

# The nucleoside transport proteins, NupC and NupG, from *Escherichia coli*: specific structural motifs necessary for the binding of ligands†

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A series of 46 natural nucleosides and analogues (mainly adenosine-based) were tested as inhibitors of [<sup>14</sup>C]uridine uptake by the concentrative, H<sup>+</sup>-linked nucleoside transport proteins NupC and NupG from *Escherichia coli*. The two evolutionarily unrelated transporters showed similar but distinct patterns of inhibition, revealing differing selectivities for the different nucleosides and their analogues. Binding of nucleosides to NupG required the presence of hydroxyl groups at each of the C-3' and C-5' positions of ribose, while binding to NupC required only the C-3' hydroxyl substituent. The greater importance of the ribose moiety for binding to NupG is consistent with the evolutionary relationship between this protein and the oligosaccharide: H<sup>+</sup> symporter (OHS) subfamily of the major facilitator superfamily (MFS) of transporters. For both proteins the natural  $\alpha$ -configuration at C-3' and the natural  $\beta$ -configuration at C-1' was mandatory for ligand binding. N-7 in the imidazole ring of adenosine and the amino group at C-6 were found not to be important for binding and both transporters showed flexibility for substitution at C-6/N<sup>6</sup>; one or both of N-1 and N-3 were important for adenosine analogue binding to NupC but significantly less so for binding to NupG. From the different effects of 8-bromoadenosine on the two transporters it appears that adenosine selectively binds to NupC in an *anti*- rather than a *syn*-conformation, whereas NupG is less prescriptive. The pattern of inhibition of NupC by differing nucleoside analogues confirmed the functional relationship of the bacterial transporter to members of the human concentrative nucleoside transporter (CNT) family and reaffirmed the use of the bacterial protein as an experimental model for these physiologically and clinically important mammalian proteins. The specificity data for NupG have been used to develop a homology model of the protein's binding site, based on the X-ray crystallographic structure of the disaccharide transporter LacY from *E. coli*. We have also developed an efficient general protocol for the synthesis of adenosine and three of its analogues, which is illustrated by the synthesis of [<sup>13</sup>C]adenosine.

## Introduction

Proteins in the membranes of all living cells are responsible for the assimilation of specific nutrients and the expulsion of waste products.<sup>1</sup> The concentrative (H<sup>+</sup>-driven) nucleoside symport (ligand and proton both migrate in the same direction into the cell) proteins NupC<sup>2</sup> and NupG<sup>3</sup> from *Escherichia coli* are two such transporters, which despite their similar functions have different amino acid sequences and are not evolutionarily related. NupC is a member of the concentrative nucleoside transporter (CNT) family, which includes three representatives in humans, hCNT1, hCNT2 and hCNT3.<sup>4</sup> This bacterial protein shows between 22 and 26% amino acid sequence identity with the human transporters and shares with hCNT3 a similar broad permeant‡ selectivity among the natural nucleosides, although the bacterial protein does not transport guanosine and transports inosine only poorly.<sup>5,6</sup> NupG, a member of the nucleoside: H<sup>+</sup> symporter (NHS) subfamily of the major facilitator superfamily (MFS) of transporters,<sup>7</sup> differs from NupC in transporting a wider range of natural nucleosides,

including both guanosine and inosine, but unlike NupC does not transport the nucleoside analogue showdomycin.<sup>2,3</sup> Unlike NupC, NupG possesses only distantly related human homologues but, interestingly, the NHS subfamily is related to the oligosaccharide: H<sup>+</sup> symporter (OHS) subfamily of di- and trisaccharide transporters.<sup>8</sup>

Our research is concerned with establishing the structure of the permeant binding sites in NupC and NupG by combining established techniques of molecular biology<sup>9</sup> with significant contributions from the results of NMR experiments.<sup>10</sup> We have now tested a rational collection of 46 nucleosides, analogues and other compounds to define the contributions of substituent atoms and groups in the ligand to the binding specificities of the two transporters.

Human nucleoside transporters are targets for drug transport and therapy.<sup>11</sup> Prominent examples include the anti-cancer and anti-AIDS drugs gemcitabine<sup>12</sup> and 3'-azido-3'-deoxythymidine<sup>13</sup> (AZT) **19**. Elucidating ligand specificity in nucleoside transporters should thus not only illuminate binding site structure but also aid in the rational development of drug therapies involving nucleosides. Most recently analogues of uridine **6** have been examined in detail as inhibitors of uridine uptake by the human transporters hCNT1 and hCNT3 expressed in yeast.<sup>14</sup> The most critical region of uridine **6** for interaction with hCNT1 was identified as the C-3'-OH, with additional contributions to binding involving the C-5'-OH

† Electronic supplementary information (ESI) available: experimental details for the synthesis of compounds **16**, **17**, **20**, **21**, **23**, **25**, **31**, **34**, **35**, **36**, **38** and **41**. See <http://www.rsc.org/suppdata/ob/b4/b414739a/>

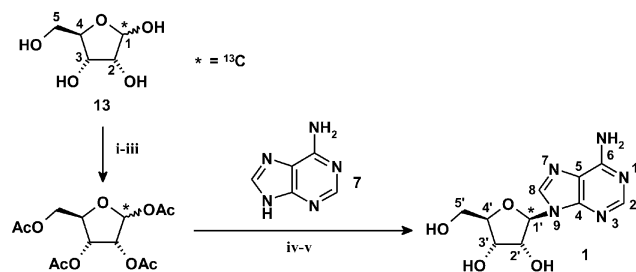
‡ A permeant is defined as a ligand that is shown to be transported by a protein.

and the N-3-H; the influence of stereochemistry was also examined.<sup>14</sup> Broadly similar results were obtained for hCNT3, although the C-5'-OH and the N-3-H appeared not to be important for binding. The results we present here further relate NupC through these motifs to members of the human CNT family and justify research on the bacterial protein as a good model for the human transporters.

Our results on structural group motifs involved in binding to NupG (Fig. 1 and Fig. 2) are consonant with the relatedness of this protein to members of the OHS family of transporters. We have exploited this similarity in order to construct an experimentally testable model for the NupG permeant binding site using these data and the recently determined X-ray crystallographic structure for the OHS family member LacY,<sup>15</sup> which transports the disaccharide lactose.

## Results and discussion

Representative pyrimidine and purine permeants for the transporters NupC and NupG are uridine **6** and adenosine **1**. The economical synthesis of [2'-<sup>13</sup>C, 1,3-<sup>15</sup>N<sub>2</sub>]uridine has been described previously together with solid-state NMR application.<sup>16</sup> We now report an efficient general procedure for the synthesis of adenosine **1**, illustrated by the preparation of [1'-<sup>13</sup>C]adenosine, beginning with [1-<sup>13</sup>C]-D-ribose (see **13**). Samples of labelled adenosine **1** were prepared for use in NMR structural studies.<sup>10,17</sup> The synthetic protocols (Scheme 1) were adapted for the synthesis of three unlabelled nucleoside analogues (**27**–**29**) that were unavailable commercially. These readily adaptable synthetic routes will be helpful to others working with the biologically pre-eminent nucleoside adenosine and we thus provide selected experimental details. We include improved, up-to-date physical data for the characterisation of the compounds that were synthesised.



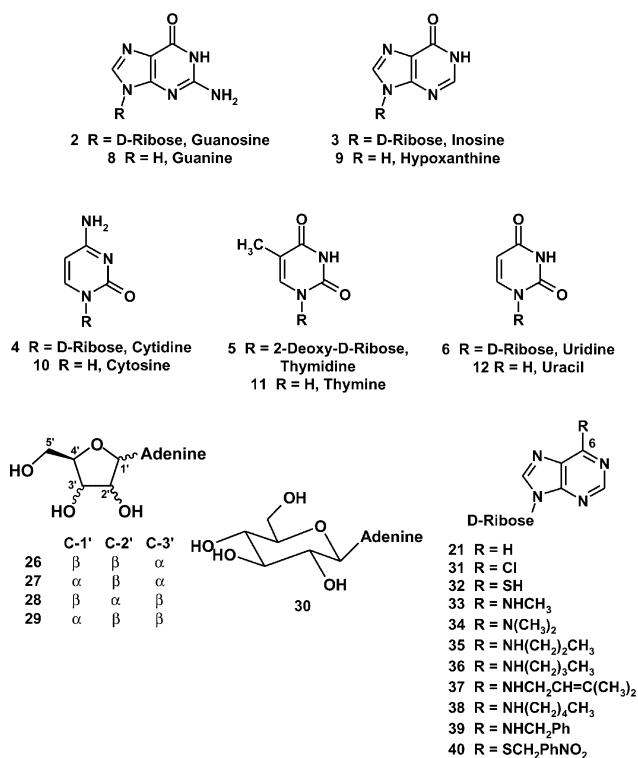
**Scheme 1** Synthesis of [1'-<sup>13</sup>C]adenosine **1** and strategy for the synthesis of the isomers of adenosine **27**–**29**. i). MeOH–Dowex (H<sup>+</sup>), 100%; ii). Ac<sub>2</sub>O–pyridine, 80%; iii). Ac<sub>2</sub>O–AcOH–H<sub>2</sub>SO<sub>4</sub>, 80%; iv). SnCl<sub>4</sub>–MeCN, 67% (oil), 27% (crystallised from EtOH); v). NaOMe–MeOH, 33%.

