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Improved Synthesis of Three Brominated Analogues of the Potent Environmental Mutagen 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone (MX)

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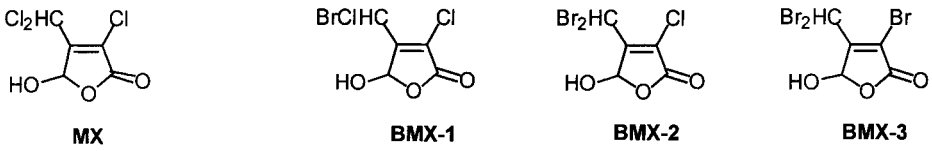
Abstract—The synthesis of three brominated analogues of the environmental mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone (**MX**), namely, 3-chloro-4-(bromochloromethyl)-5-hydroxy-2(5*H*)-furanone (**BMX-1**), 3-chloro-4-(dibromomethyl)-5-hydroxy-2(5*H*)-furanone (**BMX-2**) and 3-bromo-4-(dibromomethyl)-5-hydroxy-2(5*H*)-furanone (**BMX-3**), by employing a simple procedure from a common precursor, is described.

The occurrence of halogenated furanones in drinking water is a matter of concern due to the potential toxicity of these compounds. The most important representative of this family of toxins is 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone (**MX**, Scheme 1). This compound is a highly potent direct-acting mutagen in the *Salmonella typhimurium* assay.[1–3] The data available indicate that **MX** is originated from the reactions of chlorine with the humic substances not eliminated during the purification of water.[4,5] It has been shown that **MX** induces DNA damage in mammalian cells in vitro[6–8] and in vivo,[9,10] and that the administration of this mutagen through drinking water caused cancer effects in rats.[11] More recently, adducts from the reaction of **MX** with nucleosides and calf thymus DNA have been identified.[12,13] These findings, together with the epidemiological studies showing a link between cancer in humans and consumption of chlorinated drinking water, suggest that **MX** should be considered as a health risk factor.[14,15]

The chlorine disinfection of drinking water with high bromide content could also lead to the formation of brominated hydroxyfuranones. These compounds may arise from the generation of hypobromous acid or bromine by the reaction of chlorine with bromide ion. Horth et al. described the formation of three bromohydroxyfuranones, namely **BMX-1**, **BMX-2** and **BMX-3** (Scheme 1) as result of the chlorination of water containing bromide ions; furthermore, mutagenic activities were reported for these compounds although their rigorous chemical characterization was not given.[4,16] Suzuki et al. reported the occurrence of **BMX-1**, **BMX-2** and **BMX-3**, together with **MX**, in four different samples of Japanese chlorinated drinking water.[17]

Finally, LaLonde and coworkers have recently published the synthesis and mutagenicity in the Ames *Salmonella typhimurium* TA-100 assay of two bromohydroxyfuranones, namely **BMX-2** and **BMX-3**.[18] This work prompted us to report our results on these **MX** analogues. In the present contribution the synthesis of bromohydroxyfuranones **BMX-1**, **BMX-2** and **BMX-3** is reported. For **BMX-1**, this is the first report on its synthesis and chemical characterization. The preparation of these bromohydroxyfuranones was accomplished by using a simple synthetic pathway starting from methyl 3-methylbut-2-enoate as common precursor.



Scheme 1. Structure of **MX** and of its brominated analogues **BMX-1**, **BMX-2** and **BMX-3**.

Keywords: mutagen; synthesis; bromofuranones; polybrominated compounds.
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Results and Discussion

LaLonde and coworkers described the preparation of **BMX-2** and **BMX-3** by using 4-(hydroxymethyl)-2(5*H*)-furanone as common precursor for both bromohydroxyfuranones.[18] However, the synthetic procedures involved a high number of steps and some of them proceeded in moderate or low yields, which limit the availability of these mutagens for toxicological studies. We designed an alternative procedure which allows the preparation of all three bromohydroxyfuranones in higher yields and in a simple and shorter manner. This procedure resembles that followed by Padmapriya et al. for the synthesis of **MX**. These authors introduced the chlorine atoms into non-cyclic intermediates and the cyclisation to the hydroxyfuranone was performed in the last step.[19] By contrast, the strategy of LaLonde et al. consisted in the formation of the furanone ring first followed by its subsequent chlorination and hydroxylation at the appropriate positions. Assays carried out for the preparation of MX had indicated that the former strategy was more convenient than that by the group of LaLonde. Thus, in our procedure the readily available methyl 3-methylbut-2-enoate (**1**) was used as common precursor. As shown in Scheme 2, one route led to the formation of **BMX-1** and **BMX-2** while the second afforded **BMX-3**.

