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Synthesis of 3-(4-Bromobenzyl)-5-(aryl methylene)-5*H*-furan-2-ones and Their Activity as Inhibitors of the Photosynthetic Electron Transport Chain

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A series of 12 3-(4-bromobenzyl)-5-(arylmethylene)-5*H*-furan-2-one lactones, designed using the naturally occurring toxin nostoclides as a lead structure, were synthesized and screened as potential inhibitors of photosynthetic electron transport. The structures were confirmed by ^{1}H and ^{13}C NMR, MS, and IR analyses. Their biological activity was evaluated both in vitro, as the ability to interfere with light-driven reduction of ferricyanide by isolated spinach chloroplasts, and in vivo, as the capability to inhibite the oxygen production by intact Chlorella cells. Some of the compounds exhibited inhibitory properties in the micromolar range against basal and phosphorylating electron flow from water to $K_3[Fe(CN)_6]$, with no effect on uncoupled electron flow. Thus, they seem to behave as energy-transfer inhibitors. Although poor solubility in water may limit their effectiveness, the active derivatives could present structures to be exploited for the design of new substances endowed with herbicidal activity.

KEYWORDS: Herbicides; nostoclide analogues; photosynthetic electron transport inhibitors; solubility

INTRODUCTION

Improved agronomical techniques, rapid mechanization, artificial fertilizers, and high-yielding crop varieties have been responsible for the impressive increase of farm productivity over the years. Among these factors, the availability of chemical agents for crop protection has played a pivotal role, helping farmers to control a variety of pests and diseases, among which are weeds (*1*). Herbicide application has become the most reliable and least expensive method of weed control (*2, 3*). However, repeated use of the same active ingredients has led to herbicide resistance. Starting from the 1960s, hundreds of weed biotypes were reported to survive herbicide application (*4*). As a consequence, new herbicides must be continuously developed to overcome weed resistance. Modern pesticides should have a favorable combination of properties, including high levels of herbicidal activity, low application rates, crop tolerance, and low toxicity to mammals. Moreover, increasing public concern for the environmental pollution deriving from agricultural practice strictly demands that phytochemicals be endowed with low recalcitrancy and thus be able to be rapidly mineralized by the soil microflora. In this context, natural products may provide novel lead compounds that may be optimized using well-established chemical strategies (*5–9*).

Among the numerous phytotoxic products of microbial origin is cyanobacterin (**1**), a natural lactone isolated from the blue-green alga *Scytonema hofmanni* (*10*) (**Figure 1**). At a concentration as low as 5 μM, compound **1** is toxic to most cyanobacteria, and also inhibits the growth of eukaryotic algae and several mono- and dicotyledones (*11, 12*). It acts mainly by inhibiting the photosynthetic electron transport, with a mechanism of action similar to that of the herbicide 3-(3,4-diclorophenhyl)-1,1-dimethylurea (*13*). In isolated *Euglena gracilis* chloroplasts, such interference leads to a cascade of events that results in the disruption of the tylakoid membrane (*14*). Two lactones similar to compound **1**, namely, nostoclides I and II (**Figure 1**, compounds **2a** and **2b**), were isolated from the culture of a symbiotic cyanobacterium, *Nostoc* sp., in *Peltigera canina*, a common lichen (*15*). Contrary to compound **1**, for which several studies have been reported, the mode of action of nostoclides has not yet been fully investigated. Nostoclides has shown moderate cytotoxicity against two mouse neuroblastoma cell lines (*15*). However, because of the high structural similarity between nostoclides and cyanobacterin (they share a 3-benzyl-5-benzylidene-4-isopropyl-dihydro-furan-2-one ring), and the fact that *P. canina* cultures are usually not

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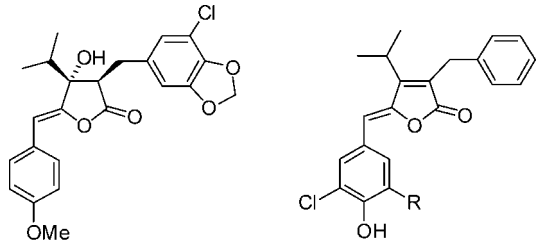

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(2a) R=Cl Nostoclide I
(2b) R=H Nostoclide II

Figure 1. Structure of cyanobacterin (**1**) and nostoclides (**2**).

contaminated with microorganisms, it has been suggested that these chlorinated compounds may play a role as allelopathic agents.

Within the frame of research programs aimed at the utilization of natural products as lead compounds to develop new herbicides (*16–19*), we previously assessed the potential phytotoxicity of several nostoclide analogues (*20*). In this paper, we describe the preparation of 12 3-(4-bromobenzyl)-5-(arylmethylene)-5*H*-furan-2-one lactones analogues to nostoclides. Their ability to interfere with the photosynthetic electron transport chain in isolated spinach chloroplasts and in intact *Chlorella* cells was also investigated.

MATERIALS AND METHODS

Chemicals. Dichloromethane, tetrahydrofuran (THF), hexamethylphosphoramide (HMPA), diethyl ether, and amines were purified according to standard methods (*21*). Analytical grade *tert*-butyldimethylsilyltrifluoromethanesulfonate (TBDMSOTf), piperonal, benzaldehyde, 2,4,6-trimethoxybenzaldehyde, 3-methylbenzaldehyde, 4-fluorobenzaldehyde, 4-bromobenzaldehyde, 4-chlorobenzaldehyde, 3-nitrobenzaldehyde, 4-trifluoromethylbenzaldehyde, 4-cyanobenzaldehyde, 2-chloro-4-dimethylaminobenzaldehyde, 4-bromobenzyl bromide, 8-diazabyciclo[5.4.0]undec-7-ene (DBU), and phosphoryl chloride ($POCl_3$) were procured from Aldrich (Milwaukee, WI) and utilized without further purification.

Synthesis. All reactions were carried out under a protective atmosphere of dry nitrogen or utilizing a calcium chloride tube. The aldehyde 2,5-dimethoxybenzaldehyde was prepared from the corresponding benzylic alcohol by Swern oxidation (*22*). Lactone **3** was synthesized employing a previously described methodology (*23*). Commercially available *n*-butyllithium hexane solutions (1.6 mol L^{-1}) were titrated prior to use (*24*). ^1H and ^{13}C NMR spectra were recorded on a Bruker AVANCE DRX 400 spectrometer at 400 and 100 MHz, respectively, using $CDCl_3$ as solvent and TMS as internal standard. Mass spectra were obtained on a SHIMADZU GCMS-QP5050A instrument by direct injection using the following temperature program: 40 °C min^{-1} until temperature reaches 60 °C, and then 80 °C min^{-1} until temperature reaches 300 °C; the detector temperature was 280 °C. Infrared spectra were recorded on a Perkin Elmer Paragon 1000 FTIR spectrophotometer, using potassium bromide (1% w/w) disks, scanning from 625 to 4000 cm^{-1}. Melting points are uncorrected and were obtained with a MQAPF-301 melting point apparatus (Microquimica, Brazil). Analytical thin layer chromatography was carried out on TLC plates recovered with $60GF_{254}$ silica gel. Column chromatography was performed over silica gel (60–230 mesh).

