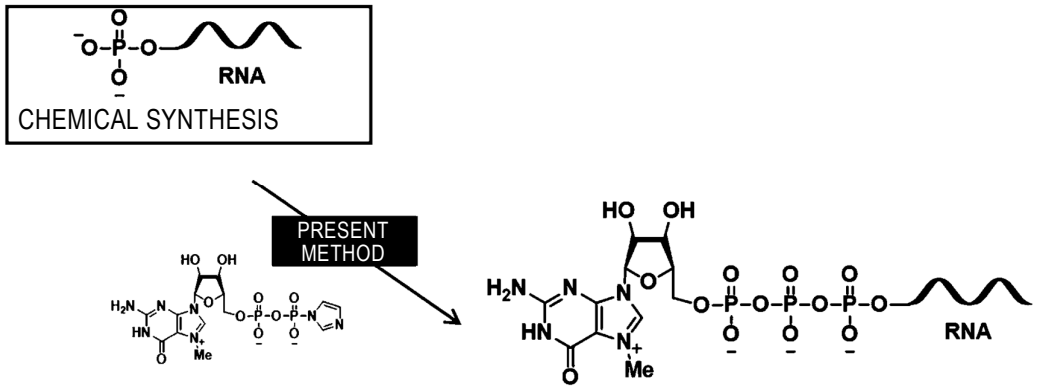
[FIG. 1]

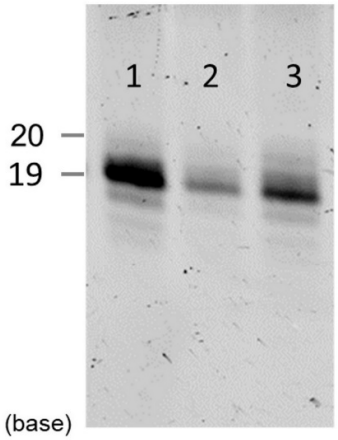


[FIG. 2]

(a)

	ul	final conc.
5'PO-RNA	1.02	40 uM
compound **13**	2	5 mM
ZnCl2	4	10 mM
anh. DMSO	40	

(b)



Analyzed by 15% denaturing PAGE
(7.5 M urea, 20% formamide, 1× TBE).
20W const. 1.5 h
The gel was stained w. SYBR Green II.

(c)

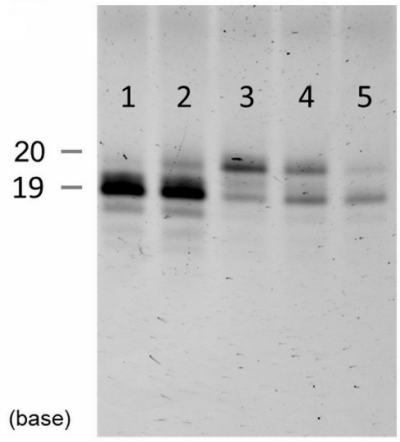
Lane	Temp. (°C)	yield (%)
1	r.t.	–
2	37	–
3	55	22

[FIG. 3]

(a)

	ul	final conc.
5'PO-RNA	1.02	40 uM
compound **13**	2	5 mM
MCl2	4	10 mM
anh. DMSO	40	

(b)



Analyzed by 15% denaturing PAGE
(7.5 M urea, 20% formamide, 1× TBE).
20W const. 1.5 h
The gel was stained w. SYBR Green II.

(c)

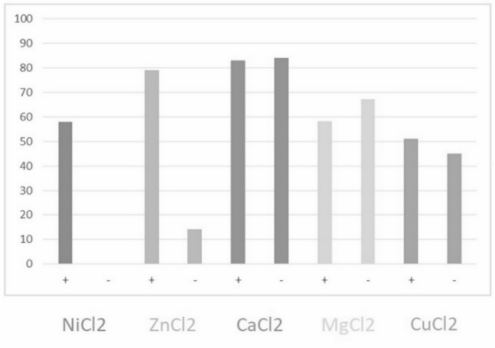
Lane	MCl_2	yield (%)
1	Ni	12
2	Zn	22
3	Ca	68
4	Mg	52
5	Cu	37

[FIG. 4]

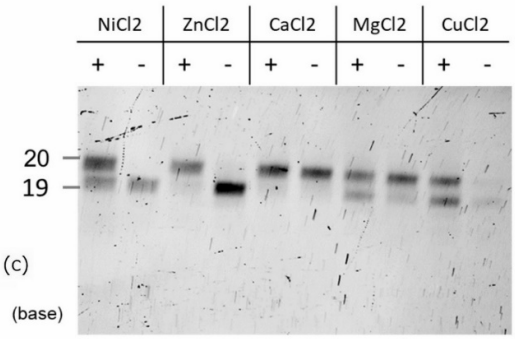
(a)

	final conc. 1	final conc. 2	
5'PO-RNA	40		(uM)
compound **13**	5		(mM)
CaCl2	10		(mM)
1-methyl imidazole	0	1	(M)
anh. DMSO	36	32	(ul)
total	40	40	(ul)