Synthesis of BMX-3

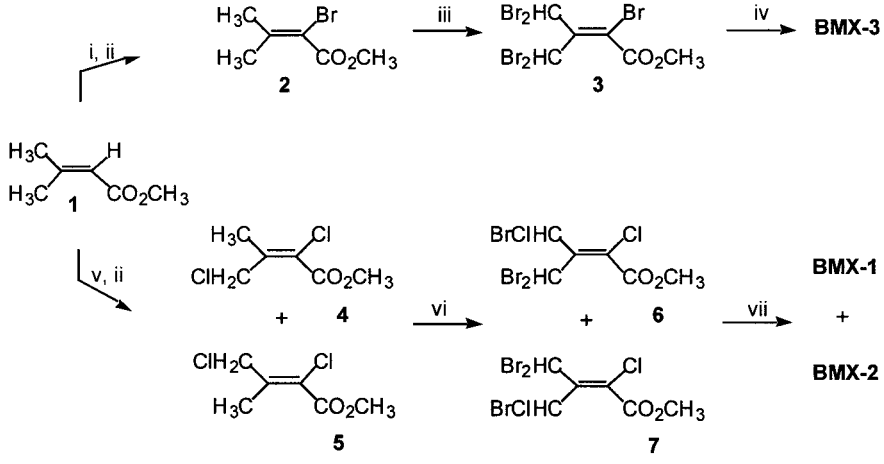
Our initial attempts were directed towards the allylic bromination of ester **1**. This reaction gave the corresponding tetrabromo derivative in 13% yield. However, all efforts to brominate or even chlorinate the conjugated double bond of this intermediate were unsuccessful and only products resulting from the rearrangement of the double bond to the β,γ-position were detected. This result suggested that steric hindrance could be operating when both methyl groups in **1** were dibrominated.[20]

Then we investigated the inverse sequence, i.e. first bromination of the double bond which was followed by the introduction of the allylic bromo substituents. Thus, the bromination of ester **1** and subsequent dehydrobromination with base led to the bromo ester **2** in 77% yield. This ester was treated with *N*-bromosuccinimide under irradiation to give the pentabromo derivative **3** in 40% yield. Whereas the first three bromine atoms were introduced in six hours in almost quantitative yields (GC and GC/MS monitoring), prolonged treatments with large excesses of the brominating agent were required to accomplish the introduction of the fourth one, and even then the conversion was not complete.

Interestingly, the ^{1}H NMR spectrum of compound **3** showed broad peaks at 7.21 and 6.79 ppm which were attributed to the hydrogen atoms *cis* and *trans* with respect to the ester moiety, respectively. In particular, the peak at 7.21 ppm was the broadest. The ^{13}C NMR spectrum of **3** showed two broad peaks at 34.3 and 34.9 ppm assigned to both dibromomethyl carbon atoms. These observations suggested the possibility of a restricted rotation around the C-3/C-4 and C-3/C-5 bonds of this derivative.

To confirm this hypothesis, the peaks shapes in the ^{1}H NMR spectrum of **3** were monitored at different temperatures in deuterochloroform and in deuterated dimethyl sulfoxide solutions (Fig. 1). The results obtained in deuterochloroform indicated that the rotation was severely restricted at low temperatures. Upon heating the rotation was possible but slow enough to give very broad peaks, especially for H-5, which suggests a more restricted rotation of the dibromomethyl moiety linked to this hydrogen atom. As expected, these peaks became narrow by increasing the temperature. While the shape of H-4 showed a similar pattern in the spectra recorded at the same temperatures in both solvents, the peak of H-5 was better resolved in deuterated dimethyl sulfoxide. This suggests that the solvent plays a role in the rotation of the corresponding dibromethyl group.

This presumption was supported by the X-ray diffraction analysis of this pentabromo derivative. As shown in Fig. 2, a hydrogen bond between H-5 and the carbonyl oxygen of the ester moiety (bond distances: H5–O1 2.18(6) Å; C5–O1 2.831(10) Å; bond angle 123(4)°) is present, which explains the higher restriction of rotation observed for this group in comparison with that of the other dibromomethyl moiety.