## Synthesis of adenosine analogues

### (a) Configurational isomers of adenosine and analogues modified in the sugar ring

The isomers **27**,<sup>18</sup> **28**<sup>19</sup> and **29**<sup>20</sup> were synthesised from D-arabinose, D-xylose and D-lyxose, respectively, by adaptation of the synthetic route outlined in Scheme 1. The synthesis of 9-(β-D-arabinofuranosyl)adenine **26** (also named ara-A and Vidarabine<sup>21</sup>) was achieved by deprotection<sup>22</sup> of 2',3',5'-tri-O-benzyl-9-(β-D-arabinofuranosyl)adenine, which is commercially available. 5'-Deoxyadenosine **16**<sup>23</sup> (see **1**)<sup>§</sup> was synthesised from adenosine and the adenine **17**, bearing only vestiges of a sugar moiety, was made by a published route.<sup>24</sup> The glucose isomer **30**<sup>25</sup> was prepared from aceto-bromo-glucose.

<sup>§</sup> Throughout, where modifications are minimal, structures are not depicted but are referred to a closely related structure, e.g. **16** and **1**.



### (b) Adenosine analogues modified in the heterocyclic moiety

Inosine **3** served as starting material for **31**,<sup>26</sup> **34**<sup>27</sup> and **36**<sup>28</sup>, and also **35** and **38**, which are new compounds. 1,3-Dideazaadenosine **23**,<sup>29</sup> 3'-azido-2',3'-dideoxyadenosine (AZA) **20**<sup>30</sup> and the "minimalist" 1-deoxy-1-phenyl-β-D-ribofuranoside **25**<sup>31</sup> were prepared following published procedures.

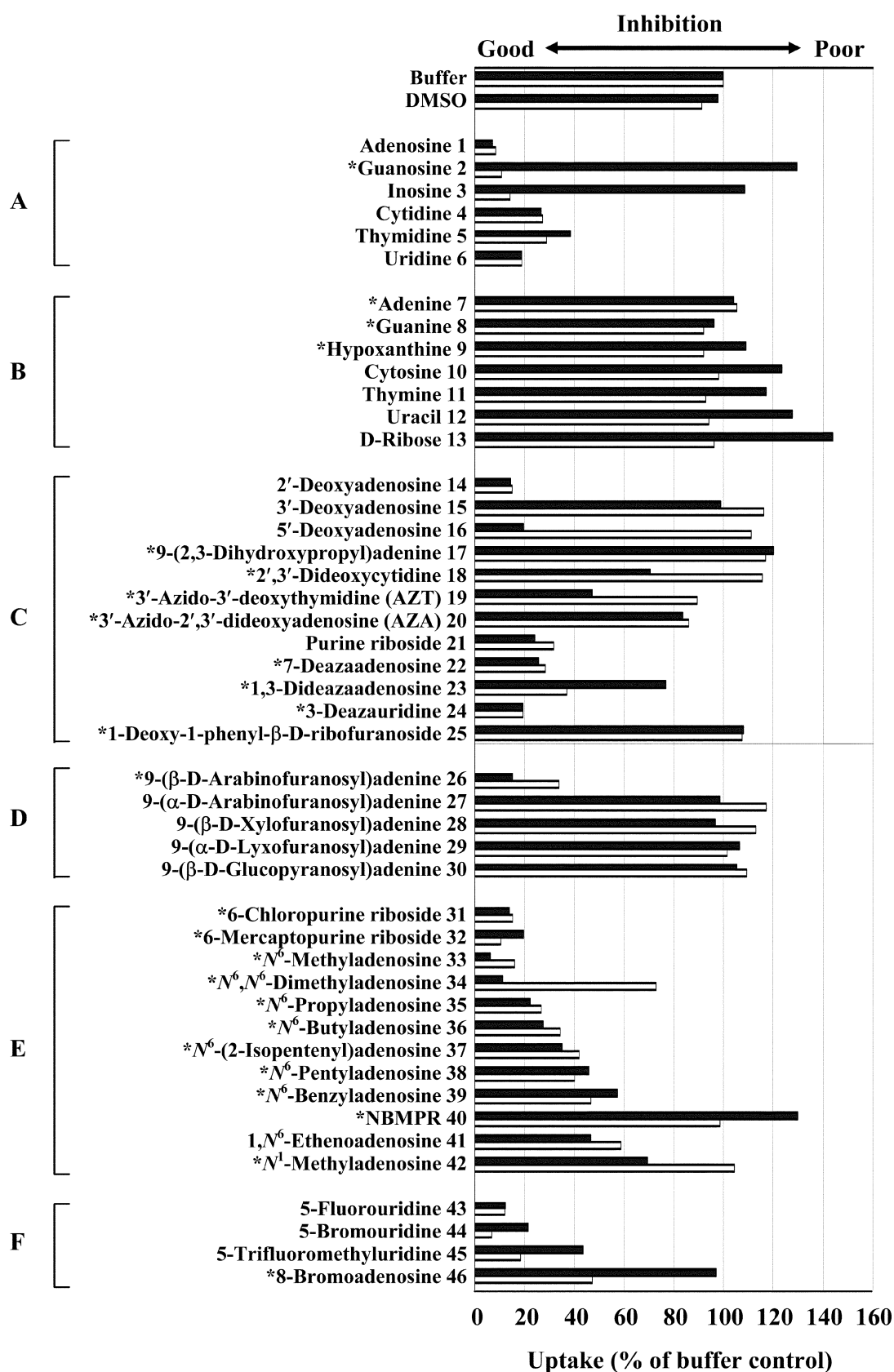
Adenosine **1** provided the source for the synthesis of purine riboside **21**,<sup>32</sup> and 1,N<sup>6</sup>-ethenoadenosine **41**.<sup>33</sup> The latter compound is fluorescent and this property may be useful in a bio-assay for nucleoside transporters. Excitation of a solution of **41** at a wavelength of 300 nm produces a fluorescence emission spectrum with a maximum at 410 nm. This long wavelength UV absorption allows excitation outside the range of absorption of proteins and nucleic acids and the intense emission at 410 nm allows detection in the presence of these molecules.

### The transport assay for NupC and NupG

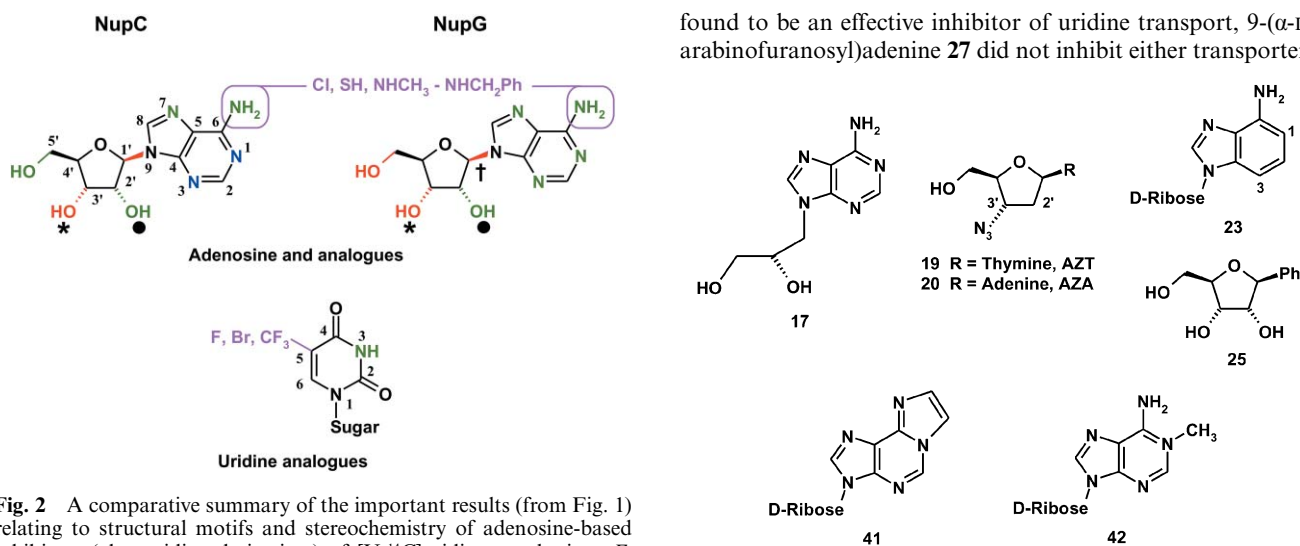
Compounds were tested for their ability to serve as ligands for NupC and NupG by their inhibition of [U-<sup>14</sup>C]uridine uptake into intact *E. coli* cells in which expression of each transporter had separately been induced during growth in liquid culture. To achieve this expression, the coding sequences of the corresponding genes were inserted into the inducible expression vector pTTQ18.<sup>8,34</sup> The cells were then energised for transport and the uptake of [U-<sup>14</sup>C]uridine (see **6**) was measured in the absence and then presence of unlabelled nucleosides and their analogues. The level of decrease in radiolabelled nucleoside uptake in the presence of the unlabelled compounds measures their ability to bind to each transporter.

