

Trámite: 11
Evento: 379
Actuación: 457



No. 14-137284-00003-0000

Fecha: 2015-01-08 16:00:01 Dep. 2020 DIR.NUEVASOR
Tra. 11 PATENTEIYII Eve. 379 FASENACIONALII
Act 457 PROCEDIPPH Folios: 104

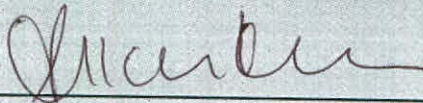
DIRECCIÓN DE NUEVAS CREACIONES
Procedimiento acelerado de exámenes de patente (pph)

1	NÚMERO DE SOLICITUD ANTE LA SIC	14-137.284	
2	OFICINA DE PRIMERA PRESENTACIÓN (OPP)	Oficina de Patentes y Marcas de Estados Unidos (USPTO)	
3	NÚMERO(S) DE SOLICITUD O PUBLICACIÓN DE LA(S) SOLICITUD(ES) (OPP):	1. Solicitud US 13/721,988	
4	☒ REPRESENTANTE LEGAL ☒ APODERADO		
APELLIDOS		NOMBRES	IDENTIFICACIÓN
LLOREDA RICAURTE		ALICIA	C.C. 39.690.713 T.P. 53.215
DIRECCIÓN	Calle 72 N° 5-83 Piso 5to	No. TELÉFONO	3264270
CIUDAD	Bogotá	CORREO ELECTRÓNICO	notificacionespatentes@lloredacamacho.com
PAÍS	Colombia	No. RADICACIÓN O PROTOCOLO DE PODER GENERAL	
5	CONDICIONES PARA ACCEDER A ESTE PROCESO:		
5.1.	DATOS DE PRIORIDAD ☒ SI ☐ NO		
(33) PAÍS DE ORIGEN		CÓDIGO PAÍS	(31) NÚMERO
1. Estados Unidos de América		US	61/579,560
2. Estados Unidos de América		US	61/613,836
			(32) FECHA (AAAA/MM/DD)
			2011/12/22
			2012/03/21
5.2.	DATOS SOLICITUD INTERNACIONAL PCT ☒ SI ☐ NO		
Solicitud Internacional No.		PCT/US2012/071063	Fecha 20/12/2012
Publicación Internacional No.		WO 2013/096679	Fecha 27/06/2013
El solicitante presenta una copia de la(s) reivindicación(es) patentable(s) u otorgable(s) de la solicitud de la Oficina de Primera Presentación que profirió el examen de patentabilidad, junto con una traducción al castellano. ☒ SI ☐ NO			
REQUISITOS PARA EL RECONOCIMIENTO DE LOS RESULTADOS DE EXÁMENES DE PATENTABILIDAD EFECTUADOS A SOLICITUDES EXTRANJERAS			
Para que la Superintendencia de Industria y Comercio pueda reconocer los resultados de exámenes de patentabilidad producidos por otras Oficinas de Propiedad Industrial se debe cumplir con las siguientes condiciones:			
(a) Al momento de solicitar el examen de patentabilidad, si el solicitante pide que se reconozcan exámenes de patentabilidad de otras oficinas extranjeras, deberá aportar junto con el pago de la tasa correspondiente, los siguientes anexos:			
(i) Copia de las resoluciones, fallos o decisiones que han sido relevantes para la determinación de la patentabilidad de la(s) solicitud(es) en la Oficina que haya proferido el examen de patentabilidad que contienen reivindicaciones patentables u otorgables que son la base de la petición, y si es del caso, su traducción al castellano. ☒ SI ☐ NO			
(ii) Copia de todas las reivindicaciones determinadas como patentables u otorgables en la Oficina que profirió el examen de patentabilidad y, si es del caso, su traducción al castellano. ☒ SI ☐ NO			

(iii) Copia de los antecedentes, información o documentos citados por el examinador de la Oficina de Primera Presentación del examen de patentabilidad, en las resoluciones, fallos o decisiones correspondientes, incluyendo la literatura no patente. Los documentos de patente deberán presentarse siempre y cuando no estén disponibles para la Superintendencia de Industria y Comercio mediante el simple acceso a bases de datos gratuitas. ☒ SI ☐ NO

(iv) Una tabla de correspondencias de las reivindicaciones que han sido determinadas como patentables u otorgables en la solicitud de la Oficina que haya proferido el examen de patentabilidad. ☒ SI ☐ NO

7	FIRMA DEL SOLICITANTE, DEL APODERADO O DEL REPRESENTANTE LEGAL <i>Junto a cada firma, indicar el nombre del firmante y su calidad (si tal calidad no es obvia al leer el petitorio)</i>
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ALICIA LLOREDA RICAURTE
(Representante – Apoderado)
C.C 39.690.713 T.P. 53.215

6	TABLA DE CORRESPONDENCIA DE REIVINDICACIONES		
	Reivindicaciones en la solicitud en Colombia 14-137.284	Reivindicaciones otorgables en la solicitud internacional US 13/721,988	Explicación sobre la suficiente correspondencia ó Comentarios que explican la correspondencia
	1	1	Las reivindicaciones son idénticas
	2	6	Parcialmente comprendida en la reivindicación 6 americana
	3	1	Parcialmente comprendida en la reivindicación 1 americana
	4	1	Parcialmente comprendida en la reivindicación 1 americana
	5	1 y 7	Parcialmente comprendida en las reivindicaciones 1 y 7 americanas
	6	1 y 7	Parcialmente comprendida en las reivindicaciones 1 y 7 americanas
	7	8	Parcialmente comprendida en la reivindicación 8 americana.
	8	8	Parcialmente comprendida en la reivindicación 8 americana.
	9	9	Parcialmente comprendida en la reivindicación 9 americana.
	10	8	Parcialmente comprendida en la reivindicación 8 americana
	11	9	Parcialmente comprendida en la reivindicación 9 americana
	12	9	Parcialmente comprendida en la reivindicación 9 americana
	13	1	Parcialmente comprendida en la reivindicación 1 americana
	14	1	Parcialmente comprendida en la reivindicación 1 americana.
	15	6	Parcialmente comprendida en la reivindicación 6 americana
	16	10	Parcialmente comprendida en la reivindicación 10 americana
	17	1 y 11	Parcialmente comprendida en las reivindicaciones 1 y 11 americanas
	18	1 y 11	Parcialmente comprendida en las reivindicaciones 1 y 11 americanas
	19	1 y 11	Parcialmente comprendida en las reivindicaciones 1 y 11 americanas
	20	1	Parcialmente comprendida en la reivindicación 1 americana
	21	1	Parcialmente comprendida en la reivindicación 1 americana

22	6	Parcialmente comprendida en la reivindicación 6 americana
23	12	Parcialmente comprendida en la reivindicación 12 americana
24	12	Parcialmente comprendida en la reivindicación 12 americana.
25	12	Parcialmente comprendida en la reivindicación 12 americana
26	1	Parcialmente comprendida en la reivindicación 1 americana
27	1	Parcialmente comprendida en la reivindicación 1 americana
28	2	Las reivindicaciones son idénticas
29	2	Parcialmente comprendida en la reivindicación 2 americana
30	2	Parcialmente comprendida en la reivindicación 2 americana
31	4	Las reivindicaciones son idénticas
32	3	Las reivindicaciones son idénticas
33	3	Parcialmente comprendida en la reivindicación 3 americana
34	3	Parcialmente comprendida en la reivindicación 3 americana
35	5	Las reivindicaciones son idénticas
36	5	Parcialmente comprendida en la reivindicación 5 americana
37	13	Las reivindicaciones son idénticas
38	13	Parcialmente comprendida en la reivindicación 13 americana
39	13	Parcialmente comprendida en la reivindicación 13 americana
40	13	Parcialmente comprendida en la reivindicación 13 americana
41	13	Parcialmente comprendida en la reivindicación 13 americana
42	14	Parcialmente comprendida en la reivindicación 14 americana
43	14	Parcialmente comprendida en la reivindicación 14 americana
44	1	Parcialmente comprendida en la reivindicación 1 americana
45	1	Parcialmente comprendida en la reivindicación 1 americana
46	1	Parcialmente comprendida en la reivindicación 1 americana
47	1	Parcialmente comprendida en la reivindicación 1 americana

48	1	Parcialmente comprendida en la reivindicación 1 americana
49	1	Parcialmente comprendida en la reivindicación 1 americana
50	1	Parcialmente comprendida en la reivindicación 1 americana
51	1	Parcialmente comprendida en la reivindicación 1 americana
52	1	Parcialmente comprendida en la reivindicación 1 americana.
53	1	Parcialmente comprendida en la reivindicación 1 americana
54	1	Parcialmente comprendida en la reivindicación 1 americana
55	1	Parcialmente comprendida en la reivindicación 1 americana
56	1	Parcialmente comprendida en la reivindicación 1 americana
57	1	Parcialmente comprendida en la reivindicación 1 americana
58	15	Las reivindicaciones son idénticas
59	22	Las reivindicaciones son idénticas
60	23	Las reivindicaciones son idénticas
61	24	Las reivindicaciones son idénticas
62	25	Las reivindicaciones son idénticas
63	16	Parcialmente comprendida en la reivindicación 16 americana
64	26	Las reivindicaciones son idénticas
65	27	Las reivindicaciones son idénticas
66	28	Las reivindicaciones son idénticas
67	29	Las reivindicaciones son idénticas
68	16 y 17	Parcialmente comprendida en las reivindicaciones 16 y 17 americanas
69	30	Las reivindicaciones son idénticas
70	31	Las reivindicaciones son idénticas
71	34	Las reivindicaciones son idénticas
72	35	Las reivindicaciones son idénticas

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73	18	Las reivindicaciones son idénticas
74	32	Las reivindicaciones son idénticas
75	33	Las reivindicaciones son idénticas
76	36	Las reivindicaciones son idénticas
77	19	Parcialmente comprendida en la reivindicación 19 americana
78	20	Parcialmente comprendida en la reivindicación 20 americana

LLOREDA CAMACHO & CO.

ABOGADOS

Calle 72 No. 5-83 Piso 5, Bogotá, D.C. Tel: 6069700 Fax 6069701

Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

DIVISION DE NUEVAS CREACIONES

E. _____ S. _____ D. _____

Trámite: 11

Evento: 379

Actuación: 457

Re: PI.-ALIOS BIOPHARMA, INC.-Solicitud de Patente de Invención en Fase Nacional PCT, solicitud de examen acelerado de patentes vía PPH para "NUCLEOSIDOS SUSTITUIDOS, NUCLEOTIDOS Y ÁNALOGOS DE LOS MISMOS".-Clase C07H 19/00.-Expediente número 14-137.284.-

1. Me refiero a mi modificación voluntaria presentada el pasado 6 de enero de 2015.
2. De conformidad con lo dispuesto en el Artículo 1 de la Resolución N° 54093 de 18 de septiembre de 2012, y en virtud del Acuerdo para el Procedimiento Acelerado de Estudio de Solicitudes de Patente (PPH) suscrito entre la Superintendencia de Industria y Comercio (SIC) y la Oficina de Patentes y Marcas de los Estados Unidos de América (USPTO) solicito a su Despacho hacer estudio de patentabilidad de la presente solicitud de conformidad con la guía de procedimiento para este tipo de trámite.

Para la solicitud de referencia específicamente, solicito se tenga en cuenta el resultado del examen de patentabilidad realizado por la Oficina de Marcas y Patentes de Estados Unidos de América (USPTO), aquí presentada bajo la notificación de admisibilidad expedida por esta entidad en relación con la patente identificada bajo el número **US 13/721,988**; la presente solicitud es equivalente a la antes mencionada patente que fue aceptada por la **USPTO** como **primera oficina de presentación**, tal y como se podrá evidenciar más adelante.

3. La equivalencia entre la materia concedida en la patente **US 13/721,988** y la materia reclamada en la presente solicitud, de acuerdo con el capítulo reivindicatorio que comprende 78 reivindicaciones presentadas el pasado 6 de enero de 2015, es explicada tal como se muestra a continuación:

Reivindicaciones de la presente solicitud	Reivindicaciones patente US13/721,988	Correspondencia de las reivindicaciones
1	1	Las reivindicaciones son idénticas

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Reivindicaciones de la presente solicitud	Reivindicaciones patente US13/721,988	Correspondencia de las reivindicaciones
2	6	Parcialmente comprendida en la reivindicación 6 americana
3	1	Parcialmente comprendida en la reivindicación 1 americana
4	1	Parcialmente comprendida en la reivindicación 1 americana
5	1 y 7	Parcialmente comprendida en las reivindicaciones 1 y 7 americanas
6	1 y 7	Parcialmente comprendida en las reivindicaciones 1 y 7 americanas
7	8	Parcialmente comprendida en la reivindicación 8 americana.
8	8	Parcialmente comprendida en la reivindicación 8 americana.
9	9	Parcialmente comprendida en la reivindicación 9 americana.
10	8	Parcialmente comprendida en la reivindicación 8 americana
11	9	Parcialmente comprendida en la reivindicación 9 americana
12	9	Parcialmente comprendida en la reivindicación 9 americana
13	1	Parcialmente comprendida en la reivindicación 1 americana
14	1	Parcialmente comprendida en la reivindicación 1 americana.
15	6	Parcialmente comprendida en la reivindicación 6 americana
16	10	Parcialmente comprendida en la reivindicación 10 americana
17	1 y 11	Parcialmente comprendida en las reivindicaciones 1 y 11 americanas
18	1 y 11	Parcialmente comprendida en las reivindicaciones 1 y 11 americanas
19	1 y 11	Parcialmente comprendida en las reivindicaciones 1 y 11 americanas
20	1	Parcialmente comprendida en la

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Reivindicaciones de la presente solicitud	Reivindicaciones patente US13/721,988	Correspondencia de las reivindicaciones
		reivindicación 1 americana
21	1	Parcialmente comprendida en la reivindicación 1 americana
22	6	Parcialmente comprendida en la reivindicación 6 americana
23	12	Parcialmente comprendida en la reivindicación 12 americana
24	12	Parcialmente comprendida en la reivindicación 12 americana.
25	12	Parcialmente comprendida en la reivindicación 12 americana
26	1	Parcialmente comprendida en la reivindicación 1 americana
27	1	Parcialmente comprendida en la reivindicación 1 americana
28	2	Las reivindicaciones son idénticas
29	2	Parcialmente comprendida en la reivindicación 2 americana
30	2	Parcialmente comprendida en la reivindicación 2 americana
31	4	Las reivindicaciones son idénticas
32	3	Las reivindicaciones son idénticas
33	3	Parcialmente comprendida en la reivindicación 3 americana
34	3	Parcialmente comprendida en la reivindicación 3 americana
35	5	Las reivindicaciones son idénticas
36	5	Parcialmente comprendida en la reivindicación 5 americana
37	13	Las reivindicaciones son idénticas
38	13	Parcialmente comprendida en la reivindicación 13 americana
39	13	Parcialmente comprendida en la reivindicación 13 americana
40	13	Parcialmente comprendida en la reivindicación 13 americana

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Reivindicaciones de la presente solicitud	Reivindicaciones patente US13/721,988	Correspondencia de las reivindicaciones
41	13	Parcialmente comprendida en la reivindicación 13 americana
42	14	Parcialmente comprendida en la reivindicación 14 americana
43	14	Parcialmente comprendida en la reivindicación 14 americana
44	1	Parcialmente comprendida en la reivindicación 1 americana
45	1	Parcialmente comprendida en la reivindicación 1 americana
46	1	Parcialmente comprendida en la reivindicación 1 americana
47	1	Parcialmente comprendida en la reivindicación 1 americana
48	1	Parcialmente comprendida en la reivindicación 1 americana
49	1	Parcialmente comprendida en la reivindicación 1 americana
50	1	Parcialmente comprendida en la reivindicación 1 americana
51	1	Parcialmente comprendida en la reivindicación 1 americana
52	1	Parcialmente comprendida en la reivindicación 1 americana.
53	1	Parcialmente comprendida en la reivindicación 1 americana
54	1	Parcialmente comprendida en la reivindicación 1 americana
55	1	Parcialmente comprendida en la reivindicación 1 americana
56	1	Parcialmente comprendida en la reivindicación 1 americana
57	1	Parcialmente comprendida en la reivindicación 1 americana
58	15	Las reivindicaciones son idénticas
59	22	Las reivindicaciones son idénticas
60	23	Las reivindicaciones son idénticas

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Reivindicaciones de la presente solicitud	Reivindicaciones patente US13/721,988	Correspondencia de las reivindicaciones
61	24	Las reivindicaciones son idénticas
62	25	Las reivindicaciones son idénticas
63	16	Parcialmente comprendida en la reivindicación 16 americana
64	26	Las reivindicaciones son idénticas
65	27	Las reivindicaciones son idénticas
66	28	Las reivindicaciones son idénticas
67	29	Las reivindicaciones son idénticas
68	16 y 17	Parcialmente comprendida en las reivindicaciones 16 y 17 americanas
69	30	Las reivindicaciones son idénticas
70	31	Las reivindicaciones son idénticas
71	34	Las reivindicaciones son idénticas
72	35	Las reivindicaciones son idénticas
73	18	Las reivindicaciones son idénticas
74	32	Las reivindicaciones son idénticas
75	33	Las reivindicaciones son idénticas
76	36	Las reivindicaciones son idénticas
77	19	Parcialmente comprendida en la reivindicación 19 americana
78	20	Parcialmente comprendida en la reivindicación 20 americana

Teniendo en cuenta la tabla de equivalencias aquí presentadas, considero viable la utilización del examen realizado por la USPTO como guía para el estudio de patentabilidad de la solicitud en trámite.

4. De igual forma me permito llamar su atención en cuanto a que la presente solicitud cumple con los requisitos para el reconocimiento de los resultados de exámenes de patentabilidad efectuados a solicitudes extranjeras, establecidos en la Resolución N° 54093 de 2012, de acuerdo con las siguientes condiciones:

a. Se está solicitando el reconocimiento del examen de patentabilidad realizado por la Oficina de Marcas y Patentes de Estados Unidos, como oficina de propiedad industrial reconocida en virtud del acuerdo suscrito con la Oficina Colombiana de Patentes.

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Además, se realiza esta solicitud junto con la petición del examen de patentabilidad y el correspondiente pago de las tasas.

b. La presente solicitud se encuentra en fase nacional en virtud del PCT y reivindica las siguientes prioridades US 61/579,560 del 22 de diciembre de 2011 y US 61/613,836 de 21 de marzo de 2012 de conformidad con lo establecido en el artículo 9 de la Decisión 486/2000.

c. La patente **US 13/721,988** corresponde con la presente solicitud, tal como se evidencia de los capítulos reivindicatorios arriba comparados, la descripción de las solicitudes y los datos bibliográficos.

No. de la solicitud	US13/721.988
Fecha de presentación	20 de Diciembre de 2012
Solicitante	Alios Biopharma, Inc.
Inventores	Leonid Beigelman, Guangyi Wang, David Bernard Smith, Jerome Deval, Marija Prhavic.
Título de la invención	SUBSTITUTED NUCLEOSIDES, NUCLEOTIDES AND ANALOGS THEREOF
Número de PCT	PCT/US2012/071063 presentada el 20 de diciembre de 2012.

d. La decisión de aceptación por parte de la Oficina de Patentes de los Estados Unidos, hace mención a que las reivindicaciones concedidas van de la (1-40) (ver numeral 3 de la Notificación de Admisibilidad adjunta).

Dichas reivindicaciones son enteramente equivalentes a las (78) reivindicaciones presentadas bajo modificación voluntaria el día 6 de enero de 2015, y corresponden suficientemente a las reivindicaciones aceptadas, toda vez que las mismas tienen un alcance igual, similar o incluso menor que las aceptadas para patentabilidad por la Oficina de Primera Presentación, tal y como se puede evidenciar en la tabla de equivalencias y al realizar el cotejo de los dos capítulos reivindicatorios.

e. Las 78 reivindicaciones de la solicitud en cuestión presentadas ante la Superintendencia de Industria y Comercio, corresponden a las reivindicaciones protegidas en la patente **US 13/721,988**, y se encuentran del todo contenidas en la materia reclamada ante la Oficina de Primera Presentación.

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5. Finalmente y en cumplimiento de los requisitos para la tramitación del Procedimiento Acelerado de Estudio de Solicitudes de Patente, me permito anexar los siguientes documentos:

- a) Formato único de procedimiento acelerado de exámenes de patentes;
- b) Copia de la Notificación de Admisibilidad (*Notice of Allowance*) debidamente emitida por la Oficina de Patentes de Los Estados Unidos, donde se detalla el número de la solicitud (13/721,988), los datos correspondientes a los inventores y el solicitante y la fecha de aceptación que data del 11 de julio de 2014.
- c) Traducción de la Notificación de Admisibilidad.
- d) Datos bibliográficos de la solicitud tal y como se presentó en Estados Unidos donde se menciona el título de la invención e inventores de la solicitud.
- e) Copia de las reivindicaciones que han sido determinadas como patentables (reivindicaciones 1-40);
- f) Traducción de las reivindicaciones que han sido determinadas como patentables (reivindicaciones 1-40), y
- g) Copia de la lista de antecedentes citados por el examinador de la Oficina de Patentes de los Estados Unidos (USPTO).
- h) Recibo No. 15-000446, expedido por la Superintendencia de Industria y Comercio, que acredita el pago de la tasa que causa el Examen de Patentabilidad.

6. De conformidad con lo anterior, ruego a usted tomar atenta nota de lo anterior y proceder de conformidad.

Señor Superintendente,


ALICIA LLOREDA RICAURTE

T.P. 53.215

Notificación de admisibilidad	Solicitud No. 13/721.988	Solicitante(s) Beigelman ET AL.	
	Examinador LAYLA BLAND	Unidad de Arte 1673	Estado AIA (Primer inventor en radicar) No

--La FECHA DE ENVÍO de esta comunicación aparece en la portada con la Dirección de correspondencia--
Siendo permisibles todas las reivindicaciones, EL PROCESAMIENTO POR MERITOS ESTÁ (O SE MANTIENE) CERRADO en esta aplicación. Si no se incluye con la presente (o no ha sido previamente enviada por correo), una Notificación de Admisibilidad (PTOL-85) u otra comunicación apropiada se enviará por correo a su debido tiempo. **ESTE AVISO DE ADMISIBILIDAD NO ES UNA CONCESIÓN DE DERECHOS DE PATENTE.** Esta solicitud está sujeta a retirada de consideración por iniciativa de la Oficina o a petición del solicitante. Ver 37 CFR 1.313 y MPEP 1308.

1. ☒ Esta comunicación responde a la Enmienda del solicitante presentada el 27 de octubre 2014.
☐ Una declaración / declaración jurada bajo 37 CFR 1.130(b) fue presentada el _____.

2. ☐ Una elección fue hecha por el solicitante en respuesta a un requisito de restricción establecido durante la entrevista en _____; el requisito de restricción y la elección se han incorporado a esta acción.

3. ☒ La(s) reivindicación(es) permitida(s) es/son 1-40. Como resultado de la(s) reivindicación(es) permitida(s), usted puede ser elegible para beneficiarse de la tramitación bajo el **Programa de patentes de pista rápida** en una oficina de propiedad intelectual participante para la aplicación correspondiente. Para obtener más información, consulte la página http://www.uspto.gov/patents/init_events/pph/index.jsp o envíe una consulta a PPHFeedback@uspto.gov.

4. ☐ Se reconoce una reivindicación de prioridad extranjera bajo 35 USC§119(a)-(d) o (f).
Copias certificadas:
a) ☐ Todas b) ☐ Algunas *c) ☐ Ninguna de las:
1. ☐ Copias certificadas de los documentos de prioridad se han recibido.
2. ☐ Copias certificadas de los documentos de prioridad se han recibido en la Solicitud No. _____.
3. ☐ Copias de las copias certificadas de los documentos de prioridad se han recibido en esta etapa nacional de la solicitud de parte de la Oficina Internacional (Regla del PCT 17.2 (a)).
* Copias certificadas no recibidas: _____.

El Solicitante tiene TRES MESES DESDE LA "FECHA DE ENVÍO" de esta comunicación para presentar una respuesta que cumpla con los requisitos indicados a continuación. La falta de cumplimiento oportuno dará lugar al abandono de esta solicitud. **ESTE PERIODO DE TRES MESES NO ES EXTENSIBLE.**

5. ☐ Deben presentarse DIBUJOS CORREGIDOS (como "hojas de reemplazo").
☐ Incluyendo los cambios requeridos por la adjunta Enmienda/Comentario del examinador o en el Acta de Oficina del Documento No. / Fecha de envío _____.

Señales identificadoras tales como el número de solicitud (ver 37 CFR 1.84(c)) deben estar escritas en los dibujos en la parte delantera (no el dorso) de cada hoja. Las Hoja(s) de reemplazo debe(n) ser etiquetada(s) como tal en la cabecera de acuerdo con 37 CFR 1.121(d).

6. ☐ Debe presentarse CONSIGNACION DE y/o INFORMACIÓN sobre el depósito de MATERIAL BIOLÓGICO. Tenga en cuenta el comentario adjunto del Examinador con respecto al REQUISITO PARA EL DEPOSITO DE MATERIAL BIOLÓGICO.

Anexo(s)

1. ☐ Notificación de las referencias citadas (PTO-892) de 2. ☒ Declaraciones de divulgación de información (PTO/SB/08), Documento No./ Fecha de envío _____. 3. ☐ Comentario del Examinador sobre los requisitos para el depósito de material biológico 4. ☐ Resumen de Entrevista (PTO-413), Documento No./ Fecha de envío _____.	5. ☐ Enmienda / Comentario del Examinador 6. ☐ Declaración del Examinador de Motivos de Admisibilidad 7. ☐ Otro _____.
--	---

/ LAYLA BLAND / Examinador Primaria, Unidad de Arte 1673	
---	--

Oficina de Patentes y Marcas de EE.UU. PTOL-37 (Rev. 08-13)	Aviso de admisibilidad	Parte del Documento No. / Fecha de envío 20141029
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Número de Solicitud/Control: 13/721,988

Unidad de Arte: 1673

La presente solicitud se examina bajo las disposiciones pre-AIA primero en inventar.

ACCIÓN DETALLADA

Esta acción del despacho es en respuesta a la enmienda del Solicitante presentada el 27 de octubre 2014.

En vista de la enmienda del Solicitante, el rechazo de las reivindicaciones 1-13, 19-21, 37, y 39 bajo 35 USC 112, párrafo segundo, por ser indefinidas se retira.

En vista de la enmienda del Solicitante, el rechazo de las reivindicaciones 2 y 12 bajo 35 USC 112, cuarto párrafo, se retira.

El rechazo de las reivindicaciones 1, 3, y 13 bajo 35 USC 102(b) como anticipadas por Ohrui se retira. El compuesto de Ohrui no cumple con la limitación de R^{4A} en las reivindicaciones modificadas.

El rechazo de las reivindicaciones 1, 4, 13, y 14 bajo 35 USC 102(b) como anticipadas por Pfundheller se retira porque el compuesto de Pfundheller no cumple con la limitación de R^{2A} en las reivindicaciones modificadas.

El rechazo de las reivindicaciones 1, 3, y 13 bajo 35 USC 102(b) como anticipadas por Sato se retira porque el compuesto de Sato no cumple con la limitación de R^{4A} en las reivindicaciones modificadas.

En consecuencia, la enmienda del Solicitante como se mencionó anteriormente es suficiente para eliminar todos los rechazos realizados en la acción anterior de este despacho y colocar la solicitud en condiciones de asignación.

Cualquier comentario que se considere necesario por el Solicitante deberá presentarse a más tardar al pago de la tarifa de expedición y, para evitar retrasos en el procesamiento, preferentemente debe acompañar a la tarifa de expedición. Dichas propuestas deberán estar claramente etiquetadas "Comentarios sobre la Declaración de Motivos de Admisibilidad".

Número de Solicitud/Control: 13/721,988

Unidad de Arte: 1673

Cualquier consulta relativa a esta comunicación o comunicaciones anteriores del examinador debe dirigirse a LAYLA BLAND cuyo número de teléfono es (571)272-9572. El examinador normalmente puede contactarse de lunes - viernes 10:00-06:30 EST.

Si los intentos de contactar al examinador por teléfono no tienen éxito, el supervisor del examinador, Anna Jiang, puede contactarse en el (571) 272-0627. El número de teléfono de fax de la organización en la que se asigna esta aplicación o proceso es 571-273-8300.

La información sobre el estado de una solicitud se puede obtener en el Sistema de Recuperación de Información de Solicitud de patente (PAIR). La información de estado para las aplicaciones publicadas se puede obtener PAIR privado o PAIR Público. La información de estado para las solicitudes no publicadas sólo está disponible a través del PAIR privado. Para obtener más información sobre el sistema PAIR, ver <http://pair-direct.uspto.gov>. Si tiene preguntas sobre el acceso al sistema PAIR privado, contacte al Centro de Comercio Electrónico (EBC) en el 866-217-9197 (llamada gratuita). Si desea la asistencia de un representante de servicio al cliente de la USPTO o acceso al sistema automatizado de información, llame al 800-786-9199 (EN EE.UU. o CANADÁ) o al 571-272-1000.

/LAYLA BLAND/

Examinador Primario, Unidad de Arte 1673

Notice of Allowability	Application No. 13/721,988	Applicant(s) BEIGELMAN ET AL.	
	Examiner LAYLA BLAND	Art Unit 1673	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to Applicant's amendment submitted October 27, 2014.
☐ A declaration(s)/affidavit(s) under 37 CFR 1.130(b) was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☒ The allowed claim(s) is/are 1-40. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:
a) ☐ All b) ☐ Some *c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).
* Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.
Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)	5. ☐ Examiner's Amendment/Comment
2. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____	6. ☒ Examiner's Statement of Reasons for Allowance
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material	7. ☐ Other _____
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____	

/LAYLA BLAND/ Primary Examiner, Art Unit 1673	
--	--

The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION

This office action is in response to Applicant's amendment submitted October 27, 2014.

In view of Applicant's amendment, the rejection of claims 1-13, 19-21, 37, and 39 under 35 U.S.C. 112, second paragraph as being indefinite is withdrawn.

In view of Applicant's amendment, the rejection of claims 2 and 12 under 35 U.S.C. 112, 4th paragraph is withdrawn.

The rejection of claims 1, 3, and 13 under 35 U.S.C. 102(b) as being anticipated by Ohruai is withdrawn. Ohruai's compound does not meet the limitation of R^{4A} in the amended claims.

The rejection of claims 1, 4, 13, and 14 under 35 U.S.C. 102(b) as being anticipated by Pfundheller is withdrawn because Pfundheller's compound does not meet the limitation of R^{2A} in the amended claims.

The rejection of claims 1, 3, and 13 under 35 U.S.C. 102(b) as being anticipated by Sato is withdrawn because Sato's compound does not meet the limitation of R^{4A} in the amended claims.

Accordingly, Applicant's amendment as discussed above is sufficient to remove all rejections made in the prior office action and to place the application in condition for allowance.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Application/Control Number: 13/721,988

Page 3

Art Unit: 1673

Any inquiry concerning this communication or earlier communications from the examiner should be directed to LAYLA BLAND whose telephone number is (571)272-9572.

The examiner can normally be reached on Monday - Friday, 10:00 - 6:30 EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anna Jiang can be reached on (571) 272-0627. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/LAYLA BLAND/

Primary Examiner, Art Unit 1673

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	ALIOS.052A
		Application Number	
Title of Invention	SUBSTITUTED NUCLEOSIDES, NUCLEOTIDES AND ANALOGS THEREOF		
The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.			

Secrecy Order 37 CFR 5.2

Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to	
☐	37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Leonid		Beigelman		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					
City	San Mateo	State/Province	CA	Country of Residence i US	
Mailing Address of Inventor:					
Address 1		991 East Grant Place			
Address 2					
City	San Mateo	State/Province	CA		
Postal Code	94402	Country i	US		
Inventor 2					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Guangyi		Wang		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					
City	Carlsbad	State/Province	CA	Country of Residence i US	
Mailing Address of Inventor:					
Address 1		5066 Millay Ct.			
Address 2					
City	Carlsbad	State/Province	CA		
Postal Code	92008	Country i	US		
Inventor 3					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	David	Bernard	Smith		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	ALIOS.052A		
		Application Number			
Title of Invention	SUBSTITUTED NUCLEOSIDES, NUCLEOTIDES AND ANALOGS THEREOF				
City	San Mateo	State/Province	CA	Country of Residence ⁱ	US
Mailing Address of Inventor:					
Address 1		218 W. 40th Avenue			
Address 2					
City	San Mateo	State/Province	CA		
Postal Code	94403	Country ⁱ	US		
Inventor 4					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Jerome		Deval		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					
City	Pacifica	State/Province	CA	Country of Residence ⁱ	US
Mailing Address of Inventor:					
Address 1		143 Carmel Avenue			
Address 2					
City	Pacifica	State/Province	CA		
Postal Code	94044	Country ⁱ	US		
Inventor 5					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Marija		Prhavo		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					
City	Encinitas	State/Province	CA	Country of Residence ⁱ	US
Mailing Address of Inventor:					
Address 1		1122 Cambria Way			
Address 2					
City	Encinitas	State/Province	CA		
Postal Code	92024	Country ⁱ	US		
All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button.					Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ An Address is being provided for the correspondence information of this application.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	ALIOS.052A
		Application Number	
Title of Invention	SUBSTITUTED NUCLEOSIDES, NUCLEOTIDES AND ANALOGS THEREOF		
Customer Number	20995		
Email Address	efiling@kmob.com	Add Email	Remove Email

Application Information:

Title of the Invention	SUBSTITUTED NUCLEOSIDES, NUCLEOTIDES AND ANALOGS THEREOF		
Attorney Docket Number	ALIOS.052A	Small Entity Status Claimed	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Suggested Class (if any)		Sub Class (if any)	
Suggested Technology Center (if any)			
Total Number of Drawing Sheets (if any)	1	Suggested Figure for Publication (if any)	

Publication Information:

☐	Request Early Publication (Fee required at time of Request 37 CFR 1.219)
☐	Request Not to Publish. I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application has not and will not be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	☒ Customer Number	☐ US Patent Practitioner	☐ Limited Recognition (37 CFR 11.9)
Customer Number	20995		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.			
Prior Application Status	Pending	Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	non provisional of	61/579560	2011-12-22
Prior Application Status	Pending	Remove	

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	ALIOS.052A
		Application Number	
Title of Invention	SUBSTITUTED NUCLEOSIDES, NUCLEOTIDES AND ANALOGS THEREOF		
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	non provisional of	61/613836	2012-03-21
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button.			Add

Foreign Priority Information:

This section allows for the applicant to claim benefit of foreign priority and to identify any prior foreign application for which priority is not claimed. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(a).			
			Remove
Application Number	Country i	Filing Date (YYYY-MM-DD)	Priority Claimed
			☐ Yes ☒ No
Additional Foreign Priority Data may be generated within this form by selecting the Add button.			Add

Authorization to Permit Access:

☒ Authorization to Permit Access to the Instant Application by the Participating Offices
If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application. In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application. In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date of filing this Authorization.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	ALIOS.052A
	Application Number	
Title of Invention	SUBSTITUTED NUCLEOSIDES, NUCLEOTIDES AND ANALOGS THEREOF	

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Remove

☒ Assignee

☐ Legal Representative under 35 U.S.C. 117

☐ Person to whom the inventor is obligated to assign.

☐ Person who shows sufficient proprietary interest

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :

If the Assignee is an Organization check here. ☒

Organization Name

Alios BioPharma, Inc.

Mailing Address Information:

Address 1

260 E. Grand Ave.

Address 2

2nd Floor

City

South San Francisco

State/Province

CA

Country ⁱ

US

Postal Code

94080

Phone Number

Fax Number

Email Address

Additional Applicant Data may be generated within this form by selecting the Add button.

Add

Signature:

Remove

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications

Signature	/Kimberly J. Miller/		Date (YYYY-MM-DD)	2012-12-20	
First Name	Kimberly J.	Last Name	Miller	Registration Number	61749

Additional Signature may be generated within this form by selecting the Add button.

Add

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	ALIOS.052A
	Application Number	
Title of Invention	SUBSTITUTED NUCLEOSIDES, NUCLEOTIDES AND ANALOGS THEREOF	

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

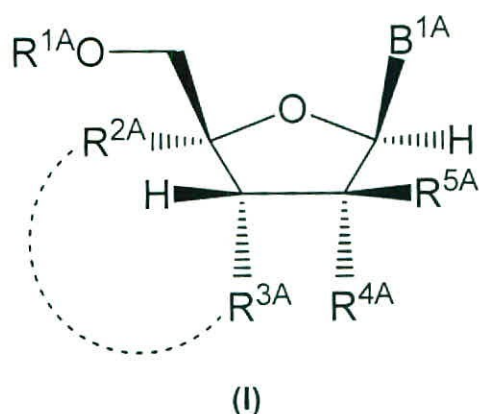
The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

REIVINDICACIONES

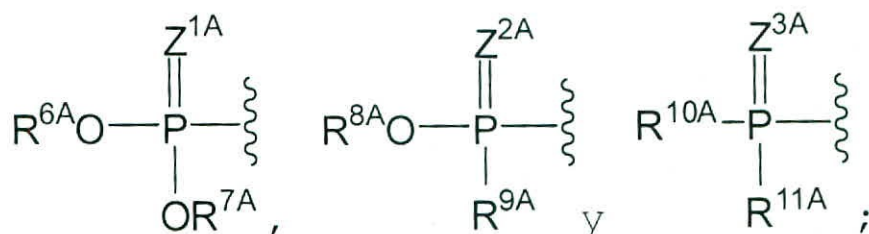
1. Un compuesto seleccionado entre la Fórmula (I) o una sal farmacéuticamente aceptable del mismo,



en la cual:

B^{1A} es una base heterocíclica opcionalmente sustituida o una base heterocíclica opcionalmente sustituida con un grupo amino protegido;

R^{1A} se selecciona del grupo que consiste de hidrógeno, un acilo opcionalmente sustituido, un aminoácido con enlace O opcionalmente sustituido,



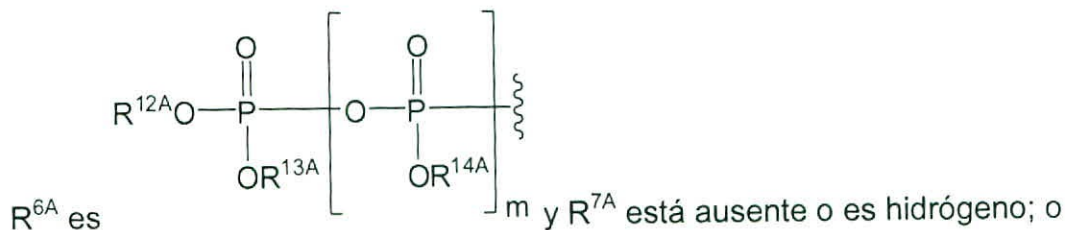
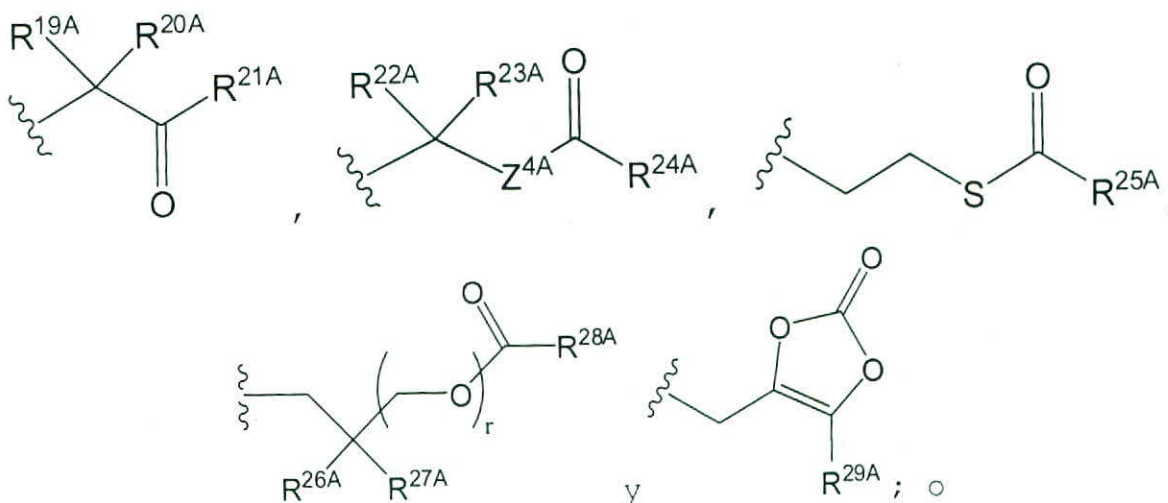
R^{2A} se selecciona del grupo que consiste de un alquilo C_{1-6} sin sustituir, un alquilo C_{1-6} sustituido con halógeno, un alquilo C_{1-6} sustituido con hidroxilo, un alquilo C_{1-6} sustituido con alcoxi, un alquilo C_{1-6} sustituido con sulfenilo, un alqueno C_{2-6} opcionalmente sustituido, un alquino C_{2-6} opcionalmente sustituido, un cicloalquilo C_{3-6} opcionalmente sustituido, un $-O-$ alquilo C_{1-6} opcionalmente sustituido, un $-O-$ alqueno C_{3-6} opcionalmente sustituido, un $-O-$ alquino C_{3-6} opcionalmente sustituido y ciano;

R^{3A} se selecciona del grupo que consiste de OH, $-OC(=O)R^{nA}$ y un aminoácido con enlace O opcionalmente sustituido;

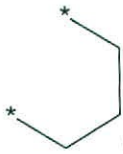
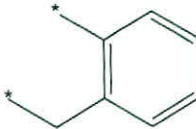
R^{4A} es fluoro o cloro;

R^{5A} es hidrógeno o halógeno;

R^{6A} , R^{7A} y R^{8A} se seleccionan, independientemente, del grupo que consiste estar ausente, ser hidrógeno, un alquilo C_{1-24} opcionalmente sustituido, un alquenilo C_{2-24} opcionalmente sustituido, un alquinilo C_{2-24} opcionalmente sustituido, un cicloalquilo C_{3-6} opcionalmente sustituido, un cicloalquenilo C_{3-6} opcionalmente sustituido, un arilo opcionalmente sustituido, un heteroarilo opcionalmente sustituido, un aril(alquilo C_{1-6}) opcionalmente sustituido, un $^{*}-(CR^{15A}R^{16A})_p-O-$ alquilo C_{1-24} opcionalmente sustituido, un $^{*}-(CR^{17A}R^{18A})_q-O-$ alquenilo C_{1-24} opcionalmente sustituido,



R^{6A} y R^{7A} se toman en conjunto para formar una fracción seleccionada del grupo

que consiste de  opcionalmente sustituido, y un  opcionalmente sustituido, en donde los oxígenos conectados a R^{6A} y R^{7A} , el fósforo y la fracción forman un sistema de anillos de seis a diez miembros;

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R^{9A} se selecciona, independientemente, del grupo que consiste de un alquilo C_{1-24} opcionalmente sustituido, un alquenilo C_{2-24} opcionalmente sustituido, un alquinilo C_{2-24} opcionalmente sustituido, un cicloalquilo C_{3-6} opcionalmente sustituido, un cicloalquenilo C_{3-6} opcionalmente sustituido, $NR^{30A}R^{31A}$, un aminoácido con enlace N opcionalmente sustituido y un derivado de éster de aminoácido con enlace N opcionalmente sustituido;

R^{10A} y R^{11A} son, independientemente, un aminoácido con enlace N opcionalmente sustituido o un derivado de éster de aminoácido con enlace N opcionalmente sustituido;

R^{12A} , R^{13A} y R^{14A} , independientemente, están ausentes o son hidrógeno;

cada R^{15A} , cada R^{16A} , cada R^{17A} y cada R^{18A} son, independientemente, hidrógeno, un alquilo C_{1-24} opcionalmente sustituido o alcoxi;

R^{19A} , R^{20A} , R^{22A} y R^{23A} se seleccionan, independientemente, del grupo que consiste de hidrógeno, un alquilo C_{1-24} opcionalmente sustituido y un arilo opcionalmente sustituido;

R^{21A} y R^{24A} se seleccionan, independientemente, del grupo que consiste de hidrógeno, un alquilo C_{1-24} opcionalmente sustituido, un arilo opcionalmente sustituido, un $-O-$ alquilo C_{1-24} opcionalmente sustituido y un $-O-$ arilo opcionalmente sustituido;

R^{25A} y R^{29A} se seleccionan, independientemente, del grupo que consiste de hidrógeno, un alquilo C_{1-24} opcionalmente sustituido y un arilo opcionalmente sustituido;

R^{26A} y R^{27A} son, independientemente, $-C\equiv N$ o un sustituyente opcionalmente sustituido, seleccionado del grupo que consiste de organilcarbonilo C_{2-8} , alcoxicarbonilo C_{2-8} y organilaminocarbonilo C_{2-8} ;

R^{28A} se selecciona del grupo que consiste de hidrógeno, un alquilo C_{1-24} opcionalmente sustituido, un alquenilo C_{2-24} opcionalmente sustituido, un alquinilo C_{2-24} opcionalmente sustituido, un cicloalquilo C_{3-6} opcionalmente sustituido y un cicloalquenilo C_{3-6} opcionalmente sustituido;

R^{30A} y R^{31A} se seleccionan, independientemente, del grupo que consiste de hidrógeno, un alquilo C_{1-24} opcionalmente sustituido, un alquenilo C_{2-24} opcionalmente sustituido, un alquinilo C_{2-24} opcionalmente sustituido, un cicloalquilo C_{3-6} opcionalmente sustituido y un cicloalquenilo C_{3-6} opcionalmente sustituido;

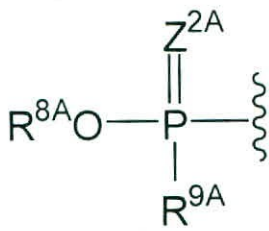
R^{nA} es un alquilo C_{1-24} opcionalmente sustituido;

m es 0 o 1;

p y q se seleccionan, independientemente, del grupo que consiste de 1, 2 y 3;

r es 1 o 2;

Z^{1A} , Z^{2A} , Z^{3A} y Z^{4A} son, independientemente, O o S; y

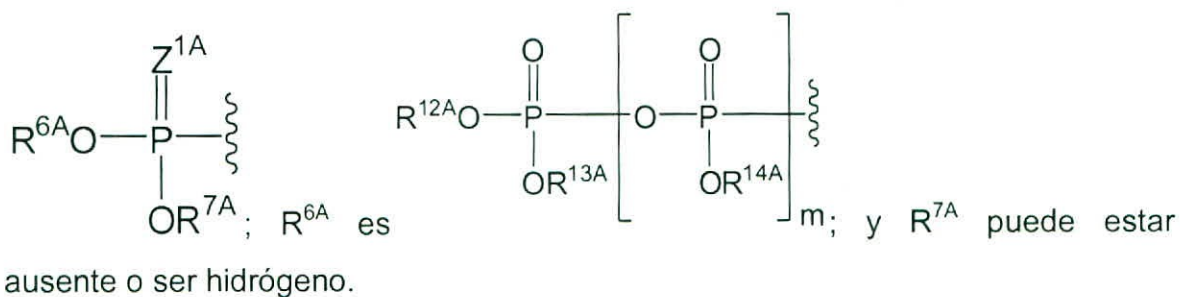


con la condición de que R^{1A} sea en la cual R^{8A} es un fenilo o alquilo C_{1-4} sin sustituir, opcionalmente para-sustituido con un halógeno o metilo y R^{9A} es éster metílico, éster etílico, éster isopropílico, éster n-butilico, éster bencílico o éster fenílico de un aminoácido seleccionado del grupo que consiste de glicina, alanina, valina, leucina, fenilalanina, triptófano, metionina y prolina; R^{3A} es OH; R^{4A} es fluoro; R^{5A} es fluoro o hidrógeno; y B^{1A} es un uracilo sin sustituir; entonces R^{2A} no pueda ser $-OCH_3$;

con la condición de que R^{1A} sea H; R^{3A} sea OH; R^{4A} sea fluoro R^{5A} sea fluoro; y B^{1A} sea una citosina sin sustituir; entonces R^{2A} no pueda ser alenilo; y

con la condición de que R^{1A} sea H; R^{3A} sea OH; R^{4A} sea fluoro R^{5A} sea fluoro; y B^{1A} sea una citosina sin sustituir; entonces R^{2A} no pueda ser etinilo.

2. El compuesto de acuerdo con la reivindicación 1, en el cual R^{1A} es

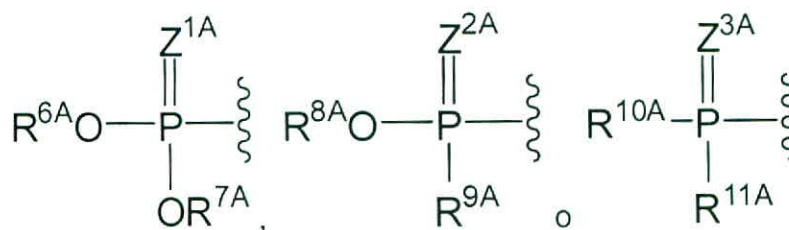


3. El compuesto de acuerdo con la reivindicación 1, en el cual R^{1A} es un acilo opcionalmente sustituido.

4. El compuesto de acuerdo con la reivindicación 1, en el cual R^{1A} es H.

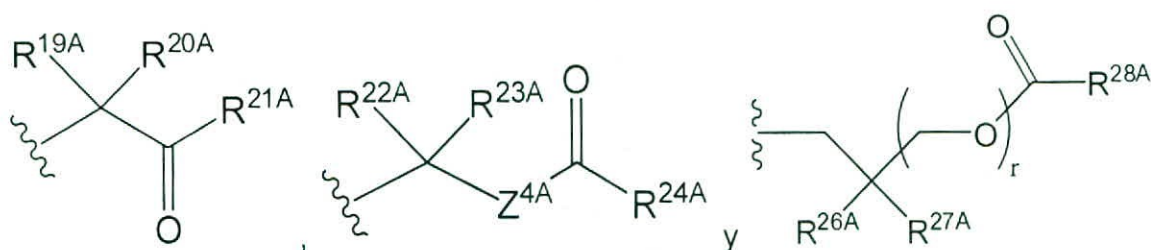
5. El compuesto de acuerdo con la reivindicación 1, en el cual R^{1A} es un aminoácido con enlace O opcionalmente sustituido.

6. El compuesto de acuerdo con la reivindicación 1, en el cual R^{1A} es

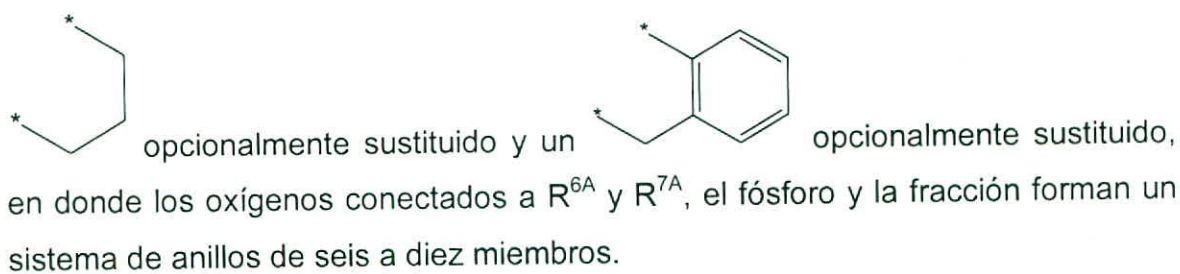
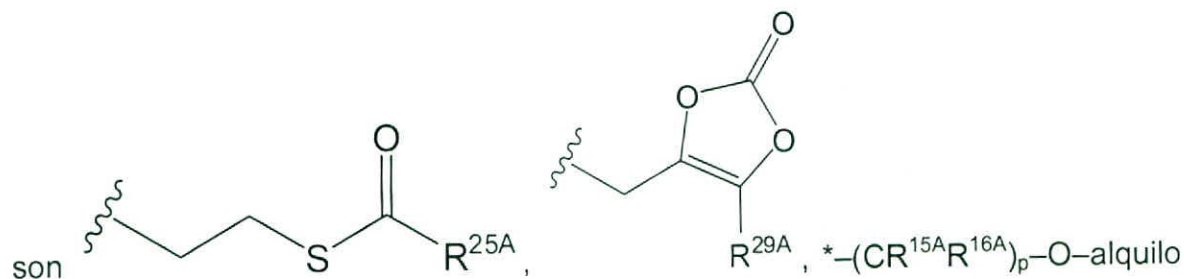


7. El compuesto de acuerdo con la reivindicación 6, en el cual ambos R^{6A} y R^{7A} se seleccionan, independientemente, del grupo del grupo que consiste de un alquilo C_{1-24} opcionalmente sustituido, un alquenilo C_{2-24} opcionalmente sustituido, un alquinilo C_{2-24} opcionalmente sustituido, un cicloalquilo C_{3-6} opcionalmente sustituido, un cicloalquenilo C_{3-6} opcionalmente sustituido, un arilo opcionalmente sustituido, un heteroarilo opcionalmente sustituido y un aril(alquilo C_{1-6}) opcionalmente sustituido.

8. El compuesto de acuerdo con la reivindicación 6, en el cual R^{6A} y R^{7A} ambos se seleccionan, independientemente, del grupo que consiste de



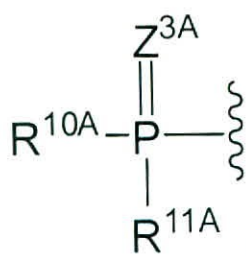
9. El compuesto de acuerdo con la reivindicación 6, en el cual R^{6A} y R^{7A} ambos



10. El compuesto de acuerdo con la reivindicación 6, en el cual R^{8A} se selecciona del grupo que consiste entre estar ausente, ser hidrógeno, un alquilo C_{1-24} opcionalmente sustituido, un alquenilo C_{2-24} opcionalmente sustituido, un alquinilo C_{2-24} opcionalmente sustituido, un cicloalquilo C_{3-6} opcionalmente sustituido y un cicloalquenilo C_{3-6} opcionalmente sustituido; y R^{9A} se selecciona, independientemente, del grupo que consiste de un alquilo C_{1-24} opcionalmente sustituido, un alquenilo C_{2-24} opcionalmente sustituido, un alquinilo C_{2-24} opcionalmente sustituido, un cicloalquilo C_{3-6} opcionalmente sustituido, un cicloalquenilo C_{3-6} opcionalmente sustituido y $NR^{30A}R^{31A}$.

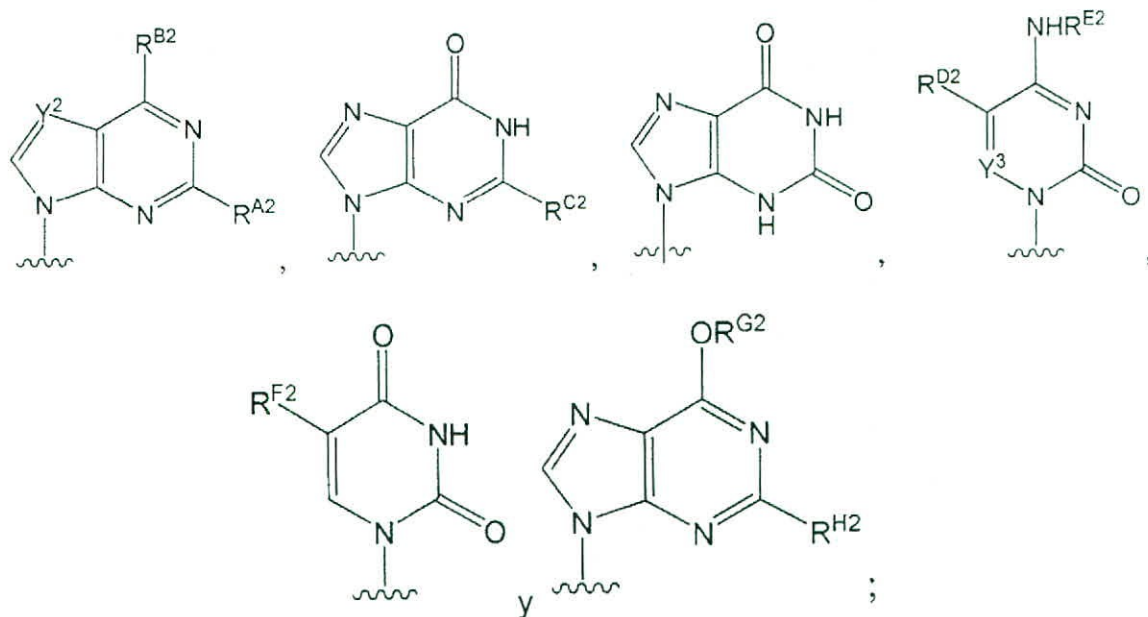
11. El compuesto de acuerdo con la reivindicación 6, en el cual R^{8A} es un arilo opcionalmente sustituido; y R^{9A} es un aminoácido con enlace N opcionalmente sustituido o un derivado de éster de aminoácido con enlace N opcionalmente sustituido.

12. El compuesto de acuerdo con la reivindicación 6, en el cual R^{1A} es



; y R^{10A} y R^{11A} ambos son un aminoácido con enlace N opcionalmente sustituido o un derivado de éster de aminoácido con enlace N opcionalmente sustituido.

