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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
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Señor
SUPERINTENDENTE DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES
E. S. D.

HM

Trámite: 11
Evento: 378
Actuación: 444

Re: PI.-**F. HOFFMANN-LA ROCHE AG**.-Solicitud de Patente de Invención en Fase Nacional PCT para **"DERIVADOS DE LOS NUCLEÓSIDOS SUSTITUÍDOS EN 4'-AZIDO, 3'-FLUORO COMO INHIBIDORES DE LA REPLICACIÓN DEL RNA DEL VHC"**.-Clase **C07H 19/06**.-Expediente número **14-133.951**.-

1. Me refiero al auto dictado por ese Despacho notificado por fijación en lista de 10 de noviembre de 2015.

2. Con el fin de responder las objeciones expuestas por el Señor Examinador en su Concepto Técnico correspondiente al Oficio No. **13083** en relación con la claridad del Capítulo Reivindicatorio y la Descripción, la Novedad y el Nivel Inventivo de la presente solicitud, me permito dirigir su atención a las siguientes modificaciones y argumentos técnicos por medio de los cuales no sólo se superan completamente dichas objeciones sino que además se establece de manera incontrovertible la patentabilidad de la solicitud en estudio.

3. **CAPÍTULO REIVINDICATORIO.**

3.1. **Modificaciones al Capítulo Reivindicatorio**

En primer lugar, me permito manifestar que es deseo del solicitante presentar un **capítulo reivindicatorio modificado**, que remplaza al actualmente presentado, con el propósito de superar las objeciones presentadas como parte del concepto técnico realizado por ese Despacho.

En conexión con el mencionado **capítulo reivindicatorio modificado**, me permito resaltar que éste no constituye de ninguna manera una ampliación al alcance de la solicitud, tal como fue originalmente presentada, toda vez que define los novedosos compuestos derivados de nucleósidos sustituidos de fórmula I con actividad inhibitoria de la replicación del RNA del virus de la Hepatitis C (VHC), los cuales son el objeto de la presente invención de acuerdo con las enseñanzas de la descripción. Dichas modificaciones se exponen a continuación:

- **Nueva Reivindicación 1:** tiene como base la reivindicación 1 actualmente presentada, la cual ha sido modificada en la definición de los sustituyentes de la fórmula I para restringir significativamente el alcance de la materia reclamada. Específicamente, se han incluido las características técnicas de las reivindicaciones 2, 3, 7, 9 y 10 actualmente presentadas y se han definido grupos funcionales particulares para los sustituyentes R^1 a R^6, según los ejemplos ilustrados en la descripción. Dichas modificaciones tienen pleno sustento en los 9 compuestos de la invención y la página 3 de la memoria descriptiva.

- **Nueva Reivindicación 2:** corresponde a la reivindicación 4 del capítulo reivindicatorio actualmente presentado.

- **Nuevas Reivindicaciones 3 y 4:** corresponden a las respectivas reivindicaciones 5 y 6 del capítulo reivindicatorio actualmente presentado, las cuales han sido modificadas para especificar realizaciones preferidas para cada sustituyente R^{2a} y R^{2b}, de manera independiente.

- **Nuevas Reivindicaciones 5 y 6:** corresponden a las reivindicaciones 8 y 9 del capítulo reivindicatorio actualmente presentado, donde se ha especificado el grupo funcional de preferencia derivado de la fórmula de la reivindicación 9 actualmente presentada.

- **Nuevas Reivindicaciones 7 y 8:** corresponden a las respectivas reivindicaciones 11 y 12 del capítulo reivindicatorio actualmente presentado.

- **Nuevas Reivindicaciones 9 y 10:** corresponden a las respectivas reivindicaciones 14 y 15 del capítulo reivindicatorio actualmente presentado.

De acuerdo a lo anterior, me permito señalar que la reivindicaciones **2, 3, 7, 10 y 13** del Capítulo Reivindicatorio actualmente presentado fueron **eliminadas** del documento modificado. Asimismo, me permito aclarar que el orden de enumeración y dependencia de las nuevas reivindicaciones se ha reajustado de conformidad con las modificaciones previamente descritas.

De esta forma, **el capítulo reivindicatorio modificado queda constituido por 10 reivindicaciones,** las cuales se refieren de manera más clara y concisa a las características técnicas esenciales de los compuestos derivados de nucleósidos sustituidos de fórmula I con actividad inhibitoria de la replicación del RNA del VHC que son objeto de la presente solicitud, y que cuentan con pleno sustento en las enseñanzas de la descripción.

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3.2. **Claridad del Capítulo Reivindicatorio**

Con respecto a la claridad del capítulo reivindicatorio, el señor Examinador afirma lo siguiente:

- *"La reivindicación 1 no es concisa, porque los sustituyentes de la fórmula al ser referidos de manera general bajo denominaciones tales como "haloalquilo inferior, arilo, alquilo inferior, alquienilo, inferior, alquinilo inferior, alcoxi inferior, entre otros", a partir de los mismos se obtienen posibles compuestos que cumplen con dichas características, pero que no se encuentran sustentados dentro de la descripción. Por lo tanto, se sugiere a la solicitante que se restrinja la materia reclamada a una generalización razonable de los sustituyentes planteados, de tal forma que a partir de los mismos se obtengan los compuestos probados y sustentados en la descripción (...)".*

Respecto al anterior señalamiento, me permito indicar que, tal y como se expuso en el numeral 3.1 del presente memorial y tomando en cuenta las sugerencias del señor Examinador, la reivindicación 1 ha sido modificada de tal forma que las definiciones de los sustituyentes de la fórmula I han sido significativamente restringidas según una generalización razonable de los compuestos debidamente soportados en los Ejemplos de la memoria descriptiva. Específicamente, dichas modificaciones reflejan el alcance de los compuestos de la nueva reivindicación 10, lo cual representa una restricción significativa sobre el objeto reclamado y evidencia que el nuevo capítulo reivindicatorio es conciso y claro.

Frente a las específicas objeciones del señor Examinador, en primer lugar me permito señalar que el nuevo pliego reivindicatorio es conciso porque se redujo sustancialmente el número de posibles definiciones generales para los diferentes sustituyentes de la fórmula I. De acuerdo con lo descrito en el numeral 3.1 del presente memorial, los siguientes sustituyentes fueron modificados para limitar el número de posibilidades y establecer el alcance del objeto reclamado:

- ✓ El sustituyente R^1 se modificó para seleccionarse exclusivamente de hidrógeno, fenilo o naftilo.

- ✓ Los sustituyentes R^{2a} y R^{2b} se modificaron de tal forma que se escogen *independientemente* de hidrógeno y metilo.

- ✓ El sustituyente R^3 fue limitado para seleccionarse exclusivamente de isopropilo, etilo o bencilo.

- ✓ El sustituyente R^4 quedo particularmente definido como hidrógeno.

- ✓ El sustituyente R^5 se modificó para ser definido exclusivamente como hidrógeno o como propioniloxilo ($C(=O)CH_2CH_3$).

- ✓ El sustituyente R^6 se modificó para recitar dos estructuras particulares que reflejan los derivados de pirimidina que hacen parte de los nucleósidos de fórmula I de la invención.

Frente a las anteriores definiciones, me permito señalar que las restricciones hechas reflejan las estructuras de los diferentes compuestos específicos usados en los ejemplos de la descripción, y por lo tanto, el nuevo capítulo reivindicatorio constituye la generalización del tipo de compuestos que han sido sintetizados.

A la luz de todo lo anterior, dichas modificaciones definen de manera clara y concisa todos los sustituyentes de los compuestos de fórmula I que son derivados de nucléosidos sustituidos con 4'-azido y 3'-fluoro, limitando la extensión del objeto de la invención y logrando la diferenciación de la materia reclamada con los compuestos del estado del arte previo. Consecuentemente, me permito manifestar que se supera completamente las objeciones a este respecto.

En vista de las anteriores consideraciones, me permito resaltar que el nuevo capítulo reivindicatorio es totalmente claro y conciso al reclamar los novedosos compuestos derivados de nucleósidos sustituidos de fórmula I con actividad inhibitoria de la replicación del RNA del VHC. Asimismo, dicho capítulo modificado supera completamente las objeciones de claridad presentadas por el Señor Examinador y, por lo tanto, cumple a cabalidad con lo estipulado por el Artículo 30 de la Decisión Andina 486 de 2000.

4. **DESCRIPCIÓN.**

En conexión con la descripción de la solicitud en estudio, el Señor Examinador resalta que la descripción no divulga la invención de manera suficientemente completa puesto que, si bien se reclama en las reivindicaciones que el sustituyente R^6 puedes ser una purina, el desarrollo de la solicitud solamente menciona compuestos donde R^6 incluye derivados de pirimidina.

Frente a la anterior objeción y de conformidad con la sugerencia del señor Examinador, me permito señalar que se ha eliminado de la definición de R^6 el grupo funcional derivado de purinas y se han dejado las estructuras correspondientes a los grupos pirimidin-2,4,-diona y 4-aminopirimidin-2-ona (*ver numeral 3.1 del presente memoria!*).

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De esta manera, me permito señalar que la solicitud de patente en cuestión comprende una descripción clara y suficiente de la invención para que pueda ser comprendida y ejecutada por una persona capacitada en la materia técnica. As su vez, vale la pena señalar que toda la información que está incluida en la descripción enseña a la persona versada en la materia los características estructurales y funcionales de los compuestos de la invención y las diferentes aplicaciones de los mismos.

En vista de todo lo anterior, me permito resaltar que la descripción de la solicitud en estudio, tal como ha sido presentada, es clara y suficiente, ajustándose completamente a las exigencias del Artículo 28 de la Decisión Andina 486 de 2000.

5. **NOVEDAD.**

Ahora bien, el Señor Examinador asegura que la reivindicación 1 (*y sus dependientes*) en estudio no cumplen con el requisito de novedad en vista de las enseñanzas del documento **WO 2008/121634 (D1).**

De manera general, el Señor Examinador asegura que las características técnicas esenciales del compuesto reclamado en la reivindicación 1 del capítulo actualmente presentado habían sido divulgadas previamente por el documento de la anterioridad.

De acuerdo con las tablas comparativas del concepto técnico, las características técnicas de la invención descritas en dicha reivindicación ya se enseñaban de manera independiente en las anterioridades del documento D1. Por lo tanto, el señor Examinador concluye que el compuesto de fórmula I reclamado en el capítulo reivindicatorio actualmente presentado ya era conocido en el estado de la técnica anterior.

Adicionalmente, el señor Examinador asegura que las reivindicaciones dependientes 2 a 15 no mencionan alguna característica que haga nuevo el compuesto de la reivindicación 1. Por lo tanto, dichas reivindicaciones también son afectadas con relación al requisito de novedad.

Sin embargo, sobre tales afirmaciones, **muy amablemente me permito señalar que las enseñanzas del documento D1 de ninguna manera pueden afectar la novedad de los compuestos de la invención debido a que dicho documento no revela explícitamente compuestos químicos particulares y ejemplificados que reúnan en su totalidad las características técnicas estructurales de los compuestos de la presente invención, tal y como se reclaman en el capítulo reivindicatorio modificado que se presenta junto con este memorial.**

En conexión con lo anterior, la presente invención cae dentro de la categoría de *patente de selección* ya que reivindican un grupo pequeño de elementos (*en este caso, una fórmula Markush basada en 9 compuestos particulares*) que pertenecen a un grupo *extenso* de elementos ya conocidos. Si bien las enseñanzas del documento D1 presentan una fórmula general que incluye las opciones de los sustituyentes de la presente invención, ninguno de los compuestos *ejemplificados y explícitamente ensayados* incluyen **todos** los grupos funcionales particulares que se definen para los sustituyentes de la fórmula I del nuevo capítulo reivindicatorio.

Frente al examen de novedad de patentes de selección, la Guía para Examen de Solicitudes de Patente de Invención y Modelo de Utilidad de la Superintendencia de Industria y Comercio, en su numeral 3.5.2.4, establece *que "(...) si el grupo específico de compuestos reivindicado, en el cual **R1** es alquilC_{3-5}:*

- *No está descrito explícitamente en algún antecedente del estado de la técnica por su nombre químico, ni por su fórmula química,*
- *Está alejado de los ejemplos del estado de la técnica (compuestos en los cuales **R1** es alquilC16-17) y de los extremos (alquilC1-20), y*
- *Exhibe un efecto técnico inesperado, no descrito en el estado de la técnica,*

Tal grupo específico reivindicado es nuevo (se considera una selección) y tiene nivel inventivo" (Resaltado fuera del texto).

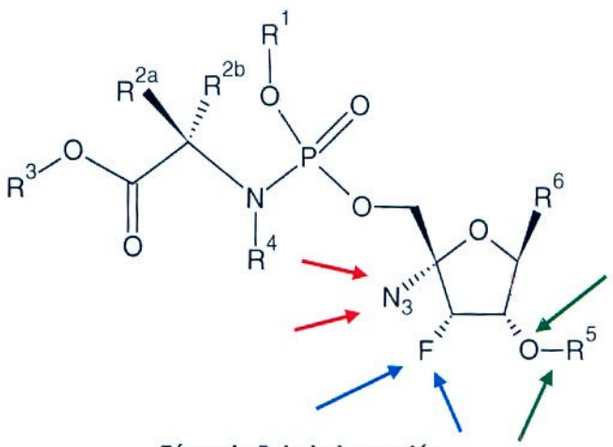
Asimismo, la misma Guía en el numeral 3.5.4 señala que *"(...) una fórmula general del estado de la técnica no anula la novedad de un compuesto o un subgrupo de compuestos reivindicados incluidos en ella. En caso contrario, un compuesto específico del estado de la técnica anula la novedad de una fórmula general reivindicada (...) Si el grupo específico de compuestos reivindicado no está descrito explícitamente en algún antecedente del estado de la técnica por su nombre químico, ni por su fórmula química y está alejado de los ejemplos del estado de la técnica y de los extremos, tal grupo específico reivindicado es nuevo (selección)"* (Resaltado fuera del texto).

En vista de lo anterior, entonces es claro que es posible reivindicar *compuestos particulares* y *fórmulas con sustituyentes significativamente restringidos* que caigan dentro del alcance de las enseñanzas de documentos de la anterioridad, siempre y cuando dichos documentos no revelen *compuestos específicos* que contengan dichas opciones definidas en la fórmula generalizada de los mismos.

Para el presente caso, me permito manifestar que no existen compuestos específicos en D1 que contengan cada una de las características técnicas específicas *requeridas* por la fórmula I de la nueva reivindicación 1. Concretamente: i) ninguno de los compuestos *ejemplificados* está sustituido con un grupo azida (N_3) en la posición 4'

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de la furanosa del núcleo central (*ver flechas rojas)*, y ii) ninguno de los compuestos sintetizados ni ensayados de D1 está sustituido con flúor en la posición 3' del mismo anillo de furanosa (*ver flechas azules*). Es más, al revisar tanto las definiciones de la fórmula I como los compuestos particulares de D1 se encontró que dicho documento no incluye la definición del sustituyente -OR^5 como *propioniloxilo*, el cual se une a la furanosa en la posición 2' del anillo central (*ver flechas verdes*).



Fórmula I de la invención

Así las cosas, es evidente que la anticipación de un compuesto (*de una patente de selección*) por parte de alguna anterioridad requiere que dicho documento del arte previo revele un compuesto debidamente *ejemplificado y soportado* que incluya todas las características esenciales que se reclaman con los compuestos de la invención. Es decir, al menos un único compuesto de la anterioridad tiene que presentar todas y cada una de las características (*sustituyentes particulares*) que se definen en la fórmula de la invención.

Teniendo en cuenta lo anterior, y para efectos de la presente solicitud, D1 no puede desvirtuar la novedad de los compuestos derivados de fórmula I puesto que, de las 559 páginas que revelan análogos de nucleósidos en D1, **ningún compuesto *ejemplificado, estudiado y/o sintetizado* contiene la sustitución con fluoro en la posición 3' de la furanosa, tal y como se reclaman en los compuestos de la invención.**

De las anteriores consideraciones, es evidente que el documento de la anterioridad sólo revela una generalización de posibles sustituyentes de una fórmula similar a la reivindicada en la presente invención. Sin embargo, falla en presentar compuestos particularmente ejemplificados y sintetizados que incluyan todas y cada una de las definiciones de los sustituyentes que se especifican en el capítulo reivindicatorio que se presenta junto a este memorial.

En vista de este señalamiento, es absolutamente evidente que **los compuestos derivados de nucleósidos sustituidos de fórmula I con actividad inhibitoria de la replicación del RNA del VHC son totalmente novedosos** frente a las enseñanzas del documento D1 porque dicha anterioridad de ninguna manera divulga compuestos particularmente ejemplificados que incluyan exactamente las mismas definiciones para los sustituyentes de fórmula I de acuerdo con el objeto reclamado.

En consecuencia, atentamente me permito manifestar ante ese Despacho que la materia de las nuevas reivindicaciones presenta un inequívoco carácter novedoso frente a las enseñanzas de los documentos de la anterioridad, cumpliendo por lo tanto a cabalidad con los requisitos de patentabilidad establecidos en el artículo 16 de la Decisión 486 de 2000.

6. **NIVEL INVENTIVO.**

Finalmente, el Señor Examinador considera que la materia reclamada en la reivindicación 1 actualmente presentada carecería de altura inventiva con respecto a las enseñanzas de los documentos **WO 2008/121634 (D1) y US 2007/0042988,** donde D2 se considera como el estado de la técnica más cercano a la invención definida en dicha reivindicación.

En conexión con lo anterior, el Señor Examinador asegura que la persona del oficio normalmente versada en la materia, basándose en las sustituciones de los compuestos del documento D1, podría modificar sin esfuerzos los compuestos de D2, modificando los sustituyentes del grupo furanosilo e incluyendo un átomo de F en la posición 3. De esta forma, la persona medianamente versada en la materia alcanzaría el objeto de la reivindicación 1 de la presente solicitud de patente.

No obstante, sobre esta particular objeción, **me permito señalar que las enseñanzas de los mencionados documento D1 y D2 de ninguna manera anticipan o sugieren de manera obvia los compuestos derivados de nucleósidos sustituidos de fórmula I, teniendo en cuenta que dichas anterioridades no revelan ni describen el efecto técnico inesperado atribuido a los compuestos de la invención.**

Tal como se expuso en el anterior numeral, la presente invención consiste en una patente de selección donde un *subgrupo* de compuestos de una fórmula Markush se reivindica. Según la Guía para Examen de Solicitudes de Patente de Invención y Modelo de Utilidad de la Superintendencia de Industria y Comercio, en su numeral 3.5.5, *"(...) una invención de selección (...) tiene nivel inventivo si todos los compuestos del subgrupo presentan un efecto o actividad técnica no descrita en el estado de la técnica y además, es inesperada"* (Resaltado fuera del texto).

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Aunado a lo anterior, la misma Guía presenta ciertos indicios sobre el nivel inventivo, y en el numeral 3.5.5.2, señalar que una selección no es obvia, y por tanto inventiva, cuando *"la invención consiste en la selección de ciertos compuestos químicos o composiciones (incluyendo combinaciones) a partir de un amplio campo, donde estos compuestos o composiciones tienen ventajas inesperadas".*

Así las cosas, es claro que un grupo de compuestos, cuyos sustituyentes pueden estar revelados dentro de la generalización de una fórmula de algún documento de la anterioridad, puede gozar de un inequívoco nivel inventivo siempre y cuando se le atribuya un efecto técnico no revelado en el estado del arte previo, y por lo tanto, inesperado. Tal es el caso de los compuestos de la invención, los cuales permiten una inhibición de la replicación del replicón del RNA del VHC con moléculas cuya estructura –*según el conocimiento de la persona versada en la materia y las enseñanzas de las anterioridades*- no exhibiría tal actividad o efecto.

Con el ánimo de demostrar ante ese Despacho que la anterior afirmación es completamente correcta, a continuación me permito presentar el siguiente análisis comparativo entre la información revelada en la presente solicitud y las enseñanzas brindadas por dichas anterioridades:

a) Las diferencias estructurales existentes entre los compuestos de la presente invención y aquellos particularmente divulgados y ejemplificados en el documento D1 no son evidentes para la persona medianamente versada en la materia.

Como se ha venido discutiendo, según el análisis de las enseñanzas del documento D1, de los compuestos análogos de nucleósidos especificados y ejemplificados en las 559 páginas de D1, **la gran mayoría no tiene ningún tipo de sustitución en la posición 4' de la furanosa del núcleo central.** Por lo tanto, son relativamente pocos los compuestos que tienen dicha sustitución, la cual se refleja con un grupo –OMetilo, -F, o –N_3. Sin embargo, es preciso resaltar que **ninguno de los compuestos ejemplificados tienen la sustitución con flúor en la posición 3' de la furanosa,** la cual es una característica esencial de los compuestos de la presente invención.

En vista de lo anterior, entonces es altamente improbable que las enseñanzas de D1 (*que no tienen menciona alguna sobre las anteriores sustituciones*) motiven a la persona versada en la materia a escoger, *particularmente*, la combinación de las sustituciones del anillo 3'-F y 4'-N_3 del sinnúmero de posibilidades genéricamente permitidas en las enseñanzas de dicho documento para construir análogos de nucleósidos optimizados.

En realidad, las sustituciones que se observan en los compuestos particulares de D1 desvían a la persona versada en la materia a obtener compuestos como los aquí reclamados. Por ejemplo, el sustituyente R^6 en la posición 2' de los compuestos de D1 es -F o $-CH_3$ y el sustituyente X es -H o -F mientras que los compuestos de la aplicación (*y los sustituyentes de la fórmula I reclamada*): a) no permiten siquiera alguna sustitución en dicha posición 2' de la furanosa o b) no definen un halógeno como tal en dicha posición. Sencillamente, ni -H ni –F pueden unirse al átomo de carbono 2', pues tal posición está ocupada por un grupo –OH u $-OC(=O)CH_2CH_3$.

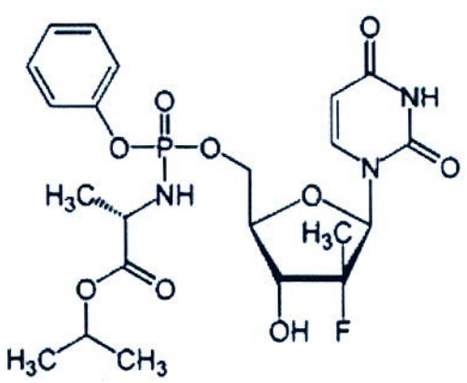
Ahora bien, es importante manifestar que, aunque la estructura genérica (*Fórmula Markush*) de D1 sí incluye el grupo N_3 como parte de sus sustituyentes *-y también otros sustituyentes potenciales en la posición 4', incluyendo varios halógenos-*, la persona versada en la materia no esperaría obtener compuestos con estabilidad deseada utilizando tales grupos funcionales en dicha posición. Como reconocería dicha persona, la posición 4' de la furanosa se puede considerar como una posición anomérica (*tal y como la posición 1'),* la cual al ser reemplazada con halógenos (C-Cl, C-Br y C-I) resultaría en enlaces anoméricos que son verdaderamente inestables.

La persona versada en la materia sabría, por ejemplo, que enlaces anoméricos de C-Br sólo duran unos días a la luz pero pueden ser resguardados a bajas temperaturas en la oscuridad por varias semanas. Efectivamente, no existen además ejemplos de nucleósidos con una sustitución 4'-I (*sólo existe un número CAS pero no hay referencia que sugiera que la molécula se sintetizó*) y solamente se han aislado como intermediaros (*aproximadamente 4 ejemplos)* nucleósidos con una sustitución 4'-Cl. A su vez, compuestos similares con la sustitución 4'-Br sólo han sido descrito como intermediarios sintéticos en 4 artículos científicos. No obstante, la actividad biológica de los anteriores compuestos ha sido debidamente evaluada.

