



No. 00-092690-00011-0000

Fecha: 2007-11-15 16:02:24 Dep. 2020 NUECREACIONE
Tra. 2 PATENTES Eve: 1 REGDEPOSITO
Act. 444 RESPUESTAREQUE Folios: 129

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Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

DIVISION DE NUEVAS CREACIONES

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CABLES: LLOREDA

CALLE 72 No. 5-83 5º. PISO

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Re: P1.-F.HOFFMANN-LA ROCHE AG.-Solicitud de Patente de Invención para "4-PIRIDINIL-N-ACIL-L-FENILALANINAS".-Clase A.61.-Expediente número 00-092.690.-

1. Me refiero al auto dictado por ese Despacho notificado por fijación en lista de 23 de Agosto de 2007.

2. Con el fin de dar respuesta a las objeciones expuestas por el señor Examinador en su Concepto Técnico número 1324 en contra de los requisitos de Claridad de la Solicitud, Novedad y Nivel Inventivo, me permito dirigir su atención a las siguientes modificaciones y argumentos técnicos, por medio de los cuales se superan completamente dichas objeciones.

3. **CLARIDAD DE LA SOLICITUD:**

En relación con la objeción en contra de la claridad de la reivindicación 49, según la cual *"los sustituyentes P_1 y P_2 están definidos en términos de adjetivos calificativos, no con significados técnicos específicos e inequívocos y debe quedar plenamente establecido que están enteramente sustentados en la descripción"*, me permito dirigir la atención del señor Examinador al hecho de que con el fin de dar cumplimiento a sus sugerencias, el solicitante ha modificado dicha reivindicación de manera tal que se ha reemplazado la expresión " P_1 y P_2 son en cada caso un grupo protector", por la expresión modificada " **P_1 es un grupo Boc o carbobenciloxi y P_2 es un alquilo C_{1-6} o alquilo C_{1-6} sustituido**", definiendo de esta forma los sustituyentes en mención mediante **significados de carácter técnico, específico e inequívoco**, tal como ha sido solicitado en su Concepto Técnico y de manera completamente clara para una persona versada en la materia.

En conexión con lo anterior, me permito señalar que el sustento para la anterior modificación se encuentra en la página 32, líneas 13 a 16, del capítulo descriptivo.

Por otra parte, el señor Examinador ha manifestado dentro de sus objeciones en contra de la claridad del capítulo reivindicatorio de la presente solicitud que *"La reivindicación 51 no es clara, porque reclama materia ya reclamada sin ninguna característica técnica particular, solamente se rellena el texto de la reivindicación con usos*

y métodos de tratamiento". En relación con la objeción anteriormente mencionada, me permito manifestar que, **debidamente autorizado por mí representado**, el solicitante ha decidido **cancelar la reivindicación 51** actualmente presentada, con el fin de dar respuesta completa y satisfactoria a sus objeciones a este respecto.

De conformidad con lo anterior, me permito manifestar que teniendo en cuenta que en el nuevo capítulo reivindicatorio presentado se han definido claramente las connotaciones que pueden tomar los sustituyentes P_1 y P_2 y que se ha **eliminado** la reivindicación 51, se entienden **superadas todas las objeciones de claridad** realizadas por el señor Examinador respecto a dicho requisito.

Finalmente, me permito manifestar que las modificaciones realizadas al capítulo reivindicatorio en mención **no amplían de manera alguna el alcance de la invención** en concordancia con lo establecido en el Art. 34 de la Decisión 486, 2000, toda vez que tal como se mencionó en el párrafo anterior, dichas modificaciones corresponden a la adecuada definición de grupos sustituyentes en términos que no resulten ambiguos y a la eliminación de una reivindicación, lo que evidentemente corresponde a una restricción de la materia objeto de la solicitud bajo estudio respecto a la forma como se encuentra actualmente presentada.

4. **ANTECEDENTES:**

En el estado de la técnica se conoce que la molécula-1 de adhesión a células vasculares (VCAM-1), correspondiente a un miembro de la familia de supergenes de la inmunoglobulina (Ig), se expresa en endotelio activado pero no en reposo. Por su parte, se ha reconocido que la integrina VLA-4 ($\alpha_4\beta_1$), que se expresa en muchos tipos de células, incluyendo los linfocitos circulantes, eosinófilos, basófilos y monocitos, pero no en neutrófilos, es el principal receptor de la VCAM-1.