(d)



(b)



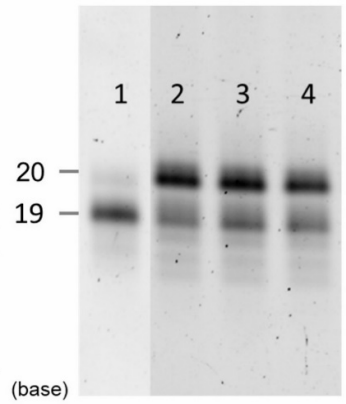
MCl_2		yield (%)
Ni	+	58
	−	0
Zn	+	79
	−	14
Ca	+	83
	−	84
Mg	+	58
	−	67
Cu	+	51
	−	45

Analyzed by 15% denaturing PAGE
(7.5 M urea, 20% formamide, 1× TBE).
20W const. 1.5 h
The gel was stained w. SYBR Green II.

[FIG. 5]

	1	2	3	4	
		final conc.			
5'PO-RNA		100			(uM)
compound **13**		5			(mM)
CaCl2		10			(mM)
1-methyl imidazole	0	0.5	1	2	(M)
anh. DMSO	40	38	36	32	(ul)
total		40			(ul)

lane	1-methyl imidazole (mM)	yield (%)
1	0	26
2	0.5	64
3	1	61
4	2	62

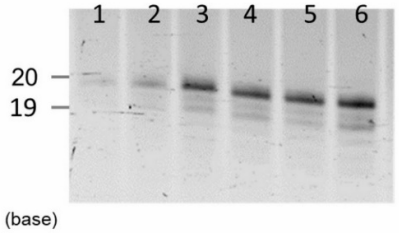


Analyzed by 15% denaturing PAGE
(7.5 M urea, 25% formamide, 1× TBE).
20W const. 1.5h
The gel was stained w. SYBR Green II.

[FIG. 6]

	1	2	3	4	5	6	
			final conc.				
5'PO-RNA	2	4	8	20	40	80	(uM)
compound **13**			5				(mM)
CaCl2			10				(mM)
1-methyl imidazole			1				(M)
anh. DMSO			34				(ul)
total			40				(ul)

lane	RNA (uM)	yield (%)
1	2	78
2	4	78
3	8	81
4	20	73
5	40	77
6	80	72



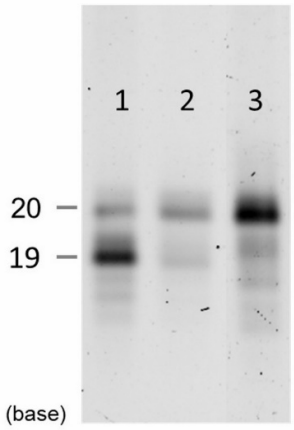
Analyzed by 10% denaturing PAGE
(7.5 M urea, 25% formamide, 1× TBE).
20W const. 1.0 h
The gel was stained w. SYBR Green II.

[FIG. 7]

	ul	final conc.
5'PO-RNA	3.44	100 uM
compound **13**	2	5 mM
CaCl2	4	10 mM
1-methyl imidazole	4	1 M
slovent	40	

lane	yield (%)
1	28
2	70
3	78

1 : anh. DMSO/MQ = 1/1
2 : anh. DMSO only
3 : MQ 3.44 ul + anh. DMSO

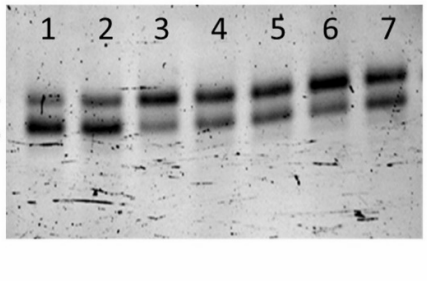


Analyzed by 15% denaturing PAGE
(7.5 M urea, 25% formamide, 1× TBE).
20W const. 1.5 h
The gel was stained w. SYBR Green II.

[FIG. 8]

	1	2	3	4	5	6	7	
				final conc.				
5'PO-RNA				40				(uM)
compound **13**				5				(mM)
CaCl2	0	0.1	1	10	20	30	40	(mM)
1-methyl imidazole				1				(M)
anh. DMSO				35				(ul)
total				40				(ul)

lane	CaCl2 (uM)	yield (%)
1	0	41
2	0.1	41
3	1	67
4	10	60
5	20	58
6	30	64
7	40	54

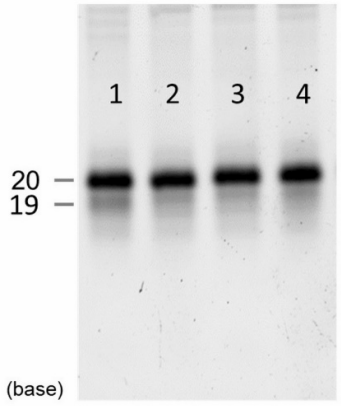


Analyzed by 15% denaturing PAGE
(7.5 M urea, 25% formamide, 1× TBE).
20W const. 1.0 h
The gel was stained w. SYBR Green II.