### Ligand specificities for NupC and NupG

The biosassay involves *inhibition* by the ligand of [U-<sup>14</sup>C]uridine uptake (transport) by NupC and NupG: *a good inhibitor is to be understood as a good ligand*. The results of the uptake studies are summarised in Fig. 1, where formulae numbers double for table entry numbers. An extensive range of 46 compounds was studied. The results establish ligand specificities that reveal broad overall similarities but also a consistent pattern of significant differences in the structural motifs and stereochemistries that are involved in ligand binding to the two transport proteins.



**Fig. 1** Normalised uptake, after 15 s, of [U-<sup>14</sup>C]uridine (50 μM) into *E. coli* cells expressing NupC (black bars) and NupG (white bars) pre-incubated with potential inhibitors 1 to 46 (500 μM); \* indicates compounds that were added in 50% or 100% DMSO. The categories of compounds are: **A** natural nucleosides, **B** natural nucleobases, **C** group-deficient nucleoside analogues, **D** configurational isomers of adenosine, **E** C-6/*N*<sup>6</sup>- or *N*<sup>1</sup>-substituted adenosines, and **F** halogenated nucleosides. The uptake values are the means of duplicate measurements that differed from the means by less than 5%. Adenosine was always used as a competitor with each set of compounds that were tested to ensure consistency between different batches of cells in the assay procedure. Compounds that were effective inhibitors and/or that demonstrated important ligand specificities for NupC and NupG were tested on different batches of cells and on different days; the results obtained from repeat experiments were always found to be consistent.



**Fig. 2** A comparative summary of the important results (from Fig. 1) relating to structural motifs and stereochemistry of adenosine-based inhibitors (also uridine derivatives) of [U-<sup>14</sup>C]uridine uptake into *E. coli* cells expressing NupC and NupG. Key: features that are essential to substrate binding (■), possibly important (■), not important (■), flexibility to substitution (■), inflexibility to configurational change (\*), flexibility to configurational change (●), flexibility to conformational change (†).

As previously reported,<sup>2,35</sup> transport by NupC was effectively inhibited by the pyrimidine nucleosides cytidine **4**, thymidine **5** and uridine **6** plus the purine nucleoside adenosine **1**, but not at all by guanosine **2** and inosine **3**. One common distinguishing feature of the latter two purine nucleosides is the oxygen function at C-6, which unlike the corresponding amino function in **1** could act only as an H-bond acceptor. However, the properties of adenosine analogues lacking the exocyclic amino group at C-6 suggest that this position is not involved in hydrogen bond formation (see below). The reason for these results is unclear. NupG shows broader substrate acceptance than NupC, being an effective transporter for all six natural purine/pyrimidine nucleosides, including **2** and **3**, as previously reported.<sup>3,8</sup> Adenosine was a slightly better inhibitor of transport than uridine for both transport proteins and we found a slightly higher affinity of adenosine for NupG than NupC, where the transport efficiencies ( $V_{max}/K_m$ ) are 0.69 and 0.34, respectively.<sup>36</sup> The transport efficiencies for uridine transport by NupC and NupG are more similar with values of 0.60 and 0.72, respectively.<sup>36</sup>

Neither NupC- nor NupG-mediated uridine transport showed any inhibition by ribose (**13**) (mixture of ring isomers), 1-*O*-methyl-D-ribofuranoside (locked in the furanose form), or by the separate nucleobase moieties (**7** to **12**) of natural nucleosides. Lack of inhibition by ribose analogues or by nucleobases indicates that both parts of the nucleoside molecule contribute to binding. Given that substrate binding to transporters is commonly weak (NupC:  $K_d = 2.6 \text{ mM}^{10}$ ; NupG:  $K_d < 2 \text{ mM}^8$ , both for uridine as ligand) the loss of binding affinity of the complete nucleoside, and also the loss of the entropic advantage associated with the binding of two linked halves, it is not surprising that undetectable inhibition was observed in the assay (*cf. ref. 37*).

The importance of the ribose portion of the nucleoside for binding to both transporters was revealed by the properties of adenosine analogues in which it was replaced by another moiety. Neither 9-(β-D-glucopyranosyl)adenine **30**, where the five-membered sugar ring is replaced with a six-membered glucose ring, nor 9-(2,3-dihydroxypropyl)adenine **17**, which lacks a cyclic sugar moiety, acted as inhibitors of uridine transport by either NupC or NupG. Similarly, for binding to the transporters it was necessary that the glycosidic bond linking the sugar and nucleobase moieties was in the natural β configuration: while 9-(β-D-arabinofuranosyl)adenine **26** was

found to be an effective inhibitor of uridine transport, 9-(α-D-arabinofuranosyl)adenine **27** did not inhibit either transporter.

To probe the involvement of the sugar in nucleoside binding in more detail, the effects of nucleosides bearing ribose analogues were next investigated. The 2'-hydroxyl group was found not to be an essential substituent in either purine or pyrimidine nucleosides: 2'-deoxyadenosine **14** (see **1**) was almost as effective as adenosine **1** in inhibiting transport in both transporters, while thymidine **5** was almost as effective as uridine **6**. Interestingly, while the configurational isomer of adenosine at the 2'-position, 9-(β-D-arabinofuranosyl)adenine **26** was as good an inhibitor of NupC-mediated uridine transport as 2'-deoxyadenosine, it was a slightly poorer inhibitor of NupG, possibly reflecting steric hindrance. In contrast to adenosine analogues modified at the 2' position, 3'-deoxyadenosine **15** (see **1**) failed to inhibit uptake by either transporter, indicating that the 3'-hydroxyl group in adenosine **1** is essential for binding to both NupC and NupG. Moreover, the correct α-stereochemistry at C-3' is essential: 9-(β-D-xylofuranosyl)adenine **28** and 9-(α-D-lyxofuranosyl)adenine **29** were not inhibitors of uridine transport mediated by either transporter (*cf. ref. 38* for similar conclusions regarding NupC). When 2',3'-dideoxycytidine **18** (see **4**) was examined there was a small inhibition of transport by NupC and no inhibition of that by NupG. This finding suggests that the 3'-hydroxyl group is less important for NupC inhibition by pyrimidine nucleosides than is the case with the purine nucleoside adenosine. In contrast, the 3'-hydroxyl group is essential for the binding of both purine and pyrimidine nucleosides to NupG.

The antiviral 3'-azido-3'-deoxythymidine (AZT) **19** was a moderately effective inhibitor of NupC, suggesting that the sugar 3'-hydroxyl group in *e.g.* adenosine and uridine acts primarily as a hydrogen bond acceptor in binding to this transporter. In the case of NupG inhibition was poor. This may be for steric reasons or because the crucial C-3' substituent (OH) in the ligand for NupG is preferentially a hydrogen-bond donor: the azido group can only act as an acceptor. The difference in the binding of AZT to the two *E. coli* transporters is notable. AZT has been found to be transported by NupC when expressed in *Xenopus laevis* oocytes.<sup>6</sup> In contrast to AZT, 3'-azido-2',3'-dideoxyadenosine (AZA) **20** was a poor inhibitor for both transporters, a finding consistent with the greater importance of the 3'-hydroxyl group for binding of purine than of pyrimidine nucleosides to NupC, as discussed above for AZT.

A striking distinction in ligand recognition between NupC and NupG was observed when 5'-deoxyadenosine **16** (see **1**) was tested for inhibition. This compound inhibited NupC transport almost as effectively as adenosine **1**, whereas it did not inhibit NupG transport at all. Clearly (Fig. 2) both of the C-3' and C-5' hydroxyl substituents in the sugar moiety of adenosine are critical for binding (recognition) to NupG but not NupC. The involvement of two adjacent pentose



hydroxyl groups in nucleoside binding to NupG suggests that the mechanism of binding may parallel that of galactosides to the homologous MFS transporter LacY, which involves the C-3' and C-4' hydroxyl groups of the hexose.<sup>15</sup> NupC, as we have seen, is unrelated in amino acid sequence to NupG and for this transporter only the C-3' hydroxyl is essential. For NupC the specificity, relatively lax in the sugar fragment, is more prescriptive in the heterocyclic moiety (see below).

Effective inhibition of NupC- and NupG-mediated uridine uptake both by purine riboside **21** and by 7-deaza-adenosine (tubercidin) **22** (see **1**) indicates that neither N-6 nor N-7 of adenosine **1** is required for binding to the transporters. Indications that the purine N-1 and/or N-3 is/are important for ligand recognition by NupC, comes from poor inhibition of uridine uptake by 1,3-dideaza-adenosine **23**. The purine nitrogens N-1 and N-3 appear to contribute to some extent to ligand binding to NupG. The putative importance of the N-1 position was highlighted by the poor or complete inability of *N*<sup>1</sup>-methyladenosine **42** to inhibit transport by NupC and NupG respectively. The steric relative of *N*<sup>1</sup>-methyladenosine, the intensely fluorescent compound 1,*N*<sup>6</sup>-ethenoadenosine **41**,<sup>33</sup> was similarly a relatively poor inhibitor of NupC and of NupG.