13. El compuesto de acuerdo con la reivindicación 1, en el cual B^{1A} se selecciona del grupo que consiste de:



en el cual:

R^{A2} se selecciona del grupo que consiste de hidrógeno, halógeno y NHR^{J2}, donde R^{J2} se selecciona del grupo que consiste de hidrógeno, -C(=O)R^{K2} y -C(=O)OR^{L2};

R^{B2} es halógeno o NHR^{W2}, en donde R^{W2} se selecciona del grupo que consiste de hidrógeno, un alquilo C₁₋₆ opcionalmente sustituido, un alquenilo C₂₋₆ opcionalmente sustituido, un cicloalquilo C₃₋₈ opcionalmente sustituido, -C(=O)R^{M2} y -C(=O)OR^{N2};

R^{C2} es hidrógeno o NHR^{O2}, en el cual R^{O2} se selecciona del grupo que consiste de hidrógeno, -C(=O)R^{P2} y -C(=O)OR^{Q2};

R^{D2} se selecciona del grupo que consiste de hidrógeno, halógeno, un alquilo C₁₋₆ opcionalmente sustituido, un alquenilo C₂₋₆ opcionalmente sustituido y un alquinilo C₂₋₆ opcionalmente sustituido;

R^{E2} se selecciona del grupo que consiste de hidrógeno, hidroxilo, un alquilo C₁₋₆ opcionalmente sustituido, un cicloalquilo C₃₋₈ opcionalmente sustituido, -C(=O)R^{R2} y -C(=O)OR^{S2};

R^{F2} se selecciona del grupo que consiste de hidrógeno, halógeno, un alquilo C₁₋₆

opcionalmente sustituido, un alquenilo C_{2-6} opcionalmente sustituido y un alquinilo C_{2-6} opcionalmente sustituido;

Y^2 e Y^3 son, independientemente, N o CR^{l2} , en el cual R^{l2} se selecciona del grupo que consiste de hidrógeno, halógeno, un alquilo C_{1-6} opcionalmente sustituido, un alquenilo C_{2-6} opcionalmente sustituido y un alquinilo C_{2-6} opcionalmente sustituido;

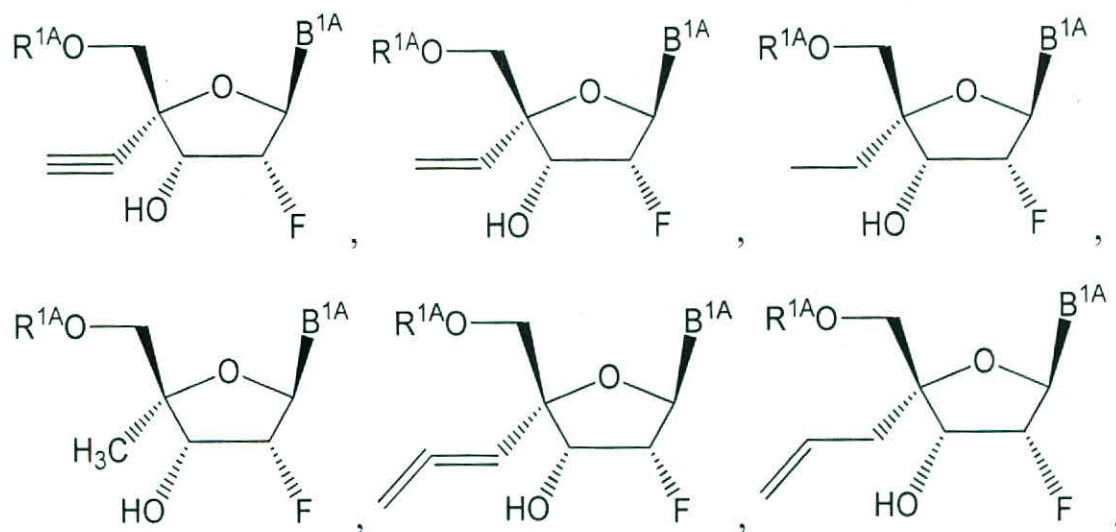
R^{G2} es un alquilo C_{1-6} opcionalmente sustituido;

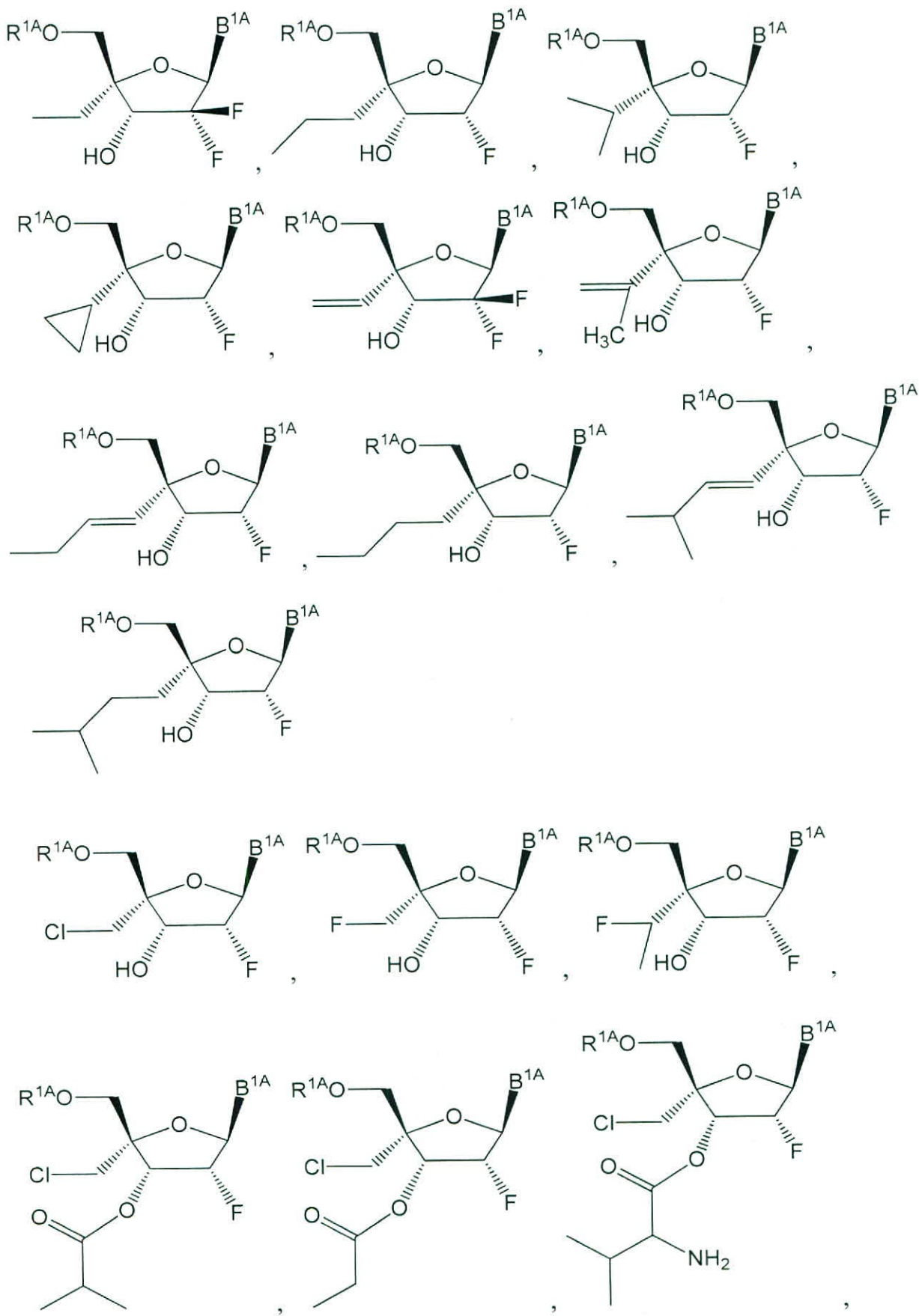
R^{H2} es hidrógeno o NHR^{T2} , en el cual R^{T2} se selecciona, independientemente, del grupo que consiste de hidrógeno, $-C(=O)R^{U2}$ y $-C(=O)OR^{V2}$; y

R^{K2} , R^{L2} , R^{M2} , R^{N2} , R^{P2} , R^{Q2} , R^{R2} , R^{S2} , R^{U2} y R^{V2} se seleccionan, independientemente, del grupo que consiste de alquilo C_{1-6} , alquenilo C_{2-6} , alquinilo C_{2-6} , cicloalquilo C_{3-6} , cicloalquenilo C_{3-6} , arilo C_{6-10} , heteroarilo, heteroalíclicilo, aril(alquilo C_{1-6}), heteroaril(alquilo C_{1-6}) y heteroalícilil(alquilo C_{1-6}).

14. El compuesto de acuerdo con la reivindicación 1, en el cual R^{2A} es un alquilo C_{1-6} sustituido con halógeno o un alquilo C_{1-6} sustituido con sulfenilo, y R^{3A} es OH o $-OC(=O)R^{nA}$.

15. El compuesto de acuerdo con la reivindicación 1, en el cual el compuesto de la Fórmula (I) se selecciona del grupo que consiste de:





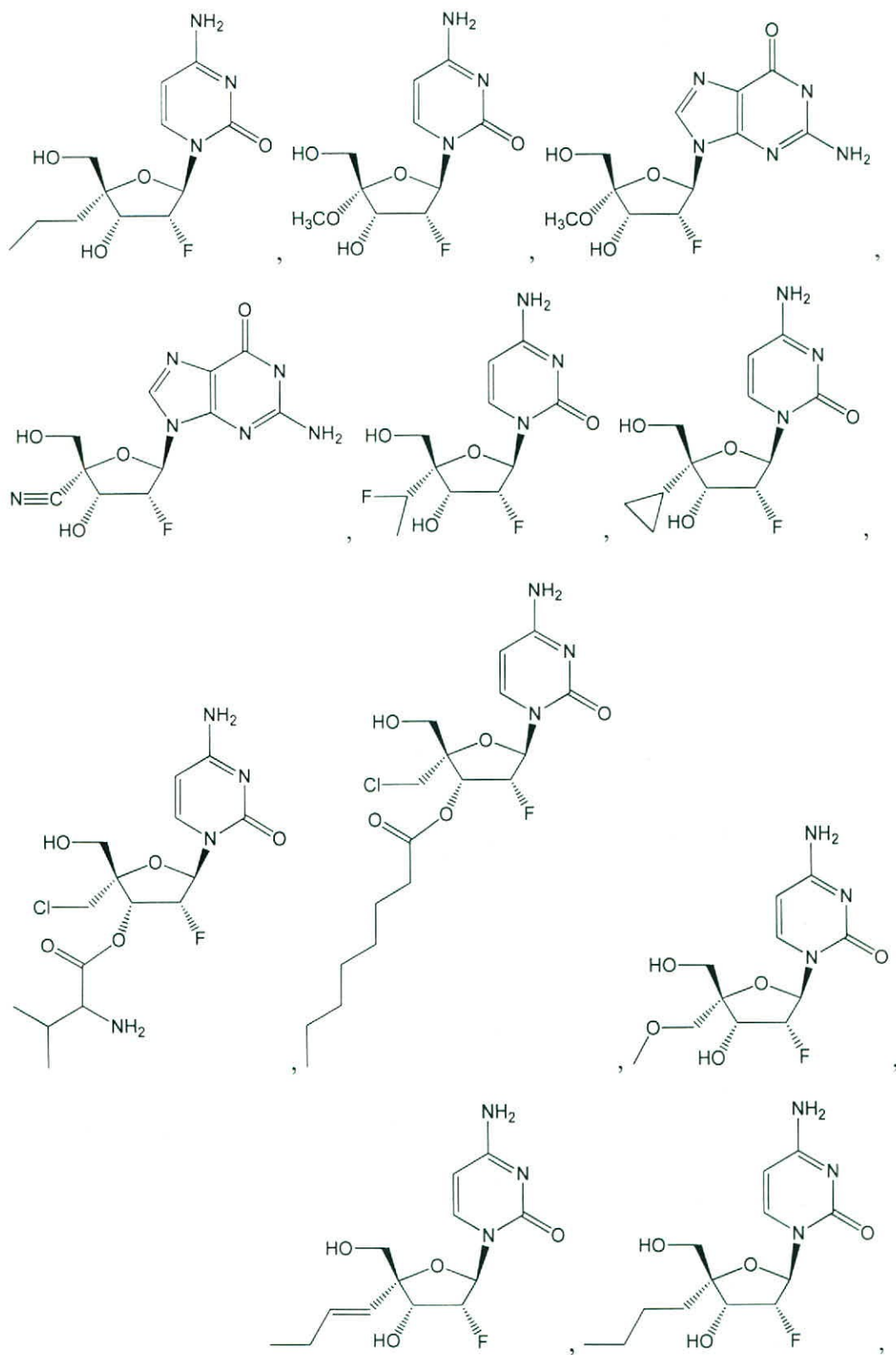
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16. El compuesto de acuerdo con la reivindicación 1, en el cual el compuesto de la Fórmula (I) se selecciona del grupo que consiste de:

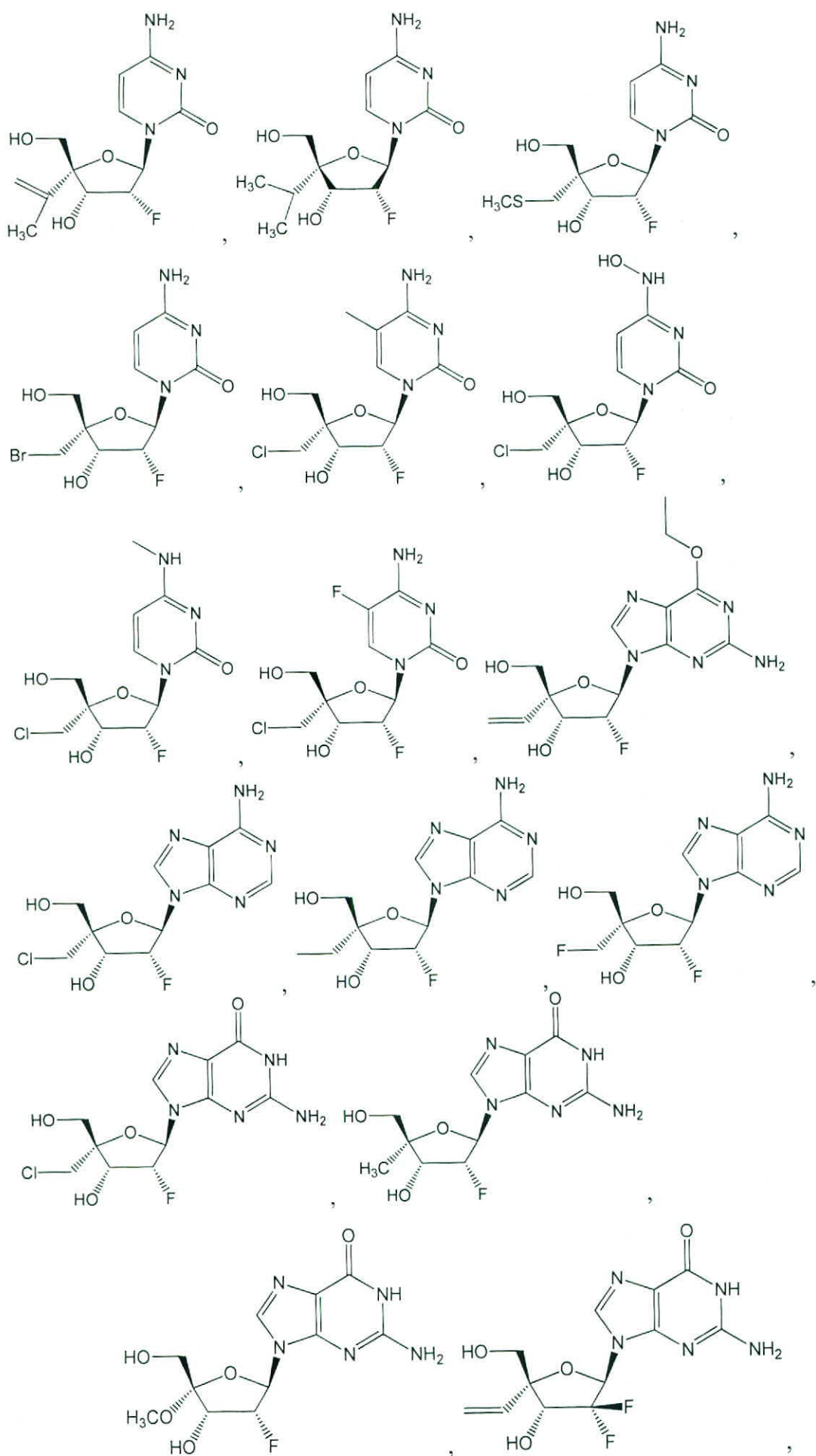
Solicitud No.: 13/721,988

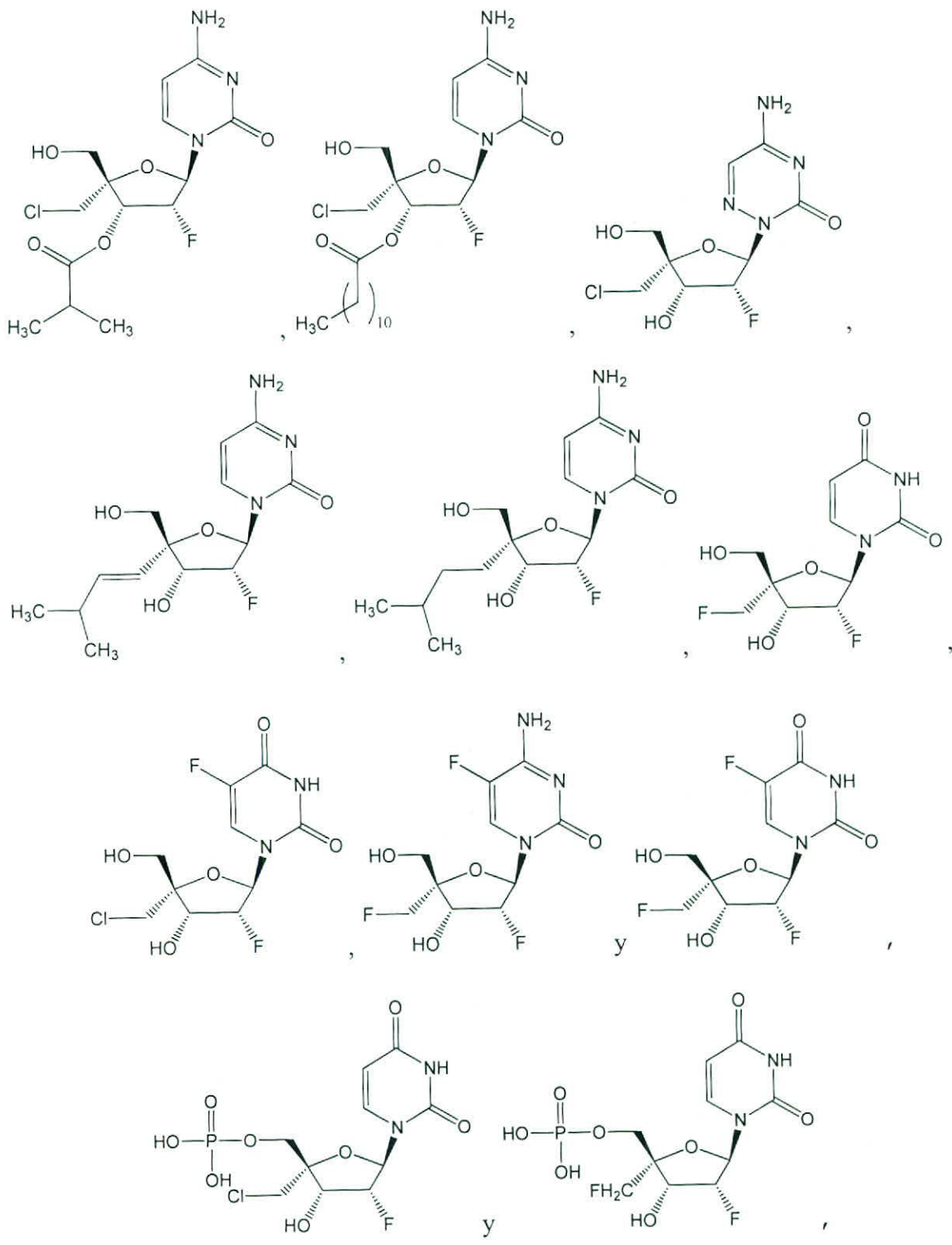
Fecha de presentación: Diciembre 20, 2012



Solicitud No.: 13/721,988

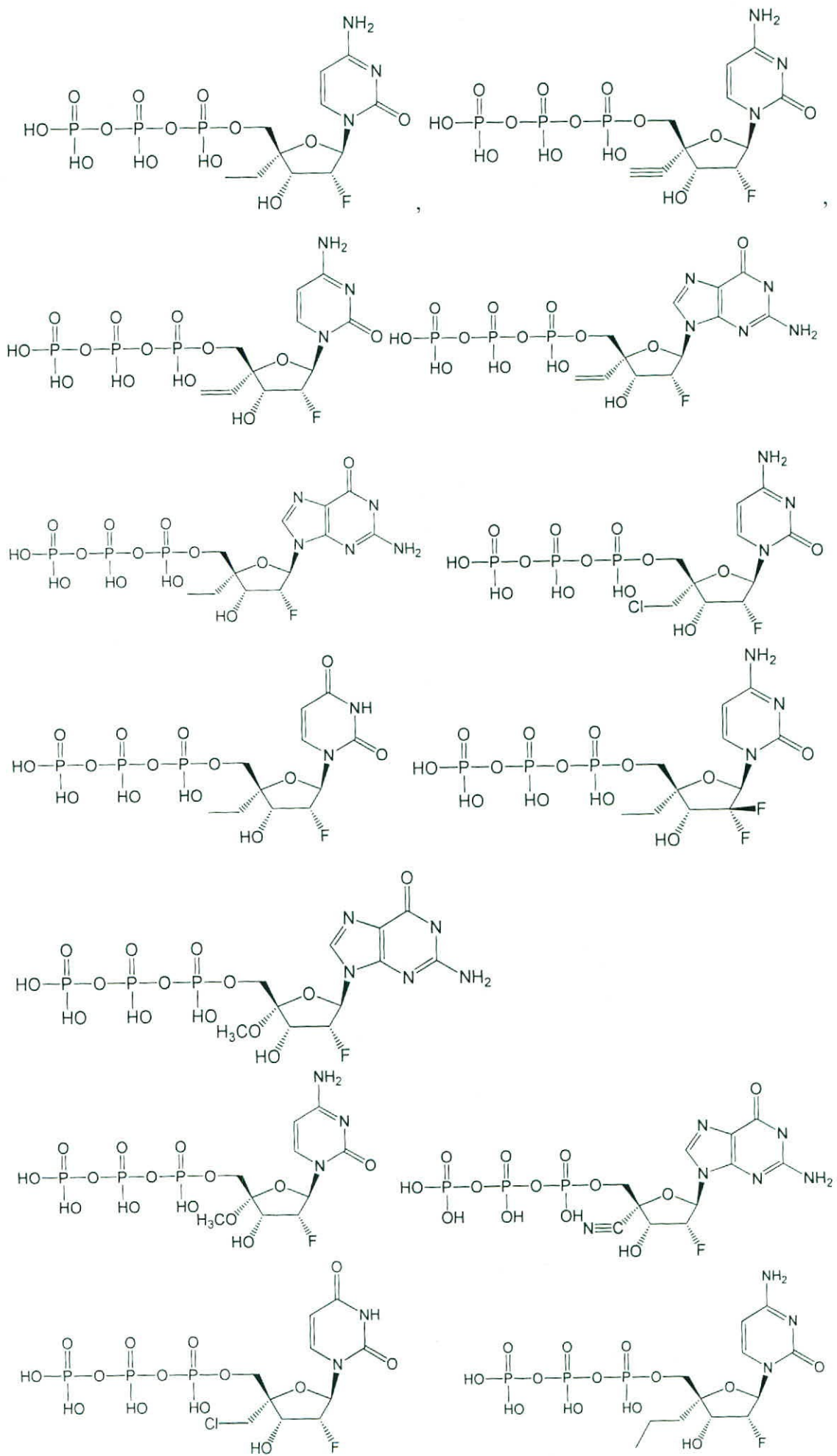
Fecha de presentación: Diciembre 20, 2012





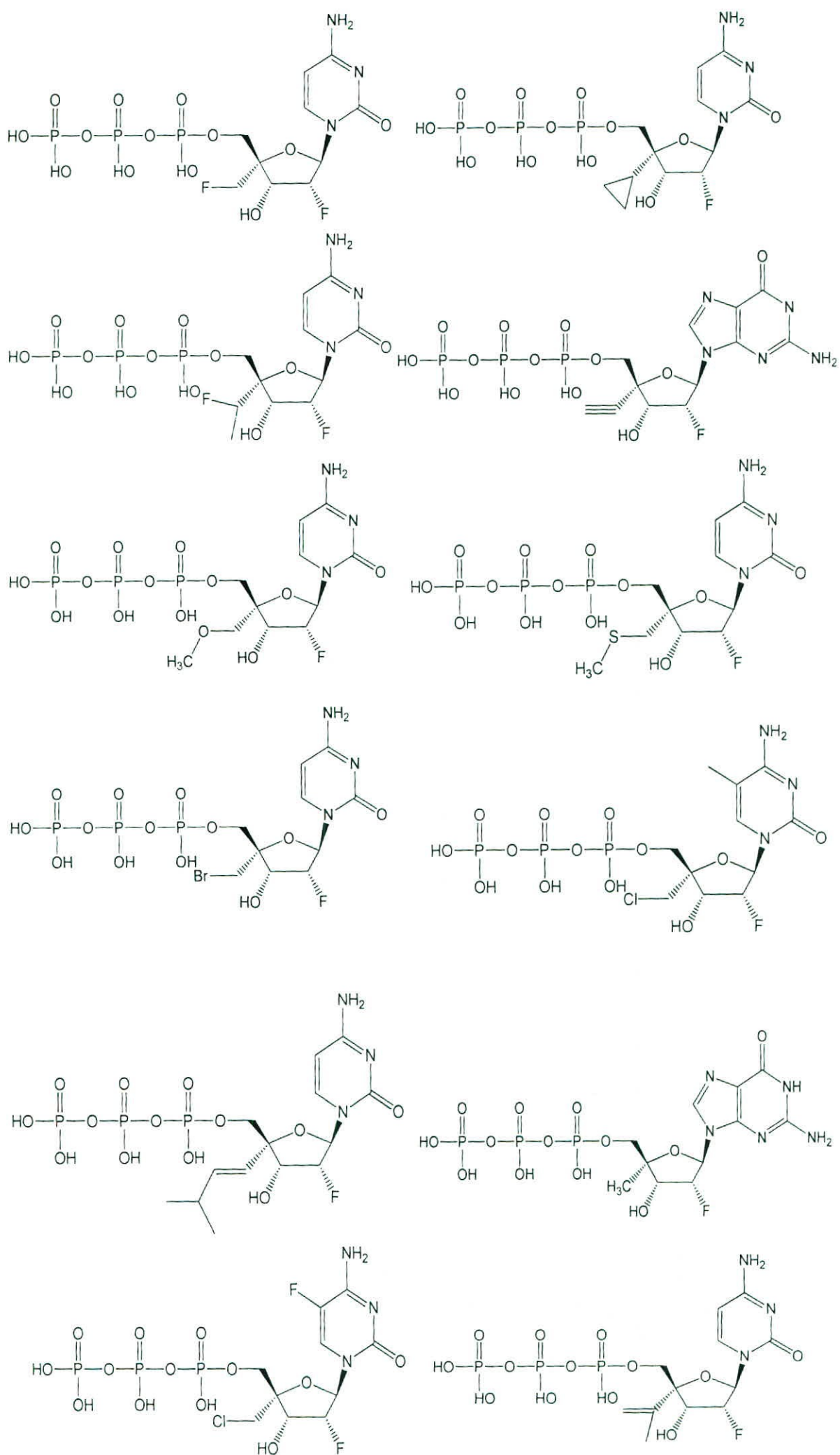
o una sal farmacéuticamente aceptable de lo que antecede.

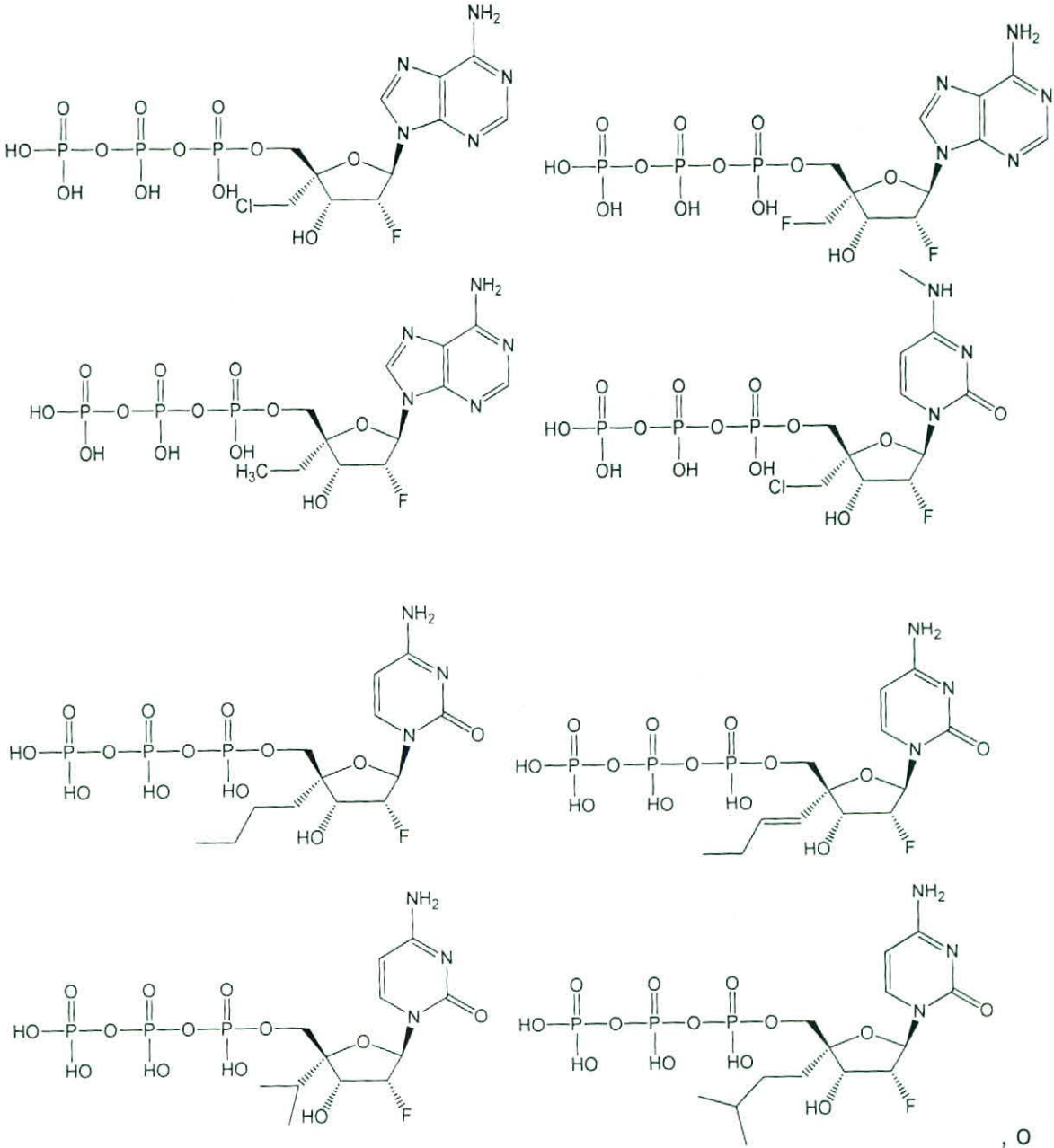
17. El compuesto de acuerdo con la reivindicación 1, en el cual el compuesto de la Fórmula (I) se selecciona del grupo que consiste de:



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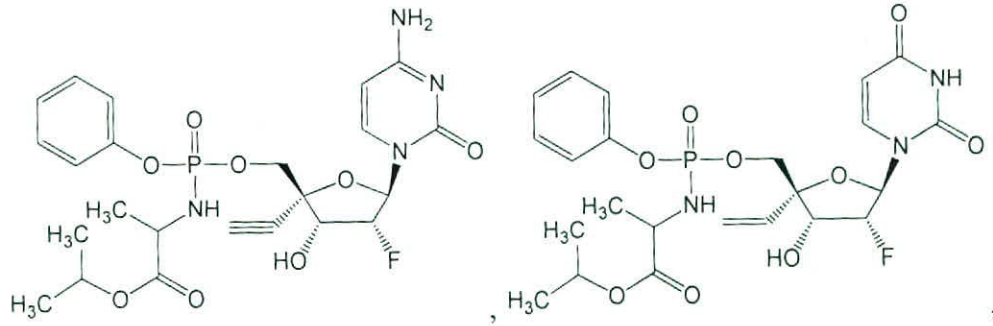
Fecha de presentación: Diciembre 20, 2012

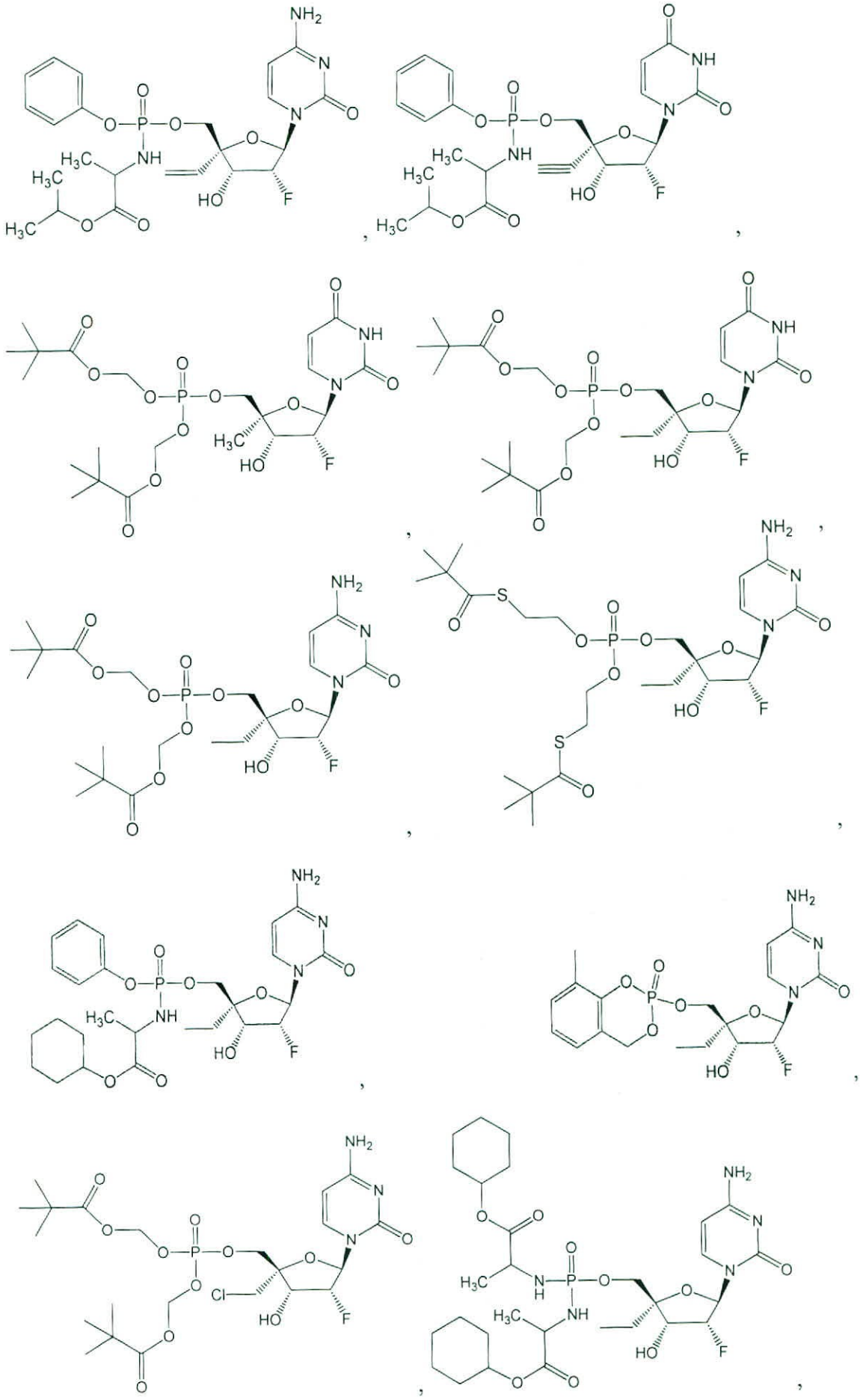


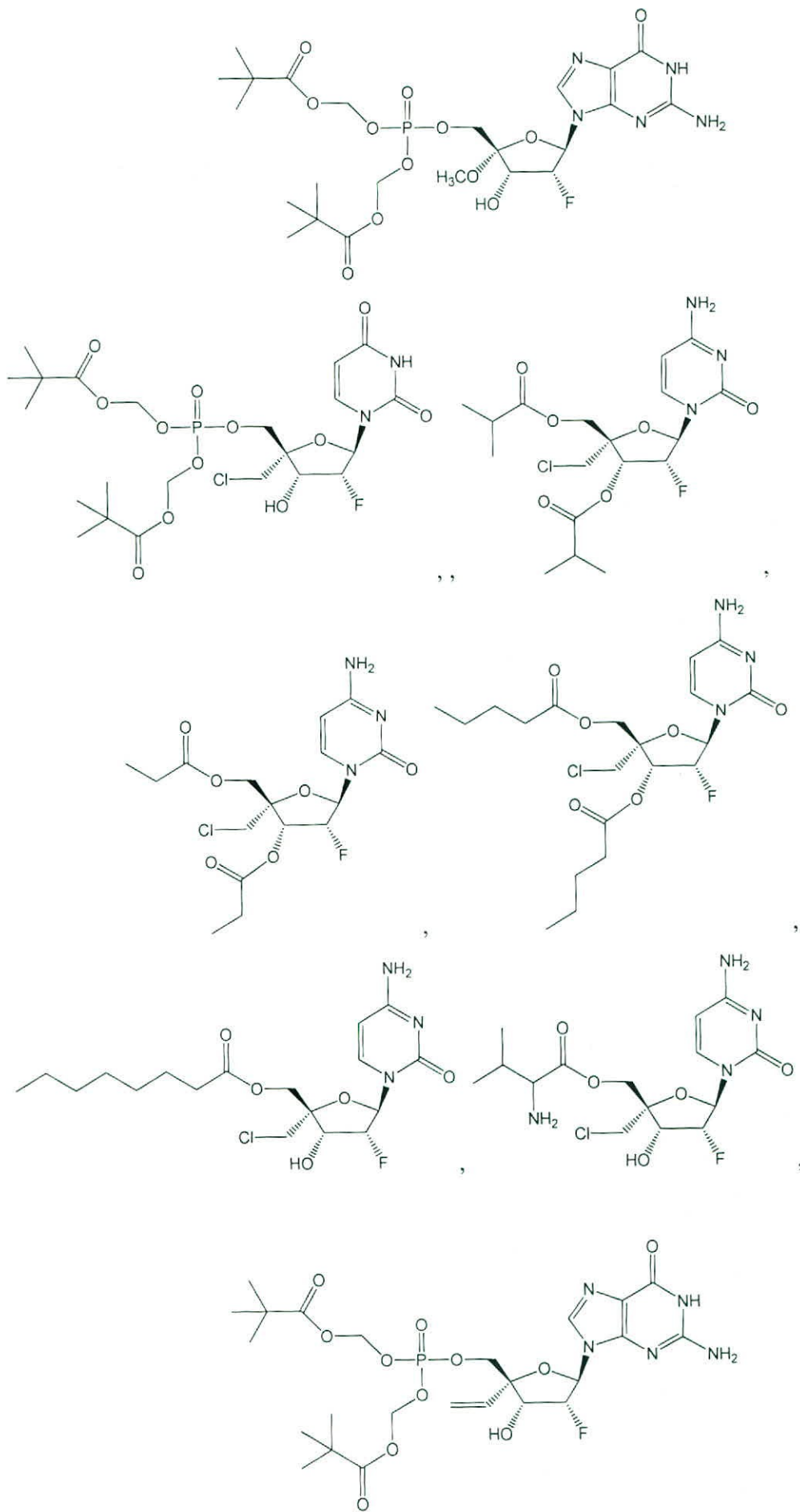


una sal farmacéuticamente aceptable de lo que antecede.

18. El compuesto de acuerdo con la reivindicación 1, en el cual el compuesto de la Fórmula (I) se selecciona del grupo que consiste de:

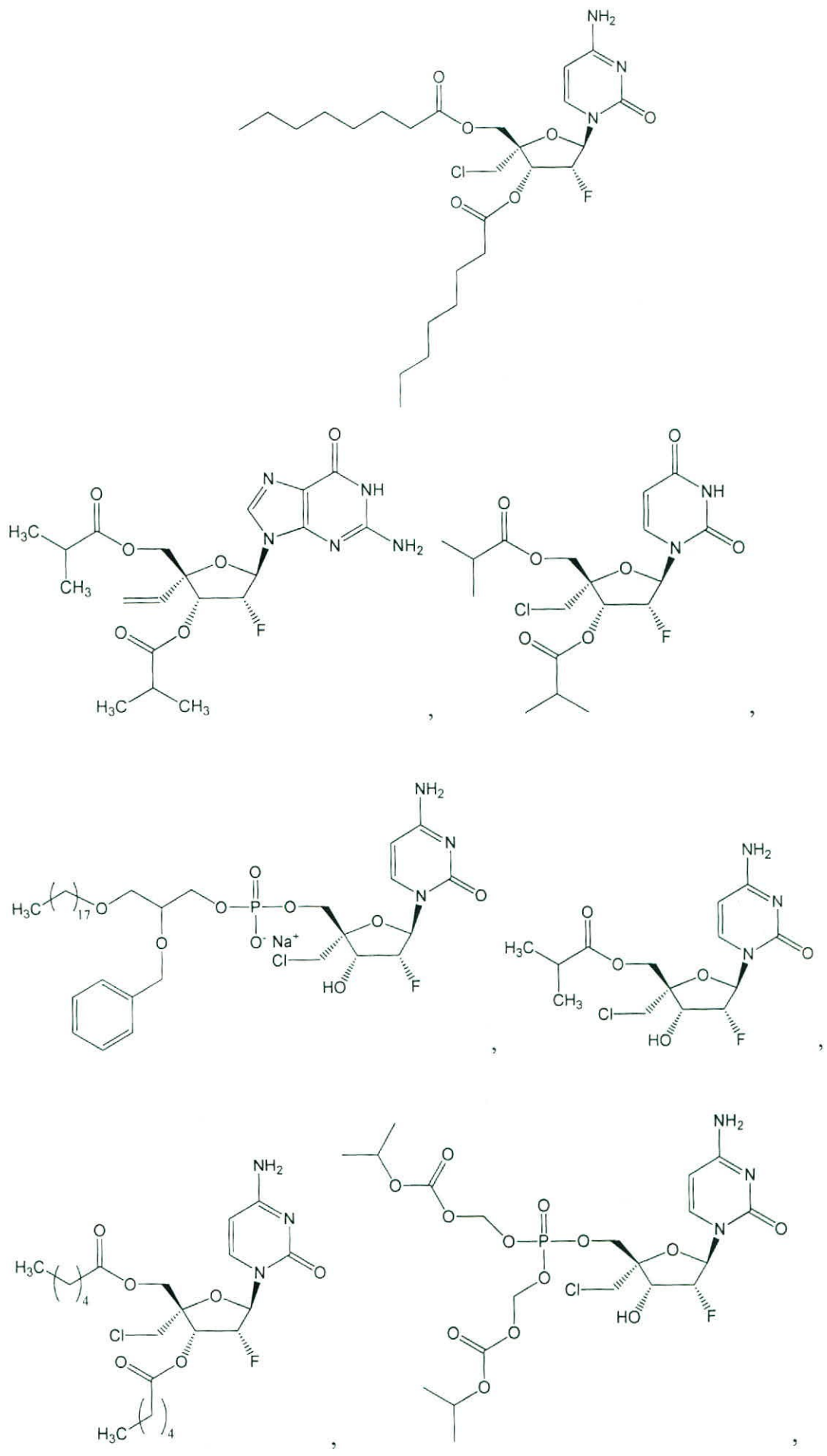


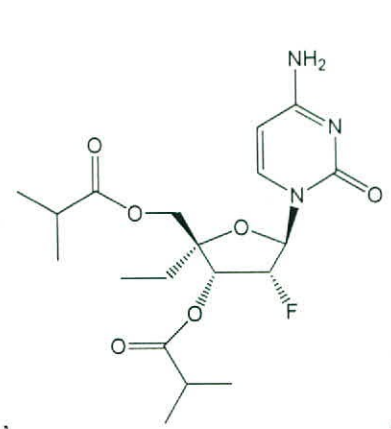
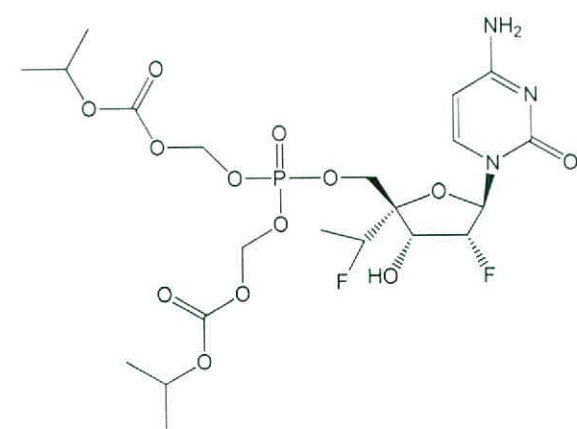
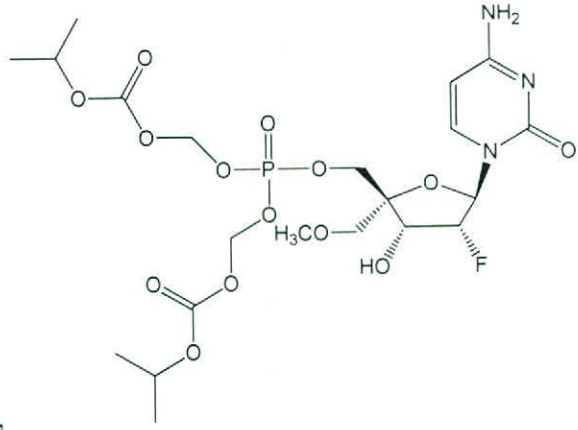
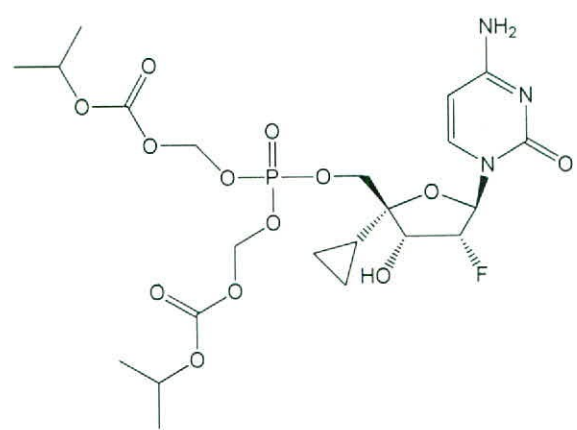
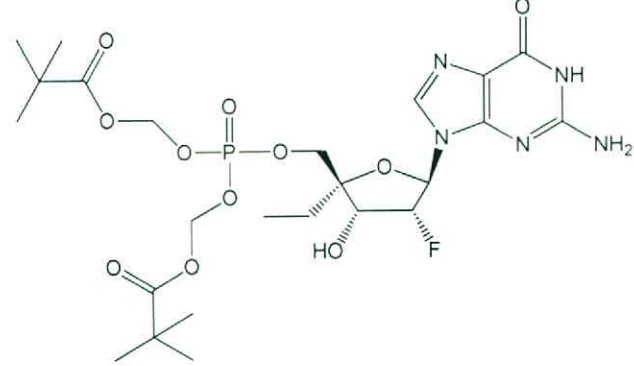
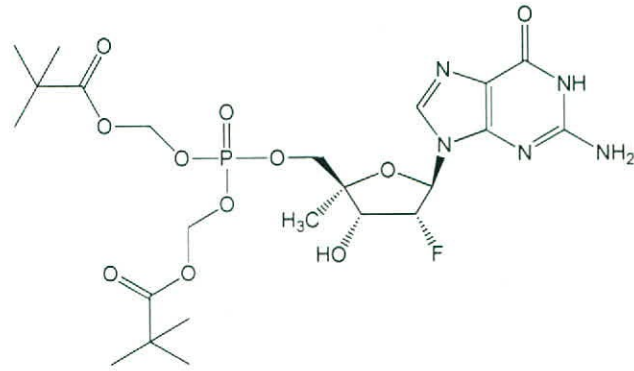


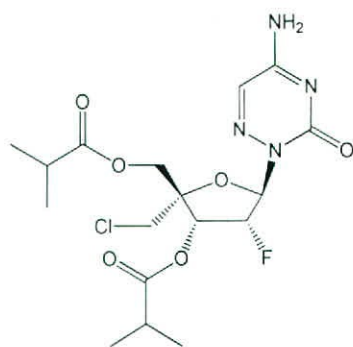


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Fecha de presentación: Diciembre 20, 2012







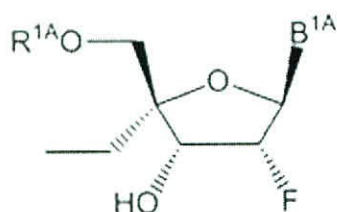
y , o una sal farmacéuticamente aceptable de lo que antecede.

19. Una composición farmacéutica que comprende una cantidad efectiva de un compuesto de acuerdo con la reivindicación 1, o una sal farmacéuticamente aceptable del mismo y un vehículo, diluyente o excipiente farmacéuticamente aceptables, o una combinación de los mismos.

20. Un método para mejorar o tratar una infección viral que comprende poner en contacto una célula infectada con un virus en un sujeto que sufre la infección viral, con una cantidad efectiva de un compuesto de acuerdo con la reivindicación 1, o una sal farmacéuticamente aceptable del mismo, o una composición farmacéutica de acuerdo con la reivindicación 217; y en el cual la infección viral se selecciona entre una infección viral por paramixovirus y una infección viral por ortomixovirus.

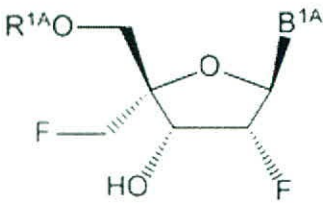
21. Un método para mejorar o tratar una infección viral en combinación con uno o más agentes, que comprende administrar a un sujeto que sufre la infección viral o poner en contacto una célula con una cantidad efectiva de un compuesto de acuerdo con la reivindicación 1, o una sal farmacéuticamente aceptable del mismo; y en el cual la infección viral se selecciona entre una infección viral por paramixovirus y una infección viral por ortomixovirus.

22. El compuesto de acuerdo con la reivindicación 1, en el cual el compuesto de la Fórmula (I) es:



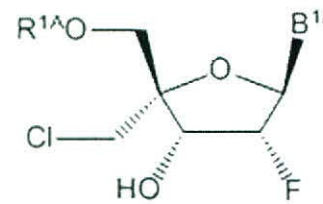
, o una sal farmacéuticamente aceptable del mismo.

23. El compuesto de acuerdo con la reivindicación 1, en el cual el compuesto de la Fórmula (I) es:



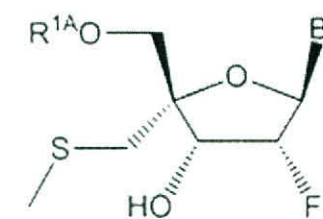
, o una sal farmacéuticamente aceptable del mismo.

24. El compuesto de acuerdo con la reivindicación 1, en el cual el compuesto de la Fórmula (I) es:



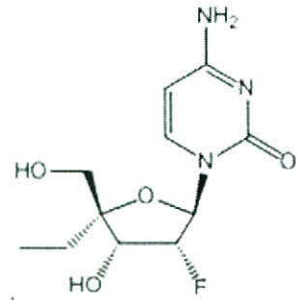
, o una sal farmacéuticamente aceptable del mismo.

25. El compuesto de acuerdo con la reivindicación 1, en el cual el compuesto de la Fórmula (I) es:



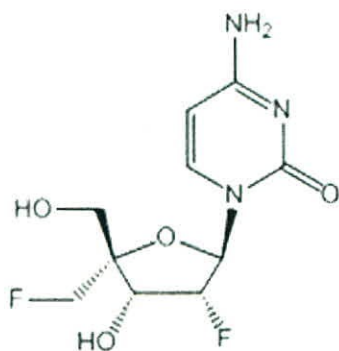
, o una sal farmacéuticamente aceptable del mismo.

26. El compuesto de acuerdo con la reivindicación 1, en el cual el compuesto de la Fórmula (I) es:



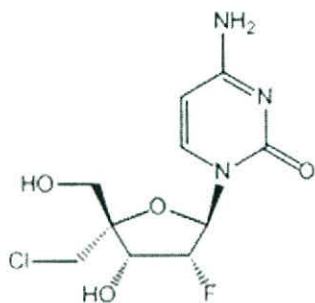
, o una sal farmacéuticamente aceptable del mismo.

27. El compuesto de acuerdo con la reivindicación 1, en el cual el compuesto de la Fórmula (I) es:



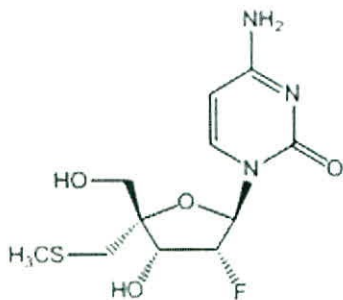
, o una sal farmacéuticamente aceptable del mismo.

28. El compuesto de acuerdo con la reivindicación 1, en el cual el compuesto de la Fórmula (I) es:



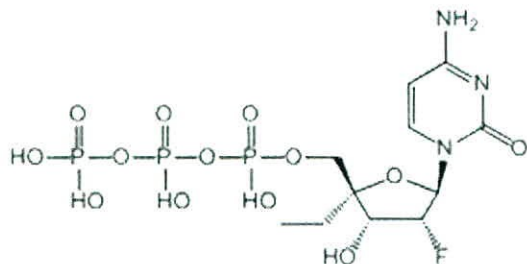
, o una sal farmacéuticamente aceptable del mismo.

29. El compuesto de acuerdo con la reivindicación 1, en el cual el compuesto de la Fórmula (I) es:



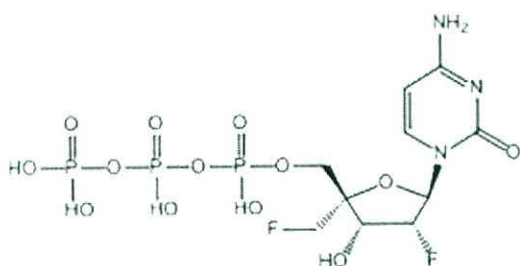
, o una sal farmacéuticamente aceptable del mismo.

30. El compuesto de acuerdo con la reivindicación 1, en el cual el compuesto de la Fórmula (I) es:



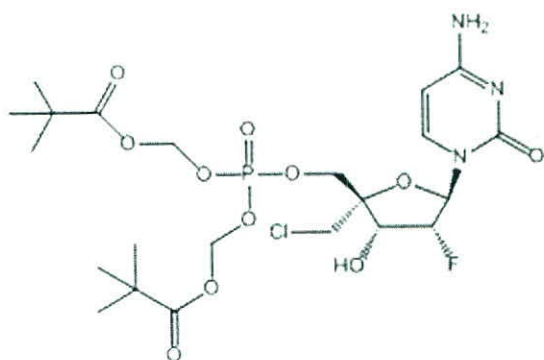
, o una sal farmacéuticamente aceptable del mismo.

31. El compuesto de acuerdo con la reivindicación 1, en el cual el compuesto de la Fórmula (I) es:



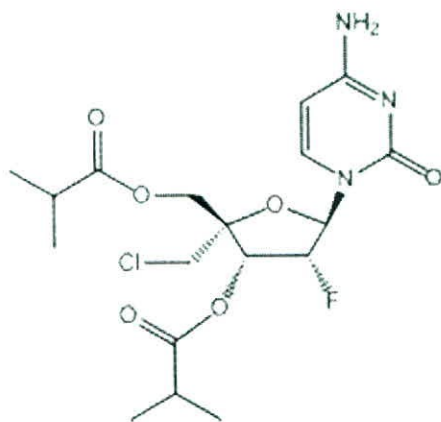
, o una sal farmacéuticamente aceptable del mismo.

32. El compuesto de acuerdo con la reivindicación 1, en el cual el compuesto de la Fórmula (I) es:



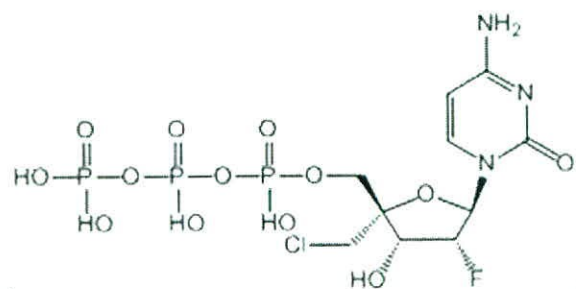
, o una sal farmacéuticamente aceptable del mismo.

33. El compuesto de acuerdo con la reivindicación 1, en el cual el compuesto de la Fórmula (I) es:



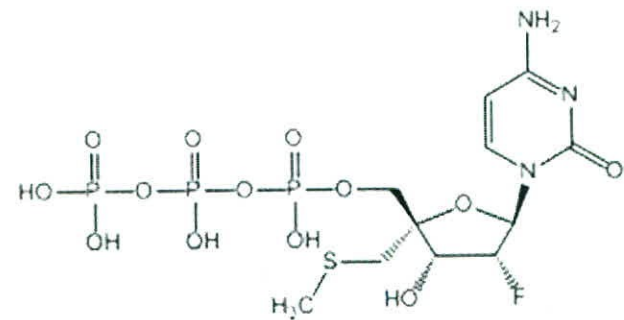
, o una sal farmacéuticamente aceptable del mismo.

34. El compuesto de acuerdo con la reivindicación 1, en el cual el compuesto de la Fórmula (I) es:



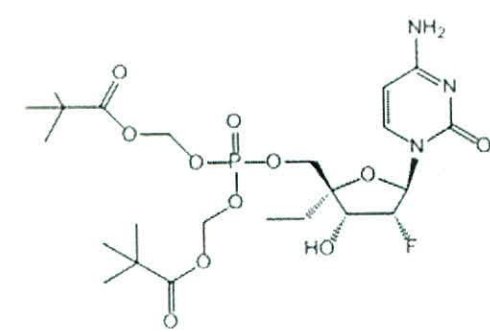
., o una sal farmacéuticamente aceptable del mismo.

35. El compuesto de acuerdo con la reivindicación 1, en el cual el compuesto de la Fórmula (I) es:



, o una sal farmacéuticamente aceptable del mismo.

36. El compuesto de acuerdo con la reivindicación 1, en el cual el compuesto de la Fórmula (I) es:



, o una sal farmacéuticamente aceptable del mismo.

37. El método de acuerdo con la reivindicación 20, en el cual la infección viral es una infección viral por paramixovirus.

38. El método de acuerdo con la reivindicación 37, en el cual la infección viral por el paramixovirus es una infección por el virus sincitial respiratorio humano;

39. El método de acuerdo con la reivindicación 21, en el cual la infección viral es una infección viral por paramixovirus.

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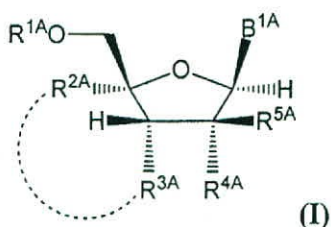
40. El método de acuerdo con la reivindicación 39, en el cual la infección viral por el paramixovirus es una infección por el virus sincitial respiratorio humano;

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AMENDMENTS TO THE CLAIMS

Claims 1, 2, 12, 14 and 17 are amended as shown herein.

