Effect of 11β-substituents on the regioselective chlorination of estrogens with 2,3,4,5,6,6-hexachloro-2,4-cyclohexadienone

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The reaction of 11β-substituted estrogens with 2,3,4,5,6,6-hexachloro-2,4-cyclohexadienone affords exclusively ortho-substituted monochlorinated products including a major 4-chloro and a minor 2-chloro derivative. In the absence of an 11β-substituent, regioselectivity is lost, resulting in a mixture of 10β- and ortho-chlorinated products. (*Steroids* **59**:498–502, 1994)

Keywords: estradiol derivative; regioselective chlorination; 2,3,4,5,6,6-hexachloro-2,4-cyclohexadienone

Introduction

Radiohalogenated estrogens have found applications as drugs in the detection and therapy of hormone-dependent breast tumors,[1–3] as inhibitors of steroid metabolizing enzymes[4] and as metabolic probes of the mechanism of estrogen carcinogenesis.[5] The regioselective chlorination of aromatic substrates in general constitutes an important challenge in organic synthesis. Chlorination of estradiol derivatives can be accomplished with a number of reagents, however most reported reactions give complex mixtures of halogenated products, from which the desired compound can only be obtained by exhaustive chromatographic procedures.[6–9] Although a number of reagents have been reported for the regioselective halogenation of estrogens at the C-2 position,[10–16] halogenation at position C-4 has received little attention.[17–19] Since C-4 substituted derivatives show better biological activities,[19–23] we sought to improve synthetic procedures for such analogues.

A few publications have appeared on the use of hexachlorocyclohexadienone as a regioselective chlorinating agent.[24–27] Selectivity was attained by tailoring the reagent, such as to permit participation in selected donor-acceptor and H-bonding interactions. Thus, 2,3,4,4,5,6-hexachlorocyclohexa-2,5-dien-1-one allows the chlorination of phenol in the para position, while 2,3,4,5,6,6-hexachlorocyclohexa-2,4-dien-1-one permits the regioselective chlorination in the ortho position.[25,26] These results prompted us to evaluate hexachlorocyclohexadienes as a reagent for the regioselective chlorination of estradiol derivatives.

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Experimental

Chemicals

Steroids were obtained from Sigma Chemical Company (St. Louis, MO, USA) or from Steraloids Inc. (Wilton, NH, USA). 17-Ethylenedioxyestra-1,3,5(10)-triene-3,11β-diol was synthesized according to Baran[28] and converted to the 11β-methoxy or 11β-ethoxy derivatives with methyl iodide or ethyl iodide.[29] The ketone derivatives were subsequently converted to the 17α-ethynyl analogues by treatment with lithium acetylide, ethylenediamine complex.[21] 2,3,4,5,6,6-Hexachloro-2,4-cyclohexadienone was obtained from TCI America (Portland, OR). All chemically pure solvents and inorganic compounds were purchased from Fisher (Montreal, Canada) and Aldrich (Milwaukee, WI, USA).

Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS)

^{1}H NMR spectra were obtained with a Brüker WM 250 spectrometer in $CDCl_3$ or in $CDCl_3$ + DMSO-d_6. Chemical shifts are reported in ppm downfield from tetramethylsilane as an internal standard (δ scale). Low resolution electron impact mass spectra (EIMS) were acquired at 70 eV with a Hewlett-Packard Model 5988A quadrupole instrument. The high resolution mass spectra (HRMS) were determined with a V9 micro-mass model ZAB-1F apparatus at 70 eV ionization voltage.

Chromatography

Analytical thin-layer chromatography (TLC) was performed on Polygram silica gel plates coated with fluorescent indicator (UV 254). Steroid visualization was achieved with short wave ultraviolet light absorbance and/or color response upon spraying with H_2SO_4/EtOH and heating at 120°C. Column chromatography was performed on silica gel (60–200 mesh). High-performance liquid chromatography (HPLC) was performed on a 25 × 0.94 cm reverse-phase column (C-18, ODS-2 spherisorb, 5 μm, CSC, Montreal, Canada) and compounds were detected at 280 nm.

