Electronic Supplementary Information

Product analysis of photooxidation in isolated quadruplex DNA; 8-oxo-7,8-dihydroguanine and its oxidation product at 3'-G are formed instead of 2,5-diamino-4*H*-imidazol-4-one

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Experiments

The structure of single-stranded and quadruplex d(TGGGGT) with riboflavin (RF).

Single-stranded and quadruplex d(TGGGGT) were oxidized with RF in this study, and the structure of d(TGGGGT) might be influenced by RF. To determine the binding of RF to d(TGGGGT), fluorescence quenching of RF by the excess of single-stranded and quadruplex d(TGGGGT) were performed. As a result, RF fluorescence was quenched by d(TGGGGT) (Fig. S8a-b). Hence, single-stranded and quadruplex d(TGGGGT) bind RF. Furthermore, influence of RF on the structure of d(TGGGGT) was determined by CD spectroscopy. As a result, the structure of d(TGGGGT) was not changed by RF (Fig. S8c-f). Thus, RF binds the d(TGGGGT), and then RF has no effect on the structure of single-stranded and quadruplex d(TGGGGT).

The HPLC analysis of the oxidation products from d(TGGGT).

Although KCl was required to form the quadruplex structure, photooxidation of guanine might be influenced by KCl. In 10 mM KCl, the structure of 5-mer DNA d(TGGGT) was determined by CD spectroscopy, and quadruplex d(TGGGT) was not detected (Fig. S9a). Therefore, d(TGGGT) was photooxidized with 75 μM RF, and subsequently these products were analyzed using HPLC. In HPLC analysis, a significant difference was not observed between 0.1 and 10 mM KCl (Fig. S9b-e). Thus, guanine oxidation products did not depend on KCl concentration.

HPLC analysis of the oligomer containing 8oxoG.

Since d(TGGGGhoxT) was detected in Fig. 1c, the peak at 20.4 min in Fig. 1c was thought to be d(TGGG8oxoGT). For confirmation, the oligomer containing 8oxoG was analyzed by HPLC, and the synthetic d(TG8oxoGGGT) and d(TGGG8oxoGT) in Fig. S6a and S6b were detected at the same time as the peak at 20.4 min in Fig. 1c. Next, the synthetic d(TG8oxoGGGT) and d(TGGG8oxoGT) were oxidized by UVA radiation with riboflavin, and the oxidation product of d(TGGG8oxoGT) in Fig. S6c and S6d was detected at the same time as d(TGGGGhoxT) in Fig. 1c. In contrast, the peak of the oxidized d(TG8oxoGGGT) in Fig. S6e and S6f was not matched with d(TGGGGhoxT) in Fig. 1c.

Formation of 8oxoG in pathway 2.

The isolated quadruplex and the single-stranded DNA were photooxidized with 20 U/μl superoxide dismutase (SOD). As a result, the addition of SOD significantly decreased the yield of the oxidation in single-stranded DNA (Fig. S7). In contrast, oxidation in quadruplex DNA was not affected by SOD. Thus, the oxidation in quadruplex DNA is not mediated by $O_2^{\bullet-}$, and we conclude that 8oxoG was formed by pathway 2.

The equilibrium constants for the deprotonation of $G^{\bullet+}$ in single-stranded and quadruplex DNA.

(a) The ratio of the equilibrium constants for the deprotonation of $G^{\bullet+}$ in single-stranded and quadruplex DNA was obtained with the acid dissociation constant for the O6 protonated guanine ($K_{a(O6)}$), and $K_{a(O6)}$ was acquired with pK_a for the N7 protonated guanine and the relative free energy of O6 protonated guanine and N7 protonated guanine in aqueous phase (see reference S1);

The localization of the HOMO in quadruplex DNA.

As calculations for the double-stranded DNA (see reference 1), we calculated the localization of the HOMO in quadruplex DNA. Geometries of stacked methylated nucleobases at N1 were constructed as follows. The geometry of the stacked bases was generated from the crystallographic coordinates of d(TGGGGT) (PDB 1S45, see reference S2a). All ions were exchanged to potassium ions, and the ends of thymines and its sugar backbones were removed except for the 2'-deoxyribose O5 and O3. One H atom was then attached to the O5 and O3 to complete the N-methylated nucleobases. The geometries of all potassium ions were fixed, and the structure was energy minimized using the MacroModel version 7.5 with the OPLS2005 force field (see reference S2b). Then, all the sugar backbones were removed except for the 2'-deoxyribose C1 carbon and C1 proton. Two H atoms were then attached to the C1 to complete the N-methylated nucleobases. The HOMO of the structure was calculated, and calculation was performed at the B3LYP/6-31G* levels utilizing Gaussian 03 (see reference S2c).

references

S1 V. Verdolino, R. Cammi, B. H. Munk, and H. B. Schlegel, J. *Phys. Chem. B*, 2008, **112**, 16860-16873.