1. (CURRENTLY AMENDED) A compound selected from Formula (I), or a pharmaceutically acceptable salt thereof:

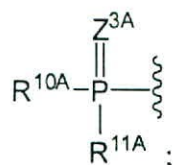
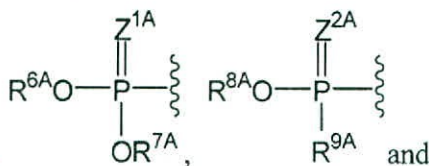


wherein:

B^{1A} is an optionally substituted heterocyclic base or an optionally substituted heterocyclic base with a protected amino group;

R^{1A} is selected from the group consisting of hydrogen, an optionally substituted

acyl, an optionally substituted O-linked amino acid,



[[when]]the dashed line (-----) of Formula (I) is absent[[,]];:

R^{2A} is selected from the group consisting of an ~~optionally~~unsubstituted C₁₋₆ alkyl, a halogen substituted C₁₋₆ alkyl, a hydroxy substituted C₁₋₆ alkyl, an alkoxy substituted C₁₋₆ alkyl, a sulfenyl substituted C₁₋₆ alkyl, an optionally substituted C₂₋₆ alkenyl, an optionally substituted C₂₋₆ alkynyl, an optionally substituted C₃₋₆ cycloalkyl, an optionally substituted -O-C₁₋₆ alkyl, an optionally substituted -O-C₃₋₆ alkynyl, an optionally substituted -O-C₃₋₆ alkynyl and cyano[[,]]; [[and]]

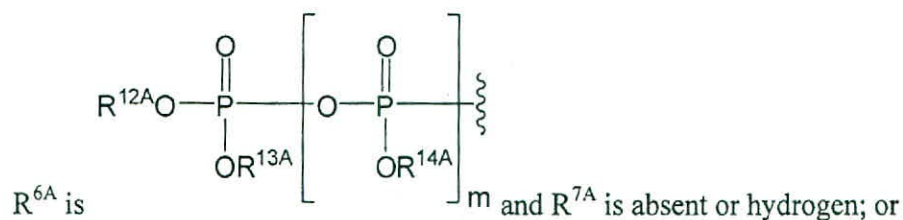
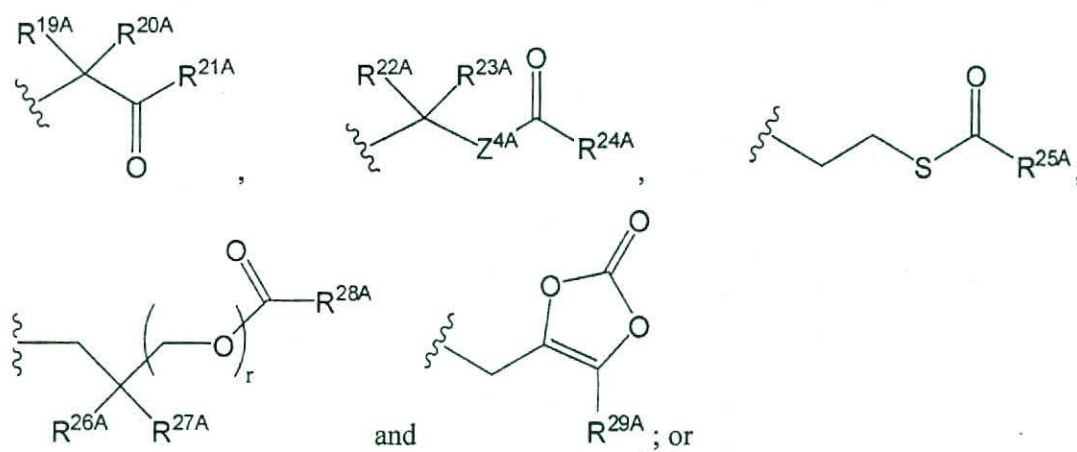
R^{3A} is selected from the group consisting of OH, -OC(=O)R^{''A} and an optionally substituted O-linked amino acid;

R^{4A} is a ~~halogen~~fluoro or chloro;

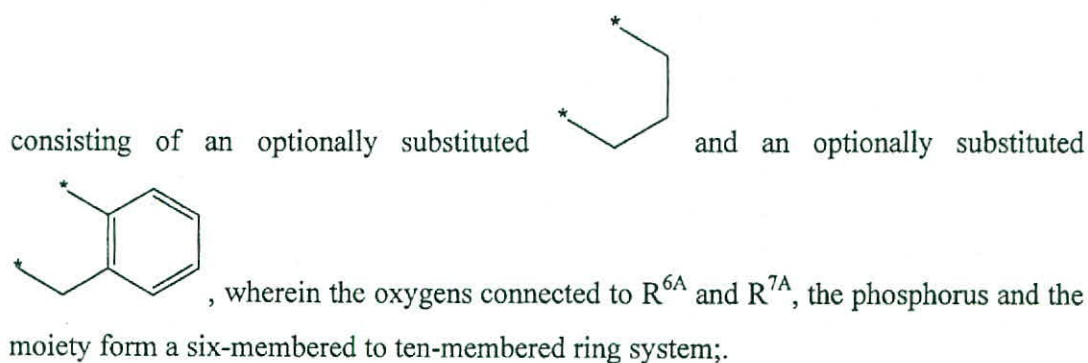
R^{5A} is hydrogen or halogen;

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R^{6A} , R^{7A} and R^{8A} are independently selected from the group consisting of absent, hydrogen, an optionally substituted C_{1-24} alkyl, an optionally substituted C_{2-24} alkenyl, an optionally substituted C_{2-24} alkynyl, an optionally substituted C_{3-6} cycloalkyl, an optionally substituted C_{3-6} cycloalkenyl, an optionally substituted aryl, an optionally substituted heteroaryl, an optionally substituted aryl(C_{1-6} alkyl), an optionally substituted $*(CR^{15A}R^{16A})_p-O-C_{1-24}$ alkyl, an optionally substituted $*(CR^{17A}R^{18A})_q-O-C_{1-24}$ alkenyl,



R^{6A} and R^{7A} are taken together to form a moiety selected from the group



R^{9A} is independently selected from the group consisting of an optionally substituted C_{1-24} alkyl, an optionally substituted C_{2-24} alkenyl, an optionally substituted C_{2-24} alkynyl, an optionally substituted C_{3-6} cycloalkyl, an optionally substituted C_{3-6}

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cycloalkenyl, $\text{NR}^{30\text{A}}\text{R}^{31\text{A}}$, an optionally substituted N-linked amino acid and an optionally substituted N-linked amino acid ester derivative;

$\text{R}^{10\text{A}}$ and $\text{R}^{11\text{A}}$ are independently an optionally substituted N-linked amino acid or an optionally substituted N-linked amino acid ester derivative;

$\text{R}^{12\text{A}}$, $\text{R}^{13\text{A}}$ and $\text{R}^{14\text{A}}$ are independently absent or hydrogen;

each $\text{R}^{15\text{A}}$, each $\text{R}^{16\text{A}}$, each $\text{R}^{17\text{A}}$ and each $\text{R}^{18\text{A}}$ are independently hydrogen, an optionally substituted C_{1-24} alkyl or alkoxy;

$\text{R}^{19\text{A}}$, $\text{R}^{20\text{A}}$, $\text{R}^{22\text{A}}$ and $\text{R}^{23\text{A}}$ are independently selected from the group consisting of hydrogen, an optionally substituted C_{1-24} alkyl and an optionally substituted aryl;

$\text{R}^{21\text{A}}$ and $\text{R}^{24\text{A}}$ are independently selected from the group consisting of hydrogen, an optionally substituted C_{1-24} alkyl, an optionally substituted aryl, an optionally substituted $-\text{O}-\text{C}_{1-24}$ alkyl and an optionally substituted $-\text{O}-\text{aryl}$;

$\text{R}^{25\text{A}}$ and $\text{R}^{29\text{A}}$ are independently selected from the group consisting of hydrogen, an optionally substituted C_{1-24} alkyl and an optionally substituted aryl;

$\text{R}^{26\text{A}}$ and $\text{R}^{27\text{A}}$ are independently $-\text{C}\equiv\text{N}$ or an optionally substituted substituent selected from the group consisting of C_{2-8} organylcarbonyl, C_{2-8} alkoxy carbonyl and C_{2-8} organylaminocarbonyl;

$\text{R}^{28\text{A}}$ is selected from the group consisting of hydrogen, an optionally substituted C_{1-24} -alkyl, an optionally substituted C_{2-24} alkenyl, an optionally substituted C_{2-24} alkynyl, an optionally substituted C_{3-6} cycloalkyl and an optionally substituted C_{3-6} cycloalkenyl;

$\text{R}^{30\text{A}}$ and $\text{R}^{31\text{A}}$ are independently selected from the group consisting of hydrogen, an optionally substituted C_{1-24} -alkyl, an optionally substituted C_{2-24} alkenyl, an optionally substituted C_{2-24} alkynyl, an optionally substituted C_{3-6} cycloalkyl and an optionally substituted C_{3-6} cycloalkenyl;

R^{mA} is an optionally substituted C_{1-24} -alkyl;

m is 0 or 1;

p and q are independently selected from the group consisting of 1, 2 and 3;

r is 1 or 2;

$\text{Z}^{1\text{A}}$, $\text{Z}^{2\text{A}}$, $\text{Z}^{3\text{A}}$ and $\text{Z}^{4\text{A}}$ are independently O or S; and

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$$\text{R}^{8\text{A}}\text{O}-\text{P}\begin{matrix} \text{Z}^{2\text{A}} \\ \parallel \\ \text{R}^{9\text{A}} \end{matrix}-\text{wavy line}$$

provided that when the dashed line (---) of Formula (I) is absent; R^{1A} is H; R^{3A} is OH; R^{4A} is fluoro; R^{5A} is fluoro; and B^{1A} is an unsubstituted cytosine; then R^{2A} cannot be ethynyl.

$$\begin{array}{c} \text{Z}^{1\text{A}} \\ \parallel \\ \text{R}^{6\text{A}}\text{O}-\text{P}-\text{OR}^{7\text{A}}; \text{R}^{6\text{A}} \text{ is} \\ \text{OR}^{7\text{A}} \end{array} \quad \text{R}^{12\text{A}}\text{O}-\text{P}(=\text{O})(\text{OR}^{13\text{A}})-\left[\text{O}-\text{P}(=\text{O})(\text{OR}^{14\text{A}})\right]_m$$

m; and R^{7A} is absent or hydrogen.

5. (ORIGINAL) The compound of Claim 1, wherein R^{1A} is an optionally substituted O-linked amino acid.

5A

$$\begin{array}{ccc} \text{R}^6\text{O}-\text{P} \begin{array}{l} \text{Z}^{1\text{A}} \\ \text{OR}^{7\text{A}} \end{array} & \text{R}^8\text{O}-\text{P} \begin{array}{l} \text{Z}^{2\text{A}} \\ \text{R}^{9\text{A}} \end{array} & \text{R}^{10\text{A}}-\text{P} \begin{array}{l} \text{Z}^{3\text{A}} \\ \text{R}^{11\text{A}} \end{array} \\ & \text{OR} & \end{array}$$

8. (ORIGINAL) The compound of Claim 6, wherein both R^{6A} and R^{7A} are

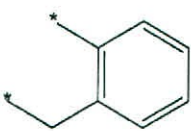
*C(R19A)(R20A)C(=O)R21A



 $-(CR^{17A}R^{18A})_q-O-C_{2-24}$ alkenyl; or wherein R^{6A} and R^{7A} can be taken together to form a moiety $-(CR^{15A}R^{16A})_p-O-C_{1-24}$ alkyl or $-(CR^{17A}R^{18A})_q-O-C_{2-24}$ alkenyl; or wherein R^{6A} and R^{7A} can be taken together to form a moiety

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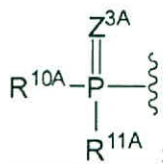
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substituted , wherein the oxygens connected to R^{6A} and R^{7A}, the phosphorus and the moiety form a six-membered to ten-membered ring system.

10. (ORIGINAL) The compound of Claim 6, wherein R^{8A} is selected from the group consisting of absent, hydrogen, an optionally substituted C₁₋₂₄ alkyl, an optionally substituted C₂₋₂₄ alkenyl, an optionally substituted C₂₋₂₄ alkynyl, an optionally substituted C₃₋₆ cycloalkyl and an optionally substituted C₃₋₆ cycloalkenyl; and R^{9A} is independently selected from the group consisting of an optionally substituted C₁₋₂₄ alkyl, an optionally substituted C₂₋₂₄ alkenyl, an optionally substituted C₂₋₂₄ alkynyl, an optionally substituted C₃₋₆ cycloalkyl, an optionally substituted C₃₋₆ cycloalkenyl and NR^{30A}R^{31A}.

11. (ORIGINAL) The compound of Claim 6, wherein R^{8A} is an optionally substituted aryl; and R^{9A} is an optionally substituted N-linked amino acid or an optionally substituted N-linked amino acid ester derivative.

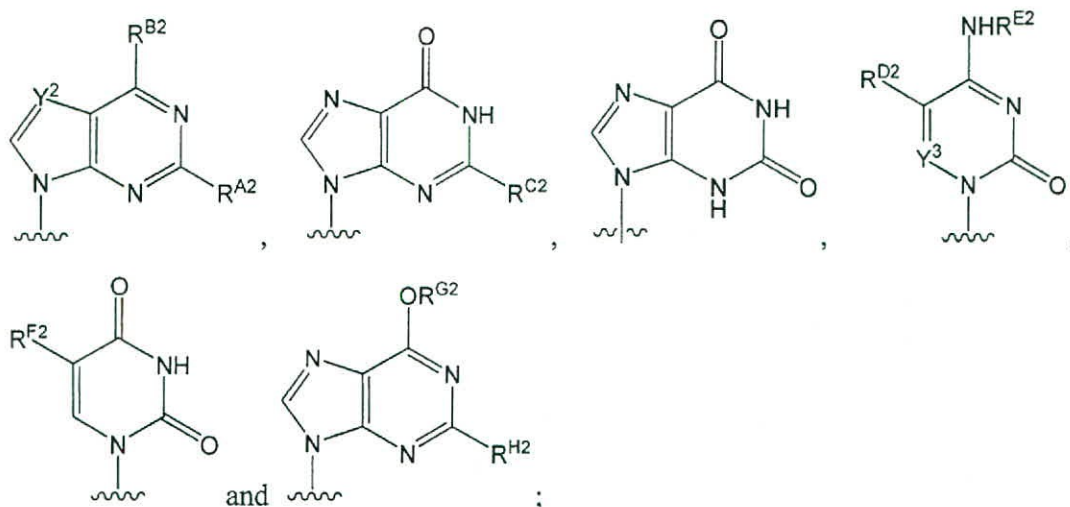
12. (CURRENTLY AMENDED) The compound of Claim 6, wherein R^{1A} is



R^{11A}; and R^{10A} and R^{11A} are both independently an optionally substituted N-linked amino acid or an optionally substituted N-linked amino acid ester derivative.

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13. (ORIGINAL) The compound of Claim 1, wherein B^{1A} is selected from the group consisting of:



wherein:

R^{A2} is selected from the group consisting of hydrogen, halogen and NHR^{J2} , wherein R^{J2} is selected from the group consisting of hydrogen, $-C(=O)R^{K2}$ and $-C(=O)OR^{L2}$;

R^{B2} is halogen or NHR^{W2} , wherein R^{W2} is selected from the group consisting of hydrogen, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl, an optionally substituted C_{3-8} cycloalkyl, $-C(=O)R^{M2}$ and $-C(=O)OR^{N2}$;

R^{C2} is hydrogen or NHR^{O2} , wherein R^{O2} is selected from the group consisting of hydrogen, $-C(=O)R^{P2}$ and $-C(=O)OR^{Q2}$;

R^{D2} is selected from the group consisting of hydrogen, halogen, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl and an optionally substituted C_{2-6} alkynyl;

R^{E2} is selected from the group consisting of hydrogen, hydroxy, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{3-8} cycloalkyl, $-C(=O)R^{R2}$ and $-C(=O)OR^{S2}$;

R^{F2} is selected from the group consisting of hydrogen, halogen, an optionally substituted C_{1-6} alkyl, an optionally substituted C_{2-6} alkenyl and an optionally substituted C_{2-6} alkynyl;

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Y^2 and Y^3 are independently N or CR^{12} , wherein R^{12} is selected from the group consisting of hydrogen, halogen, an optionally substituted C_{1-6} -alkyl, an optionally substituted C_{2-6} -alkenyl and an optionally substituted C_{2-6} -alkynyl;

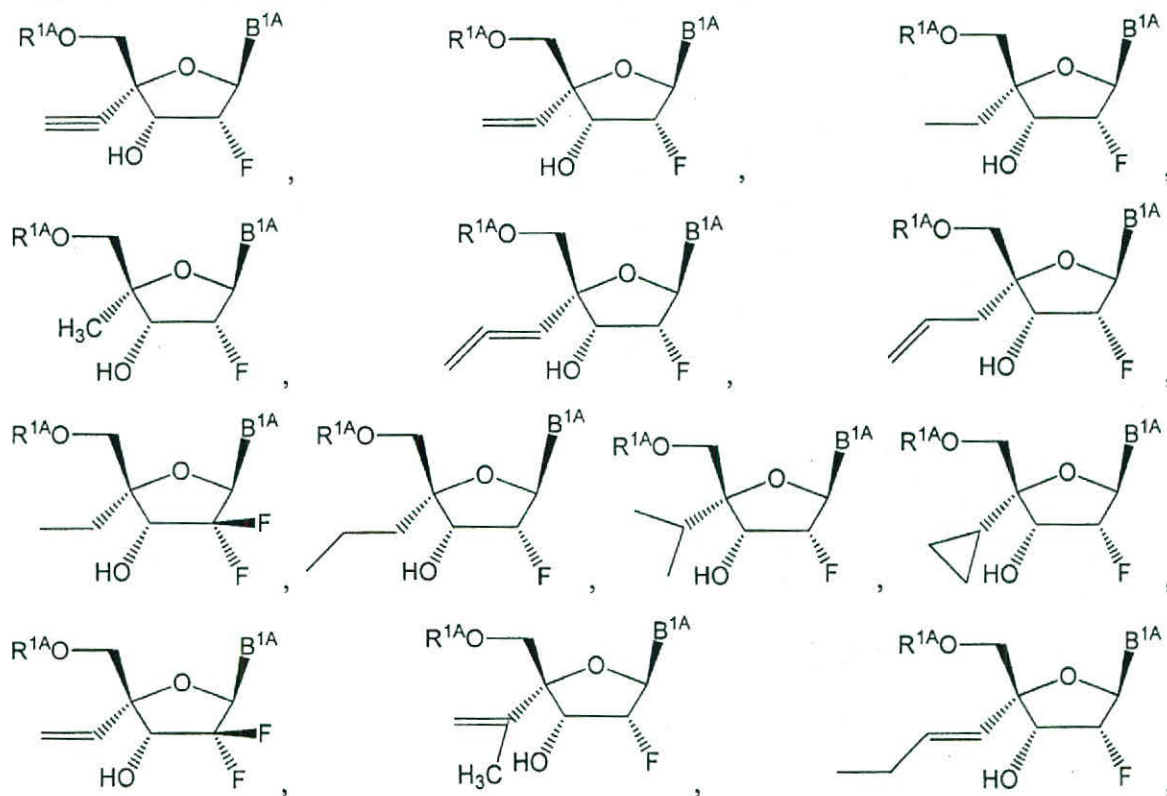
R^{G2} is an optionally substituted C_{1-6} alkyl;

R^{H2} is hydrogen or NHR^{T2} , wherein R^{T2} is independently selected from the group consisting of hydrogen, $-C(=O)R^{U2}$ and $-C(=O)OR^{V2}$; and

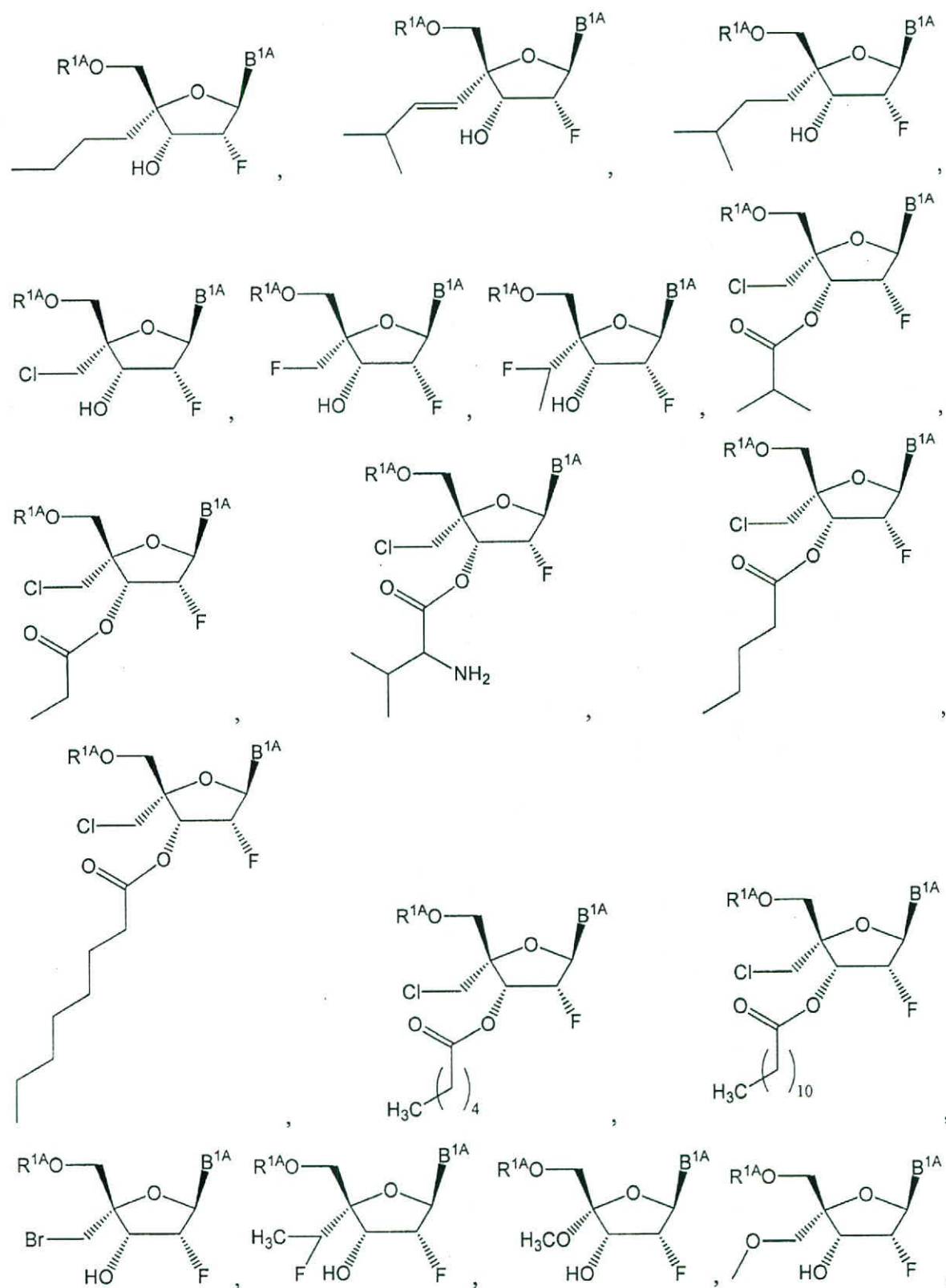
R^{K2} , R^{L2} , R^{M2} , R^{N2} , R^{P2} , R^{Q2} , R^{R2} , R^{S2} , R^{U2} and R^{V2} are independently selected from the group consisting of C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-6} cycloalkyl, C_{3-6} cycloalkenyl, C_{6-10} aryl, heteroaryl, heteroalicycyl, aryl(C_{1-6} alkyl), heteroaryl(C_{1-6} alkyl) and heteroalicycyl(C_{1-6} alkyl).

14. (CURRENTLY AMENDED) The compound of Claim 1, wherein ~~the dashed line (---) is absent, R^{2A} is an optionally substituted C_{1-6} alkyl a halogen substituted C_{1-6} alkyl or a sulfenyl substituted C_{1-6} alkyl, and R^{3A} is OH or $-OC(=O)R^A$.~~

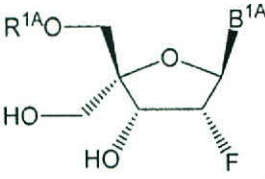
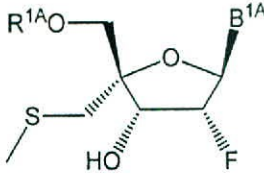
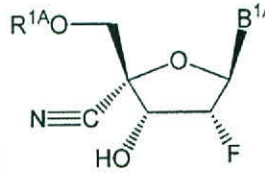
15. (PREVIOUSLY PRESENTED) The compound of Claim 1, wherein the compound of Formula (I) is selected from the group consisting of:



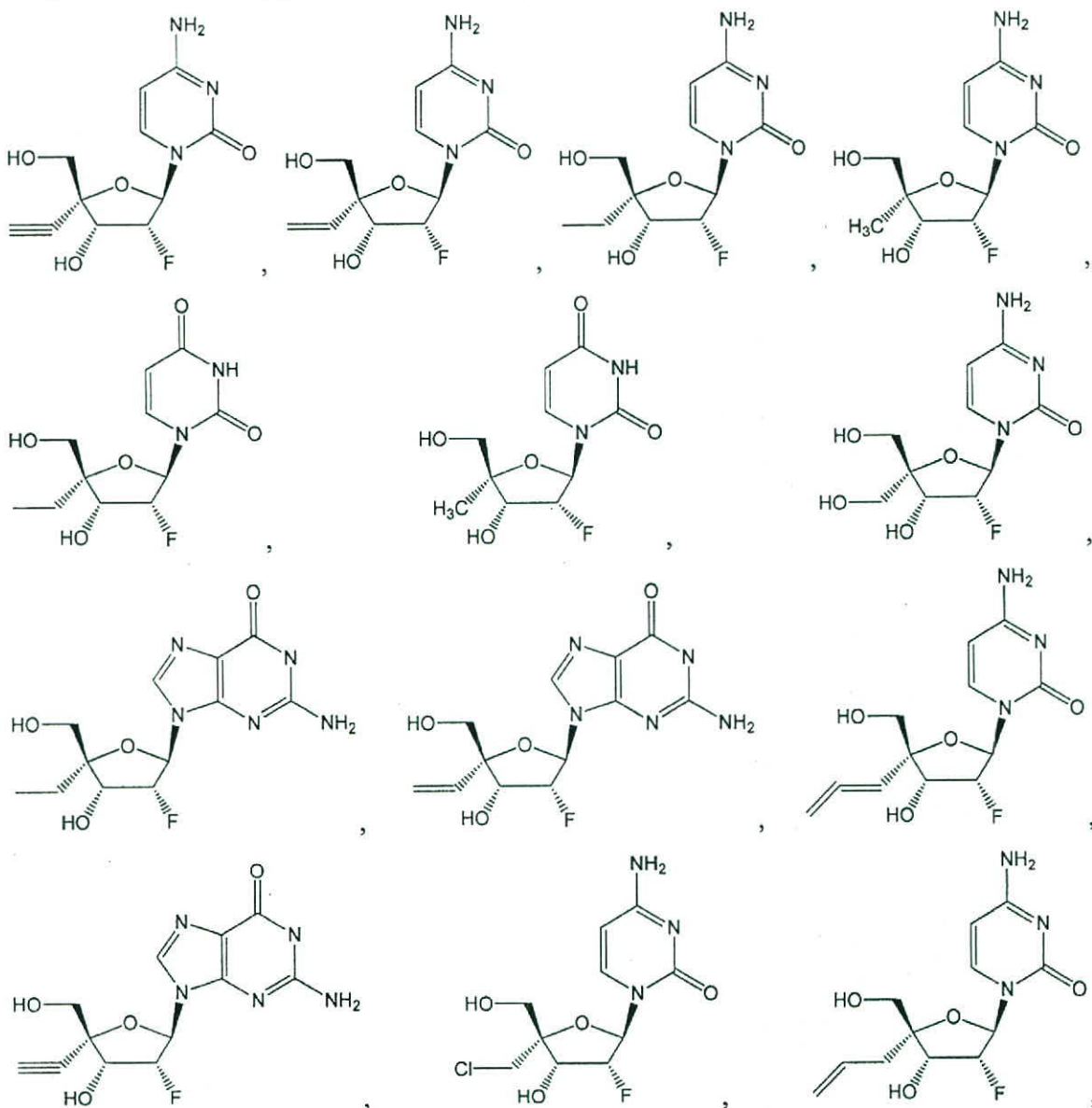
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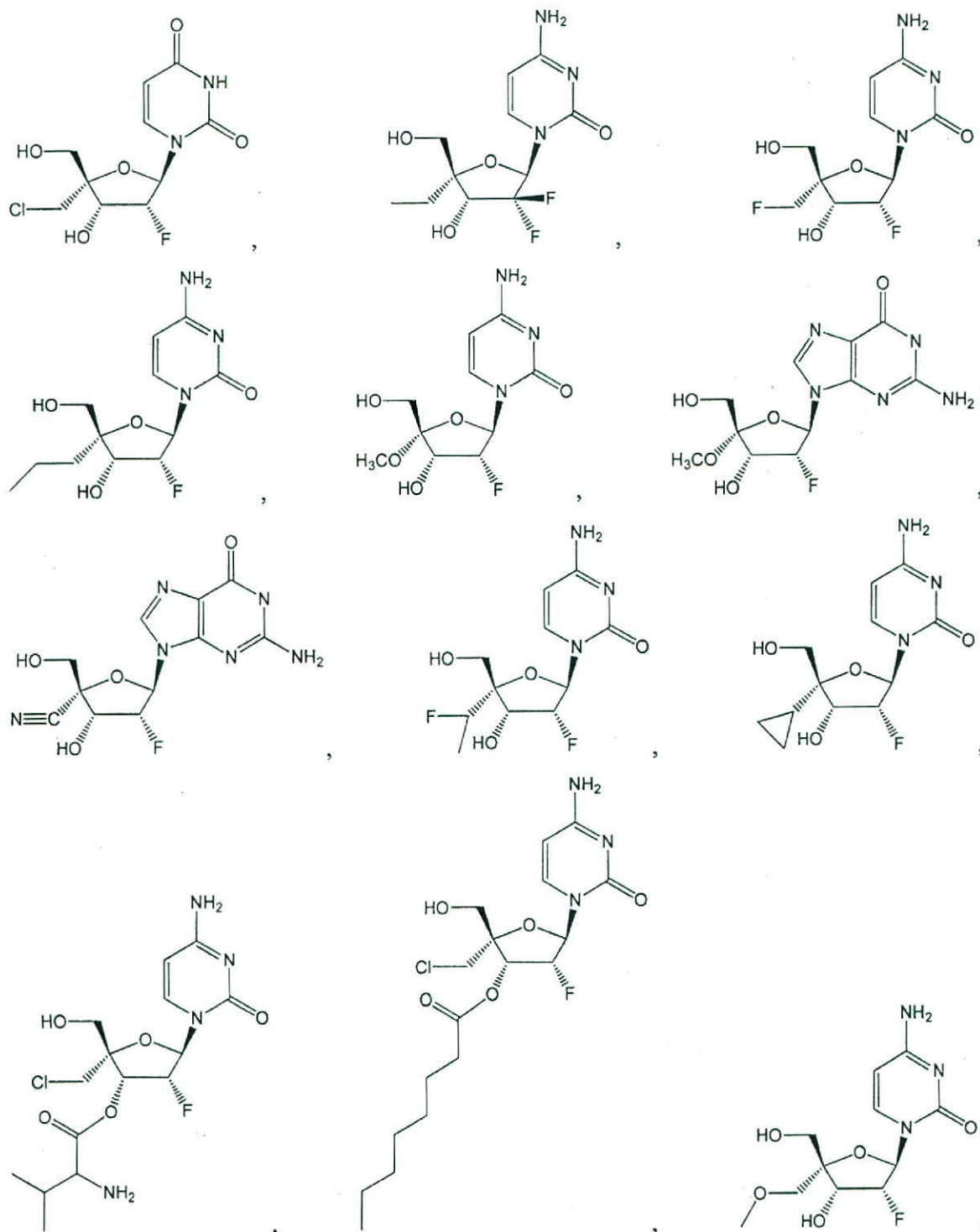
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, or a pharmaceutically acceptable salt thereof.

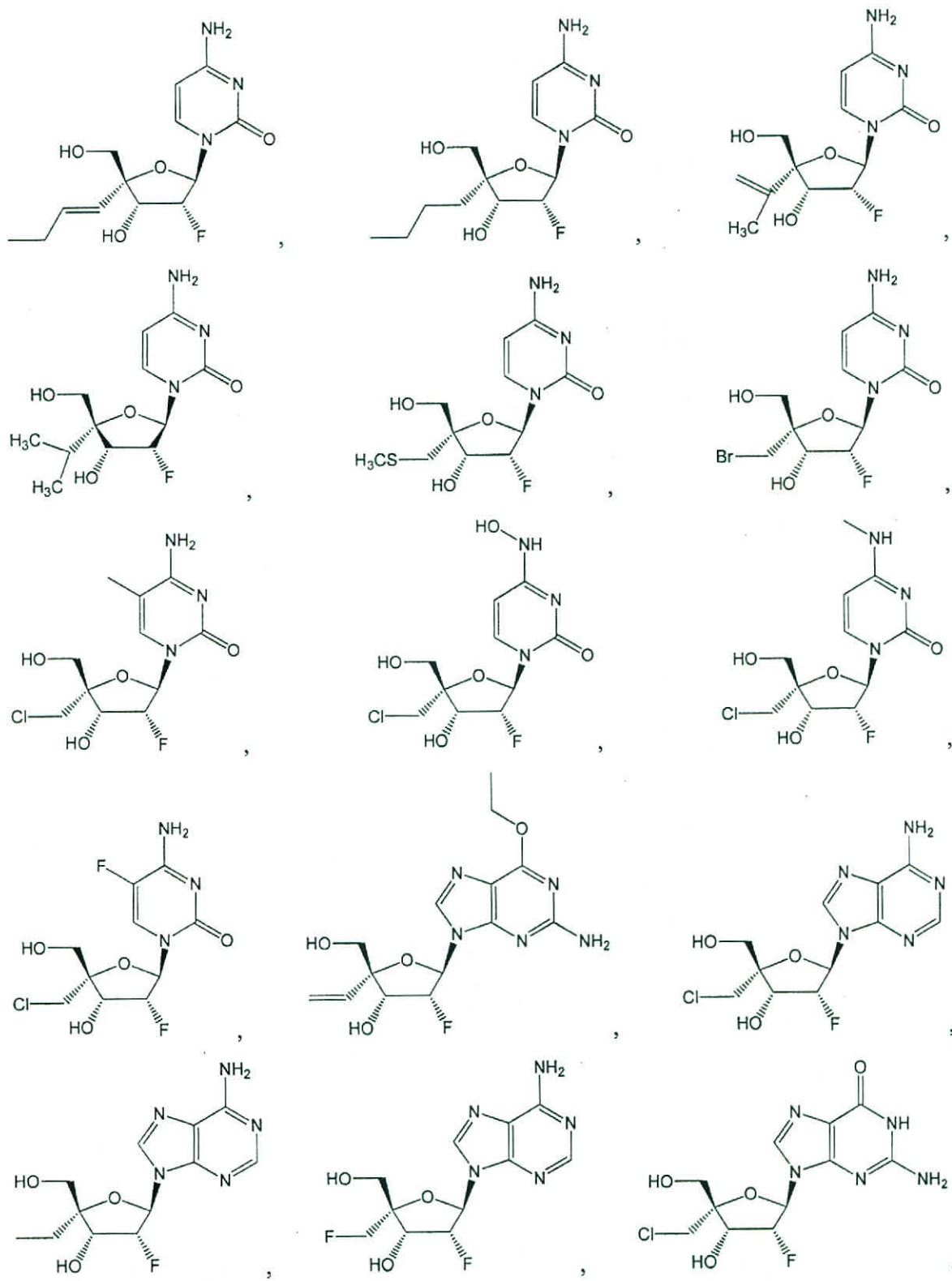
16. (PREVIOUSLY PRESENTED) The compound of Claim 1, wherein the compound of Formula (I) is selected from the group consisting of:



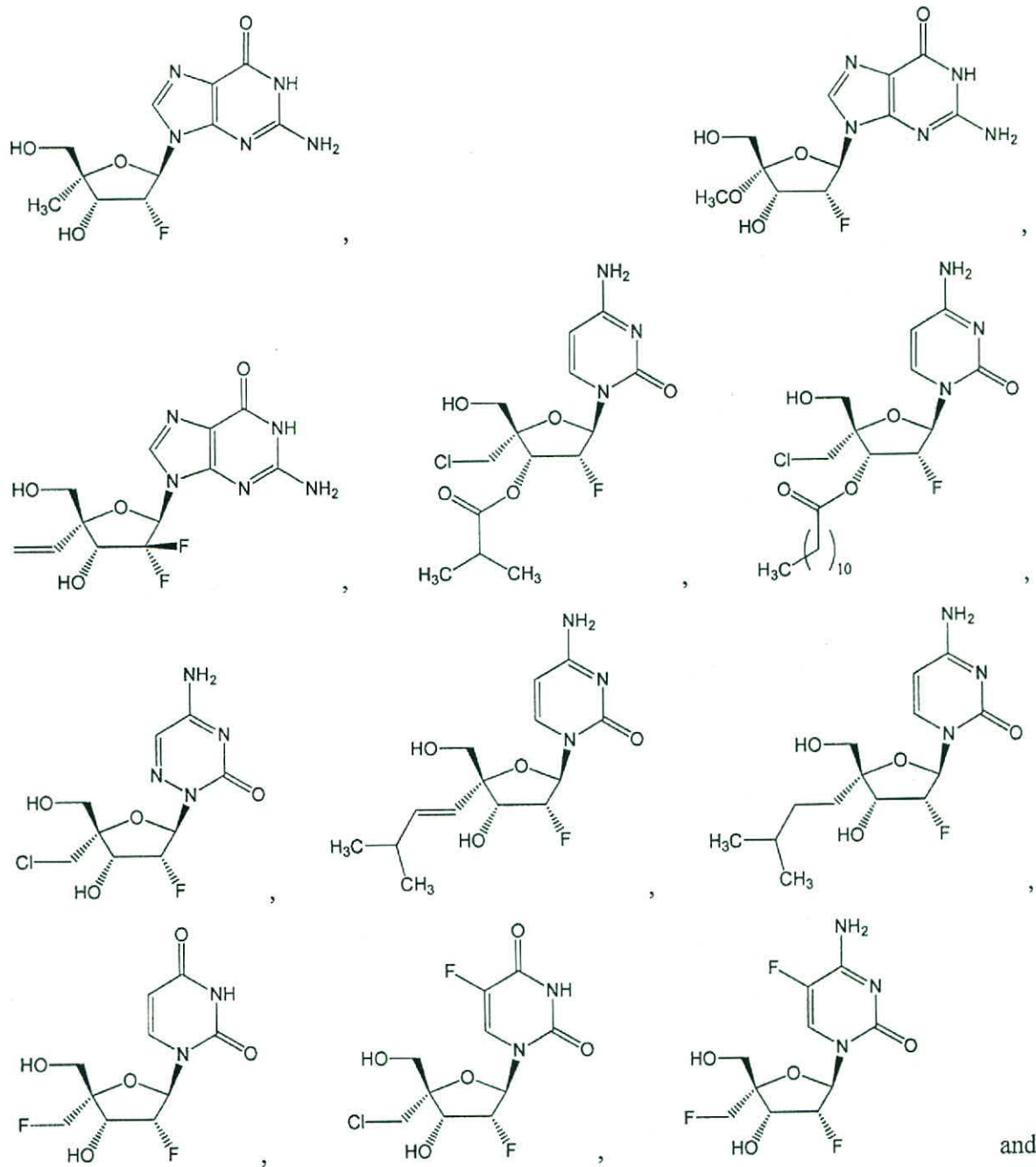
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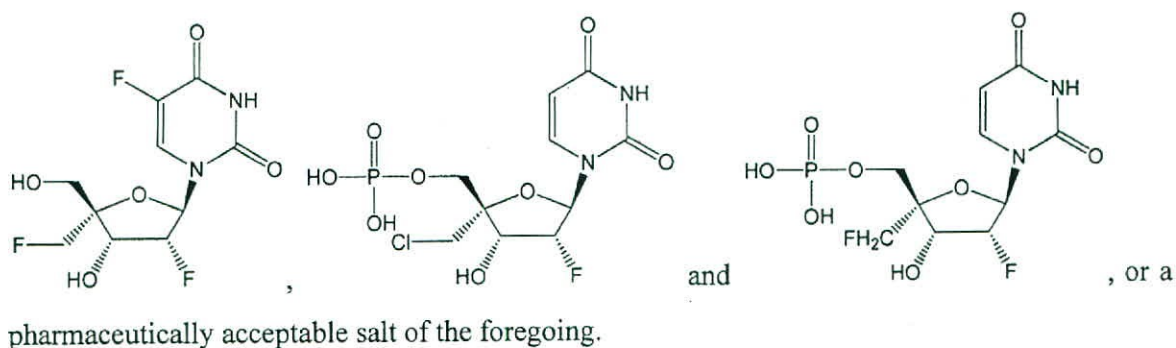
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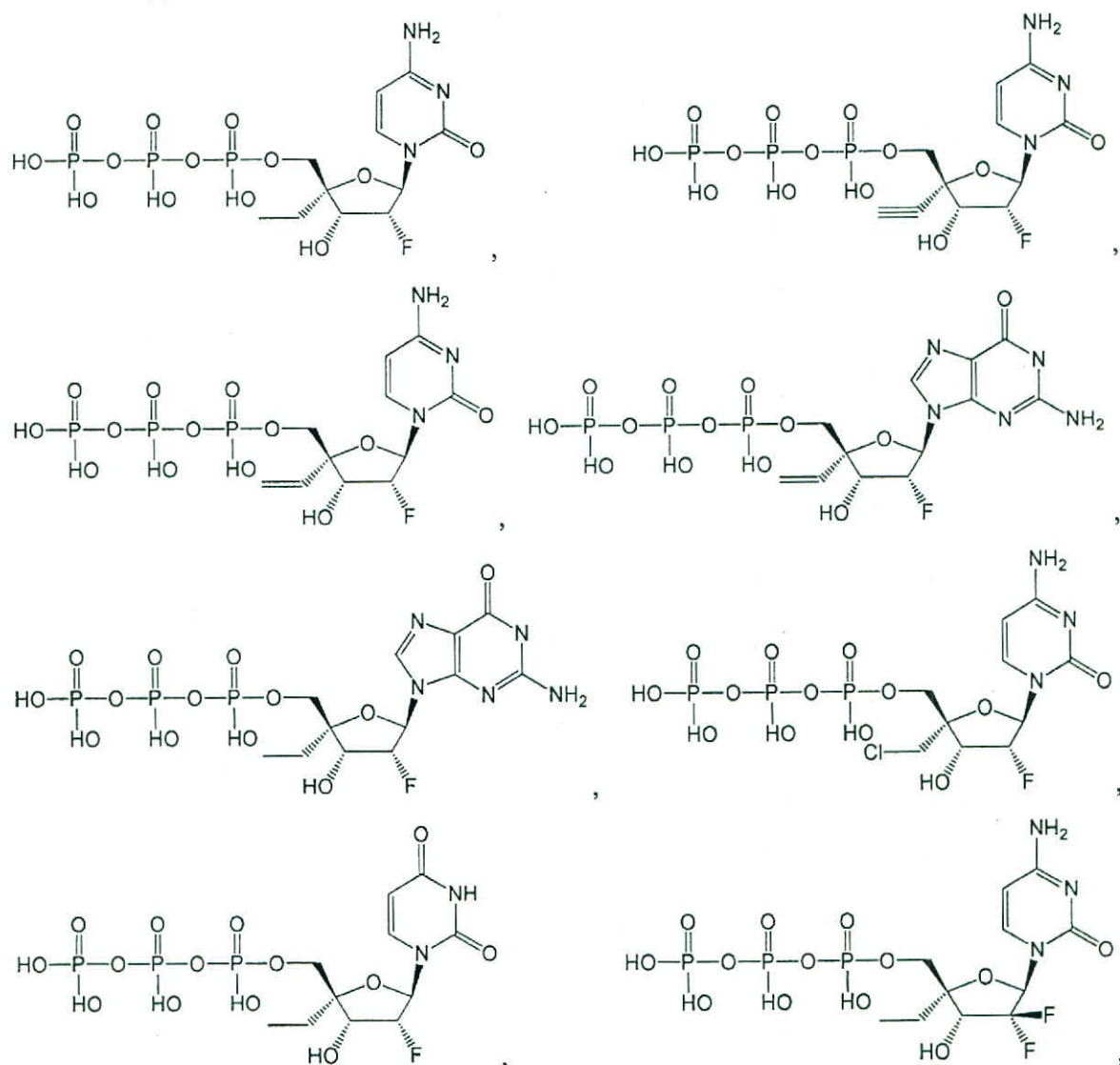
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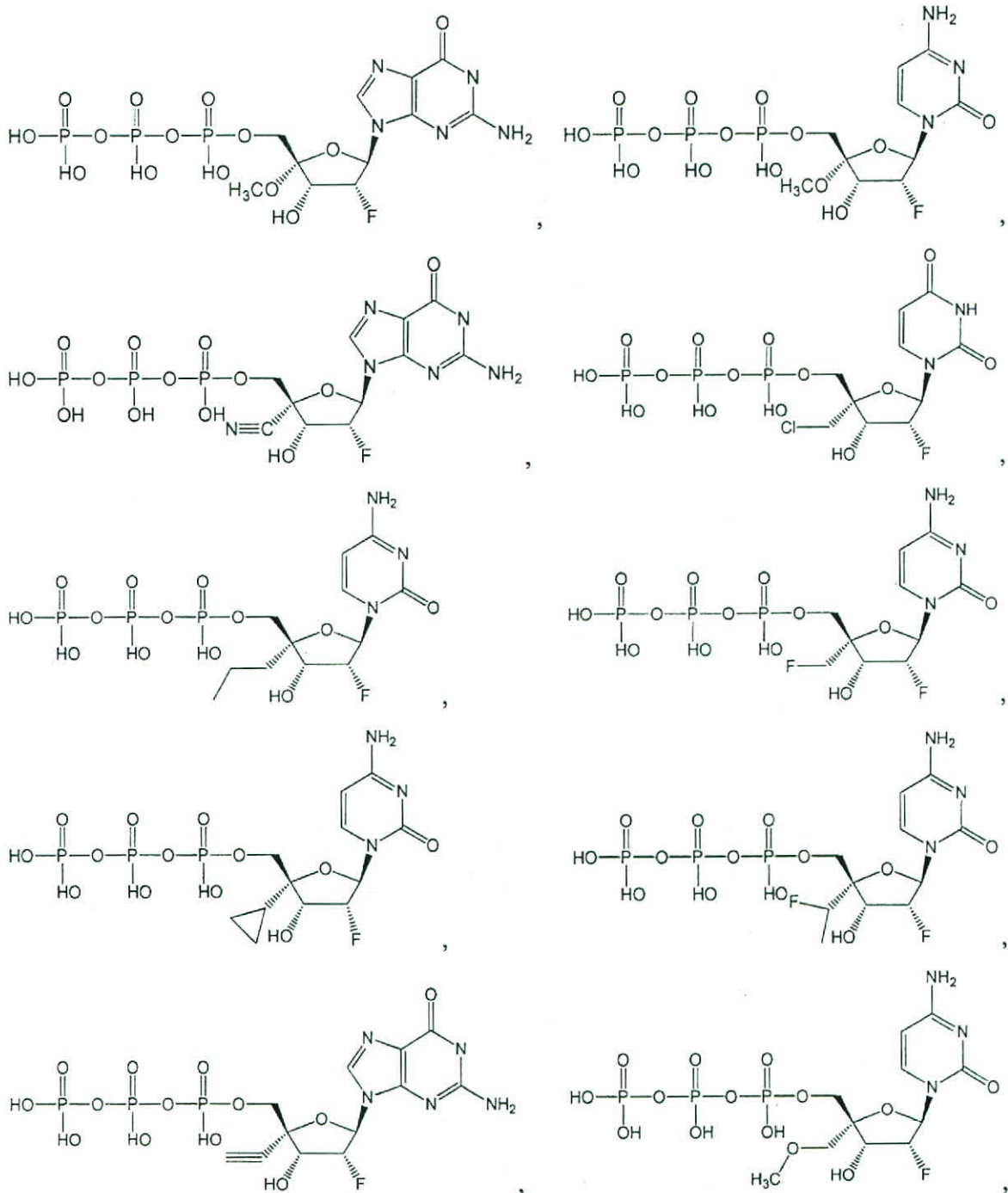
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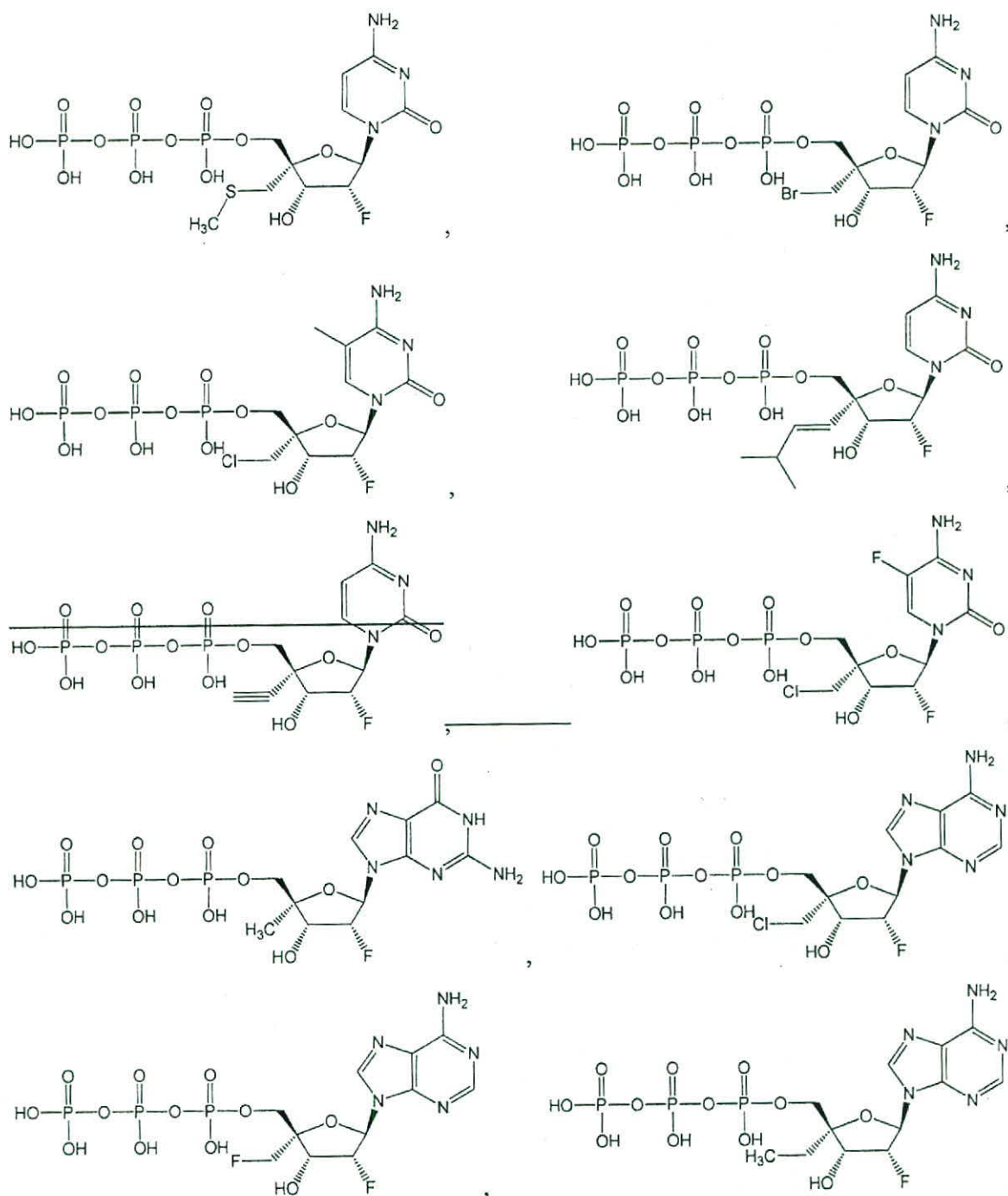
17. (CURRENTLY AMENDED) The compound of Claim 1, wherein the compound of Formula (I) is selected from the group consisting of:



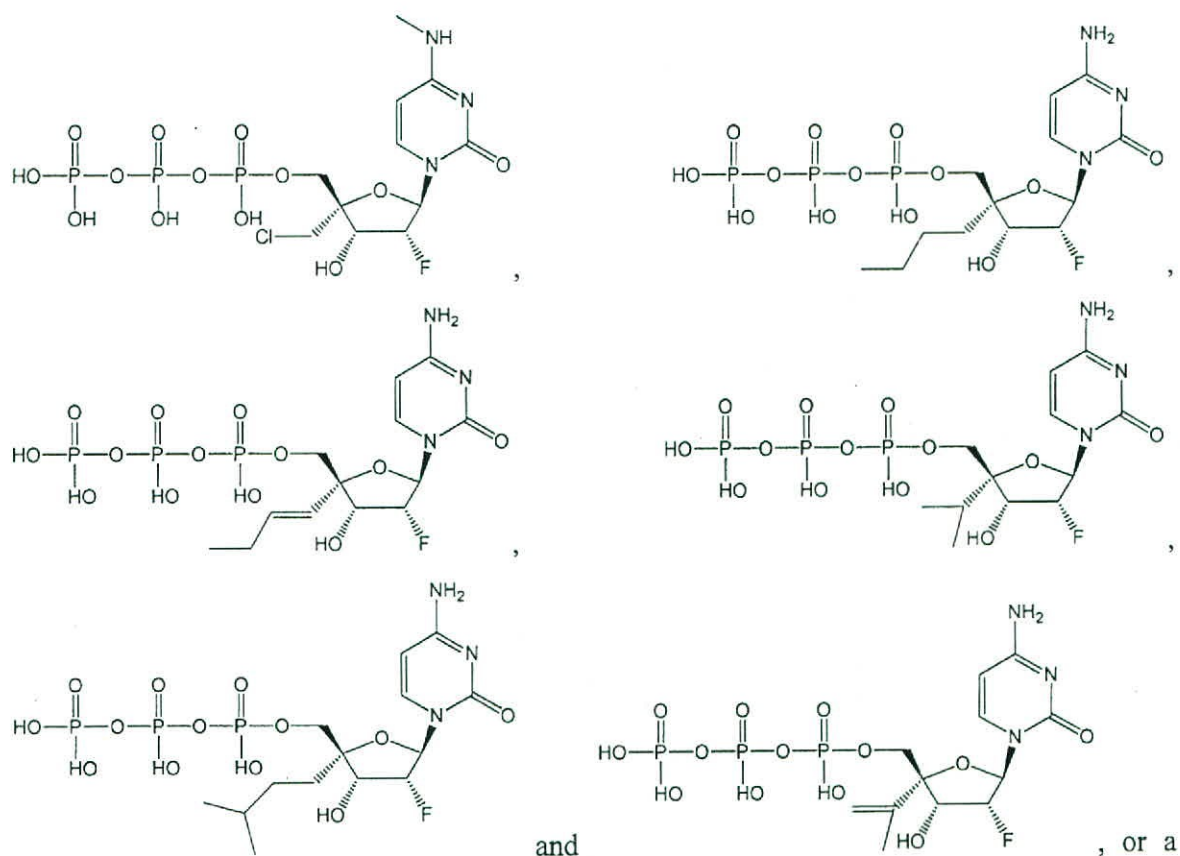
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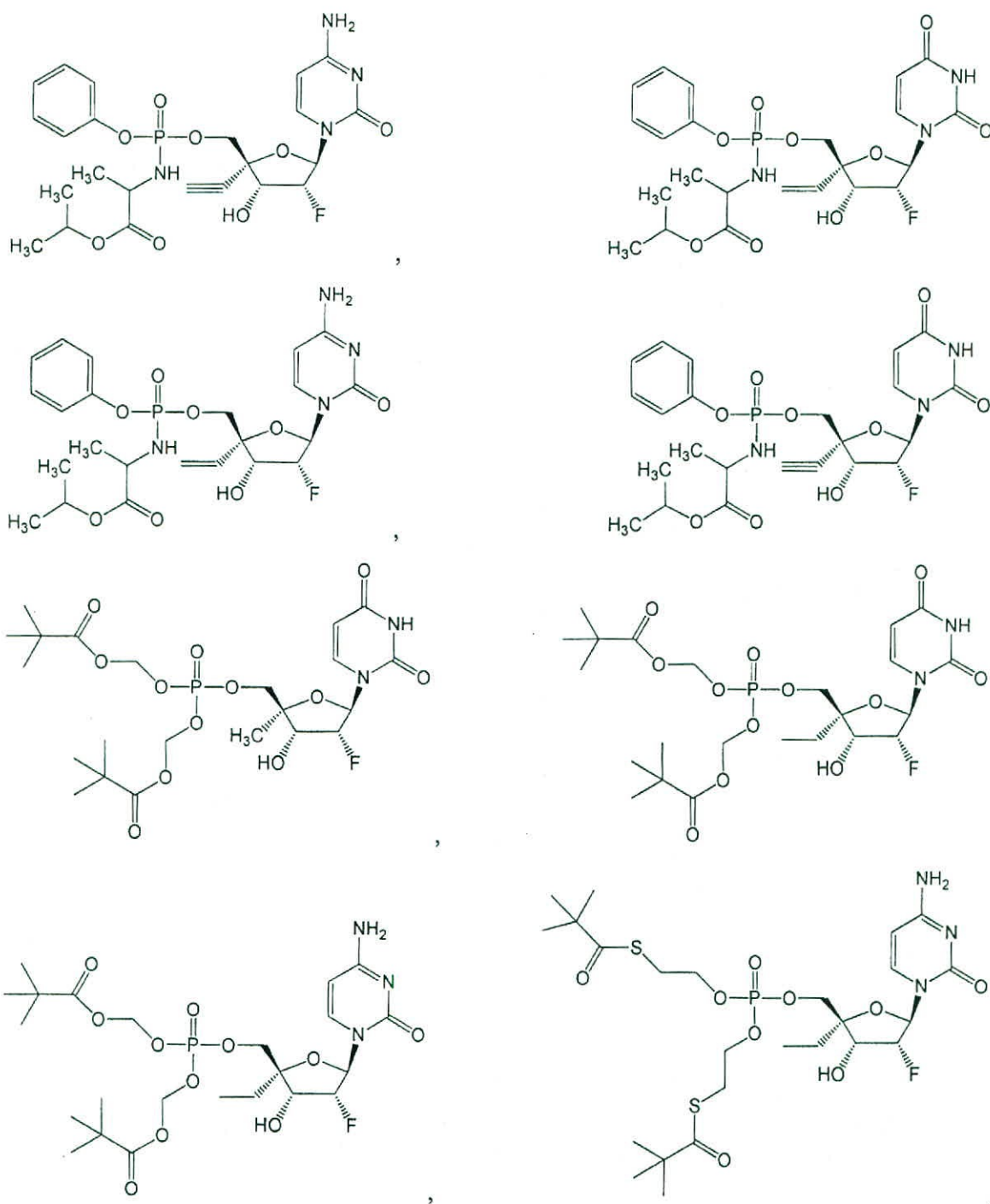
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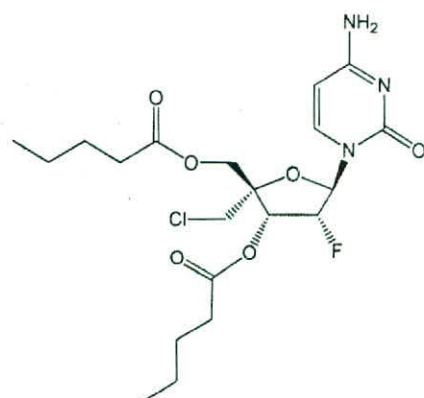
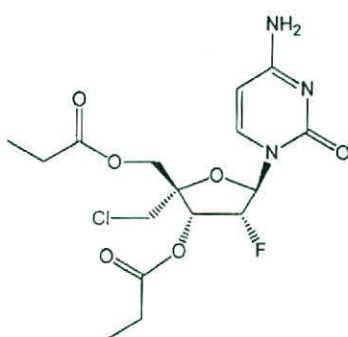
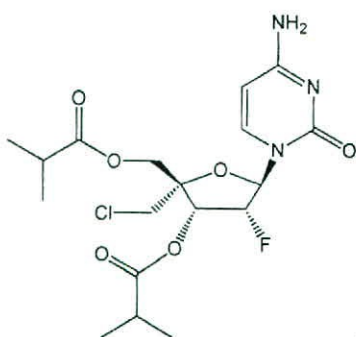
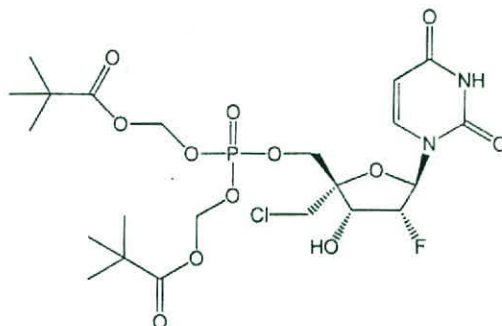
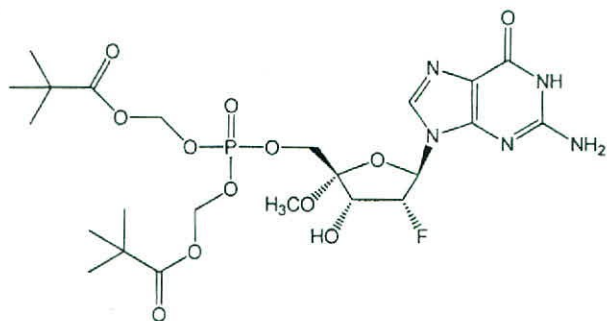
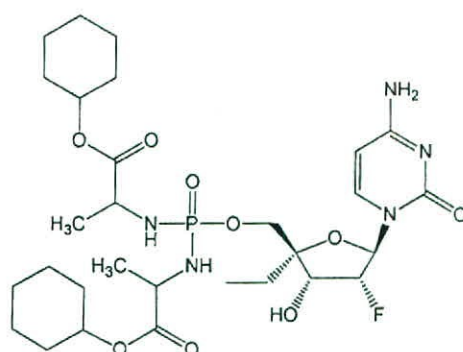
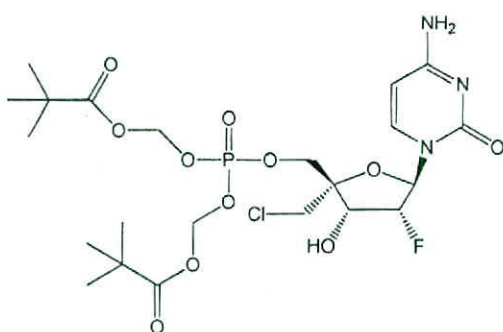
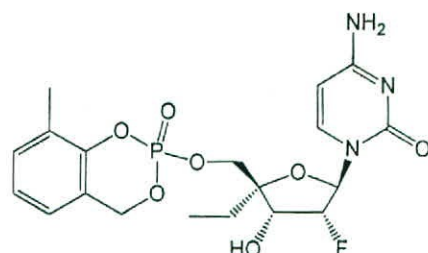
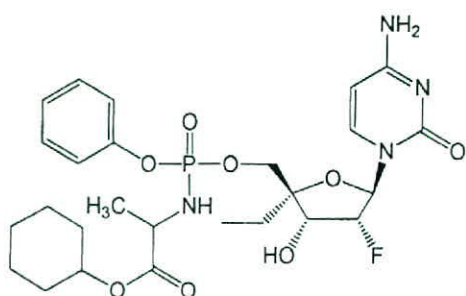
pharmaceutically acceptable salt of the foregoing.

