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Synthesis and properties of 2-azidodeoxyadenosine and its incorporation into oligodeoxynucleotides

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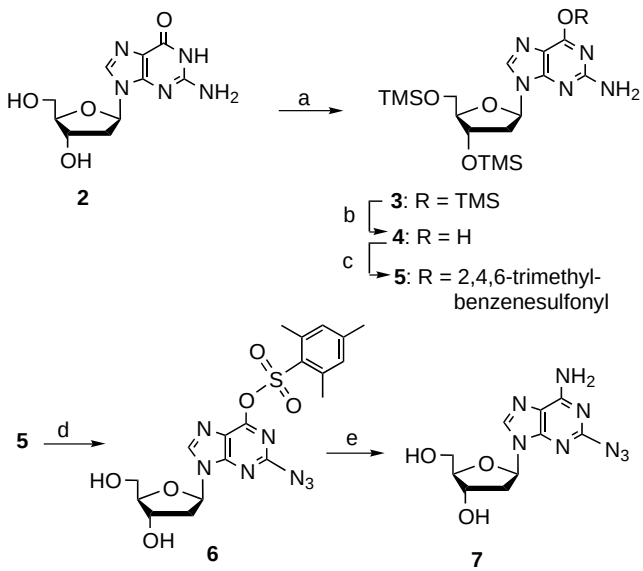
Abstract—2-Azidodeoxyadenosine (**7**) was conveniently synthesized from deoxyguanosine (**2**) by use of a combined reagent of $TMSN_3$–BuONO. The structure of the tautomer of the azido derivative was determined by ^1H NMR. Reaction of **7** with $iPr_2NP(OEt)_2$ gave an intermediate **10** of the Staudinger reaction. Incorporation of **7** into a DNA 13mer resulted in a significant decrease of the T_m value of the DNA duplex upon hybridization with the complementary strand. The thermal stability was discussed based on the hydrogen bond energy and electrostatic repulsion.

Oligonucleotides containing a nucleoside derivative capable of photo-crosslinking have proved to be useful for determination of the precise site of interaction between protein–DNR/RNA[1] and RNA–RNA.[2] A variety of photo-crosslinking reactions using oligonucleotides containing thionucleoside derivatives such as 4-thiouridine and 6-thioguanosine have been reported.[3] On the other hand, azido-nucleoside derivatives, which are expected to be photo-activated, have not been utilized at the oligonucleotide level at all.[4] Only examples of 6-azidopurine nucleoside 5′-di- and tri-phosphate derivatives have been studied in connection with the mechanism of biological reactions associated with kinases and ATPases.[5]

Recently, Higashiya et al. reported a new method for the synthesis of 2-azidoadenosine (**1**) by treatment of a 2-amino-6-chloropurine riboside derivative with $TMSN_3$ in the presence of butyl nitrate.[6] This effective reaction provides a new possibility that 2-azido-2′-deoxyadenosine could be obtained by a similar reaction and used as a deoxynucleoside component that can be incorporated into DNA and activated selectively by photo-irradiation. Here, we report the synthesis of 2-azidodeoxyadenosine (**7**) and its chemical and structural properties as well as its incorporation into oligodeoxynucleotides.

We found an effective method for the synthesis of **7** from 2′-deoxyguanosine (**2**). The reaction of **2** with hexamethyldisilazane in the presence of a catalytic amount of Me_3SiCl in acetonitrile gave a fully trimethylsilylated species **3**. It turned out that addition of ethanol to the mixture resulted in 3′,5′-*O*-



Scheme 1. (a) HMDS (3.0 equiv.), TMSCl (0.05 equiv.), CH_3CN, rt, 1 h; (b) EtOH, rt, 1 h; (c) MsCl (1.3 equiv.), Et_3N (3.0 equiv.), DMAP (0.1 equiv.), CH_2Cl_2, rt, 48 h; (d) (1) $TMSN_3$ (10 equiv.), BuONO (10 equiv.), CH_2Cl_2, –20°C to rt, 24 h, (2) H_2O–MeOH (1:1, v/v), rt, 1 h; (e) conc. NH_3 (20 equiv.), dioxane, rt, 60 h.