General method for the chlorination of estrogen derivatives (1a–c and 6a–e) with 2,3,4,5,6,6-hexachloro-2,4-cyclohexadienone

To a solution of the phenolic steroid **1** or **6** (0.1 mmol) in dimethylformamide (5 mL) was added with stirring 2,3,4,5,6,6-hexachloro- 2,4-cyclohexadienone (**2**; 0.12 mmol). The reaction mixture was stirred at room temperature for 2–6 h. During this period the color of the reaction mixture changed from dark yellow to light yellow. The mixture was poured into water (20 mL) and extracted with EtOAc (2 × 20 mL) and the combined extracts were washed with water (15 mL), dried (Na_2SO_4), and the solvent was evaporated under reduced pressure. The residue was chromatographed over a column of silica gel (20 g) in 10–30% EtOAc/hexane to yield a mixture which was analyzed on a reverse-phase C-18 HPLC column, using water/methanol as the eluting solvent. Percentage conversion and relative yield of the differently substituted products are given in Table 1 and physical and spectroscopic properties are summarized in Table 2.

Results and discussion

The reaction of estradiol (**1a**) with 2,3,4,5,6,6-hexachloro-2,4-cyclohexadienone (**2**) in a 1:1 molar ratio gave three products, which upon HPLC separation were characterized as the 2-, 4-, and 10-monochloro derivatives of **1a**, i.e., 10β-chloro-17β-hydroxy-1,4-estradien-3-one (**3a**; 76%), 2-chloroestradiol (**4a**; 8%) and 4-chloroestradiol (**5a**; 16%; Figure 1). Structures were assigned upon comparison of physical properties (m.p., TLC, HPLC, MS) with those of authentic compounds prepared by established methods.[19] The relative yield between 10β-chlorinated and ortho-substituted products is similar to that reported for the chlorination of phenol with this reagent, but differs from those obtained in the case of naphth-2-ol, whereby only the C-1 of the two possible α-positions relative to the OH group is chlorinated. The predominantly 10β-chlorination of **1a** was rather unexpected. Extending the procedure to estrone (**1b**) gave 10β-chloro-1,4-estradien-3,17-dione (**3b**), 2-chloroestrone (**4b**), and 4-chloroestrone (**5b**). 17α-Ethynylestradiol (**1c**) likewise gave mixtures of 10β-chlorinated and ortho-substituted products, 10β-chloro-17α-ethynyl-17β-hydroxy-1,4-estradien-3-one (**3c**), 2-chloro-17α-ethynylestradiol (**4c**), and 4-chloro-17α-ethynylestradiol (**5c**) in high overall yields (Table 1). The reaction of estradiol with trichloroisocyanuric acid has been reported to give 2,4,10β-trichloro-1,4-dien-3-one (25%), 2,10- and 4,10-dichloro-1,4-dien-3-one (13%, mix), and 10β-chloro-1,4-dien-3-one (20%).[8] Similarly, *N*-chlorosuccinimide reacts with estradiol to yield 2- or 4-monochloroestradiol (<10%) and 10β-chloro-1,4-dien-3-one (20%).[6] Thus, for the synthesis of the 10β-chloro derivative, reagent **2** is preferable over established reagents such as *N*-chlorosuccinimide[6] and trichloroisocyanuric acid[8] since the latter mainly yield dichlorinated and trichlorinated products. Surprisingly, with 11β-substituted estrogen derivatives (**6**) as a substrate, **2** failed to yield 10β-chlorinated products (Table 1). Instead, using identical reaction condition as described above for estradiol, the reaction of 11β-hydroxyestrone (**6a**) with **2** only gave two products which were purified by HPLC and characterized as the ortho-substituted derivatives 2-chloro-11β-hydroxyestrone (**7a**) and 4-chloro-11β-hydroxyestrone (**8a**; Figure 2). Similar type of products were obtained when the hydroxy group was substituted by methoxy or ethoxy groups. Thus 11β-methoxyestrone (**6b**) gave 2-chloro-11β-methoxyestrone (**7b**) and 4-chloro-11β-methoxyestrone (**8b**), and 11β-methoxy-17α-ethynylestradiol (**6c**) gave 2-chloro-11β-methoxy-17α-ethynylestradiol (**7c**) and 4-chloro-11β-methoxy-17α-ethynylestradiol (**8c**). Similarly 11β-ethoxyestrone (**6d**) yielded 2-chloro-11β-ethoxyestrone (**7d**) and 4-chloro-11β-ethoxyestrone (**8d**), and 11β-ethoxy-17α-ethynylestradiol (**6e**) gave 2-chloro-11β-ethoxy-17α-ethynylestradiol (**7e**) and 4-chloro-11β-ethoxy-17α-ethynylestradiol (**8e**). Increasing the molar ratio of reagent **2** over **6c** from 1 to 2.5 favored the formation of 2,4-dichloro-11β-methoxyestrone (**9c**; 90%) without affecting the 10β-position. None of the 11β-substituted estrogens gave 10β-chlorinated products with this reagent (Figure 2, Table 1). Further substitution with a 17α-ethynyl group

