N-Alkylated guanine derivatives

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Abstract: The synthesis, chemical, physical, biological, spectroscopic and miscellaneous analytical properties of *N*-alkylguanine derivatives substituted at 1-, 1,N^2-, N^2-, 3-, 3,N^2-, 7-, 7,9- and 9-positions have been surveyed, mainly from the 2003-2009 period. Beyond the synthetic methods, particular emphasis has been given to products of mutagenesis and carcinogenesis, the role of modified fluorescent guanosines (wyosine, wyebutosine), mRNA cap structures and drugs stemming from *N*-alkylguanines. The review is based on 154 references and contains 64 schemes with 355 numbered structures.

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1. INTRODUCTION. METHODICAL CONSIDERATIONS, SCOPE AND LIMITATIONS

Guanine (**1**) is a ubiquitous purine base present in (deoxy)ribonucleic acids Scheme (**1**). Beyond its 9-glycosylated derivatives in DNA and RNA it also occurs in the form of *N*-alkylated guanines. The exposure of DNA and RNA to mutagens and carcinogens results in the formation of exogenous guanine derivatives, e.g. 7-methyl- or 7-ethylguanine (**2**, **3**), acrolein-2'-deoxyguanosine adducts (**4a**, **4b**). *N*-Alkylguanines, however, also occur endogenously and play multifaceted roles. RNA is especially rich in modified nucleobases, e.g. guanosine derivatives wyosine (**5**) and wyebutosine (**6**) which are responsible for enhancing and expanding tRNA function to increase cell viability, by augmenting heat tolerance in mesophiles and hyperthermophiles, and by frameshifting and constitute a switch for the deactivation of the codon function of $tRNA^{Phe}$. 7-Methylguanosine derivatives, the so-called cap structures, e.g. (**7**), are intrinsicly involved in mRNA metabolism, including mRNA transport between the nucleus and the cytoplasm, and in control of mRNA stability. In addition to naturally occurring derivatives, the advent of antiviral drugs acyclovir (**8**), ganciclovir (**9**) and penciclovir (**10**), which are highly selective agents against both herpes simplex virus (HSV) and varicella-zoster virus (VZV) infections, has also increased the value of *N*-alkylguanines. Simple *N*-alkylguanines often serve as model compounds for interactions of substances, e.g. the anticancer agent cisplatin and its analogs, with nucleosides/nucleotides.

In this review we have surveyed the most important *N*-alkylguanines in respect of their synthesis, chemical, physical and relevant biological properties. The order of compounds treated is primarily based on the substitution pattern (1-, 1,N^2-, N^2-, 3- and 3,N^2-, 7- and 7,9-, 9-alkyl derivatives) though strict order was not always practical and cross-references to other sections have also been provided. The important class of coordination complexes of *N*-alkylguanines will be the subject of a separate review. The basic time frame (2003-2009) of this review was dictated by previously published comprehensive works, namely the reviews on 9-alkylguanines by Clausen and Juhl-Christensen *et al.* in 1993 [1] and that of Seela *et al.* on purines in 2003 (published in 2004) [2]. References to earlier articles, to a limited extent, have also been given to ensure continuity and where it was felt appropriate, unduly forgotten methods have also been included, also from the pre-2003 period. Patents, with one notable exception, have not been covered.

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2. 1-ALKYLGUANINES

tRNAs, rRNAs, snRNAs and mRNAs found in Nature contain modified nucleosides up to 25% (more than 100 different modifications, among them some 20 guanine derivatives). A comprehensive list of these modifications can be retrieved in *The RNA Modification Database* maintained by the University of Utah (http://library.med.utah.edu/RNAmods/) [3,4]. tRNA modifications are recognition determinants for aminoacyl-tRNA synthetases (aaRS) and initiation and elongation factors. A single methyl group of 1-methylguanosine in position 37, m^1G_{37}, prevents misarginylation of $tRNA^{Asp}$ in yeast [5]. Slow decoding event at codon(s) read by tRNA(s) normally containing m^1G_{37} is responsible for the *PurF*-independent thiamine synthesis [6].

1-Alkylated guanines, especially methylated derivatives, occur also in monomeric form in the natural product family of herbipolines (**11**, **12**) [2]. 1-Methylherbipoline (**12**) Scheme (**2**), isolated first from marine sponge, *Jaspis* sp. [7], has later also been found in Papua New Guinean tunicate *Eudistoma* sp. and extensively characterized spectroscopically [8]. It was tested for cytotoxicity against the p53 wild-type ($p53^{+/+}$) and p53 deficient ($p53^{-/-}$) HCT 116 human colon carcinoma cell lines but only minimal growth inhibition at 100 μg/mL was identified.

1-Alkylguanines are usually not obtained by direct alkylation of guanine because unselective and multiple alkylation takes place. Zhi-li *et al.* [9] have studied the direct alkylation of guanine with iodomethane and trimethyl phosphate in the presence of tetra-*n*-butylammonium hydroxide as a catalyst at rt to afford the mixtures of 1,7- (37-40% yield) and 1,9-dimethylguanines (31-34% yield).

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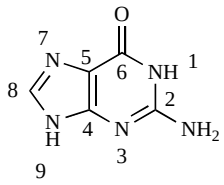
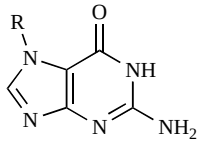
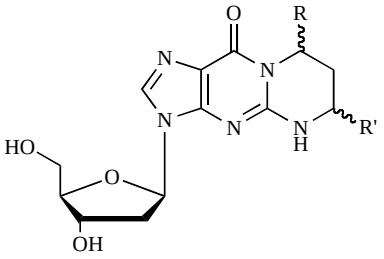


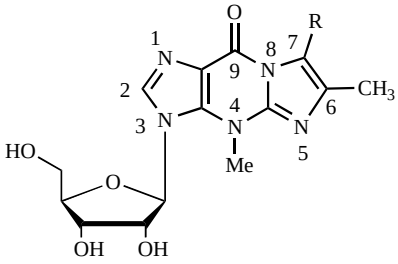
1
guanine



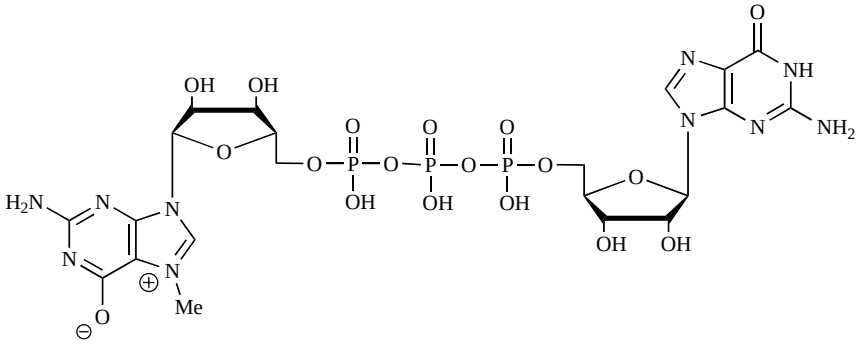
2 7-methylguanine (R = Me)
3 7-ethylguanine (R = Et)



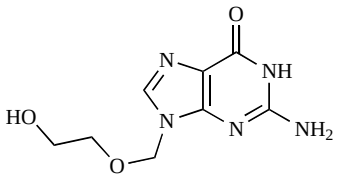
acrolein-2'-deoxyguanosine adducts
4a (R = H; R' = OH)
4b (R = OH; R' = H)



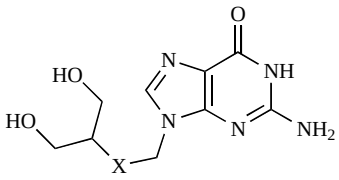
5 wyosine (R = H)
6 wybutosine
(R = $CH_2CH_2CH(NHCO_2Me)CO_2Me$)



7 mRNA cap (m^7GpppG)

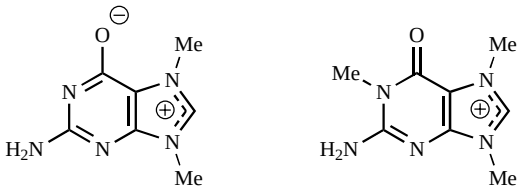


8 acyclovir



9 ganciclovir (X = O)
10 penciclovir (X = CH_2)

Scheme 1.



11 **12**

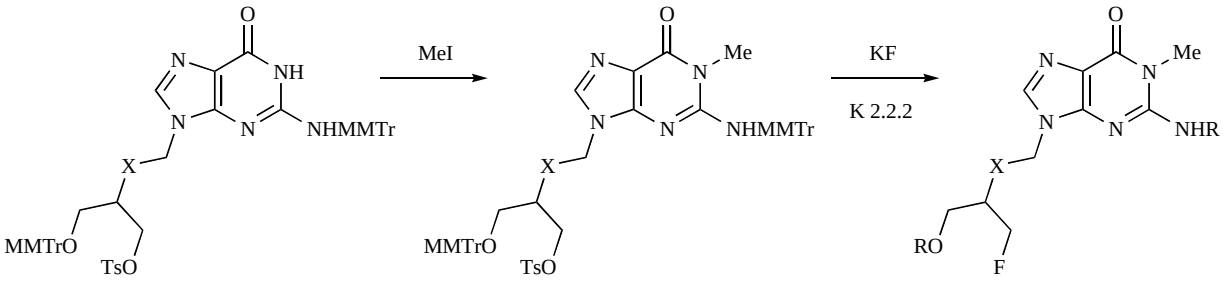
Scheme 2.

Better results are obtained when guanine is protected at N-9. Grote *et al.* [10] in an attempt to modify substrate properties of ganciclovir (**9**) and penciclovir (**10**) changed the lipophilicity of the molecules by N1-methylation Scheme (**3**). To this purpose, compounds (**13**) and (**14**) were methylated with iodomethane in the presence of tetra-*n*-butylammonium hydroxide to afford 1-methyl derivatives (**15**, **16**) in 44% yield for both cases. Radiolabeling with K[^{18}F]F/Kryptofix® 2.2.2 in the side chain of 1-methylated guanine derivatives afforded [^{18}F]-labeled derivatives (**19**, **20**) after deprotection. Ganciclovir can also be directly methylated at N-1 but penciclovir gave poor results and required extensive chromatographic purification. Chu *et al.* [11] have obtained antiviral 9-dioxolanyl nucleosides (DXG) by designing and preparing lipophilic and water-soluble DXG prodrugs with better pharmacokinetic profiles.

To this end, compound (**21**) Scheme (**4**) was methylated using diphenylmethylsulfonium tetrafluoborate in DMF in the presence of KOH, or benzylated with benzyl bromide in the presence of NaH to obtain the targets (**22**, **23**) in 77 and 66% yield, respectively. The 1-alkyl DXG prodrugs (**22**, **23**) exhibited lower potency than the parent compound (DXG, **21**). Strassmeier and Karpen [12] have prepared N7-, C8- and N1-subtituted 8-thio-cGMP analogs to study their effect on cyclic nucleotide-gated (CNG) channels that generate electrical responses to odorant- and light-induced changes in cyclic nucleotide concentration. A 1-alkylated derivative (**24**) was obtained in a reaction of an 8-(4-chlorophenylthio)-cGMP analog and phenyl vinyl sulfone in phosphate buffer at pH 7.9 in 61% yield Scheme (**4**).

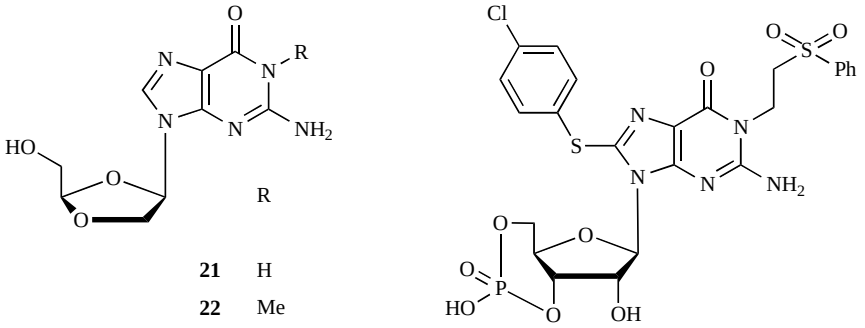
An unequivocal synthesis of 1-methylguanines can be attained using pyrimidine derivatives. Ryabukhin *et al.* [13] have obtained (**26**) Scheme (**5**) in 76% yield from 2,5,6-triamino-3-methylpyrimidin-4(3*H*)-one (**25**) in an oxidative conversion using anisaldehyde and trimethylchlorosilane.

1-Alkylguanines often appear as unwanted by-products in chemical syntheses. Theodorakis *et al.* [14] have accomplished the synthesis of protected 2'-*O*-methylguanosine (**28**)



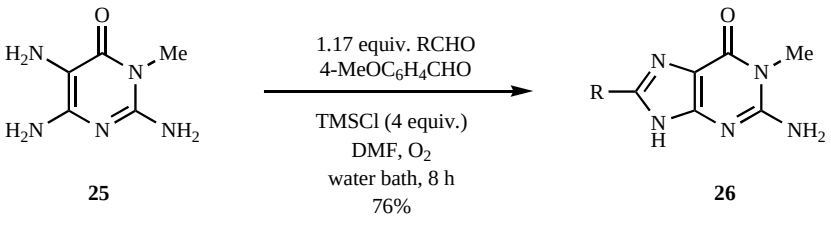
13 X = O
14 X = CH_2
15 X = O
16 X = CH_2
17 X = O; R = MMTr
18 X = CH_2; R = MMTr
19 X = O; R = H
20 X = CH_2; R = H

Scheme 3.

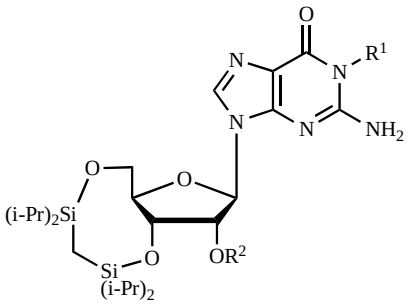


21 H
22 Me
23 Bn
24

Scheme 4.



25 **26** R = 4-$MeOC_6H_4$

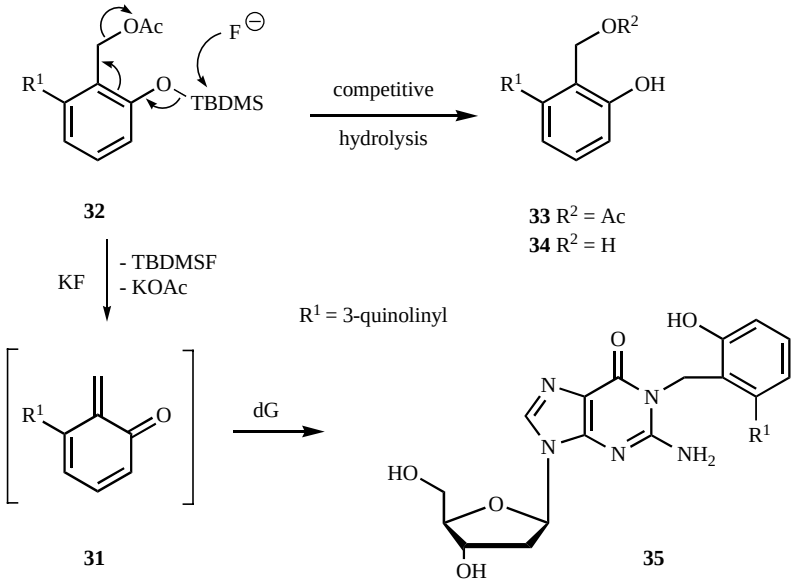


27 $R^1 = R^2 = H$
28 $R^1 = H$; $R^2 = Me$
29 $R^1 = Me$; $R^2 = H$
30 $R^1 = Me$; $R^2 = Me$

Scheme 5.

Scheme (**5**) without protecting guanine thanks to the new 3',5'-*O*-protecting group methylenebis(diisopropylsilyl). Upon attempted direct methylation of 2'-hydroxyl in compound (**27**) a mixture of 1-methylated product (**29**), along with dimethylated adduct (**30**) (at N-1 and 2'-OH), was obtained. 1-Alkylation was predominant under various conditons. An optimized procedure resulted in the formation of a 9 : 1 mixture of 2'-*O*-methyl (**28**) and 1,2'-*O*-dimethyl (**30**) derivatives using NaHMDS at -40 °C and excess liquid MeCl, the major product (**28**) was isolated in 83% yield and its structure was corroborated by X-ray as well.

The metabolism of estrogens and related drugs yield reactive quinone methide intermediates that are capable of cross-linking DNAs and give rise to nucleobase modifications. In particular, stable N1-, N^2- and reversible N7-adducts of guanine were observed and the reactivity and selectivity of quinone methides toward nucleobases are influenced by



Scheme 6.

the substituent groups. Zhou *et al.* [15] have studied the reactions of a quinolinyl quinone methide (**31**) to enhance the potential interactions with DNA through partial intercalation and charges of a quinoline moiety. The precursor (**32**) Scheme (**6**) participated a selective N1-dG alkylation in the presence of KF in 10% aqueous buffered or unbuffered DMF solution to afford compound (**35**) in 30% yield being the highest. NaCl suppressed the hydrolysis of acetate group that is vital for the formation of reactive quinomethide. Additional coordination by the quinoline moiety and steric effect may play a significant role in alkylation efficiency.

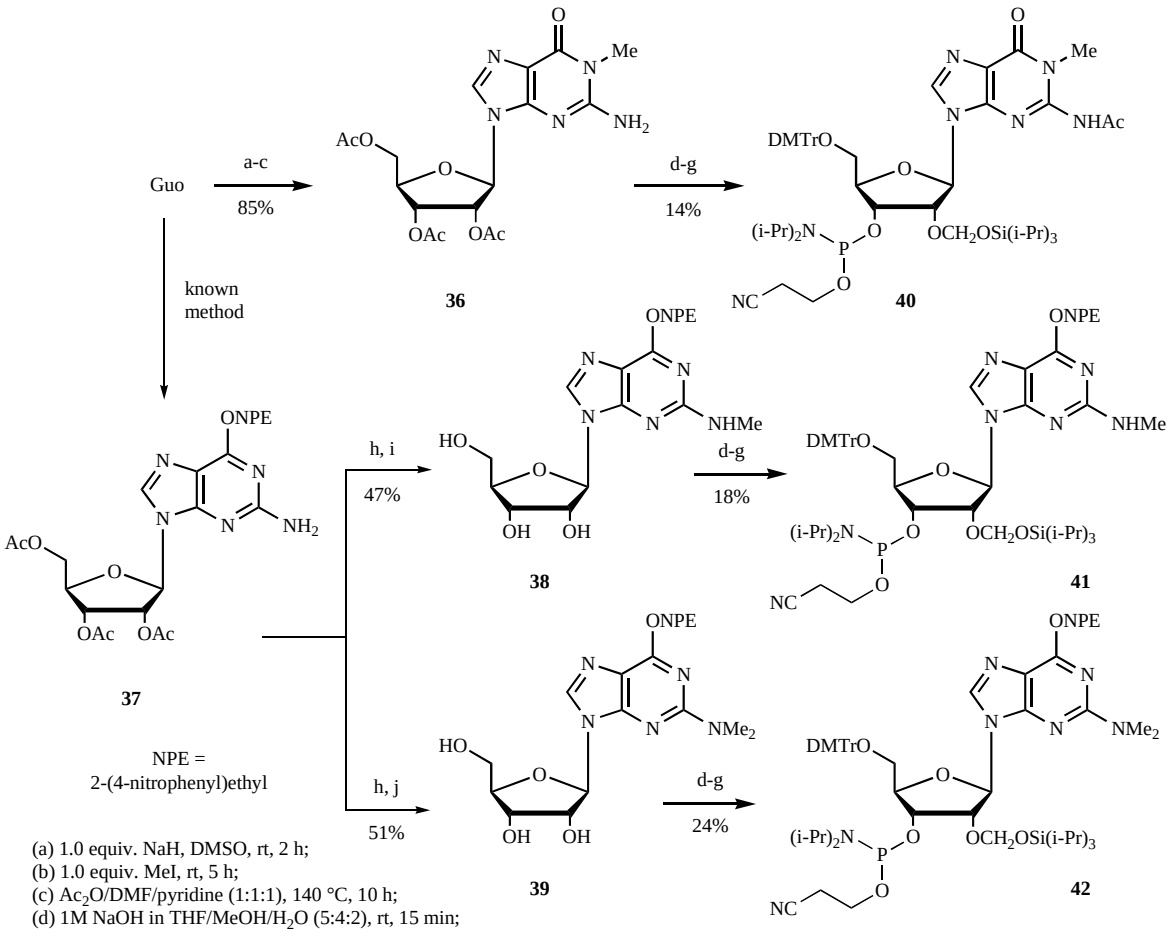
Unnatural and natural 1-alkylguanines have been introduced into ribozymes, siRNAs, and into short model RNAs to study their multifaceted role in native and artificial environments. The impact of RNA methylations is manifested on duplex hairpin equilibria, possible role in the ribosomal helix 45 with respect to secondary structure formation, stabilizing effect of methylated guanosine on codon-anticodon pairing by cyclic model compounds and other structural impacts [16].

Micura *et al.* [16] have reported on the straightforward synthesis of naturally occurring methylated ribonucleoside phosphoramidites of 1-methylguanosine (m^1G), N^2-methylguanosine (m^2G), N^2,N^2- dimethylguanosine ($m_2{}^2G$) and methylated derivatives of other nucleobases in order to incorporate them into RNA by automated solid-phase synthesis. The synthesis of monomers included methylation of guanosine with high regioselectivity at N-1 by treatment with 1 equiv of NaH in DMSO followed by addition of 1 equiv of MeI to give m^1G (**36**) (85% yield over 3 steps) Scheme (**7**). The significant insolubility of N^2-acetyl-1-methyl guanosine in pyridine resulted in poor yields of DMTr protection and that was eventually resolved by transient 2',3'-*O*-acetal protection to raise the yield of tritylation to 45%. For the synthesis of m^2G and $m_2{}^2G$ monomers Guo was transformed into 2',3',5'-*O*-triacetyl-O^6-[2-(4-nitrophenyl)ethyl]-guanosine derivative (**37**) then the amino group was subjected to fluorine displacement via diazotation and treatment with excess HBF_4, followed by substitution with $MeNH_2$ or Me_2NH to give (**38**) and (**39**), respectively (47 and 51% overall yield). The synthesis of oligomers from the corresponding phosphoramidites (**40**-**42**) followed standard conditions using TOM chemistry (97-99.5% coupling yields). The removal of NPE group proceeded along with TOM deprotection in 1 M TBAF/THF. Porcher and Pitsch [17] have attempted to improve the synthesis of m^1G, m^2G and $m_2{}^2G$ RNA monomers employed in automated oligomer synthesis.

A silylated m^1G derivative (**44**) was obtained by direct K_2CO_3-mediated methylation using MeI (63% yield) Scheme (**8**). The synthesis of m^2G (**46**) and $m_2{}^2G$ derivatives (**48**) was accomplished by transient TMS protection of O^6 and 3'-OH groups and reaction with 1,3-benzodithiolylium tetrafluoroborate to give a N^2-(1,3-benzodithiol-2-yl) derivative that was reduced with $(Me_3Si)_3SiH$/AIBN (57% overall yield after 3'-OH deprotection with ammonia). The synthesis of $m_2{}^2G$ derivative (**48**) followed the same strategy using the 1,3-benzodithiolylium reagent repeatedly (50% overall yield, *cf.* [18]).

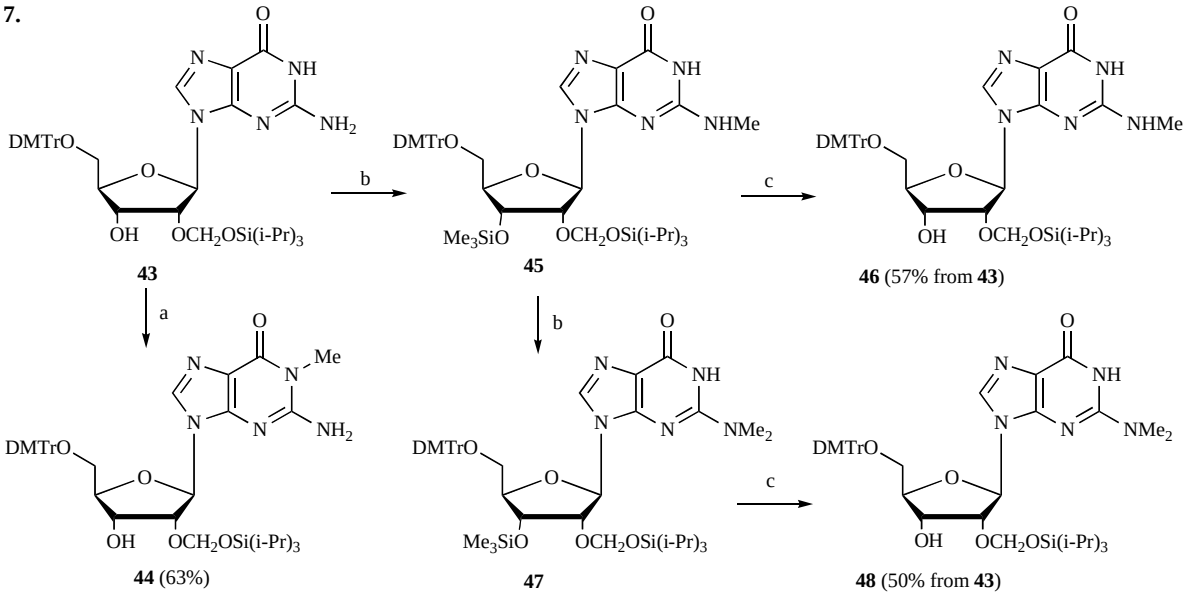
1-Alkylguanines have been used in diverse studies as model compounds. The Suzuki coupling of 8-bromo-2'-deoxyguanosine was slow, compared to 8-bromo-2'-deoxyadenosine. It was hypothesized by Western and Shaughnessy [19] that coordination of the guanine moiety to Pd and the decreased ability of electron-rich dG to oxidatively add to Pd(0) were the reasons. It was found that guanine nucleosides coordinate to Pd(II) inhibiting reduction to the Pd(0) active species, therefore an induction period and decreased coupling rate was observed. m^1dG and 8-bromo-m^1dG did not affect the rate of coupling showing that N-1 proton plays a critical role in the effect of guanine on the Suzuki arylation. dG is coordinating to Pd through N-1 (and/or O-6) rather than N-7 under the basic conditions of Suzuki coupling, as demonstrated by multinuclear ^{13}C and ^{31}P NMR measurements.

Diederichsen *et al.* [20] have aimed at the design of amphiphilic 14-helical β-peptides containing nucleobase recog-



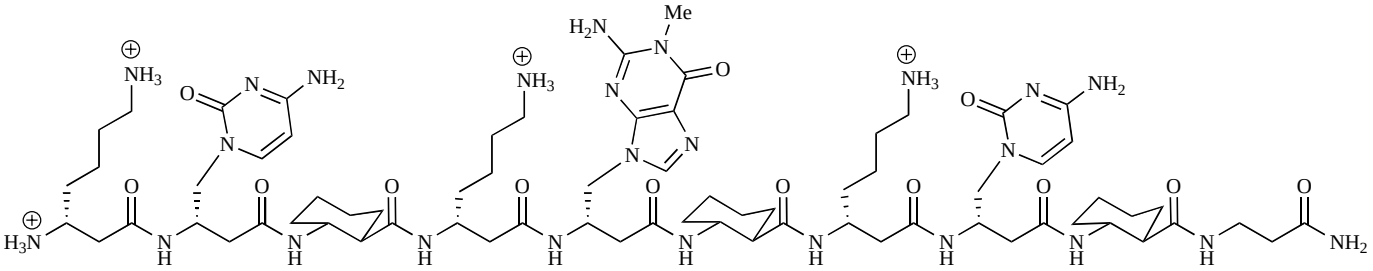
(a) 1.0 equiv. NaH, DMSO, rt, 2 h;
(b) 1.0 equiv. MeI, rt, 5 h;
(c) Ac_2O/DMF/pyridine (1:1:1), 140 °C, 10 h;
(d) 1M NaOH in THF/MeOH/H_2O (5:4:2), rt, 15 min;
(e) 1.2 equiv. dimethylformamide dimethylacetal, pyridine/DMSO (5:1), rt, 2 h, then 1.4 eq DMT-Cl, overnight;
(f) 4.0 equiv. $(i\text{-}Pr)_2NEt$, 1.2 equiv. *tert*-Bu_2SnCl_2, $ClCH_2CH_2Cl$, 70 °C, then 1.2 eq TOM-Cl, rt, 1 h, separation of 2'-*O*-isomer by chromatography;
(g) 10 equiv. Et_2NMe, 1.5 equiv. 2-cyanoethyl diisopropylphosphoramidochloridite, CH_2Cl_2, rt, 2 h;
(h) 100 equiv. HBF_4, 2.5 equiv. $NaNO_2$, aq. acetone, -20°C to rt, 3 h;
(i) 8 M $MeNH_2$, ethanol, 7 h;
(j) Me_2NH in aq. ethanol, rt, 3 h;

Scheme 7.



(a) K_2CO_3, DMF, 20 °C; then MeI, -15 °C;
(b) 1. Me_3SiCl, py, 20 °C; then 1,3-benzodithiolylium tetrafluoroborate;
2. $(Me_3Si)_3SiH$/AIBN, benzene, reflux;
(c) NH_3, MeOH, THF, 20 °C

Scheme 8.



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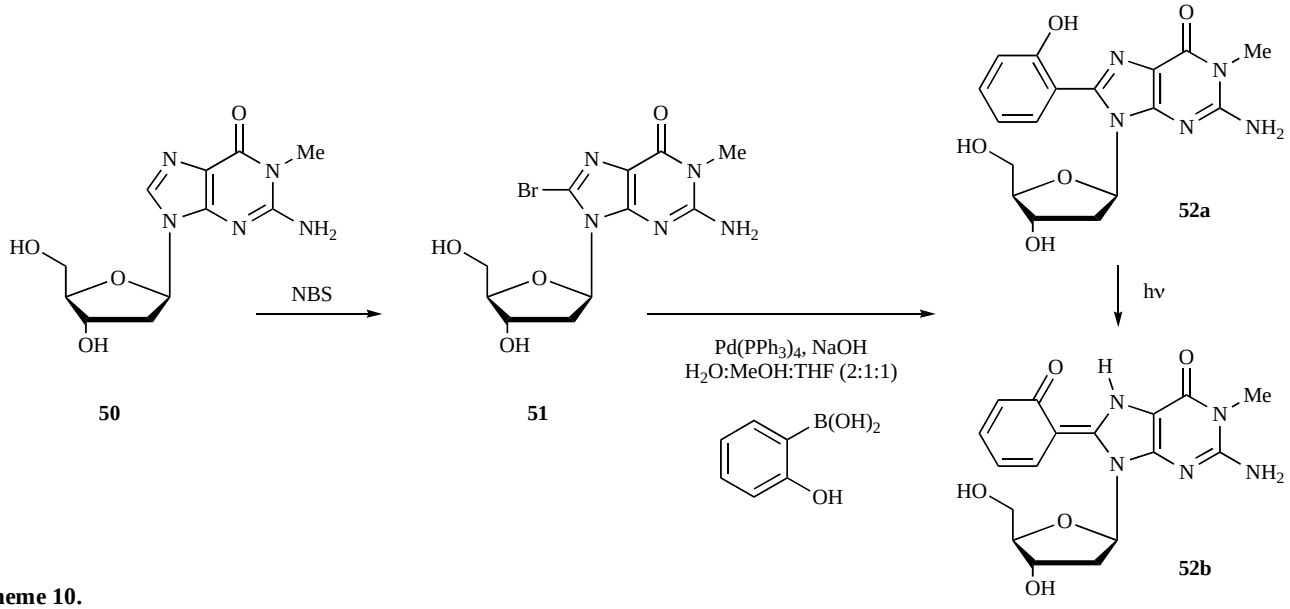
Scheme 9.



Scheme 10.

nition units in the side chains and at the investigation of their secondary structures and association by base pairing to gain more control over the geometry, stoichiometry and specificity of self-association. β-Peptides that contain nucleo-β-amino acids [e.g. (*S*)-γ-(1-methylguanin-9-yl)-β-homoalanine] were synthesized. To determine whether H-bond-mediated pairing of the nucleobases is important for the antiparallel association, 1-methylguanine oligomers Cm^1GC (**49**) Scheme (**9**) and m^1GCm^1G were prepared. Formation of a 14-helix was indicated by CD, but no interaction between the helix could be detected by temperature-dependent UV spectroscopy at low temperatures. The maximum at 270 nm suggests the preferred orientation of the nucleobases, but the Cotton effect linearly decrease with increasing temperature and does not show the sigmoidal pattern characteristic for base-paired double strands.