N,N,N′,N′-Tetraethyldiamidophosphate ***(5).*** A 25 mL flask, protected from moisture by a calcium chloride tube, was charged with 5*H*-furan-2-one (**3**) (2.00 g, 24 mmol), dichloromethane (5.0 mL), and $POCl_3$ (2.2 mL, 24 mmol). A solution of triethylamine (4.8 mL, 36 mmol) in dichloromethane (4.0 mL) was added dropwise over 1 h. The resulting mixture was stirred overnight (18 hours), after which triethylamine (0.8 mL, 6.0 mmol) was added in one portion, and stirring was continued for 24 h. The solvent was removed on a rotatory evaporator and diethyl ether (12 mL) was added carefully to the dark residue, followed by pentane (6 mL), to precipitate the amine hydrochloride. The flask was stoppered and shaken for 1–2 min. The hydrochloride was filtered by suction and washed immediately with diethyl ether (6 mL) and pentane (12 mL). The bottle was tightly stoppered and the filtrate was allowed to stand in the refrigerator (+5 °C) overnight. The clear brown ethereal phase was decanted from the lower phase, and the solvent was evaporated. Diethyl ether (10 mL) was added to the residue. The flask mixture was cooled in an ice–salt bath, and diethylamine (18.0 mL) was added to the chilled solution over 1 h, utilizing a dropping funnel connected to a calcium chloride tube. After the addition, the temperature of the reaction mixture was raised to 35 °C and stirring was continued for 20 h. The hydrochloride formed was removed by suction filtration and washed with diethyl ether (3 × 20 mL). The organic phase was concentrated under reduced pressure, affording a brown oil. This crude material was purified by column chromatography eluted with a mixture of hexane:ethyl acetate of increasing polarity (hexane:EtOAc = 3:1, 2:1, 1:1 v/v). The required compound was obtained as a yellow oil in 51% yield (3.35 g, 12.2 mmol). IR (film, NaCl, cm^{-1}) $\bar{\nu}_{max}$ 3145; 3117, 2974; 2935; 2876; 1605; 1516; 1466; 1248; 1213; 1199; 1171; 1102; 1066; 1029; 962; 847; 792. ^1H NMR (400 MHz, $CDCl_3$): δ 1.11 (t, 12H, J = 7.0 Hz; CH_3); 3.06–3.19 (m, 8H, NCH_2); 5.68 (brs, 1H; H3); 6.27 (brs, 1H, H4); 6.94 (brs, 1H; H5). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.71 (s, CH_3); 39.76 (d, $^2J_{C\text{-}P}$ = 4.5 Hz; NCH_2); 88.78 (d, $^3J_{C\text{-}P}$ = 3.2 Hz; C3); 111.13 (s, C4); 134.02 (s, C5); 152.58 (d, $^2J_{C\text{-}P}$ = 3.3 Hz; C2). MS, *m/z* (%): 274 (M^+, $C_{12}H_{23}N_2O_3P$, 3); 191 (100); 163 (8); 83 (8); 72 (51); 44 (29).

3-(4-Bromobenzyl)-5H-furan-2-one ***(7).*** Previously titrated *n*-butyllithium hexane solution (1.1 mol L^{-1}, 7.5 mL, 8.25 mmol) was added over 10 min to a solution of compound **5** (1.0 g; 3.65 mmol) in THF (15 mL) and HMPA (5 mL), chilled to −78 °C. The addition was carried out so that the temperature reached −55 °C but did not exceed this level. The resulting mixture was chilled to −78 °C for 20 min; then 4-bromobenzyl bromide (3.8 g, 15.0 mmol) dissolved in THF (15 mL) was added with a syringe over 15 min so that the temperature did not rise above −55 °C. After the addition, the temperature was raised to room temperature. Water (20 mL) and ethyl acetate (80 mL) were added, the phases were separated, and the dark inorganic phase was extracted with ethyl acetate (2 × 80 mL). The combined organic phases were washed with brine (40 mL) and dried over magnesium sulfate. The solvent was removed under reduced pressure to yield the substituted furan **6**, which need not be purified for the next reaction. To this crude material formic acid (5 mL) was added in a 50 mL round bottom flask on a water bath at room temperature, and the resulting mixture was stirred until bubbling had ceased (45 minutes). Benzene (12 mL) was added and most of the excess of formic acid was removed under reduced pressure. To the residue, ethyl acetate (40 mL) and sodium chloride-sodium carbonate solution (12 mL) were added. The organic phase was washed twice with the latter solution (2 × 12 mL). The combined inorganic phase was extracted with ethyl acetate (2 × 40 mL). The combined organic phases were dried over magnesium sulfate, and the solvent was removed under reduced pressure. The resulting material was purified by column chromatography on silica gel eluted with hexane:diethyl ether (2:1 v/v) to generate compound **7** as a yellow oil in 76% yield (704 mg, 2.78 mmol). IR (KBr, cm^{-1}) $\bar{\nu}_{max}$ 3087; 2929; 2868; 1748; 1651; 1591; 1488; 1349; 1069; 803. ^1H NMR (400 MHz, $CDCl_3$): δ 3.56 (s, 2H, H6); 4.76 (d, 2H, J = 1.8 Hz; H5); 6.95 (d, 1H, J = 1.8 Hz; H4); 7.11 (d, 2H, J = 8.5 Hz; H2′/H6′); 7.44 (d, 2H, J = 8.5 Hz; H3′/H5′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.52 (C6); 70.52 (C5); 121.02 (C4′); 130.89 (C2′/C6′); 132.10 (C3′/C5′); 133.98 (C3); 136.53 (C1′); 145.94 (C4); 173.95 (C2). MS, *m/z* (%): 252 (M^+, $C_{11}H_9BrO_2$, 8); 254 (M + 2, 7); 209 (4); 191 (2); 173 (13); 144 (4); 128 (100); 115 (36); 89 (20); 63 (22); 57 (49); 51 (65).

(5Z)-3(4-Bromobenzyl)-5-(1,3-dioxalanebenzylidene)-5H-furan-2-one ***(8a).*** To a two-neck round bottom flask, under nitrogen atmosphere, were added 3-(4-bromobenzyl)-5*H*-furan-2-one (**7**) (100 mg, 0.39 mmol), dichloromethane (3 mL), TBDMSOTf (108 μL, 0.47 mmol), diisopropyl ethyl amine (DIPEA) (200 μL, 1.2 mmol), and piperonal (120 mg, 0.47 mmol). The resulting mixture was stirred at room temperature for 1 h. After DBU (116 μL, 0.78 mmol) was added, the reaction mixture was refluxed for an additional 3 h before addition of dichloromethane (70 mL). The resulting organic layer was washed with 3 mol L^{-1} HCl aqueous solution (2 × 25

mL) and brine (25 mL). After separation, the organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting material was purified by column chromatography on silica gel eluted with hexane:dichloromethane (1:1 v/v). The procedure described afforded compound **8a** (65 mg, 0.15 mmol) as a yellow solid in 59% yield. The product was recrystalized from a mixture of hexane:dichloromethane (~1:1 v/v).