Lo anterior es importante porque los anticuerpos de VCAM-1 o de VLA-4 pueden bloquear la adhesión de estos leucocitos mononucleares, así como de las células de melanoma, al endotelio activado *in vitro*. En este sentido, los anticuerpos de ambas proteínas han sido eficaces para inhibir la infiltración de leucocitos y prevenir el daño de los tejidos en varios modelos animales de inflamación. Más aún, se ha demostrado que los anticuerpos monoclonales anti-VLA-4 bloquean la emigración de células T en la artritis inducida por adyuvantes, previenen la acumulación de eosinófilos y la bronconstricción en modelos de asma, reducen la parálisis e inhiben la infiltración de monocitos y linfocitos en encefalitis autoinmune experimental.

De la misma manera, se ha demostrado que los anticuerpos monoclonales anti-VCAM-1 prolongan el tiempo de supervivencia de los aloinjertos cardíacos, y estudios recientes han demostrado que los anticuerpos monoclonales anti-VLA-4 pueden prevenir la insulinitis y la diabetes de ratones diabéticos no obesos y pueden atenuar de modo significativo la inflamación en caso de modelo de colitis de monos tamarinos del algodón. Finalmente, se ha demostrado además que la VCAM se expresa en células del endotelio del tejido inflamado del colon en un modelo de enfermedad intestinal inflamatoria de ratas sometidas a TNB/etanol (*Gastroenterology* 1999, 116, 874-883).

Por todo lo anterior, en el estado de la técnica se reconoce que los compuestos que inhiben la interacción entre integrinas que contienen α_4 y la VCAM-1 pueden ser muy útiles como agentes terapéuticos para el tratamiento de enfermedades inflamatorias crónicas, tales como la artritis reumatoide, la esclerosis múltiple, el asma y las enfermedades intestinales inflamatorias, por lo que resulta altamente deseable poder contar con sustancias que presenten características inhibitorias de la interacción entre las integrinas mencionadas.

En este sentido, la presente solicitud surge como una alternativa novedosa e inventiva en el esfuerzo por proporcionar compuestos que tengan tal efecto inhibitorio, toda vez que tal como se desprende de las enseñanzas consignadas en la memoria descriptiva de la misma, los compuestos ahora revelados y reivindicados por el solicitante poseen efectivamente la actividad buscada como antagonistas de la integrina $\alpha_4\beta_1$.

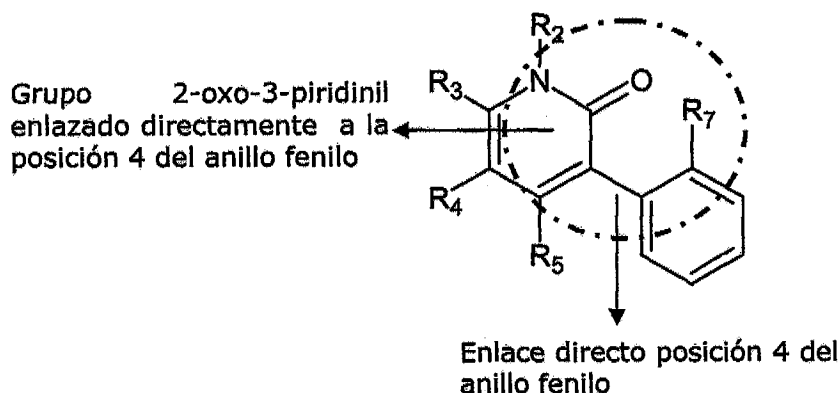
5. **NOVEDAD:**

De conformidad con las objeciones expuestas por el señor Examinador en su Concepto Técnico, la presente invención carece de novedad frente a las enseñanzas del documento citado WO 99/43642 (D1), dado que "los compuestos de a solicitud en estudio caen dentro del alcance de la materia descrita en la anterioridad, especialmente en las págs. 4 y 5; pág. 12 renglón 25; pág. 13 renglón 31; reivindicación 1 y 8. Donde se establece que **R1 es un grupo heteroaromático opcionalmente sustituido, el grupo aromático es piridil, sustituido con hidroxil**, que forma el tautometo (sic) ceto-enol. Por lo tanto esta oficina considera que la solicitud en estudio no tiene novedad" (resaltado fuera de texto).

Sobre este particular, me permito dirigir la atención del señor Examinador al hecho de que, si bien existen algunas semejanzas estructurales entre los compuestos de la fórmula (I) de la referencia **WO 99/43642 (D1)** y aquellos de la fórmula general expuesta en la reivindicación 1 de la presente solicitud, debe hacerse notar que, a diferencia de las enseñanzas reveladas en **D1**, los compuestos de la presente invención

siempre presentan un grupo 2-oxo-3-piridinil enlazado directamente a la posición 4 del anillo fenilo de la mitad fenilalanina, tal como es revelado en la presente solicitud.

