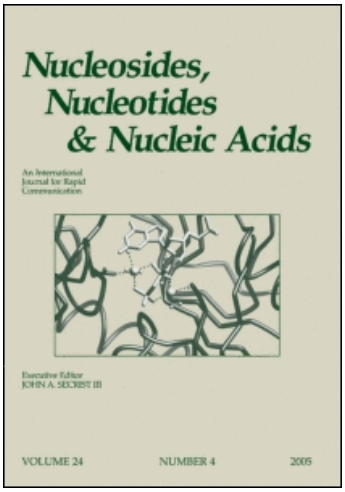

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SYNTHESIS AND PROPERTIES OF mRNA 5'-CAP ANALOGUES WITH 7-METHYLGUANINE REPLACED BY BENZIMIDAZOLE OR 3-METHYLBENZIMIDAZOLE.

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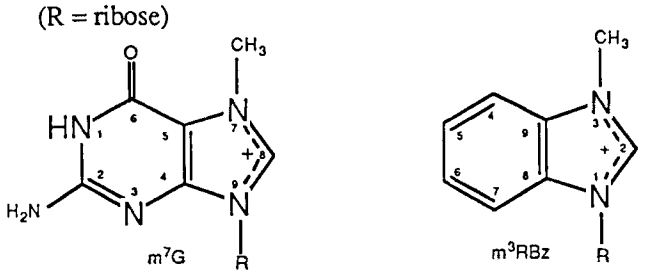
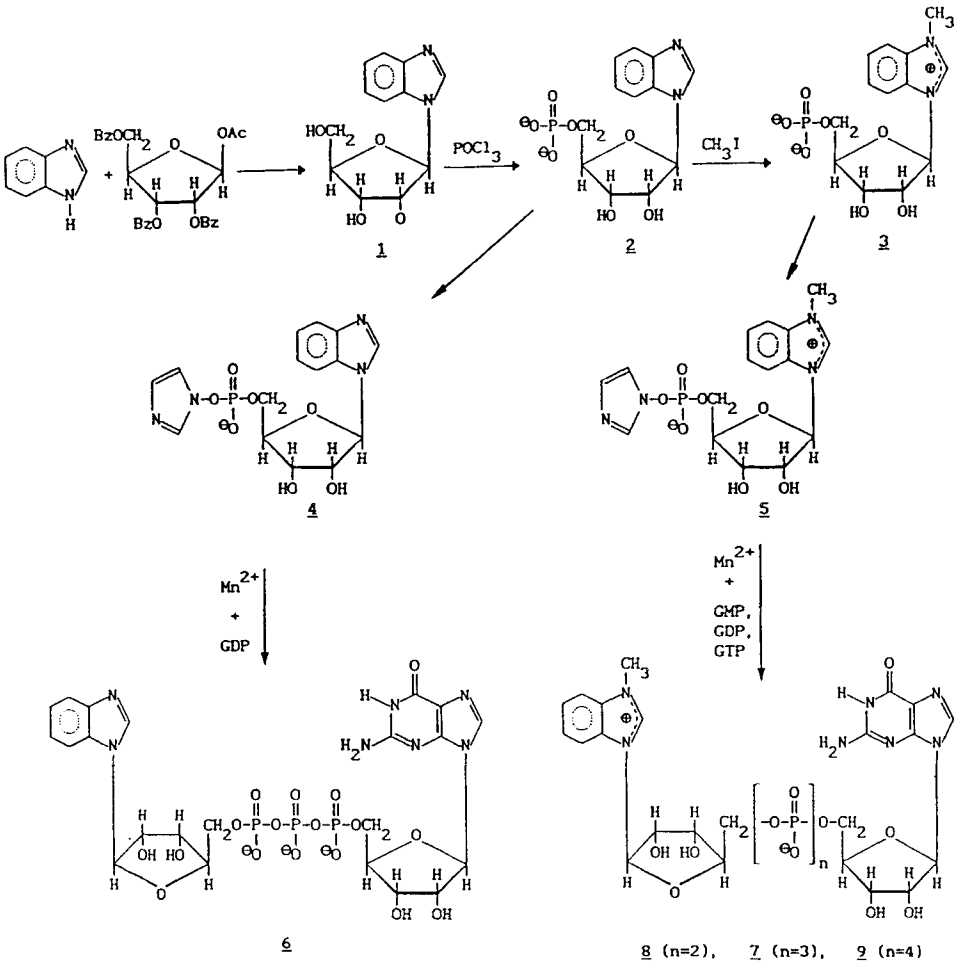
Abstract: Several new analogues of the mRNA 5'-cap structure, $m^7G(5')p_n(5')N$, with n=2-4, have been synthesized in which the m^7G component is replaced by 1-(β-D-ribofuranosyl)benzimidazole (RBz) or 3-methyl-RBz. The latter, like m^7G, has a positively charged imidazole ring and is likewise fluorescent. All compounds have been characterized by various physico-chemical and enzymatic criteria, and by ^1H and ^{31}P NMR spectroscopy .