[FIG. 9]

	1 ul	2 ul	3 ul	4 ul	final conc.
5'PO-RNA	3.44	3.44	3.44	3.44	100 uM
compound **13**	2	4	8	12	5-30 mM
CaCl2	4	4	4	4	10 mM
1-methyl imidazole	4	4	4	4	1 mM
anh. DMSO	32.56	32.56	32.56	32.56	

compound 13 (mM)	yield (%)
5	68
10	79
20	84
30	82



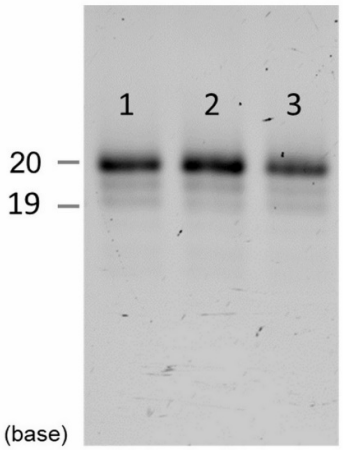
Analyzed by 10% denaturing PAGE
(7.5 M urea, 25% formamide, 1× TBE).
20W const. 1.0 h
The gel was stained w. SYBR Green II.

[FIG. 10]

		final conc.	
5'PO-RNA		40	(uM)
compound **13**		10	(mM)
CaCl2		10	(mM)
1-methyl imidazole		1	(M)
anh. DMSO	36		(ul)
total	40		(ul)

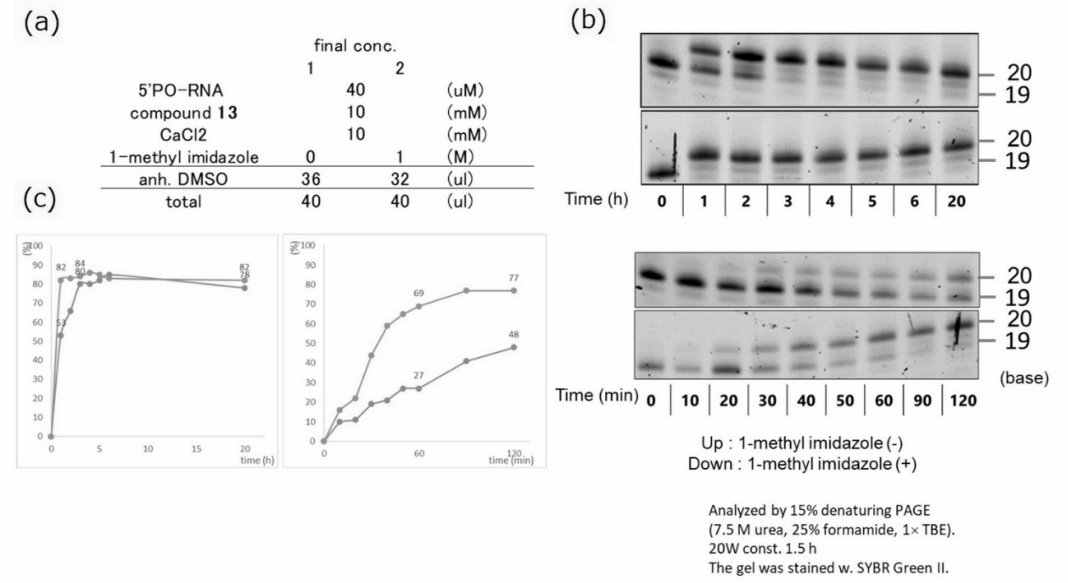
1 : 5'-PO RNA
2 : compound 13
3 : $CaCl_2$

