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Practical syntheses of antiviral nucleosides

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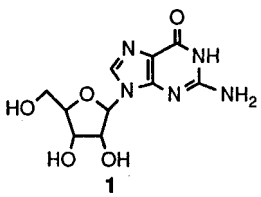
Abstract: Guanosine produced by fermentation is one of the nucleosides most readily available on an industrial scale. We have recently developed several processes leading to known antiviral agents starting with guanosine. The processes involve enzymatic transglycosylation for stavudine (d4T), chemical transpurination for acyclovir and ganciclovir, and novel alkylation for penciclovir and famciclovir.

Introduction

5'-Guanylic acid (Guanosine 5'-monophosphate, 5'-GMP) is one of the nucleotides widely distributed in nature and has been isolated from hydrolyzates of yeast RNA in 1911 (ref. 1). Since the disodium salt of 5'-GMP was recognized as a good flavor enhancer (ref. 2) and later found to be a major flavor component of shiitake mushroom (*lentinus edodes sing.*), much attention has been paid to the industrial process for 5'-GMP. Combining a biological process for the production of guanosine **1** and a direct phosphorylation process of the unprotected nucleoside, an industrial process for 5'-GMP was commercialized in Japan. Having **1** available in quantity at a reasonable cost, we envisioned establishing a practical process for manufacturing the major known antiviral nucleosides such as stavudine **2**, acyclovir **3**, ganciclovir **4**, penciclovir **5** and famciclovir **6**. In order to realize this, we carried out extensive studies on enzymatic transglycosylation, chemical transpurination and novel N9-selective alkylation of guanine derivatives. Here we wish to report the practical syntheses of antiviral nucleosides starting with **1**.



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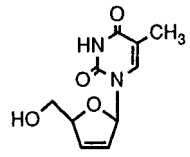

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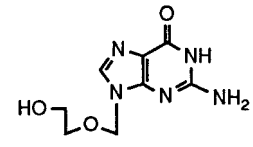

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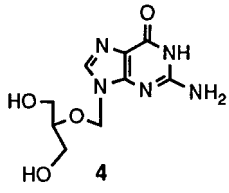

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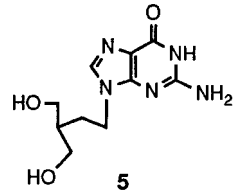

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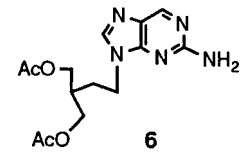

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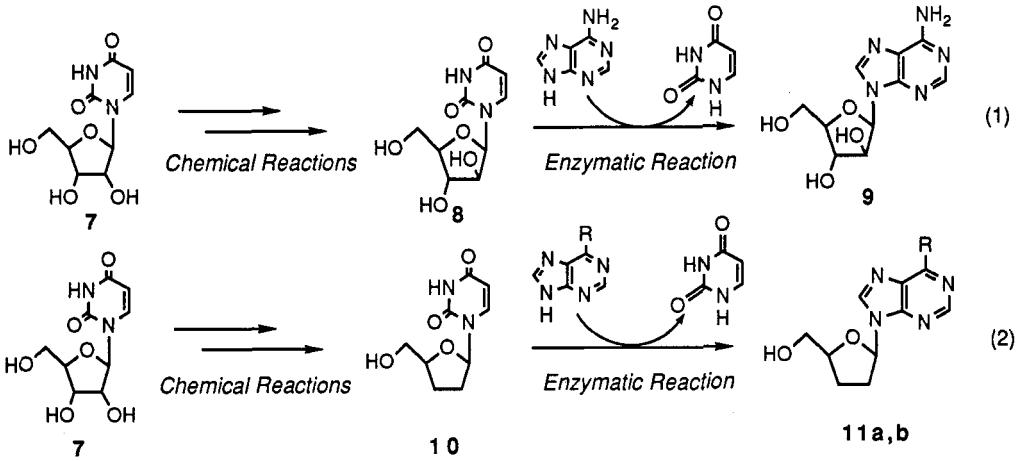
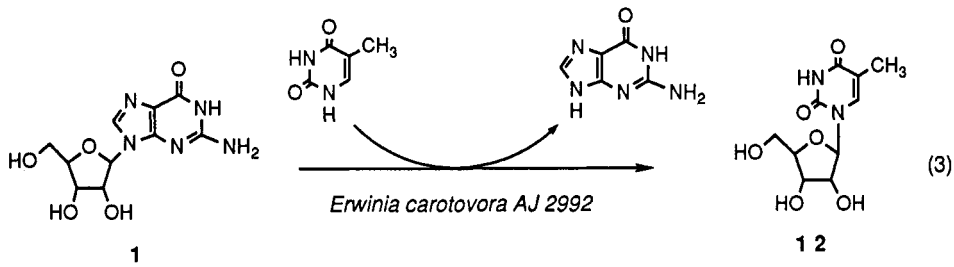
Synthesis of stavudine (d4T, 2) *via* enzymatic transglycosylation.

