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Ten-Membered Neocarzinostatin Chromophore Analogs Leading to σ,σ-Biradical via a Cumulene Intermediate

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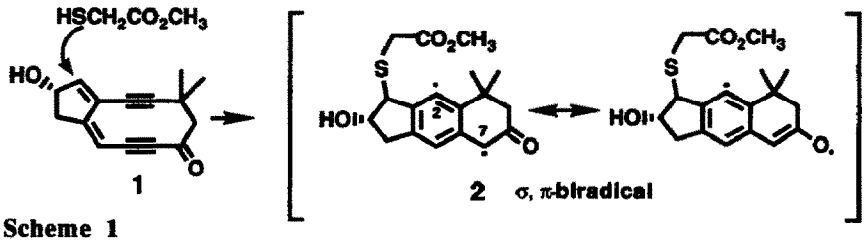
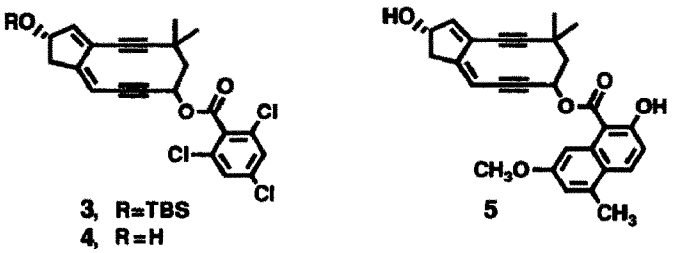
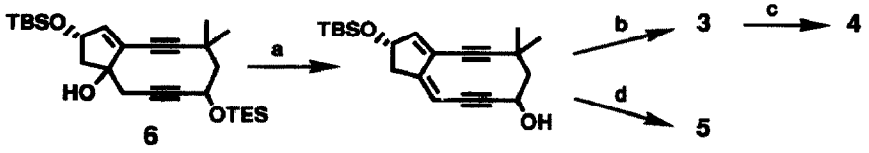
Abstract: New neocarzinostatin chromophore analogs of 10-membered ring are synthesized. Generation of σ,σ-biradical via a cumulene intermediate has been demonstrated. The DNA-cleaving activity of the hybrid **5** is found to be 50-fold more potent than **1**.

Keen efforts have been directed toward new DNA-cleaving molecules related to the enediyne anticancer antibiotics.[1] We recently reported that 10-membered ring analog (**1**) of neocarzinostatin (NCS)[2] chromophore undergoes the thiol-triggered cycloaromatization.[3] DNA-Cleaving activity of **1**, however, was proven to be not so potent as that of NCS.[4,5] Lack of DNA binding groups should be a major cause for the low activity of **1**. Another reason could be attributed to the less reactive intermediate (**2**) with resonance-stabilized C7 π-radical (Scheme 1).[5] In this communication, we describe the synthesis and cycloaromatization of new model compounds **3**, **4**, and **5**, and the intermediary formation of enynecumulene (**9**) and active σ,σ-biradical (**12**), as well as the DNA-cleaving activities.