lane	yield (%)
1	81
2	83
3	80

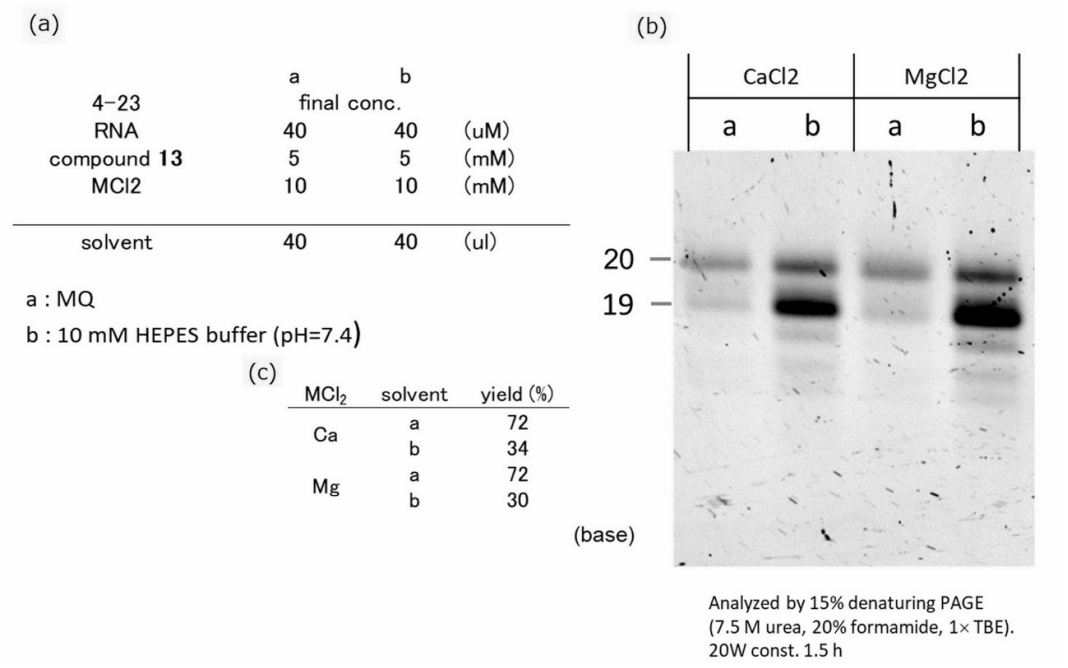


Analyzed by 15% denaturing PAGE
(7.5 M urea, 25% formamide, 1× TBE).
20W const. 1.5 h
The gel was stained w. SYBR Green II.

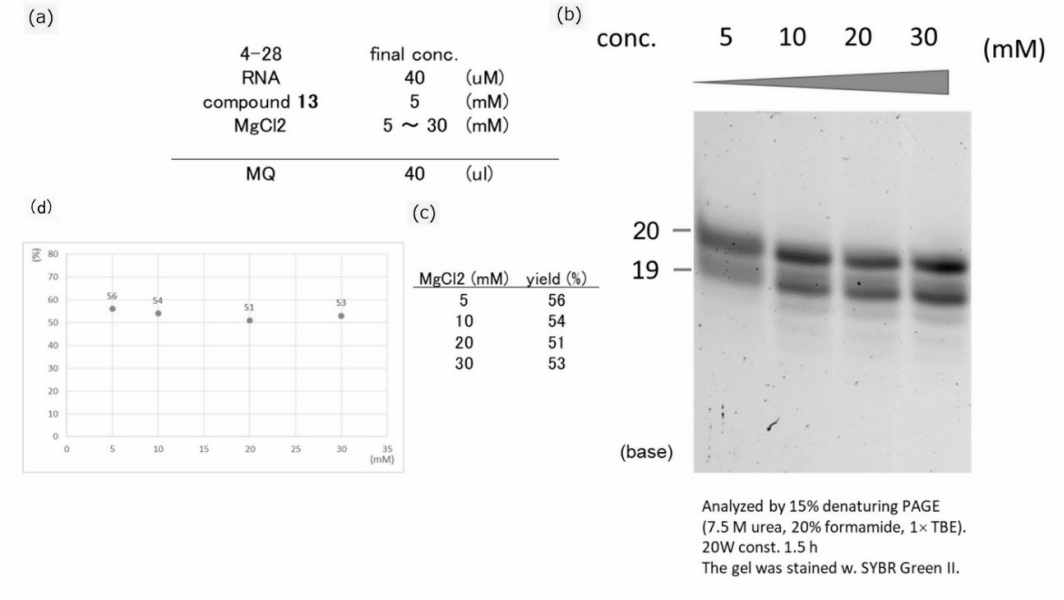
[FIG. 11]


(a)
final conc.
1 2
5'PO-RNA 40 (uM)
compound 13 10 (mM)
CaCl2 10 (mM)
1-methyl imidazole 0 1 (M)
anh. DMSO 36 32 (ul)
total 40 40 (ul)
(b)
20
19
20
19
Time (h) 0 1 2 3 4 5 6 20
20
19
20
19
(base)
Time (min) 0 10 20 30 40 50 60 90 120
Up : 1-methyl imidazole (-)
Down : 1-methyl imidazole (+)
Analyzed by 15% denaturing PAGE
(7.5 M urea, 25% formamide, 1× TBE).
20W const. 1.5 h
The gel was stained w. SYBR Green II.
(c)
time (h)
time (min)