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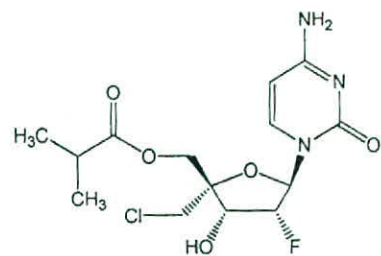
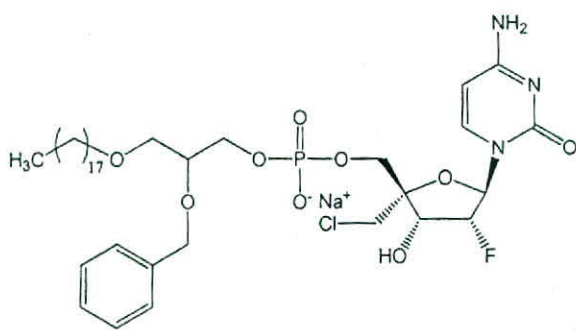
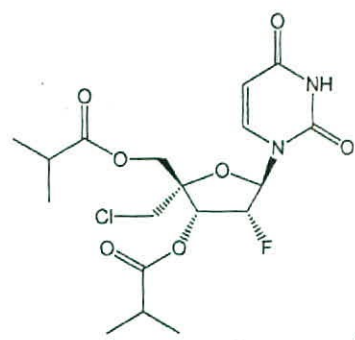
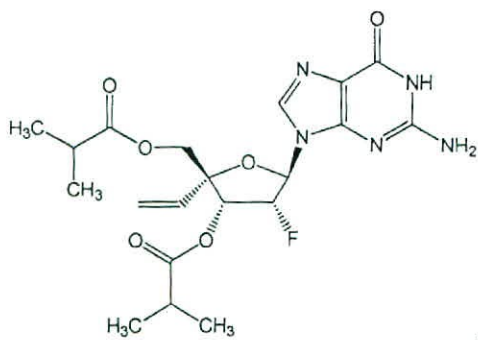
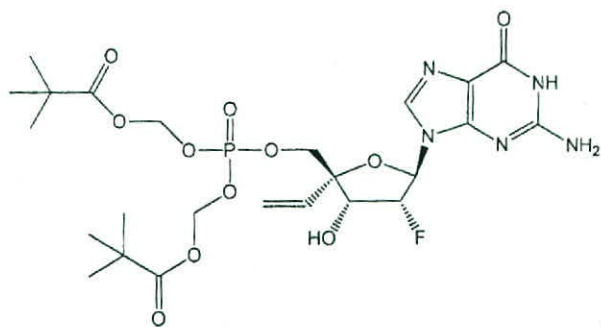
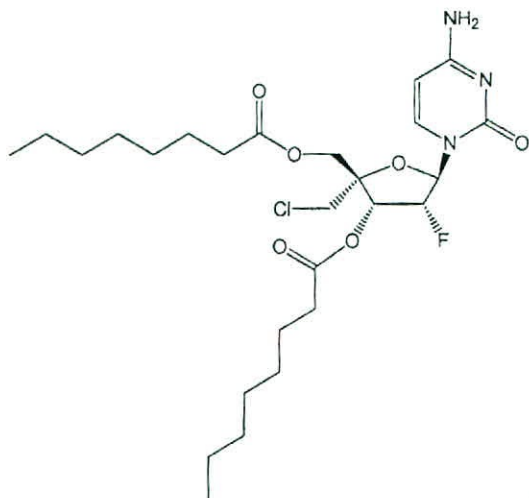
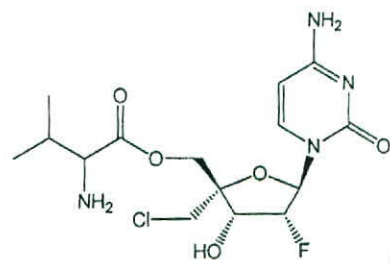
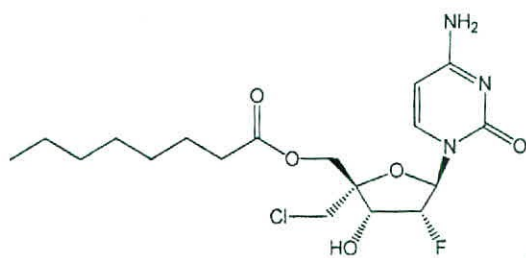
18. (ORIGINAL) The compound of Claim 1, wherein the compound of Formula (I) is selected from the group consisting of:



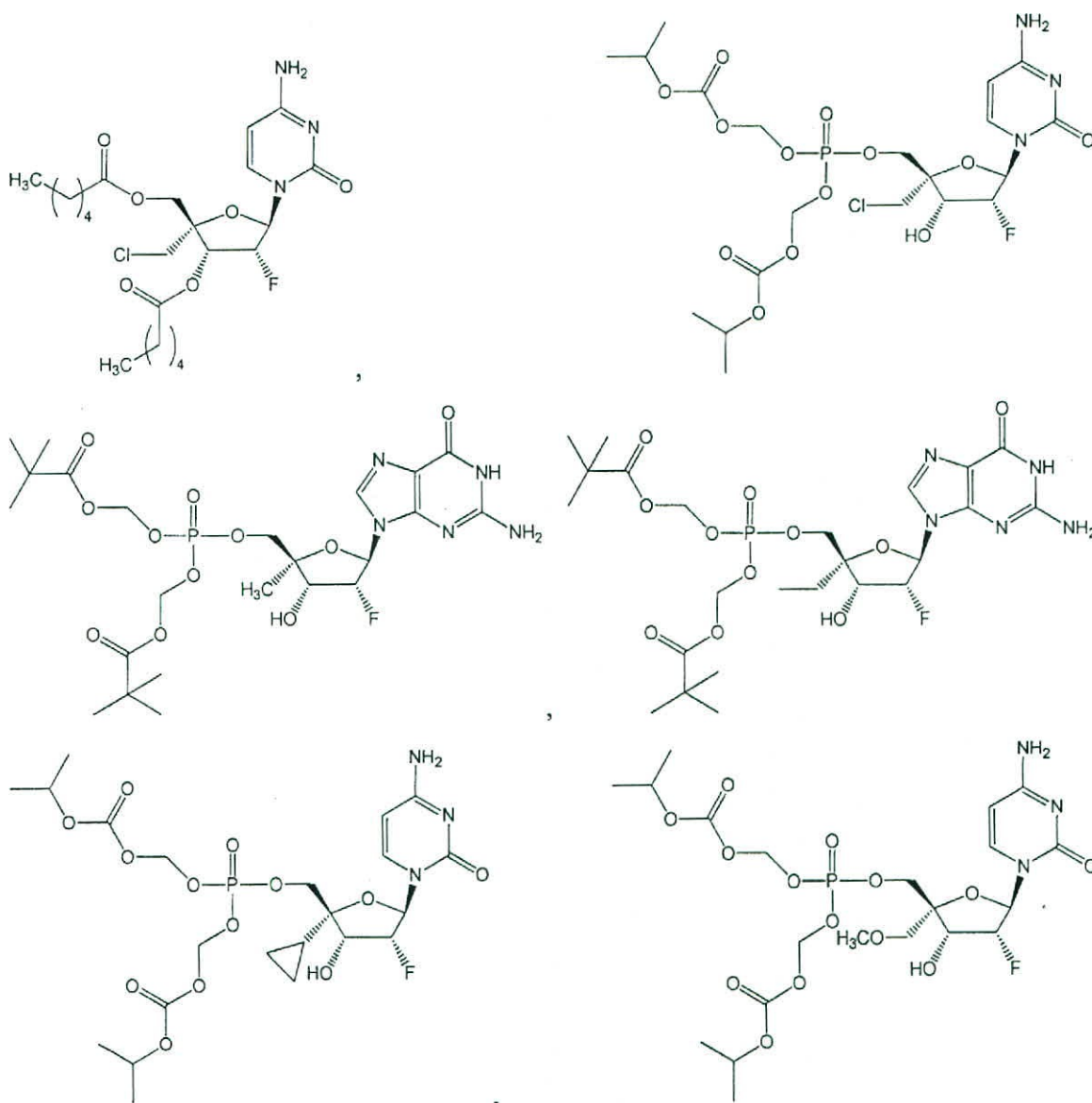
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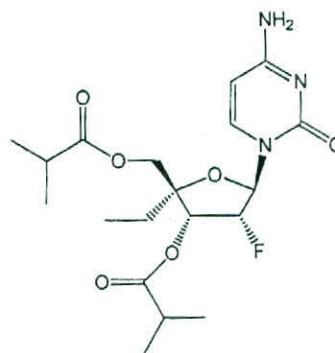
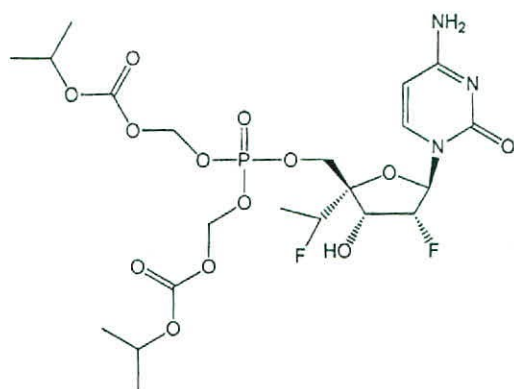
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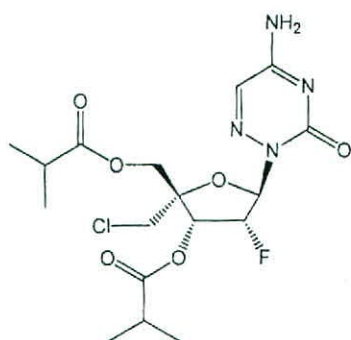
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and



, or a pharmaceutically acceptable salt of the foregoing.

19. (ORIGINAL) A pharmaceutical composition comprising an effective amount of a compound of Claim 1, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier, diluent, excipient, or combination thereof.

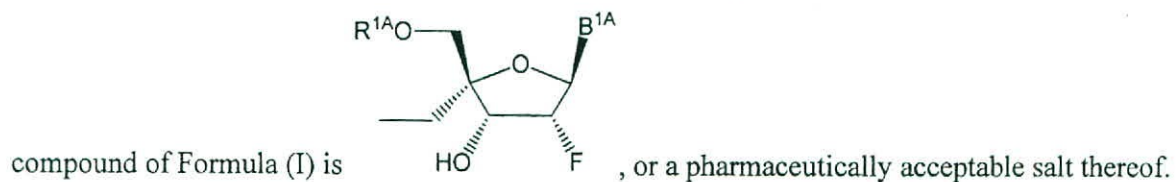
20. (ORIGINAL) A method for ameliorating or treating a viral infection comprising contacting a cell infected with the virus in a subject identified as suffering from the viral infection with an effective amount of a compound of Claim 1, or a pharmaceutically acceptable salt thereof; and wherein the viral infection is selected from a paramyxovirus viral infection and an orthomyxovirus viral infection.

21. (ORIGINAL) A method for ameliorating or treating a viral infection in combination with one or more agents comprising administering to or contacting a cell in a subject identified as suffering from the viral infection with an effective amount of a compound of Claim 1, or a pharmaceutically acceptable salt thereof; and wherein the viral infection is selected from a paramyxovirus viral infection and an orthomyxovirus viral infection.

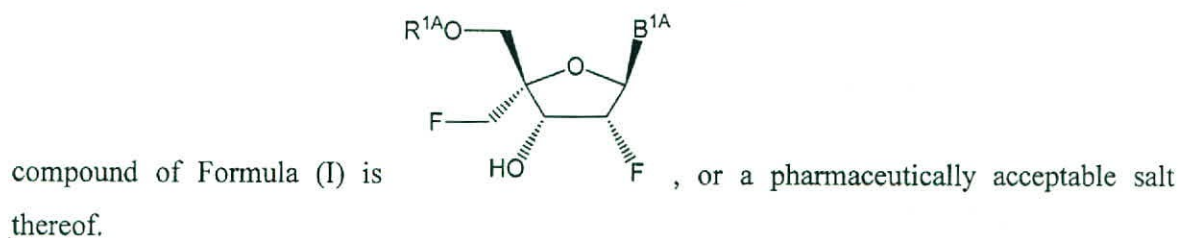
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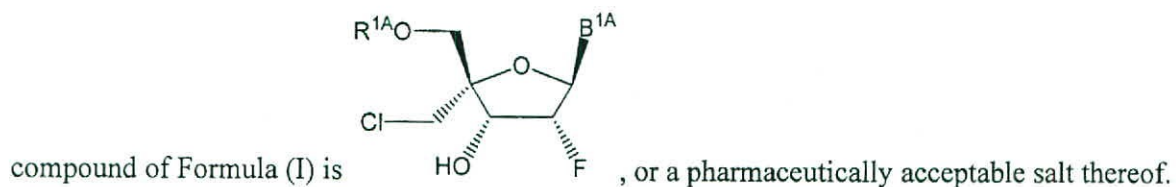
22. (PREVIOUSLY PRESENTED) The compound of Claim 1, wherein the



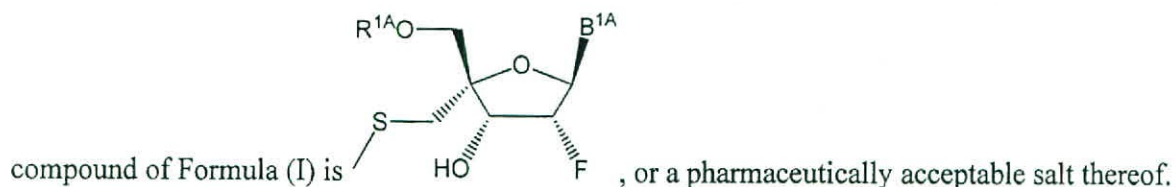
23. (PREVIOUSLY PRESENTED) The compound of Claim 1, wherein the



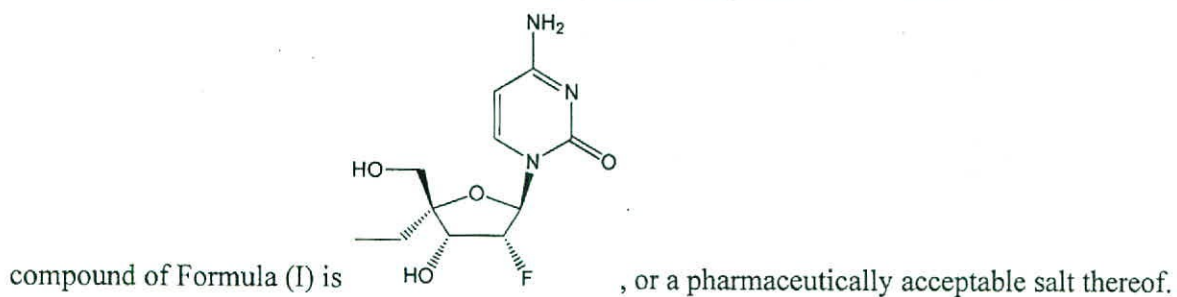
24. (PREVIOUSLY PRESENTED) The compound of Claim 1, wherein the



25. (PREVIOUSLY PRESENTED) The compound of Claim 1, wherein the



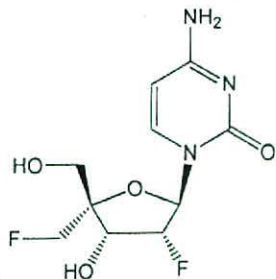
26. (PREVIOUSLY PRESENTED) The compound of Claim 1, wherein the



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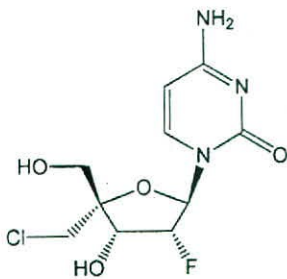
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27. (PREVIOUSLY PRESENTED) The compound of Claim 1, wherein the



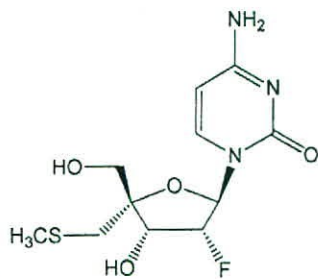
compound of Formula (I) is , or a pharmaceutically acceptable salt thereof.

28. (PREVIOUSLY PRESENTED) The compound of Claim 1, wherein the



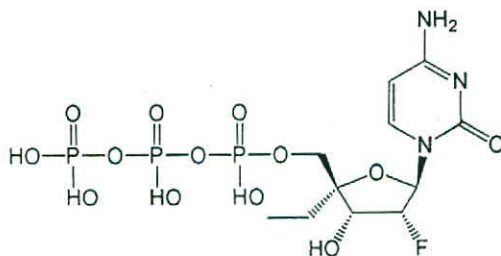
compound of Formula (I) is , or a pharmaceutically acceptable salt thereof.

29. (PREVIOUSLY PRESENTED) The compound of Claim 1, wherein the



compound of Formula (I) is , or a pharmaceutically acceptable salt thereof.

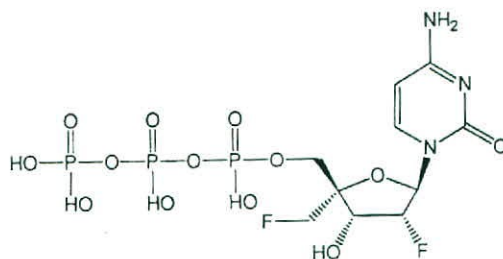
30. (PREVIOUSLY PRESENTED) The compound of Claim 1, wherein the



compound of Formula (I) is , or a pharmaceutically acceptable salt thereof.

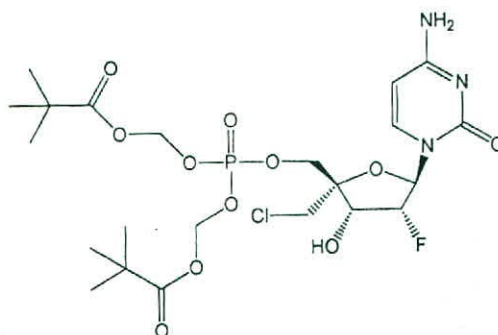
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31. (PREVIOUSLY PRESENTED) The compound of Claim 1, wherein the



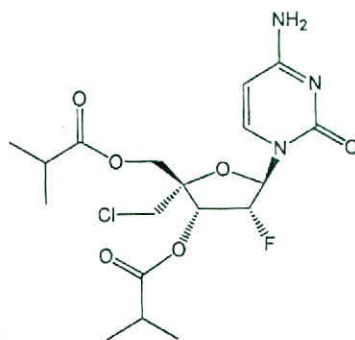
compound of Formula (I) is , or a pharmaceutically acceptable salt thereof.

32. (PREVIOUSLY PRESENTED) The compound of Claim 1, wherein the



compound of Formula (I) is , or a pharmaceutically acceptable salt thereof.

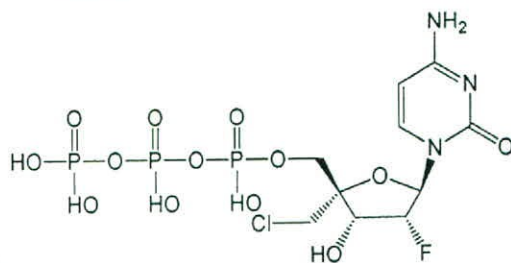
33. (PREVIOUSLY PRESENTED) The compound of Claim 1, wherein the



compound of Formula (I) is , or a pharmaceutically acceptable salt thereof.

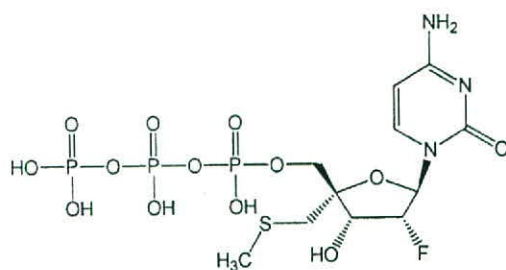
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34. (PREVIOUSLY PRESENTED) The compound of Claim 1, wherein the



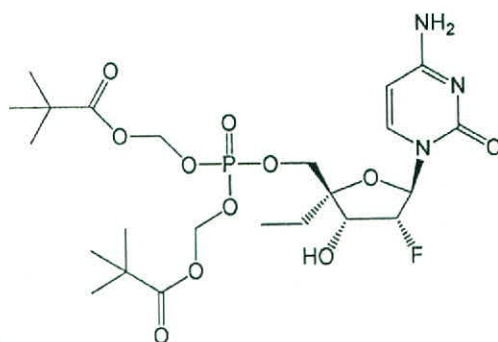
compound of Formula (I) is
acceptable salt thereof.

35. (PREVIOUSLY PRESENTED) The compound of Claim 1, wherein the



compound of Formula (I) is
acceptable salt thereof.

36. (PREVIOUSLY PRESENTED) The compound of Claim 1, wherein the



compound of Formula (I) is
acceptable salt thereof.

37. (PREVIOUSLY PRESENTED) The method of Claim 20, wherein the viral infection is a paramyxovirus viral infection.

38. (PREVIOUSLY PRESENTED) The method of Claim 37, wherein the paramyxovirus viral infection is human respiratory syncytial virus infection.

39. (PREVIOUSLY PRESENTED) The method of Claim 21, wherein the viral infection is a paramyxovirus viral infection.

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40. (PREVIOUSLY PRESENTED) The method of Claim 39, wherein the paramyxovirus viral infection is human respiratory syncytial virus infection.

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**LISTA DE ANTECEDENTES CITADOS POR EL
EXAMINADOR DE LA OFICINA DE PATENTES Y MARCAS
DE ESTADOS UNIDOS (USPTO)**

- a) U.S Patent No. 6,403,568
- b) Pfunderheller et al., *Helv. Chim. Acta* (2000) 83:128-151
- c) WO 2009/125841, y
- d) U.S. Patent No. 8,445,669



US006403568B1

(12) **United States Patent**
Ohruai et al.

(10) **Patent No.:** US 6,403,568 B1
(45) **Date of Patent:** *Jun. 11, 2002

(54) **4'-C-ETHYNYL PYRIMIDINE NUCLEOSIDES AND PHARMACEUTICAL COMPOSITIONS THEREOF**

(75) **Inventors:** Hiroshi Ohruai, Sendai; Shiro Shigeta, Fukushima; Eichi Kodama, Kyoto; Haruhiko Machida, Choshi; Satoru Kohgo, Sendai; Hiroaki Mitsuya, Kumamoto, all of (JP)

(73) **Assignee:** Yamasa Corporation, Chiba (JP)

(*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) **Appl. No.:** 09/941,845

(22) **Filed:** Aug. 30, 2001

Related U.S. Application Data

(63) Continuation of application No. 09/570,041, filed on May 12, 2000, now Pat. No. 6,291,670.

(30) Foreign Application Priority Data

May 12, 1999	(JP)	11-131539
Jun. 22, 1999	(JP)	11-174920
Nov. 9, 1999	(JP)	11-318246
Mar. 23, 2000	(JP)	2000-81117

(51) **Int. Cl.⁷** A61K 31/70; C07H 19/073; C07H 19/09; C07H 19/10

(52) **U.S. Cl.** 514/49; 514/51; 536/26.8; 536/27.4; 536/28.5; 536/28.51; 536/28.52

(58) **Field of Search** 514/49, 51; 536/27.4, 536/28.5, 28.51, 28.52, 26.8

(56) References Cited

U.S. PATENT DOCUMENTS

6,291,670 B1 * 9/2001 Ohruai et al. 536/27.4

OTHER PUBLICATIONS

I. Sugimoto et al., "Nucleosides and Nucleotides. 183. Synthesis of 4'-Branched Thymidines as a New Type of Antiviral Agent", Bioorganic & Medicinal Chemistry Letter,

vol. 9, pp. 385-388, 1999. (Issue No. 3, Feb. 8, 1999).

R. Yamaguchi et al., "Synthesis of 4'-C-Ethynyl-β-D-ribo-pentofuranosyl Pyrimidines", Biosci. Biotechnol. Biochem., vol. 63, No. 4, pp. 736-742, 1999. (Apr., 1999).

S. Kohgo et al., "Synthesis of 4'-C-Ethynyl-β-D-arabino- and 4'-C-Ethynyl-2'-deoxy-β-D-ribo-pentofuranosyl Pyrimidines, and Their Biological Evaluation", Biosci. Biotechnol. Biochem., vol. 63, No. 6, pp. 1146-1149, 1999. (Jun., 1999).

M. Nomura et al., "Nucleosides and Nucleotides. 185. Synthesis and Biological Activities of 4'-Branched-Chain Sugar Pyrimidine Nucleosides", J. Med. Chem. vol. 42, pp. 2901-2908, 1999. (Issue No. 15, Jul. 29, 1999).

S. Kohgo et al., "Synthesis of 4'-substituted nucleosides and their biological evaluation", Nucleic Acid Symposium Series, No. 42, pp. 127-128, Oxford Univ. Press, Macbashi, JP, Nov. 10-12, 1999.

* cited by examiner

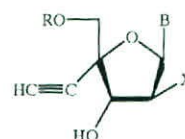
Primary Examiner—James O. Wilson

Assistant Examiner—L. E. Crane

(74) *Attorney, Agent, or Firm*—Wenderoth, Lind & Ponack, L.L.P.

(57) ABSTRACT

The invention provides 4'-C-ethynyl pyrimidine nucleosides (other than 4'-C-ethynylthymidine) represented by formula [I]:



[I]

wherein B represents a base selected from the group consisting of pyrimidine and derivatives thereof; X represents a hydrogen atom or a hydroxyl group; and R represents a hydrogen atom or a phosphate residue; and a pharmaceutical composition containing any one of the compounds and a pharmaceutically acceptable carrier. Preferably, the composition is used as an anti-HIV agent or a drug for treating AIDS.

12 Claims, No Drawings

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4'-C-ETHYNYL PYRIMIDINE NUCLEOSIDES AND PHARMACEUTICAL COMPOSITIONS THEREOF

This application is a continuation of U.S. application Ser. No. 09/570,041, filed on May 12, 2000, now U.S. Pat. No. 6,291,670.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to 4'-C-ethynyl nucleosides and the use thereof for producing pharmaceuticals, and more particularly to the use thereof in treating acquired immunodeficiency syndrome (AIDS).

2. Background Art

The clinical setting for AIDS has been dramatically changed by a multi-drug therapy called highly active antiretroviral therapy, or HAART. In this therapy, nucleoside reverse transcriptase inhibitors (NRTIs) such as zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), and lamivudine (3TC) and protease inhibitors (PIs) are employed in combination. Application of this therapy has drastically decreased the number of deaths due to AIDS in many countries (Textbook of AIDS Medicine, p751 (Williams & Wilkins, Baltimore, 1999)).

In spite of the decrease in AIDS-related deaths due to HAART, there has emerged a multi-drug resistant HIV-1 (human immunodeficiency virus-1) mutant exhibiting cross-resistance to various drugs. For example, in the early 1990s patients infected with an HIV exhibiting resistance to both AZT and 3TC were very rare, whereas the percentage of AIDS patients infected with such an HIV was as high as 42% in 1995-1996 (AIDS, 11, 1184(1997)).

It has been reported that such multi-drug resistant viruses cause 30-60% of drug failure cases in which the viremia level drops once below the detection limit and then revives to exhibit lasting viremia (AIDS, 12, 1631(1998)). Thus, the present status of AIDS treatment is serious.

Conventionally, in terms of a compound which exhibits potent antiviral activities against multi-drug resistant viruses, there have been known only a few protease inhibitors; e.g., JE-2147, which have potent antiviral activity against a multi-PI resistant HIV-1 (Proc. Natl. Acad. Sci. USA, 96,8675(1999)). However, no nucleoside derivative having such potent activities has been reported yet.

Ohrui, one of the inventors of the present invention, has synthesized 1-(4'-C-ethynyl-β-D-ribo-pentofuranosyl) thymine, 4'-C-ethynyluridine, and 4'-C-ethynylcytidine and assayed biological activities such as antiviral and antitumor activities thereof. However, no such biological activities have been observed for these compounds (Biosci. Biotechnol. Biochem., 63(4), 736-742, 1999).

Furthermore, Matsuda et al. have synthesized 4'-C-ethynylthymidine and assayed the anti-HIV activity thereof. The anti-HIV activity of the compound is weaker than that of AZT. However, the assay described by Matsuda et al. (Bioorg. Med. Chem. Lett., 9(1999), 385-388) is drawn to an ordinary assay for determining anti-HIV activity on the basis of MT-4 cells versus an HIV-1 III_b strain, and does not use a multi-drug resistant virus strain.

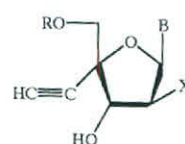
SUMMARY OF THE INVENTION

In order to find a compound having more potent antiviral activity than AZT, the present inventors have synthesized a variety of 4'-C-ethynyl nucleosides and evaluated the anti-

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viral activity thereof, and have found that: 1) a 4'-ethynyl nucleoside derivative having a specific structure exhibits potent anti-HIV activity equal to or greater than that of AZT; 2) the compound has potent antiviral activity against a multi-drug resistant virus strain exhibiting resistance to various anti-HIV drugs such as AZT, ddI, ddC, d4T, and 3TC; and 3) the compound exhibits no significant cytotoxicity. The present invention has been accomplished on the basis of these findings.

Accordingly, the present invention provides 4'-C-ethynyl nucleosides (other than 4'-C-ethynylthymidine) represented by formula [1]:



[1]

wherein B represents a base selected from the group consisting of pyrimidine, purine, and derivatives thereof; X represents a hydrogen atom or a hydroxyl group; and R represents a hydrogen atom or a phosphate residue.

The present invention also provides a pharmaceutical composition containing any one of the compounds and a pharmaceutically acceptable carrier.

Preferably, the composition is employed as an antiviral drug or a drug for treating AIDS.

The present invention also provides use, as pharmaceuticals, of compounds represented by formula [1].

The present invention also provides a method for treatment of AIDS, comprising administering a compound of formula [1] to a vertebrate, including human.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

(1) Compounds

The compounds of the present invention are represented by formula [1]. Examples of bases in formula [1] represented by B include pyrimidines; purines, including azapurines and deazapurines; and derivatives thereof.

Examples of substituents in the bases includes a halogen atom, an alkyl group, a haloalkyl group, an alkenyl group, a haloalkenyl group, an alkynyl group, an amino group, an alkylamino group, a hydroxyl group, a hydroxyamino group, an aminoxy group, an alkoxy group, a mercapto group, an alkylmercapto group, an aryl group, an aryloxy group, and a cyano group. The number and substitution site of these substituents are not particularly limited.

Examples of halogen atoms serving as substituents include chlorine, fluorine, iodine, and bromine. Examples of alkyl groups include C1-C7 alkyl group such as methyl, ethyl, and propyl. Examples of haloalkyl groups include C1-C7 haloalkyl groups such as fluoromethyl, difluoromethyl, trifluoromethyl, bromomethyl, and bromoethyl. Examples of alkenyl groups include C2-C7 alkenyl groups such as vinyl and allyl. Examples of haloalkenyl groups include C2-C7 haloalkenyl groups such as bromovinyl and chlorovinyl. Examples of alkynyl groups include C2-C7 alkynyl groups such as ethynyl and propynyl. Examples of alkylamino groups include C1-C7 alkylamino groups such as methylamino and ethylamino.

Examples of alkoxy groups include C1-C7 alkoxy groups such as methoxy and ethoxy. Examples of alkylmercapto

groups include C1-C7 alkylmercapto groups such as methylmercapto and ethylmercapto. Examples of aryl groups include a phenyl group; alkylphenyl groups having a C1-C5 alkyl such as methylphenyl and ethylphenyl; alkoxyphenyl groups having a C1-C5 alkoxy such as methoxyphenyl and ethoxyphenyl; alkylaminophenyl groups having a C1-C5 alkyl such as dimethylaminophenyl and diethylaminophenyl; and halogenophenyl groups such as chlorophenyl and bromophenyl.

Examples of pyrimidine bases and derivatives thereof include cytosine, uracil, 5-fluorocytosine, 5-fluorouracil, 5-chlorocytosine, 5-chlorouracil, 5-bromocytosine, 5-bromouracil, 5-iodocytosine, 5-iodouracil, 5-methylcytosine, 5-ethylecytosine, 5-methyluracil (thymine), 5-ethyluracil, 5-fluoromethylcytosine, 5-fluorouracil, 5-trifluorocytosine, 5-trifluorouracil, 5-vinyluracil, 5-bromovinyluracil, 5-chlorovinyluracil, 5-ethynylecytosine, 5-ethynyluracil, 5-propynyluracil, pyrimidin-2-one, 4-hydroxyaminopyrimidin-2-one, 4-aminoxypyrimidin-2-one, 4-methoxypyrimidin-2-one, 4-acetoxypyrimidin-2-one, 4-fluoropyrimidin-2-one, and 5-fluoropyrimidin-2-one.

Examples of purine bases and derivatives thereof include purine, 6-aminopurine (adenine), 6-hydroxypurine, 6-fluoropurine, 6-chloropurine, 6-methylaminopurine, 6-dimethylaminopurine, 6-trifluoromethylaminopurine, 6-benzoylamino purine, 6-acethylaminopurine, 6-hydroxyaminopurine, 6-aminoxypurine, 6-methoxypurine, 6-acetoxypurine, 6-benzoyloxypurine, 6-methylpurine, 6-ethylpurine, 6-trifluoromethylpurine, 6-phenylpurine, 6-mercaptupurine, 6-methylmercaptupurine, 6-aminopurine-1-oxide, 6-hydroxypurine-1-oxide, 2-amino-6-hydroxypurine (guanine), 2,6-diaminopurine, 2-amino-6-chloropurine, 2-amino-6-iodopurine, 2-aminopurine, 2-amino-6-mercaptupurine, 2-amino-6-methylmercaptupurine, 2-amino-6-hydroxyaminopurine, 2-amino-6-methoxypurine, 2-amino-6-benzoyloxypurine, 2-amino-6-acetoxypurine, 2-amino-6-methylpurine, 2-amino-6-cyclopropylaminomethylpurine, 2-amino-6-phenylpurine, 2-amino-8-bromopurine, 6-cyanopurine, 6-amino-2-chloropurine (2-chloroadenine), 6-amino-2-fluoropurine (2-fluoroadenine), 6-amino-3-deazapurine, 6-amino-8-azapurine, 2-amino-6-hydroxy-8-azapurine, 6-amino-7-deazapurine, 6-amino-1-deazapurine, and 6-amino-2-azapurine.

When B is a pyrimidine base and X is a hydrogen atom, examples of compounds represented by formula [I] include the following compounds:

4'-C-ethynyl-2'-deoxycytidine,
4'-C-ethynyl-2'-deoxy-5-halogenocytidine,
4'-C-ethynyl-2'-deoxy-5-alkylecytidine,
4'-C-ethynyl-2'-deoxy-5-haloalkylecytidine,
4'-C-ethynyl-2'-deoxy-5-alkenylecytidine,
4'-C-ethynyl-2'-deoxy-5-haloalkenylecytidine,
4'-C-ethynyl-2'-deoxy-5-alkynylecytidine,
4'-C-ethynyl-2'-deoxy-5-halogenouridine,
4'-C-ethynyl-2'-deoxy-5-alkyluridine (other than 4'-C-ethynylthymidine),
4'-C-ethynyl-2'-deoxy-5-haloalkyluridine,
4'-C-ethynyl-2'-deoxy-5-alkenyluridine,
4'-C-ethynyl-2'-deoxy-5-haloalkenyluridine, and
4'-C-ethynyl-2'-deoxy-5-alkynyluridine, and 5'-phosphate esters thereof.

When B is a pyrimidine base and X is a hydroxyl group, examples of compounds represented by formula [I] include the following compounds:

1-(4-C-ethynyl-β-D-arabino-pentofuranosyl)cytosine,
1-(4-C-ethynyl-β-D-arabino-pentofuranosyl)-5-halogenocytosine,
1-(4-C-ethynyl-β-D-arabino-pentofuranosyl)-5-alkylecytosine,
1-(4-C-ethynyl-β-D-arabino-pentofuranosyl)-5-haloalkylecytosine,
1-(4-C-ethynyl-β-D-arabino-pentofuranosyl)-5-alkenylecytosine,
1-(4-C-ethynyl-β-D-arabino-pentofuranosyl)-5-haloalkenylecytosine,
1-(4-C-ethynyl-β-D-arabino-pentofuranosyl)-5-alkynylecytosine,
1-(4-C-ethynyl-β-D-arabino-pentofuranosyl)-5-halogenouracil,
1-(4-C-ethynyl-β-D-arabino-pentofuranosyl)-5-alkyluracil,
1-(4-C-ethynyl-β-D-arabino-pentofuranosyl)-5-haloalkyluracil,
1-(4-C-ethynyl-β-D-arabino-pentofuranosyl)-5-alkenyluracil,
1-(4-C-ethynyl-β-D-arabino-pentofuranosyl)-5-haloalkenyluracil, and
1-(4-C-ethynyl-β-D-arabino-pentofuranosyl)-5-alkynyluracil, and 5'-phosphate esters thereof.

When B is a purine base and X is a hydrogen atom, examples of compounds represented by formula [I] include the following compounds:

4'-C-ethynyl-2'-deoxyadenosine,
4'-C-ethynyl-2'-deoxyguanosine,
4'-C-ethynyl-2'-deoxyinosine,
9-(4-C-ethynyl-2-deoxy-β-D-ribo-furanosyl)purine, and
9-(4-C-ethynyl-2-deoxy-β-D-ribo-furanosyl)-2,6-diaminopurine, and 5'-phosphate esters thereof.

When B is a purine base and X is a hydroxyl group, examples of compounds represented by formula [I] include the following compounds:

9-(4-C-ethynyl-β-D-arabino-pentofuranosyl)adenine,
9-(4-C-ethynyl-β-D-arabino-pentofuranosyl)guanine,
9-(4-C-ethynyl-β-D-arabino-pentofuranosyl)hypoxanthine,
9-(4-C-ethynyl-β-D-arabino-pentofuranosyl)purine, and
9-(4-C-ethynyl-β-D-arabino-pentofuranosyl)-2,6-diaminopurine, and 5'-phosphate esters thereof.

Examples of preferred compounds of the present invention includes the following compounds:

- (i) 4'-C-ethynyl pyrimidine nucleosides including
 - (1) a compound represented by formula [I] wherein X is a hydrogen atom,
 - (2) a compound represented by formula [I] wherein X is a hydroxyl group,
 - (3) a compound represented by formula [I] wherein B is cytosine,
 - (4) a compound represented by formula [I] wherein B is cytosine and X is a hydrogen atom,
 - (5) a compound represented by formula [I] wherein B is cytosine and X is a hydroxyl group,
 - (6) 4'-C-ethynyl-2'-deoxycytidine,
 - (7) 4'-C-ethynyl-2'-deoxy-5-fluorocytidine, and
 - (8) 1-(4-C-ethynyl-β-D-arabino-furanosyl)cytosine, and
- (ii) 4'-C-ethynyl purine nucleosides including
 - (1) a compound represented by formula [I] wherein X is a hydrogen atom,
 - (2) a compound represented by formula [I] wherein X is a hydroxyl group,
 - (3) a compound represented by formula [I] wherein B is selected from the group consisting of adenine, guanine, hypoxanthine, and diaminopurine,

- (4) a compound represented by formula [I] wherein B is selected from the group consisting of adenine, guanine, hypoxanthine, and diaminopurine and X is a hydrogen atom,
- (5) a compound represented by formula [I] wherein B is selected from the group consisting of adenine, guanine, hypoxanthine, and diaminopurine and X is a hydroxyl group,
- (6) 4'-C-ethynyl-2'-deoxyadenosine,
- (7) 4'-C-ethynyl-2'-deoxyguanosine,
- (8) 4'-C-ethynyl-2'-deoxyinosine,
- (9) 9-(4-C-ethynyl-2-deoxy-β-D-ribo-pentofuranosyl)-2,6-diaminopurine, and
- (10) 9-(4-C-ethynyl-β-D-arabino-pentofuranosyl) adenine.

The compounds of the present invention may be salts, hydrates, or solvates. When R is a hydrogen atom, examples of salts include acid-adducts such as hydrochlorides and sulfates. When R is a phosphate residue, examples of salts include alkali metal salts such as sodium salts, potassium salts, and lithium salts; alkaline earth metal salts such as calcium salts; and ammonium salts. These salts are pharmaceutically acceptable.

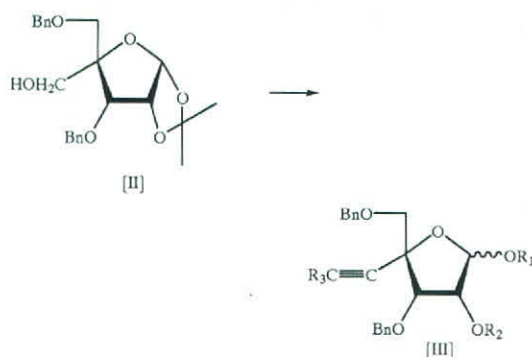
Examples of hydrates or solvates include adducts comprising one molecule of the compound of the present invention or a salt thereof and 0.1–3.0 molecules of water or a solvent. In addition, the compounds of the present invention encompass a variety of isomers thereof such as tautomers.

(2) Method of Production

One of the compounds of the present invention in which X is a hydrogen atom; i.e., a 2'-deoxy derivative, can be produced by the following steps.

First Step;

In the first step, a hydroxymethyl group at the 4-position of the compound represented by [II] is oxidized to thereby form an aldehyde, which is further converted into an alkyne to thereby yield a compound represented by formula [III]:



wherein each of R1 and R2 represents a protective group; R3 represents a hydrogen atom or a protective group; and Bn represents a benzyl group.

The starting material of the reaction is a known compound represented by formula [II] (Biosci. Biotech. Biochem., 57, 1433–1438(1993)).

Each of R1 and R2 may be a protective group which is typically employed for protecting a hydroxyl group. Examples of types of a protective moiety containing R1 or R2 include an ether type, an acyl type, a silyl type, and an acetal type. Specific examples protective groups include a

silyl group, an acetyl group, a benzyl group, and an isopropylidene group.

When the hydroxymethyl group at the 4-position of the compound represented by [II] is converted into an aldehyde group by use of an oxidizing agent, examples of oxidizing agents include a chromium-containing oxidizing agent such as chromic anhydride-pyridine-acetic anhydride composite reagent, pyridinium chlorochromate, or pyridinium dichromate; a high-valency iodine oxidizing agent such as Dess-Martin reagent; and a dimethylsulfoxide-based oxidizing agent such as a combination of dimethylsulfoxide and any one of acetic anhydride, oxalyl chloride, or dicyclohexyl carbodiimide.

Reaction conditions vary depending on an employed oxidizing agent. For example, when oxidation is carried out by use of oxalyl chloride and dimethyl sulfoxide, oxalyl chloride in an amount of 0.5–5 mol and dimethyl sulfoxide in an amount of 1.5–6 mol are added to 1 mol of a compound represented by formula [II] in an organic solvent such as dichloromethane optionally under an inert gas such as argon or nitrogen. The mixture is then allowed to react for approximately 15 minutes to two hours at –100° C. to 0° C. Subsequently, a base such as triethylamine is added in an amount of 2–10 mol to the mixture, and the resultant mixture is further allowed to react at room temperature for approximately 15 minutes to two hours.

The thus-formed aldehyde can be converted into a corresponding alkyne through carbon-increasing (i.e., C–C bond formation) reaction of the aldehyde; treating the resultant compound with a strong base to thereby form a metal alkynyl compound; and introducing a protective group to the metal alkynyl compound.

Carbon-increasing reaction may be carried out in an organic solvent such as dichloromethane or dichloroethane, optionally under an inert gas such as argon or nitrogen. Specifically, 1 mol of the above-produced aldehyde is reacted with 1–5 mol of carbon tetrabromide and 2–10 mol of triphenylphosphine at 0–50° C. for approximately 15 minutes to three hours.

Treatment with a strong base may be carried out in an organic solvent such as tetrahydrofuran, 1,4-dioxane, or dimethoxyethane, optionally under an inert gas such as argon or nitrogen. Specifically, 1 mol of a compound obtained through carbon-increasing reaction is reacted with 2–4 mol of a lithium compound such as n-butyllithium or t-butyllithium at –100° C. to –20° C. for approximately 5–60 minutes.

Furthermore, when a silyl protective group represented by R3 is introduced into an alkynyl group in the thus-obtained compound, the aforementioned treatment is followed by addition of a silylating agent such as chlorotriethylsilane. A protective group can be introduced to a hydroxyl group by use of a customary method. For example, an acetyl group may be introduced through reaction with an acetylating agent such as acetic anhydride.

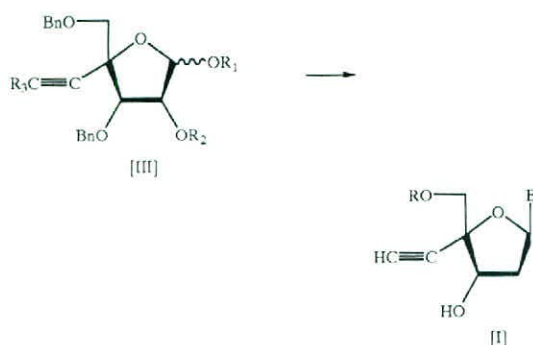
The thus-obtained compound represented by formula [III] may be isolated and purified through a manner which is employed for isolating and purifying typical protected saccharides. For example, the crude compound is partitioned by use of an ethyl acetate-saturated sodium bicarbonate solution, and the isolated compound is purified by use of a silica gel column.

Second step;

The second step includes condensation of a compound represented by formula [III] and a base represented by B; deoxygenation at the 2'-position; removing a protective group of a saccharide portion; and optionally phosphorylat-

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ing the hydroxyl group at the 5'-position, to thereby produce a compound represented by formula [I]:



wherein B represents a base selected from the group consisting of pyrimidine; purine, including azapurine or deazapurine; and a derivative thereof (other than thymine); R represents a hydrogen atom or a phosphate residue; each of R1 and R2 represents a protective group; R3 represents a hydrogen atom or a protective group; and Bn represents a benzyl group.

Condensation of a compound represented by formula [III] and a base represented by B can be carried out by reacting the compound with the base in the presence of a Lewis acid.

The base represented by B may be silylated, and silylation may be carried out through a known method. For example, a base is silylated by use of hexamethylsilazane and trimethylchlorosilane under reflux.

Examples of Lewis acids include trimethylsilyl trifluoromethanesulfonate, tin tetrachloride, zinc chloride, zinc iodide, and anhydrous aluminum chloride.

Condensation reaction may be carried out in an organic solvent such as dichloromethane, 1,2-dichloroethane, acetonitrile, or toluene, optionally under an inert gas such as argon or nitrogen. Specifically, 1 mol of a compound represented by formula [III] is reacted with 1-10 mol of a base represented by B and 0.1-10 mol of Lewis acid at -20° C. to 150° C. for approximately 30 minutes to three hours.

Deoxygenation at the 2'-position may be carried out by converting the derivative having a hydroxyl group to the derivative having a group such as halogeno, phenoxythiocarbonyl, thiocarbonylimidazolyl, or methylthiocarbonyl and reducing the converted derivative using a radical reducing agent in the presence of a radical initiator.

For example, when deoxygenation is carried out through phenoxythiocarbonate, conversion of a hydroxyl group to a phenoxythiocarbonyl group may be carried out in an organic solvent, such as tetrahydrofuran, acetonitrile, or dichloromethane, in the presence of a base such as dimethylaminopyridine or pyridine, optionally under an inert gas such as argon or nitrogen. Specifically, 1 mol of the aforementioned condensation product in which only the protective group for the hydroxyl group at the 2'-position had been eliminated is reacted under stirring with 1-10 mol, preferably 1.1-2 mol, of a phenyl chlorothionoformate derivative at 0-50° C. for approximately 0.5-5 hours. Alternatively, when deoxygenation is carried out via a bromo compound, the bromination may be carried out in an organic solvent, such as tetrahydrofuran, acetonitrile, or dichloromethane, by use of a brominating agent such as acetyl bromide at 0-150° C. for approximately 0.5-5 hours, optionally under an inert gas such as argon or nitrogen. The brominating agent is used

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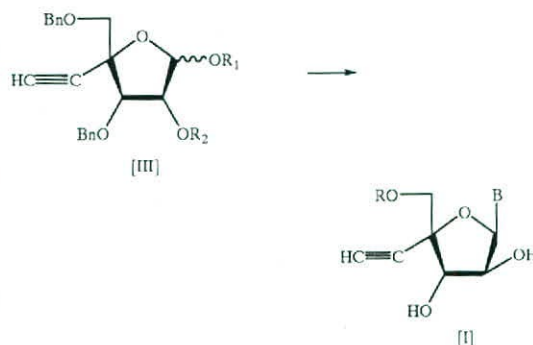
in an amount of 1-50 mol, preferably 5-20 mol, per mol of the aforementioned condensate from which a protective group at the 2'-position had been removed.

Subsequently, reduction may be carried out in an organic solvent such as toluene or benzene in the presence of a radical initiator such as azobisisobutyronitrile, optionally under an inert gas such as argon or nitrogen. Specifically, 1 mol of the aforementioned phenoxythiocarbonate or bromide is reacted under stirring with 1-10 mol, preferably 2-5 mol, of a radical reducing agent such as tributyltin hydride at 50-150° C. for approximately 1-5 hours.

One of the compounds of the present invention in which X is a hydroxyl group; i.e., an arabino derivative, can be produced by the following steps.

First step;

The first step includes condensation of a compound represented by formula [III] and a base represented by B; stereochemically inverting the hydroxyl group at the 2'-position to be an arabino form; removing a protective group of a saccharide portion; and optionally phosphorylating the hydroxyl group at the 5'-position, to thereby produce a compound represented by formula [I]:



wherein B represents a base selected from the group consisting of pyrimidine, purine including azapurine or deazapurine, and a derivative thereof; R represents a hydrogen atom or a phosphate residue; each of R1 and R2 represents a protective group; R3 represents a hydrogen atom or a protective group; and Bn represents a benzyl group.

Condensation of a compound represented by formula [III] and a base represented by B can be carried out by reacting the compound with the base in the presence of a Lewis acid.

The base represented by B may be silylated, and silylation may be carried out through a known method. For example, a base is silylated by use of hexamethylsilazane and trimethylchlorosilane under reflux.

Examples of Lewis acids include trimethylsilyl trifluoromethanesulfonate, tin tetrachloride, zinc chloride, zinc iodide, and anhydrous aluminum chloride.

Condensation reaction may be carried out in an organic solvent such as dichloromethane, 1,2-dichloroethane, acetonitrile, or toluene, optionally under an inert gas such as argon or nitrogen. Specifically, 1 mol of a compound represented by formula [III] is reacted with 1-10 mol of a base represented by B and 0.1-10 mol of Lewis acid at -20° C. to 150° C. for approximately 30 minutes to three hours.

Stereo-inversion of the hydroxyl group at the 2'-position can be carried out by converting a compound containing the hydroxyl into a corresponding 2,2'-anhydrocyclo nucleoside and hydrolyzing the nucleoside. Anhydrocyclization may be carried out through treatment with a sulfonating agent such

as methanesulfonyl chloride, or through treatment with a fluorinating agent such as diethylaminosulfur trifluoride.

For example, when diethylaminosulfur trifluoride is employed, anhydrocyclization may be carried out in an organic solvent such as dichloromethane or toluene, optionally under an inert gas such as argon or nitrogen. Specifically, 1 mol of the aforementioned condensation product in which the protective group for the hydroxyl group at the 2'-position was removed is reacted with 1.1-5 mol, preferably 1.5-2 mol, of diethylaminosulfur trifluoride at 0° C. to room temperature for approximately five minutes to 2 hours. Alternatively, when methanesulfonyl chloride is employed, anhydrocyclization may be carried out in an organic solvent such as pyridine, optionally under an inert gas such as nitrogen. Specifically, 1 mol of the aforementioned condensation product in which the protective group for the hydroxyl group at the 2'-position had been eliminated is reacted with 1.1-5 mol, preferably 1.5-2 mol, of methanesulfonyl chloride at 0-50° C. for approximately five minutes to 10 hours.

Subsequently, hydrolysis may be carried out in the presence of an appropriate base or acid catalyst. For example, when a base catalyst is employed, hydrolysis may be carried out in a solvent mixture comprising water and an alcoholic solvent such as ethanol in the presence of a base such as sodium hydroxide or potassium hydroxide at room temperature to 100° C. for approximately 30 minutes to 5 hours.

In the case in which a base represented by B in the target compound; i.e., 4'-ethynynucleoside, is a base having an amino group, the target compound may also be produced from a hydroxyl-containing base compound through a known method. For example, if the 4-position of a pyrimidine base is sought to be aminated, the hydroxyl group at the 4-position of a pyrimidine base may be converted into a group such as chloro, silyloxy, alkyloxy, sulfonyloxy, or triazolyl, and then the converted group is reacted with ammonia. For example, amination through a triazole derivative may be carried out with stirring in an organic solvent such as dichloromethane, acetonitrile, dimethylformamide, or pyridine in the presence of a base such as triethylamine (triethylamine may be omitted if pyridine is used as a solvent) and a phosphorylating agent such as 4-chlorophenylphosphorodichloridate, optionally under an inert gas such as argon or nitrogen. Specifically, 1 mol of the aforementioned condensation product is reacted with 1-20 mol, preferably 2-10 mol, of 1,2,4-triazole at 0° C. to room temperature for approximately 12-72 hours, followed by addition of aqueous ammonia in an appropriate amount and further reaction at 0° C. to room temperature for approximately 1-12 hours.

In addition, an amino group in a base may be removed through a conventional method making use of any of a variety of deaminases, such as adenosine deaminase or cytidine deaminase.

Finally, a protective group of the thus-produced nucleoside is removed, to thereby obtain the compounds (R=H) of the present invention.

A protective group may be removed through a method appropriately selected from a routine procedure such as hydrolysis under acidic conditions, hydrolysis under basic conditions, treatment with tetrabutylammonium fluoride, or catalytic reduction, in accordance with the protective group employed.

When R in a target compound is a phosphate residue such as monophosphate or diphosphate, a compound in which R is a hydrogen atom is reacted with a phosphorylating agent; e.g., phosphorus oxychloride or tetrachloropyrophosphoric

acid, which selectively phosphorylates the 5'-position of a nucleoside, to thereby produce a target compound in a free or salt form.

The compounds of the present invention may be isolated and purified through conventional methods, in appropriate combination, which are employed for isolating and purifying nucleosides and nucleotides; e.g., recrystallization, ion-exchange column chromatography, and adsorption column chromatography. The thus-obtained compounds may further be converted to a salt thereof in accordance with needs.

(3) Use

As shown in the below-described Test Examples, the compounds of the present invention exhibit excellent antiviral activity against herpesvirus or retrovirus. Thus, the compositions of the present invention containing one of the compounds of the present invention as an active ingredient can be used as therapeutic drugs. Specifically, the compositions of the present invention are useful for the treatment of infectious diseases caused by herpesvirus or retrovirus, in particular, AIDS, which is caused by HIV infection.

Examples of target viruses include viruses belonging to Herpesviridae such as herpes simplex virus type 1, herpes simplex virus type 2, or varicella-zoster virus, and Retroviridae such as human immunodeficiency virus.

The dose of the compounds of the present invention depends on and is determined in consideration of conditions such as the age, body weight, and type of disease of the patient; the severity of a disease of the patient; the drug tolerance; and the administration route. However, the dose per day and per body weight is selected typically within 0.00001-1,000 mg/kg, preferably 0.0001-100 mg/kg. The compounds are administered in a single or divided manner.

Any administration route may be employed, and the compounds may be administered orally, parenterally, enterally, or topically.

When a pharmaceutical is prepared from the compounds of the present invention, the compounds are typically mixed with customarily employed additives, such as a carrier and an excipient. Examples of solid carriers include lactose, kaolin, sucrose, crystalline cellulose, corn starch, talc, agar, pectin, stearic acid, magnesium stearate, lecithin, and sodium chloride. Examples of liquid carriers include glycerin, peanut oil, polyvinylpyrrolidone, olive oil, ethanol, benzyl alcohol, propylene glycol, and water.

The dosage form is arbitrarily selected. When the carrier is solid, examples of dosage forms include tablets, powder, granules, capsules, suppositories, and troches, whereas when it is liquid, examples include syrup, emulsion, soft-gelatin-encapsulated, cream, gel, paste, spray, and injection.

As shown in the below-described results of Test Examples, the compounds of the present invention exhibit excellent anti-HIV activity, particularly against multi-drug resistant HIV strains having resistance to various of anti-HIV drugs such as AZT, DDI, DDC, D4T, and 3TC. The compounds have no significant cytotoxicity. Thus, the compounds of the present invention are expected to be developed for producing pharmaceuticals, particularly drugs for treating AIDS.

EXAMPLES

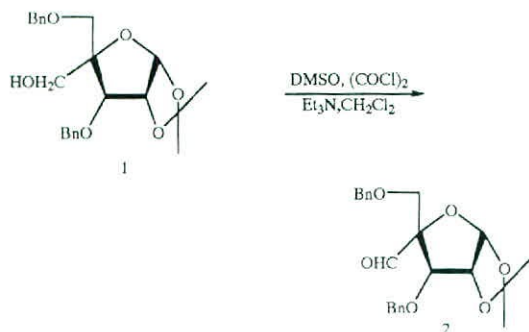
The present invention will next be described in detail by way of examples including Synthesis Examples, Test Examples, and Drug Preparation Examples, which should not be construed as limiting the invention thereto.

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Synthesis Example 1

(1) Synthesis of 4-C-formyl-3,5-di-O-benzyl-1,2-O-isopropylidene- α -D-ribo-pentofuranose (Compound 2)



Oxalyl chloride (3.38 ml, 38.7 mmol) was dissolved in dichloromethane (80.0 ml), and dimethylsulfoxide (5.50 ml, 77.5 mmol) was added dropwise to the solution at -78°C . in an argon atmosphere, followed by stirring for 15 minutes at the same temperature. A solution (100 ml) of 4-C-hydroxymethyl-3,5-di-O-benzyl-1,2-O-isopropylidene- α -D-ribo-pentofuranose (Compound 1) (10.3 g, 25.7 mmol) in dichloromethane was added dropwise to the solution at -78°C ., and the mixture was stirred for 30 minutes. After triethylamine (10.9 ml, 77.6 mmol) was added thereto, the reaction mixture was allowed to warm to room temperature, followed by stirring for 30 minutes. After water was added to the mixture with stirring, the organic layer was dried over anhydrous magnesium sulfate and was concentrated through distillation under reduced pressure. The residue was purified by means of silica gel column chromatography (silica gel 1500 ml, eluent; n-hexane:ethyl acetate=2:1), to thereby yield a colorless viscous compound (Compound 2; 9.68 g, 24.3 mmol, 94.1%).

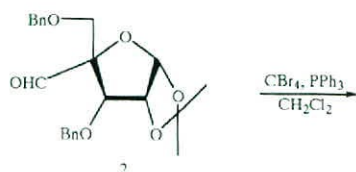
$^1\text{H-NMR}(\text{CDCl}_3)$ δ 9.92 (1H, s, formyl), 7.33-7.24 (10H, m, aromatic), 5.84 (1H, d, H-1 $J_{1,2}$ =3.30), 4.71, 4.59 (each 1H, d, benzyl, J_{gem} =12.00), 4.60 (1H, br.t, H-2), 4.52, 4.46 (each 1H, d, benzyl, J_{gem} =12.00), 4.37 (1H, d, H-3, $J_{2,3}$ =4.50), 3.68, 3.61 (each 1H, d, H-5, J_{gem} =10.95), 1.60, 1.35 (each 3H, s, acetonide)

EIMS m/z : 398(M^+).

