

# Chemical Aminoacylation of RNA by an Intermolecular Adenosine Transfer Reaction

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RNA aminoacylation, based on an adenosine transfer mechanism, was achieved by the intermolecular transfer of phenylalanyl adenosine from a donor probe to an acceptor RNA. This method can be applied to a wide variety of unnatural amino acids for tRNA aminoacylation.

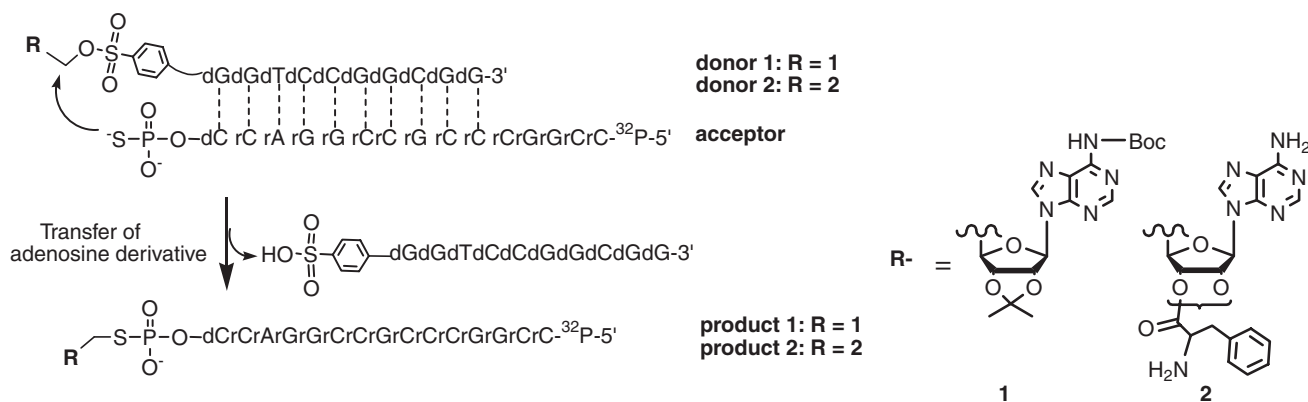
tRNA aminoacylated with unnatural amino acids has become a powerful tool for the site-specific incorporation of unnatural amino acids into proteins. This has been used for various applications, such as the investigation of protein–protein interactions, conformational changes, and signal transduction.<sup>1–8</sup> The synthesis of aminoacylated tRNA has been reported by several research groups. These approaches can be divided into two main classes: enzymatic and nonenzymatic methods. Among the enzymatic methods, Hecht's group first reported a general strategy for the preparation of aminoacylated tRNA, in which T4 RNA ligase attaches an aminoacylated pCpA derivative to tRNAs lacking a 3'-terminal dinucleotide.<sup>4</sup> Suga's group reported RNA ribozymes that catalyze the aminoacylation of tRNAs with phenylalanine derivatives as substrates.<sup>5</sup> Mutated aminoacyl tRNA synthetase catalyzing the aminoacylation of phenylalanine derivatives has been developed independently by Schultz's group and Yokoyama's group.<sup>6,7</sup> Although each of these techniques has some advantages, they also have some restrictions in their ability to selectively aminoacylate tRNA, or in their applicability to a wide range of substrate amino acids and tRNA. Recently, Sisido's group developed a novel nonenzymatic method for the aminoacylation of tRNA, which can be applied to a wide variety of unnatural amino acids. The method is based on an aminoacyl transfer mechanism using a peptide nucleic acid (PNA) probe.<sup>8</sup>

Here, we propose a new nonenzymatic strategy for tRNA-specific aminoacylation, which is based on an adenosine transfer mechanism. This can be applied to a wide range of unnatural

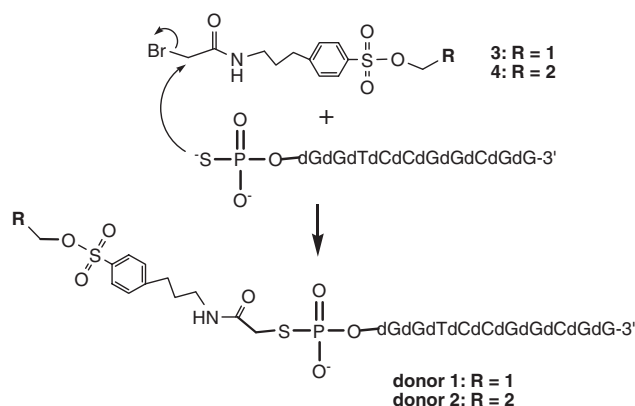
amino acids. The transfer chemistry is a nucleophilic substitution reaction, which has formerly been used for the chemical ligation of nucleic acids.<sup>9–12</sup> In the present study, we designed the model system shown in Scheme 1, to confirm the transfer of aminoacylated adenosine **2** from the donor probe to the acceptor RNA in the chemical reaction. When tRNA lacking a 3'-terminal adenosine is used as the acceptor, the aminoacylation of the tRNA can be achieved.

The structures of the donor probe and the acceptor RNA used are shown in Scheme 1. The donor consists of three parts: an oligonucleotide (DNA), a linker, and an adenosine derivative ( $R = \mathbf{1}$  or  $\mathbf{2}$ ). The donor probe binds to the acceptor to form a DNA–RNA double strand before the transfer reaction. The adenosine derivative is linked to the deoxyoligonucleotide by an electrophilic linker containing a benzenesulfonyl group, which makes the 5'-hydroxy group of the adenosine (**1** or **2**) reactive to nucleophilic substitution. The acceptor consists of an RNA strand, except for deoxycytidine at the 3'-terminus, which has a phosphorothioate group. When the donor and acceptor are hybridized, the phosphorothioate group of the acceptor attacks the 5'-carbon center of the adenosine in the donor probe. The transfer of the adenosine derivative then proceeds from the donor probe to the acceptor RNA.