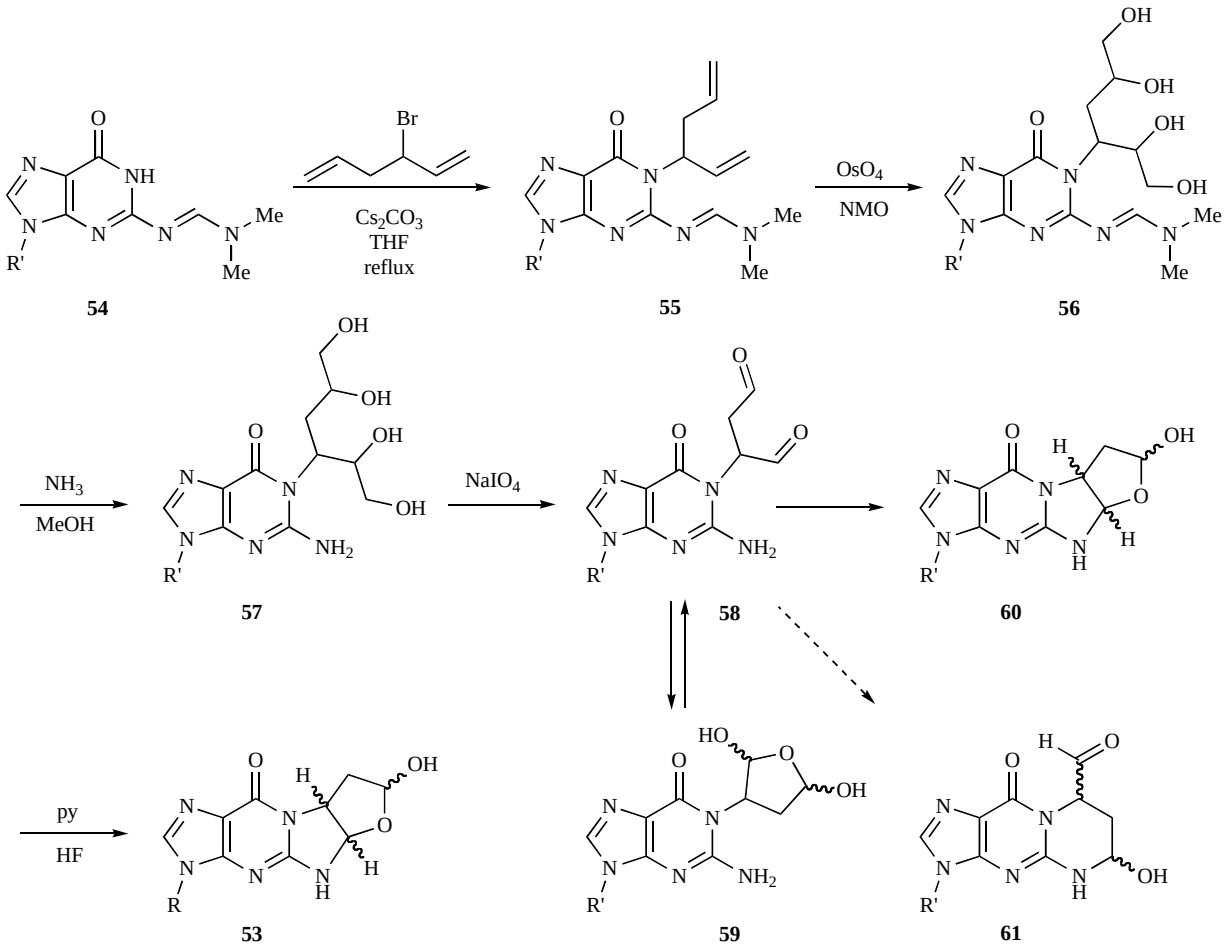
Satyamurthy *et al.* [21] have synthesized 8-fluoro-2',3',5'-tri-*O*-acetyl-guanosine, -1-methylguanosine and -inosine derivatives by direct fluorination of the corresponding protected nucleoside and studied the kinetics of their defluorination in acidic (0.01 M HCl in methanol) and basic medium (0.5 M NH_3 in methanol or isopropanol) by ^{19}F NMR. The reactions followed pseudo-first-order kinetics. The defluorination reaction rate constant in basic media is related to electron density at C-8 (determined by ^{13}C and ^{19}F NMR) while in acidic medium to the N-7 pK_a value.

Manderville *et al.* [22] have studied the conformational properties of the nucleoside adducts, 8-(2"-hydroxyphenyl)-2'-dG (**52**), and its 1-methyl and 2"-*O*-methyl derivatives, that are potential biomarkers for exposure to phenolic carcinogens. Adduct (**52a**) possesses the unique ability to phototautomerize, through an excited-state intramolecular proton transfer (ESIPT) process, to generate its keto form (**52b**). This tautomerization depends on the presence of an intramolecular hydrogen H-bond between the phenolic OH and the imine nitrogen (N-7). To unambigously determine a phenolic pK_a value, the 1-methyl analog was synthesized from the corresponding 8-bromo-m^1G compound (**51**) in a Suzuki coupling reaction Scheme (**10**). The determination of pK_a in (**52a**) yielded a value of 9.05 for deprotonation of the phenolic moiety. In a follow-up investigation, the 8-(4"-hydroxyphenyl)-2'-dG and its 1-methyl derivative were identified as pH-sensitive fluorophores [23].

3. 1,N^2-DIALKYLGUANINES

3.1. Products of Mutagenesis and Carcinogenesis

1-Alkyl-, 1,N^2- and N^2,3-dialkylguanines, along with other alkylated nucleobases, formed from the interaction of nucleobases and carbonyl derivatives (mostly aldehydes), are frequent products of mutagenesis and carcinogenesis.



R' = 3',5'-*O*-bis(*tert*-butyldimethylsilyl)-2'-deoxy-β-D-ribofuranosyl

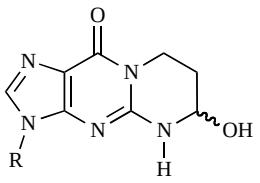
R = 2'-deoxy-β-D-ribofuranosyl

Scheme 11.

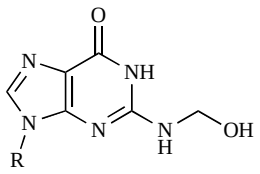
cis-2-Butene-1,4-dial is a reactive metabolite of furan, a known liver and kidney toxicant and a hepatocarcinogen in rodents. c*is*-2-Butene-1,4-dial reacts with nucleosides to form adducts *in vitro*, compound (**53**) is the major product with dG Scheme (**11**) [24]. 3',5'-*O*-Bis(*tert*-butyldimethylsilyl)-2'-deoxy guanosine derivative (**54**) was alkylated at N-1 with 3-bromo-1,5-hexadiene to afford diene (**55**) (79% yield) that was hydroxylated (**56**), deprotected (**57**) and oxidized with $NaIO_4$ to give 3 isomeric products. The 1-substituted adduct (**58**) existed primarily as the cyclic hemiacetal form (**59**) and it was expected to spontaneously cyclize to (**60**). The 1,N^2-cyclic propano adduct (**61**), unlikely to form *in vitro*, is a mixture of two diastereomers. The 1,N^2-bicyclic, thermodynamically stable adduct (**60**) is a mixture of four diastereomers and after deprotection of sugar moiety the adduct (**53**) was obtained. In an alternative route the deprotected sugar derivative was subjected to periodate oxidation at pH 8 but the major product was dGuo, thus the N-1 dialdehyde adduct readily dealkylated under basic conditions.

In the reaction of acrolein, crotonaldehyde, acetaldehyde with dGuo, partly as by-products of lipid peroxidation, sugar and ethanol metabolism, 1,N^2-propano-dGuo adducts (**62a, b**) form Scheme (**12**). Hecht *et al.* [25] have studied the reaction of dGuo and DNA with combinations of formaldehyde and acetaldehyde (the hypothetic congeners of acrolein). The interaction of dGuo and the above aldehydes resulted in the formation of adducts (**62**-**66**) with N^2-hydroxymethyl derivative (**63**) being dominant. In the reaction mixtures with DNA no acrolein adducts were detected and formaldehyde-crosslinked dAdo dominated, with only small amount of formaldehyde-crosslinked dGuo (**66**). Thus, the reactions of formaldehyde plus acetaldehyde with dGuo are dominated by newly identified cyclic adducts and formaldehyde-derived products whereas the reactions with DNA result in the formation of formaldehyde-crosslinked adducts.

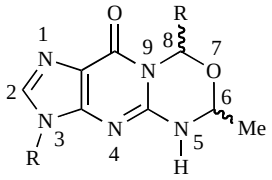
Kronberg *et al.* [26] have reported malonaldehyde-formaldehyde and malonaldehyde-acetaldehyde conjugate adducts with Guo. The major products of these interactions with malonaldehyde and acetaldehyde under physiological conditions after 6 days were the 1,N^2-cycloadduct (**67**) and an N^2-1,4-dihydropyridine derivative (**68**), isolated in 7 and 2% yield, respectively, both originating from condensation reactions between the above aldehydes Scheme (**13**). In the reaction of malonaldehyde and formaldehyde with Guo, the major product was identified as (**69**) in only 0.3% yield after 3 days. The plausible mechanism for the formation and interconversion of these products has been discussed in detail.



62a, b

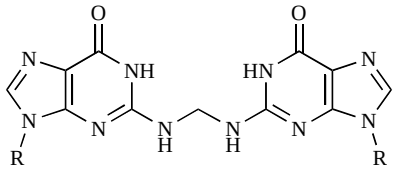


63



64a, b; R = H

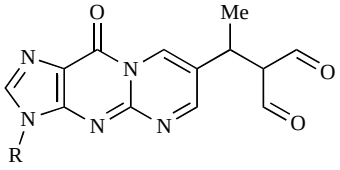
65a, b; R = Me



66

R = 2'-deoxy-β-D-ribofuranosyl

Scheme 12.



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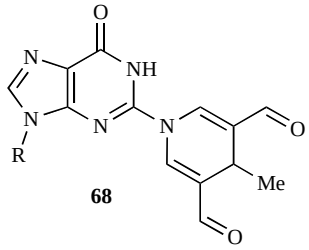
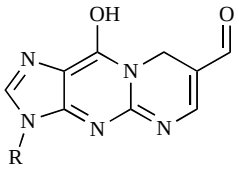


68

R = β-D-ribofuranosyl



69

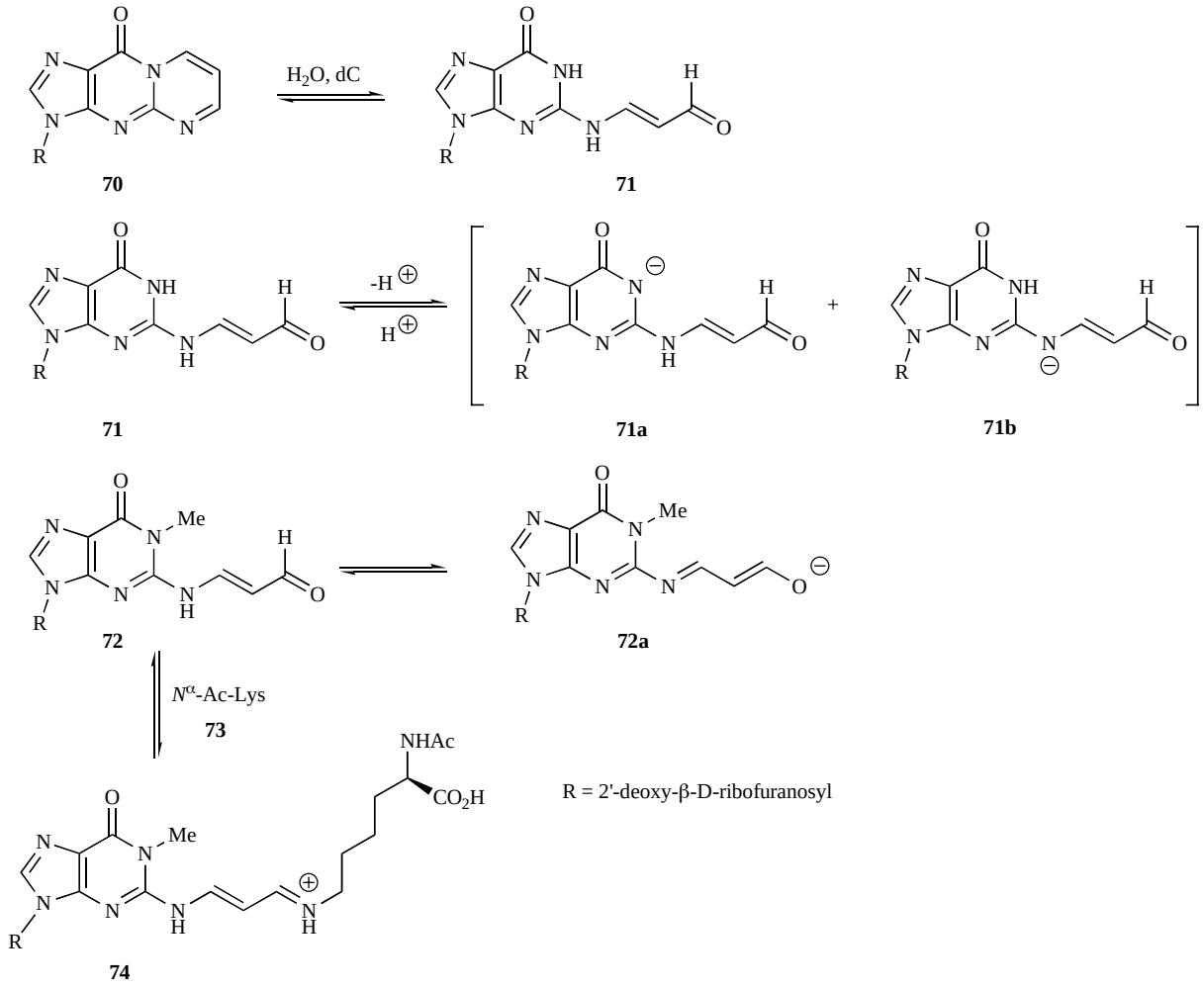
Scheme 13.

The frequency of the above lesions was later found to be 100-800/10^5 nucleotides, based on the positive ESI-MS/MS of dsDNA hydrolysates of calf thymus. The dGuo adducts were generated in smaller amounts when the incubation was performed at pH 6.0 rather than at pH 7.4, especially in the ssDNA reaction (for ssDNA the lesions occurred at the frequency 500-6000/10^5 nucleotides). In dsDNA and ssDNA, two dGuo and two dAdo conjugate adducts were detected but the guanine base was the major target for the malonaldehyde-acetaldehyde conjugates with calf thymus DNA [27,28].

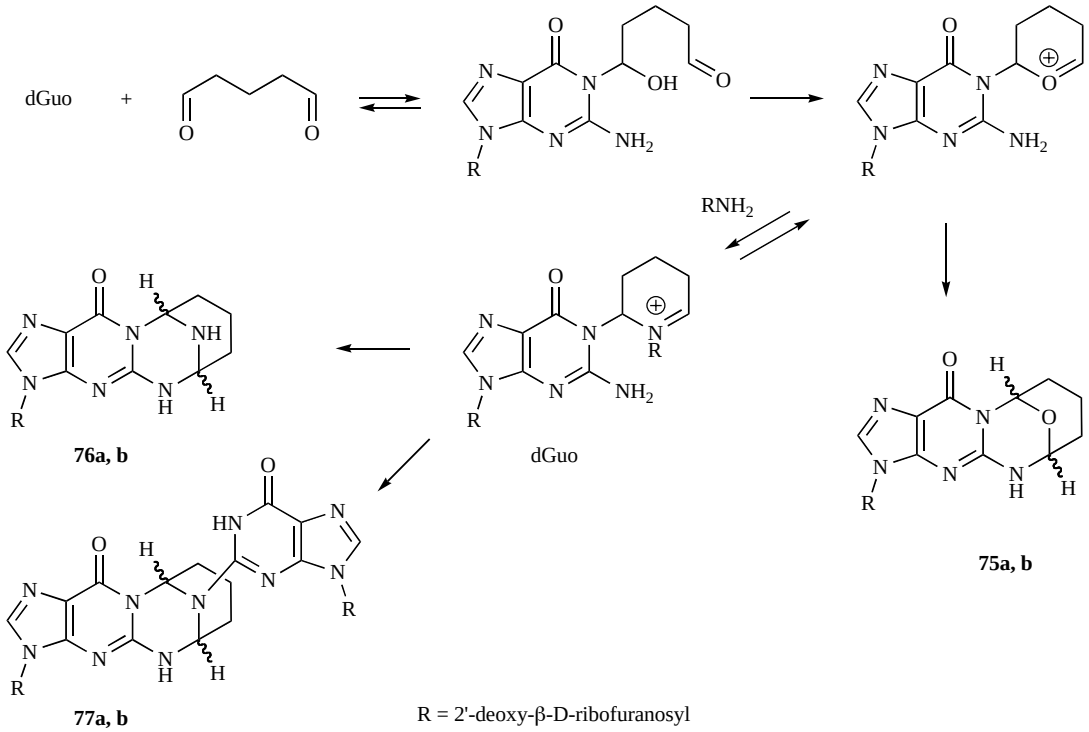
Malonaldehyde itself forms a condensed pyrimidine (**70**) and an N^2-oxopropenyl derivative (**71**) with dGuo, that are in equilibrium with each other Scheme (**14**). The acidity of (**71**) (pK_a ca. 6.9) may be an important determinant of its miscoding properties and its reactivity to nucleophiles in DNA or proteins to form cross-links. To test this hypothesis, N^2-oxopropenyl derivatives of dAdo, dCyd , and 1-methyl-dGuo (**72**) were synthesized and their pKa values and ability to form cross-links with N^{α}-acetyl-L-lysine (**73**) have been studied by Marnett *et al.* [29]. The N^2-oxopropenyl derivative of 1-methyl-Guo (**72**) exhibited a pKa of 8.2. Compound (**71**) did not react with N^{α}-acetyl-L-lysine, whereas (**72**) readily formed cross-links. The oxopropenyl side-chain is an effective electron withdrawing group that lowers the pK_a of (**71**) by ca. 4 log units relative to dGuo and the dissociable proton is the N-1-imino proton rather than N-2.

From the interaction of the known biocide glutaraldehyde and dGuo and calf thymus DNA (pH 8.8, 3 days), 6 new compounds with bridged structures at N-1 and N-2 were isolated and studied by UV, ^{1}H, ^{13}C, ^{15}N NMR and MS methods by Kronberg *et al.* [30] Scheme (**15**). Compounds (**75**) (lesion frequency: 11/10^7 nucleotides only after 7 days), (**76a**) (304/10^7 nucleotides) and (**76b**) (357/10^7 nucleotides) were formed in dsDNA, yields in ssDNA were 10 times higher. The structure dynamics of compounds (**77a,b**) has been studied in detail by ^{1}H, ^{13}C, ^{15}N NMR methods.

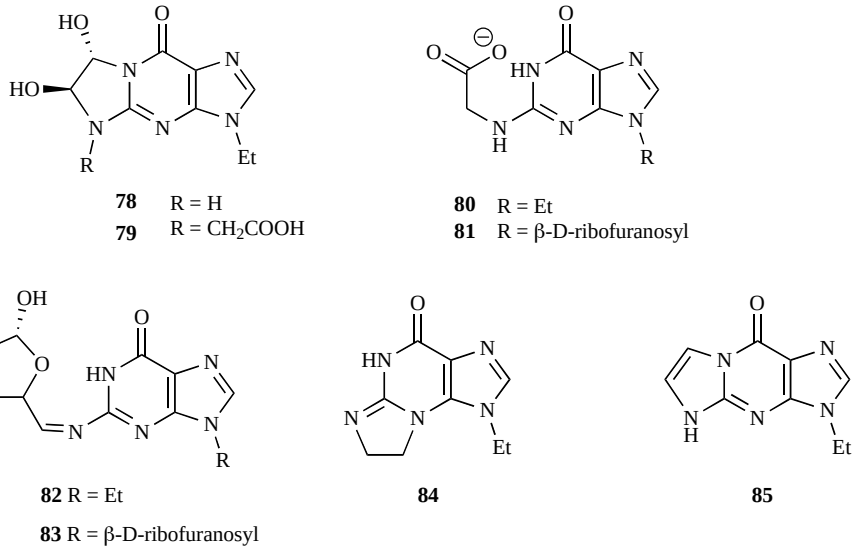
The reactions of 9-ethylguanine, 9-ethyl-1-methylguanine, dGuo and Guo with bromomalondialdehyde in aqueous buffers over a wide pH-range have been investigated using standard analytical methods by Lönnberg *et al.* [31]. The final products formed under acidic and basic conditions were different, but they were all derived from glyoxal. Among the 1 : 1 adducts, compound (**78**) predominated at pH < 6 and N^2-carboxymethylguanine adducts



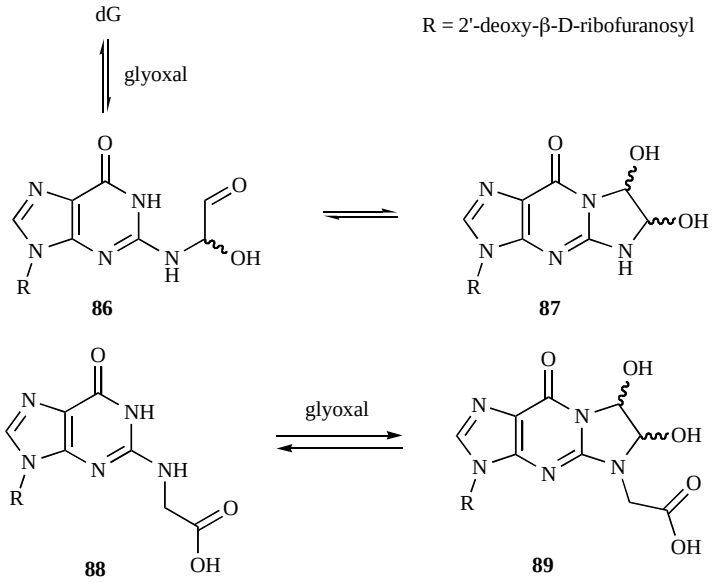
Scheme 14.



Scheme 15.



Scheme 16.

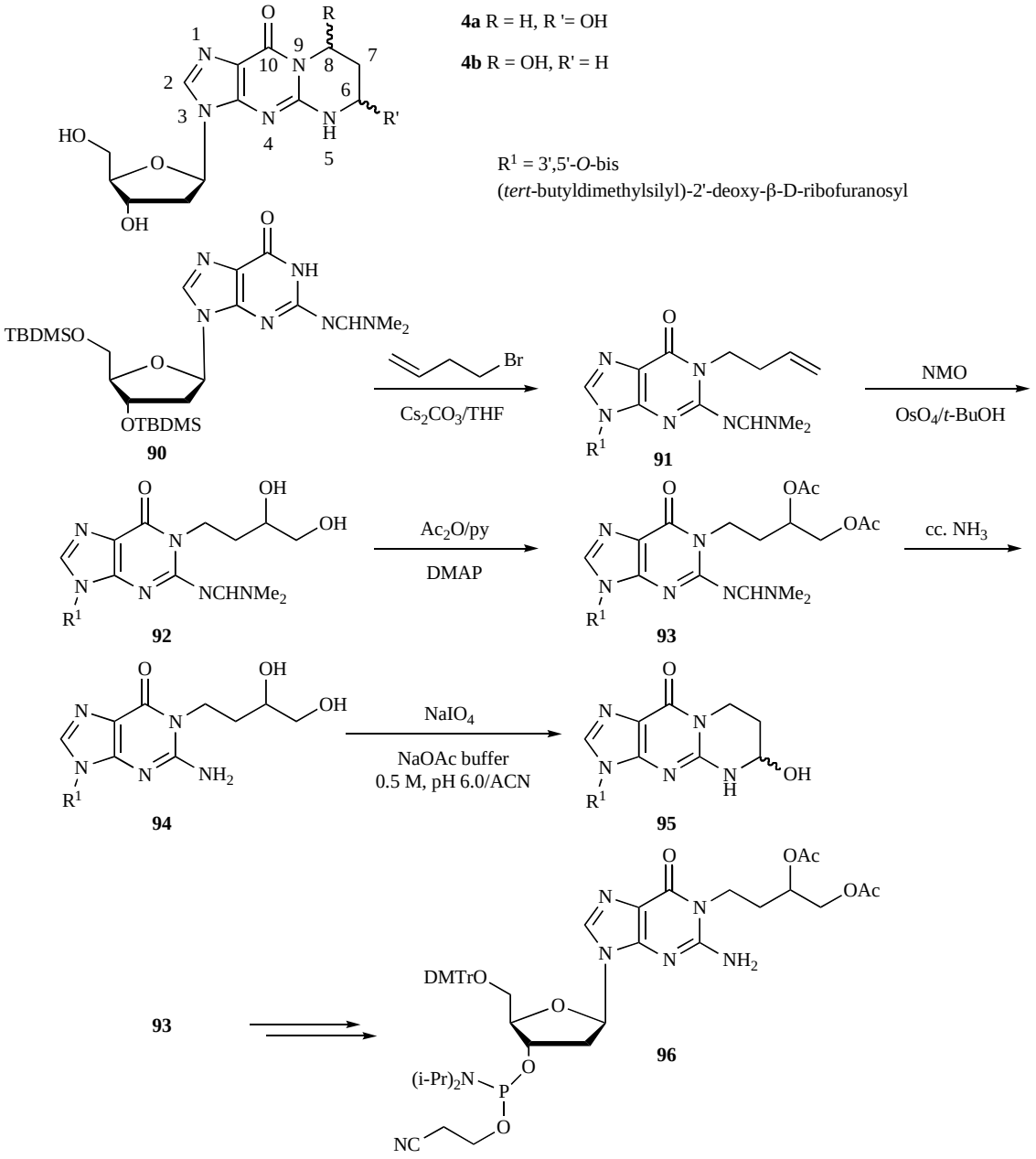


Scheme 17.

(**80**, **81**) at pH > 7 Scheme (**16**). In addition to these, 1 : 2 adducts (**82, 83**) and (**79**) were obtained at pH 10. Bromomalondialdehyde is significantly decomposed to formic acid and glycolaldehyde under the conditions required to obtain guanine adducts. Glycolaldehyde is oxidized to glyoxal, which then modifies the guanine base more readily than bromomalondialdehyde. Besides the glyoxal-derived adducts, N^2,3-ethenoguanine (**84**) and 1,N^2-ethenoguanine adducts (**85**) were formed as minor products.

Glyoxal, a widely used industrial chemical, also present in foods, beverages and cigarette smoke gives with dGuo in calf thymus ds and ssDNA the dihydroxy derivative (**87**) Scheme (**17**) with a lesion frequency of 290 adducts/10^5 nucleotides (dsDNA) and 2500 adducts/10^5 nucleotides (ssDNA). In addition, Kronberg *et al.* [32] have reported that two previously undetected adducts (**88**, **89**) were formed in the above reactions in aqueous buffered solutions under physiological conditions. The compounds isolated, purified and analyzed by UV, MS, ^{1}H and ^{13}C NMR arise from adduct (**86**). Compounds (**87**) and (**89**) are unstable in phosphate buffer and transform mainly to dGuo and partly to (**88**), a stable adduct and likely the end-product of glyoxal-DNA interaction. These adducts are relatively rare (1.7-3.2 mutations/10^6 nucleotides in dsDNA and 1.9-18/10^6 nucleotides in ssDNA).

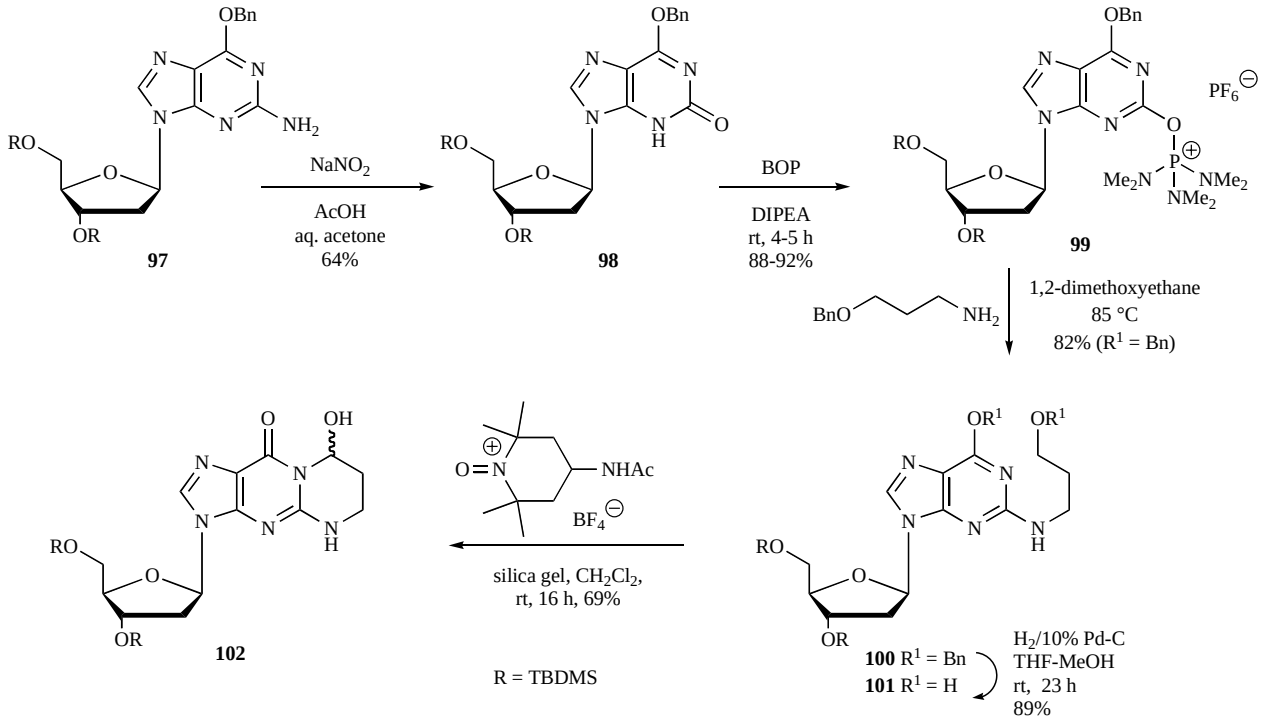
Acrolein, a known mutagen, undergoes reaction *in vitro* under physiological conditions with both dGuo and native DNA to give rise to exocyclic adducts with 5,6,7,8-tetrahydropyrimido[1,2-a]purine-10(3*H*)-one ring having a hydroxy group at either the 6 (**4a**) or the 8 position (**4b**). The synthesis of so-called distal 6-hydroxy isomer (**4a**) and its incorporation into DNA has been accomplished by Johnson *et al.* Scheme (**18**) [33]. The derivative (**95**) under the conditions of ODN synthesis is not stable and upon acetylation elimination took place therefore the protected diol (**93**) was used in oligonucleotide synthesis. The homoallyl congener



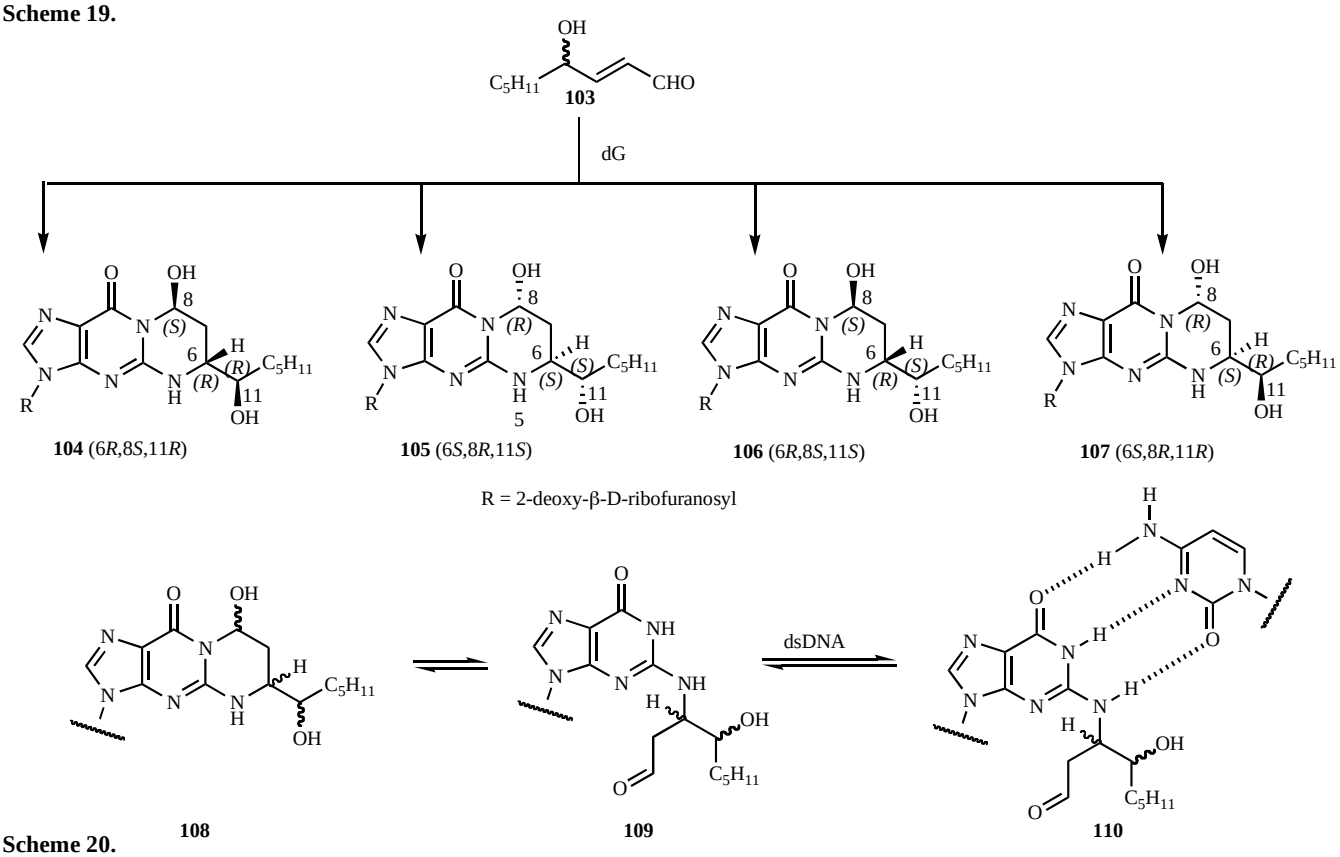
Scheme 18.

(**91**) was synthesized in the same fashion as described later by Rizzo *et al.* [34] followed by vicinal diol formation (**92**) and acetylation (**93**). The phosphoramidite (**96**) has been obtained from diacetate (**93**) using standard methods and ODNs with 7, 13 and 28 nucleotide units containing (**93**) have been synthesized with 99% coupling efficiency. Sodium periodate oxidation of (**94**) in aqueous buffer gave diastereomers of the 6-hydroxy-1,N^2-propano-dGuo derivative (**95**), corresponding to (**4a**).

