Pergamon

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Tetrahedron Letters 44 (2003) 4729–4732

TETRAHEDRON LETTERS

Studies on the construction of glycosidic linkage in guanofosfocins. Glycosylation of 8-oxoinosine and 8-oxoguanosine derivatives with mannopyranosyl bromide

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Received 31 March 2003; revised 16 April 2003; accepted 18 April 2003

Abstract—In model studies directed to the total synthesis of guanofosfocins, a unique glycosydic bond formation between the 8-oxoinosine derivatives and mannopyranosyl donor is described. The reaction employing previously reported conditions resulted in the formation of two regioisomers, the 8-*O*- and *N*7-mannosylated inosine derivatives. However, the use of tetrabutylammonium iodide as a catalyst was found to improve the chemoselectivity towards *O*-glycosylation to afford the desired 8-*O*-mannosylated products in satisfactory yields. The established procedure was successfully applied for the synthesis of 8-(mannopyranosyloxy)guanosine derivative.

Guanofosfocins are a novel family of chitin synthase inhibitors isolated from the fermentation broths of *Streptmyces sp*. and *Trichoderma sp*.[1] Its three-component structure is highly distinctive and contains a unique glycosidic bond between the 8-position of guanosine and the D-mannopyranose moiety. The potent therapeutic activity of guanofosfocin against fungous diseases makes research into this entirely new class of compounds attractive both for the benefits gained by screening new structures and as a synthetic challenge (Fig. 1).

As the first step toward the construction of its unique skeleton, we focused on the development of an efficient methodology for the synthesis of a hybrid part consisting of α-mannopyranose and the purine nucleoside. Recently, we disclosed that two different approaches were possible for the preparation of such hybrid molecules using adenosine derivatives as substrates, the first involving substitution of the 8-bromoadenosine derivative with the sodium salt of mannopyranose as a nucleophile[2] and the second consisting of the glycosylation of the 8-oxoadenosine derivative with mannopyranosyl bromide.[3] Especially, while the latter approach was efficient for obtaining only the desired mannopyranose–adenosine hybrid in good yield, we have embarked on a more extensive study of this reaction. In this letter, we documented a study of the reaction for the synthesis of the mannopyranose–inosine and guanosine hybrids as found in the guanofosfocins.

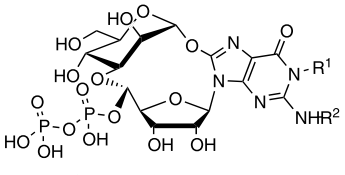
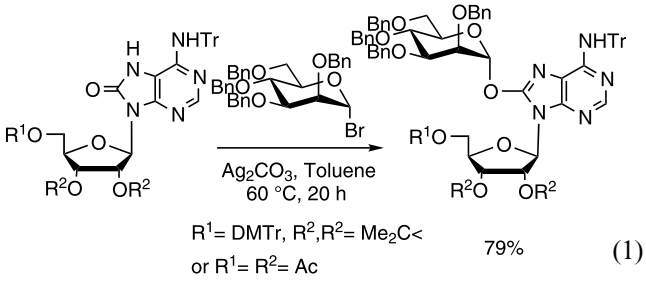


Figure 1.



(1)

In previous experiments, 8-oxoadenosine derivative was treated with mannopyranosyl bromide in the presence of silver carbonate at 60°C for 20 h to provide the

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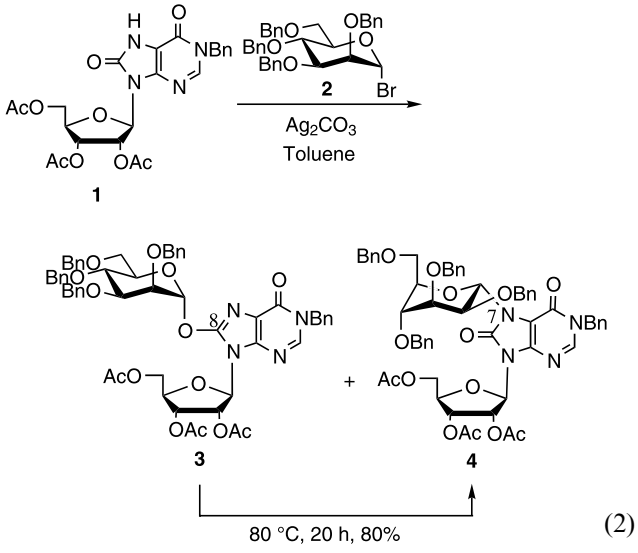
0040-4039/03/$ - see front matter
doi:10.1016/S0040-4039(03)01013-X