Scheme 2. Synthetic route for the preparation of **BMX-1**, **BMX-2** and **BMX-3**. Reaction conditions: (i) Br_2, CCl_4; (ii) Et_3N; (iii) 4 equiv. NBS; (iv) 48% HBr; (v) Cl_2, CCl_4; (vi) 3 equiv. NBS, hv; (vii) 70% CH_3SO_3H.

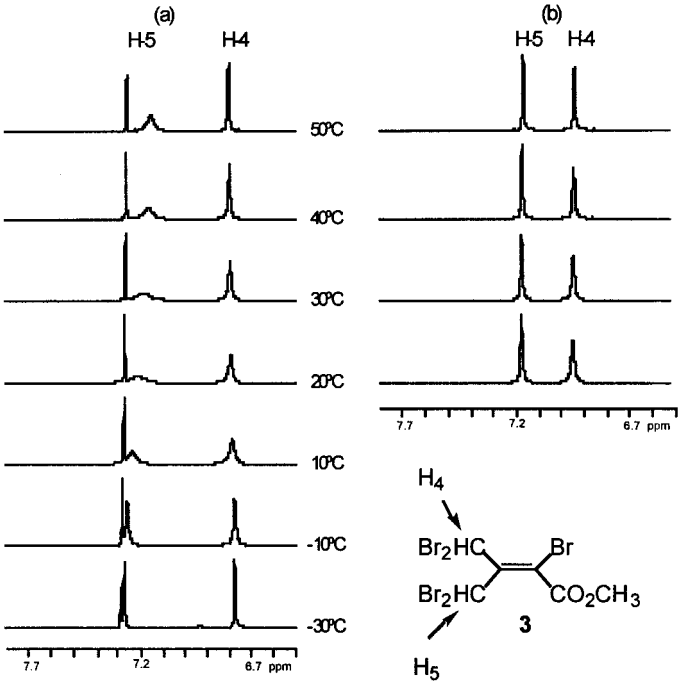


Figure 1. Profile within the 6.5–7.8 ppm region of the ^{1}H NMR spectra (300 MHz) of ester **3** at different temperatures in: (a) $CDCl_3$; and (b) d_6-DMSO solutions.

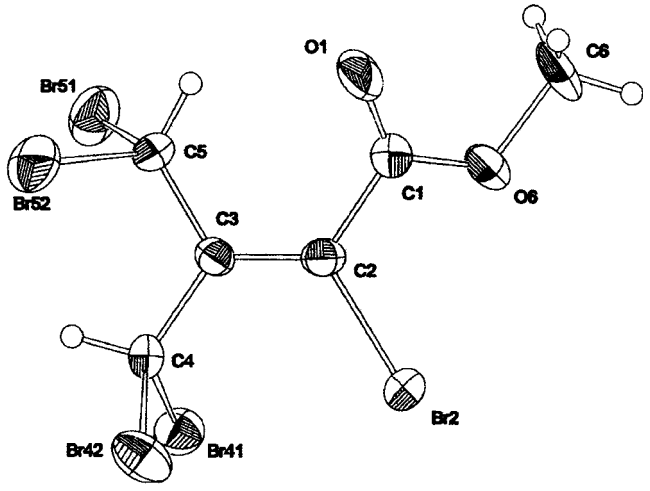


Figure 2. X-Ray structure of the pentabromo ester **3** with atom labelling.

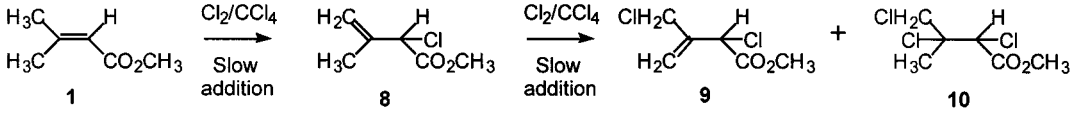
When the hydrolysis and subsequent cyclisation of **3** was attempted using the basic conditions as reported for **MX**, i.e. lithium hydroxide in tetrahydrofuran/water followed by treatment with potassium bicarbonate, **BMX-3** was formed in yields lower than 5%. Conversely, when this cyclisation was performed in 48% hydrobromic acid,[21] the expected brominated mutagen was isolated in 48% yield, which corresponds with 15% overall yield.