En consecuencia, es razonable argumentar que la inclusión de <u>*todos los halógenos*</u> (*según la generalización de la fórmula I*) como posibles sustituyentes en la posición 4' harían que los compuestos de D1 serían inoperables (*y lo más probable, inestables*). Por lo tanto, la inclusión de un sustituyente de naturaleza similar (*como N3*) tampoco sería una acción obvia para la persona versada en la materia, quien no tendría expectativa de éxito en practicar la invención como se reivindica en su generalización. Así entonces, que <u>*todos*</u> los compuestos de la presente invención tengan tal sustitución (4'-N_3) en el anillo de furanosa y exhiban una satisfactoria actividad inhibitorio, es prueba fehaciente de un efecto técnico inesperado, y por lo tanto, de una inequívoca actividad inventiva.

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Aunado a todo lo anterior, en este punto considero relevante aclarar que D1 contiene listas voluminosas de posibles combinaciones de sustituyentes en cada punto de unión del anillo de ribosa, haciendo una generalización de las posibles combinaciones sin proveer o demostrar una descripción escrita de la estructura de la gran mayoría de dichos elementos. Lo anterior equivale, efectivamente, a una lista de compuestos deseados pero no ejemplificados en una aplicación que, eventualmente, se redujo a un único compuesto de interés cuando se patentó en E.E.U.U.: Sovaldi (también conocido como *Sofosbuvir*, el cual tiene un flúor y un metilo en la posición 2' de la furanosa, un –OH en la posición 3' y ninguna sustitución en la posición 4')



Estructura química de Sovaldi

En vista de tal estructura, es claro entonces que tal compuesto –*el cual es la única selección de una lista sinnúmero de posibilidades para un compuesto ideal*- no tiene ninguna sustitución en la posición 4' de la furanosa y está sustituido en la posición 2' con un átomo de flúor y un grupo metilo. Teniendo en cuenta tales características del dicho compuesto "ideal" (*comprobado además en la práctica*), la persona versada en la materia no tendría motivación alguna para modificar algún compuesto con sustituciones 3'-F y 4'-N_3 con el fin de obtener un compuesto alternativo con actividad optimizada.

A la luz de todas las anteriores consideraciones, me permito reiterar que el documento D1 no describe ni *ejemplifica* ninguno de los compuestos reivindicados en la presente solicitud, ni tampoco la revelación hecha por D1 abre camino a los compuestos reivindicados. La persona versada en la materia tendría que escoger y elegir entre cientos de diferentes variables y las innumerables combinaciones de los mismos para llegar a la combinación reivindicada. Adicionalmente, dicho experto en la materia no se vería impulsado para seleccionar los compuestos que se reivindican al instante con la referencia D1 antes de ellos mismos. A pesar de la extensa lista de combinaciones en las 559 páginas de tablas en D1, las únicas especies con la sustitución 4'-N_3 están en tablas XIV-1 a XIV-50. Es importante destacar que ninguno de los compuestos en las tablas tiene el sustituyente Y definido como F (*en realidad, Y es OH en cada compuesto enumerado*). Además, cada compuesto en dichas tablas se define R^6 como F y X se define como H.

Por lo tanto, los compuestos de la invención (*como parte de una patente de selección*) están fuera del alcance de las combinaciones enumeradas debido a que el patrón de sustitución en las posiciones 2' y 3' son completamente diferentes y es bien conocido en este campo que aparentemente ligeras modificaciones a los sustituyentes del anillo en los nucleósidos pueden tener efectos impredecibles y dramáticos. Al parecer, el señor Examinador supone que los efectos de las ligeras modificaciones estructurales típicas en la optimización tradicional de cabezas de serie (*moléculas de partida*) en química medicinal se aplican o trasladan a las pequeñas modificaciones estructurales aparentemente similares en la química de nucleósidos.

En conexión con lo anterior, el siguiente extracto de Antiviral Research (100 500-519, 2013) detalla por qué la anterior presunción es falsa en el desarrollo de inhibidores de nucleósidos (*NI*) con actividad antiviral:

> *"Si bien es conceptualmente fácil, hay varios desafíos clave en el desarrollo de inhibidores de nucleósidos para los tratamientos terapéuticos. En primer lugar, a menudo es imposible predecir la relación estructura-actividad de los compuestos, ya que se requieren varios pasos enzimáticos para convertirlos a tri-fosfatos activos (Stein y Moore, 2001). Esto hace que sea difícil para la química médica clásica desarrollar acercamientos técnicos para mejorar la potencia de los compuestos. Por lo tanto, inhibidores de nucleósidos potentes se descubren por casualidad. En segundo lugar, la toxicidad de los análogos de nucleósidos es impredecible y a menudo se pierde in vitro. Aunque la toxicidad mitocondrial, tal como la inhibición de la gamma polimerasa, se ha implicado en la mayoría de los inhibidores de nucleósidos antivirales, especialmente para aquellos para el tratamiento de la infección por VIH-1, hay otras toxicidades asociadas con tales inhibidores (Dagan et al., 2002). Por lo tanto, a menudo son necesarios estudios toxicológicos en animales para evaluar el potencial efecto secundario de esta clase de compuestos, que es a menudo costoso en términos de compuesto y el requisito animal"* (Resaltado fuera del texto).

Así entonces, me permito señalar que ninguna de los sustituciones 4'-N_3 ni 3'-F en el anillo han sido, en D1, efectivamente ejemplificadas en un Ejemplo o incluso "proféticamente" enseñadas en combinación, a fin de permitir y/o proporcionar una descripción escrita de la producción de tales derivados de nucleósidos. Por lo tanto, es claro que su obtención debe ser extremadamente difícil y dicho patrón de sustitución no se considera eficaz, haciendo que la presente solicitud, de manera efectiva, no sea evidente. En resumen, debido a las diferencias entre los compuestos reivindicados y las especies descritas de D1 (*falta la sustitución 3'- y existe un patrón de sustitución muy diferente en la posición 2' en los compuestos ejemplificados*) son significativas, es muy poco probable que la presente invención haya sido derivada de manera evidente, y por lo tanto, no goce de un intrínseco carácter inventivo.

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b) Las enseñanzas del documento D1 tampoco permiten a la persona versada en la materia derivar de manera evidente los compuestos particulares de fórmula I de la presente invención.

En la línea de los mismos argumentos para D1, el documento D2 puede razonablemente considerarse como una enseñanza que "aleja" a la persona versada en la materia de la intención de incorporar la sustitución de 4-N_3 en nucleósidos para el tratamiento del VHC. Particularmente, la divulgación de D2 podría ser vista como un fracaso de otros en el desarrollo de nucleótidos con el patrón de sustitución 4'-N_3 para el tratamiento de VHC. Por lo tanto, en combinación con las enseñanzas de D2 que alejan a la persona versada en la materia y el ejemplo de fracaso de otros inventores proporcionados por el artículo Smith et al. (*J. Med. Chem., 52 (9) 2971-2978, 2009*), D2 no sólo puede ser ineficaz como una referencia en un rechazo contra la actividad inventiva de la presente solicitud sino también puede ser útil en la presentación de consideraciones secundarias, tales como incluir enseñanzas que alejan o "desmotivan" a la persona versada en la materia a *obtener de manera evidente* los compuestos de la invención.

Consecuentemente, la persona versada en la materia no estaría motivada a tener una expectativa de éxito en la práctica de la invención como se describe en la presente solicitud, y por lo tanto, es poco probable que las enseñanzas de D2 sean útiles, solas o en combinación, para desvirtuar el nivel inventivo de la presente solicitud.

c) Otras consideraciones técnicas que evidencian el efecto técnico inesperado, y por lo tanto, la altura inventiva de los compuestos particulares de fórmula I de la presente invención (*como patente de invención*).

Para complementar todas las anteriores consideraciones, me permito ahora presentar ciertos argumentos adicionales que demuestran que los compuestos actualmente reivindicados no eran evidentes a partir de las enseñanzas particulares de las anterioridades. Particularmente, tales compuestos no son evidente, incluso si existiese la necesidad de obtener un pro-fármaco sería, teniendo en cuenta que la obtención de un compuesto activo es una actividad compleja y, en ninguna circunstancia, obvia.

En conexión con lo anterior, por ejemplo, McGuigan y Klumpp et al., (*Bioorg. Med. Chem. Lett., 2009, 19, 4250*) describen pro-fármacos de fosforamidato de 4'-azidocitidina. De un total de 55 compuestos en dicho artículo, sólo 3 presentaron un mejoramiento en potencia 2 veces mayor. Así entonces, la mayoría de los pro-fármacos de fosforamidato (47 compuestos) eran menos activos que el nucleósido original (IC50 = 1,2 M), y la forma más eficaz de todas las opciones (CI50 = 0,38 M) comprendía un grupo funcional fenilo en combinación con un éster etílico de alanina.

Aunado a lo anterior, la Tabla 3 incluye pro fármacos derivados de fosforamidato con una fracción de 1-naftilo, combinados con un éster bencílico de alanina. Sin embargo, ningún epímero presenta mejora alguna en el nucleósido original. Así las cosas, este documento constituye el único estudio extenso de fosforamidatos aplicados a análogos de citidina, en particular4'-azidocitidina. Entonces, la falta de mejora explícitamente evidenciada en dicho documento constituye una enseñanza que "alejaría" a la persona versada en la materia de la intención de obtener de manera obvia los compuestos actualmente reivindicados.

Ahora bien, es preciso señalar que, para ser parte de una terapia a base de nucleótidos eficaz para el VHC, un análogo de nucleósido trifosfato tiene que ser altamente selectivo para NS5B del VHC, debe incorporarse de manera eficiente en una cadena en crecimiento de RNA viral y no debe ser un sustrato para las polimerasas humanas, incluyendo polimerasas de ARN mitocondriales. Por lo tanto, las siguientes consideraciones científicas son de gran importancia en la evaluación de la falta de obviedad/nivel inventivo de la presente solicitud con respecto a D1 y el estado de la técnica, incluyendo el conocimiento común, en el momento de concebir el objeto de la invención:

i) Nucleósidos con el patrón de sustitución 3'-deoxi-3'-fluoro son conocidos por ser citostáticos (*J. Med. Chem., 1991, 34, 2195 – Ver anexos*), a diferencia de los ribonucleósidos parentales, destacando el hecho de que nucleósidos 3'-desoxi-3'-fluoro inherentemente funcionan de manera opuesta a sus análogos de ribonucleósidos y, por lo tanto, no pueden ser científicamente considerados como equivalentes o evidentes los unos sobre los otros;

ii) De hecho, se encontró que los análogos de 3'-deoxi-3'-fluoro uridina y citidina son inactivos contra todos los virus ensayados, y los análogos de guanosina fueron en gran medida inactivos y sólo cuestionablemente activos contra algunos virus. Por lo tanto, la expectativa de cualquier probabilidad de éxito de los nucleósidos 3'-deoxi-3'-fluoro reivindicados siendo activos contra HCV debería haber sido muy baja para un experto en la materia (Id.).

iii) Es importante destacar que la aplicación instantánea da a conocer el primer ejemplo de un nucleósido o nucleótido activo 3'-deoxi contra el VHC sin ser citostático o citotóxico.

iv) La citidina 4'-azido es conocida por ser fácilmente fosforilada *in vivo* y es un sustrato de quinasas de deoxicitidina.

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v) **No hay evidencia en el dominio público que sugiera que la combinación de un grupo 4'-azido con una combinación de sustituyentes 3'-deoxi-3'-F proporcionaría una molécula altamente selectiva para la polimerasa del VHC y que no sea citostática o citotóxica.**

vi) Se encontró que los 3'-F-epímeros de los compuestos instantáneos son inactivos (*el análogo xilo-F de la citidina no mostró actividad y la uridina tenía una IC_{50} de más de 62 M, que generalmente se considera inactivo*) como trifosfatos contra VHC polimerasas. Lo anterior representa un hallazgo impredecible basado en la literatura y en el conocimiento común para un experto en la técnica, lo que indica, además, una falta de previsibilidad de los efectos sobre SAR prestados por ligeras modificaciones en la química de nucleósidos.

En vista de todo lo anterior, es claro que el efecto técnico *inesperado* de los compuestos de la invención entonces radica en que los mismos incluyen características estructurales (*sustituyentes con grupos funcionales particularmente definidos*) que no han demostrado una combinación lo suficientemente ventajosa en vista de la actividad inhibitoria atribuida. Es decir, no se había evidenciado que tales sustituciones lleven a una estructura que sea capaz de inhibir eficientemente la replicación del RNA de VHC, y por lo tanto, los compuestos de la invención son alternativas inesperadas frente a lo ya divulgado en el arte previo.

Aunado a lo anterior, me permito resaltar que el hecho no es que los compuestos deban mejorar obligatoriamente su actividad en el tratamiento de infecciones causadas por VHC. Por el contrario, la idea es obtener compuestos análogos de nucléosidos como alternativa que puedan lograr dicho tratamiento en comparación con compuestos equivalentes. Tal situación fue la que se logró resolver con las estructuras de la presente invención. Por lo tanto, no es adecuado afirmar que el efecto mejorado debe encontrarse en todos los compuestos, puesto que de por sí, las moléculas de fórmula I ya representan una alternativa que no fue derivada de manera obvia o evidente de los documentos del arte previo.

Frente a las anteriores consideraciones, me permito afirmar que **se descarta cualquier indicio de obviedad en el remplazo de los sustituyentes en las diferentes posiciones del grupo furanosil, y se evidencia el nivel inventivo de los compuestos derivados de nucleósidos sustituidos de fórmula I con actividad inhibitoria de la replicación del RNA del VHC de la nueva reivindicación 1 de la presente solicitud**.

Ahora bien, el punto no es si un experto en la materia podría haber llegado a la invención mediante la adaptación o modificación de las enseñanzas de los documentos D2, a partir de D1, sino que si lo hubiera hecho de esa manera, ¿por qué el documento D1 le motivó a hacerlo con la expectativa de resolver el problema técnico objetivo o en la expectativa de obtener una mejoría o ventaja?

Es más, **¿qué razón tendría la persona versada en la materia para que, a la luz del problema resuelto por la presente invención, decidiera modificar los compuestos de D2, usando las enseñanzas de D1, de manera que las estructuras resultantes comprendan compuestos de nucléosidos como los reclamados, y no otro tipo de modificación?**

En conexión con lo anterior, muy respetuosamente me permito señalar que la única motivación que tendría la persona versada en la materia para hacer esta particular modificación sería la revelación hecha por la presente invención, por lo que el análisis de nivel inventivo realizado por el Señor Examinador está enmarcado dentro de lo que en el ámbito de las patentes se conoce como *"hindsight"* o análisis *"Ex post facto"*. Conociendo de antemano la invención, el Señor Examinador está seleccionando partes específicas de las enseñanzas contenidas dentro de los documentos D1 y D2, las cuales modifica –*justificando las enseñanzas D1*- de forma que permiten construir la materia reclamada por la nueva reivindicación 1 de la presente solicitud. Dichas acciones o modificaciones de ningún modo podrían ser evidentes para la persona versada en la materia en el momento en el cual se desarrolló la invención.

En este sentido, vale la pena resaltar que, al tener conocimiento de la invención, es de esperarse que dichas modificaciones particulares de las enseñanzas de D1 o D2 parezcan obvias, toda vez que ya se sabe de antemano que efectivamente el cambio sustituyentes en ciertas posiciones de la estructura resuelven el problema técnico planteado. Sin embargo, esta información no era conocida por el inventor en el momento de desarrollar la invención, por lo que no podría tener certeza de la utilidad de realizar estos pasos.

En vista de este señalamiento, y a la luz de lo discutido en el presente memorial, me permito resaltar que al no *"saltar a la vista de manera clara y directa"* a partir la divulgación de los documentos D1 y D2 un compuesto derivado de nucleósidos sustituidos de fórmula I, como el considerado en la presente invención, y al no tenerse *"certeza clara y manifiesta"* de que dicho compuesto conservaría el efecto en la inhibición de la replicación del RNA del VHC, es claro que los documentos D1 y D2 no pueden de ninguna manera afectar el Nivel Inventivo de la solicitud en estudio.

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7. **CONCLUSIÓN.**

Para finalizar, me permito reiterar que el capítulo reivindicatorio modificado presentado junto con este memorial es totalmente claro y conciso al reclamar los novedosos compuestos derivados de nucleósidos sustituidos de fórmula I con actividad inhibitoria de la replicación del RNA del VHC, y está plenamente soportado dentro del texto de la descripción, cumpliendo así con lo exigido por la mencionada Decisión Andina 486 de 2000.

Por otro lado, en virtud de los argumentos discutidos con respecto a los documentos citados, se estableció de manera incontrovertible que dichos compuestos de fórmula I con actividad relevante en la inhibición de la replicación del RNA del VHC, los cuales son objeto de la solicitud en estudio, no pueden ser anticipados a partir de las enseñanzas de los documentos de la anterioridad, demostrando irrefutablemente la patentabilidad de la presente solicitud.

8. Anexo al presente memorial, me permito presentar:

i) Copia de las páginas **57** a **59** que corresponden al **capítulo reivindicatorio modificado**, tal como se discutió en el presente memorial, constituido por **10 reivindicaciones**;

ii) Copia del artículo Smith et al. (*J. Med. Chem., 52 (9) 2971-2978, 2009*);

iii) Copia del artículo McGuigan y Klumpp et al., (*Bioorg. Med. Chem. Lett., 2009, 19, 4250*); y

iv) Copia del artículo Mikhailopulo et al. (*J. Med. Chem., 34, 2195-2202, 1991*).

9. En vista de lo anterior, ruego a usted proceder a estudiar nuevamente la solicitud a la luz de las modificaciones y los argumentos presentados y conceder el privilegio de patente solicitado.

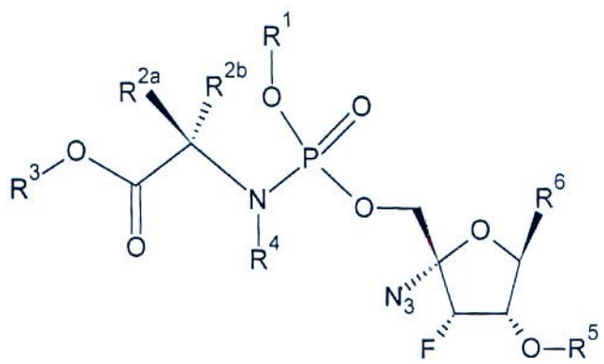
Señor Superintendente,

ALICIA LLOREDA RICAURTE
T.P. 53.215

Dirección para Notificaciones: **notificacionespatentes@lloredacamacho.com**

REIVINDICACIONES

1. El compuesto con la fórmula I:



(I)

en el que:

R^1 es H, fenilo o naftilo;

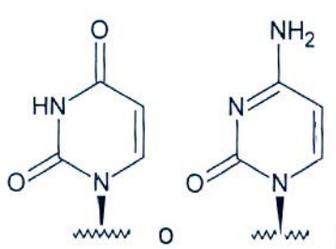
R^{2a} y R^{2b} son independientemente H y metilo;

R^3 es isopropilo, etilo o bencilo;

R^4 es H;

R^5 es H o propioniloxilo;

R^6 es



o una sal farmacológicamente aceptable de los mismos.

2. El compuesto de la reivindicación 1, en el que R^1 es un naftilo o un fenilo.

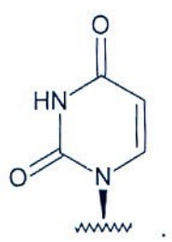
3. El compuesto de la reivindicación 2, en el que R^{2a} es metilo.

4. El compuesto de la reivindicación 3, en el que R^{2b} es H.

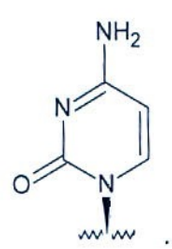
5. El compuesto de la reivindicación 4, en el que R^5 es H.

6. El compuesto de la reivindicación 4, en el que R^5 es propioniloxilo.

7. El compuesto de la reivindicación 5, en el que R^6 es



8. El compuesto de la reivindicación , en el que R^6 es



9. Un compuesto seleccionado a partir del grupo que incluye:

(S)-2-{[(2R,3S,4S,5R)-2-azido-5-(2,4-dioxo-3,4-dihidro-2*H*-pirimidin-1-ilo)-3-fluoro-4-hidroxi-tetrahidro-furan-2-ilmetoxi]-fenoxi-fosforilamino}-propionato de isopropilo;

(S)-2-{[(2R,3S,4S,5R)-2-azido-5-(2,4-dioxo-3,4-dihidro-2*H*-pirimidin-1-ilo)-3-fluoro-4-hidroxi-tetrahidro-furan-2-ilmetoxi]-fenoxi-fosforilamino}-propionato de etilo;

(S)-2-[[(2R,3S,4S,5R)-2-azido-5-(2,4-dioxo-3,4-dihidro-2*H*-pirimidin-1-ilo)-3-fluoro-4-hidroxi-tetrahidro-furan-2-ilmetoxi]-(naftalen-1-iloxi)-fosforilamino]-propionato de etilo;

(S)-2-[[(2R,3S,4S,5R)-5-(4-amino-2-oxo-2*H*-pirimidin-1-ilo)-2-azido-3-fluoro-4-hidroxi-tetrahidro-furan-2-ilmetoxi]-(naftalen-1-iloxi)-fosforilamino]-propionato de isopropilo;

(S)-2-[[(2R,3S,4S,5R)-5-(4-amino-2-oxo-2*H*-pirimidin-1-ilo)-2-azido-3-fluoro-4-hidroxi-tetrahidro-furan-2-ilmetoxi]-(naftalen-1-iloxi)-fosforilamino]-propionato de bencilo;

(S)-2-[[(2R,3S,4S,5R)-5-(4-amino-2-oxo-2*H*-pirimidin-1-ilo)-2-azido-3-fluoro-4-hidroxi-tetrahidro-furan-2-ilmetoxi]-(naftalen-1-iloxi)-fosforilamino]-propionato de etilo;

(S)-2-{[(2R,3S,4S,5R)-5-(4-amino-2-oxo-2*H*-pirimidin-1-ilo)-2-azido-3-fluoro-4-hidroxi-tetrahidro-furan-2-ilmetoxi]-fenoxi-fosforilamino}-propionato de isopropilo;

(S)-2-{[(2R,3S,4S,5R)-5-(4-amino-2-oxo-2*H*-pirimidin-1-ilo)-2-azido-3-fluoro-4-hidroxi-tetrahidro-furan-2-ilmetoxi]-hidroxi-fosforilamino}-propionato de isopropilo; y

(S)-2-[[(2R,3S,4S,5R)-5-(4-amino-2-oxo-2*H*-pirimidin-1-ilo)-2-azido-3-fluoro-4-propioniloxi-tetrahidro-furan-2-ilmetoxi]-(naftalen-1-iloxi)-fosforilamino]-propionato de isopropilo.

10. Una composición farmacéutica que incluye un compuesto de cualquiera de las reivindicaciones 1 a 9 y transportadores terapéuticamente inertes.

The Design, Synthesis, and Antiviral Activity of Monofluoro and Difluoro Analogues of 4′-Azidocytidine against Hepatitis C Virus Replication: The Discovery of 4′-Azido-2′-deoxy-2′-fluorocytidine and 4′-Azido-2′-dideoxy-2′,2′-difluorocytidine

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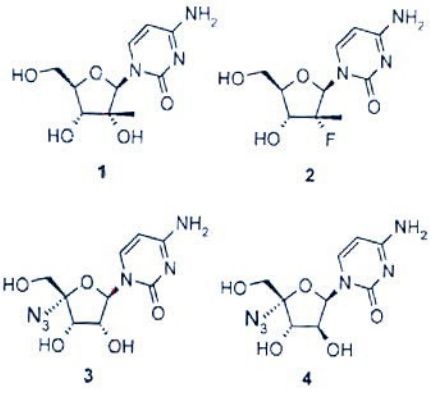
Received December 17, 2008

The discovery of 4′-azidocytidine (**3**) (R1479) (*J. Biol. Chem.* 2006, *281*, 3793; *Bioorg. Med. Chem. Lett.* 2007, *17*, 2570) as a potent inhibitor of RNA synthesis by NS5B (EC_{50} = 1.28 μM), the RNA polymerase encoded by hepatitis C virus (HCV), has led to the synthesis and biological evaluation of several monofluoro and difluoro derivatives of 4′-azidocytidine. The most potent compounds in this series were 4′-azido-2′-deoxy-2′,2′-difluorocytidine and 4′-azido-2′-deoxy-2′-fluoroarabinocytidine with antiviral EC_{50} of 66 nM and 24 nM in the HCV replicon system, respectively. The structure−activity relationships within this series were discussed, which led to the discovery of these novel nucleoside analogues with the most potent compound, showing more than a 50-fold increase in antiviral potency as compared to 4′-azidocytidine (**3**).