HRMS $m/z(\text{M}^+)$: Calcd. for $\text{C}_{23}\text{H}_{26}\text{O}_6$: 398.1729, Found: 398.1732

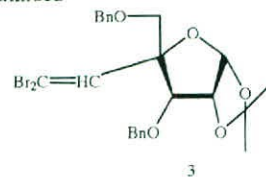
$[\alpha]_D^{+24.5^{\circ}}$ (c =1.03, CHCl_3)

(2) Synthesis of 4-C-(2,2-dibromoethenyl)-3,5-di-O-benzyl-1,2-O-isopropylidene- α -D-ribo-pentofuranose (Compound 3)



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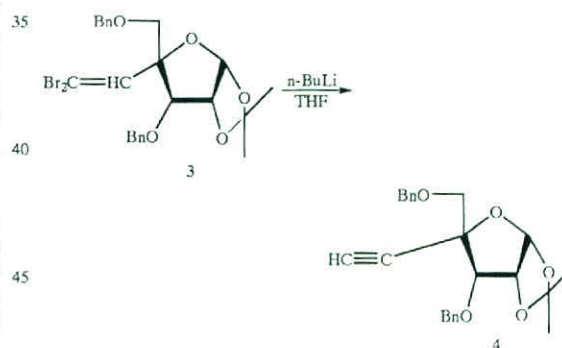
Compound 2 (9.50 g, 23.8 mmol) was dissolved in dichloromethane (200 ml), and carbon tetrabromide (15.8 g, 47.6 mmol) and triphenylphosphine (25.0 g, 95.3 mmol) were added to the solution under ice-cooling, followed by stirring at room temperature for one hour. Triethylamine (20.0 ml, 142 mmol) was added to the mixture, followed by stirring for 10 minutes. The reaction mixture was poured into n-hexane (1000 ml) and the produced precipitates were separated through filtration. The filtrate was concentrated through distillation under reduced pressure, and the residue was purified by means of silica gel column chromatography (silica gel 1500 ml, eluent; n-hexane:ethyl acetate=3:1), to thereby yield a colorless viscous compound (Compound 3; 12.6 g, 22.7 mmol, 95.4%).

$^1\text{H-NMR}(\text{CDCl}_3)$ δ 7.34-7.24 (10H, m, aromatic), 7.16 (1H, s, $\text{Br}_2\text{C}=\text{CH}-$), 5.76 (1H, d, H-1 $J_{1,2}$ =3.90), 4.72, 4.60 (each 1H, d, benzyl, J_{gem} =12.00), 4.53 (1H, br.t, H-2), 4.60, 4.42 (each 1H, d, benzyl, J_{gem} =12.00), 4.21 (1H, d, H-3, $J_{2,3}$ =4.80), 3.83, 3.39 (each 1H, d, H-5, J_{gem} =11.40), 1.59, 1.30 (each 3H, s, acetonide)

EIMS m/z : 473, 475 ($\text{M}-\text{Br}$).

$[\alpha]_D^{+6.20^{\circ}}$ (c =1.00, CHCl_3)

(3) Synthesis of 4-C-ethynyl-3,5-di-O-benzyl-1,2-O-isopropylidene- α -D-ribo-pentofuranose (Compound 4)

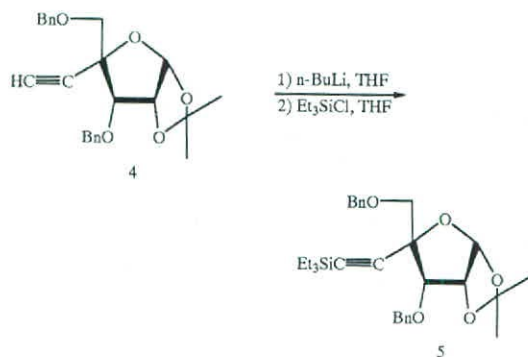


Compound 3 (12.4 g, 22.4 mmol) was dissolved in dry tetrahydrofuran (160 ml), and a 1.6 M n-butyl lithium (30.7 ml, 49.1 mmol) in n-hexane was added to the solution at -78°C . in an argon atmosphere, followed by stirring for 30 minutes at the same temperature. After water was added to the mixture with stirring, the organic layer was dried over anhydrous magnesium sulfate and concentrated through distillation under reduced pressure. The residue was purified by means of silica gel column chromatography (silica gel 1500 ml, eluent; n-hexane:ethyl acetate=3:1), to thereby yield a colorless viscous compound (Compound 4; 7.95 g, 20.2 mmol, 90.3%).

$^1\text{H-NMR}(\text{CDCl}_3)$ δ 7.39-7.22 (10H, m, aromatic), 5.70 (1H, d, H-1 $J_{1,2}$ =3.60), 4.78, 4.69 (each 1H, d, benzyl, J_{gem} =12.60), 4.55 (1H, br.t, H-2), 4.53, 4.44 (each 1H, d, benzyl, J_{gem} =12.30), 4.16 (1H, d, H-3, $J_{2,3}$ =4.50), 3.71, 3.56 (each 1H, d, H-5, J_{gem} =11.40), 1.73, 1.33 (each 3H, s, acetonide)

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EIMS m/z : 394(M^+).
 HRMS $m/z(M^+)$: Calcd. for $C_{24}H_{26}O_5$: 394.1780, Found: 394.1777
 $[\alpha]_D^{25} +22.60$ ($c=1.00$, $CHCl_3$)
 (4) Synthesis of 4-C-triethylsilylethynyl-3,5-di-O-benzyl-1,2-O-isopropylidene- α -D-ribo-pentofuranose (Compound 5)



Compound 4 (5.00 g, 12.7 mmol) was dissolved in dry tetrahydrofuran (100 ml), and a 1.6 M *n*-butyl lithium (9.50 ml, 15.2 mmol) in *n*-hexane was added to the solution at -78°C . in an argon atmosphere, followed by stirring for five minutes at the same temperature. Under the same conditions, chlorotriethylsilane (2.55 ml, 15.2 mmol) was added thereto, followed by stirring for 30 minutes. After water was added to the mixture with stirring, the organic layer was dried over anhydrous magnesium sulfate and concentrated through distillation under reduced pressure. The residue was purified by means of silica gel column chromatography (silica gel 1000 ml, eluent; *n*-hexane:ethyl acetate=3:1), to thereby yield a colorless oily compound (Compound 5; 6.32 g, 12.4 mmol, 97.6%).

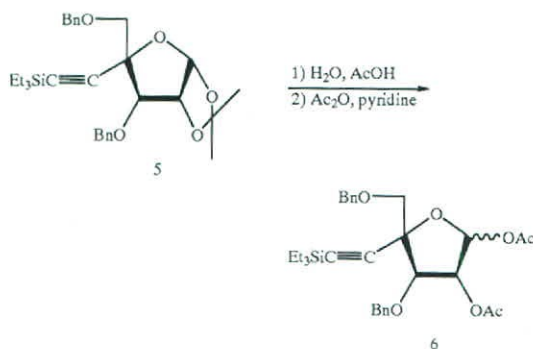
$^1\text{H-NMR}$ ($CDCl_3$) δ 7.41-7.22 (10H, m, aromatic), 5.71 (1H, d, H-1, $J_{1,2}=3.85$), 4.77, 4.65 (each 1H, d, benzyl, $J_{gem}=12.09$), 4.63 (1H, br.t, H-2), 4.57, 4.48 (each 1H, d, benzyl, $J_{gem}=12.09$), 4.23 (1H, d, H-3, $J_{2,3}=4.67$), 1.73, 1.33 (each 3H, s, acetonide), 0.98 (9H, t, Si-CH₂-CH₃, $J=7.83$), 0.60 (6H, Si-CH₂-CH₃, $J=7.97$)

EIMS m/z : 508(M^+).

HRMS $m/z(M^+)$: Calcd. for $C_{30}H_{40}O_5Si$: 508, 2645, Found: 508,2642

$[\alpha]_D^{25} -27.27^\circ$ ($c=1.045$, $CHCl_3$)

(5) Synthesis of 4-C-triethylsilylethynyl-1,2-di-O-acetyl-3,5-di-O-benzyl-D-ribo-pentofuranose (Compound 6)



Compound 5 (5.55 g, 10.9 mmol) was dissolved in acetic acid (70.0 ml), and trifluoroacetic acid (10.0 ml) and water (30.0 ml) were added to the solution, followed by stirring

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overnight at room temperature. After disappearance of Compound 5 had been confirmed by means of silica gel thin-layer chromatography, the reaction mixture was concentrated through distillation under reduced pressure. The residue was further concentrated by co-boiling with toluene three times, and then dissolved in pyridine (50.0 ml). Acetic anhydride (10.3 ml, 0.11 mol) was added thereto, followed by stirring overnight at room temperature. The reaction mixture was concentrated through distillation under reduced pressure, and the residue was dissolved in ethyl acetate. The organic layer was washed with water, dried over anhydrous magnesium sulfate, and concentrated through distillation under reduced pressure. The residue was purified by means of silica gel column chromatography (silica gel 1000 ml, eluent; *n*-hexane:ethyl acetate=5:1), to thereby yield a colorless viscous compound (Compound 6; 4.80 g, 8.68 mmol, 79.6%) as an anomer mixture ($\alpha:\beta=1:6.6$).

$^1\text{H-NMR}$ for α anomer ($CDCl_3$) δ 7.38-7.28 (10H, m, aromatic), 6.39 (1H, d, H-1, $J_{1,2}=4.67$), 5.13 (1H, dd, H-2, $J_{1,2}=4.67$, $J_{2,3}=6.87$), 4.80, 4.55 (each 1H, benzyl, d, $J_{gem}=12.09$), 4.61, 4.52 (each 1H, d, benzyl, $J_{gem}=12.09$), 4.30 (1H, d, H-3, $J_{2,3}=6.87$), 3.62 (2H, d, H-5, $J=0.55$), 2.12, 2.07 (each 3H, s, acetyl), 0.94 (9H, t, Si-CH₂-CH₃, $J=7.97$), 0.55 (6H, Si-CH₂-CH₃, $J=7.97$)

$[\alpha]_D^{25} -21.8^\circ$ ($c=1.00$, $CHCl_3$)

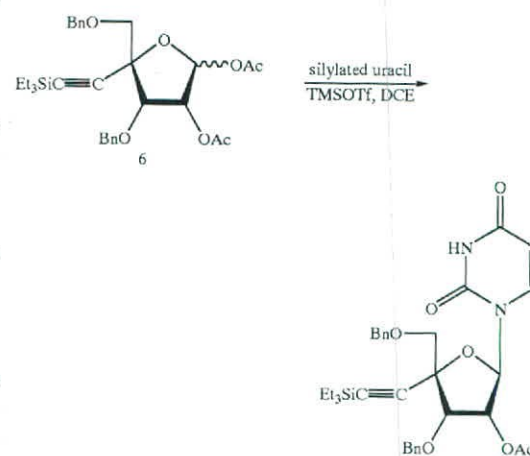
$^1\text{H-NMR}$ for β anomer ($CDCl_3$) δ 7.35-7.24 (10H, m, aromatic), 6.20 (1H, d, H-1, $J_{1,2}=0.82$), 5.33 (1H, dd, H-2, $J_{1,2}=0.82$, $J_{2,3}=4.67$), 4.66, 4.61 (each 1H, benzyl, d, $J_{gem}=11.81$), 4.56, 4.47 (each 1H, benzyl, d, $J_{gem}=11.81$), 4.48 (1H, d, H-3, $J_{2,3}=4.67$), 3.69, 3.62 (each 1H, d, H-5, $J_{gem}=10.99$), 2.09, 1.84 (each 3H, s, acetyl), 0.96 (9H, t, Si-CH₂-CH₃, $J=7.97$), 0.58 (6H, Si-CH₂-CH₃, $J=7.97$)

$[\alpha]_D^{25} -58.0^\circ$ ($c=1.00$, $CHCl_3$)

EIMS m/z : 552(M^+).

HRMS $m/z(M^+)$: Calcd. for $C_{33}H_{40}O_7Si$: 552.2543, Found: 552.2551

(6) Synthesis of 4'-C-triethylsilylethynyl-2'-O-acetyl-3',5'-di-O-benzyluridine (Compound 7)



Compound 6 (3.00 g, 5.43 mmol) was dissolved in 1,2-dichloroethane (100 ml), and uracil (1.52 g, 13.6 mmol) and *N*,*O*-bis(trimethylsilyl)acetamide (9.40 ml, 38.0 mmol) were added to the solution, followed by refluxing for one hour. After the reaction mixture was allowed to cool to room temperature, trimethylsilyl trifluoromethanesulfonate (1.97 ml, 10.9 mmol) was added thereto, followed by stirring overnight at 50°C . A saturated aqueous solution of sodium

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hydrogencarbonate was added to the mixture, and after stirring, precipitate was filtered. The organic layer was dried over anhydrous magnesium sulfate and concentrated through distillation under reduced pressure. The residue was purified by means of silica gel column chromatography (silica gel 300 ml, eluent; n-hexane:ethyl acetate=1:1), to thereby yield a colorless viscous compound (Compound 7; 2.50 g, 4.13 mmol, 76.1%).

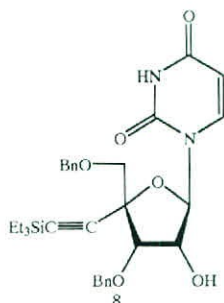
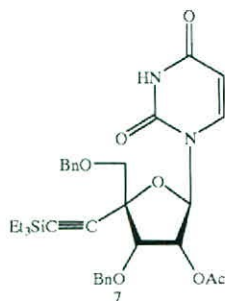
¹H-NMR(CDCl₃) δ8.63 (1H, br.s, 3-NH), 7.59 (1H, d, 6-H, J_{5,6}=8.24), 7.41-7.24 (10H, m, aromatic), 6.31 (1H, d, H-1', J_{1',2'}=4.95), 5.34 (1H, d, H-5, J_{5,6}=8.24), 5.21 (1H, dd, H-2', J_{1',2'}=4.95, J_{2',3'}=6.04), 4.71, 4.58 (each 1H, d, benzyl, J_{gem}=11.81), 4.48 (2H, s, benzyl), 4.34 (1H, d, H-3', J_{2',3'}=6.04), 3.86, 3.67 (each 1H, d, H-5', J_{gem}=10.50), 2.05 (3H, s, acetyl), 0.97 (9H, t, Si-CH₂-CH₃, J=7.95), 0.60 (6H, Si-CH₂-CH₃, J=7.95).

FABMS m/z: 605(MH⁺).

HRMS m/z(MH⁺): Calcd. for C₃₃H₄₁N₂O₇Si: 605.2683, Found: 605.2683.

[α]_D-21.97° (c=1.015, CHCl₃).

(7) Synthesis of 4'-C-triethylsilylethynyl-3',5'-di-O-benzyluridine (Compound 8)



Compound 7 (2.00 g, 3.3 mmol) was dissolved in methanol (90.0 ml), and triethylamine (10.0 ml) was added to the solution, followed by stirring for 48 hours at room temperature. The reaction mixture was concentrated through distillation under reduced pressure, and the residue was purified by means of silica gel column chromatography (silica gel 200 ml, eluent; n-hexane:ethyl acetate=1:1), to thereby yield a white powdery compound (Compound 8; 1.72 g, 3.06 mmol, 92.4%).

¹H-NMR(CDCl₃) δ8.43 (1H, br.s, 3-NH), 7.55 (1H, d, H-6, J_{5,6}=8.24), 7.41-7.25 (10H, m, aromatic), 6.10 (1H, d, H-1', J_{1',2'}=5.22), 5.37 (1H, dd, H-5, J_{5,6}=8.24), 4.96, 4.66 (each 1H, d, benzyl, J_{gem}=11.54), 4.56, 4.50 (each 1H, d, benzyl, J_{gem}=11.00), 4.21 (1H, m, H-2'), 4.17 (1H, d, H-3', J_{2',3'}=5.77), 3.87, 3.74 (each 1H, d, H-5', J_{gem}=10.44), 3.02 (1H, br.d, 2'-OH), 0.97 (9H, t, Si-CH₂-CH₃, J=7.69), 0.60 (6H, Si-CH₂-CH₃, J=7.69).

FABMS m/z: 563(MH⁺).

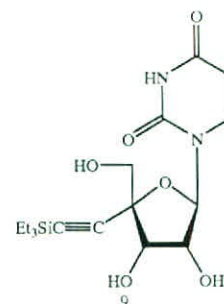
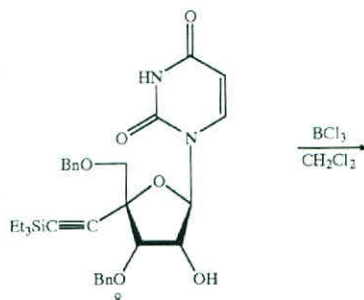
HRMS m/z(MH⁺): Calcd. for C₃₁H₃₉N₂O₆Si: 563.2577, Found: 563.2586.

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[α]_D-21.56° (c=1.025, CHCl₃)

m.p. 119-120° C.

(8) Synthesis of 4'-C-triethylsilylethynyluridine (Compound 9)



Compound 8 (1.50 g, 2.67 mmol) was dissolved in dichloromethane (75.0 ml), and a 1.0 M boron trichloride (26.7 ml, 26.7 mmol) in dichloromethane was added to the solution at -78° C. in an argon atmosphere, followed by stirring for three hours at the same temperature. A mixture of pyridine (10.0 ml) and methanol (20.0 ml) was added thereto at -78° C., followed by stirring for ten minutes. The reaction mixture was concentrated through distillation under reduced pressure, and the residue was partitioned with ethyl acetate and water. The organic layer was dried over anhydrous magnesium sulfate and concentrated through distillation under reduced pressure. The residue was purified by means of silica gel column chromatography (silica gel 200 ml, eluent; chloroform:methanol=9:1), to thereby yield a white powdery compound (Compound 9; 0.95 g, 2.48 mmol, 92.9%).

¹H-NMR(CDCl₃) δ11.36 (1H, d, 3-NH), 7.81 (1H, d, H-6, J_{5,6}=8.24), 5.92 (1H, d, H-1', J_{1',2'}=6.32), 5.68 (1H, dd, J_{5,6}=8.24), 5.55 (1H, t, 5'-OH), 5.33 (1H, d, 2'-OH), 5.16 (1H, d, 3'-OH), 4.13 (1H, dd, H-2', J_{1',2'}=6.32, J_{2',3'}=5.77), 4.07 (1H, t, H-3', J_{2',3'}=5.77), 3.58 (1H, d, H-5'), 0.96 (9H, t, Si-CH₂-CH₃, J=7.97), 0.57 (6H, Si-CH₂-CH₃, J=7.97).

FABMS m/z: 383(MH⁺).

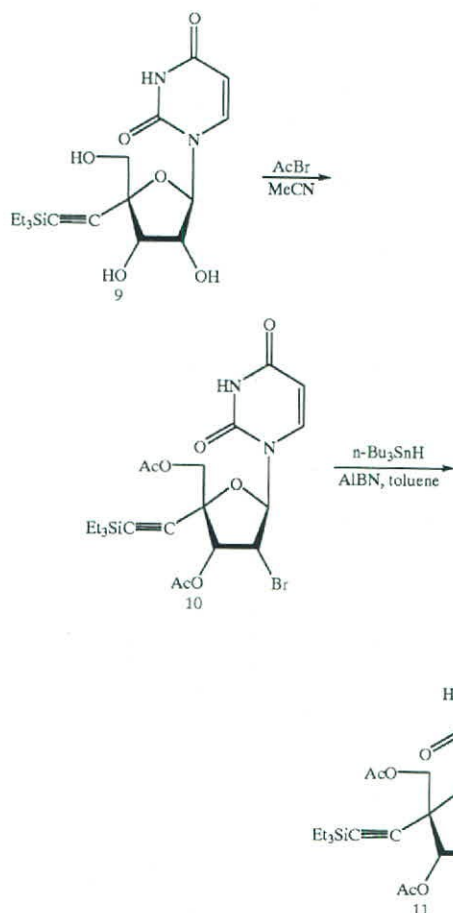
HRMS m/z(MH⁺): Calcd. for C₁₇H₂₇N₂O₆Si: 383, 1638, Found: 383.1645.

[α]_D-4.50° (c=1.00, CH₃OH)

m.p. 183-186° C.

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(9) Synthesis of 4'-C-triethylsilyl-3',5'-di-O-acetyl-2'-deoxyuridine (Compound 11)



Compound 9 (0.80 g, 2.09 mmol) was suspended in acetonitrile (20.0 ml), and a solution (20.0 ml) of acetyl bromide (1.55 ml, 21.0 mmol) in acetonitrile was added dropwise to the suspension at 85° C. over 30 minutes, followed by refluxing for one hour. After the reaction mixture was concentrated through distillation under reduced pressure, the residue was dissolved in ethyl acetate and the solution was washed with a saturated aqueous solution of sodium hydrogencarbonate and a saturated aqueous solution of sodium chloride. The organic layer was dried over anhydrous magnesium sulfate and concentrated through distillation under reduced pressure, to thereby yield 4'-C-triethylsilyl-3',5'-di-O-acetyl-2'-bromo-2'-deoxyuridine (Compound 10). After the crude product (Compound 10) was concentrated by co-boiling with toluene three times, the product was dissolved in dry toluene (50.0 ml). Hydrogenated tri(n-butyl)tin (1.08 ml, 4.19 mmol) and 2,2'-azobis(isobutyronitrile) (0.01 g) were added to the solution at 85° C., and the mixture was heated under stirring for one hour in an argon atmosphere. After the reaction mixture was concentrated through distillation under reduced pressure, the residue was purified by means of silica gel column chromatography (silica gel 300 ml, eluent; toluene: ethyl acetate), to thereby yield a colorless viscous compound (Compound 11; 0.40 g, 42.6%).

¹H-NMR(CDCl₃) δ 7.49 (1H, d, H-6, J_{5,6}=8.24), 6.34 (1H, t, H-1', J_{1',2'}=6.46), 5.77 (1H, dd, H-5, J_{5,6}=8.24), 5.37 (1H,

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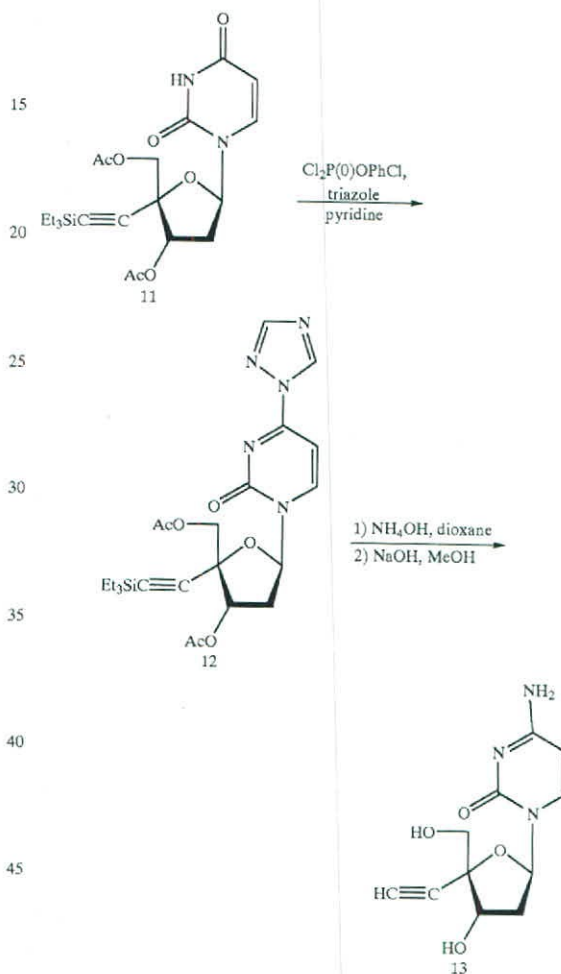
dd, H-3', J_{2',3'}=4.95, 7.42), 4.42, 4.37 (each 1H, d, H-5', J_{gem}=11.81), 2.62, 2.32 (each 1H, m, H-2'), 2.13 (6H, s, acetyl), 1.00 (9H, t, Si-CH₂-CH₃, J=7.82), 0.63 (6H, Si-CH₂-CH₃, J=7.82).

FABMS m/z: 451(MH⁺).

HRMS m/z(MH⁺): Calcd. for C₂₁H₃₁N₂O₇Si: 451.1900, Found: 451.1934.

[α]_D²⁰-11.7° (c=1.04, CHCl₃)

(10) Synthesis of 4'-C-ethynyl-2'-deoxycytidine (Compound 13)



Compound 11 (0.30 g, 0.67 mmol) was dissolved in pyridine (15.0 ml), and p-chlorophenylphosphordichloridate (0.33 ml, 2.00 mmol) was added to the solution under ice-cooling, followed by stirring for two minutes. 1,2,4-Triazole (0.46 g, 6.66 mmol) was added to the mixture, followed by stirring for seven days at room temperature. After disappearance of raw material had been confirmed by means of silica gel thin-layer chromatography, the reaction mixture was concentrated through distillation under reduced pressure, and the residue was partitioned with ethyl acetate and water. The organic layer was dried over anhydrous magnesium sulfate and concentrated through distillation under reduced pressure. The residue was purified by means of silica gel column chromatography (silica gel 50 ml, eluent; n-hexane:ethyl acetate=1:3), to thereby yield colorless viscous Compound 12: 4-(1,2,4-triazolo)-4'-C-ethynyl-2'-deoxyuridine. Compound 12 was dissolved in dioxane

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(30.0 ml), and 25% aqueous ammonia (10.0 ml) was added to the solution, followed by stirring overnight at room temperature. After disappearance of Compound 12 had been confirmed by means of silica gel thin-layer chromatography, the reaction mixture was concentrated through distillation under reduced pressure. The residue was dissolved in methanol (45.0 ml), and an aqueous 1 N solution of sodium hydroxide (5.00 ml, 5.00 mmol) was added thereto, followed by stirring for two hours at room temperature. Acetic acid (0.29 ml, 5.00 mmol) was added to the mixture, and the reaction mixture was concentrated through distillation under reduced pressure. The residue was purified by means of reversed-phase medium-pressure column chromatography (Wakosil 40C18 50 g, eluent; a 5% aqueous solution of acetonitrile). The fractions containing Compound 13 were brought to dryness under reduced pressure, and the residue was crystallized from methanol-ether, to thereby yield a white crystalline compound (Compound 13; 0.12 g, 0.48 mmol, 71.6%).

¹H-NMR(DMSO-d₆) δ7.78 (1H, d, H-6, J_{5,6}=7.50), 7.17 (2H, br.d, NH₂), 6.14 (1H, dd, H-1', J_{1',2}=4.76, 7.20), 5.72 (1H, d, H-5, J_{5,6}=7.50), 5.49 (1H, d, 3'-OH), 5.42 (1H, t, 5'-OH), 4.30 (1H, t, H-3', J_{2',3}=32.7.20), 3.64, 3.58 (each 1H, m, H-5'), 3.48 (1H, s, ethynyl), 2, 25, 2.07 (each 1H, m, H-2')

[α]_D+75.0° (c=1.00, CH₃OH)

FABMS m/z: 252(MH⁺).

HRMS m/z(MH⁺): Calcd. for C₁₁H₁₄N₃O₄: 252.0984, Found: 252.0979.

UV λ max (CH₃OH) nm (ε): 271 (9227)

m.p. 220° C. (Dec)

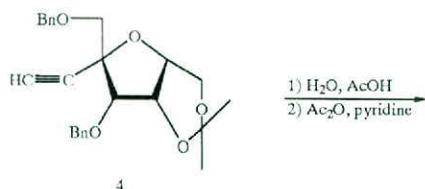
Synthesis Example 2

5-Fluorouracil, 5-ethyluracil, 5-bromovinyluracil, and 5-ethynyluracil were employed instead of uracil used in Synthesis Example 1 (6), and the reactions were carried out in the same manner as described above (if necessary, amination reaction by use of triazole described in (10) was omitted), to thereby synthesize the following compounds:

- 4'-C-ethynyl-2'-deoxy-5-fluorouridine;
- 4'-C-ethynyl-2'-deoxy-5-ethyluridine;
- 4'-C-ethynyl-2'-deoxy-5-bromovinyluridine;
- 4'-C-ethynyl-2'-deoxy-5-ethynyluridine;
- 4'-C-ethynyl-2'-deoxy-5-ethylcytidine;
- 4'-C-ethynyl-2'-deoxy-5-bromovinylcytidine; and
- 4'-C-ethynyl-2'-deoxy-5-ethynylcytidine.

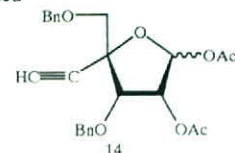
Synthesis Example 3

(1) Synthesis of 4'-C-ethynyl-1,2-di-O-acetyl-3,5-di-O-benzyl-D-ribo-pentofuranose (Compound 14)



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-continued



Compound 4 (6.00 g, 15.2 mmol) was dissolved in acetic acid (70.0 ml), and trifluoroacetic acid (10.0 ml) and water (30.0 ml) were added to the solution, followed by stirring overnight at room temperature. After disappearance of Compound 4 had been confirmed by means of silica gel thin-layer chromatography, the reaction mixture was concentrated through distillation under reduced pressure. The residue was concentrated by co-boiling with toluene three times. The treated residue was dissolved in pyridine (50.0 ml). Acetic anhydride (14.3 ml, 0.15 mol) was added thereto, followed by stirring overnight at room temperature. The reaction mixture was concentrated through distillation under reduced pressure, and the residue was dissolved in ethyl acetate. The organic layer was washed with water, dried over anhydrous magnesium sulfate, and concentrated through distillation under reduced pressure. The residue was purified by means of silica gel column chromatography (silica gel 1000 ml, eluent; n-hexane:ethyl acetate=2:1), to thereby yield a colorless viscous compound (Compound 14; 5.40 g, 12.3 mmol, 80.9%) as an anomer mixture (α:β=1:3.0).

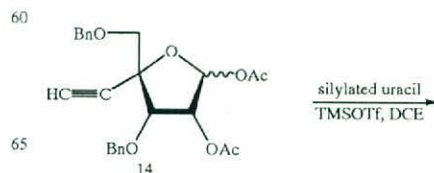
¹H-NMR for α anomer (CDCl₃) δ7.39-7.25 (10H, m, aromatic), 6.42 (1H, d, H-1, J_{1,2}=4.67), 5.13 (1H, dd, H-2, J_{1,2}=4.67, J_{2,3}=6.87), 4.81, 4.60 (each 1H, benzyl, d, J_{gem}=12.09), 4.59, 4.51 (each 1H, d, benzyl, J_{gem}=12.09), 4.30 (1H, d, H-3, J_{2,3}=6.87), 3.63 (2H, d, H-5, J=0.55), 2.73 (1H, s, ethynyl), 2.10, 2.02 (each 3H, s, acetyl).

¹H-NMR for β anomer (CDCl₃) δ7.35-7.20 (10H, m, aromatic), 6.21 (1H, d, H-1, J_{1,2}=0.82), 5.40 (1H, dd, H-2, J_{1,2}=0.82, J_{2,3}=4.67), 4.66, 4.60 (each 1H, benzyl, d, J_{gem}=11.81), 4.50, 4.47 (each 1H, benzyl, d, J_{gem}=11.81), 4.42 (1H, d, H-3, J_{2,3}=4.67), 3.70, 3.66 (each 1H, d, H-5, J_{gem}=10.99), 2.80 (1H, s, ethynyl), 2.08, 1.81 (each 3H, s, acetyl).

EIMS m/z: 438(M⁺).

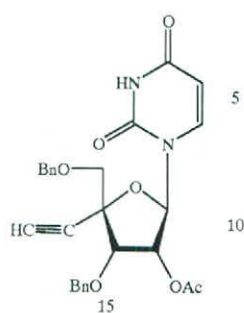
HRMS m/z(M⁺): Calcd. for C₂₅H₂₆O₇: 438.1679, Found: 438.1681

(2) Synthesis of 4'-C-ethynyl-2'-O-acetyl-3',5'-di-O-benzyluridine (Compound 15)



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-continued



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Compound 14 (2.50 g, 5.70 mmol) was dissolved in 1,2-dichloroethane (80.0 ml), and uracil (1.60 g, 14.27 mmol) and N,O-bis(trimethylsilyl)acetamide (9.86 ml, 39.74 mmol) were added to the solution, followed by refluxing for one hour. After the reaction mixture was allowed to cool to room temperature, trimethylsilyl trifluoromethanesulfonate (2.06 ml, 11.40 mmol) was added thereto, followed by stirring overnight at 50° C. A saturated aqueous solution of sodium hydrogencarbonate was added to the mixture, and after stirring, precipitate was filtered. The organic layer was dried over anhydrous magnesium sulfate and concentrated through distillation under reduced pressure. The residue was purified by means of silica gel column chromatography (silica gel 300 ml, eluent; n-hexane:ethyl acetate=2:3), to thereby yield a colorless viscous compound (Compound 15; 2.44 g, 4.97 mmol, 87.2%).

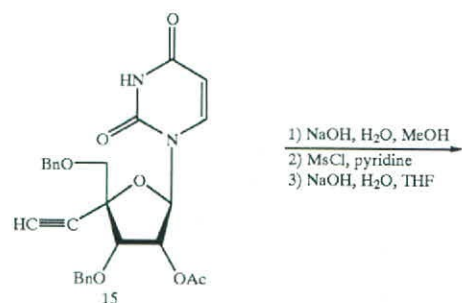
¹H-NMR(CDCl₃) δ8.52 (1H, br. s, 3-NH), 7.55 (1H, d, 6-H, J_{5,6}=8.24), 7.40-7.22 (10H, m, aromatic), 6.25 (1H, d, H-1', J_{1',2'}=4.40), 5.33 (1H, d, H-5, J_{5,6}=8.24), 5.22 (1H, dd, H-2', J_{1',2'}=4.40, J_{2',3'}=5.77), 4.63 (2H, s, benzyl), 4.45, 4.40 (each 1H, d, benzyl, J_{gem}=10.99), 4.34 (1H, d, H-3', J_{2',3'}=5.77), 3.84, 3.62 (each 1H, d, H-5', J_{gem}=10.58), 2.69 (1H, s, ethynyl), 2.11 (3H, s, acetyl).

FABMS m/z: 491(MH⁺).

HRMS m/z(MH⁺): Calcd. for C₂₇H₂₇N₂O₇: 491.1818, Found: 491.1821.

[α]_D²⁰ 29.0° (c=1.00, CHCl₃).

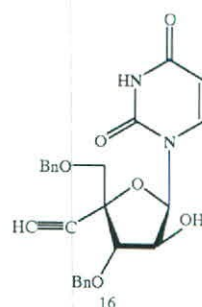
(3) Synthesis of 1-(4-C-ethynyl-2-O-acetyl-3,5-di-O-benzyl-β-D-arabino-pentofuranosyl)uracil (Compound 16)



15

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-continued



16

Compound 15 (2.30 g, 4.69 mmol) was dissolved in methanol (90.0 ml), and a 1 N aqueous solution of sodium hydroxide (10.0 ml) was added to the solution, followed by stirring for two hours at room temperature. The reaction mixture was neutralized with acetic acid and then brought to dryness under reduced pressure. The residue was dissolved in ethyl acetate. The organic layer was washed with water, dried over anhydrous magnesium sulfate, and brought to dryness under reduced pressure. The residue was concentrated by co-boiling with a small amount of pyridine three times. The product was dissolved in pyridine (50.0 ml), and methanesulfonyl chloride (0.73 ml, 9.41 mmol) was added to the solution under cooling, followed by stirring for three hours. A small amount of water was added to the reaction mixture, and the mixture was brought to dryness under reduced pressure. The residue was dissolved in ethyl acetate, followed by washing with water. The organic layer was dried over anhydrous magnesium sulfate and then brought to dryness under reduced pressure. The residue was dissolved in tetrahydrofuran (30.0 ml), and a 1 N aqueous solution of sodium hydroxide (50.0 ml) was added to the solution, followed by refluxing for one hour. After the reaction mixture was neutralized with acetic acid, the target compound was taken up through extraction with ethyl acetate. The organic layers were combined and dried over anhydrous magnesium sulfate. The organic layer was brought to dryness under reduced pressure, and the residue was purified by means of silica gel column chromatography (silica gel 250 ml, eluent; n-hexane:ethyl acetate=1:2), to thereby yield a white powder compound (Compound 16; 1.54 g, 3.43 mmol, 73.1%).

¹H-NMR(CDCl₃) δ9.82 (1H, br.s, 3-NH), 7.73 (1H, d, 6-H, J_{5,6}=8.06), 7.41-7.19 (10H, m, aromatic), 6.24 (1H, d, H-1', J_{1',2'}=5.86), 5.25 (1H, d, H-5, J_{5,6}=8.06), 4.88, 4.76 (each 1H, d, benzyl, J_{gem}=12.21), 4.78 (1H, H-2'), 4.52 (1H, 2'-OH), 4.46, 4.39 (each 1H, d, benzyl, J_{gem}=11.11), 4.19 (1H, d, H-3, J_{2',3'}=6.59), 3.834, 3.64 (each 1H, d, H-5', J_{gem}=10.62), 2.67 (1H, s, ethynyl).

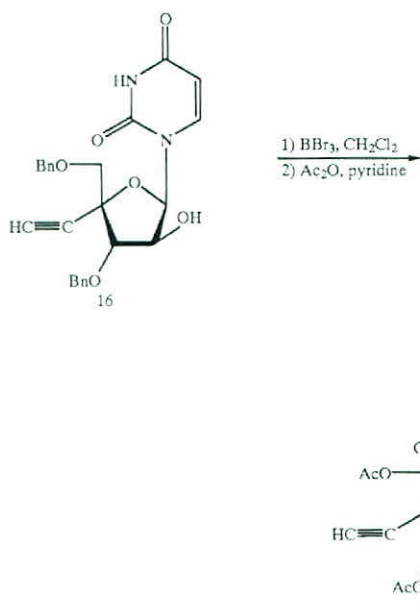
FABMS m/z: 449(MH⁺).

HRMS m/z(MH⁺): Calcd. for C₂₅H₂₅N₂O₆: 449.1712, Found: 449.1713.

[α]_D²⁰ 40.70 (c=1.00, CHCl₃).

m.p. 105-106° C.

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(4) Synthesis of 1-(4-C-ethynyl-2,3,5-tri-O-acetyl- β -D-arabino-pentofuranosyl)uracil (Compound 17)

Compound 16 (1.40 g, 3.12 mmol) was dissolved in dichloromethane (40.0 ml), and 1.0 M boron tribromide (15.6 ml, 15.6 mmol) in dichloromethane was added to the solution at -78°C . in an argon atmosphere, followed by stirring for three hours at the same temperature. A mixture of pyridine (5.00 ml) and methanol (10.0 ml) was added thereto at -78°C ., and after stirring for ten minutes, the reaction mixture was concentrated through distillation under reduced pressure. After the residue was concentrated by co-boiling with a small amount of methanol three times and by another co-boiling with a small amount of pyridine three times, the residue was dissolved in pyridine (50.0 ml), and acetic anhydride (4.42 ml, 46.7 mmol) was added to the solution, followed by stirring overnight at room temperature. The reaction mixture was brought to dryness under reduced pressure, and the residue was concentrated by co-boiling with a small amount of toluene three times and then partitioned with ethyl acetate and water. The organic layer was dried over anhydrous magnesium sulfate and concentrated through distillation under reduced pressure. The residue was purified by means of silica gel column chromatography (silica gel 150 ml, eluent; chloroform:methanol=20:1), to thereby yield a white powdery compound (Compound 17; 1.15 g, 2.92 mmol, 93.6%).

$^1\text{H-NMR}$ (CDCl_3) δ 8.99 (1H, br. s, 3-NH), 7.42 (1H, d, 6-H, $J_{5,6}$ =8.24), 6.45 (1H, d, H-1', $J_{1',2}$ =4.95), 5.76 (1H, dd, H-5, $J_{5,6}$ =8.24), 5.55 (1H, dd, H-2', $J_{1',2}$ =4.95, $J_{2',3}$ =3.57), 5.34 (1H, d, H-3', $J_{2',3}$ =3.57), 4.51, 4.42 (each 1H, d, H-5', J_{gem} =11.81), 2.73 (1H, s, ethynyl).

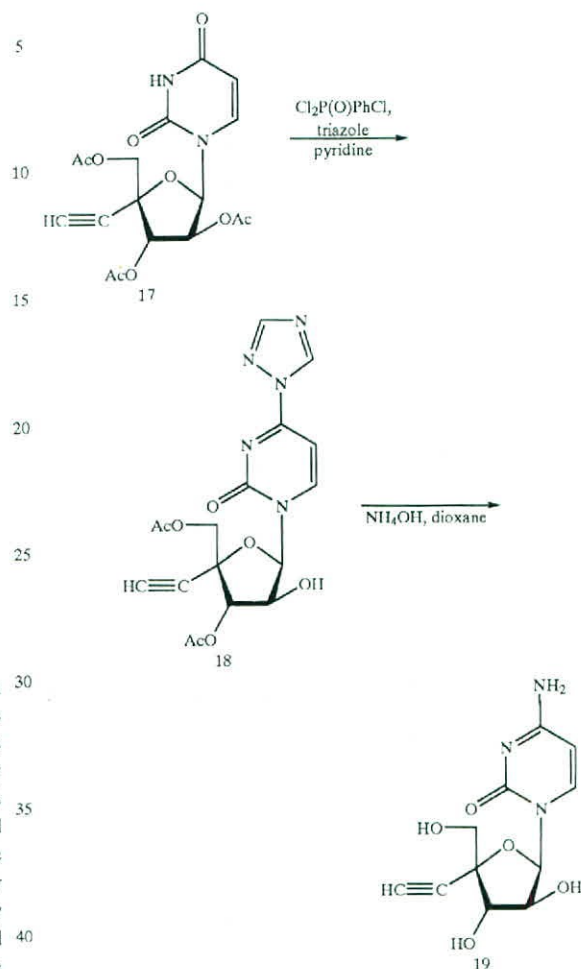
FABMS m/z : 395(MH^+).

HRMS m/z (MH^+): Calcd. for $\text{C}_{17}\text{H}_{19}\text{N}_2\text{O}_9$: 395.1090, Found: 395.1092.

$[\alpha]_D^{25}$ 18.2° (c =1.00, CHCl_3).

m.p. 160–162° C.

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(5) Synthesis of 1-(4-C-ethynyl- β -D-arabino-pentofuranosyl)cytosine (Compound 19)

Compound 17 (1.00 g, 2.54 mmol) was dissolved in pyridine (50.0 ml), and *p*-chlorophenylphosphordichloridate (1.05 ml, 6.38 mmol) was added to the solution under ice-cooling, followed by stirring for five minutes. 1,2,4-Triazole (1.75 g, 25.3 mmol) was added to the mixture, followed by stirring for seven days at room temperature. After disappearance of raw material had been confirmed by means of silica gel thin-layer chromatography, the reaction mixture was concentrated through distillation under reduced pressure, and the residue was partitioned with ethyl acetate and water. The organic layer was dried over anhydrous magnesium sulfate and concentrated through distillation under reduced pressure. The residue was purified by means of silica gel column chromatography (silica gel 50 ml, eluent; *n*-hexane:ethyl acetate=1:3), to thereby yield a colorless viscous compound (Compound 18: 1-(4-C-ethynyl-2,3,5-tri-O-acetyl- β -D-arabino-pentofuranosyl)-4-(1,2,4-triazolo)uracil. Compound 18 was dissolved in dioxane (60.0 ml), and a 25% aqueous solution of ammonia (20.0 ml) was added to the solution, followed by stirring overnight at room temperature. After disappearance of Compound 18 had been confirmed by means of silica gel thin-layer chromatography, the reaction mixture was concentrated through distillation under reduced pressure. The residue was purified by means of reversed-phase medium-pressure col-

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umn chromatography (Wakosil 40C18 50 g, eluent; a 3% aqueous solution of acetonitrile). The fractions containing Compound 19 were brought to dryness under reduced pressure, and the residue was dissolved in methanol-ether and crystallized from the same medium, to thereby yield a white crystalline compound (Compound 19; 0.51 g, 1.91 mmol, 75.2%).

$^1\text{H-NMR}$ (DMSO- d_6) δ 7.52 (1H, d, H-6, $J_{5,6}$ =7.42), 7.10 (2H, br. d, NH_2), 6.17 (1H, dd, H-1', $J_{1',2'}=6.04$), 5.66 (1H, d, H-5, $J_{5,6}$ =7.42), 5.62, 5.49 (each 1H, d, 2'-OH, 3'-OH), 5.42 (1H, t, 5'-OH), 4.16 (1H, q, H-2', $J_{1',2'}=J_{2',3'}=6.04$), 3.97 (1H, t, H-3', $J_{2',3'}=6.04$), 3.58 (2H, m, H-5'), 3.48 (1H, s, ethynyl).

$[\alpha]_D^{+95.7^\circ}$ ($c=1.00$, CH_3OH)

FABMS m/z : 268(MH $^+$).

HRMS m/z (MH $^+$): Calcd. for $\text{C}_{11}\text{H}_{14}\text{N}_3\text{O}_5$: 268.0933, Found: 268.0965.

UV λ_{max} (CH_3OH) nm (ϵ): 271 (9350)

m.p. $\sim 200^\circ\text{C}$. (Dec)

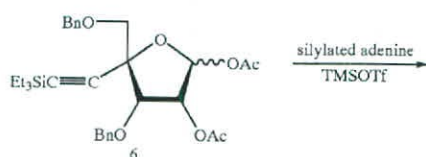
Synthesis Example 4

5-Fluorouracil, 5-ethyluracil, 5-bromovinyluracil, and 5-ethynyluracil were employed instead of uracil used in Synthesis Example 3 (2), and the reactions were carried out in the same manner as described above (if necessary, amination reaction by use of triazole described in (5) was omitted), to thereby synthesize the following compounds:

- 1-(4-C-ethynyl- β -D-arabino-pentofuranosyl)-5-fluorouracil;
- 1-(4-C-ethynyl- β -D-arabino-pentofuranosyl)-5-ethyluracil;
- 1-(4-C-ethynyl- β -D-arabino-pentofuranosyl)-5-bromovinyluracil;
- 1-(4-C-ethynyl- β -D-arabino-pentofuranosyl)-5-ethynyluracil;
- 1-(4-C-ethynyl- β -D-arabino-pentofuranosyl)-5-fluorocytosine;
- 1-(4-C-ethynyl- β -D-arabino-pentofuranosyl)-5-ethylcytosine;
- 1-(4-C-ethynyl- β -D-arabino-pentofuranosyl)-5-bromovinylcytosine; and
- 1-(4-C-ethynyl- β -D-arabino-pentofuranosyl)-5-ethynylcytosine.

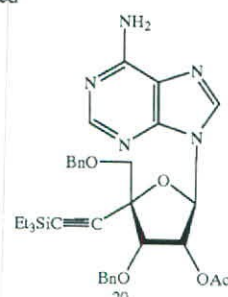
Synthesis Example 5

(1) Synthesis of 2'-O-acetyl-3',5'-di-O-benzyl-4'-C-triethylsilylethynyladenosine (Compound 20)



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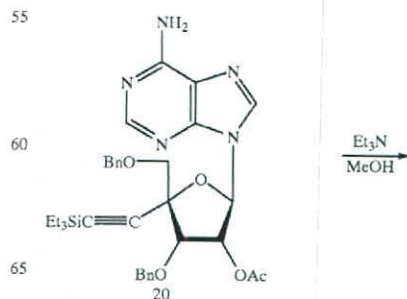
-continued



To a solution of Compound 6 (1.1 g, 2 mmol) in 1,2-dichloroethane (16.5 ml), adenine (0.405 g, 3 mmol) and N,O-bis(trimethylsilyl)acetamide (2.7 ml, 11 mmol) were added, followed by refluxing for 1.5 hours. After the mixture was allowed to cool to room temperature, trimethylsilyl trifluoromethanesulfonate (0.77 ml, 4 mmol) was added dropwise to the mixture under stirring at 0°C . in an argon atmosphere. The mixture was stirred for 15 minutes at room temperature, refluxed for 24 hours, and allowed to cool to room temperature. A saturated aqueous solution of sodium hydrogencarbonate was added thereto at 0°C ., followed by stirring for 15 minutes at room temperature. Insoluble materials were removed through filtration by use of Celite, and then the organic layer was separated from the filtrate. After an aqueous layer was extracted with chloroform, the organic layer was washed once with a saturated aqueous solution of sodium hydrogencarbonate, dried over anhydrous sodium sulfate, and concentrated through distillation under reduced pressure, so as to evaporate the solvent. The residue was applied to a silica gel column (15 g, eluent; ethyl acetate:n-hexane:ethanol=20:20:1), to thereby yield Compound 20 in an amount of 0.69 g (55%).

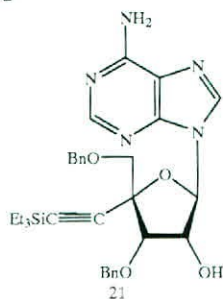
$^1\text{H-NMR}$ (CDCl_3) δ 8.32 (1H, s, purine-H), 8.01 (1H, s, purine-H), 7.27-7.37 (10H, m, $2\times\text{Ph}$), 6.37 (1H, d, $J=5.1\text{ Hz}$, H-1'), 5.60 (1H, t, $J=5.6\text{ Hz}$, H-2'), 5.59 (2H, br s, NH_2), 4.75 (1H, d, $J=11.0\text{ Hz}$, $\text{CHH}'\text{Ph}$), 4.69 (1H, d, $J=5.6\text{ Hz}$, H-3'), 4.60 (1H, d, $J=11.0\text{ Hz}$, $\text{CHH}'\text{Ph}$), 4.58 (1H, d, $J=11.2\text{ Hz}$, $\text{CHH}'\text{Ph}$), 4.51 (1H, d, $J=11.0\text{ Hz}$, $\text{CHH}'\text{Ph}$), 3.84 (1H, d, $J=11.1\text{ Hz}$, H-5'), 3.69 (1H, d, $J=11.1\text{ Hz}$, H-5') 2.03 (3H, s, Ac), 0.98 (9H, t, $J=8.7\text{ Hz}$, $3\times\text{CH}_3\text{CH}_2$), 0.61 (6H, q, $J=8.7\text{ Hz}$, $3\times\text{CH}_3\text{CH}_2$).

(2) Synthesis of 3',5'-di-O-benzyl-4'-C-triethylsilylethynyladenosine (Compound 21)



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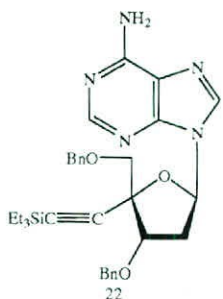
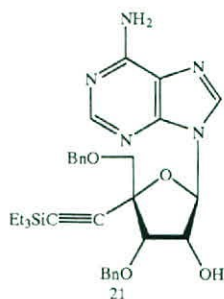
-continued



To a solution of Compound 20 (0.354 g, 0.565 mmol) in methanol (14 ml), triethylamine (3.3 ml) was added, and the mixture was stirred for one day at room temperature under air-tight condition. The mixture was concentrated under reduced pressure. The residue was applied to a silica gel column (10 g, eluent; ethyl acetate:n-hexane:ethanol=20:10:1), to thereby yield Compound 21 in an amount of 0.283 g (86%).

¹H-NMR(CDCl₃) δ8.30 (1H, s, purine-H), 8.00 (1H, s, purine-H), 7.30–7.42 (10H, m, 2×Ph), 6.17 (1H, d, J=5.6 Hz, H-1'), 5.55 (2H, br s, NH₂), 4.97 (1H, d, J=11.1 Hz, C-3'), 4.75–4.80 (1H, m, H-2'), 4.72 (1H, d, J=11.1 Hz, C-4'), 4.59 (1H, d, J=11.6 Hz, C-5'), 4.54 (1H, d, J=11.6 Hz, C-6'), 4.50 (1H, d, J=5.6 Hz, H-3'), 3.84 (1H, d, J=11.1 Hz, H-5'), 3.74 (1H, d, J=11.1 Hz, H-5'), 3.50 (1H, d, J=8.3 Hz, OH), 0.98 (9H, t, J=7.9 Hz, 3×CH₃CH₂), 0.62 (6H, q, J=7.9 Hz, 3×CH₃CH₂).

(3) Synthesis of 3',5'-di-O-benzyl-2'-deoxy-4'-C-triethylsilylthymine (Compound 22)



To a solution of Compound 21 (0.18 g, 0.308 mmol) and DMAP (0.113 g, 0.924 mmol) in acetonitrile (10.6 ml), 4-fluorophenylchlorothionoformate (0.065 ml, 0.462 mmol) was added dropwise under stirring at room temperature in an argon atmosphere and stirred for an hour at room temperature, followed by condensation under reduced pressure. Water was added to the residue, and the mixture was extracted with ethyl acetate. The organic layer was washed

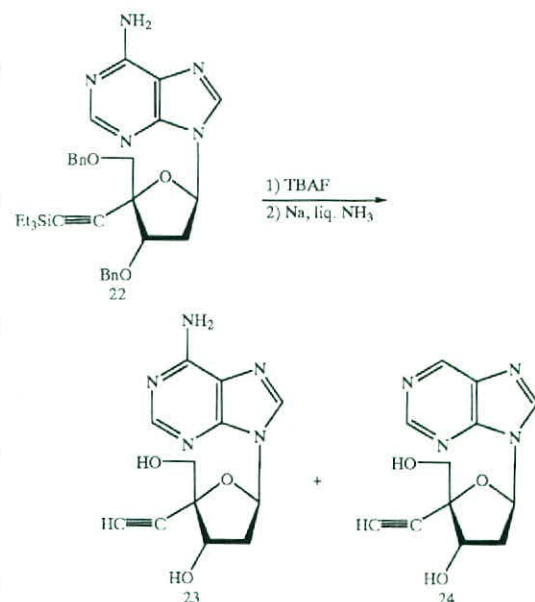
28

with water and was washed with a saturated aqueous solution of sodium chloride, dried over anhydrous sodium sulfate, and the solvent was distilled off under reduced pressure. The residue was applied to a silica gel column (eluent; ethyl acetate:n-hexane:ethanol=20:20:1), to thereby yield crude thiocarbonate.

The thiocarbonate was dissolved in toluene (9 ml), and hydrogenated tributyltin (0.41 ml, 1.85 mmol) and 2,2'-azobis(isobutyronitrile) (0.013 g, 0.077 mmol) were added to the solution. The reaction mixture was stirred at 85° C. for an hour in an argon atmosphere and allowed to cool to room temperature. The solvent was evaporated under reduced pressure. The residue was applied to a silica gel column (20 g, eluent; ethyl acetate:n-hexane:ethanol=20:10:1), to thereby yield Compound 22 in an amount of 0.10 g (57%).

¹H-NMR(CDCl₃) δ8.32 (1H, s, purine-H), 8.11 (1H, s, purine-H), 7.26–7.37 (10H, m, 2×Ph), 6.51 (1H, t, J=6.0 Hz, H-1'), 5.54 (2H, br s, NH₂), 4.72 (1H, d, J=12.0 Hz, C-3'), 4.61 (2H, d, J=10.5 Hz, CH₂Ph), 4.60 (1H, t, J=6.6 Hz, H-3'), 4.55 (1H, d, J=12.0 Hz, CH₂Ph), 3.88 (1H, d, J=10.7 Hz, H-5'), 3.76 (1H, d, J=10.7 Hz, H-5'), 2.71–2.76 (2H, m, H-2'), 0.99 (9H, t, J=7.8 Hz, 3×CH₃CH₂), 0.62 (6H, q, J=7.5 Hz, 3×CH₃CH₂).

(4) Synthesis of 2'-deoxy-4'-C-ethynyladenosine (Compound 23) and 9-(2-deoxy-4'-C-ethynyl-β-D-ribofuranosyl)purine (Compound 24)



To a solution of Compound 22 (0.23 g, 0.404 mmol) in tetrahydrofuran (9.4 ml), a 1.0 M solution of tetrabutylammonium fluoride (0.44 ml, 0.44 mmol) was added under stirring at room temperature, and after stirring for 30 minutes at the same temperature, the solvent was evaporated under reduced pressure. The residue was applied to a silica gel column and eluted with ethyl acetate, to thereby yield 0.186 g of a crude compound with no triethylsilyl group.

A solution of the above-described compound with no triethylsilyl group in tetrahydrofuran (1.8 ml) and anhydrous ethanol (0.18 ml) were fed to a flask. Ammonia gas was condensed at -78° C. to 18 ml and fed to the flask. Metallic sodium (0.047 g, 2.02 mmol) were added quickly in an argon atmosphere, followed by stirring for 15 minutes at the same temperature. In addition, metallic sodium (0.023 g) was

added to the mixture, and after stirring for 10 minutes, ammonium chloride was added. After the mixture was stirred for 1.5 hours at room temperature, ethanol was added thereto. Insoluble materials were separated through Celite, and washed with ethanol two times. The resultant filtrate and the washing liquid were concentrated under reduced pressure. The residue was applied to a silica gel column (10 g, eluent; ethyl acetate:methanol=20:1), to thereby yield a mixture of Compound 23 and Compound 24 in an amount of 0.079 g. Subsequently, the mixture was applied to a reversed-phase ODS silica gel column and eluted with a 5% aqueous solution of ethanol, to thereby yield Compound 24 in an amount of 0.028 g (27%), and further eluted with a 7.5% aqueous solution of ethanol, to thereby yield Compound 23 in an amount of 0.021 g (19%).

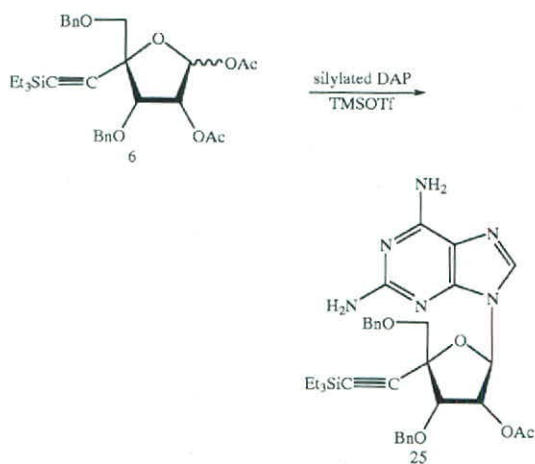
(Compound 23)
¹H-NMR(DMSO-d₆) δ8.33 (1H, s, purine-H), 8.15 (1H, s, purine-H), 7.30 (2H, br s, NH₂), 6.36 (1H, t, J=6.4 Hz, H-1'), 5.54 (1H, d, J=5.4 Hz, OH), 5.53 (1H, t, J=5.4 Hz, OH), 4.58 (1H, q, J=5.9 Hz, H-3'), 3.66 (1H, dd, J=12.2, 5.4 Hz, H-5'), 3.56 (1H, dd, J=11.7, 7.3 Hz, H-5'), 3.50 (1H, s, ethynyl-H), 2.76 (1H, dt, J=13.2, 6.4 Hz, H-2'), 2.41 (1H, dt, J=13.2, 6.8 Hz, H-2').

(Compound 24)

¹H-NMR(DMSO-d₆) δ9.18 (1H, s, purine-H), 8.96 (1H, s, purine-H), 8.79 (1H, s, purine-H), 6.50 (1H, t, J=7.3, 4.9 Hz, H-1'), 5.60 (1H, d, J=5.9 Hz, OH), 5.29 (1H, t, J=5.4 Hz, OH), 4.67 (1H, q, J=5.9 Hz, H-3'), 3.67 (1H, dd, J=11.7, 5.9 Hz, H-5'), 3.58 (1H, dd, J=11.7, 6.8 Hz, H-5'), 3.53 (1H, s, ethynyl-H), 2.85 (1H, ddd, J=13.2, 6.8, 4.9 Hz, H-2'), 2.48–2.56 (1H, m, H-2').

Synthesis Example 6

(1) Synthesis of 9-(2-O-acetyl-3,5-di-O-benzyl-4-C-triethylsilyl-β-D-ribofuranosyl)-2,6-diaminopurine (Compound 25)

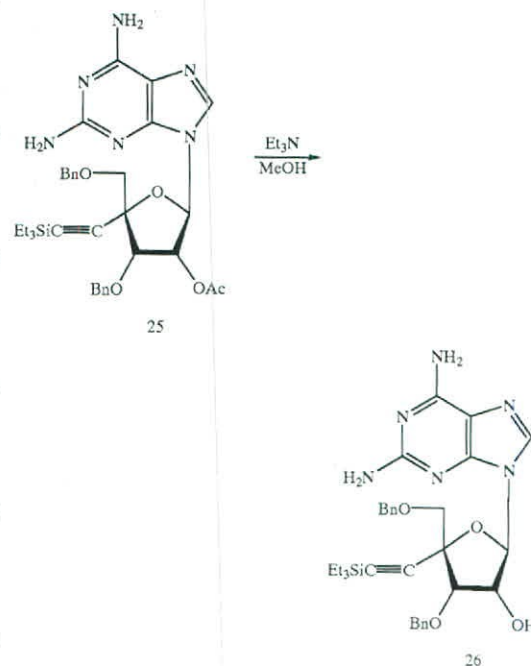


To a solution of Compound 6 (1.1 g, 2 mmol) in 1,2-dichloroethane (16.5 ml), diaminopurine (0.45 g, 3 mmol) and N,O-bis(trimethylsilyl)acetamide (4.4 ml, 18 mmol) were added, followed by refluxing for three hours. After the mixture was cooled to room temperature, trimethylsilyl trifluoromethanesulfonate (0.77 ml, 4 mmol) was added dropwise to the mixture at 0° C. in an argon atmosphere. The mixture was stirred for 15 minutes at room temperature, refluxed for 24 hours, and cooled to room temperature. A saturated aqueous solution of sodium hydrogencarbonate was added thereto at 0° C., followed by stirring for 15

minutes at room temperature. Insoluble materials were separated through filtration by use of Celite, and then the organic layer was separated from the filtrate. After an aqueous layer was extracted with chloroform once, the organic layer was washed with a saturated aqueous solution of sodium chloride, dried over anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure. The residue was applied to a silica gel column (20 g, eluent; ethyl acetate:n-hexane:ethanol=20:10:1), to thereby yield Compound 25 in an amount of 0.85 g (66%).