Por el contrario, el documento D1 no enseña ni describe al menos un compuesto específico de esta naturaleza, es decir, que incluya la mitad 4-(2-oxo-3-piridinil)fenilalanina, ya que en todos los ejemplos allí revelados, el grupo fenilo y el otro grupo heteroaromático, cualquiera sea su naturaleza y definición (piridinilo, fenilo, etc.), están siempre separados por un grupo químico que adicionalmente contiene un heteroátomo tal como O o N, correspondiente al grupo $-(Alk^1)_T(L^1)_S$ de la fórmula (I) de D1.



Finalmente, ninguno de los ejemplos de dicha referencia enseña o sugiere la presencia de un grupo oxo sobre el grupo heteroaromático, mucho menos que dicho grupo oxo se encuentre en la posición adyacente respecto al átomo de carbono del sistema heteroaromático piridinilo enlazado directamente con el anillo aromático y mucho menos que dicho sistema heteroaromático sea específicamente 2-oxo-3-piridinilo, lo cual es completamente diferente a contar con un grupo hidroxilo como un posible sustituyente sobre un grupo heteroaromático cualquiera y en cualquier posición, tal como es enseñado en las páginas 12, 13 y las reivindicaciones 1 y 8 sobre las cuales hace énfasis el señor Examinador.

En este punto, resulta importante señalar al señor Examinador que, para que se presente la posible tautomería ceto-enol que supuestamente se puede presentar, es claro que la condición necesaria para ello es que el grupo hidroxilo tiene que estar en una posición adyacente al heteroátomo del sistema heteroaromático (en nuestro caso, adyacente al átomo de N del anillo de piridilo), lo cual no es sugerido ni enseñado en ningún aparte de D1.

Teniendo en cuenta lo anterior, es completamente claro que **D1** no revela ningún compuesto específico que caiga dentro del alcance de la presente solicitud, y por lo tanto la presente invención es novedosa frente a dicho documento.

Sobre este particular, atentamente me permito dirigir la atención del señor Examinador al **Manual Andino de Patentes**, relevante publicación preparada por la Secretaría General de la Comunidad Andina, el cual establece en su acápite "Análisis de la Novedad" varios aspectos que pueden ser útiles para entender que la presente invención se ajusta al sentir de los países Andinos en lo que se relaciona con la novedad de las invenciones. Por ejemplo, el mencionado **Manual** establece que *"Para determinar la novedad de la invención, se debe comprobar si existen anticipaciones del estado de la técnica que contengan explícitamente todas las características técnicas esenciales de la invención. El examen de novedad se efectúa comparando elemento por elemento de la invención tal como está definido por las reivindicaciones con los del estado de la técnica"*¹ (resaltado fuera de texto).

En este sentido, es claro que el señor Examinador fallaría en el análisis de novedad que ha realizado para la presente invención, porque está citando un documento que no contiene explícitamente todas las características técnicas estructurales esenciales de los compuestos tal como han sido reivindicados por el solicitante. Es decir, el señor Examinador estaría considerando una referencia que, si bien se desarrolla en el mismo campo de aplicación, no constituye una anticipación relevante para desvirtuar la novedad de la presente solicitud.

Adicionalmente, el mismo **Manual** establece *"Si todas las características técnicas de la reivindicación independiente se encuentran descritas en un mismo antecedente y además se encuentran íntimamente relacionadas, el objeto de dicha reivindicación carece de novedad. Si una característica, aunque sea banal, no se encuentra contenida en el antecedente, la reivindicación es nueva"*² (resaltado fuera de texto).

A partir de los planteamientos anteriormente expuestos, es completamente claro entonces que el único documento citado como relevante para desvirtuar el requisito de novedad no cumple con tal función, dado que no revela todas las características técnicas esenciales de la invención reivindicada, características que, lejos de ser banales, son específicas y esenciales para asegurar la actividad antagonista de integrina alfa4-beta1 que presentan los compuestos revelados por el solicitante.

¹ Ibid, página 61.

² Ibid, página 62.

En consecuencia, todo lo expuesto demuestra el carácter novedoso de la presente invención con respecto a las enseñanzas del documento D1 citado, toda vez que el mismo no revela de manera directa y nada ambigua las características estructurales propias y específicas de los compuestos ahora enseñados, tal como son reivindicados en la presente invención.