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bis(trimethylsilyl)deoxyguanosine (**4**). In situ treatment of **4** with mesitylenesulfonyl chloride gave the 6-*O*-mesitylated species **5** in 83% yield (Scheme 1).

The reaction of **5** with 10 equiv. each of trimethylsilyl azide and butyl nitrite in CH_2Cl_2 followed by hydrolysis gave the product **6** in 45% yield. Further treatment of **6** with conc. NH_3 gave **7** in 89% yield.

It was reported that 2-azidoadenosine **1a** exists in equilibrium with two tricyclic tautomers **1b** and **1c** having a tetrazole ring (Fig. 1).[7]

However, the ^{1}H NMR spectrum of the deoxy counterpart **7** suggested that **7** exists in equilibrium between the actual azide derivative **7a** and a tricyclic tetrazole derivative **7b** or **7c**. At 20°C the ratio of the two tautomers was 6:4, as shown in Fig. 2. When the temperature increased to 100°C, the ratio changed to 9:1. It has been generally recognized that, in the azide–tetrazole equilibrium, higher temperatures favor the azide species.[8] Therefore, the enriched tautomer was determined to be the azido derivative **7a**. The ^{1}H NMR spectrum of **7** showed that there is only a slight difference (0.14 ppm) in the chemical shift between the anomeric protons of **7a** and the teterazole form which appeared at 6.26 and 6.40 ppm, respectively. This result suggested that the tetrazole species is the one described as **7b**, since it is expected that, if **7c** were present, the chemical shift of the anomeric proton of **7c** should be significantly shifted to a low-magnetic field compared with that of **7a** because the tetrazole ring approaches very close to the anomeric proton so that a considerable deshielding effect is expected.

This conclusion was also supported by the following theoretical calculation: the ab initio MO calculations of the tautomers **8a**–**c** of 9-methyl-2-azidoadenine were carried out at the level of MP2/6-31G*.[9] The tetrazolo[5,1*a*]-9-methyladenine derivative **8b** is 0.46 kcal/mol stable than the tetrazolo[5,1,*b*]-9-methyladenine derivative **8c**.

The detailed analysis of the thermodynamic parameters in the equilibrium between the azido form **7a** and tetrazole form **7b** was carried out by ^{1}H NMR. The thermodynamic parameters ΔH, ΔS, and ΔG were calculated to be 3.89, 1.44×10^{-2}, and –0.44 kcal/mol (27°C).

To test if 2-azidodeoxyadenosine can be incorporated into DNA, several chemical properties of this compound were studied. It was found that **7** was stable under basic conditions such as conc. NH_3–pyridine (9:1, v/v) at room temperature for 48 h and conc. NH_3–EtOH (3:1, v/v) at room temperature for 48 h. On the other hand, the glycosidic bond of **7** was found to be considerably stable under acidic conditions: (1) $t_{1/2}=$ 4 h, $t_{comp}=24$ h in 3% dichloroacetic acid in CH_2Cl_2; (2) $t_{1/2}=1$ h, $t_{comp}=8$ h in 1% trifluoroacetic acid in CH_2Cl_2. Compared with these results, *N*-benzoyldeoxycytidine showed less stability of $t_{1/2}<5$ min and $t_{comp}=$ 30 min in 3% dichloroacetic acid in CH_2Cl_2 and $t_{1/2}<5$ min and $t_{comp}=10$ min in 1% trifluoracetic acid in CH_2Cl_2.

In the current chemical synthesis of DNA, two approaches are available.[10] One is the phosphoramidite method.[11] The other is the *H*-phosphonate approach.[12] Since it is well known that azide compounds easily react with tervalent phosphorus compounds to give iminophosphorane derivatives,[13] we examined the compatibility of **7** with phosphoramidite reagents which have been used as deoxynucleotide building blocks in the phosphoramidite approach. As a model compound, diethyl *N*,*N*-diisopropylphosphoramidite (**9**) was chosen for this study.

