Products generated by amine-catalyzed strand cleavage at apurinic/apyrimidinic sites in DNA: new insights from a biomimetic nucleoside model system

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ABSTRACT: Abasic sites are common in cellular and synthetic DNA. As a result, it is important to characterize the chemical fate of these lesions. Amine-catalyzed strand cleavage at abasic sites in DNA is an important process in which conversion of small amounts of the ring-opened abasic aldehyde residue to an iminium ion facilitates β-elimination of the 3'-phosphoryl group. This reaction generates a *trans*-α,β-unsaturated iminium ion on the 3'-terminus of the strand break as an obligate intermediate. The canonical product expected from amine-catalyzed cleavage at an AP site is the corresponding *trans*-α,β-unsaturated aldehyde sugar remnant resulting from hydrolysis of this iminium ion. Interestingly, a handful of studies have reported noncanonical 3'-sugar remnants generated by amine-catalyzed strand cleavage, but the formation and properties of these products are not well understood. To address this knowledge gap, a nucleoside system was developed that enabled chemical characterization of the sugar remnants generated by amine-catalyzed β-elimination in the 2-deoxyribose system. The results predict that amine-catalyzed strand cleavage at an AP site under physiological conditions, has the potential to reversibly generate noncanonical cleavage products including *cis*-alkenal, 3-thio-2,3-dideoxyribose, and 2-deoxyribose groups alongside the canonical *trans*-alkenal residue on the 3'-terminus of the strand break. Thus, the model reactions provide evidence that the products generated by amine-catalyzed strand cleavage at abasic sites in cellular DNA may be more complex that commonly thought, with *trans*-α,β-unsaturated iminium ion intermediates residing at the hub of interconverting product mixtures. The results expand the list of possible 3'-sugar remnants arising from amine-catalyzed cleavage of abasic sites in DNA that must be chemically or enzymatically removed for completion of base excision repair and single-strand break repair in cells.

■ INTRODUCTION

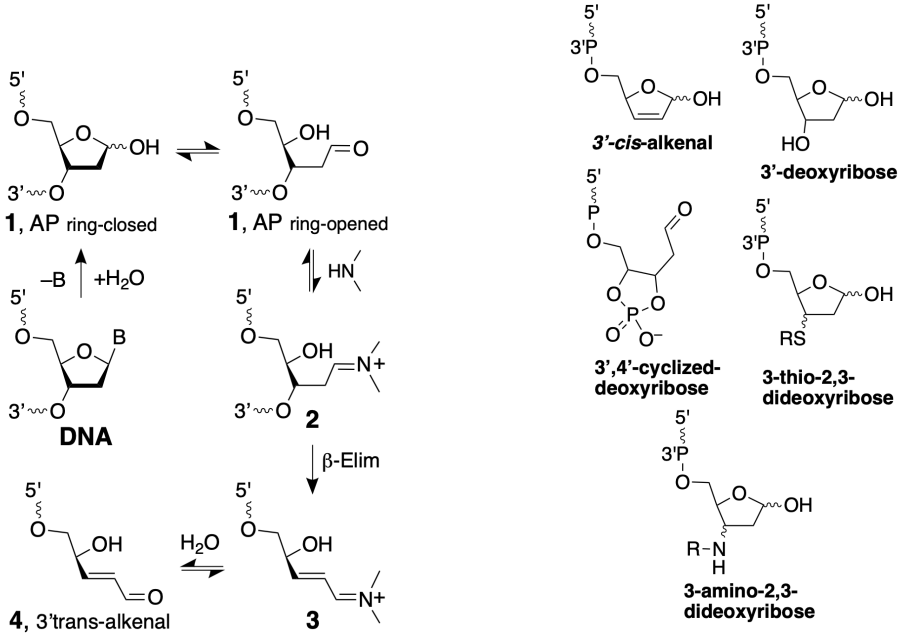
The sequence of nucleobases in DNA provides the genetic information that guides the operation of all cells and organisms.[1] In addition, the information encoded in the structure of DNA can be exploited for next-generation digital information storage[2] and the development of antisense therapeutic agents.[3] Suprisingly, the loss of coding bases is one of the most common unavoidable reactions that occurs in cellular and synthetic DNA.[4-9] Spontaneous,[10-14] chemically-induced,[12, 15] and enzyme-catalyzed[16-19] hydrolysis of the glycosidic bonds holding nucleobases to the sugar-phosphate backbone generates abasic sites (apurinic/apyrimidinic, AP, **1** in Scheme 1). It is important to understand the fate of these common DNA lesions.

AP sites in DNA exist as an equilibrium mixture of the ring-closed hemiacetal and the ring-opened aldehyde (Scheme 1).[20, 21] As with all "reducing sugars", much of the interesting chemistry associated with AP sites flows from small amounts of the ring-opened aldehyde form. One of the features that defines the chemistry and biology of AP sites is their conversion to DNA strand breaks.[22-27] In this process, the acidic character of the α-protons[28, 29] in the ring-opened AP aldehyde enables β-elimination of the 3'-phosphate residue (Scheme 1).[4, 22, 23, 26, 30, 31]

Under physiological conditions, AP sites are converted into DNA strand breaks rather slowly, with half-times in the range of 200-2000 h.[22, 32-34] However, the rate of strand cleavage at AP sites in DNA is significantly increased at higher temperatures, under alkaline conditions, or in the presence of low molecular weight amines like piperidine or spermine.[12, 22, 35-41]

Amine groups on proteins also can catalyze cleavage of the DNA backbone at AP sites.[18, 22-24, 32, 38, 39, 42-55] A series of early, incisive studies established that amine-catalyzed strand cleavage at AP sites in DNA occurs by β-elimination of the phosphoryl group[23, 24, 26, 31, 56] to generate a *trans*-α,β-unsaturated aldehyde sugar remnant **4** on the 3'-terminus of the strand break (3'*trans*-alkenal, Scheme 1).[38, 51, 57-59] Interestingly, a handful of studies have discussed "noncanonical" sugar remnants produced on the 3'-terminus of strand breaks by amine-catalyzed cleavage of AP sites in DNA. These noncanonical cleavage products include 3'-*cis*-alkenal,[60, 61] 3'-deoxyribose,[60, 62-64] 3',4'-cyclized deoxyribose,[22, 26, 39] and adducts derived from conjugate addition of nitrogen nucleophiles,[49, 65, 66] or thiols[67-69] to the 3'-*trans*-α,β-unsaturated aldehyde residue (Figure 1).

The reports of noncanonical cleavage products suggest that the chemistry of amine-catalyzed β-elimination at AP sites may be more complex than commonly expected. With this in mind, we were motivated to develop a nucleoside system that models amine-catalyzed strand cleavage at an AP site in DNA. This model system enabled rigorous chemical characterization of various sugar remnants arising from amine-catalyzed β-elimination in the 2-deoxyribose system. Our results predict that, in the cellular environment, α,β-unsaturated iminium ion intermediates (**3**, Scheme 1) generated by amine-catalyzed strand cleavage at AP sites in DNA reside at the hub of complex interconverting product mixtures that include the canonical 3'-*trans*-alkenal product alongside noncanonical products including 3'-*cis*-alkenal, 3'-deoxyribose, and diastereomeric mixtures of 3-thio-2,3-dideoxyribose residues.



Above, left side: Scheme 1. Amine-catalyzed strand cleavage at an abasic site in DNA (a simple dialkylamine is shown to represent structurally diverse primary and secondary amines that can serve this role).

Above, right side: Figure 1. Possible noncanonical sugar remnants generated on the 3'-terminus of a strand break by amine-catalyzed DNA cleavage. The wavy line represents a strand of DNA, with the noncanonical sugar remnants shown on the 3'-end.

■ EXPERIMENTAL PROCEDURES

Material and Methods. All commercial materials were used as received unless otherwise noted. All chemicals were obtained from Sigma-Aldrich, TCI, or Alfa Aesar. Flash column chromatography was performed using 230-400 mesh silica gel as a stationary phase. Thin layer chromatography was performed on silica gel plates from Sigma Chemical Co. Deuterated solvents were purchased from Cambridge Isotope Laboratories. The ^{1}H NMR spectra were recorded on a 500 MHz or a 600 MHz spectrometer while ^{13}C NMR spectra were obtained on the same instruments at 126 or 151 MHz. The chemical shift values (δ) are reported in ppm versus residual chloroform δ

= 7.26 ppm and 77.16 ppm for ^{1}H and ^{13}C respectively. The ^{1}H spectra are reported as follows δ (multiplicity, coupling constant *J*, number of protons). NMR spectra are shown in the Supporting Information. HPLC analyses were performed on a modular system equipped with a 168-diode array detector and 32 KARAT software. The HPLC system was coupled with an ion-trap mass spectrometer. A Beta-basic C18 column (150 Å, 0.46 cm × 15 cm) was used for analysis.

Synthesis of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-methoxytetrahydrofuran-3-ol (6). A solution of 2'-deoxyribose (1.84 g, 13.72 mmol), methanol (60 mL) and HCl (1 mL, 2 M) was stirred for 1 h at 24 ˚C. The reaction was quenched by the addition of pyridine (5 mL) and the crude mixture containing **5** was concentrated *in vacuo*. The residue was dissolved in pyridine (20 mL) and *t*-butyldiphenylsilyl chloride (3.74 mL, 14.4 mmol) was added, followed by stirring at 24 ˚C under an atmosphere of nitrogen gas for 20 h. The reaction was quenched by addition of water (20 mL) and then extracted with ethyl acetate (3 x 40 mL). The organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:4) gave **6** (3.70 g, 70% yield) as a colorless oil: R_f = 0.3 (1:4 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (diastereomers) 7.70 – 7.62 (m, 4H), 7.47 – 7.33 (m, 6H), 5.11 (d, *J* = 4.6 Hz, 0.6H), 5.05 (dd, *J* = 5.4, 2.1 Hz, 0.4H), 4.51 (td, *J* = 6.7, 4.4 Hz, 0.4H), 4.30 (dd, *J* = 10.3, 6.0 Hz, 0.6H), 4.16 (ddd, *J* = 5.0, 3.5, 1.5 Hz, 0.6H), 3.98 – 3.91 (m, 0.4H), 3.82 (dd, *J* = 10.2, 5.2 Hz, 0.4H), 3.75 (dd, *J* = 11.0, 3.6 Hz, 0.6H), 3.66 (dd, *J* = 10.2, 7.6 Hz, 0.4H), 3.61 (dd, *J* = 11.0, 4.9 Hz, 0.6H), 3.38 (s, 1.8H), 3.27 (s, 1.2H), 2.85 (d, *J* = 10.7 Hz, 1H), 2.24 – 2.15 (m, 1.2H), 2.03 (d, *J* = 1.1 Hz, 0.8H), 1.08

(s, 3.6H), 1.05 (s, 5.4H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ (diastereomers) 135.8(135.7), 133.4, 130.0(129.9), 127.9(127.8), 105.8(105.1), 88.0(85.8), 73.5(73.4), 65.5(64.5), 55.2(54.9), 41.3(41.2), 27.0(26.9), 19.4.; HRMS (ESI, $[M+Na]^+$) *m/z* calcd for $C_{22}H_{30}SiO_4Na$: 409.1811; found 409.1807.

