Pergamon

Tetrahedron Letters 41 (2000) 3303–3307

TETRAHEDRON LETTERS

# An unusual solvent effect on the regiochemical outcome (N-9 versus N-7) of guanine glycosylation using Robins' reagent (2-*N*-acetyl-6-*O*-diphenylcarbamoylguanine)

Adrian Wai-Hing Cheung,* Achyutharao Sidduri, Lisa M. Garofalo and Robert A. Goodnow Jr.

*Roche Research Center, Hoffmann-La Roche Inc., Nutley, New Jersey 07110, USA*

Received 31 December 1999; accepted 3 March 2000

**Abstract**

An unexpectedly low N-9/N-7 regioselectivity was obtained when Robins' reagent (2-*N*-acetyl-6-*O*-diphenylcarbamoylguanine) was coupled with a D-glucosamine derivative under trimethylsilyl trifluoromethanesulfonate activation. An unprecedented solvent effect (toluene versus dichloroethane) on the N-9/N-7 ratio was also observed in the same study. The use of 2-*N*-acetyl-6-*O*-benzylguanine to successfully overcome the above regioselectivity problem is described. 

A novel series of glucosamine-based oligonucleotide analog **1** (GNA) was recently reported in our laboratories (Fig. 1).[1] These optically pure, conformationally constrained and amide-linked oligomers were assembled from the nucleic acid building blocks (thymine **2a**, cytosine **2b**, adenine **2c** and guanine **2d**) on solid phase using *N*-Fmoc type peptide chemistry.[2]

In the synthesis of monomers **2a**–**2d**, the key step is the coupling of D-glucosamine derivative **3** with modified nucleobases which works well for thymine **4a** (80% yield), cytosine **4b** (85% yield) and adenine **4c** (79% yield) (Fig. 2). However, the coupling of **3** with persilylated 2-*N*-acetylguanine in acetonitrile gave a 2:1 mixture of the desired N-9 (**4d**, 36–43% yield) and N-7 isomers which required chromatographic separation.[1] Herein, we report our efforts to improve the regioselectivity and yield in preparing guanine derivatives related to **4d**.

The N-9 versus N-7 regioselectivity problem in guanine glycosylation is well documented.[3] 2-*N*-Acetyl-6-*O*-diphenylcarbamoylguanine **5**, introduced by Robins,[4] has been used with much success in the preparation of cyclic and acyclic nucleosides with excellent N-9 selectivity. We were surprised when the coupling of **3** with persilylated **5** (bis(trimethylsilyl)trifluoroacetamide (BSA), $ClCH_2CH_2Cl$, 80°C) under Robins' conditions[4] (trimethylsilyl trifluoromethanesulfonate (TMSOTf), toluene, 80°C, 2 h) gave after chromatography 6% of N-9 isomer **6** and 14% of N-7 isomer **7** (Scheme 1). Significant amounts of

* Corresponding author. E-mail: adrian.cheung@roche.com (A. W.-H. Cheung)

0040-4039/00/$ - see front matter 
*PII:* S0040-4039(00)00423-8

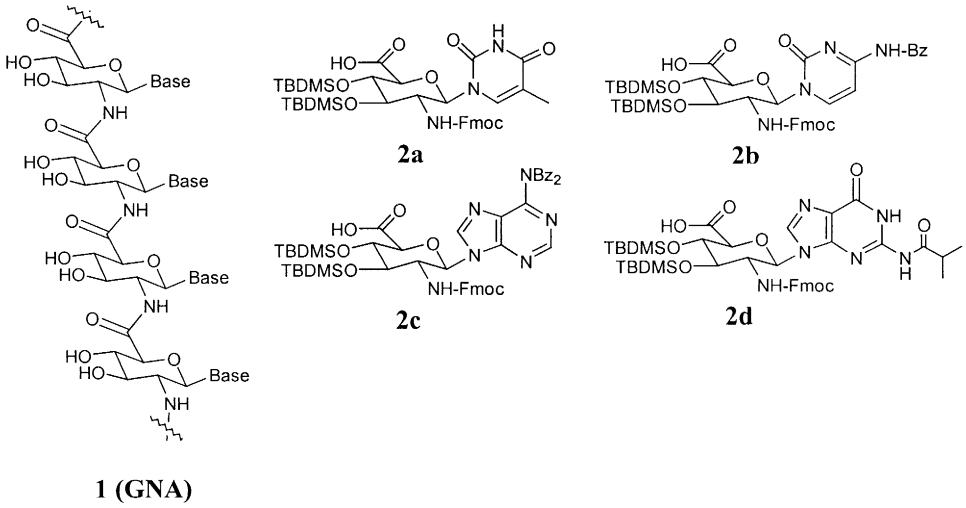



Fig. 1.

