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Review Article

Specific labelling of nucleosides and nucleotides with ^{13}C and ^{15}N

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Summary

Synthetic methods leading to nucleosides and deoxynucleosides specifically labelled with ^{13}C and ^{15}N are reviewed. The paper covers labelling the sugar part of the molecule (with ^{13}C) and the aglycone part (both with ^{13}C and ^{15}N). Synthetic methods for labelling at every relevant site of the nucleoside molecule are discussed. The paper also presents the strategies for the synthesis of multilabelled nucleosides. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: nucleosides; ^{13}C; ^{15}N

Introduction

Isotope labelling of biomacromolecules such as proteins and nucleic acids has emerged in recent years as one of the most powerful tools in elucidating their structural details[1–5]. Selective labelling with stable isotopes such as ^{2}H (deuterium),[6–11] ^{13}C carbon[12–16] or ^{15}N nitrogen[17–25] opened a possibility for application of additional heteronuclear multidimensional techniques. Crystallographic analysis of large biomolecules[26–29] usually gives a better resolution of molecular structure than NMR spectroscopy and provides good results for larger molecules. On

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the other hand, it is the NMR, not crystallography, which can give the information on the real structures present in solution at quasi-physiological conditions. Therefore, the task of improving the accuracy of NMR analyses to the level comparable with that of crystallography has become a crucial one. One possible solution is building more powerful magnets and development of new, more sophisticated pulse sequences and spectral techniques[30–37] There has been tremendous progress in this field in recent years, but as the magnetic field intensity increases, so does the intensity of the relaxation processes, which cause spectral lines to broaden and weaken, and improvements of a technological nature can soon reach their limits.[38]

The other possible solution is to prepare the molecule of interest in a way that will help reduce the undesired consequences of the size and monotonously repeating primary structure. Labelling with stable isotopes appears as a method of choice in this case. The first approach was to label the studied molecule uniformly via the biosynthetic techniques.[1,3,4,39,40] There is a great number of excellent structural studies on such labelled nucleic acid fragments which have provided important structural results.[41–44] More detailed studies, however, require site-specific labelling of nucleic acid fragments and this task cannot be achieved biosynthetically. Numerous reports on different labelling procedures can be found in the literature, but only deuteriation has been recently reviewed.[8] Deuterium is the isotope most frequently used for site-specific labelling and is the most versatile label, but selective labelling with ^{13}C or ^{15}N widens the scope of analytical techniques and opens a possibility to study structural phenomena in more detail.[44–51] The purpose of this paper is to give a general view of the reactions and procedures leading to specific ^{13}C carbon-and ^{15}N nitrogen-labelled nucleosides. Such molecules are intended to serve mainly as the starting material for the synthesis of the oligomers with strategically positioned labels. A study of isotopically modified macromolecules allows observation of the structural detail of the molecule or its functional process without the interference from the other, non-labelled parts of the molecule.

^{13}C Labelling

Spectral width of ^{13}C NMR spectrum is almost 20 times that of the proton NMR spectrum. It means that in ^{13}C NMR there is much better

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chance of avoiding line overlap and that each factor influencing the chemical shift (ionization, shielding, stereoelectronic effects) will be much more pronounced, therefore easier to observe and quantify than in the case of proton NMR. Labelled nucleus of spin number $I = \frac{1}{2}$ present in abundance close to 100% appears as a new spectral line, characteristic of the labelled site. This line is $\sim$100 times more intense than the lines of unlabelled sites, which makes possible to study heteronuclear interactions of the labelled site with the rest of the molecule while interactions of the other carbon atoms are not pronounced in the spectrum. The available methods enable the introduction of the carbon label into practically any chosen site of the nucleoside molecule, moreover the molecules containing combinations of several carbon labels or carbon/nitrogen/deuterium have been synthesized.

Labelling of the aglycone part of the nucleoside molecule introduces the carbon label into a position close to the centre of the hydrogen bonding[52–56] and to the sites responsible for the formation of association complexes with proteins,[24,25,57] drugs,[21,58] intercalators or other nucleic acid molecules.[59,60] A carbon label at such a site gives the possibility to study the kinetics and dynamics of those interactions as well as makes an excellent reference point for the NOE distance measurements.

By labelling the carbon atoms of the sugar ring, the label is introduced at the site which is directly engaged into sugar–phosphate backbone steric interactions. By using heteronuclear multiple resonance techniques, such a label can be used to impose additional angle and distance constraints in structural calculations.[30,46,49,50,61–65]

Monomeric units of the nucleic acids (nucleosides or nucleotides) are substrates of numerous enzymes. Such molecules in a specifically labelled form have found the application as probes in studying enzymatic reactions.[66] When the reaction mixture is studied by NMR spectroscopy, unlabelled substrates of enzymes are extremely difficult to observe due to the great disproportion in molecular size between nucleoside and enzyme protein, which translates into similar disproportion in line intensity. This effect is enhanced by the fact that when nucleoside–protein complex is formed, increased relaxation usually causes severe line broadening. The labelling results in increasing the line intensity by two orders of magnitude and usually provides measurable signals and the enzymatic transformations of the labelled molecule can be followed by NMR.[66]

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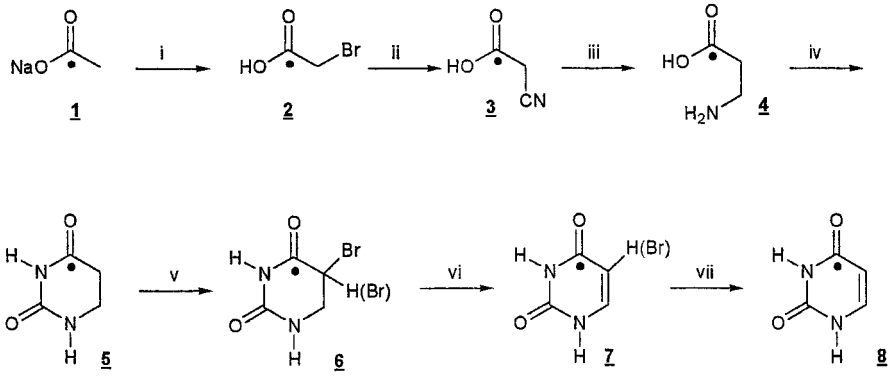
Labelling of the aglycones: pyrimidines

Uracil ring can be efficiently assembled from simple precursors, all of which are commercially available in labelled form. In the synthetic pathway described by Poulter,[67,68] the label can be placed in potentially any position of the six-membered ring simply by changing the label source. Two of these possibilities brought into reality via this method are [4-^{13}C]-uracil **8** (later converted into labelled uridine and cytidine) and [3-^{15}N]-uracil (*vide infra*) (Scheme 1).



Scheme 1. (i) 1. H^+, 2. Br_2, $(CF_3CO)_2O$; (ii) 1. KCN/K_2CO_3, 2. HCl; (iii) H_2/PtO_2; (iv) 1. KCNO, 2. HCl; (v) Br_2; (vi) K_2CO_3; (vii) $H_2/Pd/BaSO_4$

In a similar synthesis starting from [2-^{13}C-cyano] propionic acid, [6-^{13}C] thymine was obtained, and subsequently it was converted into thymidine.[69]

The methyl group of thymine plays a crucial role in the DNA-protein ineractions. The methyl group was introduced in the labelled form on the nucleoside level, starting from 5-bromodeoxyuridine **9**.[70] Bromonucleoside was converted into 5-litio derivative **10** which reacted with $^{13}CH_3I$ giving the labelled thymidine **11**. This nucleoside was also transformed into [5-^{13}C-methyl]-deoxycytidine[70] (Scheme 2).

Labelling of the aglycones: purines

In the ^{13}C labelling of purine nucleosides the syntheses reported concerned position C2 and C8 of the base ring.

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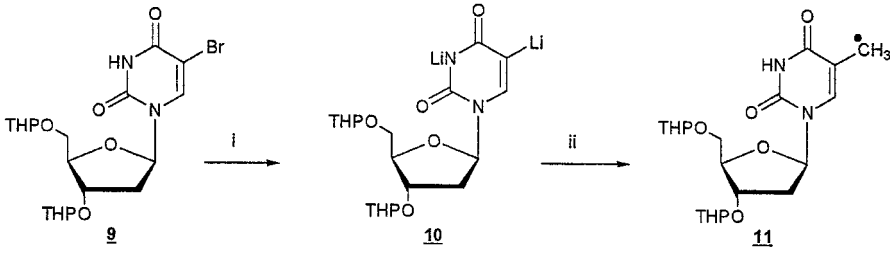

Scheme 2. (i) BuLi (2 eq.), THF/-40°C; (ii) $^{13}CH_3I$/-60°C

Labelling at C2

The chemical shift of this carbon nucleus proved to be sensitive to protonation at N1 (due to severe line broadening, the ^{15}N NMR cannot be applied in this case).[71] The ^{13}C isotope can be introduced into this position when constructing the purine ring from 5-aminoimidazole-4-carboxamide (AICA, **12**). In the synthesis[72] the label source was ethyl sodium [^{13}C]-xantate, generated *in situ* from the labelled carbon disulphide (Scheme 3).

