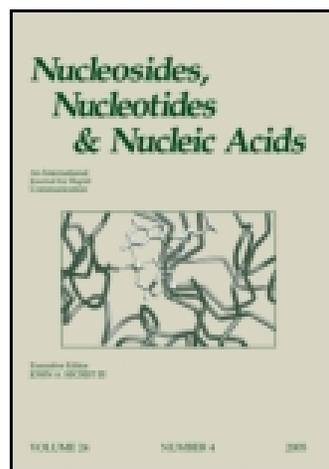


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## Nucleosides, Nucleotides and Nucleic Acids

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### DESIGN OF HIGHLY EFFICIENT AND SELECTIVE TRANSFER REACTION OF NITROSYL GROUP TO dC AND d<sup>M</sup>C RESULTING IN SPECIFIC DEAMINATION

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## DESIGN OF HIGHLY EFFICIENT AND SELECTIVE TRANSFER REACTION OF NITROSYL GROUP TO dC AND d<sup>M</sup>C RESULTING IN SPECIFIC DEAMINATION

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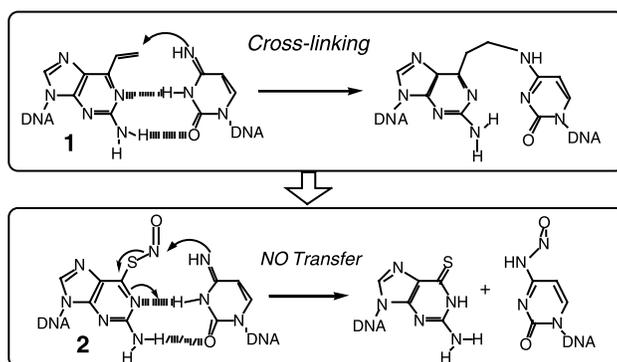
**Shigeki Sasaki** □ *CREST, Japan Science and Technology Agency, Japan*

□ *Nitric oxide (NO) is an important endogenous regulatory molecule, and S-nitrosothiols are believed to play a significant role in NO storage, transport, and delivery. Based on the ability to generate NO in vivo, S-nitrosothiols can be used as therapeutic drugs. In this study, we have developed an innovative method for sequence- and base-specific delivery of NO to a specific site of DNA followed by specific deamination.*

### INTRODUCTION

Chemical reactions to DNA may cause the point mutation to lead diseases. For example, the reaction of nitrous acid was deaminated of primary amino groups to convert dA into dI, dC into dU, and dG into dX, resulting in mutation.<sup>[1]</sup> Since chemical reactions to DNA occur nonselectively, and mutations are caused in a random manner. If we can control chemical reactions to DNA in a specific manner, they can be used as chemical tools to cause a selective point mutation on the target DNA. We have previously reported that 2-amino-6-vinylpurine skeleton (**1**) exhibited efficient cross-linking with selectivity toward cytidine.<sup>[2]</sup> The high selectivity and efficiency of this cross-linking reaction is attributable to the proximity effect. As an expansion of this design concept, we have designed a NO-transfer reaction from S-nitroso thioguanine to an imino tautomer of cytosine (Figure 1). In this article, we wish to report the development of an innovative method for sequence- and base-specific delivery of NO to a specific site of DNA followed by specific deamination.

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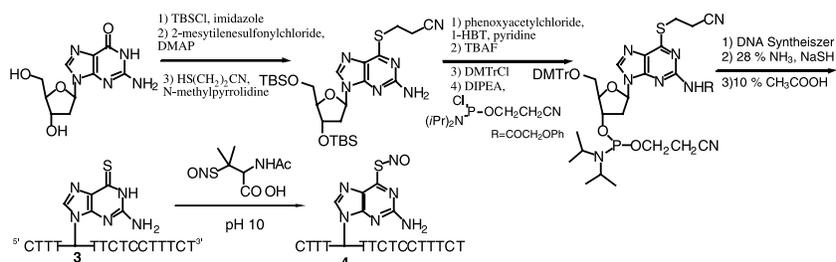
**FIGURE 1** Design of the novel NO transfer reaction.

## RESULTS AND DISCUSSION

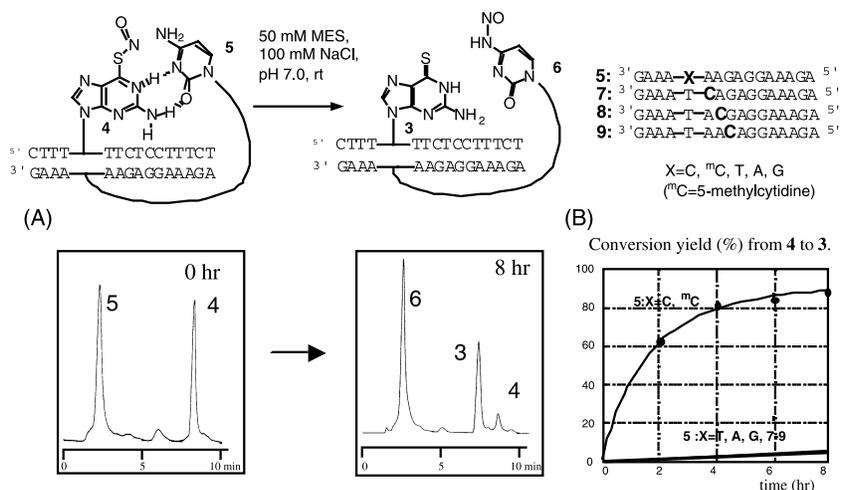
Reaction in a complex between *S*-nitroso thioguanine and an imino tautomer of cytosine was simulated by ab initio calculation, and it was suggested that NO transfer from *S* to *N* would take place together with hydrogen transfer from N3 of cytosine to N1 of thioguanine with relatively low activation energy.