Synthesis of BMX-1 and BMX-2

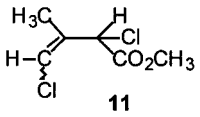
The synthesis of these mutagens was carried out as shown in Scheme 2. The chlorination of ester **1** was problematic due to the formation of products with different degree of chlorination. The optimised procedure involved the slow addition of a saturated solution of chlorine in carbon tetrachloride to the ester **1**. Even in this case, as shown in Scheme 3, the addition of the first equivalent of chlorine led to the formation of the chloro ester **8** (approx. 30% yield), while the addition of a second equivalent of chlorine afforded a mixture of products in which the dichloro ester **9** (30%) and the diastereomeric mixture of trichloro esters **10** (16%) were the major components.

In view of the complexity and anticipated instability of this reaction mixture, it was decided to perform the subsequent dehydrochlorination without any further purification. Thus, treatment of the above crude reaction mixture with base afforded a 1.8:1 mixture of the unsaturated dichloro esters **4** and **5** in 55% yield.[22] An attempt to purify this mixture by distillation led to the isolation of pure compound **5**; unfortunately, most of the dichloro ester **4** decomposed. In addition, an *E*,*Z* mixture of the rearranged dichloro esters **11** (Scheme 4), which was not detected in the crude reaction mixture, was also isolated.

When pure dichloro ester **5** was treated with an excess of



Scheme 3. Treatment of acrylate **1** with chlorine.



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Scheme 4. Major by-products isolated in the distillation of the crude reaction mixture containing dichloro esters **4** and **5**.

N-bromosuccinimide under irradiation, a 1:1.3 mixture of the tribromo esters **6** and **7** was formed. This result indicated that an extensive isomerisation occurred during the bromination.[23] This fact together with the problems encountered in the purification of the mixture of dichloro esters **4** and **5** led to the idea to brominate the crude reaction mixture containing these dichloro esters. Accordingly, this mixture was subjected to the allylic bromination under the same conditions described above to render the tribromo esters **6** and **7** in 18% overall yield. Similarly to the allylic bromination during the synthesis of **BMX-3**, the introduction of the third bromine atom was slow and difficult because of the steric hindrance exerted by the two bromo substituents already present and the chlorine atom attached to the double bond. All efforts to separate both stereoisomers were unsuccessful.

The ^{1}H NMR analysis of this mixture revealed features similar to those observed for the pentabromo derivative **3**. Especially, the proton linked to the bromochloromethyl moiety in **7** appeared as a broad peak. The peak width at half height was 33.1 Hz at 21.5°C in $CDCl_3$ and this value was reduced to 12.6 and 9.4 Hz at 30 and 40°C, respectively. This effect was considerably less pronounced for the proton linked to the dibromomethyl moiety in **6** (3.75 and 2.35 Hz at 21.5 and 40°C, respectively). These results suggest that in **7** a strong hydrogen bond interaction is present between the hydrogen atom linked to the bromochloromethyl moiety and the carbonyl oxygen atom. For both tribromo derivatives however, in contrast to **3**, the methine proton *trans* with respect to the ester moeity does not exhibit this band broadening, which suggests that the steric hindrance in these compounds is considerably reduced.

Hydrolysis and subsequent cyclisation of these intermediates in 70% methanesulphonic acid afforded the mixture of **BMX-1** and **BMX-2**, which was separated by preparative reversed phase HPLC to give the pure compounds in 25 and 20% yields, respectively. The use of hydrobromic acid to promote the cyclisation was precluded in this case since undesired halogen replacements were observed for both **BMX-1** and **BMX-2**. Although **BMX-1** should be present as a diastereomer mixture, only one peak was observed in the HPLC using different elution conditions. In constrast, the derivatization of **BMX-1** with bis(trimethylsilyl)trifluoroacetamide showed two peaks in GC with mass spectra compatible with two TMS esters. This derivatisation protocol may be of value for the identification of these diastereomers in water samples.