The initial product of condensation was [2-^{13}C]-2-mercapto-6-oxo-purine **13**, which can be transformed into hypoxanthine, adenine or guanine. All of these three aglycones were obtained and subsequently coupled with protected ribose yielding C2-labelled purine nucleosides: inosine, adenosine **16** or guanosine **18**.[72]

Another route to C2-labelled guanosine makes use of Ueda's synthesis of 2,6-diaminopurine nucleoside.[73] The synthesis started with readily available adenosine 1-oxide **19**.[73] The oxide upon reaction with labelled bromocyan (prepared from $K^{13}CN$ and Br_2) gave tricyclic intermediate **20**.[74,75] This intermediate upon methylation with iodomethane and action of sodium hydroxide underwent the Dimroth rearrangement.

During this reaction the original C2 carbon atom was split off the molecule and open-ring intermediate **21** condensed again to the six-membered aromatic ring, introducing the cyanide carbon into position C2. The cyanide nitrogen atom became *N*2-*exo*-amine group and the initial N1 and N6 nitrogen atoms exchanged their positions. The final product of this sequence is [2-^{13}C], *N*6-methoxy-2,6-diaminopurine nucleoside **22**. The methoxyamino group in the position 6 of diaminopurine can be selectively exchanged for oxygen by the action of adenosine deaminase, and this operation efficiently yielded the desired [2-^{13}C]-guanosine **18**[74,75] (Scheme 4).

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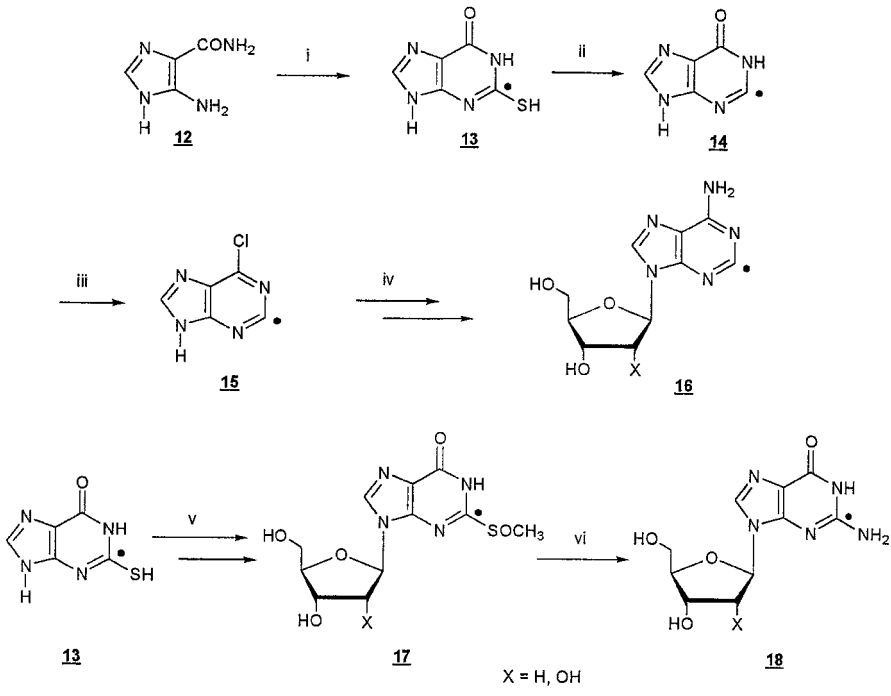
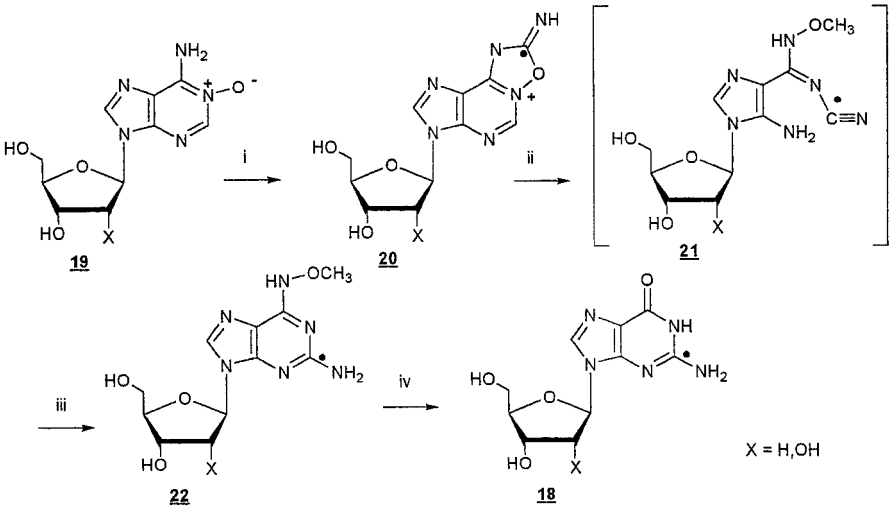


Scheme 3. (i) $NaS^{13}C(S)OEt$; (ii) Ni/Raney, HCOOH; (iii) $POCl_3/(CH_3)_2NPh$; (iv) 1. Enzymatic transglycosylation, 2. NH_3; (v) 1. CH_3I, 2. Enzymatic transglycosylation, 3. Oxone®; (vi) $KHCO_3/NH_4Cl$



Scheme 4. (i) Br_2, $K^{13}CN$, MeOH; (ii) Et_3N, CH_3I; (iii) 1. 0.25 M NaOH, 1 h, 2. pH 7; (iv) Adenosine deaminase, pH 7.4

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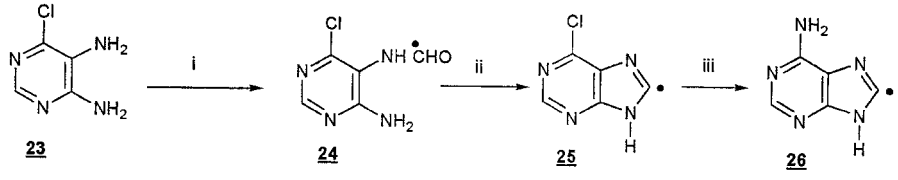
Labelling at position C8

The C8 carbon atom is situated between two nitrogen atoms, so a logical way to introduce the label into this position would be a reaction of a labelled formylating reagent with 5,6-diaminopyrimidine system, as in **23**. Among the reagents tested the most efficient was mixed formyl–pivaloyl anhydride, which can be conveniently prepared from pivaloyl chloride and sodium [^{13}C]-formate[76] (Scheme 5).



Scheme 5. (i) [^{13}C]-formyl–pivaloyl anhydride, CH_2Cl_2; (ii) $TiCl_2(OiPr)_2$, $P(OEt)_3$; (iii) NH_3 aq

Labelling of carbon atoms of the sugar ring

All the described methods of ribose/deoxyribose labelling are based on sugar transformations. Carbon exchange occurring in sugar moiety of a nucleoside would be extremely hard to achieve in a simple and economic way. All the methods presented to date start with preparation of the labelled sugar component followed by condensation with the appropriate base to yield the nucleoside.

Although selective labelling of single carbon atoms within the sugar moiety would be extremely useful for NMR studies,[77] such syntheses are scarce and limited to positions 1,2 and 5 of the furanose ring. It is in great contrast to the number of methods available for the selective deuterium labelling of sugars.[8] In the case of carbon labelling, such a selective introduction of the isotope would require reconstruction of the carbon skeleton instead of exchange of substituent without affecting the skeleton. Such a reconstruction (or assembling of the sugar molecule *de novo*) requires regio- and stereo-selective syntheses hard to realize with the necessary high yield. Isotope sources are available only in the form of simple inorganic (less frequently organic) compounds and converting them into sugars usually means multistep syntheses.[78–80] Different sugars selectively enriched with ^{13}C were synthesized for the purpose of NMR studies,[81] but the only selective ^{13}C labelling of

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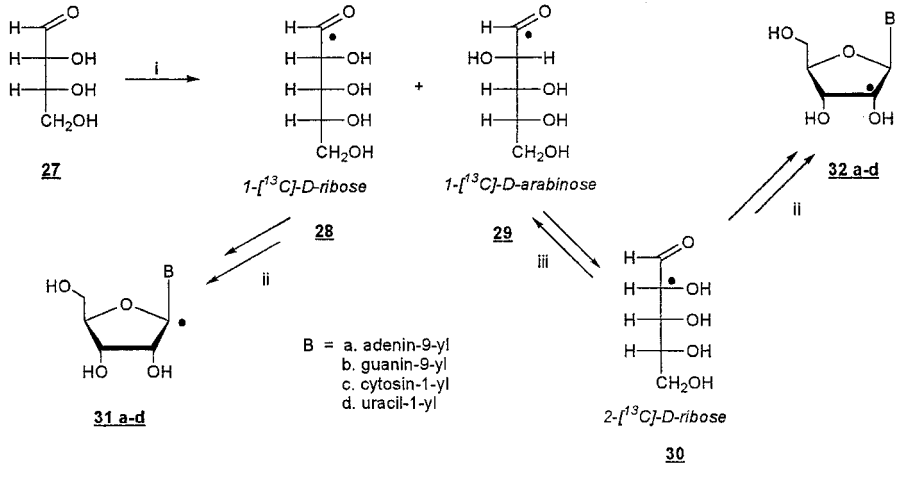
nucleosides reported to date involved 'the peripheries' of the sugar molecule i.e. the carbon atoms C1, C2 and C5.