Introduction

Hepatitis C virus (HCV) is a causative agent of chronic liver disease, estimated to affect over 170 million people worldwide.[3] Although often asymptomatic, HCV infection can progress to fibrosis, reduced liver function, and hepatocellular carcinoma. Currently, the standard treatment for HCV infection involves treatment with pegylated α interferon in combination with the nucleoside analogue ribavirin. This treatment regimen effects a response in approximately 40−60% of the genotype-1 (GT-1) population.[4] Considering the limitations in efficacy and the adverse event profile associated with the current standard of care treatment, there remains a significant unmet clinical need for the development of new antiviral medication, specific for HCV. Recently, a number of nucleoside analogues have been reported to inhibit HCV replication. Among these, 2′-deoxy-2′-*C*-methylcytidine (**1**),[5] 2′-deoxy-2′-fluoro-2′-*C*-methylcytidine (**2**),[6] and 4′-azido-cytidine (**3**)[1,2] have shown most promise and progressed into clinical development for the treatment of HCV infection. The identification of 4′-substituted ribonucleosides as potential inhibitors of HCV replication culminating in the selection of **3** as a clinical candidate suggested that further exploration of the SAR may allow further optimization of phosphorylation efficiency and antiviral potency.[1,2] Indeed, 4′-azido-arabinocytidine (**4**) (RO-9187) was later identified as an inhibitor of HCV replication with improved potency as compared to **3**, related to improved phosphorylation efficiency (NS5B EC_{50} = 171 nM).[7,8]

Further work focused on the assessment of fluorinated derivatives, on the carbohydrate moiety, of 4′-azidocytidine. Fluoronucleosides have a history of being well phosphorylated by cellular kinases and can be good substrates for RNA and DNA polymerases.[6,9−11] Here we discuss the synthesis and anti-



HCV activity of monofluoro and difluoro analogues of 4′-azidocytidine.

Chemistry

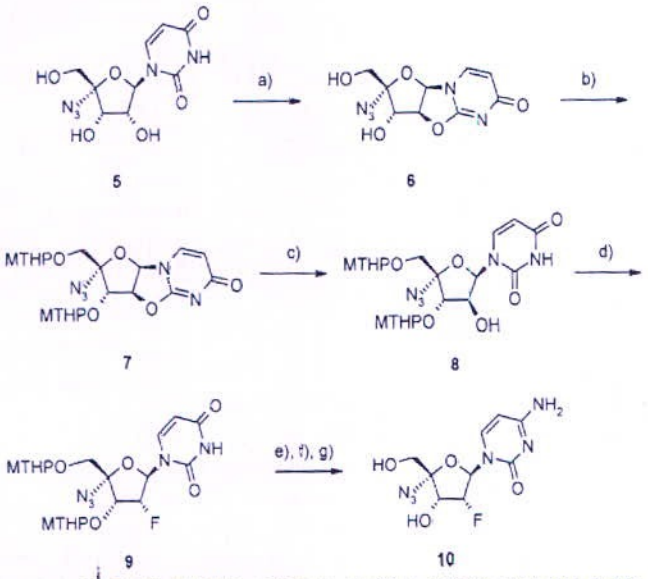
The synthesis of 4′-azido-2′-deoxy-2′-fluorocytidine (**10**) is outlined in Scheme 1. Thus, treatment of 4′-azidouridine (**5**)[8] with diphenylcarbonate, according to Reese's protocol, yielded **6** in 85%.[12] Protection with 4-methoxy-3,6-dihydro-2*H*-pyran, followed by hydrolysis with NaOH in EtOH, provided the arabino-derivative **8** in 56% yield over two steps. Fluorination with (diethylamino)sulfur trifluoride (DAST) furnished **9** as a single isomer in 56% yield. Standard conversion from uridine to cytidine was conducted with 1*H*-tetrazole and 4-chlorophenyldichlorophosphate followed by ammonia. Deprotection with acetic acid provided **10** in 26% yield over two steps.

4′-Azido-2′-deoxy-2′-fluoroarabinocytidine (**17**) was prepared as illustrated in Scheme 2. Thus, treatment of **11** with iodine and triphenylphosphine and imidazole in THF gave the iodide **12** in 76% yield. Elimination of the hydrogen iodide was performed by sodium methoxide to furnish **13** in 82%. Treatment of **13** with iodine and benzyltriethylammonium azide, prepared from the corresponding chloride and sodium azide in 4-methylmorpholine (MMP) and THF, provided **14** in 93% yield as a single isomer. Oxidative nucleophilic substitution of the

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Scheme 1[a]



[a] (a) $(PhO)_2CO$, $NaHCO_3$, DMF, Δ; (b) CSA, MTHP; (c) NaOH, EtOH; (d) DAST, pyridine, CH_2Cl_2; (e) tetrazole, $Cl_2P(O)C_6H_4Cl$, Et_3N, MeCN; (f) NH_3, dioxane; (g) AcOH.

5′-iodine with *m*-chlorobenzoic acid/*m*-chloroperoxybenzoic acid, followed by deprotection and acetylation, gave **15** in 53% yield. Protection of the 3′,5′-hydroxy groups followed by standard conversion from uridine to cytidine, via the method developed by Divakar and Reese, furnished **17** in 88% yield over two steps.[12]

Synthesis of 3′-deoxy-3′-fluoroxylocytidine (**20**) is outlined in Scheme 3. Herein, protection of **5** by treatment with triphenylmethyl chloride in pyridine at 100 °C for 3 days gave **18** as the single product in 55% after chromatographic separation. Fluorination of **18** was achieved by heating with DAST and pyridine in dichloromethane for 18 h to furnish **19** in 31% yield. Conversion of **19** to cytidine via the triazole method,[12] followed by deprotection with Amberlyst 15 (H^+) ion-exchange resin in MeOH, provided **20** in 47% yield.

4′-Azido-3′-deoxy-3′-fluorocytidine (**28**) was prepared in an analogous manner to **17** and is outlined in Scheme 4. Thus, 3′-deoxy-3′-fluorouridine (**21**), treated with iodine and triphenylphosphine and imidazole in THF, gave the iodide **22** in 93% yield. Subsequent elimination by sodium methoxide provided **23** in 92% yield. Treatment of **23** with iodine and benzyltriethylammonium azide furnished a 1:1 mixture of diastereomers that were separated by column chromatography to give the desired isomer **24** in 31% yield. This lack of selectivity has previously been observed with a 3′-deoxyuridine substrate.[8] Oxidative nucleophilic substitution of 5′-iodine with *m*-chlorobenzoic acid/*m*-chloroperoxybenzoic acid, followed by benzoylation, gave **26** in 98% yield. Standard conversion from uridine to cytidine furnished **28** in a yield of 53% over two steps.[12]

Treatment of *N*-benzoylgemcitabine, prepared according to the literature[13] with iodine and triphenylphosphine, yielded **30** in 90% yield. Elimination followed by protection with 4-methoxybenzoyl chloride and treatment with iodine and benzyltriethylammonium azide gave **32** 60% yield over three steps. Nucleophilic substitution of 5′-iodine **32** with benzoate, followed by deprotection, led to **34** in 35% yield (Scheme 5).

Results and Discussion

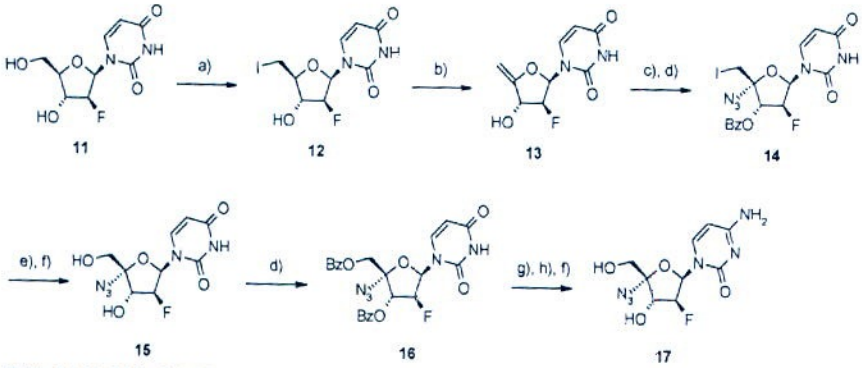
Compounds were characterized as inhibitors of HCV replication in the subgenomic replicon assay system using the 2209-23 cell line as described previously.[2,8] It was also determined if any of the compounds were cytotoxic or inhibited cell proliferation, an indication of potentially reduced selectivity against human polymerases. Cell viability was measured using the WST-1 assay (Roche), and cell proliferation was measured by quantification of the incorporation of tritiated thymidine into cellular DNA using the [^{3}H]-thymidine incorporation scintillation proximity assay (Amersham Biosciences).[5−8] Results are summarized in Table 1.

None of the compounds tested showed significant cytotoxic or cytostatic properties with the exception of the control compound, 2′deoxy-2′-fluoroarabinocytidine (dFAraC), which was a potent inhibitor of cell proliferation inhibiting tritiated thymidine incorporation with an IC_{50} value of 174 nM. Introduction of the 4′-azido moiety into dFAraC resulted in 4′-azido-2′-deoxy-2′-fluoroarabinocytidine (**17**), which did not show any measurable inhibition of cell proliferation. The introduction of the 4′-azido moiety therefore increased the selectivity of **17** by over 4000-fold as compared to dFAraC. In addition, **17** showed exceptionally high antiviral potency in the HCV replicon system. With an antiviral IC_{50} value of 24 nM, the potency of **17** was improved more than 50-fold as compared to 4′-azido-cytidine and 7-fold as compared to 4′-azido-arabinocytidine **4**.[7] Unexpectedly, the lack of a 2′-hydroxy group, in the ribo configuration, was not only acceptable for binding and incorporation by the HCV RNA polymerase, but such 2′-deoxy-nucleoside analogues could become highly potent without apparent effects on cell viability and proliferation in the HCV replicon system. Interestingly, inversion of the 2′-fluoro group to form 4′-azido-2′-deoxy-2′-fluorocytidine (**10**) reduced potency by >150-fold as compared to **17**. It has not yet been determined if this reduction in potency is due to reduced phosphorylation efficiency of **10** or whether incorporation efficiencies by HCV polymerase are different between **10** and **17** triphosphates. The related compound 2′-deoxy-2-fluorocytidine (FdC) has previously been characterized in the HCV replicon system. FdC was found to be a potent inhibitor of cell proliferation with an IC_{50} of 0.8 μM.[6] Therefore the introduction of the 4′-azido moiety into FdC to form **10** also abolished cell proliferation inhibition and increased selectivity by more than 125-fold.

Gemcitabine is a highly cytotoxic cytidine analogue and approved for the treatment of several cancer indications, including nonsmall cell lung cancer and pancreatic cancer. CC_{50} values for gemcitabine in human hepatoma cells have been reported to be in the nM range.[14,15] As the 2′-difluoro motif was accepted by kinases and polymerases, it was of interest to evaluate the effect of 4′-substitutions on the antiviral and cytotoxicity profile of such compounds. As shown in Table 1, the introduction of the 4′-azido moiety (**34**) resulted in a highly interesting compound with significant antiviral potency in the HCV replicon system (EC_{50} = 66 nM) and no apparent cytotoxicity, suggesting a substantial increase in selectivity as compared to gemcitabine. The effect of the 4′-azido moiety to confer high selectivity has therefore been consistent through **3**, **4**, **10**, **17**, and **34**.

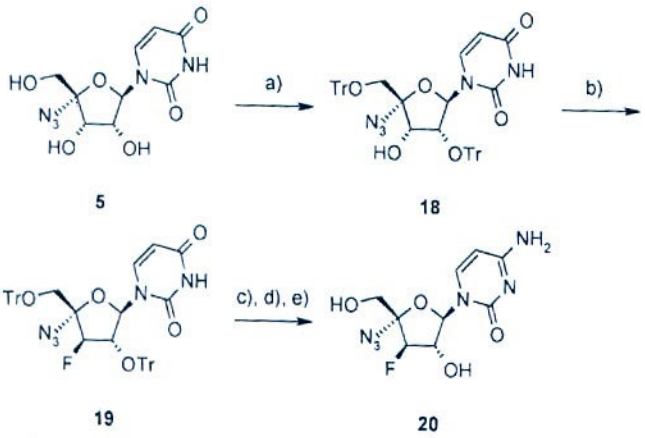
3′-Fluoro analogues of 4′-azidocytidine, **20** and **28**, were completely inactive as well as nontoxic in the replicon system. The 3′-hydroxyl group was suggested from structural studies with deoxycytidine kinase to be important for productive binding to the kinase, as hydrogen bonds are formed with E197 and Y86 in the human deoxycytidine kinase active site.[16] Removal of the 3′-hydroxy group in **20** and **28** may prevent these

Scheme 2[a]



[a] (a) I_2, PPh_3, imidazole, THF; (b) NaOMe, MeOH; (c) $[Bn(Et)_3N]N_3$, I_2, MMP, THF; (d) BzCl, DMAP, MMP, THF; (e) $(Bu_4N)HSO_4$, K_2HPO_4, mCPBA, mCBA, CH_2Cl_2; (f) NH_3, MeOH; (g) 1*H*-tetrazole, $Cl_2P(O)OC_6H_4Cl$, pyridine; (h) NH_3, dioxane.

Scheme 3[a]



[a] (a) TrCl, pyridine; (b) DAST, pyridine, CH_2Cl_2; (c) triazole, $POCl_3$, Et_3N, MeCN; (d) NH_4OH, dioxane; (e) Amberlyst 15, MeOH.

compounds from binding productively to human dCK and thus from being phosphorylated to their triphosphate derivatives.

Conclusions

We have presented data that shows both 4′-azido-2′-deoxy-2′,2′-difluorocytidine (**34**) and 4′-azido-2′-deoxyfluoroarabinocytidine (**17**) to be highly potent and selective inhibitors of HCV replication. Potency in this series was increased more than 50-fold as compared to 4′-azidocytidine (**3**), which has shown efficacy in HCV infected patients. Considerable potency is also observed with 4′-azido-2′-fluorocytidine **10**, although ~3-fold lower as compared to 4′-azidocytidine (**3**). Further characterization of these compounds is therefore warranted as potential antiviral agents with low cytotoxicity in human cells. Derivatives at the 3′ position of 4′-azidocytidine did not show antiviral potency in cell culture, presumably due to inefficient phosphorylation.

Experimental Section

All starting materials, solvents and reagents were reagent grade and used as purchased. Chromatography solvents were HPLC grade and used without further purification. Flash column chromatography was performed on Merck KGaA Silica Gel 60 F_{254} (particle size 0.040−0.063 mm) unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) analysis using precoated glass plates (Silica Gel 60 F_{254}). The purity of compounds **10**, **17**, **20**, **28**, and **34** was determined by high pressure liquid chromatography and were found to be greater than 95%, apart from compound **20** (94%).

2,2′-Anhydro-1-(4′-azido-β-D-arabinofuranosyl)uracil (6). A mixture containing **5** (1.00 g, 3.50 mmol), diphenyl carbonate (0.83 g, 3.85 mmol), sodium hydrogen carbonate (0.030 g, 0.35 mmol), and *N*,*N*-dimethylformamide (1 mL) was heated at 100 °C under N_2. After 14 h, the reaction mixture was cooled to room temperature and diluted with CH_3CN (5 mL). The resulting precipitate was collected by filtration to provide **6** (0.80 g, 85%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.94 (d, J = 7.5 Hz, 1H), 6.62 (d, J = 5.6 Hz, 1H), 6.46 (d, J = 6 Hz, 1H), 5.92 (d, J = 7.5 Hz, 1H), 5.54 (t, J = 5.7 Hz, 1H), 5.35 (dd, J = 2.4 and 6 Hz, 1H), 4.50 (br s, 1H), 3.53−3.41 (m, 2H).

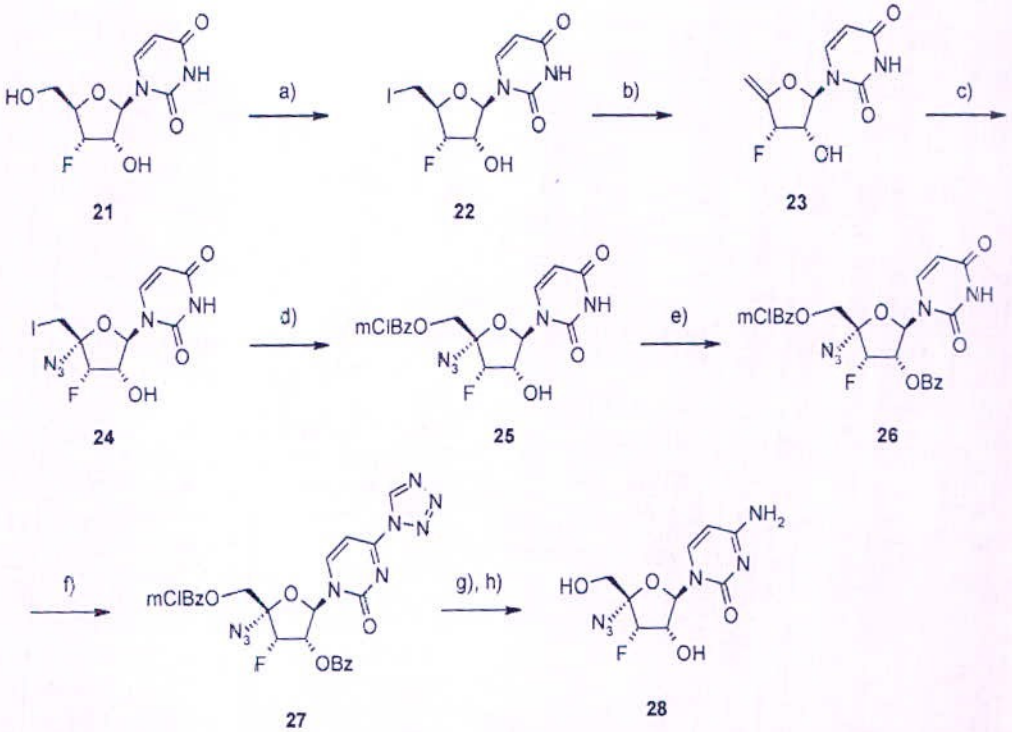
2,2′-Anhydro-1-(4′-azido-3′,5′-*O*-bis-(4-methoxy-tetrahydropyran-4-yl)-β-D-arabinofuranosyl)uracil (7). To a solution of **6** (0.77 g, 2.88 mmol) in dry *N*,*N*-dimethylformamide (15 mL) was added 4-methoxy-3,4-dihydro-2*H*-pyran (2.6 mL, 23 mmol), followed by camphorsulfonic acid (0.040 g, 0.17 mmol). The reaction mixture was stirred for 2 days at room temperature, neutralized with solid K_2CO_3, and evaporated to dryness under reduced pressure. Purification by silica gel column chromatography (10−15% MeOH in $CHCl_3$) gave **7** as an off-white solid (0.87 g, 61%). ^{1}H NMR (400 MHz, CD_3Cl): δ 7.36 (d, J = 7.5 Hz, 1H), 6.29 (d, J = 6.1 Hz, 1H), 6.09 (d, J = 7.5 Hz, 1H), 5.34 (dd, J = 2.0 and 6.1 Hz, 1H), 4.88 (d, J = 2.0 Hz, 1H), 3.89−3.79 (m, 2H), 3.69−3.56 (m, 6H), 3.45 (q, J = 10.5 Hz, 2H), 3.32 (s, 3H), 3.14 (s, 3H), 2.10−1.70 (m, 8H).

1-(4′-Azido-3′,5′-*O*-bis-(4-methoxy-tetrahydropyran-4-yl)-β-D-arabinofuranosyl)uracil (8). A solution of **7** (0.87 g, 1.76 mmol) and KOH (0.070 g, 1.25 mmol) in 9:1 mixture of EtOH/H_2O (10 mL) was refluxed for 3 h. The reaction mixture was cooled, neutralized with Amberlyst 15 ion-exchange resin, filtered, and evaporated to dryness under reduced pressure to give **8** (0.83 g, 92%) as a foam. ^{1}H NMR (400 MHz, CD_3Cl): δ 7.52 (d, J = 7.9 Hz, 1H), 6.36 (d, J = 5.1 Hz, 1H), 5.58 (d, J = 7.9 Hz, 1H), 4.53 (t, J = 5.1 Hz, 1H), 4.47 (d, J = 5.1 Hz, 1H), 3.80−3.55 (m, 8H), 3.48 (s, 2H), 3.30 (s, 3H), 3.24 (s, 3H), 1.95−1.70 (m, 8H).

1-(4′-Azido-3′,5′-*O*-bis-(4-methoxy-tetrahydropyran-4-yl)-2′-deoxy-2′-fluoro-β-D-ribofuranosyl)uracil (9). To a solution of **8** (0.41 g, 0.8 mmol) in CH_2Cl_2 (5 mL) was added pyridine (1.2 mL) followed by DAST (0.64 mL, 4.8 mmol). The reaction mixture was stirred at room temperature for 24 h, diluted with CH_2Cl_2, washed with brine, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (10−15% MeOH in $CHCl_3$) to give **9** (0.23 g, 56%) as a foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.54 (d, J = 7.4 Hz, 1H), 5.83 (d, J = 21.0 Hz, 1H), 5.80 (d, J = 7.4 Hz, 1H), 5.25 (dd, J = 5.5 and 53.9 Hz, 1H), 4.82 (dd, J = 5.5 and 22.0 Hz, 1H), 3.80−3.55 (m, 10H), 3.33 (s, 3H), 3.21 (s, 3H), 2.00−1.75 (m, 8H). MS (ES^+) m/z 516 (MH^+).

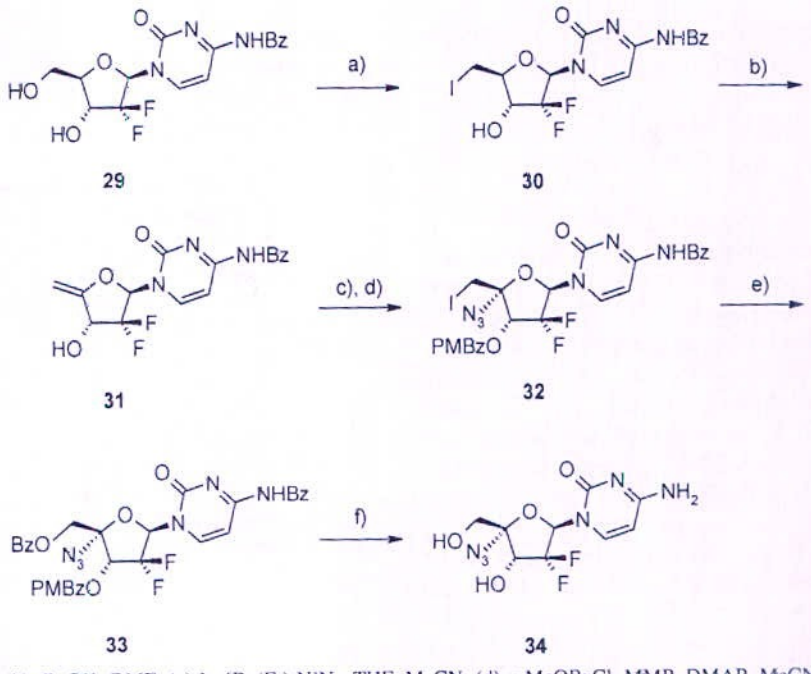
1-(4′-Azido-2′-deoxy-2′-fluoro-β-D-ribofuranosyl)cytosine (10). To a ice−water bath cooled solution of **9** (0.21 g, 0.407 mmol) and 1*H*-tetrazole (0.086 g, 1.22 mmol) in dry pyridine (10 mL) was added chlorophenyldichlorophosphate (0.1 mL, 0.61 mmol) over 5 min under stirring. The reaction mixture was stirred at room temperature for 6 h and evaporated to dryness under reduced pressure (<30 °C). The residue was partitioned between cold CH_2Cl_2 and saturated aqueous $NaHCO_3$. The organic phase was

Scheme 4[a]



[a] (a) I_2, PPh_3, Pyr, MeCN; (b) NaOMe, MeOH, 65 °C; (c) I_2, $[Bn(Et)_3N]N_3$, MeCN, THF, 5 °C; (d) $(Bu_4N)HSO_4$, K_2HPO_4, mCPBA, mCBA, CH_2Cl_2; (e) Bz_2O, Pyr; (f) 4-ClPhOP(O)Cl_2, 1*H*-tetrazole, Pyr, 5 °C; (g) 0.5 M, NH_3/dioxane; (h) 2 M NH_3/MeOH.