Synthesis of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-methoxytetrahydrofuran-3-yl acetate (7). A solution of **6** (2.0 g, 5.2 mmol) in pyridine (10 mL), and acetic anhydride (2 mL, 21.2 mmol) was stirred for 2 h at 24 ˚C. The reaction was quenched by addition of methanol (3 mL) and the crude mixture was concentrated *in vacuo*. The residue was mixed with water (30 mL) and extracted with ethyl acetate (3 x 30 mL). The combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:9) gave **7** (2.19 g, 99% yield) as a colorless oil: R_f = 0.8 (1:9 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (diastereomers) 7.69 (ddt, *J* = 8.1, 5.0, 1.8 Hz, 4H), 7.48 – 7.36 (m, 6H), 5.35 (ddd, *J* = 6.9, 4.3, 2.8 Hz, 0.4H), 5.27 (ddd, *J* = 8.0, 3.3, 1.8 Hz, 0.6H), 5.12 (dt, *J* = 5.5, 1.7 Hz, 1H), 4.23 – 4.08 (m, 1H), 3.82 (ddd, *J* = 47.1, 11.0, 3.6 Hz, 1.2H), 3.76 – 3.67 (m, 0.8H), 3.40 (s, 1.8H), 3.30 (s, 1.2H), 2.44 (ddd, *J* = 14.6, 8.0, 5.5 Hz, 0.6H), 2.32 (ddd, *J* = 14.1, 6.9, 3.1 Hz, 0.4H), 2.13 – 1.98 (m, 4H), 1.06 (s, 3.6H), 1.05 (s, 5.4H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ (diastereomers) 171.1(170.50), 135.8(135.7), 133.4(133.3), 129.8(129.8), 127.8(127.8), 105.6(105.5), 84.2(84.1), 75.5(74.8), 64.9(64.3), 55.4(55.2), 39.6(39.2), 26.9, 21.3(21.2), 19.4(19.3); HRMS (ESI $[M+Na]^+$) *m/z* calcd for $C_{24}H_{32}SiO_5Na$: 451.1916; found 451.1911.

Synthesis of (2R,3S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-5-hydroxytetrahydrofuran-3-yl acetate (8). A solution of **7** (1.0 g, 2.33 mmol) in acetone (20 mL), water (10 mL), and acetic acid (60 mL) was heated in an oil bath at 65 ˚C with stirring for 8 h. The mixture was then diluted with diethyl ether (100 mL) and saturated sodium bicarbonate was added in 20 mL aliquots until the bubbling ceased. The organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (2:3) afforded **8** (0.8 g, 84% yield) as a colorless oil: R_f = 0.3 (2:3 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (diastereomers) 7.74 – 7.61 (m, 4H), 7.49 – 7.33 (m, 6H), 5.63 (d, *J* = 4.9 Hz, 0.6H), 5.59 (dd, *J* = 5.5, 2.7 Hz, 0.4H), 5.41 (ddd, *J* = 7.0, 4.2, 2.6 Hz, 0.4H), 5.37 (ddd, *J* = 7.1, 2.2, 1.2 Hz, 0.6H), 4.32 (td, *J* = 3.5, 2.1 Hz, 0.4H), 4.16 – 4.08 (m, 0.6H), 3.86 – 3.79 (m, 1.2H), 3.72 (dd, *J* = 11.1, 3.7 Hz, 0.8H), 2.50 – 2.32 (m, 0.6H), 2.24 (ddd, *J* = 14.2, 5.5, 4.1 Hz, 0.4H), 2.17 (s, 1.2H), 2.09 (s, 1.8H), 2.07 (s, 0.4H), 2.05 (d, *J* = 5.6 Hz, 0.6H), 1.09 (s, 3.6H), 1.05 (s, 5.4H); ^{13}C-NMR ($CDCl_3$, 126 MHz): δ (diastereomers) 170.8, 135.9(135.7), 133.2(133.1), 130.2(129.9), 128.0(127.8), 99.4, 84.9(84.8), 75.5(75.4), 65.2(64.3), 41.6(40.2), 27.0(26.9), 21.3(21.1), 19.31; HRMS (ESI $[M+Na]^+$) *m/z* calcd for $C_{23}H_{30}SiO_5Na$: 437.1760; found 437.1757.

Synthesis of (S,E)-4,5-dihydroxypent-2-enal (11, where R = H). We followed the general procedure of Esterbauer,[70] with minor modifications. A solution of 2'-deoxyribose (0.50 g, 3.73 mmol) in water (20 mL) was heated at 120 ˚C for 2 h in an autoclave. The reaction mixture was concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (9:1) afforded the *trans*-

alkenal (0.08 g, 18% yield) as a pale-yellow oil: R_f = 0.3 (ethyl acetate): ^{1}H NMR (CD_3CN, 600 MHz) δ 9.54 (d, *J* = 8.0 Hz, 1H), 6.93 (dd, *J* = 15.7, 4.2 Hz, 1H), 6.26 (ddd, *J* = 15.7, 8.0, 1.8 Hz, 1H), 4.54 – 4.21 (m, 1H), 3.76 (bs, 1H), 3.60 (dd, *J* = 11.2, 4.6 Hz, 1H), 3.49 (dd, *J* = 11.2, 6.4 Hz, 1H), 3.34 (bs, 1H); ^{13}C NMR (CD_3CN, 151 MHz) δ 194.9, 158.1, 132.1, 72.3, 65.7.; HRMS (ESI $[M\text{-}H_2O]^+$) *m/z* calcd for $C_5H_6O_2$: 98.0367; found 98.0361.

Synthesis of (S,E)-5-((tert-butyldiphenylsilyl)oxy)-4-hydroxypent-2-enal (11) from 15. A solution of **15** (0.29 g, 1.86 mmol) in 80% acetic acid in water (15 mL) at 24 ˚C was stirred for 8 h and the reaction mixture concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (9:1) afforded (S,E)-4,5-dihydroxypent-2-enal as a pale-yellow oil (0.17 g, 79% yield) with spectroscopic properties matching as reported previously.[71] A solution of (S,E)-4,5-dihydroxypent-2-enal (75 mg, 0.65 mmol) in dry DMF (5 mL) and TEA (0.13 g, 1.30 mmol) was stirred at 0 ˚C under an atmosphere of argon. After 5 min, *t*-butyldiphenylsilyl chloride (0.21 g, 0.78 mmol) was added dropwise to the reaction mixture and the reaction was stirred for 6 h at 24 ˚C. The reaction was quenched by addition of water (10 mL) and extracted with ethyl acetate (5 x 10 mL). The organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:4) gave **11** (0.15 g, 59% yield) as a pale-yellow oil: R_f = 0.5 (1:2 ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.52 (d, *J* = 7.8 Hz, 1H), 7.73 – 7.60 (m, 5H), 7.41 (tdd, *J* = 8.1, 2.6, 1.5 Hz, 5H), 6.69 (dd, *J* = 15.7, 4.2 Hz, 1H), 6.37 (ddd, *J* = 15.7, 7.9, 1.8 Hz, 1H), 4.51 (dq, *J* = 7.2, 2.7 Hz, 1H), 3.82 (dd, *J* = 10.3, 4.2 Hz, 1H),

3.66 (dd, J = 10.3, 6.6 Hz, 1H), 2.78 (s, 1H), 1.08 (s, 9H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 193.3, 154.8, 135.6, 132.8, 132.4, 130.2, 128.1, 71.6, 66.7, 27.0, 19.4; HRMS (ESI $[M]^+$) *m/z* calcd for $C_{21}H_{26}SiO_3$: 354.1651; found 354.1639.

Synthesis of (3R,4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)tetrahydrofuran-2,3,4-triol (16). The solution of D-ribose (2.0 g, 13.32 mmol) in pyridine (25 mL), and t-butyldiphenylsilyl chloride (4.16 mL, 15.98 mmol) was stirred for 8 h at 24 ˚C. The reaction was diluted by addition of methanol (15 mL) and the crude mixture was concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:1) gave **16** (4.45 g, 86% yield) as a pale-yellow oil: R_f = 0.4 (2:1 ethyl acetate/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ (diastereomers) 7.65 (ddd, J = 8.0, 6.2, 1.5 Hz, 4H), 7.45 – 7.33 (m, 6H), 5.35 (d, J = 3.9 Hz, 0.7H), 5.28 (s, 0.3H), 4.23 – 4.17 (m, 3H), 3.70 (d, J = 3.2 Hz, 2H), 1.06 (s, 3H), 1.03 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ (diastereomers) 135.7(135.6), 133.1(132.9), 130.0(129.9), 128.0(127.9), 101.9(96.9), 84.6(83.9), 76.2(72.2), 71.8(71.5), 64.3(64.2), 27.0(26.9), 19.30; HRMS (ESI $[M+Na]^+$) *m/z* calcd for $C_{21}H_{28}SiO_5Na$: 411.1598; found 411.1588.