Table 1 Chlorination of estrogen derivatives with 2,3,4,5,6,6-hexachloro-2,4-cyclohexadienone

No	Substrate	Conversion (%)	Relative yield (%)		
			10β-Chloro-	2-Chloro-	4-Chloro-
1a	Estradiol	95	76	8	16
1b	Estrone	84	44	18	38
1c	17α-Ethynylestradiol	84	43	32	24
6a	11β-Hydroxyestrone	92	—	14	86
6b	11β-Methoxyestrone	83	—	25	75
6c	11β-Methoxy-17α-ethynylestradiol	92	—	20	80
6d	11β-Ethoxyestrone	80	—	30	70
6e	11β-Ethoxy-17α-ethynylestradiol	81	—	27	73
10	11β-Methoxyestrone 3-methyl ether	80	—	35	65

Table 2 Physical and spectroscopic data of different chlorinated products

Product	HPLC (t_R min)[a]	mp (°C)[b]	MS m/z (relative intensity)	^{1}H NMR ($CDCl_3$/TMS) δ, J(Hz)	HRMS
7A	A(12)	244–247	320 (M^+, 95), 322 (M^+, 27), 180 (100).	0.96 (s, 3H, 18-CH_3), 4.50 (m, 1H, 11α-H), 6.52 (s, 1H, 4-H), 7.01 (s, 1H, 1-H), 8.61 (s, 1H, OH).	$C_{18}H_{21}ClO_3$ Calculated 320.1179 Found: 320.1185
8a	A(15)	250–255	320 (M^+, 95), 322 (M^+, 27), 266 (M^+ –(HCl + H_2O)), 180 (100).	1.09 (s, 3H, 18-CH_3), 4.69 (m, 1H, 11α-H), 6.84 (d, 1H, J = 8.6, 1-H), 7.04 (d, 1H, J = 8.6, 2-H), 7.69 (s, 1H, OH).	$C_{18}H_{21}ClO_3$ Calculated 320.1179 Found: 320.1185
7b	A(16)	205	334 (M^+, 55), 336 (M^+, 21), 299 (14), 275 (36), 115 (100).	1.10 (s, 3H, 18-CH_3), 3.33 (s, 3H, 11β-OCH_3), 4.13 (m, 1H, 11α-H), 6.74 (s, 1H, 4-H), 6.97 (s, 1H, 1-H).	$C_{19}H_{23}ClO_3$ Calculated 334.1336 Found: 334.1332
8b	A(18)	200	334 (M^+, 53), 336 (M^+, 21), 299 (16), 275 (34), 115 (100).	1.09 (s, 3H, 18-CH_3), 3.29 (s, 3H, 11β-OCH_3), 4.18 (m, 1H, 11α-H), 5.76 (s, 1H, OH), 6.87 (d, 1H, J = 8.6, 2-H), 6.98 (d, 1H, J = 8.6, 1-H).	$C_{19}H_{23}ClO_3$ Calculated 334.1336 Found: 334.1332
9b	C(21)	230–232	368 (M^+, 23), 370(M^+, 17), 309 (19), 280 (9), 98 (100).	1.09 (s, 3H, 18-CH_3), 3.35 (s, 3H, 11β-OCH_3), 4.15 (m, 1H, 11α-H), 7.08 (s, 1H, 1-H).	$C_{19}H_{22}Cl_2O_3$ Calculated 368.0946 Found: 368.0934
7c	B(14)	115–120	360 (M^+, 48), 362 (M^+, 15), 283 (45), 263 (98).	1.07 (s, 3H, 18-CH_3), 3.31 (s, 3H, 11β-OCH_3), 4.13 (m, 1H, 11α-H), 6.71 (s, 1H, 4-H), 7.06 (s, 1H, 1-H).	$C_{21}H_{25}ClO_3$ Calculated 360.1492 Found: 360.1486
8c	C(16)	230–232	360 (M^+, 40), 362 (M^+, 12), 283 (45), 80 (100).	1.08 (s, 3H, 18-CH_3), 3.28 (s, 3H, 11β-OCH_3), 4.20 (m, 1H, 11α-H), 5.48 (s, 1H, OH), 6.87 (d, 1H, J = 8.6, 1-H), 7.01 (d, 1H, J = 8.6, 2-H).	$C_{21}H_{25}ClO_3$ Calculated 360.1492 Found: 360.1486
