Synthesis and X-Ray Crystal Structure of a Thymidine Derivative

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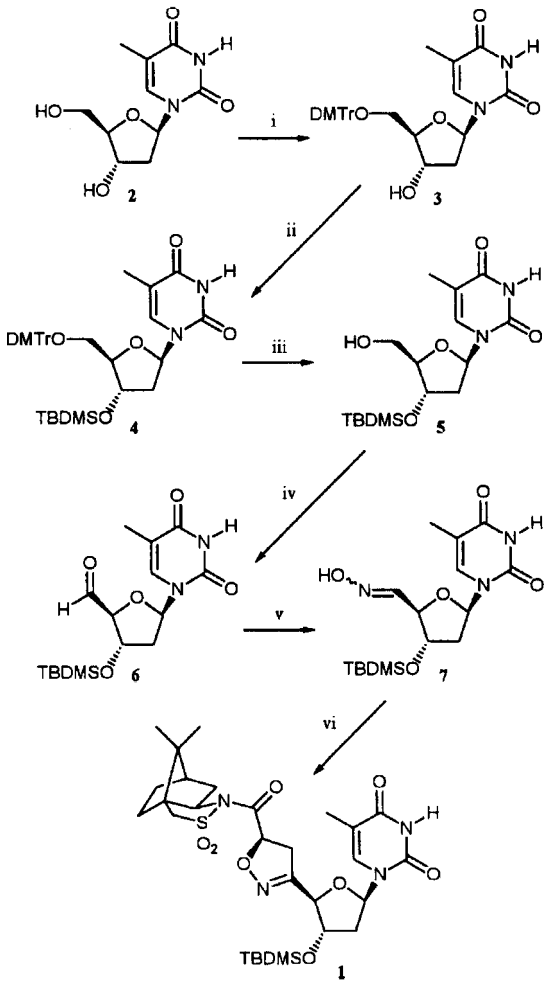
Received April 25, 1998

There have been numerous research activities in antisense oligonucleotides for the treatment of diseases at the level of gene expression.[1] For the antisense oligonucleotides with heterocyclic isoxazoline linkage,[2] we have synthesized thymidine derivative **1** from thymidine as shown in Scheme 1. Selective protection of 5'-hydroxyl group with 4,4'-dimethoxytrityl (DMTr) group followed by silylation of 3'-hydroxyl group with *t*-butyl dimethyl silyl chloride provided the fully protected compound **4**. Selective deprotection of 5'-position with $ZnBr_2$, and the modified Moffatt oxidation[3] with 1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) gave the desired aldehyde **6**. This aldehyde was further reacted with hydroxylamine hydrochloride to give the mixture of *syn* and *anti* oxime **7**. Diasteroselective cycloaddition of *in situ* generated nitrile oxide from the oxime **7** to *N*-acryloyl (2*R*)-bornane-10,2-sultam afforded 90 : 10 mixture of compound **1** and its isoxazoline diastereomer.[4] It was essential to treat the crude cycloadducts with methyl sulfide to improve the yield. Otherwise the *N*-chlorinated side product at thymine base was isolated in *ca.* 25% yield. Recrystallization from dichloromethane/diethylether/*n*-hexane (1 : 1 : 1) solution gave an enantiomerically pure compound **1**.

X-ray diffraction-grade single crystals of compound **1** were grown by slow solvent evaporation of an EtOH/EtOAc solution of the thymidine derivative. The resulting X-



Scheme 1. *Reagents and conditions*: i, DMTrCl, triethylamine, DMAP, Py, 93%; ii, TBDMSCl, diisopropylethylamine, DMF, 97%; iii, $ZnBr_2$, $CHCl_3$/MeOH (9 : 1), 89%; iv, EDC, DMSO, Py, Trifluoroacetic acid; v, NH_2OH·HCl, Na_2CO_3, MeOH/H_2O (1 : 1), 70% (iv and v, overall); vi, 1) 0 °C, NaOCl, N-acryloyl (2*R*)-bornane-10,2-sultam, CH_2Cl_2, 2) CH_3SCH_3, 3) recrystallization, 72%.