Synthesis of (3R,4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-3,4-dihydroxydihydrofuran-2(3H)-one (17). To the solution of **16** (0.60 g, 1.54 mmol) in 10 mL of a 1:1 mixture of acetonitrile and water at 24 ˚C, sodium bicarbonate (0.26 g, 3.09 mmol) and bromine (0.1 mL, 1.85 mmol) were added. The reaction mixture was stirred for 2 h followed by quenching by the addition of saturated solution of sodium thiosulfate (10 mL). The reaction mixture was extracted with ethyl acetate (3 x 15 mL) and the combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted

with ethyl acetate and hexanes (1:2) gave **17** (0.43 g, 72% yield) as a white waxy solid: R_f = 0.4 (1:1 ethyl acetate/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.66 (ddt, *J* = 13.6, 6.5, 1.7 Hz, 4H), 7.49 – 7.36 (m, 6H), 4.90 (d, *J* = 5.7 Hz, 1H), 4.57 (d, *J* = 5.5 Hz, 1H), 4.51 (t, *J* = 2.6 Hz, 1H), 3.89 (dd, *J* = 11.9, 2.9 Hz, 1H), 3.83 (dd, *J* = 11.9, 2.3 Hz, 1H), 1.05 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.2, 135.6, 135.5, 132.3, 131.9, 130.2, 130.1, 128.1, 128.0, 85.6, 70.4, 69.7, 63.3, 26.8, 19.1; HRMS (ESI $[M+Na]^+$) *m/z* calcd for $C_{21}H_{26}SiO_5Na$: 409.1442; found 409.1417.

Synthesis of (3aR,6R,6aR)-6-(((tert-butyldiphenylsilyl)oxy)methyl)-2-thioxodihydrofuro[3,4-d][1,3]dioxol-4(3aH)-one (18). The solution of **17** (0.30 g, 0.78 mmol) in dichloroethane (20 mL), and thiocarbonyldiimidazole (0.18 g, 0.93 mmol) was stirred for 8 h at 24 ˚C. The reaction was quenched by the addition of water (15 mL). The reaction mixture was extracted with dichloromethane (3 x 10 mL) and the combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:4) gave **18** (0.22 g, 66% yield) as a pale-yellow liquid: R_f = 0.3 (1:4 ethyl acetate/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.62 – 7.55 (m, 4H), 7.52 – 7.41 (m, 6H), 5.43 (s, 2H), 4.91 (dd, *J* = 2.0, 1.4 Hz, 1H), 3.98 (dd, *J* = 11.7, 2.0 Hz, 1H), 3.82 (dd, *J* = 11.8, 1.4 Hz, 1H), 1.05 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 187.9, 167.5, 135.6, 135.5, 131.7, 130.9, 130.7, 130.6, 128.4, 128.4, 83.4, 82.5, 78.0, 63.5, 26.9, 19.2; HRMS (ESI $[M+H]^+$) *m/z* calcd for $C_{22}H_{25}SO_5$: 429.1186; found 429.1188.

Synthesis of (S)-5-(((tert-butyldiphenylsilyl)oxy)methyl)furan-2(5*H*)-one (19). The solution of **18** (0.20 g, 0.47 mmol) in trimethyl phosphite (5 mL), was refluxed for 10 h at 110 ˚C. The reaction was quenched by the addition of water (5 mL). The reaction mixture

was extracted with dichloromethane (3 x 5 mL) and the combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:4) gave **19** (0.14 g, 87% yield) as a pale-yellow liquid: R_f = 0.2 (1:4 ethyl acetate/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.64 (dq, *J* = 6.7, 1.4 Hz, 4H), 7.48 – 7.43 (m, 3H), 7.43 – 7.37 (m, 4H), 6.18 (dd, *J* = 5.7, 2.0 Hz, 1H), 5.07 (tt, *J* = 4.6, 1.8 Hz, 1H), 3.90 (qd, *J* = 10.9, 4.7 Hz, 2H), 1.04 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.0, 154.2, 135.7, 135.6, 132.9, 132.6, 130.1, 128.0, 122.8, 83.4, 63.5, 26.8, 19.3. HRMS (ESI $[M+Na]^+$) *m/z* calcd for $C_{21}H_{24}SiO_3Na$: 375.1387; found 375.1381.

Synthesis of (5S)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-2,5-dihydrofuran-2-ol (12). To the solution of **19** (0.30 g, 0.85 mmol) in 10 mL of a dry dichloromethane at -78 ˚C, diisobutylaluminium hydride (0.16 g, 1.11 mmol) was added. The reaction mixture was stirred for 1 h followed by quenching by the addition of saturated solution of sodium potassium tartrate (10 mL). The reaction mixture was extracted with dichloromethane (3 x 10 mL) and the combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:4) gave **12** (0.24 g, 78% yield) as a pale-yellow liquid: R_f = 0.2 (1:4 ethyl acetate/hexanes); ^{1}H NMR (600 MHz, CD_3CN) δ (diastereomers) 7.80 – 7.58 (m, 4H), 7.54 – 7.29 (m, 6H), 6.17 – 6.11 (m, 1H), 5.98 (dd, *J* = 8.4, 4.3 Hz, 0.4H), 5.92 (ddd, *J* = 5.9, 2.2, 1.1 Hz, 0.6H), 5.90 – 5.86 (m, 1H), 4.93 (qdd, *J* = 4.6, 2.3, 1.4 Hz, 0.4H), 4.75 (dddd, *J* = 6.8, 3.2, 2.3, 1.4 Hz, 0.6H), 4.15 (d, *J* = 8.4 Hz, 0.2H), 3.81 (d, *J* = 9.8 Hz, 0.4H), 3.73 (dd, *J* = 10.6, 4.4 Hz, 0.4H), 3.70 (dd, *J* = 4.7, 2.1 Hz, 1H), 1.04 (s, 5.4H), 1.03 (s, 3.6H). ^{13}C NMR (151 MHz, CD_3CN) δ

(diastereomers) 136.5(136.4), 134.2(134.0), 132.9(132.4), 130.9(130.8), 130.5, 128.8(128.7), 103.9(103.5), 86.2, 67.1, 27.2, 19.79. HRMS (ESI $[M+Na]^+$) *m/z* calcd for $C_{21}H_{26}SiO_3Na$: 377.1543; found 377.1534.

Synthesis of tert-butyl(furan-2-ylmethoxy)diphenylsilane (20). The solution of **12** (0.10 mg, 0.03 mmol) in 0.6 ml of $CDCl_3$ was kept in an NMR tube. After 12 h, complete conversion of **12** to **20** was observed. Compound **20** was also formed quantitively when the solution of **12** (0.25 mg, 0.08 mmol) in 1.5 ml solvent mixture of 1:1 acetonitrile and 0.1 N HCl was stirred for 1 h at 24 °C. R_f = 0.4 (1:6 ethyl acetate/hexanes); ^1H NMR (500 MHz, $CDCl_3$) δ 7.81 – 7.73 (m, 4H), 7.50 – 7.42 (m, 6H), 7.42 (dd, *J* = 2.0, 0.9 Hz, 1H), 6.35 (dd, *J* = 3.2, 1.8 Hz, 1H), 6.20 (d, *J* = 3.2 Hz, 1H), 4.72 (s, 2H), 1.13 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.2, 142.2, 135.8, 133.5, 129.8, 127.8, 110.3, 107.4, 59.0, 26.9, 19.4. HRMS (ESI $[M+Na]^+$) *m/z* calcd for $C_{21}H_{24}SiO_2Na$: 359.1438; found 359.1436.

LC-MS analysis supporting the formation of (2R,3R)-1-((tert-butyldiphenylsilyl)oxy)-2-hydroxy-5-(piperidin-1-yl)pentan-3-yl acetate (13) provides evidence for the generation of the iminium ion intermediate 9 in the amine-catalyzed elimination of acetate from 8. To a solution of **8** (0.04 g, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile and phosphate buffer (50 mM, pH 7) at 24 ˚C, piperidine (0.01 g, 0.12 mmol) and $NaBH_3CN$ (25 mg, 0.4 mmol) was added. The reaction mixture was stirred for 3 h followed by addition of water (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and the combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. The reaction mixture was analyzed by LC-MS using a Beta-basic C18 column (150 Å, 0.46 cm × 15 cm) eluted with water

and acetonitrile each containing 0.1% TFA, in a gradient of 10-95% acetonitrile-water over 55 min at a flow rate of 1 mL/min. Sheath and auxiliary gases were set at 45 and 15 psi, respectively. The electrospray voltage was set at 5 kV with the heated ion transfer capillary set at 375 ˚C and 38 V. We observed a major signal in both the UV-chromatogram and the ion current chromatograph eluting at 24.8 min, with an *m/z* of 484 corresponding to the $[M+H]^+$ ion of the **13** (Figure S1).

Evidence for the generation of alkenal products 11 and 12 from the amine-catalyzed elimination of acetate from 8. To a solution of **8** (0.04 g, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile and phosphate buffer (50 mM, pH 7) at 24 ˚C, piperidine (1.0 mg, 0.015 mmol) was added. The reaction mixture was stirred for 5 min followed by quenching with saturated ammonium chloride (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and the combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. The crude ^{1}H-NMR showed resonances for the alkene and C1 protons consistent with the presence of the *trans* and *cis* alkenal products **11** and **12** in a 4:1 ratio. We found that extended incubation of **8** at 37 ˚C for 7 d in this solvent mixture, but in the absence of an amine catalyst, gave an approximately 30% yield of a 4:1 mixture of **11** and **12**.

Evidence for the isomerization of *trans*-alkenal 11 to *cis*-alkenal 12 in the amine-catalyzed elimination of acetate from 8. To a solution of **8** (40 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile and aqueous HCl (12.5 mM), piperidine (42.6 mg, 0.5 mmol) was added. The reaction mixture was stirred for 15 min followed by quenching with saturated ammonium chloride (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and the combined organic extract was washed with brine, dried over

magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:4) afforded **11** (24 mg, 68% yield). When reaction was quenched after 1 h of stirring followed by extraction and drying, a 77:23 mixture of **11** and **12** were obtained in 70% combined yield. Quenching after 3 h afforded 45:55 ratio of **11** and **12** in 45% overall yield. The lower yield of **11**/**12** at 3 h was attributed to the formation of unidentified polar product. Experiments observing the conversion of **12** (35 mg, 0.01 mmol) to **11** (Figure S3) were carried out under the same conditions using the same experimental methods described above.