6. **NIVEL INVENTIVO:**

Finalmente, en su Concepto Técnico número 1324, el señor Examinador manifiesta que la presente solicitud carece de nivel inventivo porque "es bien conocido (sic) la naturaleza química de los derivados de acil fenilalanina" y por lo tanto "es evidente para una persona del nivel medio derivar, la invención del estado de la técnica con lo ensañado en las anterioridades".

En relación con lo anterior, el solicitante considera que es de la mayor importancia dirigir la atención del señor Examinador nuevamente al hecho de que los compuestos de la presente solicitud siempre tienen **un grupo oxopiridinilo unido directamente al grupo fenilo**, así como también que **D1 no enseña ningún compuesto específico que tenga un grupo oxopiridinilo unido directamente al grupo fenilo**, siendo ésta una importante diferencia estructural que no es sugerida de ninguna forma en dicha referencia, ya que por ejemplo, en el Ejemplo 2d de D1, el cual corresponde al compuesto más cercano a los compuestos de la presente solicitud entre todos los compuestos allí revelados, el grupo piridinilo, que **no contiene un sustituyente oxo, está unido al anillo fenilo por medio de un grupo metilenoxi**, diferencia a la que, adicionalmente, no podría llegar en forma obvia o evidente una persona versada en la materia tal como se demostrará más adelante.

En conexión con lo anterior, el solicitante considera que la presente invención es inventiva frente a las enseñanzas de D1 con base en las siguientes dos razones principales:

- 1) La fórmula general (I) revelada en D1 es extremadamente amplia, y claramente cubre un considerable número de grupos de compuestos que no son activos como antagonistas de la integrina alfa4-beta1. Por ejemplo, los compuestos de la fórmula (I) **en donde m es 0 o alk² es un alquilo ramificado o R² es metilo o R³ es alquilo inferior son inactivos** (Tilley, Jefferson W., Chen, L., Sidduri, Achyutharao; Fotohui, Nader. *The Discovery of VLA-4 Antagonists*; Current Topics in Medicinal Chemistry, (2004), 4, 14, ver especialmente las páginas 1513-1515). De la misma manera, investigaciones más profundas y extensas en ésta área no han revelado

excepciones a esta regla, tal como se desprende de los artículos de Tilley, Jefferson W., *VLA4 Antagonists; Expert Opinion on Therapeutic Patents*(2002), 12(7), 991-1008; Yang, Ginger X., Hagmann, William K., *VLA-4 antagonists: potent inhibitors of lymphocyte migration*. Medicinal Research Reviews (2003), 23(3), 369-392; Tilley, Jefferson W., Chen, L., Sidduri, Achyutharao; Fotohui, Nader. *The Discovery of VLA-4 Antagonists; Current Topics in Medicinal Chemistry*, (2004), 4, 14 (de los cuales me permito anexar copia simple).

Al parecer, los autores de **D1** eran conocedores de estas consideraciones respecto a la relación estructura-actividad para derivados de acilfenilalaninas antagonistas de VLA-4, tal como se desprende del hecho de que la reivindicación 5 del citado documento (**D1**) está dirigida a compuestos en donde m es 1 y alk^2 es $-CH_2-$, y más aún cuando se señala, tal como en la reivindicación 6 del mismo, que tanto R^2 y R^3 deben ser ambos hidrógeno. (Con respecto a R^3 , resulta relevante señalar que los científicos de Celltech afirmaron que "el amino ácido α -NH es importante para la actividad". (Brand, S.; De Candole, B. C.; Brown, J. A.; Org. Lett, 2003, 5, 2343-2346, ver especialmente el segundo párrafo de la página 2344, copia anexa al presente memorial).

Por lo tanto, una persona versada en la materia que estuviera en la búsqueda de novedosos antagonistas de integrina alfa4-beta1 podría reconocer, después de un número sustancial de experimentos, **que únicamente los compuestos reivindicados en las reivindicaciones dependientes de la referencia citada son activos, a pesar del gran número de compuestos cubiertos por la fórmula general (I) expuesta en la reivindicación 1 de dicho documento D1.**

En este sentido, cobra mucha más importancia el hecho de que la reivindicación 9 de **D1** especifica **al grupo enlazante $-(Alk^1)_T(L^1)_S$ de la Fórmula (I) como $-CH_2O-$, $-SO_2NH-$, $-C(O)O-$, o $-CON(R^4)-$.** De la misma forma, resulta pertinente resaltar que los compuestos en donde $-(Alk^1)_T(L^1)_S$ **corresponda a un enlace directo**, tal como es necesario para los compuestos de la presente invención, **no es mencionado ni en la reivindicación dependiente 9 ni en ningún otro lado de la descripción de dicha referencia, como por ejemplo en las páginas 8 y 9, en donde se definen los valores de L^1 para el grupo enlazante mencionado.**