S2 (a) C. Cáceres, G. Wright, C. Gouyette, G. Parkinson, and J. A. Subirana. *Nucleic Acids Res*., 2004, **32**, 1097-1102; (b) MacroModel, version 7.5, Schrödinger, LLC, New York, 2006; (c) M. J. Frisch, et al. Gaussian 03, revision C.02, Gaussian Inc., Wallingford, CT, 1995.

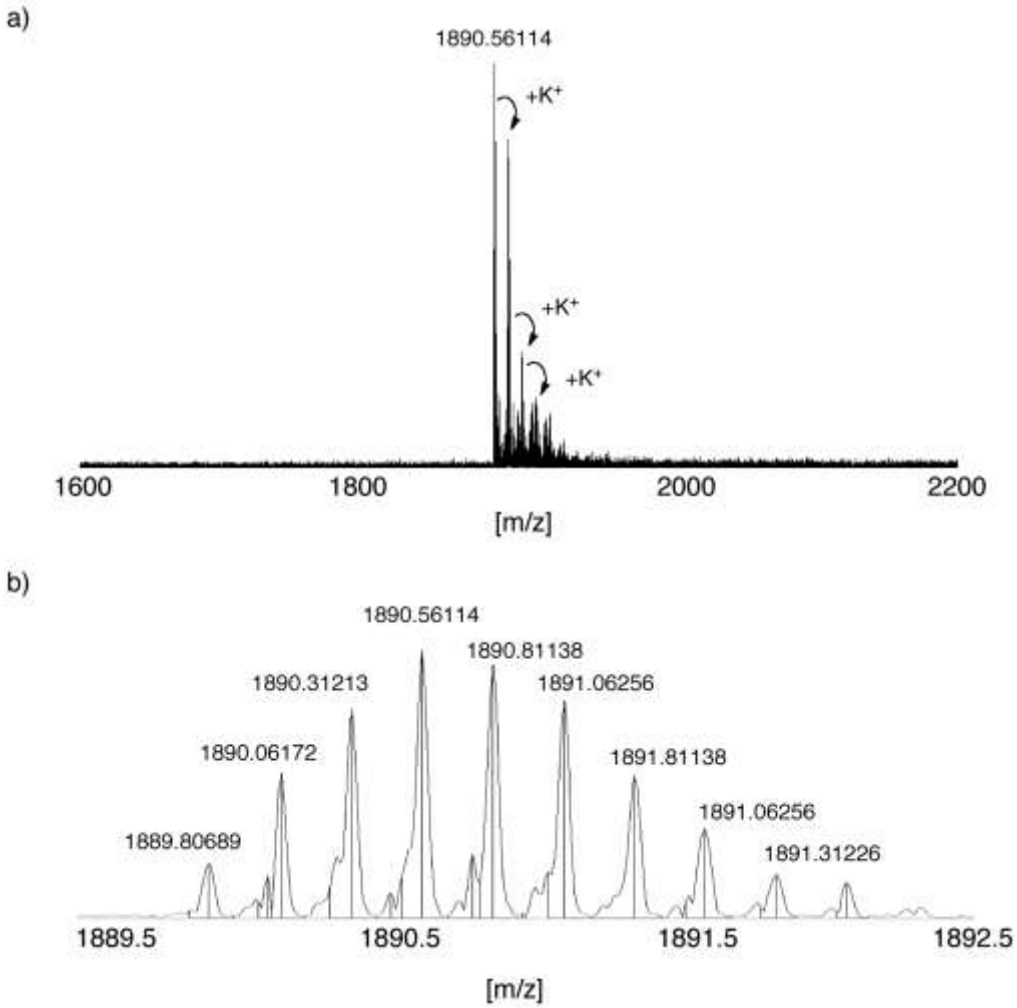

Fig. S1. The mass spectrum of the quadruplex DNA containing potassium ions. The raw data (m/z 1600-2200) were shown in panel a, and magnified charts (m/z 1889.5-1892.5) were shown in panel b.

