A Simple Solution to the Age Old Problem of Regioselective Functionalization of Guanine: First Practical Synthesis of Acyclic N^9- and/or N^7-Guanine Nucleosides Starting from N^2,N^9-Diacetylguanine

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Regioselective alkylation of guanine, a long-lasting challenge, has been overcome by understanding the role of acids as catalyst in the coupling reaction of DAG **(10)** with OBDDA **(11)**. The acid-catalyzed and noncatalyzed reactions of **10** with OBDDA which mainly give N^7 and N^9 isomers, respectively, appear to follow different mechanisms. The practical utility of the noncatalyzed reaction, which gives almost quantitative yields of N^9 derivatives, is demonstrated by synthesizing acylovir/gancyclovir in high yields.

Guanine is one of the five important bases of life (DNA and RNA). However, because of its polyfunctionality and amphoteric nature, which is accompanied by its extremely poor solubility in most solvents, this molecule is difficult to functionalize. Direct alkylation or glycosylation of N^2- or N^2,N^9-protected guanines always produce N^9/N^7 isomeric mixtures that are difficult to separate.[1−7] Since regioselective N^9 alkylation of guanine precursors is the primary route to clinically effective antiviral drugs such as 9-[2-(hydroxyethoxy)methyl]guanine[2] (acyclovir, **1**) and 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine[8] (gancyclovir, **2**), which are active against viral diseases caused by herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), varicella zoster virus (VZV), and the human cytomagalo virus,[9] studies toward achieving high regioselectivity in this transformation have been the subject of considerable interest and intensive investigation in recent years. Selective N^7 alkylation of guanine is also important for understanding the factors responsible for achieving high regioselectivity as well as for synthesizing N^7 analogues for evaluating as drug candidates. For example, 2-amino-7-[(1,3-dihydroxy-2-propoxy)-methyl]purine (**3**), the 6-deoxy derivative of the N^7 regioisomer of gancyclovir, has recently been found to display excellent antiherpes action.[10]

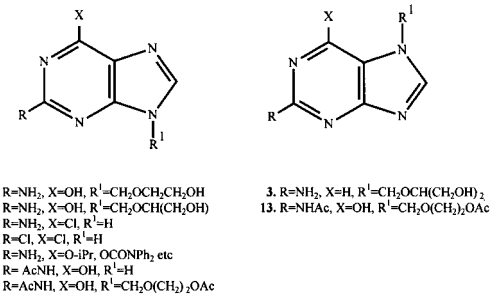

Figure 1.

Several multistep conversions which include alkylation of 2-amino-6-chloropurine[11] (**4**), 2,6-dichloropurine[2d] (**5**), and 6-enolate derivatives of guanine[12] (**6**) followed by hydrolytic cleavage of intermediates to give the corresponding 6-oxo derivatives, have been employed to give enhancement in N^9/N^7 isomer ratios (Figure 1). Even better results are reported by using persilylated 6-enolates of N^2-acetylguanine[13] (**7**) and 2-amino-6-chloropurine (**4**), and its derivatives.[10,14] Vorbruggen's group demonstrated reasonably high regioselectivity in the glycosylation[15] reaction of per(trimethylsilyl)-N^2-acetylguanine (**8**) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**9**) in the presence of TMSOTf as catalyst at elevated temperature. The alkylated product after deprotection and crystallization afforded guanosine in 66%