In summary, our procedure allows the preparation of all three bromohydroxyfuranones in sufficient amounts and purities for future toxicological assays. In addition, the overall yield obtained for **BMX-2** (2%) was slightly higher than that reported by the group of Lalonde (1.6%), and this difference was considerably greater for **BMX-3** in terms of both yields (15% versus 0.7%) and simplicity. Moreover, this procedure permitted the preparation and rigorous chemical characterization of **BMX-1** for the first time. In our case, the availability of these bromohydroxyfuranone standards, especially of **BMX-1**, allows the study of their cytotoxicity.[24] This study revealed that they are potent direct mutagens in the Ames test and that their eventual generation in drinking water must be adequately monitored. In this context, the reactivity of **BMX-1**, **BMX-2** and **BMX-3** with DNA model molecules was investigated and will be reported elsewhere.

Experimental

Caution: **MX** and its brominated analogues (**BMXs**) are hazardous due to their mutagenicity. Therefore, the use of gloves, well ventilated fume cupboards and careful destruction of residues with NaOCl is recommended for the manipulation of these compounds.

Melting points were determined with a Koffler apparatus and are uncorrected. The IR spectra were registered with a MB model 120 Bomen apparatus and absorptions are given in cm^{-1}. The ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded with a Varian Unity 300 spectrometer. Unless stated otherwise, spectra were taken in previously neutralized $CDCl_3$ solution. Chemical shifts are given in ppm related to tetramethylsilane for ^{1}H and deuterochloroform for ^{13}C as internal standards. The HPLC analyses were performed with a modular system formed by two Waters 510 pumps and an Applied Biosystems detector-gradient controller (1000S Diode Array Detector). The preparative purifications were performed by employing a 30×0.78 cm ODS, 10 μm column, from Tracer, S.A. Barcelona, Spain. The high resolution mass spectra (HRMS) were obtained with a VG Autospec Q instrument at the 'Servei d'Espectrometria de Masses del CID'. The GC/MS/EI spectra (70 eV) were obtained using a Fisons model MD 800 mass spectrometer coupled to a Fisons GC 8000 apparatus, which was equiped with a 25 m HP-5 capillary column; the halogen isotope pattern observed and the postulated fragmentation are given in parentheses. Elemental microanalyses were carried out at the 'Servei de Microanàlisi' of the CID by using a 1108 Carlo Erba analyser.

Unless stated otherwise, organic solutions obtained from the treatment of crude reaction mixtures were dried over $MgSO_4$. Purifications by flash chromatography were performed by using 40–60 mm silicagel.

Synthesis of BMX-3

Methyl 2-bromo-3-methylbut-2-enoate (2). Bromine (1.8 mL, 35.8 mmol) was added dropwise to a solution of ester **1** (4.09 g, 35.8 mmol) in CCl_4 (40 mL) and the mixture was stirred for 1 h at 25°C. The residue obtained after removal of the solvent was redissolved in CH_2Cl_2 (30 mL). Then, a solution of Et_3N (5.3 mL, 38.3 mmol) in

CH_2Cl_2 (6 mL) was added dropwise to the above solution, maintained at 0°C. When the addition was completed, the crude reaction mixture was stirred for 16 h at 25°C (GC monitoring) and filtered. The filtrate was washed with 0.3 N HCl, water, brine and dried. The residue obtained from the elimination of solvents (5.9 g) was purified by flash chromatography on silicagel eluting with hexane–EtOAc mixtures to give pure acrylate **2** as a pale yellow oil (5.50 g, 77% yield). **2**:[25] IR (film): 1716; ^{1}H NMR δ: 3.79 (s, 3 H), 2.14 (s, 3 H, *Z*-CH_3), 2.05 (s, 3 H, *E*-CH_3); ^{13}C NMR δ: 164.5 (C-1), 149.1 (C-3), 108.2 (C-2), 52.7 (CH_3), 27.2 (*E*-CH_3), 23.2 (*Z*-CH_3); MS (EI): *m/z*, 192, 194 (M, Br); 177, 179 (M−Me, Br); 161, 163 (M−OMe, Br); 160, 162 (M−[OMe+H], Br); 133, 135 (M−[OMe+CO], Br); 132, 134 (M−[OMe+CO+H], Br); 53 (base peak, M−[OMe+CO+Br+H]).