Scheme 5[a]



[a] (a) I_2, PPh_3, MeCN, Pyr; (b) tBuOK, DMF; (c) I_2, $[Bn(Et)_3N]N_3$, THF, MeCN; (d) *p*-MeOBzCl, MMP, DMAP, MeCN, THF; (e) NaBz, 15-crown-5, DMF, 60 °C; (f) 2 M NH_3/MeOH.

washed with brine and then dried over $MgSO_4$, filtered, and evaporated. The residue was treated with 0.5 M NH_3 in 1,4-dioxane (20 mL), and the resulting solution was stirred for 4 h at room temperature and evaporated to dryness under reduced pressure. The residue was dissolved in AcOH/MeOH/H_2O [4:2:1 (20 mL)], heated at 50 °C for 5 h, cooled, and evaporated to dryness under reduced pressure. Purification by preparative HPLC (Hypercarb column, H_2O in CH_3CN) provided **10** (0.03 g, 26%) as a white solid. ^1H NMR (400 MHz, methanol-d_4): δ 7.87 (d, J = 7.5 Hz, 1H), 6.12 (dd, J = 1.3 and 19.4 Hz, 1H), 5.89 (d, J = 7.5 Hz, 1H), 5.13 (ddd, J = 1.4, 5.3, and 53.9 Hz, 1H), 4.57 (dd, J = 5.3 and 22.1 Hz, 1H), 3.80 (dd, J = 12.1 and 39.3 Hz, 2H). HRMS (ES^+) calcd for $C_9H_{12}FN_6O_4$ $[M + H]^+$ 287.0904; found 287.0908.

1-(2′-Deoxy-2′-fluoro-5′-iodo-β-D-arabinofuranosyl)uracil (12). A suspension of **11** (1.61 g, 6.54 mmol) and pyridine (7 mL) in anhydrous CH_3CN (142 mL) was cooled on an ice−water bath. Iodine (2.00 g, 7.88 mmol) and triphenylphosphine (2.23 g, 8.50 mmol) were added, and the reaction mixture was stirred at 0 °C for 30 min. After stirring at room temperature for 24 h, the reaction mixture was again cooled on an ice−water bath and more iodine (0.40 g, 1.58 mmol) and triphenylphosphine (0.45 g, 1.71 mmol) were added. The reaction mixture was stirred at room temperature

Table 1. EC_{50} and CC_{50} Determination in the HCV Replicon System

compd	GT-1b stable replicon (2209-23) EC_{50} (μM)	cytotoxicity (2209-23) CC_{50} (μM)	proliferation (^{3}H-Thy incorporation) IC_{50} (μM)
3	1.28	>2000	>100
4	0.171	>1000	>100
10	4.02	>100	>100
17	0.024	>100	>100
20	>100	>100	nd
28	>100	>100	nd
34	0.066	>100	nd
dFAraC	0.114	>100	0.174

for a further 41 h and then concentrated to 1/4 of the volume under reduced pressure, diluted with CH_2Cl_2 (200 mL), and washed with 0.5 M $Na_2S_2O_3$ in saturated aqueous $NaHCO_3$ solution. The aqueous layer was washed with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), treated with activated carbon, and filtered through celite. The filter cake was then washed with CH_2Cl_2 (200 mL), followed by azeotropic distillation of the filtrate with toluene. Chromatography (1−5% MeOH in CH_2Cl_2) gave **12** as an off-white solid (1.78 g, 76%). ^{1}H NMR (400 MHz, methanol-d_4): δ 7.71 (dd, J = 1.8 and 8.2 Hz, 1H), 6.21 (dd, J = 3.3 and 19.9 Hz, 1H), 5.71 (d, J = 8.2 Hz, 1H), 5.02 (ddd, J = 1.8, 3.4, 51.9, 1H), 4.29 (ddd, J = 1.7, 3.3, 17.5, 1H), 3.99 (td, J = 3.4, 6.3, 1H), 3.53−3.45 (m, 2H). MS (ES$^+$) $[M + H]^+$ m/z 357.

1-(2′,5′-Dideoxy-2′-fluoro-erytho-β-D-pent-4-enofuranosyl)uracil (13). Sodium methoxide (0.76 g, 14.1 mmol) was added to a suspension of compound **12** (1.00 g, 2.81 mmol) in anyhdrous methanol (21 mL), and the reaction mixture was stirred at 65 °C for 5 h. The reaction mixture was cooled to room temperature and diluted with methanol (10 mL). Pyridinium form DOWEX-H$^+$ (4 g) was added, and the mixture stirred until a clear solution was obtained (ca. 20 min). The mixture was filtered and the resin washed with methanol and then evaporated to dryness under reduced pressure. Purification by silica gel column chromatography (10% MeOH in CH_2Cl_2 in) to give **13** (0.52 g, 82%) as a white solid. ^{1}H NMR (methanol-d_4): δ 7.46 (dd, J = 2.1 and 8.2 Hz, 1H), 6.50 (dd, J = 3.3 and 19.4 Hz, 1H), 5.71 (d, J = 8.2 Hz, 1H), 5.03 (ddd, J = 1.9, 3.1, and 51.5 Hz, 1H), 4.67 (d, J = 2.3 Hz, 1H), 4.66 (dd, J = 1.7 and 11.7 Hz, 1H), 4.46 (d, J = 2.3 Hz, 1H). MS (ES$^+$) $[M + H]^+$ m/z 229.

1-(4′-Azido-3′-O-benzoyl-2′-deoxy-2′-fluoro-5′-iodo-β-D-arabinofuranosyl)uracil (14). Benzyltriethylammonium chloride (0.98 g, 4.29 mmol) and sodium azide (0.28 g, 4.29 mmol) were suspended in anhydrous CH_3CN (22 mL). The suspension was stirred vigorously for 15 h and was then filtered. The filtrate containing quarternary ammonium azide was added to a solution of compound **13** (0.48 g, 2.10 mmol) and 4-methylmorpholine (0.07 mL, 0.64 mmol) in anhydrous THF (15 mL). A solution of iodine (0.90 g, 3.58 mmol) in anyhdrous THF (19 mL) was added dropwise over 1 h under stirring at 0 °C. The reaction mixture was stirred at 0 °C for a further 4 h. *N*-Acetyl-L-cysteine (0.034 g, 0.21 mmol), 4-methylmorpholine (1.16 mL, 10.5 mmol), and DMAP (0.26 g, 2.10 mmol) were added, followed by a dropwise addition of benzoyl chloride (0.54 mL, 4.63 mmol). Stirring at 0 °C continued for 1 h. A solution of 0.1 M Na_2SO_3 in saturated aqueous $NaHCO_3$ (50 mL) was added, and the mixture was shaken. The mixture was diluted with CH_2Cl_2 (150 mL) and washed with saturated aqueous $NaHCO_3$ and water. The organic layer was separated and the aqueous layer washed with CH_2Cl_2 (2 × 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (3% MeOH in CH_2Cl_2) to give **14** (0.98 g, 93%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (br s, 1H), 8.10−7.50 (m, 6 H), 6.59 (dd, J = 4.3 and 17.9 Hz, 1H), 5.87 (dd, J = 2.1, 11.3 Hz, 1H), 5.84 (t, J = 1.9 Hz, 1H), 5.43 (ddd, J = 2.1, 3.9, and 51.7 Hz, 1H), 3.88−3.79 (m, 2H). MS (ES$^+$) $[M + H]^+$ m/z 501.9.

1-(4′-Azido-2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)uracil (15). 3-Chloroperoxybenzoic acid [55% in balance with 3-chlorobenzoic acid (10%) and water (35%)] (4 × 3.33 g) was added portion-wise over 20 h to a two-phase suspension containing **14** (0.97 g, 1.94 mmol), tetra-*n*-butylammonium hydrogensulfate (0.70 g, 2.05 mmol), and 3-chlorobenzoic acid (0.33 g, 2.13 mmol) in CH_2Cl_2 (125 mL) and 1.7 M aqueous K_2HPO_4 (45 mL) under stirring at rt. A solution of Na_2SO_3 (7.5 g, 59.5 mmol) in saturated aqueous $NaHCO_3$ (150 mL) was added. The mixture was shaken for 5 min. The organic layer was separated and the aqueous layer washed with CH_2Cl_2 (2 × 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure. The residue was treated with saturated ammonia in MeOH (150 mL) for 19 h at room temperature, after which time the solution was evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (10% MeOH in CH_2Cl_2) followed by reversed phase column chromatography (5−10% CH_3CN in H_2O) to give **15** (0.31 g, 56%) as a white solid. ^{1}H NMR (DMSO-d_6): δ 7.64 (dd, J = 1.7 and 8.1 Hz, 1H), 6.40 (d, J = 6.0 Hz, 1H), 6.34 (dd, J = 5.7 and 10.6 Hz, 1H), 5.71 (t, J = 6.0 Hz, 1H), 5.67 (d, J = 8.1 Hz, 1H), 5.25 (dt, J = 5.5 and 53.9 Hz, 1H), 4.45 (dt, J = 5.6 and 23.1 Hz, 1H), 3.78−3.69 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 163.5 (C-4), 150.8 (C-2), 141.8 (C-6), 102.5 (C-5), 97.34 and 97.26 (C-4′), 95.9 and 94.4 (C-2′), 81.7 (C-1′), 75.3 and 75.1 (C-3′), 62.8 (C-5′). MS (ES$^+$) $[MH]^+$ m/z 288.1.

1-(4′-Azido-2′-deoxy-3′,5′-O-dibenzoyl-2′-fluoro-β-D-arabinofuranosyl)uracil (16). A solution of compound **15** (0.244 g, 0.850 mmol), 4-methylmorpholine (0.65 mL, 5.95 mmol), and DMAP (0.05 g, 0.425 mmol) in anhydrous THF (25 mL) was cooled to 0 °C and benzoyl chloride (0.247 mL, 2.12 mmol) was added dropwise over 5 min. The reaction mixture was stirred at 0 °C for a further 1 h and was then diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The aqueous layer was washed with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by silica gel column chromatography (2−5% MeOH in CH_2Cl_2) to give compound **16** (0.406 g, 96%) as a white solid. ^{1}H NMR (methanol-d_4): δ 8.10−7.39 (m, 11H), 6.63 (dd, J = 5.1 and 14.4 Hz, 1H), 6.08 (dd, J = 3.1 and 20.3 Hz, 1H), 5.74−5.72 and 5.61−5.59 (m, 1H), 5.67 (d, J = 8.2 Hz, 1H), 4.96−4.84 (m, 2H). MS (ES$^+$) $[M + H]^+$ m/z 496.0.

1-(4′-Azido-2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)cytosine (17). 4-Chlorophenyldichlorophosphate (0.20 mL, 1.22 mmol) was added dropwise over 5 min to a solution, cooled on an ice−water bath of **16** (0.40 g, 0.815 mmol) and 1*H*-tetrazole (0.17 g, 2.45 mmol) in anhydrous pyridine (15 mL). After stirring for 5 min, the reaction mixture was allowed to warm to room temperature. After 5 h, the reaction mixture was evaporated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ and water, dried (4 Å molecular sieves), and evaporated to dryness under reduced pressure. Azeotropic distillation with toluene removed the remaining traces of pyridine from the product. The residue was then treated with 0.5 M ammonia in 1,4-dioxane (40 mL) for 5.5 h at room temperature. The solution was evaporated to dryness under reduced pressure, and the residue treated with saturated NH_3 in methanol (55 mL) for 15 h at rt. The mixture was evaporated to dryness under reduced pressure and the residue purified by silica gel column chromatography [saturated NH_3 in MeOH (0.5%), MeOH (19.5%), CH_2Cl_2 (80%)] to give **17** as an off-white solid (0.21 g, 93%): mp 99.0−100.0 °C. ^{1}H NMR (methanol-d_4): δ 7.75 (dd, J = 1.2 and 7.6 Hz, 1H), 6.48 (dd, J = 5.0 and 12.4 hz, 1H), 5.91 (d, J = 7.4 Hz, 1H), 5.19 (dt, J = 4.3 and 56.0 Hz, 1H), 4.47 (dd, J = 4.3 and 21.6 Hz, 1H), 3.83 (s, 2H). ^{13}C NMR (DMSO-d_6): δ 166.3 (C-4), 155.3 (C-2), 142.3 (C-6), 97.5 and 97.4 (C-4′), 94.8 (C-5), 96.2 and 94.3 (C-2′), 82.9 (C-1′), 75.8 and 75.6 (C-3′), 63.0 (C-5′). MS (ES$^+$) $[2 M + Na]^+$ m/z 595.2. HRMS (ES$^+$) calcd for $C_9H_{12}FN_6O_4$ $[M + H]^+$ 287.0904; found 287.0911.

1-(4′-Azido-2′,5′-di-O-trityl-β-D-ribofuranosyl)uracil (18). Triphenylmethyl chloride (19.30 g, 69.23 mmol) was added to a solution

of **5** (5.00 g, 17.53 mmol) in pyridine (50 mL). After stirring at 100 °C under N_2 for 72 h, the reaction mixture was cooled to room temperature and then evaporated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2, washed with brine, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. Chromatography (EtOAc in hexanes) provided **18** (7.43 g, 55%) as an off-white foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.02−8.97 (br s, 1H), 7.32−7.25 and 7.54−7.45 (m, 30H), 6.23 (d, J = 6 Hz, 1H), 5.18 (d, J = 8 Hz, 1H), 4.72 (t, J = 5.5 Hz, 1H), 3.36 (t, J = 4.5 Hz, 1H), 3.10 (s, 2H), 2.79 (d, J = 4.1 Hz, 1H). MS (ES^-) $[M - H]^-$ m/z 768.2.

1-(4′-Azido-3′-deoxy-3′-fluoro-2′,5′-di-*O*-trityl-β-D-xylofuranosyl)uracil (19). (Diethylamino)sulfur trifluoride (1.6 mL, 12.40 mmol) was carefully added to a flask containing a solution of **18** (6.40 g, 8.31 mmol) in pyridine (6 mL) and CH_2Cl_2 (90 mL). Once complete, the reaction mixture was heated at 50 °C for 18 h under N_2. The cooled reaction mixture was poured onto ice−water and extracted with CH_2Cl_2. The organics were washed with brine, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. Chromatography (EtOAc in hexanes) provided **19** (2.0 g, 31%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.16−8.14 (br s, 1H), 7.47−7.16 (m, 30H), 6.85 (dd, J = 1.6 and 8 Hz, 1H), 6.66 (d, J = 3.1 Hz, 1H), 5.60 (dd, J = 2.2 and 8 Hz, 1H), 4.14 (dd, J = 3.2 and 21.1 Hz, 1H), 3.72 (d, J = 49 Hz, 1H), 3.41−3.33 (m, 2H). MS (ES^-) $[M - H]^-$ m/z 770.2.

1-(4′-Azido-3′-deoxy-3′-fluoro-β-D-xylofuranosyl)cytosine (20). $POCl_3$ (1 mL, 11.82 mmol) was slowly added to a flask, cooled to 0 °C, containing **19** (2.28 g, 2.95 mmol), triazole (3.06 g, 44.31 mmol), triethylamine (8.2 mL, 59.08 mmol), and CH_3CN (90 mL). The resulting mixture was stirred at 0 °C for 15 min and then allowed to warm to room temperature. After 2.5 h, the crude reaction mixture was evaporated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ solution, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. The resulting solid was dissolved in dioxane (60 mL) and treated with NH_4OH (10 mL) and left to stir at room temperature for 20 h. The crude reaction mixture was evaporated to dryness under reduced pressure. Chromatography (15% MeOH in CH_2Cl_2) provided 1-(4′-azido-3′-deoxy-3′-fluoro-2′,5′-di-*O*-trityl-β-D-xylofuranosyl)cytosine (1.78 g, 2.39 mmol) as a light-brown solid. This material was dissolved in MeOH (60 mL) and treated with 2 M NH_3 in EtOH (7.2 mL). After stirring for 3 h, the precipitate was removed by filtration and the filtrate evaporated to dryness under reduced pressure. Chromatography (0−10% MeOH in CH_2Cl_2) provided **20** (0.40 g, 47%) as an off-white solid; mp 171.8−172.9 °C. ^{1}H NMR (300 MHz, methanol-d_4): δ 7.66 (d, J = 7.5 Hz, 1H), 6.26 (d, J = 4.2 Hz, 1H), 5.95 (d, J = 7.5 Hz, 1H), 4.77 (dd, J = 2.5 and 51 Hz, 1H), 4.44 (ddd, J = 2.5, 4, and 21 Hz, 1H), 3.99−3.87 (m, 2H). HRMS (ES^+) calcd for $C_9H_{12}FN_6O_4$ $[M + H]^+$ 287.0904; found 287.0896.

1-(3′-Deoxy-3′-fluoro-5′-iodo-β-D-ribofuranosyl)uracil (22). 3′-Deoxy-3′-fluorouridine[17] **21** (0.31 g, 1.28 mmol) and triphenylphosphine (0.47 g, 1.79 mmol) were dissolved in CH_3CN/pyridine [95:5 (13 mL)]. Iodine (0.422 g, 1.66 mmol) was added, and the reaction mixture was stirred for 18 h at room temperature. Water (5 mL) was added, and the solvents were evaporated to dryness under reduced pressure. Azeotropic distillation with CH_3CN and then with $CHCl_3$ was performed to remove the water. The residue was purified by silica gel column chromatography (2−6% EtOH in CH_2Cl_2) to give **22** (0.426 g, 93%) as a white solid. ^{1}H NMR (DMSO-d_6): δ 11.43 (br s, 1H), 7.70 (d, J = 8.1 Hz, 1H), 5.95 (d, J = 6.1 Hz, 1H), 5.85 (d, J = 8.0 Hz, 1H), 5.72 (d, J = 8.1 Hz, 1H), 4.90 (dd, J = 4.6 and 53.0 Hz, 1H), 4.55−4.40 (m, 1H), 4.25 (dt, J = 7.5 and 13.7 Hz, 1H), 3.48 (ddd, J = 6.9, 10.4, 16.5, 2H). MS (ES^+) $[M + H]^+$ m/z 357.1.

1-(3′,5′-Dideoxy-3′-fluoro-erytho-β-D-pent-4-enofuranosyl)uracil (23). Compound **22** (0.426 g, 1.2 mmol) was dissolved into a solution of 0.4 M sodium methoxide in MeOH (27 mL). The reaction mixture was stirred for 5 h at 65 °C. Pyridinium form DOWEX H^+ was added portionwise until neutral pH (2−3 g), and the mixture was stirred at room temperature for 10 min. The resin was removed by filtration and washed with MeOH, and the filtrate was evaporated to dryness under reduced pressure. The residue was slurried in 2% EtOH in CH_2Cl_2 and purified by silica gel column chromatography (2−6% EtOH in CH_2Cl_2) to give **23** as a white solid (0.251 g, 92%). ^{1}H NMR (DMSO-d_6): δ 11.50 (br s, 1H), 7.75 (d, J = 8.1 Hz, 1H), 6.10 (d, J = 6.2, 1H), 6.04 (d, J = 7.6 Hz, 1H), 5.70 (dd, J = 2.2, 8.1 Hz, 1H), 5.26 (dd, J = 4.4 and 55.8 Hz, 1H), 4.68−4.55 (m, 1H), 4.55 (ddd, J = 2.2, 6.4, and 18.9 Hz, 2H). MS (ES^+) $[M + H]^+$ m/z 229.1.

1-(4′-Azido-3′-deoxy-3′-fluoro-5′-iodo-β-D-ribofuranosyl)uracil (24). Benzyltriethylammonium chloride (0.296 g, 1.3 mmol) and sodium azide (0.084 g, 1.3 mmol) were suspended in anhydrous CH_3CN (7 mL) and sonicated for several minutes. The resulting fine suspension was stirred for 3 h at room temperature and filtered under nitrogen into a dry THF solution (4.4 mL) of **23** (0.148 g, 0.65 mmol). 4-Methylmorpholine (0.021 mL, 0.19 mmol) was added, the resulting solution was cooled on an ice−water bath, and a solution of iodine (0.28 g, 1.1 mmol) in anhydrous THF (6 mL) was added dropwise over 60 min. The reaction mixture was stirred for 16 h at 0−9 °C. *N*-Acetyl-L-cysteine (0.011 g, 0.065 mmol) was added, and the solution was stirred until bubbling subsided. The solvent was concentrated under reduced pressure to half-volume, and then a solution of 0.1 M $Na_2S_2O_3$ in saturated aqueous $NaHCO_3$/brine was added under stirring. The mixture was extracted with 10% EtOH in CH_2Cl_2, and the organic layers were dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The 4′-epimeric mixture (~1:1) was separated by silica gel column chromatography (2−3% EtOH in CH_2Cl_2) and then by semi-preparative Hyper Carb HPLC column purification (10−90% CH_3CN in H_2O) to give **24** as a white solid (0.081 g, 31%). ^{1}H NMR ($CDCl_3$): δ 8.50 (br s, 1H), 7.34 (d, J = 8.1 Hz, 1H), 5.81 (d, J = 8.1 Hz, 1H), 5.70 (d, J = 4.1 Hz, 1H), 5.38 (dd, J = 5.7 and 52.1 Hz, 1H), 4.84−4.76 (m, 1H), 3.55 (dd, J = 11.3 and 28.8 Hz, 2H). MS (ES^-) $[M - H]^-$ m/z 395.9.

1-(4′-Azido-5′-*O*-(4-chloro)benzoyl-3′-deoxy-3′-fluoro-β-D-ribofuranosyl)uracil (25). A solution of **24** (0.081 g, 0.2 mmol) in CH_2Cl_2 (12 mL) was combined with a mixture of tetrabutylammonium hydrogensulfate (0.073 g, 0.22 mmol) and *m*-chlorobenzoic acid (0.048 g, 0.31 mmol) in 1.75 M aqueous K_2HPO_4 (4.7 mL). The two-phase system was stirred vigorously at room temperature, and one portion of *m*-chloroperbenzoic acid [55% in balance with 3-chlorobenzoic acid (10%) and water, (35%)] (0.360 g) was added. After 1.5 h, 3 × 120 mg of this reagent mixture was added at 1.5 h intervals. After the last addition, the mixture was vigorously stirred for another 18 h at rt. A solution of 0.1 M Na_2SO_3 in saturated aqueous $NaHCO_3$ (10 mL) was added, and the mixture was stirred vigorously at rt for 15 min. The organic layer was separated, and the water layer was extracted with CH_2Cl_2. The combined organic layers were washed with saturated aqueous $NaHCO_3$. The organic layer was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (2−4% EtOH in CH_2Cl_2) to give **25** as a white solid (0.032 g, 37%). ^{1}H NMR ($CDCl_3$): δ 8.04 (t, J = 1.8 Hz, 1H), 7.96−7.91 (m, 1H), 7.57 (ddd, J = 1.1, 2.2, and 8.0 Hz, 1H), 7.42 (t, J = 7.9 Hz, 1H), 7.29 (d, J = 8.1 Hz, 1H), 5.75 (d, J = 8.1 Hz, 1H), 5.74 (d, J = 2.9 Hz, 1H), 5.52 (dd, J = 5.7 and 51.8 Hz, 1H), 4.83−4.75 (m, 1H), 4.61 (s, 2H). MS (ES^-) $[M - H]^-$ m/z 424.

