1-CHLOROBENZOTRIAZOLE-MEDIATED RING CLOSURE OF 1,3,5-TRIARYLFORMAZANS: IMPROVED SYNTHESES OF 2,3,5-TRIARYL-2H-TETRAZOLIUM SALTS

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Abstract - Oxidative ring closure of mono- and bis-triarylformazans mediated by easily available 1-chlorobenzotriazole leads to the corresponding 2,3,5-triaryl-2H-tetrazolium chlorides in yields of 70-97%.

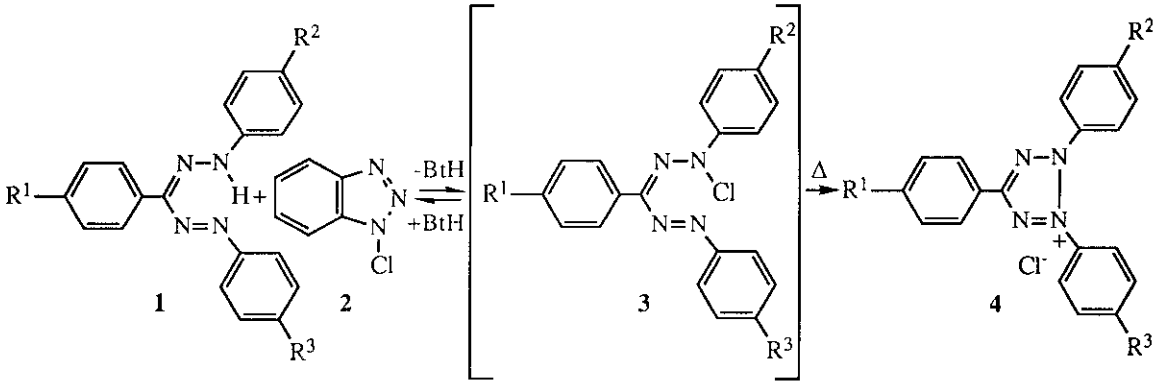
INTRODUCTION

2,3,5-Triaryl-2H-tetrazolium chlorides (**4**) are reagents which have found widespread use in histochemistry, medicine (oncology, virology and enzymology), analytical chemistry etc.[1] Various derivatives of (**4**) have been synthesized by oxidation of the initial 1,3,5-triarylformazans (**1**). The oxidizing agents most often used include mercuric oxide, iso-amyl nitrite,[2] lead tetraacetate,[3] and more recently potassium permanganate/5% HCl,[4] thionyl chloride[5] and nitrous acid (formed in situ from sodium nitrite/HCl).[6] However, none of the previous methods are general, and all are subject to limitations and disadvantages. Most of the oxidizing agents used cause undesirable side reactions such as halogenation, sulfonation, nitrosation. When this is not the case, the reaction mixtures contain inorganic side products (manganese dioxide, sodium chloride etc.) which hinder the isolation of the tetrazolium salts in a pure state. Because of the ease of formation of adducts between tetrazolium salts and water, alcohols or HCl, the choice of solvents for the oxidation is limited. These factors have resulted in a wide range of oxidizing agents, that have seemingly been chosen almost in a random fashion.

RESULTS AND DISCUSSION

1-Chlorobenzotriazole (**2**) is a mild oxidizing agent for the conversion of alcohols into aldehydes/ketones[7] and aldoximes into nitrile oxides.[8] 1-Chlorobenzotriazole is easily synthesized by reacting 1H-benzotriazole with sodium hypochlorite in aqueous acetic acid [7] We now find that treatment of triarylformazans (**1**) with 1-chlorobenzotriazole (**2**) in organic solvents yields the corresponding triaryl-2H-tetrazolium chlorides (**4**) (Scheme 1).

Scheme 1



Formazans (**1**) and salts (**4**):

1,4:	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**
R^1	H	MeO	Cl	NO_2	Br	H	MeO	MeO	NO_2	H
R^2	H	H	H	H	H	H	H	H	H	NHAc
R^3	H	H	H	H	Br	Me	Br	NO_2	Br	NHAc

Since (**2**) contains a "positive" halogen, the first stage of the reaction is probably formation of the N-chlorotriarylformazan (**3**) with the elimination of a benzotriazole molecule, and this is supported by the nmr spectra of the crude reaction mixtures. The first stage is probably reversable, because the reaction rate is accelerated by an excess of (2). Nevertheless, the use of equimolar amounts of formazan and (**2**), in the case of (**4a**), gave the expected salt in good yield (75%).