Methyl 2,4,4-tribromo-3-(dibromomethyl)but-2-enoate (3). A soln. of the bromo ester **2** (3.42 g, 17.7 mmol) in CCl_4 (150 mL) was treated with NBS (13.2 g, 74.2 mmol) and the mixture was stirred while irradiating with a 300 W mercury lamp for 15 h (GC monitoring). The crude reaction mixture was filtered, the filtrate was washed with CCl_4, concentrated and treated with additional NBS (4.09 g, 23 mmol) and irradiation for 3 more days. The crude reaction mixture was filtered and the precipitate was washed thoroughly with CCl_4. The residue obtained from the elimination of the solvent was purified by flash chromatography on silicagel eluting with 30:1 hexane:EtOAc solvent mixture, to give compound **3** as a solid (3.45 g, 40% yield). **3**: mp103–104°C (hexane:$CHCl_3$); IR (KCl): 1710, 734; ^{1}H NMR δ: 7.21 (br, 1 H, *Z*-$CHBr_2$), 6.79 (br, 1 H, *E*-$CHBr_2$), 3.94 (s, 3 H, CH_3); ^{13}C NMR δ: 163.0 (C-1), 142.5 (C-3), 120.4 (C-2), 54.2 (CH_3), 34.9 (*E*-$CHBr_2$), 34.3 (*Z*-$CHBr_2$); MS (EI): *m/z*, 473, 475, 477, 479, 481, 483 (Br_5, M-OCH_3), 445, 447, 449, 451, 453, 455 (Br_5, M-CO_2CH_3), 425, 427, 429, 431, 433 (Br_4, M-Br), 397, 399,401, 403, 405 (Br_4), 50 (base peak). Elemental analysis for $C_6H_5Br_5O_2$: C, 14.12; H, 0.98; Br, 78.56. Found: C, 13.95; H, 0.91, Br, 78.30.

Cyclisation of the pentabromo ester 3. A suspension of **3** (0.51 g, 1 mmol) in 48% HBr (5 mL) was heated for 8 h at gentle reflux (TLC monitoring). The crude reaction mixture was diluted with water and extracted with EtOAc. The organic fraction was washed with brine and dried. The brownish residue obtained after the elimination of the solvent (0.33 g) was purified by flash chromatography on silicagel, eluting with a hexane:EtOAc solvent mixture to give an enriched sample of **BMX-3**. A final purification by cristallyzation ($CHCl_3$) afforded the pure bromohydroxyfuranone as a colorless solid (170 mg, 48% yield). **BMX-3**:[18] mp 71–73°C ($CHCl_3$); IR (KBr): 1797, 1780; ^{1}H NMR δ: 6.47 (s, 1 H, $CHBr_2$), 6.42 (br, 1H, C*H*OH), 4.97 (s, 1 H, OH); ^{13}C NMR δ: 164.9 (C-1), 155.7 (C-3), 114.1 (C-2), 98.6 (C-5), 26.4 (C-6). Elemental analysis for $C_5H_3Br_3O_3$: C, 17.11, H, 0.85, Br, 68.35. Found: C, 17.12, H, 0.76, Br, 68.25. Silylation of **BMX-3** using bis(trimethylsilyl)trifluoroacetamide led to the TMS ester. MS (EI): *m/z*, 419, 421, 423, 425 (Br_3, M-1), 405, 407, 409, 411 (Br_3, M−Me), 223, 225, 227 (Br_2, base peak, M−[Br+CHO+O-$SiMe_3$]), 195, 197, 199 (Br_2, M−[Br+CHO+$OSiMe_3$+CO]).

