Synthesis of Long-Chain Triazine Aldehydes – Substrates of Bacterial Luciferase and Photosynthetic Inhibitors

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S-triazines are photosynthetic inhibitors. They have been substituted with ω-aminoundecanoic acid. The coupling products have been transformed into triazine aldehydes. These compounds displace radioactive terbutryn and have inhibitory effects on photosynthesis in plants and bacteria. Triazine aldehydes were shown to be effective substrates for bacterial luciferase. A competitive assay between photosystem-II-herbicides and aldehyde-labeled triazines is discussed.

Herbicides are an important pillar of modern agriculture. However, their application over decades has brought about increasing problems for our water. From the commercial point of view, photosystem-II-herbicides (PS-II-herbicides) are the most important herbicides [1, 2]. For this reason, the development of a biosensor for PS-II-herbicides is planned. Although their chemistry is different, these compounds inhibit photosynthetic electron flow at the Q_B-site of PS-II by displacing plastoquinone [3, 4].

The herbicide sensor is based on the detection of displaced triazines by bacterial luciferase. First, the Q_B-site is occupied by labeled triazines, which are then displaced by PS-II-herbicides. Displaced labeled triazines will be detected by bacterial luciferase. The amount of luminescent light emitted by bacterial luciferase is proportional to the substrates and can be easily monitored with a luminometer [5, 6].

$$FMNH_2 + O_2 + \text{aldehyde} \longrightarrow FMN + H_2O + \text{acid} + h\nu$$

One substrate of bacterial luciferase is a long-chain aliphatic aldehyde with an optimal chain length ranging from C_{10} to C_{12} [6]. For this reason, triazines were labeled with long-chain aliphatic aldehydes. The displaced triazine aldehydes should be determined by bacterial luciferase.

We report here about the synthesis of triazine aldehydes, especially terbutryn derivatives and 11-aminonundecanals. Their binding to Q_B and their substrate properties to bacterial luciferase are also described.

Abbreviations: DCPIP, 2,6-dichlorophenol-indophenol; RC, reaction centre; *R. sphaeroides, Rhodobacter sphaeroides;* PS II, photosystem II; Chl, chlorophyll.

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Materials and Methods

General procedure I: reaction of mono-chloro triazines with amino acids

A mixture of the corresponding amino acid (12 mmol) and N,N-diisopropylethylamine (24 mmol) in 10 ml ethylen glycol was slowly added to a well-stirred solution of the corresponding triazine (10 mmol) in 30 ml ethylene glycol. After 2 h at 120 °C, 160 ml of 5% aqueous $NaHCO_3$ was added and the mixture washed with CH_2Cl_2. The aqueous layer was acidified to pH 1 with 6 N HCl, NaCl-saturated and extracted 3 times with CH_2Cl_2. The CH_2Cl_2-phases were dried over $MgSO_4$ and after concentration under vacuum the residue was purified by thin-layer chromatography.

General procedure II: converting triazine acids into aldehydes

Boran-dimethyl sulfide (BMS) (5 mmol) is added dropwise from a syringe under nitrogen at room temperature to a solution of the corresponding acid (5 mmol) in 20 ml dry tetrahydrofuran. After adding an initial 2.5 mmol BMS, and after the gas evolution has ceased, the mixture is heated under gentle reflux to complete the evolution of hydro-

gen. Then, the remainder of the BMS is added and the mixture heated under reflux for 1 h. Additional BMS (5 mmol) is added and heated for another hour. The solvent and dimethyl sulfide is removed under vacuum and 30 ml dry CH_2Cl_2 is introduced to suspend the product. Pyridinium chlorochromate (PCC) (7.5 mmol) in 20 ml CH_2Cl_2 is added and the stirred mixture is heated for 1 h and then diluted with 40 ml diethyl ether. The supernatant liquid is filtered through silica gel and the solid residue is triturated with diethyl ether (3 × 50 ml) and passed through the same column. The filtrate is concentrated and purified by thin-layer chromatography.

Remarks to NMR spectra:

1) b = broad triplet of nitrogen-bound hydrogen in $-NH-CH_2-$.