Por lo tanto, considerando el gran número de compuestos cubiertos por la fórmula general (I), el hecho de que un número sustancial de compuestos de dicha fórmula general (I) es inactivo y la tendencia de que los compuestos que realmente son activos se mencionan únicamente en las reivindicaciones dependientes, es claro que una persona versada en la materia no pensaría en nuevos antagonistas de integrina alfa4-beta1 en donde el enlazante $-(Alk^1)_T(L^1)_S$ **sea un enlace sencillo.**

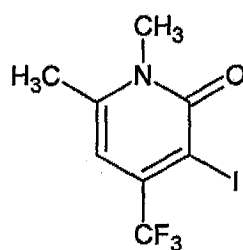
De otra parte, los compuestos de la presente invención siempre tienen **un grupo oxopiridinilo unido directamente al grupo fenilo mediante un enlace sencillo**, siendo ésta una condición que no solo **no puede desprenderse en forma evidente del documento D1**, sino a la que la persona versada en la materia no podría llegar a partir de las enseñanzas que el estado de la técnica pone a su disposición, tal como los artículos anteriormente mencionados, en los que **no existe indicación o sugerencia alguna acerca de esta particularidad estructural, única de los compuestos de la presente invención.** En efecto, a partir de la revisión de tales artículos científicos, el señor Examinador podrá apreciar que **no existe un solo ejemplo en donde se presente la combinación de sustituciones estructurales que den origen a un grupo 4-(2-oxo-3-piridinil)fenilalanina**, lo que corrobora en forma contundente que para llegar a dicho tipo de derivados, la persona versada en la materia **tendría que realizar un esfuerzo intelectual y experimental considerable, demostrando así que el objeto de la presente sí posee el nivel inventivo requerido, no solamente frente al documento D1, sino sobre las enseñanzas generales del estado de la técnica relacionado con los compuestos de acilfenilalanina antagonistas de VLA-4.**

- 2) Ahora bien, de otra parte, el documento **D1** revela ciertos métodos específicos para la preparación de los compuestos de la fórmula general (I) **en donde el enlazante $-(Alk^1)_T(L^1)_S$ es $-CH_2O-$, $-SO_2NH-$, $-C(O)O-$, o $-CON(R_4)-$** (páginas 24 a 25 de la descripción del documento **D1**), más no hace mención alguna a las condiciones de síntesis específicas que son requeridas para preparar compuestos en donde dicho grupo enlazante corresponde a un enlace directo, y menos aún, en donde el sistema heteroaromático corresponde a una mitad 2-oxo-3-piridinilo. En este sentido, es claro que **D1 no revela métodos para la preparación de compuestos como los de la presente solicitud, especialmente derivados de 4-(2-oxo-3-piridinil)fenilalanina,** y mucho menos de manera tal que una persona versada en la materia pueda prepararlos de forma evidentemente derivada de las enseñanzas de dicho documento.

En este sentido, el solicitante desea resaltar que es claro el hecho de que los métodos generales para la formación de enlaces arilo-arilo existían en el estado de la técnica incluso antes de **D1**, tal como por ejemplo, el artículo de revisión de Stanforth, Stephen P. *Catalytic cross-coupling reactions in biaryl síntesis*, Tetrahedron (1998), 54 (3/4), 263-303, del cual me permito anexar copia como referencia de este hecho.

Sin embargo, con el fin de preparar los compuestos de la presente invención, **el acoplamiento tiene que ocurrir en un sitio adyacente a un grupo carbonilo, hecho que no se encuentra bien referenciado ni documentado en la literatura.** Más aún, cada compuesto requiere cuidado particular y atención en los detalles para su síntesis. La elección particular de los reactivos, catalizador y condiciones de reacción no son de carácter rutinario y esto es particularmente cierto para aquellos anillos que implican una cierta demanda estereoquímica, una poderosa atracción electrónica o la presencia de grupos sensibles, tal como es el caso de los compuestos de la presente invención.

Por ejemplo, aunque la investigación cuidadosa condujo a la síntesis exitosa de los compuestos de la presente solicitud en donde R^5 es hidrógeno, este proceso fue demasiado exigente y, más aún, los procedimientos que fueron exitosos para una clase de derivados, no resultaban extrapolables a otras clases de compuestos en donde, por ejemplo, R^5 corresponda a $-CH_3-$ o $-CF_3$. Más aún, con el fin de preparar los compuestos de la presente solicitud, algunos de los materiales de partida, como por ejemplo, la iodopiridona 2, no habían sido previamente descritos y, en consecuencia, tuvieron que ser sintetizados.