Amine-catalyzed generation of the thiol adducts (R)-4-((4-(tert-butyl)phenyl)thio)-5-(((tert-butyldiphenylsilyl)oxy)methyl) tetrahydrofuran-2-ol (22) from 8: evidence for conjugate addition of 4-*tert*-butylbenzenethiol to the α,β-unsaturated iminium ion 21. To a solution of **8** (40 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile and phosphate buffer (50 mM, pH 7) at 37 ˚C, DMEDA (0.88 mg, 0.01 mmol) and 4-*tert*-butyl benzenethiol (0.17 g, 1.0 mmol) were added. The reaction mixture was stirred for 0.5 h followed by quenching with saturated ammonium chloride (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and the combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:4) afforded a 9:1 mixture of the diastereomers **22** (44 mg, 85% yield) as a colorless oil: R_f = 0.4 (1:4 ethyl acetate/hexanes); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ (diastereomers) 7.69 – 7.27 (m, 14H), 5.68 – 5.41 (m, 1H), 4.58 – 3.08 (m, 4H), 2.84 – 1.87 (m, 2H), 1.30 (d, *J* = 2.1 Hz, 9H), 1.14 – 0.98 (m, 9H); ^{13}C-NMR ($CDCl_3$, 126 MHz): δ (diastereomers) 135.9(135.8), 132.4, 131.9, 130.1(130.0), 129.9, 128.0(127.9), 126.4(126.3), 99.6(98.5),

85.7(84.6), 64.7(64.5), 45.4, 43.7, 42.9, 31.4, 27.1(27.0), 19.4; HRMS (ESI $[M+Na]^+$) *m/z* calcd for $C_{31}H_{40}SiSO_3Na$: 543.2365; found 543.2362. The ratio of **22a**:**22b** changed as a function of reaction time as described in the Results section. At shorter reaction times, such as 5 min, the diastereomeric ratio of the mixture **22** is 3:2 as indicated by the distinct C1 resonances in the ^{1}H-NMR (see inset on the NMR of **22** in Figure S4).

Thiol exchange reaction: conversion of 22 to (R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-4-(hexylthio)tetrahydrofuran-2-ol (23): evidence that amine-catalyzed addition of thiols to 21 is reversible. To a solution of **22** (50 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile and phosphate buffer (50 mM, pH 7) at 37 ˚C, DMEDA (0.88 mg, 0.01 mmol) and hexanethiol (0.12 mg, 1.0 mmol) was added. The reaction mixture was stirred for 5 min followed by quenching with saturated ammonium chloride (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and the combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:4) afforded an diastereomeric mixture of **23** (38 mg, 81% yield) as a colorless oil: R_f = 0.4 (1:4 ethyl acetate/hexanes); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ (diastereomers) 7.87 – 7.32 (m, 10H), 5.68 – 5.39 (m, 1H), 4.49 – 2.91 (m, 4H), 2.68 – 2.44 (m, 2H), 2.42 – 1.89 (m, 2H), 1.62 – 1.20 (m, 8H), 1.16 – 1.03 (m, 9H), 0.89 (tdd, *J* = 7.2, 2.1, 1.2 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 126 MHz): δ (diastereomers) 136.1(135.9, 135.8, 135.7), 133.6(133.5, 132.8, 132.7), 130.2(130.1, 129.9, 129.7), 128.0(127.9, 127.8, 127.7), 99.4(99.1, 98.6, 97.8), 86.2(84.9, 81.9, 81.3), 64.7(64.6), 44.7(44.2, 43.4), 42.2(42.1, 40.6, 40.5), 33.3(32.8, 32.0), 31.9(31.7, 31.6, 31.5),

30.2(29.9, 29.8, 29.5), 28.7(28.6), 27.1(27.0, 26.9), 22.8(22.7), 19.4(19.3), 14.3(14.2); HRMS (ESI [M+Na]$^+$) *m/z* calcd for $C_{27}H_{40}SiSO_3Na$: 495.2365; found 495.2359.

Synthesis of (4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)tetrahydrofuran-2,4-diol (24b). A solution of **6** (1.0 g, 2.6 mmol) in acetone (20 mL), water (10 mL), and acetic acid (60 mL) was heated in an oil bath at 65 ˚C with stirring for 8 h. The mixture was then diluted with diethyl ether (100 mL) and saturated sodium bicarbonate was added in 20 mL aliquots until the bubbling ceased. The organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (3:7) gave **24b** (0.78 g, 84% yield) as a colorless oil: R_f = 0.3 (3:7 ethyl acetate/hexanes); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ (diastereomers) 7.67 (dddt, *J* = 11.8, 6.7, 3.8, 1.5 Hz, 4H), 7.48 – 7.34 (m, 6H), 5.59 (s, 0.4H), 5.58 (s, 0.6H), 4.39 (dt, *J* = 5.7, 1.2 Hz, 0.4H), 4.30 (ddd, *J* = 5.0, 3.7, 1.2 Hz, 0.6H), 4.03 – 3.79 (m, 0.6H), 3.79 – 3.75 (m, 0.4H), 3.71 (dd, *J* = 11.0, 3.8 Hz, 1.2H), 3.56 (dd, *J* = 10.9, 5.1 Hz, 0.8H), 2.23 – 2.08 (m, 1.2H), 2.07 – 2.01 (m, 0.8H), 1.12 – 1.07 (m, 3.6H), 1.05 (d, *J* = 1.6 Hz, 5.4H); ^{13}C-NMR ($CDCl_3$, 126 MHz): δ (diastereomers) 135.7(135.6), 133.3(133.2), 123.0(129.9), 127.9(127.8), 99.6(99.0), 87.9(86.4), 73.6(73.0), 65.2(64.5), 41.6, 27.0(26.9), 19.3; HRMS (ESI [M +Na]$^+$) *m/z* calcd for $C_{21}H_{28}SiO_4Na$: 395.1655; found 395.1652.

Amine-catalyzed generation of 5-(((tert-butyldiphenylsilyl)oxy)methyl)tetrahydrofuran-2,4-diol (24) from 8: evidence for conjugate addition of water to the α,β-unsaturated iminium ion 21 generated by amine-catalyzed elimination of acetate from 8. A solution of **8** (40 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile and aqueous NaOH (10 mM) and DMEDA (0.88 mg,

0.01 mmol) was stirred for 12 h at 37 ˚C. The reaction was quenched by the addition of saturated ammonium chloride (2 mL) and extracted with ethyl acetate (3 x 5 mL). The combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (3:7) afforded **24** (32 mg, 85% yield) with spectroscopic properties of the major isomer match that of the authentic material synthesized by hydrolysis of **6** (see above). The C1 region of the ^{1}H-NMR provides evidence for a small, inseparable amount of the 2-deoxyxylose isomer **24a** (see inset of NMR **24** in Figure S4).

Evidence that the addition of water to the α,β-unsaturated iminium ion 21 is reversible: amine-catalyzed conversion of 24 to 22. To the solution of **24** (80 mg, 0.22 mmol) in 4 mL of a 1:1 mixture of acetonitrile and phosphate buffer (50 mM, pH 7) at 37 ˚C, DMEDA (1.94 mg, 0.022 mmol) and 4-*tert*-butyl benzenethiol (0.37 g, 2.20 mmol) were added. The reaction mixture was stirred for 24 h followed by quenching by the addition of saturated ammonium chloride (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:4) afforded a diastereomeric mixture of **22** (32 mg, 28% yield) with spectroscopic properties matching the material generated by amine-catalyzed conversion of **8** to **22** (see above).

Amine-catalyzed generation of (5S)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-4-(piperidin-1-yl)tetrahydrofuran-2-ol (25): evidence for conjugate addition of piperidine to the α,β-unsaturated iminium ion generated by amine-catalyzed

elimination of acetate from 8. The solution of **8** (40 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile and phosphate buffer (50 mM, pH 7) at 37 ˚C, and piperidine (0.1 mL, 1.0 mmol) was stirred for 12 h at 37 ˚C. The reaction was quenched by the addition of saturated ammonium chloride (2 mL) and extracted with ethyl acetate (3 x 5 mL). The combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (4:1) afforded **25** (22 mg, 53% yield) as a pale-yellow liquid: R_f = 0.1 (ethyl acetate). ^{1}H NMR (600 MHz, CD_3CN) δ (diastereomers) 7.70 (dddt, J = 7.8, 6.6, 5.4, 2.7 Hz, 4H), 7.50 – 7.37 (m, 6H), 5.36 (dd, J = 5.3, 1.7 Hz, 0.6H), 5.30 (dd, J = 4.4, 2.8 Hz, 0.4H), 4.20 (q, J = 4.5 Hz, 0.4H), 4.00 (q, J = 5.0 Hz, 0.6H), 3.77 – 3.60 (m, 2H), 3.06 (td, J = 7.7, 5.1 Hz, 0.6H), 2.91 – 2.85 (m, 0.4H), 2.42 (d, J = 10.0 Hz, 1H), 2.28 (q, J = 7.8, 5.5 Hz, 2H), 2.02 – 1.95 (m, 2H), 1.87 (ddd, J = 13.1, 7.9, 1.7 Hz, 1H), 1.56 – 1.33 (m, 6H), 1.05 (s, 5.4H), 1.04 (s, 3.6H). ^{13}C NMR (151 MHz, CD_3CN) δ (diastereomers) 136.6(136.5), 134.4(134.3, 134.2), 130.9(130.8), 128.8(128.7), 99.4(99.2), 82.5(80.4), 68.3, 66.8(66.5), 53.1(52.6), 37.6, 35.4, 27.3(27.2, 27.0), 25.4(25.3), 19.8. HRMS (ESI $[M+H]^+$) *m/z* calcd for $C_{26}H_{38}SiNO_3$: 440.2615; found 440.2616.

Evidence that the formation of 25 is reversible: amine-catalyzed conversion of the piperidine adduct 25 to the thiol adducts 22. To the solution of **25** (70 mg, 0.16 mmol) in 4 mL of a 1:1 mixture of acetonitrile and phosphate buffer (50 mM, pH 7) at 37 ˚C, 4-*tert*-butyl benzenethiol (0.26 g, 1.59 mmol) and DMEDA (1.40 mg, 0.016 mmol) were added. The reaction mixture was stirred for 30 min followed by quenching by the addition of saturated ammonium chloride (2 mL). The mixture was extracted with ethyl

acetate (3 x 5 mL) and combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:4) afforded **22** (56 mg, 68% yield) with spectroscopic properties matching the same material synthesized by thiolation of **8** (see above).