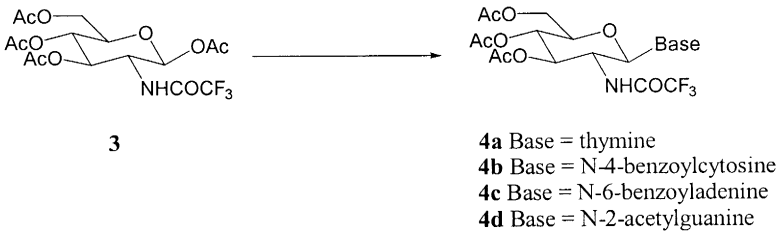
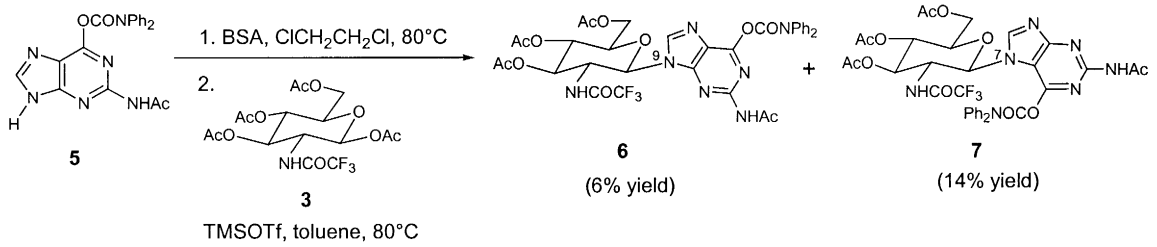



Fig. 2.

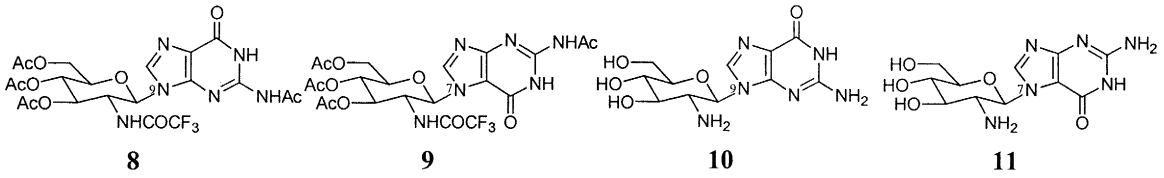
an inseparable mixture (~20–30% yield) of compounds **8** and **9** (~1:1 ratio) were also isolated from the product mixture demonstrating the lability of the C-6 diphenylcarbamoyl group of **5** under the reaction conditions. The low N-9/N-7 regioselectivity in the above coupling was in stark contrast to the excellent N-9 regioselectivity of Robins' reagent reported in the literature and in our own previous experience in the ribose series. Even more surprisingly, when the TMSOTf-mediated coupling of **3** and persilylated **5** was carried out in dichloroethane instead of toluene, 45–55% yield of **7** was isolated without any trace of **6**.[5] This is, to the best of our knowledge, the only example in the literature involving the use of Robins' reagent **5** in which a solvent switch led to a drastic change in the regiochemical outcome (N-9:N-7 ratio) of guanine glycosylation.[6–10]




Scheme 1.

When N-7 isomer **7** was heated with TMSOTf in toluene at 80°C, no trace of N-9 isomer **6** could be detected.[11] Using tin(IV) chloride ($SnCl_4$, 2 equiv., refluxing dichloroethane) in place of TMSOTf in the coupling of **3** and **5** gave only N-7 isomer **9** (the C-6 diphenylcarbamoyl group was lost) in 40–50%