The synthesis of the silylated derivative of so-called proximal 8-hydroxy isomer (**4b**) has been accomplished in a novel fashion by Lakshman and Bae [35] using a nucleoside phosphonium salt Scheme (**19**). O^6-Benzyl derivative (**97**) was diazotized to afford 2'-deoxyxanthosine (**98**) in 64% yield. The reaction of the latter with peptide coupling agent 1*H*-benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) resulted in the formation of stable nucleoside phosphonium salt (**99**) in 88-92% yield. This fact is in sharp contrast with the behaviour of inosine nucleosides with BOP, where the *in situ* formed phosphonium salts undergo subsequent reaction with the 1*H*-benzo[d][1,2,3] triazol-1-olate anion to yield O^6-(benzotriazol-1-yl)inosine derivatives. The structure of phosphonium salt (**99**) has been corroborated by ^{1}H and ^{31}P NMR spectral data as well. The 2'-deoxyguanosine-acrolein adduct has been obtained by allowing (**99**) to react with the *O*-benzyl protected derivative of 3-amino-1-propanol to give compound (**100**) in 82% yield. Catalytic debenzylation afforded the alcohol (**101**) that was subjected to oxidation. Out of the various reagents examined, the TEMPO-derived 4-acetylamino-2,2,6,6-



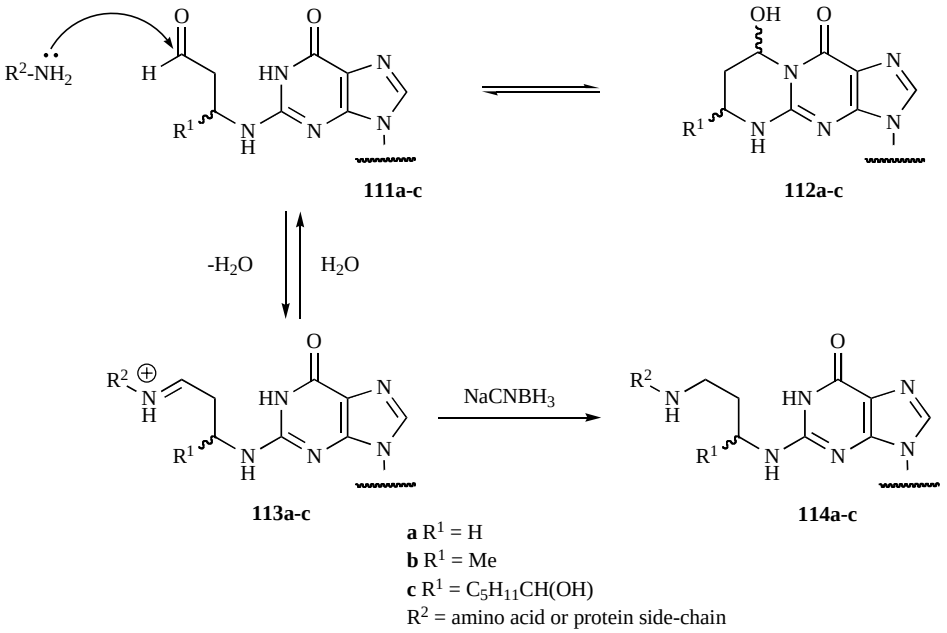
Scheme 19.



Scheme 20.

tetramethylpiperidine-1-oxoammonium tetrafluoroborate on silica gel support gave the compound (**102**) in 69% yield, other oxidants were less effective. The displacement reaction of phosphonium salt (**99**) with various amines to give N^2,N^2-substituted 2'-deoxyguanosines will be treated in *section 4*.

Polyunsaturated ω-3 and ω-6 fatty acids (PUFAs) can be converted under oxidative stress into various endogeneous lipid hydroperoxides that eventually undergo metal ion mediated fragmentation to yield bifunctional aldehydes [malonaldehyde, acrolein, crotonaldehyde, and *trans*-4-hydroxynon-2-enal (HNE, **103**) *etc*.] that are potential mutagens and human exposure to these reactive aldehydes is virtually unavoidable. The mutagenicity of these compounds is supposed to be exerted by the formation of various DNA



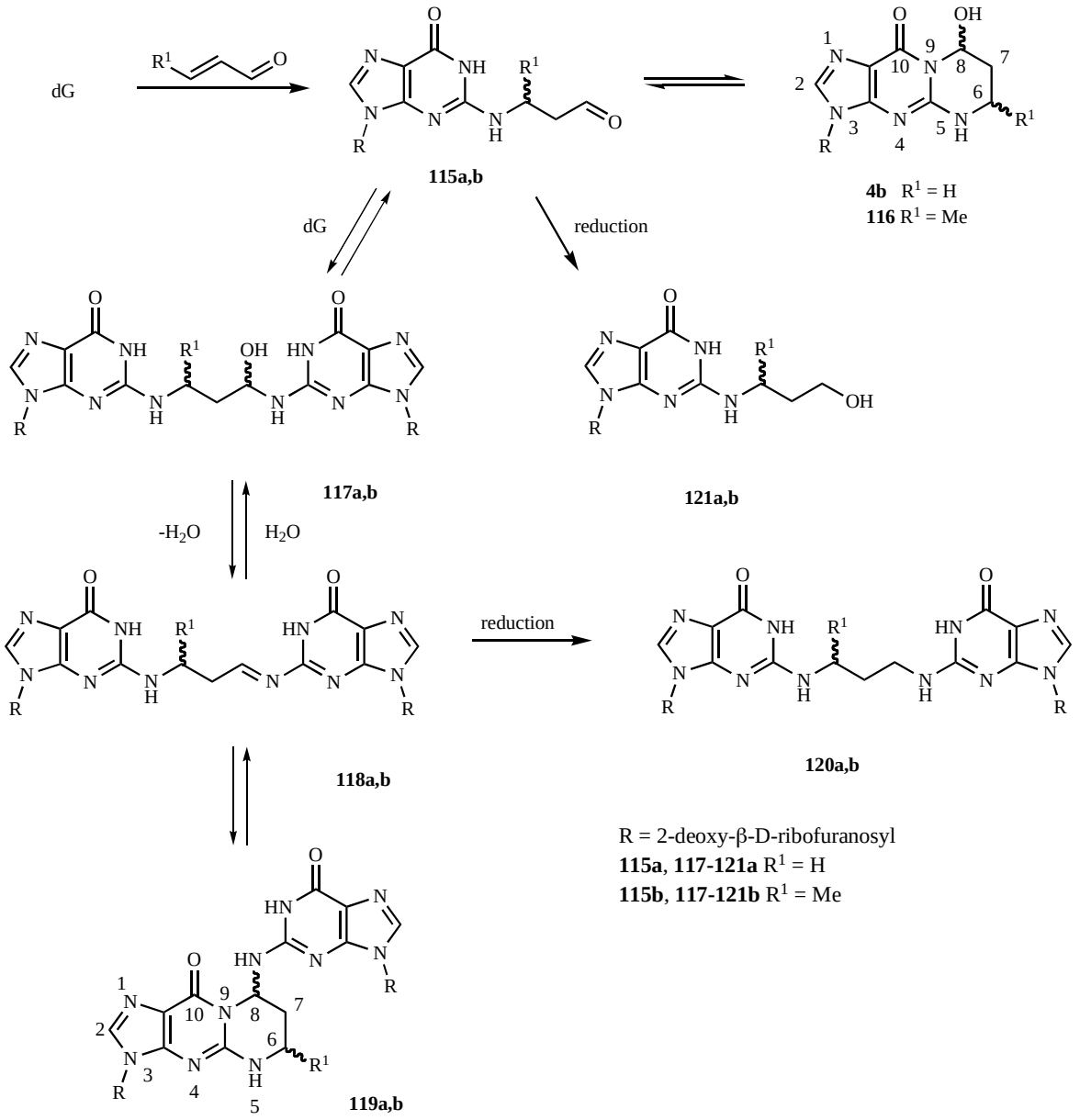
Scheme 21.

and protein adducts as well as DNA-protein cross-links (DPCs) *in vivo* [36].

HNE (**103**) is derived from the oxidation of ω-6 polyunsaturated fatty acids, such as arachidonic acid, linoleic acid, or their hydroperoxides. HNE is capable of exhibiting a wide range of biological effects, from alteration in gene expression and cell signaling to cell proliferation and apoptosis. The presence of high levels of HNE and HNE-protein adducts has been implicated in a number of human diseases caused by oxidative stress, including Alzheimer's disease, Parkinson's disease, arteriosclerosis, and hepatic ischemia reperfusion injury. HNE also exhibits genotoxic effects. It is mutagenic in Chinese hamster lung cells and causes G:C to T:A transversions at codon 249 of a wild-type p53 lymphoblastoid cell line. Racemic HNE (**103**) can specifically react with 2'-deoxyguanosine in DNA, by Michael addition, to yield four diastereomeric propano 1,N^2-deoxyguanosine adducts (**104**-**107**), the 8-hydroxyl group and the 6-substituents occur exclusively in *trans* relative configuration [37] Scheme (**20**). Lloyd *et al.* [36] have incorporated the above HNE-dGuo adducts into dodecamer oligodeoxynucleotides 5' GCTAGCG*AGTCC 3' (where G* = modified dGuo adducts **104**-**107**), inserted into a DNA shuttle vector and evaluated for the ability of each stereoisomer to induce mutagenesis when replicated through mammalian cells. The resultant mutagenicity of these adducts was related to their stereochemistry, HNE-dGuo adducts (**104**) (*6R,8S,11R*) and (**105**) (*6S,8R,11S*), were significantly (by one order of magnitude) more mutagenic than adducts (**106**) (*6R,8S,11S*) and (**107**) (*6S,8R,11R*). The predominant mutation for the adducts (**104**) (*6R,8S,11R*) and (**105**) (*6S,8R,11S*) was the G → T transversion, with no evidence for adduct-induced deletions. The ring-closed HNE-dGuo adducts (*syn* or *anti* conformation) (**108**) are hypothesized to undergo ring-opening (**109**) in duplex DNA, thus displacing the aldehydic moiety in the minor groove and facilitating conventional Watson–Crick base pair formation with an incoming 2'-deoxycytosine (**110**). The spatial disposition of the chiral groups appears to govern mutagenesis and influences the polymerase's ability to bypass the HNE-adduct in a mutagenic or nonmutagenic fashion.

Lloyd and Kurtz [38] have extended their studies on the formation of DPCs and have found that oligodeoxynucleotides containing 1,N^2-deoxyguanosine adducts (**111a-c**) of acrolein, crotonaldehyde, and *trans*-4-hydroxynon-4-enal can form cross-links with the tetrapeptide Lys-Trp-Lys-Lys Scheme (**21**). DNA-peptide complexes were covalently trapped following reduction with sodium cyanoborohydride (**114a-c**), and pre-reduction of adducted DNAs inhibited complex formation. The adducts (**111a-c**) were more reactive in dsDNA than in ssDNA, and the ring-open aldehydic moiety is the induced tautomer in duplex DNA for adducts exhibiting high relative reactivity. Adducted DNA cross-linked to peptides Arg-Trp-Arg-Arg and Lys-Trp-Lys-Lys with comparable efficiency, and N^{α}-acetylation of peptides dramatically inhibited trapping. Thus, the reactive nucleophile is located at the *N*-terminal α-amine of the peptide, consistent with the lower intrinsic pK_a of an α-amine (pK_a ca. 7.6) compared with an ε-amine (pK_a ca. 10.3) in a random-coil peptide because nucleophilic attack at the ring-open aldehyde adducts requires a neutral deprotonated amine.

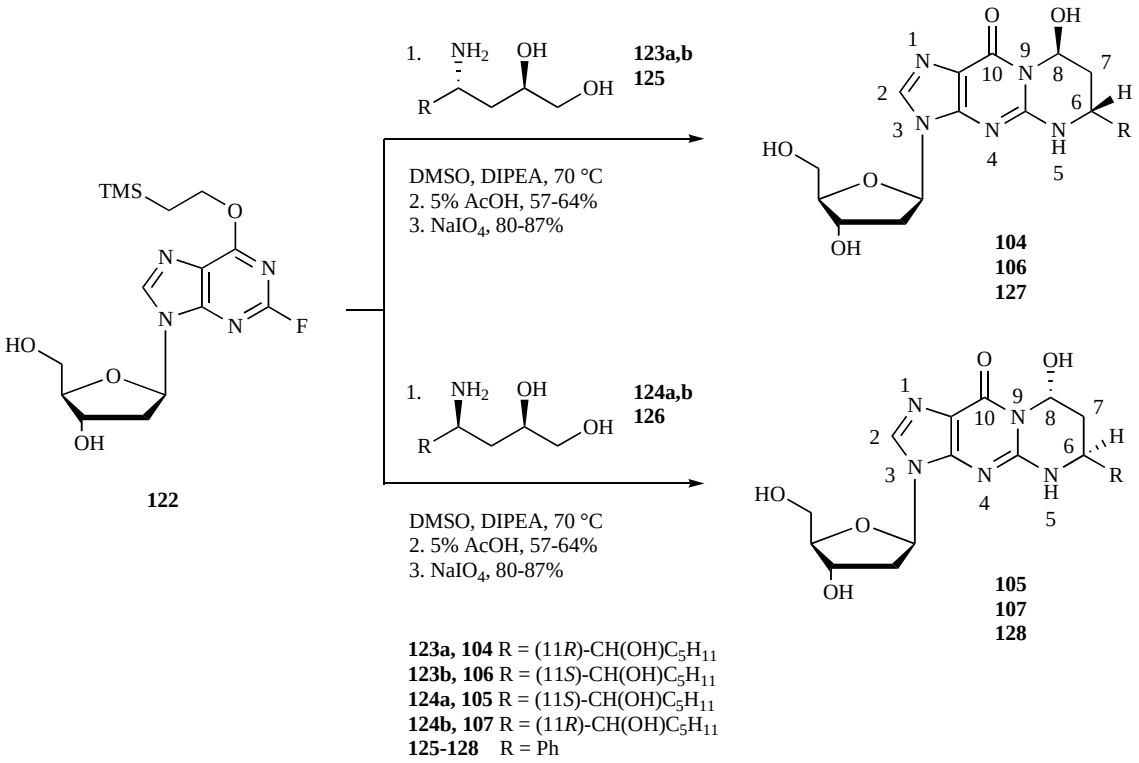
Rizzo *et al.* [39] have scrutinized the effect of configuration of chiral adducts on DNA-DNA cross-links. First, acrolein and crotonaldehyde DNA adducts were investigated. 8-Hydroxy-1,N^2-propanodeoxyguanosine (**4b**) is a major DNA adduct formed by acrolein Scheme (**22**). The potential for oligodeoxynucleotide duplexes containing (**4b**) to form interchain cross-links was evaluated by HPLC, CZE, MALDI-TOF and melting phenomena. In dodecamer oligodeoxynucleotides containing a sequence 5'-dC-(**4b**) (CpG context) cross-linking occurred in a slow, reversible manner to the extent of 50% after 7 days of incubation at 37 °C. Enzymatic digestion to form (**119a**) and reduction with $NaCNBH_3$ followed by enzymatic digestion (DNAse I, snake



Scheme 22.

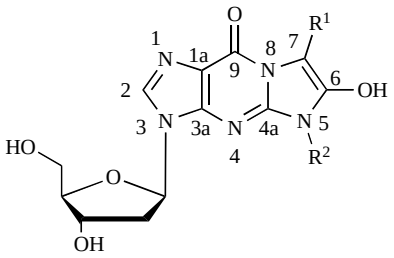
venom phosphodiesterase, alkaline phosphatase) to give 1,3-bis(2'-deoxyguanosin-N^2-yl)propane (**120a**) established that cross-linking had occurred with the exocyclic amino group of 2'-deoxyguanosine. It is concluded that the cross-link is a mixture of imine (**118a**) and carbinolamine (**117a**) structures. With oligodeoxynucleotide duplexes containing the sequence 5'-(**4b**)-dC (GpC context), cross-links were not detected by the techniques enumerated above. In addition, ^{15}N-^{1}H HSQC and HSQC-filtered NOESY spectra carried out with a duplex having ^{15}N-labeling of the target amino group established unambiguously that a carbinolamine cross-link (**117a**) was not formed. The potential for interchain cross-link formation by the analogous crotonaldehyde adduct (**116**) was evaluated in a 5'-dC-(**116**) sequence context. Cross-link formation was strongly dependent on the configuration of the methyl group at C6 of (**116**). The (6*R*) diastereomer of (**116**) formed a cross-link to the extent of 38%, whereas the (6*S*) diastereomer cross-linked only 5% after incubation for 21 days at 37 °C.

Next, the HNE-derived adducts were investigated by the same group [37]. Stereospecific syntheses of these four adducts at the nucleoside level (**104-107**) have been accomplished using Sharpless asymmetric epoxidation and homologation. The aminodiols (**123a,b**) and (**124a,b**) were individually condensed with 2-fluoro-O^6-(2-trimethylsilylethyl)-2'-deoxyinosine (**122**) to give the corresponding diol derivatives in 57-64% yield after deprotection of the O^6-(2-trimethylsilylethyl) group Scheme (**23**). The diol intermediates served as masked aldehydes and periodate oxidation afforded the proximal adducts (**104-107**) in 80-87% yield. The structure of nucleoside adducts (**104-107**) has been corroborated by 2D ^{1}H-^{13}C NMR (HMBC) and CD studies. In addition, a versatile post-oligomerization strategy for their site-specific incorporation into oligonucleotides has

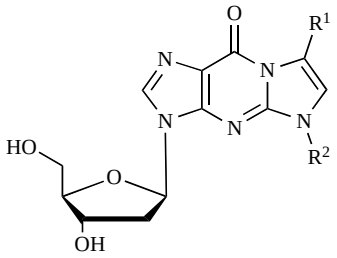


123a, 104 R = (11*R*)-CH(OH)C_5H_{11}
123b, 106 R = (11*S*)-CH(OH)C_5H_{11}
124a, 105 R = (11*S*)-CH(OH)C_5H_{11}
124b, 107 R = (11*R*)-CH(OH)C_5H_{11}
125-128 R = Ph

Scheme 23.



129 R^1= $CH_2CO(CH_2)_6COOH$; R^2 = H
130 R^1= $CH_2CO(CH_2)_6COOMe$; R^2 = Me

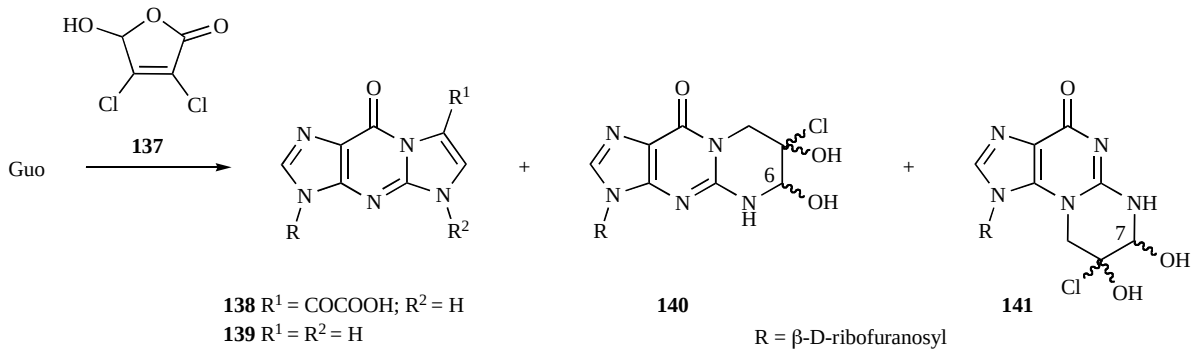


131 R^1 = $CH_2COC_5H_{11}$; R^2 = Me
132 R^1 = R^2 = H
133 R^1 = $CH_2COC_5H_{11}$; R^2 = H
134 R^1 = $CH_2CO(CH_2)_3COOH$; R^2 = H
135 R^1 = CH=CHCH(OH)$(CH_2)_3COOH$; R^2 = H
136 R^1 = $CH_2COCH_2CH_3$; R^2 = H

Scheme 24.

been developed. It was found that these adducts are destabilizing as measured by melting temperature when compared to an unadducted strand. The thermal destabilization of the adducted dodecamers ranged from 5 to 16 °C and is dependent on the absolute stereochemistry of the adduct. The HNE adducts were also examined for their ability to form interstrand DNA-DNA cross-links when incorporated into a CpG sequence. Only one of the HNE stereoisomers (**105**) (*6S,8R,11S*) formed interstrand DNA-DNA cross-links in 50% yield after 7 days of incubation at 37 °C (this cross-linking adduct shares the same C6 configuration with the crotonaldehyde adduct that also forms interstrand cross-links). Essentially the same strategy was used for the synthesis of *trans*-cinnamaldehyde adducts (**127**) and (**128**) Scheme (**23**) [40]. *trans*-Cinnamaldehyde, a common constituent of essential oil and the principal component of cinnamon, has been shown to induce neoplastic transformations in Chinese hamster ovary cells and cause lethal mutations in *Drosophila*. Interestingly, cinnamaldehyde has also been shown to significantly reduce the spontaneous mutation frequency in *Salmonella typhimurium*. The chiral aminodiols (**125**, **126**) have been obtained in a metal-promoted intramolecular C–H insertion reaction of nitrogen of an enantiomerically pure sulfamate ester.

ω-6 PUFA-derived lipid hydroperoxides such as 13-hydroperoxy-(9*Z*,11*E*)-octadecadienoic acid (13-HPODE) can undergo Fe(II)-, Cu(I)-, or vitamin C-mediated homolytic decomposition into α,β-unsaturated aldehydes which react with DNA bases. Blair *et al.* [41] have studied the reaction of 9,12-dioxo-(10*E*)-dodecenoic acid (DODE), also a lipid hydroperoxide-derivative, with dGuo. The structure of carboxynonanone-1,N^2-etheno-dGuo derivative (**129**) has now been established by a combination of ^{1}H and



138 R^1 = COCOOH; R^2 = H
139 R^1 = R^2 = H

Scheme 25.

^{13}C NMR spectroscopy studies of its bis-methylated (**130**) derivative Scheme (**24**). The site of dGuo methylation was first established as being at N-5 rather than at O-9 from NMR analysis of a methyl derivative of the model compound, heptanone-etheno-1,N^2-dGuo (**131**).

In a combined LC/MS and NMR study of adducts from the reaction dGuo with (5*S*)-hydroperoxy-6,8,11,14-(*E*,*Z*,*Z*,*Z*)-eicosatetraenoic acid [(5*S*)-HpETE], another PUFA-derived lipid hydroperoxide, in the presence of transition metal ions [Fe(II) or Fe(III) or vitamin C, Blair *et al.* [42] have isolated four products (**132-135**) Scheme (**24**). Adduct (**134**) was characterized as its mono- and bismethylated derivative by NMR. The adducts were expected to be formed through a 4-oxo-2-nonenal-like molecule that contained the carboxy terminus therefore the formation of major products etheno-1,N^2-dGuo (**132**) and heptanone-etheno-1,N^2-dGuo (**133**) was surprising. This suggested that the etheno-1,N^2-adducts had arisen by a previously unrecognized pathway of (5*S*)-HpETE decomposition, and a mechanism for this transformation was proposed by the authors.

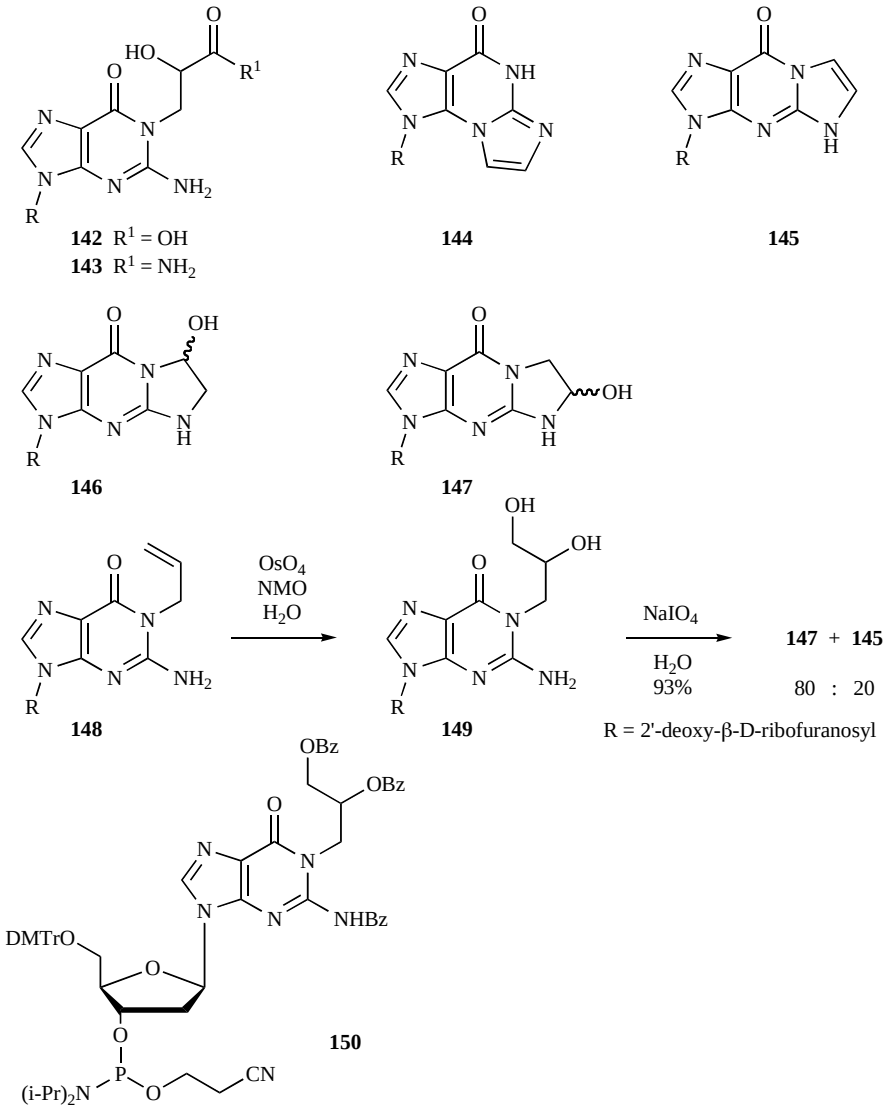
Maekawa *et al.* [43] have also studied the reactions of possible mutagens, derived from lipid peroxidation, with dGuo in model systems. Methyl linoleate (model of ω-6 fat), methyl α-linolenate (MLN) (model of ω-3 fat), and commercial salad oil were incubated at pH 7.4 with hemin (models of high-fat and red meat diets) and the unstable mutagens in the model reactions were trapped as dGuo adducts. The compounds observed comprised 1,N^2-etheno-dGuo derivatives of glyoxal, glyoxylic acid, ethylglyoxal, 4-oxo-2-hexenal (4-OHE, a lipid peroxidation product of ω-3 fatty acids), respectively, and 8-hydroxy-dGuo Scheme (**24**). The structure of 4-OHE adduct (**136**) has been elucidated by MS and 2D NMR methods and by comparison with synthesized 9-ethyl-10-(2-oxobutyl)-1,N^2-ethenoguanine. At concentrations exceeding 10 μg/plate, 4-OHE was quite toxic to *Salmonella* bacteria. These results raise the concern that ω-3 fats are more toxic than ω-6 fats, in that ω-3 fats produce larger amounts of mutagens by lipid peroxidation, even if ω-3 fats themselves in the diet inhibit carcinogenesis.

Mucochloric acid (**137**) is a chlorinated hydroxyfuranone, renowned for its mutagenicity and ubiquitous in domestic waters as a consequence of waste-water treatment by chlorination. It produces with guanosine 7-oxalyl-1,N^2-ethenoguanosine (**138**) (20-40% yield) as the major product together with a minor amount of its degradation product 1,N^2-ethenoguanosine (**139**) Scheme (**25**) [44]. Two sets of 1,N^2- and N^2,3-regioisomeric α-hydroxy chlorohydrins (**140, 141**), present in trace amounts, consisting of interconverting C-6(7) epimers for both C-7(6) stereoisomers, *i.e.* a total of four stereoisomers were also present for each regioisomeric set. The structural elucidation of the compounds was based on ^{1}H, ^{13}C, and ^{15}N NMR studies and a plausible mechanism for their formation was also presented.

Acrylamide, a high production volume chemical with a wide variety of industrial applications and also ocurring in starchy foodstuffs, is neurotoxic, clastogenic, and carcinogenic in animal experiments, and probably carcinogenic in humans. Acrylamide reacts slowly with nucleobases but its mutagenicity in human and mouse cells is attributed to the epoxide metabolite glycidamide. Backman and Kronberg [45] have investigated the reaction of glycidamide with nucleosides. At pH 7 the reaction of acrylamide with dG afforded an N7-substituted derivative while at pH 9 alkylation took place at N-1 although the yields of products (**142, 143**) were quite low Scheme (**26**).

The formation of N-7- and N-1-substituted carboxamides was rationalized by a direct attack of the endocyclic N-7 and N-1 on the β-carbon of the oxirane ring. The plausible mechanism for the hydrolysis of amide (**143**) involves exocyclic amino groups of dG that attack the carboxamide in glycidamide followed by deamination and ring closure through reaction of the oxirane ring with the nucleophilic ring N of dG. It was found earlier by Beland *et al.* [46] that in mice glycidamide typically produced higher levels of DNA adducts (1700-2500 adducts/10^8 nucleotides) than observed with acrylamide (840-2100 N7-glycidamide-guanine lesions/10^8 nucleotides). The preferred site of alkylation of guanosine with acrylamide and glycidamide in neutral medium (N-7) using semiempirical quantum mechanical calculations was corroborated by Varnali *et al.* [47].

Chlorooxirane, formed from vinyl chloride, acrylonitrile, vinyl carbamate and other potentially carcinogenic compounds, rapidly rearranges to chloroacetaldehyde that reacts at the N-1, N-2, N-3 and N-7 positions of dGuo to give 2-oxoethyl adducts. Except for an N7-adduct they undergo immediate cyclization to give hydroxyethano derivatives, from which N1- and N3-alkylated dGuo derivatives dehydrate and tautomerize and produce N^2,3- and 1,N^2-etheno-dGuo adducts (**144, 145**) Scheme (**26**). Compound (**146**) is highly resistant to dehydration to give (**145**). An intermediate in the formation of 1,N^2-ethenoguanine is 6-hydroxy-1,N^2-ethanoguanine (**147**) (half-life 24-48 h). The chemical properties and miscoding potential of the latter were studied

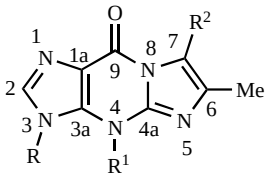


Scheme 26.

by Rizzo *et al*. [34] by its synthesis and site-specific incorporation into ODNs and the synthesis of ε-dGuo phosphoramidite was also improved. Alkylation of dGuo with allyl bromide at N-1 afforded compound (**148**) that was hydroxylated using OsO_4/NMO. Periodate cleavage of the resulting diol (**149**) gave an aldehyde that spontaneously cyclized to (**147**) (74%) and (**145**) (19%). Prolonged reaction times gave by-products. Alternatively, alkylation with (*R*)-glycidol also afforded (**149**). Incorporation of (**147**) into ODN in the form of phosphoramidite (**150**) relied on the generation of the aldehyde by periodate oxidation after cleavage and deprotection of ODN that contains the diol precursor (**149**). The miscoding properties and rates of nucleotide incorporation of adducts (**147**) and (**145**) were significantly different. For the replicative polymerase T7 dATP was preferentially incorporated opposite adduct (**145**) while dATP and dGTP were incorporated opposite (**147**). Both adducts were very strong blocks to further extension. Lesion bypass polymerase Dpo4 incorporated dATP opposite (**147**) and dGTP opposite (**145**). In the later case a one-base deletion resulted through base pairing of the incoming dGTP with a dC on the 5'-side of (**145**) sequence-dependently. In conclusion, compound (**147**) has a sufficient lifetime to contribute to the genotoxic spectrum of vinyl chloride.

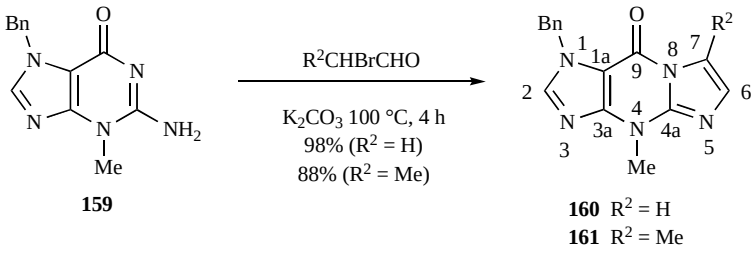
3.2. The Wyosine-Wyebutosine Family

Naturally occurring 1,N^2-dialkylguanines include the tricyclic fluorescent, so-called Y (or wye) bases, wye (**151**) and wybutine (**153**), and the corresponding ribonucleosides wyosine (**5**) and wyebutosine (**6**), respectively Scheme (**27**). The biological role of these unusual modifications has attracted much attention since their isolation and structure elucidation in the 1970s. Wye nucleosides occur specifically at position 37 in the anticodon loop of eukaryal and archeal phenylalanine transfer RNA ($tRNA^{Phe}$) and are generally thought to enhance and expand tRNA function to increase cell viability [48] by augmenting heat tolerance in mesophiles and hyperthermophiles [49], and by -1 frameshifting [50]. Mild acidic treatment of wyosine causes the loss of its codon recognition property required for protein biosynthesis, thus constituting a switch for the deactivation of the codon function of $tRNA^{Phe}$ [51].

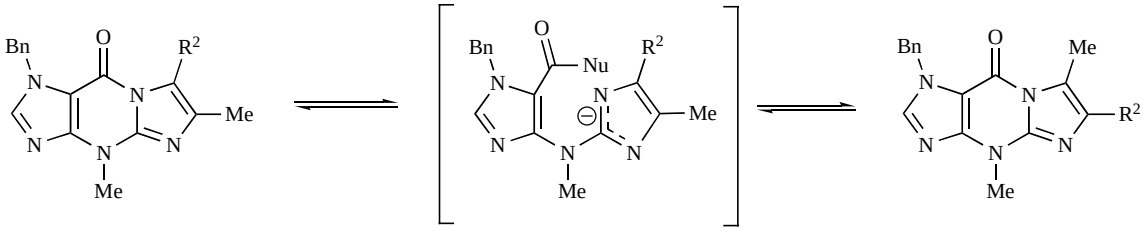


	Symbol	*Common name*	*Substituents*
151		wye	R = H, R^1 = Me, R^2 = H
5	imG	wyosine	R = β-D-Rib*f*, R^1 = Me , R^2 = H
152	mymG	methylwyosine	R = β-D-Rib*f*, R^1 = Me , R^2 = Me
153		wybutine	R = H, R^1 = Me, R^2 = $CH_2CH_2CH(NHCOMe)CO_2Me$
6	yw	wybutosine	R = β-D-Rib*f*, R^1 = Me, R^2 = $CH_2CH_2CH(NHCO_2Me)CO_2Me$
154	OHyW	hydroxywybutosine	R = β-D-Rib*f*, R^1 = Me, R^2 = $CH_2CH(OH)CH(NHCO_2Me)CO_2Me$
155	O_2yW	peroxywybutosine	R = β-D-Rib*f*, R^1 = Me, R^2 = $CH_2CH(OOH)CH(NHCO_2Me)CO_2Me$
156	OHyW*	undermodified hydroxywybutosine	R = β-D-Rib*f*, R^1 = Me, R^2 = $CH_2CH(OH)CH(NH_2)COOH$
157	imG-14	4-demethylwyosine	R = β-D-Rib*f*, R^1 = H, R^2 = H
158	imG2	isowyosin	R = β-D-Rib*f*, R^1 = H, R^2 = Me

Scheme 27.