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Por lo tanto, el solicitante considera que los compuestos de la presente invención no pueden prepararse con base en la descripción del documento **D1** de manera evidente, más aún, sin una carga considerable de dificultad, incluso si la persona versada en la materia utiliza el conocimiento general que existe en

este campo para ello. En consecuencia, si bien la fórmula general (I) de **D1** presenta algunas semejanzas estructurales con los compuestos de la presente invención, dichas semejanzas no pueden ser consideradas como una revelación técnica relevante capaz de desvirtuar la altura inventiva que efectivamente poseen los compuestos de la presente solicitud.

7. Anexo me permito presentar:

- (a) Recibo número 07 - 90,494 expedido por la Superintendencia de Industria y Comercio, que acredita el pago de las modificaciones mencionadas;
- (b) Copia del nuevo capítulo reivindicatorio, **que reemplaza el originalmente presentado** en el cual se han realizado las modificaciones expuestas anteriormente;
- (c) Copia informal de las páginas 8, 9, 24 y 25 del documento **D1** citado, en las que se resalta que dadas las definiciones expuestas para el grupo enlazante, no es posible tener al menos una sugerencia acerca de un compuesto como los revelados por el solicitante, así como tampoco acerca del método de síntesis de los mismos; y
- (d) Copia informal de los artículos científicos de Tilley, Jefferson W., *VLA4 Antagonists*; Expert Opinion on Therapeutic Patents (2002), 12(7), 991-1008; Yang, Ginger X., Hagmann, William K., *VLA-4 antagonists: potent inhibitors of lymphocyte migration*. Medicinal Research Reviews (2003), 23(3), 369-392; Tilley, Jefferson W., Chen, L., Sidduri, Achyutharao; Fotohui, Nader. *The Discovery of VLA-4 Antagonists*; Current Topics in Medicinal Chemistry, (2004), 4, 14, en los cuales se resaltan los apartes mencionados a lo largo del presente memorial en cuanto a las enseñanzas estructurales del estado de la técnica para los derivados de acilfenilalanina antagonistas de VLA-4, y del artículo de revisión de Stanforth, Stephen P. *Catalytic cross-coupling reactions in biaryl síntesis*, Tetrahedron (1998), 54 (3/4), 263-303, relacionado con los métodos de formación de enlaces arilo-arilo.

JOSE LLOREDA CAMACHO & CO.

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

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8. En vista de todo lo anterior, ruego a usted proceder a estudiar nuevamente la solicitud a la luz de las argumentaciones presentadas y de las modificaciones realizadas y conceder el privilegio de patente solicitado.

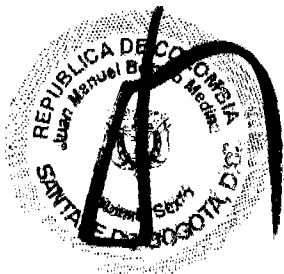
Señor Superintendente,

Gustavo Tamayo
GUSTAVO TAMAYO ARANGO
T. P. 37.172



NOTARIA SEXTA DE BOGOTÁ
RECONOCIMIENTO Y PRESENTACION PERSONAL
Bogotá, D.C. **15 NOV 2007**
Ante mi JUAN MANUEL BOTERO MEDINA
NOTARIO SEXTO DEL CIRCULO DE BOGOTÁ
15 NOV 2007
Comparecieron:
Gustavo Tamayo
Tamayo Arango
quien (as) exhibió (n) el (los) C.C.
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y declaró (n) que la (s) firma (s) que aparece (n)
en el presente documento es (son) la (s) suya (s) y
que el contenido del mismo es cierto. En constan-
cia se firma esta diligencia

Gustavo Tamayo
GUSTAVO TAMAYO ARANGO
C.C. No. 79'152.549 de Usaquén



00-092.690

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 07 - 90,494
FECHA : NOVIEMBRE 6 DE 2007

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	Vr. PAGO
LLOREDA Y CIA S. A	CONSIGNACION	BANCO POPULAR	050-00110-6	72074899	06/11/2007	7,130,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-03	CAMBIO DE MODALIDAD Y MODIFICA	
		12 MARCAS Y LEAS COMERCIALES	99,000.00
		TOTAL :	99,000.00