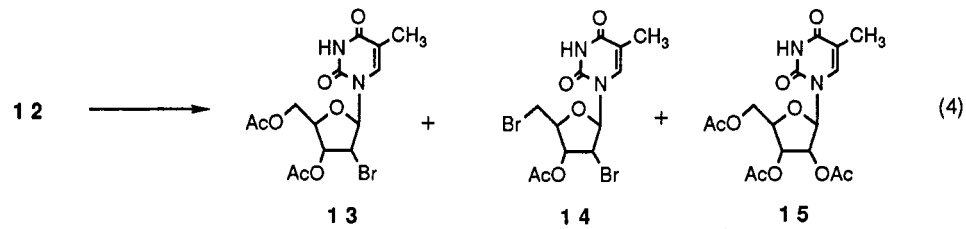
Enzymatic transglycosylation reactions were extensively studied by Utagawa *et al.* in these laboratories (ref. 3). Utilizing this enzymatic process, Ara-A (adenine arabinoside, **9**) has been commercially produced from Ara-U (uracil arabinoside, **8**) as a sugar donor and adenine as a sugar acceptor (eq. 1, ref. 4). Enzymatic transglycosylation was also applicable to the synthesis of 2',3'-dideoxynucleosides, i.e. ddU **10** was converted into ddA **11a** (R=NH_2) and ddI **11b** (R=OH) (eq. 2, ref. 5).



Stavudine (d4T, **2**) was recently approved as an effective treatment for HIV (human immunodeficiency virus) infection. There are several known methods for the production of **2** from thymidine (ref. 6). However, thymidine may be considered as an inappropriate starting material due to its relatively high price and low availability from natural sources such as salmon sperm. Aiming at a more economical synthesis of **2**, we selected 5-methyluridine **12** as an alternative starting material as it is easily prepared by enzymatic transglycosylation using guanosine as a sugar donor and thymine as a sugar acceptor (eq. 3, ref. 7).

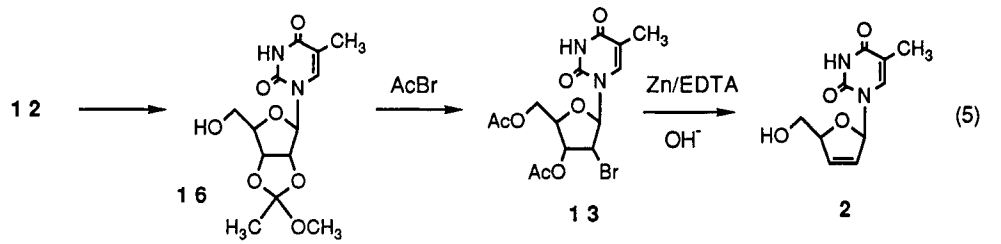


Previously Mansuri *et al.* reported an efficient and convenient method to synthesize **2** from **12** prepared by chemical coupling of silylated thymine and 1-*O*-acetyl-2,3,5-tri-*O*-benzoylribose (ref. 8). In order to make a key intermediate, 1-(3,5-di-*O*-acetyl-2-bromo-2-deoxy-β-D-ribofuranosyl)thymine **13**, they utilized the direct reaction of **12** with acetyl bromide according to Marumoto's method (ref.9). Although we carefully re-examined the same reaction conditions including Chu's improvement method (ref.10), the bromoacetate **13** could not be obtained in a high yield (maximum 63%) and the 5'-brominated compound **14**, the triacetate **15** and thymine were found as major by-products (eq. 4). The formation of **14** was found to be troublesome because it finally gave 5'-bromo-5'-deoxy-d4T which could not be removed effectively by suitable industrial purification methods such as extraction and recrystallization.



