Chemical Evidence for Thiyl Radical Addition to the C6-Position of a Pyrimidine Nucleoside and Its Possible Relevance to DNA Damage Amplification

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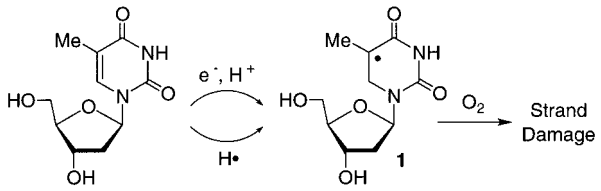
Received May 15, 2000

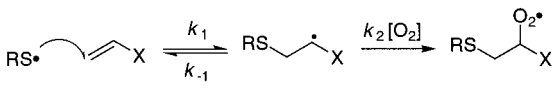
Thiols are potent antioxidants and have been used as radioprotecting agents on account of their proficiency to act as hydrogen atom donors in radical reactions and to participate in electron-transfer processes.[1,2] Perhaps less well appreciated are observations that link physiological levels of thiols to mutagenicity in bacteria.[3,4] Under some conditions, thiols are believed to enhance DNA damage through their involvement in the formation of reactive oxygen species.[2] Alternatively, one could consider the possibility that thiols and/or a species derived from them react directly with DNA. In this regard, there is chemical precedent which suggests that under the appropriate conditions thiyl radicals may abstract hydrogen atoms from carbon−hydrogen bonds or add to π-bonds.[5,6] We wish to report chemical evidence for thiyl radical addition to the C6-position of a pyrimidine double bond, resulting in the formation of an intermediate 5,6-dihydropyrimidin-5-yl radical. Based upon investigations of the interaction of DNA with ionizing radiation, where the similar radical, 5,6-dihydrothymidin-5-yl (**1**, Scheme 1), has been shown to participate in a novel DNA damage pathway that involves the formation of a tandem lesion, this result presents the possibility that thiols may initiate DNA damage by adding to a pyrimidine double bond.[7,8]

The involvement of thiyl radicals in reaction processes is gaining increasing recognition. Hydrogen atom abstraction by thiyl radicals has been appreciated for almost half a century,[5a,9] and has been receiving increased notoriety due to the ability of these species to abstract hydrogen atoms from carbon−hydrogen bonds during enzyme mediated processes.[5d,10] Rapid and reversible addition of thiyl radicals to alkenes is probably the most well studied family of reactions involving thiyl radicals (Scheme 2).[11] Although the equilibrium constants for these additions are typically less than unity, the β-sulfido alkyl radicals can be trapped by O_2. Of particular relevance to the current discussion is the isolation of thiol-pyrimidine nucleobase photoaddition products.[12] These nucleoside adducts are believed to result from predominant addition of the electrophilic thiyl radical to the more electron rich C5-position of the pyrimidine. Thiyl radical addition to the C6-position of the pyrimidines has been inferred, but the expected 5,6-dihydropyrimidine products resulting from addition to this position have not been isolated.[13] The absence of such products can be rationalized by extrapolating from the unstable nature of the respective C6-hydrates which readily undergo dehydration to reconstitute the nucleobases, and/or fragmentation.[1a,14,15]

Scheme 1



Scheme 2



Results and Discussion

Synthesis and Application of a Probe for Thiyl Radical Addition to the C6-Position of a Pyrimidine. Given the anticipated instability of C6-pyrimidine-thiyl radical adducts (**2**), we sought to provide evidence for this pathway by designing a substrate that would leave a fingerprint of the initial thiyl radical addition, regardless of whether a formal addition product could be isolated (which we considered unlikely). Consequently,

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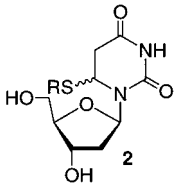
10.1021/jo0007433 CCC: $19.00
Published on Web 10/28/2000



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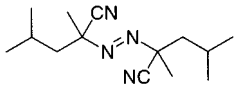
we sought to take advantage of the rapid cyclopropyl carbinyl radical rearrangement (Scheme 3).[16] We anticipated that even if the initial product (**5**) derived from the C6-thiyl adduct (**4**) was unstable, the cleaved cyclopropane ring would serve as an indication of radical addition to the C6-position of the pyrimidine ring. A phenylcyclopropane was chosen as a trigger following consideration of previous measurements made on reactions of alkenes with thiyl radicals. Using these reports as a guide, we assumed that the rate of elimination of a thiyl radical from **4** would be $\leq 10^8$ s^{-1}.[11] A phenyl-substituted cyclopropane was incorporated into the radical substrate in order to maximize the probability that the reverse reaction (k_{-1}, Scheme 3) would not compete with ring opening (k_3). The *trans*-phenylcyclopropyl carbinyl radical rearrangement occurs with a rate constant equal to 3×10^{11} s^{-1} at 25 °C.[16] Although conflicting reports on the effects of substituents on the rate of cyclopropyl carbinyl radical ring opening have appeared, any retardation was expected to be significantly less than 10^3 s^{-1}. Hence, regardless of the effect (if any) of the substituents on the rate of cyclopropyl carbinyl radical ring opening of **4**, we were confident that k_3 would be significantly larger than k_{-1}.[17]