2) If not otherwise specified NMR spectra were recorded in $CDCl_3$ at room temperature.

3) Because of the existence of rotamers [7] one atom may give several NMR signals and quaternary carbon atoms of the triazine ring may be difficult to detect in ^{13}C NMR spectra.

Synthesis of 2-chloro-4-ethylamino-6-methylthio-s-triazine (**1**)

was carried out according to [8].

Synthesis of 2-chloro-4-(1',1'-dimethylethylamino)-6-methylthio-s-triazine (**2**)

was carried out according to [8].

Synthesis of 11-(4-ethylamino-6-methylthio-s-triazine-2-yl)undecanoic acid (**6**)

According to general procedure **I,** 2.04 g (10 mmol) 2-chloro-4-ethylamino-6-methylthio-s-triazine **1,** 2.41 g (12 mmol) 11-aminoundecanoic acid (**5**) and 3.07 g (24 mmol) DIPEA yielded 2.4 g (6.5 mmol, 65%) **6** (m.p. 77 °C). – ^1H NMR (400 MHz): δ = 1.19–1.22 (m; 3H, CH_3CH_2), 1.25–1.40 (m; 12H, CH_2), 1.55–1.67 (m; 4H, 3-H_2, 10-H_2), 2.33 (t, $J_{2,3}$ = 7.4 Hz; 2H, 2-H_2), 2.41, 2.46 (2s; 3H, SCH_3), 3.20–3.50 (m; 4H, 11-H_2, CH_2-CH_3), 5.90–6.52, 8.50–9.20 (b; 2H, 2NH). – ^{13}C NMR (100 MHz): δ = 12.73 (SCH_3), 14.74 (CH_3CH_2), 24.93, 26.82, 29.09 (C-3 to C-10), 34.55 (C-2), 35.56 (CH_2-CH_3), 40.63 (C-11), 178.85 (C-1). – MS (FAB): *m/z* (%) = 369 (100) [M^+], 307 (16), 289 (9), 154 (53), 136 (38). –

$C_{17}H_{31}N_5O_2S$ (369.5)

Calcd	55.25%	C 8.45%	H 18.95%	N
Found	54.48%	C 8.25%	H 18.74%	N

Synthesis of 11-(4-ethylamino-6-methylthio-s-triazine-2-yl)undecanal (**7**)

According to general procedure **II,** 738 mg (2.0 mmol) 11-(4-ethylamino-6-methylthio-s-triazine-2-yl)undecanoic acid (**6**) yielded 431 mg (1.22 mmol, 61%) **7** (oil). – ^1H NMR (400 MHz): δ = 1.17–1.22 (m; 3H, CH_3CH_2), 1.25–1.38 (m; 12H, CH_2); 1.50–1.69 (m; 4H, 3-H_2, 10-H_2), 2.42 (dt, $J_{1,2}$ = 1.9 Hz, $J_{2,3}$ = 7.3 Hz; 2H, 2-H_2), 2.45 (s; 3H, CH_3S), 3.25–3.50 (m; 4H, 11-H_2, CH_2-CH_3), 5.00–5.35 (b; 2H, 2NH), 9.77 (t, $J_{1,2}$ = 1.9 Hz; 1H, 1-H). – ^{13}C NMR (100 MHz): δ = 12.98 (CH_3S), 14.90 (CH_3CH_2), 22.07, 26.83, 29.14, 29.27, 29.31, 29.33, 29.45 (C-3 to C-10), 35.49 (CH_2CH_3), 40.59 (C-11), 43.90 (C-2), 202.91 (C-1). – MS (90 °C): *m/z* (%) = 353 (0.7) [M^+], 338 (0.7) [M^+-CH_3], 325 (7), 310 (5), 212 (7), 199 (14), 71 (100). –

$C_{17}H_{31}N_5OS$ (353.5)

Calcd	57.76%	C 8.84%	H
Found	57.78%	C 8.81%	H

Synthesis of 11-(4-(1',1'-dimethylethylamino)-6-methylthio-s-triazine-2-yl)aminoundecanoic acid (**8**)