9c	C(21)	110–115	394 (M^+, 24), 396 (M^+, 20), 317 (24), 268 (29), 85 (100).	1.07 (s, 3H, 18-CH_3), 3.30 (s, 3H, 11β-OCH_3), 4.14 (m, 1H, 11α-H), 7.08 (s, 1H, 1-H).	$C_{21}H_{24}Cl_2O_3$ Calculated 394.1102 Found: 394.1094
7d	B(18)	203–215	348 (M^+, 87), 350 (M^+, 34), 275 (46), 180 (100).	1.07 (t, 3H, J = 7, 11β-OCH_2CH_3), 1.10 (s, 3H, 18-CH_3), 3.45 (dq, 1H, J = 4.6 and 7, 11β-OCH_2CH_3), 3.66 (dq, 1H, J = 4.6 and 7,11β-OCH_2CH_3), 4.22 (m, 1H, 11α-H), 5.36 (s, 1H, OH), 6.72 (s, 1H, 4-H), 7.07 (s, 1H, 1-H).	$C_{20}H_{25}ClO_3$ Calculated 348.1492 Found: 248.1488
8d	B(21)	155–157	348 (M^+, 98), 350 (M^+, 32), 275 (51), 180 (100).	1.03 (t, 3H, J = 7, 11β-OCH_2CH_3), 1.10 (s, 3H, 18-CH_3), 3.31 (dq, 1H, J = 4.6 and 7, 11β-OCH_2CH_3), 3.65 (dq, 1H, J = 4.6 and 7,11β-OCH_2CH_3), 4.27 (m, 1H, 11α-H), 5.60 (s, 1H, OH), 6.86 (d, 1H, J = 8.6, 1-H), 6.99 (d, 1H, J = 8.6, 2-H).	$C_{20}H_{25}ClO_3$ Calculated 348.1492 Found: 248.1488
7e	B(15)	158–160	374 (M^+, 26), 376 (M^+, 8), 283 (47), 145 (100).	1.07 (t, 3H, J = 7, 11β-OCH_2CH_3), 1.08 (s, 3H, 18-CH_3), 3.32 (dq, 1H, J = 4.6 and 7, 11β-OCH_2CH_3), 3.65 (dq, 1H, J = 4.6 and 7,11β-OCH_2CH_3), 4.22 (m, 1H, 11α-H), 6.71 (s, 1H, 4-H), 7.07 (s, 1H, 1-H).	$C_{22}H_{27}ClO_3$ Calculated 374.1649 Found: 374.1643
8e	B(18)	144–145	374 (M^+, 94), 376 (M^+, 31), 283 (78), 115 (100).	1.04 (t, 3H, J = 7, 11β-OCH_2CH_3), 1.08 (s, 3H, 18-CH_3), 3.29 (dq, 1H, J = 4.6 and 7, 11β-OCH_2CH_3), 3.65 (dq, 1H, J = 4.6 and 7,11β-OCH_2CH_3), 4.28 (m, 1H, 11α-H), 6.85 (d, 1H, J = 8.6, 1-H), 6.99 (d, 1H, J = 8.6, 2-H).	$C_{22}H_{27}ClO_3$ Calculated 374.1649 Found: 374.1643
11	C(15)	153–155	348 (M^+, 53), 350 (M^+, 18), 288 (M^+, -Cl, 334), 288 (23), 194 (72), 115 (100).	1.09 (s, 3H, 18-CH_3), 3.32 (s, 3H, 11β-OCH_3), 3.84, (s, 3H, -OCH_3), 4.15 (m, 1H, 11α-CH), 6.63 (s, 1H, 4-H), 7.12 (s, 1H, 1-H).	$C_{20}H_{25}ClO_3$ Calculated 348.1492 Found: 348.1488
12	C(18)	200	348 (M^+, 53), 350 (M^+, 18), 288 (30), 194 (75), 115 (100).	1.09 (s, 3H, 18-CH_3), 3.30 (s, 3H, 11β-OCH_3), 3.80 (s, 3H, -OCH_3), 4.21 (m, 1H, 11α-H), 6.82 (d, 1H, J = 9, 2-H), 7.05 (d, 1H, J = 9, 1-H).	$C_{20}H_{25}ClO_3$ Calculated 348.1492 Found: 348.1488