Labelling at carbon atoms C1 and C2

The [1-^{13}C]-D-ribose is commercially available, but its high price discourages the use of this compound as the starting material for nucleoside synthesis, although such a transformation was reported.[82,83] Most syntheses start with a simple label source and unlabelled chiral precursor of the D-ribose core. D-Ribose labelled at the carbon atom C1 was obtained in cyanohydrin synthesis from D-erythrose **27** and ^{13}C-enriched cyanide.[16] After the reduction a mixture of anomeric labelled sugars, D-ribose **28** and D-arabinose **29**, was separated. Arabinose was used as a substrate for synthesis of [2-^{13}C]-D-ribose **30** by isomerization reaction catalysed by molybdate ion[84] (Scheme 6).



Scheme 6. (i) 1. K^{13}CN, pH 8.0–8.3, 2. Pd/BaSO$_4$, H$_2$; (ii) 1. MeOH/H$_2$SO$_4$ cat., 2. BzCl/Pyridine, 3. AcOH/Ac$_2$O/H$_2$SO$_4$, 4. Silylated base, TfOTMS, (CH$_2$Cl)$_2$, 5. NH$_3$/MeOH; (iii) Molybdic acid 0.005 M, pH 4.5, 90°C

This route to the [2-^{13}C]-labelled sugar is quite tedious – due to unfavourable equilibrium, the mixture after the isomerization had to be separated and the recovered arabinose recycled. Having repeated this procedure twice, a 50% yield of [2-^{13}C]-D-ribose **30** was achieved.[16]

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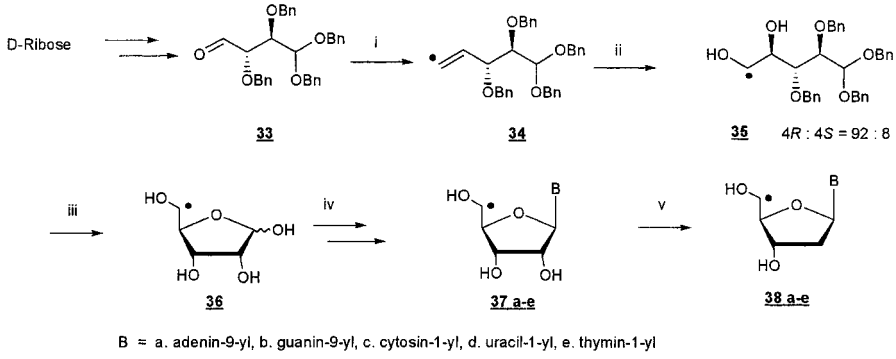
Another possible route to this form of labelled ribose was realized by double cyanohydrin reaction: at first D-glyceraldehyde was converted into a mixture of [1-^{13}C]-D-erythrose and [1-^{13}C]-D-threose with the use of labelled cyanide and the reaction was repeated on the separated isomers with cyanide of natural isotopic abundance. The labelled pentoses obtained in this way were not, however, intended for the synthesis of nucleosides.[85]

Labelling at carbon atom C5

This position on the other end of the pentose molecule can be labelled relatively easily by reacting the label source with the substrate of appropriate stereochemistry[15,86] (Scheme 7)



Scheme 7. (i) BuLi, Ph_3P,$^{13}CH_3I$; (ii) 1. OsO_4, 4-Methylmorpholine *N*-oxide, 2. quench; (iii) 1. H_2, Pd/C; (iv) 1. MeOH/H_2SO_4cat., 2. BzCl/Pyridine, 3. AcOH/Ac_2O/H_2SO_4, 4. Silylated base, TfOTMS, $(CH_2Cl)_2$, 5. NH_3/MeOH; (v) 1. PhOC(S)Cl, 1-methylimidazole, 2. Bu_3SnH, AIBN

The (2*S*,3*R*)2,3,4,4-tetrabenzyloxybutanal **33** was prepared from D-ribose and subjected to the Wittig synthesis with the ^{13}C-labelled ylide prepared from $^{13}CH_3I$. Terminal olefin **34** obtained in this way was oxidized to a diol **35** with high stereoselectivity. After debenzylation, the labelled [5-^{13}C]-D-ribose **36** was converted into nucleosides **37a–e** and **38a–e** using standard methods. This synthetic route was accomplished with much higher overall yield than those of the earlier methods[87,88] starting with [1-^{13}C]-L-ribonic acid or [6-^{13}C]-D-glucose.

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Labelling at all carbon atoms of the sugar moiety

This labelling procedure means in fact the conversion of [$^{13}C_6$]-D-glucose into [$^{13}C_5$]-D-ribose. The labelled glucose can be obtained biosynthetically and is evidently the most economic starting material for the synthesis of labelled [$^{13}C_5$]-D-ribose.[89] Two operations on the molecule of glucose are necessary in order to achieve transformation into ribose. The first of these operations is the inversion of the absolute configuration of the C3 carbon atom (from *S* '*gluco*-' into *R* '*allo*-') and the second is the shortening of the molecule by one carbon atom. In principle, due to mutual stereochemical relation of allose and ribose, the second operation can be accomplished in two ways; either the C1 carbon atom can be split off with simultaneous oxidation of C2 to aldehyde, or the C6 carbon atom can be removed. Although both transformations were described for unlabelled D-glucose,[90–92] only the second way was applied for the labelled compounds.

All the syntheses[13,14,93,94] begin with converting of the glucose into 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose ('diacetonoglucose') **39**. In this derivative all the hydroxyl groups of glucose, except one, are protected. The group which remains free is 3-OH, the group requiring inversion of configuration. This operation can be achieved by oxidation to 3-oxo derivative **40** and hydride reduction. Because the 3-carbonyl group of **40** is hindered by the dioxolane ring formed from 2-OH and 1-OH, the hydride attack occurred from the *β*-side of the furanose ring. This led to the *allo*- configuration of the 3-OH (route A + C)[14,93,94] in the resulting product **41** (Scheme 8).

The other possibility is the classical S_N2 inversion; the glucose derivative **39** was converted into 3-*O*-mesylate **43** and subjected to substitution with the benzoate ion (route B) to give **44**.[3b]

The second operation (shortening of the molecule) was achieved by selective oxidative cleavage of the C5–C6 bond. It is possible to deprotect glycol system on C5–C6 by mild acid hydrolysis without affecting the 1–2 acetal system.[91] The C5–C6 glycol **45** was oxidised with periodate,[92] giving as a result a molecule with one carbon atom less (**46**).

The above basic operations on the glucose molecule can be performed in a different order and several possible synthetic routes have been tested.[13,14,89,93,94]

All the syntheses gave comparable yields of acylated [$^{13}C_5$]-ribofuranose, sugar component for the Vorbrüggen synthesis of nucleosides. Although the syntheses consisted of eight to ten steps

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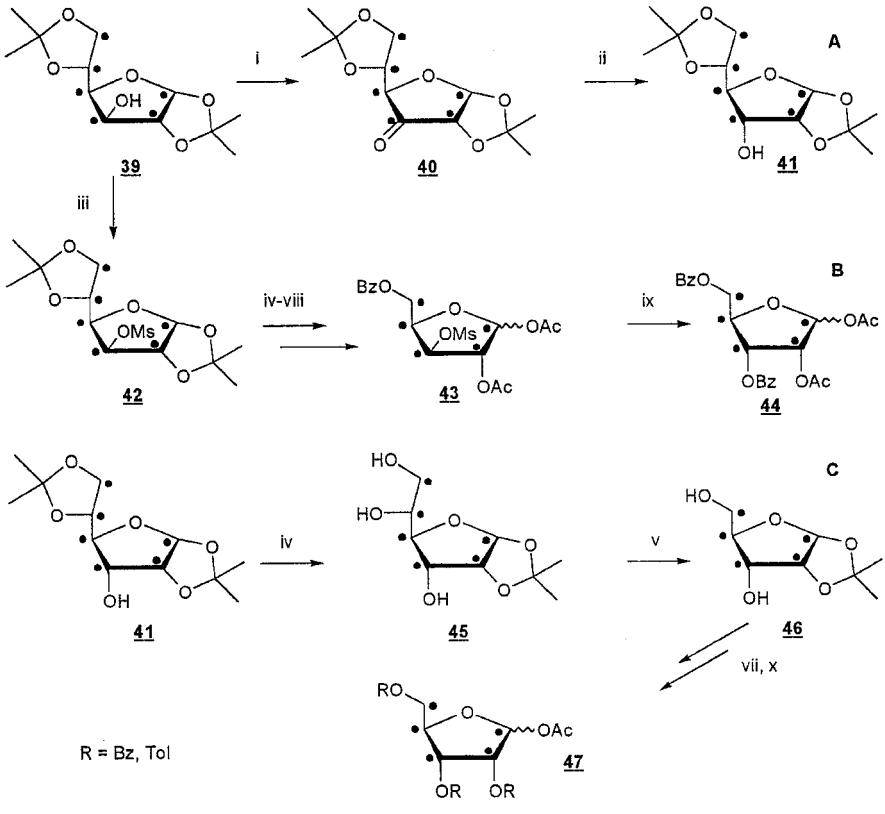

Scheme 8. (i) PDC/Ac_2O; (ii) $NaBH_4$, EtOH; (iii) MsCl, DMAP, Et_3N; (iv) 80% AcOH, r.t.; (v) 1. $NaIO_4$, 2. $NaBH_4$, EtOH/H_2O; (vi) BzCl, pyridine; (vii) 80% AcOH, 100°C; (viii) Ac_2O, pyridine; (ix) Tetrabutylammonium benzoate, toluene; (x) 1. MeOH/H_2SO_4 cat., 2. BzCl (TolCl), pyridine, 3. Ac_2O/AcOH/H_2SO_4

from the labelled glucose to the protected ribofuranose, their overall yields were reasonably high (~45–50%) and it appears that the practical limit of efficiency was achieved.

