chromatographed on a preparative TLC plate [silica gel, 1500 μm, 20 × 20 cm, 3:1:1 *i*-PrOH–MeOH–NH_4OH (concd)]. The main yellow band was eluted with 4:1 EtOH–NH_4OH (concd), and the elutions were rotary evaporated to near dryness. Coevaporation of remaining solvent with 1:1 benzene–EtOH afforded 81.9 mg (75.3%) of 4 as a yellow powder, which was essentially homogeneous on silica gel TLC [3:1:1 *i*-PrOH–MeOH–NH_4OH (concd), R_f 0.28; ninhydrin positive]: UV (0.1 N KOH) λ_{max} 258, 302, 370 nm (ε 25 100, 24 800, 8000); UV (0.1 M potassium phosphate, pH 7.0) λ_{max} 258, 302, 370 nm (ε25 600, 25 100, 8200); UV (30% CH_3COOH, pH 1.8) λ_{max} 304 nm (ε 27 100).

The product was further purified by chromatography on a CM-Sephadex C-25 column. The major peak from the gradient elution was isolated and repurified by HPLC on a preparative C-18 column. Compound 4 emerged from the HPLC column with a retention time of 31 min. Amino acid analysis of the HPLC-purified 4 indicated the presence of lysine (recovery >85%) following hydrolysis in 6 N HCl (110 °C, 20 h).

N^{α}-(4-Amino-4-deoxy-N^{10}-methylpteroyl)-N^{ϵ}-[5-(dimethylamino)-1-naphthalenesulfonyl]-L-lysine (5). A solution of dansyl chloride (144 mg, 0.53 mmol) in 7 mL of acetone was added to a solution of 4 (40 mg, 0.09 mmol) in a 0.5 M $Na_2CO_3/NaHCO_3$ buffer (6 mL, pH 11.5). The homogeneous reaction was allowed to stand for 1.5 h (pH 10.24), and the solvents were removed by rotary evaporation. Triturating with benzene removed excess dansyl chloride. The residue was taken up in DMF and purified by preparative reversed-phase TLC (Whatman KC18F, with preadsorbent area, 3:1 methanol–2% aqueous acetic acid). A sharp yellow band at R_f 0.64 (ninhydrin negative; precursor R_f 0.72) was isolated, and the product was separated from the stationary phase with methanol. Removal of the solvent gave 13 mg (22%) of 5: mp 190–198 °C (glassy melt); UV (0.1 N KOH) λ_{max} 252, 304, 370 nm (ε 15 300, 13 200, 3600); UV (0.1 M potassium phosphate, pH 7.0) λ_{max} 252, 302, 370 nm (ε 17 000, 12 100, 3800); UV (30% CH_3COOH, pH 1.8) λ_{max} 296 nm (ε 15 300). Fluorescence: λ excitation 338 nm, λ emission 560 nm in 0.1 M potassium phosphate, pH 7.0. Mass spectrum[20] calcd for $C_{33}H_{38}N_{10}O_6S$ plus potassium, 725.23145; found, 725.23843.

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Registry No. 2, 19741-14-1; 3, 83399-36-4; 4, 80407-56-3; 5, 83416-29-9; N^{ϵ}-(*tert*-butyloxycarbonyl)-L-lysine *tert*-butyl ester hydrochloride, 13288-57-8; dansyl chloride, 605-65-2; dihydrofolate reductase, 9002-03-3.

(20) This spectrum was run on a Finnigan MAT 731 field-desorption instrument, which was funded by Grant RR-00317 from the Division of Research Resources, National Institutes of Health, to the Massachusetts Institute of Technology (K. Biemann, principal investigator). We thank Dr. Catherine E. Costello for performing the analysis.

