typhimurium* TA100).[7] Similar results were also obtained by Wright et al. among 88 samples taken from 36 towns in Massachusetts (USA).[8]

MX, in some model systems, was particularly potent relative to other halogenated compounds in inducing DNA damage and altering pathways involved in cell growth.[4] MX was also found to be mutagenic in mammalian cell assays in vitro and in vivo.[9] In studies performed by Komulainen et al. MX was found to be a potent carcinogen in rodents.[10] There has been speculation that MX reacts directly with the aminopurine functionality of adenosines.[5] Because we observed that MX reacted covalently with the active site lysine of an enzyme in heme biosynthesis that we are investigating, we sought to prepare larger quantities of MX to explore its chemical reactivity and stability.

Two distinct methods have been reported to synthesize MX. The first, by Padmapriya et al. in 1985, involved five steps starting from tetrachloroacetone (**3**, Scheme 1).[11] In 1995, Franzén et al. modified Padmapriya's synthesis by the addition of $H_2SO_4$ to the metal catalyst in the olefin chlorination of **4** to give **5**, and Jinqu et al. later used UV-light instead of a metal catalyst in the chlorination procedure.[12] We found that the methods for the chlorination of olefin **4** were not generally reproducible, even repeating exactly the methods and stoichiometry of reagents used. The second




Mucochloric acid (**1**) MX(**2**)

**Figure 1.** Mucochloric acid (**1**) and Mutagen X (**2**).

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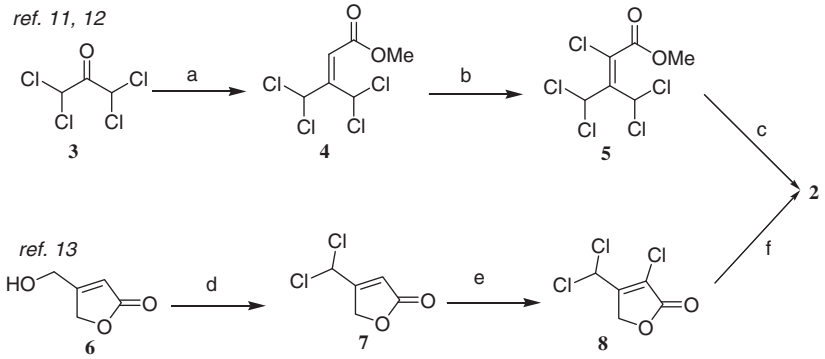
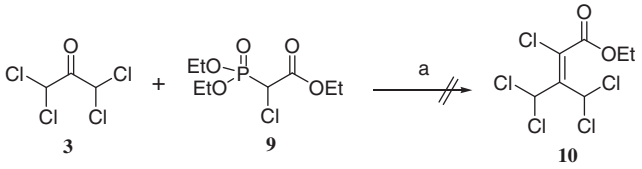



**Scheme 1.** Reagents and conditions: (a) $Ph_3P = CHCO_2Me$, THF, 84%; (b) (i) $Cl_2$ gas, $FeCl_3$. (ii) $Et_3N$, 80%; (c) (i) LiOH, quantitative. (ii) $KHCO_3$, 65%; (d)(i) PCC. (ii) $PCl_5$, 60%; (e) (i) $Cl_2$ gas, $FeCl_3$. (ii) $Et_3N$, 48%; (f) (i) NBS, light. (ii) $Hg(OAc)_2$, $H_2O$, acetone, 20%.




**Scheme 2.** Reagents and conditions: (a) NaH, THF, 0 °C; or *n*-BuLi, −78 °C.

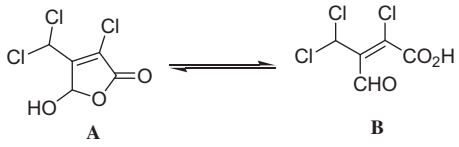



**Figure 2.** MX in ring-closed (**A**) and open chain (**B**) forms.

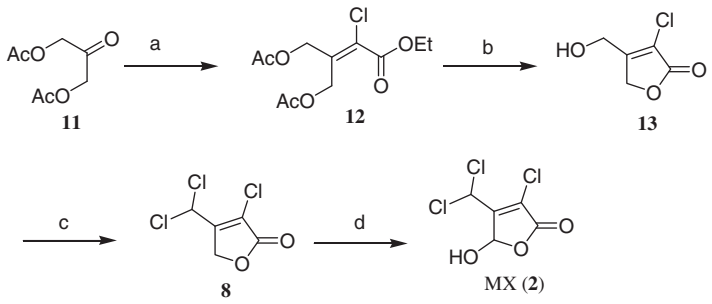
general procedure for the preparation of MX was reported by Lalonde et al., in 1990.[13] Key intermediate 4-(hydroxymethyl)-2(5*H*)-furanone (**6**), made in two steps, was utilized to assemble MX in eight steps going through olefin **7** and vinylchloride **8** with an overall yield of 4%. In our work, we sought to improve the overall yield and reduce the number of chemical steps, while removing the use of chlorine gas altogether.