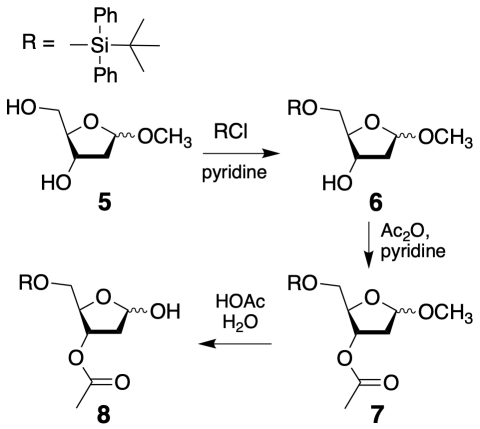
Conjugate addition of 4-*tert*-butylbenzenethiol to the *cis*- and *trans*-alkenal (11 and 12) for the synthesis of 22. To a solution of **11** or **12** (35 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of acetonitrile and phosphate buffer (50 mM, pH 7) at 37 ˚C, 4-*tert*-butyl benzenethiol (0.17 g, 1.0 mmol) were added. The reaction mixture was stirred for 1.5 h followed by quenching with saturated ammonium chloride (2 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and the combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. Column chromatography of the residue on silica gel eluted with ethyl acetate and hexanes (1:4) afforded a 55:45 mixture of **22** (42 mg, 81% yield) with spectroscopic properties matching the same material synthesized using **8** (see above).

Comparison of the rate of the reaction of 11 and 12 with the water soluble thiol 2-mercaptoethanol via HPLC analysis. To a solution of **11** or **12** (71 µg, 0.2 µmol) in 5 mL of a 1:1 mixture of acetonitrile and phosphate buffer (50 mM, pH 7) at 24 ˚C, 2-mercaptoethanol (3.9 mg, 50 µmol) was added. The reaction mixture was stirred at 24 °C. Aliquots of the reaction mixture at different time interval was taken for HPLC analysis using C8 column (250 mm, 5 micron, 4.6 mm) and eluted with 0.1% TFA containing acetonitrile-water (40% to 95% acetonitrile over 20 min followed by 95% acetonitrile for 5 min).

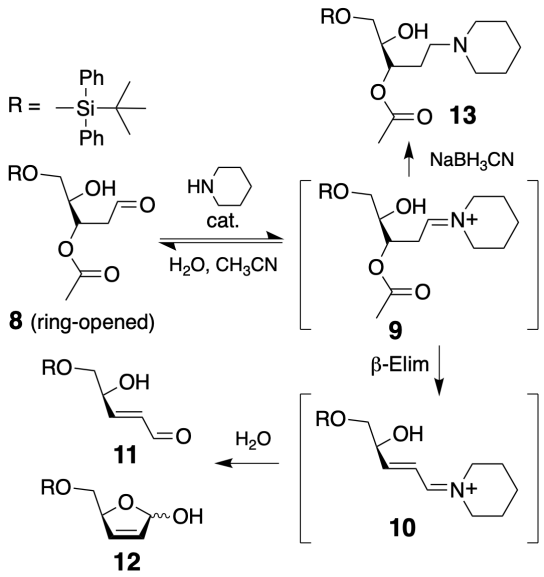
■ RESULTS AND DISCUSSION

Design and synthesis of a nucleoside analog that mimics amine-catalyzed strand cleavage at an AP site in DNA. Our approach involved preparation of a 2-deoxyribose derivative bearing a leaving group at the 3-position that would be stable against spontaneous elimination in aqueous buffer, while readily undergoing amine-catalyzed β-elimination that mimics strand cleavage at an AP site in DNA. We anticipated that an acetyl group might provide the desired balance of stability and reactivity. Our design incorporated a silyl protecting group on the 5-hydroxyl to lock the sugar into the 5-membered, furanose form found in DNA and to facilitate chromatography on silica gel, aqueous-organic extractions, and monitoring of reactions using thin-layer chromatography.

We converted 2-deoxy-D-ribose to the corresponding methyl acetal **5**,[72] followed by reaction with *t*-butyldiphenylsilyl chloride in pyridine to obtain the silylated derivative **6** as a 1.5:1 mixture of the β and α anomers (Scheme 2).[73, 74] Treatment of **6** with acetic anhydride in pyridine gave the acetylated product **7**, again as a mixture of anomers.[73] Finally, removal of the methyl acetal protecting group in acid provided the desired AP model compound **8** in good yield as a mixture of β and α anomers.



Scheme 2. Synthesis of the DNA abasic site model compound **8**.



Scheme 3. Amine catalyzed generation of alkenal products by β-elimination of acetate from the AP model compound **8**.

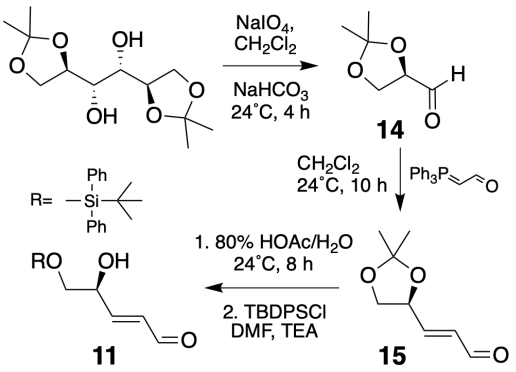
Authentic spectroscopic standards of the anticipated *cis*- and *trans*-alkenal β-elimination products (11 and 12). Amine-catalyzed β-elimination of acetate from **8** has the potential to generate either the *cis*- or *trans*-alkenal products (**11** or **12**, Scheme 3). The vinylic resonances in the proton NMR are diagnostic for these isomers.[51, 70, 75] To facilitate conclusive spectroscopic identification of these products in our model reactions, we prepared authentic samples of **11** and **12**.

We initially undertook synthesis of the *trans*-alkenal **11** by the method of Esterbauer involving thermolysis of 2-deoxyribose.[70] This approach successfully gave the unprotected analog of **11** (R = H), but the yield was low. Therefore, we employed an extension of our recently reported[71] synthesis involving periodate oxidation of 1,2:5,6-di-*O*-isopropylidene-D-mannitol to give the aldehyde **14**, followed by a Wittig condensation

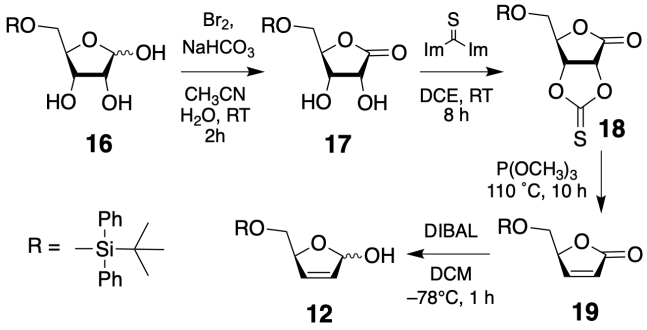
with (formylmethylene)triphenylphosphorane (Scheme 4). Removal of the acetonide protecting group from **15** using 80% acetic acid in water, followed by reaction with *t*-butyldiphenylsilyl chloride in dry DMF containing triethylamine gave **11** in good yield (Scheme 4).

An authentic sample of the *cis*-alkenal **12** was prepared by reaction of D-ribose with *t*-butyldiphenylsilyl chloride in pyridine to give **16**, followed by oxidation to the lactone **17** and conversion to the unsaturated lactone **19** via the thiocarbonate derivative **18** (Scheme 5). Reduction of the unsaturated lactone using diisobutylaluminum hydride (DIBAL) gave the *cis*-alkenal **12**.

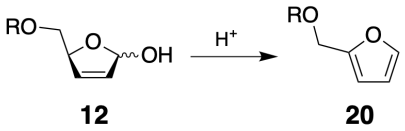
Unexpectedly, we observed a slow conversion of **12** to the furan derivative **20** in chloroform (Scheme 6). Further investigation showed that this reaction was favored under acidic conditions. For example, **12** was transformed to **20** quantitatively within 1 h at 24 ˚C, in a solvent mixture composed of 1:1 acetonitrile and aqueous HCl (0.1 N). In a solvent mixture composed of 1:1 acetonitrile and pH 5 sodium acetate buffer (50 mM), **12** gave **20** in 15-20% yield after 1 h at 24 ˚C. This reaction may be mechanistically related to the conversion of 4-hydroxynonenal to 2-pentylfuran reported by Sayre et al.[76] The potential relevance of this reaction to nucleic acid chemistry is discussed in the Conclusions.



Scheme 4. Synthesis of an authentic sample of the *trans*-alkenal **11**.



Scheme 5. Synthesis of an authentic sample of the *cis*-alkenal **12** (Im = imidazol-1-yl).



Scheme 6. Conversion of the *cis*-alkenal **12** to the furan derivative **23** (R = TBDPS group).

Validation of the model system: amine-catalyzed β-elimination of acetate from 8 generates mixtures of the *cis*- and *trans*-alkenal products (11 and 12) via iminium ion intermediates. In the absence of an amine catalyst, the AP model compound **8** (25 mM) was stable for 24 h at 24 ˚C in a solvent mixture composed of 1:1 acetonitrile/buffer (50 mM sodium phosphate, pH 7). On the other hand, **8** was completely consumed within 2 h when catalytic amounts (0.1-10 mol%) of an amine catalyst such as piperidine,

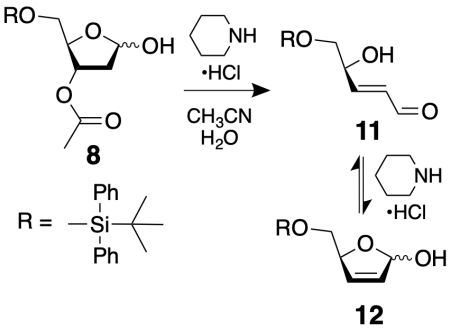
N,*N*'-dimethylethylenediamine (DMEDA), or spermine was present in the reaction mixture. Unless noted otherwise, all of the reactions described below were carried out in our standard solvent composed of 1:1 acetonitrile/buffer (50 mM sodium phosphate, pH 7) at 24 ˚C, with the model compound at a concentration of 25 mM. Likewise, 10 mol% of the amine catalyst DMEDA was used, unless otherwise noted.