1-(4′-Azido-2′-*O*-benzoyl-5′-*O*-(4-chloro)benzoyl-3′-deoxy-3′-fluoro-β-D-ribofuranosyl)uracil (26). Benzoic anhydride (0.021 g, 0.09 mmol) was added to a solution of **25** (0.032 g, 0.076 mmol) in pyridine (0.75 mL). After stirring for 16 h at rt, MeOH (5 mL) was added. The solvents were evaporated to dryness under reduced pressure, and the residue was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. The organic phase was separated, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. Purification by silica gel column chromatography (1% EtOH in CH_2Cl_2) gave **26** (0.040 g, 98%) as a white foam. ^{1}H NMR ($CDCl_3$): δ 8.86 (br s, 1H), 8.12−8.08 (m, 3H), 8.00−7.96 (m, 1H), 7.66−7.40 (m, 5H), 7.22 (d, J = 8.1 Hz, 1H), 5.89−5.76 (m, 4H), 4.75−4−64 (m, 2H). MS (ES^+) $[M + H]^+$ m/z 530.6.

1-(4′-Azido-3′-deoxy-3′-fluoro-β-D-ribofuranosyl)cytosine (28). Compound **26** (0.040 g, 0.075 mmol) and 1*H*-tetrazole (0.016 g, 0.23 mmol) were azeotroped with pyridine to remove moisture and then redissolved in anhydrous pyridine (1.3 mL). The solution was cooled on an ice−water bath, and 4-chlorophenylphosphorodichloridate (0.018 mL, 0.11 mmol) was added. The reaction mixture was stirred at 0−5 °C for 5 min and then allowed to warm to room temperature. After 5 h, the reaction mixture was evaporated to dryness under reduced pressure and the residue was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. The organic phase was separated, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The intermediate **27** was then treated with 0.5 M NH_3 in 1,4-dioxane (5 mL) for 4 h at rt and then evaporated to dryness under reduced pressure. The residue was dissolved in saturated NH_3 in MeOH (15 mL) and the mixture was stirred for 16 h at room temperature and then evaporated to dryness under reduced pressure. Purification by semipreparative Hyper Carb HPLC column purification (10−90% CH_3CN in H_2O) gave **28** (0.011 g, 53%) as a white solid. ^{1}H NMR (DMSO-d_6): δ 7.71 (d, J = 7.5 Hz, 1H), 7.32 (br d, J = 4.8 Hz, 2H), 6.18 (d, J = 7.6 Hz, 1H), 5.98 (br s, 1H), 5.78 (d, J = 7.5 Hz, 1H), 5.72 (br s, 1H), 5.07 (dd, J = 4.5 and 53.6 Hz, 1H), 4.45 (ddd, J = 4.4, 7.3, and 22.2 Hz, 1H), 3.49 (q, J = 12.1 Hz, 2H). MS (ES$^+$) [M + H]$^+$ m/z 287. HRMS (ES$^+$) calcd for $C_9H_{12}FN_6O_4$ [M + H]$^+$ 287.0904; found 287.0909.

1-(4-*N*-Benzoyl-2′-dideoxy-2′,2′-difluoro-5′-iodo-β-D-ribofuranosyl)cytosine (30). A solution of 4-*N*-benzoylgemcitabine (**29**)[15] (3.50 g, 9.54 mmol) in dry CH_3CN/pyridine [95:5 (150 mL)] was cooled on an ice−water bath. Iodine (3.15 g, 12.4 mmol) and triphenylphosphine (3.38 g, 12.9 mmol) were added, and the reaction mixture was stirred at 0 °C for 30 min. The ice−water bath was then removed, and stirring continued at room temperature for 64 h. The reaction mixture was diluted with CH_2Cl_2 (300 mL) and washed with 0.5 M $Na_2S_2O_3$ in saturated aqueous $NaHCO_3$ (200 mL). The aqueous layer was washed with CH_2Cl_2 (4 × 25 mL). The combined organic layers were dried (Na_2SO_4) and filtered. The filtrate was diluted with toluene (150 mL) and concentrated under reduced pressure. Purification by silica gel column chromatography (1−5% MeOH in CH_2Cl_2) gave **30** (4.10 g, 90%) as a white solid. ^{1}H NMR (methanol-d_4): δ 8.20−7.53 (m, 7H), 6.34 (t, J = 8.2 Hz, 1H), 4.19−4.06 (m, 1H), 3.88−3.82 (m, 1H), 3.72−3.68 (m, 1H), 3.61−3.57 (m, 1H). MS (ES$^+$) [M + H]$^+$ m/z 478.

1-(4-*N*-Benzoyl-2′,5′-dideoxy-2′,2′-difluoro-β-D-pent-4-enoribofuranosyl)cytosine (31). Compound **30** (4.10 g, 8.59 mmol) was dissolved in anhydrous *N*,*N*-dimethylformamide (125 mL), and the solution was cooled to 0 °C. A solution of potassium *tert*-butoxide (1.93 g, 17.2 mmol) in anhydrous *N*,*N*-dimethylformamide (25 mL) was added dropwise over 20 min. The reaction mixture was stirred at 0 °C for another 60 min, after which time HPLC analysis showed some remaining compound **30**. A solution of potassium *tert*-butoxide (0.19 g, 1.72 mmol) in anhydrous *N*,*N*-dimethylformamide (2.5 mL) was added dropwise over 5 min, and stirring continued for another 5 min at 0 °C. Pyridinium form DOWEX H$^+$ (16 g) was added, and the mixture was stirred at room temperature for 10 min. The resin was removed by filtration and washed with DMF. The filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10% MeOH in CH_2Cl_2) to give **31** (2.37 g, 79%) as a white solid. ^{1}H NMR (methanol-d_4): δ 7.99−7.52 (m, 7H), 6.47 (t, J = 7.3 Hz, 1H), 4.89 (t, J = 9.7 Hz, 1H), 4.78 (s, 1H), 4.57 (s, 1H). MS (ES$^+$) [M + H]$^+$ m/z 350.

1-(4′-Azido-4-*N*-benzoyl-2′-dideoxy-2′,2′-difluoro-5′-iodo-β-D-ribofuranosyl)cytosine (32). Benzyltriethylammonium chloride (3.08 g, 13.5 mmol) and NaN_3 (0.878 g, 13.5 mmol) were suspended in anhydrous CH_3CN (150 mL) and sonicated for 3 min. The resulting fine suspension was stirred for 3 h at room temperature and was then filtered under nitrogen into a dry THF solution (47 mL) of **31** (2.36 g, 6.76 mmol). 4-Methylmorpholine (0.223 mL, 2.03 mmol) was added and the resulting solution cooled on an ice−water bath. A solution of iodine (2.92 g, 11.5 mmol) in anhydrous THF (62 mL) was added dropwise over 60 min and then left to stir for 16 h at 0−9 °C. The mixture was concentrated to half-volume under reduced pressure and the remaining mixture left to stir for 24 h at 0−9 °C. *N*-Acetyl-L-cysteine (0.11 g, 0.68 mmol) was added, and the solution was stirred until bubbling subsided. The mixture was kept at 5 °C and 4-methylmorpholine (3.72 mL, 33.8 mmol) and DMAP (826 mg, 6.76 mmol) were added, followed by anisoylchloride (3.45 g, 20.3 mmol) dissolved in MeCN (14 mL). The resulting mixture was stirred for 1 h. MeOH (15 mL) was added and the solvents were concentrated to half-volume under reduced pressure, and then a solution of 0.1 M $Na_2S_2O_3$ in saturated aqueous $NaHCO_3$ (500 mL) was added and the mixture was stirred until colorless. The mixture was extracted with CH_2Cl_2, and the organic layer was separated and then washed with 5% citric acid, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was separated twice by silica gel column chromatography (0.5−2% EtOH in CH_2Cl_2) to give **32** (3.33 g, 76%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (d, J = 8.6 Hz, 2H), 7.95 (br s, 1H), 7.90 (d, J = 8.0 Hz, 2H), 7.70 (br s, 1H), 7.65 (t, J = 7.2 Hz, 1H), 7.55 (t, J = 8.0 Hz, 2H), 6.98 (d, J = 8.6 Hz, 2H), 6.72 (br s, 1H), 5.90 (br s, 1H), 3.91 (s, 3H), 3.87 (s, 2H). MS (ES$^+$) [M + H]$^+$ m/z 484.

1-[4′-Azido-4-*N*-5′-*O*-dibenzoyl-3′-*O*-(4-methoxybenzoyl)-2′-dideoxy-2′,2′-difluoro-β-D-ribofuranosyl)cytosine (33). Compound **32** (3.33 g, 5.1 mmol) was dissolved in DMF (43 mL), together with 15-crown-5 (1.72 mL, 8.67 mmol) and sodium benzoate (1.25 g, 8.67 mmol), and the mixture was stirred for 65 h at 60 °C. Further sodium benzoate (2.5 g, 17.34 mmol) and 15-crown-5 (3.4 mL, 17.34 mmol) were added, and the mixture was stirred for 5 d. As the reaction was not complete, further quantities of sodium benzoate (1.25 g, 8.67 mmol), 15-crown-5 (1.72 mL, 8.67 mmol), and DMF (5 mL) were added. Stirring continued for a further 4 d. The thick suspension was filtered through celite and the filtrate evaporated to dryness under reduced pressure. The residue was treated with EtOAc, and the mixture was extracted with 5% aqueous $NaHCO_3$. The organic layer was separated, washed with water, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. Purification by silica gel column chromatography (20−40% EtOAc in hexanes) provided **33** as an off-white solid (1.46 g, 44%). ^{1}H NMR ($CDCl_3$): δ 8.12−7.43 and 6.97−6.95 (m, 16 H), 6.77 (m, 1H), 5.86 (m, 1H), 4.87 (s, 2H), 3.89 (s, 3H). MS (ES$^+$) [M + H]$^+$ m/z 647.

4′-Azido-2′-dideoxy-2′,2′-difluoro-β-D-ribofuranosyl)cytosine (34). Compound **33** (1.46 g, 2.26 mmol) was dissolved in dioxane (10 mL) and then saturated NH_3 in methanol (150 mL) was added and the mixture was stirred for 16 h at room temperature. The residue after evaporation of the solvents was subjected to purification by silica gel column chromatography (5−20% EtOH in CH_2Cl_2) and by semipreparative Hyper Carb HPLC column chromatography (10−90% CH_3CN in H_2O) to give **34** as a white solid (0.548 g, 80%); mp 143.0−144.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.58 (d, J = 7.6 Hz, 1H), 7.46 (s, 2H), 6.73 (br s, 1H), 6.30 (br s, 1H), 5.81 (br s, 1H), 5.80 (d, J = 7.6 Hz, 1H), 4.50 (br s, 1H), 3.80 (s, 2H). MS (ES$^+$) [M + H]$^+$ m/z 305. HRMS (ES$^+$) calcd for $C_9H_{11}F_2N_6O_4$ [M + H]$^+$ 305.0810; found 305.0812.

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Supporting Information Available: Biology methods, analytical methods, and HPLC spectral data for compounds **10**, **17**, **20**, **28**, and **34**. This material is available free of charge via the Internet at http://pubs.acs.org.

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The application of phosphoramidate ProTide technology to the potent anti-HCV compound 4′-azidocytidine (R1479)

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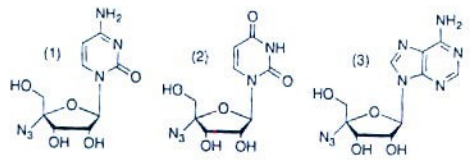
ABSTRACT

We report the design, synthesis and evaluation of a family of ca 50 phosphoramidate ProTides of the potent anti-HCV compound 4′-azidocytidine (R1479), with variation on the ester, amino acid and aryl moiety of the ProTide. Sub-μM inhibitors of HCV emerge. The compounds are all non-cytotoxic in the replicon assay. We herein report detailed SARs for each of the regions of the ProTide.

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The discovery of new inhibitors of the hepatitis C virus (HCV) is a major current activity of both the pharmaceutical industry and academia. The Roche group have previously reported that various 4′-substituted ribonucleosides were endowed with potent and selective inhibitory activity against HCV[1] with 4′-azidocytidine (R1479) (**1**) emerging as a promising clinical candidate.[2]

As with the majority of nucleoside therapeutics (**1**) requires intracellular phosphorylation to its 5′-triphosphate form to interact at its viral (RNA polymerase) target.[2] As HCV lacks any suitable nucleoside kinase expression, the necessary phosphorylation steps must be host kinase mediated. Since such phosphorylations may be inefficient for modified nucleoside analogues a number of laboratories have developed phosphate pro-drug methods to allow the direct use of the masked monophosphate.[3] The Cardiff group first introduced phosphoramidate ProTides in 1996[4] and this motif has been reviewed by us[5] and now adopted for example by Gilead for phosphonate delivery[6] and Pharmasset for their 2′-modified anti-HCV family.[7] In a collaboration between the Cardiff and Roche laboratories we have previously reported the significant enhancement in the potency of the uridine analogue of **1**, compound **2** by the application of phosphoramidate ProTide methods.[8] Indeed, the triphosphate of **2** is a similarly potent inhibitor of HCV RNA polymerase as the triphosphate of **1**[2,8] even though the parent nucleoside (**2**) is inactive versus the virus; presumably due to poor phosphorylation. Thus, the ProTide method boosted **2** from inactive to sub-μM potency.[8] We subsequently noted a similar effect on the purine analogue 4′-azidoadenosine (**3**).[9]



Given the clinical progression of **1** as its ester pro-drug[10] we were interested to consider whether the antiviral activity of **1** might be further enhanced by our ProTide methodology and we herein report the early results of this study.

The synthesis of **1** was achieved entirely as has been recently reported,[11] involving the stereo- and regio-selective addition of TMS-azide to the appropriate 4′,5′-olefin related to **1**. We prepared a family of 5′-ProTides of **1** using phosphorochloridate chemistries we have extensively reported.[4,8,9]

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In the first instance, the synthesis of phosphoramidates was carried out in the presence of **1** with phosphorochloridate prepared from phenyl phosphorochloridates and amino acid ester hydrochlorides[4] in THF in the presence of *t*BuMgCl followed by repeated column chromatography gave the initial family of phenyl aminoacyl ProTides in low to moderate yield (Table 1, route 1).[12] Thirty phosphoramidates were synthesized in this way with problems of purification and solubility of the starting material (**1**) leading to low yields. In order to overcome this problem and considering our previous work, the phosphoramidate synthesis was performed with 2′/3′ protected nucleoside. In the first instance, the 2′,3-isopropylidine nucleoside was synthesized under standard conditions (Fig. 1, route 2). The synthesis of the phosphoramidates was then performed as above followed by deprotection in the presence of acetic acid (Fig. 1). In this way 10 phosphoramidates were synthesized with improvements in the purification and consequently in the overall yield. In order to further improve the yields of this synthetic pathway, the cyclopentylidine group was used as an alternative protecting group in 2′- and 3′-positions (Fig. 1, route 3).

The subsequent phosphoramidate synthesis was performed under standard condition followed by deprotection reaction in the presence of formic acid (80% v/v) at room temperature for 5 h. In this way 10 phosphoramidates were synthesized with a purification method that required only one column chromatography for each step and with the consequent improvements in the overall yield compared to routes 1 and 2.

Each of the phenyl ProTides **4a–at** was tested for inhibition of HCV in replicon as previously detailed with data being shown in Table 1.

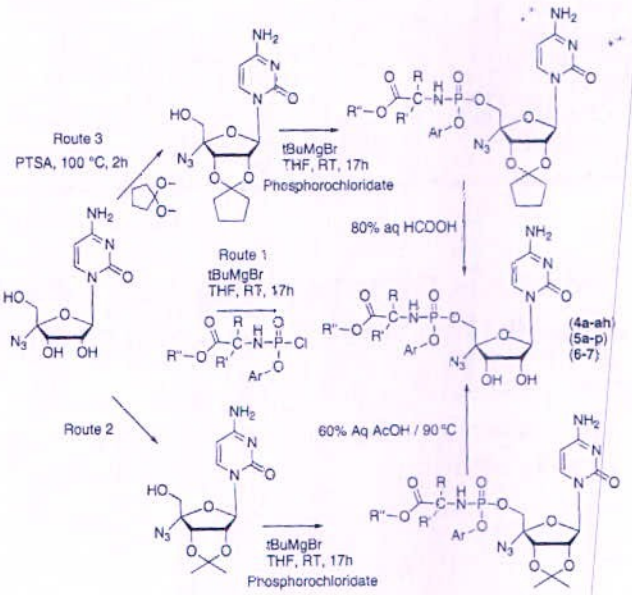


Figure 1.

Thus, the parent nucleoside (**1**) was active versus HCV replicon with a mean IC_{50} value of 1.28 μM. We have often noted the benzyl alanine phenyl ProTide to be a reasonable motif to embark with[4,8,9] and we did so on this occasion, and with some variation of the amino acid moiety.

Thus **4a–g** represent the benzyl esters of L-Ala, D-Ala, α,α-dimethylglycine, Gly, L-Val, L-Leu and L-Phe, respectively. Compound

Table 1
Anti-HCV activity and cytotoxicity of **4a–ah**

Route	Compd	Amino acid	R″	HCV replicon EC_{50}[a] (μM)	HCV replicon CC_{50} (μM)
1	**4a**	Ala	Bn	6.0	>100
1	**4b**	D-Ala	Bn	2.2	>100
1	**4c**	Me2Gly	Bn	9.2	>100
2	**4d**	Gly	Bn	2.1	>100
1	**4e**	Val	Bn	5.6	>100
1	**4f**	Leu	Bn	88% (4.2)[b]	>100
1	**4g**	Phe	Bn	89% (7.7)[b]	>100
2	**4h**	Ala	Et	0.38	>100
2	**4i**	D-Ala	Et	2.3	>100
1	**4k**	Leu	Et	11	>100
1	**4l**	Ile	Et	>100	>100
1	**4m**	Phe	Et	11	>100
1	**4n**	Met	Et	18	>100
1	**4o**	EtAsp	Et	88% (4.4)[b]	>100
1	**4p**	EtGlu	Et	74% (44)[b]	>100
1	**4q**	CycloPntGly	Et	69% (56)[b]	>100
2	**4r**	Pro	Et	>100	>100
1	**4s**	*N*-MeGly	Et	72% (48)[b]	>100
3	**4t**	BetaAla	Et	77% (47)[b]	>100
2	**4u**	Ala	*i*Pr	0.99	>100
1	**4v**	Me2Gly	*i*Pr	3.1	>100
1	**4w**	Leu	*i*Pr	>100	>100
1	**4x**	Phe	*i*Pr	2.9	>100
3	**4y**	Gly	*i*Pr	15	>100
3	**4y′**	Gly	*i*Pr	4.5	>100
2	**4z**	Ala	Me	3.1	>100
1	**4aa**	Ala	*n*Bu	11	>100
1	**4ab**	D-Ala	*n*Bu	0.94	>100
1	**4ac**	Ala	2-Bu	0.62	>100
1	**4ad**	Ala	*t*Bu	0.72	>100
1	**4ae**	D-Ala	*t*Bu	>100	>100
3	**4af**	Phe	*t*Bu	84% (6.7)[b]	>100
3	**4ag**	Ala	Ph	2.9	>100
1	**4ah**	D-Ala	C12H25	30	>100
R1479	**1**	—	—	1.28	—

[a] HCV Con 1 (GT1b) replicon inhibition. All data are means from ⩾2 experiments.
[b] Level of inhibition at top concentration (100 μM) in % (approximate IC_{50}).

4a is slightly less potent than **1** in this assay, implying a slightly lower eventual level of the triphosphate of (**1**) being delivered by (**4a**) as compared to (**1**) under the assay conditions. This is in contrast to the corresponding ProTide of (**2**), where we noted a >200-fold boost in potency.[8] This most likely relates to the relative efficiency of phosphorylation of (**1**) and (**2**) in the replicon system, with (**1**) being a good substrate for deoxycytidine kinase.[20] Although we have previously noted that D-amino acids, such as D-Ala, were poorly effective as ProTide motifs[13] we did observe that the benzyl D-Alanine derivative of (**2**) showed similar potency with only a twofold increased IC_{50} as compared to the L-analogue.[8] In the case of the cytidine analog (**1**), the D-Ala analogue (**4b**) was slightly (2.7-fold) more active as compared to the L-Ala analog (**4a**). Also, replacement of both glycine hydrogen atoms by methyl groups only led to a rather small loss of potency for the dimethylglycine compound (**4c**), with 4.2-fold as compared to the D-Ala analog (**4b**), similar to what had been seen with analogous prodrugs of (**2**).[8]

The glycine analogue (**4d**) showed similar potency as compared to the D-Ala analog (**4b**). The Valine analogue (**4e**) was similarly potent as compared to L-Ala (**4a**). Longer amino acids such as Leu (**4f**) and Phe (**4g**) could also be introduced without a large impact on apparent EC_{50}, although these compounds did not reach 90% inhibition levels under the assay conditions.

It was also of interest to explore other esters. Firstly, we prepared a series of ethyl esters of varying amino acids (**4h–t**). The ethyl alanine compound (**4h**) was the most potent of the series with a mean EC_{50} of 380 nM, thus 3.4-fold more potent than R1479 (**1**) and 16-fold more potent than the benzyl equivalent (**4a**). In the case of Et esters, the D-Ala analogue (**4i**) was sixfold less potent as compared to the L-Ala analogue. The introduction of bulky amino acid side chains significantly reduced potency in the Et-ester subseries; Leu (**4k**), and Phe (**4m**) showed 29-fold reduced potency as compared to the L-Ala analogue, whereas Ile (**4l**) was inactive. These results are in contrast to the case of nucleoside **2** where the Et-Phe analogue, was similarly potent as the Et-L-Ala analogue.[8] Several other amino acids were also explored as their ethyl esters. Thus, the Met (**4n**), ethyl aspartate (**4o**), and particularly ethyl glutamate (**4p**) analogues were relatively poorly active. The unusual spiro fused cyclopentyl glycine motif in (**4q**) has been found to be effective in other ProTide programmes in our laboratory, but was poorly active here, as was the Proline compound (**4r**). The poor activity of the latter could correspond to its unique cyclic structure amongst natural amino acids, or could arise from the need for a free NH. The poor activity of the *N*-methylglycine compound (**4s**) supports the latter point at least, although the Proline ring may also be detrimental. Lastly in this series we prepared a chain extended beta amino acid; beta alanine (**4t**), which was poorly active. Alpha amino acids have been considered essential by us for the putative activation mechanism of ProTides[14] and indeed early chain-extended examples were inactive when applied to the anti-HIV agent d4T.[15] Thus, it appears here too that an alpha amino acid with a free NH and a small amino acid motif is essential for potent activity.

Gilead have found success in applying our approach to acyclic nucleoside phosphonates[6] and have found particular advantage from alanines with branched aliphatic esters such as *i*Pr. For compound **2** we found that iPr esters were roughly equiactive with Et examples.[8] Thus, here we prepared iPr examples of Ala (**4u**), dimethylglycine (**4v**), Leu (**4w**), Phe (**4x**), and Gly (**4y**). In general activities fell within the range observed with the ethyl and benzyl series above. Notably, the Phe compound which showed 29-fold reduced potency as compared to L-Ala in the Et ester series had less of a detrimental effect in the benzyl and iPr series. In the case of the iPr glycine compound we observed a separation of the two diastereoisomers arising from mixed stereochemistry at the phosphate centre. Such an isomeric pair was present in every case, and roughly 1:1 as judged by ^{31}P NMR. In most cases the isomers were tested as mixtures. However, the fortuitous separation of the isomers in the case of **4y** lead to two fractions being evaluated separately. Although not fully resolved, **4y** was primarily the more polar isomer, based on reverse phase HPLC (9:1 'fast'/'slow') while **4y′** was primarily the less polar isomer (3.5:6.5 'fast'/'slow'). The 3.3-fold difference in potency noted between **4y** and **4y′** suggests a ca. sevenfold difference for the pure separate isomers, with the more lipophilic compound being more potent. In the case of two diastereomers of a ProTide of **2** we previously noted no significant difference in activity,[8] although we[16] and others[6] have seen more substantial isomers differences in some cases.

Shortening (**4z**) and linear extension (**4aa–ac**) of the ester chain was also explored. The activities and SARs noted were rather similar to those captured above, although the *n*-butyl case was anomalous, with the D-Ala example (**4ab**) being unusually more active than the L-compound (**4aa**).