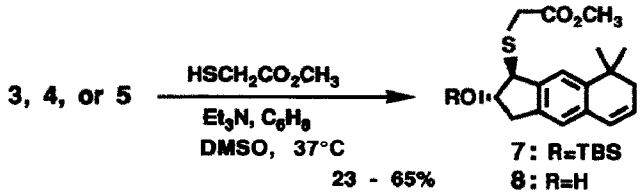
Scheme 1





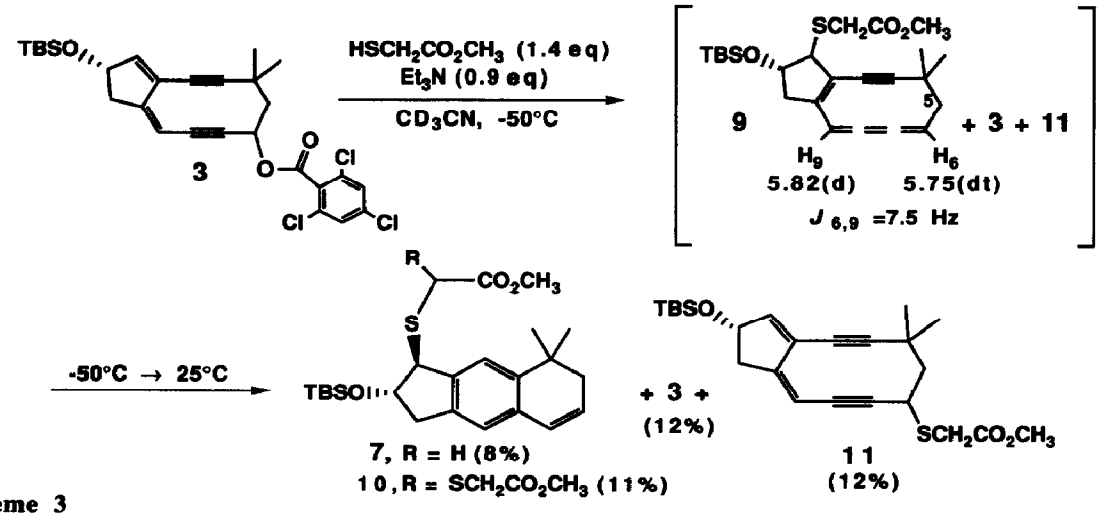
Scheme 2. Reagents and conditions: (a) (i) MsCl (3.5 eq), DMAP (2.2 eq), Et_3N (10 eq), CH_2Cl_2, 0°C; (ii) K_2CO_3 (0.5 eq), MeOH, rt, 10 h. (b) 2,4,6-trichlorobenzoyl chloride (1.5 eq), DMAP (3.0 eq), CH_2Cl_2, rt, 3 h. (c) THF-H_2O-CF_3CO_2H (10:5:1), 0°C, 5.5 h. (d) (i) 2-hydroxy-7-methoxy-5-methylnaphthalene-1-carboxylic acid[13] (2.3 eq), $Me_2HN^+(CH_2)_3N{=}C{=}NEt,Cl^-$ (2.7 eq), CH_2Cl_2, rt, 3 h; (ii) THF-H_2O-CF_3CO_2H (10:5:1), rt, 2.5 h.

The new model compounds **3**-**5**[6] were synthesized in the manner shown in Scheme 2 from the common intermediate **6** prepared previously.[3a] Cycloaromatization of **3** proceeded instantaneously when triggered by methyl thioglycolate (1.5 eq) in the presence of amine (2.0 eq) and 1,4-cyclohexadiene (200 eq) at 37°C in DMSO to give **7**[6] in 65% yield. Similarly, the aromatized thiol adduct **8**[6] was isolated from **4** and **5** in 28% and 23% yield, respectively. Very recently several papers proposed the intermediacy of 10-membered cyclic enynecumulenes.[7] However, none of them presented a spectroscopic evidence for it except for 1,6-dehydro[10]annulene.[7a]