Table 1. 1,3,5-Triarylformazans (**1a-j**)

Comp. **1**	Yield, % (lit., yield); Solvt. of recryst.[a]	mp °C[b] (lit., mp)	^{1}H Nmr, δ_{NH}, ($CDCl_3$) ppm/TMS	Molecular formula	Elemental analysis Found, % (Required) C	H	N
a	63 (23[10]); A	173-174 (170[10])	-	$C_{19}H_{16}N_4$	-	-	-
b	57 (30[11]); B	160 (156-158[11])	-	$C_{20}H_{18}N_4O$	-	-	-
c	79; A	187-188(190[12])	-	$C_{19}H_{15}N_4Cl$	-	-	-
d	68 (40[2]); C	200-203 (204[2])	-	$C_{19}H_{15}N_5O_2$	-	-	-
e	73(80[2]); C	204-206(203[2])	-	$C_{19}H_{14}N_4Br_2$	-	-	-
f	75(63[13]); D	157-158(156[13])		$C_{20}H_{18}N_4$	-	-	-
g	71; A	151-153	15.1	$C_{20}H_{17}N_4OBr$	58.77 (58.69	4.15 4.19	13.60 13.69)
h	70; C	182-183	13.5[c]	$C_{20}H_{17}N_5O_3$	64.12 (63.99	4.54 4.57	18.59 18.66)
i	59; C	228-230	9.1[c]	$C_{19}H_{14}N_5O_2Br$	53.79 (53.79	3.27 3.33	16.11 16.50)
j	32 (17[2]); C	253-255 (237[2])	-	$C_{23}H_{22}N_6O_2$	-	-	-

[a]A - aq. 1,4-dioxane; B - benzene; C - aq. dimethylformamide; D - benzene:hexane (1:1 v/v)

[b]With decomp. [c]With DMSO-d_6 (1:1 v/v)

To study the influence of substituents on the selectivity of the formazan oxidation, we have synthesized a series of formazans (Table 1), including some already described, and some new.

Those containing electron-withdrawing substituents are (i) easier to isolate and obtain in higher yield; (ii) they have higher melting points; and (iii) crystallize readily. Formazan (**1j**) and the corresponding salt (**4j**) were chosen because both have been successfully used as intermediates in the syntheses of stearic acid redox derivatives for use with the Langmuir-Blodgett technique,[9] but salt (**4j**) was previously obtained only in poor yield (ca.12%) by oxidation of (**1j**) with nitrous acid.

An appropriate solvent was chosen for the oxidation depending on the solubility of the initial formazan and the nature of the substituents. With a strong electron-withdrawing substituent in the formazan, a solvent of high

Table 2. 2,3,5-Triaryl-2H-tetrazolium salts (**4a-j**)

Comp. **4** (molar)	Ratio[a] **(1):(2)**	Time, min	Yield, %[b] (lit., yield)	mp, °C[c] (lit., mp)	Molecular formula	Elementary analysis Found (Required), % C	H	N
a	1:1 (A)	180	75 (58[10])	241-243 (245[10])	$C_{19}H_{15}N_4Cl$	-	-	-
a	1:2 (A)	15	88	243-245	$C_{19}H_{15}N_4Cl$	-	-	-
b	1:2 (A)	30	92	235-236	$C_{20}H_{17}N_4OCl$	65.83 (65.84	4.75 4.70	15.30 15.36)
c	1:2 (A)	20	88	254-255	$C_{19}H_{14}N_4Cl_2$	61.76 (61.79	4.01 3.82	15.07 15.17)
d	1:4 (B)	5	95 (72[2])	246-248 (250[2])	$C_{19}H_{14}N_5O_2Cl$	-	-	-
e	1:2 (A)	15	82	193-195 (183-185[2])	$C_{19}H_{13}N_4Br_2Cl$	-	-	-
f	1:2 (A)	20	90	225-227 (229[13])	$C_{20}H_{17}N_4Cl$	-	-	-
g	1:2 (A)	20	84	189-190	$C_{20}H_{16}N_4OBrCl$	54.04 (54.13	3.94 3.63	12.33 12.63)
h	1:4 (A)	30	97	184-185	$C_{20}H_{16}N_5O_3Cl$	58.50 (58.61	4.05 3.93	17.04 17.09)
i	1:6 (A)	60	71	205-206	$C_{19}H_{13}N_5O_2BrCl$	49.69 (49.75	3.08 2.85	15.33 15.27)
j	1:3 (B)	60	70	235-237	$C_{23}H_{21}N_6O_2Cl$	61.48 (61.53	4.92 4.72	18.53 18.72)

[a]Solvents used: A - benzene; B - 1,4-dioxane [b]Yield of isolated products [c]With decomp.

boiling point and lipophilicity is required. The greater influence is exerted by those of the substituents in the aryl ring attached to the meso-position of the heterocyclic ring: salt (**4d**) was formed after 5 min in high yield, while the production of (**4b-c**) required longer times. Substitution in the N-aryl rings affects the reaction time less dramatically (salts **4e,j**). Tests of various combinations of electron-withdrawing and electron-releasing substituents in the molecule of the initial formazans showed that the best results are obtained if the meso-aryl ring contains the donor whilst the N-aryl ring contains the acceptor substituents (salts **4g-i**).