2-(Hydroxyphenyl)indoles: A New Class of Mammary Tumor Inhibiting Compounds

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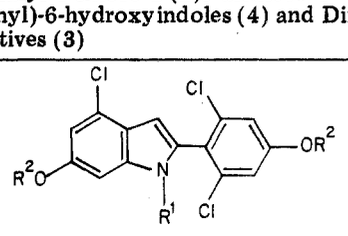
1-Alkyl-4-chloro-2-(2,6-dichloro-4-hydroxyphenyl)-6-hydroxyindoles (4, alkyl = CH_3, C_2H_5, C_3H_7) were synthesized by thermolysis of the corresponding *N,N'*-dialkyl-1,2-diphenylethylenediamines and subsequent ether cleavage. They showed an affinity for the estrogen receptor (1% of 17β-estradiol) and inhibited the growth of the 9,10-dimethyl-1,2-benz[*a*]anthracene (DMBA) induced mammary carcinoma of the Sprague-Dawley (SD) rat. The best result was obtained by the ethyl compound (**4b**), which reduced the original tumor area by 50% after 4 weeks administration of 6 × 18 (mg/kg)/week. Since **4a** and **4b** show uterotrophic activity and cytostatic effects against hormone-independent cells, a dual mode of action has to be considered for the tumor inhibition.

In previous papers we have reported attempts to develop antiestrogens for the treatment of hormone-dependent mammary carcinoma by replacing the methylene groups in the synthetic estrogen hexestrol by isosteric imino groups. The resulting 1,2-diarylethylenediamines showed either a too weak antitumor activity[1,2] or proved to be true estrogens.[3] Starting from these ethylenediamines, we searched for another appropriate structure with an affinity for the estrogen receptor and a growth-inhibiting effect on the mammary carcinoma.

From previous studies,[4] we knew that *o*-chloro-substituted diphenylethylenediamines can be converted into 2-phenylindoles. Thermolysis of *meso*- or (±)-*N,N'*-dialkyl-1,2-bis(2,6-dichloro-4-methoxyphenyl)ethylenediamines (1)[3] at 215 °C afforded 1-alkyl-4-chloro-2-(2,6-dichloro-4-methoxyphenyl)-6-methoxyindoles (3). The free phenolic compounds **4a–c** were obtained by ether cleavage with BBr_3 (Scheme I).

Table I. 1-Alkyl-4-chloro-2-(2,6-dichloro-4-hydroxyphenyl)-6-hydroxyindoles (4) and Dimethyl Ether Derivatives (3)



compd	R^1	R^2	mp, °C	formula	RBA,[a] %
3a	CH_3	CH_3	145–146	$C_{17}H_{14}Cl_3NO_2$	
3b	C_2H_5	CH_3	157–158	$C_{18}H_{16}Cl_3NO_2$	
3c	C_3H_7	CH_3	134–136	$C_{19}H_{18}Cl_3NO_2$	
4a	CH_3	H	215–216	$C_{15}H_{10}Cl_3NO_2$	1.3
4b	C_2H_5	H	126–129	$C_{16}H_{12}Cl_3NO_2$	1.9
4c	C_3H_7	H	70–72	$C_{17}H_{14}Cl_3NO_2$	1.0

[a] Relative binding affinities for the calf uterine estrogen receptor = ratio of molar concentrations of 17β-estradiol (E2) and inhibitor required to decrease the amount of bound [^{3}H]E2 by 50% × 100.

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(2) von Angerer, E.; Egginger, G.; Kranzfelder, G.; Bernhauer, H.; Schönenberger, H. *J. Med. Chem.* **1982**, *25*, 832.
(3) von Angerer, E. *J. Med. Chem.* **1982**, *25*, 1374.
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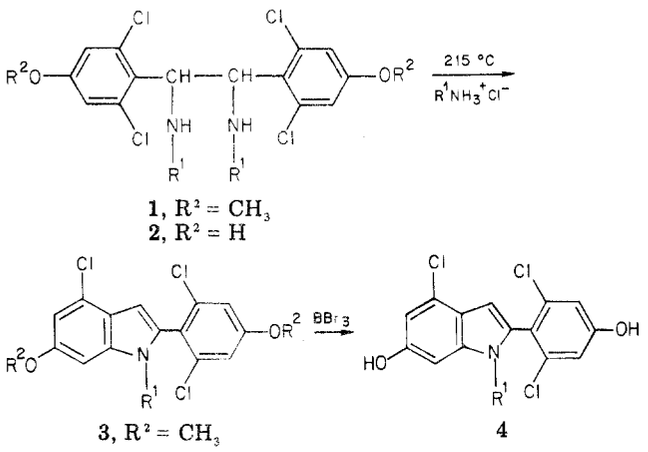
0022-2623/83/1826-0113$01.50/0

Scheme I[a]



1, $R^2 = CH_3$
2, $R^2 = H$

3, $R^2 = CH_3$ **4**

[a] For **1a-4a**, $R^1 = CH_3$; for **1b-4b**, $R^1 = C_2H_5$; for **1c-4c**, $R^1 = C_3H_7$

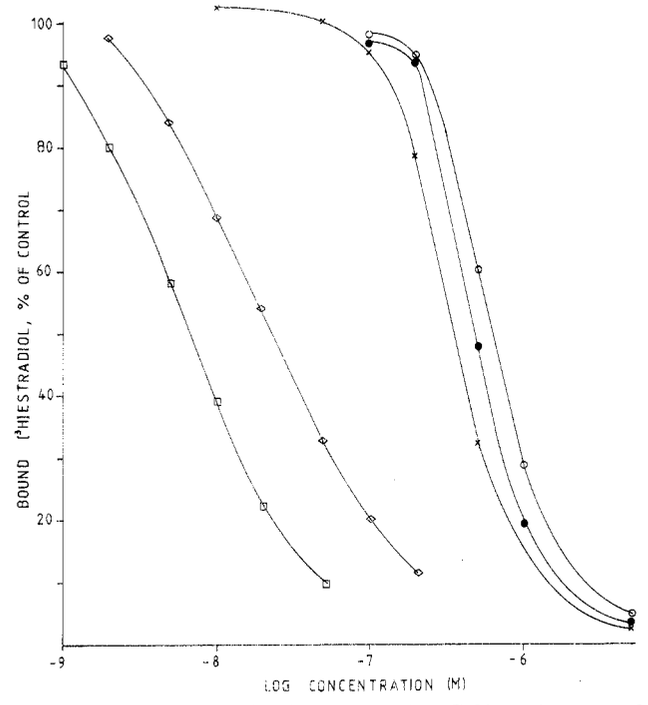


Figure 1. Competitive binding assay of **4**. Calf uterine cytosol was incubated for 16 h at 0–4 °C with the stated concentration of competitor and 5×10^{-9} M [^{3}H]estradiol. After incubation, dextran-coated charcoal was added to adsorb unbound ligand (90 min, 0–4 °C), and radioactivity was determined in the supernatant. Competing ligands were 17β-estradiol (□), hexestrol (◊), **4a** (●), **4b** (X), and **4c** (○).

Biological Properties. The affinities of the three homologous indoles **4a–c** for the estrogen receptor were determined by a competitive binding assay using 17β-[^{3}H]-estradiol and the dextran-coated charcoal method:[5] All three compounds exhibited a binding affinity, with RBA values of ca. 1% of 17β-estradiol. The receptor affinity can be rationalized by the presence of the basic structure of diethylstilbestrol in the molecule. We observed an interesting difference to other synthetic estrogens and antiestrogens in the semilogarithmic plot of receptor-bound 17β-[^{3}H]estradiol vs. concentration of inhibitor: Instead of a curve of **4** parallel to the binding curve of estradiol, a line with a larger slope was obtained (Figure 1). The same effect was noticed with a cytosol of the DMBA-induced mammary carcinoma of the rat.