The *t*-Butyl case was even more intriguing. Historically, we have generally observed a reduction, and in some cases a loss, of activity for *t*-Bu esters,[17] this being attributed to poor hydrolysis of these systems in vitro. In the case of ProTides of (**2**) we did see activity for a *t*-Bu ester,[8] although it was a log less active than the benzyl lead. In the case of (**1**), the *t*BuAla compound (**4ad**) was surprisingly similarly potent as compared to the Et-Ala lead, while the *t*Bu-D-Ala analogue (**4ae**) was inactive, as was the Phe compound (**4af**), thus differing from the SAR in the Et ester series. This is amongst the most striking and specific example of the apparent

Table 2
Anti-HCV activity and cytotoxicity of **5a–p**

Route	Compd	Ph subst	Amino acid	R″	HCV replicon EC_{50} (μM)	HCV replicon CC_{50} (μM)
3	**5a**	*o*-Cl	Ala	Bn	3.5	>100
2	**5b**	*m*-Cl	Ala	Bn	3.7	>100
1	**5c**	*p*-Cl	Ala	Bn	6.2	>100
3	**5d**	*o*-OMe	Ala	Bn	2.3	>100
3	**5e**	*m*-OMe	Ala	Bn	8.8	>100
1	**5f**	*p*-OMe	Ala	Bn	>100	>100
2	**5g**	*o*-Me	Ala	Bn	1.3	>100
3	**5h**	*p*-Me	Ala	Bn	0.51	>100
3	**5i**	*p*-Br	Ala	Bn	6.5	>100
2	**5j**	3,4-Cl_2	Ala	Bn	9.2	>100
2	**5k**	*p*-Me	Ala	Et	2.9	>100
3	**5l**	2,6-(OMe)	Ala	Et	4.1	>100
1	**5m**	*p*-Cl	Leu	Et	2.5	>100
1	**5n**	*p*-OMe	Leu	Et	9.3	>100
1	**5o**	*p*-Me	Leu	Et	4.3	>100
1	**5p**	3,4-Cl_2	Leu	Et	2.3	>100

Table 3
Anti-HCV activity and cytotoxicity of **6–7**

Route	Compd	Nap	Isomer	HCV replicon EC_{50} (μM)	HCV replicon CC_{50} (μM)
3	**6**	1-Nap	Polar	0.95	>100
3	**6′**	1-Nap	Non-polar	1.2	>100
1	**7**	2-Nap	Polar	4.3	>100
1	**7′**	2-Nap	Non-polar	2.9	>100

interplay between ester and amino acid motifs in ProTide SAR that we have observed.

In the case of ProTides of the anti-HIV agent d4T we have previously studied the effect of substitutions in the phenyl moiety,[18] and noted the beneficial effect of a *p*-Cl group for example. Thus we pursued Ph substitution in the present case. The same synthetic access was used as for the parent Ph systems **4a–ah** but it was now necessary to prepare the appropriate substituted phenyl phosphorodichloridates by reaction of the corresponding phenol and $POCl_3$ in inert solvent. The dichloridates were assayed by ^{31}P NMR and used without purification in most cases. Thus, as noted in Table 2, we explored a number of *o*-, *m*-, and *p*-mono- and di- substitutions on the Ph ring, with electron-withdrawing and donating groups, and examples in the BnAla, EtAla and EtLeu series.

Overall, phenyl substitution did not have a great affect on potency in this series, although **5h** did emerge as slightly more active than the parent nucleoside **1** (ca threefold) and its parent ProTide **4a** (ca 10-fold).

We previously noted a boost in efficacy for anti-cancer ProTides of BVDU[19] and also for the anti-HCV activity of ProTides of (**2**) on substitution of the phenyl moiety entirely by a 1-naphthyl unit. Thus, we prepared the 1-naphthyl analogue of (**4a**), using the phosphorochloridate prepared from naphthyl phosphorodichloridate and benzylalanine hydrochloride. The product was separated into its polar (**6**) and non-polar (**6′**) diastereomers by reverse phase HPLC. The compounds were essentially pure by ^{31}P NMR, with **6** resonating upfield of **6′** (δ_P 3.75, 3.87). We also prepared the corresponding 2-naphthyl analogue and also resolved it into its separate isomers **7** and **7′**. Data on the naphthyl family are presented in Table 3.

As we note in Table 2, the 1-naphthyl compound showed ~6-fold improved potency as compared to the parent Phenyl BnAla (**4a**) compound, with no significant difference in potency between the two diastereomers **6** and **6′**. By contrast, the 2-naphthyl compounds (**7** and **7′**) were slightly less potent (3–5-fold).

In conclusion, we herein report the application of the phosphoramidate ProTide method to the potent anti-HCV agent 4′-azidocytidine (**1**). Approximately 50 new compounds were prepared, with variations in the ester, amino acid, and aryl moiety. Structure activity relationships support the notion that an α-amino acid is essential for significant activity and a free NH on the amino acid also seems important. The ester moiety can be varied significantly. Interestingly, in this study *t*-butyl is an acceptable ester in the case of L-alanine, but not other amino acids. Small amino acids were best tolerated; L and (in most cases) D-alanine, and glycine. Larger and branched amino acids were generally less or poorly effective. Substitution in the phenyl ring was tolerated by a variety of substituents at various positions, though no substantial enhancements in potency were seen. 1-Naphthyl provided a small benefit over phenyl, whereas 2-naphthyl could substitute with no loss of potency. Lastly, in three cases the phosphate diastereoisomers were resolved and tested separately in replicon assay, and little difference in potency noted between the stereoisomers.

Many of the ProTides herein described retained or slightly (up to 3–4 fold) enhanced the potency of **1** in replicon, but the true potential of such ProTides is only likely to become apparent with further in vitro and in vivo study. For example, it is notable that the lead agent **5h** has a calculated lipophilicity (Clog *P*, ChemDraw Ultra 11.0) of 1.2 versus −1.9 for parent nucleoside **1**; the almost 3-log lipophilicity boost may well lead to more efficient passive diffusion into cells and independence from nucleoside transporters, which could convey significant in vivo advantage. Nucleoside deaminase resistance is also likely for these ProTides, based on prior examples.[21] Also, notably two cytidine analogues for HCV, NM 283 and R1626, have recently shown efficacy in HIV infected subjects, but have been withdrawn due to safety reasons.[22] Potential targeting of these ProTides to liver is quite likely based on recent data on some of our 2′-modified nucleoside ProTides,[23] and this could well lead to enhanced clinical safety profiles for such agents. As a sub-μM lead, the *p*-MePh analogue **5h** may represent a useful point of departure.

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12. *Synthesis of 4′-Azidocytidine-5′-O- [phenyl-(benzyloxy-L-alaninyl)]-phosphate* [**4a**] ^{t}BuMgCl (4.11 mL, 1 M solution in THF, 4.11 mmol) and 4′-azidocytidine (500 mg, 1.643 mmol) were dissolved in dry THF (15 mL) and stirred for 15 min. Then phenyl(benzyloxy-L-alaninyl)-phosphorochloridate (4.11 mmol, 4.11 mL, 1 M in THF) was added dropwise at RT and stirred at room temperature overnight. Then a solution of sat. NH_4Cl was added to quench the reaction. The solvent was removed under reduced pressure to yield a yellow solid which was purified by repeat column chromatography, using 10–20% MeOH/DCM eluent gradients each time, then a preparative TLC using a DCM/MeOH (9:1) solvent mixture. The obtained pure product was a white solid (49 mg, 5%). ^{31}P NMR (121.5 MHz, MeOH-d_4): δ_P 4.70, 4.49; ^{1}H NMR (300 MHz, MeOH-d_4): δ_H 7.65–7.58 (1H, m, H-6), 7.36–7.34 (7H, m, Ph-*CH*), 7.26–7.19 (3H, m, Ph-*CH*), 6.20–6.13 (1H, dd, J = 4.7 and 14.3 Hz, H-1′), 5.92–5.85 (1H, m, H-5), 5.20 (2H, s, Ph-CH_2), 4.37–4.29 (2H, m, H-2′ and H-3′), 4.23–4.11 (2H, m, H-5′), 4.01 (1H, m, Ala-CH), 1.41–1.25 (3H, m, Ala-CH_3). ^{13}C NMR (75.5 MHz, MeOH-d_4): δ_C 174.94, 174.88, 174.62, 174.56 (C=O), 167.62 (C-4), 158.34 (C-2), 152.05, 151.97 (Ph-C), 143.12, 142.93 (C-6), 137.41, 137.25 (Ar-C), 130.96, 130.17, 130.31, 130.171 (Ar-C), 129.66, 129.59, 129.41, 129.36, 129.29 (Ar-C), 126.44 (Ar-C), 124.34 (Ar-C), 123.92 (Ar-C) 121.66, 121.60, 121.47, 121.41 (Ar-C), 121.28, 121.22 (Ar-C), 98.83, 98.72, 98.60 (C-5), 97.04 (C-4′), 93.87, 93.42 (C-1′), 74.57, 74.37 (C-3′), 73.50 (C-2′), 68.82, 68.75 (Bn-CH_2), 68.12, 67.74 (Ala-CH), 20.52, 20.43, 20.30, 20.20 (Ala-CH_3).
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Synthesis and Antiviral and Cytostatic Properties of 3′-Deoxy-3′-fluoro- and 2′-Azido-3′-fluoro-2′,3′-dideoxy-D-ribofuranosides of Natural Heterocyclic Bases

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A series of 3′-deoxy-3′-fluoro- and 2′-azido-2′,3′-dideoxy-3′-fluoro-D-ribofuranosides of natural heterocyclic bases have been synthesized with the use of universal carbohydrate precursors, viz., 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-deoxy-3-fluoro-D-ribofuranose and methyl 2-azido-5-*O*-benzoyl-2,3-dideoxy-3-fluoro-β-D-ribofuranoside, respectively. The cytostatic and antiviral activity of the compounds was evaluated against a variety of tumor cell lines and DNA/RNA viruses, respectively. As the most active compound, from both a cytostatic and antiviral activity viewpoint, emerged 3′-deoxy-3′-fluoroadenosine. It inhibited the proliferation of some tumor cell lines (i.e. murine leukemia L1210 and human T-lymphocyte MT-4) at a concentration of 0.2–2 μg/mL, and proved inhibitory to the replication of positive-stranded RNA viruses (i.e. polio, Coxsackie, Sindbis, Semliki forest), double-stranded RNA viruses (i.e. reo), and some DNA viruses (i.e. vaccinia) at a concentration of 1–4 μg/mL, which is well below the cytotoxicity threshold (40 μg/mL).

Introduction

The analogues of natural 2′-deoxyribonucleosides in which the hydroxy group at C3′ is substituted by a fluorine atom in the ribo configuration exhibit a wide range of biological properties[1-9] following their intracellular conversion to 5′-triphosphates.[10-13] The latter act as competitive inhibitors[10-13] and/or alternate substrates[10,14-17] of some DNA polymerases, depending on the experimental conditions used.[11,14,15] When being substrates, the analogues of natural compounds are incorporated into a growing DNA chain and terminate it at a site that is strictly complementary to the corresponding template base. Hence, they can be used as an effective tool for DNA sequencing by the method of Sanger et al.[10-17]

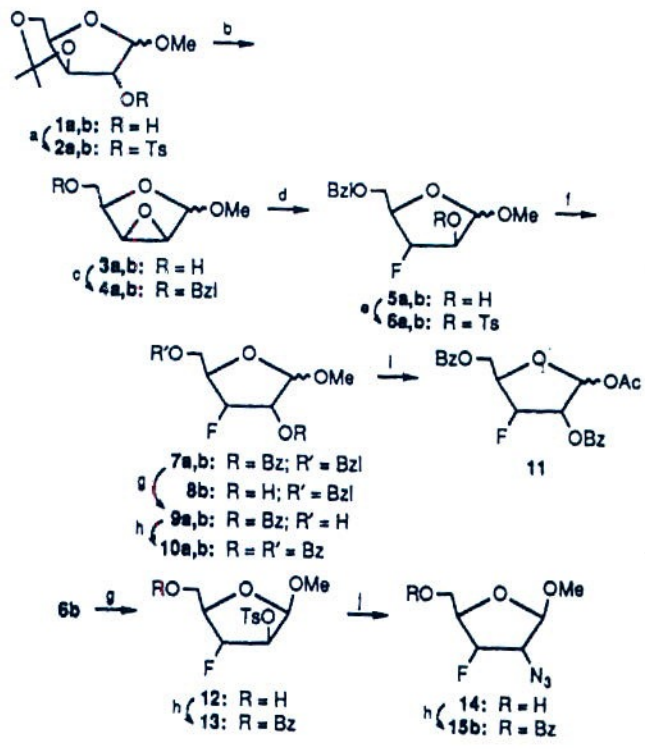
The synthesis and investigation of the biological activity of 3′-deoxy-3′-fluoro analogues of ribonucleosides have received relatively little attention.[18] Thus, Langen et al. briefly described two approaches to the synthesis of 3′-deoxy-3′-fluorouridine and did not find an inhibitory effect of the compound on RNA synthesis in vitro.[25] Later, another group of investigators gave a more detailed account on the above approach (without reference to the original paper of Langen et al.[25]). From the physicochemical data[26] provided for 3′-deoxy-3′-fluorouridine it appears that the ^{1}H NMR results and, in particular $J_{2',F}$, differ significantly from those reported by Langen et al.[25] Also, Wiebe and his co-workers[26] failed to explain the mechanism by which 3′-deoxy-3′-fluorouridine was formed. Recently, two other groups reported the synthesis of 3′-fluoro-3′-deoxyadenosine.[20,27] The compound proved active against a broad range of viruses encompassing vaccinia, polio, Coxsackie B, Sindbis, Semliki forest, and reovirus.[27]

The present study was undertaken to develop an universal method for the synthesis of 3′-fluoro-3′-deoxyribonucleosides and various 2′-derivatives thereof, and to provide a detailed account of their biological properties.

Chemistry

1-*O*-Acetyl-2,5-di-*O*-benzoyl-3-deoxy-3-fluoro-D-ribofuranose (**11**) was used as the key glycosylating agent for the synthesis of the 3′-fluoro-3′-deoxy analogues of natural ribonucleosides (Scheme I). Initially, starting from D-xylose, individual α- and β-methyl glycosides (**1a** and **1b**) of 3,5-*O*-isopropylidene-D-xylofuranose were prepared by using a modification[28] of the method described by Baker et al.[29] Tosylation of **1a** and **1b** in pyridine gave the

Scheme I[a]



[a] (a) TsCl, pyr, room temperature, 10 h (**2a**, 87.5%; **2b**, 86%). (b) (1) AcOH/H_2O, 50 °C, 2 h; (2) MeONa/MeOH, 0–4 °C, 18–24 h (**3a**, 89%; **3b**, 80%). (c) NaH, dioxane, room temperature, 0.5 h; BzlBr, 0 °C, 1 h, room temperature, 1 h (**4a**, 89%; **4b**, 80%). (d) KHF_2/NaF, 1,2-ethylene glycol, reflux, 1.5–4 h (**5a**, 45%; **5b**, 31%). (e) TsCl, pyr, room temperature, 28 h (**6a**, 93%; **6b**, 89%). (f) BzONa, DMSO, dibenzo-18-crown-6, reflux, 4–7 h (**7a**, 33.6%; **7b**, 39.8%, + **8b**, 38.5%). (g) H_2, 10% Pd/C, EtOH, room temperature, 72 h (**9a**, 97%; **9b**, 98%). (h) BzCl, pyr, room temperature, 12 h (**10a**, 94%; **10b**, 81%). (i) AcOH/Ac_2O/H_2SO_4, room temperature, 20 h (89–94%). (j) NaN_3, DMSO, 180 °C, 1 h (**15b**, 61%).

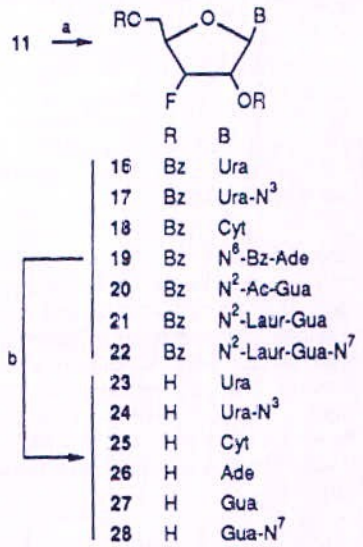
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crystalline tosylates **2a**[30] and **2b**, respectively; these compounds were subsequently converted to the corresponding crystalline epoxides **3a** and **3b** in a two-step procedure.[29] Epoxides **3a** and **3b** were benzylated with benzyl bromide in dioxane in the presence of NaH[31] to give upon workup compounds **4a** and **4b** as oils in high yields. Treatment of the α-anomer **4a** with KHF_2 and NaF in 1,2-ethylene glycol,[32,33] followed by silica gel column chromatography, afforded the fluoride **5a** in 45% yield. Contrary to published data,[33] under similar conditions with β-anomer **4b** as the starting material, fluoride **5b** was obtained in 31% yield, but no 2-fluoro-2-deoxy xylo isomer was isolated. The treatment of tosylate **6b** with potassium benzoate in boiling DMSO in the presence of dibenzo-18-crown-6, followed by silica gel column chromatography, afforded the compounds **7b** and **8b** in 40% and 37% yield, respectively. Benzoylation of the latter compound gave **7b**. As could be expected, a combination of steric and electronic effects of α-OMe group of **6a** proved unfavorable to nucleophilic substitution of the 2-tosyloxy group. As a result, conversion of **6a** to **7a** required longer heating of reagents, and benzoate **7a** was isolated by chromatography in 35% yield. Catalytic hydrogenolysis of **7a** and **7b** in the presence of 10% Pd/C in EtOH gave rise to crystalline **9a** and oily **9b**, respectively; these compounds were subsequently converted to **10a** and **10b** by benzoylation. The total yield of **10a** and **10b** (from **7a** and **7b**, respectively) was greater than 90%. Acetolysis[34] of both **10a** and **10b** afforded a

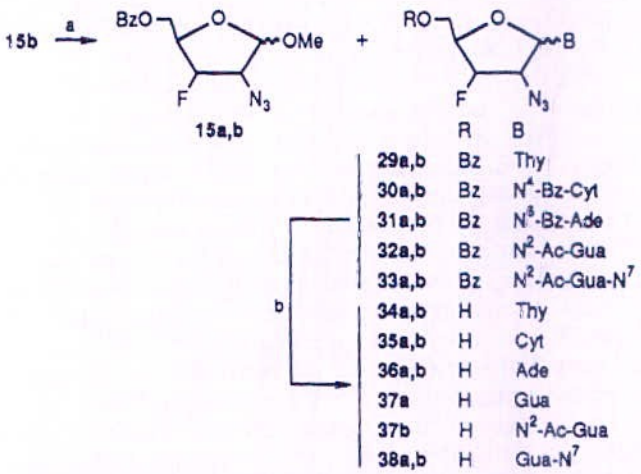
Scheme II[a]



	R	B
16	Bz	Ura
17	Bz	Ura-N^3
18	Bz	Cyt
19	Bz	N^6-Bz-Ade
20	Bz	N^2-Ac-Gua
21	Bz	N^2-Laur-Gua
22	Bz	N^2-Laur-Gua-N^7
23	H	Ura
24	H	Ura-N^3
25	H	Cyt
26	H	Ade
27	H	Gua
28	H	Gua-N^7

[a] (a) Persilylated base, (1) $SnCl_4$, 1,2-dichloroethane, room temperature, 18–24 h; (2) TMS-Tfl, 1,2-dichloroethane or CH_3CN, reflux, 1–3 h. (b) NH_3/MeOH, room temperature, 48 h.

Scheme III[a]



	R	B
29a,b	Bz	Thy
30a,b	Bz	N^4-Bz-Cyt
31a,b	Bz	N^6-Bz-Ade
32a,b	Bz	N^2-Ac-Gua
33a,b	Bz	N^2-Ac-Gua-N^7
34a,b	H	Thy
35a,b	H	Cyt
36a,b	H	Ade
37a	H	Gua
37b	H	N^2-Ac-Gua
38a,b	H	Gua-N^7

[a] (a) Persilylated base, (1) $SnCl_4$, CH_3CN, reflux, 5 h; (2) TMS-Tfl, CH_3CN, reflux, 3 h. (b) NH_3/MeOH, room temperature, 48 h.

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mixture of the anomeric acetates 11 (α/β ratio $\approx$ 1:1) in a high yield.

As the first step in the synthesis of the 2'-azido-2',3'-dideoxy-3'-fluoro derivatives, nucleophilic displacement of the tosyloxy group in **6a** and **6b** by an azido group was studied. The reaction could not be accomplished with α-anomer **6a** under different conditions due to the reasons mentioned above. On the contrary, when **6b** was treated with NaN_3 in DMSO at 200 °C, nucleophilic displacement with inversion of the configuration occurred. However, the yield of the desired compound did not exceed 34%. The following reaction sequence was found to be more effective. Catalytic hydrogenolysis of fluoride **6b** gave **12**, which was converted to the benzoate **13** by benzoylation in 85% yield. Nucleophilic displacement of the tosyloxy group in **13** by treatment with NaN_3 in DMSO led to partial debenzoylation yielding compound **14**. Therefore, the reaction products were treated with benzoyl chloride in pyridine, and after column chromatography methyl 2-azido-5-*O*-benzoyl-2,3-dideoxy-3-fluoro-β-D-ribofuranoside (**15b**) (61% yield) and the starting tosylate **13** were isolated. The methyl glycoside **15b** was as such used for the synthesis of the nucleosides.

The condensation of the silylated bases with sugars **11** and **15b** in the presence of Friedel–Crafts catalysts was used for the synthesis of nucleosides[35] (Schemes II and III). Thus, the reaction of persilylated uracil with compound **11** in the presence of excess $SnCl_4$ [ratio of the reagents (mol) 1.7:1.0:4.16] in 1,2-dichloroethane for 22 h at room temperature followed by column chromatography gave 1-(2,5-di-*O*-benzoyl-3-deoxy-3-fluoro-β-D-ribofuranosyl)-uracil (**16**) and the N^3-isomer **17** in 70% and 8% yield, respectively. It should be emphasized that a decrease of $SnCl_4$ amount resulted in a higher yield of N^3-isomer and lower total yield of nucleoside products. The condensation of persilylated cytosine with acetate **11** in the presence of trimethylsilyl triflate (TMS-Tfl) (1:1:1, mol) in refluxing 1,2-dichloroethane for 1 h gave the nucleoside **18** in 75% yield.

For the synthesis of the adenine and guanine nucleosides, the results obtained earlier for related compounds were taken into consideration.[36-38] Thus, to minimize the formation of N^7-adenine nucleoside, persilylated N^6-benzoyladenine was glycosylated with compound **11** in the presence of excess $SnCl_4$ (1:1:2, mol) in 1,2-dichloroethane at room temperature.[36,37] After column chromatography on silica gel, N^9-glycoside **19** was obtained in 82% yield. As demonstrated earlier,[37,38] the use of TMS-Tfl instead of $SnCl_4$ as a condensing agent led to the formation of the guanine N^9-glycosides as principal product along with formation of the N^7-isomers. Indeed, the reaction of persilylated N^2-acetylguanine with **11** and TMS-Tfl (1.2:1.0:1.16, mol) followed by chromatography gave the N^9-glycoside **20** in 62% isolated yield. The TLC data indicated that after deprotection minor amounts of the N^7-isomer were present in the reaction products. However, when silylated N^2-acetylguanine was replaced by silylated N^2-lauroylguanine in the above condensation, the N^9-glycoside **21** was obtained as the main product of the reaction along with the N^7-glycoside **22** (the ratio of N^9/N^7 was 2:1) in 53% yield (combined).

Treatment of the protected nucleosides **16–22** with methanolic ammonia afforded in good to excellent yield the free nucleosides **23–28**, respectively.