In contrast to these findings with purine analogues, effective transport inhibition by the uridine analogue 3-deaza-uridine **24** (see **6**) suggests that N-3 is not important for pyrimidine binding to either protein. However, mere aromaticity of the nucleobase moiety is not sufficient for binding, since 1-deoxy-1-phenyl- $\beta$ -D-ribofuranoside **25**, where no heterocyclic nitrogen atoms are present, was not an inhibitor.

In contrast to the N-1 position of the purine ring, both transporters showed greater flexibility with regard to substituents at the N-6 position. 6-Chloropurine riboside **31**, 6-mercaptapurine riboside **32** and *N*<sup>6</sup>-methyladenosine **33** all inhibited uridine transport to extents similar to or greater than those of purine riboside **21**. Introduction of increasingly bulky substituents up to the size of a benzyl moiety at the 6-position (see **31** to **39**) increasingly diminished, but did not completely abolish, the inhibitory activities of the analogues. An exception was *N*<sup>6</sup>,*N*<sup>6</sup>-dimethyladenosine **34**, which was a much poorer inhibitor of NupG than of NupC, possibly as a result of steric hindrance. NBMPR **40** was the only *N*<sup>6</sup>-substituted analogue tested that did not inhibit transport by NupC or NupG: as discussed previously, the parent molecule inosine is a poor inhibitor of NupC, while in the case of NupG the large *N*<sup>6</sup>-substituent may simply have exceeded the space available for binding.

The substitution at C-5' of uridine **6** with fluorine or bromine (**43** and **44**) failed to diminish inhibition of transport of either NupC or NupG; indeed some enhancement was observed (Fig. 1). 5-Trifluoromethyluridine **45** was likewise as good an inhibitor as uridine in the case of NupG-mediated transport, and was a moderately effective inhibitor of NupC, indicating that there is steric flexibility in the corresponding regions of the transporter binding sites. Whilst 8-bromo-adenosine **46** (see **1**) failed to inhibit transport by NupC it was a moderately effective inhibitor of NupG. The normally preferred conformation for adenosine is *anti*,<sup>39</sup> but this will be distorted towards *syn* by the presence of the large bromine substituent in **46**. The results lead to the conclusion that NupG is more tolerant of deviations toward the *syn*-conformation in purine ligands, whereas NupC is more demanding for conformations approximating to *anti*.

### Modelling the ligand-binding site of NupG

Understanding the molecular basis for the differing patterns of permeant recognition by NupG and NupC is hampered by the lack of a high resolution structure for either protein. However, as indicated above, NupG is distantly related to the OHS family of oligosaccharide transporters, the structure of one of which, the lactose-H<sup>+</sup> symporter LacY, has recently been determined in a mutant (C154G) arrested in its cytoplasmic-facing conformation.<sup>15</sup> We therefore used the latter as a template to construct a homology model for NupG. Because the sequences of NupG and LacY are only about 10% identical, the predicted locations of transmembrane helices and the patterns of sequence conservation within the NHS and OHS families were used to guide the alignment of the two proteins, as described in the Experimental section. A similar approach has recently been employed in modelling other distant homologues of LacY, the related Tn10-encoded metal-tetracycline/H<sup>+</sup> antiporter TetAB and the rat vesicular monoamine transporter rVMAT2.<sup>40</sup> While the resultant model must be regarded as provisional, it provides a useful starting point for future experiments aimed at identifying residues important for permeant binding, both by site-directed mutagenesis and by solid-state NMR approaches. To this end, Fig. 3 shows the arrangement of residues in NupG that may be involved in adenosine binding, by comparison with the known permeant binding site of LacY.<sup>15</sup> Of particular note is NupG residue R136, located at a position in putative transmembrane (TM) helix 5 that is occupied by arginine in all members of the OHS and NHS families analysed in the present study. In

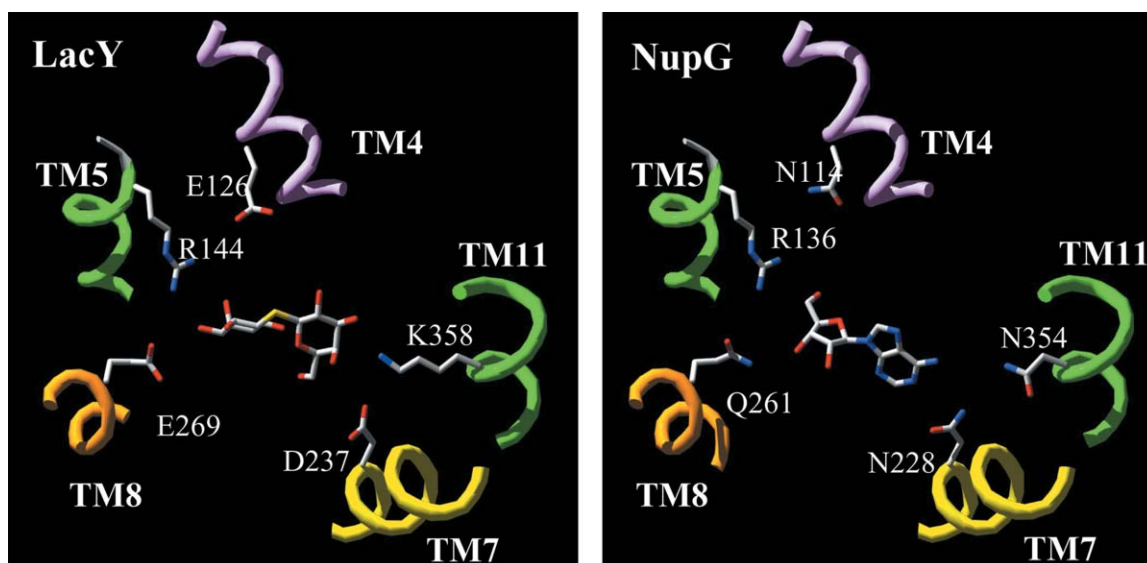


Fig. 3 Comparison of the permeant binding site of LacY, with bound TDG (taken from PDB ID 1PV7<sup>15</sup>), with that of a model of NupG, with bound adenosine.

LacY the corresponding residue, R144, is essential for permeant binding and forms a bifurcated hydrogen bond with the O-3 and O-4 atoms of the galactopyranosyl ring of thiodigalactoside (TDG).<sup>15</sup> The galactosyl moiety of the galactoside permeants of LacY determines binding specificity,<sup>41</sup> paralleling the importance of the ribose moiety for permeant binding to NupG. The absolute conservation of the TM5 arginine residue in the NHS family suggests that it plays a similar role in NupG, possibly forming hydrogen bonds with the C-3' and/or the C-5' hydroxyls, both of which play key roles in ligand binding, as described above. In Fig. 3 the adenosine molecule has therefore been oriented in the NupG binding site such that its ribose moiety is adjacent to R136. In the cytoplasmic-facing conformation of LacY, R144 is believed to form a salt bridge with TM8 residue E269. The latter is thought to play a particularly critical set of roles both in permeant binding and proton translocation, and to be vital for the energetic link between the N- and C-terminal domains of the protein.<sup>15</sup> The corresponding location in NupG, Q261, is occupied by a glutamine in all members of the NHS family and so, while not involved in salt-bridge formation, may play a similar role.

A third key residue known to be directly involved in permeant binding in LacY is K358 in TM11, which forms a hydrogen bond with the O-4' atom of TDG. The corresponding position in NupG, N354, is occupied by an asparagine or glutamine residue in 11 out of the 14 NHS family sequences analysed. It may therefore play a similar functionally important role, possibly in interaction with the base moiety of nucleoside ligands. Two other conserved positions in NupG and other members of the NHS family also correspond to residues implicated in ligand binding in LacY. An absolutely conserved TM4 asparagine residue (NupG N114) corresponds in location to an absolutely conserved OHS family residue (LacY E126) believed to interact with the O-4, O-5 or O-6 atoms of TDG *via* water molecules.<sup>15</sup> Similarly N228 of NupG, a position occupied by asparagine in 9 out of the 14 NHS sequences, corresponds in location to TM7 residue D237 of LacY. In the crystal structure of the LacY/TDG complex this residue lies close to the O-4' of TDG and is thought to interact with the permeant *via* a water molecule.<sup>15</sup> The NupG TM7 residue may similarly be involved in interaction with the nucleobase moiety of the nucleoside.

## Conclusions

Although NupC and NupG are unrelated in sequence, these two transporters from *E. coli* show broad similarities in ligand acceptability (Fig. 1). For example, the two transporters have similar stereochemical requirements: the glycosidic bond must be  $\beta$  and the normal  $\alpha$ -configuration at C-3' is essential. Similarly neither N-6 nor N-7 of adenosine **1** are of major importance for binding to either transporter. However, there are significant differences in the detail of the binding specificities, which are summarised diagrammatically in Fig. 2.

Structural group motifs important for adenosine binding by NupC are associated with the heterocyclic ring of nucleoside ligands, notably N-1 and/or N-3, and a single hydroxyl group in the sugar ring (C-3'); adenosine **1** is the best ligand. The C-5' hydroxyl does not appear to be involved in ligand recognition. NupC is a member of the concentrative nucleoside family of transporters and is thus evolutionarily related to the three human members of this family, hCNT1-3. The critical structural motifs in uridine and adenosine required for recognition by the latter transporters<sup>14</sup> satisfactorily correlate with those for NupC, affirming the suitability of the bacterial transporter as an experimentally amenable model for the human proteins.