The reaction is not accompanied by any chlorination of the phenyl group (**4a**) or the methyl substituent (**4f**). Bromine atoms (**4e,g,i**), esters (**4b,g,h**), and amides (**4j**) are unaffected. Our reaction was successfully applied to obtain the commercially important bis-tetrazolium salts, such as Tetrazolium Blue (**4k**) and Nitro Tetrazolium

Table 3. ^{1}H Nmr data of compounds (**4a-j**) (DMSO-d_6; δ, ppm/TMS)

Comp. **4**	Aromatic protons	Others
a	7.60-7.90 (m, 8H); 7.94-8.14 (m, 4H); 8.26-8.42 (m, 3H)	-
b	7.31 (d, 2H, *J*=8.5 Hz); 7.64-7.86 (m, 6H); 7.93-8.20 (m, 4H); 8.28 (d, 2H, *J*=8.5 Hz)	3.91 (s, 3H, OMe)
c	7.60-7.88 (m, 8H); 7.98-8.09 (m, 4H); 8.35 (d, 2H, *J*=8.3 Hz)	-
d[a]	7.70-8.87 (m, 6H); 8.00 (d, 4H, *J*=8.0 Hz); 8.55-8.70 (m, 4H)	-
e	7.62-7.83 (m, 3H); 7.86-7.95 (m, 4H); 8.05-8.18 (m, 4H); 8.24 (d, 2H, *J*=7.7 Hz)	-
f	7.54 (d, 2H, *J*=5.9 Hz); 7.68-7.94 (m, 10H); 8.36 (d, 2H, *J*=5.3 H)	2.41 (s, 3H, Me)
g	7.24-7.35 (m, 2H); 7.66-7.86 (m, 3H); 7.92-8.13 (m, 6H), 8.22-8.30 (m, 2H)	3.92 (s, 3H, OMe)
h	7.30 (d, 2H, *J*=9.1 Hz); 7.69-7.80 (m, 3H), 8.05-8.65 (m, 8H)	3.93 (s, 3H, OMe)
i	7.66-7.88 (m, 3H); 7.91-8.18 (m, 6H); 8.49-8.65 (m, 4H)	-
j	7.71-7.84 (m, 6H); 7.89-7.97 (m, 4H); 8.27-8.34 (m, 3H)	2.14 (s, 6H, Me); 10.92 (s, 2H, NH)

[a]Mixture DMSO-d_6:CD_3OD (1:1 v/v)

Blue (**4l**) (see Experimental) In their usually available form these compounds contain either alcohol or water of crystallization, but in our case (**4k,l**) were isolated directly from the reaction mixture.

Formazans have also been oxidized by sodium hypochlorite, but only chlorate salts were isolated from this procedure.[2] 1-Chlorobenzotriazole acts as a mild transfer agent of a "positive" halogen and is stable in a solid state. Hence, the ratio of reagents is easily controlled whilst hypochlorite exists only as unstable water solutions. The benzotriazole side product is readily soluble in the solvents used for oxidation. Nmr spectra of (**4a-l**) showed no signals of either (**2**) or benzotriazole. Experimental details are summarized in Tables 1 and 2. Table 1 lists the starting formazans, Table 2 the tetrazolium products. Unlike previous procedures, our formazan oxidation requires a smaller excess of oxidizing agent and is completed in 5-60 min depending on the type of substituents in the aryl rings; yields range from 70% to 97%. Elemental analyses and ^{1}H and ^{13}C nmr data (Tables 2-4) confirm the formation of salts (**4**).

Table 4. ^{13}C Nmr data of compounds (**4a-j**) (DMSO-d_6; d, ppm/TMS)

