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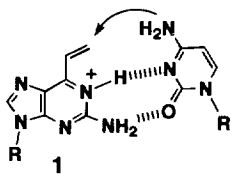
6-Vinylated Guanosine as a Novel Cross-Linking Agent and its Versatile Synthesis from the 6-O-Tosylate by Pd(0)-Catalyzed Cross-Coupling

Fumi Nagatsugi, Kengo Uemura, Shouji Nakashima, Minoru Maeda and Shigeki Sasaki*

Faculty of Pharmaceutical Sciences, Kyushu University
3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan

Summary 6-Vinylated guanosine (**1**) was designed as a novel cross-linking agent, and synthesized by a new Pd(0)-catalyzed cross-coupling reaction using guanosine 6-O-tosylate and vinyltributylstannane. Its potential as a cross-linking agent was demonstrated by adduct formation with guanosine and cytidine at the 7-N and 4-N positions, respectively.

Selective complexation of "antisence" oligonucleotides with complementary nucleotides (RNA or duplex DNA) have become a new biological method for inhibition of gene expression, and its *in vivo* applications have attracted great attention for therapeutic use.[1] Such applications require stabilization of the complementary duplex or triplex complexes, which has been attempted through sequence-specific covalent cross-linking by antisence oligonucleotides with an alkylating group.[1-5] However, it has been pointed out that most of such modified oligonucleotides have afforded slow cross-linking,[2] and that the use of a reactive alkylating group is not suitable for *in vivo* use because of its chemical instability.[3] Thus, recent studies have focused on the development of new cross-linking agents applicable to *in vivo* use.[4] We designed a 6-vinylated guanosine analog (**1**) as a new cross-linking agent, with the following characteristic: 1) conjugated vinyl group may act as an alkylating group, 2) reactivity and stability of the vinyl group may be adjustable with an additional functional group, and 3) cross-linking is expected to occur at a matched base pair of guanosine-cytidine. We describe here that the 6-vinylated guanosine analog (**1**) has potential as a new cross-linking agent as demonstrated by adduct formation between **1** and cytidine or guanosine in the presence of an acid catalyst. In addition, the versatile synthesis of **1** *via* a new Pd(0)-catalyzed cross-coupling reaction between guanosine 6-O-tosylate and vinyltributylstannane is also described.



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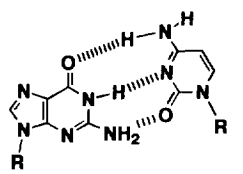

Fig. 1. Expected Complexation Between Protonated Guanosine Derivative and Cytidine

Natural G-C Pair

The protonated form of the 6-vinylated guanosine analog (**1**) is expected to form a complex with cytidine to bring vinyl and amine groups into proximity (Fig.1), although hydrogen bondings may be weaker than those in the normal G-C pair. A planar and small vinyl group would not destabilize the complementary complex when it is incorporated into the oligonucleotides.

We found that 6-O-triflate afforded 6-vinylated compounds (**1**) in good yields by the $Pd(PPh_3)_4$-catalyzed reaction with vinyltributylstannane in the presence of LiCl.[6] Interestingly, 6-O-tosylates were found to be superior substrates in this cross-coupling reaction under the same conditions (Table 1). Because 6-O-tosylates can be easily prepared without 2-amino protection, this coupling reaction may become a versatile method for the synthesis of the 6-alkylated guanosine derivative.[7] Some examples of the synthesis of guanine derivatives are summarized in Table 1. The cross-coupling reaction did not take place when $PdCl_2(PPh_3)_2$ was used as a catalyst.

Scheme 1

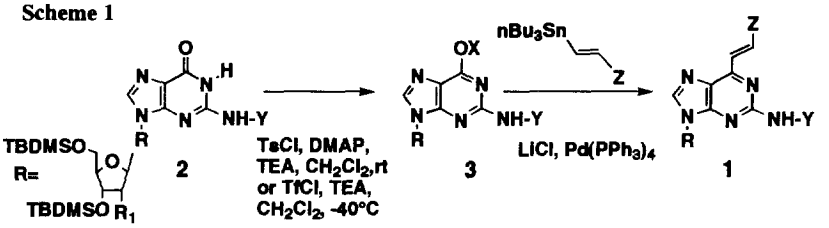

Table 1. Cross-Coupling Reaction with $Pd(PPh_3)_4$ as a Catalyst[a]

Substrate				Conditions	Yield (%)
R1	X	NHY	Z		
TBDMSO	Tf	NHAc	H	toluene, 60°C, 24 h	45
TBDMSO	Tf	NHAc	H	dioxane, 100°C, 0.75 h	74
TBDMSO	Tf	$N(Boc)_2$	H	dioxane, 100°C, 3 h	70
TBDMSO	Ts	NH_2	TMS	dioxane, 100°C, 1 h	70
TBDMSO	Ts	NH_2	H	dioxane, 100°C, 3 h	82
H	Ts	NH_2	H	dioxane, 100°C, 3 h	94

a) A typical procedure: a mixture of guanosine 6-O-tosylate (**3**), $Pd(PPh_3)_4$, and LiCl in dioxane was stirred for 30 minutes followed by the addition of vinyltributylstannane. The mixture was heated at 100°C in an argon atmosphere for 3 hours. The reaction mixture was diluted with AcOEt and successively washed with 10% aqueous NH_3 and brine.

We then investigated nucleophilic addition to the terminal olefin of 6-vinyl guanosine (**1**) (Scheme 2). It was found that the alkylation occurred in the presence of an acid catalyst such as CSA (camphor sulfonic acid), although no adduct was observed in the absence of the catalyst. PPTS (pyridinium *p*-toluenesulfonate) could also be used as the acid catalyst, but it gave

somewhat slower reaction rates. The adducts were isolated and the structures were determined by 1D-, 2D-NMR, MS/MS FAB and high resolution FAB mass spectra. The reactivity was in the order of thiol>amine>alcohol, the same as the order of nucleophilicity (entries 1-3). Interestingly, nucleobases showed a different reactivity toward **1**. Adducts were formed with cytidine at N4 (entry 4, Fig.2A) and with guanosine at N7 (entry 5, Fig.2B). But alkylation did not occur with adenosine and thymidine (entries 6 and 7). Thymidine stabilized **1** probably due to base-pairing with **1** (Fig.2C). Adducts formation seemed to attain equilibrium, since the adduct-to-**1** ratios did not change after 4 hours, and the isolated adducts were decomposed to the mixture of each component in solution in the presence of CSA. These results clearly suggest that **1** may become a new cross-linking agent. In addition, because it is known that alkylation of guanosine at N7 causes depurination,[5] **1** is also expected to be applicable to oligonucleotides for sequence specific depurination.

Scheme 2. Nucleophilic Attack to 6-Vinyl Guanosine (1)

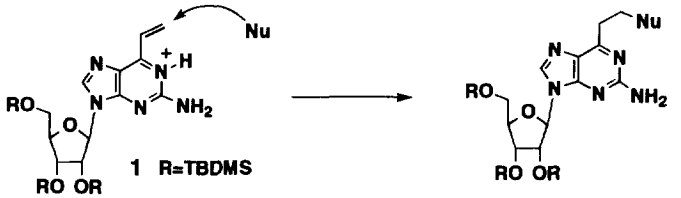

Table 2. Adduct Formation Between **1** and Several Nucleophiles[a]

Entry	Nucleophile	Time (hour)	Adduct (%)[b]	Recovered **1** (%)[b]
1[c]	L-Cys-OMe•HCl	1	90	0
2	aniline	1	90	0
3[d]	MeOH	30	44	0
4[e]	Cytidine	4	17 (30)	41 (70)
5[e]	Guanosine	4	6 (35)	10 (65)
6	Thymidine	24	no adduct	quantitative
7	Adenosine	24	no adduct	decomposition

a) The reaction was performed using 0.08 M each of **1** and nucleophile in CH_2Cl_2 in the presence of 0.8-1.0 equivalent of camphor sulfonic acid (CSA). b) Isolated yields. Data in parenthesis indicate the ratios obtained by ^{1}H-NMR. c) The reaction was carried out in ethanol solution in the absence of CSA. d) Methanol was used as solvent. e) Repetitive purification lowered the isolated yields.

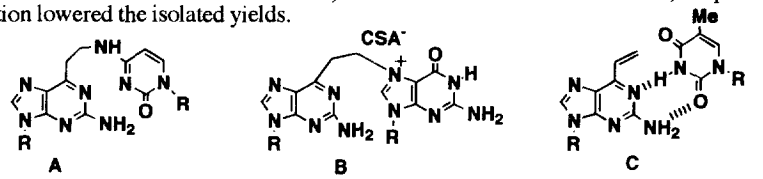

Fig. 2. The Structure of Adducts with Cytidine (**A**), Guanosine (**B**) and a Possible Base-Pairing with Thymidine (**C**).

In conclusion, we synthesized 6-vinyl guanosine (**1**), and successfully demonstrated its potential as a new cross-linking agent by achieving adduct formation with cytidine and guanosine in the presence of an acid catalyst. Acceleration using an acid catalyst may be beneficial, since the major and minor grooves of DNA are in acidic environments.[8] Further study is on going for sequence specific cross-linking and depurination using oligonucleotides incorporated with **1**.

Acknowledgments

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