Compounds **8b**–**8l** were prepared employing a procedure similar to that described for compound **8a**, and yields are presented in the Results. The synthesized compounds were fully characterized by IR, NMR (^{1}H and ^{13}C), and MS spectrometry. HMBC and HSQC bidimensional experiments helped in the ^{13}C assignments. Structures of lactones **8a**–**8l** were supported by the following spectroscopic data.

*Data for (5Z)-3(4-Bromobenzyl)-5-(1,3-dioxalanebenzylidene)-5H-furan-2-one **(8a)**.* Yellow solid; m.p. 175.6–176.0 °C. IV (KBr, cm^{-1}): $\bar{\nu}_{max}$ 3102; 2891; 1729; 1650; 1599; 1499; 1488; 1267; 1045; 1034; 940. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.66 (s, 2H, H7); 5.81 (s, 1H, H6); 5.99 (s, 2H, OCH_2O); 6.80 (d, 1H, J = 8.2 Hz, H5″); 6.93 (s, 1H, H4); 7.11 (dd, 1H, J = 8.2, 1.6 Hz, H6″); 7.13 (d, 2H, J = 8.3 Hz, H2′/H6′); 7.42 (d, 1H, J = 1.6 Hz, H2″); 7.46 (d, 2H, J = 8.3 Hz, H3′/H5′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.11 (C7); 101.50 (OCH_2O); 108.58 (C5″); 109.98 (C2″); 113.07 (C6); 120.87 (C4′); 125.87 (C6″); 127.39 (C1″); 130.63 (C2′/C6′); 130.75 (C3); 131.95 (C3′/C5′); 136.33 (C1′); 139.69 (C4); 146.06 (C5); 148.28 (C3″); 148.53 (C4″); 170.21 (C2). MS, *m/z* (%): 384 (M$^+$, $C_{19}H_{13}BrO_4$, 82); 386 (M + 2, 80); 339 (6); 304 (8); 276 (7); 259 (10); 219 (11); 189 (17); 169 (15); 162 (70); 134 (78); 115 (47); 104 (39); 89 (29); 76 (100); 63 (25); 50 (55).

*Data for (5E)-3(4-Bromobenzyl)-5-(2,4,6-trimethoxybenzylidene)-5H-furan-2-one **(8b)**.* Yellow solid; m.p. 155.8–156.5 °C. IV (KBr, cm^{-1}): $\bar{\nu}_{max}$ 3142; 2990; 2946; 2840; 1733; 1598; 1573; 1462; 1337; 1218; 1160; 1060; 821. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.64 (s, 2H, H7); 3.74 (s, 6H, 2″/6″-OCH_3); 3.83 (s, 3H, 4″-OCH_3); 6.12 (s, 2H, H3″/H5″); 6.56 (s, 1H, H6); 7.07 (s, 1H, H4); 7.11 (d, 2H, J = 8.3 Hz, H2′/H6′); 7.43 (d, 2H, J = 8.3 Hz, H3′/H5′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.25 (C7); 55.44 (4″-OCH_3); 55.64 (2″/6″-OCH_3); 90.81 (C3″/C5″); 103.73 (C1″); 106.12 (C6); 120.64 (C4′); 130.66 (C2′/C6′); 131.77 (C3′/C5′); 132.09 (C3); 136.76 (C1′); 137.90 (C4); 148.53 (C5); 158.72 (C2″/C6″); 162.00 (C4″); 170.05 (C2). MS, *m/z* (%): 430 (M$^+$, $C_{21}H_{19}BrO_5$, 98); 432 (M + 2, 100); 387 (2); 361 (6); 265 (3); 233 (6); 208 (15); 205 (9); 193 (22); 181 (30); 166 (54); 165 (24); 151 (23); 138 (52); 115 (39); 109 (13); 69 (23); 63 (19); 53 (15).

*Data for (5Z)-3(4-Bromobenzyl)-5-(benzylidene)-5H-furan-2-one **(8c)**.* White solid; m.p. 127.3– 27.9 °C. IV (KBr, cm^{-1}): $\bar{\nu}_{max}$ 3102; 3058; 3022; 1764; 1649; 1609; 1486; 1362; 1026; 930; 759. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.68 (brs, 2H, H7); 5.89 (s, 1H, H6); 6.96 (t, 1H, J = 1.4 Hz, H4); 7.14 (d, 2H, J = 8.4 Hz, H2′/H6′); 7.30 (t, 1H, J = 7.2 Hz, H4″); 7.39 (t, 2H, J = 7.2 Hz, H3″/H5″); 7.46 (d, 2H, J = 8.4 Hz, H3′/H5′); 7.73 (d, 2H, J = 7.2 Hz, H2″/H6″). ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.14 (C7); 113.06 (C6); 120.93 (C4′); 128.81 (C3″/C5″); 129.03 (C4″); 130.49 (C2″/C6″); 130.62 (C2′/C6′); 131.75 (C3); 131.97 (C3′/C5′); 133.02 (C1″); 136.16 (C1′); 139.74 (C4); 147.29 (C5); 170.18 (C2). EM, *m/z* (%): 340 (M$^+$, $C_{18}H_{13}BrO_2$, 41); 342 (M + 2, 38); 322 (3); 295 (5); 261 (9); 243 (23); 233 (13); 217 (16); 216 (30); 215 (41); 202 (18); 189 (3); 171 (8); 156 (3.1); 143 (13); 115 (56); 101 (13); 91 (15); 90 (100); 77 (9); 63 (27); 51 (15).

*Data for 5(Z)-3(4-Bromobenzyl)-5-(3-methylbenzylidene)-5H-furan-2-one **(8d)**.* White solid; m.p. 111.2–111.9 °C. IV (KBr, cm^{-1}): $\bar{\nu}_{max}$ 3108; 1747; 1648; 1488; 1419; 1341; 1072; 1034; 914; 899; 793. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (s, 3H, 3″-CH_3); 3.68 (brs, 2H, H7); 5.86 (s, 1H, H6); 6.95 (t, 1H, J = 1.3 Hz, H4); 7.12 (d, 1H, J = 7.6 Hz, H4″); 7.14 (d, 2H, J = 8.3 Hz, H2′/H6′); 7.26 (t, 1H, J = 7.6 Hz, H5″); 7.47 (d, 2H, J = 8.3 Hz, H3′/H5′); 7.54 (d, 1H, J = 7.6 Hz, H6″); 7.55 (s, 1H, H2″). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.41 (3″-CH_3); 31.13 (C7); 113.32 (C6); 120.92 (C4′); 127.77 (C6″); 128.71 (C5″); 129.97 (C4″); 130.65 (C2′/C6′); 131.01 (C2″); 131.56 (C3); 131.97 (C3′/C5′); 132.94 (C1″); 136.22 (C1′); 138.48 (C3″); 139.82 (C4); 147.16 (C5); 170.30 (C2). MS, *m/z* (%): 354 (M$^+$, $C_{19}H_{15}BrO_2$, 95); 356 (M + 2, 93); 336 (7); 311 (9); 275 (11); 257 (35)247 (21); 230 (44); 229 (45); 215 (35); 204 (18); 169 (16); 143 (26); 132 (50); 115 (89); 104 (69); 89 (47); 78 (100); 63 (34); 51 (31).