[a] High performance liquid chromatography (HPLC) was conducted on a 25 cm long × 0.94 cm i.d. column packed with C-18 ODS-2 on 5 μm spherosorb (CSC, Montreal) operated at 2 mL/min with a mixture of methanol and water (A, 70:30; B, 75:25; C, 80:20).
[b] Uncorrected, measured with Fisher-Johns apparatus.

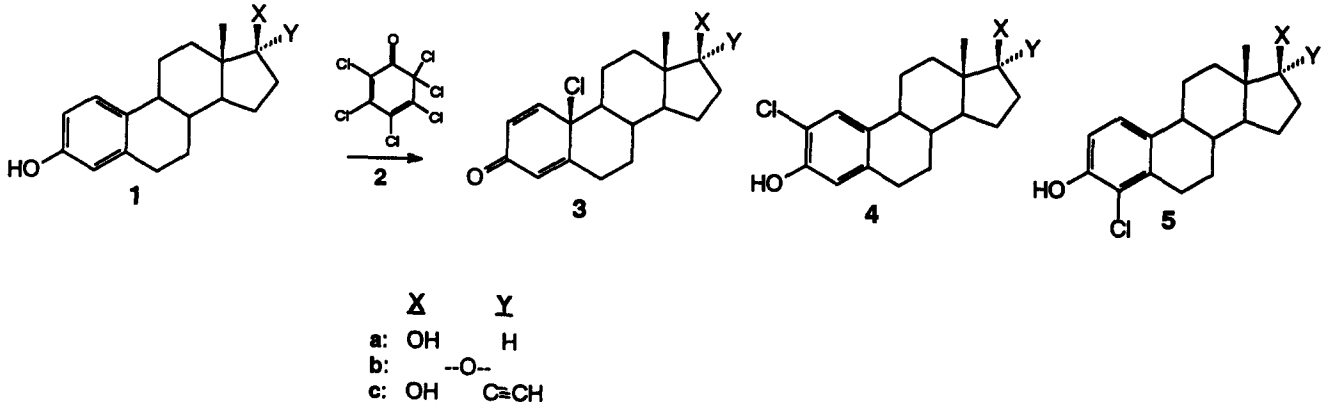

Figure 1 Reaction of estrogen derivative as with 2,3,4,5,6,6-hexachloro-2,4-cyclohexadienone in DMF at room temperature.

