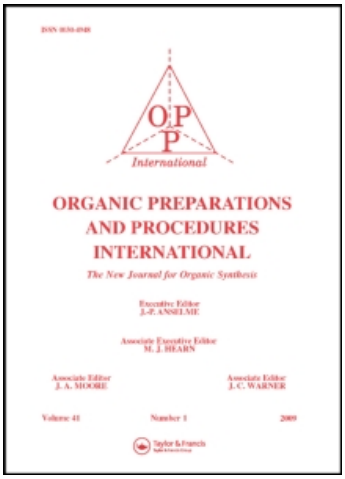

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SYNTHESIS OF SPIN-LABELED TRIAZENES

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SYNTHESIS OF SPIN-LABELED TRIAZENES

Submitted by (03/23/92) Zahary Raikov*†, Vesselina Gadzheva†, Mario Koch†† and George Kolar††

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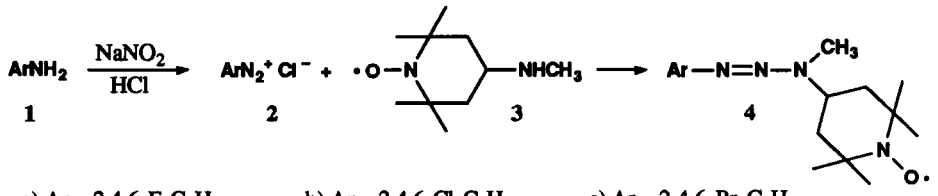
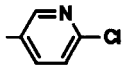
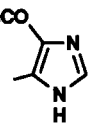
5-(3,3-Dimethyl-1-triazenyl)imidazole-4-carboxamide (dacarbazine, DTIC) is the single most effective agent in the treatment of human disseminated malignant melanoma.[1,2] A serious disadvantage of DTIC as a cancer chemotherapeutic agent derives from its photosensitivity that leads to a rapid decomposition and development of a red color.[3] The replacement of the imidazole ring with an aryl- or other heteroaryl ring stabilizes the triazenes and, in most of cases, does not adversely affect their activity.[4,5] On the other hand, the presence of stable nitroxyl radicals in biologically active compounds reduces their toxicity,[6] and they possess antitumour[7] and radiosensitizing properties[8] and accumulate mainly in pigment melanomas.[9] Hence, we assumed that a combination of triazene and the nitroxyl moieties could lead to a more viable drug. The present work reports the synthesis and structure elucidation of triazenyl substituted 2,2,6,6-tetramethylpiperidine-1-oxyls which are structurally related to biologically active 1-aryl- or 1-heteroaryl-3,3-dimethyltriazenes (Schemes 1 and 2).

A general method of triazene synthesis involves the N-coupling of arenediazonium salts with aliphatic or aromatic amines.[10] Due to the acid-labile nature of a nitroxide radical center, diazonium salts have not been used in the chemistry of nitroxide radicals and attempts to synthesize spin-labeled triazenes have so far not been described. We investigated the conditions of coupling of the arenediazonium salts with the nitroxylamine and established that high yields of spin-labeled triazenes could be obtained in basic solution (pH = 10-12) and temperature -5° to 0°. Compound **3** was prepared by the reductive amination of 4-oxo-2,2,6,6-tetramethylpiperidine-1-oxyl with methylamine in the presence of sodium cyanoborohydride.[11] The compounds **4a-i** were synthesized by diazotization of the corre-

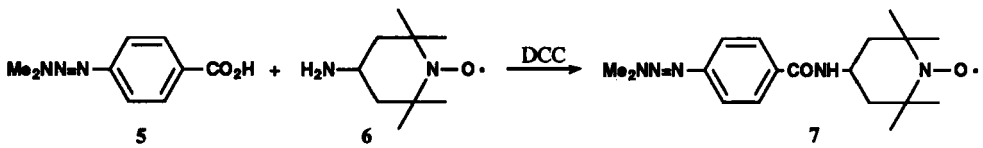
sponding aromatic or heteroaromatic amines **1a-i** in hydrochloric acid with sodium nitrite followed by interaction of the aryl- or heteroaryldiazonium salts **2a-i** with 2,2,6,6-tetramethyl-4-methylamino piperidyl-1-oxyl **3** (Scheme 1).



a) Ar = 2,4,6-$F_3C_6H_2$ b) Ar = 2,4,6-$Cl_3C_6H_2$ c) Ar = 2,4,6-$Br_3C_6H_2$
d) Ar = 4-$CF_3C_6H_4$ e) Ar = 2-CH_3-4-ClC_6H_3 f) Ar = 4-ClC_6H_4
g) Ar = 4-$HO_2CC_6H_4$ h) Ar =  i) Ar = 

Scheme 1

Compound **7** was synthesized by the condensation of 3,3-dimethyl-(4-carboxyphenyl)triazene (**5**) with 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl (**6**) with N,N-dicyclohexylcarbodiimide (DCC) (Scheme 2). The spin-labeled triazenes are crystalline products and their structures were characterized by elemental and spectroscopic analyses. Storage in the dark is not necessary.