159 **160** R^2 = H **161** R^2 = Me



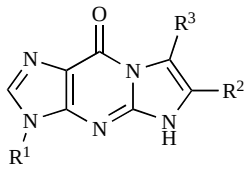
162a-i **163e** R^2 = CHO, Nu=OH **164a-i**

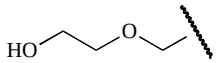
R^2 = $CH_2CH_2CHMe_2$ (**a**); (*E*)-CH=CHCHMe$_2$ (**b**); CH_2OH (**c**); CH_2OMe (**d**); CHO (**e**); CO_2Me (**f**); Cl (**g**); Br (**h**); I (**i**)

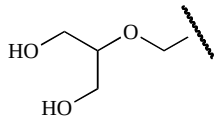
Scheme 28.

Wyosine (**5**) has been isolated in large scale from torula yeast (*Candida utilis*). Comparison of the tri-*O*-acetyl-d_9 derivative of this nucleoside with a chemically synthesized authentic sample has unambiguously established its structure by means of MS, ^{1}H-NMR and CD measurements [52]. The number of known wyosine and wybutosine modifications (**151-158**), along with their parent nucleobases (**5, 6**) amounts to ten to date Scheme (**27**) [53]. The ^{1}H, ^{13}C, ^{15}N NMR spectral properties, thermodynamic stability, tautomeric equilibria, chemical reactivity, calculated structural properties [51], photophysical characteristics (absorption, steady-state and dynamic fluorescence as a function of pH in aqueous solution) of wyosine and its analogs have been studied in detail [54].

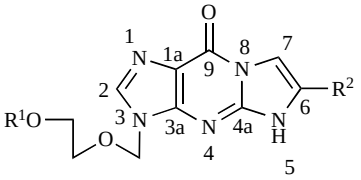
The wyosine analog base 1-benzyl-6-demethylwye (**160**) and 1-benzyl-6-demethyl-7-methylwye (**161**) have been synthesized from 7-benzyl-3-methylguanine (**159**) and bromoacetaldehyde or 2-bromopropanal by Itaya *et al*. Scheme (**28**) [55]. The transformations of (**160**) using various electrophiles to afford 7-substituted derivatives have also been studied in detail. The effect of 7-substituent on rearrangement through fission of pyrimidine ring in compounds (**162a-i**) and recyclization of imidazolium salts (**163a-i**) to 6-substituted derivatives (**164a-i**) upon nucleophilic attack was



165a-g R^1 =  R^2 = 4-FC_6H_4, Ph, 4-$F_3CC_6H_4$, 4-$MeOC_6H_4$; R^3 = H, Me, 4-FC_6H_4, Bn

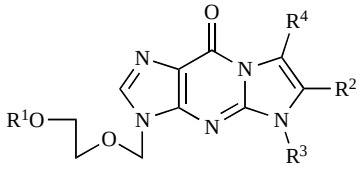
166a, b R^1 =  R^2 = 4-FC_6H_4, 4-$F_3CC_6H_4$; R^3 = H

Scheme 29.

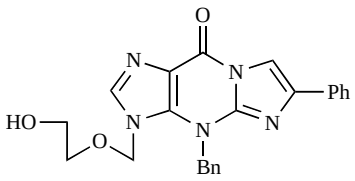


167 R^1 = H, R^2 = Me
168 R^1 = TBDMS, R^2 = Me

TEA, TrCl, DCM
or K_2CO_3, TrCl, DMF

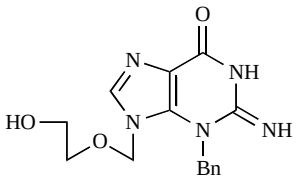


169 $R^1 = R^3$ = H, R^2 = Me, R^4 = Tr
170 R^1 =TBDMS, R^2 = Me, R^3 = H, R^4 = Tr
171 $R^1 = R^2$ = H, R^3 = Tr, R^4 = H
172 R^1 = TBDMS, $R^2 = R^4$ = H, R^3 = Tr
173 $R^1 = R^2$ = H, $R^3 = R^4$ = Tr
174 R^1 = TBDMS, R^2 = H, $R^3 = R^4$ = Tr
175 $R^1 = R^2 = R^3$= H, R^4 = Tr



176

a-c
36%



177

(a) NBS, 0.5 M acetate buffer, pH 4.8/dioxane 1:1, rt, 90 min, 25% aq. NH_3, 90 min
(b) Ac_2O, py, rt, 12 h, chromatography.
(c) NH_3/MeOH, rt, 24 h

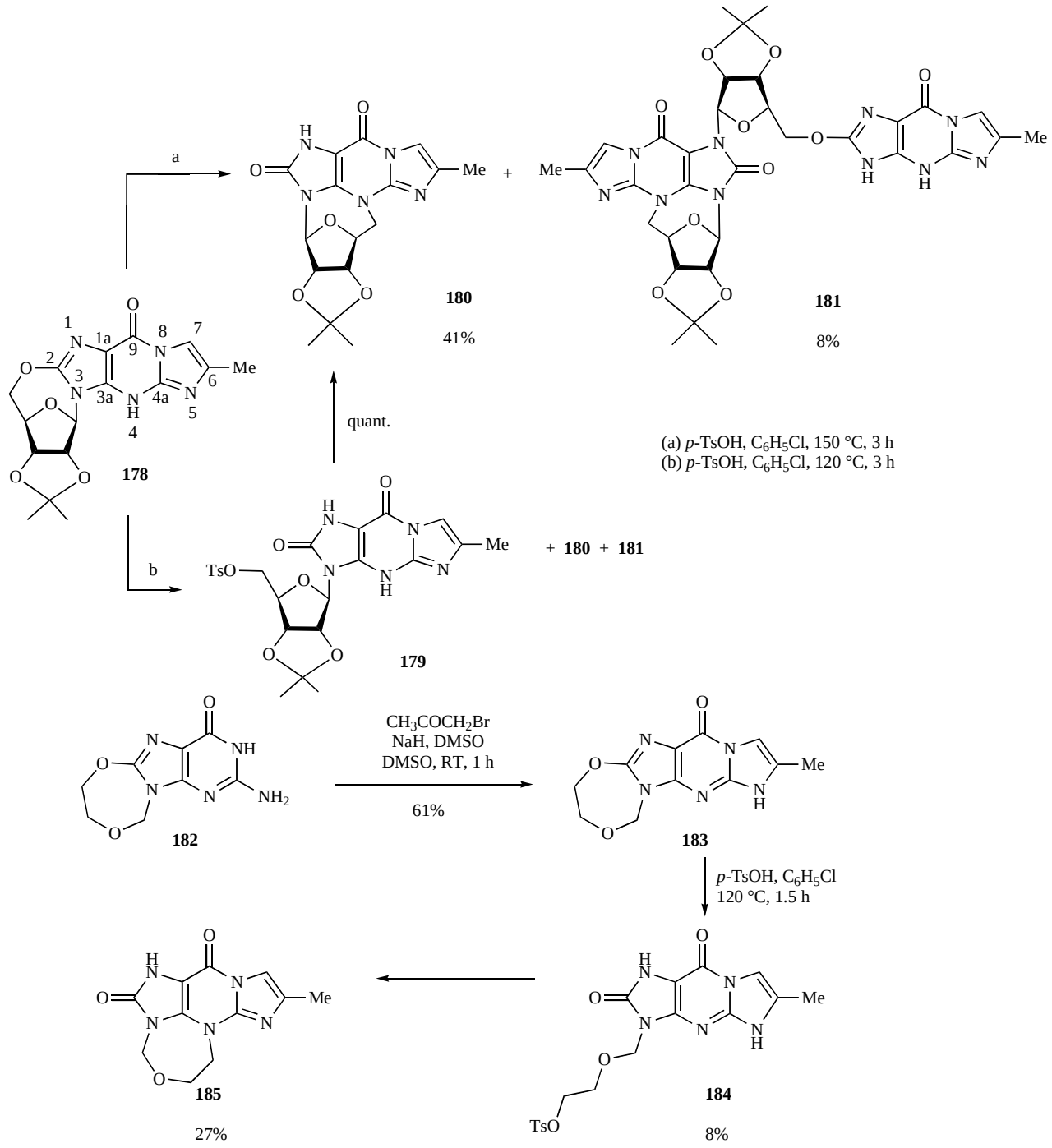
Scheme 30.

investigated and it was found that either equilibrium or irreversible change happened and the position of equilibrium was affected by electronic factor of the substituent rather than a steric one. The reaction is accelerated by electron-withdrawing substituents.

Baranowski *et al.* [56] have reported that substitution of wyosine 2',3',5'-triacetate at position C-2 with electronegative groups MeO and BnO results in a noticeable electron distribution disturbance in the ''extra'' imidazole ring and a significant increase in the *North* (C3'-*endo*-C2'-*exo*) conformer population of the sugar moiety. These substituents significantly decrease the aromatic character of the ''extra'' imidazole ring (^{13}C and ^{15}N NMR data).

Wyosine also served as a lead compound in the synthesis of tricyclic acyclovir (ACV) analogs. Golankiewicz *et al.* [57] have described the synthesis of new fluorine-substituted tricyclic analogs (**165a-g, 166a,b**) of acyclovir (ACV) (**8**) and ganciclovir (GCV) (**9**) as selective cytostatic agents Scheme (**29**). All mono-, and the majority of 6,7-disubstituted tricyclic analogs, were synthesized by alkylation-condensation reaction of ACV and GCV. The fluorine derivatives obtained deemed to be good canditates for non-invasive *in vivo* ^{19}F NMR identifying and monitoring of fluorine-containing metabolites. Of the 6,7-disubstituted compounds, 7-Me derivatives are highly potent antiherpetic agents.

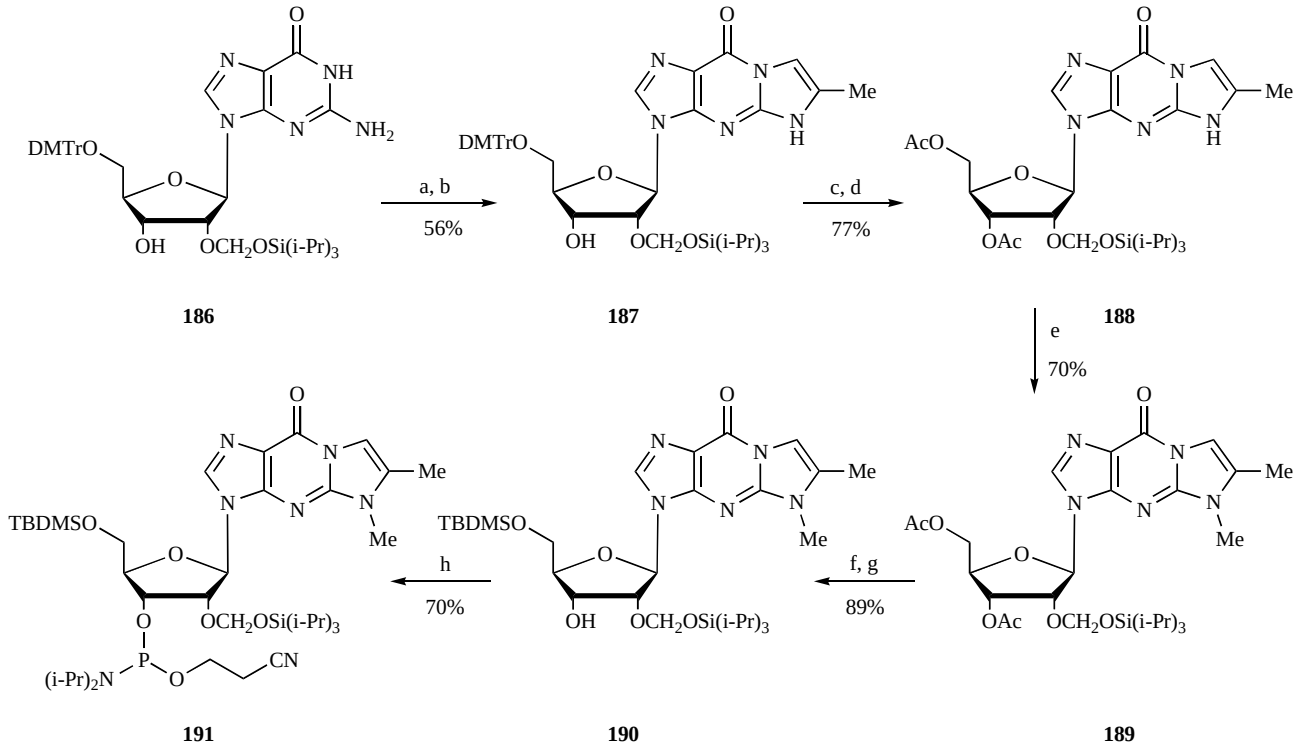
Golankiewicz *et al.* [58] have reported that attempted N-5-tritylation of ACV analogs (**167, 168**) led unexpectedly to C7-tritylation (**169, 170**). Substrates lacking 6-Me group yielded 5-tritylated (**171, 172**) and 5,7-ditritylated (**173, 174**) major products and a 7-tritylated (**175**) minor product Scheme (**30**). The regioselectivity of the reaction seems to be driven mainly by steric hindrance of the 6-Me substituent. It was found in further investigations [59,60] that an aryl or *tert*-butyl substituent in position 6 of ACV analogs partly directs aralkylation reactions into unusual positions to give N4-substituted or N-5,7-disubstituted or N-4,7-disubstituted



Scheme 31.

derivatives. In the case of alkylation the effect is limited to aryl substituent and position N-4. Replacement of acyclic moiety with a bulkier ribofuranosyl moiety at N-3 prevents N-4 substitution. Cleavage of the third ring of (**176**) employing NBS in aqueous buffer [60] to give 3-benzylacyclovir (**177**, dominant tautomer) (36% yield) constitute an access to 3-aralkyl-9-substituted guanines. Alternatively, 30% aq. H_2O_2 can also be used for the same purpose in a sluggish reaction [59].

1,N^2-Isopropenoguanosine derivatives (e.g. 4-demethylwyosine) in the presence of acidic catalysts undergo a reversible intermolecular 7 ↔ 9 transglycosylation via unstable 7,9-diglycosylpurine intermediates and guanine 5',8-cyclo-8-oxo-nucleosides are obtained in intramolecular transglycosylation. In this context Zandecki and Boryski [61] have investigated the rearrangement reactions of 1,N^2-isopropenoguanosine derivatives. 5',8-Cyclo-8-oxo-2',3'-*O*-isopropylidene-1,N^2-isopropenoguanosine (**178**) rearranged at 150 °C via intermediate (**179**) to 3,5'-cyclonucleoside (**180**) and bis-nucleoside (**181**) under acidic conditions Scheme (**31**). The mechanism of this rerrangement was studied and an unsubstituted H-(N-2) and 1, N^2-etheno tricyclic



186 **187** **188** **189** **190** **191**

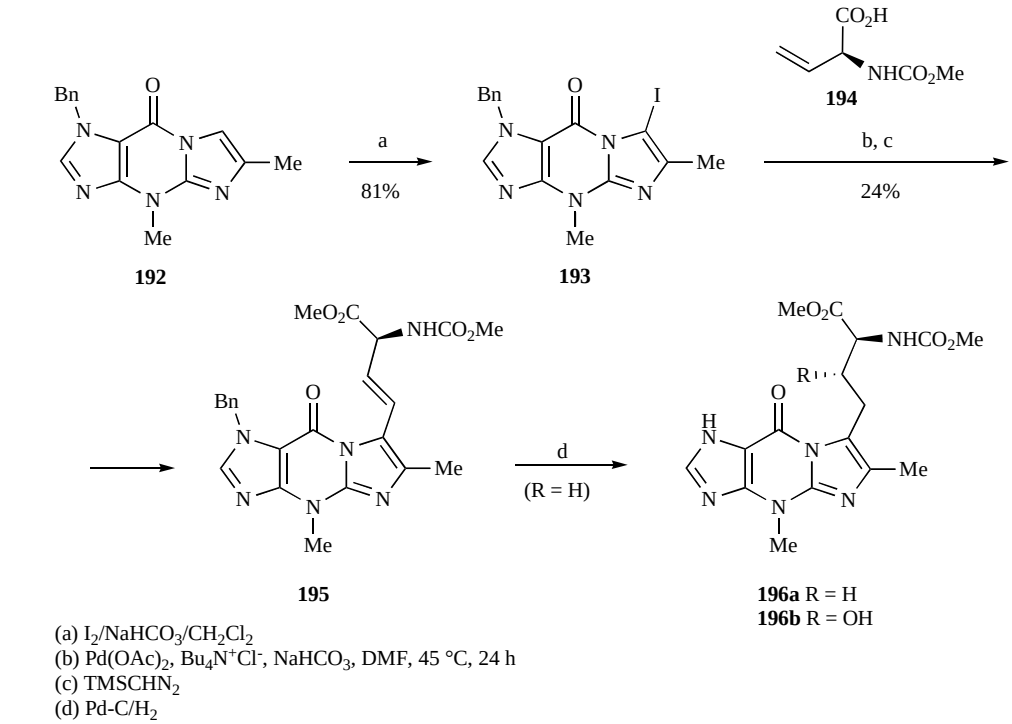
(a) bromoacetone/K_2CO_3/KI, -15 °C
(b) 4 Å molecular sieves in DCM, 20 °C
(c) $Cl_2CHCOOH$, CH_2Cl_2, 20 °C, 85%
(d) 1. Ac_2O, py, 2. MeOH/py, N-4 deacetylation with MeOH/H_2O/py 1:1:1
(e) N-4 methylation: CH_2I_2/Et_2Zn in Et_2O, 4 °C
(f) NH_3, MeOH, 20 °C
(g) TBDMS-Cl, imidazole, DMF, CH_2Cl_2, 4 °C
(h) 2-cyanoethyl diisopropylphosphoramidochloridite, $(i\text{-}Pr)_2NEt$

Scheme 32.

system were identified as necessary requirements. Dioxazepine (**183**) exhibited similar reactivity and its *p*-TsOH-catalyzed rearrangement at 120 °C gave 3,4-disubstituted compound (**185**) (27% yield) and 3-tosyl derivative (**184**) (8% yield).

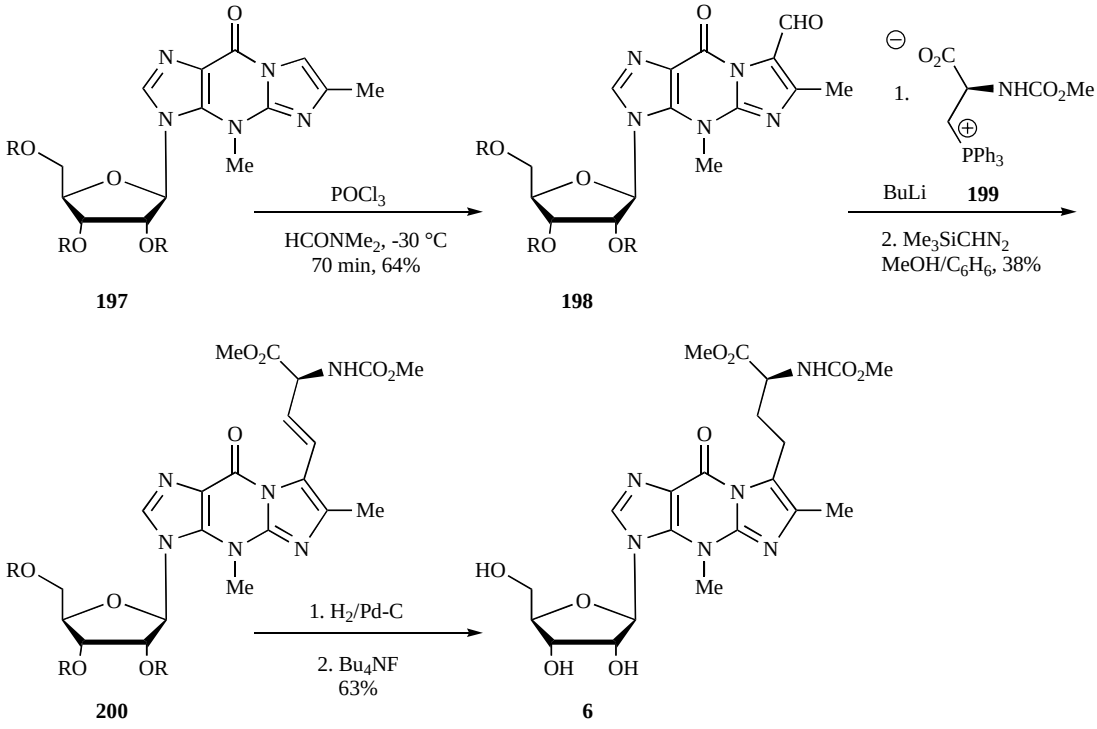
The replication of *Moloney murine leukaemia* virus relies on the formation of a stable homodimeric 'kissing complex' of a highly conservative GACG tetraloop interacting through only two C·G base pairs flanked of 5'-adjacent unpaired adenosines A9. To understand this phenomenon, a 2'-*O*-TOM protected phosphoramidite building block of wyosine (**191**) Scheme (**32**) and its incorporation into a model oligonucleotide sequence by means of automated RNA synthesis and enzymatic ligation were reported by Porcher [62]. Direct 2'-*O*-TOM protection of unprotected wyosine results in depurination therefore the monomer synthesis started from 2'-*O*-TOM-5'-*O*-DMTr-guanosine (**186**). 1-Alkylation employing bromoacetone followed by dehydration (4 Å molecular sieves at 20 °C) afforded 4-demethylwyosine analog (**187**) in 56% overall yield from (**186**). Methylation at N-4 required the exchange of protecting groups on the sugar moiety and eventually was achieved by CH_2I_2 and Et_2Zn to give (**189**) in 54% overall yield from (**187**). Protecting group manipulations and standard phosphitylation yielded the amidite monomer (**191**) in 62% yield from (**189**). The inherent lability of wyosine towards acid, $MeNH_2$ and iodine under the conditions of oligonucleotide synthesis allows its incorporation only at the 5' end and template-assisted enzymatic ligation by T4 DNA ligase was needed to secure its presence in the interior position. The 18mer RNA hairpin r(GGUGGGAG-imG-CGUCCCACC) was analyzed by NMR but no kissing interaction was observed (also confirmed by computational simulation). The investigation of additional analogs (1-methyl-Guo, N^2,N^2-dimethyl-Guo) revealed that $NH_2\cdots$N-7 interactions may play role in the structuration and the rigidification of the loop.

The extremely minute amounts of wybutosine available for structural elucidation and biological studies required its chemical synthesis. Itaya *et al.* [63] have improved the synthesis of the key intermediate (**195**) for the chiral syntheses of wybutine (**196a**) and β-hydroxywybutine analogs (**196b**) by Pd-catalyzed Heck vinylation of iodo derivative (**193**) Scheme (**33**). The same conditions have been used for the synthesis of nucleoside wybutosine (**6**) and under the mild reaction conditions neither glycoside bond cleavage, nor racemization of chiral vinylglycine derivative (**194**) was observed but the final product was contaminated to some extent with its diastereoisomer and the yield of coupling step was



(a) $I_2/NaHCO_3/CH_2Cl_2$
(b) $Pd(OAc)_2$, $Bu_4N^+Cl^-$, $NaHCO_3$, DMF, 45 °C, 24 h
(c) $TMSCHN_2$
(d) Pd-C/H_2

Scheme 33.

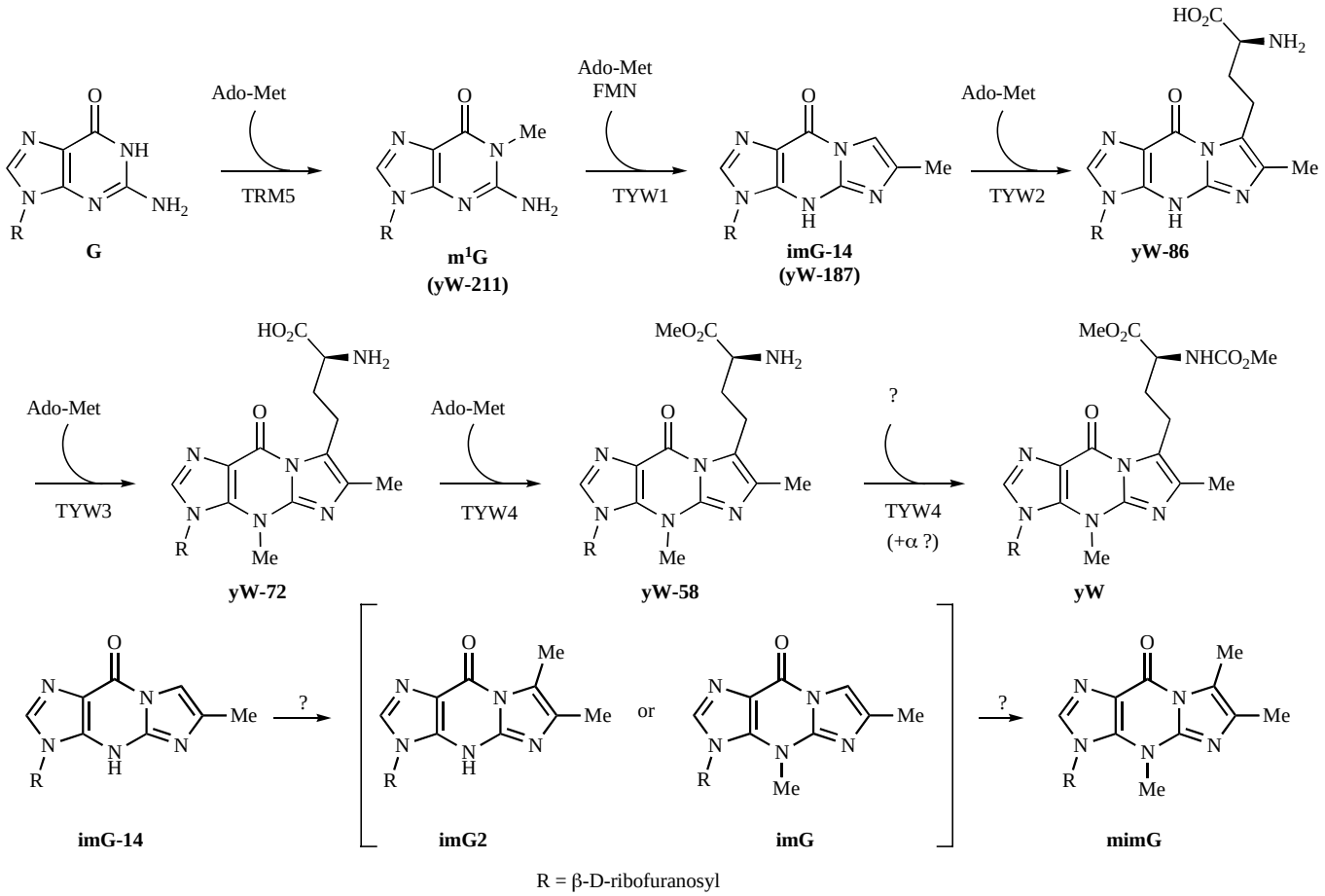


R = TBDMS

Scheme 34.

relatively low (19%). Earlier synthesis of the key intermediate in a Wittig reaction from 1-benzyl-7-formylwye gave only 5% yield. The extreme acid lability of wyebutosine (**6**) is demonstrated by rapid hydrolysis of the glycosyl bond in 0.1 M HCl, 25 °C (half life 88 s). The Heck reaction, as the source of diastereomer contamination in the above synthesis was identified, during which racemization took place in the amino acid side chain [64]. Essentially the same procedure was used later to synthesize β-hydroxywyebutosine, isolated from rat liver $tRNA^{Phe}$ [65]. The β-hydroxyl group was introduced by vicinal diol formation and triphosgene-catalyzed elimination followed by hydrogenolysis (yields not reported).

Itaya *et al*. [66] have isolated wyebutosine (**6**) from unfractionated tRNA of baker's yeast on a scale of 80 μg. The synthesis of wyebutosine was further refined Scheme (**34**). Low-temperature Vilsmeier-Haack reaction of persilylated wyosine (**197**) afforded the 7-carboxaldehyde deriva-



Scheme 35.

tive (**198**) in 64% yield with 26% recovery of (**197**). Perbenzylated or peracetylated wyosine failed to afford the desired alkene. Wittig reaction of inner phosphonium salt (**199**) and subsequent methylation gave the alkene intermediate (**200**) in 38% yield. Standard hydrogenation and removal of silyl protecting groups gave rise to wyebutosine (**6**) in 63% yield from (**200**). The epimer contamination was determined to be 1% (HPLC).

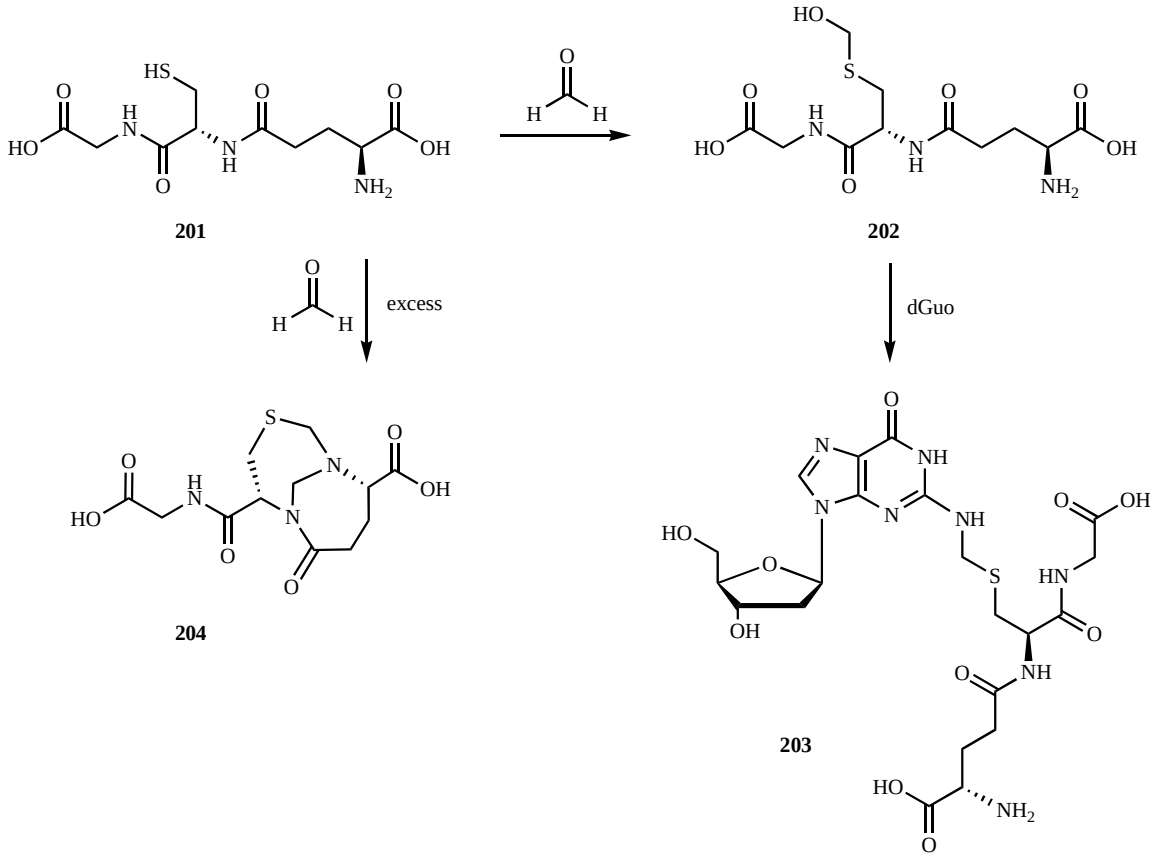
The biosyntheses of wye nucleosides start from guanosine, the transformations leading to wyebutosine are better characterized than those yielding wyosine [67]. In eukaryotes, wyebutosine is synthesized in five or six steps Scheme (**35**) [67-69]. Some of the enzymes responsible for the individual steps have been characterized recently (TYW1 [67-70], TYW2 [69], TYW3 [69,71], TYW4 [69]).

4. N^2-ALKYLGUANINES

The N-2 atom of guanine is susceptible to modification by various carcinogens and, along with additional reactions at N-1 (*cf. section 3.1*), this can seriously impair the biological functions of nucleic acids, most notably base pair formation and transcription fidelity.

Oligonucleotides with increasing bulk at position N-2 were analyzed for fidelity and catalytic efficiency by the replicative DNA polymerases RT and T7$^-$ by Choi and Guengerich [18]. N^2-Alkyl adducts were synthesized by two methods, either by reductive alkylation (RCHO/$NaBH_3CN$) of dGuo to yield N^2-Me-dGuo (m^2dG) and N^2-benzyl-dGuo (bn^2dG) or via replacement of fluorine in 2-fluoro-2'-deoxyinosine by amines to furnish N^2-ethyl-dGuo (e^2dG) and N^2-isobutyl-dGuo (ibu^2dG) (yields were higher in the second method, cf. [16,17]). 24- and 36-mer DNA oligomers were prepared from the phosphoramidites of dGuo N^2-alkyl adducts. It was found that RT and T7$^-$ effectively bypassed m^2dG and readily extended primers, but were strongly blocked by e^2dG, ibu^2dG, bn^2dG, N^2-(9-anthracenylmethyl)-dG. Single nucleotide incorporation by RT and T7$^-$ showed a decrease of 10^3 in k_{cat}/K_m for dCTP incorporation opposite m^2dG and a further large decrease opposite e^2dG (preference for dATP). Misincorporation frequency was increased 10^2-10^3-fold by Me group and another 10^3-fold by Et. Thus, even a Me group can cause a profound interfering effect on the fidelity and catalytic efficiency, Et or larger groups causes preferential misincorporation and strong blockage of replicative polymerases.

Fishbein *et al.* [72] have investigated how efficiently e^2dG blocks polymerization by mammalian DNA polymerases α and η. According to their results DNA polymerase η efficiently and accurately bypasses e^2dG. Insertion of Cyt opposite e^2dG by DNA polymerase α is 10^4-fold less efficient than opposite dGuo, by DNA polymerase η 370-fold more efficient than by DNA polymerase α and by DNA polymerase η with nearly the same level of accuracy as opposite dGuo thus minimizing the mutagenic potential of this lesion. Extension from e^2dG : dC is 10^3-fold less efficient



201 **202** **204** **203**

Scheme 36.