According to general procedure **I,** 2.32 g (10 mmol) 2-chloro-4-(1',1'-dimethylethylamino)-6-methylthio-s-triazine (**2**), 2.41 g (12 mmol) 11-aminoundecanoic acid (**5**) and 3.07 g (24 mmol) DIPEA yielded 3.7 g (9.5 mmol, 95%) **8** (m.p. 77 °C). – ^1H NMR (d_6-DMSO, 400 MHz): δ = 1.21–1.29 (m; 9H, CH_3), 1.33–1.38 (m; 12H, CH_2), 1.44–1.53 (m; 4H, 3-H_2, 10-H_2), 2.18 (t, $J_{2,3}$ = 7.4 Hz; 2H, 2-H_2), 2.34, 2.37 (2s; 3H, SCH_3), 3.10–3.29 (m; 2H, 11-H_2), 6.59–6.84, 7.00–7.32 (b; 2H, 2NH). – ^{13}C NMR (100 MHz): δ = 12.16, 12.60 (SCH_3), 24.68, 26.60, 28.73, 29.02, 29.13, 29.28 (C-3 to C-10), 28.68 ($CH_3)_3$, 33.85 (C-2), 39.91 (C-11), 50.32, 55.05 ($C(CH_3)$), 163.81, 174.65 (triazine ring), 177.56 (C-1). – MS (105 °C): *m/z* (%) = 397 (46) [M^+], 382 (100) [M^+-CH_3], 353 (39), 338 (87), 282 (80), 227 (76), 171 (81). –

$C_{19}H_{35}N_5O_2S$ (397.6)

Calcd	56.77%	C 8.87%	H 17.61%	N
Found	56.69%	C 8.69%	H 17.48%	N

Synthesis of 11-(4-(1′,1′-dimethylethylamino)-6-methylthio-s-triazine-2-yl)aminoundecanal (**9**)

According to general procedure **II,** 796 mg (2 mmol) 11-(4-(1′,1′-dimethylethylamino)-6-methylthio-s-triazine-2-yl)aminoundecanoic acid (**8**) yielded 462 mg (1.21 mmol, 61%) **9** (yellow oil). – ^{1}H NMR (400 MHz): δ = 1.27, 1.29 (2s, 9H, C**H**$_3$), 1.37–1.48 (m; 12H, CH$_2$), 1.50–1.67 (m; 4H, 3-H$_2$, 10-H$_2$), 2.42 (dt, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 7.4 Hz; 2H, 2-H$_2$), 2.47 (s; 3H, CH$_3$S); 3.20–3.50 (m; 2H, 11-H$_2$), 4.50–5.30, 5.40–5.60 (b; 2H, 2NH), 9.76 (t, $J_{1,2}$ = 1.8 Hz; 1H, 1-H). – ^{13}C NMR (100 MHz): δ = 12.74, 13.04 (CH$_3$S), 21.99, 26.80, 29.00, 29.06, 29.23, 29.25, 29.62 (C-3 to C-10), 29.39 (CH$_3$)$_3$, 40.70 (C-11), 43.82 (C-2), 50.96 (**C**(CH$_3$)), 163.91, 164.11 (triazine ring), 202.82 (C-1). – MS (85 °C): *m/z* (%) = 381 (3) [M$^+$], 380 (6), 379 (6), 366 (100) [M$^+$–CH$_3$], 353 (82), 338 (55), 324 (19), 282 (65), 198 (74), 170 (75). –

C$_{19}$H$_{35}$N$_5$OS (381.6)

Calcd	59.80%	C 9.24%	H 18.35%	N
Found	59.62%	C 9.03%	H 18.17%	N

Synthesis of 11-(4-(1′,1′-dimethylethylamino)-6-ethylamino-s-triazine-2-yl)aminoundecanoic acid (**10**)