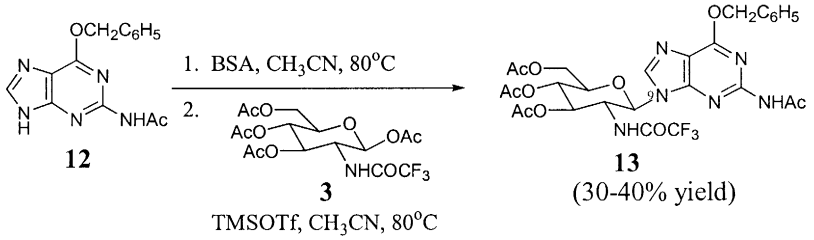
yield.[12] The N-9 assignment of **6** and N-7 assignment of **7** was established by analysis of $^{1}H$, $^{13}C$ NMR and UV spectra of the corresponding deprotected derivatives **10** and **11**, respectively (Fig. 3). In addition, an X-ray crystal structure of a derivative of **10** unambiguously confirmed its N-9 assignment.[1]



**8** **9** **10** **11**

Fig. 3.

2-*N*-Acetyl-6-*O*-benzylguanine **12**,[13,14] in which the labile diphenylcarbamoyl group at C-6 of **5** is replaced with a benzyl group, was prepared from 2-amino-6-chloropurine (step 1: NaH, BnOH, dioxane, reflux; step 2: $Ac_2O$, toluene, reflux; 72% over two steps). Compound **12** was persilylated and coupled with **3** under TMSOTf activation in acetonitrile to give exclusively the desired N-9 isomer **13** in 30–40% yield (Scheme 2). Dichloroethane was a suitable replacement for acetonitrile as solvent and about 35% yield of **13** was isolated when the reaction was carried out in dichloroethane at 80°C. The glycosylation reaction between **3** and **12** did not proceed at room temperature; running the reaction at 110°C (1,1,2,2-tetrachloroethane as solvent) led to complete consumption of **3** but no trace of **13** was detected. The N-9 linkage of **13** was confirmed by its exhaustive deprotection (step 1: $H_2$, Pd/C, $CH_3OH$; step 2: $Et_3N$, $CH_3OH$, $H_2O$; step 3: $NH_4OH$, $CH_3OH$, 60°C) to give **10** which was identical in all aspects to a sample prepared from **6**. Also, **13** was converted into **6** in two steps (step 1: $H_2$, Pd/C, $CH_3OH$; step 2: $Ph_2NCOCl$, $(i\text{-}Pr)_2EtN$, pyridine) in 50% overall yield. Over 10 g of **13** were prepared in this manner and were converted into guanine monomer **2d** using similar procedures as previously described.[1]




Scheme 2.

It's been reported by Imbach that only N-9 products (87% total yield) were obtained in the coupling of peracetylated β-D-galactopyranose with Robins' reagent **5**.[15] Since the reaction conditions used by Imbach are identical to our conditions in the coupling of glucosamine derivative **3** with **5** (TMSOTf catalysis in toluene at 80°C), this led us to postulate that the trifluoroacetamido group of **3** is likely to be responsible for the failure of Robins' reagent **5** to regioselectively give the N-9 isomer **6**. This dependence of the regiochemical outcome of guanine glycosylation (N-9 versus N-7) on the nature of the sugar protecting group (2-*O*-acetyl versus 2-*N*-trifluoroacetamido) is precedented.[16] While both 2-*O*-acetyl and 2-*N*-trifluoroacetamido groups could form the corresponding cyclic acyloxonium and oxazolinium ions, respectively, *N*-trifluroacetamide group is believed to participate less strongly at C-1.[17] One possible scenario is that the coupling of **3** and **5** is irreversible (supported by the failure of **7** to isomerize to **6** under TMSOTf activation, vide supra) and is under kinetic control. In dichloroethane, the rate of N-7 isomer formation (giving **7**) might be much higher than that of N-9 isomer formation (giving **6**) and only **7** would be isolated.[18] In toluene, the rate of N-9 isomer formation might become comparable to that of N-7 isomer formation and a mixture of **6** and **7** would be formed (**8** and **9** were formed through

the loss of diphenylcarbamoyl group from **6** and **7**).[6b] In the coupling of **3** and **12**, N-9 isomer **13** might be either the kinetic product (if the reaction is irreversible) or thermodynamic product (if the reaction is reversible). In either case, **13** would be isolated as the exclusive product using either dichloroethane or acetonitrile as solvent.