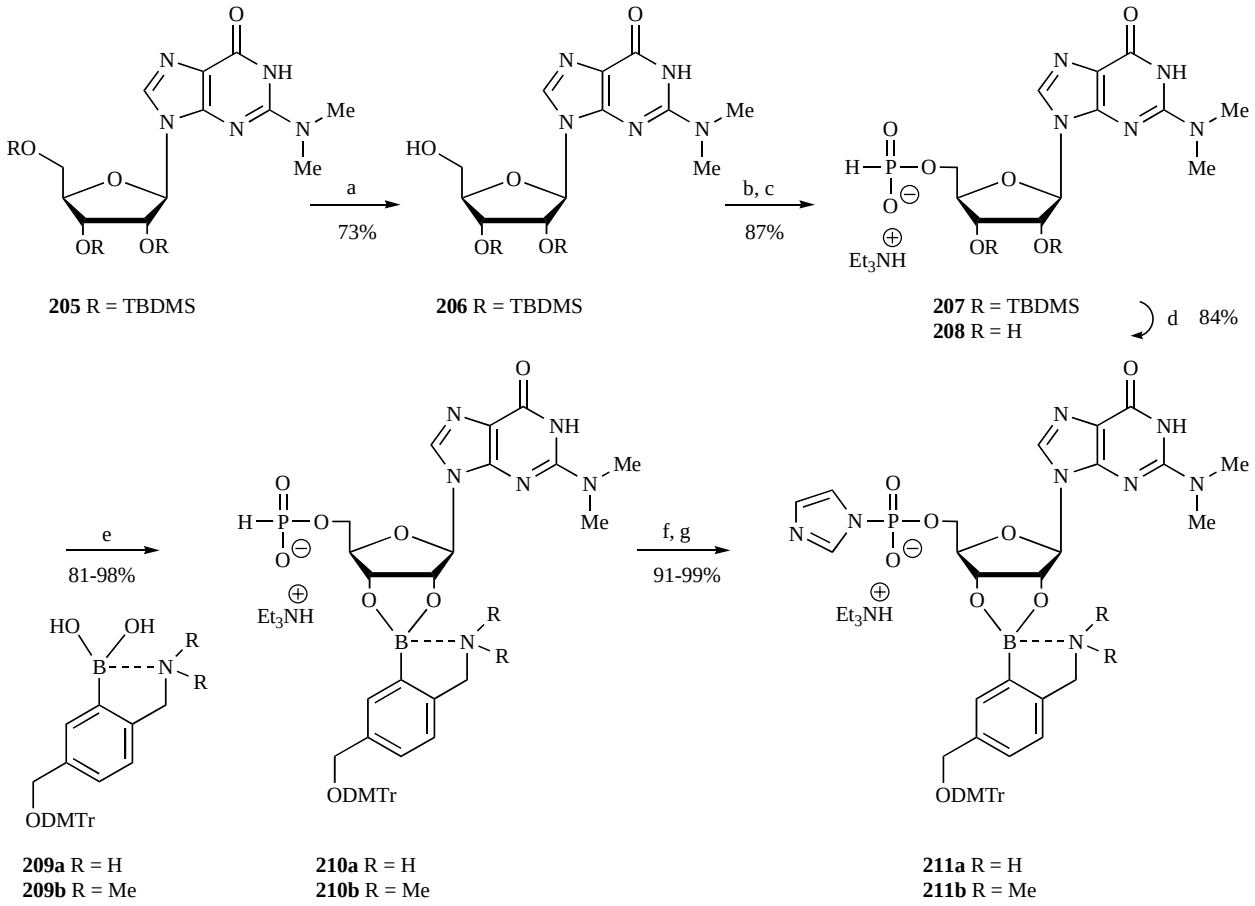
than from dGuo : dC by DNA polymerase α,by DNA polymerase η 3-fold more efficient than DNA polymerase α.

Isopropyl diazonium ion, derived from a number of carcinogenic tobacco specific nitrosamines, decomposes to a *sec*-carbocation that readily reacts with the exocyclic amino groups of purines. Fishbein *et al.* [73] have aimed at unveiling the effect of this lesion on DNA polymerases α, η and ι. They have found dramatic differences, namely N^2-isopropyl-dGuo represents a powerful block to DNA synthesis catalyzed by DNA polymerase α, with nearly undetectable levels of bypass synthesis, but it is efficiently and accurately bypassed by the TLS (translesion synthesis) DNA polymerases η and ι.

Quirk and Seley [74] have studied the substrate discrimination by the human GTP fucose pyrophosphorylase from nonsubstrates. They found that discrimination depends primarily by sensing a potential H-bonding face exerted by the carbonyl oxygen at C-6, the N-1 ring nitrogen and the exocyclic amino group of guanine. Of three base determinants (O^6-methylguanine, 1-methylguanine, N^2-methylguanine) the primary determinant seems to be the exocyclic amino group (m^2G exhibits a discrimination factor of ca. 50,000).

Formaldehyde is an essential metabolic intermediate in human cells and can also enter into the body through environmental exposures. It is also a human and animal carcinogen by forming nucleoside adducts *in vitro* at N^6 (2'-deoxyadenosine), N^2 (2'-deoxyguanosine), and N^4 (2'-deoxycytidine). This can be partially attributed to the rapid metabolism of formaldehyde by glutathione (GSH)-dependent enzyme systems. Among the intermediates in the pathway of formaldehyde detoxication, *S*-hydroxymethyl-glutathione (**202**) is a reactive species and has the potential to further conjugate with DNA bases. Swenberg *et al.* [75] have recently demonstrated the formation of an exogenous formaldehyde-induced DNA adduct *S*-[1-(N^2-deoxyguanosinyl) methyl]glutathione (**203**) between glutathione (**201**) and dGuo in the presence of formaldehyde Scheme (**36**). The structure of (**203**) was elucidated by ESI-MS/MS and 2D NMR measurements. The *in vivo* presence of this adduct was demonstrated by incubating DNA with GSH in the presence of formaldehyde in a dose-dependent manner. Excess formaldehyde (up to 50 mM) was consumed in the form of bis-adduct (**204**).

N^2,N^2-Dimethylguanosine- ($m_2^{2,2}G$) and N^2,N^2,7-trimethylguanosine-cap ($m_3^{2,2,7}G$) structures play an important role in the transport of these capped RNAs between the cytoplasm and the nucleus (*cf. section 6.2*). To improve the solubility and colorimetric detectability of capping reagents in organic solvents lipophilic 2-aminomethyl- and 2-dimethylaminomethyl-5-[(4,4'-dimethoxytrityl)oxy]methyl] phenylboranylidene groups were used as the protecting group for the 2',3'-*cis*-diol function of $m_2^{2,2}G$ in the solid phase synthesis (SPS) of $m_2^{2,2}G$-capped RNAs by Sekine *et al.* Scheme (**37**) [76]. This protecting group was stabilized by coordination of amino group to the boron atom. The synthesis of 2',3'-*O*-boronated N^2,N^2-dimethylguanosine capping units (**211a,b**) was accomplished through the key intermediate (**205**) obtained by reductive methylation of 2',3',5'-tri-*O*-(*tert*-butyldimethylsilyl)guano-sine with paraformaldehyde and $NaBH_3CN$ in the presence of acetic acid.



205 R = TBDMS
206 R = TBDMS
207 R = TBDMS
208 R = H
209a R = H
209b R = Me
210a R = H
210b R = Me
211a R = H
211b R = Me

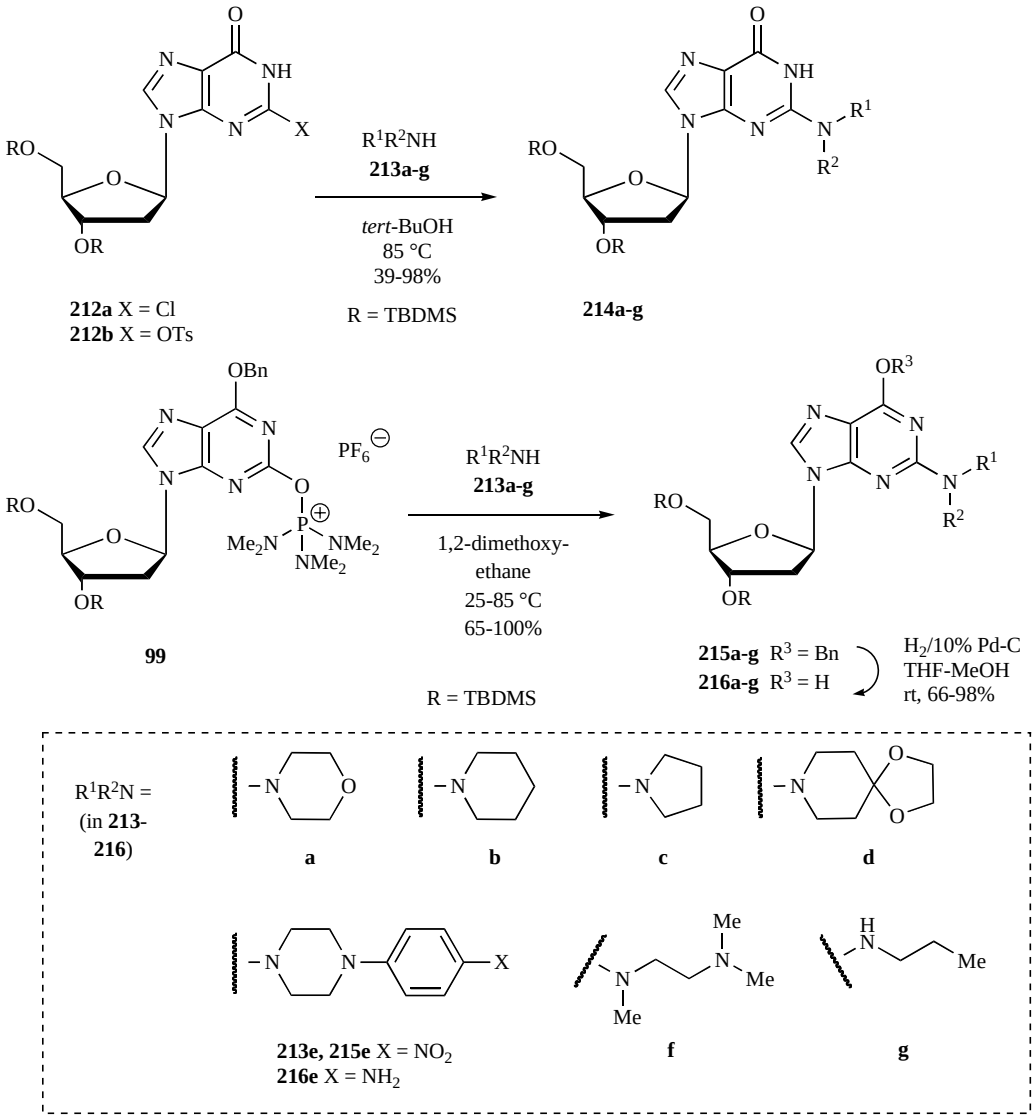
(a) AcOH-THF (3:1:1, v/v/v), 80 °C, 28 h;
(b) $(PhO)_2P(O)H$ (7 equiv.), pyridine, rt, 4 h;
(c) Et_3N-H_2O (1:1, v/v), rt, 20 min;
(d) 80% formic acid, rt, 46 h;
(e) **209a** or **209b** (1.2 equiv.), dioxane, 100 °C, 1 h;
(f) TMS-Im (4.0 equiv.), MeCN-CCl_4 (1:1, v/v), rt, 30 min;
(g) MeOH, rt, 10 min

Scheme 37.

In a synthesis employing (**211b**) the major product was $m_2^{2,2}G^{5'}$ppT (50% based on HPLC and colorimetric assay of $DMTr^+$ cation). Overphosphorylation to give branched phosphate esters owing to excess of reagents required for solid phase synthesis of oligonucleotides should be kept in mind.

Competitive inhibitors of herpes simplex virus thymidine kinases (HSV TK) prevent viral reactivation *in vitro* and *in vivo* and expression of viral DNA in the infected nerve ganglia is reduced. In an effort to synthesize new candidate inhibitors starting from the lead compound N^2-phenyl-9-(4-hydroxy-*n*-butyl)-guanine (HBPG) a series of N^2-phenylguanines were prepared by Wright *et al.* [77]. Of the 19 derivatives obtained by standard methods analogs containing tetra- or decahydroquinoline or 2-phenylpiperidine moieties at the terminus of *n*-butyl side-chain were the most potent. The mechanism of inhibition was studied with the decahydroquinoline derivative. N^2-Methylation decreased the antiviral activity of N^2-phenylguanines. However, *in vivo* studies did not show activity in relevant mouse infection models, HBPG was uniquely active. The results suggest that TK inhibition alone is not responsible for the activities.

The synthesis of N^2,N^2-disubstituted 2'-deoxyguanosines, if the two aforementioned substituents are part of a ring, can be best accomplished using a nucleophilic displacement reaction of 2'-deoxyinosine derivatives with a leaving group in the purine ring at position 2. The method of Lakshman *et al.* Scheme (**38**) [78] relied on a silylated O^6-allyl-2'-deoxyguanosine that was subjected to diazotization-chlorination to furnish (**212a**) after considerable experimentation (50% overall yield). The 2-tosyloxy derivative (**212b**) was obtained from a silylated O^6-benzyl-2'-deoxyguanosine by diazotization and subsequent tosylation (31% overall yield). The nucleophilic displacement of leaving group by primary and secondary amines (**213a-g**) proceeded in good yields with the 2-chloro- and in moderate yields with the 2-tosyloxy-2'-deoxyguanosine derivative to afford compounds (**214a-g**). Alternatively, the reaction of O^6-allyl-2-chloro-2'-deoxyguanosine derivative with secondary amines in the presence of $Pd_2(dba)_3$/(±)BINAP, for simultaneous deallylation, compared favorably with the previous versions.

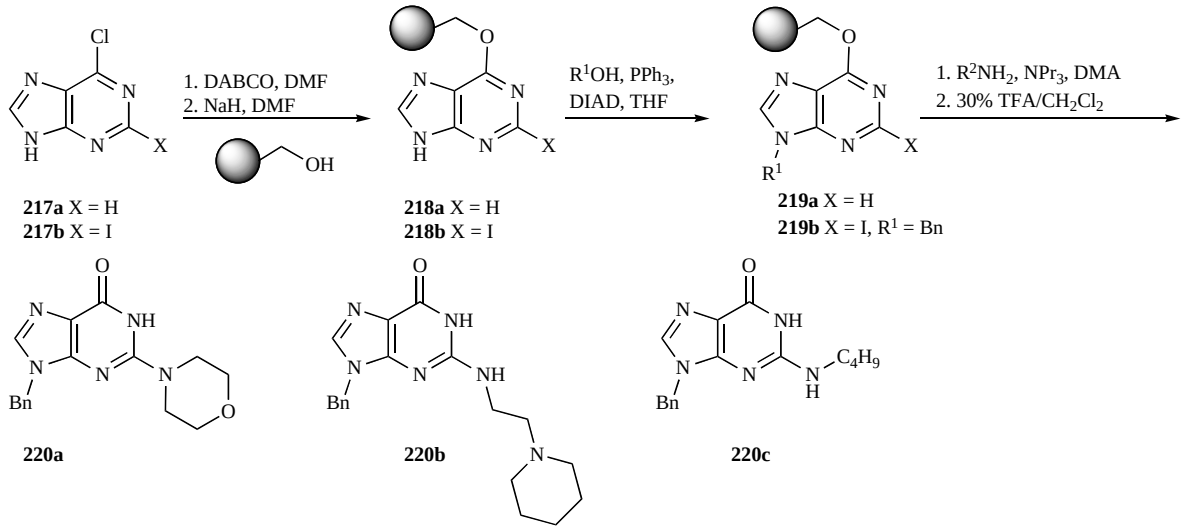


Scheme 38.

The nucleoside phosphonium salt (**99**) [*cf. section 3.1*, Scheme (**19**) [35] also serves as an excellent starting material for displacement reactions with the above amines (**213a-g**) to furnish derivatives (**215a-g**) in 65-100% yield that could be catalytically debenzylated to afford N^2(,N^2)-(di)substituted nucleosides (**216a-g**) in 66-98% yield Scheme (**38**). In the case of nitro derivative (**215e**) hydrogenation also reduced the nitro group to the corresponding amino derivative (**216e**).

A similar approach was used by Tan *et al*. [79] for the traceless solid phase synthesis of 6-oxopurines on Wang resin to obtain inhibitors of multidrug resistance protein 4 (MRP4/ABCC4) Scheme (**39**). The purine ring was attached to the resin through an O^6-linkage. Alkylation of resin (**218**) with *n*-butanol or benzyl alcohol under Mitsunobu conditions afforded resin (**219**). Replacement of 2-iodo substituents with amines and acidic cleavage from the resin gave derivatives (**220a-c**) none of which were effective against efflux of Bimane-glutathione conjugate.

Highly ordered structures from guanosine-rich DNA and RNA oligomers often manifest in the form of quadruplexes/quartets. Base-modifications to date occurred almost exclusively at C-8 but Wu *et al*. [80] have reported that the lipophilic analog N^2-(4-*n*-butylphenyl)-2',3',5'-*O*-triacetylguanosine (**221**) in the presence of either K^+ or Na^+, self-assembles into a D_4-symmetric octamer consisting of two stacking all-*syn* G-quartets in a tail-to-tail (or head-to-head) fashion and a central ion. The octamer formation was evidenced by ESI-MS, ^{1}H and ^{23}Na NMR studies. ^{1}H NMR and molecular modeling also suggest a possible π-π stacking between the phenyl rings from the two different G-quartets. Lack of N^2-substituent results in the formation of polymeric columnar aggregates (2D ^{1}H NOESY spectra). The ESI-MS spectra for 2',3',5'-*O*-triacetylguanosine/K^+ aggregate show clearly the presence of octamer, dodecamer, and hexadecamer. Thus, the N^2-alkylation changes the G-quadruplex structure from an extended polymer to a discrete octamer. Kaucher and Davis [81] have prepared an 8-vinyl-N^2-pent-4-enylguanosine derivative (**224**) by reductive alkylation and Stille coupling by starting from protected 8-bromoguanosine (**222**) Scheme (**40**). Barium picrate mediated the formation



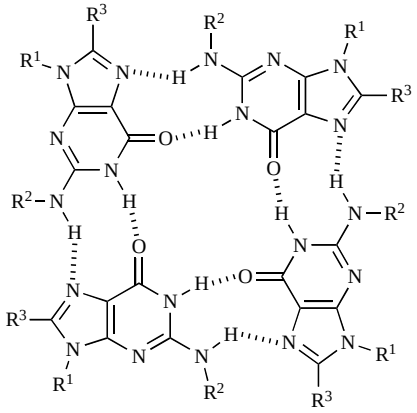
217a X = H
217b X = I

218a X = H
218b X = I

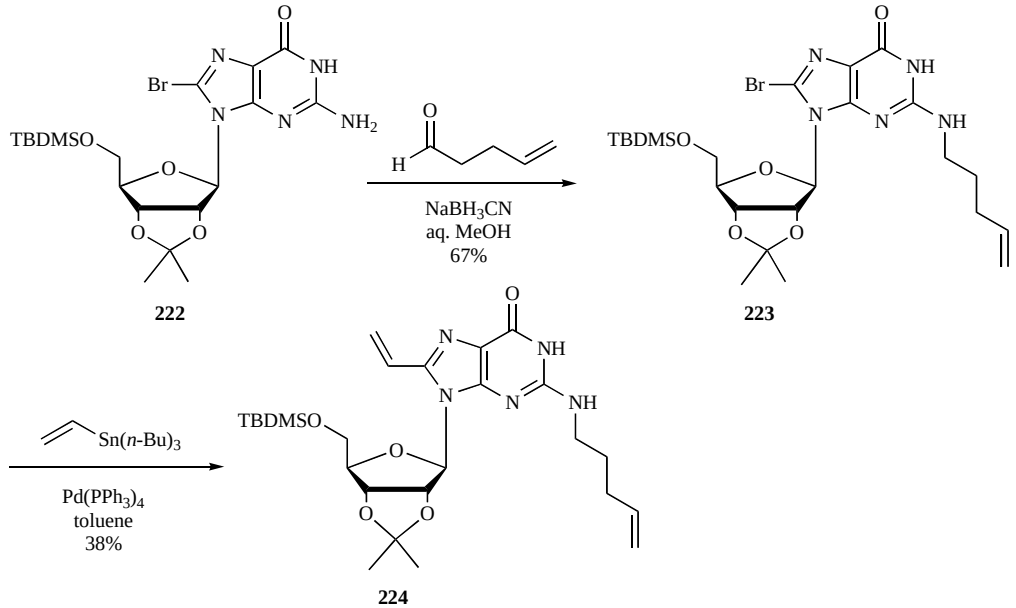
219a X = H
219b X = I, R^1 = Bn

220a **220b** **220c**

Scheme 39.



221 R^1 = 2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl; R^2 = 4-*n*-butylphenyl; R^3 = H
224 R^1 = 2',3',-*O*-isopropylidene-5'-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl; R^2 = pent-4-enyl; R^3 = vinyl

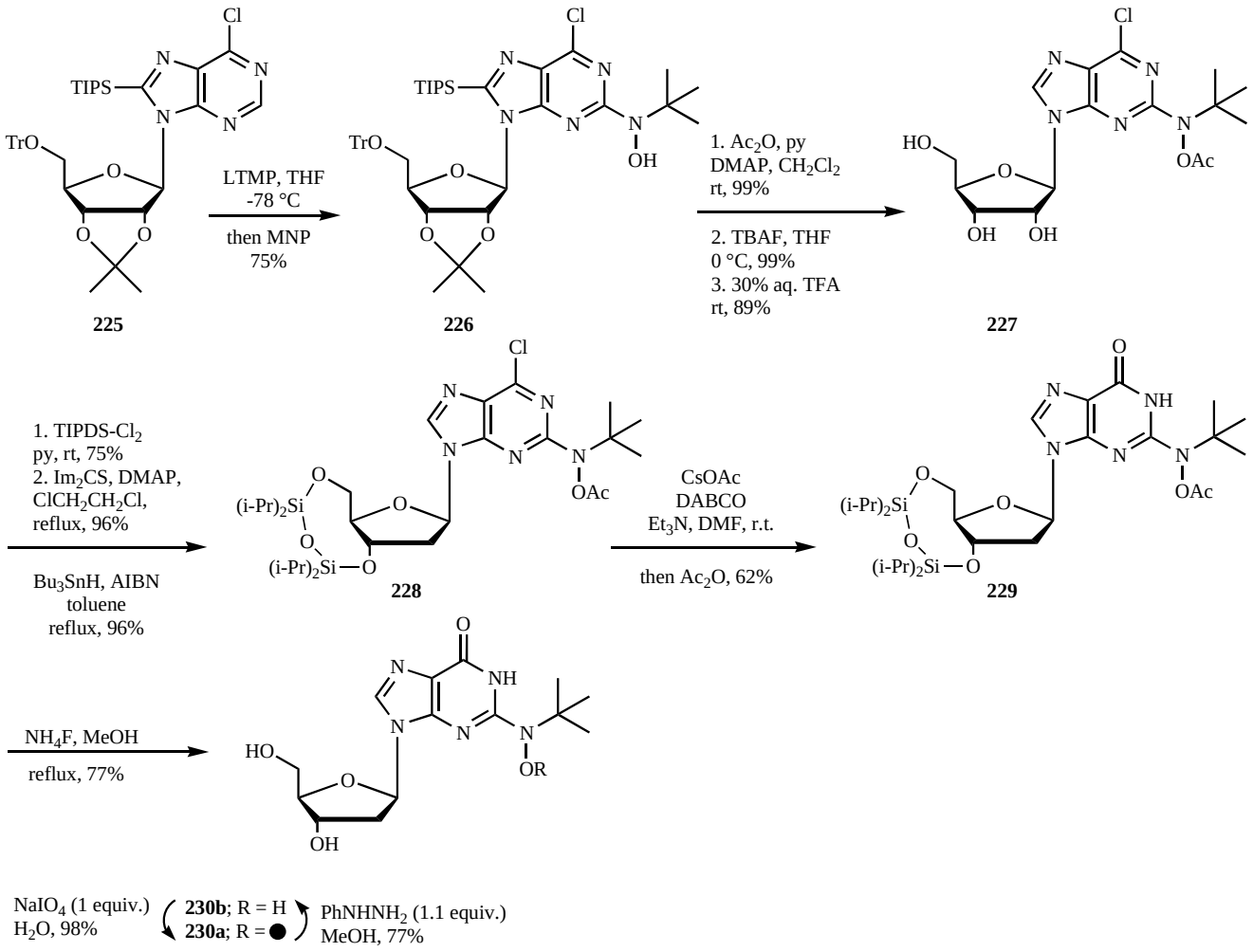


222 **223** **224**

Scheme 40.

of an octamer with G-quartet structure (NMR, ESI-MS, UV data). Attempted covalent cross-linking via alkene metathesis of the complex failed.

The quantification of DNA damage and repair requires specifically labelled alkyl-nucleotides, -nucleosides or -bases. Rajewsky *et al*. [82] have prepared N^2-(5-

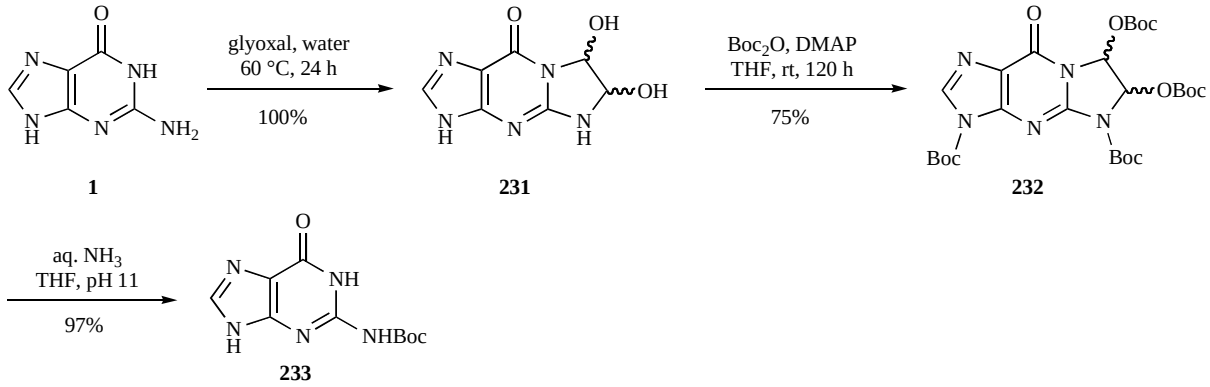


Scheme 41.

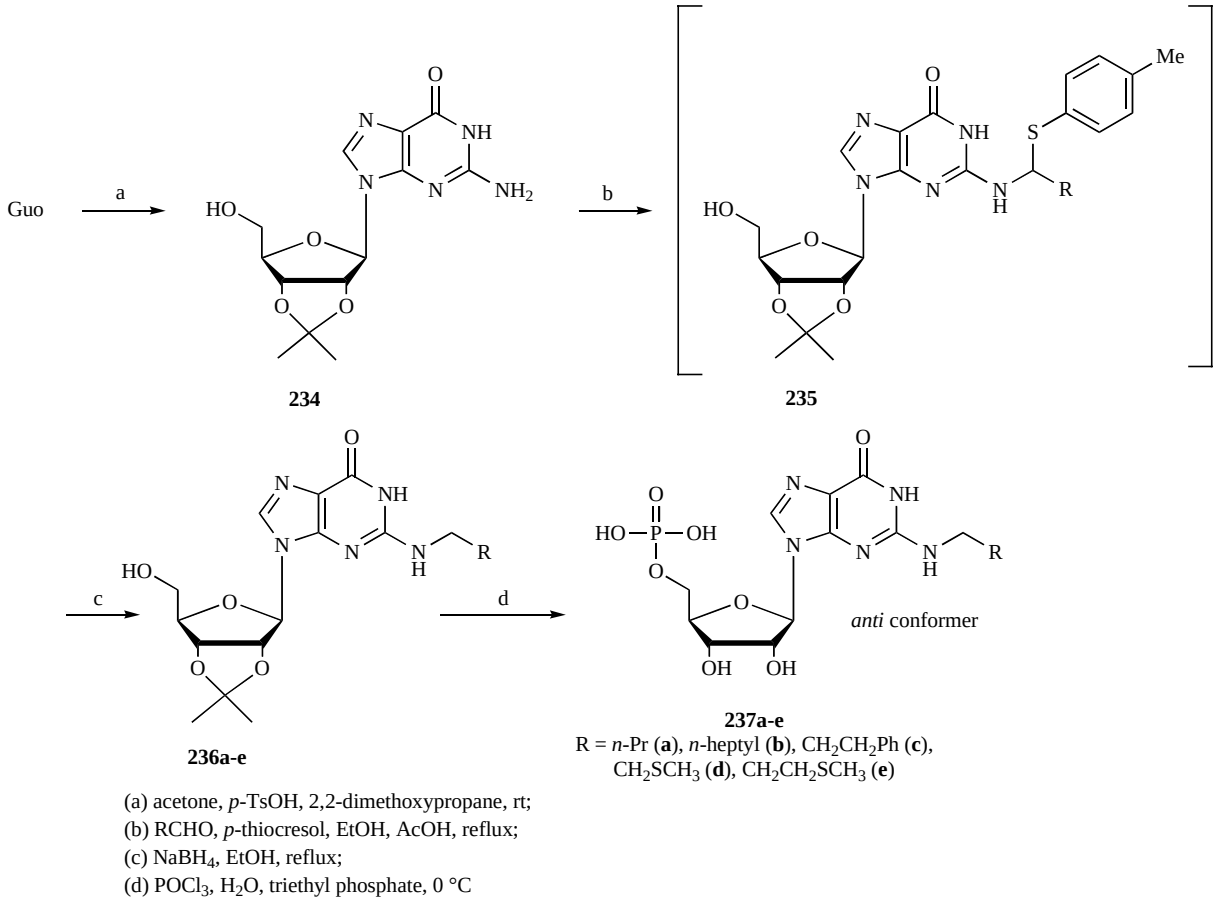
aminopentyl)-9-ethylguanine along with other 9-alkyl- or 9-glycosylguanine derivatives conjugated to [^{3}H]-labelled phenylalanine. 7-Ethyl-[8,5'-^{3}H]guanosine-3':5'-cyclic monophosphate was also obtained. The radiotracers prepared were successfully employed in competitive radioimmunoassays for the quantification of defined DNA alkylation products in DNA repair analyses. Suemune *et al*. [83,84] have synthesized spin-labelled nucleosides, e.g. (**230a**), with N^2-*tert*-butylaminoxyl label Scheme (**41**). The compounds were obtained by lithiation of 8-TIPS-6-chloropurine nucleoside (**225**) at the 2-position and the following reaction with 2-methyl-2-nitrosopropane (MNP). Compound (**226**) was acetylated, desilylated, the acid-sensitive protecting groups were removed (**227**), the 3',5'-hydroxyls were protected and after radical deoxygenation compound (**228**) was obtained. Exchange of 6-chloro substituent to hydroxyl and desilylation gave precursor (**230b**). The aminoxyl group in (**230a**) was generated by $NaIO_4$ oxidation. This reporter group was used as a pH probe thanks to the sensitivity of aminoxyl nitrogen hyperfine splitting constants (a_N values) due to dipolar effects between the β-substituent and the aminoxyl function.

The protection of the exocyclic amine groups of nucleobases by acid-labile groups that can be removed under acidic or neutral conditions and are compatible with Fmoc-mediated solid-phase synthesis protocols of peptide nucleic acids has been investigated by Dey and Garner [85]. They proposed the N^2-Boc protecting group that can be installed by glyoxal condensation of guanine, forming a cyclic 1,N^2-adduct (**231**), followed by the formation of a highly lipophilic tetra-Boc derivative (**232**), isolated in 75% yield Scheme (**42**). N^2-Boc-guanine (**233**) was obtained in almost quantitative yield by exposing (**232**) to aq. ammonia in THF. The removal of N^2-pivaloyl group in 7-methyl-N^2-pivaloylguanine can be accomplished to afford 7-methylguanine in 63% yield using catalytic amount of $Fe(NO_3)_3 \cdot 9\ H_2O$ in MeOH [86].

Monosodium glutamate (MSG) is a known flavour enhancer and considered to be the prototypical umami substance. Nucleoside 5'-monophosphates increase the umami taste exponentially therefore combinations of MSG, IMP and GMP sodium salts have extensively been used in the food industry. Manitto *et al*. [87] have synthesized a series of N^2-alkylguanosines in the modified Kernal-Reese procedure by condensing 2',3'-*O*-isopropylideneguanosine (**234**) with aldehydes in the presence of *p*-thiocresol Scheme (**43**). The in situ formed *S*,*N*-acetals (**235**) were reduced and the N^2-alkyl derivatives (**236a-e**) were phosphorylated to give GMP analogs (**237a-e**) (29-52% yield from guanosine). Guanosines



Scheme 42.



R = *n*-Pr (**a**), *n*-heptyl (**b**), CH_2CH_2Ph (**c**), CH_2SCH_3 (**d**), $CH_2CH_2SCH_3$ (**e**)

(a) acetone, *p*-TsOH, 2,2-dimethoxypropane, rt;
(b) RCHO, *p*-thiocresol, EtOH, AcOH, reflux;
(c) $NaBH_4$, EtOH, reflux;
(d) $POCl_3$, H_2O, triethyl phosphate, 0 °C

Scheme 43.