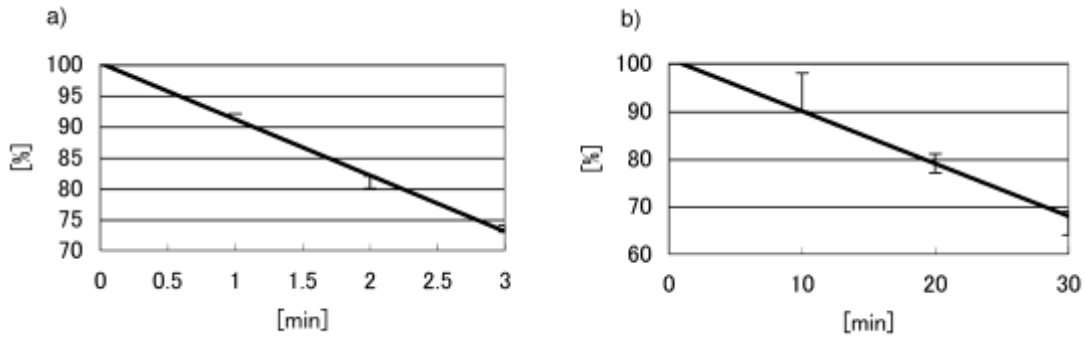

Fig. S2. Time course of guanine oxidation with UVA. a) Single-stranded d(TGGGGT) (700 μM) with 75 μM RF in 0.1 mM KCl and 5 mM cacodylate buffer (pH 7) was irradiated at 365 nm for 1, 2, and 3 min. b) Quadruplex d(TGGGGT) (700 μM) with 75 μM RF in 10 mM KCl and 5 mM cacodylate buffer (pH 7) was irradiated at 365 nm for 10, 20, and 30 min.

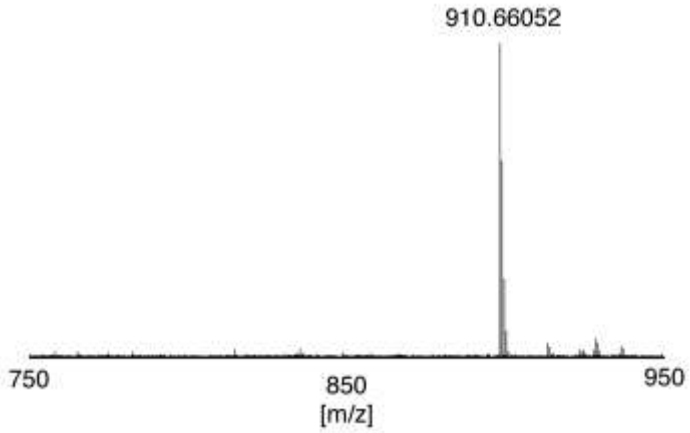

Fig. S3. The mass spectrum of the oligomer containing Iz.