In summary, we encountered an unexpectedly low N-9/N-7 regioselectivity in the coupling of Robins' reagent (2-*N*-acetyl-6-*O*-diphenylcarbamoylguanine) with a D-glucosamine derivative and the N-9:N-7 ratio was also strongly influenced by the solvent used in the glycosylation. 2-*N*-Acetyl-6-*O*-benzylguanine was used successfully as a replacement of Robins' reagent in the above glycosylation to give selectively the desired N-9 product in either acetonitrile or dichloroethane in modest yield. Further studies to probe the underlying cause of the above described solvent effect and investigation of the scope in the use of 2-*N*-acetyl-6-*O*-benzylguanine are currently in progress and will be reported in due course.

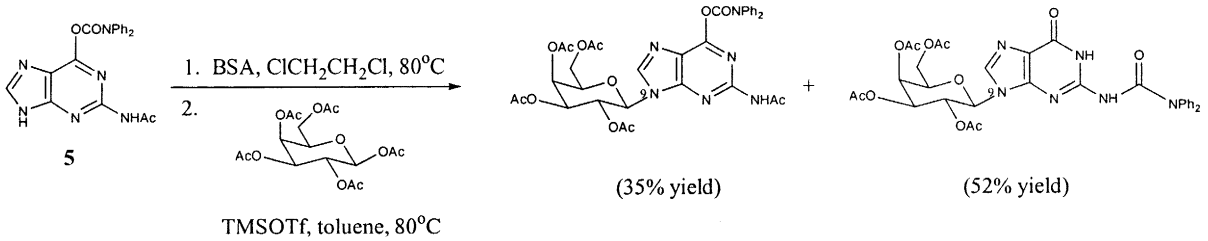
## Acknowledgements

The authors are grateful to Roche Physical Chemistry Department for spectroscopic measurements and interpretations. Advice and encouragement from Drs. Christopher Exon, Masami Okabe, Ramakanth Sarabu, Steve Tam and Steve Wolff are gratefully acknowledged.

## References

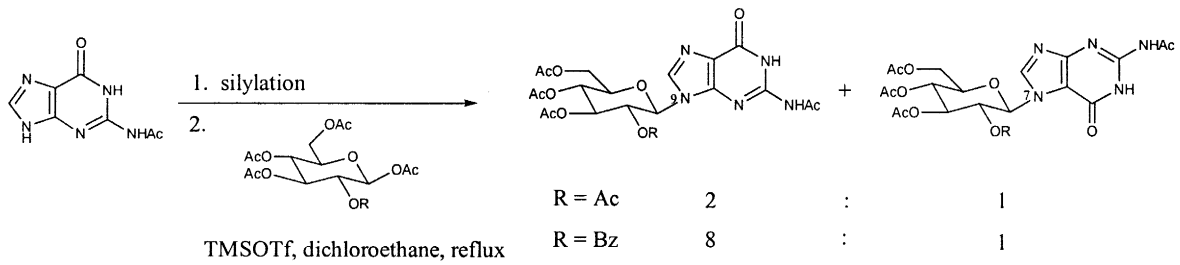
1. Goodnow Jr., R. A.; Richou, A.-R.; Tam, S. *Tetrahedron Lett.* **1997**, *38*, 3195–3198.
2. Goodnow Jr., R. A.; Tam, S.; Pruess, D. L.; McComas, W. W. *Tetrahedron Lett.* **1997**, *38*, 3199–3202.
3. For leading references, see: (a) Robins, M. J.; Zou, R.; Hansske, F.; Madej, D.; Tyrrell. D. L. J. *Nucleosides Nucleotides* **1989**, *8*, 725–741. (b) Jenny, T. F.; Benner, S. A. *Tetrahedron Lett.* **1992**, *33*, 6619–6620. (c) Garner, P.; Ramakanth, S. *J. Org. Chem.* **1988**, *53*, 1294–1298.
4. (a) Zou, R.; Robins, M. J. *Can. J. Chem.* **1987**, *65*, 1436–1437. (b) Robins, M. J.; Zou, R.; Guo, Z.; Wnuk, S. F. *J. Org. Chem.* **1996**, *61*, 9207–9212.
5. Ref. 3a stated that "our procedure is very sensitive to moisture and has been found to proceed well only in anhydrous toluene" but it was not further elaborated. Dichloroethane used in our studies was freshly distilled from calcium hydride under argon.
6. (a) Ref. 3c stated that in glycosylation studies using 2-*N*-acetylguanine that "a variety of modifications involving solvent composition, different catalysts, and nucleoside precomplexation were tried but did not increase the proportion of N-9 isomer significantly". (b) For an example in which coupling with 2-*N*-acetylguanine gave a higher N-9:N-7 ratio when a less polar solvent was used, see: Chamberlain, S. D.; Biron, K. K.; Dornsife, R. E.; Averett, D. R.; Beauchamp, L.; Koszalka, G. W. *J. Med. Chem*. **1994**, *37*, 1371–1377.
7. We are aware of only two published examples involving persilylated **5** under TMSOTf activation (Robins' conditions) which did not use toluene as solvent. (a) Bednarski, K.; Dixit, D. M.; Mansour, T. S.; Colman, S. G.; Walcott, S. M.; Ashman, C. *Bioorg. Med. Chem. Lett*. **1995**, *5*, 1741–1744. Methylene chloride was used as solvent, product yield was 25–30% and only N-9 regioisomers were formed. (b) Sells, T. B.; Nair, V. *Tetrahedron* **1994**, *50*, 117. Acetonitrile was used as solvent, yield of N-9 product was 21% and it was not mentioned in the paper if any N-7 regioisomer was formed.
8. For an example involving the use of **5** in which trimethylsilyl perchlorate ($TMSClO_4$) was used as activator and dichloroethane was used as solvent, see: Herdewijn, P.; Van Aerschot, A.; Busson, R.; Claes, P.; De Clercq, E. *Nucleosides Nucleotides* **1991**, *10*, 1525–1549. The yield of N-9 product was 55% and it was not mentioned in the paper if any N-7 regioisomer was formed.
9. For an example involving the use of **5** in which iodotrimethylsilane (TMSI) was used as activator and dichloroethane was used as solvent, see: Tse, H. L. A.; Knight, D. J.; Coates, J. A. V.; Mansour, T. S. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 1387–1392. The reaction regioselectively gave the N-9 product in 49% yield.
10. For an example involving the use of **5** in which the yield of N-9 glycosylation product depended strongly on the choice of solvent (acetonitrile versus dichloroethane; 80% versus 40% yield), see: Janardhanam, S.; Nambiar, K. P. *J. Chem. Soc., Chem. Commun*. **1994**, 1009–1010.