Table 1. Mannosylation of 8-oxoinosine **1**

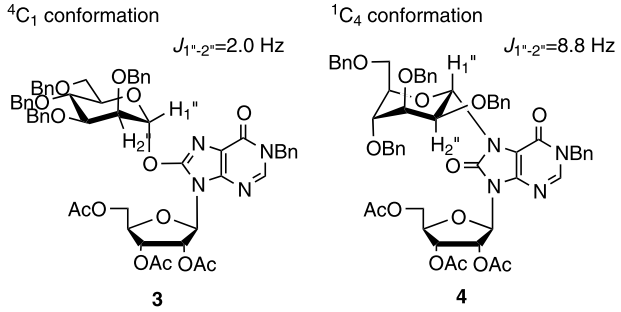
Run	Temp. (°C)/time (h)	Additive (equiv.)	Yields (%)		
			3	**4**	**1**
1	60/20	None	21	8	23
2	85/20	None	Trace	56	Trace
3	60/20	Bu_4NI (0.1)	61	18	8
4	60/20	Bu_4NI (0.2)	68	22	Trace
5	60/20	Bu_4NI (1.0)	26	18	31
6	40/20	Bu_4NI (0.2)	54	9	11
7	20/20	Bu_4NI (0.2)	47	7	22

8-(α-mannopyranosyloxy)adenosine derivative in 79% yield (Eq. (1)). We first envisioned a simple extension of these conditions to the mannosylation of the *N*1-benzyl-8-oxoinosine derivative **1** (Eq. (2)). As a result, the yield of the desired 8-(mannopyranosyloxy)inosine derivative **3** was poor, and a side-product, the regioisomeric *N*7-mannosylated inosine **4**, was also produced (run 1 in Table 1).[4] Since the starting 8-oxoinosine **1** was recovered from this reaction, the reaction temperature was next raised to 85°C in order to complete the reaction. However, although the starting 8-oxoinosine **1** was almost consumed, only the *N*7-mannosylated product **4** was isolated in 56% yield (run 2). This result implies that 8-(mannopyranosyloxy)inosine **3** is a relatively labile substance, which may be readily converted to the more stable *N*7-(mannopyranosyl)inosine **4** under the stated thermodynamic conditions. This hypothesis was supported by the fact that merely heating of the isolated 8-(mannopranosyloxy)inosine **3** at 80°C for 20 h caused complete conversion to *N*7-(mannopyranosyl)inosine **4** in 80% yield.



(2)

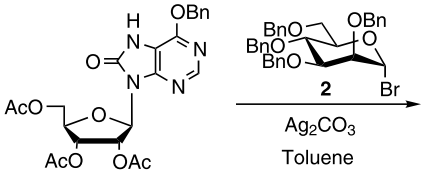
It is also worth noting that the conformation of the α-mannopyranose moiety of compound **4** is the $^1C_4$ form, which was confirmed by the ^1H NMR spectrum, showing a large coupling constant for the anomeric proton diaxial relationship. A similar observation has been reported in the synthetic study of the 9-(mannopyranosyl)hypoxanthine derivative and assumed by the authors that it is due to both the steric interaction and the reverse anomeric effect.[5] The structure and stereochemistry of compound **3** were confirmed in a similar manner as previously reported.[3]