When compound **7** was allowed to react with 1.5 equiv. of **9** in DMF–CD_3CN (9:1, v/v), a new resonance signal at 34.0 ppm in the ^{31}P NMR spectrum appeared at the initial stage, as shown in Fig. 3. After 6 h, this peak disappeared and a new resonance signal at 13 ppm was observed as the main peak. The FAB mass of the mixture was analyzed. Consequently, it was found that the initial product has a molecular weight of 513 and the final product has a molecular weight of 485. From these results, we concluded that the initial product is the actual intermediate **10** of the Staudinger reaction, as shown in Scheme 2.[13] The final product is the iminophosphorane type product **11** (Fig. 3).

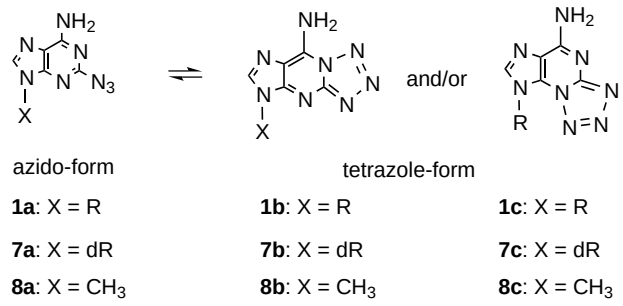


Figure 1. Tautomerism of 2-azidoadenosine (**1a**), 2-azido-2′-deoxyadenosine (**7a**), and 9-methyl-2-azidoadenine (**8a**).

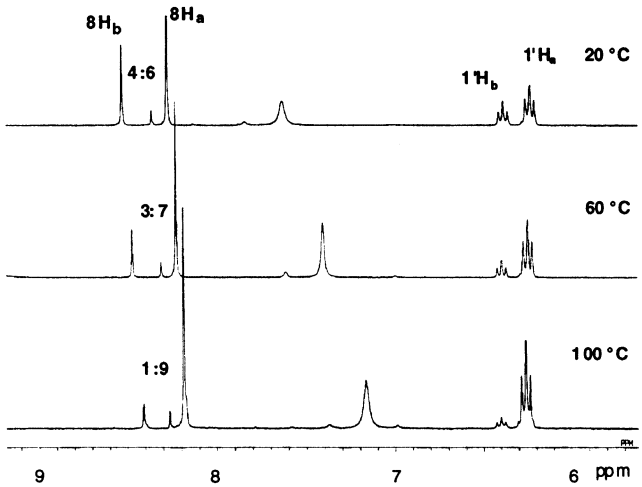
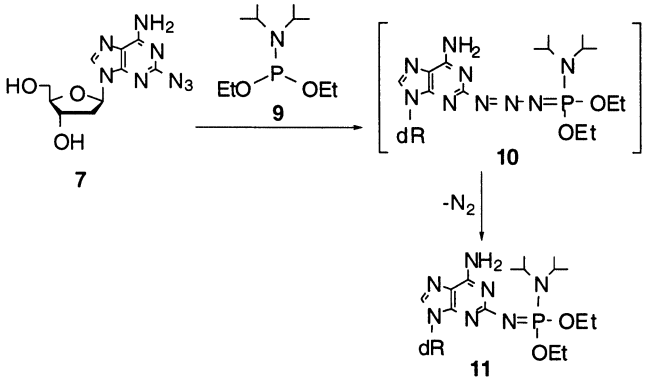


Figure 2. ^{1}H NMR spectra of a tautomeric mixture of 2-azido-2′-deoxyadenosine **7a** and its tetrazole derivative **7b** at various temperatures in DMSO-d_6.



Scheme 2. Reaction of 2-azidodeoxyadenosine (**7**) with diethyl *N*,*N*-diisopropylphosphoramidite (**9**) in DMF–CD_3CN (9:1, v/v).

From the viewpoint of the synthesis of oligodeoxynucleotides incorporating **7**, these results indicated that it is impossible to synthesize 2′-azidodeoxyadenosine 3′-phosphoramidite derivatives required for the standard phosphoramidite approach. On the other hand, the *H*-phosphonate method involves the use of pentavalent *H*-phosphonate building blocks. Quite recently, we have demonstrated that the 2-azidobenzoyl group can be used as the base protecting group that is compatible with the *H*-phosphonate approach.[14] Therefore, we tried to synthesize 2-azido-2′-deoxyadenosine 3′-*H*-phosphonate building block **13** (Scheme 3).