We postulated that the formation of **14** might be formed from the 5'-hydroxy intermediate either by direct bromination or indirectly *via* the O^2-5'-anhydro compound at a rather high reaction temperature (80°C). The reaction of **12** with acetyl bromide apparently produces water *in situ* which may prevent acetylation of the 5'-position and consequently cause the formation of **14**. Based on this hypothesis, we investigated an alternative indirect route to the synthesis of **13** which consists of the methoxyethlidenation of **12** followed by acetoxybromination under milder conditions. We anticipated that the reaction of the methoxyethylidene compound **16** with acetyl bromide dose not produce water so that the formation of **14** should be diminished. **12** was reacted with trimethyl orthoacetate in acetic acid. The methoxyethylidene derivative **16**

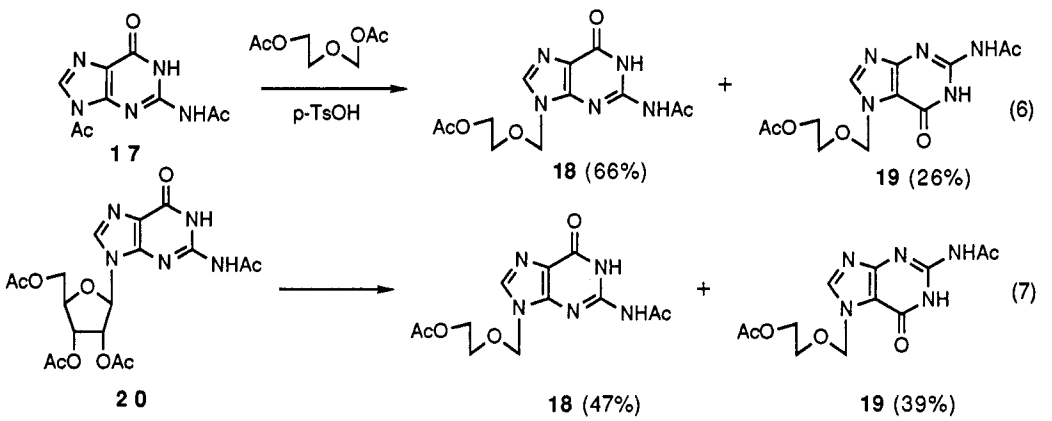
was isolated from the reaction mixture although it could be used for further reaction simply after concentration. The residue was then reacted with acetyl bromide and/or HBr/AcOH at a lower temperature (50°C). As a result, **13** was obtained in a high yield and the formation of **14** was successfully reduced (0 - 1.3%). The best result (90%) was obtained by the application of Chu's conditions (AcBr and HBr/AcOH) to the methoxyethylidene derivative **16**.



Several methods for reductive β-elimination of bromoacetate nucleosides have been reported (ref. 11). Considering the question of scaling up, we adopted a zinc mediated reductive elimination process due to the mildness of the reaction and the ease of waste treatment. The reductive elimination proceeded smoothly by adding zinc powder to **13** and afforded more than a 90% yield of 5'-*O*-acetyl-d4T. After completion of the reaction, the mixture became homogeneous and was treated with aqueous EDTA sodium salt yo remove zinc. The d4T acetate thus obtained was then hydrolyzed with aqueous NaOH, purified with a resin and crystallized from water to give d4T, **2** in a 72% yield from **13** (eq. 5, ref. 12). The four step reaction sequence described above gave **2** in a 65% overall yield from **12** as opposed to 45% overall yield using Mansuri's direct acetoxybromination approach.