Another tested procedure is the cascade of enzymatic reactions presented by Williamson *et al.*[95] By careful adjustment of composition of the reaction mixture it was possible to perform a sequence of transformations leading from [$^{13}C_6$]-D-glucose **48** to a mixture of three ribonucleoside 5′-triphosphates: ATP, GTP and UTP **54a–c**, labelled at all sugar carbon atoms with ^{13}C (Scheme 9).

The whole sequence was achieved without the need to separate the intermediates **49**–**53**. By an additional enzymatic reaction performed on

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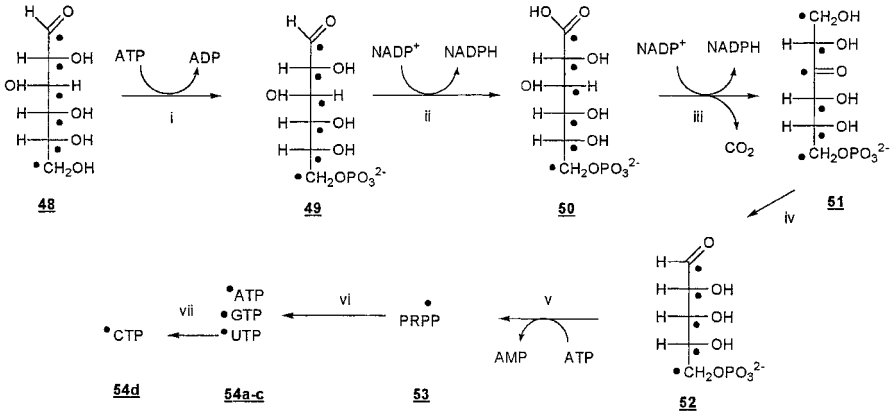

Scheme 9. (i) Hexokinase; (ii) Glucose-6-phosphate dehydrogenase; (iii) 6-phosphogluconate dehydrogenase; (iv) Ribose-6-phosphate isomerase; (v) PRPP synthetase; (vi) Adenine, uracil and xantine–guanine phosphoribosyltransferases; (vii) CTP synthetase

part of the above mixture of triphosphates, the UTP **53c** present in it was transformed into CTP (**53d**).

After combining the two mixtures, the four triphosphates were used as a substrate pool for the T7 ribonuclease transcription furnishing the all-ribose ^{13}C-labelled TAR RNA molecule, which was isolated and purified for the NMR studies.[95]

Labelling with ^{15}N

Many of the procedures developed for specific labelling of selected ring for ^{13}C labelling of particular carbon atom can be used as well for ^{15}N labelling of the neighbouring nitrogen atoms simply by changing the isotopes in the label source. Therefore, the same synthetic procedures are often applied in both cases. Introduction of ^{15}N atom into the molecule of nucleoside (and consequently, oligonucleotide) has an effect comparable to that of substituting ^{12}C with ^{13}C. Although the magnetic moment of the naturally abundant ^{14}N isotope is not equal to zero, other properties of this isotope (spin number $I = 1$, electric quadrupole moment $eQ \neq 0$) render the NMR signals of ^{14}N practically useless because of extensive line broadening and signal splitting. Contrary to this, the other isotope, ^{15}N, has very favourable properties ($I = \frac{1}{2}$, $eQ = 0$) and gives narrow spectral lines.[96] As the nitrogen atoms are

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involved in the fundamental process of base-pairing, as well as in other association processes, the adequately positioned ^{15}N label gives the possibility of directly following these processes. The ^{15}N isotope labelling was helpful in studying duplexes,[54,68] triplexes[52] and G-rich quadruplexes,[59] interactions with proteins[53] (particularly when labelling concerned the N7 nitrogen positioned in the major groove), with drugs[20,58] (N7 as well as N3 located in minor groove) or intercalators. Ionization (both protonation and dissociation of proton) of the base ring proceeds on the nitrogen atom. Selective labelling in combination with isotope filtering techniques enables a study of microionization processes concerning particular base residues, not the global result of ionization.[54] It helps to follow local changes in the base's pK_a and attributes them to structural features of the molecule. There are synthetic methods able to introduce the ^{15}N label into any chosen position of any nucleoside aglycone.

Labelling at the *exo*-amine groups (adenosine N6, cytidine N4, guanosine N2)

Introduction of the label into the *exo*-amine group appears as the simplest and most straightforward of labelling operations. Usually, it is possible to perform it without affecting the heterocyclic ring and, therefore, it is often done on the nucleoside level or even on the oligonucleotide (post-synthetic labelling). Groups attached at the positions 4 of pyrimidine and 6 of purine ring are prone to undergo nucleophilic substitution[97] and there are numerous examples of such reactions. With the appropriate selection of leaving groups post-synthetic N4 labelling of cytidine and N6 labelling of adenosine was achieved with good yield.[98] Usually the source of ^{15}N was labelled ammonia (either as a water solution,[70,98–103] or generated *in situ* from its salt[22,74,75,104–107]). Another possibility is the use of ^{15}N-labelled phthalimide[108,109] or ^{15}N-benzylamine[110-114] as a nucleophile (Scheme 10).

The latter is able to directly substitute oxygen in uridine (O4) or inosine (O6) in refluxing hexamethyldisilazane.[114]

The substituent in the position 2 of purine is less susceptible to the nucleophilic attack, and only good leaving group, as for example methylsulphinyl in **59**, on reaction with $^{15}NH_3$ gives substitution product **60** with reasonable yield.[72] Fluorine atom at position 2 proved to be a proper leaving group as well[98] (Scheme 11).

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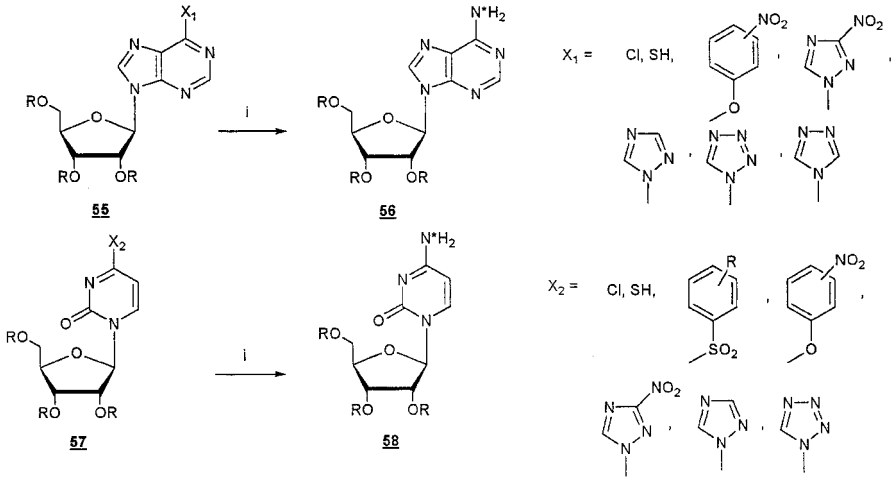
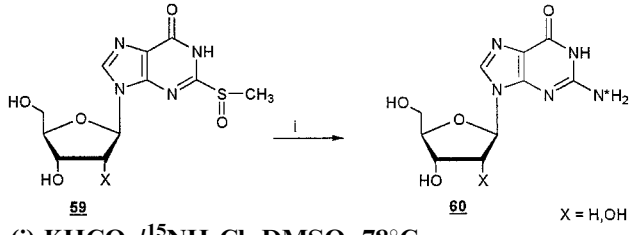
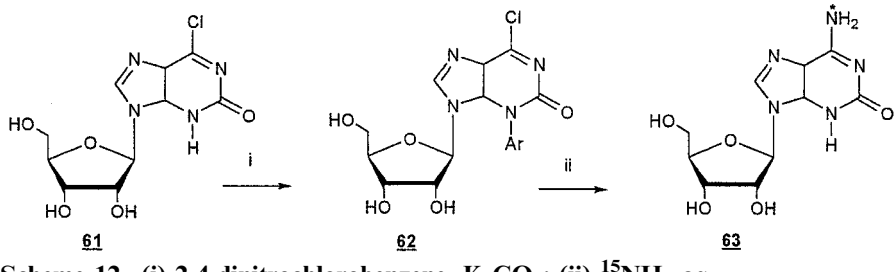


Scheme 10. (i) $^{15}NH_3$aq or $^{15}NH_3$/MeOH, or 1. $PhCH_2{}^{15}NH_2$, 2. H_2/Pd, or 1. [^{15}N]-phthalimide, 2. NaOH/H_2O–EtOH



Scheme 11. (i) $KHCO_3$/$^{15}NH_4Cl$, DMSO, 78°C

The substitution at the position 6 of purine smoothly leads to adenine, but when the synthesis aimed at isoguanosine (synthesis started with 6-chloro-2-oxo-purine riboside **61**), an activation of the purine ring was necessary. It was achieved by substituting an electron-withdrawing group at nitrogen atom N3, which increased the nucleophilic properties of the position 6. In such a derivative **62** the chlorine atom was smoothly substituted with labelled ammonia leading to [6-^{15}N]-isoguanosine **63**[103] (Scheme 12).