In contrast to NupC, a hydroxyl group is required at *each* of the C-3' and C-5' positions for binding to NupG (see Fig. 1 and Fig. 2). The heterocyclic ring appears to play a lesser role in binding, for example the N-1 and N-3 positions appear to be less important than is the case for NupC. Overall, the greater

importance of the ribose moiety for binding is consistent with the evolutionary relationship between NupG and the galactoside transporter LacY, where permeant specificity is determined by the galactosyl moiety. A second notable difference between the two transporters is that NupG is tolerant of the *anti*- and *syn*-conformations between the heterocyclic and ribose rings in adenosine, whereas NupC requires an *anti*-conformation.

Our results for the two transporters provide essential information on the structural motifs necessary for the binding of nucleoside ligands to NupC and NupG. The results (Fig. 1 and Fig. 2) have allowed us to develop a homology model for the binding site in NupG (Fig. 3). Differences in structure associated with ligand binding to the two transporters have been shown to fit with the different families to which they belong.

Motif identification and the homology model for NupG provide a firm foundation for further research into nucleoside transporter structure, not least those approaches involving mutants and solid-state NMR spectroscopy.

## Experimental

For general experimental procedures, see ref. 16. Nucleosides and their analogues that were obtained from commercial sources were purchased from Aldrich or Sigma.

### Homology modelling

To build a homology model of NupG, separate alignments were made of the sequences of 14 members of the OHS and 14 members of the NHS families using Clustal X.<sup>8,42</sup> The patterns of residue conservation within the two families, and the locations of transmembrane helices predicted using the TMHMM algorithm,<sup>43</sup> were then used to guide a manual alignment of the NupG and LacY sequences. Modelling was performed using SWISS-MODEL<sup>44</sup> with the LacY structure (PDB ID 1PV7)<sup>15</sup> as template and the resultant sequence alignment as input. The structure of adenosine, taken from that of the nucleoside in site 1 of the human adenosine kinase-adenosine complex (PDB 1BX4), was manually introduced into the putative NupG permeant binding site. The model was visualised using Swiss PDB Viewer.<sup>45</sup>

### Transport assay for NupC and NupG

The assay used for the measurement of nucleoside transport was based on the method of Henderson *et al.*<sup>46</sup>, for measuring sugar transport into *E. coli*. The *E. coli* strain BL21(DE3), containing the plasmid vector DNA pGJL16 (for expressing NupC) or pGJL25 (for expressing NupG) was grown in M9 minimal liquid medium containing glycerol (20 mM). The cells were induced for overexpression with 0.05 mM isopropyl- $\beta$ -D-thiogalactoside (IPTG) after reaching an  $A_{600}$  of *ca.* 0.6 (*ca.* 3 h) and then grown for a further 1 h to an  $A_{600}$  of *ca.* 0.9–1.2. The cells were collected by centrifugation, washed three times by resuspending in transport buffer (150 mM KCl, 5 mM MES, pH 6.6), with centrifugation between each wash, and then resuspended in transport buffer to an accurately determined  $A_{600}$  of *ca.* 2.0. The cell suspension was used in the following transport assay: glycerol (5  $\mu$ l, 2 M, final concentration 20 mM) was introduced into a 5 cm<sup>3</sup> Bijou bottle and to this an aliquot of cells (482.5  $\mu$ l) was added. After vortexing briefly, the bottle was transferred to a water jacket at 25 °C and air was bubbled into the suspension to energise the cells. After precisely 3 min [<sup>14</sup>C]uridine (12.5  $\mu$ l, 2 mM, 5.0  $\mu$ Ci cm<sup>-3</sup>, final concentration 50  $\mu$ M) was added. After vortexing briefly, the bottle was returned to the water jacket with aeration. An aliquot (200  $\mu$ l) was removed from the suspension and the cells were collected by pipetting onto a pre-wetted 0.45  $\mu$ m cellulose nitrate membrane filter on a vacuum manifold at exactly 15 s after addition of the radiolabelled substrate. The filter was immediately washed with 3  $\times$  2 cm<sup>3</sup> transport buffer and then transferred to a scintillation vial for

counting. Background control samples ( $\times 2$ ) were prepared by pipetting aliquots (200  $\mu\text{l}$ ) of the original cell suspension onto pre-wetted filters on the manifold, which were then transferred to scintillation vials. Radioactivity standards ( $\times 3$ ) were prepared by pipetting 4  $\mu\text{l}$  of the radiolabelled substrate solution onto a filter in a scintillation vial. Competing unlabelled compounds (500  $\mu\text{M}$ ) were introduced by the addition of 10  $\mu\text{l}$  of a 25 mM solution to the Bijou bottle before introduction of the cells.

## Synthesis

In the following  $^{13}\text{C}$  NMR data, a resonance with  $^{13}\text{C}$  enrichment is indicated by \*. References 50–54 relate to supplementary material.†

### Synthesis of [1'- $^{13}\text{C}$ ]adenosine 1a

**1-O-Methyl-D-[1- $^{13}\text{C}$ ]ribofuranoside.** Dowex 8  $\times$  50 W, 200–400,  $\text{H}^+$  cation-exchange resin (300 mg) was washed with anhydrous methanol and then stirred for 4.5 h at room temperature with D-[1- $^{13}\text{C}$ ]ribose **13** (260 mg, 1.72 mmol) in anhydrous methanol (6  $\text{cm}^3$ ). The resin was removed by filtration and washed with anhydrous methanol. The combined filtrate and washings were evaporated to leave 1-O-methyl-D-[1- $^{13}\text{C}$ ]ribofuranoside<sup>47</sup> (284 mg, quant.) as a very pale yellow oil;  $\delta_{\text{H}}$  (300 MHz;  $\text{D}_2\text{O}$ ) 4.80 (*ca.* 0.2H, d,  $J_{1\alpha,2\alpha}$  4.2, H-1 $\alpha$ ), 4.72 (*ca.* 0.8H, d,  $J_{1\beta,2\beta}$  *ca.* 1, H-1 $\beta$ ), 4.04 (dd,  $J_{2\beta,3\beta}$  4.6,  $J_{3\beta,4\beta}$  6.9, H-3 $\beta$ ), 3.98 (dd,  $J_{1\alpha,2\alpha}$  4.2,  $J_{2\alpha,3\alpha}$  6.4, H-2 $\alpha$ ), 3.93–3.86 (m, overlapping H-2 $\beta$ , H-3 $\alpha$ , H-4 $\alpha$  and H-4 $\beta$ ), 3.68 (dd,  $J_{4\beta,5\beta\text{a}}$  3.3,  $J_{5\beta\text{a},5\beta\text{b}}$  12.3, H-5 $\beta\text{a}$ ), 3.58 (dd,  $J_{4\alpha,5\alpha\text{a}}$  3.5,  $J_{5\alpha\text{a},5\alpha\text{b}}$  12.3, H-5 $\alpha\text{a}$ ), 3.49 (dd,  $J_{4\beta,5\beta\text{b}}$  6.4,  $J_{5\beta\text{a},5\beta\text{b}}$  12.3, H-5 $\beta\text{b}$ ) (H-5 $\alpha\text{b}$  overlapping), 3.26 (0.72H, s,  $\text{OCH}_3$ ,  $\alpha$ ) and 3.23 (2.28H, s,  $\text{OCH}_3$ ,  $\beta$ );  $\delta_{\text{C}}$  (62.9 MHz;  $\text{D}_2\text{O}$ ) 108.2 (C-1 $\beta$ )\*, 103.5 (C-1 $\alpha$ )\*, 84.8 (C-4 $\alpha$ ), 83.1 (C-4 $\beta$ ), 74.5 (C-2 $\beta$ ), 71.3 (C-2 $\alpha$ ), 71.0 (C-3 $\beta$ ), 70.0 (C-3 $\alpha$ ), 62.9 (C-5 $\beta$ ), 61.6 (C-5 $\alpha$ ), 55.6 ( $\text{OCH}_3$ ,  $\alpha$ ) and 55.4 ( $\text{OCH}_3$ ,  $\beta$ );  $R_f$  (90 : 10 acetone–water) 0.61 and 0.75 ( $\alpha$  and  $\beta$ ).

