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An Unprecedented Arylcarbene Formation in Thermal Reaction of Non-Conjugated Aromatic Enetetraynes and DNA Strand Cleavage

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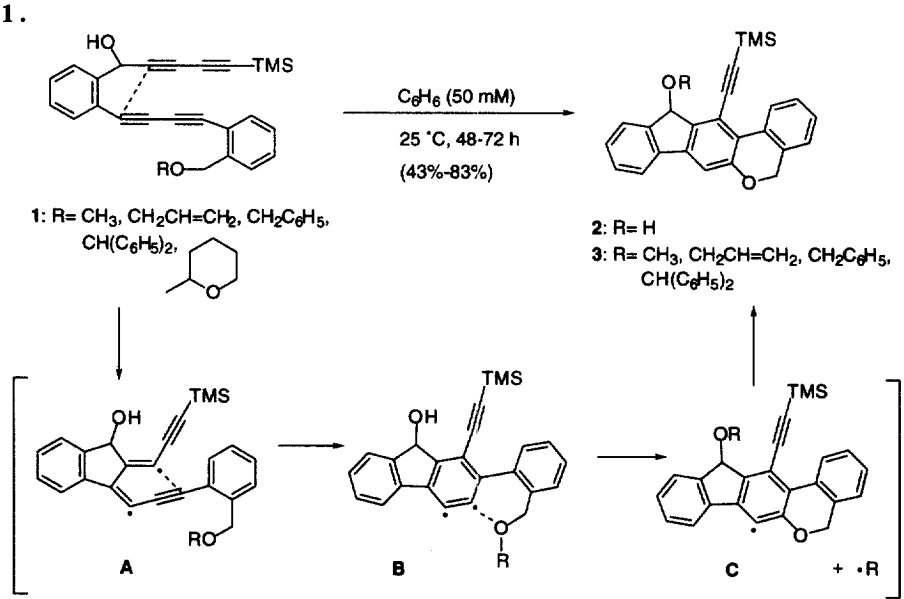
Abstract: The thermal cyclization of non-conjugated aromatic enetetrayne (**4**) led to the final products (**2** and **10**) affording 5*H*-12-hydroxybenzo[*d*]fluoreno[3,2-*b*]pyran radical (**C**) and arylcarbene (**D**) intermediates. DNA strand cleavage was observed.

We recently reported a novel thermal cyclization of non-conjugated aromatic enetetraynes **1** to 5*H*-12-hydroxybenzo[*d*]fluoreno[3,2-*b*]pyran (**2**) via radical intermediates (**A**, **B**, **C**) along with its *O*-alkyl derivatives **3**[1] (Scheme 1). To account for the formation of **3** we postulated a carbene intermediate (:CHR)[2] although, in principle, **3** may also be formed via an ionic intermediate ($^{+}CH_2R$)[3, 4].

Scheme 1.



During our investigation on the thermal reactions of non-conjugated aromatic enetetraynes we discovered

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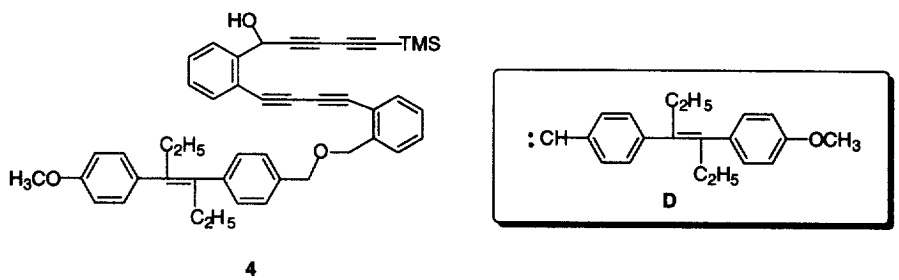
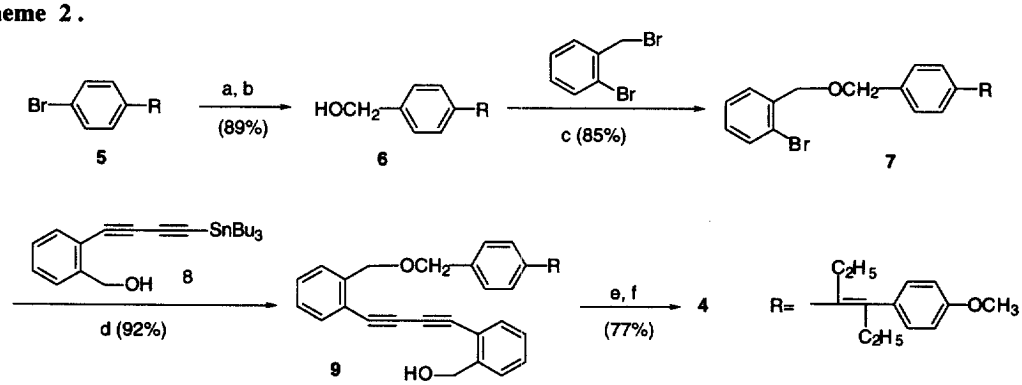
that **4** bearing a diethylstilbestrol moiety (the estrogen receptor agonist) as a delivery vehicle undergoes thermal cyclization in benzene at 25 °C for 72 h to afford 7-arylcyclohepta-1,3,5-triene derivative (**11**) along with **2** and its *O*-alkyl derivative (**10**) (Scheme 3). Furthermore, we obtained evidence that the arylcarbene (**D**) is formed *in situ* with a lifetime long enough to allow trapping by external reagents. Herein we report the thermal reaction and DNA strand cleavage of **4**.