Literature precedents indicate that the amine-catalyzed β-elimination of acetate from **8** likely proceeds via iminium ion intermediates (**9** and **10**, Scheme 3).[46, 54, 55, 77-83] We and others have used hydride reagents to trap iminium ion intermediates (e.g. **9**) generated in the reactions of low molecular weight amines with AP sites in DNA.[46, 77-79, 82, 84-88] To probe the involvement of iminium ion intermediates in the amine-catalyzed decomposition of **8**, we incubated the AP model compound with piperidine (30 mM) in the presence of the water-compatible hydride reagent $NaBH_3CN$ (4 equiv).[89] LC-MS analysis of the resulting reaction mixture revealed that the major peak in the UV and ion current chromatograms displayed an *m*/*z* of 484, consistent with the $[M+H]^+$ ion of the reduced iminium ion **13** (Scheme 3, Figure S1). We did not observe the reduced form of **10**, consistent with earlier results in the context of duplex DNA.[46]

We next characterized the initial products generated by the amine-catalyzed elimination of acetate from **8**. Incubation of **8** with piperidine (10 mol%), followed by extraction with ethyl acetate after 5 min, gave a product mixture displaying NMR signals consistent with a 4:1 mixture of the *trans*-alkenal **11** and the *cis*-alkenal **12** (Scheme 3, Figure S2). The ^{1}H-NMR resonances of these products matched those of the corresponding synthetic standards described above. When the amine-catalyzed decomposition of **8** was allowed to proceed until all starting material was consumed

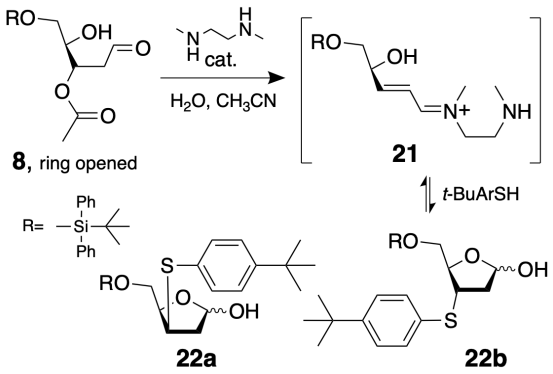
(approximately 1 h) in the absence of any added nucleophile to intercept the α,β-unsaturated iminium ion intermediate, we observed small amounts of **11** and **12** embedded in a complex mixture. This is consistent with literature precedents indicating that complex mixtures can arise by oligomerization of α,β-unsaturated iminium ions.[90-92]

Under a different set of conditions, we were able to observe amine-catalyzed interconversion of the *cis* and *trans* isomers (**11** and **12**, Scheme 7). When **8** (25 mM) was treated with excess piperidine (125 mM) at 24 ˚C, in a solvent mixture composed of 1:1 acetonitrile and aqueous HCl (12.5 mM), the reaction gave a good yield (68%) of the *trans*-alkenal **11** within 15 min (Figure S3). At longer reaction times, the products evolved into a mixture of the *trans* and *cis* isomers (**11** and **12**). For example, after 1 h, a 77:23 mixture of **11** and **12** was isolated by column chromatography in 70% combined yield. After 3 h, a mixture containing a 45:55 ratio of **11** and **12** was obtained (Figure S3). A control reaction showed that the amine catalyst was required to induce the conversion of **8** into **11** and **12** under these conditions. A separate experiment showed that the *cis*-alkenal **12** was converted to a mixture of the *cis* and *trans*-alkenals (**11** and **12**) over the course of 12 h under these reaction conditions.



Scheme 7. Equilibration of the *cis*- and *trans*-alkenal isomers **11** and **12**.

These results provided evidence that the amine-catalyzed elimination of acetate from **8** generates the *trans*-alkenal **11** as the major initial product, via iminium ion intermediates (Scheme 3). The product **11** is analogous to the canonical 3'-*trans*-alkenal sugar remnant generated by amine-catalyzed strand cleavage at an AP site in DNA (**4**, Scheme 1).[38, 51, 57] Importantly, our results provide evidence that the *cis*- and *trans*-alkenals can undergo equilibrium interconversion in the presence of an amine catalyst (Scheme 7).



Scheme 8. Conjugate addition a thiol to the α,β-unsaturated iminium ion **21** generates a diastereomeric mixture of thiol adducts **22**.

Reversible conjugate addition of thiols to the α,β-unsaturated iminium ion generated by amine-catalyzed elimination of acetate from 8. Cells contain millimolar concentrations thiols, such as the tripeptide glutathione.[93-95] Importantly, thiols readily undergo conjugate addition to α,β-unsaturated aldehydes in neutral aqueous solution.[96-99] The addition of thiols to the *trans*-alkenal **4** derived from strand cleavage at an AP site in DNA has been reported,[67-69] but the structures and properties of the resulting products have not been well characterized. In this section, we describe the formation and

properties of products resulting from the amine-catalyzed elimination of acetate from **8** in the presence of thiols.

We found that **8** was stable when stirred with *t*-butylbenzenethiol (10 equiv) in the *absence* of an amine catalyst for 24 h. On the other hand, when **8** was mixed with *t*-butylbenzenethiol (10 equiv) in the presence of the amine catalyst DMEDA (10 mol%), the starting material was completely consumed in less than 5 min. This reaction generated an 85% yield of the diastereomeric 3-phenylthio-2,3-dideoxyribose products **22**, envisioned to arise from conjugate addition of the thiol to the α,β-unsaturated iminium ion **21** (Scheme 8). Further examination of this reaction revealed that the diastereomeric ratio of isomers in **22** changes over time, suggestive of an initial kinetic product mixture evolving to a thermodynamic mixture (Figure S4). Precedents from closely-related systems indicate that the thermodynamic product of these reactions is likely to be **22b**.[100, 101]

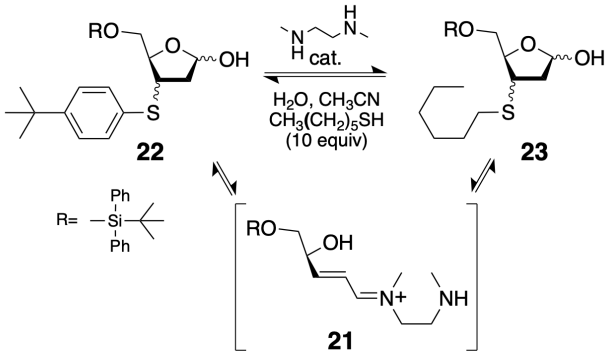
Analogous mixtures of thiol addition products were obtained from reactions with a variety of structurally diverse thiols (Supporting Information, pp S19-21). Various amine catalysts including DMEDA, piperidine, and spermine were able to similarly catalyze the reaction of **8** with *t*-butylbenzenethiol to generate the diastereomeric mixtures **22**. The reaction induced by lysine was somewhat slower (about 2 h for complete disappearance of the starting material), while reactions triggered by valine, tryptophan, and threonine were sluggish, with most starting material remaining intact after 24 h.

The isolated thiol adducts **22** were stable in the *absence* of an amine catalyst, in either organic solvents or neutral aqueous buffer (48 h, 24 ˚C). On the other hand, we

found that formation of the thiol adducts **22** was reversible in the presence of an amine catalyst. The reversible nature of the thiol addition reaction was highlighted by an experiment in which the 3-phenylthio-2,3-dideoxyribose adducts **22** were incubated with DMEDA (10 mol%) in the presence of an excess of hexanethiol (10 equiv). This generated an 81% yield of the diastereomeric thiol exchange products **23** within 5 min (Scheme 9). No thiol-exchange was observed when the diastereomeric mixture **22** was incubated with excess hexanethiol in the absence of the DMEDA catalyst for 1 h.

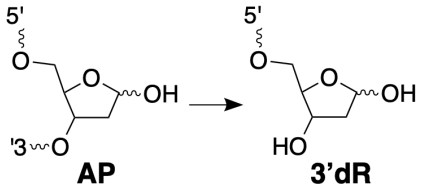
The spectroscopic data indicated that the thiol-adducts **22** exist predominantly in the ring-closed form. Presumably, the thiol-exchange reaction proceeds via reaction of the amine catalyst with small amounts of the ring-opened aldehyde to generate an iminium ion that enables a retro-thia-Michael elimination reaction to give **21** (Scheme 9).

Overall, the results provided evidence for reversible addition of thiols to the α,β-unsaturated iminium ion **21**. A role for amine catalysis in the reversible conjugate addition of acrolein to protein thiol groups has previously been proposed.[102] In a separate vein, the generation of thiol-addition products such as **22** from the easily accessible precursor **8** could serve as part of a concise synthetic route to 3-arylthio- and 3-alkylthio-2,3-deoxyribose nucleosides.[103]



Scheme 9. A thiol exchange reaction shows that amine-catalyzed formation of thiol-addition products is reversible.

Reversible conjugate addition of water to the α,β-unsaturated iminium ion generated by amine-catalyzed elimination of acetate from 8. There are a handful of reports indicating that cleavage of AP sites in DNA can give rise to a 2-deoxyribose residue on the 3'-terminus of the strand break (3'dR in Scheme 10).[35, 60, 62, 63] This cleavage product could also be referred to as a 3'-terminal AP site. Motivated by these precedents, we explored whether the 3'-dR residue can arise by conjugate addition of water to the α,β-unsaturated iminium ion generated by amine-catalyzed elimination of acetate from **8**.



Scheme 10. Conversion of an AP site in DNA to a strand break with a 3'-deoxyribose (3'dR) terminus.

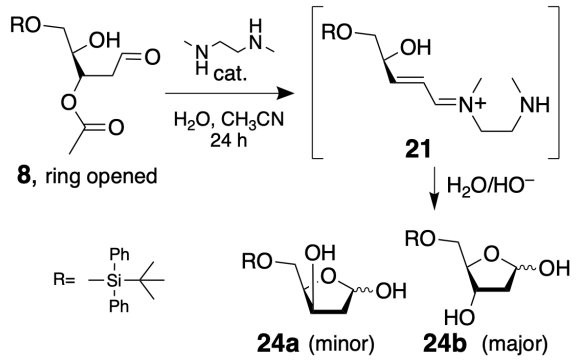
Treatment of **8** with DMEDA (10 mol%) at 37 ˚C for 12 h in a solvent composed of 1:1 acetonitrile and aqueous NaOH (10 mM), produced an 85% yield of the 2-deoxyribose derivative **24b** containing small, inseparable amounts of the 2-deoxyxylose isomer **24a** (Scheme 11). The biogenic amine, spermine (10 mol%), similarly catalyzed formation of **24** from **8** under these conditions. The structure of the major isomer **24b** generated in this reaction was confirmed by comparison of its NMR spectra to that of an authentic sample prepared independently by treatment of the methyl acetal **6** with mild aqueous acid (Scheme 12).

