Synthesis of novel nucleoside–carbohydrate hybrids

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A substitution approach for the synthesis of 8-(mannosyloxy)-adenosines, novel nucleoside–carbohydrate hybrids found in a new family of chitin synthase inhibitor—guanofosfocins, has been investigated. When an N^6-benzoyl-8-bromoadenosine derivative was exposed to the sodium alkoxides derived from di-*O*-isopropylidene-*β*-mannoses or cyclohexanols, substitution occurred at room temperature to yield 8-(mannosyloxy)-adenosines and their 8-cyclohexyloxy derivatives. The 8-mannosyloxy products were isolated solely as α anomers and adopted a *syn* conformation with respect to the anomeric C—N bond. Whilst the hybrid compounds were obtainable in good yields, the new glycosidic linkage was acid sensitive to furnish 8-oxo derivatives under deprotecting conditions for the dimethoxytrityl group.

The synthesis of structurally unique nucleoside analogs is of considerable importance in the development of antiviral, antibiotic, antitumor and antifungal agents.[1] Naturally occurring compounds often provide inspiration for the creation of such analogs and a recently isolated family of chitin synthase inhibitor, guanofosfocins, stimulated our interest. The guanofosfocins, isolated from the fermentation broths of *Streptomyces sp.* and *Trichoderma sp.*, have a unique cyclic structure containing a glycosidic bond between the 8-position of guanosine and a D-mannose moiety (Fig. 1).[2] Despite their promising therapeutic effects against fungal diseases, further investigation of these fascinating molecules has been hindered by their low stability.[3] Since the ethereal bond between a glycosyl moiety and the 8-position of a purine nucleoside found in guanofosfocin is previously unknown, we planned to develop methodology for the efficient construction of this linkage in order to synthesise novel nucleoside–carbohydrate hybrids for screening and to evaluate their stability.

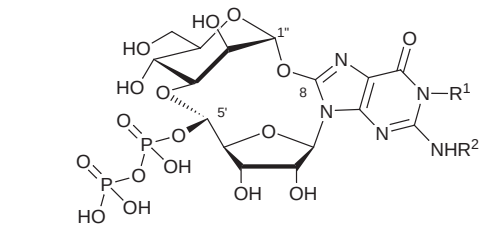

Fig. 1 Structure of guanofosfocin A–C.

One possible strategy for the synthesis of an 8-(glycosyloxy) purine nucleoside would be to substitute a protected carbohydrate-type nucleophile into a purine substrate bearing a leaving group at the 8-position. To date, simple 8-alkoxypurine nucleosides (8-methoxy and 8-benzyloxy substituted derivatives) have been prepared by such a method.[4–6] However, the reaction conditions (*e.g.* non-protected nucleosides are treated with excess alcohol and sodium metal in DMSO at 65 °C or higher) are unsuitable for sensitive carbohydrate-type nucleophiles. Therefore, prior to the introduction of a carbohydrate moiety, we endeavored to establish a general substitution procedure using an easily accessible purine nucleoside substrate, 8-bromoadenosine, and a simple secondary alcohol, cyclohexanol.

As the substitution reaction conditions will be basic, the protecting groups employed on 8-bromoadenosine must be base stable. We first prepared a 2′,3′-*O*-isopropylidene-5′-*O*, N^6-dityrityl substrate **1a**, which was treated with sodium cyclohexyloxide generated *in situ* from cyclohexanol and sodium hydride in DMF.‡ Whilst no reaction occurred at room temperature, on heating the mixture at 70 °C the reaction proceeded and, after 24 h, the starting nucleoside was almost consumed to provide 8-(cyclohexyloxy)adenosine derivative **2a** in 65% yield along with a 22% yield of 8-oxoadenosine **4** (run 1 in Table 1). A similar tendency was observed in the reaction of N^6 free substrate **1b** (run 2). By contrast, the reaction of

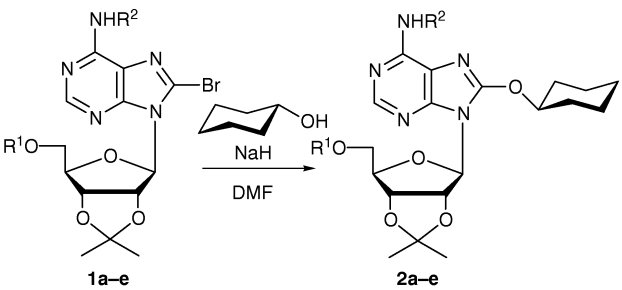

Table 1 Substitution reaction of cyclohexanol with 8-bromoadenosines

Run	Substrate	R^1	R^2	Temp., Time	Product	Yield (%)[a]
1	**1a**	Tr	Tr	70 °C, 24 h	**2a**	65 (22)
2	**1b**	Tr	H	70 °C, 24 h	**2b**	50 (11)
3	**1c**	Tr	Bz	r.t., 4 h	**2c**	75 (10)
4	**1d**	TBDPS	Bz	r.t., 4 h	**2d**[b]	40
5	**1e**	DMTr	Bz	r.t., 4 h	**2e**	83 (5)

[a] The number in parentheses shows the yield of 8-oxo byproduct **4**. [b] R^1=H.