The synthesis of **4** is outlined in Scheme 2; the starting material (**5**), prepared by the method described in the literature[5] was converted into **6** by reaction with ^{t}BuLi followed by formylation and $NaBH_4$-reduction. Compound **7**, prepared from 2-bromobenzyl bromide and **6**, was coupled with **8** under the conditions described in the literature[1] to afford **9**. Oxidation of **9** with 1-hydroxy-1,2-benziodoxol-3(1*H*)-one 1-oxide (IBX), followed by the reaction with 4-trimethylsilyl-1,3-butadiyn-1-yl lithium afforded **4**.[1] The total yield from **5** was 54%. The structure of **4** was determined on the basis of IR and NMR spectral data.

Figure 1.



Scheme 2.



Reagents and Conditions: a) ^{t}BuLi/Et_2O, -78 °C and DMF; b) $NaBH_4$/MeOH, 0 °C; c) NaH/Bu_4NI/DMF, r.t.; d) $PdCl_2(PPh_3)_2$/toluene, 110 °C; e) IBX/DMSO, r.t.; f) Li—≡—≡—TMS/Et_2O, r.t.

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[1] All new compounds in this paper gave satisfactory IR, NMR, Mass spectra and elementary analyses. Selected physical data are as follows: **4**: yellow powder, ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.71 (d, 1H, *J*=7.8 Hz), 7.60-7.56 (m, 3H), 7.45-7.40 (m, 4H), 7.37-7.25 (m, 2H), 7.21 (d, 2H, *J* =7.8 Hz), 7.12 (d, 2H, *J* =8.8 Hz), 6.90 (d, 2H, *J*=8.5 Hz), 5.96 (d, 1H, *J*=5.6 Hz), 4.84 (s, 2H), 4.68 (s, 2H), 3.83 (s, 3H), 2.56-2.52 (m, 1H), 2.17-2.09 (m, 4H), 0.78-0.73 (m, 6H), 0.19 (s, 9H). ^{13}C-NMR (100Mz, $CDCl_3$) δ 157.99, 142.41, 142.28, 141.87, 138.95, 138.82, 135.93, 134.84, 133.59, 133.27, 129.88, 129.70, 129.61, 128.81, 128.67, 127.89, 127.60, 127.45, 127.00, 120.26, 120.20, 113.36, 88.77, 87.13, 81.01, 79.23, 79.09, 77.75, 76.24, 72.90, 71.59, 70.53, 63.16, 55.18, 28.57, 28.49, 13.35, -0.53. IR (KBr) ν 3411, 2361, 2343, 2216, 2107 cm^{-1}. FABMS m/z 684 [(M+Na)$^+$].

Thermolysis of **4** (30 mM) in purified benzene at 25 °C for 72 h afforded **11** in 16% yield along with the expected products (**2** and **10**) in 44% and 13% yield. When **4** (3.0 mM and 0.3 mM) in benzene was stirred under the same conditions, **11** was obtained in increasing yields of 23% and 37% along with **2** in respective yields of 47% and 55% and **10** in reduced yields of 13% and 4%. Reaction of **4** (126 mM) in styrene afforded an addition product (**12**) in a low yield of 5% along with **2** and **10** in 33% and 7 % yields, respectively, with a large amount of a styrene polymer. Reaction of **4** (3.0 mM) in methanol afforded **13** in 60% yield with **2** in 62 % yield without giving **10**. Treatment of **4** (3.0 mM) in acetic acid afforded **14** in 66% yield with **2** in 71% yield without giving **10**. The results are summarized in Table 1.

Scheme 3.