¹H-NMR(CDCl₃) δ7.68 (1H, s, H-8), 7.26–7.37 (10H, m, 2×Ph), 6.17 (1H, d, J=6.5 Hz, H-1'), 5.78 (1H, dd, J=6.5, 6.0 Hz, H-2'), 5.34 (2H, br s, NH₂), 4.76 (1H, d, J=11.4 Hz, C-HH'Ph), 4.69 (1H, d, J=6.0 Hz, H-3'), 4.61 (1H, d, J=11.4 Hz, CHH'Ph), 4.60 (1H, d, J=11.9 Hz, CHH'Ph), 4.55 (2H, br s, NH₂), 4.52 (1H, d, J=11.9 Hz, CHH'Ph), 3.83 (1H, d, J=10.7 Hz, H-5'), 3.70 (1H, d, J=10.7 Hz, H-5'), 2.04 (3H, s, Ac), 0.99 (9H, t, J=8.3 Hz, 3×CH₃CH₂), 0.61 (6H, q, J=8.3 Hz, 3×CH₃CH₂).

(2) Synthesis of 2,6-diamino-9-(3,5-di-O-benzyl-4-C-triethylsilyl-β-D-ribofuranosyl)purine (Compound 26)

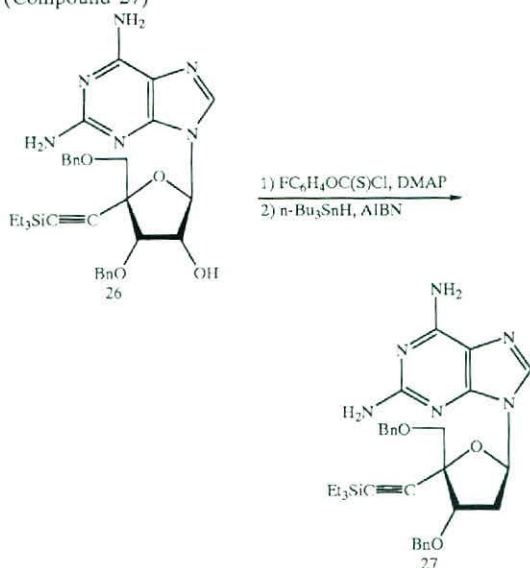


Compound 25 (0.85 g, 1.32 mmol) was treated in the same manner as in the synthesis of Compound 21, and the resultant residue was applied to a silica gel column (15 g, eluent; ethyl acetate:n-hexane:ethanol=30:10:1), to thereby yield Compound 26 in an amount of 0.74 g (93%).

¹H-NMR(CDCl₃) δ7.70 (1H, s, H-8), 7.29–7.42 (10H, m, 2×Ph), 6.00 (1H, d, J=4.9 Hz, H-1'), 5.35 (2H, br s, NH₂), 4.93 (1H, d, J=11.5 Hz, CHH'Ph), 4.74 (1H, d, J=11.5 Hz, CHH'Ph), 4.73 (1H, t, J=5.8 Hz, H-2'), 4.60 (1H, d, J=12.0 Hz, CHH'Ph), 4.55 (2H, br s, NH₂), 4.54 (1H, d, J=12.0 Hz, CHH'Ph), 4.49 (1H, d, J=5.9 Hz, H-3'), 3.81 (1H, d, J=10.7 Hz, H-5'), 3.72 (1H, d, J=10.7 Hz, H-5'), 3.62 (1H, br s, OH), 0.99 (9H, t, J=7.8 Hz, 3×CH₃CH₂), 0.62 (6H, q, J=7.8 Hz, 3×CH₃CH₂).

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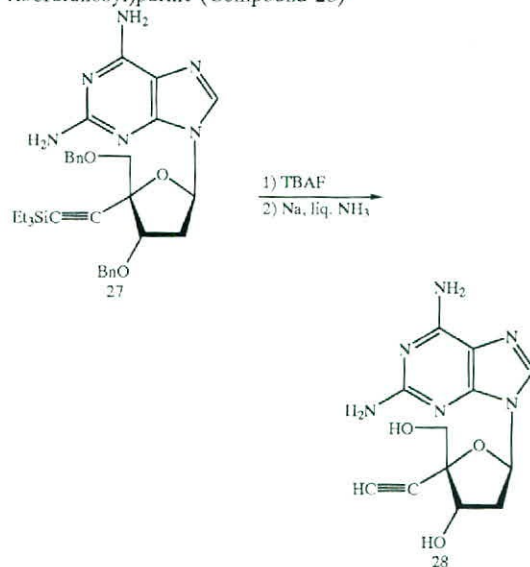
(3) Synthesis of 2,6-diamino-9-(3,5-di-O-benzyl-2-deoxy-4-C-triethylsilyl-ethynyl-β-D-ribofuranosyl)purine (Compound 27)



Compound 26 (0.103 g, 0.171 mmol) was treated in the same manner as in the synthesis of Compound 22, and the resultant residue was applied to a silica gel column (10 g, eluent; ethyl acetate:n-hexane:ethanol=30:10:1), to thereby yield Compound 27 in an amount of 0.055 g (55%).

¹H-NMR(CDCl₃) δ7.79 (1H, s, H-8), 7.26–7.37 (10H, m, 2×Ph), 6.34 (1H, dd, J=6.6, 5.5 Hz, H-1'), 5.36 (2H, br s, NH₂), 4.72 (1H, d, J=11.7 Hz, CHH'Ph), 4.56–4.63 (5H, m, CH₂Ph, H-3'), 4.57 (1H, d, J=11.7 Hz, CHH'Ph), 3.85 (1H, d, J=10.1 Hz, H-5'), 3.75 (1H, d, J=10.6 Hz, H-5'), 2.62–2.73 (2H, m, H-2'), 0.99 (9H, t, J=7.9 Hz, 3×CH₂CH₃), 0.62 (6H, q, J=7.9 Hz, 3×CH₂CH₃).

(4) Synthesis of 2,6-diamino-9-(2-deoxy-4-C-ethynyl-β-D-ribofuranosyl)purine (Compound 28)



To a solution of Compound 27 (0.263 g, 0.45 mmol) in tetrahydrofuran (10.3 ml), a 1.0 M solution of tetrabutylam-

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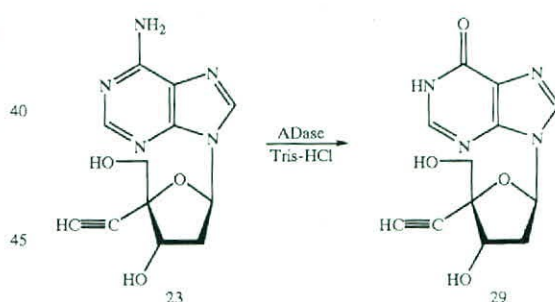
monium fluoride (0.5 ml, 0.5 mmol) was added at room temperature, and the mixture was stirred for 30 minutes at the same temperature. The solvent was evaporated under reduced pressure. The residue was applied to a short silica gel column (eluent; ethyl acetate:ethanol=30:1), to thereby yield 0.214 g of a crude compound with no triethylsilyl group.

The above-described compound with no triethylsilyl group in tetrahydrofuran (2 ml) and anhydrous ethanol (0.1 ml) were fed to a flask. Ammonia gas was condensed at -78° C. to 20 ml and fed to the flask. Metallic sodium (0.062 g, 2.7 mmol) was added quickly in an argon atmosphere, followed by stirring for 30 minutes at the same temperature. After ammonium chloride was added thereto, the mixture was stirred for two hours at room temperature, and ethanol was added to the mixture. Insoluble materials were separated through filtration by use of Celite and washed with ethanol two times. The resultant filtrate and the washing liquid were concentrated under reduced pressure. The residue was applied to a silica gel column (13 g, eluent; ethyl acetate:methanol=10:1), to thereby yield Compound 28 in an amount of 0.099 g (76%).

¹H-NMR(DMSO-d₆) δ7.89 (1H, s, H-8), 6.71 (2H, br s, NH₂), 6.20 (1H, t, J=6.3 Hz, H-1'), 5.74 (2H, br s, NH₂), 5.59 (1H, t, J=5.9 Hz, OH), 5.47 (1H, d, J=4.9 Hz, OH), 4.50 (1H, q, J=5.9 Hz, H-3'), 3.65 (1H, dd, J=11.7, 5.4 Hz, H-5'), 3.56 (1H, dd, J=11.7, 7.3 Hz, H-5'), 3.46 (1H, s, ethynyl-H), 2.64 (1H, dt, J=12.7, 6.4 Hz, H-2'), 2.32 (1H, dt, J=13.2, 6.4 Hz, H-2').

Synthesis Example 7

Synthesis of 2'-deoxy-4'-C-ethynylinosine (Compound 29)



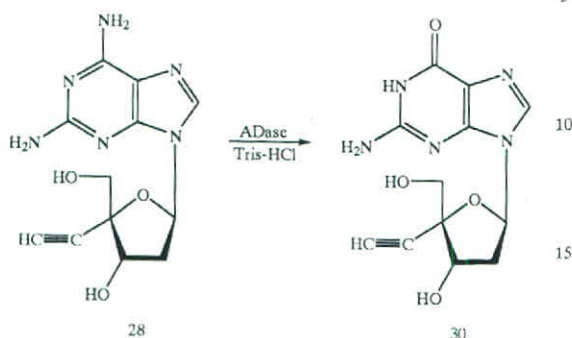
To a Tris-HCl buffer solution (6 ml, pH 7.5) of Compound 23 (0.022 g, 0.08 mmol), adenosine deaminase (0.044 ml, 20 unit) was added, and the mixture was stirred for 2.5 hours at 40° C., followed by cooling to room temperature. The reaction mixture was applied to a reverse-phase ODS silica gel column (50 g), desalted by water (500 ml) flow, and through use of a 2.5% aqueous ethanol, Compound 29 was eluted. Subsequently, the Compound 29 was pulverized with isopropanol, to thereby yield 0.016 g of Compound 29 (72%).

¹H-NMR (DMSO-d₆) δ12.28 (1H, brs, NH), 8.29 (1H, s, purine-H), 8.06 (1H, s, purine-H), 6.32 (1H, dd, J=6.8, 4.9 Hz, H-1'), 5.57 (1H, d, J=5.4 Hz, OH), 5.32 (1H, t, J=5.9 Hz, OH), 4.56 (1H, dt, J=6.4, 5.4 Hz, H-3'), 3.65 (1H, dd, J=12.2, 5.9 Hz, H-5'), 3.57 (1H, dd, J=11.7, 6.4 Hz, H-5'), 3.50 (1H, s, ethynyl-H), 2.66 (1H, dt, J=12.2, 5.9 Hz, H-2'), 2.46 (1H, dt, J=13.2, 6.9 Hz, H-2').

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Synthesis Example 8

Synthesis of 2'-deoxy-4'-C-ethynylguanosine (Compound 30)



To a Tris-HCl buffer solution (7.8 ml, pH 7.5) of Compound 28 (0.03 g, 0.103 mmol), adenosine deaminase (0.057 ml, 20 unit) was added, and the mixture was stirred for 2 hours at 40° C., followed by cooling to room temperature. The reaction mixture was applied to a reverse-phase ODS silica gel column (50 g), desalted by water (500 ml) flow, and through use of aqueous 2.5% ethanol, Compound 30 was eluted. Recrystallization from water yielded Compound 30 in an amount of 0.015 g (50%).

¹H-NMR(DMSO-d₆) δ 10.61 (1H, br s, NH), 7.90 (1H, s, H-8), 6.48 (2H, br s, NH₂), 6.13 (1H, dd, J=7.3, 5.9 Hz, H-1'), 5.51 (1H, d, J=4.9 Hz, OH), 5.30 (1H, t, J=5.9 Hz, OH), 4.47 (1H, dt, J=6.4, 5.4 Hz, H-3'), 3.62 (1H, dd, J=12.2, 6.4 Hz, H-5'), 3.54 (1H, dd, J=12.2, 6.4 Hz, H-5'), 3.47 (1H, s, ethynyl-H), 2.56 (1H, dt, J=12.2, 6.4 Hz, H-2'), 2.36 (1H, dt, J=12.7, 6.8 Hz, H-2').

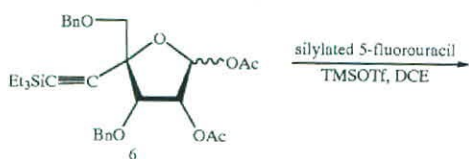
Synthesis Example 9

Adenine, guanine, and 2,6-diaminopurine were employed instead of uracil used in Synthesis Example 3 (2), and reaction is carried out in a manner similar to that described above (amination by use of triazole described in (5) omitted), to thereby synthesize the following compounds:

9-(4-C-ethynyl-β-D-arabino-pentofuranosyl)adenine;
9-(4-C-ethynyl-β-D-arabino-pentofuranosyl)guanine; and
9-(4-C-ethynyl-β-D-arabino-pentofuranosyl)-2,6-diaminopurine.

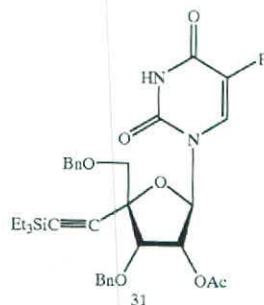
Synthesis Example 10

(1) Synthesis of 2'-O-acetyl-3',5'-di-O-benzyl-4'-C-triethylsilyl-5-fluorouridine (Compound 31)



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-continued



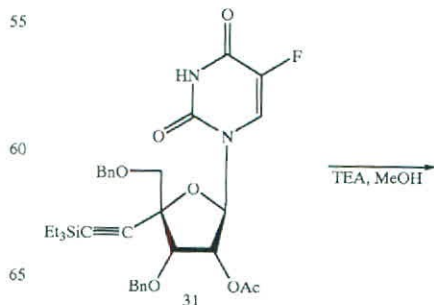
Compound 6 (2.00 g, 3.62 mmol) was dissolved in 1,2-dichloroethane (60.0 ml), and 5-fluorouracil (0.71 g, 5.46 mmol) and N,O-bis(trimethylsilyl)acetamide (5.37 ml, 21.7 mmol) were added to the solution, followed by refluxing for one hour. After the reaction mixture was allowed to cool to room temperature, trimethylsilyl trifluoromethanesulfonate (0.85 ml, 4.70 mmol) was added thereto, followed by stirring overnight at 50° C. A saturated aqueous solution of sodium hydrogencarbonate was added to the mixture, and after stirring, the organic layer was dried over anhydrous magnesium sulfate. The residue was purified by means of silica gel column chromatography (silica gel 300 ml, eluent; n-hexane:ethyl acetate=3:1), to thereby yield a colorless viscous compound (Compound 31; 0.80 g, 1.28 mmol, 35.4%).

¹H-NMR(CDCl₃) δ 8.78 (1H, d, H-6, J_{6,7}=6.35), 7.37-7.29 (10H, m, aromatic), 6.32 (1H, dd, H-1', J=5.62, 1.47), 5.17 (1H, t, H-2', J_{2',3'}=5.62), 4.73, 4.55 (each 1H, d, benzyl, J_{gem}=11.72), 4.55, 4.50 (each 1H, d, benzyl, J_{gem}=11.72), 4.32 (1H, d, H-3', J_{2',3'}=5.86), 3.87, 3.63 (each 1H, d, H-5 J_{gem}=10.50), 2.04 (3H, s, acetyl), 0.96 (9H, t, Si-CH₂-CH₃, J=8.06), 0.59 (6H, Si-CH₂-CH₃, J=7.81).

FABMS m/z: 623 (MH⁺).

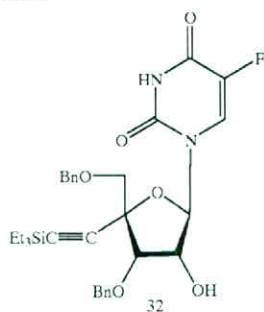
HRMS m/z(MH⁺): Calcd. for C₃₃H₄₀FN₂O₇Si: 623.2589, Found: 623.2589.

[α]_D²⁰ -23.3° (c=0.18, CHCl₃). ps (2) Synthesis of 3',5'-di-O-benzyl-4'-C-triethylsilyl-5-fluorouridine (Compound 32):



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-continued



Compound 31 (0.77 g, 1.24 mmol) was dissolved in methanol (45.0 ml), and triethylamine (5.00 ml) was added to the solution, followed by stirring for 48 hours at 30° C. The reaction mixture was concentrated through distillation under reduced pressure, and the residue was purified by means of silica gel column chromatography (silica gel 100 ml, eluent; n-hexane:ethyl acetate=2:1), to thereby yield a white powdery compound (Compound 32; 0.68 g, 1.17 mmol, 94.4%).

¹H NMR(CDCl₃) δ 8.42 (1H, br. s, 3-NH), 7.80 (1H, d, J_{6,7}=6.10), 7.38-7.29 (10H, m, aromatic), 6.10 (1H, dd, H-1', J=5.98, 1.47), 5.00, 4.63 (each 1H, d, benzyl, J_{gem}=11.23), 4.58, 4.54 (each 1H, d, benzyl, J_{gem}=10.99), 4.20 (1H, m, H-2'), 4.13 (1H, d, H-3', J_{2,3}=5.86), 3.88, 3.70 (each 1H, d, H-5', J_{gem}=10.25), 2.99 (1H, d, 2'-OH, J=9.77), 0.96 (9H, t, Si-CH₂-CH₃, J=8.06), 0.58 (6H, Si-CH₂-CH₃, J=7.82).

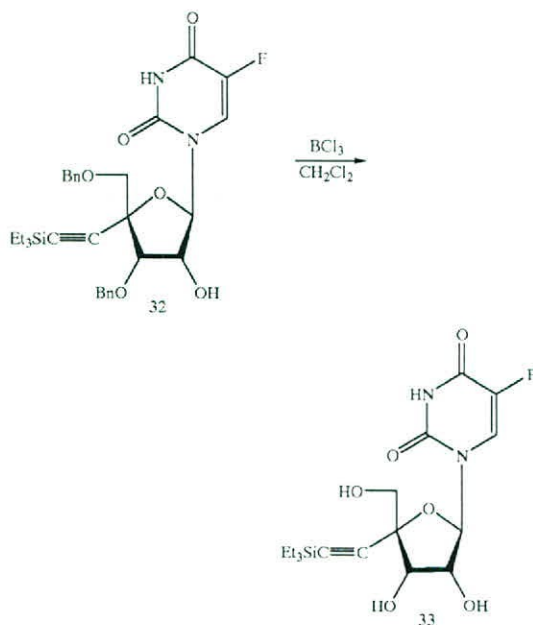
FABMS m/z: 581 (MH⁺).

HRMS m/z(MH⁺): Calcd. for C₃₁H₃₈FN₂O₆Si: 581.2483, Found: 581.2484.

[α]_D²⁰ -16.3° (c=1.05, CHCl₃)

m.p. 138-139° C.

(3) Synthesis of 4'-C-triethylsilyl-5-fluorouridine (Compound 33):



Compound 32 (1.00 g, 1.72 mmol) was dissolved in dichloromethane (50.0 ml), and a solution (26.7 ml, 26.7

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mmol) of 1.0 M boron trichloride in dichloromethane was added thereto at -78° C. in an argon atmosphere, followed by stirring for three hours at the same temperature. A mixture of pyridine (10.0 ml) and methanol (20.0 ml) was added at -78° C., followed by stirring for 30 minutes. The reaction mixture was concentrated through distillation under reduced pressure, and the residue was partitioned with ethyl acetate and water. The organic layer was dried over anhydrous magnesium sulfate and concentrated through distillation under reduced pressure. The residue was purified by means of silica gel column chromatography (silica gel 150 ml, eluent; chloroform:methanol=10:1), to thereby yield a white powdery compound (Compound 33; 0.64 g, 1.60 mmol, 93.0%).

¹H NMR(DMSO-d₆) δ 11.93 (1H, d, 3-NH, J=5.13), 8.13 (1H, d, H-6, J_{6,7}=7.08), 5.89 (1H, dd, H-1', J=6.35, 1.95), 5.71 (1H, t, 5'-OH, J=5.37), 5.37, 5.23 (each 1H, d, 2'-OH, 3'-OH, J=6.35), 4.12 (1H, q, H-2, J=6.35), 4.05 (1H, t, H-3, J=5.61), 3.61-3.57 (2H, m, H-5), 3.35 (1H, s, ethynyl), 0.95 (9H, t, Si-CH₂-CH₃, J=7.81), 0.55 (6H, Si-CH₂-CH₃, J=7.81).

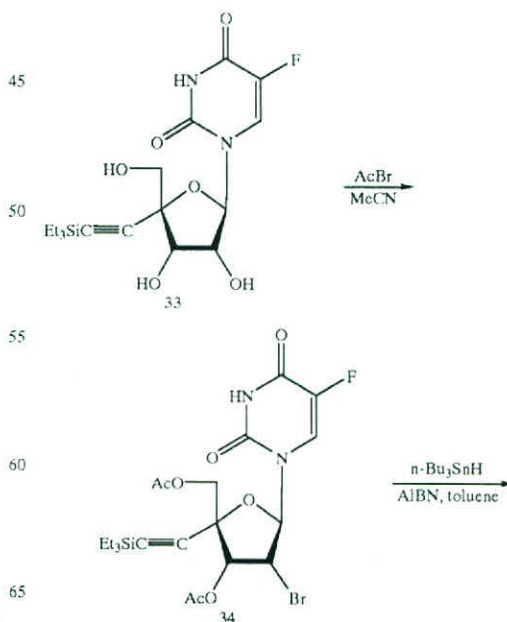
FABMS m/z: 401 (MH⁺).

HRMS m/z(MH⁺): Calcd. for C₁₇H₂₆FN₂O₆Si: 401.1544, Found: 401.1550.

[α]_D²⁰ -2.30° (c=1.00, CH₃OH)

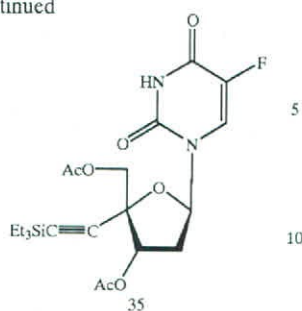
m.p. 180-183° C.

(4) Synthesis of 3',5'-di-O-acetyl-2'-deoxy-4'-C-triethylsilyl-5-fluorouridine (Compound 34):



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-continued



Compound 33 (0.54 g, 1.35 mmol) was suspended in acetonitrile (30.0 ml), and a solution (20.0 ml) of acetyl bromide (1.00 ml, 13.5 mmol) in acetonitrile was added dropwise to the suspension at 85° C. over one hour, followed by refluxing for a further three hours. After the reaction mixture was concentrated through distillation under reduced pressure, the residue was dissolved in ethyl acetate and the solution was washed sequentially with a saturated aqueous sodium hydrogencarbonate solution and a saturated aqueous sodium chloride solution. The organic layer was dried over anhydrous magnesium sulfate and concentrated through distillation under reduced pressure, to thereby yield 3',5'-di-O-acetyl-2'-bromo-2'-deoxy-4'-C-triethylsilyl-5-fluorouridine in crude form (Compound 34). After the crude product (Compound 34) was concentrated by co-boiling with toluene three times, the product was dissolved in dry toluene (20.0 ml). Hydrogenated tri(n-butyl)tin (0.75 ml, 2.91 mmol) and 2,2'-azobis(isobutyronitrile) (0.01 g) were added to the solution at 85° C., and the mixture was heated under stirring for 30 minutes in an argon atmosphere. After the reaction mixture was concentrated through distillation under reduced pressure, the residue was purified by means of silica gel column chromatography (silica gel 200 ml, eluent; n-hexane ethyl acetate), to thereby yield a white powdery compound (Compound 35; 0.41 g, 0.88 mmol, 65.2%).

¹H-NMR(CDCl₃) δ: 9.23 (1H, br.s, 3-NH), 7.70 (1H, d, H-6, J_{6,5}=6.10), 6.35 (1H, t, H-1, J_{1,2}=7.08), 5.36 (1H, t, H-3', J_{2',3'}=7.57), 4.43, 4.39 (each 1H, d, H-5', J_{gem}=12.21), 2.65, 2.33 (each 1H, m, H-2'), 2.17, 2.13 (each 3H, s, acetyl), 1.00 (9H, t, Si-CH₂-CH₃, J=7.82), 0.63 (6H, Si-CH₂-CH₃, J=7.82).

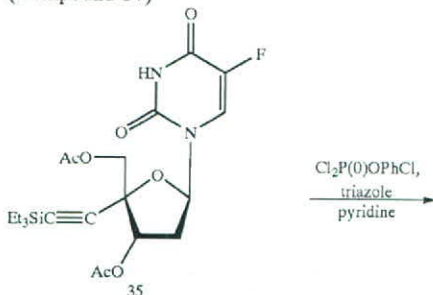
FABMS m/z: 469 (MH⁺).

HRMS m/z(MH⁺): Calcd. for C₂₁H₃₀FN₂O₇Si: 469.1806, Found: 469.1810.

[α]_D²⁰ -12.9° (c=1.00, CHCl₃)

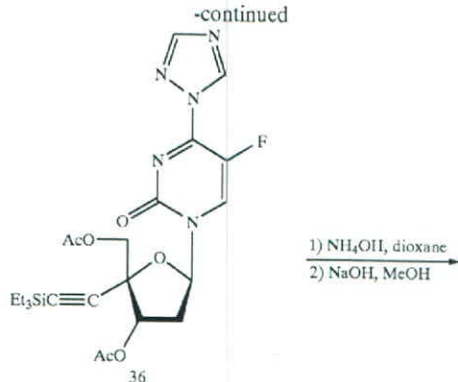
m.p. 111-112° C.

(5) Synthesis of 4'-C-ethynyl-2'-deoxy-5-fluorocytidine (Compound 37)

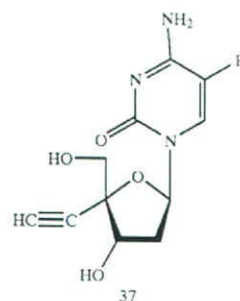


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-continued



1) NH₄OH, dioxane
2) NaOH, MeOH



Compound 35 (0.35 g, 0.75 mmol) was dissolved in pyridine (5.00 ml), and p-chlorophenylphosphordichloride (0.62 ml, 3.77 mmol) was added to the resultant solution under ice-cooling, followed by stirring for five minutes. 1,2,4-Triazole (0.78 g, 11.3 mmol) was added to the mixture, followed by stirring for 24 hours at 30° C. The reaction mixture was concentrated through distillation under reduced pressure, and the residue was partitioned with ethyl acetate and water. The organic layer was dried over anhydrous magnesium sulfate, and concentrated through distillation under reduced pressure. The residue was purified by means of silica gel column chromatography (silica gel 50 ml, eluent; ethyl acetate), to thereby yield a colorless viscous compound (4'-C-triethylsilyl-5-ethynyl-2'-deoxy-5-fluoro-4-(1,2,4-triazolo)uridine; Compound 36). Compound 36 was dissolved in dioxane (15.0 ml), and 25% aqueous ammonia (5.00 ml) was added to the resultant solution, followed by stirring overnight at room temperature. After disappearance of Compound 36 had been confirmed by means of silica gel thin-layer chromatography (chloroform:methanol=10:1), the reaction mixture was concentrated through distillation under reduced pressure. The residue was dissolved in methanol (45.0 ml), and an aqueous 1 N sodium hydroxide solution (5.00 ml, 5.00 mmol) was added thereto, followed by stirring for 24 hours at room temperature. Acetic acid (0.29 ml, 5.00 mmol) was added to the mixture, and the reaction mixture was concentrated through distillation under reduced pressure. The residue was purified by means of silica gel column chromatography (silica gel 50 ml; chloroform:ethanol=4:1). The fraction containing Compound 37 was brought to dryness under reduced pressure, and the residue was crystallized from methanol-ether, to thereby yield a white crystalline compound (Compound 37; 0.12 g, 0.45 mmol, 60.0%).

¹H-NMR(DMSO-d₆) δ: 8.06 (1H, d, H-6, J_{6,5}=7.08), 7.79, 7.54 (each 1H, br.s, NH₂), 6.05 (1H, m, H-1'), 5.57, 5.50 (each 1H, br, 3'-OH, 5'-OH), 4.31 (1H, br.q, H-3), 3.66, 3.60 (each 1H, d, H-5', J_{gem}=11.72), 3.51 (1H, s, ethynyl), 2.25, 2.12 (each 1H, m, H-2')

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$[\alpha]_D +77.9^\circ$ ($c=1.00$, CH_3OH)
FABMS m/z : 270 (MH^+).
HRMS $m/z(\text{MH}^+)$: Calcd. for $\text{C}_{11}\text{H}_{13}\text{FN}_3\text{O}_4$: 270.0890,
Found: 270.0888.
m.p. -225°C . (Dec)

Drug Preparation Example 1

Tablets

Compound of the present invention	30.0 mg
Cellulose micropowder	25.0 mg
Lactose	39.5 mg
Starch	40.0 mg
Talc	5.0 mg
Magnesium stearate	0.5 mg

Tablets are prepared from the above composition through a customary method.

Drug Preparation Example 2

Encapsulated Drug

Compound of the present invention	30.0 mg
Lactose	40.0 mg
Starch	15.0 mg
Talc	5.0 mg

Encapsulated drugs are prepared from the above composition through a customary method.

Drug Preparation Example 3

Injections

Compound of the present invention	30.0 mg
Glucose	100.0 mg

Injections are prepared by dissolving the above composition in purified water for preparing injections.

Test Examples will next be described. Employed in tests were the following seven compounds of the present invention and two known compounds:

- Compound 13: 4'-C-ethynyl-2'-deoxycytidine;
Compound 19: 1-(4-C-ethynyl- β -D-arabino-pentofuranosyl)cytosine;
Compound 23: 9-(2-deoxy-4-C-ethynyl- β -D-ribo-pentofuranosyl)adenine (4'-C-ethynyl-2'-deoxyadenosine);
Compound 28: 9-(2-deoxy-4-C-ethynyl- β -D-ribo-pentofuranosyl)-2,6-diaminopurine;
Compound 29: 9-(2-deoxy-4-C-ethynyl- β -D-ribo-pentofuranosyl)hypoxanthine (4'-C-ethynyl-2'-deoxyinosine);
Compound 30: 9-(2-deoxy-4-C-ethynyl- β -D-ribo-pentofuranosyl)guanine (4'-C-ethynyl-2'-deoxyguanosine);
Compound 37: 4'-C-ethynyl-2'-deoxy-5-fluorocytidine; and
Known compounds: 4'-C-ethynylthymidine and AZT.

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Test Examples

Test Methods

(1) Anti-HSV-1 Activity

- Human embryonic lung cells are subcultured by splitting at 1:2 to 1:4 in an Eagle's MEM supplemented with 10% bovine serum (Mitsubishi Chemical Corporation) at intervals of 4-5 days.
- The suspension of cells obtained from parent cells by splitting at 1:2 is added to a 96-well-microplate at 200 μl /well, and the cells are cultured in a CO_2 -incubator for four days at 37°C .
- After culture medium is removed, a test agent (100 μl) in serial fivefold dilution with a Hanks' MEM is added to the wells.
- An Eagle's MEM (100 μl) supplemented with 5% bovine serum containing 100-320 TCID_{50} of herpes simplex virus type-1, VR-3 strain is added to the wells to thereby seed the virus, and the infected cells are cultured at 37°C in a CO_2 -incubator.
- After the cells are cultured for 2-3 days, the degree of CPE of each well is observed under a microscope for evaluation on a scale of 0 to 4. When the cells in test agent-free controls are completely degenerated through infection with the virus, the CPE score is 4.
- The antiviral activity is expressed as ED_{50} at which HSV-induced CPE were expressed at least 50%.

(2) Anti-human Immunodeficiency Virus (HIV) Activity

1) MTT Method Using MT-4 Cells

- A test agent (100 μl) is diluted on a 96-well microplate. MT-4 cells infected with HIV-1 (III_b strain; 100 TCID_{50}) and non-infected MT-4 cells are added to the microplate such that the number of cells in each well becomes 10,000. The cells are cultured at 37°C for five days.
- MTT (20 μl , 7.5 mg/ml) is added to each well, and the cells are further cultured for 2-3 hours.
- The cultured medium (120 μl) is sampled, and MTT terminating solution (isopropanol containing 4% Triton X-100 and 0.04N HCl) is added to the sample. The mixture is stirred to form formazane, which is dissolved. The absorbance at 540 nm of the solution is measured. Since the absorbance is proportional to the number of viable cells, the test agent concentration at which a half value of the absorbance is measured in a test using infected MT-4 cells represents EC_{50} , whereas the test agent concentration at which a half value of the absorbance is measured in a test using non-infected MT-4 cells represents CC_{50} .

2) MAGI Assay Using HeLa CD4/LTR-beta-Gal Cells

- HeLa CD4/LTR-beta-Gal cells are added to 96 wells such that the number of cells in each well is 10,000. After 12-24 hours, the culture medium is removed, and a diluted test agent (100 μl) is added.
- A variety of HIV strains (wild strain: WT, drug-resistant strain: MDR, M184V, NL4-3, 104pre, and C; each equivalent to 50 TCID_{50}) are added, and the cells are further cultured for 48 hours.
- The cells are fixed for five minutes using PBS containing 1% formaldehyde and 0.2% glutaraldehyde.
- After the fixed cells are washed with PBS three times, the cells are stained with 0.4 mg/ml X-Gal for one hour, and the number of blue-stained cells of each well is counted under a transmission stereoscopic microscope. The test agent concentration at which blue-stained cells decrease to 50% and 90% in number represents EC_{50} and EC_{90} , respectively.
- In a manner similar to that employed in the MTT method, cytotoxicity is measured by use of HeLa CD4/LTR-beta-Gal cells.

The test results are shown in Tables 1 to 7.

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Results

(1) Anti-HSV-1 Activity

TABLE 1

Drug	HSV-1 (ED ₅₀ μg/ml)
Compound 13	33

(2) Anti-human Immunodeficiency Virus (HIV) Activity and Cytotoxicity

Each value shown in Tables 2 to 7 represents an average of two to five assayed values.

1. MTT Method Using MT-4 Cells

TABLE 2

MT-4 cells		
Drugs	HIV-1 (EC ₅₀ μg/ml)	Cytotoxicity (CC ₅₀ μg/ml)
Compound 13	0.0012	0.56
Compound 19	0.0115	0.53
4'-C-ethynyl thymidine	0.22	>100
AZT	0.0016	>0.27

TABLE 3

MT-4 cells		
Drugs	HIV-1 (EC ₅₀ μg/ml)	Cytotoxicity (CC ₅₀ μg/ml)
Compound 23	0.0027	4.4
Compound 28	0.0001	0.26
Compound 29	0.037	38
Compound 30	0.00044	0.41
AZT	0.0011	9.08

TABLE 4

MT-4 cells		
Drugs	HIV-1 (EC ₅₀ μM)	Cytotoxicity (CC ₅₀ μM)
Compound 37	0.033	>500
AZT	0.055	

2. MAGI Assay Using HeLa CD4/LTR-beta-Gal Cells

TABLE 5

HeLa CD4/LTR-beta-Gal cells				
HIV				
Drugs	WT	MDR (EC ₅₀ μg/ml)	M184V	Cytotoxicity (CC ₅₀ μg/ml)
Compound 13	0.00031	0.00030	0.00054	>100
Compound 19	0.0019	0.021	0.19	>100
4'-C-ethynyl thymidine	0.097	0.033	0.049	>100
AZT	0.0059	4.1	0.0083	>26.7

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TABLE 6

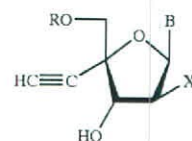
HeLa CD4/LTR-beta-Gal cells				
HIV				
Drugs	WT	MDR (EC ₅₀ μg/ml)	M184V	Cytotoxicity (CC ₅₀ μg/ml)
Compound 23	0.0012	0.0017	0.013	>100
Compound 28	0.00028	0.00029	0.0017	2.7
Compound 29	0.22	0.14	4.6	>100
Compound 30	0.002	0.0014	0.0023	15.2
AZT	0.0027	5.34	0.0013	>26.7

TABLE 7

HeLa CD4/LTR-beta-Gal cells			
HIV			
Drugs	NL-43	104 pre EC ₅₀ μM (EC ₉₀ μM)	C
Compound 37	0.021 (0.25)	0.022 (0.19)	0.122 (3.44)
AZT	0.109 (4.96)	0.059 (9.66)	3.269 (>10)

What is claimed is:

1. A 4'-C-ethynyl pyrimidine represented by the following formula (1):



wherein B represents a pyrimidin-1-yl or a derivative thereof, wherein the derivative is a pyrimidin-1-yl having a substituent selected from the group consisting of a halogen atom, an alkyl group, a haloalkyl group, an alkenyl group, a haloalkyl group, an alkynyl group, an amino group, an alkylamino group, a hydroxy group, a hydroxyamino group, an aminoxo group, an alkoxy group, a mercapto group, an alkylmercapto group, an aryl group, an aryloxy group, and a cyano group, and R represents a hydrogen atom or a phosphate ester group, and X represents a hydrogen atom or a hydroxyl group, or a pharmaceutically acceptable salt, hydrate, or solvate thereof,

provided that the compound is not 4'-C-ethynylthymidine, 4'-C-ethynyl-2'-deoxycytidine, 4'-C-ethynyl-2'-deoxy-5-fluorocytidine, or 1-(4'-C-ethynyl-β-D-arabinopentofuranosyl)cytosine.

2. A compound according to claim 1, wherein X is a hydrogen atom.

3. A compound according to claim 1, wherein X is a hydroxyl group.

4. The compound according to claim 1, wherein B is cytosin-1-yl or said derivative thereof.

5. The compound according to claim 1, wherein B is cytosin-1-yl or said derivative thereof, and X is a hydrogen atom.

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- 6. The compound according to claim 1, wherein B is cytosin-1-yl or said derivative thereof, and X is a hydroxyl group.
- 7. A pharmaceutical composition comprising a 4'-C-ethynyl pyrimidine nucleoside as recited in claim 1 and a pharmaceutically acceptable carrier.
- 8. A pharmaceutical composition comprising a 4'-C-ethynyl pyrimidine nucleoside as recited in claim 2 and a pharmaceutically acceptable carrier.
- 9. A pharmaceutical composition comprising a 4'-C-ethynyl pyrimidine nucleoside as recited in claim 3 and a pharmaceutically acceptable carrier.

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- 10. A pharmaceutical composition comprising a 4'-C-ethynyl pyrimidine nucleoside as recited in claim 4 and a pharmaceutically acceptable carrier.
- 11. A pharmaceutical composition comprising a 4'-C-ethynyl pyrimidine nucleoside as recited in claim 5 and a pharmaceutically acceptable carrier.
- 12. A pharmaceutical composition comprising a 4'-C-ethynyl pyrimidine nucleoside as recited in claim 6 and a pharmaceutically acceptable carrier.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

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Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 42,

Line 29, please replace "ethynyl pyrimidine represented" with -- ethynyl pyrimidine nucleoside represented --.

Signed and Sealed this

Twenty-second Day of July, 2003

A handwritten signature in black ink, appearing to read "James E. Rogan", with a horizontal line drawn underneath it.

JAMES E. ROGAN
Director of the United States Patent and Trademark Office

Oligonucleotides Containing Novel 4'-C- or 3'-C-(Aminoalkyl)-Branched Thymidines¹⁾

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Dedicated to Prof. Dr. Frank Seela on the occasion of his 60th birthday

The synthesis of four novel 3'-C-branched and 4'-C-branched nucleosides and their transformation into the corresponding 3'-O-phosphoramidite building blocks for automated oligonucleotide synthesis is reported. The 4'-C-branched key intermediate **11** was synthesized by a convergent strategy and converted to its 2'-O-methyl and 2'-deoxy-2'-fluoro derivatives, leading to the preparation of novel oligonucleotide analogues containing 4'-C-(aminomethyl)-2'-O-methyl monomer **X** and 4'-C-(aminomethyl)-2'-deoxy-2'-fluoro monomer **Y** (Schemes 2 and 3). In general, increased binding affinity towards complementary single-stranded DNA and RNA was obtained with these analogues compared to the unmodified references (Table 1). The presence of monomer **X** or monomer **Y** in a 2'-O-methyl-RNA oligonucleotide had a negative effect on the binding affinity of the 2'-O-methyl-RNA oligonucleotide towards DNA and RNA. Starting from the 3'-C-allyl derivative **28**, 3'-C-(3-aminopropyl)-protected nucleosides and 3'-O-phosphoramidite derivatives were synthesized, leading to novel oligonucleotide analogues containing 3'-C-(3-aminopropyl)thymidine monomer **Z** or the corresponding 3'-C-(3-aminopropyl)-2'-O,5-dimethyluridine monomer **W** (Schemes 4 and 5). Incorporation of the 2'-deoxy monomer **Z** induced no significant changes in the binding affinity towards DNA but decreased binding affinity towards RNA, while the 2'-O-methyl monomer **Z** induced decreased binding affinity towards DNA as well as RNA complements (Table 2).

1. Introduction. – A large number of chemically modified oligonucleotides (ONs) have been synthesized and investigated to improve the binding affinity towards complementary RNA and the stability towards nucleases [1b][2]. As important examples, 2'-deoxy-2'-fluoro-modified ONs [3][4], and 2'-O-alkylated ONs, including 2'-O-methyl derivatives, have shown increased binding affinity towards RNA [5–8]. The increased binding affinity towards RNA has been attributed to the tendency of these nucleotides to adopt a C(3')-endo conformation, giving thermally stable A-type duplexes [9].

ONs containing 4'-C-substituted nucleotide monomers have likewise been reported to show, in general, minor decreases in binding affinity towards RNA and small increases in binding affinity towards DNA [10–15], presumably originating from the C(2')-endo-like conformation preferentially adopted by these nucleotides [16], in analogy with what is found in B-type DNA·DNA duplexes [9]. Furthermore, ONs containing 2'-O-alkylated or 4'-C-substituted nucleotides have shown increased

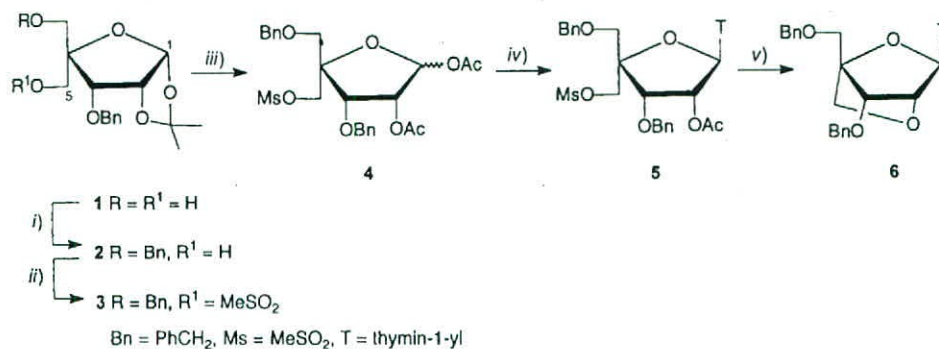
¹⁾ Part of this work has been published in a preliminary form [1a].

resistance towards enzymatic degradation, most pronounced for ONs containing modified nucleotides with an ionizable amino group in the sugar moiety, *e.g.*, 2'-*O*-(aminopropyl)- [17] and 4'-*C*-(aminoalkyl)-substituted thymidines [12][13][15]. Based on the results described above, we decided to evaluate the thermal stability of duplexes formed between ONs containing 2',4'-*dimodified* nucleotide monomers and complementary RNA and DNA. Thus, in an attempt to maximize the binding affinity of ONs containing 4'-*C*-substituted nucleotides towards RNA and to increase the nuclease stability, we decided to synthesize and evaluate ONs containing 4'-*C*-(aminomethyl)-2'-*C*-methoxy- and 4'-*C*-(aminomethyl)-2'-fluoro-modified thymidines.

In addition, we and others have been interested in obtaining modified ONs with a DNA-selective binding, *e.g.* for diagnostics applications. Thus, 2'- β -ethynyl-modified ONs were recently shown to preferentially hybridize with complementary DNA [18] which was explained by the tendency of these modified nucleotides to adopt a C(2')-*endo*-type conformation. Likewise, we have reported the synthesis and evaluation of ONs containing 3'-*C*-(3-hydroxypropyl)- [19] and 3'-*C*-allyl-branched [20] thymidines showing better binding affinities towards DNA than towards RNA. However, this was not observed for ONs containing similar nucleotides with a C₁ 3'-*C*-branch, *e.g.* 3'-*C*-(hydroxymethyl) [21], 3'-*C*-methyl [22][23], and 3'-*C*-(aminomethyl) [23]. A 3'-*C*-branch is expected to be oriented in a pseudoequatorial position, driving the sugar pucker towards a C(2')-*endo* conformation. An explanation for the results described above could be that a larger C₃ 3'-*C*-branch is not as well accommodated in the DNA · RNA duplex as a smaller C₁ branch. Herein we report the synthesis and evaluation of ONs containing 3'-*C*-(3-aminopropyl)thymidine and 3'-*C*-(3-aminopropyl)-2'-*O*,5-dimethyluridine in an attempt to improve the thermal stability of duplexes formed between ONs containing 3'-*C*-branched monomers and complementary DNA.

2. Results and Discussion. – 2.1. 2'-Substituted 4'-*C*-(Aminomethyl) Nucleosides and ONs. For the synthesis of 4'-*C*-(aminomethyl)-2'-*C*-methoxy- and 4'-*C*-(aminomethyl)-2'-fluoro-derivatized thymidine nucleosides, a strategy starting from the known 3-*O*-benzyl-4-*C*-[(benzyloxy)methyl]-1,2-di-*O*-isopropylidene- β -L-lyxofuranose (**2**), which can be synthesized from 3-*O*-benzyl-4-*C*-(hydroxymethyl)-1,2-di-*O*-isopropylidene- β -L-lyxofuranose (**1**) *via* regioselective benzylation [24][25], was chosen (*Scheme 1*). In an attempt to improve the regioselectivity of the monobenzylation, furanose **1** was benzylation by the dibutyltin oxide method [26][27]. However, equal amounts of two dibenzylated regioisomers were obtained in moderate yields, and the reported [24][25] regioselective monobenzylation method was therefore used. It was decided to use an azido substituent as a group which could be converted to an amino functionality later at an appropriate step in the synthesis. For the introduction of this azido group, furanose **2** was mesylated under standard conditions to give furanose **3** in 92% yield, as reported [28]. However, treatment of **3** with NaN₃ in hot DMF was unsuccessful resulting in complete recovery of **3** that might be ascribed to the 'neopentyl' character of the mesyloxy group of **3** and its position on the concave face of the oxabicyclo[3.3.0]octane system [24]. Instead, both by isopropylidene group hydrolysis in 80% AcOH/H₂O followed by basic acetylation, and by direct one-pot acidic acetolysis [29], the 1,2-di-*O*-acetyl derivative **4** was obtained in 54% yield. Stereoselective reaction with silylated thymine [30][31] afforded in 75% yield the nucleoside **5**, which was heated under

Scheme 1



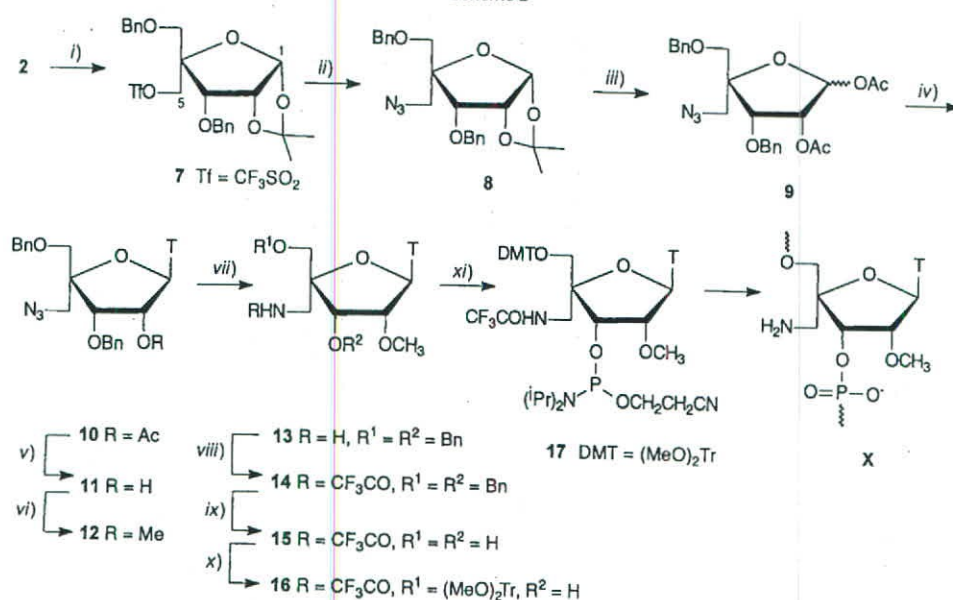
i) BnBr , NaH , DMF ; ii) MsCl , Py ; iii) 1. 50% AcOH ; 2. Ac_2O , Py or $\text{AcOH}/\text{Ac}_2\text{O}/\text{conc. H}_2\text{SO}_4$ soln. 100:10:0.1.
 iv) Thymine, N,O -bis(trimethylsilyl)acetamide (BSA), $\text{Me}_3\text{SiOSO}_2\text{CF}_3$, MeCN . v) LiEt_3BH , THF .

reflux in DMF with NaN_3 to give several products of which none was identified. This disappointing result is consistent with another report describing the reaction of 4'-C-[(mesyloxy)methyl]thymidine derivatives with LiN_3 to give the desired 4'-C-(azido-methyl)nucleoside in only low yields [32]. At this point, it was realized that it was impossible to use the mesyloxy group as a leaving group with azide as the nucleophile. Instead, we used hydride as the nucleophile in an attempt to obtain a 4'-C-methylnucleoside. However, treatment of nucleoside 5 with lithium triethylborohydride yielded the 2,5-dioxabicyclo[2.2.1]heptane locked nucleic acid (LNA) monomer 6 [25][33] in 86% yield, presumably *via* reduction of the acetate followed by intramolecular attack of the resulting alkoxide on the 'neopentyl' C-atom.

In an attempt to introduce the azido group while taking advantage of the excellent leaving-group ability of the triflate ion, the 4-C-(triflyloxy)methyl derivative 7 was synthesized using standard chemistry and was reacted with NaN_3 in DMF at 60° to give furanose 8 in 59% yield (two steps) (Scheme 2). Acidic hydrolysis and basic acetylation ($\rightarrow 9$) followed by coupling with silylated thymine as described above ($\rightarrow 10$) gave, after deacetylation, nucleoside 11 in 83% yield from 8. To obtain the 2'-O-methyl derivative 12, nucleoside 11 was chemoselectively O-methylated in 90% yield by the reaction conditions described by Wang and Seifert [12]. We have successfully performed methylation of 3'-C-allyl-3',5'-di-O-benzyl-5-methyluridine under similar conditions [34]. The structure of 12 was verified by ^1H - and ^{13}C -NMR data (NH of thymine at δ 9.35 and $\text{CH}_3\text{O}-\text{C}(2')$ at δ 59). Reduction of the azido group with Lindlar catalyst ($\rightarrow 13$) followed by trifluoroacetylation of the resulting amino group afforded nucleoside 14 in 73% yield from 12. Debenzylation to give diol 15 in 92% yield followed by dimethoxytritylation with $(\text{MeO})_2\text{TrCl}$ to give the 5'-O-(4,4'-dimethoxytrityl)-protected analogue 16 in 91% yield, and subsequent phosphitylation afforded the phosphoramidite derivative 17 in 43% yield.

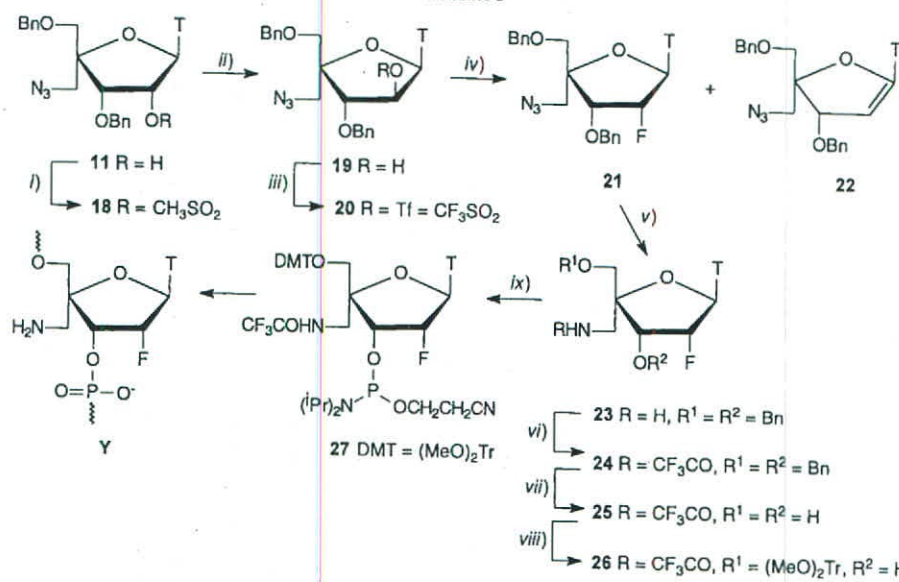
To obtain the analogous 4'-C-(aminomethyl)-2'-deoxy-2'-fluoro phosphoramidite derivative 27, the configuration at C(2') of nucleoside 11 was inverted by the anhydro approach [35]. Thus, mesylation of L-lyxo-configured 11 followed by reaction with aq. base afforded the L-xylo-configured nucleoside 19 in 94% yield from 11 (Scheme 3). Several attempts to prepare the 2'-fluoronucleoside 21 directly from 19 with

Scheme 2



i) (CF₃SO₂)₂O, Py, CH₂Cl₂. ii) NaN₃, DMF. iii) 1. 50% AcOH; 2. Ac₂O, Py. iv) Thymine, BSA, Me₃SiOSO₂CF₃, MeCN. v) NH₃, MeOH. vi) MeI, NaH, THF. vii) H₂, Lindlar catalyst, EtOH. viii) CF₃COOEt, Et₃N, CH₂Cl₂. ix) H₂, 5% Pd/C, EtOH. x) (MeO)₂TrCl, Py. xi) NC(CH₂)₂OP(Cl)NⁱPr₂, ⁱPr₂EtN, CH₂Cl₂.

Scheme 3



i) MeSO₂Cl, Py. ii) NaOH, EtOH, H₂O. iii) Tf₂O, 4-(dimethylamino)pyridine (DMAP), Py, CH₂Cl₂. iv) Bu₄NF, THF. v) H₂, Lindlar catalyst, dioxane, EtOH. vi) CF₃COOEt, NEt₃, CH₂Cl₂. vii) H₂, 5% Pd/C, EtOH. viii) (MeO)₂TrCl, Py. ix) NC(CH₂)₂OP(Cl)NⁱPr₂, ⁱPr₂EtN, CH₂Cl₂.

(diethylamino)sulfur trifluoride (DAST) [36][37] and different temperatures and solvents (CH_2Cl_2 or DMF) were unsuccessful. An attempt to react tris(dimethylamino)sulfur (trimethylsilyl)difluoride (TASF) [38][39] with the (trifluoromethyl)sulfonyl derivative **20**, synthesized in 77% yield from **19** under standard conditions, was likewise unsuccessful. However, under strictly anhydrous conditions, 2'-fluoronucleoside **21** was obtained with dried 'anhydrous' tetrabutylammonium fluoride (Bu_4NF) [40][41]. Thus, nucleoside **20** was reacted with 10 equiv. of Bu_4NF in anh. THF to give **21** in 37% yield and ketene *N,O*-acetal **22** in 30% yield, the latter formed *via trans*-elimination of the 2'-*C*-(triflyloxy) group. The structure of **22** was verified by MS and NMR and by comparison with other structurally similar 1',2'-unsaturated compounds [42][43]. This type of elimination reaction has been reported earlier [44], but the analogous reaction of the 4'-*C*-unbranched 2'-*O*-(triflyloxy) derivative of **20** afforded exclusively the desired 2'-fluoro product in a very high yield [45]. The *gauche* effect of the 2'-*O*-(triflyloxy) group positioned at the α -face of the furanose ring should drive the sugar pucker towards a *C*(2')-endo-like conformation in both **20** and its 4'-*C*-unbranched analogue. However, also the 4'-*C*-(azidomethyl) group shifts the pentofuranose sugar pucker towards a *C*(2')-endo conformation. Accordingly, the $^3J(\text{H}-\text{C}(1'), \text{H}_\beta-\text{C}(2'))$ coupling constant of 4.3 Hz observed for **20**, corresponding to a $\text{H}-\text{C}(1')-\text{C}(2')-\text{H}_\beta$ torsion angle of ca. 45° , as obtained from a simplified *Karplus* equation [46], is in accordance with a *C*(2')-endo-like conformation. Thus, $\text{H}-\text{C}(1')$ and the 2'-*C*-(triflyloxy) group exist in a *trans*-diaxial geometry advantageous for *E2* elimination, offering an explanation for the nearly equal amounts of substitution and elimination observed in the reaction. Following similar synthetic procedures as for the 2'-*O*-methylnucleosides (**12** $\rightarrow$ **16**, Scheme 1), 4'-*C*-(azidomethyl)-2'-deoxy-2'-fluoro derivative **21** was reduced to give the 4'-*C*-(aminomethyl)nucleoside **23**, trifluoroacetylated ($\rightarrow$ **24**), debenzylated ($\rightarrow$ **25**) and 5'-*O*-[(MeO)₂Tr]-protected to give **26** in an overall yield of 46% from **21**. Nucleoside **26** was further transformed into the 3'-*O*-phosphoramidite derivative **27** in 76% yield.

The phosphoramidites **17** and **27** were incorporated into ONs as monomers **X** and **Y**, respectively, on an automated DNA synthesizer. The stepwise coupling efficiency obtained for **17** was only 40% when 1*H*-tetrazole was used as activator (2×12 min coupling). With pyridinium hydrochloride as activator [47][48], the coupling efficiency was >90% (10 min coupling). The ONs were deprotected by heating (55° for 12 h) in aqueous NH_3 solution followed by reversed-phase cartridge purification. Analysis by capillary gel electrophoresis verified the purity to be >90%, and the composition of the ONs was confirmed by MALDI-MS. The phosphoramidite **17** was incorporated into three types of ONs: 14-mer oligothymidylates **II–IV**, mixed 9-mer sequences **VI–VIII**, and a sequence **XIII** containing 2'-*O*-methyl-modified ribonucleotides instead of unmodified 2'-deoxynucleotides. The hybridization properties of these **X**-modified oligonucleotides and of their reference strands **I**, **V**, and **XII** towards complementary DNA and RNA were measured both in a medium salt buffer and in a high salt buffer (Table 1).