*Data for (5Z)-3(4-Bromobenzyl)-5-(4-fluorobenzylidene)-5H-furan-2-one **(8e)**.* White solid; m.p. 138.2–138.9 °C. IV (KBr, cm^{-1}): $\bar{\nu}_{max}$ 3088; 1758; 1727; 1595; 1507; 1485; 1237; 1044; 822; 800. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3,68 (s, 2H, H7); 5.85 (s, 1H, H6); 6.96 (t, 1H, J = 1.3 Hz, H4); 7.06 (t, 2H, J = 8.8 Hz, H3″/H5″); 7.13 (d, 2H, J = 8.3 Hz, H2′/H6′); 7.46 (d, 2H, J = 8.3 Hz, H3′/H5′); 7.72 (dd, 2H, J = 8.8, 5.5 Hz, H2″/H6″). ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.14 (s, C7); 111.75 (s, C6); 115.98 (d, $^1J_{C\text{-}F}$ = 21.5 Hz, C3″/C5″); 120.96 (s, C4′); 129.33 (s, C1″); 130.63 (s, C2′/C6′); 131.72 (s, C3); 131.99 (s, C3′/C5′); 132.37 (d, $^2J_{C\text{-}F}$ = 8.3 Hz, C2″/C6″); 136.11 (s, C1′); 139.63 (s, C4); 146.92 (s, C5); 162.92 (d, $J_{C\text{-}F}$ = 250; C4″); 170.08 (s, C2). MS, *m/z* (%): 358 (M$^+$, $C_{18}H_{12}BrFO_2$, 64); 360 (M + 2, 62); 340 (4); 313 (10); 279 (15); 261 (28); 235 (18); 233 (61); 220 (19); 203 (7); 183 (5); 169 (10); 143 (23); 136 (39); 115 (66); 108 (100); 89 (33); 75 (12); 63 (23); 51 (13).

*Data for (5Z)-3(4-Bromobenzyl)-5-(4-bromobenzylidene)-5H-furan-2-one **(8f)**.* White solid; m.p. 164.3–165.2 °C. IV (KBr, cm^{-1}): $\bar{\nu}_{max}$ 3111; 1769; 1756; 1643; 1577; 1487; 1405; 1311; 1279; 1144; 1033; 813. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.67 (s, 2H, H7); 5.82 (s, 1H, H6); 6.95 (s, 1H, H4); 7.13 (d, 2H, J = 8.3 Hz, H2′/H6′); 7.47 (d, 2H, J = 8.3 Hz, H3′/H5′); 7.49 (d, 2H, J = 8.6 Hz, H3″/H5″); 7.59 (d, 2H, J = 8.6 Hz, H2″/H6″). ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.19 (C7); 111.65 (C6); 121.01 (C4′); 123.33 (C4″); 130.63 (C2′/C6′); 131.79 (C2″/C6″); 131.93 (C3); 132.02, 132.04 (C3′/C5'and C3″/C5″); 132.28 (C1″); 135.98 (C1′); 139.55 (C4); 147.65 (C5); 169.87 (C2). MS, *m/z* (%): 418 (M$^+$, $C_{18}H_{12}Br_2O_2$, 29); 420 (M + 2, 60); 422 (M+4, 26); 375 (3); 339 (2); 323 (8); 311 (1); 295 (4); 260 (9); 232 (10); 215 (24); 203 (11); 169 (9); 143 (11); 115 (32); 101 (16); 89 (100); 75 (6); 63 (27); 50 (8).

*Data for (5Z)-3(4-Bromobenzyl)-5-(4-chlorobenzylidene)-5H-furan-2-one **(8g)**.* White solid; m.p. 148.9–150.5 °C. IV (KBr, cm^{-1}) $\bar{\nu}_{max}$ 3110; 2928; 1770; 1758; 1645; 1584; 1488; 1407; 1091; 1034; 1012. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.68 (s, 2H, H7); 5.84 (s, 1H, H6); 6.95 (s, 1H, H4); 7.13 (d, 2H, J = 8.2 Hz, H2′/H6′); 7.34 (d, 2H, J = 8.4 Hz, H3″/H5″); 7.47 (d, 2H, J = 8.2 Hz, H3′/H5′); 7.66 (d, 2H, J = 8.4 Hz, H2″/H6″). ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.18 (C7); 111.60 (C6); 121.01 (C4′); 129.08 (C3″/C5″); 130.64 (C2′/C6′); 131.53 (C3); 131.60 (C2″/C6″); 132.02 (C3′/C5′); 132.19 (C4″); 134.96 (C1′); 136.02 (C1″); 139.55 (C4); 147.55 (C5); 169.90 (C2). MS, *m/z* (%): 374 (M$^+$, $C_{18}H_{12}BrClO_2$, 43); 376 (M + 2, 56); 378 (M + 4, 14); 321 (9); 311 (1); 295 (9); 277 (11); 259 (5); 232 (13); 215 (31); 203 (15); 193 (4); 169 (7); 152 (22); 143 (15); 124 (33); 115 (5); 101 (26); 89 (100); 63 (40); 49 (21); 44 (91); 32 (36).

*Data for (5Z)-3(4-Bromobenzyl)-5-(2,5-dimetoxybenzylidene)-5H-furan-2-one **(8h)**.* Fluorescent yellow solid; m.p. 84.0–85.3 °C. IV (KBr, cm^{-1}): $\bar{\nu}_{max}$ 2954; 2835; 1758; 1645; 1607; 1581; 1498; 1236; 1023; 884. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.67 (s, 2H, H7); 3.81, 3.80 (s, 6H, 3″-OCH_3 and 6″-OCH_3); 6.41 (s,1H, H6); 6.80 (d, 1H, J = 9.0 Hz, H3″); 6.85 (dd, 1H, J = 9.0, J = 2.9 Hz, H4″); 6.98 (s, 1H, H4); 7.14 (d, 2H, J = 8.3 Hz, H2′/H6′); 7.46 (d, 2H, J = 8.3 Hz, H3′/H5′); 7.72 (d, 1H, J = 2.9 Hz, H6″). ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.13 (C7); 55.92, 56.25 (2″-OCH_3 and 5″-OCH_3); 106.85 (C6); 111.80 (C3″); 115.74 (C6″); 116.65 (C4″), 120.87 (C4′); 122.60 (C1″); 130.65 (C2′/C6′); 131.15 (C3); 131.94 (C3′/C5′); 136.33 (C1′); 140.13 (C4); 147.28 (C5); 152.16 (C2″); 153.77 (C5″); 170.26 (C2). MS, *m/z* (%): 400 (M$^+$, $C_{20}H_{17}BrO_4$, 100); 402 (M + 2, 98); 357 (7); 329 (7); 306 (4); 278 (23); 261 (7); 250 (14); 234 (9); 178 (10); 163 (39); 153 (8); 136 (38); 123 (15); 115 (31); 92 (20); 77 (33); 63 (21); 51 (17); 44 (75); 32 (33).