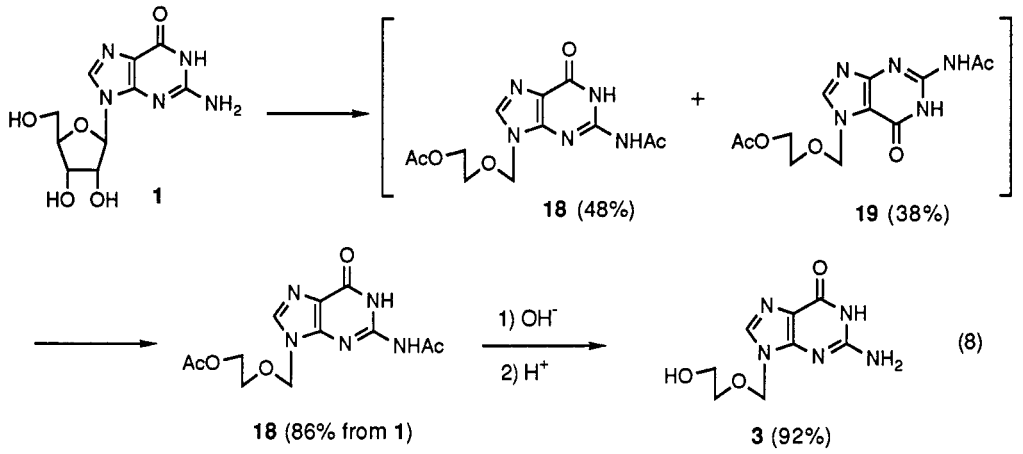
Synthesis of Acyclovir 3 *via* Chemical Transpurination followed by Isomerization.

Acyclovir **3** is an orally active acyclic nucleoside with inhibitory activity against herpes viruses which is used widely throughout the world. Several methods for the synthesis of acyclovir have already been reported (ref. 13). Amongst these, one of the most convenient and efficient methods is the one which involves the condensation reaction of N,N'-diacetylguanine **17** with (2-acetoxyethoxy)methyl acetate in the presence of p-toluenesulfonic acid (eq. 6, ref. 14). The drawbacks in this procedure are the accompanying formation of considerable amounts of N7-isomer **19** and the difficulty of its separation by an industrial process. Boryski and Golankiewicz studied the transpurination of tetraacetylguanosine **20** and obtained 9-(2-acetoxyethoxymethyl)-N^2-acetylguanine **18** and its 7-isomer **19** in a ratio of 9/7 = 47/39 (eq. 7). It is of interest that the same authors observed that **19** isolated by chromatography could be transformed in a similar ratio (9/7=55/45) of the mixture applying thermal isomerization in chlorobenzene at 230°C (ref. 15).



In order to establish a more economical process for the synthesis of acyclovir **3**, we intended to use guanosine **1** as a starting material and find out the conditions leading to the exclusive formation of the desired 9-isomer **18**. After several unsuccessful attempts, we examined the reaction of **1** with (2-acetoxyethoxy)methyl acetate in acetic anhydride as a solvent and in the presence of p-toluenesulfonic acid as a catalyst. As a result, it was found that diacetylacyclovir (**18** and **19**) was obtained in a satisfactory yield (86%) as a mixture of 9/7 isomers in a ratio of 1.45. Surprisingly, the ratio was much improved to 17.1, after concentrating the reaction mixture and then heating the residue at 100°C under reduced pressure for 24 hours. The isomerization conditions were considerably milder than those reported by Boryski *et al.*

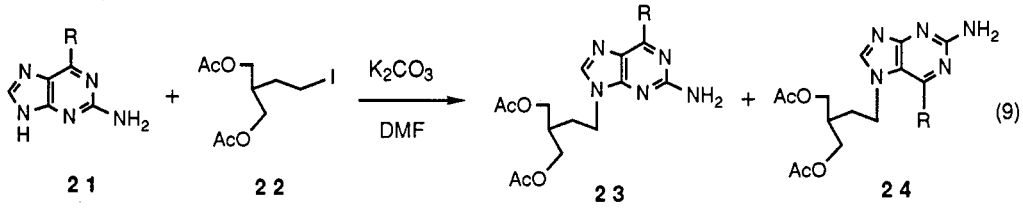
By adding an appropriate solvent such as ethyl acetate and acetonitrile to the residue, almost pure **18** (9/7 = 120) was obtained in an 86% yield from **1**.