**1-O-Methyl-2,3,5-tri-O-acetyl-D-[1- $^{13}\text{C}$ ]ribofuranoside.** A cooled (ice-bath) solution of 1-O-methyl-D-[1- $^{13}\text{C}$ ]ribofuranoside (284 mg, 1.72 mmol) in anhydrous pyridine (5  $\text{cm}^3$ ) was treated with acetic anhydride (0.5  $\text{cm}^3$ , 564 mg, 5.48 mmol) and then stirred at room temperature for 4 h. The reaction mixture was cooled (ice-bath), diluted with water (25  $\text{cm}^3$ ) and the resultant aqueous solution was extracted with chloroform (4  $\times$  25  $\text{cm}^3$ ). The combined organic extracts were washed with water (4  $\times$  50  $\text{cm}^3$ ), dried and then co-evaporated with toluene to give 1-O-methyl-2,3,5-tri-O-acetyl-D-[1- $^{13}\text{C}$ ]ribofuranoside<sup>48</sup> (403 mg, 80%) as a pale yellow oil;  $\delta_{\text{H}}$  (250 MHz;  $\text{CDCl}_3$ ) 5.33 (dd,  $J_{2\beta,3\beta}$  4.7,  $J_{3\beta,4\beta}$  6.6, H-3 $\beta$ ), 5.23 (dd,  $J_{1\beta,2\beta}$  < 1,  $J_{2\beta,3\beta}$  4.7, H-2 $\beta$ ), 5.18 (dd,  $J_{2\alpha,3\alpha}$  4.5,  $J_{3\alpha,4\alpha}$  7.2, H-3 $\alpha$ ), 5.14 (d,  $J_{1\alpha,2\alpha}$  4.4, H-1 $\alpha$ ), 4.98 (dd,  $J_{1\alpha,2\alpha}$  4.4,  $J_{2\alpha,3\alpha}$  4.5, H-2 $\alpha$ ), 4.91 (d,  $J_{1\beta,2\beta}$  < 1, H-1 $\beta$ ), 4.44–4.20 (m, overlapping H-4 $\alpha$ , H-4 $\beta$ , H-5 $\alpha$   $\alpha$  and  $\beta$ , and H-5 $\beta\text{a}$ ), 4.10 (dd,  $J_{4\beta,5\beta\text{b}}$  5.1,  $J_{5\beta\text{a},5\beta\text{b}}$  11.2, H-5 $\beta\text{b}$ ), 3.45 (0.58H, s,  $\text{OCH}_3$ ,  $\alpha$ ), 3.38 (2.42H, s,  $\text{OCH}_3$ ,  $\beta$ ), 2.14, 2.09, 2.05 and 2.12, 2.11, 2.07 (9H, 6  $\times$  s, 3  $\times$   $\text{CH}_3$   $\alpha$  and 3  $\times$   $\text{CH}_3$   $\beta$ , respectively);  $\delta_{\text{C}}$  (62.9 MHz;  $\text{CDCl}_3$ ) 171.0, 170.1, 170.0, (3  $\times$  C=O,  $\beta$ ) (3  $\times$  C=O,  $\alpha$  overlapping), 106.6 (C-1 $\beta$ )\*, 101.9 (C-1 $\alpha$ )\*, 79.9 (C-4 $\alpha$ ), 78.9 (C-4 $\beta$ ), 75.0 (C-3 $\beta$ ), 71.2 (C-3 $\alpha$ ), 71.9 (C-2 $\beta$ ), 70.2 (C-2 $\alpha$ ), 64.8 (C-5 $\beta$ ), 63.9 (C-5 $\alpha$ ), 56.0 ( $\text{OCH}_3$ ,  $\alpha$ ), 55.7 ( $\text{OCH}_3$ ,  $\beta$ ) and 21.2, 21.0, 20.9 (3  $\times$   $\text{CH}_3$ ,  $\beta$ ) (3  $\times$   $\text{CH}_3$ ,  $\alpha$  overlapping);  $R_f$  (95 : 5 acetone–water) 0.79 ( $\alpha$  and  $\beta$ ).

**1,2,3,5-Tetra-O-acetyl-D-[1- $^{13}\text{C}$ ]ribofuranoside.** A cooled (ice-bath) solution of 1-O-methyl-2,3,5-tri-O-acetyl-D-[1- $^{13}\text{C}$ ]ribofuranoside (403 mg, 1.39 mmol) and acetic anhydride (1.4  $\text{cm}^3$ , 1.56 g, 15.16 mmol) in glacial acetic acid (10  $\text{cm}^3$ ) was treated dropwise with conc. sulfuric acid (0.2  $\text{cm}^3$ ) and then stirred at room temperature for 3 h. The reaction mixture was cooled (ice-bath) and ice (10 g) was added with continued stirring. When the ice had melted, the resultant aqueous solution was extracted with chloroform (4  $\times$  25  $\text{cm}^3$ ). The

combined organic extracts were washed successively with water (2  $\times$  50  $\text{cm}^3$ ), a saturated aqueous solution of sodium hydrogen carbonate (2  $\times$  50  $\text{cm}^3$ ) and water (50  $\text{cm}^3$ ), then dried and evaporated to give 1,2,3,5-tetra-O-acetyl-D-[1- $^{13}\text{C}$ ]ribofuranoside<sup>48</sup> (355 mg, 80%) as a virtually colourless oil which solidified after standing at room temperature;  $\delta_{\text{H}}$  (250 MHz;  $\text{CDCl}_3$ ) 6.43 (0.29H, d,  $J_{1\alpha,2\alpha}$  3.8, H-1 $\alpha$ ), 6.17 (0.71H, d,  $J_{1\beta,2\beta}$  < 1, H-1 $\beta$ ), 5.36 (dd,  $J_{2\beta,3\beta}$  =  $J_{3\beta,4\beta}$  4.9, H-3 $\beta$ ), 5.34 (dd,  $J_{1\beta,2\beta}$  < 1,  $J_{2\beta,3\beta}$  4.9, H-2 $\beta$ ), 5.28 (dd,  $J_{2\alpha,3\alpha}$  6.8,  $J_{3\alpha,4\alpha}$  2.5, H-3 $\alpha$ ), 5.24 (dd,  $J_{1\alpha,2\alpha}$  3.8,  $J_{2\alpha,3\alpha}$  6.8, H-2 $\alpha$ ), 4.45 (dd,  $J_{4\beta,5\beta\text{a}}$  3.5,  $J$  6.1, H-4 $\beta$ ), 4.34 (dd,  $J_{4\beta,5\beta\text{a}}$  3.5,  $J_{5\beta\text{a},5\beta\text{b}}$  11.6, H-5 $\beta\text{a}$ ) (H-4 $\alpha$  overlapping), 4.15 (dd,  $J_{4\beta,5\beta\text{b}}$  5.0,  $J_{5\beta\text{a},5\beta\text{b}}$  11.6, H-5 $\beta\text{b}$ ) (H-5 $\alpha$   $\alpha$  and  $\beta$  overlapping), 2.14, 2.13, 2.12, 2.09 and 2.14, 2.11, 2.10, 2.08 (12H, 8  $\times$  s, 4  $\times$   $\text{CH}_3$ ,  $\alpha$  and  $\beta$ , respectively);  $\delta_{\text{C}}$  (62.9 MHz;  $\text{CDCl}_3$ ) 170.9, 170.1, 169.8, 169.7 (4  $\times$  C=O,  $\beta$ ), 170.3, 170.2, 170.1, 168.7 (4  $\times$  C=O,  $\alpha$ ), 98.6 (C-1 $\beta$ )\*, 94.4 (C-1 $\alpha$ )\*, 82.0 (C-4 $\alpha$ ), 79.7 (C-4 $\beta$ ), 74.5 (C-2 $\beta$ ), 70.9 (C-3 $\beta$ ), 70.4 (C-2 $\alpha$ ), 70.1 (C-3 $\alpha$ ), 64.0 (C-5 $\beta$ ), 63.7 (C-5 $\alpha$ ) and 21.4, 21.1, 20.9, 20.8 (4  $\times$   $\text{CH}_3$ ,  $\beta$ ) (4  $\times$   $\text{CH}_3$ ,  $\alpha$  overlapping);  $m/z$  (EI) no  $\text{M}^+$ , 259 (11%,  $\text{M}^+$  –  $\text{OCOCH}_3$ ), 245 (4,  $\text{M}^+$  –  $\text{CH}_2\text{OCOCH}_3$ ), 156 (9,  $\text{M}^+$  –  $\text{CH}_2\text{COCH}_3$  – 2  $\times$   $\text{CH}_3$  –  $\text{OCOCH}_3$ ), 128 (4, 156 – C=O), 43 (100,  $\text{CH}_3\text{CO}^+$ );  $R_f$  (70 : 30 diethyl ether–petroleum ether) 0.24 and 0.31 ( $\alpha$  and  $\beta$ ).