Our initial concept was to utilize triethyl-2-chloro-2-phosphonoacetate (**9**) in a Horner–Wadsworth–Emmons (HWE) reaction[14] to install the chlorine in the desired α-position. Compound **3**[15] was reacted with **9** under basic conditions but reaction was not observed to give **10** (Scheme 2), which we attributed to competition between deprotonation of the acidic hydrogens and quenching of the reactivity of **3** with deprotonation of phosphonoacetate **9**.

Installation of the α-chlorine on the olefin was achieved first by the HWE reaction of **9** with 1,3-diacetoxyacetone (**11**) to yield α-chloroester **12** in 80% yield (Scheme 3),[16] followed by treatment of **12** with catalytic PTSA under reflux conditions[17] in EtOH to furnish lactone **13** in 85% yield.[18] Primary alcohol **13** was oxidized using PCC and then treated with $PCl_5$ to afford dichloromethyl compound **8** in 80% yield.[19] Bromination of **13** at the anomeric center was achieved with refluxing in $CCl_4$, with 2 equiv of N-bromosuccinimide (NBS) and a catalytic amount of azobisisobutyronitrile (AIBN). The crude reaction mixture was then treated with HCl/dioxane in water under reflux conditions to hydrolyze the anomeric bromide to the corresponding alcohol, thus affording **2** in 50% yield over the two steps.[20] The use of HCl/dioxane did not result in the formation of appreciable side products, and purification was relatively straight-forward.

Evaluation of the $^1H$ NMR (300 MHz) of MX in $CDCl_3$, $D_2O$, and DMSO-$d_6$ confirmed the dependency of solvent on the equilibrium of the open-chain and the ring-closed forms of MX (Fig. 2). MX exists as a 1:1 ratio of the ring-closed (A) and the open-chain forms (B) in DMSO-$d_6$ at ambient temperature, and in $CDCl_3$ and $D_2O$ the predominant form observed was the ring closed form at ambient temperature. In contrast to this previous report,[21] we observed that the only form of MX at pH of 7.4 in $D_2O$ was the closed ring lactone **A**. In fact, in all tested acidic and neutral solutions of $D_2O$, MX existed as the closed ring lactone.

There are three sites in MX that can react with nucleophiles such as amines, namely the lactone carbonyl, hemiacetal carbon, and the dichloromethyl substituent. When MX was treated with 3-phenylpropylamine under conditions of reductive amination,[22] five membered ring lactam **14** was isolated in 50% yield.[23] Formation of these lactams was similar to the reductive amination of mucochloric acid as previously reported.[24] These results suggested




**Scheme 3.** Reagents and conditions: (a) $(EtO)_2P(O)CH(Cl)CO_2Et$ (**10**), NaH, THF, 80%; (b) PTSA, EtOH, 85%; (c) (i) PCC. (ii) $PCl_5$, 80% over two steps; (d) (i) NBS, AIBN (cat), $CCl_4$. (ii) HCl, dioxane, 50% over two steps.

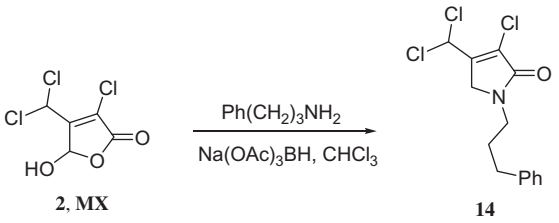



**Figure 3.** Reductive amination of MX with phenylpropylamine.

that in biological systems, **1** or **2** could react with nucleophiles (DNA or aminoacids) to form a Schiff base as the first step followed by subsequent modifications (Fig. 3).[25]

In conclusion a facile synthesis of MX has been developed with an overall yield of 27% in six steps, starting with **9**. A favorable aspect of the synthetic route presented is that a variety of MX analogs can be prepared, without the use of chlorine gas.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.04.044.