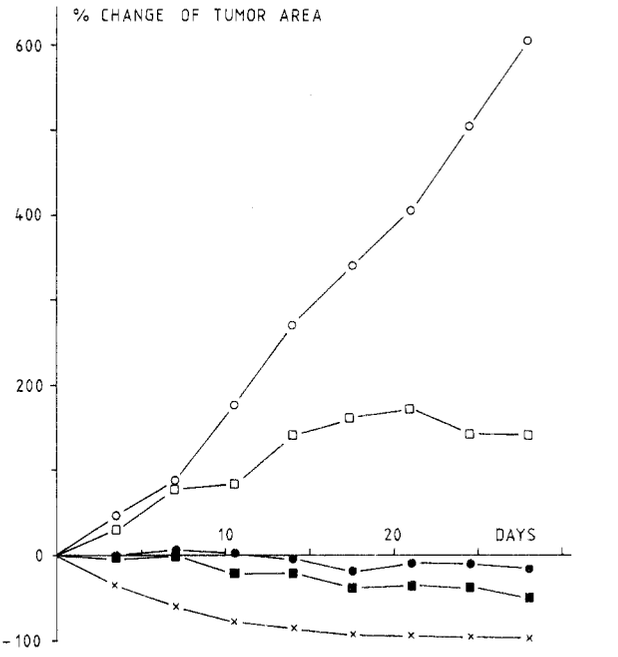


Figure 2. The effect of various doses of **4b** and of ovariectomy on the tumor area of SD rats bearing DMBA-induced mammary carcinoma. Administration scheme: from Monday to Thursday, a single daily dose; on Friday, a double dose sc: control, vehicle alone (○); **4b**, 2.0 mg/kg (□); **4b**, 6.0 mg/kg (●); **4b**, 18.0 mg/kg (■); ovariectomy (X).

Compounds **4a–c** were tested for their inhibitory activity against the established DMBA-induced mammary carcinoma of the rat, which is believed to have many similarities with hormone-dependent human breast cancer.[6] For this experiment we used animals that developed tumors between the 5th and 10th week after DMBA administration. The estrogen dependence of these tumors was demonstrated by ovariectomy, leading to a complete regression of most of the tumors (Figure 2). The ethyl compound **4b** inhibited the growth of tumors, depending on the dose used (Figure 2). Administration of 6 × 6 (mg/kg)/week reduced the tumor area by 18% after 4 weeks and led to the regression of a high percentage of tumors (Table II). The threefold dose (18 mg) affected only the tumor size but not the remission rate. According to the lower binding affinities, the methyl (**4a**) and propyl (**4c**) compounds showed a less marked growth inhibition. The great difference in the tumor growth of the control groups (235% vs. 610%) cannot be rationalized, since the starting area was the same. A similar difference has been observed previously.[3,7]

Compounds **4a** and **4b** were tested for their uterotrophic activity in the immature mouse as a measure of estrogenicity. Both compounds stimulated the uterine growth but reached the maximum effect at rather high doses (**4a**, 625 μg/animal; **4b**, 25 μg/animal; Table III). Since the dose–response curve is relatively flat in the case of **4a**, this compound can be considered as an "impeded" estrogen; the character of **4b** is similar but not so distinct. Like other compounds with diminished estrogenicity,[8] **4a** and **4b**

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Table II. Effect of 4 on the DMBA-Induced Mammary Carcinoma of the Sprague-Dawley Rat

compd	dose,[a] mg	no. of animals	no. of tumors[b]	new tumors	complete remission,[c] %	partial remission,[d] %	static tumors,[e] %	progr tumors,[f] %	change of body wt,[g] %	change of tumor area,[h] %
control		9	17	42	0	2	20	78	+4	+610
4b	2	9	22	15	43	14	19	24	+2	+138
4b	6	9	22	4	62	15	8	15	+1	−18
4b	18	9	20	5	56	24	4	16	+0	−50
control		10	25	41	3	18	38	41	+5	+235
4a	6	8	19	24	19	28	25	28	−2	+24
4c	6	8	17	9	8	19	50	23	−0	+57

[a] Dose per kilogram of body weight, dissolved in olive oil. The animals received a single dose daily from Monday to Thursday and a double dose on Friday. [b] At the beginning of the test. [c] Tumor not palpable. [d] Reduction of initial tumor size ≤ 50%. [e] Tumor size 51–150% of the initial size. [f] Tumor size > 150% of the initial size. [g] Average on the 7th day of therapy. [h] Average on the 28th day of therapy. The U test according to Wilcoxon, Mann, and Whitney was used to determine the significance; all results are significant ($p < 0.01$).

showed antiuterotrophic properties. Both compounds reduced the estrone-stimulated uterine growth by a third, but this effect should not be overestimated, since comparatively high doses were required. The indole **4b** was also tested in the rat: The uterotrophic curve was similar to the one obtained in the mouse, reaching the level of 5 μg of estradiol at a dose of 125 μg per animal. No antiuterotrophic effect was observed between 25 and 625 μg (data not shown).