According to general procedure **I,** 2.75 g (12 mmol) 2-chlor-4-(1′,1′-dimethylethylamino)-6-ethylamino-s-triazine (**3**), 2.89 g (14.4 mmol) 11-aminoundecanoic acid (**5**) and 3.69 g (28.8 mmol) DIPEA yielded 4.52 g (11.5 mmol, 96%) **10** (m.p. 50–53 °C). – ^{1}H NMR (400 MHz): δ = 1.16–1.22 (m; 3H, C**H**$_3$CH$_2$), 1.26–1.35 (m; 12H, CH$_2$), 1.43 (s; 9H, CH$_3$), 1.51–1.66 (m; 4H, 3-H$_2$, 10-H$_2$), 2.26 (t, $J_{2,3}$ = 7.3 Hz; 2H, 2-H$_2$), 3.22–3.47 (m; 4H, 11-H$_2$, C**H**$_2$–CH$_3$), 7.70–7.97 (b; 3H, 3NH). – ^{13}C NMR (100 MHz): δ = 14.88 (**C**H$_3$CH$_2$), 25.69, 26.70, 29.25, 29.71 (C-3 to C-10), 29.13 ((**C**H$_3$)$_3$), 35.68, 36.32 (**C**H$_2$–CH$_3$ und C-2), 40.49 (C-11), 52.41 (**C**(CH$_3$)), 160.20, 160.33, 164.72 (triazine ring), 180.64 (C-1). – MS (120 °C): *m/z* (%) = 394 (85) [M$^+$], 379 (100) [M$^+$–CH$_3$], 350 (25), 335 (75), 279 (70), 237 (65). –

C$_{20}$H$_{38}$N$_6$O$_2$ (394.6)

Calcd	60.88%	C 9.71%	H 21.30%	N
Found	59.47%	C 9.41%	H 21.12%	N

Synthesis of 11-(4-(1′,1′-dimethylethylamino)-6-ethylamino-s-triazine-2-yl)aminoundecanal (**11**)

According to general procedure **II,** 792 mg (2 mmol) 11-(4′-(1′,1′-dimethylethylamino)-6-ethylamino-s-triazine-2-yl)aminoundecanoic acid (**10**) yielded 100 mg (0.26 mmol, 13%) **11** (oil). – ^{1}H NMR (400 MHz): δ = 1.17 (t, J = 7.2 Hz; 3H, C**H**$_3$CH$_2$), 1.24–1.36 (m; 12H, CH$_2$), 1.42 (s; 9H, CH$_3$), 1.50–1.68 (m; 4H, 3-H$_2$, 10-H$_2$), 2.42 (dt, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 7.4 Hz; 2H, 2-H$_2$), 3.27–3.42 (m; 4H, 11-H$_2$, C**H**$_2$–CH$_3$), 4.70–5.00 (b; 3H, 3NH), 9.77 (t, $J_{1,2}$ = 1.8 Hz; 1H, 1-H). – ^{13}C NMR (100 MHz): δ = 15.18 (**C**H$_3$CH$_2$), 22.11, 26.98, 29.16, 29.34, 29.50, 29.72, 29.89 (C-3 to C-10), 29.31 ((**C**H$_3$)$_3$), 43.93 (C-2), 50.85 (**C**(CH$_3$), 202.96 (C-1). – MS (100 °C): *m/z* (%) = 378 (12) [M$^+$], 363 (100) [M$^+$–CH$_3$], 350 (83), 335 (47), 321 (25), 237 (57). –

C$_{20}$H$_{38}$N$_6$O (378.6)

Calcd	63.45%	C 10.12%	H	
Found	62.70%	C 9.98%	H	

Synthesis of 11-(4-chloro-6-methylthio-s-triazine-2-yl)aminoundecanoic acid (**12**)