Likewise, oligonucleotides **IX–XI** and **XIV** (Table 1), containing the monomer **Y**, were synthesized with a stepwise coupling efficiency for the amidite **27** of 80% with pyridinium hydrochloride as activator (10 min coupling). *Krug et al.* [49] have reported that when 2'-deoxy-2'-fluorouridine is incorporated as the 3'-terminal residue of an ON,

Table 1. Melting Experiments of Oligonucleotides Containing Monomers **X** and **Y**^{a)}

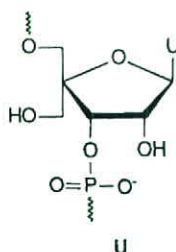
		Complementary ssDNA <i>T_m</i> ^{b)} or ΔT_m ^{b)} per mod. ^{b)}		Complementary ssRNA <i>T_m</i> ^{b)} or ΔT_m ^{b)} per mod. ^{b)}	
		[Na ⁺]: 150 mM ^{c)}	[Na ⁺]: 750 mM ^{d)}	[Na ⁺]: 150 mM ^{c)}	[Na ⁺]: 750 mM ^{d)}
I	5'-T ₁₄	33.0	n.d.	31.0	n.d.
II	5'-T ₇ XT ₆	-4.0	n.d.	-4.0	n.d.
III	5'-T ₆ XTXT ₅	-3.0	n.d.	-3.0	n.d.
IV	5'-T ₁₂ XT	±0.0	n.d.	-1.0	n.d.
V	5'-G ₄ TG ₄ A ₄ TA ₄ TG ₄ C ₄	31.0	36.5	29.7	34.0
VI	5'-G ₄ TG ₄ A ₄ XA ₄ TG ₄ C ₄	+1.5	-0.5	+2.5	+2.5
VII	5'-G ₄ TG ₄ A ₄ XA ₄ XG ₄ C ₄	+1.5	-0.3	+1.3	+1.3
VIII	5'-G ₄ XG ₄ A ₄ XA ₄ XG ₄ C ₄	-0.3	-1.7	+0.8	+1.0
IX	5'-G ₄ TG ₄ A ₄ YA ₄ TG ₄ C ₄	+1.5	-0.5	+0.5	±0.0
X	5'-G ₄ TG ₄ A ₄ YA ₄ YG ₄ C ₄	+1.8	±0.0	+1.5	+0.8
XI	5'-G ₄ YG ₄ A ₄ YA ₄ YG ₄ C ₄	+0.3	-0.8	+1.0	+0.7
XII	5'-G ^m U ^m G ^m A ^m U ^m A ^m U ^m G ^m C	31.5	37.0	42.5	50.5
XIII	5'-G ^m XG ^m A ^m XA ^m XG ^m C	24.0	28.0	34.5	40.5
XIV	5'-G ^m YG ^m A ^m YA ^m YG ^m C	19.0	23.0	29.0	37.0

^{a)} **X** = 1-[4-C-(aminomethyl)-2-O-methyl- α -L-lyxofuranosyl]thymine unit; **Y** = 1-[4-C-(aminomethyl)-2-deoxy-2-fluoro- α -L-lyxofuranosyl]thymine unit, G^m = 2'-O-methylguanosine unit, U^m = 2'-O-methyluridine unit, A^m = 2'-O-methyladenosine unit. ^{b)} *T_m* is shown for the oligonucleotides **I**, **V**, and **XII–XIV**. ΔT_m is shown for oligonucleotides **II–IV** with **I** as reference and for oligonucleotides **VI–XI** with **V** as reference. *T_m* = melting temperature determined as the maximum of the first derivative of the absorbance vs. temperature curve; ΔT_m /° per mod. = change in *T_m* per modification compared to the unmodified reference strand; n.d. = not determined. ^{c)} Measured at 260 nm in medium salt buffer: 1 mM EDTA, 10 mM sodium phosphate, 140 mM sodium chloride, pH 7.1. ^{d)} Measured at 260 nm in high salt buffer: 1 mM EDTA, 10 mM sodium phosphate, 740 mM sodium chloride, pH 7.1.

it is converted to arabinosyluracil via internal nucleophilic attack from the pyrimidine nucleobase and ring opening of the 2,2'-anhydro intermediate during the deprotection step with hot aqueous NH₃ solution. However, when 2'-deoxy-2'-fluorouridine was incorporated at other positions in an ON, a similar conversion to arabinosyluracil was not observed which is consistent with results reported by others [50]. A similar kind of degradation was reported for oligophosphoramidates containing 2'-deoxy-2'-fluorouridine monomers [51]. Furthermore, Kawasaki *et al.* [3] reported a significant degradation of 2'-deoxy-2'-fluoropyrimidine nucleosides under the usual ON deprotection conditions. They suggested the alternative use of methanolic NH₃ solution for 24 h at room temperature for ON deprotection. However, when oligonucleotide **XI** was deprotected under the latter conditions, MALDI-MS showed that ca. 50% of the ON products still contained the trifluoroacetyl protecting group. Furthermore, the *N*²-isobutyryl protecting group of the guanine bases was not removed. Substitution of methanolic NH₃ with aqueous NH₃ solution did induce removal of the trifluoroacetyl protecting group after 24 h, but only ca. 50% of the *N*²-isobutyryl protecting groups. Therefore, the usual deprotecting conditions (aqueous NH₃ solution at 55° for 12 h) were used in the present study. In fact, MALDI-MS did not indicate any kind of degradation of the ONs, as further verified by capillary gel electrophoresis showing the ONs to be > 90% pure. The MALDI-MS data were determined with an experimental error of less than 0.4 Da with internal standards. The hybridization properties of the

modified oligonucleotides **IX–XI** and **XIV** and of their reference strands **V** and **XII** towards complementary DNA and RNA were measured both in a medium salt buffer and in a high salt buffer (*Table 1*).

Upon single and two-fold incorporation of 2'-*O*-methyl monomer **X** in the middle of a 14-mer oligothymidylate (see ONs **II** and **III**, resp.), the stability of the duplexes with DNA and RNA was significantly decreased ($\Delta T_m/\text{mod.} = -3$ and -4°) compared to the unmodified reference strand **I** when measured in a medium salt buffer, whereas the presence of a single **X** towards the 3'-end of sequence **IV** had no significant effect on the binding affinity towards complementary DNA and RNA. These results are consistent with results obtained with ONs containing 4'-*C*-(hydroxymethyl)uridine [52], although this analogue (see **U**) displayed even lower binding affinity towards complementary DNA ($\Delta T_m/\text{mod.} = -8^\circ$ for the sequence 5'-T₇UT₆). Due to the disappointing melting results, the hybridization properties of ONs **II–IV** were not evaluated in a high salt buffer.



More encouraging results were obtained when monomers **X** and **Y** were incorporated into 9-mer mixed oligonucleotide sequences (*Table 1*, **VI–XI**). Thus, the presence of a single **X** or **Y** (ONs **VI** and **IX**) or of two **X** or **Y** (ONs **VII** and **X**) in the 9-mer caused an increase ($\Delta T_m/\text{mod.} \approx +1.5^\circ$) in the stability of the resulting duplex with DNA when measured in a medium salt buffer. Incorporation of monomer **X** three times (ON **VIII**) caused a minor decrease ($\Delta T_m/\text{mod.} = -0.3^\circ$) in the thermal stability of the resulting duplex with DNA compared with the reference strand **V**, while the presence of **Y** in the same sequence (ON **XI**) caused a small increase ($\Delta T_m/\text{mod.} = +0.3^\circ$). When the corresponding ONs containing 2'-*O*-methyl derivative **X** (ONs **VI–VIII**) were hybridized with complementary RNA in a medium salt buffer, increases in the thermal stability of the resulting duplexes ($\Delta T_m/\text{mod.} = +2.5$, $+1.3$, and $+0.8^\circ$) were observed. Similar results were obtained with ONs containing the 2'-deoxy-2'-fluoro derivative **Y** though the presence of **Y** in the singly modified ON **IX** caused a significant smaller increase in T_m compared to **VI** containing the 2'-*O*-methyl monomer **X**. In relation to the main purpose of this work, namely evaluation of the possibility of maximizing the thermal affinity of *C*-branched ONs, comparison with results obtained with the 4'-*C*-(hydroxymethyl)uridine monomer **U** appeared relevant. In the mixed-sequence 9-mer context, monomer **U** in general induced decreased T_m values compared to the reference ON **V** with both DNA and RNA complements ($\Delta T_m/\text{mod.}$ against DNA = 0, -2.0 and -2.0° and $\Delta T_m/\text{mod.}$ against RNA = $+2.0$, 0 and -0.3° for the 9-mer sequences containing one, two or three **U** monomers, respectively). It is thus evident that the combined exchange of the HO–C(2') and

the $\text{HOCH}_2\text{-C}(4')$ groups with a $\text{F-C}(2')/\text{MeO-C}(2')$ group and a $\text{NH}_2\text{CH}_2\text{-C}(4')$ group leads to increased thermal stability of duplexes with both DNA and RNA complements. The apparent sequence dependence on the influence of the different modifications on the thermal stability of the resulting duplexes stresses the importance of comparing only in identical sequence contexts and excludes direct comparison of the effect induced by, e.g., monomers **X** and **U** with effects reported in the literature for, e.g., 2'-*O*-alkyl-substituted ONs [5–8] or 4'-*C*-(aminomethyl)-modified ONs [23].

For ONs **VI–VIII** containing the 2'-*O*-methyl monomer **X**, $\Delta T_m/\text{mod.}$ for duplexes with DNA as well as RNA was reduced with increasing number of **X** monomers incorporated (medium salt buffer). The same tendency, though less pronounced, could be seen for ONs **IX–XI** containing the 2'-deoxy-2'-fluoro monomer **Y** when hybridized with complementary DNA in a medium salt buffer. The 2'-*O*-methyl group and the 2'-fluoro substituent both direct the sugar pucker towards a $\text{C}(3')$ -*endo* conformation (indicated by $^3J(\text{H-C}(1'), \text{H-C}(2'))$ values of *ca.* 1.5–4.5 Hz), which in general is expected to induce an increased thermal stability of duplexes with an RNA complement. However, compared to other 2'-*O*-methyl- and 2'-deoxy-2'-fluorouridines, 2'-modified 4'-*C*-(aminomethyl) nucleotides **X** and **Y** probably display a less pronounced tendency towards adopting a $\text{C}(3')$ -*endo*-like conformation, as indicated by the relatively high $^3J(\text{H-C}(1'), \text{H-C}(2'))$ values. Thus, the increase in stability of the duplexes **VI**, **VII**, and **IX–XI** with DNA is, most likely, caused by a reduction in the electrostatic repulsion between phosphate moieties due to the presence of the positively charged aminomethyl group under the experimental conditions used. To confirm an effect of the protonated aminomethyl group, the duplex stabilities were measured under conditions of high ionic strength ($[\text{Na}^+] = 750 \text{ mM}$, Table 1). As anticipated, the stabilities of the duplexes involving ONs **VI–VIII** containing monomer **X** or ONs **IX–XI** containing monomer **Y** and complementary DNA were decreased relative to the stability of the duplex involving the unmodified reference strands **V** under high ionic strength conditions. This confirms the positive effect of the protonated aminomethyl group regarding duplex stability with complementary DNA. However, for ONs **VI** and **VII** in experiments towards complementary RNA, the presence of the positively charged aminomethyl group does not seem to affect the duplex stability.

Due to the larger *gauche* effect of the 2'-F-atom compared to the 2'-*O*-methyl group, we anticipated that substitution of the 2'-*O*-methyl monomer **X** with the 2'-deoxy-2'-fluoro monomer **Y** would cause an increase in binding affinity towards RNA. However, comparable binding affinities towards RNA were obtained for ONs **VII** and **VIII** containing 2'-*O*-methyl monomer **X** and ONs **X** and **XI** containing 2'-deoxy-2'-fluoro monomer **Y** ($[\text{Na}^+] = 150 \text{ mM}$). When the hybridization affinities were measured under conditions of high ionic strength, the increases in binding affinity towards RNA for ONs **IX–XI** containing **Y** were somewhat reduced compared to the corresponding oligonucleotides **VI–VIII** containing **X**.

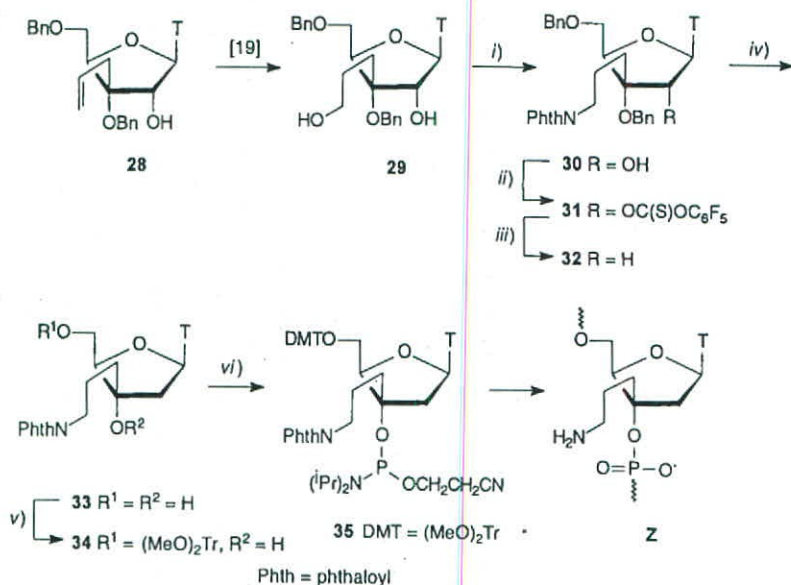
We also evaluated the effect of the presence of the 4'-*C*-(aminomethyl) group in an otherwise uniformly modified 2'-*O*-methyl-RNA ON. For the 2'-*O*-methyl-RNA reference strand **XII** (Table 1), containing uracil as nucleobase instead of thymine, no change in the thermal stability towards a DNA complement was observed compared to

the 2'-deoxy counterpart **V** confirming results reported earlier [5] [53]. However, when monomer **X** was incorporated three times in the 9-mer 2'-*O*-methyl-RNA strand (ON **XIII**), the thermal stability of the duplex with DNA was reduced significantly (ΔT_m value -7.5°). Thus, the positive effect of the 4'-*C*-(aminomethyl) substituent in the thymine monomer **X** observed earlier for ONs **VI**–**VIII** regarding binding affinity towards DNA is not seen with ON **XIII**. In fact, substitution of a thymine base with a uracil base generally results in a decrease in T_m of *ca.* 0.5° per substitution [54], which further emphasizes the detrimental effect of the 4'-*C*-(aminomethyl) group on the T_m value obtained for **XIII**. As expected, 2'-*O*-methyl-modified oligonucleotide **XII** showed increased binding affinity towards RNA ($\Delta T_m/2'$ -methylnucleotide $= +1.6^\circ$) compared to the 2'-deoxy analog **V**. ON **XIII** showed significantly reduced binding affinity towards RNA in both salt buffers compared to the reference strand **XII**, but still increased binding affinity towards RNA compared to the completely unmodified 2'-deoxy analog **V**. Likewise, the 2'-deoxy-2'-fluoro monomer **Y** was incorporated in an otherwise 2'-*O*-methyl modified ON (**XIV**, Table 1). The negative effect of the presence of the 2'-deoxy-2'-fluoro monomer **Y** on the thermal stability of the 2'-*O*-methyl-RNA strand was even more pronounced than observed for the similar oligonucleotide containing **X** with resulting ΔT_m values of *ca.* -13° for duplexes with complementary DNA and RNA. Based on these results, it appears that the incorporation of branched 4'-*C*-alkylnucleotide monomers in a 2'-*O*-methyl-RNA ON causes a detrimental disruption of the regular duplex structure.

2.2. 3'-C-(Aminoalkyl)-Substituted Nucleosides and ONs. We have recently published the synthesis and evaluation of ONs containing 3'-*C*-(3-hydroxypropyl)thymidine [19]. We have also been interested in evaluating ONs containing the corresponding amino derivative, 3'-*C*-(3-aminopropyl)thymidine, which could potentially increase the stability of the DNA·DNA and DNA·RNA duplexes by lowering the electrostatic repulsion between the two anionic strands. Thus, 3'-*C*-(hydroxypropyl)nucleoside **29** was synthesized *via* hydroboration/oxidation of the 3'-*C*-allyl derivative **28** as reported earlier [19] (Scheme 4). Standard Mitsunobu reaction [55] afforded the phthaloyl(Phth)-protected primary-amino-substituted nucleoside derivative **30** contaminated with triphenylphosphine oxide. Several attempts to remove the phosphine oxide by-product, *e.g.*, trituration with toluene, precipitation in Et₂O and extensive column chromatography, failed. Impure **30** was, therefore, thioacylated to give 2'-*O*-[(pentafluorophenoxy)thiocarbonyl]nucleoside **31** in 27% yield (two steps). Deoxygenation afforded the 2'-deoxynucleoside **32** in disappointing 25% yield (after extensive column-chromatographic purification and prep. TLC to remove tin impurities) and nucleoside **30** in 19% yield. Removal of the benzyl protecting groups of **32** by hydrogenation afforded the debenzylated diol **33** in only 18% yield. TLC showed the formation of several products upon addition of additional Pd(OH)₂/C during the reaction. Nevertheless, the small amount of diol **33** was 5'-*O*-(MeO)₂Tr-protected to give nucleoside **34**, which was subsequently phosphitylated to give the 3'-*O*-phosphoramidite building block **35** [20] in 23% yield (two steps).

The corresponding 3'-*C*-(3-aminopropyl)-2'-*O*-methylnucleoside derivative **42** was likewise synthesized and evaluated as a monomeric substitution in ONs. As the first step, by the same procedure as for the methylation of **11**, nucleoside **28** was methylated

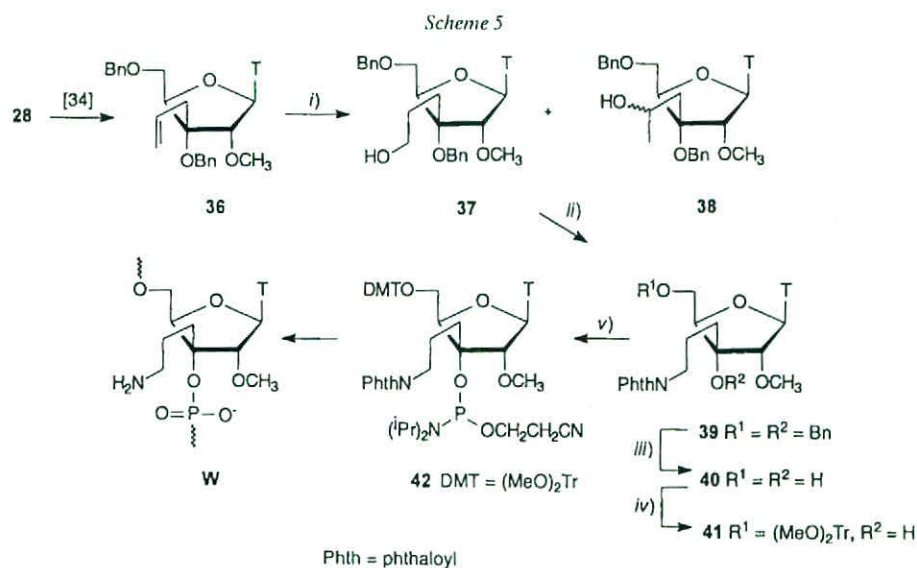
Scheme 4



i) Phthalimide, PPh₃, diethyl diazenedicarboxylate (DEAD), THF. ii) C₆F₅OC(S)Cl, DMAP, CH₂Cl₂. iii) 2,2'-azobis[isobutyronitrile] (AIBN), Bu₃SnH, benzene. iv) H₂, 20% Pd(OH)₂/C, EtOH, dioxane. v) (MeO)₂TrCl, Py. vi) NC(CH₂)₂OP(Cl)NⁱPr₂, ⁱPr₂EtN, CH₂Cl₂.

to give the 2'-O-methyl derivative **36** in 84% yield as reported earlier [34] (Scheme 5). Nucleoside **36** was subjected to a hydroboration/oxidation reaction sequence to afford the desired primary alcohol **37** in 54% yield. Furthermore, we isolated a mixture of the *Markovnikov* diastereoisomers **38** in 13% yield. *Mitsunobu* reaction of **37** with phthalimide [55] afforded the phthaloyl-protected derivative **39** in 20% yield in addition to a fraction contaminated with triphenylphosphine oxide. Subsequent debenzoylation with BCl₃ afforded diol **40** whereupon derivative **41** was obtained by standard 5'-O-(MeO)₂Tr protection in 65% yield (two steps). Eventual phosphitylation yielded the 3'-O-phosphoramidite derivative **42** in 33% yield after extensive column-chromatographic purification.

ONs containing 3'-C-(3-aminopropyl)thymidine monomer **Z** were synthesized from the phosphoramidite **35**. With pyridinium hydrochloride as activator and *t*-BuOOH [56] [57] as oxidizing agent, **35** was coupled in >95% stepwise yield (10 min coupling time). Standard deprotection with aq. NH₃ solution at 55° and cartridge purification afforded the ONs **XVI** and **XIX** (Table 2). The purity (>85%) and composition of the ONs was verified as described earlier for the ONs containing monomers **X** and **Y**. To avoid formation of ONs containing partially ring-opened phthaloyl functionalities under standard deprotection conditions, the use of methylamine for deprotection has been reported [17]. However, according to MALDI-MS this was not a problem for ONs **XVI** and **XIX**. Instead, a minor peak at $[M + 54]^+$ in the MALDI-MS of oligomer **XVI** was seen, presumably originating from *Michael* addition of the amino group to acrylonitrile during deprotection. Similarly, ONs **XVII** and **XX**



i) 1. BH_3 in 1,4-oxathiane, THF; 2. aq. NaOH soln., H_2O_2 . ii) Phthalimide, PPh_3 , DEAD, THF. iii) BCl_3 in hexane, CH_2Cl_2 . iv) $(\text{MeO})_2\text{TrCl}$, Py. v) $\text{NC}(\text{CH}_2)_2\text{OP}(\text{Cl})\text{N}(\text{iPr})_2$, $^i\text{Pr}_2\text{EtN}$, CH_2Cl_2 .

containing monomer **W** were synthesized with a stepwise coupling efficiency for the phosphoramidite **42** of > 80% (10 min coupling) with pyridinium hydrochloride as activator and *t*-BuOOH as oxidizing agent. Deprotection, purification, capillary gel electrophoresis, and MALDI-MS were performed as described earlier to yield ONs **XVII** and **XX** with a purity of > 85%.

The hybridization of the modified ONs **XVI** and **XIX** containing the 3'-C-(3-aminopropyl) nucleoside monomer **Z** were evaluated in comparison to the reference strands **XV** and **XVIII**, respectively, in a medium salt buffer, and for duplexes involving a DNA complement furthermore in a high salt buffer (Table 2). Incorporation of 3'-C-

Table 2. Melting Experiments of Oligonucleotides Containing Monomers **Z** and **W**^{a)}

		Complementary ssDNA				Complementary ssRNA	
		[Na ⁺] = 150 mM ^{b)}		[Na ⁺] = 750 mM ^{c)}		[Na ⁺] = 150 mM ^{b)}	
		T_m /° ^{d)}	ΔT_m /° per mod. ^{d)}	T_m /° ^{d)}	ΔT_m /° per mod. ^{d)}	T_m /° ^{d)}	ΔT_m /° per mod. ^{d)}
XV	5'-T ₁₄	33.0	reference	42.5	reference	31.0	reference
XVI	5'-T ₇ ZT ₆	33.0	± 0.0	41.5	- 1.0	28.5	- 2.5
XVII	5'-T ₇ WT ₆	23.5	- 9.5	n.d.	n.d.	n.d.	n.d.
XVIII	5'-G ₄ TG ₄ A ₄ TA ₄ TG ₄ C ₄	31.0	reference	36.5	reference	29.5	reference
XIX	5'-G ₄ TG ₄ A ₄ ZA ₄ TG ₄ C ₄	31.5	+ 0.5	36.5	± 0.0	25.5	- 4.0
XX	5'-G ₄ TG ₄ A ₄ WA ₄ TG ₄ C ₄	27.5	- 3.5	n.d.	n.d.	26.0	- 3.5

^{a)} **Z** = 3'-C-(3-aminopropyl)thymidine unit; **W** = 3'-C-(3-aminopropyl)-2'-O-methyl-5-methyluridine unit.

^{b)} Measured at 260 nm in medium salt buffer: 1 mM EDTA, 10 mM sodium phosphate, 140 mM sodium chloride, pH 7.1. ^{c)} Measured at 260 nm in high salt buffer: 1 mM EDTA, 10 mM sodium phosphate, 740 mM sodium chloride, pH 7.1. ^{d)} T_m = melting temperature determined as the maximum of the first derivative of the absorbance vs. temperature curve; ΔT_m /° per mod. = change in T_m per modification compared to the unmodified reference strand; n.d. = not determined.

(3-aminopropyl)thymidine monomer **Z** in the middle of an oligothymidylate (ON **XVI**) or in the middle of a mixed sequence 9-mer (ON **XIX**) induced no significant change in the thermal stability involving a DNA, while the presence of **Z** had a destabilizing effect on the duplexes involving complementary RNA ($\Delta T_m/\text{mod.} = -2.5$ and -4.0°) when measured in a medium salt buffer. The hybridization results with DNA are in contrast to the results obtained with ONs containing 3'-C-(3-hydroxypropyl)thymidine [19] which showed small decreases in binding affinity towards DNA. An interaction between the positively charged amino group of **Z** and the anionic phosphate backbone could be an immediate explanation of the better hybridization results towards DNA obtained for ONs containing the 3'-C-(aminopropyl) branch. We, therefore, measured the T_m values of ONs **XVI** and **XIX** in a buffer with high salt concentration (Table 2). As the resulting changes in the T_m values for ONs **XVI** and **XIX** ($+8.5$ and $+5.0^\circ$, resp.) are slightly smaller than for the reference strands **XV** and **XIII** ($+9.5$ and $+5.5^\circ$, resp.), an electrostatic interaction between the amino group in **Z** and the phosphate backbone appears likely. Compared with the few reports describing ONs containing 3'-C-branched monomers, ONs containing the 3'-C-(aminopropyl)-branched monomer **Z** show quite promising hybridization properties towards DNA. Thus, in general, ONs containing 3'-C-(hydroxymethyl)- [21], 3'-C-methyl- [22] [23], 3'-C-(aminomethyl)- [23], and 3'-C-allylnucleotide [20] monomers hybridize with complementary DNA with $\Delta T_m/\text{mod.}$ values of approximately 0 to -1.0° under medium salt conditions.

The significant decrease in binding affinity of mid-modified ONs containing monomer **Z** towards RNA compared to DNA is noticeable (Table 2). Similar results were observed with ONs containing 3'-C-allylthymidine [20] or 3'-C-(3-hydroxypropyl)thymidine [19] and can be explained by the conformation of the sugar moiety. Thus, the presence of a 3'- β -substituent on the pentofuranose ring shifts the sugar pucker towards a C(2')-endo type conformation through its preference to adopt a pseudo-equatorial orientation [58]. For monomer **Z**, coupling constants between H-C(1') and H_a-C(2')/H_β-C(2') are ca. 5.5 and ca. 9.5 Hz, respectively, as seen, e.g., for **34**, confirming the C(2')-endo-type conformation, as these coupling constants correspond to a H-C(1')-C(2')-H_a torsion angle of ca. 41° and a H-C(1')-C(2')-H_β torsion angle close to 160° according to a simplified Karplus equation [46]. The presence of the monomer **Z** with C(2')-endo conformation in the otherwise unmodified DNA strand might cause a rather significant distortion of the structure of the DNA-RNA duplex. For ONs containing 3'-C-(hydroxymethyl)- [21] [59], 3'-C-methyl- [22] [23], and 3'-C-(aminomethyl)thymidine [23] monomers, smaller decreases in $\Delta T_m/\text{mod.}$ values were in general observed for duplexes towards complementary RNA. The C₃-alkyl branch in **Z**, 3'-C-(3-hydroxypropyl)thymidine [19], and 3'-C-allylthymidine [20] monomers might be involved in some unfavorable steric interactions which are less pronounced for the modified monomers with only one C-atom in the 3'-C-branch. Thus, apparently a more selective DNA binding is observed when the 3'-C-branch contains three C-atoms. However, the limited amount of experimental data should be kept in mind before any general conclusions are drawn.

The hybridization properties of the modified oligonucleotides **XVII** and **XX**, containing monomer **W**, towards complementary DNA and RNA measured in a medium salt buffer are depicted in Table 2. Incorporation of a single 3'-C-(3-

aminopropyl)-2'-O,5-dimethyluridine monomer **W** in a 14-mer oligothymidylate induces a major decrease in the thermal stability of the duplex formed with complementary DNA with a $\Delta T_m/\text{mod.}$ value of -9.5° . More encouraging results were obtained when a single **W** was incorporated in the mixed 9-mer ON **XX**, inducing a $\Delta T_m/\text{mod.}$ value of -3.5° towards both complementary DNA and RNA. The binding affinities towards complementary DNA obtained for **XVII** and **XX** are in a striking contrast to the results obtained with the 2'-deoxy analogue **Z**. However, ONs containing 3'-C-allyl-2'-O,5-dimethyluridine [34], 3'-C,2'-O,5-trimethyluridine [22], the conformationally restricted 2'-O-methyl-6,3'-ethanouridine [60], and 2'-O-methyl-3'-C,2'-O-linked bicyclic nucleosides [34] displayed similar pronounced decreases in the thermal stability of duplexes towards DNA and RNA.

Despite the finding that the 2'-O-methyl modification in general has shown RNA-affinity-enhancing properties, probably by shifting the pentofuranose pucker towards the C(3')-endo conformation due to the *gauche* effect, the presence of the 2'-O-methyl modification in monomer **W** does not lead to an increased affinity of the oligonucleotides towards RNA compared to DNA. An explanation might be found in the conformation of the sugar ring since the coupling constant $^3J(\text{H}-\text{C}(1'), \text{H}-\text{C}(2'))$ for both monomers (as seen, e.g., for nucleoside **41**) is ca. 8 Hz, thus indicating a C(2')-endo conformation of the pentofuranose ring and additionally highlighting the dominating effect of the 3'- β -branch on the sugar conformation. Consequently, the 2'-O-methyl group is oriented in a pseudoequatorial position, probably causing a significant distortion of the structure of the duplexes with DNA as well as RNA, as seen in molecular-modeling studies of oligonucleotides containing 3'-C-allyl-2'-O,5-dimethyluridine [34].

3. Conclusion. – 4'-C-(Aminomethyl)-2'-deoxy-2'-fluoro- and 4'-C-(aminomethyl)-2'-O-methylnucleosides have been efficiently synthesized by a convergent approach involving substitution of a 2'-[(methylsulfonyl)oxy] group or chemoselective 2'-O-methylation, respectively. The corresponding 3'-O-phosphoramidite derivatives were used in syntheses of partly modified 14-mer oligothymidylates and 9-mer mixed sequences by means of an automated DNA synthesizer. Compared with the corresponding 4'-C-branched 2'-deoxy derivatives, increased binding affinities towards complementary DNA and RNA were in general obtained, and a positive effect of the positively charged aminomethyl group was indicated at a physiologically relevant ionic strength. The introduction of the 2'-fluoro or 2'-methoxy substituents induces a conformational shift towards a C(3')-endo-type furanose conformation. However, no additional effect was observed after incorporation of the 2'-substituted 4'-C-(aminomethyl) monomers in a 9-mer 2'-O-methyl-RNA strand. On the contrary, significantly reduced binding affinities resulted, indicating an unfavorable steric interaction involving the 4'-C-(aminomethyl) branch. The 3'-C-(3-aminopropyl)-2'-deoxy- and 4'-C-(aminomethyl)-2'-O-methylnucleosides were likewise synthesized by a convergent approach involving either free-radical deoxygenation at C(2') or chemoselective 2'-O-methylation, respectively. The corresponding 3'-O-phosphoramidite derivatives were used for syntheses of partly modified 14-mer oligothymidylates and 9-mer mixed sequences. Compared with the corresponding unmodified reference ONs, the 2'-unsubstituted and 2'-substituted 3'-C-(3-aminopropyl)thymidine monomers induce

unchanged or decreased thermal stabilities of duplexes towards complementary DNA and decreased thermal stabilities towards RNA. Thus, one of the aims of the present research, namely to develop oligonucleotide analogues displaying DNA-selective hybridization, has been accomplished, but neither here nor earlier has it been possible to obtain significantly increased binding affinities for 3'-C-branched ONs. Noteworthy are, however, the affinity-enhancing properties of the 2'-substituted 4'-C-(amino-methyl)thymidine monomers reported herein. The somewhat troublesome synthetic routes devised for the relevant nucleosides and the nonideal oligomerizations of the corresponding phosphoramidite derivatives on automated synthesizers have so far precluded the evaluation of fully modified 3'-C- and 4'-C-branched ON derivatives. However, 4'-C- and 3'-C-branched ON analogues are likely to find utility as monomeric constituents, e.g., conjugation sites, in antisense oligonucleotides.

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Experimental Part

General. Reactions were conducted under Ar when anh. solvents were used. Petroleum ether was of distillation range 60–80°. On workup, org. phases were dried (Na_2SO_4) and filtered. Column chromatography (CC): glass columns; silica gel 60 (0.040–0.063 mm). NMR spectra: chemical shift values δ in ppm rel. to SiMe_4 as internal reference (^1H and ^{13}C) and rel. to 85% H_3PO_4 soln. as external reference (^{31}P). MS: in m/z . Microanalyses were performed at The Microanalytical Laboratory, Department of Chemistry, University of Copenhagen.

3-O-Benzyl-4-C-[(benzyloxy)methyl]-1,2-di-O-isopropylidene- β -L-lyxofuranose (2). To a soln. of **1** (570 mg, 1.83 mmol) in anh. MeOH (50 ml), dibutyltin oxide (478 mg, 1.92 mmol) was added. The mixture was heated under reflux for 1 h, and the resulting clear soln. was cooled to r.t. and evaporated to give a yellow/white solid material which was redissolved in anh. toluene (25 ml). BnBr (0.65 ml, 5.5 mmol) was added to the mixture followed by Bu_4NBr (295 mg, 0.92 mmol), and the mixture was heated under reflux for 15 h. Additional Bu_4NBr (325 mg, 1.01 mmol) was added followed by BnBr (0.65 ml, 5.5 mmol), but no further conversion was observed according to anal. TLC. After additional heating under reflux for 24 h, the mixture was cooled to r.t. CH_2Cl_2 (30 ml) was added followed by a sat. aq. NaHCO_3 soln. (30 ml). The resulting precipitate was filtered off and the separated org. phase washed with sat. aq. NaCl soln. (3×20 ml), dried (Na_2SO_4), and evaporated. Purification by CC (10–50% AcOEt /petroleum ether, then 5% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) afforded **2** (139 mg, 19%), another dibenzyl derivative (151 mg, 21%), and a tribenzyl derivative (206 mg, 23%) as oils, in addition to starting material **1** (ca. 128 mg, ca. 23% (contaminated with Sn-alkyls)) as a yellowish solid material. **2**: ^1H - and ^{13}C -NMR: in accordance with [24].

1,2-Di-O-acetyl-3-O-benzyl-4-C-[(benzyloxy)methyl]-5-O-(methylsulfonyl)-L-lyxofuranose (4). **Method A:** Furanose **3** [28] (630 mg, 1.32 mmol) was heated in 50% aq. AcOH soln. (5.5 ml) at 90° for 3 h and subsequently cooled to r.t. Evaporation followed by co-evaporation with abs. EtOH, toluene, and pyridine afforded an oil which was redissolved in anh. pyridine (3 ml). Ac_2O (2 ml) was added and the mixture stirred under Ar for 12 h at r.t. The mixture was poured into ice-cold H_2O (10 ml), and sat. aq. NaHCO_3 soln. (10 ml) was added followed by AcOEt (20 ml). The org. phase was washed several times with sat. aq. NaHCO_3 soln. The aq. phase was extracted with AcOEt (2×10 ml) and the combined org. phase dried (Na_2SO_4) and evaporated. Purification by CC (0–2% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) afforded **4** (α/β -L 3:1; 370 mg, 54%).

Method B: To a soln. of **3** [28] (118 mg, 0.25 mmol) in AcOH (2 ml) and Ac_2O (0.2 ml), conc. H_2SO_4 (2 μl) was added. The soln. was stirred at r.t. for 12 h, poured into ice-cold H_2O (5 ml), and extracted with CH_2Cl_2 (3×10 ml). The org. phase was washed successively with sat. aq. NaHCO_3 soln. (3×10 ml) and sat. aq. NaCl soln. (10 ml), dried (MgSO_4), and evaporated. Purification by CC (0–2% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) afforded furanose **4** (α/β -L 4:1) (70 mg, 54%). Oil. ^1H -NMR (CDCl_3): 7.37–7.21 (m, Bn); 6.38 (d, $J=4.7$, H-C(1)(β -L)); 6.14 (s, H-C(1)(α -L)); 5.35 (d, $J=5.0$, H-C(2)(α -L)); 5.17 (dd, $J=4.6$, 6.3, H-C(2)(β -L)); 4.69–4.35 (m, Bn, H-C(3)); 4.25 (d, $J=11.3$, Bn); 3.64, 3.52, 3.45, 3.39 (4d, $J=9.7$, 10.0, 9.7, 10.0, 2 H-C(5), CH_2 -C(4)); 2.98, 2.95 (2s, 2 MeSO_2), 2.13, 2.06, 2.04, 1.90 (4s, 4 MeCO). ^{13}C -NMR (CDCl_3): 170.1, 170.0, 169.6, 169.0 (4 C=O); 137.7,

137.0, 128.6, 128.5, 128.2, 128.1, 128.0, 127.9, 127.8 (Bn); 97.5 (C(1)(α -L)); 94.2 (C(1)(β -L)); 86.3 (C(4)(β -L)); 84.3 (C(4)(α -L)); 79.1, 74.5, 74.1, 73.7, 73.5, 71.6, 70.6, 70.2, 69.8 (C(2), C(3), C(5), CH₂-C(4), Bn); 37.6, 37.4 (2 MeSO₂); 21.0, 20.7, 20.6, 20.4 (4 MeCO). FAB-MS: 545 ([M + Na]⁺). Anal. calc. for C₂₅H₃₀O₁₀S: C 57.46, H 5.79; found: C 57.72, H 5.74.

1-(2-O-Acetyl-3-O-benzyl-4-C-[(benzyloxy)methyl]-5-O-(methylsulfonyl)- α -L-lyxofuranosyl)thymine (5). To a soln. of **4** (460 mg, 0.88 mmol) in anh. MeCN (2 ml), thymine (0.20 g, 1.59 mmol) was added followed by *N,O*-bis(trimethylsilyl)acetamide (BSA; 1.31 ml, 5.30 mmol). The mixture was heated under reflux until the soln. became clear (5 min). After cooling to 0°, Me₃SiOSO₂CF₃ (Me₃SiOTf; 0.27 ml, 1.50 mmol) was added dropwise, and the soln. was stirred for 12 h at r.t. As anal. TLC showed that the reaction was not complete, identical amounts as specified above of more thymine and BSA were further added, and the soln. was heated under reflux for 15 min, whereupon Me₃SiOTf (0.54 ml, 3.0 mmol) was added dropwise at 0°. After stirring at r.t. for 1.5 h, sat. aq. NaHCO₃ soln. (10 ml) was added, the mixture extracted with CH₂Cl₂ (3 × 20 ml), and the org. phase washed with sat. aq. NaHCO₃ soln. (2 × 10 ml), dried (Na₂SO₄), and evaporated. Purification by CC (0–2% MeOH/CH₂Cl₂) afforded **5** (386 mg, 75%). White solid material. ¹H-NMR (CDCl₃): 8.92 (br. s, NH); 7.40–7.26 (m, 11 H, H-C(6), Bn); 6.14 (d, *J* = 5.0, H-C(1')); 5.48 (t, *J* = 5.4, H-C(2')); 4.62 (d, *J* = 11.1, 1 H, Bn); 4.58 (m, 5 H, H-C(3'), 2 H-C(5'), Bn); 4.27 (d, *J* = 11.3, 1 H, Bn); 3.89 (d, *J* = 10.1, 1 H, CH₂-C(4')); 3.59 (d, *J* = 10.2, 1 H, CH₂-C(4')); 2.92 (s, MeSO₂); 2.10 (s, COMe); 1.59 (d, *J* = 0.5, Me). ¹³C-NMR (CDCl₃): 170.2 (C=O); 163.6 (C(4)); 150.4 (C(2)); 137.0, 136.9, 136.1, 128.9, 128.8, 128.7, 128.5, 128.3, 128.2, 127.8 (C(6), Bn); 111.6 (C(5)); 87.8, 85.5 (C(1'), C(4')); 77.8, 74.8, 74.4, 73.8, 70.8, 69.0 (C(2'), C(3'), C(5'), CH₂-C(4'), Bn); 37.2 (MeSO₂); 20.6 (MeCO); 11.9 (Me). FAB-MS: 589 ([M + H]⁺). Anal. calc. for C₂₈H₃₂N₂O₁₀S: C 57.13, H 5.48, N 4.76; found: C 57.17, H 5.38, N 4.36.

(1S,3R,4R,7S)-7-(Benzyloxy)-1-[(benzyloxy)methyl]-3-(thymine-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (6) [25]. A soln. of **5** (73 mg, 0.124 mmol) in anh. THF (3 ml) was cooled to 0°. Then, 1.0M LiEt₃BH in THF (0.25 ml, 0.25 mmol) was added dropwise, and the mixture was stirred at r.t. for 3 h and then cooled to 0°. An identical amount as specified above of LiEt₃BH was additionally added dropwise, and the mixture was stirred for 12 h at r.t., and cooled to 0°. The reaction was quenched by slow addition of 35% aq. H₂O₂ soln./2N NaOH 1:1 (0.5 ml). The mixture was diluted with CH₂Cl₂ (10 ml) and washed with sat. aq. NaCl soln. (3 × 10 ml). The org. phase was dried (Na₂SO₄) and evaporated. Purification by CC (1% MeOH/CH₂Cl₂) afforded **6** (48 mg, 86%). White solid material. ¹H-NMR, ¹³C-NMR, and FAB-MS: in accordance with [25].

3-O-Benzyl-4-C-[(benzyloxy)methyl]-5-O-[(trifluoromethyl)sulfonyl]-1,2-O-isopropylidene- β -L-lyxofuranose (7). To a soln. of **2** (6.34 g, 15.8 mmol) in anh. CH₂Cl₂ (60 ml), anh. pyridine (3.83 ml, 47.5 mmol) was added. The mixture was cooled to –50° and Tf₂O (4.15 ml, 25.3 mmol) added dropwise. The mixture was stirred for 1 h, then allowed to warm up to 0°, and stirred for 1 h, whereupon the reaction was quenched by slow addition of sat. aq. NaHCO₃ soln. (15 ml). Additional CH₂Cl₂ (40 ml) and sat. aq. NaHCO₃ soln. (50 ml) were added. The org. phase was successively washed with ice-cold 1M HCl (2 × 75 ml) and sat. aq. NaHCO₃ soln. (75 ml). The aq. phases were separately extracted with CH₂Cl₂ (25 ml each), and the combined org. phase was dried (Na₂SO₄) and evaporated: **7** as a white solid material which was used directly in the next step without further purification. ¹H-NMR (CDCl₃): 7.38–7.26 (m, 10 H, Bn); 5.74 (d, *J* = 3.6, H-C(1)); 5.00 (d, *J* = 10.7, 1 H, Bn); 4.81 (d, *J* = 10.7, 1 H, Bn); 4.75 (d, *J* = 12.1, 1 H, Bn); 4.61–4.54 (m, H-C(2), 2 H-C(5), Bn); 4.22 (d, *J* = 5.2, H-C(3)); 3.54 (d, *J* = 10.0, 1 H, CH₂-C(4)); 3.49 (d, *J* = 10.3, 1 H, CH₂-C(4)); 1.64, 1.35 (2s, Me₂C). ¹³C-NMR (CDCl₃): 137.8, 137.3, 128.8, 128.7, 128.5, 128.4, 128.3, 128.1, 128.0 (Bn); 118.8 (q, *J* = 320, CF₃); 114.1 (Me₂C); 104.5 (C(1)); 84.2 (C(4)); 79.5, 78.8 (C(2), C(3)); 76.4, 74.0, 72.8, 70.5 (C(5), CH₂-C(4), Bn); 26.5, 25.9 (Me₂C). Anal. calc. for C₂₄H₂₇F₃O₈S·0.25 H₂O: C 53.67, H 5.12; found: C 53.73, H 4.91.

5-Azido-3-O-benzyl-4-C-[(benzyloxy)methyl]-5-deoxy-1,2-O-isopropylidene- β -L-lyxofuranose (8). To a soln. of **7** in anh. DMF (230 ml), NaN₃ (8.22 g, 0.126 mol) was added in one portion and the mixture stirred at 60° for 6 h. The soln. was cooled to r.t., filtered, and evaporated to give an oil which was redissolved in AcOEt (120 ml). The org. phase was washed with sat. aq. NaCl soln. (3 × 75 ml), dried (Na₂SO₄), and evaporated. Purification by CC (10–20% AcOEt/petroleum ether) afforded **8** (3.99 g, 59% from **2**). Clear oil. IR (NaCl): 2103 (–N₃). ¹H-NMR (CDCl₃): 7.38–7.26 (m, 10 H, Bn); 5.79 (d, *J* = 3.8, H-C(1)); 4.76 (d, *J* = 12.0, 1 H, Bn); 4.63 (dd, *J* = 3.8, 5.1, H-C(2)); 4.58 (s, 1 H, Bn); 4.54 (s, 1 H, Bn); 4.48 (d, *J* = 12.0, 1 H, Bn); 4.20 (d, *J* = 5.5, 1 H, H-C(3)); 4.05 (d, *J* = 13.4, 1 H, CH₂-C(4)); 3.58 (d, *J* = 10.2, 1 H, H_b-C(5)); 3.46 (d, *J* = 10.2, 1 H, H_a-C(5)); 3.33 (d, *J* = 13.4, 1 H, CH₂-C(4)); 1.67, 1.36 (2s, Me₂C). ¹³C-NMR (CDCl₃): 138.0, 137.6, 128.5, 128.4, 128.0, 127.9, 127.7 (Bn); 113.5 (Me₂C); 104.2 (C(1)); 86.4 (C(4)); 78.9, 78.3, 73.6, 72.4, 71.3 (C(2), C(3), C(5), Bn); 52.1 (CH₂-C(4)); 26.6, 25.8 (Me₂C). FAB-MS: 448 ([M + Na]⁺). Anal. calc. for C₂₃H₂₇N₃O₅: C 64.93, H 6.40, N 9.88; found: C 64.76, H 6.32, N 9.72.

1,2-Di-O-acetyl-5-azido-3-O-benzyl-4-C-[(benzyloxy)methyl]-5-deoxy-L-lyxofuranose (9). Furanose **8** (5.93 g, 13.9 mmol) was heated in 50% aq. AcOH soln. (120 ml) at 100° for 1 h and cooled to r.t. Evaporation followed by co-evaporation successively with abs. EtOH, toluene, and pyridine afforded an oil which was redissolved in anh. pyridine (30 ml). Ac₂O (20 ml) was added, and the mixture was stirred under Ar for 12 h at r.t. The mixture was cooled to 0° and poured into ice-cold H₂O (50 ml). Sat. aq. NaHCO₃ soln. (50 ml) was added followed by AcOEt (100 ml). The org. phase was washed with sat. aq. NaHCO₃ soln., the aq. phase extracted with AcOEt (2 × 50 ml), and the combined org. phase dried (Na₂SO₄) and evaporated. Purification by CC (CH₂Cl₂) afforded **9** (α/β-L 1:4; 5.86 g, 90%). Clear oil. ¹H-NMR (CDCl₃): 7.39–7.24 (m, Bn); 6.42 (d, *J* = 4.7, H–C(1)(α-L)); 6.18 (s, H–C(1)(β-L)); 5.35 (d, *J* = 4.8, H–C(2)(β-L)); 5.25 (dd, *J* = 4.8, 6.6, H–C(2)(α-L)); 4.63–4.48 (m, Bn); 4.36 (d, *J* = 5.0, H–C(3)(β-L)); 4.29 (d, *J* = 6.5, H–C(3)(α-L)); 3.74 (d, *J* = 13.1, 1 H, CH₂–C(4)(α-L)); 3.64–3.43 (m, H–C(5), 2 H–C(4)); 3.37 (d, *J* = 13.3, 1 H, CH₂–C(4)(α-L)); 2.13, 2.12, 2.05, 1.90 (4s, 4 MeCO). ¹³C-NMR (CDCl₃): 169.7, 169.6, 168.9 (4 C=O); 137.8, 137.3, 128.5, 128.4, 128.0, 127.9, 127.8, 127.7, 127.6 (Bn); 97.5 (C(1)(β-L)); 94.3 (C(1)(α-L)); 87.8 (C(4)(α-L)); 86.1 (C(4')(β-L)); 79.0, 77.4, 74.3, 74.2, 73.7, 73.5, 73.4, 71.6, 71.5, 71.0 (2 C(2), 2 C(3), 2 C(5), Bn); 53.0 (CH₂–C(4)(β-L)); 52.7 (CH₂–C(4)(α-L)); 21.0, 20.7, 20.6, 20.3 (4 MeCO). FAB-MS: 492 ([*M* + Na]⁺). Anal. calc. for C₂₄H₂₇N₃O₇: C 61.40, H 5.80, N 8.95; found: C 61.64, H 5.81, N 8.96.

1-[2-O-Acetyl-5-azido-3-O-benzyl-4-C-[(benzyloxy)methyl]-5-deoxy-α-L-lyxofuranosyl]thymine (10). To a soln. of **9** (5.86 g, 12.5 mmol) in anh. MeCN (50 ml), thymine (3.15 g, 25 mmol) and BSA (24.7 ml, 0.1 mol) were added. The soln. was heated under reflux for 30 min, cooled to 0°, and Me₃SiOTf (4.5 ml, 25 mmol) was added dropwise. The mixture was heated under reflux for another 30 min and then cooled to 0°. The reaction was quenched by addition of a sat. aq. NaHCO₃ soln. (60 ml). The mixture was filtered and evaporated, the oily residue dissolved in CH₂Cl₂ (75 ml), and the soln. washed with sat. aq. NaHCO₃ soln. (3 × 50 ml), dried (Na₂SO₄), and evaporated. Purification by CC (0–1% MeOH/CH₂Cl₂) afforded **10** (6.22 g, 93%). White solid material. ¹H-NMR (CDCl₃): 9.22 (br. s, NH); 7.40 (d, *J* = 1.3, H–C(6)); 7.39–7.26 (m, 10 H, Bn); 6.21 (d, *J* = 5.1, H–C(1')); 5.46 (t, *J* = 5.6, H–C(2')); 4.63 (d, *J* = 11.3, 1 H, Bn); 4.56 (m, 4 H, H–C(3'), Bn); 3.77 (d, *J* = 9.9, H_b–C(5')); 3.65 (d, *J* = 13.0, 1 H, CH₂–C(4')); 3.51 (d, *J* = 10.3, H_a–C(5')); 3.38 (d, *J* = 13.1, 1 H, CH₂–C(4')); 2.10 (s, COMe); 1.57 (d, *J* = 1.1, Me). ¹³C-NMR (CDCl₃): 170.2 (C=O); 163.8 (C(4)); 150.5 (C(2)); 137.2, 137.1, 135.8 (C(6), Bn); 128.7, 128.6, 128.3, 128.2, 128.1, 127.8 (Bn); 111.5 (C(5)); 87.1 (C(1')), C(4')); 77.5, 74.8, 74.6, 73.7, 71.4 (C(2'), C(3'), C(5'), Bn); 52.6 (CH₂–C(4')); 20.5 (MeCO); 11.9 (Me). FAB-MS: 536 ([*M* + H]⁺). Anal. calc. for C₂₇H₂₉N₃O₇: C 60.55, H 5.46, N 13.08; found: C 60.34, H 5.39, N 12.87.

1-[5-Azido-3-O-benzyl-4-C-[(benzyloxy)methyl]-5-deoxy-α-L-lyxofuranosyl]thymine (11). Nucleoside **10** (6.18 g, 11.54 mmol) was stirred in a sat. NH₃ soln. in MeOH (100 ml) at r.t., and after 5 h, the mixture was evaporated. Purification by CC (1% MeOH/CH₂Cl₂) afforded **11** (6.18 g, 97%). White solid material. ¹H-NMR (CDCl₃): 10.3 (br. s, NH); 7.54 (d, *J* = 1.1, H–C(6)); 7.38–7.26 (m, 10 H, Bn); 5.99 (d, *J* = 4.4, H–C(1')); 4.91 (d, *J* = 11.8, 1 H, Bn); 4.61 (d, *J* = 11.5, 1 H, Bn); 4.54–4.49 (m, 3 H, H–C(2'), Bn); 4.31 (d, *J* = 5.5, H–C(3')); 3.81 (d, *J* = 13.5, 1 H, CH₂–C(4')); 3.80 (d, *J* = 10.3, H_b–C(5')); 3.52 (d, *J* = 10.1, H_a–C(5')); 3.31 (d, *J* = 13.3, 1 H, CH₂–C(4')); 1.50 (d, *J* = 0.8, Me). ¹³C-NMR (CDCl₃): 164.0 (C(4)); 151.4 (C(2)); 137.5, 137.3, 136.1 (C(6), Bn); 128.7, 128.5, 128.2, 128.1, 127.8 (Bn); 110.7 (C(5)); 90.3, 87.2 (C(1'), C(4')); 77.9, 74.7, 73.9, 73.6, 71.4 (C(2'), C(3'), C(5'), Bn); 52.9 (CH₂–C(4')); 11.9 (Me). FAB-MS: 494 ([*M* + H]⁺). Anal. calc. for C₂₅H₂₇N₅O₆: C 60.84, H 5.51, N 14.19; found: C 60.80, H 5.38, N 14.10.

1-[5-Azido-3-O-benzyl-4-C-[(benzyloxy)methyl]-5-deoxy-2-O-methyl-α-L-lyxofuranosyl]thymine (12). A soln. of **11** (0.99 g, 2.01 mmol) in anh. THF (20 ml) was cooled to 0°. NaH (0.241 g of a 60% dispersion in mineral oil, 6.03 mmol) was added and the mixture stirred for 30 min. MeI (0.63 ml, 10.1 mmol) was added dropwise, and the mixture was stirred at 0° for 10 h. Ice-cold H₂O (10 ml) was added followed by AcOEt (25 ml). The org. phase was washed with sat. aq. NaHCO₃ soln. (3 × 30 ml), dried (Na₂SO₄), and evaporated. Purification by CC (0.5% MeOH/CH₂Cl₂) afforded **12** (0.92 g, 90%). White solid material. ¹H-NMR (CDCl₃): 9.35 (br. s, NH); 7.67 (d, *J* = 1.3, H–C(6)); 7.41–7.22 (m, 10 H, Bn); 6.07 (d, *J* = 2.4, H–C(1')); 4.74 (d, *J* = 11.7, 1 H, Bn); 4.68 (d, *J* = 11.4, 1 H, Bn); 4.52 (s, 1 H, Bn); 4.49 (d, *J* = 11.7, 1 H, Bn); 4.35 (d, *J* = 5.5, 1 H, H–C(3')); 3.94 (d, *J* = 10.4, H_b–C(5')); 3.93 (d, *J* = 13.6, 1 H, CH₂–C(4')); 3.87 (dd, *J* = 2.4, 5.7, H–C(2')); 3.58 (s, MeO); 3.55 (d, *J* = 10.0, H_a–C(5')); 3.34 (d, *J* = 13.7, 1 H, CH₂–C(4')); 1.48 (d, *J* = 1.2, Me). ¹³C-NMR (CDCl₃): 164.0 (C(4)); 150.3 (C(2)); 137.3, 137.2, 135.8 (C(6), Bn); 128.7, 128.6, 128.2, 127.9, 127.8 (Bn); 110.8 (C(5)); 88.5, 87.2, 83.4 (C(1'), C(2'), C(4')); 75.9, 73.6, 73.1, 70.2 (C(3'), C(5'), Bn); 59.2 (MeO); 53.0 (CH₂–C(4')); 11.7 (Me). FAB-MS: 508 ([*M* + H]⁺). Anal. calc. for C₂₆H₂₉N₅O₆: C 61.53, H 5.76, N 13.80; found: C 61.26, H 5.78, N 13.62.

1-[5-Amino-3-O-benzyl-4-C-[(benzyloxy)methyl]-5-deoxy-2-O-methyl-α-L-lyxofuranosyl]thymine (13). To a soln. of **12** (440 mg, 0.87 mmol) in abs. EtOH (13 ml), Lindlar catalyst (130 mg) was added. The mixture was stirred at r.t. under H₂ for 8 h, filtered through a thin layer of silica gel, and evaporated. Purification by

CC (2–5% MeOH/CH₂Cl₂) afforded **13** (316 mg, 75%). White solid material. ¹H-NMR (CDCl₃): 7.60 (*d*, *J* = 0.9, H–C(6)); 7.38–7.21 (*m*, 10 H, Bn); 6.12 (*d*, *J* = 3.3, H–C(1')); 4.79 (*d*, *J* = 11.9, 1 H, Bn); 4.56–4.46 (*m*, 2 H, Bn); 4.48 (*d*, *J* = 11.7, 1 H, Bn); 4.33 (*d*, *J* = 5.9, H–C(3')); 3.88 (*dd*, *J* = 3.6, 5.9, H–C(2')); 3.82 (*d*, *J* = 10.4, H₂–C(5')); 3.59 (*d*, *J* = 10.5, H₂–C(5')); 3.50 (*s*, MeO); 3.12 (*d*, *J* = 13.8, 1 H, CH₂–C(4')); 2.83 (*d*, *J* = 13.8, 1 H, CH₂–C(4')); 1.49 (*d*, *J* = 0.5, Me). ¹³C-NMR (CDCl₃): 164.0 (C(4)); 150.5 (C(2)); 137.7, 137.4, 135.9 (C(6), Bn); 128.5, 128.1, 128.0, 127.8, 127.7 (Bn); 111.0 (C(5)); 88.0, 87.8, 83.9 (C(1'), C(2'), C(4')); 76.8, 73.6, 73.4, 71.8 (C(3'), C(5'), Bn); 59.1 (MeO); 44.2 (CH₂–C(4')); 11.8 (Me). FAB-MS: 482 ([*M* + H]⁺). Anal. calc. for C₂₆H₃₁N₃O₆ · 0.25 H₂O: C 64.25, H 6.53, N 8.65; found: C 64.27, H 6.38, N 8.55.

1-[3-O-Benzyl-4-C-[(benzyloxy)methyl]-5-deoxy-2-O-methyl-5-[(trifluoroacetyl)amino]-α-L-lyxofuranosyl]-thymine (14). To a soln. of nucleoside **13** (300 mg, 0.62 mmol) in anhyd. CH₂Cl₂ (8 ml), ethyl trifluoroacetate (0.15 ml, 1.25 mmol) and Et₃N (0.086 ml, 0.62 mmol) were added. The mixture was stirred for 12 h at r.t. and then evaporated. Purification by CC (1% MeOH/CH₂Cl₂) afforded **14** (346 mg, 97%). White solid material. ¹H-NMR (CDCl₃): 8.86 (br. *s*, NH); 7.52 (*d*, *J* = 1.3, H–C(6)); 7.39–7.18 (*m*, 11 H, NHCOCF₃, Bn); 5.90 (*d*, *J* = 1.6, H–C(1')); 4.72 (*d*, *J* = 11.5, 1 H, Bn); 4.54–4.44 (*m*, 3 H, Bn); 4.43 (*d*, *J* = 6.1, H–C(3')); 3.95 (*dd*, *J* = 1.7, 6.3, H–C(2')); 3.77–3.68 (*m*, CH₂–C(4')); 3.55–3.48 (*m*, 2 H–C(5')); 3.58 (*s*, MeO); 1.51 (*d*, *J* = 1.0, Me). ¹³C-NMR (CDCl₃): 164.1 (C(4)); 157.4 (*q*, *J* = 37, COCF₃); 150.2 (C(2)); 136.8, 136.1 (C(6), Bn); 128.6, 128.4, 128.2, 128.1, 127.9, 127.8, 127.7 (Bn); 115.9 (*q*, *J* = 288, COCF₃); 110.9 (C(5)); 90.2, 86.8, 83.0 (C(1'), C(2'), C(4')); 76.0, 73.6, 73.2, 70.8 (C(3'), CH₂–C(4'), Bn); 59.3 (MeO); 41.4 (C(5')); 11.8 (Me). FAB-MS: 578 ([*M* + H]⁺). Anal. calc. for C₂₈H₃₀F₃N₃O₇: C 58.23, H 5.23, N 7.28; found: C 58.27, H 5.21, N 7.34.