**2',3',5'-Tri-O-acetyl-[1'- $^{13}\text{C}$ ]adenosine.** At room temperature and under an atmosphere of nitrogen a stirred mixture of 1,2,3,5-tetra-O-acetyl-D-[1'- $^{13}\text{C}$ ]ribofuranoside (355 mg, 1.11 mmol) and adenine **7** (161 mg, 1.19 mmol) in anhydrous acetonitrile (30  $\text{cm}^3$ ) was treated dropwise with tin(IV) chloride (0.28  $\text{cm}^3$ , 620 mg, 2.38 mmol) on which the adenine dissolved to give a pale yellow solution. Stirring was continued for *ca.* 18 h. The reaction mixture was concentrated by evaporation to *ca.* 5  $\text{cm}^3$ , then, with cooling (ice-bath), sodium hydrogen carbonate (0.69 g) in water (2.35  $\text{cm}^3$ ) was added dropwise with stirring, which was continued until frothing had ceased. This left a white precipitate. The solvents were removed by evaporation under vacuum to leave a white powdery residue, which was repeatedly extracted with hot chloroform until no UV-absorbing material was detected in the extract. The combined chloroform extracts were filtered and then evaporated to leave a pale yellow oil (539 mg). The protected adenosine was isolated on a silica column (95 : 5 chloroform–methanol) as a colourless oil (293 mg, 67%); crystallisation from ethanol gave 2',3',5'-tri-O-acetyl-[1'- $^{13}\text{C}$ ]adenosine<sup>49</sup> (118 mg, 27%) as a white powder (found: C, 48.7; H, 5.0; N, 16.75.  $\text{C}_{15}^{13}\text{CH}_9\text{N}_5\text{O}_7$  requires C, 48.85; H, 4.9; N, 18%);  $\delta_{\text{H}}$  (250 MHz;  $\text{CDCl}_3$ ) 8.37 (1H, s, H-8), 7.96 (1H, s, H-2), 6.19 (1H, d,  $J_{1',2'}$  5.4, H-1'), 5.94 (1H, t,  $J_{2',3'}$  =  $J_{3',4'}$  5.4, H-3'), 5.77 (2H, br s,  $\text{NH}_2$ ), 5.68 (1H, t,  $J_{1',2'}$  =  $J_{2',3'}$  5.4, H-2'), 4.48–4.36 (3H, m, overlapping H-4', H-5'a and H-5'b) and 2.15, 2.13, 2.09 (9H, 3  $\times$  s, 3  $\times$   $\text{CH}_3$ );  $\delta_{\text{C}}$  (62.9 MHz;  $\text{CDCl}_3$ ) 170.8, 170.0, 169.8 (3  $\times$  C=O), 156.0 (C-6), 153.8 (C-2), 150.0 (C-4), 139.2 (C-8), 120.3 (C-5), 86.6 (C-1')\*, 80.6 (C-4'), 73.5 (C-3'), 71.0 (C-2'), 63.5 (C-5') and 21.2, 21.0, 20.8 (3  $\times$   $\text{CH}_3$ );  $m/z$  (EI) 393 (3%,  $\text{M}^+$ ), 350 ( $\text{M}^+$  –  $\text{CH}_3\text{CO}$ ), 334 (14,  $\text{M}^+$  –  $\text{CH}_3\text{CO}$  –  $\text{NH}_2$ ), 259 (28,  $\text{M}^+$  – base), 43 (100,  $\text{CH}_3\text{CO}^+$ );  $R_f$  (90 : 10 chloroform–methanol) 0.38.

**[1'- $^{13}\text{C}$ ]Adenosine 1a.** A solution of 2',3',5'-tri-O-acetyl-[1'- $^{13}\text{C}$ ]adenosine (100 mg, 0.25 mmol) in anhydrous methanol (12.5  $\text{cm}^3$ ) was treated with sodium methoxide (109 mg, 2.02 mmol = 2.7  $\text{cm}^3$  of a freshly prepared solution of sodium (62.5 mg, 2.72 mmol) in anhydrous methanol (12.5  $\text{cm}^3$ )) and then stirred at room temperature for 1 h. The pH of the reaction mixture was lowered from *ca.* 12 to 5.5 by careful addition of a solution of sulfuric acid in methanol (5%). The resultant white precipitate was removed by filtration over Celite and washed with anhydrous methanol; the combined filtrate and washings were evaporated to leave a colourless powder (71 mg, *ca.* 100%). Recrystallisation from water with drying under vacuum over  $\text{P}_2\text{O}_5$  gave **1a** (22 mg, 33%) as colourless needles (found: C, 44.45; H, 4.9; N, 25.95.  $\text{C}_9^{13}\text{CH}_{13}\text{N}_5\text{O}_4$  requires C, 44.2; H, 4.9;



N, 27.25%);  $\delta_{\text{H}}$  (250 MHz; DMSO- $d_6$ ) 8.38 (1H, s, H-8), 8.15 (1H, s, H-2), 7.42 (2H, br s,  $\text{NH}_2$ ), 5.89 (1H, dd,  $J_{1,2}$  6.2,  $J_{1,3}$  164.3, H-1'), 5.51 (2H, m overlapping OH-2' and OH-5'), 5.25 (1H, d,  $J_{3,3'-\text{OH}}$  4.5, OH-3'), 4.63 (1H, m,  $J_{1,2}$  6.2,  $J_{2,3}$  3.6, H-2'), 4.15 (1H, m,  $J_{3,4}$  3.1,  $J_{3,3'-\text{OH}}$  4.7, H-3'), 3.98 (1H, unresolved m, H-4'), 3.69 (1H, dt,  $J_{4,5a}$  4.1,  $J_{5a,5b}$  12.1, H-5'a) and 3.56 (1H, dt,  $J_{5b,4}$  3.7,  $J_{5a,5b}$  12.1, H-5'b); (unlabelled: 8.37 (1H, s, H-8), 8.15 (1H, s, H-2), 7.41 (2H, br s,  $\text{NH}_2$ ), 5.89 (1H, d,  $J_{1,2}$  6.2, H-1'), 5.49 (2H, m, overlapping OH-2' and OH-5'), 5.25 (1H, d,  $J_{3,3'-\text{OH}}$  4.6, OH-3'), 4.63 (1H, m,  $J_{1,2}$  6.2,  $J_{2,3}$  4.9, H-2'), 4.16 (1H, m,  $J_{2,3}$  4.9,  $J_{3,4}$  3.0, H-3'), 3.98 (1H, m,  $J_{3,4}$  3.0 and  $J$  6.0, H-4'), 3.69 (1H, dt,  $J_{4,5a}$  3.9,  $J_{5a,5b}$  12.0, H-5'a) and 3.56 (1H, dt,  $J_{4,5b}$  3.7,  $J_{5a,5b}$  12.0, H-5'b));  $\delta_{\text{C}}$  (62.9 MHz; DMSO- $d_6$ ) 156.5 (C-6), 152.7 (C-2), 149.4 (C-4), 140.3 (C-8), 119.7 (C-5, d,  $J_{5,1}$  2.2), 88.3 (C-1')\*, 86.3 (C-4'), 73.8 (C-2', d,  $J_{1,2}$  42.6), 71.0 (C-3', d,  $J_{1,3}$  3.5) and 62.0 (C-5');  $m/z$  (EI) 269 ( $\text{M}^+$  + H), 251 ( $\text{M}^+$  - OH), 238 ( $\text{M}^+$  -  $\text{CH}_2\text{OH}$ ), 179 (36%), 165 (74), 135 (100,  $\text{M}^+$  - sugar), 119 (34, 135 -  $\text{NH}_2$ );  $m/z$  (ES) 291 (100%,  $\text{M}^+$  + Na);  $m/z$  (ES) 291.0896 ( $\text{M}^+$  + Na, required for  $\text{C}_9^{13}\text{H}_{13}\text{N}_5\text{O}_4\text{Na}$  291.0899, difference 1.0 ppm);  $R_f$  (90 : 10 acetone-water) 0.82, (80 : 20 chloroform-methanol) 0.20.

**Synthesis of 9-( $\beta$ -D-arabinofuranosyl)adenine 26<sup>21</sup> (adapted from ref. 22).** At room temperature a solution of 9-(2',3',5'-tri-*O*-benzyl- $\beta$ -D-arabino-furanosyl)adenine (1.0 g, 1.86 mmol) in methanol containing 10% formic acid (100 cm<sup>3</sup>) was added to a stirred suspension of 10% Pd on carbon (2.0 g = 200 mg, 1.42 mmol Pd) in methanol containing 10% formic acid (100 cm<sup>3</sup>). With monitoring by TLC, further additions of formic acid (5 cm<sup>3</sup>) were made after 25 h and 66 h. After a total of 72 h the catalyst was removed by filtration and washed with methanol (*ca.* 500 cm<sup>3</sup>). The combined filtrates were evaporated to leave a colourless oil (584 mg), which solidified on cooling to room temperature. This was recrystallised from water and then dried under vacuum over  $\text{P}_2\text{O}_5$  to give **26** (387 mg, 78%) as colourless needles (found: C, 42.35; H, 5.25; N, 25.5.  $\text{C}_{10}\text{H}_{13}\text{N}_5\text{O}_4$  requires C, 44.9; H, 4.9; N, 26.2%);  $\delta_{\text{H}}$  (250 MHz; DMSO- $d_6$ ) 8.20 (1H, s, H-8), 8.14 (1H, s, H-2), 7.27 (2H, br s,  $\text{NH}_2$ ), 6.26 (1H, d,  $J_{1,2}$  4.4, H-1'), 5.68 (1H, d,  $J_{2,2'-\text{OH}}$  4.1, OH-2'), 5.61 (1H, d,  $J_{3,3'-\text{OH}}$  4.1, OH-3'), 5.20 (1H, t,  $J_{5a,5'-\text{OH}} = J_{5b,5'-\text{OH}}$  5.2, OH-5'), 4.13 (2H, m, overlapping H-2' and H-3'), 3.78 (1H, m,  $J$  4.3 and 8.3, H-4') and 3.67 (2H, m,  $J_{4,5a}$  4.1,  $J_{4,5b}$  5.1,  $J_{5a,5b}$  9.2, H-5'a and H-5'b);  $\delta_{\text{C}}$  (75.5 MHz; DMSO- $d_6$ ) 156.2 (C-6), 152.8 (C-2), 149.7 (C-4), 140.6 (C-8), 118.5 (C-5), 84.4 (C-4'), 83.9 (C-1'), 76.0 (C-2'), 75.3 (C-3') and 61.2 (C-5');  $m/z$  (FAB) 268 (100%,  $\text{M}^+$  + H);  $m/z$  (EI) 268.106 ( $\text{M}^+$  + H,  $\text{C}_{10}\text{H}_{13}\text{N}_5\text{O}_4$  requires 268.105, difference 3.7 ppm);  $R_f$  (90 : 10 acetone-water) 0.60.