The 5'-terminal cap of eukaryotic mRNA, $m^7G(5')ppp(5')N$, is necessary for optimal protein translation, pre-mRNA splicing and efficient transport of mRNA from nucleus to the cytoplasm. In a continuation of ongoing studies aimed at development of potentially useful cap analogues for examining the nature of the interactions between the mRNA cap and specific protein factors (e.g. cap binding proteins, CBP), we have prepared a series of cap analogues in which the m^7G component has been replaced by 1-β-D-ribofuranosylbenzimidazole (RBz) or by 3-methyl-RBz (m^3RBz). Neither of these is capable of base pairing. Furthermore, m^3RBz partially mimics m^7G in that it also carries a positive charge on the imidazole ring (Scheme 1) and is highly fluorescent.

The synthetic procedures are depicted in the accompanying Scheme 2. RBz (1) was prepared according to the procedure of Niedballa & Vorbruggen[1] from benzimidazole and 1-O-acetyl-2,3,5-tri-O-benzoyl-β-ribofuranose. The product was phosphorylated according to the procedure of Yoshikawa[2] to give the 5'-phosphate, RBz-5'-phosphate (2). Methylation of 2 with methyl iodide led to m^3RBZ-5'-



Scheme 1



Scheme 2

Table 1. TLC and HPLC characteristics of products:
R_f values on cellulose F_{254} plates (Merck) with 2 solvents:
A: saturated ammoniumsulfate, potassium hydrogenphosphate (0.1M) pH 7.4, 2-propanol (79:19:2 v/v/v)
B: aqueous ammonium sulfate (1%, w/v), 2-propanol (1:2 v/v)
R_T were measured on Spectra-Physics HPLC equipment with Supelco LC-18-T column in 0-100% B gradient in 15 minutes. Mobile phases:
A: 0.1M KH_2PO_4 at pH 6.0
B: 75% 0.1M KH_2PO_4 pH 6.0 + 25% MeOH
Flow rate 1.3 ml/min. Detection by UV absorption at 260nm.

Compound	R_f with solvent		R_T [min]
	A	B	
RBzMP (2)	0.45	0.39	13.73
RBzpppG (6)	0.26	0.13	10.72
RBzTP (10)	0.56	0.19	9.39
m^3RBzMP (3)	0.63	0.36	9.79
m^3RBzppG (8)	0.41	0.11	9.49
m^3RBzpppG (7)	0.44	0.06	8.75
m^3RBzppppG (9)	0.50	0.03	8.61
m^3RBzTP (11)	0.78	0.10	8.42

Table 2. Spectroscopic data of products at pH 7:

Compound	λ_{max}	$\varepsilon_{max} \times 10^3$
RBz [a]	281	3.5
	273	3.7
	245	6.8
RBzMP	279.8	3.3
	272.5	3.7
	245.2	7.4
RBzpppG	250.4	19.8
RBzTP	280.1	3.3
	272.4	3.8
	245.3	7.0
m^3RBzMP	276.5	5.9
	269.5	7.0
	263.2	6.3
	255.7	6.1
m^3RBzppG	254.9	21.4
m^3RBzpppG	254.7	21.0
m^3RBzppppG	269.0	17.4
	254.0	21.0
m^3RBzTP	276.4	5.8
	269.6	6.9

[a] at pH 8.7

phosphate (3). The two monophosphates 2 and 3, on treatment with phosphatase or 5'-nucleotidase, were quantitatively converted to the parent nucleosides 1 and m^3RBz, respectively.