1-[5-Deoxy-2-O-methyl-5-[(trifluoroacetyl)amino]-α-L-lyxofuranosyl]thymine (15). To a soln. of **14** (346 mg, 0.60 mmol) in abs. EtOH (10 ml), 5% Pd/C catalyst (150 mg) was added. The mixture was stirred at r.t. under H₂ for 1.5 h, filtered through a thin layer of silica gel, and evaporated. Purification by CC (3% MeOH/CH₂Cl₂) afforded **15** (223 mg, 92%). White solid material. ¹H-NMR (CD₃OD): 7.86 (*d*, *J* = 1.1, H–C(6)); 6.05 (*d*, *J* = 4.6, H–C(1')); 4.51 (*d*, *J* = 5.8, H–C(3')); 4.03 (*dd*, *J* = 4.8, 5.8, H–C(2')); 3.70–3.62 (*m*, CH₂–C(4'), 2 H–C(5')); 3.50 (*s*, MeO); 1.49 (*d*, *J* = 1.0, Me). ¹³C-NMR (CD₃OD): 166.3 (C(4)); 157.4 (*q*, *J* = 35, COCF₃); 152.4 (C(2)); 138.4 (C(6)); 117.5 (*q*, *J* = 286, COCF₃); 111.7 (C(5)); 89.0, 88.9, 84.9 (C(1'), C(2'), C(4')); 71.1, 64.3 (C(3'), CH₂–C(4')); 59.1 (MeO); 42.1 (C(5')); 12.4 (Me). EI-MS: 397 (*M*⁺). Anal. calc. for C₁₄H₁₈F₃N₃O₇ · 0.25 H₂O: C 41.85, H 4.64, N 10.58; found: C 41.93, H 4.42, N 10.40.

1-[5-Deoxy-4-C-[(4,4'-dimethoxytrityl)oxy]methyl]-2-O-methyl-5-[(trifluoroacetyl)amino]-α-L-lyxofuranosyl]thymine (16). To a soln. of **15** (223 mg, 0.56 mmol) in anhyd. pyridine (8 ml), (MeO)₂TrCl (361 mg, 1.07 mmol) was added and the mixture stirred at r.t. for 6 h. MeOH (2 ml) was added and the mixture evaporated. The oily residue was redissolved in CH₂Cl₂ (20 ml) and the soln. washed with sat. aq. NaCl soln. (3 × 15 ml), dried (Na₂SO₄), and evaporated. Purification by CC (0.5–1.5% MeOH/CH₂Cl₂ containing 0.5% pyridine) afforded **16** (382 mg, 91%). Yellowish solid material. ¹H-NMR (CDCl₃): 9.54 (br. *s*, NH); 7.45 (*d*, *J* = 0.9, H–C(6)); 7.39–7.08 (*m*, 10 H, NHCOCF₃, (MeO)₂Tr); 6.84 (*dd*, *J* = 1.7, 9.0, 4 H, (MeO)₂Tr); 6.10 (*d*, *J* = 5.6, H–C(1')); 4.49 (*d*, *J* = 5.1, H–C(3')); 4.20 (*t*, *J* = 5.8, H–C(2')); 3.82–3.75 (*m*, CH₂–C(4')); 3.63–3.61 (*m*, 2 H–C(5')); 3.79, 3.78 (2*s*, (MeO)₂Tr); 3.42 (br. *s*, OH–C(3')); 3.36 (*s*, MeO); 1.48 (*d*, *J* = 1.0, Me). ¹³C-NMR (CDCl₃): 164.0 (C(4)); 159.0 ((MeO)₂Tr); 157.5 (*q*, *J* = 37, COCF₃); 150.7 (C(2)); 143.9 ((MeO)₂Tr); 137.9, 135.7, 134.8, 134.7 (C(6), (MeO)₂Tr); 130.2, 130.1, 129.1, 128.3, 128.1, 127.4 ((MeO)₂Tr); 115.7 (*q*, *J* = 288, COCF₃); 113.4 ((MeO)₂Tr); 112.0 (C(5)); 87.7, 87.2, 87.0, 83.0 (C(1'), C(2'), C(4'), C(Ar₁)); 71.1, 65.9 (C(3'), CH₂–C(4')); 59.0 (MeO); 55.1 ((MeO)₂Tr); 41.3 (C(5')); 11.6 (Me). FAB-MS: 699 (*M*⁺). Anal. calc. for C₃₅H₃₆F₃N₃O₉ · 0.5 C₂H₆: C 62.01, H 5.41, N 5.63; found: C 62.04, H 5.50, N 5.50.

1-[5-Deoxy-4-C-[(4,4'-dimethoxytrityl)oxy]methyl]-2-O-methyl-5-[(trifluoroacetyl)amino]-α-L-lyxofuranosyl]thymine 3'-(2-Cyanoethyl Diisopropylphosphoramidite) (17). To a soln. of **16** (160 mg, 0.23 mmol) in anhyd. CH₂Cl₂ (4 ml), ⁱPr₂EtN (0.16 ml, 0.92 mmol) was added, and the mixture was cooled to 0°. 2-Cyanoethyl diisopropylphosphoramidochloridite (0.11 ml, 0.46 mmol) was added dropwise, and the mixture was stirred for 4 h at r.t., diluted with AcOEt (25 ml), and washed with sat. aq. NaCl soln. (3 × 10 ml). The org. phase was dried (Na₂SO₄) and evaporated. After purification by CC (AcOEt/CH₂Cl₂/petroleum ether/Et₃N 15:50:35:1), the product was redissolved in anhyd. toluene and precipitated in petroleum ether (50 ml) at –30° under vigorous stirring. A second purification by CC (1% Et₃N/CH₂Cl₂) followed by precipitation in petroleum ether as described above afforded phosphoramidite **17** (89 mg, 43%). White solid material. ³¹P-NMR (CDCl₃): 153.0, 152.5. FAB-MS: 900 (*M*⁺).

1-[5-Azido-3-O-benzyl-4-C-[(benzyloxy)methyl]-5-deoxy-2-O-(methylsulfonyl)-α-L-lyxofuranosyl]thymine (18). A soln. of **11** (1.16 g, 2.35 mmol) in anhyd. pyridine (6 ml) was cooled to 0°, and MsCl (0.36 ml, 4.7 mmol) was added dropwise. The mixture was stirred at r.t. for 2 h, poured into ice-cold H₂O (10 ml) and CH₂Cl₂ (50 ml), and a sat. aq. NaHCO₃ soln. (20 ml) was added. The org. phase was separated and washed successively

with sat. aq. NaHCO_3 soln. (3×25 ml) and ice-cold 1M HCl (3×25 ml), dried (Na_2SO_4), and evaporated to give **18** as a red foam which was used directly in the next step without further purification. $^1\text{H-NMR}$ (CDCl_3): 9.28 (br. s, NH); 7.52 (d , $J = 1.2$, $\text{H-C}(6)$); 7.35–7.22 (m , 10 H, Bn); 6.13 (d , $J = 3.9$, $\text{H-C}(1')$); 5.35 (dd , $J = 4.1$, 5.5, $\text{H-C}(2')$); 4.87 (d , $J = 11.4$, 1 H, Bn); 4.54–4.47 (m , 3 H, Bn); 4.41 (d , $J = 5.7$, $\text{H-C}(3')$); 3.85 (d , $J = 10.4$, $\text{H}_b\text{-C}(5')$); 3.73 (d , $J = 13.4$, 1 H, $\text{CH}_2\text{-C}(4')$); 3.49 (d , $J = 10.4$, $\text{H}_a\text{-C}(5')$); 3.39 (d , $J = 13.5$, 1 H, $\text{CH}_2\text{-C}(4')$); 3.15 (s , MeSO_2); 1.49 (s , Me). $^{13}\text{C-NMR}$ (CDCl_3): 163.7 ($\text{C}(4)$); 150.7 ($\text{C}(2)$); 136.9, 135.4 (Bn); 128.5, 128.4, 127.8 ($\text{C}(6)$, Bn); 111.6 ($\text{C}(5)$); 87.7, 87.5 ($\text{C}(1')$, $\text{C}(4')$); 79.5, 76.3, 74.1, 73.7, 70.8 ($\text{C}(2')$, $\text{C}(3')$, $\text{C}(5')$, Bn); 52.7 ($\text{CH}_2\text{-C}(4')$); 38.8 (MeSO_2); 11.8 (Me). FAB-MS: 572 ($[M + \text{H}]^+$).

1-[5-Azido-3-O-benzyl-4-C-[(benzyloxy)methyl]-5-deoxy- α -L-xylofuranosyl]thymine (19). Nucleoside **18** was stirred in 90% aq. EtOH soln. (80 ml), and 1N NaOH (5.17 ml, 5.17 mmol) was added. The mixture was heated under reflux for 30 min, cooled to r.t., neutralized with AcOH and evaporated. The resulting oil was redissolved in CH_2Cl_2 (50 ml), and a sat. aq. NaHCO_3 soln. (50 ml) was added. The org. phase was washed with sat. aq. NaHCO_3 soln. (3×30 ml), dried (Na_2SO_4), and evaporated. Purification by CC (1.5% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) afforded **19** (1.09 g, 94%). White solid material. $^1\text{H-NMR}$ (CDCl_3): 10.8 (br. s, NH); 7.45 (d , $J = 0.9$, $\text{H-C}(6)$); 7.39–7.26 (m , 10 H, Bn); 6.24 (d , $J = 3.8$, $\text{H-C}(1')$); 4.97 (d , $J = 5.1$, $\text{OH-C}(2')$); 4.84 (m , $\text{H-C}(2')$); 4.72 (d , $J = 11.8$, 1 H, Bn); 4.56 (s , 1 H, Bn); 4.55 (s , 1 H, Bn); 4.49 (d , $J = 11.5$, 1 H, Bn); 4.14 (d , $J = 2.1$, $\text{H-C}(3')$); 3.73 (d , $J = 9.8$, $\text{H}_b\text{-C}(5')$); 3.71 (d , $J = 9.8$, $\text{H}_a\text{-C}(5')$); 3.66 (d , $J = 12.8$, 1 H, $\text{CH}_2\text{-C}(4')$); 3.49 (d , $J = 12.7$, 1 H, $\text{CH}_2\text{-C}(4')$); 1.61 (s , Me). $^{13}\text{C-NMR}$ (CDCl_3): 165.7 ($\text{C}(4)$); 150.8 ($\text{C}(2)$); 138.7, 137.5, 137.3 ($\text{C}(6)$, Bn); 128.5, 128.4, 128.1, 128.0, 127.7 (Bn); 108.2 ($\text{C}(5)$); 86.6, 86.0, 83.7 ($\text{C}(1')$, $\text{C}(2')$, $\text{C}(4')$); 73.9, 73.5, 72.0, 69.9 ($\text{C}(3')$, $\text{C}(5')$, Bn); 51.7 ($\text{CH}_2\text{-C}(4')$); 12.0 (Me). FAB-MS: 494 ($[M + \text{H}]^+$). Anal. calc. for $\text{C}_{25}\text{H}_{27}\text{N}_5\text{O}_6$: C 60.84, H 5.51, N 14.19; found: C 60.81, H 5.52, N 14.05.

1-[5-Azido-3-O-benzyl-4-C-[(benzyloxy)methyl]-5-deoxy-2-O-[(trifluoromethyl)sulfonyl]- α -L-xylofuranosyl]thymine (20). A soln. of **19** (450 mg, 0.911 mmol) in anhyd. CH_2Cl_2 (20 ml) was cooled to 0° , 4-(dimethylamino)pyridine (DMAP; 445 mg, 3.64 mmol) and anhyd. pyridine (0.74 ml, 9.1 mmol) were added, followed by dropwise addition of trifluoromethanesulfonic anhydride (0.33 ml, 2.0 mmol). The mixture was stirred at r.t. for 2 h and poured into an ice-cold sat. aq. NaHCO_3 soln. (10 ml). The org. phase was washed successively with 1M ice-cold HCl (2×20 ml) and sat. aq. NaHCO_3 soln. (2×20 ml), dried (Na_2SO_4), and evaporated. Purification by CC (silica gel, 1.5% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) afforded **20** (437 mg, 77%). Yellowish solid material which was used directly in the next step without further purification. $^1\text{H-NMR}$ (CDCl_3): 8.71 (br. s, NH); 7.40–7.21 (m , 11 H, $\text{H-C}(6)$, Bn); 6.39 (d , $J = 4.4$, $\text{H-C}(1')$); 5.54 (t , $J = 4.2$, $\text{H-C}(2')$); 4.81 (d , $J = 12.0$, 1 H, Bn); 4.56–4.47 (m , 3 H, $\text{H-C}(3')$, Bn); 4.52 (d , $J = 11.6$, 1 H, Bn); 3.67 (d , $J = 12.7$, 1 H, $\text{CH}_2\text{-C}(4')$); 3.54–3.44 (m , 2 H, $\text{C}(5')$); 3.43 (d , $J = 12.9$, 1 H, $\text{CH}_2\text{-C}(4')$); 1.76 (d , $J = 1.0$, Me). FAB-MS: 626 ($[M + \text{H}]^+$).

1-[5-Azido-3-O-benzyl-4-C-[(benzyloxy)methyl]-2,5-dideoxy-2-fluoro- α -L-lyxofuranosyl]thymine (21) and 1-[4-C-(Azidomethyl)-3,5-di-O-benzyl-2,5-dideoxy-D-erythro-pent-1-enofuranosyl]thymine (22). To a soln. of **20** (437 mg, 0.70 mmol) in anhyd. THF (10 ml) at 0° , Bu_4NF (2.38 g, dried for 48 h at 0.3 Torr at 40° , ca. 9.11 mmol) in anhyd. THF (4 ml) was added slowly, and the mixture was stirred for 24 h at 5° . The mixture was evaporated, the resulting oil redissolved in AcOEt (20 ml), and the soln. washed with sat. aq. NaCl soln. (3×20 ml), dried (Na_2SO_4), and evaporated. Purification by CC (1. 10–25% AcOEt/petroleum ether; 2. 0–2% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) afforded **21** (130 mg, 37%). White solid material. $^1\text{H-NMR}$ (CDCl_3): 8.28 (br. s, NH); 7.43 (d , $J = 1.1$, $\text{H-C}(6)$); 7.40–7.22 (m , 10 H, Bn); 6.20 (dd , $J = 3.5$, 15.6, $\text{H-C}(1')$); 5.18 (ddd , $J = 3.6$, 5.0, 52.9, $\text{H-C}(2')$); 4.82 (d , $J = 11.7$, 1 H, Bn); 4.58–4.49 (m , 3 H, Bn); 4.37 (dd , $J = 5.0$, 14.9, $\text{H-C}(3')$); 3.84 (d , $J = 10.5$, $\text{H}_b\text{-C}(5')$); 3.69 (d , $J = 13.3$, 1 H, $\text{CH}_2\text{-C}(4')$); 3.57 (d , $J = 10.5$, $\text{H}_a\text{-C}(5')$); 3.43 (d , $J = 13.3$, 1 H, $\text{CH}_2\text{-C}(4')$); 1.57 (s , Me). $^{13}\text{C-NMR}$ (CDCl_3): 163.9 ($\text{C}(4)$); 150.4 ($\text{C}(2)$); 137.0, 136.9, 136.0 ($\text{C}(6)$, Bn); 128.7, 128.6, 128.3, 128.2, 128.0, 127.8 (Bn); 111.3 ($\text{C}(5)$); 92.3 (d , $J = 194$, $\text{C}(2')$); 88.3 (d , $J = 34$, $\text{C}(1')$); 87.1 ($\text{C}(4')$); 76.4 (d , $J = 14.7$, $\text{C}(3')$); 73.7, 73.5, 70.9 (2s and d (73.5), $J = 2.1$, $\text{C}(5')$, Bn); 52.6 (d , $J = 3.2$, $\text{CH}_2\text{-C}(4')$); 11.8 (Me). FAB-MS: ($[M + \text{H}]^+$). Anal. calc. for $\text{C}_{25}\text{H}_{26}\text{FN}_5\text{O}_5$: C 60.60, H 5.29, N 14.13; found: C 60.71, H 5.06, N 13.96.

In addition, the less polar derivative **22** was isolated as a yellowish gum (100 mg, 30%). $^1\text{H-NMR}$ (CDCl_3): 9.33 (br. s, NH); 7.50 (d , $J = 0.9$, $\text{H-C}(6)$); 7.39–7.26 (m , 10 H, Bn); 5.73 (d , $J = 2.4$, $\text{H-C}(2')$); 4.78 (d , $J = 2.7$, $\text{H-C}(3')$); 4.60 (d , $J = 11.6$, 1 H, Bn); 4.58 (s , 1 H, Bn); 4.56 (s , 1 H, Bn); 4.50 (d , $J = 11.9$, 1 H, Bn); 3.91 (d , $J = 12.9$, 1 H, $\text{CH}_2\text{-C}(4')$); 3.74 (d , $J = 9.5$, $\text{H}_b\text{-C}(5')$); 3.68 (d , $J = 13.2$, 1 H, $\text{CH}_2\text{-C}(4')$); 3.40 (d , $J = 9.3$, $\text{H}_a\text{-C}(5')$); 1.94 (d , $J = 0.9$, Me). $^{13}\text{C-NMR}$ (CDCl_3): 163.0 ($\text{C}(4)$); 148.1, 146.1 ($\text{C}(2)$, $\text{C}(1')$); 137.7, 137.3 (Bn); 134.4 ($\text{C}(6)$); 128.6, 128.5, 128.4, 128.3, 127.9, 127.8, 127.7, 127.6 (Bn); 111.5 ($\text{C}(5)$); 88.5, 88.4, 83.1 ($\text{C}(2')$, $\text{C}(3')$, $\text{C}(4')$); 73.6, 71.3, 68.5 ($\text{C}(5')$, Bn); 50.3 ($\text{CH}_2\text{-C}(4')$); 12.4 (Me). FAB-MS: 476 ($[M + \text{H}]^+$). Due to the instability of **22**, elemental analysis could not be obtained.

1-[5-Amino-3-O-benzyl-4-C-[(benzyloxy)methyl]-2,5-dideoxy-2-fluoro- α -L-lyxofuranosyl]thymine (23). As described for **13**, with **21** (390 mg, 0.79 mmol), abs. EtOH/anhyd. dioxane 1:4 (11 ml), and Lindlar catalyst

(120 mg). Purification by CC (2–5% MeOH/CH₂Cl₂) afforded **23** (240 mg, 64%). White solid material. ¹H-NMR (CDCl₃): 7.45 (*d*, *J* = 1.4, H–C(6)); 7.38–7.23 (*m*, 10 H, Bn); 6.26 (*dd*, *J* = 4.0, 15.1, H–C(1')); 5.28–5.08 (*ddd*, *J* = 4.4, 5.1, 52.1, H–C(2')); 4.85 (*d*, *J* = 11.6, 1 H, Bn); 4.53–4.50 (*m*, 3 H, Bn); 4.36 (*dd*, *J* = 5.3, 12.8, H–C(3')); 3.90 (*br. s.*, NH₂); 3.77 (*d*, *J* = 10.5, H_b–C(5')); 3.61 (*d*, *J* = 10.4, H_a–C(5')); 3.00 (*d*, *J* = 13.7, 1 H, CH₂–C(4')); 2.90 (*d*, *J* = 13.9, 1 H, CH₂–C(4')); 1.58 (*d*, *J* = 0.8, Me). ¹³C-NMR (CDCl₃): 164.0 (C(4)); 150.6 (C(2)); 137.3, 135.9 (C(6), Bn); 128.7, 128.6, 128.2, 128.1, 127.9, 127.7 (Bn); 111.3 (C(5)); 92.7 (*d*, *J* = 195, C(2')); 87.9 (C(4')); 87.5 (*d*, *J* = 33, C(1')); 77.1 (*d*, *J* = 14, C(3')); 73.6, 72.2 (C(5'), Bn); 44.1 (CH₂–C(4')); 11.9 (Me). FAB-MS: 470 ([*M* + H]⁺). Anal. calc. for C₂₅H₂₈FN₃O₅ · 0.25 H₂O: C 63.35, H 6.06, N 8.86; found: C 63.46, H 5.95, N 8.85.

1-[3-O-Benzyl-4-C-[(benzyloxy)methyl]-2,5-dideoxy-2-fluoro-5-[(trifluoroacetyl)amino]-α-L-lyxofuranosyl]-thymine (24). As described for **14**, with **23** (230 mg, 0.49 mmol), anhyd. CH₂Cl₂ (6 ml), ethyl trifluoroacetate (0.18 ml, 1.48 mmol), and Et₃N (0.14 ml, 0.98 mmol). Purification by CC (1% MeOH/CH₂Cl₂) afforded **24** (248 mg, 89%). White solid material. ¹H-NMR (CDCl₃): 9.55 (*br. s.*, NH); 7.40–7.22 (*m*, 11 H, H–C(6), Bn); 7.10 (*t*, *J* = 6.3, NHCOCF₃); 5.90 (*dd*, *J* = 2.3, 19.3, H–C(1')); 5.42–5.21 (*ddd*, *J* = 2.3, 5.3, 54.0, H–C(2')); 4.79 (*d*, *J* = 11.5, 1 H, Bn); 4.54–4.45 (*m*, 4 H, H–C(3'), Bn); 3.77–3.67 (*m*, 3 H, 2 H_b–C(5'), CH₂–C(4')); 3.57 (*d*, *J* = 10.5, 1 H, CH₂–C(4')); 1.67 (*d*, *J* = 1.2, Me). ¹³C-NMR (CDCl₃): 164.0 (C(4)); 157.8 (*q*, *J* = 37, COCF₃); 150.3 (C(2)); 137.4, 136.9, 136.6 (C(6), Bn); 128.7, 128.6, 128.5, 128.4, 128.2, 128.0, 127.8 (Bn); 115.8 (*q*, *J* = 288, COCF₃); 111.5 (C(5)); 92.0 (*d*, *J* = 192, C(2')); 91.1 (*d*, *J* = 36, C(1')); 86.3 (C(4')); 77.6 (C(3')); 73.7, 73.5, 72.1 (2*s* and *d* (73.5), *J* = 2.0, CH₂–C(4'), Bn); 41.4 (C(5')); 11.8 (Me). FAB-MS: 566 ([*M* + H]⁺). Anal. calc. for C₂₇H₂₇F₃N₃O₆: C 57.34, H 4.81, N 7.43; found: C 57.37, H 4.74, N 7.37.

1-[2,5-Dideoxy-2-fluoro-5-[(trifluoroacetyl)amino]-α-L-lyxofuranosyl]-thymine (25). As described for **15**, with **24** (242 mg, 0.43 mmol), abs. EtOH (7 ml), and 5% Pd/C (110 mg). Purification by CC (2–5% MeOH/CH₂Cl₂) afforded **25** (154 mg, 92%). White solid material. ¹H-NMR (CD₃OD): 7.82 (*d*, *J* = 1.1, H–C(6)); 6.17 (*dd*, *J* = 3.1, 17.2, H–C(1')); 5.31–5.10 (*ddd*, *J* = 3.2, 5.4, 53.8, H–C(2')); 4.64 (*dd*, *J* = 5.6, 17.2, H–C(3')); 3.75–3.38 (*m*, 2 H–C(5'), CH₂–C(4')); 1.67 (*d*, *J* = 1.0, Me). ¹³C-NMR (CD₃OD): 166.6 (C(4)); 159.5 (*q*, *J* = 37, COCF₃); 152.4 (C(2)); 138.3 (C(6)); 117.6 (*q*, *J* = 287, COCF₃); 111.8 (C(5)); 95.0 (*d*, *J* = 190, C(2')); 89.7 (*d*, *J* = 34, C(1')); 88.8 (C(4')); 71.2 (*d*, *J* = 15, C(3')); 63.7 (CH₂–C(4')); 41.7 (*d*, *J* = 4.1, C(5')); 12.3 (Me). FAB-MS: 386 ([*M* + H]⁺). Anal. calc. for C₁₃H₁₃F₃N₃O₆ · 0.25 H₂O: C 40.06, H 4.01, N 10.78; found: C 40.09, H 3.89, N 10.48.

1-[2,5-Dideoxy-5-O-(4,4'-dimethoxytrityl)-2-fluoro-5-[(trifluoroacetyl)amino]-α-L-lyxofuranosyl]-thymine (26). As described for **16**, with **25** (145 mg, 0.38 mmol), anhyd. pyridine (4 ml), and (MeO)₂TrCl (206 mg, 0.61 mmol). Purification by CC (1% MeOH/CH₂Cl₂ containing 0.5% of pyridine) afforded **26** (246 mg, 87%). Yellowish solid material. ¹H-NMR (CDCl₃): 9.69 (*br. s.*, NH); 7.38–7.14 (10 H, (MeO)₂Tr, H–C(6)); 7.09 (*t*, *J* = 6.3, NHCOCF₃); 6.82 (*dd*, *J* = 3.6, 9.2, 4 H, (MeO)₂Tr); 6.05 (*dd*, *J* = 4.0, 16.8, H–C(1')); 5.44–5.23 (*ddd*, *J* = 4.4, 4.6, 54.1, H–C(2')); 4.65 (*dd*, *J* = 5.5, 11.9, H–C(3')); 4.30 (*br. s.*, OH–C(3')); 3.76, 3.77 (2*s*, 2 MeO); 3.72–3.62 (*m*, 2 H–C(5')); 3.41 (*d*, *J* = 10.8, 1 H, CH₂–C(4')); 3.34 (*d*, *J* = 10.5, 1 H, CH₂–C(4')); 1.60 (*s*, Me). ¹³C-NMR (CDCl₃): 164.0 (C(4)); 158.9 ((MeO)₂Tr); 159.5 (*q*, *J* = 37, COCF₃); 150.6 (C(2)); 144.0 ((MeO)₂Tr); 136.9, 136.5, 134.8 (C(6), (MeO)₂Tr); 130.1, 129.1, 128.3, 128.1, 125.3 ((MeO)₂Tr); 115.7 (*q*, *J* = 288, COCF₃); 113.3 ((MeO)₂Tr); 112.0 (C(5)); 92.7 (*d*, *J* = 191, C(2')); 89.1 (*d*, *J* = 33, C(1')); 87.6, 86.8 (C(4'), C(Ar₃)); 71.8 (*d*, *J* = 16, C(3')); 66.0 (CH₂–C(4')); 55.1 (2 MeO); 41.1 (C(5')); 11.6 (Me). FAB-MS: 687 (*M*⁺). Anal. calc. for C₃₄H₃₃F₃N₃O₈ · 0.6 C₂H₆: C 61.75, H 5.13, N 5.66; found: C 61.96, H 5.16, N 5.89.

1-[5-Deoxy-4-C-[(4,4'-dimethoxytrityl)oxy]methyl]-2-fluoro-5-[(trifluoroacetyl)amino]-α-L-lyxofuranosyl]-thymine 3'-(2-Cyanoethyl Diisopropylphosphoramidite) (27). As described for **17**, with **26** (246 mg, 0.36 mmol), anhyd. CH₂Cl₂ (4 ml), ¹Pr₂EtN (0.16 ml, 0.94 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (0.11 ml, 0.47 mmol). After purification by CC (1% Et₃N/CH₂Cl₂), the product was redissolved in anhyd. toluene and precipitated in vigorously stirred petroleum ether (40 ml) at –30°. A second purification by CC (0.5% Et₃N/CH₂Cl₂) afforded **27** (245 mg, 76%). White solid material. ³¹P-NMR (CDCl₃): 154.2 (*dd*, *J* = 9.4, 18.2); 152.9 (*d*, *J* = 18.7). FAB-MS: 888 ([*M* + H]⁺).

1-[3,5-Di-O-benzyl-3-C-[3-(phthalimido)propyl]-β-D-ribofuranosyl]-thymine (30). To a soln. of **29** [19] (3.47 g, 6.99 mmol) in anhyd. THF (10 ml), phthalimide (1.23 g, 8.39 mmol) and PPh₃ (2.29 g, 8.74 mmol) were added, and the mixture was cooled to 0°. A soln. of diethyl diazenedicarboxylate (1.37 ml, 8.67 mmol) in anhyd. THF (3 ml) cooled to 0° was added dropwise within 15 min, and the mixture was stirred for 12 h at r.t. AcOEt (60 ml) was added, and the mixture was washed successively with sat. aq. NaCl soln. (2 × 50 ml) and H₂O (2 × 50 ml). The aq. phases were extracted with AcOEt (2 × 50 ml), and the combined org. phase was dried (Na₂SO₄) and evaporated. Purification by CC (30–90% AcOEt/petroleum ether) afforded **30** (4.34 g), contaminated with PPh₃(O). ¹H-NMR (CDCl₃): 8.35 (*s*, NH); 7.81–7.19 (*m*, Bn, H–C(6), phth, PPh₃(O)); 6.10

(*d*, *J* = 7.8, H-C(1')); 4.51 (*m*, 4 H, Bn); 4.37 (br. *s*, H-C(4')); 4.16 (*dd*, *J* = 7.8, 11.5, H-C(2')), 3.83 (*dd*, *J* = 2.9, 10.7, H_b-C(5')); 3.75 (*t*, *J* = 6.4, 2 H-C(3'')); 3.52 (*d*, *J* = 9.9, H_a-C(5')); 2.92 (*d*, *J* = 11.4, OH-C(2'')); 2.21–1.88 (*m*, 2 H-C(1''), 2 H-C(2'')); 1.44 (3 H, *d*, *J* = 0.8, Me). ¹³C-NMR (CDCl₃): 168.6 (phth); 163.5 (C(4)); 151.1 (C(2)); 137.1, 136.7, 136.1, 134.1, 133.3 (Bn, C(6), phth); 132.2–131.9 (several signals, Bn, phth, PPh₃(O)); 128.8–127.4 (several signals, Bn, phth, PPh₃(O)); 123.3 (phth); 111.3 (C(5)); 87.3, 82.6, 81.0, 79.1 (C(1'), C(2'), C(3'), C(4')); 73.6, 69.8, 65.6 (Bn, C(5')); 38.1 (C(3'')); 27.6, 22.4 (C(1''), C(2'')); 11.7 (Me). FAB-MS: 626 ([*M* + H]⁺). HR-FAB-MS: 626.2584 (C₃₅H₃₆N₃O₈⁺; calc. 626.2502).

1-[3,5-Di-O-benzyl-2-O-(pentafluorophenoxy)thiocarbonyl]-3-C-[3-(phthalimido)propyl]-β-D-ribofuranosyl]thymine (31). To a soln. of **30** (3.96 g, contaminated with PPh₃(O)) in anh. CH₂Cl₂ (20 ml) under Ar, DMAP (1.39 g, 11.4 mmol) was added and the soln. cooled to –15°. *O*-(Pentafluorophenyl) carbonochloridothioate (1.32 ml, 8.2 mmol) was added dropwise, and the soln. was stirred for 12 h at r.t. The mixture was poured into ice-cold H₂O (70 ml), stirred for 30 min, and filtered through a thin layer of *Celite*. The mixture was successively washed with H₂O (30 ml), sat. aq. CuSO₄ soln. (2 × 50 ml), and sat. aq. NaCl soln. (2 × 25 ml). All the aq. phases were extracted with CH₂Cl₂ (25 ml each), and the combined org. phase was dried (Na₂SO₄) and evaporated. Purification by CC (0–3% MeOH/CH₂Cl₂ containing 0.25% of pyridine) afforded **31** (1.23 g, 27%, two steps) as a yellowish solid material. In addition, a fraction (1.78 g) consisting of unreacted starting material and PPh₃(O) was isolated. **31**: ¹H-NMR (CDCl₃): 8.47 (*s*, NH); 7.78–7.62 (*m*, 5 H, H-C(6), phth); 7.38–7.24 (*m*, 10 H, Bn); 6.59 (*d*, *J* = 8.1, H-C(2'')); 6.16 (*d*, *J* = 8.0, H-C(1'')); 4.71 (*d*, *J* = 11.4, 1 H, Bn); 4.58 (*d*, *J* = 11.5, 1 H, Bn); 4.54 (*s*, 2 H, Bn); 4.42 (br. *s*, H-C(4'')); 3.92 (*dd*, *J* = 2.4, 11.2, H_b-C(5'')); 3.73–3.61 (*m*, H_a-C(5'), 2 H-C(3'')); 2.10–1.80 (*m*, 2 H-C(1''), 2 H-C(2'')); 1.35 (*d*, *J* = 0.7, Me). ¹³C-NMR (CDCl₃): 191.8 (C=S); 168.4 (phth); 163.6 (C(4)); 150.6 (C(2)); 137.4, 136.4, 135.9, 134.1, 131.9, 128.7, 128.6, 128.3, 127.8, 127.4, 127.1 (Bn, C(6), phth); 123.2 (phth); 111.4 (C(5)); 87.6, 83.9, 83.4, 82.6 (C(1'), C(2'), C(3'), C(4')); 73.6, 69.5, 65.1 (Bn, C(5')); 37.7 (C(3'')); 26.9, 22.3 (C(1''), C(2'')); 11.5 (Me). FAB-MS: 852 ([*M* + H]⁺).

3',5'-Di-O-benzyl-3'-C-[3-(phthalimido)propyl]thymidine (32). Through a soln. of **31** (1.18 g, 1.39 mmol) in anh. benzene (4 ml), Ar was bubbled for 15 min with stirring. Then, 2,2'-azobis[isobutyronitrile] (AIBN; 0.114 g, 0.70 mmol) was added, and bubbling with Ar was continued for further 15 min. The temp. was raised to 90°, Bu₃SnH (0.56 ml, 2.1 mmol) added, and the mixture heated under reflux for 1 h, then allowed to cool to r.t., and subsequently evaporated. Purification by CC (1. petroleum ether, then 0–2% MeOH/CH₂Cl₂; 2. petroleum ether, then 0–1% MeOH/CH₂Cl₂) and prep. TLC (1. 2% MeOH/CH₂Cl₂; 2. 4% MeOH/CH₂Cl₂) afforded **32** (208 mg, 25%). White solid material. ¹H-NMR (CDCl₃): 8.52 (*s*, NH); 7.81–7.61 (*m*, 5 H, H-C(6), phth); 7.36–7.15 (*m*, 10 H, Bn); 6.39 (*dd*, *J* = 5.3, 9.5, H-C(2'')); 4.50–4.38 (*m*, 4 H, Bn); 4.27 (br. *s*, H-C(4'')); 3.82 (*dd*, *J* = 3.0, 11.0, H_b-C(5'')); 3.74–3.67 (*m*, 2 H-C(3'')); 3.56 (*d*, *J* = 9.9, H_a-C(5'')); 2.53 (*dd*, *J* = 5.0, 13.0, H_b-C(2'')); 2.00–1.77 (*m*, H_a-C(2'), 2 H-C(1'')); 1.48 (*s*, Me). ¹³C-NMR (CDCl₃): 168.5 (phth); 163.8 (C(4)); 150.4 (C(2)); 137.8, 136.9, 136.3, 134.1, 131.9, 128.7, 128.5, 128.1, 127.7, 127.4, 123.3 (Bn, C(6), phth); 110.7 (C(5)); 86.4, 84.3, 83.1 (C(1'), C(3'), C(4'')); 73.5, 70.2, 64.1 (Bn, C(5'')); 41.6 (C(2'')); 37.8 (C(3'')); 27.6, 23.5 (C(1''), C(2'')); 11.8 (Me). HR-FAB-MS: 610.2505 (C₃₅H₃₆N₃O₇⁺; calc. 610.2553).

3'-C-[3-(Phthalimido)propyl]thymidine (33). To a soln. of **32** (0.326 mg, 0.53 mmol) in abs. EtOH (1 ml) and anh. dioxane (0.5 ml), 20% Pd(OH)/C (100 mg) was added. The mixture was degassed and H₂ introduced via a balloon. The black soln. was stirred for 3 days under H₂ at r.t. (additional catalyst (100 mg) was added after 24 h and 48 h). Then 10% MeOH/CH₂Cl₂ was added and the mixture stirred for 12 h. After filtration through a *Celite* pad, which was washed thoroughly with MeOH, the filtrate was evaporated. Purification by CC (0–3% MeOH/CH₂Cl₂) afforded **33** (42 mg, 19%). White solid material. ¹H-NMR (CD₃OD): 8.04 (*d*, *J* = 1.0, H-C(6)); 7.83–7.75 (*m*, 4 H, phth); 6.30 (*dd*, *J* = 5.3, 9.3, H-C(1'')); 3.86 (*d*, *J* = 3.0, H-C(4'')); 3.74–3.63 (*m*, 2 H-C(5'), 2 H-C(3'')); 2.19 (*dd*, *J* = 5.4, 12.7, H_b-C(2'')); 2.03 (*dd*, *J* = 9.4, 12.6, H_a-C(2'')); 1.83 (*d*, *J* = 1.2, Me); 1.78–1.71 (*m*, 2 H-C(1''), 2 H-C(2'')). ¹³C-NMR (CD₃OD): 170.1 (phth); 166.6 (C(4)); 152.7 (C(2)); 138.8, 135.5, 133.5 (C(6), phth); 124.2 (phth); 111.4 (C(5)); 90.4, 86.1, 81.8 (C(1'), C(3'), C(4'')); 62.5 (C(5'')); 44.0 (C(2'')); 39.1 (C(3'')); 33.6, 24.6 (C(1''), C(2'')); 12.4 (Me). FAB-MS: 430 ([*M* + H]⁺).

5'-O-(4,4'-Dimethoxytrityl)-3'-C-[3-(phthalimido)propyl]thymidine (34) [20]. To a soln. of **33** (55 mg, 0.13 mmol) in anh. pyridine (1 ml) at r.t., (MeO)₂TrCl (95 mg, 0.28 mmol) was added in one portion, and the mixture was stirred at r.t. for 12 h. MeOH (0.20 ml) was added to quench the reaction, and the mixture was evaporated. The residue was redissolved in CH₂Cl₂ (5 ml) and the org. phase washed with sat. aq. NaCl soln. (3 × 5 ml), dried (Na₂SO₄), and evaporated. Purification by CC (0–2% MeOH/CH₂Cl₂ containing 1% of pyridine) afforded **34** (54 mg, 57%). White solid material. ¹H-NMR (CDCl₃): 9.06 (br. *s*, NH); 7.84–7.63 (*m*, 5 H, H-C(6), phth); 7.38–7.22 (*m*, 9 H, (MeO)₂Tr); 6.83 (*dd*, *J* = 2.2, 9.0, 4 H, (MeO)₂Tr); 6.49 (*dd*, *J* = 5.0, 9.6, H-C(1'')); 4.05 (br. *s*, H-C(4'')); 3.78 (*s*, 2 MeO); 3.61 (*dd*, *J* = 3.5, 10.5, H_b-C(5'')); 3.58–3.47 (*m*, 2 H-C(3'')); 3.14 (*dd*, *J* = 2.4, 10.8, H_a-C(5'')); 2.38 (*dd*, *J* = 5.3, 12.8, H_b-C(2'')); 2.09 (*dd*, *J* = 9.8, 12.4,

$H_a-C(2'')$; 1.75–1.45 (m , 2 $H-C(1'')$, 2 $H-C(2'')$); 1.21 (s , Me). ^{13}C -NMR ($CDCl_3$): 168.6 (phth); 163.9 ($C(4)$); 158.9 ((MeO) $_2$ Tr); 150.7 ($C(2)$); 143.8 ((MeO) $_2$ Tr); 136.2, 136.1, 135.0, 134.8, 134.1, 132.0, 130.3, 130.3, 128.5, 128.0, 127.4, 123.3 ($C(6)$, (MeO) $_2$ Tr, phth); 113.3 ((MeO) $_2$ Tr); 111.3 ($C(5)$); 87.9, 87.3, 84.2, 81.0 ($C(1')$, $C(3')$, $C(4')$, $C(Ar)_3$); 62.7 ($C(5')$); 55.2 (2 MeO); 44.0 ($C(2')$); 37.9 ($C(3'')$); 31.8, 23.4 ($C(1'')$, $C(2'')$); 11.2 (Me). FAB-MS: 731 (M^+). NMR Data: in accordance with [20].

5'-O-(4,4'-Dimethoxytrityl)-3'-C-[3-(phthalimido)propyl]thymidine 3'-(2-Cyanoethyl Diisopropylphosphoramidite) (35). To a mixture of 34 (54 mg, 0.074 mmol), anh. CH_2Cl_2 (2 ml), and iPr_2EtN (0.05 ml, 0.30 mmol) at 0°, 2-cyanoethyl diisopropylphosphoramidochloridite (0.036 ml, 0.15 mmol) was added dropwise, and the mixture was stirred for 12 h at r.t. The reaction was quenched with sat. aq. $NaHCO_3$ soln. (0.1 ml) and diluted with AcOEt (5 ml). The mixture was washed with sat. aq. $NaHCO_3$ soln. (4×5 ml) and the org. phase dried (Na_2SO_4) and evaporated. Purification by CC (AcOEt/ CH_2Cl_2 /petroleum ether 10:35:1:54) afforded 35 (28 mg, 41%). White solid material. ^{31}P -NMR ($CDCl_3$): 141.0, 140.3. FAB-MS: 932 (M^+).

1-[3,5-Di-O-benzyl-3-C-(3-hydroxypropyl)-2-O-methyl- β -D-ribofuranosyl]thymine (37) and 1-[3,5-Di-O-benzyl-3-C-(RS)-2-hydroxypropyl]-2-O-methyl- β -D-ribofuranosyl]thymine (38). To a soln. of 36 [34] (703 mg, 1.43 mmol) in anh. THF (6 ml), 78M BH $_3$ in 1,4-oxathiane (0.22 ml, 1.72 mmol) was added dropwise at r.t., and stirring was continued for 90 min. After cooling to 0° 2M aq. NaOH (0.86 ml, 1.72 mmol) was dropwise added, followed by the addition of 35% aq. H_2O_2 soln. (w/w, 0.2 ml). The mixture was allowed to warm to r.t. and, after stirring for 1 h, poured into ice-cold H_2O (20 ml). The mixture was filtered and extracted with CH_2Cl_2 (3×10 ml). The combined org. phase was washed successively with H_2O (3×5 ml) and sat. aq. $NaHCO_3$ soln. (5 ml), dried (Na_2SO_4), and evaporated. Purification by CC (0–2.5% MeOH/ CH_2Cl_2) afforded as the less polar product 38 (98 mg, 13%) as a diastereoisomer mixture and 37 (400 mg, 54%) as the most polar compound.

Data of mixture 38: 1H -NMR ($CDCl_3$): 8.91 (br. s); 7.82 (d , $J=1.2$); 7.80 (d , $J=1.3$); 7.42–7.26 (m); 6.54 (d , $J=7.8$); 6.40 (d , $J=7.7$); 4.85 (d , $J=11.5$); 4.67–4.51 (m); 4.31 (br. s); 4.21–4.17 (m); 4.00 (d , $J=7.7$); 3.81–3.77 (m); 3.66 (dd , $J=1.6$, 11.0); 3.51–3.34 (m); 3.44 (s , MeO); 3.39 (s , MeO); 3.19 (d , $J=1.5$); 2.05–1.91 (m); 1.83 (br. s); 1.62 (d , $J=1.0$, Me); 1.57 (d , $J=0.9$, Me); 1.23, 1.21 (2s, 2 $H-C(3'')$). ^{13}C -NMR ($CDCl_3$): 163.8, 163.7 (2 $C(4)$); 150.6 (2 $C(2)$); 138.0, 137.8, 136.8, 136.5, 136.2, 136.0 (Bn, 2 $C(6)$); 128.9, 128.8, 128.6, 128.5, 128.0, 127.8, 127.7, 127.6, 127.3, 126.9 (Bn); 111.7, 111.5 (2 $C(5)$); 86.4, 86.3, 85.5, 85.2, 84.7, 84.2, 82.6, 81.5 (2 $C(1')$, 2 $C(2')$, 2 $C(3')$, 2 $C(4')$); 73.7, 73.6, 69.8, 69.5, 65.7, 64.9, 64.4, 62.7, 59.0, 58.2 (2 MeO, Bn, 2 $C(5')$, 2 $C(2'')$); 40.5, 37.9 (2 $C(1'')$); 23.6, 23.2, (2 $C(3'')$); 12.0, 11.9 (2 Me). FAB-MS: 511 ($[M+H]^+$). Anal. calc. for $C_{28}H_{34}N_2O_7 \cdot 0.25 H_2O$: C 65.29, H 6.75, N 5.44; found: C 65.41, H 6.80, N 5.10.

Data of 37: 1H -NMR ($CDCl_3$): 8.75 (br. s, NH); 7.77 (d , $J=1.0$, $H-C(6)$); 7.42–7.25 (m , 10 H, Bn); 6.36 (d , $J=7.9$, $H-C(1')$); 4.92 (d , $J=11.9$, 1 H, Bn); 4.61 (s , 2 H, Bn); 4.59 (d , $J=9.8$, 1 H, Bn); 4.20 (d , $J=2.6$, $H-C(4')$); 4.00 (d , $J=7.9$, $H-C(2')$); 3.78 (dd , $J=2.8$, 11.0, $H_b-C(5')$); 3.65 (m , $H_a-C(5')$, 2 $H-C(3'')$); 3.39 (s , MeO); 2.22–2.16 (m , $H_b-C(2'')$); 2.00–1.78 (m , 2 $H-C(1'')$); 1.62 (m , $H_a-C(2'')$); 1.57 (d , $J=1.0$, Me). ^{13}C -NMR ($CDCl_3$): 163.8 ($C(4)$); 150.7 ($C(2)$); 138.9, 137.0, 136.5 (Bn, $C(6)$); 128.8, 128.4, 128.3, 127.8, 127.4, 127.1 (Bn); 111.3 ($C(5)$); 87.7, 85.7, 83.6, 83.5 ($C(1')$, $C(2')$, $C(3')$, $C(4')$); 73.6, 69.6, 65.6, 62.6 (Bn, $C(5')$, $C(3'')$); 59.4 (MeO); 26.2, 26.1 ($C(1'')$, $C(2'')$); 11.9 (Me). FAB-MS: 511 ($[M+H]^+$). Anal. calc. for $C_{28}H_{34}N_2O_7 \cdot 0.25 H_2O$: C 65.29, H 6.75, N 5.44; found: C 65.36, H 6.66, N 5.32.

1-[3,5-Di-O-benzyl-2-O-methyl-3-C-[3-(phthalimido)propyl]- β -D-ribofuranosyl]thymine (39). To a soln. of 37 (560 mg, 1.10 mmol) in anh. THF (2 ml) under Ar, phthalimide (0.194 g, 1.32 mmol) and PPh_3 (0.362 g, 1.38 mmol) were added, and the mixture was cooled to 0°. A soln. of diethyl diazenedicarboxylate (0.24 ml, 1.36 mmol) in anh. THF (0.3 ml) cooled to 0° was added dropwise during 15 min, and the mixture was stirred for 12 h at r.t. AcOEt (10 ml) was added and the mixture washed successively with a sat. aq. NaCl soln. (2×10 ml) and H_2O (2×10 ml). The aq. phases were extracted with AcOEt (2×5 ml). The combined org. phase was dried (Na_2SO_4) and evaporated. Purification by CC (40–80% AcOEt/petroleum ether) afforded 39 (140 mg, 20%), a fraction (417 mg) consisting of 39 and $PPh_3(O)$, and a fraction (137 mg) consisting of unreacted 37 and $PPh_3(O)$. 1H -NMR ($CDCl_3$): 8.69 (br. s, NH); 7.80–7.63 (m , 5 H, $H-C(6)$, phth); 7.36–7.20 (m , 10 H, Bn); 6.36 (d , $J=8.0$, $H-C(1')$); 4.89 (d , $J=11.6$, 1 H, Bn); 4.57 (d , $J=11.6$, 1 H, Bn); 4.48 (d , $J=11.6$, 1 H, Bn); 4.42 (d , $J=11.5$, 1 H, Bn); 4.18 (br. s, $H-C(4')$); 3.94 (d , $J=8.0$, $H-C(2')$); 3.73 (m , $H_b-C(5')$, 2 $H-C(3'')$); 3.57 (d , $J=10.8$, $H_a-C(5')$); 3.36 (s , MeO); 2.20–1.80 (m , 2 $H-C(1'')$, 2 $H-C(2'')$); 1.48 (s , Me). ^{13}C -NMR ($CDCl_3$): 168.5 (phth); 163.7 ($C(4)$); 150.6 ($C(2)$); 138.6, 136.8, 136.2, 134.1, 131.9, 128.8, 128.4, 128.3, 127.5, 127.4, 127.2 (Bn, $C(6)$, phth); 123.3 (phth); 111.3 ($C(5)$); 87.5, 85.5, 83.3 ($C(1')$, $C(2')$, $C(3')$, $C(4')$); 73.5, 69.8, 65.8 (Bn, $C(5')$); 59.3 (MeO); 38.0 ($C(3'')$); 27.2, 22.5 ($C(1'')$, $C(2'')$); 11.8 (Me). FAB-MS: 640 ($[M+H]^+$). Anal. calc. for $C_{36}H_{37}N_3O_8 \cdot 0.5 H_2O$: C 66.65, H 5.90, N 6.48; found: C 66.69, H 5.84, N 6.53.

1-[2-O-Methyl-3-C-[3-(phthalimido)propyl]- β -D-ribofuranosyl]thymine (40). To a soln. of 39 (120 mg, 0.19 mmol) in anh. CH_2Cl_2 (5 ml) at -78° , 1M BCl_3 in hexane (0.75 ml, 0.75 mmol) was added dropwise during

15 min with stirring. After 2 h, the mixture was allowed to slowly warm up to -20° , and after further stirring for 6 h, MeOH (3 ml) was added. The mixture was stirred for 12 h at r.t. followed by evaporation and co-evaporation several times with MeOH. Purification by CC (0–4% MeOH/ CH_2Cl_2) afforded **40** (73 mg, 85%). White solid material. $^1\text{H-NMR}$ ((D_6) DMSO): 11.3 (br. s, NH); 7.97 (d, $J=0.8$, H–C(6)); 7.90–7.81 (m, 4 H, phth); 5.94 (d, $J=7.9$, H–C(1')); 5.24 (t, $J=3.8$, OH–C(5')); 4.81 (s, OH–C(3')); 3.76 (d, $J=7.4$, H–C(2')); 3.73 (br. s, H–C(4')); 3.61–3.53 (m, 2 H–C(5'), 2 H–C(3'')); 3.23 (s, MeO); 1.76 (d, $J=0.8$, Me); 1.81–1.68 (m, 2 H–C(1''), 2 H–C(2'')). $^{13}\text{C-NMR}$ ((D_6) DMSO): 168.4 (phth); 164.0 (C(4)); 151.2 (C(2)); 136.9, 134.7, 132.0, 123.4 (C(6), phth); 110.2 (C(5)); 86.8, 85.6, 85.3, 78.4 (C(1'), C(2'), C(3'), C(4')); 61.0, 58.6 (MeO, C(5')); 38.4 (C(3'')); 31.0, 22.8 (C(1''), C(2'')); 12.7 (Me). HR-FAB-MS: 460.1780 ($\text{C}_{22}\text{H}_{26}\text{N}_3\text{O}_8$; calc. 460.1720).

1-[5-O-(4,4'-Dimethoxytrityl)-2-O-methyl-3-C-{3-(phthalimido)propyl}- β -D-ribofuranosyl]thymine (41). To a soln. of **40** (85 mg, 0.19 mmol) in anhyd. pyridine (1.5 ml) at r.t., $(\text{MeO})_2\text{TrCl}$ (125 mg, 0.37 mmol) was added in one portion, and the mixture was stirred for 12 h, whereupon MeOH (0.25 ml) was added to quench the reaction. After stirring for 10 min, the mixture was evaporated, the residue dissolved in CH_2Cl_2 (5 ml), and the soln. washed with sat. aq. NaCl soln. (3×5 ml), dried (Na_2SO_4), and evaporated. Purification by CC (0–1.5% MeOH in CH_2Cl_2 /pyridine 99:1) afforded **41** (117 mg, 77%). Yellowish solid material. $^1\text{H-NMR}$ (CDCl_3): 8.73 (br. s, NH); 7.83–7.69 (m, 5 H, H–C(6), phth); 7.40–7.16 (m, 9 H, $(\text{MeO})_2\text{Tr}$); 6.86 (dd, $J=1.6$, 9.1, 4 H, $(\text{MeO})_2\text{Tr}$); 6.19 (d, $J=6.6$, H–C(1')); 4.10 (br. s, H–C(4')); 3.91 (d, $J=6.8$, H–C(2')); 3.80, 3.79 (2s, 2 MeO); 3.70 (dd, $J=3.0$, 10.9, H_b–C(5')); 3.51 (s, MeO); 3.41–3.24 (m, H_a–C(5'), 2 H–C(3'')); 2.87 (s, OH–C(3')); 1.69–1.50 (m, 2 H–C(1''), 2 H–C(2'')); 1.14 (d, $J=0.8$, Me). $^{13}\text{C-NMR}$ (CDCl_3): 168.4 (phth), 163.7 (C(4)); 159.1 ($(\text{MeO})_2\text{Tr}$); 150.6 (C(2)); 143.5 ($(\text{MeO})_2\text{Tr}$); 136.1, 134.6, 134.0, 132.1, 130.5, 129.1, 128.3, 128.1, 127.6, 136.1, 125.4, 123.3 (C(6), $(\text{MeO})_2\text{Tr}$, phth); 113.3 ($(\text{MeO})_2\text{Tr}$); 111.8 (C(5)); 87.7, 86.2, 85.7, 85.1, 78.7 (C(1'), C(2'), C(3'), C(4'), C(Ar)); 62.2 (C(5')); 59.1 (MeO); 55.2 (2 MeO); 37.9 (C(3'')); 30.5, 22.6 (C(1''), C(2'')); 11.0 (Me). FAB-MS: 761 (M^+). Anal. calc. for $\text{C}_{43}\text{H}_{43}\text{N}_3\text{O}_{10} \cdot 0.6 \text{ C}_7\text{H}_8$: C 69.41, H 5.90, N 5.14; found: C 69.30, H 6.07, N 5.03.

1-[5-O-(4,4'-Dimethoxytrityl)-2-O-methyl-3-C-{3-(phthalimido)propyl}- β -D-ribofuranosyl]thymine 3'-(2-Cyanoethyl Diisopropylphosphoramidite) (42). To a soln. of **41** (109 mg, 0.14 mmol) in anhyd. CH_2Cl_2 (2 ml) and Pr_2EtN (0.20 ml, 1.20 mmol) under Ar at 0° , 2-cyanoethyl diisopropylphosphoramidochloridite (0.14 ml, 0.58 mmol) was added dropwise, and the mixture was stirred for 12 h at r.t. The reaction was quenched by addition of sat. aq. NaHCO_3 soln. (0.1 ml), and the resulting mixture was diluted with AcOEt (5 ml) and washed with a sat. aq. NaHCO_3 soln. (4×5 ml). The org. phase was dried (Na_2SO_4) and evaporated. After purification by CC (1.0–1% $\text{Et}_3\text{N}/\text{CH}_2\text{Cl}_2$; 2. AcOEt/ $\text{CH}_2\text{Cl}_2/\text{Et}_3\text{N}$ /petroleum ether 15:45:1:39; 3. AcOEt/ $\text{CH}_2\text{Cl}_2/\text{Et}_3\text{N}$ /petroleum ether 15:35:1:49), the residue was dissolved in anhyd. toluene (1 ml) and precipitated in vigorously stirred petroleum ether (250 ml) at -50° : **42** (45 mg, 34%). White solid material. $^{31}\text{P-NMR}$ (CDCl_3): 141.3, 141.2. FAB-MS: 984 ($[M + \text{Na}]^+$).

Oligonucleotide Synthesis. All ONs were prepared on a *Biosearch 8700* DNA synthesizer by the phosphoramidite approach as described earlier [61] with *tert*-butyl hydroperoxide as oxidant for ONs containing units **Z** or **W**, and with a mixture of I_2 , pyridine, and H_2O as oxidant for ONs containing units **X** or **Y**. After completion of the sequences, deprotection with conc. NH_3 in MeOH (32% (w/w), 55° , 12 h) of 5'-O- $(\text{MeO})_2\text{Tr}$ -on ONs and reversed-phase cartridge purification yielded the final ON products, which by capillary gel electrophoresis were shown to be $>85\%$ pure. The composition of the modified ONs was verified by MALDI-MS analysis.

Thermal Stability Studies. Melting temperatures (T_m values) were determined as described earlier [25]. A medium salt buffer and a high salt buffer with compositions as indicated in Table 1 were used.

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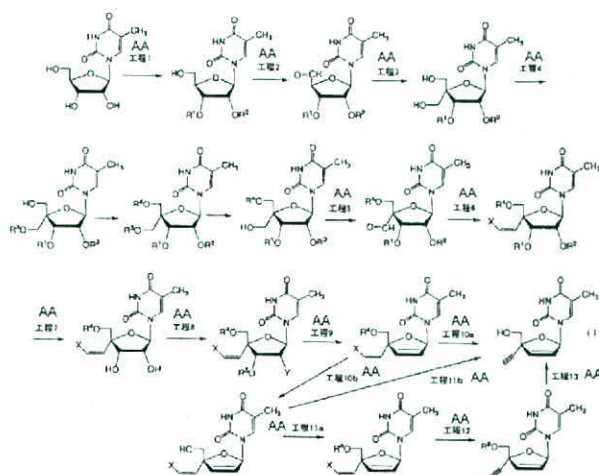
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[続葉有]

(54) Title: PROCESS FOR PRODUCTION OF ETHYNYLTHYMIDINE COMPOUND USING 5-METHYLURIDINE AS STARTING RAW MATERIAL

(54) 発明の名称: 5-メチルウリジンを出発原料とするエチニルチミン化合物の製造方法



AA Process

(57) Abstract: Disclosed is a process for producing 2',3'-didehydro-3'-deoxy-4'-ethynylthymidine, which is a compound useful as a pharmaceutical agent, with high efficiency and in an industrially advantageous manner. Specifically disclosed is a process for producing 2',3'-didehydro-3'-deoxy-4'-ethynylthymidine, which is represented by scheme (I). [In the scheme, R¹ and R² independently represent a protective group for a hydroxy group, or R¹ and R² together form a protective group for a hydroxy group; R³ and R⁴ independently represent a protective group for a hydroxy group; R⁵ represents a protective group for a hydroxy group; R⁶ represents a protective group for a hydroxy group; X represents a leaving group; and Y represents a halogen atom.]

(57) 要約: 本発明は、医薬として有用な 2',3'-ジデヒドロ-3'-デオキシ-4'-エチニルチミンを効率的にかつ工業的に製造する方法であり、具体的には、下記式で示される 2',3'-ジデヒドロ-3'-デオキシ-4'-エチニルチミンの製造方法を提供する。(式中、R¹およびR²はそれぞれ独立して又はR¹とR²が一体となって、ヒドロキシ基の保護基を表し、R³およびR⁴はヒドロキシ基の保護基を表し、R⁵はヒドロキシ基の保護基を表し、R⁶はヒドロキシ基の保護基を表し、Xは脱離基を表し、Yはハロゲン原子を表す。)

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WO 2009/125841 A1



CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, — 補正された請求の範囲 (条約第 19 条(1))
TG).

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Sato et al.

(10) **Patent No.:** US 8,445,669 B2
(45) **Date of Patent:** May 21, 2013

(54) **PRODUCTION PROCESS OF
ETHYNYLTHYMIDINE COMPOUNDS FROM
5-METHYLURIDINE AS A STARTING
MATERIAL.**

(75) **Inventors:** Tatsunori Sato, Hyogo (JP); Tetsuya
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(73) **Assignee:** Hamari Chemicals, Ltd., Osaka (JP)

(*) **Notice:** Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
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C07H 19/167 (2006.01)

(52) **U.S. Cl.**
USPC 536/28.2; 536/28.54

(58) **Field of Classification Search**
None
See application file for complete search history.

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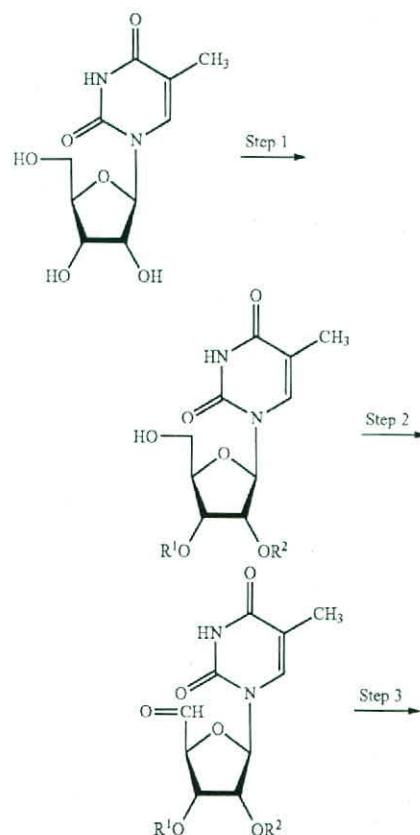
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Primary Examiner — Lawrence E Crane

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(57) **ABSTRACT**

The present invention provides a process for producing 2',3'-
didehydro-3'-deoxy-4'-ethynylthymidine, which is useful as
a medicine, in an efficient and industrially advantageous manner,
and more specifically, provides a process for producing
2',3'-didehydro-3'-deoxy-4'-ethynylthymidine as shown
below.



SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

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