Scheme 12. (i) 2,4-dinitrochlorobenzene, K_2CO_3; (ii) $^{15}NH_3$ aq

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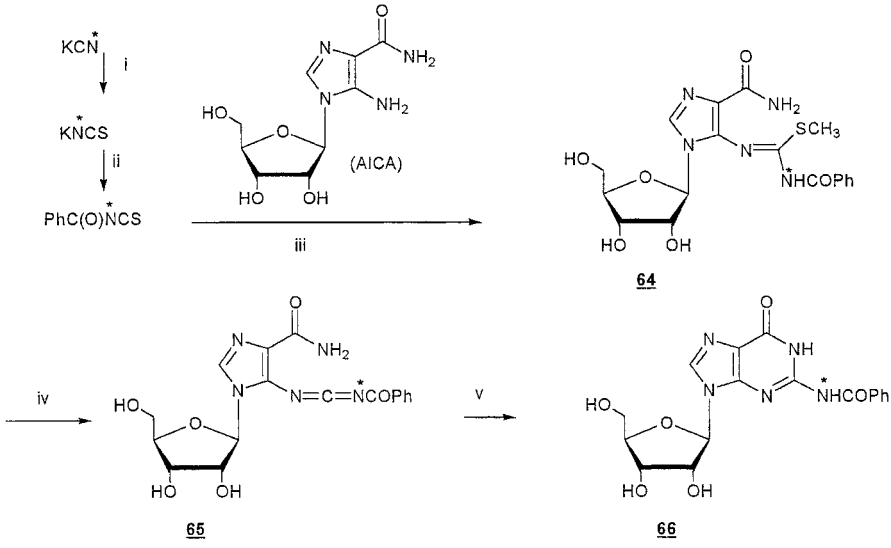
The N2-labelled guanosine can be also prepared by the already mentioned synthetic pathway employing the Dimroth rearrangement.[74,75] Another route is to synthesize guanosine **66** from AICA riboside.[115] In both cases, labelled cyanide is the primary source of the isotope (Scheme 13).



Scheme 13. (i) S_8, EtOH; (ii) BzCl; (iii) 1. AICA, 2. CH_3I; (iv) EtONa; (v) EtOH reflux

Labelling at positions N1 and N3 of pyrimidine

Introduction of the nitrogen isotope to the base ring usually proceeds through a cyclization reaction in which the ring is assembled. The reaction leads to the free base, which is subsequently condensed with ribose to give nucleoside. The synthetic route, reported by Roberts and Poulter,[67,68] originally intended for ^{13}C labelling at the C4 carbon atom (see Scheme 1), seems to be a universal procedure. All the reagents needed to assemble the uracil ring are very simple (acetate, cyanide and cyanate) and commercially available in isotope-enriched forms (^{13}C and ^{15}N as well). The original synthesis was repeated with a different label source ($KC^{15}NO$), giving, with excellent yield, [3-^{15}N]-uracil, later converted into uridine.[68]

Strazewski *et al.* used [$^{15}N2$,^{17}O] urea **67** and reacted it with propiolic acid to yield labelled uracil **68**, this was subsequently transformed into

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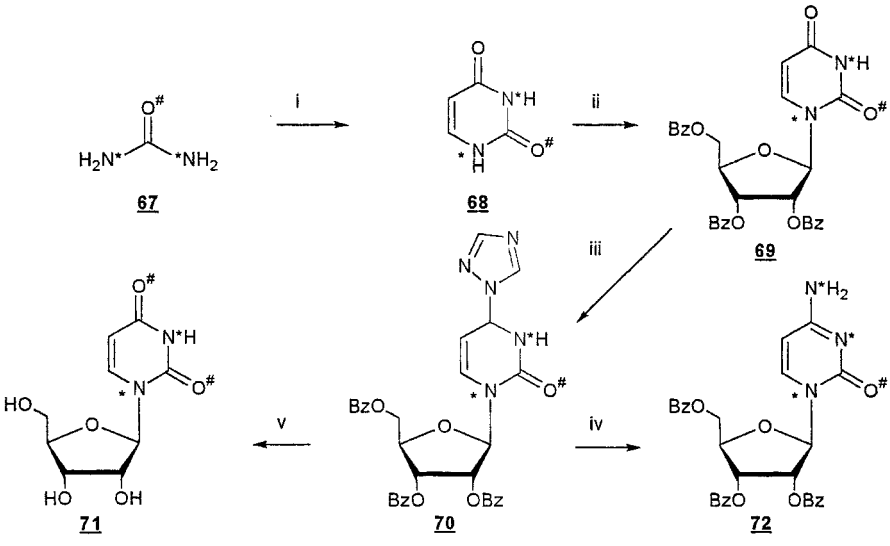
uridine **71** and cytosine **72**. The labelled urea was prepared from $^{15}NH_4Cl$ and $H_2^{17}O$[102]. A similar procedure was earlier used by Niu[100] (Scheme 14).



Scheme 14. (i) Propiolic acid, PPA; (ii) 1. HMDS, 2. 1-*O*-acetyl-2,3,5-tri-*O*-benzoylribofuranose, $SnCl_4$; (iii) $POCl_3$, Et_3N, 1,2,4-triazole; (iv) $^{15}NH_3$ aq./THF; (v) $Na^{17}OH$/THF

It is not necessary to assemble the pyrimidine ring to label nitrogen atom N3 in uracil. Substitution of the lactam hydrogen atom with the electron-withdrawing group increases C2 reactivity towards amines. When the nitro group was used as an electron-withdrawing group (**74**), the amine (or ammonia) attack on the C2 carbon atom of **74** resulted in ring opening and cyclization with the expulsion of the N3 and NO_2 nitrogen atoms. The N3 atom was replaced with the nitrogen of the attacking amine or ammonia. This reaction creates an excellent opportunity to perform the labelling on the nucleoside level. Several examples of application of this labelling method have been published[105–107,116] (Scheme 15).

Labelling at N1 position of purine

The above-mentioned nitration–substitution route can be applied also to purine nucleosides. Starting from the 1-nitroinosine **78**, [1-^{15}N]-

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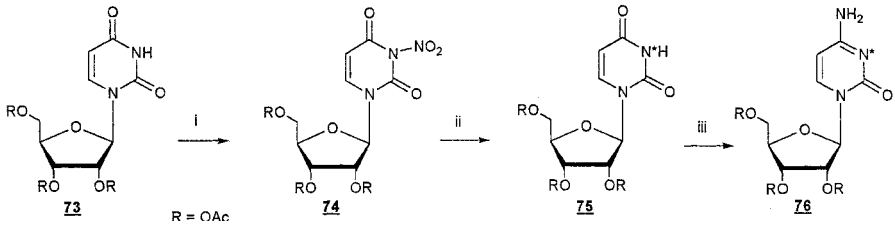
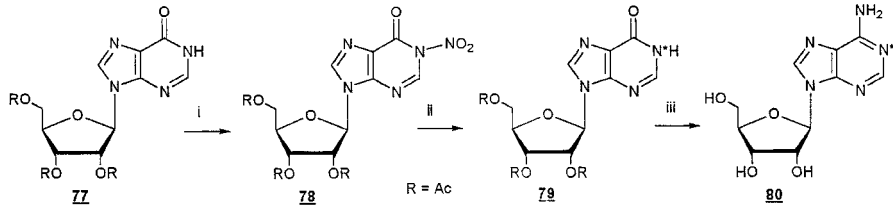


Scheme 15. (i) $NH_4NO_3/(CF_3CO)_2O$; (ii) $^{15}NH_4Cl$, Et_3N, NaOH 1 eq.; (iii) 1. Et_3N, $PhOP(O)Cl_2$, 1,2,4-triazole, 2. NH_3/MeOH

inosine **79** and subsequently [1-^{15}N] adenosine **80** were obtained.[116] A variety of electron-withdrawing substituents (apart from nitro group different nitro- and halogenated aromatic groups[117,118]) were tested in this reaction. It was possible to achieve a high yield of [1-^{15}N]-2′-deoxyinosine and [4-^{15}N]-AICA-2′-deoxyriboside (later converted into [1-^{15}N]-2′-deoxyguanosine) (Scheme 16).



Scheme 16. (i) $NH_4NO_3/(CF_3CO)_2O$; (ii) $^{15}NH_4Cl$, Et_3N, NaOH 1 eq.; (iii) 1. $SOCl_2$, DMF, 2. NH_3/MeOH

Another technique for N1 labelling is the application of the Dimroth rearrangement (see Scheme 4).[104,114] During the reaction, the nitrogen atom N1 and *exo*-amine nitrogen switch their positions. If the synthesis starts with the [6-^{15}N]-labelled adenosine, the label migrates to N1. As the N6-labelled adenosine is easy to synthesize, it is a good starting material and the whole process is reasonably efficient (Scheme 17).