Scheme 2

In addition to the known fragmentation pattern of the open chain dialkyl aryltriazenes, the mass spectra of the spin-labeled triazenes exhibited additional intense ion fragments at m/e 169, 154 and 124 which may be accounted for by fragmentation of the 2,2,6,6-tetramethylpiperidine-1-oxyl moiety. As we expected, the ^{1}H-NMR spectra of the paramagnetic triazenes showed rather broad resonances which could not be resolved. This difficulty was overcome in part by reduction of the iminoxyl groups with phenylhydrazine directly in the NMR resonator[12] leading to the formation of the corresponding hydroxylamine derivatives; this treatment resulted in the narrowing of the resonance lines so that an additional information on the molecular structure could be obtained. The EPR spectra of all spin-labeled compounds showed three lines spectrum: aN=15-16G.

EXPERIMENTAL SECTION

All melting points were measured on a Kofler apparatus and are not corrected. IR spectra were determined with UR-20 Carl Zeiss Jena spectrometer. Mass spectra were obtained on a Finnigan-MAT 711 spectrometer (100 ev). ^{1}H NMR spectra were measured in $CDCl_3$ using a Bruker AM

500 spectrometer operated at 70 ev ionizing energy. EPR spectra were recorded in toluene on a Bruker ESR-300 spectrometer at 300 K. Elemental analyses were performed on Perkin-Elmer Model 240-elemental analyzer.

2,2,6,6-Tetramethyl-4-[3-(aryl)-1-methyl-2-triazenyl]-piperidyl-1-oxyls (4a-f) and 2,2,6,6-Tetramethyl-2-triazenylpiperidyl-1-oxyl (4h). General Procedure.- The corresponding aromatic or heteroaromatic amine (**1a-f**) and **1h**, 2 mmol) in conc. hydrochloric acid (2 mL) and water (10 mL) was diazotized with sodium nitrite (0.206 g, 3 mmol) in water (3 mL) at -5-0°; diazonium solution was treated with urea (0.031 g) to destroy any unreacted nitrite. The clarified diazonium solution was mixed with a solution of 4-methylamino-2,2,6,6- tetramethylpiperidine-1-oxyl (0.481 g, 2.6 mmol) in water (10 mL) , cooled to -5° for 20 min. During coupling, the pH of the reaction mixture was gradually raised to 10-12 by the addition of solid sodium carbonate. The separated product was collected, dried and recrystallized from diethyl ether/benzene (1:1, v/v) to give the products as yellow crystalline solids in pure form.

2,2,6,6-Tetramethyl-4-[3-(2,4,6-trifluorophenyl)-1-methyl-2-triazenyl]piperidyl-1-oxyl (4a); yield: 85%, mp. 104-106°. ^{1}H NMR: δ 6.6-6.8 (m. 2H arom) , 3.5 (s, 1H, CH) , 3.2 (s, 3H, N-CH_3), 1.8-2.1 (m, 4H, CH_2), 1.3 [s, 6H, C-$(CH_3)_2$]. MS m/z (%): 343 (42), 159 (100), 131 (70), 169 (8), 154 (26), 124 (66). IR (KBr): 1445, 1480, 1340 cm^{-1}.

Anal. Calcd. for $C_{16}H_{22}F_3N_4O$: C, 55.96; H, 6.45; N, 16.31. Found: C, 55.97; H, 6.47; N, 16.06

2,2,6,6-Tetramethyl-4-[3-(2,4,6-trichlorophenyl)-1-methyl-2-triazenyl]piperidyl-1-oxyl (4b); yield: 77%, mp. 124-125°. ^{1}H NMR: δ 7.4 (s, 2H arom), 3.5 (s, 1H, CH), 3.2 (s, 3H, N-CH_3), 1.9-2.3 (m, 4H, CH_2), 1.4 [s, 6H, C$(CH_3)_2$]. MS m/z (%): 393 (56), 207 (100), 181 (88), 169 (19), 154 (81), 124 (95). IR (KBr): 1440, 1490, 1340 cm^{-1}.