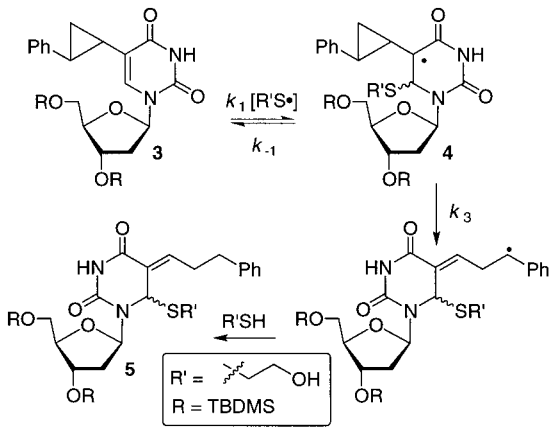
The cyclopropane substrate (**3**) was prepared as a single diastereomer from 5-*trans*-styrenyl-2′-deoxyuridine.[18] Following bis-silylation, cyclopropanation (60%) was carried out using a modified Simmons−Smith procedure.[19] The bis-silylated nucleoside (**3**) was employed as a substrate in order to facilitate isolation of potential products. Extended reaction of **3** with β-mercaptoethanol (BME) in the presence of azo initiator (VAZO-52) in



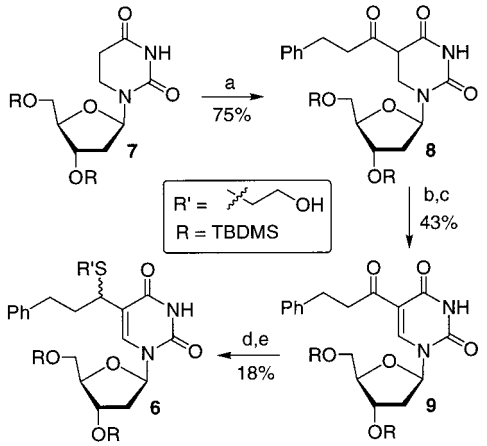
VAZO-52

benzene (50 °C) provided a single isolated product in 46% yield. Spectral analysis of this product indicated that it was a mixture of diastereomers of **6**. The assignment of the isolated product as **6** was confirmed via its independent synthesis starting from the bis-5′,3′-*O*-*tert*-butyldimethylsilyloxy ether of 5,6-dihydro-2′-deoxyuridine (**7**, Scheme 4). The dianion of **7** was acylated with hydrocinnamoyl chloride.[20] The readily epimerizable center in **8** was then quaternized and the resulting phenyl selenide was subjected to oxidative elimination conditions. Following reduction of **9** under Luche conditions, the respective crude mesylate was displaced by BME to afford a diastereomeric mixture of **6**.

Scheme 3

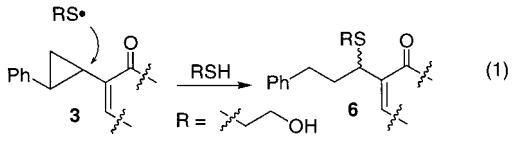


Scheme 4[a]



[a] Reagents: (a) *s*-BuLi, DMPU, hydrocinnamoyl chloride, THF, −78 °C; (b) PhSeCl, pyridine, CH_2Cl_2; (c) H_2O_2, CH_2Cl_2; (d) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH; (e) (i) MsCl, Et_3N, CH_2Cl_2; (ii) BME, K_2CO_3, DMF.