Two control experiments suggested that **24** arose via conjugate addition of water to **21** rather than by hydrolysis of the acetyl group in **8**. First, **24** was not generated from **8** in the absence of the amine catalyst, consistent with the idea that the iminium ion **21** is an obligate intermediate in the generation of **24**. Second, incubation of the methyl acetal **8** with the amine catalyst DMEDA (10 mol%) in this solvent mixture did not produce **24**, simultaneously providing evidence that the reaction conditions do not induce hydrolysis of the acetyl group and that the C1-aldehyde is required for generation of **24**.

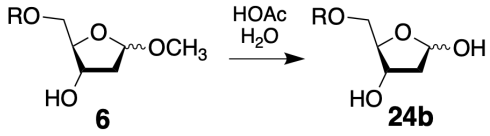
We next examined whether the formation of **24** is reversible. For these experiments, we used *t*-butylbenzenethiol to capture the possible elimination products **11** or **21**. We first demonstrated that, in the absence of an amine catalyst, **24** was stable to extended incubation with *t*-butylbenzene thiol (10 equiv, 37 ˚C, 48 h). On the other hand, incubation of **24** with DMEDA (10 mol%) in the presence of *t*-butylbenzene thiol (10 equiv), generated the corresponding thiol adducts **22** in 28% yield after 24 h at 37 ˚C (Scheme 13). The amine-catalyzed generation of **22** from **24** presumably results from small amounts of the ring-opened aldehyde that can react with the amine catalyst to generate **21**. The yield of **22** generated in this process was lower than that obtained via the amine-catalyzed thiol-exchange reaction described above (Scheme 9), but nonetheless provides evidence that the formation of **24** is reversible in the presence of an amine-catalyst.

In a broader chemical context, our observation that water can add to the iminium ion **21** is consistent with early literature describing piperidine- and sarcosine-catalyzed conjugate addition of water to crotonaldehyde.[104] In addition, this chemistry may explain the generation of 3'dR from the cleavage of an AP-containing oligodeoxynucleotide in

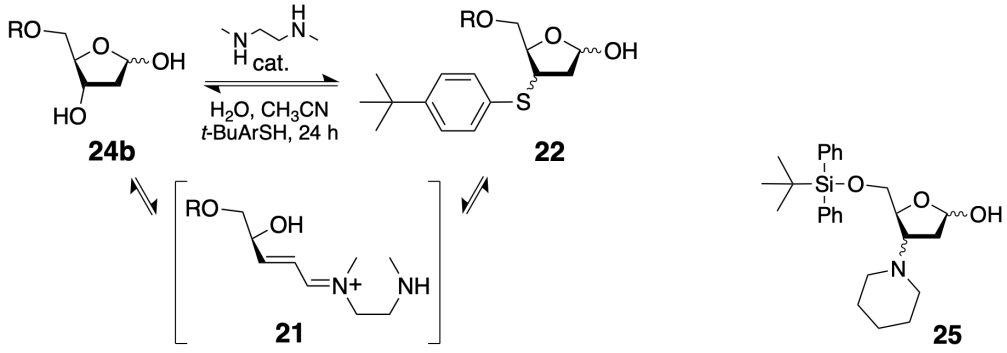
tris buffer (2-amino-2-(hydroxymethyl)propane-1,3-diol) reported by Kushida et al.[60] In that case, the amine group of the tris buffer may catalyze conjugate addition of water. Similarly, the generation of 3'dR from the AP lyase action of the base excision repair glycosylase Endo III [62-64, 105] may involve conjugate addition of water to an enzyme-bound α,β-unsaturated iminium intermediate attached to the active site lysine 120.[55]



Scheme 11. Conjugate addition of water to the α,β-unsaturated iminium ion **21**.



Scheme 12. Independent synthesis of **24** for spectral comparison (R = TBDPS group).



Scheme 13. Amine-catalyzed conversion of **24** to the diastereomeric thiol mixture **22**.

Reversible conjugate addition of an amine to the α,β-unsaturated iminium ion generated by amine-catalyzed elimination of acetate from 8. Incubation of **8** with a large excess of piperidine (10 equiv) for 12 h generated the 3-piperidino-2,3-dideoxyribose adduct **25** in 53% yield, consistent with an aza-Michael-type reaction. Formation of this product is reversible, as shown by an experiment in which incubation of **25** with DMEDA (10 mol%) in the presence of excess *t*-butylbenzenethiol (10 equiv) led to generation of the 3-phenylthio-2,3-dideoxyribose adducts **22** (68%). In contrast, **25** was stable in the absence of an amine catalyst. These results are consistent with reversible conjugate addition of an amine to the α,β-unsaturated iminium ion generated by amine-catalyzed elimination of acetate from **8**. This type of reaction may be relevant to the *cis-trans* isomerization of the alkenals **11** and **12** described above. Literature precedents indicate that amine-catalyzed cis-trans isomerization of α,β-unsaturated ketones proceeds via reversible conjugate addition of the amine catalyst.[106, 107]

Exploring the reactivity of the *cis*- and *trans*-alkenals 11 and 12 with thiols and water. For the most part, the experiments described above defined the reactivity of the α,β-unsaturated iminium ion **21**. For comparison, in this section, we describe experiments examining the analogous reactions of the *trans*- and *cis*-alkenals **11** and **12** with *t*-butylbenzenethiol, 2-mercaptoethanol, and water.

We found that both the *cis*- and *trans*-alkenals **11** and **12** (25 mM) generated the 3-phenylthio-2,3-dideoxyribose products **22** in 81% and 83% yields, respectively, after 1.5 h, when mixed with a high concentration of *t*-butylbenzenelthiol (10 equiv, 250 mM)

in our standard solvent mixture. Generation of the 3-phenylthio-2,3-dideoxyribose products from **11** and **12** was slower than generation of the same products via the amine-catalyzed process, with starting material completely consumed after about 1.5 h compared to 5 min for the amine-catalyzed reaction of **8** in the presence of thiol. The diastereomeric ratio of **22a** and **22b** arising from the reaction of **11** with the thiol did not change over the course of an additional 24 h incubation time (Scheme 14). This suggests that the reaction of **11** with the thiol in the absence of an amine catalyst is largely irreversible and under kinetic control under the conditions used here.

When a lower concentration of the water-soluble thiol 2-mercaptoethanol was used, we were able to determine that the *trans* isomer **11** is substantially more reactive than the *cis* isomer **12**. Specifically, HPLC analysis showed that incubation of the *trans*-alkenal **11** (40 μM) with 2-mercaptoethanol (10 mM) generated the 3-alkylthio-2,3-dideoxyribose addition products with a half-time of approximately 16 min (Figure 2). Generation of the 3-alkylthio-2,3-dideoxyribose products **22** from the *cis*-alkenal **12** was about four times slower, occurring with a half-time of approximately 69 min (Figure 2). The lower reactivity of the *cis*-alkenal **12** can be attributed to the fact that this isomer exists predominantly with the electrophilic aldehyde residue masked as a cyclic hemiacetal.

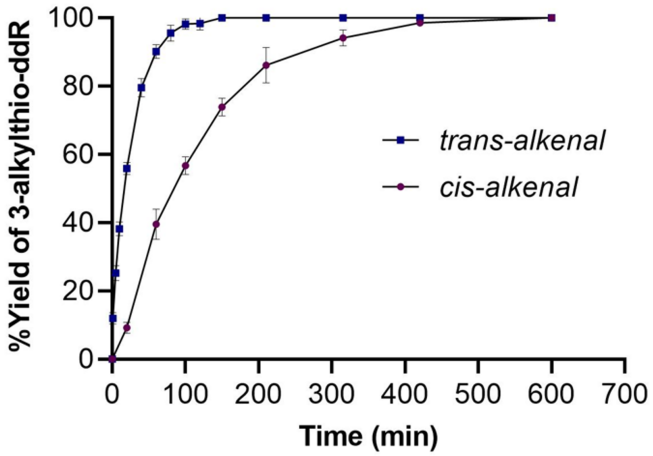
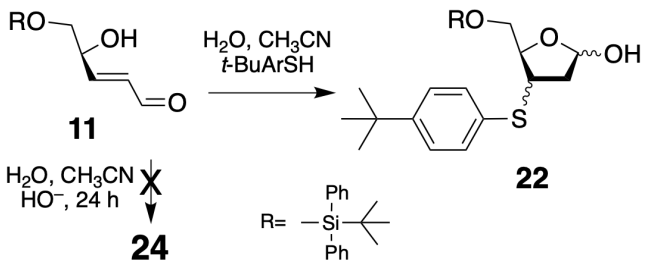


Figure 2. The reaction of 2-mercaptoethanol with the *trans*-alkenal **11** is approximately four times faster than with the *cis*-alkenal **12**.

We also examined whether water can undergo conjugate addition with **11** and **12**. Existing precedents show that water can add to the simple α,β-unsaturated aldehyde acrolein in neutral aqueous solution, with polymerization as a competing process.[108] Nonetheless, we did not observe generation of the 2-deoxyribose product **24** from either the *cis*- or *trans*-alkenals **11** and **12** in a solvent mixture composed of 1:1 acetonitrile and aqueous NaOH (10 mM). Rather, we noted decomposition of **11** to unidentified (presumably polymeric[91]) products over the course of several hours.

Overall, the results indicate that both the *cis*- and *trans*-alkenals **11** and **12** have the capacity to react with thiols, but the *trans* isomer **11** is substantially more reactive than the *cis* isomer **12**, in this regard. Under our reaction conditions, neither **11** nor **12** generate **24** via conjugate addition of water.



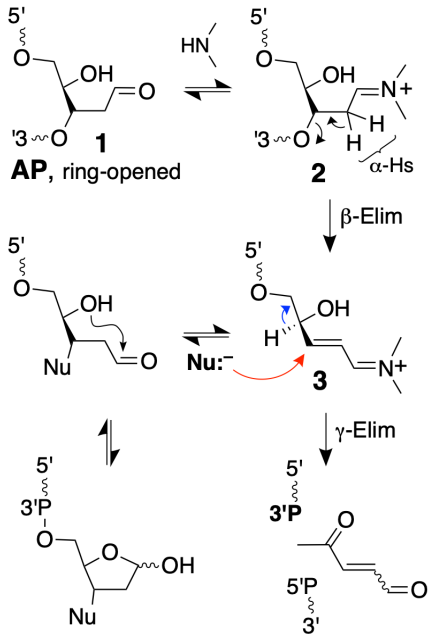
Scheme 14. The *trans*-alkenal **11** is subject to conjugate addition of thiols, but not water.