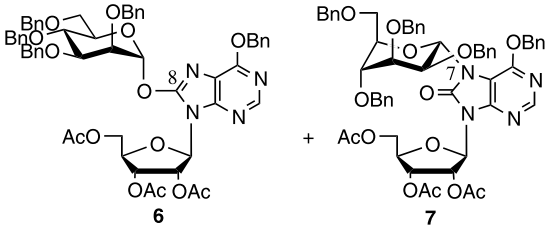


As the desired 8-(mannopyranosyloxy)inosine **3** has proved to be easily converted into thermodynamically more stable **4**, we next tried the use of a catalyst for this glycosylation in order to make the reaction favorable for the kinetically controlled product by lowering the activation energy in the transition state. Tetrabutylammonium iodide was chosen as a suitable catalyst because it is usually used for the activation of alkylation with alkyl chlorides or bromides.[6] When the mannosylation was conducted in the presence of 0.1 equiv. of tetrabutylammonium iodide at 60°C for 20 h, the total yields of the mannosylated products were remarkably improved in favor of the 8-*O*-mannoside **3** (run 3 in Table 1). Furthermore, the use of 0.2 equiv. of the ammonium salt led to a 90% conversion, providing the desired 8-*O*-mannosylated inosine **3** in 68% isolated yield (run 4).[7] However, the addition of an equimolar amount of tetrabutylammonium iodide resulted in the incomplete reaction due to production of a tarry material (run 5). Lowering the reaction temperature tends to increase the ratio of the 8-*O*-mannosylated product **3**, apparently showing that it is the kinetically preferable product though the reaction was not complete at such a lower temperature after 20 h (runs 6 and 7).

We next investigated the effect of the position of the protecting group on the purine ring by using the 6-*O*-benzyl-protected 8-oxoinosine derivative **5** as a substrate (Eq. (3)). When the reaction was carried out at 60°C for 20 h in the absence of the ammonium salt, only *N*7-(mannopyranosyl)-8-oxoinosine **7** was produced in 20% yield, along with recovery of the starting 8-oxoinosine **5** (run 1 in Table 2). When the reaction was stopped for 3 h, 8-*O*-mannoside **6** was obtained in

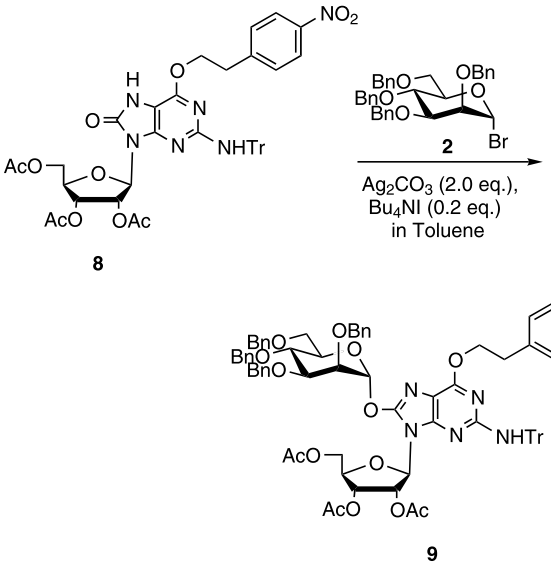
23% yield accompanied by an 11% yield of *N*7-mannoside **7** (run 2).[4] This result suggests that 8-(mannopyranosyloxy)inosine **6** is also unstable under the stated conditions, converting to the more stable *N*7-mannosylinosine **7** and, in parts, decomposing to the starting materials **5** for the long reaction time. However, we happily found that this mannosylation proceeded even at room temperature to afford the desired product **6** in a satisfactory yield after 64 h (run 3), and the reaction was again accelerated by the addition of 0.1 equiv. of tetrabutylammonium iodide (run 4).[7] Prolonging the reaction time or increasing the amount of the catalyst in this reaction did not lead to any improvement in the product yield.