A mixture of 1.33 g (6.6 mmol) 11-aminoundecanoic acid (**5**), 1.70 g (13.6 mmol) N,N-diisopropylethylamine (DIPEA) and ethanol was added to a vigourously stirred solution of 1.176 g (6 mmol) 2,4-dichloro-6-methylthio-s-triazine (**4**) in 45 ml ethanol at room temperature. After 3 h at 35 °C, the residual amino acid was filtered and the solvent of the filtrate was removed by rotary evaporation. The residue was purified by thin-layer chromatography and yielded 1.67 g (4.6 mmol, 62%) **12** (m.p. 62 °C). – ^{1}H NMR (400 MHz): δ = 1.25–1.40 (m; 12H, CH$_2$), 1.55–1.69 (m; 4H, 3-H$_2$, 10-H$_2$), 2.35 (t, $J_{2,3}$ = 7.5 Hz; 2H, 2-H$_2$), 2.48, 2.51 (2s; 3H, SCH$_3$), 3.37–3.50 (m; 2H, 11-H$_2$), 6.37–6.45, 6.77–6.87, 7.09–7.16 (b; 1H, 1NH). – ^{13}C NMR (100 MHz): δ = 13.00, 13.29, 13.52, 14.35 (SCH$_3$), 24.71, 26.67, 26.73, 26.76, 28.95, 29.03, 29.13, 29.24, 29.35, 29.68 (C-3 to C-10), 34.13 (C-2), 40.83, 41.10, 41.21 (C-11), 163.69, 164.27, 167.51 (triazine ring), 179.23 (C-1). – MS (100 °C): *m/z* (%) = 360 (12) [M$^+$], 345 (17) [M$^+$–CH$_3$], 301 (55), 203 (48), 189 (100), 74 (42). –

C$_{15}$H$_{25}$N$_4$O$_2$SCl (360.9)

Calcd	49.92%	C 6.98%	H 15.52%	N
Found	50.88%	C 7.25%	H 15.44%	N

Synthesis of 11-(4-chloro-6-methylthio-s-triazine-2-yl)aminoundecanal (**13**)

According to general procedure **II,** 720 mg (2 mmol) 11-(4-chloro-6-methylthio-s-triazine-2-yl)aminoundecanoic acid (**12**) yielded 440 mg (1.3 mmol, 63%) **13** (wax). – ^{1}H NMR (400 MHz): δ = 1.23–1.40 (m; 12H, CH_2), 1.54–1.68 (m; 4H, 3-H_2, 10-H_2), 2.43 (t, $J_{2,3}$ = 7.3 Hz, $J_{1,2}$ = 1.8 Hz; 2H, 2-H_2), 2.47, 2.51 (2s, 3H, SCH_3), 3.37–3.50 (m; 2H, 11-H_2), 5.75–5.91, 6.28–6.40 (b; 1H, NH), 9.77 (t, $J_{1,2}$ = 1.8 Hz; 1H, 1-H). – ^{13}C NMR (100 MHz); δ = 13.33, 13.49 (SCH_3), 22.03, 26.65, 26.70, 29.10, 29.11, 29.16, 29.21, 29.28, 29.39 (C-3 to C-10), 41.05, 41.19 (C-11), 43.87 (C-2), 164.06, 164.45, 167.91, 169.01, 182.53, 183.56 (triazine ring), 202.94 (C-1). – MS (170 °C): *m/z* (%) = 345 (9) [M^++H], 329 (15) [M^+–CH_3], 316 (38), 301 (65), 203 (46), 189 (100). –

$C_{15}H_{25}N_4OSCl$ (344.9)

Calcd	52.23%	C 7.31%	H 16.24	N
Found	52.61%	C 7.29%	H 16.64	N

Inhibitory potency in photosynthetic reactions and luciferase assay

Cultivation of *Rhodobacter sphaeroides* (DSM 158) and purification of RCs were carried out according to [9].

Liposomes were prepared according to [10] with slight modifications. A chloroform solution of 4 mg L-α-phosphatidylcholine (Typ III-B, 4 mg L-α-phosphatidyl-D-L-glycerol and 8 mg L-α-phosphatidylethanolamine (dipalmitoyl) was dried at reduced pressure. The residue was resuspended in 8 ml 50 mM tricine buffer (N-[2-hydroxymethyl-1,1-bis(hydroxymethyl)ethyl]glycine), pH 7.8, and 1% *n*-octyl-β-d-glucopyranoside and 24 nmol bacterial RC were added. This mixture was dialyzed against 1 l of 50 mM tricine-NaOH buffer, pH 7.8, 5 mM $MgCl_2$ and 0.1 mM dithiothreitol with two buffer changes. The collected liposomes were centrifuged at 2,500 × *g* for 15 min (Heraeus Sepatech, Labofuge GL), resuspended in tricine-NaOH buffer, pH 7.8, 5 mM $MgCl_2$ and stored at –20 °C until use.