Anal. Calcd. for $C_{16}H_{22}Cl_3N_4O$: C, 48.92; H, 5.64; N, 14.26; Cl, 27.08
Found: C, 48.86; H, 5.65; N, 13.98; Cl, 27.05

2,2,6,6-Tetramethyl-4-[3-(2,4,6-tribromophenyl)-1-methyl-2-triazenyl]piperidyl-1-oxyl (4c); yield: 73%, mp. 123-125°. ^{1}H NMR: δ 7.8 (s, 2H arom), 3.5 (s, 1H, CH), 3.2 (s, 3H, N-CH_3), 1.9-2.1 (m, 4H, CH_2), 1.4 [s, 6H, C$(CH_3)_2$]. MS m/z (%): 527 (71), 343 (93), 313 (87), 169 (38 ; 154 (81), 124 (100). IR (KBr): 1442, 1485, 1340 cm^{-1}.

Anal. Calcd. for $C_{16}H_{22}Br_3N_4O$: C, 36.52; H, 4.21; N, 10.64; Br, 45.56
Found: C, 36.41; H, 4.22; N, 10.46; Br, 45.57

2,2,6,6-Tetramethyl-4-[3-(4-trifluoromethylphenyl)-1-methyl-2-triazenyl]piperidyl-1-oxyl (4d); yield: 61%, mp.144-145°. ^{1}H NMR: δ 7.5-7.7 (m, 4H arom), 3.9 (s.1H, CH), 3.2 (s, 3H, N-CH_3), 1.9-2.1 (d, 4H, CH_2), 1.6[s, 6H, C$(CH_3)_2$]. MS m/z (%): 357 (38), 173 (79), 145 (100), 169 (10), 154 (18), 124 (40). IR (KBr): 1445, 1485, 1340 cm^{-1}.

Anal. Calcd. for $C_{17}H_{24}F_3N_4O$: C, 57.12; H, 6.76; N, 15.67. Found: C, 56.92; H, 6.88; N, 15.44

2,2,6,6-Tetramethyl-4-[3-(4-chloro-2-methylphenyl)-1-methyl-2-triazenyl]piperidyl-1-oxyl (4e); yield: 77%, mp. 112-114°. ^{1}H NMR: δ 7.1-7.4 (m, 3H arom),3.9 (s, 1H, CH), 3.2 (s, 3H, N-CH_3), 1.7-2.4 (m, 4H, CH_2), 1.4 (s, 6H, C$(CH_3)_2$). MS m/z (%): 337 (69), 169 (8), 153 (85), 125 (100). IR

(KBr): 1445, 1490, 1340 cm^{-1}.

Anal. Calcd. for $C_{17}H_{27}ClN_4O$: C, 60.25; H, 8.03; N, 16.53; Cl, 10.46
Found: C, 60.13; H, 8.03; N, 16.28; Cl, 10.38

2,2,6,6-Tetramethyl-4-[3-(4-chlorophenyl)-1-methyl-2-triazenyl]piperidyl-1-oxyl (4f); yield: 78%, mp. 156-157°. MS m/z (%): 323 (68), 138 (85), 115 (100), 169 (8), 153 (85), 125 (100). IR (KBr): 1440, 1480, 1340 cm^{-1}.

Anal. Calcd. for $C_{16}H_{24}ClN_4O$: C, 59.38; H, 7.41; N, 17.30; Cl, 10.95
Found: C, 59.31; H, 7.10; N, 17.37; Cl, 11.00

2,2,6,6-Tetramethyl-4-[3-(2-chloro-5-pyridyl)-1-methyl-2-triazenyl]piperidyl-1-oxyl (4h); yield: 83%, mp. 167-168°. ^1H NMR: δ 7.5-8.4 (d, 3H arom), 3.7 (s, 1H, CH), 3.2 (s, 3H, N-CH_3), 1.9-2.0 (m, 4H, CH_2), 1.5 [s.6H, $C(CH_3)_2$]. MS m/z (%): 324 (70), 140 (100), 112 (96), 169 (21), 154 (42), 124 (73). IR (KBr): 1440, 1490, 1340 cm^{-1}.

*Anal.*Calcd.for $C_{15}H_{23}ClN_5O$: C, 55.46; H, 7.13; N, 21.56; Cl, 10.91
Found: C, 55.15; H, 7.24; N, 21.36; Cl, 11.16