N^6-benzoylated substrate **1c** was completed at room temperature in 4 h to afford 8-cyclohexyloxy product **2c** in 75% yield (run 3). It has been reported that an N^2-acyl group on guanosine derivatives enhances the rate of intramolecular cyclization at the 8-position.[7] The benzoyl group at N^6 of adenosine increases the rate of substitution by a similar electronic effect.

The formation of 8-oxo byproduct **4** is due to hydrolysis of an addition intermediate **3** (Fig. 2) as attempts to form **4** directly by treating the substrates with sodium hydride alone in wet DMF were unsuccessful. Prolonged reaction, however, didn't lead to improvement in the yield of **2**.

Since selective deprotection at the 5′-position is required for further studies, other protecting groups at this position were briefly examined. 5′-*O*-*tert*-Butyldiphenylsilyl-protected substrate **1d** underwent desilylation under the reaction conditions to yield 5′-hydroxyl product **2d** (run 4). 5′-*O*-Dimethoxytrityl (DMTr) derivative **1e**, in which the DMTr group can be removed more readily than the Tr group,[8] reacted with cyclohexanol to furnish the desired product **2e** in good yield (run 5).

Several other base–solvent combinations were also investigated (DMSO as an alternative solvent and Bu^{t}OK/18-crown-6, Et_3N as an alternative base), but sodium hydride in DMF gave the most satisfactory results.

With a suitable substrate in hand, we turned our attention to the substitution reaction of carbohydrate-type nucleophiles (Table 2). When 2,3,4,6-tetra-*O*-benzylmannopyranose (**5**) was allowed to react with **1e**, 8-(benzyloxy)adenosine **9** was isolated (run 1). From this result, it was deduced that base catalyzed ring opening of **5** was occurring and subsequent β-elimination of benzyloxide resulting in the formation of the 8-benzyloxy product. We anticipated that protection of the 2- and 3-hydroxy groups as a cyclic acetal would restrain this process, allowing the desired reaction to proceed. Indeed, when 2,3;4,6-di-*O*-isopropylidene-D-mannopyranose (**6**), prepared by a known route,[9] was used as a nucleophile, 8-(mannopyranosyloxy)adenosine derivative **11** was successfully obtained in good yield (run 2). Similarly, the corresponding 8-(mannofuranosyloxy)adenosine derivative **12** and the 8-(mannopyranosylthio)adenosine derivative **13** have been prepared using 2,3;5,6-di-*O*-isopropylidene-D-mannofuranose (**7**) and 2,3;4,6-di-*O*-isopropylidene-1-thio-D-mannopyranose (**8**), respectively (runs 3 and 4).

The mannose–adenosine hybrids obtained here were formed as single isomers. Generally, the configuration of glycosidic linkages in pyranoses can be determined from ^{13}C NMR spectra by measuring the $^1J_{CH}$ coupling at C-1.[10] In hybrid **11**, the coupling constant is 178 Hz, showing the new linkage to be exclusively α[10,11] which is consistent with the mannosyl bond in guanofosfocins.

In addition to the new carbohydrate–nucleoside hybrids, a substrate derived form *myo*-inositol was employed in the reaction to create a novel 8-inositol substituted adenosine compound **14** (run 5).

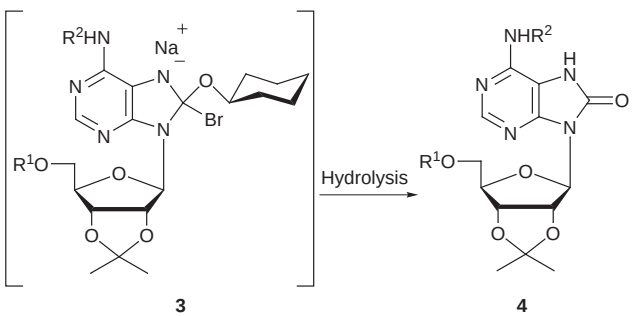

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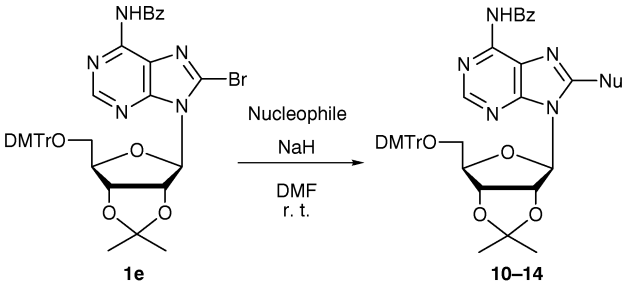
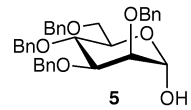
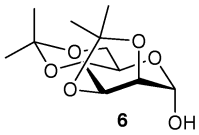
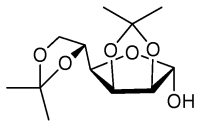
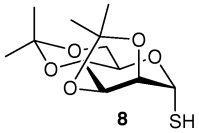
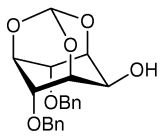
Fig. 2 A plausible mechanism for the formation of 8-oxo byproduct.