*Data for (5Z)-3(4-Bromobenzyl)-5-(3-nitrobenzylidene)-5H-furan-2-one **(8i)**.* Pale yellow solid; m.p. 122.8–123.9 °C. IV (KBr, cm^{-1}): $\bar{\nu}_{max}$ 3097; 2936; 2892; 2838; 1754; 1653; 1609; 1570; 1530; 1352; 1034; 907. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.71 (s, 1H, H7); 5.93 (s, 1H, H6); 7.01 (s, 1H, H4); 7.15 (d, 2H, J = 8.3 Hz, H2′/H6′); 7.48 (d, 2H, J = 8.3 Hz, H3′/H5′); 7.56 (t, 1H, J = 8.1 Hz, H5″); 8.13 (d, 1H, J = 8.0 Hz, H4″); 8.16 (d, 1H, J = 8.0 Hz, H6″); 8.42 (s, 1H, H2″). ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.26 (C7); 109.82 (C6); 121.16 (C4′); 123.17 (C4″); 124.74 (C2″); 129.83 (C5″); 130.65 (C2′/C6′); 132.10 (C3′/C5′); 133.65 (C1′); 134.65 (C1″); 135.62 (C6″); 135.67 (C3); 139.22 (C4); 148.59 (C3″); 148.98 (C5); 169.32 (C2). MS, *m/z* (%): 485 (M$^+$, $C_{18}H_{12}BrNO_4$, 45); 487 (M + 2, 45); 370 (43); 368 (44);

350 (6); 306 (11); 288 (6); 278 (13); 259 (7); 231 (15); 216 (16); 202 (34); 193 (8); 169 (15); 143 (17); 115 (99); 89 (100); 63 (74); 51 (19); 44 (67); 39 (49); 30 (9).

Data for (5Z)-3(4-Bromobenzyl)-5-(4-trifluoromethylbenzylidene)-5H-furan-2-one ***(8j).*** White solid; m.p. 110.7–111.4 °C. IV (KBr, cm^{-1})" $\bar{\nu}_{max}$ 3110; 2938; 1766; 1647; 1615; 1488; 1417; 1323; 1163; 1133; 1069; 1014. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.70 (s, 2H, H7); 5.91 (s, 1H, H6); 6.98 (s, 1H, H4); 7.14 (d, 2H, J = 8.3 Hz, H2′/H6′); 7.48 (d, 2H, J = 8.3 Hz, H3′/H5′); 7.61 (d, 2H, J = 8.2 Hz, H3″/H5″); 7.83 (d, 2H, J = 8.2 Hz, H2″/H6″). ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.23 (s, C7); 110.98 (s, C6); 121.10 (s, C4′); 125.66 (d, $^3J_{C\text{-}F}$ = 3.9 Hz; C3″/C5″); 130.44 (s, C2″/C6″); 130.65 (s, C2′/C6′); 132.07 (s, C3′/C5′); 133.18 (s, C3); 135.80 (s, C1′); 136.38 (s, C1″); 139.45 (s, C4); 148.66 (s, C5); 169.65 (s, C2). MS, *m/z* (%): 408 (M^+, $C_{19}H_{12}BrF_3O_2$, 59); 410 (M + 2, 61); 391 (6); 363 (9); 329 (23); 311 (26); 285 (16); 283 (4); 265 (7); 233 (10); 215 (32); 204 (17); 186 (14); 158 (56); 143 (21); 126 (11); 115 (100); 90 (20); 89 (50); 63 (39); 49 (34); 39 (30).

Data for (5Z)-3(4-Bromobenzyl)-5-(4-cyanobenzylidene)-5H-furan-2-one ***(8k).*** White solid; m.p. 178.0–179.0 °C. IV (KBr, cm^{-1}): $\bar{\nu}_{max}$ 3098; 3062; 2229; 1767; 1650; 1609; 1505; 1487; 1027; 937; 899; 796. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.70 (s, 2H, H7); 5.88 (s, 1H, H6); 6.99 (s, 1H, H4); 7.14 (d, 2H, J = 8.3 Hz; H2′/H6′); 7.48 (d, 2H, J = 8.3 Hz, H3′/H5′); 7.64 (d, 2H, J = 8.4 Hz, H3″/H5″); 7.81 (d, 2H, J = 8.4 Hz, H2″/ H6″). ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.24 (C7); 110.40 (C6); 111.84 (C4″); 118.57 (4″-CN); 121.14 (C4′); 130.56, 130.61 (C2′/C6′ and C2″/C6″); 132.07 (C3′/C5′); 132.40 (C3″/C5″); 133.73 (C3); 135.60 (C1′); 137.35 (C1″); 139.27 (C4); 149.27 (C5); 169.32 (C2). MS, *m/z* (%): 365 (M^+, $C_{19}H_{12}BrNO_2$, 25); 367 (M + 2, 24); 347 (2); 320 (5); 286 (14); 268 (16); 240 (26); 230 (12); 215 (5); 196 (5); 169 (7); 143 (33); 128 (8); 115 (100); 114 (32); 101 (16); 89 (24); 75 (8); 63 (23); 51 (14).

Data for (5Z)-3(4-Bromobenzyl)-5-(2-chloro-4-dimethylaminobenzylidene)-5H-furan-2-one ***(8l).*** Orange solid; m.p. 155.2–156.0 °C. IV (KBr, cm^{-1}): $\bar{\nu}_{max}$ 3094; 2990; 2906; 2888; 2820; 1777; 1751; 1613; 1594; 1516; 1485; 1370; 1295; 1155; 1030; 833. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.02 (s, 6H, 4″-N(CH_3)$_2$); 3.66 (brs, 2H, H7); 6.33 (s, 1H, H6); 6.65 (dd, 1H, J = 9.1, J = 2.7 Hz; H5″); 6.71 (d, 1H, J = 2.7 Hz, H3″); 6.99 (t, 1H, J = 1.3 Hz, H4); 7.14 (d, 2H, J = 8.4 Hz, H2′/H6′); 7.45 (d, 2H, J = 8.4 Hz, H3′/H5′); 8.17 (d, 1H, J = 9.1 Hz, H6″). ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.09 (C7); 40.31 (4″-(CH_3)$_2$); 109.26 (C6); 111.38 (C5″); 112.52 (C2″); 119.10 (C3″); 120.80 (C4′); 129.51 (C3); 130.65 (C2′/C6′); 131.91 (C3′/C5′); 132.77 (C6″); 136.05 (C1″); 136.56 (C1′); 140.09 (C4); 145.71 (C5); 150.62 (C4″); 170.60 (C2). MS, *m/z* (%): 417 (M^+, $C_{20}H_{17}BrClNO_2$, 81); 419 (M + 2, 100); 421 (M + 4, 25); 382 (2); 338 (5); 303 (2); 275 (4); 231 (4); 209 (4); 195 (48); 192 (23); 167 (39); 152 (20); 140 (9); 132 (43); 115 (40); 101 (12); 89 (45); 75 (12); 63 (18); 51 (14).