Synthesis of BMX-1 and BMX-2

Methyl (*E,Z*)-2,4-dichloro-3-methylbut-2-enoate (4 and 5). A satd. soln. of chlorine in CCl_4 was added dropwise and at room temperature to a solution of ester **1** (5 g, 44 mmol) in the same solvent (50 mL) until the reaction was completed (GC monitoring) (72 mL, approx. 2.4 molar equivalents of chlorine were required). The crude reaction mixture was washed with $NaHCO_3$ satd. solution, water, brine and dried. The elimination of the solvent under vaccum rendered a pale yellow oil residue. Triethylamine (3.1 mL, 22 mmol) was added dropwise to a solution of this residue in CH_2Cl_2, maintained at 0–5°C, and the mixture was stirred for 1 h at this temperature and for 20 h at 20°C (GC monitoring). The crude reaction mixture was washed with 0.5 N HCl, water, brine and dried. The dark brown residue obtained from the elimination of solvents was purified by filtration through silicagel eluting with hexane–ethyl acetate (98:2) to give a 1.8:1 mixture of dichloro esters **4** and **5** (5.8 g, 76% purity, 55% overall yield). IR (film): 1726; ^{1}H NMR δ: 4.57 (s, 2 H, CH_2Cl, **4**), 4.30 (s, 2 H, CH_2Cl, **5**), 3.85 (s, 6 H, CO_2Me, **4** and **5**), 2.25 (s, 3 H, CH_3, **5**), 2.15 (s, 3H, CH_3, **4**); ^{13}C NMR δ: 163.3 (C-1, **5**), 162.9 (C-1, **4**), 144.4 (C-3, **4**), 143.1 (C-3, **5**), 123.2 (C-2, **4**), 121.3 (C-2, **5**), 53.0 (CO_2CH_3, **4**), 52.9 (CO_2CH_3, **5**), 45.9 (CH_2Cl, **5**), 44.1 (CH_2Cl, **4**), 20.7 (CH_3, **4**), 19.1(CH_3, **5**); MS (EI): *m/z*, 182, 184, 186 (M^+, Cl_2), 147, 149 (M−Cl), 146, 148 (M−HCl), 87, 89 (base peak). Elemental analysis for $C_6H_8Cl_2O_2$. Calcd: C, 39.37; H, 4.41; Cl, 38.74. Found: C, 39.44; H, 4.41; Cl, 39.16. HRMS: calcd, 181.9901. Found, 181.9903.

Methyl (*E,Z*)-4-bromo-3-dibromomethyl-2,4-dichlorobut-2-enoate (6 and 7). A solution of the dichloro esters **4** and **5** (1.9 g, 10 mmol) in CCl_4 (70 mL) was treated with *N*-bromosuccinimide (6.5 g, 37 mmol) and the mixture was irradiated with a 300 W mercury lamp, under stirring, for 110 h divided in three different periods (GC monitoring). At the end of the first period (30 h), the crude reaction mixture was filtered and the precipitate was washed with CCl_4. The residue obtained after the elimination of the solvent was purified by flash chromatography (95:5 hexane:EtOAc) to give a fraction which was dissolved in CCl_4 (30 mL) and irradiated in the presence of NBS (2.2 g, 12 mmol) for 30 h. The whole process was repeated with a new addition of NBS (1.2 g, 7 mmol) and a final irradiation period of 50 h. The crude reaction mixture was filtered and the precipitate was washed thoroughly with CCl_4. The residue obtained from the elimination of the solvent was purified by filtration through silicagel eluting with hexane–EtOAc (from 99:1 to 95:5) to give the expected tribromo derivatives which were isolated after crystallization from $CHCl_3$–hexane as a 1:1.3 mixture of **6** and **7**, respectively (0.79 g, 18% yield). All attempts to separate this mixture by further fractional crystallization were unsuccessful. Mp 78–80°C (hexane–$CHCl_3$); IR (KBr): 1716; ^{1}H NMR ($CDCl_3$), δ: 7.57 (br, 1 H, CHBrCl, **7**), 7.51 (br, 1 H, $CHBr_2$, **6**), 6.81 (s, 1 H, CHBrCl, **6**), 6.78 (s, 1 H, $CHBr_2$, **7**), 3.94 (s, 6 H, CH_3, **6** and **7**); ^{13}C NMR ($CDCl_3$, 25°C), δ: 162.1 (C-1), 142.5 (C-3), 128.5 (C-2), 54.1 (CH_3), 51.0 (CHBrCl), 50.8 (CHBrCl), 34.0 ($CHBr_2$), 30.8 ($CHBr_2$); MS (EI): *m/z*, 385, 387, 389, 391, 393 (Cl_2Br_3, M−CH_3O), 380, 382, 384, 386, 388 ($ClBr_3$, M−HCl), 337, 339, 341, 343 (Cl_2Br_2, M−Br),

258, 260, 262 (base peak, Cl_2Br, M−2 Br). Elemental analysis for $C_6H_6Br_3Cl_2O_2$. Calcd: C, 17.16; H, 1.19; Br, 57.12; Cl, 16.98. Found: C, 17.26; H, 1.08; Br, 57.15; Cl, 17.00.