Reaction of **7** with 4,4′-dimethoxytrityl chloride in pyridine gave the 5′-*O*-tritylated product **12** in 84% yield. Reaction of **12** with 7 equiv. of diphenyl phosphonate in pyridine followed by hydrolysis gave the *H*-phosphonate building block **13** in 77% yield without damage to the azide function.

Condensation of **13** with 3′-*O*-acetylthymidine (**14**) in the presence of BOP-Cl[15] (2 equiv.) in pyridine at room temperature for 1.5 h gave the *H*-phosphonate dimer **15** in 88% yield, as shown in Scheme 4. This product was oxidized by I_2 treatment, and the protecting groups were removed by successive treatments with 80% AcOH and conc. NH_3–pyridine. Thus, the fully deprotected dimer **16** was obtained in an overall yield of 43%. This product showed a characteristic peak (2155 cm^{-1}) of the azido group in its IR spectrum and a single resonance peak at −0.38 ppm in its ^{31}P NMR spectrum. The structure of the dimer was also confirmed by ^1H and ^{13}C NMR (Fig. 4).

These results strongly suggested that it is possible to synthesize oligodeoxynucleotides containing **7** by the *H*-phosphonate method. Therefore, we synthesized $d(T_6A^{N3}T_6)$ on a T-loaded CPG gel support by the *H*-phosphonate method using a powerful condensing reagent BOMP.[16] This modified 13mer was isolated in 22% yield by anion exchange HPLC. Hybridization of the 13mer with the complementary $d(A_6TA_6)$ resulted in a sharp drop ($\Delta T_m = -7.4°C$) of the T_m value down

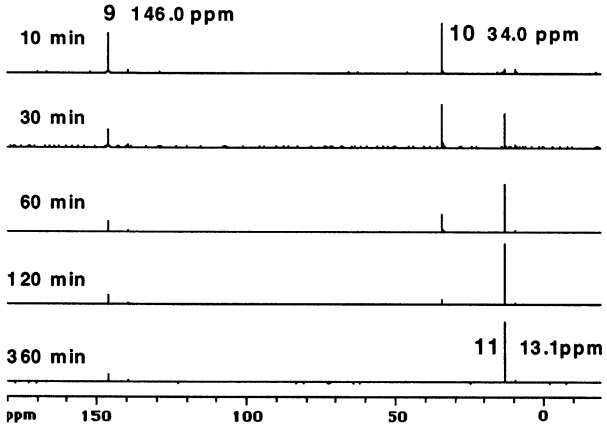
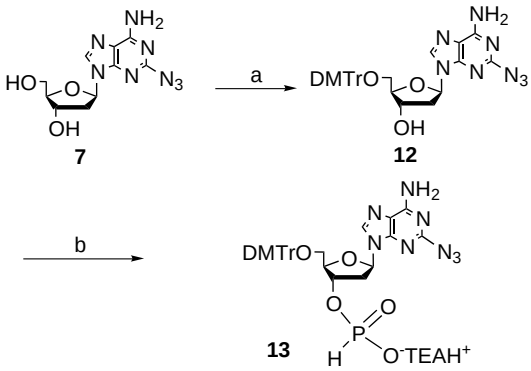
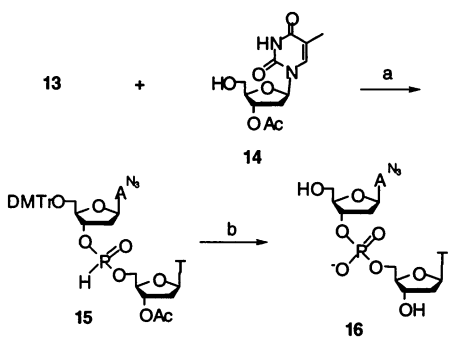


Figure 3. ^{31}P NMR analysis of the reaction of 2-azidodeoxyadenosine (**7**) with diethyl *N*,*N*-diisopropylphosphoramidite (**9**) in DMF–CD_3CN (9:1, v/v).



Scheme 3. Synthesis of 2-azidodeoxyadenosine 3′-*H*-phosphonate unit. (a) DMTrCl (1.2 equiv.), pyridine, rt, 1 h; (b) diphenyl phosphonate (7 equiv.), pyridine, rt, 1.5 h; (c) H_2O–Et_3N (1:1, v/v), rt, 1 h.