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yield. It is, however, relevant to highlight that the synthesis of pure N^9 isomer was the result of fortuitous enhanced partitioning of the N^9 isomer from an aqueous solution of the N^7/N^9 mixture. These results were further confirmed by the studies of Dudycz and Wright[16] who later established that N^7 isomers are formed as kinetic products in glycosylation of **8** and its derivatives and mixtures rich in the more thermodynamically stable N^9 isomers are obtained upon heating. The synthesis of both N^9 ($N^9/N^7 = 6/1$) and N^7 ($N^7/N^9 = 95/1$) isomers of guanine nucleosides, in the coupling of glycosyl acetate with the same substrate (**8**), under thermodynamic (TMSOTf/1,2-DCE) and kinetic ($SnCl_4/CH_3CN$/rt) conditions, respectively, is the best example of regioselective functionalization of guanine published so far.[17] Similar studies to give variable isomer ratios have been carried out by several groups.[18,20] The present report for the first time describes reaction conditions, for the alkylation of N^2,N^9-diacetylguanine[13] (DAG, **10**) with 2-oxa-1, 4-butanedioldiacetate[7] (OBDDA, **11**), by which both N^9 and N^7 isomers, namely N^2-acetyl-9-{2-acetoxyethoxymethyl}-guanine (**12**) and N^2-acetyl-7-{2-acetoxyethoxymethyl}-guanine (**13**), can be synthesized from the same substrate (**10**) in exceptionally high regioselectivity.[21] The use of acylated guanines without further derivatization is most desirable for practical as well as economical reasons. Also the selection of OBDDA (a *seco*-analogue of acylated sugars) for detailed investigations was based on the fact that its N^9 coupling product (**12**) with **10** is the key intermediate in the acyclovir manufacture and can be considered as a model compound for studying glycosylation reactions.

Results and Discussion

Since the literature reveals the use of various solvents and acids as catalysts in similar studies, we envisaged the study of the coupling reaction of DAG with OBDDA in the absence of solvent and catalyst to probe their roles in the regioselectivity of alkylation. The reaction of DAG[22] with 1−6 molar equiv of OBDDA was started at room temperature but could proceed only after the reaction temperature was raised to 100−110 °C. The monitoring of the reaction by HPLC from the very beginning indicated the formation of both the regioisomers ($N^9/N^7 \approx$ 45/55). On the other hand, a PTSA-catalyzed coupling of **10** with OBDDA was found to be much faster and was initiated at room temperature. However, the first few minutes of the reaction monitoring showed the formation of only the N^7 isomer (**13**). This finding is in agreement with the one published by Boryski[6] on the ribosylation of DAG in the presence of PTSA.

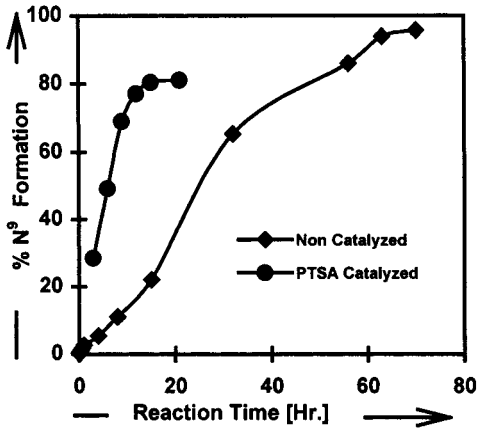

Figure 2. Plot showing comparison of N^9 isomer formation in the presence and absence of PTSA.

The rate of the noncatalyzed reaction was found to be much slower when compared with that of the PTSA-catalyzed coupling; however, both reactions gave an improved isomeric ratio of **12**/**13** at completion. The noncatalyzed reaction, which gave complete conversion on DAG after ~80 h, was found to be much superior as far as N^9/N^7 ratio (~24/1) was concerned. The observed N^9/N^7 ratio in the PTSA (0.025 molar equiv) catalyzed reaction was around 4.88/1 (see Figure 2).

The improvement in the N^9/N^7 isomeric ratio during the course of both reactions gives clear-cut evidence of the isomerization of the kinetic product **13** to **12** under thermodynamic reaction conditions. Since PTSA-catalyzed coupling initially results in the exclusive synthesis of N^7 isomer (**13**), the formation of **12** as a major product at the end of the reaction appears to be mainly due to $N^7 \rightarrow N^9$ isomerization only. However, the independent formation of the N^9 isomer (**12**) along with **13** in the absence of an acid catalyst strongly suggests that apart from the $N^7 \rightarrow N^9$ transformation, direct coupling of DAG with **11** is also taking place in this case. The latter finding gets further substantiated by the fact that the isomerization of the N^7 isomer (as studied separately on pure **13**) in OBDDA at about 100 °C is too slow to be observable in the first 10 min and even after 1 h, only ≈25% of the N^7 isomer gets converted to its thermodynamically stable counterpart[23] (see Figure 3).