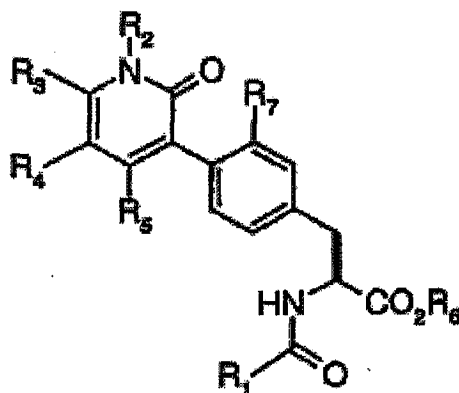
SON: NOVENTA Y NUEVE MIL PESOS

RESPONSABLE :

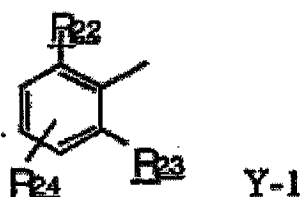
RECIBO DE CAJA APLICADO AL EXPEDIENTE No.

REIVINDICACIONES

1. Un compuesto de la fórmula



en la que R_1 es un grupo de la fórmula Y-1

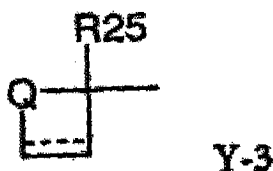


en la que R_{22} y R_{23} , con independencia entre sí, son hidrógeno, alquilo C_{1-6} , alcoxi C_{1-6} , cicloalquilo C_{3-7} , fenilo o naftilo, arilalquilo (en donde arilo es fenilo o naftilo), nitro, ciano, (alquilo C_{1-6})-tio, (alquilo C_{1-6})-sulfinilo, (alquilo C_{1-6})-sulfonilo, alquilcarbonilo C_{1-6} , halógeno o perfluor-(alquilo C_{1-6}), y por lo menos uno de R_{22} y R_{23} es diferente de hidrógeno, y R_{24} es hidrógeno, alquilo C_{1-6} , alcoxi C_{1-6} , fenilo o naftilo, nitro, ciano, (alquilo C_{1-6})-sulfonilo o halógeno, o bien

R_1 es un grupo de la fórmula Y-2 que es un anillo heteroaromático de cinco o seis eslabones, unido a través del átomo de carbono al carbonilo de la amida, dicho anillo contiene uno, dos o tres heteroátomos elegidos entre N, O y S y uno o dos átomos de dicho anillo están sustituidos con independencia entre sí por alquilo C_{1-6} , cicloalquilo C_{3-7} , halógeno, ciano, perfluor-(alquilo C_{1-6}), fenilo o naftilo y

por lo menos uno de dichos átomos sustituidos está adyacente al átomo de carbono unido al carbonilo de la amida, o bien

R₁ es un grupo de la fórmula Y-3 que es un anillo de 3-7 eslabones de la fórmula



en la que R₂₅ es alquilo C₁₋₆, alqueno C₂₋₁₀ sin sustituir o sustituido por flúor, o un grupo de la fórmula R₂₆-(CH₂)_e-, R₂₆ es fenilo, naftilo, heteroarilo, azido, ciano, hidroxilo, alcoxi C₁₋₆, (alcoxi C₁₋₆)-carbonilo, alquilcarbonilo C₁₋₆, (alquilo C₁₋₆)-tio, (alquilo C₁₋₆)-sulfonilo, (alquilo C₁₋₆)-sulfinilo, perfluor-(alquilcarbonilo C₁₋₆), nitro, o bien R₂₆ es un grupo de la fórmula -NR₂₈R₂₉, en la que R₂₈ es hidrógeno o alquilo C₁₋₆, R₂₉ es hidrógeno, alquilo C₁₋₆, (alcoxi C₁₋₆)-carbonilo, alquilcarbonilo C₁₋₆, aroilo, perfluor-(alquilcarbonilo C₁₋₆)-amino, (alquilo C₁₋₆)-sulfonilo, (alquilo C₁₋₆)-aminocarbonilo, arilaminocarbonilo, o bien R₂₈ y R₂₉ junto con el átomo de nitrógeno al que están unidos forman un anillo heterocíclico saturado de 4, 5 ó 6 eslabones que contiene un heteroátomo adicional, elegido entre O, S y N-R₄₀, Q es -CH₂)_fO-, -(CH₂)_fS-, -(CH₂)_fN(R₂₇)-, -(CH₂)_f-,

R₂₇ es H, alquilo C₁₋₆, fenilo, naftilo, alquilcarbonilo C₁₋₆, aroilo o (alcoxi C₁₋₆)-carbonilo,