Biological Tests. *Evaluation of Lactones* **8a**–**8l** *as Inhibitors of the Photosynthetic Electron Transport by the Hill Reaction.* The ability of the compounds to interfere in vitro with the electron transport chain was evaluated as previously described (*25*). Briefly, photosynthetically active thylakoid membranes were isolated from market spinach (*Spinacea oleracea* L.) leaves. Deveined plant material was resuspended in 5 mL g^{-1} of ice-cold 20 mM *N*-tris(hydroxymethyl)methylglycine (Tricine)-NaOH buffer (pH 8.0) containing 10 mM NaCl, 5 mM $MgCl_2$ and 0.4 M sucrose and homogenized for 30 s in a blender at maximal speed. The homogenate was filtered through surgical gauze, and the filtrate was centrifuged at 4 °C for 1 min at 500 g; the supernatant was further centrifuged for 10 min at 1500 g. Pelleted chloroplasts were osmotically swollen by resuspension in sucrose-lacking buffer. The suspension was immediately diluted 1:1 with sucrose-containing buffer, kept on ice in the dark and used within a few hours from the preparation. Following proper dilution with 80% (v/v) acetone, the absorbance of each sample was determined at 645 and 663 nm, and the chlorophyll content was calculated on the basis of Arnon's formula. The basal rate of photosynthetic electron transport was measured following light-driven ferricyanide reduction. Aliquots of membrane preparations corresponding to 20 μg of chlorophyll were incubated at 24 °C in 1 mL cuvettes containing 20 mM Tricine-NaOH buffer (pH 8.0), 10 mM NaCl, 5 mM $MgCl_2$, 0.2 M sucrose, and 1 mM $K_3Fe(CN)_6$. The assay was initiated by exposure to saturating light (800 μmol m^{-2} s^{-1}), and the rate of ferricyanide reduction was measured at 30 s intervals for 10 min against an exact blank at 420 nm. Activity was calculated over the linear portion of the curve from a molar extinction coefficient of 1,000 M^{-1} cm^{-1}. Nostoclide analogues were dissolved in DMSO so as to obtain 25 or 50 mM solutions that were then diluted with water, as appropriate. Their effect upon the Hill reaction was evaluated in parallel assays in which the compounds were added to the reaction mixture to concentrations of 5 μM or 10 μM. Each dose was carried out at least in triplicate, and results were expressed as percentage of untreated controls. Phosphorylating electron flow was assessed under the same conditions, but in the presence of 0.5 mM ADP and 2 mM K_2HPO_4. Unless otherwise indicated, uncoupled activity was measured following the addition of 2 mM NH_4Cl to the basal reaction mixture containing aliquots of membrane preparations corresponding to 10 μg of chlorophyll.

In vivo Inhibition of Photosynthetic Activity Rate. The ability of the most active compounds to interfere with the photosynthetic process in vivo was evaluated on eukaryotic algal cells, as described in the literature (*26*). *Chlorella protothecoides* Kruger, ATCC 30411 strain, was grown at 24 ± 1 °C in 250 mL Erlenmeyer flasks containing 50 mL of Proteose Peptone medium under continuous light (500 μmol m^{-2} s^{-1}). Mid-log-grown cells (10 ± 2.5 mg L^{-1} chlorophyll) were harvested by centrifugation for 1 min at 14000 g, and pellets were resuspended in a proper volume of Bg11 medium to a cellular density of 8.0 ± 0.2 mg L^{-1} Chl. The rate of oxygen evolution under saturating light (>2 mmol m^{-2} s^{-1} photosynthetic active radiation) was measured polarographically using a Hansatech (King's Lynn, Norfolk, England) system consisting of a DW2/2 electrode unit, an A1 stirrer, an LS2 light source with A8 fiber optic, and a CB1-D control box. All assays were performed at 24 °C. Reaction was started by switching the light on, and the oxygen concentration (arbitrary units) in the 1.0 mL cell was recorded every 15 s up to 8 min. The light was then switched off, and oxygen consumption in the dark was followed for a further 5 min, providing a measure of the cell respiratory activity. The rate of photosynthetic oxygen production was calculated as the sum of oxygen evolution in the light and oxygen consumption in the dark. The inhibitory activity of the nostoclide analogues was evaluated by adding 100 μL of freshly prepared 1.1 mM water solutions to the stirred electrode cell (final concentration 0.1 mM). The resulting rate was expressed as percent of that of controls to which only water was added. At least three independent measurements were performed, and means ± SE are presented. To improve the solubility of the compounds and increase the rate of translocation to the chloroplast, assays were also carried out in the presence of 0.001% (v/v) Triton X-100; at this level, well below the critical micelle concentration (0.2 mM, corresponding to about 0.013%), the detergent is thought to be unable to interfere with membrane functionality and was found to be ineffective on algal photosynthetic activity (data not shown).

RESULTS AND DISCUSSION

Synthesis of Lactones 8a–8l. For the preparation of compounds **8a**–**8l**, the synthesis of furyl **5** was required. This was accomplished, with a fair yield, employing a slightly modified procedure described in the literature (*23*) and having lactone **3** as a starting material. The treatment of lactone **3** with $POCl_3$ in the presence of triethylamine afforded compound **4**, which was not isolated. Subsequent reaction of **4** with diethylamine furnished **5** in 51% yield (**Figure 2**). Treatment of compound **5** with *n*-butyllithium, followed by the addition of 4-bromobenzyl bromide, resulted in the formation of the substituted furan **6** (**Figure 3**). The crude product was treated with formic acid (*27*) to provide lactone **7**, with an overall yield of 76%.

The vinylogous aldol reaction (*28*–*31*) between the silyloxy diene furan synthon and the relevant aldehydes was then employed to achieve the synthesis of compounds **8a**–**8l**. The reaction of lactone **7** with the corresponding aldehydes in the presence of *tert*-butyl-dimethylsilyltrifluoromethanesulfonate and

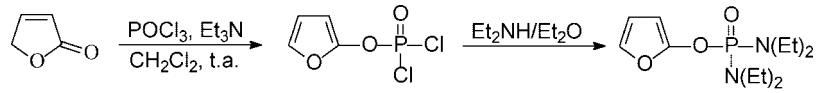
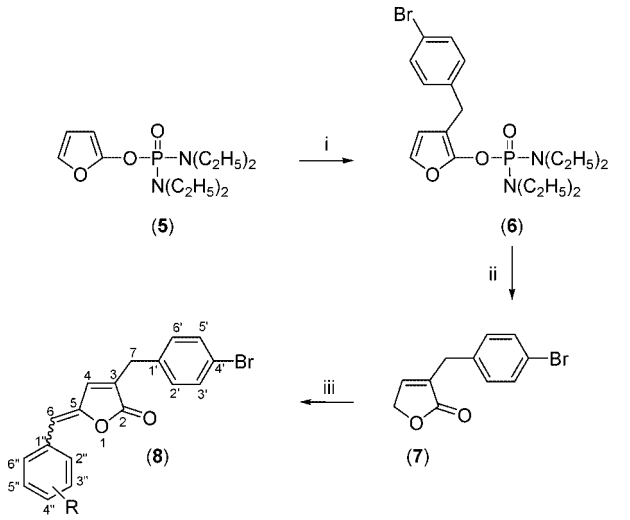


Figure 2. Synthesis of *N,N,N′,N′*-tetraethyldiamidophosphate (compound **5**).