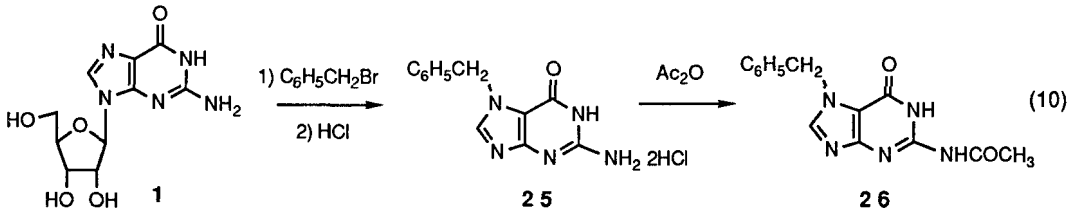
Since the catalyst in the synthesis of the acyclic sugar moiety could be the same as that used in the transpurination, the one pot reaction of **1** with 1,3-dioxolane and acetic anhydride in the presence of acid catalyst gave similar results. Treating **18** with aqueous NaOH followed by neutralization with hydrochloric acid gave acyclovir **3** in a 92% yield to a high purity, which means that the overall yield of **3** from **1** was 78% (eq. 8, ref. 16). It was confirmed that the method is also satisfactorily applicable to the synthesis of ganciclovir **4** which is used for clinical treatment of cytomegalovirus infection in immunodeficient patients.

Novel Regioselective Synthesis of 9-Alkylated Guanine Derivatives.

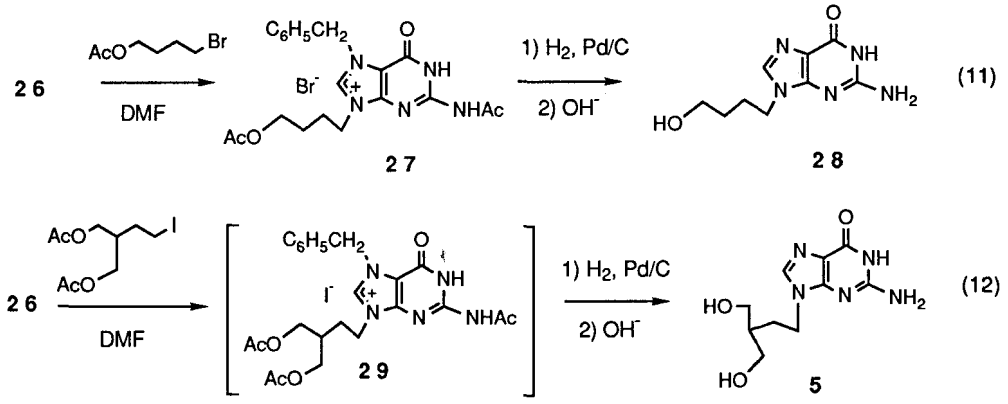
Since the first report describing that acyclovir has potent activity against herpes virus, acyclic nucleoside analogs have been the focus of much attention in the development of new antiviral therapeutic agents (ref. 17). Among them, penciclovir **5** and its orally active form famciclovir **6** were recently approved as new types of anti-hepesvirus agents. Since **6** has a 2-aminopurine nucleus which is easily transformed from the guanine nucleus, we first attempted to develop new methodology for the synthesis of 9-alkylated guanine derivatives, in particular that of penciclovir **5**. Although the structure of **5** is closely related to ganciclovir **4**, oxygen in the side chain of **4** is replaced with methylene in **5**. Accordingly, the transpurination-isomerization method employed for the synthesis of acyclovir **3** and ganciclovir **4** by us can not be applied to the synthesis of **5**. In order to obtain acyclic guanine nucleoside derivatives, 2-amino-6-substituted purine **21**(often 2-amino-6-chloro-: R=Cl) is commonly used for alkylation (ref. 18). A problem associated with this method is that the alkylation is rarely regiospesific, and although the desired N9 isomer **23** is the major product, the N7 isomer **24** is also formed to a significant extent (eq. 9). From an industrial viewpoint, it should be noted that there is another serious disadvantage using 2-amino-6-halo-purine **21** (R=halogen) as a starting material. Namely, the synthesis of **21** is not so efficient because tedious operational steps and a rather low yield render the cost high.