Displacement studies were performed as described [11] with slight modifications. Liposomes were incubated with 0.2 nmol [^{14}C]terbutryn (spec. activity 6.4 mCi/mmol) for 20 min in 1 ml tricine-NaOH buffer, pH 7.8, 5 mM $MgCl_2$ followed by the addition of inhibitors. After incubating for 30 min, liposomes were separated by centrifugation for 10 min in an Eppendorf centrifuge at 12,000 × *g*. The pellet was assayed for radioactivity.

Oxidation of reduced cytochrome c (from horse heart) by isolated RCs under anaerobic conditions in the light (> 610 nm) was monitored spectrophotometrically at 550 nm according to Oettmeier *et al.* [12] and Clayton *et al.* [13]. The reaction mixture contained in a volume of 1 ml 20 mM Trizma buffer (tris(hydroxymethyl)aminomethane) pH 7.5, 20 μg reduced cytochrome c, 50 μM ubiquinone-0 and 50 nM RC.

Spinach chloroplasts were prepared according to a procedure by Nelson *et al.* [14].

Photosynthetic activity was assayed using DCPIP as electron acceptor. DCPIP reduction was monitored at 600 nm. The 3 ml of reaction medium contained 30 mM Hepes buffer (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid), pH 7.0, 10 mM $MgCl_2$, 60 μM DCPIP, 10.5 μg gramicidin and chloroplasts at a concentration corresponding to 20 μg Chl.

Activity of bacterial luciferase was performed by measuring luminescent light with a luminometer (Berthold, Lummat LB 9501). The reaction was started by injecting 50 μl of 100 μM $FMNH_2$ (chemically reduced with sodium dithionite) into 450 μl of 0.1 M phosphate buffer, pH 6.8, 0.2% BSA, 0.1 mM dithiothreitol, 5 μg bacterial luciferase from *Photobacterium fischeri* (Boehringer, Mannheim) and 5 μl of ethanolic aldehyde solution. Light emitted during the first second after injection was integrated.

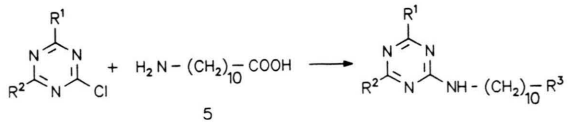
Results

Synthesis

Triazine aldehyde synthesis was based on terbutryn (2-(1′,1′-dimethylethylamino)-4-ethylamino-6-methylthio-s-triazine), a potent inhibitor of photosystem-II in bacteria and plants. Each side chain of the triazine ring system of terbutryn was substituted by undecanal, resulting in **7, 9, 11.** Two side chains of terbutryn were substituted in **13** by undecanal and chlorine, respectively.

Cyanuric chloride is frequently used as a starting compound for substituted triazines because the

Table I. Summary of synthetic reactions.



	R^1	R^2		R^3
1	SCH_3	Et-NH	**6/7**	COOH/CHO
2	SCH_3	*t*-Bu-NH	**8/9**	COOH/CHO
3	*t*-Bu-NH	Et-NH	**10/11**	COOH/CHO
4	SCH_3	Cl	**12/13**	COOH/CHO

relative electron deficiency of the ring carbon atoms and the electron withdrawing chlorine make them susceptible to nucleophilic attack. Methods involving compounds with acidic hydrogen (−OH, $-SH_2$, NH_2) as well as alkylation according to Friedel-Crafts and Grignard have been described [15]. For our work, amino compounds were selected because each of the three chlorine atoms can be substituted step by step by controlling the reaction temperature. Ω-amino acids as well as ω-amino alcohols are commercially available, but amino acids were chosen for substitution because alcohols themselves are able to react with triazine chlorides.