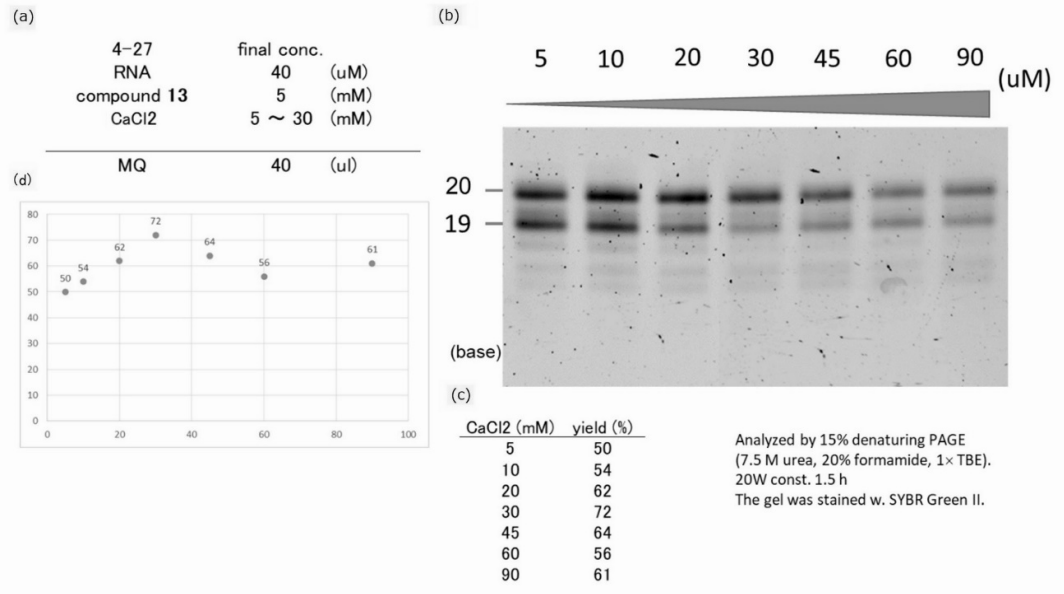
[FIG. 12]


(a)
a b
4-23 final conc.
RNA 40 40 (uM)
compound 13 5 5 (mM)
MCl2 10 10 (mM)
solvent 40 40 (ul)
a : MQ
b : 10 mM HEPES buffer (pH=7.4)
(b)
CaCl2 MgCl2
a b a b
20
19
(base)
Analyzed by 15% denaturing PAGE
(7.5 M urea, 20% formamide, 1× TBE).
20W const. 1.5 h
(c)
MCl2 solvent yield (%)
Ca a 72
b 34
Mg a 72
b 30

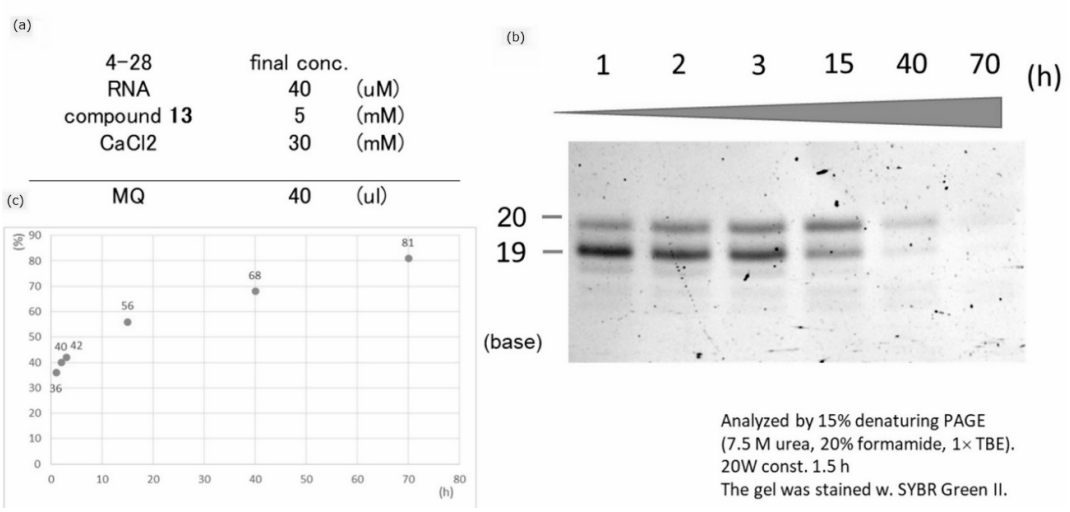
[FIG. 13]


(a)
4-28 final conc.
RNA 40 (uM)
compound 13 5 (mM)
MgCl2 5 ～ 30 (mM)
MQ 40 (ul)
(b)
conc. 5 10 20 30 (mM)
20
19
(base)
Analyzed by 15% denaturing PAGE
(7.5 M urea, 20% formamide, 1× TBE).
20W const. 1.5 h
The gel was stained w. SYBR Green II.
(c)
MgCl2 (mM) yield (%)
5 56
10 54
20 51
30 53
(d)
(%)
(mM)