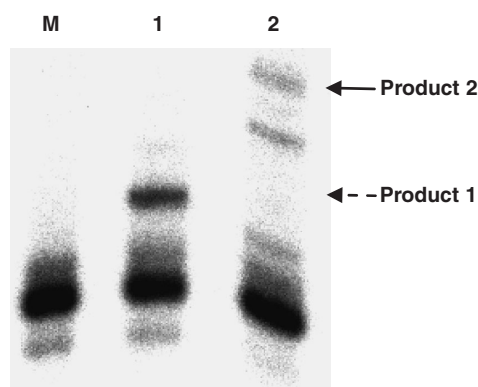
First, the donor probe was synthesized according to Scheme 2. Key compounds **3** and **4** were designed to contain two electrophilic reactive centers, a bromoacetyl group and a benzenesulfonyl group. The reactivity of the bromoacetyl group in nucleophilic substitution is significantly higher than that of the benzenesulfonyl group. Therefore, when the phosphorothioate probe is mixed with compound **3** or **4**, the bromoacetyl group selectively reacts with the thioxide anion of the phosphorothioate group, generating the donor probe (Scheme 2). The synthesis of the protected adenosine derivative **3** and the phenylalanyl adenosine derivative **4** is described in the Supporting Information. Both compounds were synthesized from commercially available



**Scheme 1.** Principle of intermolecular transfer of adenosine derivative from donor to acceptor.



Scheme 2. Synthesis of adenosine donor.



**Figure 1.** Acidic nonnatural polyacrylamide gel electrophoresis analysis of the transfer of the adenosine derivative from the donor DNA to the acceptor RNA ( $^{32}\text{P}$ -labeled at the 5' end). Lane M, acceptor RNA only; lane 1, transfer of adenosine derivative (1) from donor 1 to the acceptor; lane 2, transfer of aminoacylated adenosine (2) from donor 2 to the acceptor. Reaction conditions: [donor 1/2] = 10  $\mu\text{M}$ , [acceptor] = 100 nM, [Tris-borate pH 6.5] = 70 mM,  $[\text{MgCl}_2]$  = 10 mM,  $[\text{NaCl}]$  = 100 mM, in 20  $\mu\text{L}$ , at 35  $^\circ\text{C}$ , for 2 h.

2',3'-*O*-isopropylideneadenosine.<sup>13</sup> The coupling reaction of the 5' phosphorothioated oligonucleotide with 3 or 4 was carried out at room temperature.<sup>14</sup> After reaction for 1 h, the reaction mixture was extracted twice with *n*-butanol, and then extracted with chloroform to isolate the donor probe, which was used immediately in the transfer reaction without additional purification.<sup>15</sup>

The transfer reaction was carried out by hybridizing the donor probe with the acceptor RNA and incubating them at 35  $^\circ\text{C}$  for 2 h (Scheme 1). The reaction was analyzed by acidic polyacrylamide gel electrophoresis.<sup>16</sup> The acceptor RNA was labeled with  $^{32}\text{P}$  for the trace. The results are shown in Figure 1. The band in lane M shows the acceptor. Lane 1 contains donor 1 ( $R = 1$ ). The band indicated by the dashed arrow indicates adenosine derivative 1 successfully transferred from donor 1 to the acceptor, resulting in the formation of Product 1. In lane 2, the transfer of the phenylalanine-acylated adenosine 2 from donor 2 to the acceptor is also observed. The band indicated by the solid arrow shows Product 2, with phenylalanine at its terminus.<sup>17</sup> Although aminoacylation was achieved by the transfer reaction, hydrolysis of the amino acid from Product 2 also occurred, resulting in the formation of a hydrolyzed product, in the band visi-

ble between Product 2 and the acceptor. Therefore, the amount of Product 2 formed was less than that of Product 1.<sup>18</sup> To improve the conversion of the transfer reaction, optimization of the structure of the linker in the donor, as well as the reaction conditions, is proceeding.<sup>19</sup>

In conclusion, the aminoacylation of RNA was achieved using an adenosine transfer method. The phenylalanyl-adenosine transferred from the donor DNA to the acceptor RNA was confirmed. By using tRNA lacking a 3'-terminal adenosine, instead of the acceptor RNA, the chemical aminoacylation of tRNA by unnatural amino acid is possible.

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- 13 Detail of synthesis is described in Supporting Information.<sup>19</sup>
- 14 Reaction was carried out under the following conditions: 20  $\mu\text{M}$  5'-phosphorothioated oligonucleotide, 10 mM 3 or 4, 40% DMF in 160 mM triethylammonium acetate (TEAA) buffer (pH 6.7) containing 1 mM dithiothreitol (DTT).
- 15 Donors 1 and 2 were characterized by MALDI-TOF mass spectrometry. On the mass spectral chart for donor 2, apart from a peak derived from donor 2 (Obsd:  $m/z$  3833.2), a by-product (Obsd:  $m/z$  3686.0) was also detected. This indicates that the phenylalanine was hydrolyzed partially from donor 2 (Figure S1).<sup>19</sup>
- 16 The transfer reaction was analyzed on 20% polyacrylamide gel containing 7.5 M urea at 4  $^\circ\text{C}$  in acidic buffer (pH 4.95) containing 100 mM Tris-acetate and 2.5 mM EDTA.
- 17 Detailed analysis of transfer reaction indicated that Product 2 was aminoacylated transfer product (Figure S2).<sup>19</sup>
- 18 The yield of product 2 is about 5%.
- 19 Supporting Information is also available electronically on the CSJ-Journal website, <http://www.csj.jp/journals/chem-lett/index.html>.