Initially, one chlorine had to be replaced by 11-aminoundecanoic acid (**5**). Goodrow *et al.* [8] have described the conversion of a dichlorine triazine with 6-aminohexanoic acid in the presence of N,N-diisopropylethylamine in ethanol under reflux. Since under these conditions, it was impossible to replace the third chlorine of monochlorine triazines **1, 2** and **3** with 11-aminoundecanoic acid (**5**), which was unsoluble in ethanol, ethylene glycol was introduced here as solvent. Then the reaction temperature could be increased to 120 °C. Under these conditions, the amino acid **5** was soluble and the reaction was completed after 2 h. Triazine acids **6, 8** and **10** were obtained in a yield of 95, 65 and 96%, respectively.

The reaction of the dichlorine triazine **4** with amino acid **5** was possible in ethanol at 35 °C. Under these conditions, double substitution could be avoided and triazine acid **12** was obtained at a yield of 61%.

Secondly, triazine acids had to be converted into the corresponding aldehydes. Selective reduction with boran-dimethyl sulfide (BMS) was previously introduced by Brown *et al.* [16]. Pyridinium chlorochromate has been shown to be the reagent of choice for the oxidation of trialkyl borates to carbonyl compounds [17].

Triazine aldehydes as substrates for bacterial luciferase

The effectiveness of the synthesized triazine aldehydes as substrates of bacterial luciferase is shown (Fig. 1). Aldehydes **7, 9** and **11** are all about 100 times less effective then *n*-decanal (standard aldehyde) while aldehyde **13** is only 10 times. All linear ranges of aldehydes showed equivalent slopes. Due to their effectiveness loss of 10 or 100 times, the detection limit is also decreased 10 or 100 times.

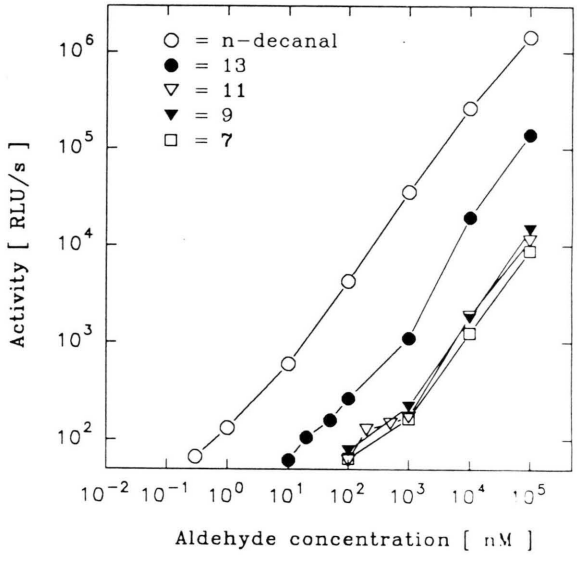


Fig. 1. Calibration curve of the luciferase activity from Photobacterium fischeri with several aldehydes. N-decanal was chosen as standard. RLU/s = relative light units per second. **13** = 11-(4-chloro-6-methylthio-s-triazine-2-yl)aminoundecanal, **11** = 11-(4-(1′,1′-dimethylethylamino)-6-ethylamino-s-triazine-2-yl)aminoundecanal, **9** = 11-(4-(1′,1′-dimethylethylamino)-6-methylthio-s-triazine-2-yl)aminoundecanal, **7** = 11-(4-ethylamino-6-methylthio-s-triazine-2-yl)undecanal.

Affinity of triazine aldehydes to the Q_B-site

The affinity of triazine aldehydes to the Q_B-site was tested by displacement studies with radioactively labeled terbutryn in liposomes containing

bacterial RCs. Furthermore, inhibition of steady state electron transport in spinach chloroplasts and bacterial RCs was also determined. The displacement of [^{14}C]terbutryn and the inhibition of photosynthetic electron flow by **7** are shown in Fig. 2. The pI_{50} values for all triazine aldehydes synthesized are given in Table II. They show a range of inhibitory potency from 5 to above 8, depending on the substituents and bacterial or plant system.