The synthesis of ODNs incorporating *S*-nitroso-thioguanosine (**2**) is summarized in Scheme 1. The ODN bearing thioguanosine (**3**) was prepared according to the methods reported previously.<sup>[3]</sup> Nitrosation of **3** was carried out by a NO transfer reaction with *S*-nitroso-*N*-acetylpenicillamine (SNAP) under alkaline conditions. This reaction proceeded in good yields to give the ODN bearing *S*-nitroso guanosine.

An interstrand NO transfer reaction was investigated with ODN (**4**) and its complementary ODN (**5**; X = dC or d<sup>m</sup>C) in 50 mM MES buffer containing 100 mM NaCl at pH 7 at 25°C, and followed by HPLC (Figure 2A). The transfer reaction yields were obtained by the conversion ratio of **4** to **3**, as shown in Figure 2B. A rapid transfer reaction of **4** was observed with dC or d<sup>m</sup>C bearing ODN (**5**). On the other hand, the NO transfer reaction did not take place with the ODNs (**5**) having dT, dA, or dG at the target site or with ODN (**7–9**) having dC at a non-target site. These results have suggested that the transfer reaction proceeds with high



**SCHEME 1** Synthesis of the ODN incorporating *S*-nitroso guanosine.



**FIGURE 2** NO transfer reaction using 4 and 5. (A) HPLC analysis of NO-transfer reaction between 4 and 5 (X = C), HPLC conditions: column: X Terra MS C18 (2.1 × 150 mm), solvent: A: 0.1 M TEAA Buffer B: CH<sub>3</sub>CN, B: 10% to 20%/10 min, 20% to 100%/20 min linear gradient, flow rate: 0.3 mL/min, monitored at 254 nm. (B) Conversion yield from 4 to 3; Closed circles and curve correspond to the reaction with 5 (X = dC or d<sup>m</sup>C); the thick line corresponds to the reaction with 5 (X = dT, dA, or dG) or 7–9.

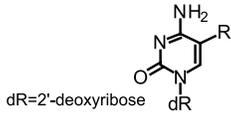
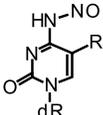
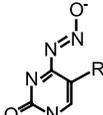
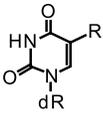
selectivity due to the efficient proximity effect between the *S*-NO thioguanosine and the target dC or d<sup>m</sup>C in the duplex.

As 5 and 6 could not be separated by HPLC, the peak containing 5 and 6 was isolated. Their nitrosyl species were analyzed by a method established by the Nagano group,<sup>[4]</sup> in which the diaminofluorescein derivative (DAF2) reacts with the NO<sup>+</sup> species to generate fluorescent DAF2-triazole. The increase of DAF2-triazole obtained from the isolated peak including 5 and 6 correlates well with the conversion of 4 to 3.

Since nitrosylation of an amino group of a nucleoside is reported to cause deamination,<sup>[1]</sup> we next analyzed deaminated products. After the NO transfer reaction with ODN (5; X = dC or d<sup>m</sup>C), the mixture was kept under acidic conditions, and the peak of ODN (6; X = dC or d<sup>m</sup>C) was isolated by HPLC. A portion of the isolated ODN (6) was subjected to enzymatic hydrolysis with VPDE and BAP and was analyzed by HPLC. In addition to the major peaks corresponding to dA, dG and dC, dC-diazoate and dU (for ODN 6; X = dC) or d<sup>m</sup>C, d<sup>m</sup>C-diazoate, and dT (for ODN 6; X = d<sup>m</sup>C) were observed.

Table 1 summarizes the fates of the nitrosylated dC or d<sup>m</sup>C, showing specific deamination, especially of d<sup>m</sup>C. Another portion of the ODN (6) isolated after acidic treatment was analyzed with DAF2 to show that the NO level decreased to 80% for X = dC and to 50% for d<sup>m</sup>C compared to those analyzed immediately after NO-transfer reaction. These results suggest that NO-species in ODN (6) remain after acidic treatment and spontaneously return to dC or d<sup>m</sup>C when the ODN is hydrolyzed to nucleosides.

**TABLE 1** The Fates of the Nitrosylated dC or d<sup>m</sup>C after Enzyme Digestion

				
R=H <sup>a</sup>	78%	0%	14% <sup>c</sup>	8% (dU)
R=Me <sup>b</sup>	45%	0%	13% <sup>c</sup>	42% (dU)

<sup>a</sup>The NO-transfer reaction mixture was kept at pH 5 for 4 d.

<sup>b</sup>The NO-transfer reaction mixture was kept at pH 5 for 1 d in the presence of CaCl<sub>2</sub>.

<sup>c</sup>The peak of HPLC was identified by comparison with the authentic sample.<sup>[5,6]</sup>

## CONCLUSION

We have developed the innovative method for nitrosyl group transfer to dC and d<sup>m</sup>C with sequence and base specificity under mild conditions.<sup>[7]</sup> The selectivity and efficiency of NO transfer followed by deamination exhibited in this study are extremely high compared to the conventional methods of using NO gas or other nitrosating agents. Investigation on effects of the nitrosylated dC in DNA polymerization reactions is now ongoing in our group.

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