(3)

Finally, we adapted these findings for the reaction of 8-oxoguanosine derivative with mannopyranosyl bromide **2**. We chose the appropriately protected guanosine derivative **8**, prepared according to the reported procedure.[8] The glycosylating reaction was carried out under two different conditions—at 60 and 20°C—in the presence of 0.2 equiv. of tetrabutylammonium iodide (Eq. (4)). In both cases, the starting materials **8** and **2** were not consumed completely. However, the isolated yields of the desired 8-*O*-mannosylated guanosine derivative **9** were acceptable,[4,7] and the *N*7-mannosylated by-product was not detected in these reactions.



conditions	Yield of **9**	Recovery of **8**
60 °C, 20 h	60%	12%
20 °C, 70 h	70%	27%

(4)

In conclusion, mannosylation of 8-oxoinosine derivatives **1** and **5** using the previously reported conditions resulted in the production of the undesirable *N*7-mannosylated products. This difficulty, however, was overcome by the addition of a catalytic amount of tetrabutylammonium iodide, providing the desired 8-(mannopyranosyloxy)inosine derivatives in satisfactory yields. Although silver salts or tetraalkyl ammonium salts are often used individually as a promotor for the glycosylation using glycosyl bromides,[9] the use of a silver salt in conjunction with a catalytic amount of ammonium iodide is, to the best of our knowledge, the first example for the activation of glycosyl bromides. Finally, the conditions found have been successfully applied in the preparation of the 8-(mannopyranosyloxy)guanosine derivative. Further study on the application of these results to more advanced guanofosfocin intermediates is now in progress.

Table 2. Mannosylation of 8-oxoinosine **5**

Run	Temp. (°C)/time (h)	Additive (equiv.)	Yields (%)		
			6	**7**	**5**
1	60/20	None	0	20	58
2	60/3	None	23	11	35
3	20/64	None	68	Trace	30
4	20/20	Bu_4NI (0.1)	72	Trace	22
5	20/20	Bu_4NI (0.2)	58	Trace	26

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2. Stansfield, K.; Kanamori, H.; Sugimura, H. *New J. Chem.* **1999**, 9.
3. Sugimura, H.; Stansfield, K. *Synlett* **1998**, 985.
4. Assignments of the 8-*O* and *N*7 glycosides were made by ^{13}C NMR chemical shifts of the anomeric carbon (C-1″) of the mannose moieties. The ^{1}H and ^{13}C NMR data of C-1″ in compounds **3**, **4**, **6**, **7** and **9**, which were confirmed by H–H and C–H COSY, are shown below.
3: ^{1}H NMR ($CDCl_3$) δ 6.68 (d, $J=2.0$ Hz), ^{13}C NMR δ 98.4; **4**: ^{1}H NMR ($CDCl_3$) δ 6.51 (d, $J=8.8$ Hz), ^{13}C NMR δ 78.2; **6**: ^{1}H NMR ($CDCl_3$) δ 6.66 (d, $J=1.7$ Hz), ^{13}C NMR δ 98.5; **7**: ^{1}H NMR ($CDCl_3$) δ 6.24 (d, $J=9$ Hz), ^{13}C NMR δ 79.0; **9**: ^{1}H NMR ($CDCl_3$) δ 6.54 (d, $J=1.7$ Hz), ^{13}C NMR δ 97.8 [cf. Guanofosfocin A: ^{1}H NMR (D_2O) δ 5.39 (d, $J=2$ Hz), ^{13}C NMR δ 99.9 from Ref. 1].
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6. For instance: (a) Czernecki, S.; Georgoulis, C.; Provelenghiou, C. *Tetrahedron Lett.* **1976**, *17*, 3535; (b) Kanai, K.; Sakamoto, S.; Ogawa, S.; Suami, T. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 1529; (c) Lipshutz, B. H.; Moretti, R.; Crow, R. *Tetrahedron Lett.* **1989**, *30*, 15.
7. **General procedure**: To a solution of **1**, **5** or **8** (0.2 mmol) in toluene (10 mL) was added Ag_2CO_3 (0.22 mmol) under Ar. After the solution was refluxed for 15 min, a solution of **2** (0.3–0.4 mmol) in toluene (3 mL) and Bu_4NI was added successively at room temperature. The reaction mixture was stirred at the designated temperature for the stated time, and then filtrated through Celite. The filtrate was evaporated under reduced pressure. The residue was purified by silica gel TLC (hexane–AcOEt) to afford the mannosylated products **3**, **4**, **6**, **7** and **9**.
8. 8-Oxopurine nucleosides were prepared according to the procedures described in the following literature: Schulz, B. S.; Pfleiderer, W. *Helv. Chim. Acta* **1987**, *70*, 210.
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