Triazine aldehyde **7** (11-(4-ethylamino-6-methylthio-s-triazine-2-yl)undecanal) showed the highest affinity to Q_B, in the bacterial as well as in the plant system. Additionally, triazine aldehyde **7** was the only synthesized derivative to be as effective as terbutryn itself.

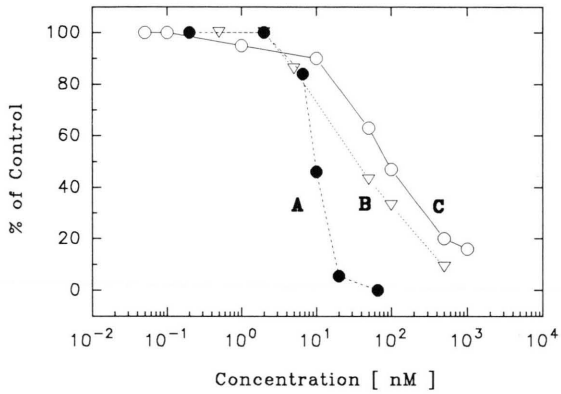


Fig. 2. Affinity of **7** to the Q_B-site. **A** = Inhibition of photosynthetic PS-II electron transport in spinach chloroplasts (activity of control: 75 µmol DCPIP/h mg Chl). **B** = Inhibition of photosynthetic electron transport in RC from *R. sphaeroides* (activity of control: 61 nmol cytochrom c/h in the presence of 50 pmol bacterial RC). **C** = Displacement of 0.2 nmol [^{14}C]terbutryn in liposomes containing RC from *R. sphaeroides*.

Table II. Affinity of triazine aldehydes to the Q_B-site. pI_{50} = negative logarithm of the concentration that displaces 50% of bound [^{14}C]terbutryn or inhibits 50% of photosynthetic electron flow in isolated chloroplast from *Spinatia spec.* or isolated RCs of *R. sphaeroides*. For rates of controls see Fig. 2. Numbers of compounds as in Table I.

Compound	Displacement of [^{14}C]terbutryn in RC-liposomes pI_{50}	Inhibition of electron flow in bact. RC pI_{50}	PS II pI_{50}
14	6.70	7.32	8.04
11	6.10	6.00	5.55
13	5.04	>5	5.72
7	7.05	7.35	8.00
9	5.52	5.40	5.82

Discussion

The results show that triazine aldehydes are able to carry out its bifunctional task. Triazine aldehydes are accepted by bacterial luciferase as substrates. However, the effectiveness is 10 to 100 times less than that of *n*-decanal. Jockers *et al.* [18] have shown that quinone aldehydes are also suitable substrates for bacterial luciferase. By optimizing aldehyde chain-length, aldehydes as effective as *n*-decanal were obtained. Therefore, this method may also be suitable for optimizing the luciferase system.

The affinity for the Q_B-site of the bacterial and the plant system of the six triazine aldehydes synthesized ranged from 5 to 8. Triazine aldehyde **7** revealed an affinity as high as terbutryn itself. This observation is in good agreement with X-ray data from Michel *et al.* [19]. Co-crystals of RCs from *Rhodopseudomonas viridis* and terbutryn showed specific interactions of the thiomethyl and ethylamine group of terbutryn. The tert.-butylamine group was oriented towards the channel where the polyisoprenyl side-chain of ubiquinone is normally located. Due to specific interactions of the thiomethyl and ethylamine group with the amino acid backbone, triazine aldehyde **7** is the only compound synthesized which can probably bind to Q_B in the same way as terbutryn, whilst the long-chain aldehyde is oriented towards the polyisoprene channel.

The affinity of triazine aldehydes is slightly higher for the plant system than for the bacterial system. Nevertheless, until now only the isolated bacterial RC fulfills the requirements for biosensor application. Chloroplasts or isolated RC from plants are unstable at room temperature. Therefore, further efforts will concentrate on this system. Easily displaceable triazine aldehydes are advantageous when creating a potential detection system for PS-II-herbicides. Therefore, the bacterial system may turn out to be more convenient than the plant system. Finally, the wide range of pI_{50} values between 5 and 8 for the synthesized triazine aldehydes offers numerous choices.

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