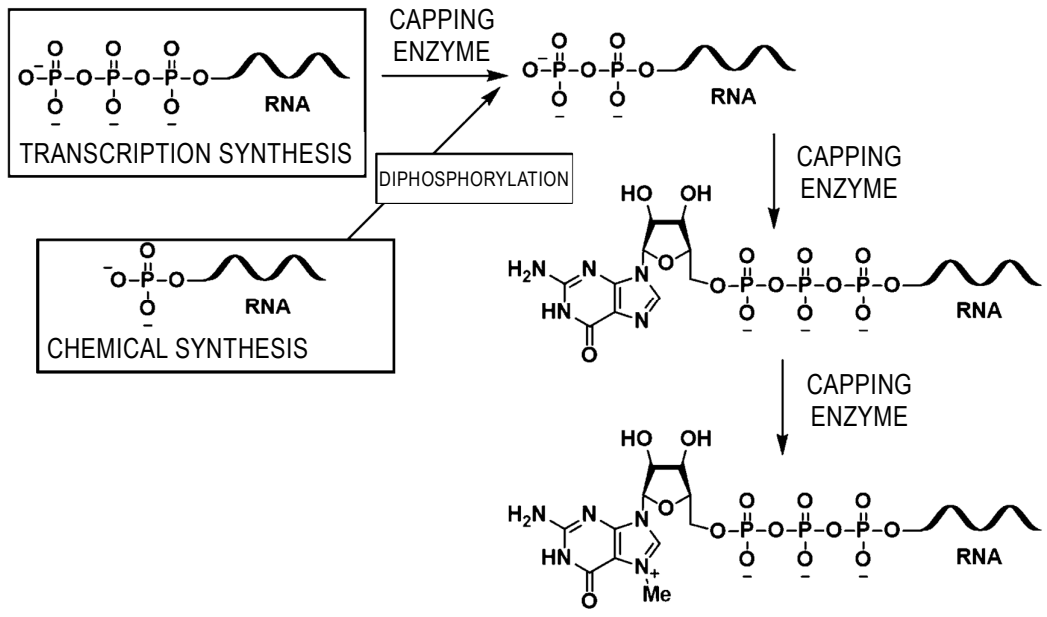
[FIG. 14]


(a)
4-27 final conc.
RNA 40 (uM)
compound 13 5 (mM)
CaCl2 5 ～ 30 (mM)
MQ 40 (ul)
(b)
5 10 20 30 45 60 90 (uM)
20
19
(base)
(c)
CaCl2 (mM) yield (%)
5 50
10 54
20 62
30 72
45 64
60 56
90 61
Analyzed by 15% denaturing PAGE
(7.5 M urea, 20% formamide, 1× TBE).
20W const. 1.5 h
The gel was stained w. SYBR Green II.
(d)

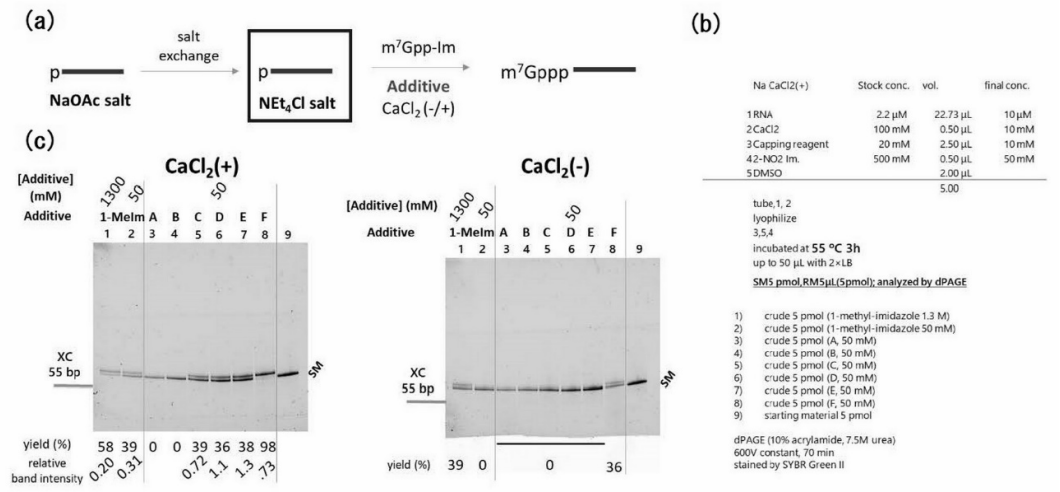
[FIG. 15]


(a)
4-28 final conc.
RNA 40 (uM)
compound 13 5 (mM)
CaCl2 30 (mM)
MQ 40 (ul)
(c)
(%)
(h)
81
68
56
40 42
36
(b)
1 2 3 15 40 70 (h)
20
19
(base)
Analyzed by 15% denaturing PAGE
(7.5 M urea, 20% formamide, 1× TBE).
20W const. 1.5 h
The gel was stained w. SYBR Green II.