Having confirmed the formation of coupling product **12** in the absence of an acid catalyst, the study to understand the effect of the concentration of PTSA as well as OBDDA on the N^9/N^7 ratio was next undertaken. As shown in Figure 2, it was observed that the higher concentrations of acid bring down the N^9/N^7 ratio substantially. The reason for this is the faster isomerization of $N^9 \rightleftharpoons N^7$ products under acid-catalyzed conditions. The rate of the coupling reaction in the presence of a large amount of OBDDA, as expected, was found to be faster[24] but resulted in a poor N^9/N^7 ratio. The same effect[7] was

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(22) **10** was perpared following Zou's condition (ref 13): mp 300 °C (dec); ^{1}H NMR (200 MHz, d_6-DMSO, 25 °C, TMS) $\delta = 2.22$ (s, 3H, N^2-$COCH_3$), 2.8 (s, 3H, N^9-$COCH_3$), 8.45 (s, 1H, H-8); ^{13}C NMR(50 MHz, d_6-DMSO, 25 °C, TMS) $\delta = 23.9$ (N^2-$COCH_3$), 24.7 (N^9-$COCH_3$), 121.5 (C-5), 137.5 (C-8), 147.9 (C-4), 148.4 (C-2), 154.7 (C-6), 168.0 (N^2-$COCH_3$), 173.8 (N^9-$COCH_3$).
(23) The transformation of the $N^7 \rightarrow N^9$ regioisomer without solvent and in the absence of an acid catalyst at 230 °C is reported to give a mixture having an isomeric ratio of N^7/N^9 = 45/55 (Boryski J.; Golankiewicz, B. *Nucleosides Nucleotides* **1989**, *8*, 529.)
(24) Heating the N^7 isomer **13** alone at 100−110 °C in solvent (DMF) with or without acid catalyst (PTSA), which leads to an insignificant amount of **12** formation, directly suggests the importance of OBDDA in $N^7 \rightarrow N^9$ transformation.

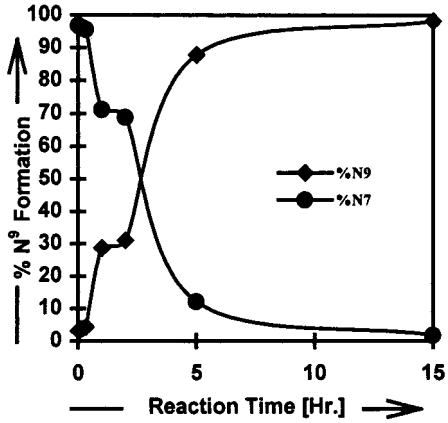
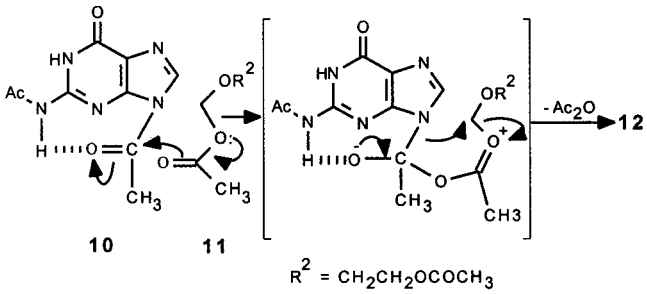


Figure 3. Plot highlighting isomerization of $N^7 \rightarrow N^9$ isomer in the absence of an acid catalyst.