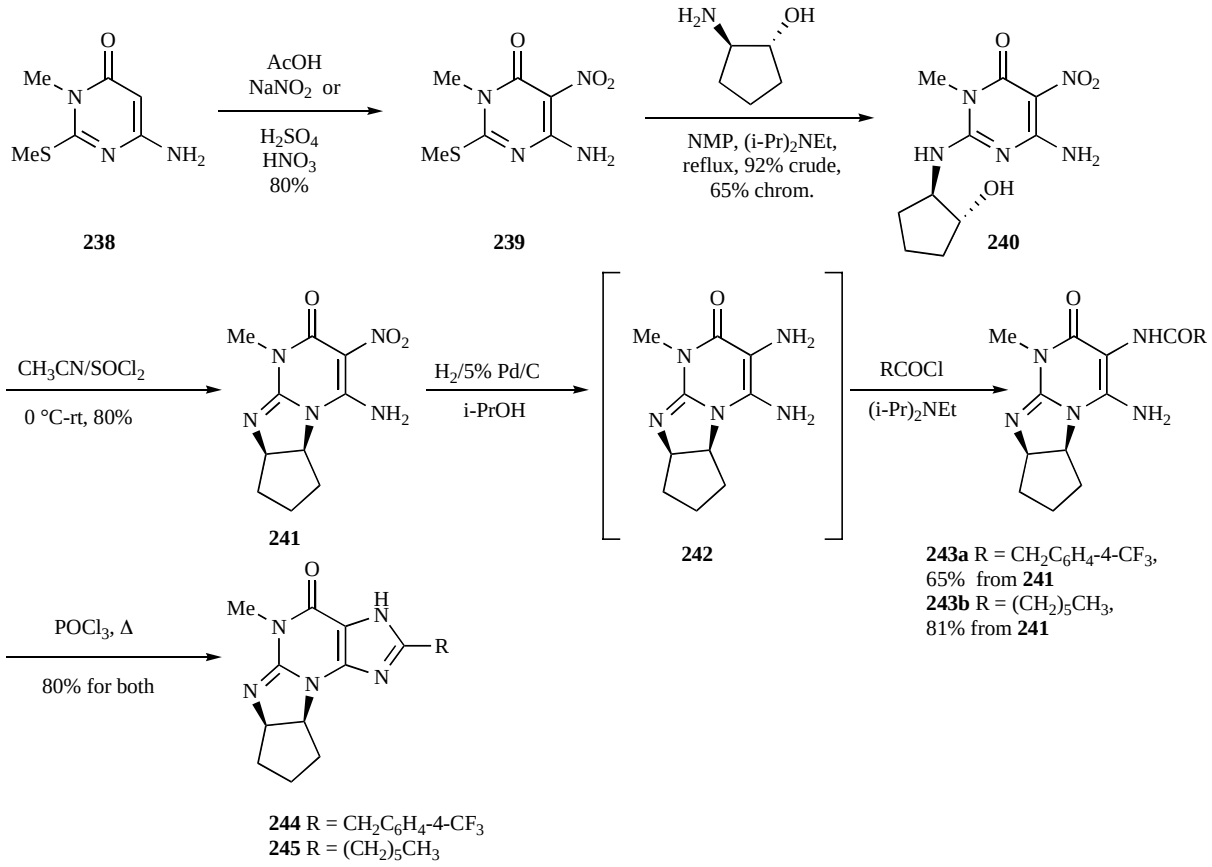
(**237a-e**) exerted synergistic taste effect with MSG (2.9- to 5.7-fold increase compared to IMP). The conformational analysis (MacroModel) of dianionic (**237a-e**) suggested for each compound two minima (*anti* and *syn*, relative to the sugar ring), the former being energetically favored. It is generally assumed that the nucleotides while interacting with the receptor protein adopt *anti* conformation.

5. 3-ALKYL- AND N^2,3-DIALKYLGUANINES

Direct alkylation of guanine at N-3 has been observed only as a minor process of mutagenesis and carcinogenesis [88]. The yield of 3-methylguanine is normally < 0.01% of 7-methylguanine in DNA from diffusible methylating agents because the N-3 site of guanine is the least accessible one in B-DNA. *In vivo*, 3-methylguanine is rapidly removed (half-life in DNA is 3.6 ± 0.3 h in *E. coli*, as compared to an *in vitro* half-life of 105 h in double-stranded DNA).

9-Substituted wye derivatives may undergo alkylation at N-4 (corresponding to N-3 of guanine ring) depending on the substitution pattern of wye ring and the 4-alkyl derivatives can be cleaved to furnish 3-alkylguanines (*cf. section 3.2*) [58-60].

3,5'-Cyclo-N^2-triphenylphosphoranylideneguanosine, containing an eight-membered ring, was obtained from the Mitsunobu reaction of guanosine in 65% yield [89]. A 3,2'-cycloguanosine with a five-membered ring was also ob-



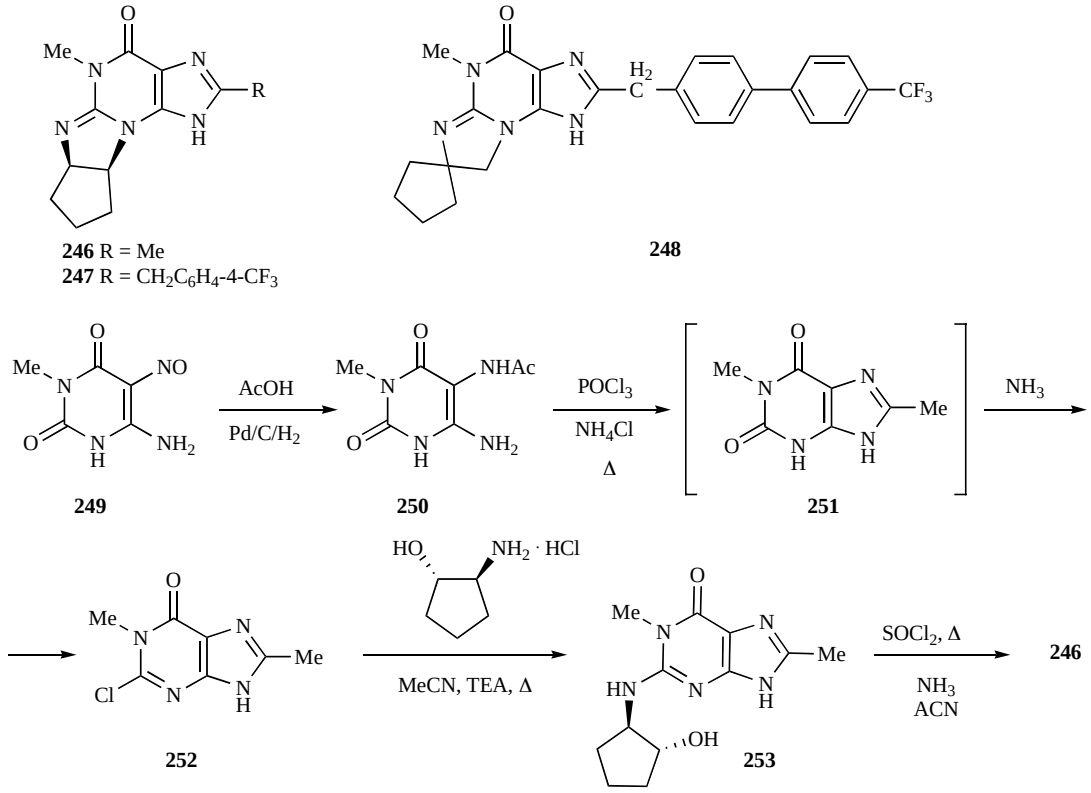
Scheme 44.

served as a byproduct in the DAST-mediated fluorination of a 3'-bromo-3'-deoxyxyloguanosine [90].

Cyclic N^2,3-dialkylguanines have been extensively investigated after the discovery of their inhibitory effect of phophodiesterase (PDE) enzymes. PDE5 inhibitors increase levels of cyclic guanylate cyclase (cGMP) in the nitric oxide (NO) pathway of penile erection, and have been developed for the treatment of male erectile dysfunction (ED). There are three marketed PDE5 inhibitors: sildenafil (Viagra), vardenafil (Levitra) and tadalafil (Cialis). In order to develop potent and selective PDE5 inhibitors that improve upon the isozyme selectivity profile over e.g. PDE6, Gala *et al*. have accomplished the synthesis of potent PDE inhibitory guanines Sch 59498 (**244**) and 51866 (**245**), Their attempted synthesis from 6-amino-3-methyluracil failed [91] therefore an alternative route was envisaged Scheme (**44**) [92]. 2-Thiomethylpyrimidine derivative (**238**), obtained from *S*-methylthiourea, was nitrated to give the 5-nitro compound (**239**) and then subjected to reaction with *trans*-2-aminocyclopentanol to furnish alcohol (**240**). The five-membered intramolecular cyclization of (**240**) to form the *cis*-bridged tricyclic compound (**241**) was very facile and afforded good yields (80%) of isolated (**241**). After hydrogenation compound (**242**) was allowed to react with appropriate acid chlorides in the presence of Hünig base to form (**243a**) (65 %) and (**243b**) (80%). Cyclization was accomplished in refluxing $POCl_3$ to afford PDE inhibitors (**244, 245**) in 80 % yield. The so-called 2-chloropurine route was developed by the same group and led to the kilogram scale synthesis of novel PDE inhibitors (**246-248**) Scheme (**45**), yields not reported] [93].

Thus, 5-nitrosouracil derivative (**249**) obtained from 6-amino-3-methyluracil was reduced to 5,6-diaminouracil (**250**) and subsequently was allowed to react with $POCl_3$ to obtain 2-chlorohypoxanthine derivative (**252**) without isolating the intermediate xanthine (**251**). Replacement of 2-chloro substituent with *trans*-2-aminocyclopentanol and $SOCl_2$-mediated ring closure yielded the target compound (**246**) via compound (**253**). Analogous transformations led to compounds (**247, 248**).

Boyle *et al*. [94] have performed further SAR studies by synthesizing novel cyclic N^2,3-dialkylguanines modified at C-7/N-5, and N-1, N-3, and C-2 positions of guanine analogs (**259a,b**) Scheme (**46**). The synthesis of C-7 and N-5 derivatives started from imidazole (**254**) that was allowed to react with alkyl isocyanates and the obtained xanthines (**255**) were chlorinated by $POCl_3$. Substitution of 2-chloro substituent in (**256**) with aminoalcohols, activation through mesylation and ring closure afforded tricyclic derivatives (**258**). The synthesis of N-1/N-3/C-2 derivatives was accomplished through halogenation (NBS, NCS or NIS) of (**260**) at C-2 followed by a subsequent Sonagashira coupling reaction. After BBr_3 treatment of (**261**), the desired product (**262**) was isolated along with its debenzylated derivative (**263**) and its N-3 regioisomer (**264**). A compound (**265**) with PDE5 inhibition and *in vivo* activity similar to sildenafil was discovered from this effort.



Scheme 45.

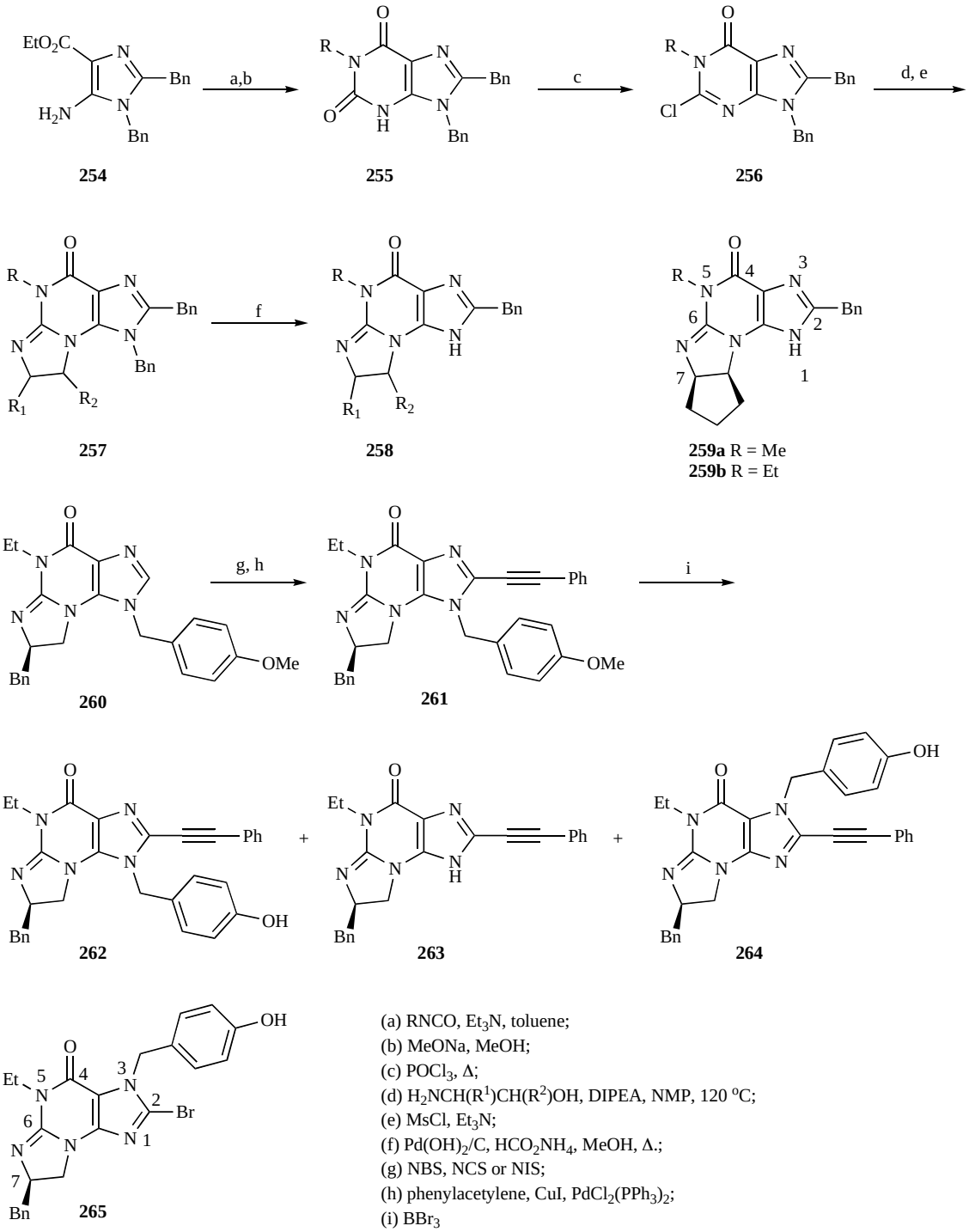
1,N^6-Ethenoadenine (ε-A), nucleos(t)ides have been previously applied as fluorescent probes in numerous biochemical systems, however, these ε-A analogs lack the H-bonding capability of adenine. To improve the fluorescence characteristics while preserving the H-bonding pattern required for molecular recognition, N^2,3-etheno-adenosine (N^2,3-ε-A, **272**) and N^2,3-etheno-guanosine (N^2,3-ε-G, **267**) have been synthesized and studied by Fischer *et al.* Scheme (**47**) [95]. Nucleobases treated with α-haloaldehydes at pH 4.5 afford N^2,3-etheno derivatives in poor yield while at an elevated pH, a nonfluorescent linear isomer, 1,N^2-ε-G was obtained from guanosine. Compound N^2,3-ε-G (**267**) was obtained in 86% yield from 2-amino-6-chloropurine nucleoside (**266**) at pH 4.5. This substance was also used in the preparation of N^2,3-etheno-adenosine (N^2,3-ε-A, **272**) but the best synthetic pathway for the preparation of (**272**) was a three-step synthesis starting from 6-thioguanosine (**268**). Thus, methylation of (**268**) afforded 6-thiomethyl derivative (**269**) the reaction of which with bromoacetaldehyde gave rise to N^2,3-etheno derivative (**271**) at pH 4.5 (64% yield) or 1,N^2-derivative (**270**) at pH 6.4 (47% yield). Ammonolysis of (**271**) furnished compound (**272**). N^2,3-ε-A (**272**) has superior fluorescent properties compared to those of adenosine, even at acidic pH. The wavelength of the emission maximum of N^2,3-ε-G does not change with solvent polarity. The quantum yield of N^2,3-ε-G increases with decreasing solvent polarity.

Similar results of cyclization have been obtained by Holý *et al*. [96]. They have allowed 2-amino-6-chloro-9-methylpurine (**274**) to react with chloroacetaldehyde at pH 6. The linear 1,N^2-ethenoguanine derivative(**275**) was obtained in 17% yield Scheme (**48**). Replacing the 6-chloro substituent with primary or secondary amines afforded 2,6-diaminopurines (**276a-d**) that gave rise to linear 1,N^2-etheno derivatives (**277a-d**) in 0-30% yield along with angular N^2,3-etheno derivatives (**278a-d**) in 0-56% yield at pH 6 using chloroacetaldehyde. With cyclopropylamino substituent the linear product (**277c**) was formed alone in 30% yield. Neither the linear nor the angular products exhibited any significant fluorescence. They were also devoid of cytostatic activity against several leukemia, human cervical carcinoma and human T lymphoblastoid cell lines.

6. 7-ALKYL- AND 7-ALKYL-9-GLYCOSYLGUANINES

In principle all nitrogen and oxygen atoms of guanine residues in DNA and RNA, except N-9, can be the subject of alkylation but these possibilities do not occur with the same frequency. Soft electrophiles (methyl halides, dimethyl sulfate, methyl methanesulfonate) are reacting in an S_N2 fashion to give mainly 7-alkyl derivatives. Hard electrophiles (*N*-methyl-*N*-nitrosourea and its ethyl homologue) are S_N1-like alkylating agents and give an increasing proportion of O-6 alkylated guanines. The overall frequency of alkylation in guanine is N-7 >> O-6 > N-3 [97]. Nucleobase alterations are generally removed by excision repair pathways that counteract the mutagenic effects of DNA lesions. In some cases, DNA damage is not repaired but instead bypassed by specialized DNA polymerases [98].

Thus, a significant portion of guanine alkylation cases are associated with potential mutagenesis and carcinogenesis although bifunctional DNA-DNA cross-linking alkylating agents can lead to antitumor drugs [99]. Methylation of gua-



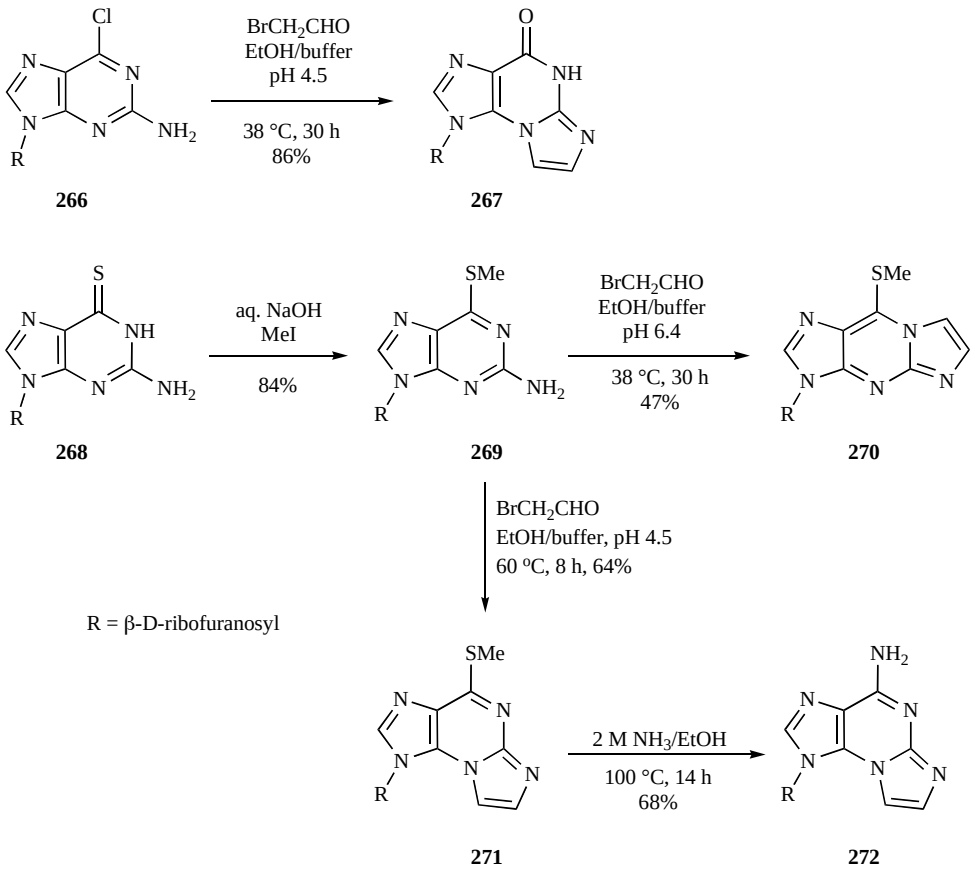
Scheme 46.

nine residues at N-7 in DNA also constitutes the basis of Maxam-Gilbert sequencing of oligodeoxynucleotides [97].

6.1. 7-Alkylguanines as Products of Mutagenesis and Carcinogenesis

The elevated level of 7-methyl-dGuo and 7-ethylguanine in smokers, compared to non-smokers, as an indicator of alkylation exposure damage has been recently confirmed [100,101].

1,2,3,4-Diepoxybutane stereoisomers (DEB) (**280**), suspected carcinogenic metabolites of 1,3-butadiene (BD) (**279**), are bifunctional electrofiles capable of forming DNA-DNA and DNA-protein cross links Scheme (**49**). The two-step process of alkylation in DNA at N-7 produces 7-(2'-hydroxy-3',4'-epoxybut-1'-yl)-guanine monoadducts (N7-HEBG) (**281**), which can then be hydrolyzed to 7-(2',3',4'-trihydroxybut-1'-yl)-guanine (N7-THBG) (**282**) or can alkylate neighboring nucleobases within the major groove of

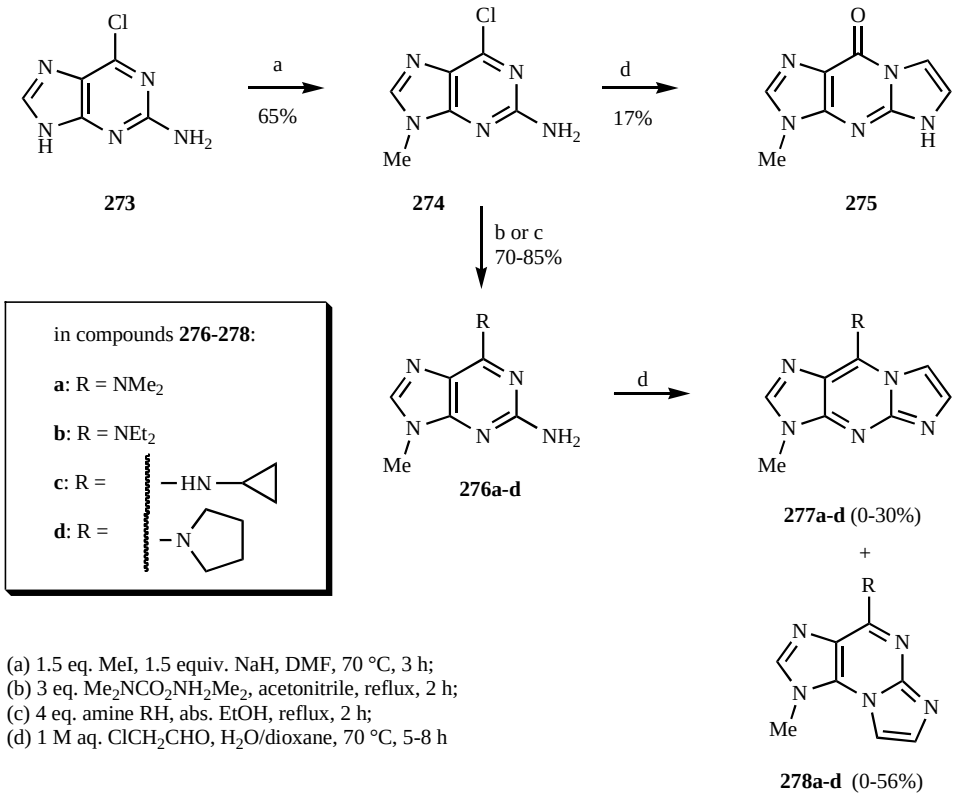


Scheme 47.

DNA to form bifunctional 1,4-bis-(guan-7-yl)-2,3-butanediol DNA adducts after hydrolysis. Interstrand and intrastrand DNA-DNA crosslinking by DEB stereoisomers was investigated by combination of HPLC-ESI$^+$-MS/MS-PAGE methods and stable isotope labeling of DNA (guanine ^{15}N-3, ^{13}C-1) by Tretyakova *et al*. [102,103]. These lesions are hydrolytically labile and can be selectively released from the DNA backbone as free bases upon heating at neutral pH and then quantified by the above methods. Bis-N7G-BD (**283**) amounts increased in a dose-dependent manner in double-stranded DNA (lesion frequency: 1-300 per 10^6 normal guanines). Interstrand cross-linking efficiency differed significantly for the stereoisomers in the order following from the highest to the lowest: (*S*,*S*)- > (*R*,*R*)- > *meso*-diepoxide. (*S*,*S*)-DEB (**280**) makes preferentially interstrand crosslinks, (*R*,*R*) and *meso*-DEB form intrastrand bis-N7G-BD lesions. DEB-induced interstrand lesions involve distal guanines within 5'-GGC sequence. First-order kinetic analysis indicated that the half-life of interstrand N7G-N7G DEB cross-links in double-standed DNA is 147 h, while for intrastrand bis-N7G-BD adducts is 35 h. This big difference in stability can be explained by a greater charge density in intrastrand bis-N7G-BD adducts which contain two neighboring, positively charged N7-guanine adducts. Beyond the guanine-guanine cross-links adenine-guanine adducts (**284**-**287**) were also identified Scheme (**49**) [104]. A quantitative HPLC-ESI$^+$-MS/MS method was also developed for measuring the level of bis-N7G-BD adducts [105]. *In vivo* analysis of bis-N7G-BD (**283**) formation after 5 days exposure to butadiene (0-625 ppm) showed that the liver DNA of treated mice contained 3.17 ± 0.35 racemic adducts per 10^6 guanines. Lung DNA isolated from treated animals contained significantly lower amounts of racemic bis-N7G-BD (1.79 ± 0.54 per 10^6 guanines) than liver tissues.

HPLC-ESI$^+$-MS/MS analysis of hydrolysates of DEB-treated DNA revealed the presence of hypoxanthine-guanine conjugates. Because of the known propensity of N-1-adenine lesions to undergo hydrolytic deamination at physiological conditions, it was proposed that the hypoxanthine-guanine conjugate (N1HX-N7G-BD) (**288**) is formed by deamination of (N1A-N7G-BD) (**287**) Scheme (**49**) [106]. The conjugate 1-(hypoxanth-1-yl)-4-(guan-7-yl)-2,3-butanediol (**288**) was synthesized from by cross-linking of N^2-acetyl-7-(4-chloro-2-butenyl)guanine and 2'-deoxyinosine and subsequent dihydroxylation. The NMR, MS/MS/MS, HPLC, UV results have been compared to DEB-treated calf tymus DNA fragments to corroborate that hypoxanthine-guanine DEB cross-links were present in acid hydrolysates of DEB-treated DNA.

7-Methyl-8-oxo-2'-deoxyguanosine (MOdG) is an analog of the abundant promutagen 8-oxo-2'-deoxyguanosine (OdG) that is one of the most common damaged nucleotides in mammalian cells. OdG forms stable base pairs with both dC and dA and it can lead to dG → T transversions. OdG forms *anti* conformation with dC utilizing Watson-Crick hydrogen bonds, and forms *syn* conformation with dA with Hoogsteen edge for hydrogen bonding. Steric bulk of the oxygen at C8 destabilizes the *anti* conformation and destabilizing OdG(*anti*) : dC base pairs. N7-hydrogen act as H-bond donor to stabilize OdG-dA mismatches. Hamm and Billig



Scheme 48.

[107] have accomplished the synthesis of MOdG that started by methylation of protected guanosine (**289**) then oxidation at C8 with H_2O_2 to furnish (**291**) Scheme (**50**). Radical deoxygenation of (**292**) at C2' provided 2'-deoxyribo derivative (**293**) that was transformed into phosphoramidite (**294**) using standard methods. This monomer was incorporated into oligonucleotides and melting point measurements were carried out to determine the strength of interactions with nucleotides in the middle positions of ODNs. It was found that the presence of the O-8 destabilizes the *anti* conformation, thereby destabilizing base pairs that contain MOdG in the *anti* conformation. The *anti*-to-*syn* nucleoside conformational change resulted in a strong downfield and upfield shifts in the H-2' and C-2' signals, respectively, in the NMR spectra.

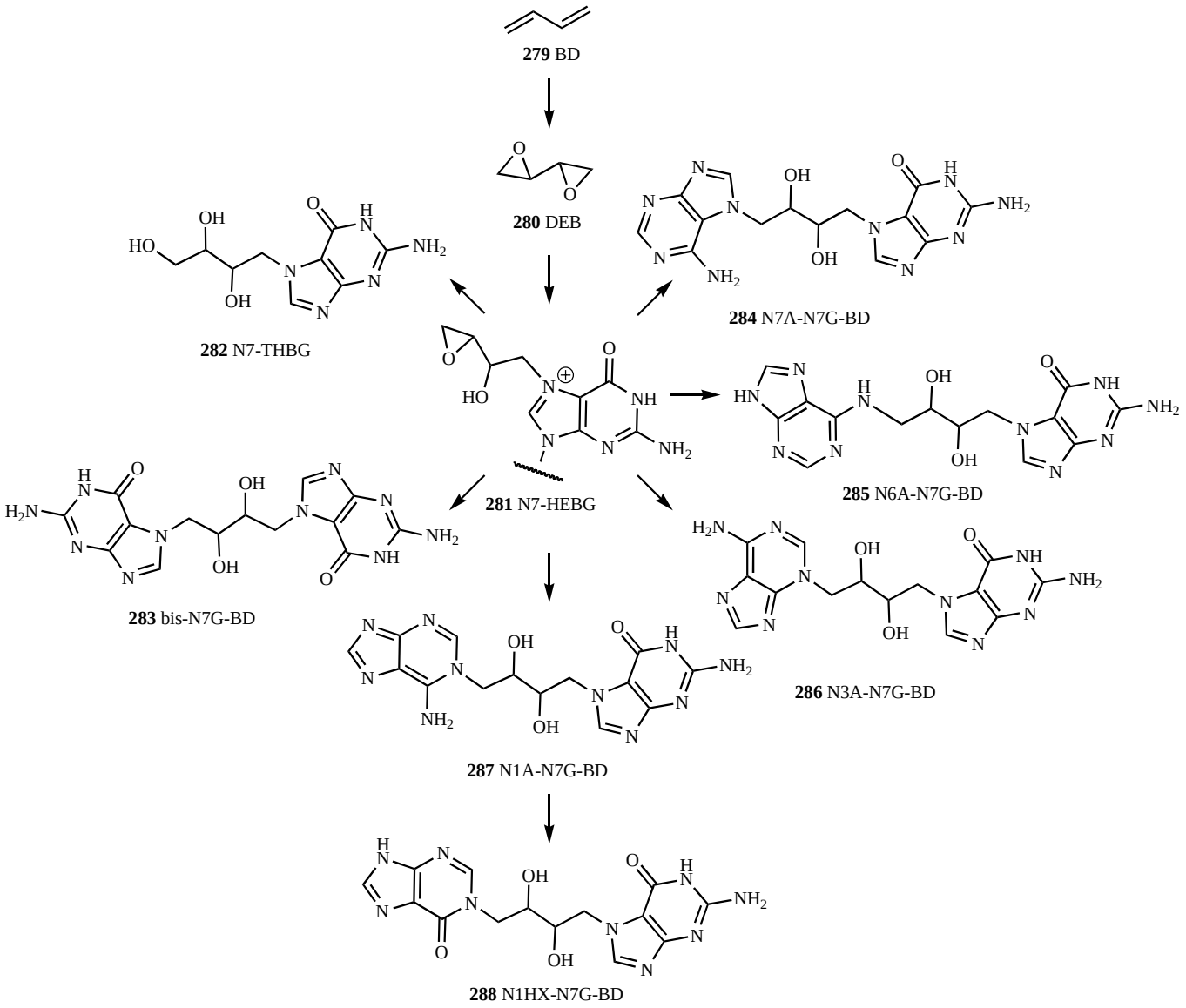
The regioselective synthesis of 7-alkylguanines can be accomplished efficiently only indirectly, among others, by using the knowledge obtained from the alkylation lesions of DNA and RNA. These procedures will be treated in detail in *section 7*. It should be mentioned in passing that the best methods rely on the acid-lability of 7-alkyl-9-glycosylguanines. This method also allows the efficient synthesis of 9-alkylguanines.

6.2. mRNA CAP Structures

Eukaryotic messenger RNAs are modified at their 5'-ends by addition of a 7-methylguanosine attached by a 5'-5' triphosphate bridge to the first nucleotide of the mRNA chain. This cap structure, $m^7G(5')ppp(5')X$ (X = any nucleoside), plays a pivotal role in mRNA metabolism, including mRNA transport between the nucleus and the cytoplasm, control of mRNA stability, involving various cap binding proteins. Among them, the nuclear cap binding complex (CBC) takes part in pre-RNA splicing, polyadenylation of the 3' terminus and nuclear export. The eukaryotic translation initiation 4E factor (eIF4E) regulates initiation of protein biosynthesis, and poly(A)-specific exoribonuclease (PARN) is responsible for cap-dependent mRNA degradation [108]. Further modifications (methylations at guanine N-2 or in other nucleotides, at sugar O-2' or O-3' positions of other nucleotides, polyA tail *etc.*) are also frequent and specific for a given RNA and organism [97]. The mechanism of recognition and removal of cellular mRNA 7-methyl-G caps by a viral capsid protein of L-A virus has recently been deciphered [109].

The biosynthesis of 7-methylguanosine derivatives is carried out by m^7G methyltransferases (m^7G MTases) using *S*-adenosyl-L-methionine (AdoMet) as a methylating agent and afford *S*-adenosyl-L-homocysteine (AdoHcy) as a by-product. These m^7G MTases are not limited to mRNA but they do methylate other RNAs as well [e.g. YggH (tRNA in *E. coli*), Agr (rRNA in *Streptomyces kanamyceticus* Kmr) and Abd1 (mRNA in *S. cerevisiae*)] [110]. Enzymatic syntheses of capped RNAs have been realized on a 100 nmol scale by Alvarez *et al.* [111].