N1-Labelling of guanosine by this method requires inclusion of cyanide into the process and conversion of the initially obtained [1-^{15}N]-2,6-diaminopurine riboside into guanosine. The latter operation can be simply and efficiently completed with adenosine deaminase, a commercially available and inexpensive enzyme.[74,75]

The label can be placed at position 1 of the purine ring during the assembly of the heterocycle from simple compounds.[119] The labelled

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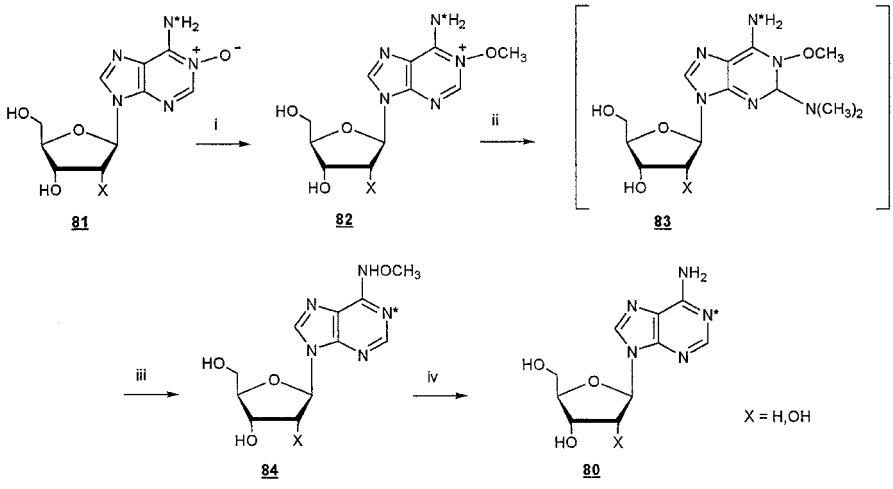
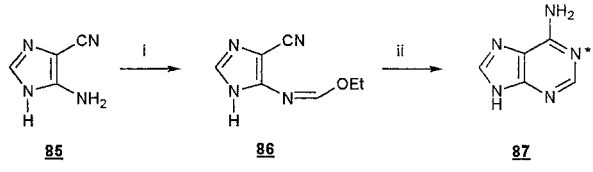


Scheme 17. (i) CH_3I; (ii) $HN(CH_3)_2$; (iii) MeOH reflux; (iv) Ni-Raney

[1-^{15}N]-adenine **87** for mass fragmentation studies was synthesized in two steps starting from 4(5)-amino-5(4)-cyanoimidazole **85** (Scheme 18).



Scheme 18. (i) $AcOCH(OEt)_2$; (ii) $^{15}NH_3$ 160°C

Labelling at position N3 of the purine

The label was introduced during the assembly of the six-membered ring.[72,120] The starting material for this synthesis was 4,5-imidazole dicarboxylate **88**. The label source sodium nitrite $Na^{15}NO_2$ was used. The nitrite was converted into arenediazonium salt (no nitrogen migration in this salt was observed) which substituted the 5-carboethoxy group in **89**. After subsequent transformations and cyclization, the labelled nitrogen atom took the position of N3 atom in hypoxantine **93**. The base was coupled to ribose, giving inosine, which was transformed into [3-^{15}N]-adenosine nucleosides **94**[72] (Scheme 19).

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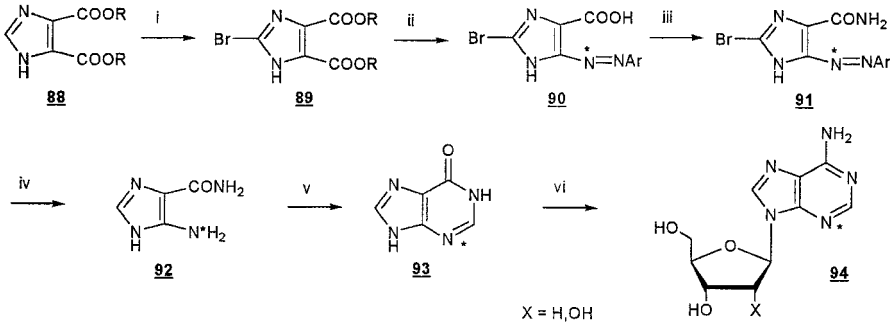

Scheme 19. (i) NBS; (ii) 1. NaOH, 2. 4-Br–C_6H_4–N=$^{15}N^+$(from $Na^{15}NO_2$ and 4-bromoaniline); (iii) 1. EtO_2CCl, 2. NH_3; (iv) H_2/Pd, KOH/MeOH; (v) $AcOCH(OEt)_2$; (vi) 1. $POCl_3$, 2. NH_3, 3. Enzymatic transglycosylation

Labelling at position N7 of the purine

If the purine molecule would be considered as a substituted pyrimidine ring, then the purine N7 atom appears as a pyrimidine substituent at the position 5. It is the position at which electrophilic substitution reactions can be achieved easily. This feature of the pyrimidine ring opens a convenient possibility of the ^{15}N labelling. The electrophilic reagent employed in the labelling reaction can be either nitronium cation[121] or arenediazonium cation.[22,122] Both these ions can be conveniently derived from sodium nitrite, a commercially available source. The immediate substitution product ([5-^{15}N-nitroso]- or [5-^{15}N-aryldiazo]-pyrimidine, **96** or **100**) can be smoothly reduced to a [5-^{15}N-amino] derivative (**97** or **101**), which is just a formylation reaction away from the desired N7-labelled purine (**98**, **102**)[22,121,122] (Scheme 20).

Labelling at position N9 of the purine

Substitution of one of the chlorine atoms in the 5-amino-4,6-dichloropyrimidine **103**[76,123] or 5-nitro-4,6-dichloropyrimidine **104**[99] with the $^{15}NH_2$ group gives (directly[76,123] or after reduction[99]) a precursor of the N9-labelled 6-chloropurine **106**. There are numerous formylating reagents available, which can be applied in this reaction. Initially diethoxymethyl acetate was used, giving good yields when used in excess (Scheme 21).

When the same reaction was used for the synthesis of double labelled [8-^{13}C, 9-^{15}N]-adenine, the formylating agent became the source of the

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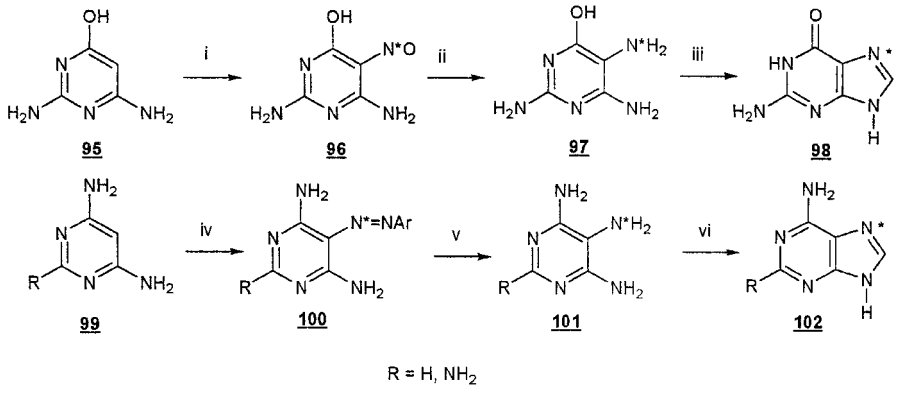
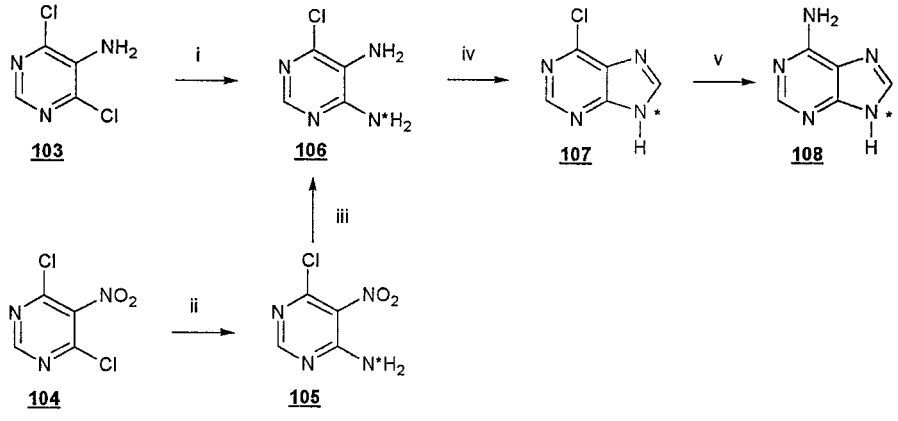


Scheme 20. (i) $Na^{15}NO_2/H^+$; (ii) $Na_2S_2O_4$; (iii) $HC(O)NH_2$, reflux; (iv) Arenediazonium salt (from $Na^{15}NO_2$ and aniline or 4-bromoaniline); (v) H_2/Pd; (vi) $AcOCH(OEt)_2$



Scheme 21. (i) $^{15}NH_3$ aq. 140°C; (ii) $^{15}NH_3$/MeOH, DIPEA, 25°C; (iii) Zn, H_2O; (iv) $AcOCH(OEt)_2$; (v) NH_3 aq

^{13}C label. In order to avoid losses of the carbon isotope, another formylating agent had to be used. When the mixed anhydride, [^{13}C]-formyl–pivaloyl anhydride was applied in this role (see Scheme 5), stoichiometric amounts of the reagent ensured good yield of the formylation reaction. The reagent was conveniently prepared from the labelled sodium [^{13}C]-formate and pivaloyl chloride[76] (Scheme 22).