Ring-Opened Nucleoside Product 6 Is Produced via Thiyl Addition to the C6-Position of 3. Ring-opened product **6** is the formal product resulting from direct attack of the thiyl radical derived from β-mercaptoethanol at C1 of the cyclopropane, followed by thiol trapping of the benzyl radical (eq 1). High-level compu-



(1)

tational studies indicate that release of cyclopropane strain energy may facilitate thiyl attack on a carbon−carbon bond.[21] However, low product yields and poor regioselectivity observed in reactions of phenylcyclopropane and *trans*-2-methyl-1-phenylcyclopropane indicate

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(21) QCISD/aug-cc-pVDZ//MP2/aug-cc-pVDZ calculations reveal that homolytic substitution by methylthiyl radical at the carbon atom in cyclopropane is predicted to be exothermic and to have an associated energy barrier of about 90 kJ mol^{-1}. In contrast, reaction between methylthiyl radical and ethane is calculated to be endothermic at the same level of theory and to have an associated energy barrier of about 215 kJ mol^{-1} higher.

that it is unlikely that homolytic substitution of the cyclopropane in **3** yields the observed product **6**.[22] Furthermore, thiyl radicals typically add into the alkene group of vinyl cyclopropanes, as opposed to attacking the cyclopropane component.[6] Another mechanistic alternative to explain the formation of **6**, nucleophilic addition of the thiolate to **3** can be ruled out on the basis that no reaction occurs between **3** and BME in the absence of the azo initiator under these conditions.[23] We propose that **6** is derived from **5**, and that its formation is driven by reconstitution of the uracil base. Although we have no evidence for the mechanism of the transformation of **5** into **6** at this time, we envision 3 possibilities. Rearrangement could occur stepwise by an ionic pathway in which thiolate is initially eliminated to form the resonance stabilized carbocation. This pathway is analogous to that postulated for the decomposition of pyrimidine C6-hydrates and C6-hydroperoxides, and under the relatively elevated temperature (50 °C) would be expected to occur at a reasonable rate.[14,15] Alternatively, **6** can be produced from **3** in a single step by either a S_N2' pathway, or its radical equivalent.

Summary

These studies support the proposal that a thiyl radical can add to the C6-position of a pyrimidine nucleoside. The potential significance of this observation with regard to naturally occurring DNA damage and the design of agents to do so which utilize this pathway remains to be explored.

Experimental Section

All reactions were carried out in oven-dried glassware under an atmosphere of argon or nitrogen unless otherwise noted. THF was freshly distilled from Na^0/benzophenone ketyl. Pyridine, methylene chloride, DMF, benzene, methanol, triethylamine, and methane sulfonyl chloride were distilled from CaH_2. β-Mercaptoethanol was distilled from itself.

Dihydroaryl Ketone 8. To a solution of TBDMS-protected 5,6-dihyrodo-2′-deoxyuridine (**7**, 2.00 g, 4.36 mmol) in THF at −78 °C was added 10.0 mL (3.0 equiv) of a 1.3 M solution of *s*-BuLi in cyclohexane. The reaction was stirred 1 h at −78 °C, and DMPU (2.78 g, 21.8 mmol) was added. After 15 min, hydrocinnamoyl chloride (1.46 g, 8.72 mmol) was added. The reaction was stirred overnight and allowed to warm to ambient temperature. The reaction was quenched with saturated aqueous NH_4Cl, taken up in EtOAc, washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified via silica gel flash chromatography (20−50% EtOAc/hexanes) to afford **8** (1.94 g, 75%) as a tautomeric/diastereomeric mixture:; ^{1}H NMR ($CDCl_3$) δ 7.67 (s, 1 H), 7.32−7.17 (m, 5 H), 4.42−3.36 (m, 1 H), 3.91−3.45 (m, 5 H), 3.10−2.90 (m, 3 H), 2.65−2.50 (m, 1 H), 2.19−2.16 (m, 1 H), 1.98−1.84 (m, 2 H), 0.91 (s, 18 H), 0.09−0.01 (m, 12 H); IR (film) 3208, 3064, 2857, 1713, 1471, 1454, 1362 cm^{-1}.

Aryl Ketone 9. To a solution of phenylselenyl chloride (164 mg, 0.857 mmol) in CH_2Cl_2 (7 mL) at O °C was added pyridine (68 mg, 0.857 mmol). The reaction was stirred at 0 °C for 15 min, and a solution of **8** (460 mg, 0.779 mmol) in CH_2Cl_2 (3 mL) was added. The reaction was allowed to warm to ambient temperature and stirred overnight. The reaction was diluted with EtOAc, washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude reaction mixture was purified via silica gel flash chromatography (10−40% EtOAc/CH_2Cl_2) to afford a diastereomeric mixture of the selenylated ketone (276 mg, 47%) as a yellow foam. The phenyl selenide (50 mg, 0.0670 mmol) was dissolved in CH_2Cl_2 (1 mL) and cooled to 0 °C. A 30% solution of H_2O_2 (7 μL) in H_2O was added, and the reaction was allowed to warm to ambient temperature overnight. The reaction was taken up in Et_2O, washed with saturated $NaHCO_3$, H_2O, brine, dried over Na_2SO_4, and concentrated in vacuo. The crude reaction mixture was purified via silica gel flash chromatography (10−30% EtOAc/Hexanes) to afford **9** (36 mg, 91%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 8.84 (s, 1 H), 7.28−7.38 (m, 5 H), 6.24 (dd, J = 7.8, 7.6 Hz, 1 H), 4.44−4.42 (m, 1 H), 4.07−4.05 (m, 1 H), 3.84−3.81 (m, 2 H), 3.36 (t, J = 6.9 Hz, 2 H), 2.97 (t, J = 7.8 Hz, 2 H), 2.42−2.40 (m, 1 H), 2.09−2.07 (m, 1 H), 0.92−0.86 (m, 18 H), 0.12−0.10 (m, 12 H); IR (film) 3061, 2953, 2856, 1702,1471 cm^{-1}.