Preparation of 2,2,6,6-tetramethyl-4-[3-(4-carboxyphenyl)-1-methyl-2-triazenyl]piperidyl-1-oxyl (4g).- *p*-Aminobenzoic acid **1g** (0.274 g, 2 mmol) in hydrochloric acid (37%, 2 mL) and water (10 mL) was diazotized with sodium nitrite (0.206 g, 3 mmol) in water (3 mL) at -5 - 0°. Stirring was continued for another 30 min and then the reaction mixture was treated with a pinch of urea to destroy unreacted nitrite. The diazonium solution **2g** was added dropwise to a cooled to 0° solution of 4-methylamino-2,2,6,6-tetramethylpiperidine-1-oxyl (**3**) (0.481 g, 2.6 mmol) and triethylamine (3 mL) in water (10 mL). The reaction mixture was stirred for 30 min at 0° and then was adjusted to pH 5 with hydrochloric acid. The reaction mixture was extracted with diethyl ether (4 x 20 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated on a rotating evaporator. The resulting product was recrystallized from ethyl alcohol and water (1:1 v/v). Yield: 57%, mp. 185-187°. ^1H NMR: δ 7.7-8.4 (m, 4H arom), 3.9 (s, 1H, CH), 3.2 (s, 3H, N-CH_3), 2.0-2.2 (m, 4h, CH_2), 1.5[s, 6H, C $(CH_3)_2$]. MS m/z (%): 333 (45), 149 (82), 121 (100), 169 (20), 154 (30), 124 (71). IR (KBr): 1445.1490.1340 cm^{-1}.

Anal. Calcd. for $C_{17}H_{25}N_4O_3$: C, 61.24; H, 7.56; N, 16.80. Found: C, 61.01; H, 7.43; N, 17.03

Preparation of 2,2,6,6-tetramethyl-4-[4-(3,3-dimethyl-1-triazenyl)-benzoylamino]piperidyl-1-oxyl (7).- 3,3-Dimethyl-1-(4-carboxyphenyl)triazene **5** (0.193g, 1 mmol) and N.N-dicyclohexylcarboxamide (0.412g, 2 mmol) were dissolved in tetrahydrofuran (3 mL). The solution was placed in a refrigerator (-4°) for 30 min. 4-Amino-2,2,6,6-tetramethylpiperidine-1-oxyl **6** (0.170g, 1 mmol) dissolved in tetrahydrofuran (3 mL) was added dropwise to the above mentioned solution. The reaction mixture was stirred for 24h at 20° and filtered. The filtrate was concentrated on a rotating evaporator. The resulting red product was washed with ether and recrystallized from ether and benzen (3:1, v/v). Yield: 47%, mp. 182-183°. ^1H NMR: δ 7.5-7.8 (m, 4H arom), 3.9 (s, 1H, CH), 3.3 (s, 3H, N-CH_3), 2.1-2.2 (m, 2H, CH_2), 1.5 [s, 6H, C $(CH_3)_2$]. MS m/z (%): 315 (26), 346 (80), 274 (24), 176 (100), 145 (80), 124 (89). IR (KBr): 1442, 1485, 1340 cm^{-1}.

Anal. Calcd. for $C_{18}H_{28}N_5O_2$: C, 62.40; H, 8.14; N, 20.21. Found: C, 62.31; H, 8.10; N, 20.17

Preparation of 2,2,6,6-tetramethyl-4-[5-(4-carboxamidoimidazole)-1-methyl-2-triazenyl]-piperidyl-1-oxyl (4i).- 5-Aminoimidazole-4-carboxamide (AIC)•HCl **1i** (1g, 6.16 mmol) in 8 mL of a cold 1N hydrochloric acids was diazotized with sodium nitrite (0.47g, 6.8 mmol) in water (12 mL) at 0-5°. The precipitate of 5-diazoimidazole-4-carboxamide **2i** was collected and washed three times with 2 mL portions of water and dried *in vacuo* over P_2O_5. The 5-diazoimidazole-4-carboxamide **2i** (0.46g, 2.7 mmol) was added in small portions to a solution of 4-methylamino-2,2,6,6-tetramethylpiperidine-1-oxyl **3** (0.5g, 2.7 mmol) in 6 mL water for 1.5 hr at 0°. After adding, the reaction mixture was stirred for 3 hrs at 0°. During the reaction pH 10-12 was kept with solid sodium carbonate. The separated product was collected, dried and recrystallized from diethyl ether as a pink cristalline material. Yield: 57%, mp. 170-172° ^{1}H NMR: δ 6.7-7.4 (m, 5H), 3.5 (s, 1H), 3.1 (s, 3H), 1.7-2.0 (m, 4H), 1.2 (s, 6H), 1.3 (s, 6H). MS m/z (%): 323 (75), 131 (62), 112 (24), 169 (33), 154 (62), 124 (95). IR (KBr): 1445, 1480, 1340 cm^{-1}.

Anal. Calcd. for $C_{14}H_{24}N_7O_2$: C, 48.12; H, 7.73; N, 28.07. Found: C, 48.22; H, 7.63; N, 28.13

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