The anhydrous triethylammonium salts of 2 and 3 were, in turn, converted by the procedure of Lohrmann & Orgel[3] to the phosphorimidazolidates 4 and 5 in high yield. These were then condensed according to the recently improved procedure of Sawai et al.[4,5] with an equimolar mixture of GDP in the presence of $MnCl_2$ in 0.2M N-ethylmorpholine buffer pH 7 to give P^1-RBz-P^3-Guo-5',5'-triphosphate (6, 29% yield) and P^1-m^3RBz-P^3-Guo-5',5'-triphosphate (7, 23% yield). Condensation of 5 with equimolar mixtures of $MnCl_2$ and the tributylammonium salts of GMP and GTP, respectively, gave P^1-m^3RBz-P^2-Guo-5',5'-diphosphate (8, 11.5% yield) and P^1-m^3RBz-P^4-Guo-5',5'-tetraphosphate (9, 14% yield)

The triphosphates of RBz (10) and m^3RBz (11) were also prepared from 4 and 5, respectively, by condensation with anhydrous tributylammonium pyrophosphate in DMF, according to Hoard & Ott[6] in yields of 60%.

The products 2, 3 and 6-11, were isolated by gradient elution with triethylammonium bicarbonate on a DEAE-Sephadex A25 (HCO_3^-) column. Compound 7 required additional desalting on XAD resin. The products were then converted to their Na^+ salts on Dowex 50 Wx2. They were characterized by TLC, HPLC (Table 1) and UV spectroscopy (Table 2), phosphodiesterase digestion (which quantitatively released 2 from 6, and 3 from 7, 8 and 9), and ^1H and ^{31}P NMR spectroscopy .

NMR spectroscopy. ^1H NMR spectra were run on a Bruker AM 500 at 0.005 M in D_2O and chemical shifts recorded vs internal TSP. ^{31}P spectra were recorded under the same conditions on a Varian XL 200. and chemical shifts recorded vs external H_3PO_4. The structures 2, 3 and 8 were fully confirmed by the ^1H NMR spectra. In the case of 7 and 9 additional confirmation was provided by the ^{31}P spectra. For 7 (m^3RBzp_3G), P^1 and P^3 gave a common peak at -10.915 ppm, and P^2 at -22.405 ppm, while the mean value of the geminal coupling constants ^{31}P-^{31}P was 18.5 Hz. With 9 (m^3RBzp_4G) the phosphorus atoms form an AA'XX' system, with chemical shifts for P^1,P^4 of -10.890 ppm and for P^2,P^3 of -22.620 ppm, while coupling constants $J(P^1,P^2) = J(P^3,P^4) = 15.7$ Hz, $J(P^2,P^3) = 20.6$ Hz, and $J(P^1,P^3) = J(P^2,P^4) = 2.4$ Hz. Complete NMR data, and their application to conformational analysis of the products, will be described elsewhere. It is worth noting that the conformations of caps with an m^3RBz component were similar to the corresponding ones with an m^7G component.

Fluorescence emission properties. Both RBz and m^3RBz, as well as their nucleotides, exhibit appreciable fluorescence, e.g. for m^3RBzMP (3) excitation at 255 nm leads to an emission band at 370 nm with a quantum yield of 0.30. With the cap analogues containing RBz (e.g. 6) or m^3RBz (7, 8, 9) the emission is strongly quenched as a result of base stacking, so that enzymatic cleavage of the oligophosphate bridge leads to an almost 10-fold increase in fluorescence emission, a highly sensitive method for following the kinetics of the reactions.

The biological properties of the foregoing new cap analogues are presently under investigation.

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