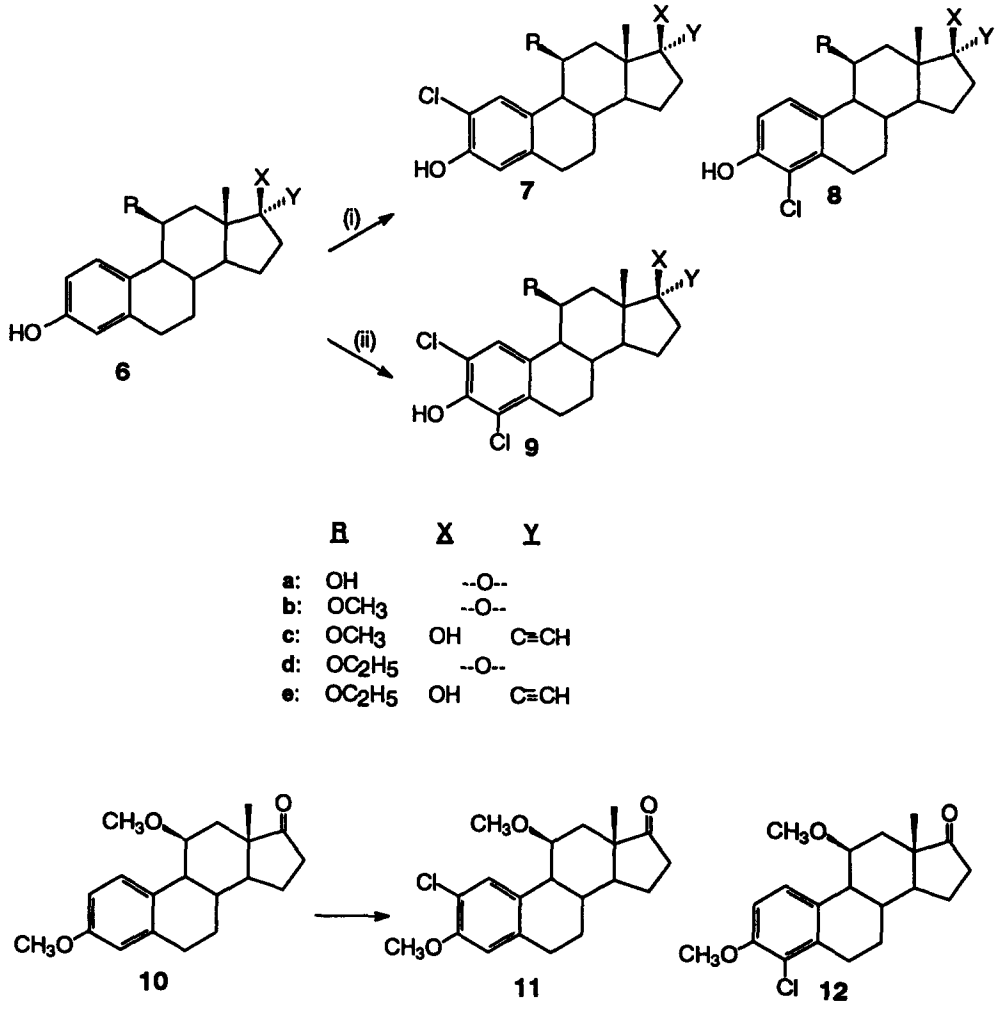

Figure 2 Reaction of 11β-substituted estrogen derivative as with (i) 1 : 1 molar ratio of **2** (ii) 2.5 molar ratio of **2**, in DMF at room temperature.

did not affect product distribution. Structures were assigned from the characteristic ^{1}H NMR spectra which showed the C-1 and C-2 protons as two doublets in the case of the 4-chloro derivative **8**, and the C-1 and C-4 protons as two singlets in the case of the 2-chloro derivative **7**. The mass spectrum revealed the parent molecular ion peaks in a ratio of 3:1, which is characteristic of the presence of chloro substituents.

The relative ratio of the isomeric products changed when the 11β-substituent was altered. The yield of 4-chloro products decreased accordingly to the nature of the 11β-substituent in the following order, OH > OCH_3 > OC_2H_5.

We also investigated the reaction of **2** with estrone 3-methyl ether analogues. The reaction of 11β-methoxyestrone 3-methyl ether (**10**) with **2** in a 1:1 molar

ratio gave 80% conversion. The products were isolated and characterized as 2-chloro-11β-methoxyestrone 3-methyl ether (**11**) and 4-chloro-11β-methoxyestrone 3-methyl ether (**12**). Interestingly, no differences in product pattern were observed between the 3-hydroxy and 3-methyl ether derivatives in the 11β-substituted estrogen series. However, the reaction of estradiol 3-methyl ether or 17α-ethynylestradiol 3-methyl ether with **2** gave a complex mixture. In the latter case only one chloro-substitution product (m/z 344/346 in a 3:1 ratio) was isolated. Similarly from the reaction of **2** with estradiol 3-methyl ether, only 4-chloroestradiol 3-methyl ether (m/z 320/322, 3:1 ratio; ^{1}H NMR δ two doublets at 7.18, 6.76, J = 9 Hz, C-1-H and C-2-H) was isolated in low yield. Chlorination of the α-position to methoxy groups has previously been reported with this reagent.[26]

Whereas the earlier reported chlorination methods gave mixtures of monochlorinated, dichlorinated, and trichlorinated products, controlling the amount of reagent as in the current procedure, provided either monosubstituted or disubstituted products only.

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