1e **10–14**

Table 2 Substitution reaction of carbohydrate-type nucleophiles with **1e**

Run	Nucleophile	Time	Product	Yield (%)[a]
1	 **5**	16 h	**10** (Nu=BnO)	45 (20)
2	 **6**	6 h	**11**	62 (17)
3	 **7**	6 h	**12**	71 (14)
4	 **8**	6 h	**13**	68 (12)
5	 **9**	4 h	**14**	67 (10)

[a] The number in parentheses shows the yield of **4** (R^1=DMTr, R^2=Bz).

Next, we briefly examined deprotection at the 5′-position. Dimethoxyltrityl group can be removed by trichloroacetic acid in nitromethane–methanol solvent system with little or no concomitant depurination.[12] However, when these conditions were applied to the nucleoside–carbohydrate hybrids **11**–**13**, the glycosidic bond was cleaved to give the 8-oxo or 8-thioxo product **17** (runs 1–3 in Table 3). The 8-cyclohexyloxy derivatives **2e** and **14** were acid stable, so that the dimethoxytrityl group could be removed without difficulty (runs 4 and 5). Efforts to investigate the selective deprotection conditions, not affecting the glycosidic bond, are currently underway.

One intriguing feature of the guanofosfocins (Fig. 1) is that they clearly adopt an *anti* conformation about the anomeric C—N bond though purine nucleosides with bulky substituent at C-8 are known to have a *syn* conformation.[13–15] It is well documented that the chemical shifts of H(2′) protons in *syn* and *anti* adenosines differ through changes in the torsion angle around the glycoside bond.[13–15] It is therefore possible to predict the glycosyl conformation of the hybrids by comparing their shifts with compounds of known orientation. 8-Bromoadenosines are known to adopt *syn* conformations with the H(2′) signal characteristically located 0.5–0.6 ppm downfield from the H(3′) signal.[13] In this respect, hybrid compounds **11** and **12** show patterns similar to **1e**, indicating a *syn* orientation. The 8-(cyclohexyloxy)adenosine **2e** also shows a typical *syn* pattern. However, on removal of the bulky dimethoxytrityl group, the H(2′) signal of compound **15** shifted

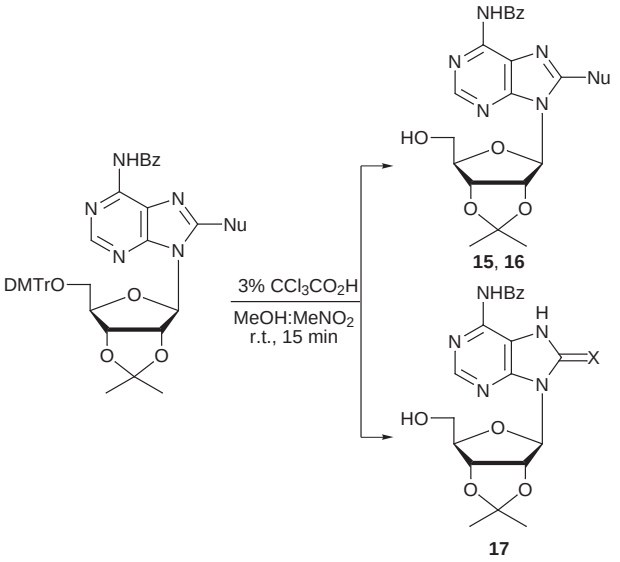
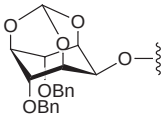


Table 3 Deprotection of DMTr group

Run	Compound	Product	Nu	Yield (%)
1	**11**	**17** (X=O)	—	63
2	**12**	**17** (X=O)	—	64
3	**13**	**17** (X=S)	—	75
4	**2e**	**15**		95
5	**14**	**16**		88

upfield to give a ^{1}H NMR spectra consistent with an *anti* relationship. This finding is significantly important because an *anti* relationship is beneficial for future attempts to prepare guanofosfocin type compounds.

In summary, by successfully forming the purine–carbohydrate bond found in guanofosfocin we have prepared a new type of nucleoside–carbohydrate hybrid. The approach will be compatible with the use of a variety of nucleophiles. The glycosidic linkage is extremely acid labile and this may contribute to the instability of the natural products. Further study on the application of this approach to the preparation of a wide variety of nucleoside hybrid molecules, including the use of guanosine substrates, is in progress.

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Notes and references

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‡ The following general procedure was used for the substitution reaction: an alcohol (0.2 mmol) was added to a suspension of sodium hydride (60% suspension in oil, 0.2 mmol) in DMF (3 ml) at room temperature and the mixture stirred for 15 minutes. An 8-bromoadenosine derivative (0.1 mmol) was added in a single portion to the stirred solution. The reaction was quenched with water (5 ml). The mixture was extracted with ethyl acetate (3 × 15 ml). The organic layer was washed with a saturated solution of sodium hydrogen carbonate (30 ml) and then dried ($MgSO_4$). The solvent was concentrated *in vacuo*. Purification by column chromatography afforded the corresponding 8-alkoxyadenosine derivative.

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