Compound	Arylidene Group	Yield (%)
8a	1,3-dioxalanebenzylidene	59 (*Z*)
8b	2,4,6-trimethoxybenzylidene	39 (*E*)
8c	benzylidene	98 (*Z*)
8d	3-methylbenzylidene	59 (*Z*)
8e	4-fluorobenzylidene	47 (*Z*)
8f	4-bromobenzylidene	24 (*Z*)
8g	4-chlorobenzylidene	27 (*Z*)
8h	2,5-dimethoxybenzylidene	68 (*Z*)
8i	3-nitrobenzylidene	31 (*Z*)
8j	4-trifluoromethylbenzylidene	42(*Z*)
8k	4-cyanobenzylidene	23 (*Z*)
8l	2-chloro-4-dimethylamino benzylidene	41 (*Z*)

Figure 3. Synthetic methodology employed to prepare nostoclide analogues **8a**–**8l**. (i) *n*-BuLi, THF/HMPA (5:1), –78 °C; *p*-$C_6H_4BrCH_2Br$ (ii) HCOOH (76% yield for steps i and ii); (iii) ArylCHO, TBDMSOTf, DIPEA, CH_2Cl_2, r.t., 1 h; DBU, reflux, 1–3 h.

Hunig's base, followed by treatment of the silyl ether generated in situ with DBU, afforded the lactones **8a**–**8l**, with yields ranging from 23 to 98% (**Figure 3**). Reaction times were not optimized, but most reactions were complete within 3 h or less. The direct β-elimination of the silyl ether intermediates, under the reaction conditions, led to the formation of the corresponding *Z* stereoisomers. However, in the case of compound **8b**, the formation of the *E* isomer was observed. This fact is probably due to a destabilizing steric repulsion between the lactone oxygen lone pair and the methoxy groups at both C2″ and C6″. In all cases, the geometry of the double bond was confirmed by bidimensional nuclear Overhauser enhancement spectroscopy (NOESY).

Table 1. In vitro Effects of Nostoclide Analogues on Ferricyanide Reduction by Functionally Intact Chloroplasts Isolated from *Spinacia oleracea* Leaves[a]

compd	arylidene group	5 μM	10 μM
8a	1,3-dioxalanebenzylidene	91.1 ± 2.5	91.0 ± 1.3
8b	2,4,6-trimethoxybenzylidene	84.2 ± 1.2	79.9 ± 3.6
8c	benzylidene	54.1 ± 2.4	42.9 ± 2.2
8d	3-methylbenzylidene	72.8 ± 0.9	69.7 ± 3.7
8e	4-fluorobenzylidene	74.0 ± 1.5	68.5 ± 4.9
8f	4-bromobenzylidene	95.4 ± 1.1	92.0 ± 0.6
8g	4-chlorobenzylidene	94.1 ± 0.9	89.4 ± 0.1
8h	2,5-dimethoxybenzylidene	94.1 ± 4.5	71.0 ± 0.5
8i	3-nitrobenzylidene	72.6 ± 2.2	66.9 ± 0.2
8j	4-trifluoromethylbenzylidene	71.7 ± 1.3	48.8 ± 0.9
8k	4-cyanobenzylidene	100.4 ± 0.9	101.4 ± 2.6
8l	2-chloro-4-dimethylamino benzylidene	94.7 ± 8.2	91.0 ± 2.0
diuron		6.8 ± 0.5	4.9 ± 0.7

[a] Basal activity was measured as described in Materials and Methods in either the absence or presence of nostoclide analogues at a concentration of 5 or 10 μM. Each sample was carried out in triplicate, and values were expressed as percentage ± SD of untreated controls.

Biological Activity. Because of their structural similarity to other nostoclide analogues, some of which were shown to inhibit the photosynthetic electron transport chain (*20*), the synthesized compounds were suspected to be endowed with biological activity against photosynthetic organisms. To verify such a hypothesis, we evaluated their ability to lower the rate of ferricyanide reduction by isolated spinach chloroplasts. Results, summarized in **Table 1**, showed that most compounds are indeed able to interfere with the light-driven electron transport chain. With the exception only of compound **8k**, in all cases, the rate of the electron transfer from water to ferricyanide was significantly ($P < 0.05$) reduced at 10 μM. The effectiveness even of the most active compounds was strikingly lower than that of the commercial herbicide diuron, taken as a comparison term. However, several of them were more effective than two nostoclide analogues previously characterized as Hill reaction inhibitors (*20*), and equipotent to some pyrazole derivatives recently described as a new class of potential inhibitors of oxygenic photosynthesis (*25*, *26*). From a qualitative point of view, their effect varies with the substituent of the benzylidene ring, but at this initial stage of the research, a structure–activity relationship analysis would be limited by the low number of derivatives available. The two most active compounds, **8c** and **8j**, bear no substituent and a trifluoromethyl moiety at position 4 of the benzylidenic ring, respectively. When the concentration of these nostoclide analogues was raised above 10 μM, in all cases, the resulting inhibitory effect rapidly reached a plateau and did not increase further (**Figure 4**). The lack of a dose-effect relationship for the active compounds above a certain threshold may depend upon their low solubility in water. Millimolar solutions of nostoclides were obtainable only with organic solvents, such as DMSO. However, those solutions had to be diluted with water, in order to avoid any interference of the solvent with the integrity (and thus the functionality) of the thylakoid membranes. A certain degree of lipophilicity is usually required for a photosynthetic inhibitor, given that in order

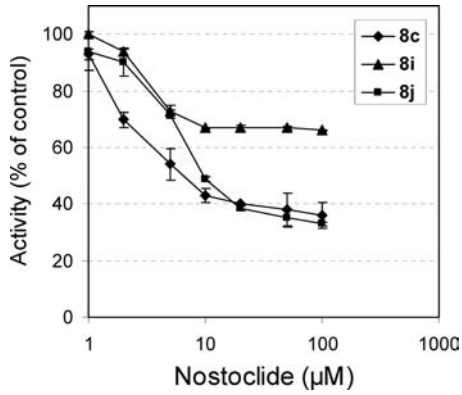


Figure 4. Effect of increasing concentrations of the most active compounds upon ferricyanide reduction by functionally intact chloroplasts isolated from spinach leaves.