Interesting synthesis of the 5-amino-4-carbethoxylimidazole ribofuranoside (**112a** and its anomer **112b**) labelled at the imidazole ring N1 atom should be mentioned.[124] An unusual approach starting from

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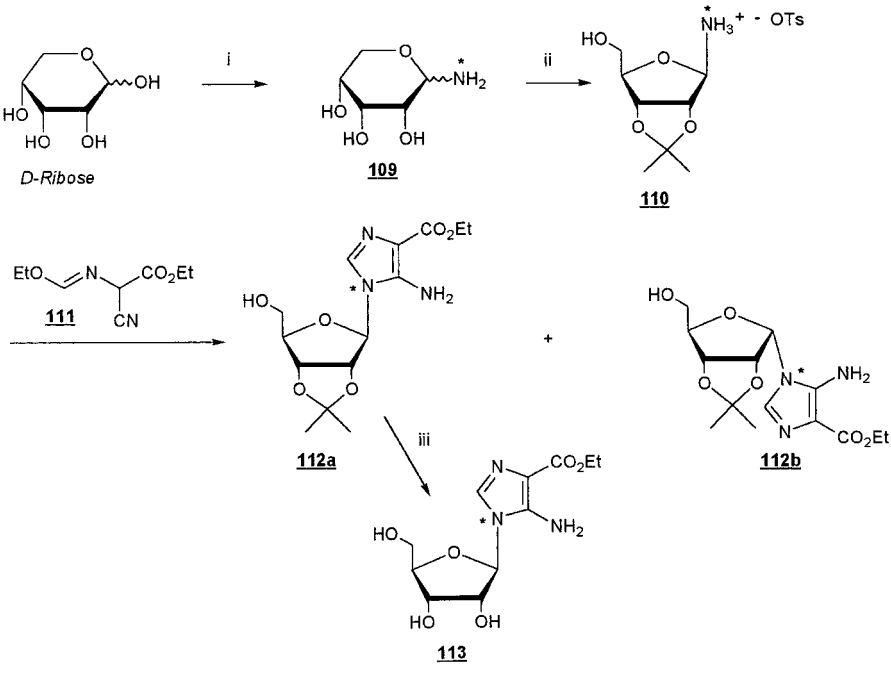

Scheme 22. (i) $^{15}NH_3$/MeOH; (ii) Acetone, 2,2-dimethoxyethane, TsOH; (iii) AcOH, 100°C

D-ribose and constructing the heterocycle ring on the basis of the labelled ribofuranosylamine **109** was chosen. The compound is a potential starting point for the synthesis of N9-labelled purines, but this possibility was not followed by the authors.

The condensation reaction leading to the imidazole ring gives also an opportunity to place the ^{15}N label at position N3 or 5-NH_2 by using an appropriately labelled 2-(ethoxymethylideneamino)cyanoacetate ester **111**.[124]

Multiple labelling

Introducing more than one isotope atom into the nucleoside molecule usually requires the use of two or more labelling syntheses in sequence. The exception is the situation, when the label source contains more than one isotope atom and several labels are introduced simultaneously. An example of such a case are the syntheses starting from [$^{13}C_6$]-D-glucose[13,14,93,94] or the synthesis of [1,3-$^{15}N_2$, 2-^{17}O]-uracil (Scheme

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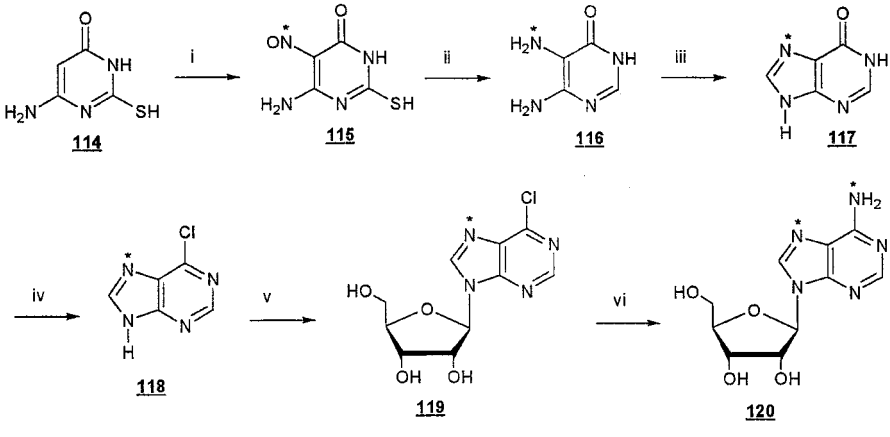
14),[102] where the authors applied a multiple-isotope-labelled urea as a substrate. Usually, however, multilabelling (either with several atoms of the same isotope or with different isotopes) means a combination of single-labelling syntheses described above. Since the economy of the incorporation is often the crucial factor in planning of the syntheses, such a combination requires careful and skilful planning of procedures. Several successful syntheses of multilabelled heterocyclic bases can serve as examples.

[6,7-$^{15}N_2$]-Adenosine and [6,7-$^{15}N_2$]-2′deoxyadenosine

In this synthesis the first operation was the construction of purine system with the N7 atom labelled with ^{15}N. The subsequent introduction of ^{15}N into the position N6 occurs readily and can be performed as the second labelling operation.[121] The synthesis was accomplished according to Scheme 23.



Scheme 23. (i) $Na^{15}NO_2$, 1 M HCl; (ii) 1. $Na_2S_2O_4$, 2. Ni-Raney; (iii) $AcOCH(OEt)_2$; (iv) $POCl_3$, $PhNMe_2$; (v) Enzymatic transglycosylation; (vi) $^{15}NH_4Cl/KHCO_3$, DMSO

[3,4-$^{15}N_2$]-Cytidine

A similar strategy was applied in this synthesis—at first the labelling of endocyclic nitrogen atom was performed (see also Scheme 15), then the *exo*-amine $^{15}NH_2$ group was introduced during the amination reaction[105] (Scheme 24).

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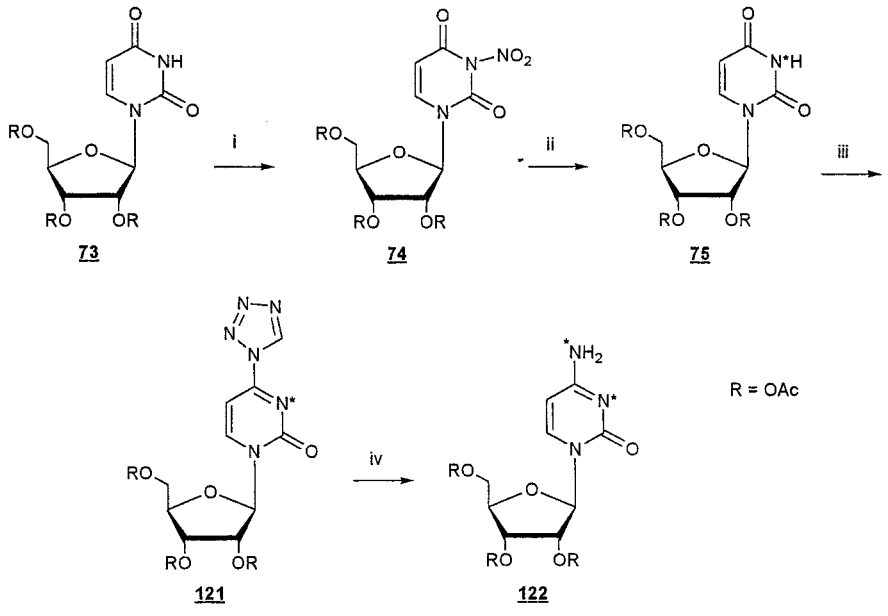

73 74 75 121 122

Scheme 24. (i) $(CF_3CO)_2O/NH_4NO_3$; (ii) $^{15}NH_4Cl/KOH/Et_3N$, MeCN–H_2O; (iii) tetrazole, TsCl, diphenyl phosphate, pyridine; (iv) $^{15}NH_4Cl/KOH/Et_3N$, MeCN–H_2O

Multilabelled. adenosine or guanosine via the Dimroth rearrangement

The use of the Dimroth rearrangement enables a very versatile strategy to be used. By changing the labelled position in the starting cyanide or using starting adenosine in N6,(N7)-labelled or non-labelled form, any combination of labels at the positions N1,C2,N2 or N7 can be obtained. This complicated approach was achieved with good yield and without the need of tedious protection–deprotection steps[75] (Scheme 25).

If the bromocyan reaction is omitted, the rearrangement opens the way to migration of the labelling ^{15}N nitrogen atom in the adenine system (Scheme 26).

An example of the versatility of this approach is the synthesis of tri-labelled adenosine in positions N1, N3 and N6[104]. The ^{15}N label migrated from the position N6 into N1 and the *exo*-amine nitrogen was back-labelled by the method of Robins.[125]

Adenosine and guanosine labelled at N1, N3, exo-amine and C2

Labelling of the N3 position requires construction of the six-membered ring of the purine starting from 4,5-dicarbethoxy-imidazole. By