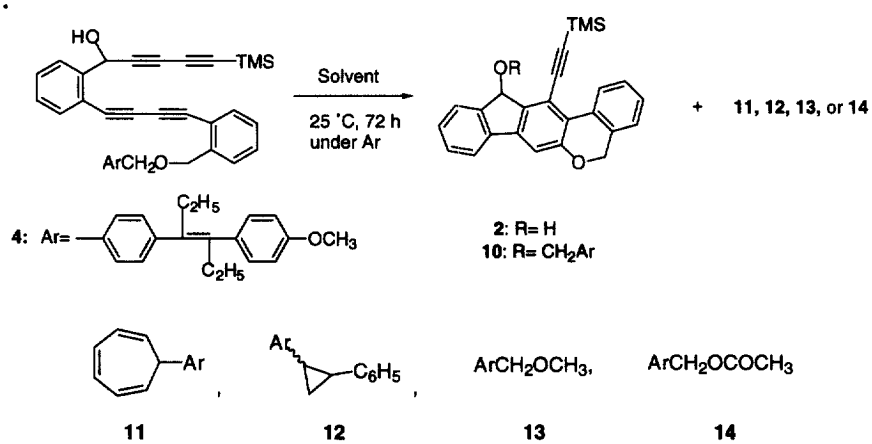


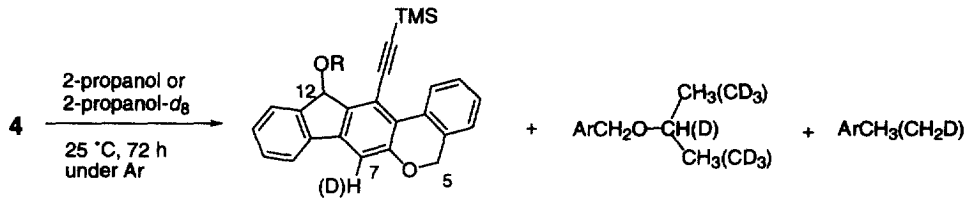
Table 1.Thermal Reaction of **4**

Compd	Conditions[a] Solvent	(Concentration of **4**)	Product (%)[b]		
4	C_6H_6	(30 mM)	**2** (44),	**10** (13),	**11**(16)
	C_6H_6	(3.0 mM)	**2** (47),	**10** (13),	**11** (23)
	C_6H_6	(0.3 mM)	**2** (55),	**10** (4),	**11** (37)
	Styrene	(126 mM)	**2** (33),	**10** (7),	**12** (5)
	CH_3OH	(3.0 mM)	**2** (62),	**10** (n.d.)[c],	**13** (60)
	CH_3COOH	(3.0 mM)	**2** (71),	**10** (n.d.)[c],	**14** (66)

a) Reactions were carried out at 25 °C for 72 h in the dark in the specified solvent with stirring under argon atmosphere. All solvents were purified by the usual procedure before use. b) Yield: Isolated yield. c) n.d.: not detected.

In order to obtain insight into the formation of the carbene, thermal reaction of **4** was carried out in both solvents of 2-propanol and 2-propanol-d_8 (Scheme 4). Thermolysis of **4** (3.0 mM) in 2-propanol afforded *iso*-propyl ether derivative (**15**) and arylmethane derivative (**16**) in 65% and 3.8% yields along with **2** in 72% yield. Thermolysis in 2-propanol-d_8 led to the formation of **15**-d_7 and **16**-d_1 containing a deuterium atom (>90 % by ^{2}H-NMR spectrometry) in 65% and 3.6% yields along with **2**-d_1 in 77% yield containing a deuterium atom exclusively in the 7-position (>95% by ^{2}H-NMR spectrometry). These findings will provide evidence that the arylcarbene (**D**) is formed *in situ*, although the question why **4** easily generates the reactive carbene remains to be solved.

Scheme 4.



2 (72%) **15** (65%) **16** (3.8%)
2-d_1 (77%) **15**-d_7(65%) **16**-d_1(3.6%)

The thermolysis of **4** was shown to induce DNA strand cleavage when incubated with the covalently closed supercoiled Bluescript II KS$^+$ form I DNA at pH 5.0 and 37 °C (Figure 2). Compound **4** clearly cleaved the DNA (form I) to the open circular DNA (form II) in concentrations from 500 μM to 2,000 μM. The DNAs (form I and form II) were completely destroyed at concentration of 2,000 μM without affording a linear DNA (form III).

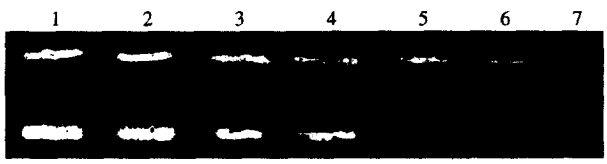


Figure 2. DNA cleavage with **4**; Bluescript II KS$^+$ form I DNA (0.75 μg) was incubated for 24 h at 37 °C with **4** in 50 μl of 10% dimethylsulfoxide-Tris-acetate buffer (pH 5.0, 50 mM) and analyzed by electrophoresis (0.7% agarose gel, ethidium bromide stain): lane 1, DNA alone; lane 2, 10 μM; lane 3, 50 μM; lane 4, 100 μM; lane 5, 500 μM, lane 6, 1,000 μM, lane 7, 2,000 μM.

In conclusion, we have found that non-conjugated aromatic enetetrayne derivative (**4**) undergoes a thermal radical cyclization to yield **2** and **10**, forming the carbene (**D**) along with the 5*H*-12-hydroxybenzo-[*d*]fluoreno[3,2-*b*]pyran radical (**C**). The radical and carbene intermediates thus generated may be utilized to effect DNA strand cleavage similar to the biradical intermediates in the Bergman and Myers-Saito cycloaromatization protocols. Further studies on the mechanism and application of this radical-forming reaction are underway.

Acknowledgments

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