Cyclization of the mixture of halo esters 6 and 7. A suspension of this mixture of esters (0.38 g, 0.9 mmol) in 70% methanesulfonic acid (30 mL) was heated for 6 h at 140°C (TLC monitoring). The crude reaction mixture was cooled, poured into water and extracted with ethyl acetate. The organic fraction was washed with brine and dried. The residue obtained after the elimination of the solvent (0.230 g) was prepurified by flash chromatography on silicagel eluting with a 70:20:10 hexane–EtOAc–AcOH mixture, to give an enriched mixture of **BMX-1** and **BMX-2**. A 50 mg fraction of this mixture was redissolved in MeOH (400 μL) and separated by preparative HPLC (30×0.78 cm ODS-2, 10 μm column). The elution conditions were 20:80 MeOH: 50 mM HCOOH–Et_3N buffer (pH 3.16) at 2.5 mL/min. The collected eluates corresponding to the bromohydroxyfuranones were independently extracted with EtOAc and the organic fractions were washed with brine and dried. Final purification of each sample by preparative TLC on silicagel, eluting with a 1:1 hexane–EtOAc solvent mixture led to the isolation of pure **BMX-1** and **BMX-2**.

3-Chloro-4-(bromochloromethyl)-5-hydroxy-2(5*H*)-furanone (BMX-1). 11 mg (25%); ^{1}H NMR, δ: 6.63, 6.62 (s, 1 H, CHBrCl), 6.43 (br, 1 H, H-5), 4.75 (0.7 H, OH), 4.32 (0.3 H, OH); ^{13}C NMR δ: 163.8 (CO), 151.0 (C-4), 124.4 (C-3), 96.5 (CHOH), 43.73 (CHBrCl); HRMS for $C_5H_2BrCl_2O_2$: calcd, 242.8615. Found: 242.8618. Silylation of **BMX-1** using bis(trimethylsilyl)trifluoroacetamide led to a mixture of TMS esters in a 1:2 diastereomeric ratio (GC/MS monitoring). MS (EI): *m/z*, 317, 319, 321, 323 ($BrCl_2$, M-15), 239, 241, 243 (Cl_2, M-CHBr), 179, 181, 183 [BrCl,M−(Cl+Me_3SiO+CHO)], 73, 75 [base peak, Cl, M−(Br+Cl+Me_3SiO+2CO)].

3-Chloro-4-(dibromomethyl)-5-hydroxy-2(5*H*)-furanone (BMX-2).[18] 18 mg (20%); ^{1}H NMR, δ: 6.49 (s, 1 H, $CHBr_2$), 6.43 (br, 1 H, H-5), 4.41 (s, 1 H, OH); ^{13}C NMR, δ: 163.6 (CO), 151.6 (C-4), 123.8 (C-3), 97.1 (CHOH), 24.80 ($CHBr_2$); HRMS for $C_5H_2Br_2ClO_2$: calcd, 286.811001. Found: 286.810829. Silylation of **BMX-2** using bis-(trimethylsilyl)trifluoroacetamide led to the TMS ester. MS (EI): *m/z*, 375, 377, 379, 381 ($ClBr_2$, M−1), 361, 363, 365, 367 ($ClBr_2$, M−Me), 223, 225, 227 (Br_2, M−[$OSiMe_3$+CHO+Cl]), 179, 181, 183 (ClBr, M−[$OSiMe_3$+CHO+Br]), 151, 153, 155 (ClBr, M−[$OSiMe_3$+CHO+Br+CO]), 73, 75 (Cl, base peak, M−[2Br+$OSiMe_3$+2CO]).

Single crystal X-ray diffraction analysis

All data were collected using an Enraf-Nonius CAD4 diffractometer with MoK_α radiation (λ=0.71073 Å) and were processed with the program WINGX.[26] The structures were solved by direct methods using the SHELX97 program[27] and refined by full-matrix least squares methods on F^2 using the SHELX97 program.[28]

Methyl 2,4,4-tribromo-3-(dibromomethyl)but-2-enoate (3). empirical formula: $C_6H_5Br_5O_2$, M=508.65, triclinic, space group P-1, a=6.236 Å, b=8.480 Å, c=11.502 Å, α=93.03°, β=101.73°, γ=94.25°, V=592.5 Å^3, Z=2, D_c=2.851 g/cm^3, μ=16.925 mm^{-1}, 3793 reflections collected (θ-range=1.81–29.98°), 3453 independent reflections (R_{int}=0.0380), 129 parameters, R_1=0.0543, wR_2=0.1239 (all reflections), residual electron density 1.210 and −1.049 e Å^{-3}.

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