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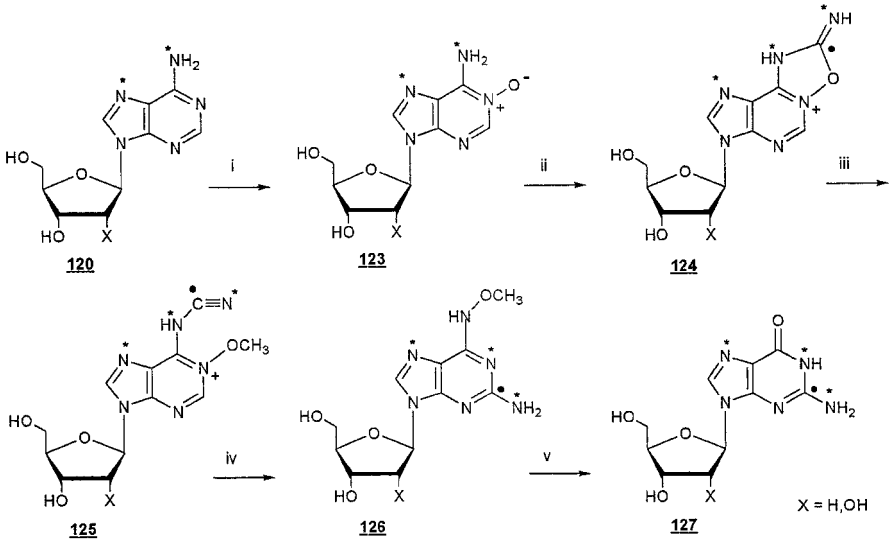
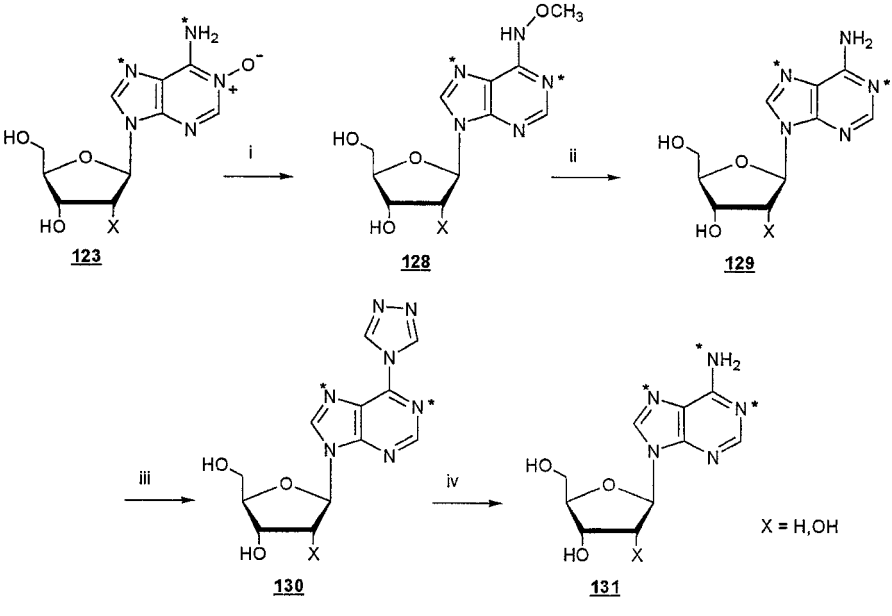


Scheme 25. (i) MCPBA; (ii) [^{13}C,^{15}N]KCN, Br_2, MeOH; (iii) Et_3N, CH_3I; (iv) 1. NaOH 0.25 M, 2. pH 7; (v) Adenosine deaminase



Scheme 26. (i) 1. Me_2SO_4, 2. Me_2NH, MeOH, reflux; (ii) Ni-Raney; (iii) *N*,*N*-dimethyl-formamide azine; (iv) $^{15}NH_4Cl/K_2CO_3$/DMSO, 80°C

diazotization of a bromoderivative of this compound, singly labelled azo-acid **90** was obtained, and next the labelling nitrogen was introduced into the molecule on transformation to the amide **132**.

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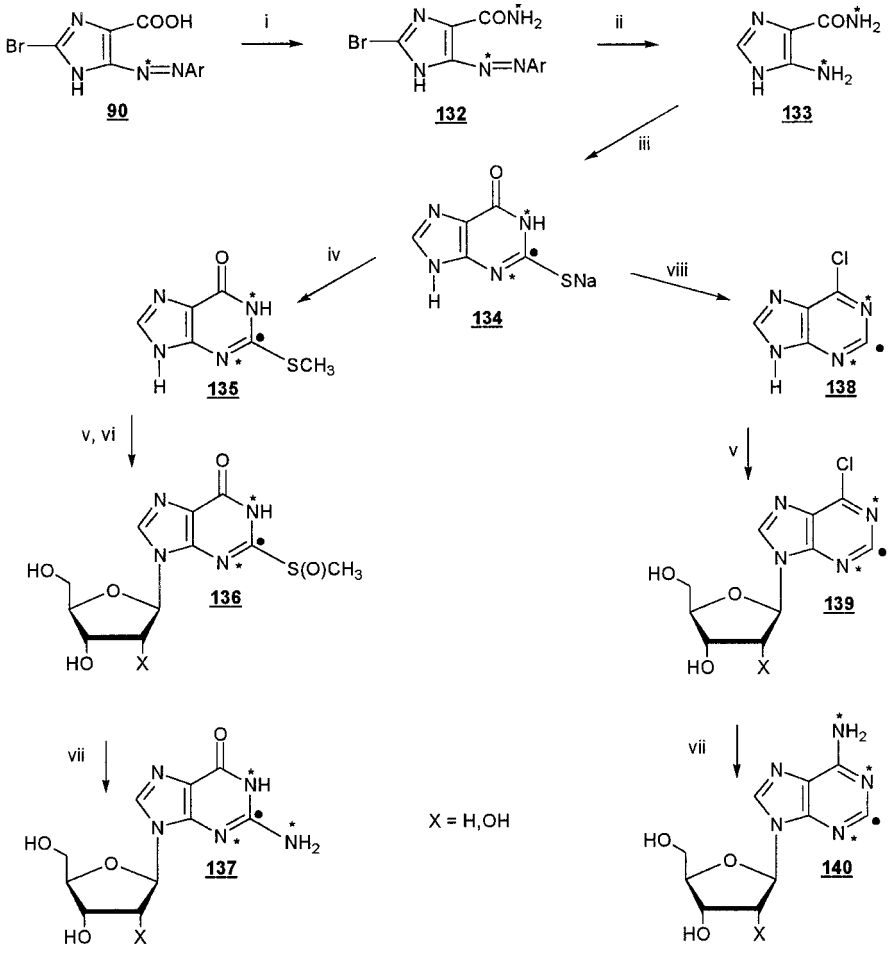
Further transformations can lead to labelling of positions C2, N2, or N6, depending on the reagents used[72] (Scheme 27).



Scheme 27. (i) DEC, $^{15}NH_4Cl$; (ii) H_2/Pd; (iii) $NaS^{13}C(S)OEt$; (iv) CH_3I; (v) Enzymatic transglycosylation; (vi) Oxone®; (vii) $^{15}NH_4Cl/KHCO_3$; (viii) 1. Ni-Raney, HCOOH, 2. $POCl_3$, $PhNMe_2$

Adenosine labelled at N9, N6 and C8

5-Amino-4,6-dichloropyrimidine **103** appears as a versatile starting material for the synthesis of the adenosine labelled at these positions. Labels were introduced sequentially, using simple isotope sources.[76] In

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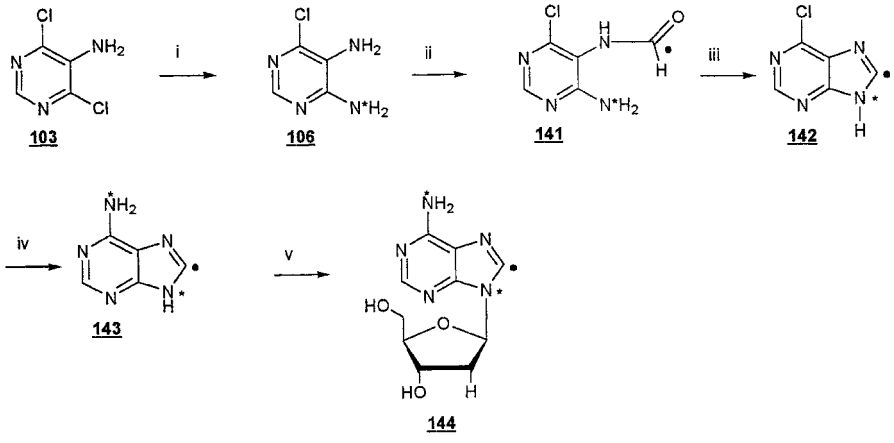
principle, adenosine with any combination of labels at sites N6, N9 and C8 can be synthesized (Scheme 28).



Scheme 28. (i) $^{15}NH_3$ aq., 120°C; (ii) [^{13}C]-formyl–pivaloyl anhydride; (iii) $TiCl_2(OiPr)_2$, $P(OEt)_3$; (iv) $^{15}NH_3$ aq., 140°C; (v) Enzymatic transglycosylation

Labelling at the base (^{15}N-exo-amine) and ribose ($^{13}C_5$) moieties

Labelled ribose[94] was condensed with the base, which contained labelling isotope (purine nucleosides).[126] Such an approach of convergent labelling ensured more economic use of the isotopes. [6-^{15}N] Adenine[121] and [2-^{15}N] guanine[75] were obtained via the reported procedures (see Schemes 10 and 12). In the case of pyrimidine nucleosides, the [4-^{15}N] label was introduced after the coupling of [$^{13}C_5$] ribose with uracil.[126] The pyrimidine nucleosides were additionally deuteriated at the position 5.

Acknowledgements

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Nomenclature

AIBN	2,2′-azobis(isobutyronitrile)
AICA	5-aminoimidazole-4-carboxamide
DEC	1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride
DIPEA	*N*,*N*-di(isopropyl)ethylamine
DMAP	4-(*N*,*N*-dimethylamino) pyridine
HMDS	hexamethyldisilazane
MCPBA	3-chloroperbenzoic acid
MsCl	methanesulphonyl chloride
NBS	*N*-bromosuccinimide
NOE	nuclear Overhauser effect
PDC	pyridinium dichromate
PPA	polyphosphoric acid
PRPP	(5-phosphoribosyl)-pyrophosphate
TfOTMS	trimethylsilyl trifluoromethanesulphonate
•	^{13}C
*	^{15}N
#	^{17}O

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