Scheme 4. Synthesis of 2-azidodeoxyadenosine 3′-*H*-phosphonate unit **13** and 2-azido-2′-deoxyadenyl(5′-3′)thymidine (**16**). (a) BOP-Cl (2.0 equiv.), pyridine, rt, 1.5; (b) (1) 2% I_2, pyridine–H_2O (98:2, v/v), rt, 1 h, (2) conc. NH_3–pyridine (9:1, v/v), rt, 1 h.

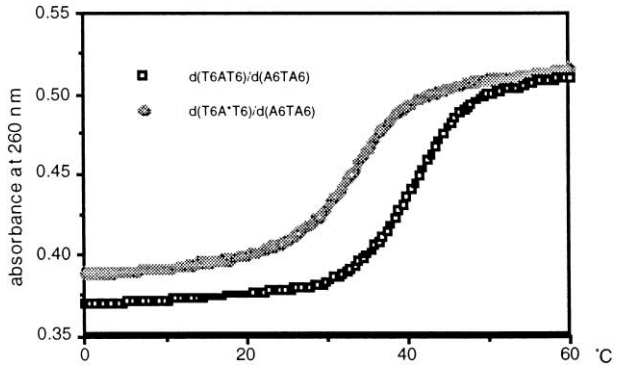


Figure 4. Melting temperature curve of $d(T_6AT_6)/d(A_6TA_6)$ duplex. Conditions: 10 mM $NaHPO_4$, 1 M NaCl, 0.1 mM EDTA (pH 7.9), conc. of an oligonucleotide 2.0 mM.

to 33.7°C compared with the normal DNA duplex $d(T_6AT_6)/d(A_6TA_6)$, which had a T_m value of 41.1°C.

This destabilization reflects formation of an unfavorable base pair between T and A^{N3}. Theoretically, we also calculated the hydrogen bond energy ΔE of this unfavored base pair at the level of MP2/6-31G*[9] using 2-azido-9-methyladenine and 1-methylthymine. As a result, the hydrogen bond energy was calculated to be −10.63 kcal/mol. The hydrogen bond energy of 9-methyladenine and 1-methylthymine was also calculated to be −12.50 kcal/mol. Therefore, the effect of the 2-azido group on the destabilization of the hydrogen bonding is estimated to be 1.87 kcal/mol. Moreover, the electron density of the nitrogen attached to C2 was estimated to be −0.515e. Even if the sterically favorable 2-azidoadenine base can form a base pair with the thymine base, this local charge is responsible for the considerable destabilization of the modified base pair since there must be electrostatic repulsion between this nitrogen and the carbonyl oxygen of the 1-methylthymine. If this is not the case, it is obvious that the destabilization observed is essentially due to the presence of the tricyclic tetrazole form like **7b** that cannot form a base pair with the thymine base (Fig. 5).

In conclusion, the present results strongly imply that oligonucleotides containing 2-azidoadenosine derivatives would be generally possible by use of the *H*-phosphonate method. RNAs containing its ribonucleoside counterpart would be more important in molecular biology since much attention has been paid to clarification of RNA–protein interaction.[2] The chemical synthesis of such modified RNAs would be realized in the near future using the present method. During this study, we were also able to detect the intermediate of the Staudinger reaction. This observation is noteworthy in phosphorus chemistry.

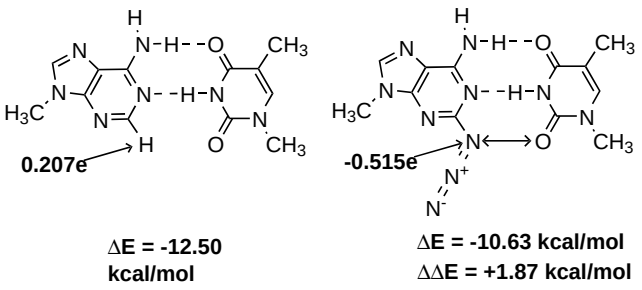


Figure 5. Hydrogen bond energy of the base pair formed between 2-azido-9-methyladenine and 1-methylthymine calculated at the MP2/6-31G*//HF/6-31G* level.

Acknowledgements

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