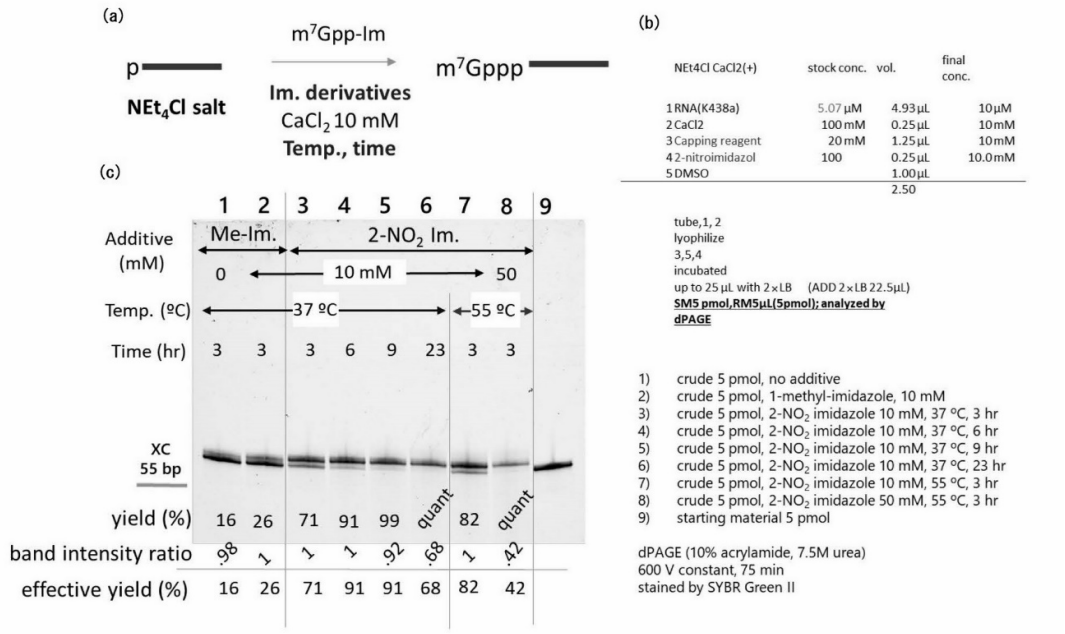
[FIG. 16]


TRANSCRIPTION SYNTHESIS
RNA
CAPPING ENZYME
DIPHOSPHORYLATION
CHEMICAL SYNTHESIS
CAPPING ENZYME
CAPPING ENZYME
Me

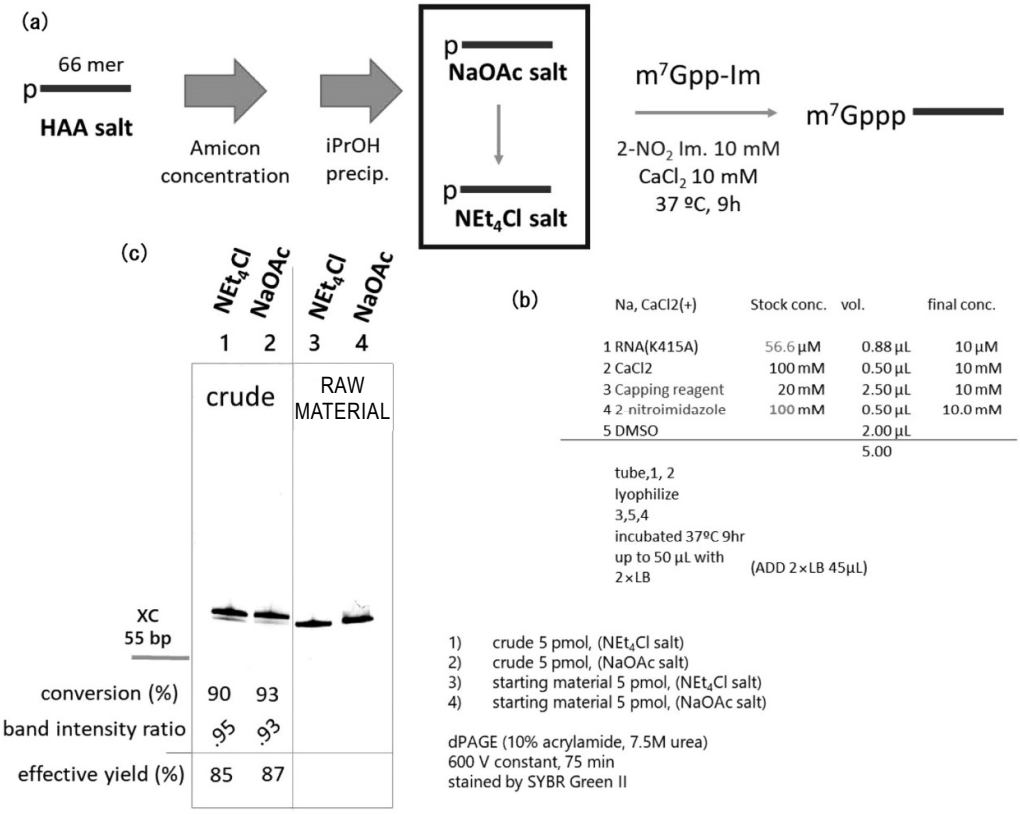
[FIG. 17]