A convenient, 10 gram-scale synthesis of 7-methylguanosine 5'-diphosphate (m^7Gpp), an important intermediate in the synthesis of cap analogs, was reported by Kore and Parmar Scheme (**51**) [112]. Dissolving guanosine 5'-diphosphate (**295**) in water and adding dimethyl sulfate dropwise over a period of 1 h at room temperature at pH 4 gave the product (**296**) within 2 h after purification on a DEAE ion exchange resin (> 96% yield). Acidic environment prevented the formation of phosphate methyl esters. This method has frequently been used, also for the synthesis



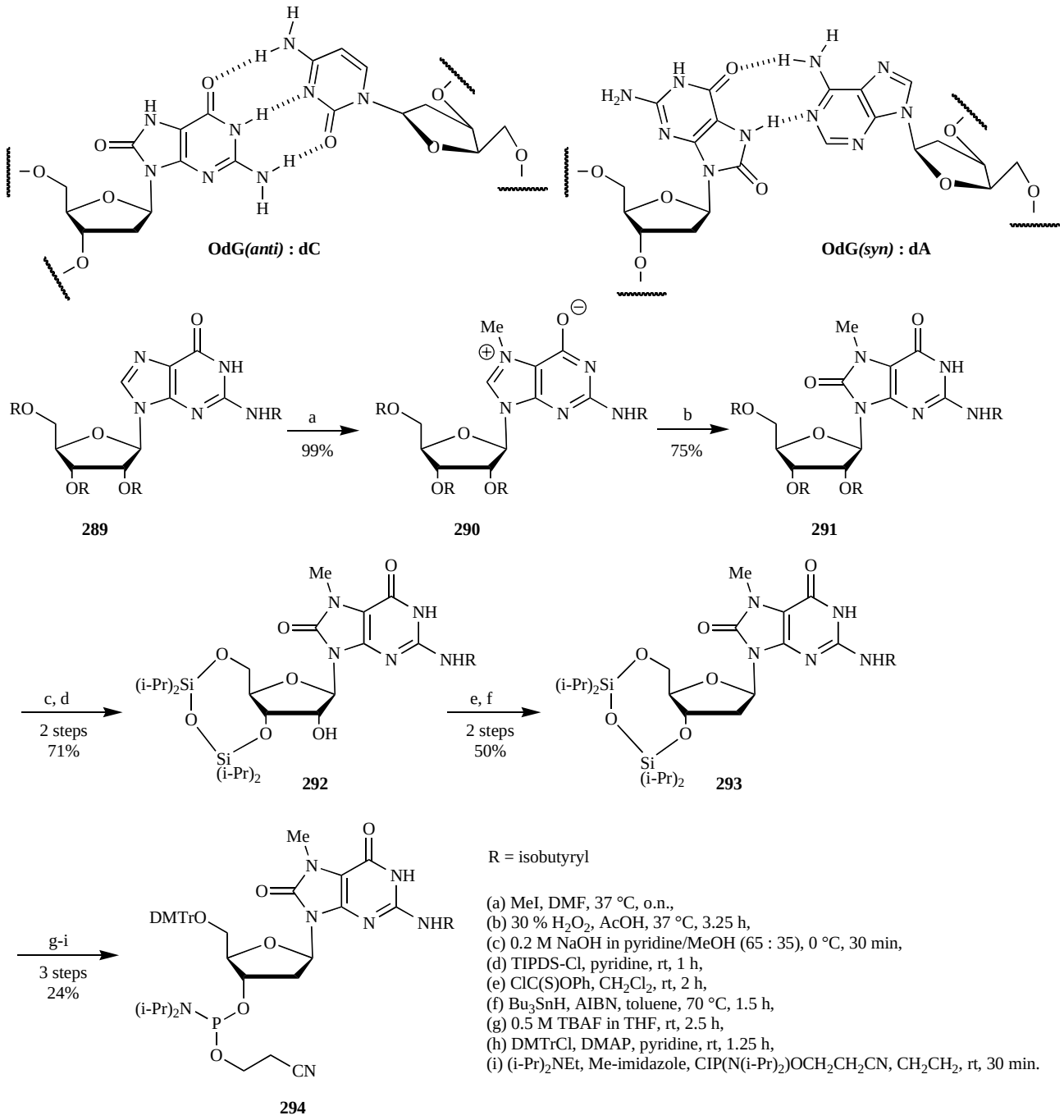
Scheme 49.

of m^7Gp, or other analogs [113-116]. Alternative methylating agents (MeI/DMSO) work with similar efficiency [117-122].

The most often employed method for obtaining cap analogs relies on the $ZnCl_2$-mediated coupling of a diphosphate-imidazolide (**297**) and monophosphate (e.g. **299**) or monophosphate-imidazolide and diphosphate (*e.g.* m^7Gpp-imidazolide and pX or Xpp-imidazolide and m^7Gp or m^7Gp-imidazolide and ppX or Xp-imidazolide and m^7Gpp, X = any nucleotide) or their analogs in anhydrous DMF to afford the desired trinucleotide m^7GpppX (e.g. X = G, **7**) in moderate to good yields Scheme (**51**) [108,113,116-118,120,122-128]. Analogously to the application of m^7Gpp-imidazolide intermediate (**297**), Kokhareva and Lebedev [115] have proposed the use of an 8-(5-chloroquinolyl)pyrophosphate derivative in the synthesis of cap analogs. Thus, 8-hydroxy-5-chloroquinoline was phosphorylated with $POCl_3$ and the unisolated intermediate was allowed to react with mono-tri-*n*-octylammonium salt of GDP to give P^1-guanosine-5'-*O*-yl,P^2-*O*-8-(5-chloroquinolyl) pyrophosphate (GppQ) in 30% yield after isolation by DEAE anion-exchange chromatography. Methylation of the G moiety (Me_2SO_4) gave the m^7GppQ (**298**) derivative in 67% yield. The capped pentaribonucleotide $m^7GpppGpApCpU$ was obtained in 37% yield after reverse phase and ion exchange HPLC purification using a $CuCl_2$-mediated coupling of tetranucleotide pGpApCpU with m^7GppQ.

Potential inhibitors of eIF4E include m^7GTP analogs. Wagner *et al*. [119,129] have synthesized a small library comprising several 7-alkylguanosines and their 5'-*H*-phosphonates (**300a-e**) Scheme (**52**). Affinity studies underlined that the presence of at least one phosphate or *H*-phosphonate group is necessary for binding. No significant difference in the binding affinity of 7-benzyl-GMP and 7-benzyl-G *H*-phosphonate (**300c**) was observed, thus confirming the ability of this substitution to replace 5'-monophosphate.

Halperin *et al*. [121] have also studied the interaction between eIF4E and cap, and to identify small molecule inhibitors of their binding, by synthesizing a fluorescent-labeled

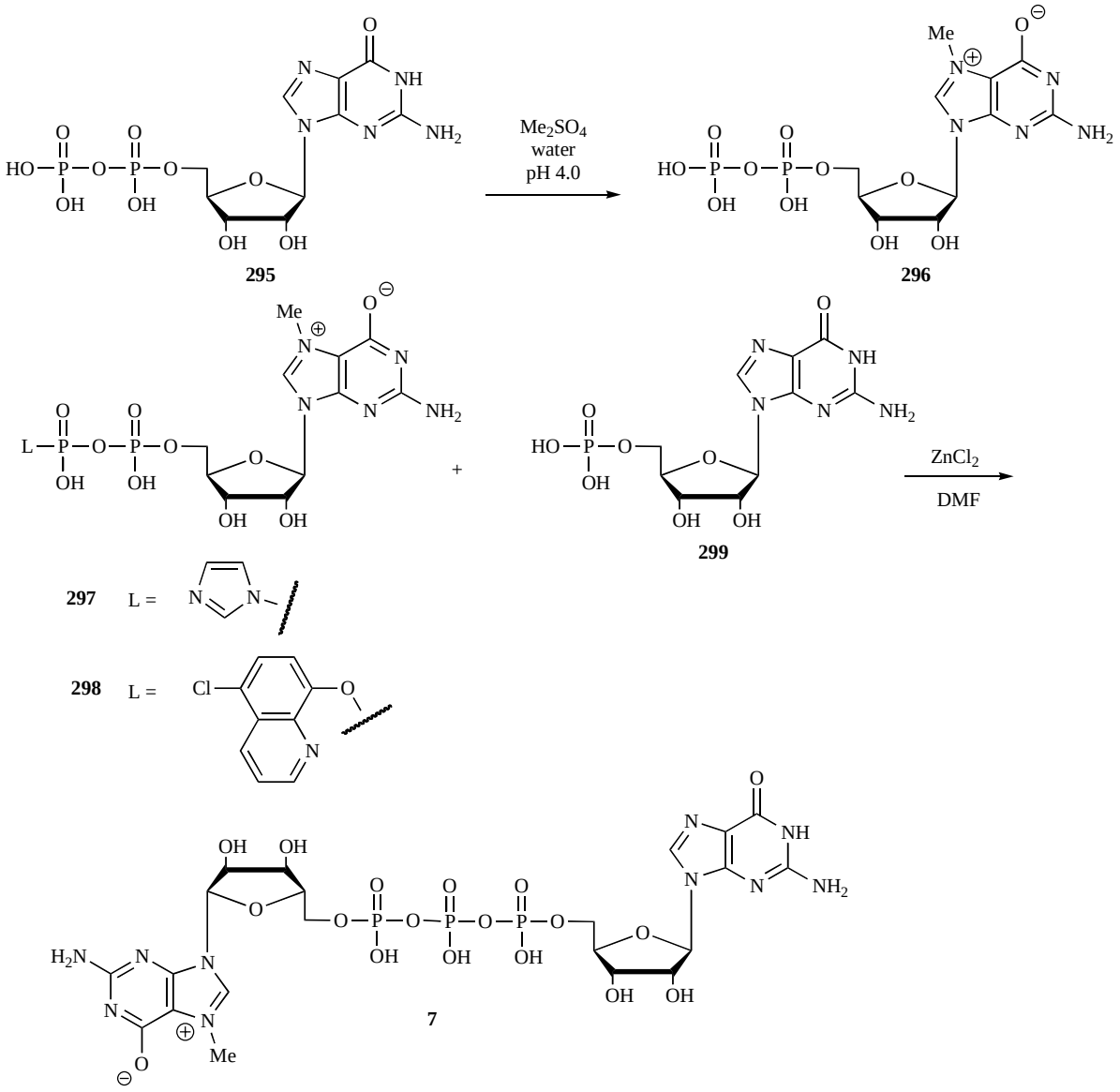


Scheme 50.

cap analog (**301**) Scheme (**53**) and developed a fluorescence-polarization assay. Briefly, protected guanosine and commercially available pivaloyl protected 5'-fluorescein phosphoramidate containing a 6-carbon linker was coupled (65% yield). Then the base labile protecting groups were removed (97% yield) and the guanine residue was quaternized with methyl iodide to afford (**301**) (96% yield). m^7GTP competitively inhibited the binding of the fluorescently labeled cap analog to human eIF4E, in a dose-dependent manner.

Ribavirin, a broad-spectrum antiviral nucleoside is also an inhibitor of eIF4E. In cells, it is converted to mono-, di- and triphosphates (RMP, RDP, RTP). Ribavirin and m^7G are structurally similar, and ribavirin interferes with the function of cap-binding proteins. RTP binds to recombinant eIF4E with similar affinity as m^7GTP. Ribavirin incorporated into mRNA as 5'-5' dinucleotide RpppG and other mRNAs, capped with m^7GpppG and GpppG (the latter not binding to eIF4E), were studied by Preiss *et al*. [130]. It was found that m^7GpppG-capped mRNA was translated 15-fold more efficiently than RpppG and GpppG so RpppG does not bind to eIF4E, thus ribavirin does not mimic the 7-methylguanosine moiety of the mRNA cap structure *in vitro*.

A stable spin-labeled cap analog, m^7Gppp-TEMPO (**302**), has recently been obtained by Niedzwicka *et al*. [108]. Thus, m^7Gpp-imidazolide (**297**) Scheme (**51**) was coupled with TEMPO-4'-*O*-phosphate in the presence of $MnSO_4$ at

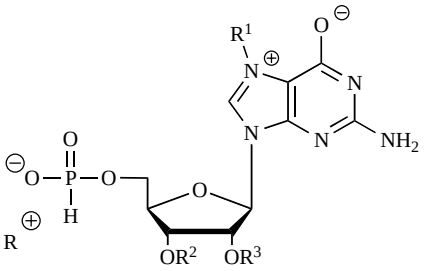


Scheme 51.

pH 7 to afford the product (**302**) in 20% yield after purification Scheme (**54**). m^7Gppp-TEMPO has been extensively characterized using 1D and 2D NMR, ESR and ESI-MS methods.

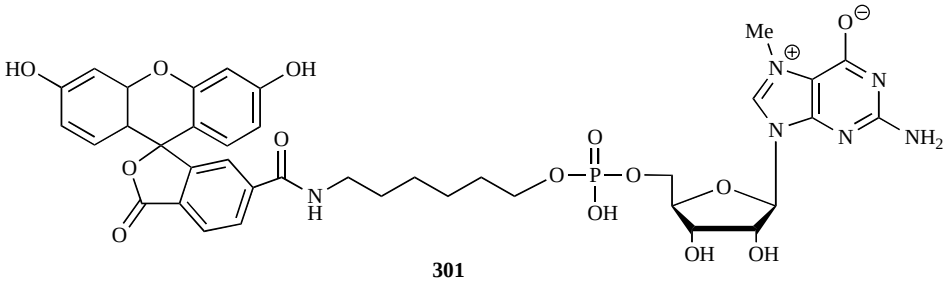
A series of cap analogs of type m^7Gp_x (x = 1-5), m^7Gp_yG (y = 3-5) and $m^7Gp_zm^7G$ (z = 3-5) have been prepared by Darzynkiewicz *et al.* [117] using the $ZnCl_2$ condensation method of imidazolides [118]. Methylation of the guanosine oligophosphates (MeI/ DMSO) gave the appropriate 7-methylguanosine oligophosphates as the main products. The analogs were used to determine their binding affinities to eIF4E mutated at different positions. It was found that phosphorylation at Ser209 creates electrostatic repulsion between the protein and the negatively charged cap structure. The inhibition of the ability to bind cap analogs by the K159A mutant and its phosphorylated counterpart shows significant participation of Lys159 in the binding of the capped mRNA. Both structural modifications, phosphorylation and the replacement of lysine with alanine, result in an increase in the negative Gibbs free energy of association that is proportional to the length of the cap phosphate chain.

Novel 5' mRNA cap analogs with one of the pyrophosphate bridge oxygen atoms of the triphosphate linkage replaced with a methylene group [methylenebis(phosphonates)] have been synthesized by Darzynkiewicz *et al.* Scheme (**55**) [118]. The analogs (**303-306**) were prepared via reaction of nucleoside phosphor/phosphon-1-imidazolidates with nucleoside phosphate/phosphonate in the presence of $ZnCl_2$ and methylation of guanosine (MeI/DMSO). Three of the new cap analogs (**304-306**) were completely resistant to degradation by human pyrophosphatase DcpS, the enzyme responsible for hydrolysis of free cap resulting from 3' to 5' cellular mRNA decay. Analog (**304**) had very high affinity for binding to human DcpS. Two of these analogs are anti reverse cap analogs ensuring that they are incorporated into mRNA chains exclusively in the correct orientation. The structures of DcpS in ligand-free form and in a complex with m^7GDP have been reported recently [131]. Further



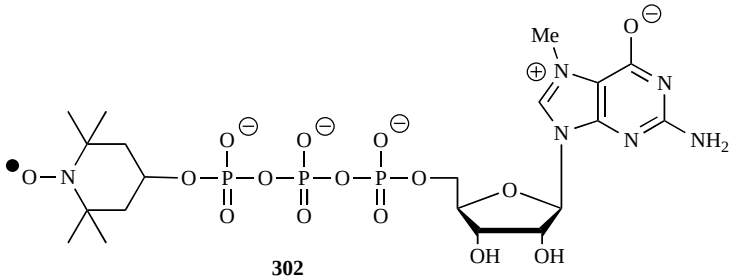
300a-d $R^+ = TEAH^+$; R^1 = Me (**a**), Et (**b**), Bn (**c**), $C_{10}H_8CH_2$ (**d**); R^2 and R^3 = isopropylidene
300e $R^+ = Na^+$; R^1 = Bn; R^2 and R^3 = H

Scheme 52.



301

Scheme 53.



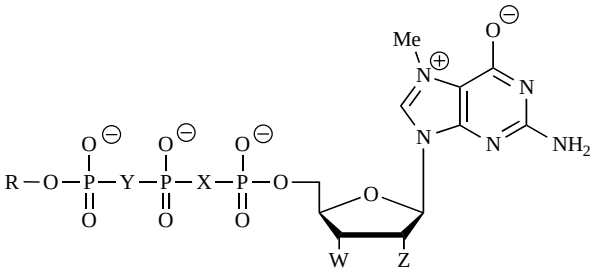
302

Scheme 54.

guanosine bisphosphonates $GppCH_2p$, $GpppCH_2p$, m^7GppCH_2p (**307**), $m^7GpppCH_2p$ along with adenosine derivatives $AppCH_2p$, $ApppCH_2p$ have also been synthesized by the reaction of bisphosphonate and GMP, GDP, AMP and ADP in a two-step procedure (imidazolide activation, $ZnCl_2$-mediated coupling of phosphates, Scheme (**55**) [122]. High overall yields were obtained when the methyl group was introduced at the final step of the reaction (MeI/DMSO). Enzymatic studies confirmed that analogs pCH_2pppG, pCH_2pppm^7G, pCH_2pppA were substrates for neither specific nucleoside tetraphosphatase from yellow lupin seeds nor the corresponding NTPs. The analogs did not inhibit these hydrolases either; however they were strong inhibitors of both the asymmetrical and symmetrical Ap_4A hydrolases. The analogs with methylene bridge between α (m^7GpCH_2ppG, **304**) and β phosphorus atoms (m^7GpCH_2ppG, **305**) have been described as well Scheme (**55**) [120]. All the compounds were resistant to the human scavenger decapping hydrolase, DcpS. Binding constants of the modified caps to eIF4E are comparable to those obtained for m^7GpppG. This suggests that these methylene modifications in the pyrophosphate chain do not significantly affect cap-binding, at least for eIF4E. These cap analogs were also good inhibitors of *in vitro* translation. mRNAs capped with novel analogs were translated similarly to the mRNA capped with the parent m^7GpppG.

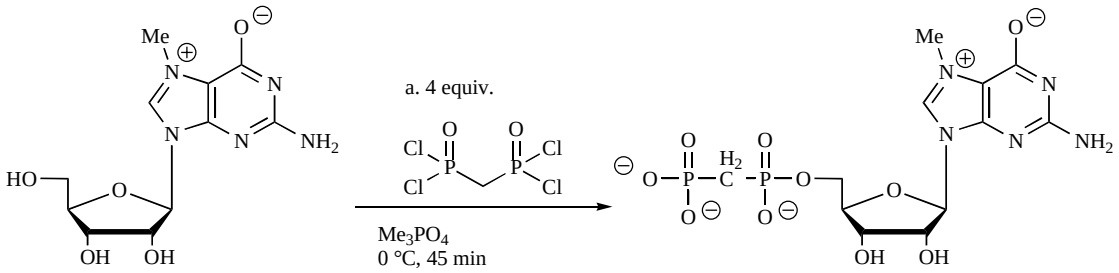
An optimized, alternative synthesis of unprotected nucleotides via phosphonylation directly with methylene-bis(phosphonic dichloride) has also been elaborated Scheme (**56**) [132]. In this way, m^7GpCH_2p (**309**) was obtained in 81% yield from m^7G (**308**) and 4 equiv. of $CH_2(POCl_2)_2$.

The triphosphate bridge of the 5' cap mRNA can be hydrolyzed by metal complexes quite dramatically, *e. g.* Cu^{2+}-bypiridine complex in 2 mM concentration at pH 8 and 60 °C enhances the hydrolysis at least by a factor 20,000. Lönnberg *et al.* [133] have studied the hydrolytic reaction of cap model m^7GpppG (**7**) in the presence of macrocyclic 18-24-membered azacrown amines in buffered neutral solutions at 60 °C Scheme (**57**). The only products observed in the absence of macrocycles resulted from the base-catalysed imidazole ring-opening (*path c*) (**311-314**) and the acid-catalysed cleavage of the 7-methylguanosine base, whereas in the presence of these catalysts hydrolysis of the triphosphate bridge predominated. The latter reaction afforded m^7GMP (**310**) and GDP (**295**) (*path a*), GMP (**299**) and m^7GDP (**296**) (*path b*), and several other products, too. The overall catalytic activity of all three macrocycles was comparable.



303 W = OH, X = O, Y = CH_2, Z = OH, R = guanosin-5'-yl
304 W = OH , X = CH_2, Y = O, Z = OH, R = guanosin-5'-yl
305 W = OMe, X = O, Y = CH_2, Z = OH, R = guanosin-5'-yl
306 W = OMe, X = CH_2, Y = O, Z = OH, R = guanosin-5'-yl
307 Y = CH_2, X = O, Z = W = OH, R = H

Scheme 55.



308 **309**

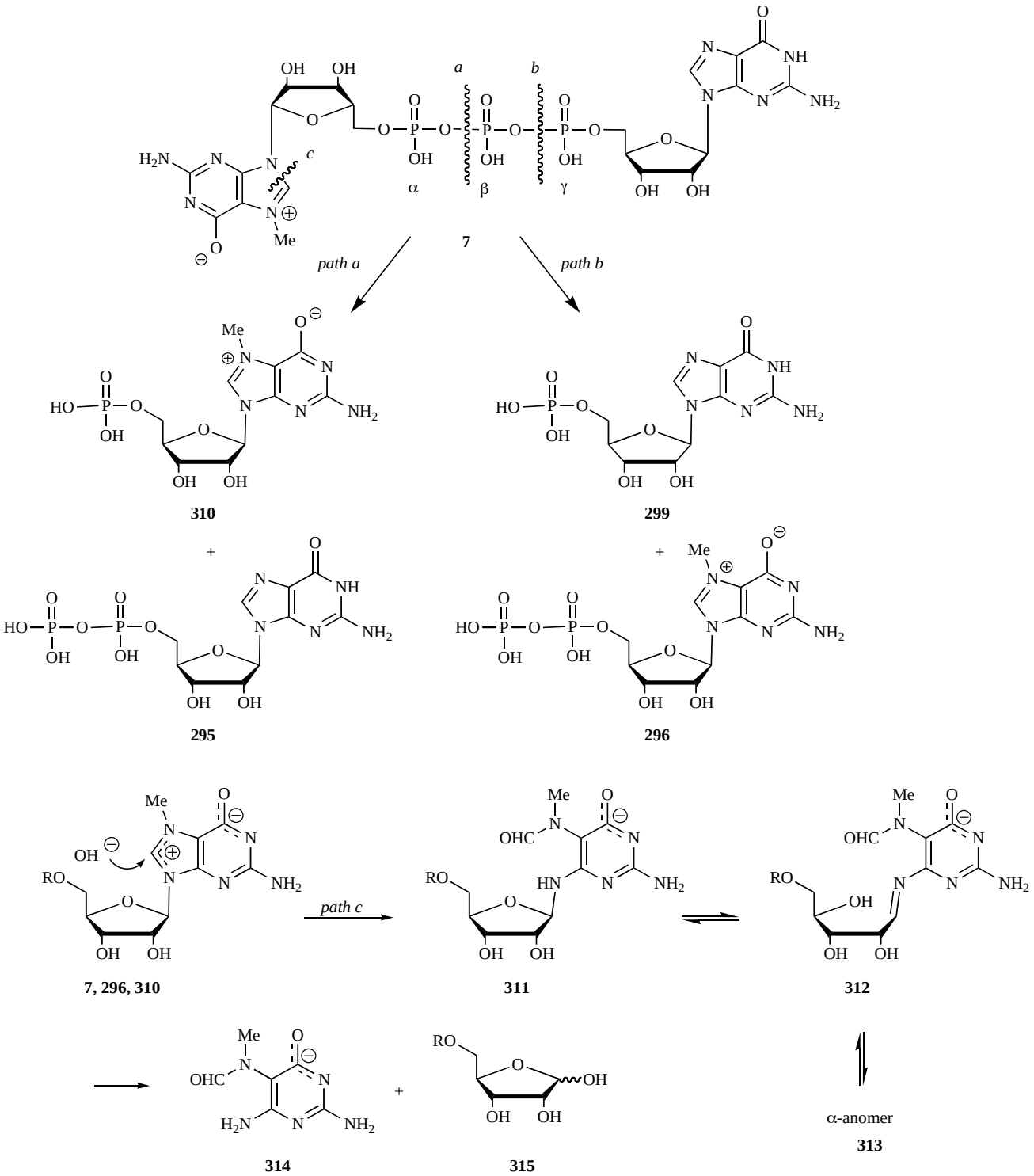
Scheme 56.

Efimtseva *et al*. [114] have synthesized masked zwitterionic AZT phosphate derivatives, prodrugs of AZT that bypass the first phosphorylation step. The synthesis of guaninyl-(5'-5')-AZT (GpAZT) was achieved by standard methods and the dimer GpAZT was methylated by a large excess of dimethyl sulfate at pH 5.0 to give m^7GpAZT in 85-90% yield. In addition, the corresponding periodate-oxidized derivatives were also prepared in high overall yield. The conjugate m^7GpAZT exhibited antiviral potency and cytotoxicity similar to that of AZT, it was completely inactive against HIV replication. m^7GpAZT is not a pronucleotide of AZT-MP as it is unable to deliver the AZT-MP inside the cells.

The sensitivity of the pyrophosphate bridge in cap structures towards cellular enzymes (ecto-NTPdiphosphohydrolase or ecto-nucleotide pyrophosphatase/diesterase) renders the *in vivo* application of cap analogs as potential drugs problematic. To overcome this problem, Jemielity *et al*. [134] have prepared mRNA cap analogs containing phosphorothioate moiety in the 5',5'-triphosphate chain in α, β or γ positions [m^7Gp(s)ppG (**316**), m^7Gpp(s)pG (**317**), and m^7Gppp(s)G (**318**) Scheme (**58**). Incorporation of a sulfur atom in α or γ position within the dinucleotide cap analog was achieved using $PSCl_3$ in a nucleoside phosphorylation reaction followed by coupling the phosphorothioate of nucleoside with a second nucleotide. Synthesis of cap analogs with the phosphorothioate moiety in β position was performed using an organic phosphorothioate salt in a coupling reaction with an activated nucleotide. The late methylation of guanine was not compatible with the phosphorothioate residue therefore m^7GMP was used in the above coupling steps. The interaction of analogs with translation initiation factor eIF4E and enzymatic hydrolysis with human and nematode DcpS scavengers was studied. The association constants (K_{as}) for the complexes m^7Gp(s)ppG-D2 and m^7Gppp(s)G-D2 do not differ considerably from K_{as} for m^7GpppG, however, m^7Gp(s)ppG-D1 binds eIF4E with a 3-fold higher affinity (D1 and D2 denote diastereomers with an opposite configuration on the asymmetric P atom, D1 corresponds to a diastereoisomer with shorter t_R on a RP column). m^7Gp(s)ppG-D1 and m^7Gp(s)ppG-D2 were resistant to cleavage by both enzymes. For m^7Gppp(s)G-D2, hydrolysis was observed using both the human and nematode DcpS enzymes with a rate comparable to that observed for the standard cap m^7GpppG.

The terminal phosphorothioate moiety significantly improves resistance to phosphatases, but usually does not affect the substrate properties towards kinases. Therefore several nucleoside 5'-(2-thiodiphosphates) and 5'-(3-thiotriphosphates), including the cap analogs m^7Gpp(s) (**319**) and m^7Gppp(s) (**320**), were synthesized by Jemielity *et al*. Scheme (**58**) [123] treating the appropriate nucleotide imidazolide derivative with a ca. 4-fold excess of triethylammonium thiophosphate salt in DMF in the presence of $ZnCl_2$ in 80-100% yields. This approach avoids basic conditions that are incompatible with 7-methylguanine derivatives.

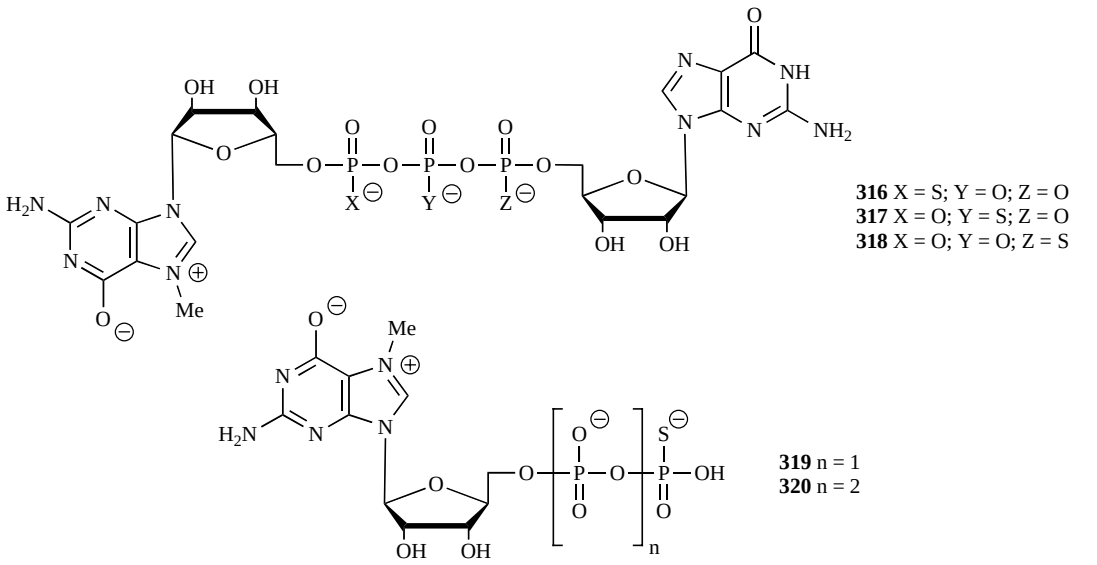
The *in vitro* synthesis of capped mRNA employs m^7GpppG (mCAP) as an initiator of transcription. The drawback of mCAP analog is that the 3'-OH of either the G or m^7G can serve as the initiating nucleophile for transcriptional elongation leading to the synthesis of two isomeric RNAs of either forward or reverse form in approximately equal proportions depending upon the ionic conditions of the transcription reaction. The reverse form of capped mRNAs, *i.e*., Gpppm^{7}G(pN)$_n$ will not be recognized during the translation process, only forward oriented sequences, i.e., m^7GpppG(pN)$_n$ will be translated. By chemical modification at either 2'- or 3'-OH of m^7G, the cap incorporated only in the forward orientation, even though the 2'-OH group does not participate in the phosphodiester linkage as demonstrated



Scheme 57.

by Kore *et al*. [113] in the synthesis of 2'-fluoro-substituted cap analogs m^7,2'-F-GpppG (**321**) and m^7,2'-F-Gpppm^{7}G (**322**) obtained by standard methods Scheme (**59**).

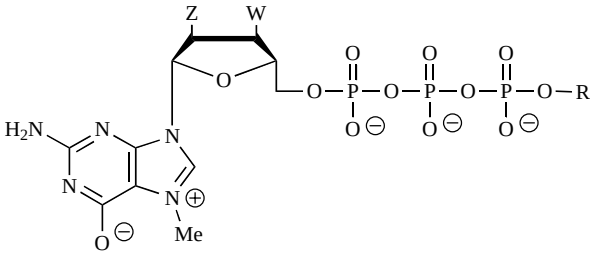
The amount of translation of the 2'-fluoro capped mRNA was increased 2.4-2.5-fold compared to m^7GpppG but the presence of the 2'-fluoro group is not responsible for this improvement, rather the increase in correctly capped mRNA molecules. In an effort to prepare anti-reverse-cap analogs (ARCAs), Rhoads *et al*. [128] have synthesized new cap analogs modified in the C2' and C3' positions of m^7Guo and in the number of phosphate residues, $m_2^{7,2'}$GpppG (**323**), $m^7$2'-dGpppG (**324**), $m^7$2'-dGppppG, $m_2^{7,2'}$GppppG, $m_2^{7,3'}$GppppG, m^7GpppppG, and $m_2^{7,3'}$GpppppG using standard methods Scheme (**59**). The analogs were analyzed for conformation in solution, binding affinity to eIF4E, inhibition of *in vitro* translation, degree of reverse capping during *in vitro* transcription, capping efficiency, and the ability to stimulate cap-dependent translation *in vitro* when incorpo-



316 X = S; Y = O; Z = O
317 X = O; Y = S; Z = O
318 X = O; Y = O; Z = S

319 n = 1
320 n = 2

Scheme 58.



321 R = guanosine-5'-yl, Z = F, W = OH
322 R = 7-methylguanosine-5'-yl, Z = F, W = OH
323 R = guanosine-5'-yl, Z = OMe, W = OH
324 R = guanosine-5'-yl, Z = H, W = OH
325 R = 7-methylguanosine-5'-yl, Z = OH, W = OMe
326 R = formycin A-5'-yl, Z = W = OH
327 R = 3'-*O*-metylguanosine-5'-yl, Z = W = OH
328 R = 9-β-D-arabinofuranosyladenine-5'-yl, Z = W = OH
329 R = isoguanosine-5'-yl, Z = W = OH

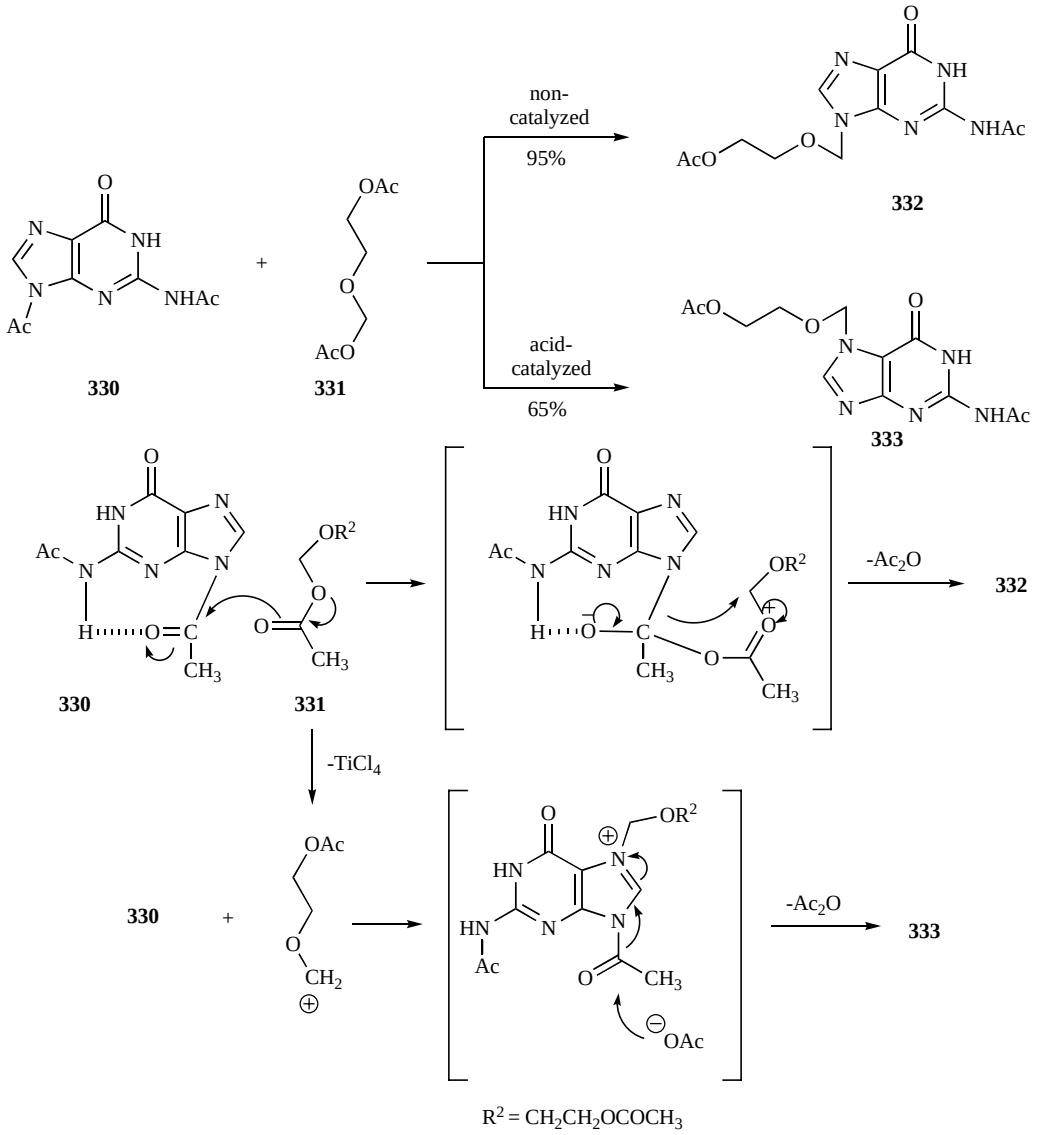
Scheme 59.

rated into mRNA. The results indicate that modifications at C2', like those at C3', prevent reverse incorporation, that tetra- and pentaphosphate cap analogs bind eIF4E and inhibit translation more strongly than their triphosphate counterparts, and that tetraphosphate ARCAs promote cap-dependent translation more effectively than previous cap analogs.