Table 2. Effects of the Most Active Nostoclide Analogues on Basal, Phosphorylating, and Uncoupled Electron Flow from water to Ferricyanide in Spinach Chloroplasts.[a]

	compd **8c**	compd **8j**	activity[b]
basal electron flow (μM)	0	0	100 ± 3.1
	10	0	44.2 ± 2.5
	0	10	47.7 ± 3.8
phosphorylating electron flow (μM)	0	0	100 ± 3.8
	10	0	47.9 ± 3.0
	0	10	52.1 ± 2.8
uncoupled electron flow (μM)	0	0	100 ± 4.1
	10	0	95.6 ± 3.1
	0	10	79.8 ± 3.0

[a] Basal activity was measured as described in Materials and Methods. Phosphorylating electron flow was assessed under the same conditions in the presence of 0.5 mM ADP and 2 mM K_2HPO_4. Activity was uncoupled by adding 2 mM NH_4Cl to the reaction mixture. [b] Activity was expressed as percent of untreated samples. Values are mean ± SE over at least six replications in two independent experiments. Control value rates were 50.0 ± 1.6, 92.9 ± 3.5, and 141.9 ± 5.8 nmol of ferricyanide reduced s^{-1} (mg of chlorophyll)$^{-1}$ under basal, phosphorylating, and uncoupled conditions, respectively.

to exert its effect, it needs to diffuse through both the plasmalemma and the chloroplast envelopes. Moreover, it has to penetrate the thylakoid membrane to reach its molecular target, and in several cases, the presence of a hydrocarbon side chain has been reported to greatly impact the activity of a given scaffold, as it provides the required lipophilicity (*26*, *32*). Consistently, some of the most effective commercial herbicides targeting photosynthesis show a relatively low solubility in water, such as lenacil. Nothwithstanding, the opposite is also true, because sufficient hydrophilicity is needed to allow herbicides to be translocated within the plants and reach their target inside the chloroplast. The recorded effect may thus be ascribed to the small concentration of a given nostoclide analogue that is able to dissolve in the buffer in which chloroplasts are resuspended.

To gain more information about their mode of action, we also assessed the inhibitory properties of the two most active compounds (**8c** and **8j**) under phosphorylating or uncoupled conditions. Results are shown in **Table 2**. In the presence of substrates of ATP synthase, both compounds retained their ability to inhibit ferricyanide reduction, and the residual activity did not differ significantly from that evaluated under basal conditions. On the contrary, the uncoupled electron flow was found to be almost insensitive for the presence of the compounds at a concentration at which the basal rate was inhibited by 50%. Because the actual concentration of the

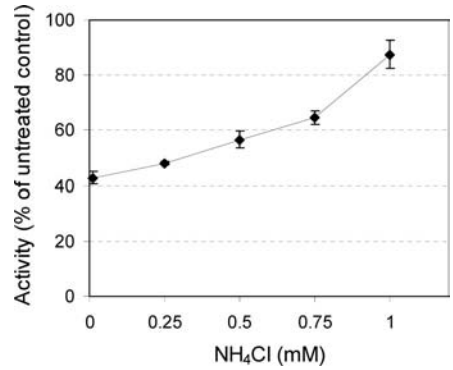


Figure 5. Reversal of the inhibition brought about by compound **8c** at increasing ammonium concentration. Basal electron flow was enhanced by the progressive addition of NH_4Cl, which acts as an uncoupler. As a consequence, the inhibitory effect exerted by the nostoclide at 10 μM was progressively reduced. Data are expressed as percent of the activity in untreated samples containing the same amount of uncoupler. Results are mean ± SE over at least six replications from two independent experiments.

Table 3. In vivo Effects of the Most Active Nostoclide Analogues on the Photosynthetic Oxygen Evolution Rate by Intact Cells of *Chlorella protothecoides*[a]

	compd **8c**	compd **8j**	diuron	activity[b]
in the absence of detergent (μM)	0	0	0	100 ± 5.9
	0	0	10	49.9 ± 3.5***
	0	0	100	38.5 ± 4.8***
	100	0	0	82.6 ± 5.7^{NS}
	0	100	0	94.5 ± 0.7^{NS}
+0.001% (v/v) Triton X-100 (μM)	0	0	0	100 ± 5.1
	0	0	10	32.2 ± 3.3***
	0	0	100	20.7 ± 4.9***
	100	0	0	74.2 ± 6.9*
	0	100	0	89.5 ± 2.1^{NS}

[a] Activity was evaluated as detailed in Materials and Methods on cells harvested during the mid-log phase of growth. The compounds were added to a concentration of 0.1 mM, in either the presence or absence of detergent. Values are expressed as percent of the rate measured in untreated controls and are means ± SE over three replications (six for untreated controls). [b] Results were subjected to Anova: *, $P < 0.10$; **, $P < 0.05$; ***, $P < 0.01$; NS, not significant.

inhibitors could not be raised above such a level because of their poor solubility (**Figure 4**), the effect of the same fixed concentration was investigated at increasing levels of ammonium, which acts as the uncoupler. Results (**Figure 5**) confirmed that the inhibition brought about by compound **8c** is progressively reverted when increasing NH_4Cl concentrations dissipate the proton gradient across the tylakoid membrane. This suggests that these nostoclide analogues may inhibit phosphorylation in chloroplasts, acting as energy-transfer inhibitors through an interaction with the coupling factor, CF_0-CF_1. A similar mode of action has been reported, for instance, in the case of euparin, a phytotoxic substance isolated from *Helianthella quinquenervis* (*33*), and chalepensin, a coumarin isolated from *Stauranthus perforatus* (*34*), which also inhibit basal and phosphorylating electron flow without affecting the uncoupled rate.

Finally, the actual ability of compounds **8c** and **8j** to interfere with the photosynthetic activity was evaluated in vivo by measuring the light-driven oxygen production in intact *Chlorella* cells. The results are outlined in **Table 3**. Even though a higher dose was used (100 μM), one at which the maximal level of compound that can dissolve in water is most likely present, the effect of nostoclide analogues was

quite poor. In the absence of detergents, the mild reduction of the rate of oxygen evolution with respect to controls was not statistically significant. However, it was consistent with the efficacy previously shown in vitro by the two compounds with respect each other, and with respect to the reference herbicide diuron. The addition of a detergent at a concentration unable to damage membrane integrity significantly improved the activity of both nostoclides, as well as that of diuron. This is not unexpected, because small amounts of detergents may increase the solubility of lipophilic compounds in aqueous media and therefore increase the rate of their translocation to the active site. Although not conclusive, such evidence seems thus to support the possibility that the two most active nostoclide analogues (**8c** and **8j**) may be able to interfere with the photosynthetic electron transport chain in vivo.

Conclusions. Several 3-(4-bromobenzyl)-5-(arylmethylene)-*5H*-furan-2-one lactones, analogues to the naturally-occurring toxins nostoclides, were synthesized and purified. Their potential as inhibitors of the photosynthetic process in the eukaryotic cell was investigated. Several of them were able to interfere with the light-driven ferricyanide reduction by isolated chloroplasts, most likely at the level of the coupling factor. However, their poor solubility in aqueous environments severely limited their efficacy, as significant rates of the electron transport chain were still present at saturating concentrations. Because of the higher concentration needed for Hill reaction inhibition, these compounds seem, therefore, not to be satisfactory for use as agrochemicals. However, their inhibitory potential is higher than that of other nostoclide analogues previously characterized in our laboratories (*20*). Thus, the most active derivatives could represent structures to be further exploited for the design of new substances endowed with herbicidal activity. Work is currently under way to achieve the synthesis of new nostoclide analogues showing better photosynthesis-inhibiting properties.

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