Scheme 1

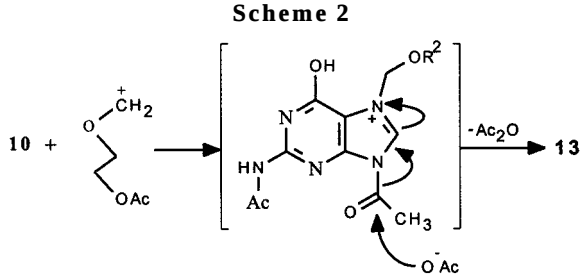


observed when this reaction was examined in the presence of solvents such as DMF/DMSO or at higher temperature (>110 °C).

The observation that the acid-catalyzed reaction proceeds with the formation of a higher ratio of N^7 isomer led us to reason that the coupling using higher concentrations of acids under kinetically controlled conditions should give a high N^7/N^9 ratio even if $N^7 \rightarrow N^9$ isomerization is not completely avoidable, which proved to be correct when a reaction of DAG with OBDDA (molar ratio 1:6) in AcOH (as solvent) was carried out in the presence of $TiCl_4$ at 25−30 °C. The N^7/N^9 ratio (4/1) observed was further improved to ≥ 9.4/1 (based on ^{1}H NMR monitoring) when a similar reaction was carried out at lower temperature (10−15 °C). The latter reaction took almost 5−6 h for ≈ 95% conversion on DAG.

It has recently been suggested that initial glycosylation of N^2,N^9-DAG (**10**) under acid-catalyzed conditions occurs at the unsubstituted imidazole ring nitrogen.[6,25] The same also appears to be true in the acid-catalyzed coupling of DAG with OBDDA under our conditions. However, since the independent formation of the N^9 isomer **12**[26] in a noncatalyzed coupling is difficult to explain following the same rationale,[25] we herein suggest a plausible mechanism for the synthesis of **12** in which activation of the electrophile is achieved with the help of the N^9-$COCH_3$ group of DAG, before giving the desired product through a six-membered transition state, as shown in Scheme 1. This mechanism receives support from an important observation that N^2-acetylguanine (**7**) under identical conditions does not react with OBDDA.

Scheme 2



The possibility that at least 1 molar equiv of AcOH can get easily generated by the hydrolytic cleavage of the N^9-$COCH_3$ group of DAG and may consequently make this transformation acid catalyzed was eliminated by performing the condensation of MAG (**7**) with OBDDA in the presence of AcOH (up to 10 molar equiv) as solvent. This reaction, as expected, did not proceed at ∼110 °C and led to the complete recovery of the starting material. Moreover, a reaction of DAG with OBDDA in the presence of 1 molar equiv of AcOH (with respect to DAG), which resulted mainly in the hydrolysis of the former to give MAG and a very small amount (∼10%) of **12**, further confirms that the reported condition is truely noncatalyzed and the N^9-$COCH_3$ group palys an important role in activating OBDDA (see Scheme 1) and giving **12** independently.

The formation of the kinetic product **13**, which is more favorable in the presence of an acid catalyst, possibly proceeds by direct alkylation of the unsubstituted imidazole ring nitrogen[6,25] with the reactive oxonium electrophile $AcOCH_2CH_2O^+{=}CH_2 \leftrightarrow AcOCH_2CH_2OCH_2^+$, generated when almost equimolar amounts of $TiCl_4$ and OBDDA are used, via an N^7,N^9-disubstituted intermediate, as shown in Scheme 2. It is difficult to directly correlate the role of the 6-enolate of DAG in giving higher N^7-regioselectivity, as observed in the $TiCl_4$-catalyzed reaction; however, a marked shift of C-5 carbon (from 121.5 to 111.3) and C-6 carbon (from 154.7 to 151.8) in the ^{13}C NMR spectrum[27] of DAG in the presence of equimolar amounts of PTSA gives an indication for the formation of the 6-enolate of DAG, under strong acidic conditions.

Almost exclusive formation of N^9 product **12**[21] at the completion of the reaction under the present condition is explainable on the basis of the observation that $N^7 \rightarrow N^9$ isomerization (see Figure 3) is almost an irreversible process in the absence of an acid, and the high temperature (100−110 °C) reaction ultimately gives the thermodynamic product (N^9 isomer) in a very high N^9/N^7 ratio (≥95:≥4).