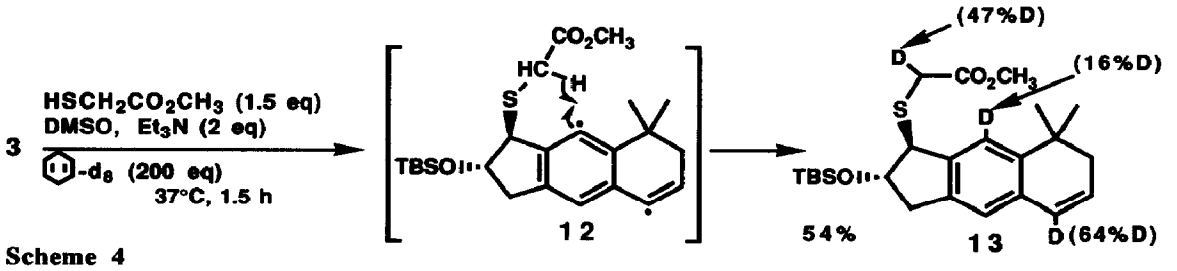


Therefore, the reaction of **3** was monitored by ^1H NMR spectroscopy at the low temperature. Since DMSO is not a proper solvent for the low temperature NMR measurement, acetonitrile was used instead while the thiol attack was retarded considerably.[5a] When **3** was treated with methyl thioglycolate (1.4 eq)-Et_3N (0.9 eq) at -50°C in CD_3CN, the thiol attack occurred slowly and four new signals rose at δ5.82, 5.75, 2.46, and 2.42 ppm, assigned for H9, H6, H5, and H5 of **9**, respectively. The coupling constant, J =7.5 Hz, between the two olefinic protons at δ5.82 and 5.75 agreed well with the [3]-cumulene structure.[8,9] Besides **9** and the unreacted **3**, the thio ether **11** was also detected at this temperature. The ratio of **9**, **3**, and **11** became about 1:4:1 after 3 h. Then the reaction mixture was warmed up to 25°C, yielding the aromatized **7**[6] (8%) and **10**[6] (11%) in addition to **3** (12%) and **11**[6] (12%) (Scheme 3). The 10-membered enynecumulene **9** was thermally stable at -50°C, and its cycloaromatization took place slowly above -40°C. The thio ether **11**[6] appears to be in equilibrium with **9** at -50°C.[5a]

The intermediacy of the σ,σ-biradical intermediate **12** was supported by deuterium incorporation at the relevant positions. When **3** was reacted with methyl thioglycolate (1.5 eq)-Et_3N (2 eq) in DMSO in the presence of 1,4-cyclohexadiene-d_8 (200 mol eq; 96.2% deuterium contents at the allyic positions)[10] at 37°C, deuterium was incorporated at C2 and at C7 of the aromatized **13** (54% yield) to the extent of 16% and 64%, respectively.[5] The low deuterium content at C2 is likely due to the intramolecular hydrogen abstraction from the thioglycolate group, because deuterium (47%) was distributed to its methylene position (Scheme 4).[11]



Scheme 3



Scheme 4

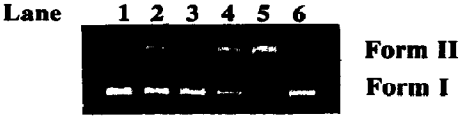


Fig.1. Agarose gel electrophoretic patterns of ethidium bromide stained pBR322 DNA, after treatment with **1** and **5** in a total volume of 20 μl containing 10% dimethylsulfoxide, 20 mM Tris-HCl (pH 7.5) at 37°C for 20 h. Lane 1: intact DNA alone, lane 2: **5** (0.1 mM) and methyl thioglycolate (MTG, 0.1 mM), lane 3: **1** (5 mM) and MTG (5 mM), lane 4: **5** (1 mM) and MTG (1 mM), lane 5: **5** (1 mM), lane 6: MTG (5 mM).

While DNA-cleavage activity of **4** turned out to be similar to **1**, their activities should not be correlated simply to the nature of the biradical generated from **4** and **1** because many other factors such as DNA affinity difference due to the structure and hydrophobicity should also influence the activities. Moreover, we do not know the exact mechanism of their DNA cleavage at this moment. Interestingly, the DNA-cleavage activity of **5** found to be 50-fold more potent than **4** (Fig. 1).[3,12] This higher activity of **5** should arise mainly from its naphthoate group, since the 2-hydroxy-7-methoxy-5-methylnaphthoate moiety[13] is believed to be a DNA intercalator.[14] On the other hand, in the absence of methyl thioglycolate, **1**, **4**, and **5** showed higher DNA-cleaving activity. This would suggest their alkylation mechanism of DNA cleavage and that those compounds

might be degraded by the thiol before they are bound to DNA. The base and sequence selectivity and mechanism of their DNA scission are currently investigated and will be reported in due course.

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