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16. *Procedure and spectral data for* ***12****:* To a suspension of sodium hydride (60% dispersion in oil, 1.0 g, 25.73 mmol) in THF (150 mL) at 0 °C was added triethyl-2-chloro-2-phosphonoacetate (6.35 g, 24.58 mmol) dropwise. The reaction solution was allowed to stir for 1 h at 0 °C and 0.5 h at room temperature. The resulting yellow solution was cooled to 0 °C and 1,3-diacetoxyacetone (4.21 g, 24.58 mmol) was added. The resulting solution was slowly allowed to warm to ambient temperature and stirred for 20 h. The reaction was quenched with sat $NH_4Cl$ (50 mL) and the aqueous layer was separated and worked up in EtOAc (2 × 100 mL). The combined organic layers were washed with water (50 mL), brine (50 mL) and dried over $Na_2SO_4$. The solvent was reduced under reduced pressure and purified via flash column chromatography (hexanes/EtOAc 5:1) to obtain 5.4 g of **12** as a yellow oil in 80% yield. IR (film) 1730, 1640, 739 $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 5.01 (s, 2H), 4.92 (s, 2H), 4.31 (q, $J$ = 7.1 Hz, 2H), 2.11 (d, $J$ = 13.1 Hz, 3H), 2.05 (s, 3H), 1.45–1.22 (m, 3H); $^{13}C$ NMR $\delta$ 170.0, 169.9 162.0, 138.3, 126.3, 62.5, 61.6, 60.4, 20.3 (2C), 13.7. HRMS (FAB) calcd for $C_{11}H_{15}C_lO_6+H^+$ = 279.0629, found 279.0637.
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18. *Procedure and spectral data for* ***13****:* To a solution of **12** (5.5 g, 19.73 mmol) in 90% EtOH (100 mL) was added PTSA (0.38 g, 2 mmol). The resulting solution was heated to reflux for 72 h. The solution was concentrated and dissolved in EtOAc (100 mL) and washed with sat.$NaHCO_3$ (30 mL), water (20 mL), brine (20 mL) and dried over $Na_2SO_4$. The solvent was reduced under reduced pressure and purified via flash column chromatography (hexanes/EtOAc 1:4) to obtain 2.5 g of **13** as a yellow oil in 85% yield. IR (film) 3340, 1722, 744 $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 4.99 (s, 2H), 4.73 (d, $J$ = 5.1 Hz, 2H), 3.46(br s, 1H); $^{13}C$ NMR $\delta$ 169.4, 160.3, 117.2, 70.8, 57.5. HRMS (FAB) calcd for $C_5H_5C_lO_3+H^+$ = 148.9999, found 149.0000.
19. *Procedure for* ***8****:* PCC (3.2 g, 14.7 mmol) was dispersed on solid NaCl (15 g) by grinding together in a mortar and pestle and then suspended into DCM (50 mL). A solution of **13** (1 g, 6.71 mmol) in DCM (15 mL) was then added to the above suspension. After 3 h the DCM layer was filtered through a short plug of silica and washed with DCM (25 mL). To this solution $PCl_5$ (6 g, 28.85 mmol) was added and stirred for 20 min at rt. Solid $NaHCO_3$ (20 g) was then added followed by water (200 mL) slowly. This biphasic solution was stirred approximately 4 h until all the $CO_2$ evolution ceased. The DCM layer was separated and the aqueous layer was extracted with DCM (30 mL). The combined organic layers were washed with water (30 mL), brine (30 mL) and dried over $Na_2SO_4$. The solvent was concentrated under reduced pressure and filtered through a plug of silica using ether (50 mL). After concentration of the organic layer 1 g of **8** was obtained as brown color oil in 80% yield. Spectral data of eight matched those reported in Ref. 13.
20. *Procedure for* ***2****:* To a solution of **8** (0.5 g, 2.48 mmol) in $CCl_4$ (25 mL) was added NBS (0.88 g, 4.96 mmol), cat AIBN (10–20 mg), and refluxed for 24 h under $N_2$. The resulting solution was cooled filtered through a plug of silica using DCM (10 mL). This solution was concentrated and redissolved in 80% dioxane (15 mL) and 5% HCl (5 mL) solution. This solution was refluxed for 2 h after which the solution was concentrated and the aqueous layer was extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with water (5 mL), brine (5 mL), and dried over $Na_2SO_4$. The solvent was evaporated under reduced pressure and purified via flash column chromatography (hexanes/EtOAc 1:4, four drops of HCl) to obtain 0.26 g of **2** as a yellow oil in 50% yield. Spectral data of **2** matched those reported in Ref. 13.
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23. *Procedure and spectral data for* ***14****:* Sodium triacetoxyborohydride (25 mg, 0.16 mmol) was slowly added to a mixture of MX (25 mg, 0.11 mmol) and 3-phenylpropylamine (16 mg, 0.12 mmol) in chloroform (1 mL), acetic acid (0.01 mL), and 22 mg of molecular sieves. The reaction mixture was stirred at rt for 24 h. The reaction mixture was partitioned between water (1 mL), dichloromethane (2 mL), the phases were separated and the organic phase was washed once with water (1 mL). The organic phase was concentrated under reduced pressure and purified via flash column chromatography (hexanes/EtOAc 4:1) to obtain 16 mg of **14** as a yellow oil in 50% yield. IR (film) 3068, 3033, 1684, 1496,1446 $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 7.32–7.17 (m, 5H), 6.74 (s, 1H), 4.19 (s, 1H), 3.57 (t, $J$ = 7.5 Hz, 2H), 2.70–2.71 (m, 2H), 2.15–1.94 (m, 2H); $^{13}C$ NMR $\delta$ 164.1, 144.3, 140.8, 128.5 (2C), 128.3 (2C), 126.2, 126.1, 62.8, 48.2, 43.1, 33.0, 29.7. HRMS (FAB) calcd for $C_{14}H_{14}C_{l3}NO+H^+$ = 318.0219 found 318.0216.
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