Conclusion

In summary, we have discovered reaction conditions that for the first time allow the alkylation of DAG with OBDDA to give **12** and/or **13** in exceptionally high regioselectivity. We have also proposed mechanisms to explain the formation of N^7(**13**) as well as the N^9 isomer (**12**) from **10** in the presence and absence of an acid catalyst, respectively. The mechanistic aspects and scope of this new methodology for the synthesis of N^7- and N^9-

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(26) The independent and simultaneous formation of N^9 isomer with its kinetically controlled counterpart (**13**) was established by repeated experimentation and continuous monitoring of the reaction starting from zero hour.

(27) ^{13}C NMR (50 MHz, d_6-DMSO, 25 °C, TMS): **10** + PTSA δ = 21.4 (N^2-$COCH_3$), 24.0 (N^9-$COCH_3$), 111.3 (C-5), 138.6(C-8), 148.2 (C-4), 150.2 (C-2), 151.7 (C-6), 172.2 (OCO), 174.2 (N^2-CO).

guanine nucleosides are currently under investigation; however, it is pertinent to highlight that the procedure disclosed in this article is the best available so far for the practical synthesis of antiviral drugs such as acyclovir and gancyclovir.[21]

Experimental Section

***N*²-Acetyl-9-(2-acetoxyethoxymethyl)guanine (12).** A mixture of DAG[22] (10.0 g, 0.0425 mol) and OBDDA (18.7 gm, 0.106 mol) was heated in a RB flask at about 105 °C [bath temperature] for about 80 h until complete disappearance of DAG. The reaction mixture was concentrated under vacuum, and the residue thus obtained was column chromatographed on a SiO_2 column using CH_2Cl_2:MeOH (6:4, v/v) to give a 12.5 g (95.1%) isolated yield of **12**; mp 189−90 °C (lit.[7] mp 189−90 °C).

***N*²-Acetyl-7-(2-acetoxyethoxymethyl)guanine** (**13**). A mixture of DAG (5.0 g, 0.0212 mol), OBDDA (22.4 gm, 0.127 mol), and $TiCl_4$ (11.67 mL, 0.106 mol) in AcOH was stirred at 10−15 °C for 5−6 h to give almost complete conversion on DAG. The reaction mixture was poured in water (150 mL) and extracted with a mixture of benzene and dichloromethane (1:1). The combined extract was concentrated to give an oily residue which was column chromatographed on a SiO_2 column using a CH_2Cl_2:MeOH (6:4, v/v) solvent system to give the N^7 isomer; isolated yield 4.3 g (65.4%); mp 184−5 °C (lit.[7] mp 184−5 °C).

9-[2-(Hydroxyethoxy)methyl]guanine (1). To a solution of NaOH (3.8 g, 0.097 mol) in 100 mL of water was added **12** (10.0 g, 0.323 mol) at room temperature. The reaction mixture was heated at 85−95 °C for 3.0 h. After bringing the temperature down to room temperature, the pH of the clear solution was adjusted to 7 using 35% HCl and filtered to give 7.1 g (97.5%) of **1** of high purity, mp 256−7 °C (lit.[2d] mp 256.5−257 °C).

All the reactions described above were monitored by HPLC {column = C_{18} ODS, 150 × 3.9 mm (reverse phase); solvent system = CH_3CN:water, 20:80 adjusted to pH 2 using H_3PO_4; detector = UV 254 nm; flow rate = 2 mL/min} or TLC using silica gel 60 F_{254} coated aluminum sheets purchased from Merck. The eluent system used for running TLC plates was prepared by thorough mixing of $CHCl_3$, CH_3OH, and aqueous NH_3 in a 6:4:1 ratio followed by layer separation and discarding the upper aqueous layer.

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Supporting Information Available: 200 MHz ^{1}H NMR and 50 MHz ^{13}C NMR spectral data of compound numbers **12**, **13**, and **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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