***β*-Mercaptoethanol Nucleoside Adduct (6).** To a solution of **9** (36 mg, 0.061 mmol) and $CeCl_3 \cdot 7H_2O$ in MeOH (0.6 mL) was added $NaBH_4$ (2.5 mg, 0.067 mmol). The reaction was stirred for 15 min, taken up in Et_2O, washed with saturated NH_4Cl, H_2O, and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude reaction mixture was purified via silica gel flash chromatography (15−30% EtOAc/Hexanes) to afford the alcohol (21 mg, 58%) as a clear oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (s, 1 H), 7.51 (d, J = 5.1 Hz), 7.27−7.16 (m, 6 H), 6.30−6.25 (m, 1 H), 4.45−4.39 (m, 2 H), 3.95−3.94 (m, 1 H), 3.79−3.72 (m, 2 H), 2.88−2.68 (m, 3 H), 2.29−1.94 (m, 4 H), 0.93−0.91 (m, 18 H), 0.11−0.083 (m, 6 H); IR (Film) 3456, 3181, 3027, 2857, 1693, 1471 cm^{-1}. The alcohol (150 mg, 0.254 mmol) was dissolved in CH_2Cl_2 (2 mL) and cooled to O °C. Triethylamine (41 mg, 0.40 mmol), and then MsCl (44 mg, 0.38 mmol) was added. The reaction was stirred for 2.5 h at 0 °C, quenched with saturated $NaHCO_3$. The crude muxture was taken up in Et_2O, washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was immediately dissolved in DMF (2 mL) with K_2CO_3 (62 mg, 0.44 mmol) and β-mercaptoethanol (34.5 mg, 0.44 mmol) and stirred at room-temperature overnight. The reaction was taken up in taken up in Et_2O, washed with H_2O, brine, dried over Na_2SO_4, and concentrated in vacuo. The crude reaction mixture was purified via silica gel flash chromatography (10−40% EtOAc/Hexanes) to afford **6** (51 mg, 31%) as a clear oil: ^{1}H NMR ($CDCl_3$) δ 9.48−9.46 (m, 1 H), 7.51 (s, 1 H), 7.30−7.18 (m, 5 H), 6.34−6.30 (m, 1 H), 4.43−4.42 (m, 1 H), 4.01−3.97 (m, 2 H), 3.81−3.77 (m, 4 H), 2.81−2.70 (m, 4 H), 2.32−2.30 (m, 1 H), 2.17−2.01 (m, 3 H), 0.91−0.89 (m, 18 H), 0.13−0.097 (m, 12 H); ^{13}C NMR ($CDCl_3$) 163.2, 163.0, 149.7, 141.0, 137.2, 136.9, 128.6, 128.5, 126.3, 116.3, 116.1, 88.2, 88.1, 85.8, 85.6, 72.8, 72.6, 63.5, 63.4, 61.5, 61.4, 41.3, 41.1, 40.8, 40.2, 37.0, 36.9, 35.4, 35.2, 34.1, 26.3, 26.0, 18.7, 18.3, −4.3, −4.5, −4.9, −5.0; IR (film) 3418, 3186, 2857, 1713, 1682, 1496 cm^{-1}; HRMS (FAB) calcd 651.3319 (M + H), found 651.3301.