As glycosylating agents, methyl glycosides have, in particular cases, certain advantages over peracyl derivatives of sugars and peracyl glycosyl halides, although their use requires more vigorous reaction conditions (cf. the data[39,40]). Thus, the reaction of persilylated thymine with methyl glycoside **15b** and TMS-Tfl (2.0:1.0:3.0, mol) in refluxing acetonitrile for 5 h followed by chromatography afforded a mixture of the α- and β-anomers **29a,b** (the ratio of α/β was 1:4 according to ^1H NMR data) in 58% yield and a mixture of the methyl glycosides **15a,b** in 10% yield. Treatment of **29a,b** with methanolic ammonia and subsequent chromatography on silica gel afforded 1-(2-azido-2,3-dideoxy-3-fluoro-β-D-ribofuranosyl)thymine (**34b**) and its α-anomer **34a** in 68% and 22% yield, respectively. Along the same line, the synthesis of cytosine nucleosides after standard workup and subsequent chromatography gave a mixture of the methyl glycosides **15a,b** (18%), the β-anomer **30b** (30%), and the α-anomer **30a** (8%). When carried out in the presence of $SnCl_4$ instead of TMS-Tfl, the reaction led to the desired β-nucleoside **30b** and its α-anomer **30a** in yields of 40% and 10%, along with the mixture of **15a,b** (16%). In a related way, the condensation of silylated N^6-benzoyladenine with methyl glycoside **15b** in the presence of excess $SnCl_4$ (2:1:5, mol) in refluxing 1,2-dichloroethane/acetonitrile (1:2, v/v) mixture for 5 h proceeded analogously, and the products **15a,b**, **31b**, and **31a** were isolated in yields of 13%, 57%, and 19%, respectively. Finally, the reaction of silylated N^2-acetylguanine with methyl glycoside **15b** and TMS-Tfl (2.0:1.0:5.0, mol) in refluxing acetonitrile for 3 h afforded a complex mixture of reaction products, the chromatography of which gave the N^9-α-nucleoside **32a** (22%) and the mixture of **32b** and **33a,b** in 70% yield (combined). Removal of the acyl protecting group from the mixture of nucleosides and subsequent chromatographical separation gave 7-(2-azido-2,3-dideoxy-3-fluoro-β-D-ribofuranosyl)-guanine (**38b**), its α-anomer **38a**, and 9-(2-azido-2,3-dideoxy-3-fluoro-β-D-ribofuranosyl)-N^2-acetylguanine (**37b**). Long-time treatment of the latter nucleoside with saturated ammonia in methanol at room temperature did not lead to deacylation. On the contrary, treatment of protected nucleosides **30a,b**, **31a,b**, and **32a** with methanolic ammonia afforded in high yield the free nucleosides **35a,b**, **36a,b**, and **37a**, respectively.

It is noteworthy that the formation of the β-anomers of nucleosides as principal products in the reaction of methyl glycoside **15b** with the silylated bases may be ascribed to steric hindrances and/or electronic effects by the 2-azido group in the formation of the α-anomers. The anomeric mixture of the methyl glycosides **15a,b** could be used for the synthesis of the corresponding nucleosides, the α/β ratio of the latter being unchanged.

The structure of all the compounds that were synthesized was proven by ^1H NMR data (Tables I–IV) and UV and CD spectroscopy, taking into account previous remarks.[15,36-38] The ^1H NMR data recorded for 3'-fluoro-3'-deoxyuridine (**23**) are in agreement with those reported

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Table I. ^{1}H NMR Spectral Data of Sugars

no.	chemical shifts						others
	H-1	H-2	H-3	H-4	H-5	H-5′	
5a	4.87 s	4.04 d	4.77 dd	4.33 ddt	3.66 dd	3.61 dd	7.35–7.26 (m, *Ph*CH$_2$), 4.65 (br s, 2-OH), 4.59 (dd, PhC*H*$_2$), 3.36 (s, OCH$_3$)
5b	~4.92 d	~4.31 m	4.86 dm	4.24 ddt	3.58 m		7.39–7.26 (m, *Ph*CH$_2$), 4.49 (dd, PhC*H*$_2$), 3.43 (s, OCH$_3$)
6a	496 s	4.83 dm	4.86 ddd	4.27 ddt	3.63 dd	3.59 dd	7.80–7.28 (m, *Ph*CH$_2$ + CH$_3$*Ph*SO$_2$), 4.57 (dd, *Ph*CH$_2$), 3.30 (s, OCH$_3$), 2.45 (s, C*H*$_3$PhSO$_2$)
6b	~4.86 m	~4.95 ddd	~5.06 ddd	4.19 ddt	3.58 dd	3.54 dd	7.85–7.25 (m, *Ph*CH$_2$ + CH$_3$*Ph*SO$_2$), 4.55 (s, PhC*H*$_2$), 3.25 (s, OCH$_3$), 2.45 (s, C*H*$_3$PhSO$_2$)
7a	5.30 d	5.08 dm	5.23 ddd	4.50 ddt	3.70 dd	3.63 dd	8.15–7.30 (m, *Ph*CH$_2$ + Bz), 4.61 (dd, PhC*H*$_2$), 3.47 (s, OCH$_3$)
7b	5.15 t	5.35 m	5.28 dt	4.49 ddt	3.66 m		7.61–7.27 (m, *Ph*CH$_2$ + Bz), 4.63 (dd, PhC*H*$_2$), 3.43 (s, OCH$_3$)
8b	4.87 t	4.17 m	5.02 dt	4.37 ddt	3.61 dd	3.57 dd	7.38–7.27 (m, *Ph*CH$_2$), 4.59 (dd, PhC*H*$_2$), 3.39 (s, OCH$_3$)
9a	5.33 d	5.04 ddd	5.19 ddd	4.50 ddt	3.91 dd	3.83 dd	8.14–7.44 (m, Bz), 3.50 (s, OCH$_3$)
9b	5.19 t	5.35 m	5.36 ddd	4.49 ddt	3.87 dd	3.74 dd	8.12–7.42 (m, Bz), 3.50 (s, OCH$_3$)
10a	5.35 d	5.12 ddd	5.30 ddd	4.68 ddt	4.60 dd	4.53 dd	8.15–7.44 (m, 2Bz), 3.50 (s, OCH$_3$)
10b	5.13 t	5.45 m	5.44 m	4.71	4.46 m		8.12–7.39 (m, 2Bz), 3.40 (s, OCH$_3$)
11b	6.41 t	5.58 ddd	5.46 dt	4.74 ddd	4.68 dd	4.48 dd	1.98 (s, OAc) 8.11–7.45 (m, Bz)
11a	6.65 d	5.35 dt	5.37 ddd	4.85 ddt	4.58 dd	4.51 dd	2.17 (s, OAc)
12	~4.90	~4.93 ddd	5.21 ddd	4.17 ddt	3.73 dd	3.66 dd	7.83–7.38 (m, CH$_3$*Ph*SO$_2$), 3.40 (s, OCH$_3$), 2.47 (s, C*H*$_3$PhSO$_2$)
13	~4.98	4.93 ddd	5.25 ddd	4.35 ddt	4.42 m		8.06–7.34 (m, Bz + CH$_3$*Ph*SO$_2$), 3.30 (s, OCH$_3$), 2.45 (s, C*H*$_3$PhSO$_2$)
15a	5.15 d	3.30 ddd	5.16 ddd	4.68 ddt	4.53 dd	4.47 dd	8.00–7.48 (m, Bz), 3.60 (s, OCH$_3$)
15b	4.88 t	4.06 m	5.36 dt	4.61	m	4.41	8.10–7.43 (m, Bz), 3.35 (s, OCH$_3$)

Table II. Coupling Constants

no.	1,2	2,3	3,4	1,F	2,F	3,F	4,F
5a	<1.0	<1.0	1.8		12.9	51.6	26.2
5b		4.8	4.2		~21.0	55.8	22.3
6a	1.2	1.2	4.8		15.6	51.6	20.4
6b	4.2	6.0	4.8		~22.0	54.0	~21.9
7a	4.8	5.4	1.2		23.4	55.2	26.4
7b	1.8	3.6	3.6	1.8		54.0	20.4
8b	1.8	3.6	3.6	1.2	9.2	54.6	21.6
9a	4.8	6.0	2.4		22.8	56.4	26.4
9b[a]	1.8	4.8	3.6	1.8	9.0	53.4	21.0
10a	4.8	6.0	2.4		22.3	55.2	25.2
11a	4.8	4.8	1.2		26.4	55.2	25.8
11b	1.8	4.8	4.8	1.8	8.4	51.6	18.6
12	4.6	6.0	4.8		23.4	56.4	21.6
13	4.8	6.6	4.8		21.0	56.1	
15a	4.2	5.4	1.8		27.0	56.4	26.4
15b	1.8	4.8	4.8	1.8	7.2	53.4	

[a] $^4J_{5,F}$ = 1.2.

by Kowollik[25] and are actually opposite to the data of Misra et al.[26] Analysis of the J and δ values suggests that the compound, to which was assigned the structure of 3′-fluoro-3′-deoxyuridine,[26] probably corresponds to 1-(3-fluoro-3-deoxy-β-D-arabinopyranosyl)uracil. This suggestion is based on the following features of ^{1}H NMR spectrum: (a) the supposed pyranoside is in conformational equilibrium ($_4C^1 \rightleftarrows {}^4C_1$) in solution, as indicated by the values of the coupling constants: viz. H4′:H5′, F3′:H2′, and F3′:H4′ as well as H1′:H2′, H2′:H3′, and H3′:H4′ (see, e.g., refs 41, 42); (b) the values of $^4J_{F,1'}$ and $^4J_{F,3'}$ agree well with a near-planar W arrangement of the corresponding nuclei in the $_4C^1$ conformation of the pyranose ring (see, e.g., refs 42, 43).

The ^{1}H NMR spectra of 3′-fluoro-3′-deoxyadenosine (**26**) and -guanosine (**27**) are in accord with those obtained for the same compounds obtained by alternative procedures.[20,21,23,24] However, the ^{1}H NMR data of 3′-fluoro-3′-deoxyadenosine differ from the ^{1}H NMR data of the compound[22] obtained by transglycosylation of 3′-fluoro-3′-deoxyuridine,[25,26] with adenine.

The site of glycosylation of uracil and guanine was determined by comparison of the ^{1}H NMR, UV, and CD spectra of the corresponding compounds with those of the pairs of related N^1- and N^3-, and N^9- and N^7-isomers,[15,37,44,45] respectively. The most informative feature of the ^{1}H NMR spectra of the α-anomers is the 0.38–0.41 ppm shift of H4′ resonance signal to a lower field when going from β- to α-anomers (see, e.g., refs 15, 45).

Cytostatic and Antiviral Properties of the Test Compounds in Vitro

The 3′-fluoro-3′-deoxynucleosides were evaluated for their inhibitory effect on the proliferation of different tumor cell lines in vitro. 3′-Fluoro-3′-deoxyadenosine (**26**) proved invariably more cytostatic than its uridine (**23**), cytidine (**25**), and guanosine (**27**) counterparts. However, the cytostatic effects of the compounds varied markedly from one tumor cell line to another (Table V). Compound **26** was 7–17-fold more cytostatic to MT-4 than L1210 or Raji cells and at least 100-fold more cytostatic to MT-4 than Molt/4F cells. In contrast, the guanine derivative proved at least 20-fold more effective against L1210 than MT-4 cells (Table V).

The compounds listed in Table VI were also evaluated for their inhibitory effects on a number of viruses including the DNA viruses herpes simplex virus type 1, herpes simplex virus type 2, and vaccinia virus and the RNA viruses vesicular stomatitis virus, Sindbis virus, Coxsackie virus type B 4, polio virus type 1, reovirus type 1, Semliki forest virus, parainfluenza virus type 3, and human immunodeficiency virus type 1. None of the 2′-azido-3′-fluoro-substituted 2′,3′-dideoxynucleoside analogues (i.e. **34a**, **34b**, **35b**, and **36b**) showed significant antiviral activity at subtoxic concentrations. However, among the 3′-fluoro-substituted 3′-deoxynucleoside analogues, 3′-fluoro-3′-deoxyadenosine (**26**) proved to be markedly inhibitory to the replication of a number of viruses including vaccinia virus, polio virus-1, Coxsackie virus B4, Sindbis virus,

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Table III. ^{1}H NMR Spectral Data of Nucleosides

	chemical shifts								
no.	H-8 (H-6)	H-2 (H-5)	H-1'	H-2'	H-3'	H-4'	H-5'	H-5''	others
23	7.83 d	5.71 d	5.90 d	4.26 ddd	4.96 dd	4.18 dt	3.63 dd	3.57 dd	
24	7.58 d	5.56 d	6.17 d	4.85 m	5.05 ddd	4.03 ddt	3.72 dd		
25	7.73 d	5.78 d	5.91 d	4.26 ddd	4.92 dd	4.13 dt	3.63 dd	3.50 dd	7.24 (br s, NH), 7.18 (br s, NH), 5.70 (d, 2'-OH), 5.21 (t, 5'-OH)
26	8.37 s	8.15 s	5.96 d	4.95 m	5.10 dd	4.31 dt	3.68 m		7.40 (br s, NH_2), 5.92 (d, 2'-OH), 5.78 dd, 5'-OH)
27	7.92 s		5.75 d	4.76 ddd	5.04 dd	4.21 dt	3.59 d		6.54 (br s, NH_2), 5.88 (d, 2'-OH), 5.42 (t, 5'-OH)
28	8.30 s		6.01 d	4.74 ddd	4.99 dd	4.18 dt	3.62 dd	3.56 dd	6.25 (br s, NH_2), 5.86 (d, 2'-OH), 5.20 (t, 5'-OH)
34a	7.27 d		6.29 d	4.78 ddd	5.36 ddd	4.69 ddt	3.52 m		11.40 (s, NH), 5.18 (t, 5'-OH), 1.80 (d, 5-CH_3)
34b	7.68 d		6.06 d	4.37 ddd	5.35 dd	4.28 ddt	3.65 m		11.40 (s, NH), 5.37 (t, 5'-OH), 1.80 (d, 5-CH_3)
35a	7.44 d	5.75 d	6.31 d	4.69 ddd	5.34 ddd	4.57 ddt	3.53 m		8.27 (s, NH), 5.19 (t, 5'-OH)
35b	7.75 d	5.80 d	6.15 d	4.22 ddd	5.32 dd	4.25 m	3.65 m		7.33 (s, NH), 7.31 (s, NH), 5.28 (t, 5'-OH)
36a	8.15 s	8.03 s	6.55 d	4.90 ddd	5.46 ddd	4.77 ddt	3.56 m		7.27 (s, NH_2), 5.19 (t, 5'-OH)
36b	8.41 s	8.17 s	6.16 d	5.11 ddd	5.53 dd	4.39 dt	3.68 m		7.42 (s, NH_2), 5.64 (t, 5'-OH)
37a	7.62 s		6.28 d	4.69 ddd	5.46 dd	4.60 dt	3.55 m		10.65 (br s, NH), 6.49 (br s, NH_2)
37b	8.00 s		5.89 d	4.93 ddd	5.47 dd	4.30 dt	3.62 m		5.67 (s, NH), 5.35 (t, 5'-OH), 1.76 (s, Ac)
38a	7.93 s		6.68 d	4.78 m	5.40 dd	4.78 m	3.56 m		6.20 (br s, NH_2), 5.25 (t, 5'-OH)
38b	8.40 s		6.26 d	4.92 ddd	5.44 dd	4.29 dt	3.64 m		11.02 (s, NH), 6.30 (br s, NH_2), 5.27 (t, 5'-OH)

Table IV. Coupling Constants

no.	1',2'	2',3'	3',4'	4',5'	4',5''	2',F	3',F	4',F	others
23	7.8	4.2	<1.0	3.0	3.6	25.2	54.0	28.2	7.8 (5,6), 12.0 (5',5'')
24	5.4	4.8	3.6	6.0	6.0	31.2	54.0	23.4	7.8 (5,6), 10.8 (5',5'')
25	7.8	4.8	<1.0	3.6	3.6	24.0	54.6	27.0	7.2 (5,6), 6.0 (2',OH-2'), 12.0 (5',5'')
26	7.8	4.2	<1.0	3.0	3.0	26.4	54.6	27.6	
27	8.4	4.8	<1.0	3.7	3.7	25.2	54.0	27.6	
28	8.6	5.8	<1.0	4.3	4.3	25.2	55.7	27.8	12.5 (5',5'')
34a	6.9	4.8	1.2	4.2	4.2	25.2	54.0	23.4	1.2 (6, CH_3-5), 5.4 (5'-OH-5', 5'',OH-5')
34b	8.4	4.8	<1.0	3.0	3.0	24.0	53.4	27.6	1.2 (6, CH_3-5), 4.8 (5',OH-5', 5'',OH-5')
35a	6.0	4.8	1.8	3.6	3.6	24.0	54.0	22.8	7.8 (5,6), 5.4 (5',OH-5', 5'',OH-5')
35b	7.8	3.6	<1.0			24.6	54.0	26.4	7.8 (5,6), 5.4 (5',OH-5', 5'',OH-5')
36a	6.6	4.8	1.2	3.9	3.9	26.1	54.3	24.6	12.0 (5,5''), 5.4 (5',OH-5', 5'',OH-5')
36b	8.4	4.8	<1.0	3.6	3.6	25.8	53.4	27.6	12.0 (5',5''), 6.0 (5',OH-5', 5'',OH-5')
37a	6.3	4.8	<1.0	3.6	3.6	26.4	55.2	24.0	
37b	8.4	4.2	<1.0	4.2	4.2	24.6	54.0	27.6	5.4 (5',OH-5', 5'',OH-5')
38a	6.6	4.8	<1.0				54.0		
38b	8.4	4.2	<1.0	4.2	4.2	25.2	54.0	27.6	6.0 (5',OH-5', 5'',OH-5')

Table V. Cytostatic Activity of Test Compounds against Different Tumor Cell Lines

	50% inhibitory concentration (IC_{50}),[a] μg/mL			
no.	murine leukemia L1210	human B-lymphoblast Raji	human T-lymphoblast Molt/4F	human T-lymphoblast MT-4
23	>100	>100	>100	6.9
25	39	98	65	7.9
26	1.6	3.9	28	0.22
27	5.0	29	75	>100

[a] Required to reduce the viability of the tumor cells by 50%.

reovirus-1, and Semliki forest virus. Its minimum inhibitory concentration (MIC) for these viruses ranged from 1 to 7 μg/mL, a concentration that is well below the cytotoxicity threshold of the compound (MCC ≥ 40 μg/mL) (Table VI). 3'-Fluoro-3'-deoxyguanosine (**27**) was inhibitory to vaccinia virus, Coxsackie virus B4, Semliki forest virus, and Sindbis virus at a 10-fold higher concentration than **26**. Compounds **23** and **25** were only marginally active against those viruses that were sensitive to **26**.

None of the compounds was inhibitory to HIV-1 replication in MT-4 cells at subtoxic concentrations (data not

Table VI. Activity of Test Compounds against Different Viruses in Different Cell Systems

	minimum cytotoxic concentration[b] (MCC), μg/mL			minimum inhibitory concentration (MIC),[a] μg/mL										
				HeLa cells										
				primary rabbit kidney (PRK) cells					African green monkey kidney (Vero B) cells					
no.	PRK cells	HeLa cells	Vero B cells	herpes simplex virus-1 (KOS)	herpes simplex virus-2 (G)	vaccinia virus	vesicular stomatitis virus		polio virus-1	Coxsackie virus-B4	Sindbis virus	para-influenza virus-3	reo-virus-1	Semliki forest virus
23	>400	>400	>400	>400	>400	>400	>400	>400	150	300	300	>400	150	>400
25	>400	>400	>400	>400	>400	>400	>400	(300)	150	150	300	>400	150	>400
26	>40	40	40	>10	>10 (20)	2	>40	>10	4	2	7	4	>4	1	4
27	200	>400	400	>100	>100	10	>200	>200	150	150	40	40	>100	>200	40
34a	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
34b	>400	>400	>400	>400	>400	>400	<400	>400	>400	>400	>400	>400	>400	>400	>400
35b	>400	>400	>400	>400	>400	>400	>300	>400	>400	>400	>400	>400	>400	>400	>400
36b	>400	>400	>400	>400	>200	300	300	>200	>200	300	>200	>200	>200	>200	>200

[a] Required to reduce virus-induced cytopathogenicity by 50%. Viral cytopathogenicity was recorded as soon as it reached completion in the control virus-infected cell cultures. The multiplicity of infection (MOI) was invariably 100 × $CCID_{50}$, that is 100 times the virus dose needed to infect 50% of the cell cultures. [b] Required to cause a microscopically detectable alteration of normal cell morphology.

shown). The mechanism of antiviral action of **26** and **27** remains to be resolved. It should be worth pursuing the synthesis of closely related derivatives of **26** in attempts to decrease its toxicity and increase its antiviral potency.

The antiviral activity spectrum of **26** and **27** includes some (±)DNA viruses such as pox (vaccinia), (+)RNA viruses such as picorna (polio, Coxsackie), toga (Sindbis, Semliki forest), and (±)RNA viruses such as reo. This spectrum is clearly different from the activity spectrum of the acyclic and carbocyclic adenosine analogues [i.e. (*S*)-9-(2,3-dihydroxypropyl)adenine (DHPA), carbocyclic 3-deazaadenosine (C-c^{3}Ado), neplanocin A, 3-deazaneplanocin A] which encompasses (−)RNA viruses [i.e. paramyxo (parainfluenza), arena (Junin, Tacaribe), rhabdo (vesicular stomatitis)] instead of (+)RNA viruses.[46] The antiviral activity of these adenosine analogues has been attributed to an inhibition of *S*-adenosylhomocysteine hydrolase,[47] and, consequently, inhibition of transmethylation reactions starting from *S*-adenosylmethionine as the methyl donor. From the antiviral activity spectrum shown by compound **26**, it is unlikely that this adenosine analogue would achieve its antiviral action through inhibition of *S*-adenosylhomocysteine hydrolase.

The synthesis and antiviral activity of compound **26** have been the subject of a previous report.[27] The results that were independently obtained with the present preparation confirm those obtained previously.

Experimental Section

Melting points were determined with a Boethius (GDR) apparatus and are uncorrected. IR spectra were recorded with a UR-20 (GDR) spectrophotometer, UV spectra were recorded with a Specord UV-vis (GDR) spectrophotometer, and ^{1}H NMR spectra were recorded with a Bruker WM-360 (FRG) spectrometer with tetramethylsilane as an internal standard (s = singlet; d = doublet, t = triplet, m = multiplet). Standard Silufol UV_{254} (Czechoslovakia), Kieselgel 60F (Merck, FRG) plates were used for TLC of sugars and nucleosides, respectively. The following solvent systems were used: hexane–ethyl acetate, 4:1, v/v (A), $CHCl_3$–MeOH, 19:1 (B), $CHCl_3$–MeOH, 4:1 (C), $CHCl_3$–MeOH, 9:1 (D), *i*-PrOH–H_2O–20% NH_4OH, 7:2:1 (E). Column chromatography was performed on silica gel L 40 × 100 (Chemapol, Czechoslovakia). Anhydrous solvents were obtained as follows: pyridine was successively refluxed and distilled over potassium hydroxide and calcium hydride; dimethyl sulfoxide was heated at 100 °C over KOH for 8 h and distilled in vacuo; 1,2-dichloroethane was kept over phosphorous anhydride for 12 h and distilled; acetonitrile was distilled twice over phosphorous anhydride, kept over calcium hydride, and distilled. In all conversion reactions, freshly distilled $SnCl_4$ and trimethylsilyl trifluoromethanesulfonate (Fluka, Switzerland) were used. The solutions of compounds in organic solvents were dried with anhydrous sodium sulfate for 4 h. The reactions were performed at 20 °C, unless stated otherwise. Elemental analyses were carried out in Microanalytical Laboratorium at the Institute of Organic Chemistry, Ukrainian Academy of Sciences (Kiev, USSR).

Methyl 5-*O*-benzyl-3-fluoro-3-deoxy-α-D-arabinofuranoside (5a) and its β-anomer (5b) were obtained[32,33] in 40–45% and 30% yield, respectively.

Methyl 5-*O*-Benzyl-2-*O*-tosyl-3-fluoro-3-deoxy-α-D-arabinofuranoside (6a) and Its β-Anomer (6b). A mixture of 2.9 g (11.31 mmol) of **5a** and 5.0 g (26.22 mmol) of tosyl chloride in 50 mL of anhydrous pyridine was stirred for 28 h. The solvent was evaporated, and the residue was dissolved in $CHCl_3$ (300 mL) and washed with saturated $NaHCO_3$ solution (2 × 100 mL). The organic layer was separated, evaporated, coevaporated with toluene, and purified by column chromatography, using a linear EtOAC gradient (0–50%, v/v; 2 L) in hexane to yield 4.32 g (93%) of **6a** as a syrup: R_f 0.44 (A).