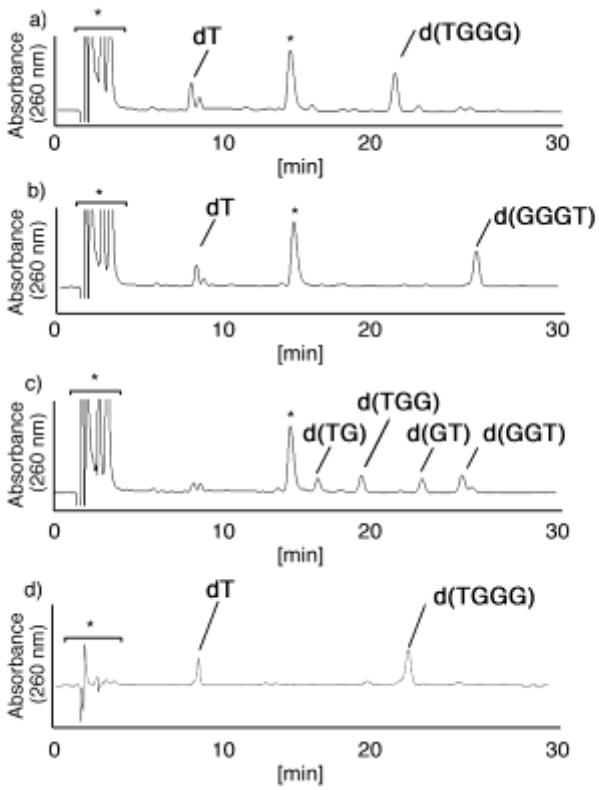

Fig. S4. Piperidine treatment of the oligomers containing Iz or Ghox. To determine the locations of Iz and Ghox in the oligomers in Fig 1b and 1c, each products was isolated, and then was heated with 1 M piperidine at 90ºC for 20 min, and subsequent dephosphorylation of all phosphate monoesters with alkaline phosphatase was performed. Further, the products were analyzed by HPLC, and the results compared with the HPLC charts of purchased standard samples, dT, d(TG), d(TGG), d(TGGG), d(GT), d(GGT), and d(GGGT). Samples were analyzed by HPLC with a CHEMCOBOND 5-ODS-H column (Chemcopak, 5 μm, 150 × 4.6 mm, elution with a solvent mixture of 50 mM $AcONH_4$ (pH 7), 3-5% CH_3CN/30 min at a flow rate of 1.0 ml/min) and monitored at 260 nm absorbance. The HPLC profiles of a) - d) represent the piperidine treatment products from the peak at a) 20.5 min, b) 20.7 min, c) 21.1 min in Fig. 1b, and d) 18.6 min in Fig. 1c. "*" in panel a-d indicates the products which was not formed from DNA.

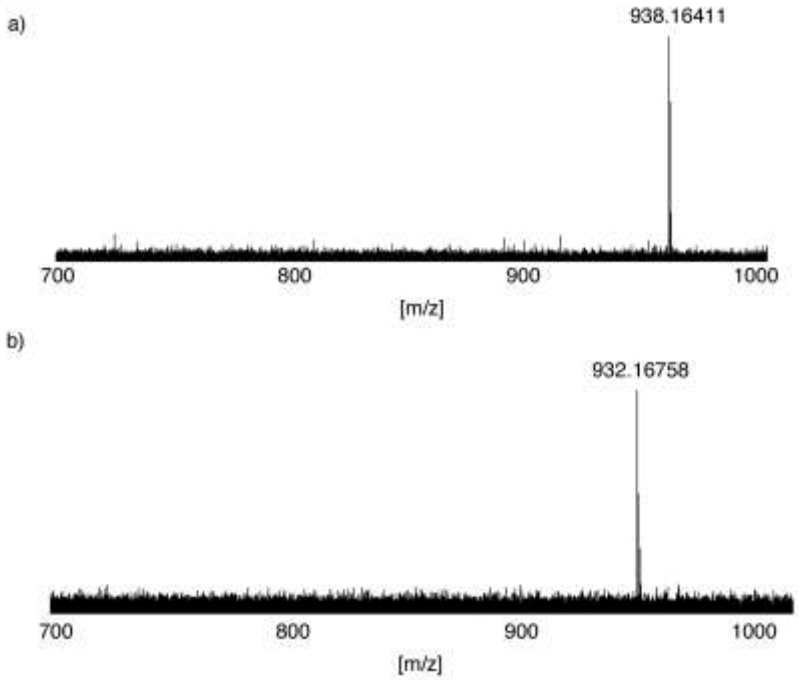

Fig. S5. The mass spectrum of the oligomers containing 8oxoG or Ghox. The oligomers containing (a) 8oxoG or (b) Ghox was detected using ESI-MS.

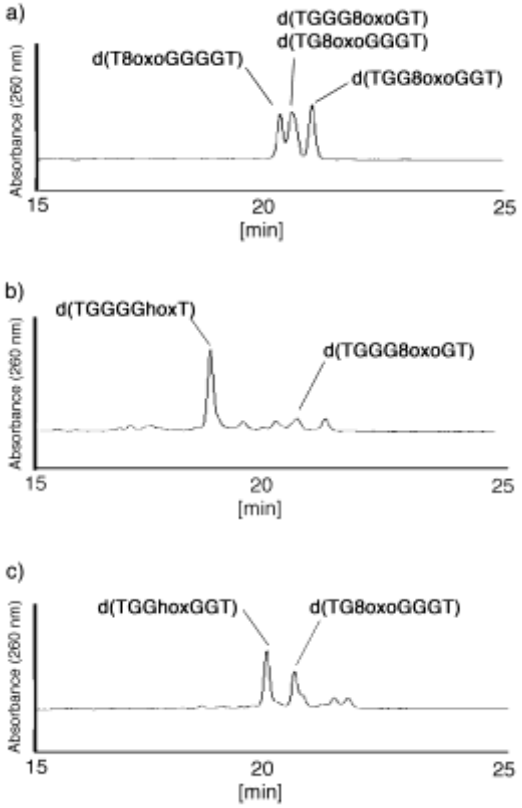

Fig. S6. HPLC analysis of four oligomers containing 8oxoG. Samples were analyzed by HPLC with a CHEMCOBOND 5-ODS-H column (Chemcopak, 5 μm, 150 × 4.6 mm, elution with a solvent mixture of 50 mM $AcONH_4$ (pH 7), 0-10%/30 min, CH_3CN at a flow rate of 1.0 ml/min) and monitored at 260 nm absorbance. d(T8oxoGGGGT), d(TG8oxoGGGT), d(TGG8oxoGGT), and d(TGGG8oxoGT) were synthesized, and the standard samples were shown in panel a. The oligomers containing 8oxoG (700 μM) with 75 μM RF in 10 mM KCl and 5 mM cacodylate buffer (pH 7) was irradiated at 365 nm for 2 min. The oxidation products of d(TGGG8oxoGT) in panel b. The oxidation products of d(TG8oxoGGGT) in panel c.

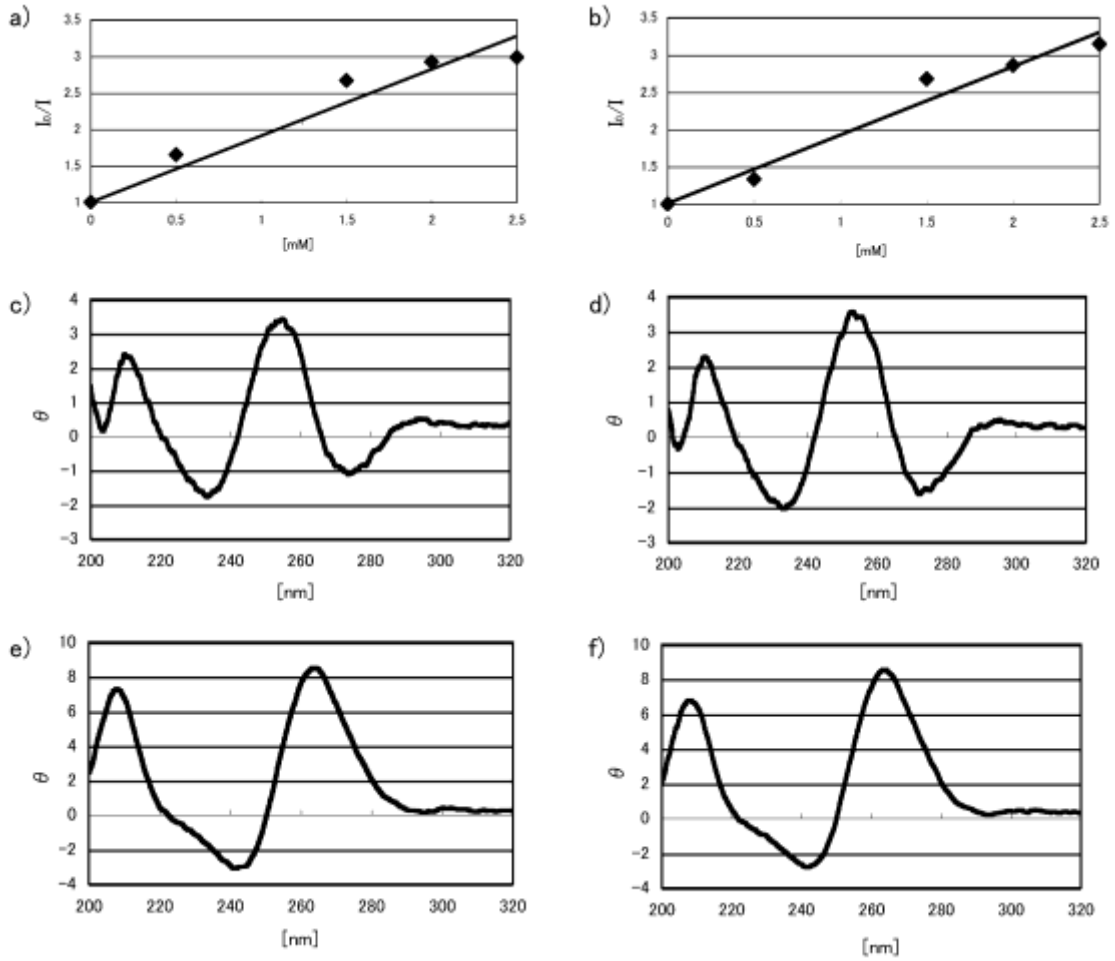

Fig. S7. The fluorescence quenching and the CD spectroscopy of single-stranded and quadruplex d(TGGGGT) with RF. Fluorescence quenching of 6.25 μM RF by a) 0-2.5 mM single-stranded d(TGGGGT) in 0.1 mM KCl and 5 mM cacodylate buffer (pH 7) or b) 0-2.5 mM quadruplex d(TGGGGT) in 10 mM KCl and 5 mM cacodylate buffer (pH 7) was performed. I_0/I indicates the ratio of fluorescence intensities. CD spectroscopy of 70 μM single-stranded d(TGGGGT) in 0.1 mM KCl and 5 mM cacodylate buffer (pH 7) c) without RF or d) with 7.5 μM RF, and quadruplex d(TGGGGT) in 10 mM KCl and 5 mM cacodylate buffer (pH 7) e) without RF or f) with 7.5 μM RF were analyzed.

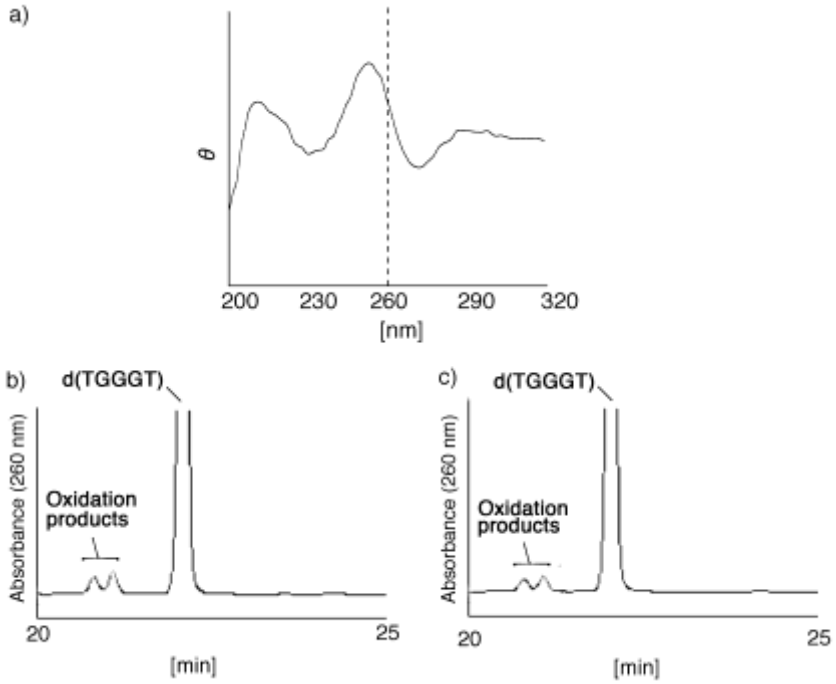

Fig. S8. The CD of 70 μM d(TGGGT) in 10 mM KCl and HPLC profiles of the oxidation products from d(TGGGT). CD spectroscopy was shown in panel a. HPLC analysis of the oxidation products were performed in 0.1 mM KCl was shown in panel b. HPLC analysis of the oxidation products were performed in 10 mM KCl was shown in panel c. Samples were analyzed by HPLC with a CHEMCOBOND 5-ODS-H column (Chemcopak, 5 μm, 150 × 4.6 mm, elution with a solvent mixture of 50 mM $AcONH_4$ (pH 7), 0-10% during 0-30 min, 10-30% during 30-35 min, CH_3CN at a flow rate of 1.0 ml/min) and monitored at 260 nm absorbance in panel b-c.