Phenylcyclopropane Nucleoside Substrate (3). A solution of diethylzinc (8.98 mL, 1.0 M, 5.0 equiv) in hexanes was added to CH_2Cl_2 (9 mL) and the resulting solution cooled to 0 °C. A solution of TFA (1.02 g, 8.98 mmol) in CH_2Cl_2 (4 mL) was added *very slowly* via syringe. After 20 min of stirring, a solution of CH_2I_2 (2.40 mL, 8.98 mmol) in CH_2Cl_2 (4 mL) was added. After an additional 20 min of stirring, a solution of 5′,3′-bis-*O*-*tert*-butyldimethylsilyloxy-5-styrenyldeoxyuridine[18] (1.00 g, 1.80 mmol) in CH_2Cl_2 (4 mL) was added. The reaction was allowed to warm to ambient temperature and, after 20 h, quenched with saturated NH_4Cl and diluted with hexanes. The aqueous layer was extracted with hexanes, and the combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified via silica gel flash chromatography (5−50% EtOAc/Hex) to yield **3** (611 mg, 60%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 8.26 (s, 1 H), 7.39−7.16 (m, 6 H), 6.31 (m, 1 H), 4.44−4.41 (m, 1 H), 3.98−3.96 (m, 1 H), 3.82−3.79 (m, 2 H), 2.29−2.26 (m, 2 H), 2.04−1.92 (2, H), 1.45−1.42, (m, 1 H), 1.28−1.22 (m, 1 H), 0.91 (s, 18 H), 0.089 (s, 12 H); ^{13}C NMR ($CDCl_3$) δ 163.1, 163.0, 150.0, 141.9, 134.8, 128.5, 126.5, 126.3, 126.0, 115.4, 88.2, 87.9, 85.5, 85.3, 72.8, 72.4, 63.4, 63.2, 41.5, 41.3, 26.2, 26.0, 24.5, 24.2, 21.0, 20.8, 18.7, 18.3, 15.0,

(22) Reaction of the BME thiyl radical (2-hydroxyethylthiyl) with phenylcyclopropane and *trans*-2-methyl-1-phenylcyclopropane produces ring opened products in low yields (<1%). Furthermore, the regioselectivity for attack at C2/C3 in and *trans*-2-methyl-1-phenylcyclopropane (2:1) is much lower than that observed for reaction with **3**.

(23) For a postulated example of such a process, see: Vega, E.; Rood, G. A.; de Waard, E. R.; Pandit, U. K. *Tetrahedron* **1991**, *47*, 4361.

14.4, − 4.3, − 4.5, −5.1; IR (film) 3184, 2856, 2359, 1686 cm^{-1}; HRMS (FAB) calcd. 573.3180 (M + H), found 573.3168.

***β*-Mercaptoethanol Nucleoside Adduct (6) via Thermolysis of 3.** Phenylcyclopropane **3** (50 mg, 0.088 mmol), BME (137 mg, 1.75 mmol), and Vazo 52 (2.2 mg, 0.0088 mmol) were dissolved in benzene (1 mL), sparged with N_2 for 20 min, and heated to 50 °C. Additional Vazo 52 (2.2 mg, 0.0088 mmol) and BME (137 mg, 1.75 mmol) were added after 24 h. The reaction was stirred for an additional 24 h, cooled to ambient temperature, taken up in Et_2O, washed with H_2O and brine, dried over Na_2SO_4, and concentrated. The crude product was purified via silica gel flash chromatography (10−50% EtOAc/hexanes) to yield unreacted **3** (42 mg, 84%) and **6** (4.2 mg, 46% based upon unrecovered starting material) as a clear residue: ^{1}H NMR ($CDCl_3$) δ 8.35 (s, 1 H), 7.58 (s, 1 H), 7.30−7.15 (m, 5 H), 6.27 (t, J = 6 Hz, 1 H), 4.40−4.38 (m, 1 H), 3.96−3.91 (m, 2 H), 3.75−3.73 (m, 4 H), 2.81−2.66 (m, 5 H), 2.30−1.93 (m, 3 H), 0.91 (m, 18 H), 0.91 (m, 12 H); ^{13}C NMR ($CDCl_3$) 162.8, 162.7, 149.6, 141.1, 137.3, 137.0, 128.7, 128.6, 126.4, 115.9, 88.3, 88.1, 85.8, 85.6, 72.8, 72.6, 63.6, 63.5, 61.5, 61.3, 41.3, 41.1, 40.9, 40.4, 37.0, 36.8, 35.5, 35.4, 34.1, 26.3, 26.2, 26.0, 18.7, 18.3, −4.4, −4.5, −4.9,−5.0; IR (film) 3410, 3189, 2857, 1713, 1681, 1496 cm^{-1}; HRMS (FAB) calcd 651.3319 (M + H), found 651.3324.

Acknowledgment. Financial support of this work from the National Institutes of Health (GM-54996) and the Melbourne Advanced Research Computing Center is appreciated. M.M.G. is grateful for a fellowship from the Alfred P. Sloan foundation.

Supporting Information Available: Gaussian archive entries for all optimizations in this study and ^{1}H NMR spectra of compounds **3**, **6**, **8**, and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0007433