■ CONCLUSIONS

The cell nucleus is rich in proteinaceous amines (e.g. histones)[109] and low molecular weight polyamines (e.g. spermine).[43] As a result, amine-catalyzed strand cleavage at AP sites in cellular DNA is likely a biologically important process. Amines catalyze strand cleavage via a covalent mechanism involving the formation of iminium ion intermediates that possess amplified reactivity.[46-48, 54-56, 77-82] For example, conversion of the ring-opened aldehyde **1** to the corresponding iminium ion **2** dramatically increases the acidity of the α-protons due to the formal positive charge on the nitrogen atom (Scheme 15).[110] The increased acidity of the α-protons, in turn, facilitates β-elimination of a leaving group from the 3-position of the sugar.[51, 55, 81, 111] Similarly, the α,β-unsaturated iminium ion generated by this elimination reaction. (**3**, Scheme 15) displays amplified reactivity relative to the corresponding α,β-unsaturated aldehyde, with respect to conjugate (1,4-addition) of nucleophiles ($Nu:^-$ in Scheme 15).[104, 112, 113] Iminium ion catalysis is central to the formation and reversible interconversion of the canonical and noncanonical 3'-sugar remnants characterized in this work (Schemes 15 and 16).

The results of our model reactions offer several interesting predictions regarding the identity and properties of the products arising from the α,β-unsaturated iminium ion intermediate generated by amine-catalyzed strand cleavage (illustrated in Scheme 16).

The major initial product generated by amine-catalyzed β-elimination of acetate from our model compound **8** is the *trans*-alkenal **11**, mirroring the canonical sugar remnant generated on the 3'-terminus of a DNA strand break generated by amine-catalyzed strand cleavage at an AP site.[38, 51, 57, 58]



Scheme 15. Conversion of the AP aldehyde to an iminium ion facilitates β-elimination by increasing the acidity of the α-protons. The resulting α,β-unsaturated iminium ion also displays amplified reactivity with respect to both γ-elimination of the 5'-phosphoryl group (blue arrow on 3) and conjugate addition of nucleophiles (red arrow on 3).

We find that the *trans*-alkenal can undergo amine-catalyzed isomerization to give an equilibrating mixture of the *cis*- and *trans*-alkenal products. This isomerization reaction likely proceeds via reversible conjugate addition of an amine to the α,β-unsaturated iminium ion or α,β-unsaturated aldehyde.[106, 107] In the few cases where the *cis*-alkenal has been observed previously, its formation may have been catalyzed by conjugate addition of an enzyme amino group,[61] the amino group in tris buffer,[60] or hydroxide at high temperature (90 ˚C).[35] We found that the *cis*-alkenal is less reactive

than the *trans*-alkenal and, therefore, could be a persistent product arising from the cleavage of AP sites in DNA.

We observed that the *cis*-alkenal **12** transforms to the furan derivative **20** under acidic conditions (Scheme 6). The possible relevance of this aromatization process in the context of DNA remains uncertain. The microenvironment of the DNA double helix is acidic[114] which could favor furan formation, but it is unclear whether elimination of water to generate the furan can occur in DNA where competing elimination of the 5'-phosphoryl group is possible.

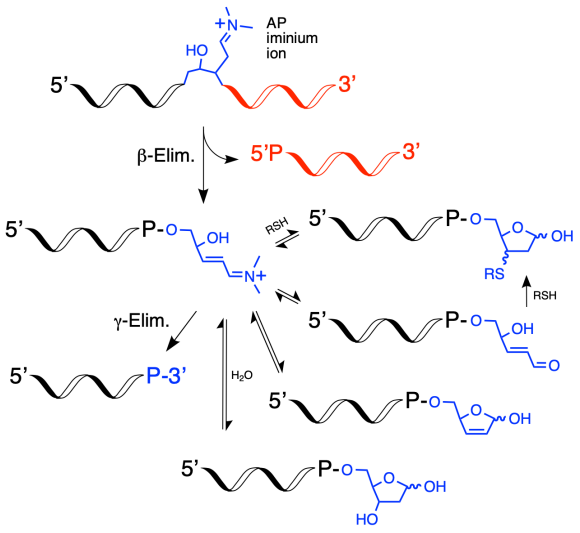
Cells are rich in thiols including millimolar concentrations of the tripeptide glutathione and cysteine residues on proteins.[93, 94, 115, 116] The results of our model reactions predict that amine-catalyzed strand cleavage in the presence of thiols can reversibly generate diastereomeric mixtures of 3-thio-2,3-dideoxyribose adducts on the 3'-terminus of an AP-derived strand break (Scheme 16). The addition of thiols to the 3'-α,β-unsaturated iminium ion or 3'-α,β-unsaturated aldehyde is rapid, suggesting that, in the cellular environment, 3-glutathionyl-2,3-dideoxyribose products may be major products generated by the cleavage of AP sites in cellular DNA. More than 30 years ago, Bailly and Verly provided evidence for 3'-thiol adducts arising from amine-catalyzed strand cleavage of a DNA AP site in the presence of thiols.[67] They further showed that the formation of 3'-thiol adducts inhibited γ-elimination reactions that remove the sugar remnant to generate a 3'P end group (Scheme 16). This led them to suggest that thiol adducts in cellular DNA could influence DNA repair processes.[67] Surprisingly, the possible formation and significance of thiol adducts generated from AP-derived strand breaks in cellular DNA seems to have been overlooked since Bailly and Verly's work.

In addition, our results suggest that the amplified reactivity of the α,β-unsaturated iminium ion intermediate generated by amine-catalyzed strand cleavage may enable conjugate addition of water. This generates an abasic, 2-deoxyribose sugar remnant (3'dR) on the 3'-terminus of the strand break (3'dR, Scheme 16). Our results show that the amine-catalyzed reversion of the 3'dR product to the iminium ion is relatively slow, suggesting that, once formed, the 3'dR sugar remnant could be persistent in DNA. In the few cases where the 3'dR cleavage product has been observed previously, its formation likely involved conjugate addition of water to an α,β-unsaturated iminium ion derived from the amino group of tris buffer,[60] or Lys 120 of the enzyme Endo III.[55, 62] Sugiyama showed that high temperature (90 ˚C) can induce formation of 3'dR in pH 7 cacodylate buffer (even in the absence of an amine catalyst).[35]

Overall, our studies forecast that amine-catalyzed strand cleavage at AP sites in DNA can generate dynamic, equilibrating mixtures of previously unrecognized complexity (Scheme 16). In the presence of an amine catalyst, the *trans*-alkenal, *cis*-alkenal, 3'-dR, 3-amino-2,3-dideoxyribose, and 3-thio-2,3-dideoxyribose products may form and interconvert on the 3'-terminus of an AP-derived strand break. Studies of amine-catalyzed strand cleavage in DNA substrates will be required to determine which of these products form and how the product mixtures evolve over time under biological conditions. It seems clear that the interconverting collection of 3'-deoxyribose sugar remnants will ultimately give way to the 3'-phosphoryl group (3'P) via an irreversible γ-elimination reaction (Schemes 15 and 16).

From a biological perspective, it is important to recognize that each of the structurally diverse 3'-sugar remnants generated by strand cleavage at an AP site presents

a distinct blocking group (a "dirty end") that must be either chemically or enzymatically removed ("trimmed" or "cleaned") from the 3'-terminus of an AP-derived strand break in DNA to generate the 3'OH terminus that is required for the gap-filling step of base excision repair (BER) or single-strand break repair pathways (SSBR).[117-122] Cleaning of the 3'-ends at DNA strand breaks can be carried out by the 3'-exonuclease activity of enzymes such as apurinic endonuclease (APE1).[52, 71, 120, 123-126] Alternatively, the lyase action of amine residues on some proteins has the potential to catalyze γ-elimination to give the 3'P product that can be trimmed to the requisite 3'OH by polynucleotide kinase phosphatase (PNKP).[117-122] The results reported here expand the list of 3'-sugar remnants that must be trimmed from AP-derived strand breaks in order to carry out BER and SSBR.



Right side: Scheme 16. The model reactions reported here predict that α,β-unsaturated iminium ions generated by amine-catalyzed cleavage of an AP site in DNA under physiological conditions reside at the hub of multiple, equilibrating cleavage products (a simple dialkylamine catalyst is shown here to represent structurally diverse primary and secondary amines that can serve this role). Ultimately, the interconverting sugar remnants on the 3'-terminus of the strand break give way to the 3'-phosphoryl end product via irreversible γ-elimination. P in the diagram represents either a phosphodiester linkage or a terminal phosphoryl group. For simplicity, this diagram does

not show all the mechanistic steps and intermediates involved in the transformations. For example, iminium ion hydrolysis reactions, cyclization of the 4'-OH onto the aldehyde residue of the ring-opened sugar, and multiple roles for the amine catalytst in the *cis-trans* isomerization process are not shown.

■ Supporting Information

The Supporting Information is available free of charge via the Internet at http://pubs.acs.org.

LC-MS analysis of the products resulting from treatment of **8** with piperidine in the presence of $NaBH_3CN$, *trans*- and *cis*-alkenals **11** and **12** is generated by amine-catalyzed elimination of acetate from **8**, alkene region in ^{1}H-NMRs revealing *trans-cis* isomerization of the alkenals **11** and **12**, and all ^{1}H-NMR and ^{13}C-NMR spectra.

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J.S.J and C.N: These authors contributed equally to this work.

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Notes

The authors declare no conflicts of interest

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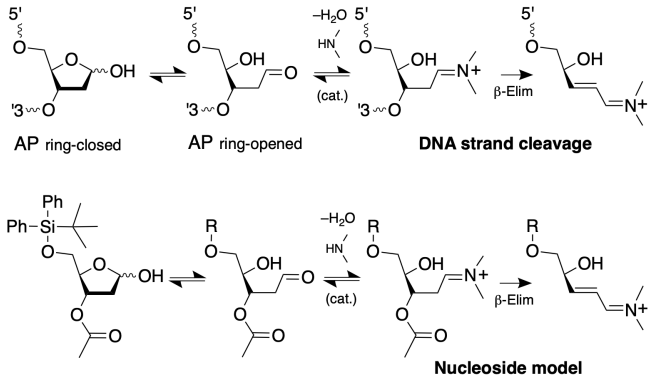
TOC Graphic


5'
O
O
OH
'3
O
AP ring-closed
AP ring-opened
–H2O
HN
(cat.)
N+
β-Elim
DNA strand cleavage
Ph
Si
R
Nucleoside model