Comp. **4**	Aromatic carbons	Meso-carbon	Others
a	123.2; 126.8; 127.3; 130.1; 133.2; 133.4; 134.0	164.0	-
b	115.1; 115.6; 126.5; 129.2; 130.2; 133.1; 133.9; 163.0	164.0	55.7(OMe)
c	122.0; 126.7; 129.1; 130.1; 130.2; 133.0; 134.0; 138.2	163.2	-
d[a]	125.9; 127.3; 130.1; 130.3; 131.7; 134.6; 135.6; 152.1	165.7	-
e	122.1; 126.6; 127.1; 127.8; 128.7; 129.1; 130.0; 132.0; 132.9; 133.0; 133.1; 134.0	163.3	-
f	123.0; 126.0; 126.3; 127.2; 130.1; 130.4, 130.5; 130.7; 133.0; 133.5; 134.1; 144.8	164.0	21.0 (Me)
g	115.0; 115.4; 126.6; 127.7; 128.6; 129.1; 130.1; 132.1; 132.9; 133.2; 134.0; 163.0	164.2	55.7 (OMe)
h	114.8; 115.5; 125.4; 126.6; 128.7; 129.2; 130.3; 132.8; 134.2; 137.0; 150.0;163.1	164.3	55.7 (OMe)
i	125.0; 126.6; 128.1; 128.4; 128.9; 130.2; 132.0; 132.8; 133 3; 134.2; 139.6, 150.1	162.5	-
j	119.2; 123.2; 126.8; 127.1; 127.2; 130.1; 133.4; 143 9	162.0	24.1 (Me) 169.5 (amide)

[a]In CD_3OD

The proton nmr spectra of the isolated salts (**4**) have no peaks in the region of 9-15 ppm, i.e., the compounds have no N-H protons. Depending on the type and position of the substituents in the aryl rings, the number and position of peaks in the ^{13}C nmr spectra vary because of restricted rotation and overlapping effects.

CONCLUSION

The reaction discussed here could probably be considered as a first example of an oxidative heteroring closure by means of interaction with 1-chlorobenzotriazole. In general, this reaction does not represent a typical case of substrate oxidation by 1-chlorobenzotriazole directly, since the first stage is just simple introduction of a "positive" halogen into a molecule of formazan (slow proton-chlorine exchange) and, only after that, a rapid oxidative ring closure takes place.

EXPERIMENTAL

^1H And ^{13}C nmr spectra were recordered on a Varian XL-200 (FT-mode) spectrometer (internal standard - TMS). Melting points were determined on a Bristoline hot-stage microscope and are uncorrected. The initial formazans (**1a-l**) were synthesized using standard procedures[2,10] from arylaldehyde arylhydrazones by azo-coupling with aryldiazonium salts. 1-Chlorobenzotriazole was obtained by the literature procedure.[7]

The general procedure for the preparation of 2,3,5-triaryl-2H-tetrazolium chlorides is as illustrated for salt (**4a**). To a stirred solution of 300 mg (1 mmol) of 1,3,5-triphenylformazan (**1a**,[10]) in 40 ml of benzene at 70°C 307 mg (2 mmol) of 1-chlorobenzotriazole in 10 ml of benzene were added at one portion. The reaction mixture was then refluxed with stirring for 15 min. During the reaction, the color changed from red to yellow and a precipitation of salt (**4a**) was observed. After cooling, the solvent was evaporated under reduced pressure and the crude product was dissolved in a minimum amount of methanol, followed by precipitation of (**4a**) upon subsequent addition of ether. Filtration of the mixture gave 296 mg (88%) of 2,3,5-triphenyl-2H-tetrazolium chloride in the form of yellow-white needles. Using the above procedure, the other salts (**4b-j**) were obtained; their properties are given in Tables 2-4.

3,3'-(3,3'-Dimethoxy-4,4'-biphenylene)bis(2,5-diphenyl-2H-tetrazolium chloride), BT (**4k**) was obtained in an analogous fashion after refluxing the initial bis-formazan (**1k**) (329 mg, 0.5 mmol) and 1-chlorobenzotriazole (461 mg, 3 mmol) in 30 ml of dioxane with stirring during 15 min. The sticky solid was collected, washed with a dioxane:acetone mixture (1:1 v/v) and dried yielding 318 mg (87%) of salt (**4k**). An analytical sample was obtained after re-precipitation of the salt from chloroform by dioxane. Elemental analysis confirmed the formation of (**4k**). mp 246-247°C (lit.,[14] mp 245-247°C). ^1H Nmr (DMSO-d_6:D_2O, 1:1 v/v, δ, ppm): 3.74 (s, 6H, OCH_3); 7.57-8.00 (m, 16H); 8.17 (d, 4H, J=8.8 Hz); 8 30-8.40 (m, 6H)

3,3'-(3,3'-Dimethoxy-4,4'-biphenylene)bis[2-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride], NBT (**4l**) has been obtained under the same conditions with 86% yield. An analytical sample of the salt was prepared by re-precipitation from ethanol by ether. Elemental analysis confirmed the compound to be (**4l**). mp 159-161°C (lit.,[15] mp 162°C). ^1H Nmr (DMSO-d_6, δ, ppm): 3 79 (s, 6H, OCH_3); 7.68-7.88 (m, 10H); 8.24-8.39 (m, 10H); 8.59 (d, 4H, J=7.7 Hz).

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