In a similar way, starting from 3.9 g (15.21 mmol) of **5b** and 6.0 g (31.47 mmol) of tosyl chloride, 5.54 g (89%) of **6b** was obtained: mp 73–74 °C (EtOH); R_f 0.32 (A). Anal. ($C_{20}H_{23}FO_6S$) C, H, F, S.

Methyl 5-*O*-Benzyl-2-*O*-benzoyl-3-fluoro-3-deoxy-α-D-ribofuranoside (7a). A mixture of 0.44 g (1.07 mmol) of **6a**, 1.83 g (12.67 mmol) of potassium benzoate, and 4.6 g (12.67 mmol) of dibenzo-18-crown-6 in 10 mL of anhydrous DMSO was heated at 205–210 °C for 7 h. After cooling, the reaction mixture was diluted with 300 mL of $CHCl_3$ and washed with H_2O (3 × 100 mL). The organic layer was separated, dried, and evaporated. The residue was chromatographed with a linear EtOAC gradient (0–50%, v/v, 1 L) in hexane to yield 0.13 g (33.6%) of **7a** as a syrup: R_f 0.42 (A).

Methyl 5-*O*-Benzyl-2-*O*-benzoyl-3-fluoro-3-deoxy-β-D-ribofuranoside (7b) and Methyl 5-*O*-Benzyl-3-fluoro-3-deoxy-β-D-ribofuranoside (8b). A mixture of 1.23 g (2.99 mmol) of **6b**, 4.5 g (31.2 mmol) of potassium benzoate, and 4.5 g (12.4 mmol) of dibenzo-18-crown-6 in 45 mL of anhydrous DMSO was heated at 205–210 °C for 4 h. The workup and isolation, performed as described above, yielded 0.43 g (39.8%) of **7b** as a syrup (R_f 0.35 (A)) and 0.28 g (38.5%) of **8b** as a syrup (R_f 0.13 (A)).

Methyl 2-*O*-Benzoyl-3-fluoro-3-deoxy-α-D-ribofuranoside (9a) and Its β-Anomer (9b). A mixture of 1.7 g of 10% Pd/C and 1.7 g (4.71 mmol) of **7a** in 150 mL of EtOH was stirred in H_2 atmosphere for 72 h. The catalyst was filtered off and washed with EtOH (2 × 50 mL). The filtrates were evaporated, and the residue was crystallized from Et_2O to give 1.24 g (97%) of **9a**: mp 104–105 °C, R_f 0.12 (A). Anal. ($C_{13}H_{15}FO_5$) C, H, F.

Similarly, starting from 1.36 g (3.77 mmol) of **7b** and 1.4 g of 10% Pd/C in 130 mL of EtOH, 1.0 g (98%) of **9b** as a syrup was produced: R_f 0.1 (A).

Methyl 2,5-Di-*O*-benzoyl-3-fluoro-3-deoxy-α-D-ribofuranoside (10a) and Its β-Anomer (10b). A mixture of 1.3 mL (1.58 g; 11.23 mmol) of benzoyl chloride and 1.3 g (4.81 mmol) of **9a** in 12 mL of anhydrous pyridine was stirred for 12 h and then poured into 50 mL of H_2O, containing 10 g of ice, and extracted by $CHCl_3$ (3 × 100 mL). The organic extracts were combined, washed, dried, evaporated, and coevaporated with toluene (2 × 50 mL). The residue was purified by column chromatography, using a linear EtOAc gradient (0–50%, v/v, 1 L) in hexane to yield 1.7 g (94%) of **10a** as a syrup: R_f 0.44 (A).

Similarly, starting from 1.0 g (3.7 mmol) of **9b** and 0.54 mL (0.65 g, 4.6 mmol) of benzyl chloride, 1.12 g (81%) of **10b** was obtained as a syrup: R_f 0.42.

1-*O*-Acetyl-2,5-di-*O*-benzoyl-3-fluoro-3-deoxy-α,β-D-ribofuranose (11). Concentrated H_2SO_4 (0.7 mL) was added to the solution of 1.3 g (3.34 mmol) of **10a** in 10 mL of AcOH and 1.2 mL of Ac_2O, the mixture was stirred for 20 h and poured into 5% aqueous solution of $NaHCO_3$ (100 mL), containing 50 g of ice, and after the ice melted, it was extracted with $CHCl_3$ (2 × 100 mL). The organic extracts were combined, evaporated, and coevaporated with toluene (2 × 50 mL). The residue was purified with column chromatography, using a linear EtOAc gradient (0–50%, v/v; 1 L) in hexane to yield 1.31 g (94%) of **11** as a syrup: R_f 0.37 (A).

In a similar way, starting from 0.5 g (1.33 mmol) of **10b**, 0.48 g (89%) of **11** was obtained.

3′-Fluoro-3′-deoxyuridine (23) and Its 3-N-Isomer (24). A solution of $SnCl_4$ (2.35 mL, 5.22 g, 20.03 mmol), 1.94 g (4.82 mmol) of **11**, and 1.71 g (2.1 mL, 5.48 mmol) of bis(trimethylsilyl) derivative of uracil in anhydrous 1,2-dichloroethane (90 mL) was stirred for 22 h. The reaction mixture was diluted with $CHCl_3$ (60 mL) and washed with 5% aqueous $NaHCO_3$ (150 mL). The water layer was washed with $CHCl_3$ (2 × 50 mL). The organic extracts were combined, dried, and evaporated. The residue was chromatographed, using a linear EtOAc gradient (20–80%, v/v; 2 L) in hexane to yield 1.54 g (70%) of **16** as foam (R_f 0.70 (B)) and 0.18 g (8%) of its isomer **17** (mp 170–171 °C (EtOH); R_f 0.55 (B)).

The solution of 0.4 mL (0.88 mmol) of **16** in methanol (20 mL), saturated with ammonia at 0 °C, was kept for 48 h and evaporated. The residue was treated with Et_2O (50 mL): the precipitate was filtered off and crystallized from EtOH to yield 0.16 g (74%) of **23**: mp 197–198 °C (lit.[27] mp 198–199 °C); R_f 0.49 (C); UV (EtOH) λ_{max} 263 nm (ε 9800). Anal. ($C_9H_{11}FN_2O_5$) C, H, F, N.

(46) De Clercq, E. *Biochem. Pharmacol.* **1987**, *36*, 2567.
(47) Cools, M.; De Clercq, E. *Biochem. Pharmacol.* **1989**, *38*, 1061.

Similarly, starting from 60 mg (0.13 mmol) of **17**, 27 mg (87%) of **24** was obtained: mp 165 °C (EtOH); R_f 0.24 (D); UV (EtOH) λ_{max} 262 nm (ε 6200); UV (0.1 n NaOH) λ_{max} 298 nm.

3'-Fluoro-3'-deoxycytidine (25). A solution of TMS-Tfl (5 mL, 0.61 g, 2.7 mmol), 1.1 g (2.73 mmol) of **11**, and 0.67 g (2.7 mmol) of bis(trimethylsilyl) derivative of cytosine in anhydrous 1,2-dichloroethane (25 mL) was refluxed for 1 h. After standard treatment, the residue was purified by column chromatography, using a linear MeOH gradient (2–10%, v/v, 2 L) in $CHCl_3$. The fractions, containing **18**, were collected, evaporated, and crystallized from EtOH to yield 0.92 g (75%) of **18**: mp 203–205 °C; R_f 0.25 (B).

Standard debenzoylation of 0.41 g (0.9 mmol) of **18** followed by crystallization from EtOH yielded 0.18 g (81%) of **25**: mp 221–223 °C; (lit.[27] mp 217–218 °C); R_f 0.16 (C); UV (EtOH) λ_{max} 270 nm (ε 8400). Anal. ($C_9H_{12}FN_3O_4$) C, H, F, N.

3'-Fluoro-3'-deoxyadenosine (26). A solution of $SnCl_4$ (0.97 mL, 2.16 g, 8.31 mmol), 1.7 g (4.22 mmol) of **11** and 1.6 g (4.17 mmol) of bis(trimethylsilyl) derivative of N^6-benzoyladenine in anhydrous 1,2-dichloroethane (30 mL) was stirred for 18 h. After standard workup, the residue was purified with column chromatography, using a linear MeOH gradient (2–10%, v/v; 2 L) in $CHCl_3$. The fractions, containing **19**, were collected and evaporated to yield 1.99 g (82%) of **19** as a foam: R_f 0.85 (B); UV (EtOH) λ_{max} 280 nm (ε 20 000).

Standard debenzoylation of 1.1 g (1.89 mmol) of **19** followed by crystallization from EtOH yielded 0.38 g (75%) of **26**: mp 211–212 °C; (lit.[27] mp 205 °C); R_f 0.52 (C); UV (EtOH) λ_{max} 260 nm (ε 14 600). Anal. ($C_{10}H_{12}FN_5O_3$) C, H, F, N.

3'-Fluoro-3'-deoxyguanosine (27) and 7-N-Isomer (28). (a) A solution of TMS-Tfl (0.91 mL, 1.11 g, 5.0 mmol), 1.74 g (4.32 mmol) of **11**, and 2.22 g (5.10 mmol) of silyl derivative of N^2-acetylguanine in anhydrous 1,2-dichloroethane (50 mL) was refluxed for 1.5 h. After standard workup, the residue was chromatographed, using a linear MeOH gradient (1–6%, v/v; 2 L) in $CHCl_3$. The fractions, containing nucleoside **20**, were collected and evaporated to yield 1.44 g (62%) of **20** as a foam: R_f 0.42 (B).

Standard deprotection of 1.2 g (2.24 mmol) of **20** followed by crystallization from H_2O yielded 0.45 g (70%) of **27**: mp 289–291 °C dec (lit.[24] mp 275–277 °C); R_f 0.73 (E); UV (H_2O) λ_{max} 253 nm (ε 14 100), 269 nm shoulder (ε 10 100); UV (0.1 N NaOH) λ_{max} 257–270 nm (ε 8600); UV (0.1 N HCl) λ_{max} 259 nm (ε 15 400), 275 shoulder (ε 9200). Anal. ($C_{10}H_{12}FN_5O_4$) C, H, F, N.

(b) A solution of TMS-Tfl (0.225 mL, 0.8 g, 1.35 mmol), 1.12 g (2.73 mmol) of **11**, and 1.78 g (3.24 mmol) of bis(trimethylsilyl) derivative of N^2-lauroylguanine in anhydrous acetonitrile (30 mL) was refluxed for 3 h. The reaction mixture was evaporated, and the residue was dissolved in $CHCl_3$ (150 mL). After standard workup the residue was chromatographed, using a linear EtOAc gradient (0–60%, v/v; 3 L) in benzene. The fractions, containing the individual products, were collected and evaporated to yield 0.33 g (18%) of **22** as a foam and 0.64 g (35%) of **21** as a foam.

Standard deprotection of 0.58 g (0.86 mmol) of **21** followed by crystallization from H_2O yielded 0.125 g (51%) of a product that was identical with **27** in all respects.

In a similar way, starting from 0.28 g (0.41 mmol) of **22**, 55 mg (46.6%) of **28** was produced: mp 284–286 °C dec (H_2O); R_f 0.71 (E); UV (H_2O) λ_{max} 239 nm (ε 7600), 289 nm (ε 8800); UV (0.1 N NaOH) λ_{max} 285 nm (ε 8700); UV (0.1 N HCl) λ_{max} 255 nm (ε 17 100), 266 nm shoulder (ε 15 400).

Methyl 2-*O*-tosyl-3-fluorodeoxy-β-D-arabinofuranoside (12) was obtained, as described above, starting from 2.46 g (5.99 mmol) of **6b** and 2.5 g of 10% Pd/C in 250 mL of EtOH. The residue was crystallized from Et_2O with hexane added until slight turbidity: 1.7 g (88%) of **12** was obtained: mp 60 °C; R_f 0.1 (A). Anal. ($C_{13}H_{17}FO_6S$) C, H, F, S.

Methyl 5-*O*-benzoyl-2-*O*-tosyl-3-fluoro-3-deoxy-β-D-arabinofuranoside (13) was obtained, as described above, starting from 0.8 g (2.49 mmol) of **12** and 0.32 mL (0.38 g, 2.73 mmol) of benzoyl chloride in 20 mL of pyridine to yield 0.86 g (81%) of **13**: mp 85–86 °C (EtOH); R_f 0.53 (A). Anal. ($C_{20}H_{21}FO_7S$) C, H, F, S.

Methyl 2-Azido-5-*O*-benzoyl-3-fluoro-2,3-dideoxy-β-D-ribofuranoside (15b). A mixture of 5.2 g (80 mmol) of sodium azide and 4.1 g (9.65 mmol) of **13** in 70 mL of anhydrous DMSO was stirred for 1 h at 180 °C. After standard workup, the residue was benzoylated with a standard procedure and chromatographed, using a linear Et_2O gradient (10–50%, v/v; 2 L) in hexane. The fractions, containing individual products, were collected and evaporated to yield 1.75 g (61%) of azide **15b** as a syrup: R_f 0.8 (A); IR (film) 2125 cm^{-1} (N_3) and 0.92 g of the starting material **13**.

2'-Azido-3'-fluoro-2',3'-dideoxythymidine (34b) and Its α-Anomer (34a). A mixture of 2.87 mL (5.51 g, 15.81 mmol) of TMS-Tfl, 1.56 (5.28 mmol) of **15b**, and 2.85 g (10.54 mmol) of bis(trimethylsilyl) derivative of thymine in acetonitrile (60 mL) was refluxed for 3 h. After standard workup, the residue was chromatographed with a linear EtOH gradient (10–90%, v/v; 2 L) in hexane to yield 0.16 g (10%) of mixture of starting azide **15b** and its α-anomer **15a**, 1.2 g (58%) of mixture of nucleosides **29a** and **29b**, and 0.25 g of a product with undetermined structure. A mixture of **29a,b** (1.0 g, 2.09 mmol) was debenzoylated with a standard procedure and chromatographed with a linear MeOH gradient (1–10%, v/v; 1 L) in $CHCl_3$ to give 0.5 g (68%) of **34b**: mp 147 °C (EtOH); R_f 0.59 (D); UV (EtOH) λ_{max} 266 nm (ε 9900); IR (KBr) 2140 cm^{-1} (N_3) [Anal. ($C_{10}H_{12}FN_5O_4$) C, H, F, N] and 0.165 g (22%) of its α-anomer **34a**: mp 130–131 °C (EtOH); R_f 0.48 (D); UV (EtOH) λ_{max} 269 nm (ε 10 000); IR (KBr) 2125 cm^{-1} (N_3). Anal. ($C_{10}H_{12}FN_5O_4$) C, H, F, N.

2'-Azido-3'-fluoro-2',3'-dideoxycytidine (35b) and Its α-Anomer 35a. A mixture of 0.74 mL (1.64 g, 6.3 mmol) of $SnCl_4$, 0.62 g (2.09 mmol) of **15b**, and 1.5 g (4.2 mmol) of bis(trimethylsilyl) derivative of N^4-benzoylcytosine in anhydrous acetonitrile (20 mL) was refluxed for 5 h. After standard workup, the residue was chromatographed with a linear EtOAc gradient (10–19%, v/v; 2 L) in hexane to yield 0.1 g (16%) of the mixture of starting sugar **15b** and its α-anomer **15a**, 0.4 g (40%) of **30b** as a foam: R_f 0.70 (B), and 0.1 g (10%) of its α-anomer **30a** as a foam: R_f 0.53 (B).

Standard debenzoylation of 0.3 g (0.62 mmol) of **30b** and subsequent chromatography with a linear MeOH gradient (0–25%, v/v; 0.5 L) in $CHCl_3$, afforded 0.15 g (89%) of **35b**: mp 193–194 °C (EtOH); R_f 0.41 (C); UV (EtOH) λ_{max} 270 nm (ε 8200). Anal. ($C_9H_{11}FN_6O_3$) C, H, F, N.

Similarly, starting from 70 g (0.15 mmol) of **30a**, 36 mg (91%) of the α-anomer **35a** was obtained as a syrup: R_f 0.3 (C); UV (EtOH) λ_{max} 272 nm (ε 8600).

2'-Azido-3'-fluoro-2',3'-dideoxyadenosine (36b) and Its α-Anomer (36a). A mixture of $SnCl_4$ (1.22 mL, 2.72 g, 10.44 mmol), 0.62 g (2.09 mmol) of **15b**, and 1.63 g (4.18 mmol) of bis(trimethylsilyl) derivative of N^6-benzoyladenine in acetonitrile/1,2-dichloroethane mixture (60 mL, 2:1; v/v) was refluxed for 5 h. After standard workup, the residue was chromatographed with a linear EtOAc gradient (10–90%, v/v; 2 L) in hexane to yield 0.08 g (12%) of the mixture of starting azide **15b** and its α-anomer **15a**, 0.6 g (57%) of **31b** as a foam: R_f 0.55 (B).

From 0.5 g (1 mmol) of **31b**, following the above procedure, 0.25 g (85%) of **36b** was obtained: mp 198–199 °C (EtOH); R_f 0.54 (D) (EtOH) λ_{max} 258 nm (ε 14 900). Anal. ($C_{10}H_{11}FN_8O_2$) C, H, F, N.

In a similar way, starting from 0.2 g (0.4 mmol) of **31a**, 0.1 g (85%) of **36a** was obtained: mp 94–95 °C (absolute EtOH + Et_2O); R_f 0.49 (D); UV (EtOH) λ_{max} 258 nm (ε 14 600). Anal. ($C_{10}H_{11}FN_8O_2$) C, H, F, N.

9-(2-Azido-3-fluoro-2,3-dideoxy-β-D-ribofuranosyl)-2-*N*-acetylguanine (37b), 9-(2-Azido-3-fluoro-2,3-dideoxy-α-D-ribofuranosyl)guanine (37a), 7-(2-Azido-3-fluoro-2,3-dideoxy-β-D-ribofuranosyl)guanine (38b), and Its α-Anomer (38a). A mixture of TMS-Tfl (0.94 mL, 1.16 g, 5.1 mmol), 0.31 g (1.05 mmol) of **15b**, and 0.7 g (2.09 mmol) of silyl derivative of N^2-acetylguanine in anhydrous acetonitrile (40 mL) was refluxed for 3 h. After standard workup, the residue was applied to a chromatographic column, which was eluted with a linear MeOH gradient (0–5%, v/v; 1 L) in $CHCl_3$. The fraction containing the mixture of **32b**, **33a**, and **33b** [0.315 g (70%)] and the fraction containing 0.1 g (22%) of individual **32a** were isolated. Each of the fractions was treated with $MeOH/NH_3$ (for 3 days), evaporated, and chromatographed with a linear MeOH gradient (0–25%, v/v; 1 L) in $CHCl_3$ as eluent. Starting from 0.315 g of a mixture of nucleosides **32b**, **33a**, and **33b**, 0.16 g of the mixture [R_f 0.42 (C)] of **37b** and **38b** was obtained in the ratio of 1:1.5 (according to ^1H NMR) and 55 mg of **38a**. The additional chromatography

of **37b** and **38b** with a linear MeOH gradient (0–25%, v/v; 0.6 L) in $CHCl_3$ gave the individual nucleosides **37b** and **38b**.

38a: R_f 0.29 (C); UV (20% EtOH in H_2O) 241 nm shoulder (ϵ 7020), λ_{max} 280 nm (ϵ 7400); UV (0.1 N NaOH) 237 nm shoulder (ϵ 8600), λ_{max} 274 nm (ϵ 6200); UV (0.1 N HCl) λ_{max} 258 nm (ϵ 9200), λ_{max} 269 nm (ϵ 8600).

37b: UV (20% EtOH in H_2O) λ_{max} 254 nm (ϵ 15 200), 268 nm shoulder (ϵ 11 500); UV (0.1 N NaOH) broad max 258–270 nm (ϵ 13 700); UV (0.1 N HCl) λ_{max} 258 nm (ϵ 15 000), 274 nm shoulder (ϵ 10 960).

38b: UV (20% EtOH in H_2O) λ_{max} 287 nm (ϵ 7400), 238 nm shoulder (ϵ 6150); UV (0.1 N NaOH) λ_{max} 283 nm (ϵ 6900) 236 nm shoulder (ϵ 9100); UV (0.1 N HCl) λ_{max} 259 nm (ϵ 8600) and 267 nm (ϵ 8070).

Deprotection of 0.1 g of **32a** afforded 40 mg of **37a**: R_f 0.2 (C); UV (20% EtOH in H_2O) λ_{max} 254 nm (ϵ 14 500), 269 nm shoulder (ϵ 12 000); UV (0.1 N NaOH), broad max 260–270 nm (ϵ 9600); UV (0.1 N HCl) λ_{max} 259 nm (ϵ 12 400), 274 nm shoulder (ϵ 8900).

Inhibition of L1210, Raji, Molt/4F, and MT-4 Cell Proliferation. All assays were performed in flat-bottomed microtests III Plates (96 wells) as previously described.[48] Briefly, the cells were suspended in growth medium and added to the microplate wells at a density of 5×10^4 L1210 or Molt/4F cells/well (200 μL), 6.25×10^4 MT-4 cells/well or 7.5×10^4 Raji cells/well in the presence of varying concentrations of the test compounds. The cells were then allowed to proliferate for 48 h (L1210 cells), 72 h (Molt/4F and Raji cells), or 120 h (MT-4 cells) at 37 °C in a humidified CO_2-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter (L1210, Raji, Molt/4F) or a blood cell counting chamber by trypan blue dye exclusion (MT-4). The IC_{50} was defined as the concentration of compound that reduced the number of viable cells by 50%.

Antiviral Assays. The antiviral assays, other than HIV-1, were based on an inhibition of virus-induced cytopathogenicity in either HeLa cell, Vero cell, or primary rabbit kidney cell cultures, following previously established procedures.[49] Briefly, confluent cell cultures in microtiter trays were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures. After a 1-h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (400, 200, 100, ... μg/mL) of the test compounds. Viral cytopathogenicity was recorded as soon as it reached completion in the control virus-infected cell cultures.

Inhibition of HIV-1-Induced Cytopathogenicity in MT-4 Cells. Human 5×10^5 MT-4 cells were infected with 100 $CCID_{50}$ HIV-1 (strain HLTV-III_B)/mL and seeded in 200 μL wells of a microtiter plate, containing appropriate dilutions of the test compounds.[50] After 5 days of incubation at 37 °C, the number of viable cells was determined in a blood cell counting chamber by trypan blue dye exclusion.

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Design, Synthesis, and Pharmacological Evaluation of Ultrashort- to Long-Acting Opioid Analgetics

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In an effort to discover a potent ultrashort-acting μ opioid analgetic that is capable of metabolizing to an inactive species independent of hepatic function, several classes of 4-anilidopiperidine analgetics were synthesized and evaluated. One series of compounds displayed potent μ opioid agonist activity with a high degree of analgesic efficacy and an ultrashort to long duration of action. These analgetics, 4-(methoxycarbonyl)-4-[(1-oxopropyl)phenylamino]-1-piperidinepropanoic acid alkyl esters, were evaluated in vitro in the guinea pig ileum for μ opioid activity, in vivo in the rat tail withdrawal assay for analgesic efficacy and duration of action, and in vitro in human whole blood for their ability to be metabolized in blood. Compounds in this series were all shown to be potent μ agonists in vitro, but depending upon the alkyl ester substitution the potency and duration of action in vivo varied substantially. The discrepancies between the in vitro and in vivo activities and variations in duration of action are probably due to different rates of ester hydrolysis by blood esterase(s). The SAR with respect to analgesic activity and duration of action as a function of the various esters synthesized is discussed. It was also demonstrated that the duration of action for the ultrashort-acting analgetic, **8**, does not change upon prolonged infusion or administration of multiple bolus injections.

Fentanyl, a potent short-acting analgetic, is used clinically during surgical procedures as an adjunct to gaseous anesthesia.[1] As a result of fentanyl's clinical success and the desire to more clearly define the structural require-

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