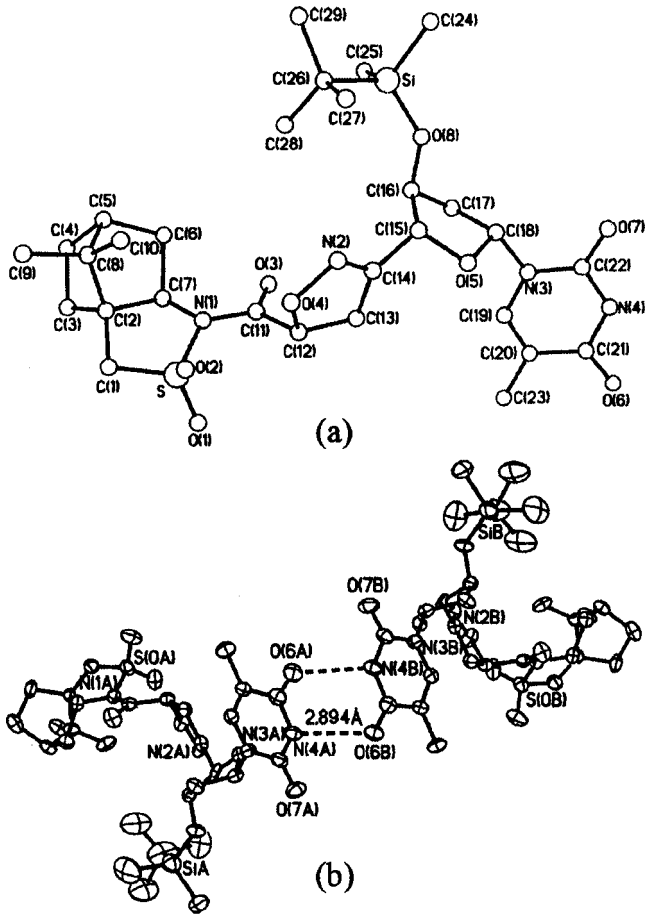


Figure 1. (a) The asymmetric unit of compound **1** with atomic numbering scheme. (b) the dimeric structure of self-assembled **1**.

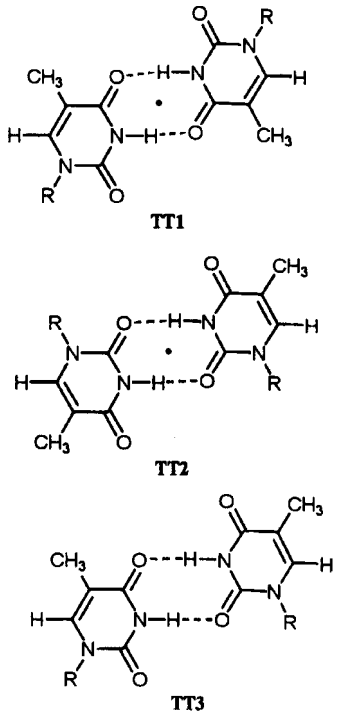


Figure 2. The possible dimeric arrangements of 1-substituted thymines.

ray crystal structure[5] (Figure 1) reveals a rare example of self-association in the crystal structure of nucleosides.[6] The number of crystal structures in which self-base pairing occurs is relatively small compared with the number of possibilities. Among the possible dimeric arrangements (Figure 2) of 1-substituted thymine, the **TT1** centrosymmetrical cyclic dimer was mainly formed in the case of 1-methyl thymine.[7]

Molecules of compound **1** are linked *via* N-H···O hydrogen bonds to produce dimers across centers of symmetry (Figure 1(b)). The distance between N and O is 2.894 (0.017)Å, which is slightly longer than the reported values (2.841 Å and 2.830 Å) of 1-methyl thymine[7] that is closely related to the nucleoside thymidine. As found in most crystal structures involving thymine and uracil derivatives, oxygen O (6) is favored in hydrogen bond formation rather than oxygen O (7). This may be attributable to the higher double bond character of C (22)-O (7) than C (21)-O (6). The preference to centrosymmetrical configuration in thymine-thymine base pairs is due to the antiparallel orientation of the dipole moments.[6] This results in a favorable cancellation of the total electric field over the crystal volume.

X-ray crystallographic study of thymidine derivative **1** clearly indicates that self-assembly with thymine-thymine base pairing is still applicable in thymidine nucleosides and cyclic dimerization occurs based on the hydrogen bonding between N (4)-H···O (6). However, this type of cyclic self-assembly was not observed in the crystal structure of the thymidine itself.[8] For thymidine, the carbonyl and N-H groups, which undergo Watson-Crick hydrogen bonding in DNA, are found to hydrogen bonded to 3' or 5' hydroxyl groups of the sugar moiety. Thus 3' and 5'-hydroxyl groups of sugar play an important role in formimg intermolecular hydrogen bonds. Because both 3' and 5'-hydroxyl groups of the sugar are protected in the thymidine derivative **1**, only self-assembly through hydrogen bonding between thymine-thymine base is possible and observed in the crystal structure. For the favorable homo base pairs with centrosymmetrical configuration in thymidine nucleoside, both 3' and 5'-hydroxyl groups should be modified appropriately.

In summary, a thymidine derivative **1** has been synthesized from thymidine in 6 steps (40.5% overall yield) and X-ray crystallographic study of compound **1** shows that this thymidine derivative forms a centrosymmetrical cyclic dimer through hydrogen bonding between thymine-thymine bases.

Acknowledgment. This research was supported by the Ministry of Education (BSRI 97-3437) and POSTECH (1RB9711501). We thank Professor Kimoon Kim and Dr. Dongmok Whang for the X-ray crystallography and helpful comments.

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5. *Crystal data* for **1**: $C_{29}H_{44}N_4O_8SSi$, M=636.83, crystal system: monoclinic, space group: *C2*, *a*=24.491(5) Å, *b*=7.705(2) Å, *c*=22.131(4) Å, β=118.52(3)o, *V*=3520 (1) Å^3, *Z*=4, d_{calc}=1.202 g cm^{-3}, *T*=296 K, Enraf-Nonius CAD4 diffractometer, Mo Ka (*l*=0.71073 Å), *m*=1.75 cm^{-1}. Structure was solved by Patterson method (SHELXS-86). All nonhydrogen atoms were refined anisotropically (SHELXL-93). Final full matrix least squares refinement on F^2 with all 2341 reflections and 388 variables converged to *R1* ($I > 2s(I)$)=0.096, *wR2* (all data)=0.2966 and GOF=1.075.
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