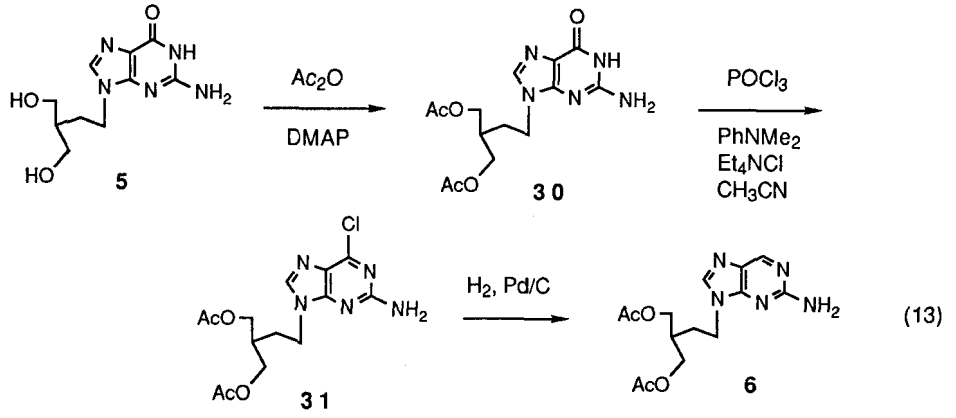
Guanosine **1** can be considered as N9-protected guanine. It is well known that **1** is alkylated at the 7-position under neutral conditions and that the ribose moiety is then readily removed by acid treatment to give N7-alkylguanine. We thought that it might be possible to obtain N9-alkylated guanine exclusively, if the alkylation of N7-alkylguanine selectively takes place at the N9-position and then the N7-alkyl group is selectively removed. Thus, N7-benzylguanine **25** was selected as our starting material since the N7-benzyl group could be selectively removed by catalytic hydrogenolysis after the alkylation reaction. **25** was obtained in more than a 90% yield from **1** according to the modification of reported procedure (eq.10, ref. 19).



The alkylation of **25** by 4-bromobutyl acetate did not proceed smoothly probably due to its low solubility in solvent (DMF). The reaction of N^2-acetyl-7-benzylguanine **26**, however, gave rise to N^2-acetyl-7-benzyl-9-(4-acetoxybutyl)guanine **27** as a bromide salt in an 87% yield. After completion of reaction, the product was easily obtained by simply adding ethyl acetate and filtering. Deprotection of the 7-benzyl group was achieved by catalytic hydrogenolysis in MeOH to give N^2-acetyl-9-(4-acetoxybutyl)guanine which was further treated with aqueous NaOH to give 9-(4-hydroxybutyl)guanine **28** in a 94% yield (eq. 11). It is worthy of note that the product was not contaminated with the 7-isomer at all. It was confirmed that the reaction sequence took place in a highly regioselective manner. Furthermore, it was found that the alkylation of **26** and the reductive removal of benzyl group proceeded in one pot. A similar reaction sequence using 2-acetoxymethyl-4-iodobutyl acetate afforded penciclovir **5** in a 75% yield in one isolated step from **26** (eq. 12).



Conversion of **5** to famciclovir **6** was rather straightforward. **5** was first acetylated with acetic anhydride and subsequently chlorinated with $POCl_3$ to give the 6-chloro derivative **31**. It should be noted that the chlorination of diacetylpenciclovir **30** went on more smoothly than that of guanine under similar conditions. The final transformation of **31** into famciclovir **6** was achieved in a highly efficient manner according to the reported procedure (eq. 13, ref. 20).



This approach should offer new routes to a variety of 9-substituted guanine and purine derivatives which are under development in several pharmaceutical companies.

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