In order to find out if general cytostatic effects contribute to the antitumor activity of **4**, we tested **4a** and **4b** in vitro using hormone-independent, human mammary carcinoma cells (MDA cells). Both compounds inhibited the growth of these cells in concentrations greater than 10^{-6} M (Table IV). Cell number and [^{3}H]thymidine incorporation were used as measure of cell growth.

Discussion

It has been shown that the conversion of bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamines (**2**) into 2-arylindoles reduces the affinity for the estrogen receptor only slightly (RBA, %: *meso*-**2b**, 8.6; (±)-**2b**, 2.1; **4b**, 1.9), despite the considerable change in structure (Scheme I, **2b**, R = C_2H_5). In the competitive binding assay, we noticed that the curves for the compounds **4a–c** have slopes different from the estradiol and hexestrol curves (Figure 1); therefore, the term "relative binding affinity" ought not to be used in this case but should be replaced by the expression "competition factor (KF)".[9] We are not able to offer an explanation for this lack of parallelism, since the binding of **4** to the receptor is completely reversible (data not shown). From the Lineweaver–Burk analysis, a competitive inhibition has to be assumed.

Like the ethylenediamines **2**, the 2-arylindoles showed a strong inhibitory activity against DMBA-induced, hormone-dependent mammary tumors of the rat. In two respects, the biological character of the indoles **4** differed from the corresponding ethylenediamines **2**: First, the estrogenic potency is considerably decreased. This fact is probably due not so much to the somewhat lower binding affinity of **4** for the estrogen receptor compared to **2b**, but to the transition from a true estrogen (**2b**) to a kind of impeded estrogen. Secondly, in higher concentrations the compounds **4a** and **4b** exhibited cytostatic effects on hormone-independent mammary tumor cells in vitro. A similar nonspecific effect was observed with 17β-estradiol and derivatives of it in hormone-responsive (MCF-7) and hormone-independent (MDA, Evsa T or E) mammary tumor cells and concentrations greater than 10^{-6} M.[10] Since other estrogenic compounds like *meso*-**2b** did not show any inhibitory activity against MDA cells up to a concentration of 10^{-5} M, a general cytostatic effect can not necessarily be associated with estrogens.

Because of the high doses required for the good antitumor activity of **4**, we assume a dual mode of action for the growth inhibition of DMBA-induced mammary tumors: The arylindoles accumulate in the target cells by the binding to the estrogen receptor and develop their antineoplastic activity via an estrogenic mechanism and nonspecific cytostatic effects. In order to support this assumption and to find out which share is more important, we are presently investigating 2-phenylindoles without chlorine substituents. The possibility of an antiestrogenic action cannot be ruled out and remains to be examined, too.

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Table III. Estrogenic and Antiestrogenic Activity of Compounds 4a and 4b in the Mouse Uterine Weight Test

	uterotrophic test		antiuterotrophic test		
compd	dose,[a] μg	effect[b]	dose,[a,c] μg	effect[b]	inhibn,[d] %
control		14.3 ± 4.8		17.0 ± 3.5	
4a	1	16.0 ± 2.8	1	48.5 ± 5.5	−6
	5	18.2 ± 3.2	5	45.4 ± 7.3	5
	25	26.0 ± 4.4	25	42.7 ± 8.3	14
	125	34.4 ± 6.0	125	37.7 ± 7.8	30[e]
	625	40.1 ± 5.7	625	39.6 ± 4.5	24[e]
estrone	1	44.8 ± 7.2	0.4	46.8 ± 7.6	
control		19.3 ± 3.4		17.0 ± 3.5	
4b	0.04	19.8 ± 5.0	1	48.7 ± 5.0	−7
	0.2	21.7 ± 7.1	5	46.3 ± 5.0	2
	1	21.5 ± 3.9	25	39.3 ± 4.5	25[e]
	5	22.2 ± 2.4	125	40.0 ± 7.7	23[f]
	25	45.8 ± 7.6	625	35.6 ± 3.2	38[e]
	125	40.6 ± 1.5			
estrone	1	50.0 ± 5.9	0.4	46.8 ± 7.6	

[a] Dose per animal, administered at 3 consecutive days sc. [b] Uterus dry weight (milligrams)/body weight (grams) × 100, determined 24 h after the last injection; mean of 10 animals ± SD. [c] Simultaneous administration of 0.4 μg of estrone per animal and day. [d] The *U* test according to Wilcoxon, Mann, and Whitney was used. [e] Significant, $p < 0.01$. [f] Significant, $p < 0.05$.