The strategy for the synthesis of the following compounds **27** to **29** from the free sugar is the same as that shown for adenosine **1** in Scheme 1.

**Synthesis of 9-( $\alpha$ -D-arabinofuranosyl)adenine 27<sup>18</sup>.** This was prepared from D-arabinose with final purification on a silica column (80 : 20 chloroform-methanol) to give **27** (34 mg) as a colourless powder;  $\delta_{\text{H}}$  (250 MHz; DMSO- $d_6$ ) 8.24 (1H, s, H-8), 8.06 (1H, s, H-2), 7.22 (2H, br s,  $\text{NH}_2$ ), 5.75 (1H, d,  $J_{1,2}$  5.1, H-1'), 4.59 (1H, t,  $J_{2,3'} = J_{3,4'}$  5.0, H-3'), 4.08 (1H, m, H-4'), 3.89 (1H, t,  $J$  6.0, H-2') and 3.52 (2H, m,  $J_{4,5a}$  3.7,  $J_{4,5b}$  4.9, H-5'a and H-5'b);  $\delta_{\text{C}}$  (75.5 MHz; DMSO- $d_6$ ) 156.4 (C-6), 152.9 (C-2), 149.6 (C-4), 140.4 (C-8), 119.6 (C-5), 88.7 (C-1'), 85.6 (C-4'), 79.7 (C-3'), 75.6 (C-2') and 61.5 (C-5');  $m/z$  (EI) 267 ( $\text{M}^+$ ), 250 ( $\text{M}^+$  - OH), 236 ( $\text{M}^+$  -  $\text{CH}_2\text{OH}$ ), 135 (100%,  $\text{M}^+$  -  $\text{C}_5\text{H}_9\text{O}_4$  + H) and 43 (88,  $\text{CH}_3\text{CO}^+$ );  $m/z$  (ES) 268.0956 ( $\text{M}^+$  + H,  $\text{C}_{10}\text{H}_{14}\text{N}_5\text{O}_4$  requires 268.1046, difference 33.6 ppm);  $R_f$  (90 : 10 chloroform-methanol) 0.08, (90 : 10 acetone-water) 0.66.

**Synthesis of 9-( $\beta$ -D-xylofuranosyl)adenine 28<sup>19</sup>.** This was prepared from D-xylose with final recrystallisation from 95% ethanol and drying under vacuum over  $\text{P}_2\text{O}_5$  to give **28** (52 mg) as a colourless powder (found: C, 45.2; H, 5.0; N, 26.05.

$\text{C}_{10}\text{H}_{13}\text{N}_5\text{O}_4$  requires C, 44.9; H, 4.9; N, 26.2%);  $\delta_{\text{H}}$  (300 MHz; DMSO- $d_6$ ) 8.35 (1H, s, H-8), 8.19 (1H, s, H-2), 7.55 (2H, br s,  $\text{NH}_2$ ), 5.90 (2H, m, overlapping OH-2' and OH-3'), 5.87 (1H, d,  $J_{1,2}$  1.6, H-1'), 4.73 (1H, t,  $J_{5a,5'-\text{OH}} = J_{5b,5'-\text{OH}}$  5.5, OH-5'), 4.31 (1H, unresolved m, H-2'), 4.16 (1H, m,  $J_{3,4'}$  3.8,  $J_{4,5'a}$  4.9,  $J_{4,5'b}$  6.1, H-4'), 4.04 (1H, m, H-3'), 3.77 (1H, dd,  $J_{4,5'a}$  4.9,  $J_{5a,5'b}$  11.5, H-5'a) and 3.65 (1H, dd,  $J_{4,5'b}$  6.1,  $J_{5a,5'b}$  11.5, H-5'b);  $\delta_{\text{C}}$  (62.9 MHz; DMSO- $d_6$ ) 155.1 (C-6), 151.2 (C-2), 149.0 (C-4), 140.4 (C-8), 119.2 (C-5), 89.9 (C-1'), 84.1 (C-4'), 81.2 (C-2'), 75.5 (C-3') and 59.8 (C-5');  $m/z$  (ES) 268 (100%,  $\text{M}^+$  + H) and 136 ( $\text{M}^+$  -  $\text{C}_5\text{H}_9\text{O}_4$  + H);  $R_f$  (90 : 10 chloroform-methanol) 0.06, (90 : 10 acetone-water) 0.65.

**Synthesis of 9-( $\alpha$ -D-lyxofuranosyl)adenine 29<sup>20</sup>.** This was prepared from D-lyxose with final purification on a silica column (70 : 30 chloroform-methanol) to give **29** (94 mg) as a very pale yellow powder;  $\delta_{\text{H}}$  (300 MHz; DMSO- $d_6$ ) 8.37 (1H, s, H-8), 8.14 (1H, s, H-2), 7.28 (2H, br s,  $\text{NH}_2$ ), 5.85 (1H, d,  $J_{1,2}$  7.0, H-1'), 5.49 (1H, br unresolved d, OH-2'), 5.18 (1H, br unresolved d, OH-3'), 5.02 (1H, dd,  $J_{1,2}$  7.0,  $J_{2,3}$  4.3, H-2'), 4.64 (1H, br unresolved d, OH-5'), 4.43 (1H, m, H-4'), 4.15 (1H, m, H-3'), 3.66 (1H, dd,  $J_{4,5'a}$  4.8,  $J_{5a,5'b}$  11.1, H-5'a) and 3.53 (1H, m, overlapping water, H-5'b);  $\delta_{\text{C}}$  (62.9 MHz; DMSO- $d_6$ ) 158.9 (C-6), 155.4 (C-2), 152.4 (C-4), 143.3 (C-8), 122.3 (C-5), 97.2 (C-1'), 90.4 (C-4'), 85.4 (C-2'), 77.4 (C-3') and 62.7 (C-5');  $m/z$  (FAB) 268 ( $\text{M}^+$  + H), 251 ( $\text{M}^+$  -  $\text{NH}_2$ );  $m/z$  (ES) 268.1049 ( $\text{M}^+$  + H,  $\text{C}_{10}\text{H}_{14}\text{N}_5\text{O}_4$  requires 268.1046, difference 1.1 ppm);  $R_f$  (95 : 5 chloroform-methanol) 0.00, (90 : 10 acetone-water) 0.48.

**Synthesis of 9-( $\beta$ -D-glucopyranosyl)adenine 30<sup>25</sup>.** 1,2,3,4,6-Penta-*O*-acetyl- $\beta$ -D-glucopyranoside (2.0 g, 5.12 mmol) was coupled with adenine **7** (0.74 g, 5.51 mmol) using tin(IV) chloride conditions with purification on a silica column (96 : 4 chloroform-methanol) to give 9-(2',3',4',6'-tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl)adenine (546 mg, 23%) as a colourless semi-crystalline oil. Deprotection with sodium methoxide afforded **30** (132 mg, 44%) as a colourless powder;  $\delta_{\text{H}}$  (300 MHz; DMSO- $d_6$ ) 8.33 (1H, s, H-8), 8.16 (1H, s, H-2), 7.29 (1H, s,  $\text{NH}_2$ ), 5.41 (1H, d,  $J_{1,2}$  9.2, H-1'), 4.01 (1H, t,  $J_{1,2}$  9.2,  $J_{2,3}$  8.9, H-2'), 3.71 (1H, d,  $J_{5,6'a}$  10.4, H-6'a) and 3.5 - 3.2 (4H, m, overlapping H-3', H-4', H-5' and H-6'b);  $\delta_{\text{C}}$  (75.5 MHz; DMSO- $d_6$ ) 156.3 (C-6), 153.0 (C-2), 150.1 (C-4), 140.1 (C-8), 119.0 (C-5), 83.1 (C-1'), 80.3 (C-5'), 77.6 (C-3'), 71.6 (C-2'), 70.1 (C-4') and 61.2 (C-6');  $m/z$  (EI) 297 ( $\text{M}^+$ ), 280 ( $\text{M}^+$  - OH), 267 ( $\text{M}^+$  -  $\text{CH}_2\text{OH}$  + H), 250 ( $\text{M}^+$  -  $\text{CH}_2\text{OH}$  - OH + H), 135 (100%,  $\text{M}^+$  - sugar), 119 (16, 135 -  $\text{NH}_2$ );  $m/z$  (ES) 298.1144 ( $\text{M}^+$  + H,  $\text{C}_{11}\text{H}_{16}\text{N}_5\text{O}_5$  requires 298.1151, difference 2.3 ppm);  $R_f$  (90 : 10 acetone-water) 0.36.

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