R₄₀ es H, alquilo C₁₋₆, fenilo, naftilo, alquilcarbonilo C₁₋₆, aroilo o (alcoxi C₁₋₆)-carbonilo, los átomos de carbono del anillo están sin sustituir o sustituidos por alquilo C₁₋₆ o halógeno, "e" es un número entero de 0 a 4 y "f" es un número entero de 0 a 3;

R₂ es hidrógeno o alquilo C₁₋₆, alquilo C₁₋₆ sustituido, fenilo, naftilo, fenilo-(alquilo C₁₋₆) o naftilo-(alquilo C₁₋₆);

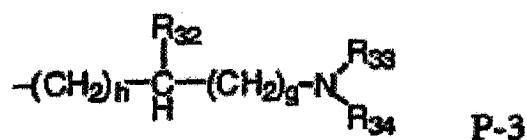
R₃ es hidrógeno, halógeno, alquilo C₁₋₆, fenilo o naftilo y puede incluir además al trifluormetilo;

R₄ es hidrógeno, halógeno, alquilo C₁₋₆, fenilo o naftilo;

R₅ es hidrógeno, alquilo C₁₋₆, alcoxi C₁₋₆, trifluormetilo u OH;

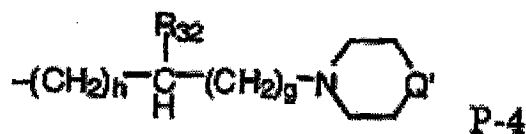
R₆ es hidrógeno, alquilo C₁₋₆, (alquilo C₁₋₆)-carboniloxi-(alquilo C₁₋₆),

o bien R₆ es un grupo de la fórmula P-3



en la que R₃₂ es hidrógeno o alquilo C₁₋₆, R₃₃ es hidrógeno, alquilo C₁₋₆, fenilo o naftilo, R₃₄ es hidrógeno o alquilo C₁₋₆, "h" es un número entero de 0 a 2, "g" es un número entero de 0 a 2, la suma de h y g se sitúa entre 1 y 3;

o bien R₆ es un grupo de la fórmula P-4



en la que R₃₂, g y h tienen los significados recién definidos,

Q' es O, S, -(CH₂)_j-O un grupo de la fórmula N-R₃₅, R₃₅ es hidrógeno, alquilo C₁₋₆, alquilcarbonilo C₁₋₆, (alcoxi C₁₋₆)-carbonilo, "j" es el número 0, 1 ó 2, y

R₇ es hidrógeno, cloro, alcoxi C₁₋₆ o alquilo C₁₋₆; o bien las sales farmacéuticamente aceptables de los mismos.

2. Un compuesto según la reivindicación 1, en el que R₃ es hidrógeno, halógeno, alquilo C₁₋₆, fenilo o naftilo.

3. Un compuesto según la reivindicación 1 ó 2, en el que R₁ es Y-3 y Q es -(CH₂)_fO-, -(CH₂)_fS- o -(CH₂)_f-.

4. Un compuesto según la reivindicación 3 en el que R₅ es hidrógeno, alquilo C₁₋₆ o trifluormetilo.

5. Un compuesto según la reivindicación 1 ó 2, en el que R_1 es un grupo Y-1 en el que R_{22} y R_{23} con independencia entre sí son hidrógeno, alquilo C_{1-6} o halógeno y R_{24} es hidrógeno, alquilo C_{1-6} o alcoxi C_{1-6} .

6. Un compuesto según la reivindicación 5 en el que R_{22} y R_{23} con independencia entre sí son hidrógeno, alquilo C_{1-6} o halógeno y R_{24} es hidrógeno o alcoxi C_{1-6} y R_2 es fenilo o naftilo-(alquilo C_{1-6}).

7. Un compuesto según la reivindicación 1 ó 2, en el que R_1 es un grupo de la fórmula Y-3 en la que R_{25} es un grupo de la fórmula $R_{26}-(CH_2)_e-$, en la que R_{26} es alcoxi C_{1-6} , Q es $-(CH_2)_f-$, "e" es un número entero de 0 a 4 y "f" es un número entero de 0 a 3.

8. Un compuesto según la reivindicación 1 ó 2, en el que R_1 es un grupo de la fórmula Y-3 y R_2 es hidrógeno, alquilo C_{1-6} , alquilo C_{1-6} sustituido, fenilo-(alquilo C_{1-6}) o naftilo-(alquilo C_{1-6}).

9. Un compuesto según la reivindicación 8, en el que R_1 es un grupo de la fórmula Y-3 en la que R_{25} es un grupo de la fórmula $R_{26}-(CH_2)_e-$, en la que R_{26} es alcoxi C_{1-6} , