Supplementary Material for Organic & Biomolecular Chemistry

Supplementary Information

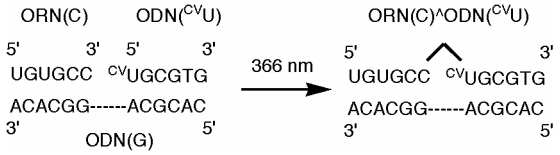
Highly sequence specific RNA terminal labeling by DNA photoligation

Yoshinaga Yoshimura, Yuuki Noguchi and Kenzo Fujimoto*

Prof. Dr. K. Fujimoto, Dr. Y. Yoshimura, Dr. Y. Noguchi
School of Materials Science, Japan Advanced Institute of Science and Technology, and
PRESTO, Japan Science and Technology Agency, Ishikawa 923-1292, Japan
Fax: (+81) 761-51-1671
E-mail: kenzo@jaist.ac.jp

Photoligation of ODNs as monitored by HPLC. The reaction mixture (total volume 60 μL) containing ODN(^{CV}C) and ORN(C) (each 20 μM, strand concn) in the presence of template ODN(G) (24 μM, strand concn) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride was irradiated with a 25 W transilluminator (366 nm) at 0 °C for 16 min. After irradiation, the progress of the photoreaction was monitored by HPLC on a Cosmosil 5C18AR column (4.6 × 150 mm, elution with a solvent mixture of 50 mM ammonium formate, pH 7.0, linear gradient over 30 min from 4% to 10% acetonitrile at a flow rate 0.8 mL/min).

Scheme S1. DNA photoligation with ORN containing C at the 3'-terminal site.



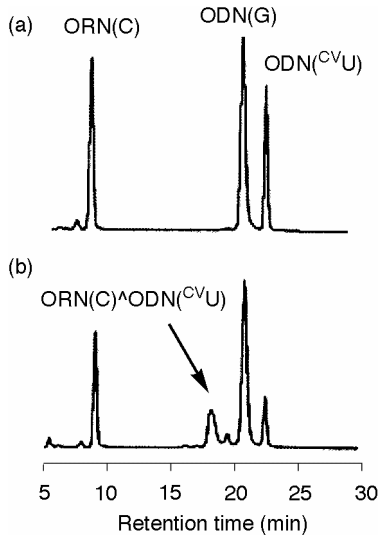


Figure S1. HPLC analysis of the irradiated ODN(^{CV}U) and ORN(C) in the presence of ODN(G): (a) before irradiation; (b) irradiated at 366 nm for 16 min.

Enzymatic digestion of the photoligated product. An aliquot of purified ORN(U)^ODN(^{CV}U) or ORN(C)^ODN(^{CV}U) was fully digested with calf intestine alkaline phosphatase, and P1 nuclease at 37 °C for 4 h. Digested solution was analyzed by HPLC on a Cosmosil 5C18AR column (4.6 × 150 mm, elution with a solvent mixture of 50 mM ammonium formate, pH 7.0, linear gradient over 30 min from 3% to 20% acetonitrile at a flow rate 1.0 mL/min).

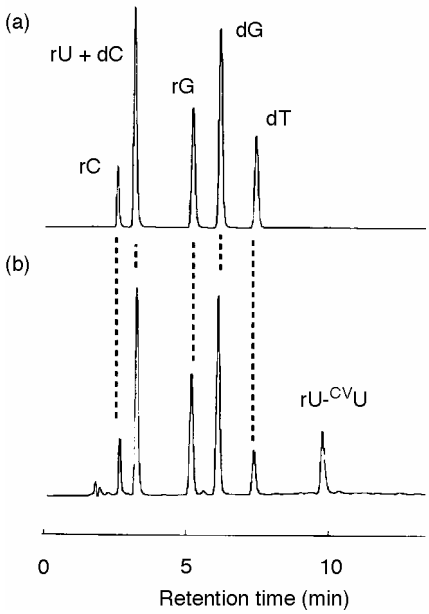


Figure S2. HPLC analysis of the enzymatic digestion: (a) authentic ORN(U)pODN(T) (5′-r(UGUGCU)d(TGCGTG)-3′); (b) ORN(U)^ODN(^{CV}U).

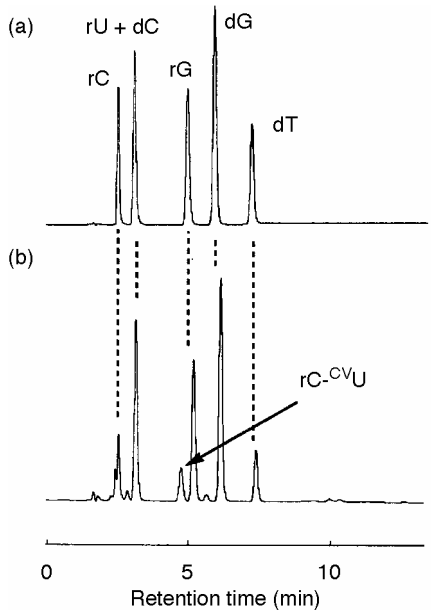


Figure S3. HPLC analysis of the enzymatic digestion: (a) authentic ORN(C)pODN(T) (5′-r(UGUGCC)d(TGCGTG)-3′); (b) ORN(C)^ODN(^{CV}U).