Table IV. Growth Inhibition of Hormone Independent MDA-MB 231 Human Breast Cancer Cells by Compounds 4a and 4b

				[^{3}H]thymidine incorporation	
compd	concn, M	cell no./dish[a] × 10^5 (mean ± SD)	% T/C[b]	cpm/dish × 10^3 (mean ± SD)	% T/C[b]
control		10.71 ± 2.12		41.2 ± 5.0	
4a	1×10^{-5}	4.12 ± 0.52	38	2.2 ± 0.1	5
	1×10^{-6}	10.12 ± 2.44	95	36.4 ± 3.6	88
4b	1×10^{-5}	2.71 ± 0.99	25	6.6 ± 0.1	16
	5×10^{-6}	5.44 ± 0.88	51	5.8 ± 0.6	14
	2×10^{-6}	9.38 ± 2.28	88	36.2 ± 4.2	88
	1×10^{-6}	11.35 1.49	106	40.2 ± 8.8	98

[a] Cell number based on coulter counts on day 3. [b] Percent T/C = test compound/control × 100.

Experimental Section

Melting points were determined on a Büchi 510 apparatus and are uncorrected. Elemental analyses were performed by the Mikroanalytisches Laboratorium, University of Regensburg, and were within ±0.40% of the calculated values. NMR spectra were obtained on a Varian EM 360A spectrometer and were consistent with the assigned structures.

General Preparation of 1-Alkyl-4-chloro-2-(2,6-dichloro-4-methoxyphenyl)-6-methoxyindoles (3). *meso-N,N'*-Dialkyl-1,2-bis(2,6-dichloro-4-methoxyphenyl)ethylenediamine (1;[3] 2.0 g) was heated to 215 °C and kept for 15 min at this temperature. After cooling, the residue was dissolved in CH_2Cl_2 and chromatographed over SiO_2 with CH_2Cl_2/ligroin (1:1) as eluent. The product was recrystallized from EtOH; the yields ranged from 70 to 85%. Melting points are reported in Table I.

General Procedure of Ether Cleavage. 1-Alkyl-4-chloro-2-(2,6-dichloro-4-hydroxyphenyl)-6-hydroxyindoles (4). A solution of **3** (0.01 mol) in dry CH_2Cl_2 (150 mL) was cooled to −60 °C under a nitrogen atmosphere, and then BBr_3 (0.04 mol) was added with stirring. After 30 min, the cooling bath was removed, and the reaction mixture was kept at room temperature for 15 h. With cooling, the mixture was poured into a aqueous solution of $NaHCO_3$. The organic layer was separated, and the aqueous phase was extracted 3 times with EtOAc. The combined organic layers were dried ($MgSO_4$). After the solvent was removed, the residue was recrystallized from MeOH/H_2O; the yields ranged from 78 to 94%. Melting points are reported in Table I.

Biological Methods. Growth Inhibition of Hormone-Independent MDA-MB 231 Human Breast Cancer Cells. This cell line was derived in the laboratory of Dr. R. Cailleau[11] from the pleural effusion of a patient with a poorly differentiated papillary carcinoma. Cells were grown in McCoy 5a medium (Boehringer, Mannheim) supplemented with 10% newborn calf serum (Gibco) and Gentamycin (40 μg/ml, Sigma Chemical Co.). [^{3}H]Thymidine incorporation into TCA-precipitable material was performed as outlined previously by Lippman;[12] 1 h prior to the start of the experiment, replicately plated cells were changed to medium supplemented with 5% serum. Compounds were then added dissolved in Me_2SO, leading to a final solvent concentration of 0.1%.

Complete experimental details for other applied procedures can be found in ref 5.

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Registry No. **3a**, 83363-99-9; **3b**, 83364-00-5; **3c**, 83364-01-6; **4a**, 83364-02-7; **4b**, 83364-03-8; **4c**, 83364-04-9.

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