11. See Ref. 4b for an example of a successful N-7 to N-9 isomerization.
12. For examples of selective N-9 guanine isomer formation using **5** activated by $SnCl_4$, see: (a) Ref. 10. (b) Janardhanam, S.; Nambiar, K. P. *Tetrahedron Lett.* **1994**, *35*, 3657–3660.
13. (a) Before our work described herein started, **12** was shown to couple with ribose derivatives under Robins' conditions in high yield and high N-9 selectivity. Li, W.-R.; Tam, S., Hoffmann-La Roche, unpublished results. (b) It was mentioned in the footnotes of Ref. 4b that in the studies using **12** and other analogs, "difficulties were encountered during preliminary investigations with preparation of and/or coupling" of these compounds.
14. After our work described herein was initiated, three reports concerning the use of 2-*N*-isobutyrl-6-*O*-benzylguanine in glycosylation reactions were published. (a) Martin, P. *Helv. Chim. Acta.* **1995**, 78, 486–504. (b) Martin, P. *Helv. Chim. Acta.* **1996**, 79, 1930–1938. (c) Martin, P. EP-626387, **1994**.
15. El-Kattan, Y.; Gosselin, G.; Imbach, J.-L. *J. Chem. Soc. Perkin Trans. 1* **1994**, 1289–1297.




We could not rule out the possibility that the change in chirality at the C-4 O-acetate group (glucose versus galactose) is responsible/partially responsible for our observed anomalous behavior.

16. Ref. 3c.




17. (a) zu Reckendorf, W. M.; Wassiliadou-Micheli, N. *Chem. Ber.* **1970**, *103*, 1792–1796. (b) Wolfrom, M. L.; Conigliaro, P. J. *Carbo. Res.* **1969**, *11*, 63–76.
18. For an example in which the N-7 isomer of a ribonucleoside was first formed which later on isomerized to give the N-9 isomer, see: Dudycz, L. W.; Wright, G. E. *Nucleosides Nucleotides* **1984**, *3*, 33–44.