(a)
salt exchange
p
NaOAc salt
p
NEt4Cl salt
m7Gpp-Im
Additive
CaCl2 (-/+)
m7Gppp
(b)
Na CaCl2(+) Stock conc. vol. final conc.
1 RNA 2.2 µM 22.73 µL 10 µM
2 CaCl2 100 mM 0.50 µL 10 mM
3 Capping reagent 20 mM 2.50 µL 10 mM
4 2-NO2 Im. 500 mM 0.50 µL 50 mM
5 DMSO 2.00 µL
5.00
tube,1, 2
lyophilize
3,5,4
incubated at 55 °C 3h
up to 50 µL with 2×LB
SM5 pmol,RM5µL(5pmol); analyzed by dPAGE
1) crude 5 pmol (1-methyl-imidazole 1.3 M)
2) crude 5 pmol (1-methyl-imidazole 50 mM)
3) crude 5 pmol (A, 50 mM)
4) crude 5 pmol (B, 50 mM)
5) crude 5 pmol (C, 50 mM)
6) crude 5 pmol (D, 50 mM)
7) crude 5 pmol (E, 50 mM)
8) crude 5 pmol (F, 50 mM)
9) starting material 5 pmol
dPAGE (10% acrylamide, 7.5M urea)
600V constant, 70 min
stained by SYBR Green II
(c)
CaCl2(+)
[Additive] (mM)
Additive
1300 50 50
1-MeIm A B C D E F
1 2 3 4 5 6 7 8 9
XC 55 bp
SM
yield (%) 58 39 0 0 39 36 38 98
relative band intensity 0.20 0.31 0.72 1.1 1.3 .73
CaCl2(-)
[Additive] (mM)
Additive
1300 50 50
1-MeIm A B C D E F
1 2 3 4 5 6 7 8 9
XC 55 bp
SM
yield (%) 39 0 0 36

[FIG. 18]


(a)
m7Gpp-Im
p
NEt4Cl salt
Im. derivatives
CaCl2 10 mM
Temp., time
m7Gppp
(b)
NEt4Cl CaCl2(+) stock conc. vol. final conc.
1 RNA(K438a) 5.07 µM 4.93 µL 10 µM
2 CaCl2 100 mM 0.25 µL 10 mM
3 Capping reagent 20 mM 1.25 µL 10 mM
4 2-nitroimidazol 100 0.25 µL 10.0 mM
5 DMSO 1.00 µL
2.50
tube,1, 2
lyophilize
3,5,4
incubated
up to 25 µL with 2×LB (ADD 2×LB 22.5µL)
SM5 pmol,RM5µL(5pmol); analyzed by dPAGE
1) crude 5 pmol, no additive
2) crude 5 pmol, 1-methyl-imidazole, 10 mM
3) crude 5 pmol, 2-NO2 imidazole 10 mM, 37 °C, 3 hr
4) crude 5 pmol, 2-NO2 imidazole 10 mM, 37 °C, 6 hr
5) crude 5 pmol, 2-NO2 imidazole 10 mM, 37 °C, 9 hr
6) crude 5 pmol, 2-NO2 imidazole 10 mM, 37 °C, 23 hr
7) crude 5 pmol, 2-NO2 imidazole 10 mM, 55 °C, 3 hr
8) crude 5 pmol, 2-NO2 imidazole 50 mM, 55 °C, 3 hr
9) starting material 5 pmol
dPAGE (10% acrylamide, 7.5M urea)
600 V constant, 75 min
stained by SYBR Green II
(c)
1 2 3 4 5 6 7 8 9
Additive
Me-Im. 2-NO2 Im.
(mM)
0 10 mM 50
Temp. (ºC) 37 ºC 55 ºC
Time (hr) 3 3 3 6 9 23 3 3
XC 55 bp
yield (%) 16 26 71 91 99 quant 82 quant
band intensity ratio .98 1 1 1 .92 .68 1 .42
effective yield (%) 16 26 71 91 91 68 82 42

[FIG. 19]


(a)
66 mer
p
HAA salt
Amicon concentration
iPrOH precip.
p
NaOAc salt
p
NEt4Cl salt
m7Gpp-Im
2-NO2 Im. 10 mM
CaCl2 10 mM
37 ºC, 9h
m7Gppp
(c)
NEt4Cl
NaOAc
NEt4Cl
NaOAc
1 2 3 4
crude
RAW MATERIAL
XC
55 bp
conversion (%) 90 93
band intensity ratio 95 93
effective yield (%) 85 87
(b)
Na, CaCl2(+) Stock conc. vol. final conc.
1 RNA(K415A) 56.6 µM 0.88 µL 10 µM
2 CaCl2 100 mM 0.50 µL 10 mM
3 Capping reagent 20 mM 2.50 µL 10 mM
4 2-nitroimidazole 100 mM 0.50 µL 10.0 mM
5 DMSO 2.00 µL
5.00
tube,1, 2
lyophilize
3,5,4
incubated 37ºC 9hr
up to 50 µL with 2×LB
(ADD 2×LB 45µL)
1) crude 5 pmol, (NEt4Cl salt)
2) crude 5 pmol, (NaOAc salt)
3) starting material 5 pmol, (NEt4Cl salt)
4) starting material 5 pmol, (NaOAc salt)
dPAGE (10% acrylamide, 7.5M urea)
600 V constant, 75 min
stained by SYBR Green II