Trimethylated cap analog with methyl groups on the N-7 of both guanine moieties, as well as the 3'-OH of one of the ribose moieties ($m_2^{7,3'}$Gpppm^{7}G) (**325**) has been prepared, employing conventional procedures, by Kore *et al*. Scheme (**59**) [116]. The function of this new analog was compared with those of three other, less-methylated cap analogs: one omitting the ribose methylation (m^{7}Gpppm^{7}G), one omitting the N7-methylation linked to the unmodified ribose ($m_2^{7,3'}$GpppG), and the standard cap analog, m^{7}GpppG. These cap modifications were assayed with respect to their effects on capping efficiency, yield of RNAs during *in vitro* transcription, and the translational activity of these RNAs upon transfection into HeLa cells. The translational activity was monitored by measuring the luciferase activity of a luciferase-fusion protein produced from the *in vitro* synthesized RNAs. The RNA capped with the trimethylated analog $m_2^{7,3'}$Gpppm^{7}G (**325**) was translated the most efficiently, with ca. 2.6-fold more activity than the conventional cap m^{7}GpppG. The other two variants were also more efficient ($m_2^{7,3'}$GpppG: ca. 2.2-fold; m^{7}Gpppm^{7}G: ca. 1.6 -fold activity) than the conventional cap.

New triphosphate dinucleotide mRNA 5'-cap analogs with four different nucleosides, formycin A (m^{7}GpppF), (**326**), 3'-*O*-methylguanosine (m^{7}Gpppm$^{3'}$G) (**327**), 9-β-D-arabinofuranosyladenine (m^{7}Gppp^{ara}A) (**328**) and isoguanosine (m^{7}Gppp^{iso}G) (**329**), have been synthesized by standard methods and spectroscopically characterized by Darzynkiewicz *et al*. Scheme (**59**) [127]. These cap analogs were tested *in vitro* for their capacity to substitute for the native form of mRNA 5' end. The binding affinity of murine eIF4E(28-217) was determined using fluorescence titration. All the cap analogs bind to eIF4E with similar affinities, m^{7}GpppF (**326**) being the most effective. Methylation of sugar moiety at position O-3' has not influenced the binding affinity. The equilibrium association constant (K_{as}) for the formycin analog is about four-fold higher than that for m^{7}GpppA or m^{7}Gppp^{ara}A and two-fold higher than for m^{7}GpppG.

The cap structures of Leishmania and other trypanosomatids include heavily methylated nucleotides, resulting in the cap-4 structure m^{7}Gppp-$m_3^{6,6,2'}$Ap-m$^{2'}$Ap-m$^{2'}$Cp-$m_2^{3,2'}$U. Darzynkiewicz *et al.* [124] have synthesized a library of analogs that mimic this cap structure. The 5' terminal mRNA tetranucleotide fragment p-$m_3^{6,6,2'}$Ap-m$^{2'}$Ap-m$^{2'}$Cp-$m_2^{3,2'}$U was synthesized by the phosphoramidite solid phase method. After deprotection and purification, the 5'-phosphorylated tetranucleotide was chemically coupled with m^{7}Gpp-imidazolide in the presence of $ZnCl_2$ [118] to yield the cap-4 structure. The compound m^{7}Gppp-$m_3^{6,6,2'}$A, called cap-1,



Scheme 60.

was also obtained in the same fashion. Biological activity of this newly synthesized compound was confirmed in binding studies with recently cloned LeishIF4E-1 from Leishmania, using the fluorescence time-synchronized titration method.

The solid phase approach to cap structure m^7Gppp-$m^{2'}$Ap-$m^{2'}$Up-$m^{2'}$Ap was also preferred by Darzynkiewicz and Lönnberg *et al*. [125] who have accomplished the synthesis of this tetranucleotide on a disulfide-tethered solid support that allowed manipulations under basic conditions and could be reductively cleaved with 1,4-dithio-D,L-threitol (DTT). The highly base-sensitive m^7G unit was introduced at the late stage of the syntesis in a $ZnCl_2$-promoted coupling of m^7Gpp-imidazolide. DTT in combination with 0.01% Et_3N released the target compoud from the solid support and also promoted the cleavage of tethering 2-mercaptoethyl unit in the form of episulfide. The same tetranucleotide lacking one phosphate group at the 3' teminus was prepared in solution phase, using the $ZnCl_2$-promoted coupling method, by Darzynkiewicz *et al*. [126]. Fluorescence titration-based association studies with cap binding complex (CBC) revelead that only the first two nucleotides at mRNA 5' terminus are responsible for the specific interaction with the CBC.

The cap structures may also contribute to intracellular delivery of proteins or nucleic acids. Smith *et al*. [135] have recently reported that with hypermethylated cap analogs containing the small nuclear RNA (snRNA) moiety $m_3^{2,2,7}$Gppp-$m^{2'}$Ap-$m^{2'}$Up-$m^{2'}$Ap, attached to biotin or antisense oligonucleotides directly or through a long linker, at least 6-fold enhancement in nuclear import of cargoes, as compared to the uncapped counterpart, could be observed in a *Xenopus* oocyte system. Moreover, $m_3^{2,2,7}$G-capped RNA oligonucleotides are able to direct nuclear accumulation of a cargo protein in mammalian cells after cytosolic delivery by a transfection reagent and $m_3^{2,2,7}$G-capped 2'-*O*-methyl RNA antisense oligonucleotides show increased efficiency in a splice correction assay. The synthesis of capped structures has been achieved using N^2,N^2,7-trimethylguanosine 5'-pyrophosphorylimidazolide in a Mn(II)-promoted capping procedure.

7. 9-ALKYLGUANINES

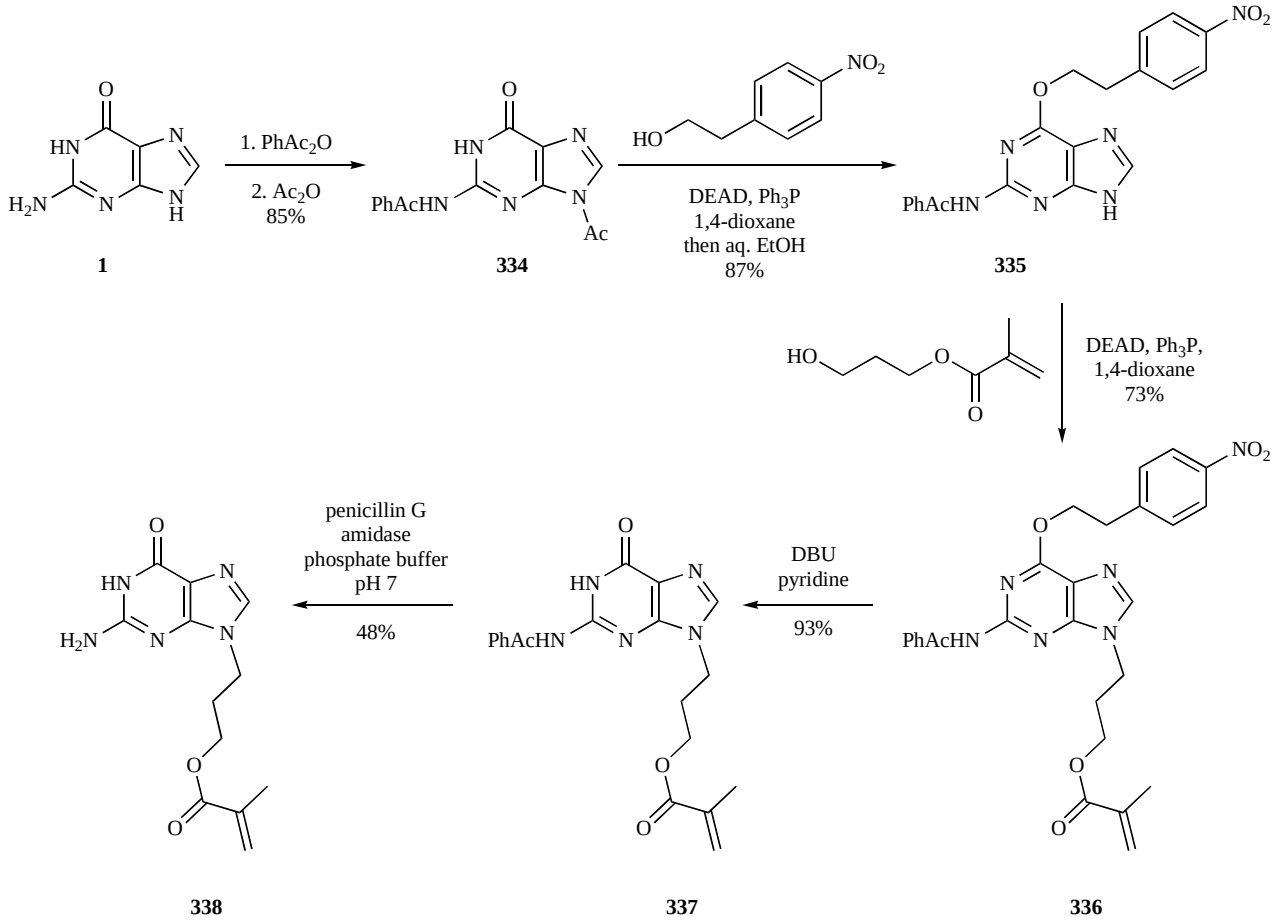
The 7/9-regioselectivity in the direct alkylation of guanines not protected at N-9 has long been an unsolved prob-

lem [1,2]. The capricious behaviour of guanine is often complicated by poor solubility as well. Unlike other nucleobases (e.g. adenine), usually almost equimolar amounts of the two regiosiomers are formed from guanine and alkylating agents under basic conditions. From time to time there are claims in the literature that this problem has been satisfactorily solved but these methods often lack generality and are limited to a particular combination of compounds and/or conditions.

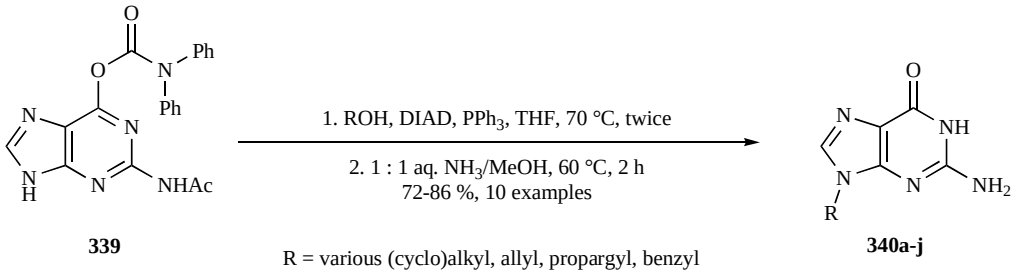
Kumar *et al*. [136] have observed that the non-catalyzed and Lewis acid-catalyzed reactions of N^2,9-diacetylguanine (**330**) with 2-oxa-1,4-butanediol diacetate (**331**) to mainly give N-9 (**332**) and N-7 (**333**) isomers, respectively, seem to follow different mechanisms Scheme (**60**). The non-catalyzed reaction likely proceeds through activation of the electrophile that is achieved with the help of the N^2- and 9-acetyl groups of (**330**) before giving the desired product through a six-membered transition state. This mechanism receives support from the important observation that N^2-acetylguanine does not react under identical conditions. The acid-catalyzed reaction seems to start by alkylation with a carbocation, when almost equimolar amounts of $TiCl_4$ and (**331**) are used, at N-7 while releasing the 9-acetyl group in the form of Ac_2O. The practical utility of the non-catalyzed reaction, which gives almost quantitative yields of N-9 derivatives, is demonstrated by synthesizing acyclovir (**8**) in 95% yield for the alkylation step.

Constraining guanine into its 6-lactim form by equipping with bulky substituents [2-(4-nitrophenyl(ethyl), *N,N*-diphenylcarbamoyl] often results in an increased ratio of 9/7-regioisomers. In fact, guanines protected with *N,N*-diphenylcarbamoyl groups at O-6 give excellent results in the synthesis of 9-glycosylguanines under acidic conditions of glycosylation (ratio of 9- to 7-substituted products may reach 100 : 1) [137]. Alkylation, however, affords diminished ratios. We have observed [138] that alkylation of N^2-isobutyryl-O^6-(*N,N*-diphenylcarbamoyl)guanine with *tert*-butyl bromoacetate (NaH, DMF, 0 °C) gave 1 : 1 ratio of the corresponding 9- and 7-isomers. The best ratio obtained for 9-/7-methoxycarbonymethylguanines was 5 : 1 when *N,N*-diisopropylethylamine and methyl bromoacetate were employed in the same reaction. The application of *tert*-butyl glycolate and various phosphines under Mitsunobu conditions gave 31-36% yield of 9-substituted guanines largely owing to the difficult chromatographic purification.

The Mitsunobu reaction was also used by Tsai *et al*. [139] to obtain nucleobase-substituted 1,6-heptadienes for cyclization and cyclopolymerization studies. The corresponding guanine derivative was synthesized from 1,6-heptadien-4-ol and N^2-isobutyryl-O^6-[2-(4-nitrophenyl (ethyl)]guanine (37% yield). van Hest *et al*. [140] have reported the synthesis and application in atom transfer radical polymerization of methacrylate monomers functionalized with nucleobases and prepared them by alkylation of the nucleobases with 3-bromopropyl methacrylate (for thymine, adenine and cytosine) or 3-hydroxypropyl methacrylate (for guanine) Scheme (**61**). Thus, for selective N-9 alkylation,



Scheme 61.



339 **340a-j**

Scheme 62.

N^2-phenylacetyl-O^6-[2-(4-nitrophenyl)ethyl]guanine (**335**) was chosen to overcome the problem of unwanted hydrolysis of the methacrylate ester function in the final monomer. Therefore, freshly prepared phenylacetic anhydride was condensed with guanine (**1**) in refluxing DMF, followed by acetylation at N-9 to obtain 9-acetyl-*N*-phenylacetylguanine (**334**) (85% overall yield). Subsequent Mitsunobu coupling with 2-(4-nitrophenyl)ethanol, followed by N-9-deacetylation upon refluxing in a water/ethanol mixture gave O^6-[2-(4-nitrophenyl)ethyl] derivative (**335**) (87% overall yield). 3-Hydroxypropylmethacrylate, prepared by esterification of 1,3-propanediol with methacryloyl chloride, was allowed to react under the conditions of a second Mitsunobu condensation under strictly anhydrous conditions resulting in the protected guanine methacrylate ester (**336**). Smooth deprotection of the 2-(4-nitrophenyl)ethyl group with DBU via (**337**), followed by enzymatic removal of the phenylacetyl protective group using penicillin G acylase, resulted in the unprotected 3-(guanin-9-yl)propyl methacrylate (**338**) in an overall yield of 24%.

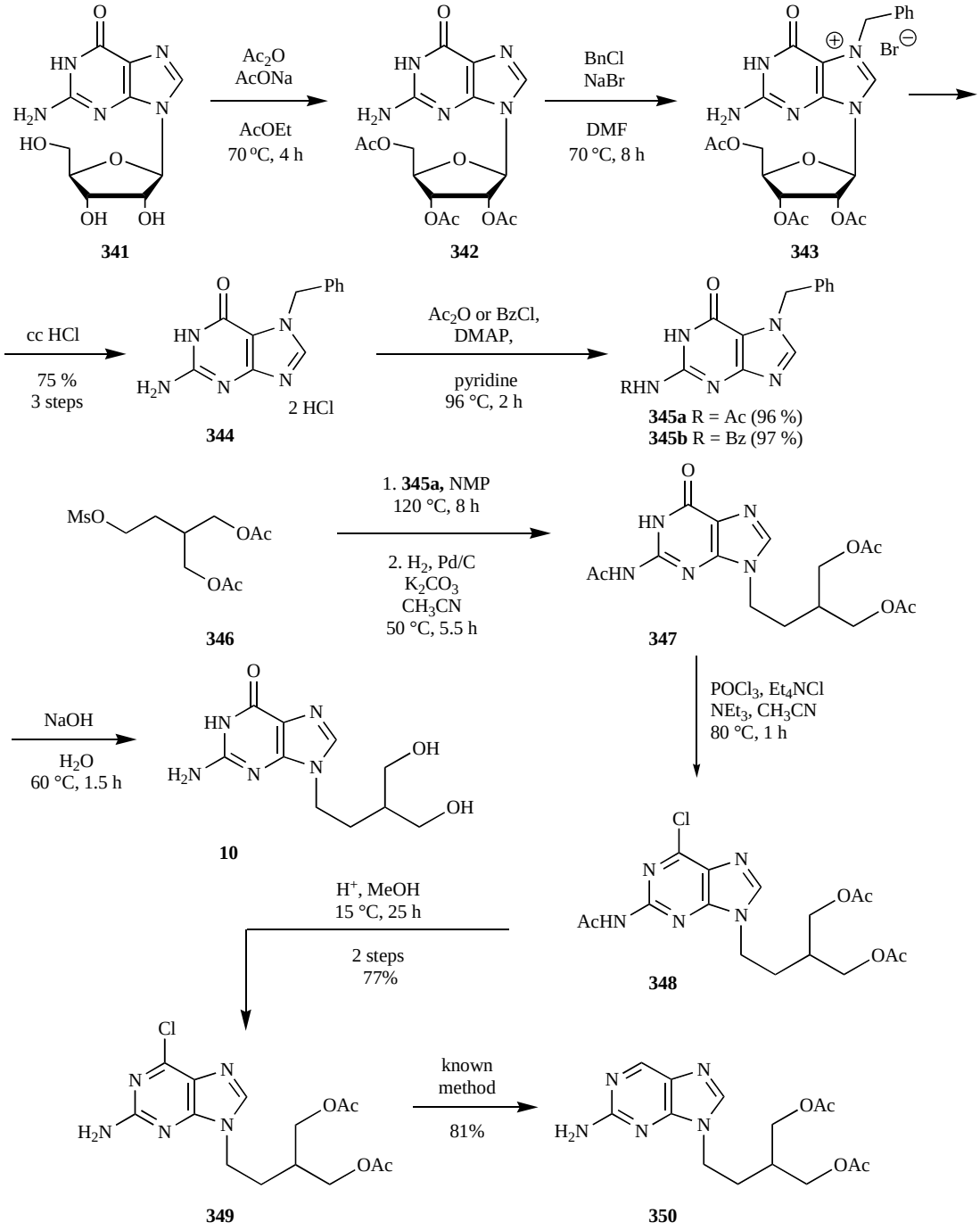
Shi *et al.* [141] have optimized the Mitsunobu coupling of guanine derivatives and different alcohols to gain 9-alkylpurines with excellent N-9 selectivity and good-to-excellent yields Scheme (**62**). To increase the solubility of purine bases in anhydrous THF, the best solvent for the Mitsunobu reaction, the reaction was carried out at 70 °C and the lipophilic derivative N^2-acetyl-O^6-(*N*,*N*-diphenylcarbamoyl)guanine (**339**) was used in model reactions with 2-*O*-(*tert*-butyldimethylsilyl)ethane-1,2-diol to give the 9-alkylguanine in a moderate yield. An alternative reaction condition includes the addition of one more equivalent of the activated alcohol after 6 h of reaction and this method gave the desired product in 93 % yield in THF. Using the optimized conditions, different primary, secondary, allylic, propargylic and benzylic alcohols were used in the Mitsunobu reactions to afford 9-alkylguanines (**340a-j**) in 72-86% yield. The reactions proceeded with complete inversion for chiral alcohols. These transformation were extended to other purines and for 2,6-dichloropurine 7-alkyl compounds were observed as the minor products in some cases.

One of the best routes to obtain exclusively 9-(ar)alkylguanines relies on the easy formation of 7-(ar)alkylguanosines that afford 7-(ar)alkylguanines upon acidic hydrolysis. 7-(Ar)alkylguanines in turn can be alkylated exclusively at N-9. If the 7-substituent can conveniently be removed from the 7,9-disubstituted guanines then 9-(ar)alkylguanines can be accessed. For the latter purpose benzyl group and its derivatives are the most advantageous and this principle has been exploited by several groups. Izawa *et al*. have optimized the synthesis of 7-benzylguanine derivatives (**345a**) Scheme (**63**) [142]. Owing to the bad solubility of guanosine (**341**), 2',3',5'-tri-*O*-acetylguanosine (**342**) was used as starting material the benzylation of which was carried out at 70 °C with BnCl, preventing higher temperatures that can cause deglycosylation of guaninium salt (**343**). Acidic treatment of (**343**) afforded 7-benzylguanine (**344**) as its dihydrochloride in 75% yield for the three steps. For improving the solubility of (**344**), acetylation or benzoylation was carried out to give (**345a**) and (**345b**), respectively. For the synthesis of penciclovir (**10**), a potent and highly selective anti-viral agent, (**345a**) was alkylated using mesylate (**346**). After debenzylation and deacetylation, penciclovir (**10**) was synthesized in good yield without the purification of any intermediates from (**345a**). Famciclovir (**350**), a 6-deoxy prodrug of penciclovir, was also obtained from intermediate (**347**) by chlorination to afford (**348**). Finally, the *N*-selective deacetylation of (**348**) was accomplished under acidic conditions to give (**349**) in 77 % overall yield. Earlier, famciclovir (**350**) was obtained using the same approach with the application of a cyclopropane derivative [143].

Kobe *et al*. have elaborated a very similar approach to obtain 9- and 7- alkylguanines from 7- and 9-benzylguanine, respectively, through the corresponding guaninium salts [144]. It is noteworthy that transfer hydrogenation of 7,9-dibenzyl-N^2-acetylguaninium bromide (10% Pd/C, NH_4OOCH, MeOH, reflux) gave a mixture containing 9- and 7-benzylguanines in ratio 7 : 1 (80% yield) from which the 9-isomer could be obtained by filtration through Celite (91% yield).

Essentially the same principle has been used by Vidal *et al.* [145] to obtain 7-(ar)alkylguanines in 51-97% yield with 11 alkylating agents in DMA or acetic acid.

In our hands the debenzylation of 9-alkyl-7-benzylguanines to obtain 9-alkylguanine derivatives proved to be very capricious therefore 4-methoxy- and 4-nitrobenzyl derivatives were used instead Scheme (**64**) [146]. The 4-methoxybenzylguaninium salts resisted oxidative, catalytic or transfer hydrogenolytic or acidic removal. The 4-nitrobenzyl derivatives were deprotected with sodium dithionite in neutral medium to afford the peptide nucleic acid building block *tert*-butyl [N^2-(pent-4-enoyl)guanin-9-yl]-acetate (**355**) in 36% overall yield for four steps. This method avoids N-7 regioisomer formation, solubility problems and any chromatographic purification. The guaninium salts displayed unusually large coupling constants $^3J_{C\text{-}8,H\text{-}8}$ (> 220 Hz), typical for imidazolium substructures, and were

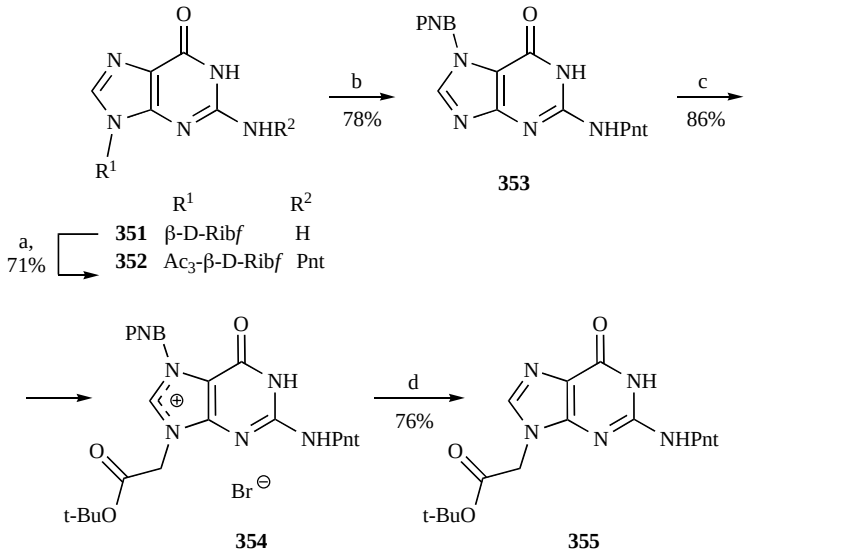


Scheme 63.

prone to undergo imidazole ring opening under basic conditions {*cf.* Scheme (**57**), *section 6.2* [133,142]}.

8-Bromoguanine was functionalized using its acid-labile 9-tetrahydrofuranyl derivative by Madre *et al.* [147]. Several derivatives protected with O^6-(*N*,*N*-diphenylcarbamoyl) and/or N^2-benzoyl, N^2-dimethylaminomethylene, 1-benzoyl, 1-benzyl, O^6-tosyl groups have been obtained. The tetrahydrofuranyl protecting group could be easily removed by mild acidic (*p*-TsOH/EtOH) or neutral (EtOH) treatment.

To circumvent the problem of often poor regioselectivity in direct alkylation of N^2- and/or N^2,O^6-disubstituted guanine derivatives the expensive and mutagenic 2-amino-6-chloropurine has also been used [96,148-150]. Toyokuni *et al.* have elaborated a selective and practical method for the synthesis of penciclovir in gram quantities involving a highly N-9 selective alkylation of 2-amino-6-chloropurine with 2-(2-phenyl-1,3-dioxane-5-yl)ethanol in the presence of K_2CO_3 in DMF at room temperature as a key step giving a 94% yield [148]. Holý *et al.* observed that methylation of 2-amino-6-chloropurine (MeI, NaH, DMF, 70 °C, 3 h) [*cf. section 5*, Scheme (**48**), (**273**) afforded 9- and 7-methyl derivatives in 65 and 9% yields, respectively [96]. Using 4 equiv. of dibenzyl carbonate and 2-amino-6-chloropurine, benzyl 2-



β-D-Rib*f* = β-D-ribofuranosyl, Ac_3-β-D-Rib*f* = 2',3',5'-tri-O-acetyl-β-D-ribofuranosyl, Pnt = pent-4-enoyl, PNB = 4-nitrobenzyl

(a) 1. TMSCl, pyridine, rt, 1 h; 2. 1.25 equiv. pent-4-enoic anhydride, pyridine, rt, 16 h; 3. water, 0–5 °C, 5 min; 4. aq. NH_3, rt, 30 min; 5. Ac_2O, pyridine, DMF, rt, 16 h;
(b) 4 equiv. 4-nitrobenzyl bromide, DMF, rt, 60 h;
(c) 3 equiv. *tert*-butyl bromoacetate, DMF, 70 °C, 16 h;
(d) 1. 4 equiv. $Na_2S_2O_4$, aq. acetone, pH 7.0, rt, 30 min; 2. 70 °C, 16 h.

Scheme 64.

amino-6-chloro-9*H*-purine-9-carboxylate was obtained in 26% yield by Howarth *et al.* [149].

8. SPECTROSCOPIC PROPERTIES AND MISCELLANEOUS ANALYTICAL STUDIES OF ALKYLGUANINES

Ascertaining the site of alkylation in alkylated guanines is not a trivial task. Among the spectroscopic methods UV is of limited use and usually restricted to special compound classes. Most often sophisticated MS [53] and 2D NMR methods (COSY, HSQC, HMBC, NOE) [15,43,95,96, 104,106,138] are invoked for unequivocal structural assignment.

Over the years we have developed three methods that are operationally simple and often require less efforts than the above methods. The statistical analysis of ^{13}C NMR chemical shift parameters of over 50 compounds [138,146] revealed that $\delta_{C\text{-}5}$ is the most sensitive to the N-9/N-7 substitution pattern (for the compounds studied $\delta_{C\text{-}5}$(N-9): 113.75–123.70 ppm; $\delta_{C\text{-}5}$(N-7): 104.56–115.09 ppm). However, due to the overlapping of chemical shift ranges for regioisomers (especially for glycosides) this chemical shift might not be sufficient for unambiguous assignment. The utility of differential parameters $a = \delta_{C\text{-}4}\text{-}\delta_{C\text{-}5}$, $b = \delta_{C\text{-}8}\text{-}\delta_{C\text{-}5}$, and $c = \delta_{C\text{-}5}\text{-}\delta_{C\text{-}1}$ was assessed and it was concluded that parameters *a*, *b* [both for (cyclo)alkyl and glycosylated derivatives] and *c* (for glycosylated derivatives) are useful for characterizing the N-9/N-7 substitution pattern of guanines. From a practical point of view the parameter *b* is the most convenient one as $\delta_{C\text{-}5}$ is unmistakable among the skeletal carbons and $\delta_{C\text{-}8}$ can simply be located in a *J*-modulated spin-echo experiment.

The study of protons and carbons conveys only indirect information about the nitrogen backbone, which is certainly the most sensitive to the pattern of N-9/N-7 substitution. The ^{15}N NMR chemical shifts of 12 N-7- and N-9-substituted purines were investigated systematically at the natural abundance level of the ^{15}N isotope [151]. The ^{15}N chemical shifts in purines (especially N-3) were found to be very sensitive to structural changes, for guanine regioisomers $\delta_{N\text{-}3}$ differed by at least 20 ppm, for guanine lactam and lactim structures differences of up to 60 ppm could be observed. The change in the chemical shielding of the N-3 nitrogen atom for an N-7/N-9 pair of regioisomers is associated mainly with a change in the $\sigma_{N\text{–}C\text{-}\pi^*}$ transition that dominates the δ_{22} component of the shift tensor.

N-9- and N-7-substituted (guaninyl)acetic esters were studied by electrospray ionization tandem mass spectrometry (ESI-MS/MS) in order to determine their ratio in alkylation reactions [152]. The intensity of ammonia loss is significantly different for the N-9- and N-7-alkylated guanine regioisomer pairs, deammoniation is characteristic for N-9 isomers. More importantly, the abundance of the $[MH\text{-}17]^+$ ion (ammonia loss) is in linear correlation with the N-9 isomer content. Therefore, the ratio of regioisomers can be determined in a mixture containing these compounds.

The analytical methods for obtaining reliable data on the quantity of different alkylguanines, particularly in biogenic samples, have also evolved. Wu *et al.* [153] have elaborated a sophisticated method for the analysis of endogeneous and exogeneous 7-methylguanine (**2**) levels using isotope dilution and gas chromatography/electron-capture negative chemical ionization mass spectrometry (GC/EC-ID-MS). [^{13}C-4]-Labeled 7-methylguanine was synthesized to serve

as an internal standard to improve accuracy of quantitation and 7-methylguanine (**2**) in tissue DNA can be measured using this GC/EC-ID-MS method with excellent sensitivity and specificity. Administration of an increasing amount of dacarbazine, a known chemotherapeutic agent, in mice led to dose-dependent increases in the formation of (**2**). The procedure used a sequential derivatization reaction of (**2**) (deamination and alkylation) to give 1,3-bis(pentafluorobenzyl)-7-methylxanthine. The electrophoric properties of the pentafluorobenzyl group allowed to develop an extremely sensitive method with detection limit in the 20-100 attomol range at a signal-to-noise (S/N) ratio >10.

Buszewski *et al.* [154] have investigated the influence of mobile phase pH and stationary phase on the separation of several modified nucleosides (e.g. 1- and 7-methylguanosine). 1-Methylguanosine showed a progressive increase in capacity coefficient (*k*) values with decreasing polar character of the packing material, the maximum value was observed in the case of cholesterol-modified silica gel. For 7-methylguanosine pH effects both retention and *k* values and the latter increases with elevating pH and also with stationary phase hydrophobicity, *k* was the largest for C_{18}-derivatized silica gel.

Wu *et al.* [101] have elaborated an isotope dilution LC/MS/MS method for measuring urinary levels of 7-ethylguanine in non-smokers and smokers with dietary control and they recorded a detection limit of 0.59 pg/ml (0.33 pmol) on-column with the use of an isotope-labeled internal standard [^{15}N-5]-7-ethylguanine and on-line enrichment techniques. The study demonstrated that cigarette smoke was highly responsible for the increased urinary excretion of 7-ethylguanine. It is estimated that there are 0.9–3.6 7-ethylguanine lesions per 10^7 dGp residues in DNA of non-tumorous lung tissues. An immunoslot blot technique used for the quantitation of 7-methyl-dGuo concentrations in cervical tissues also confirmed that smokers have ca. 3 times higher level of 7-methyl-dGuo lesions than non-smokers [100].

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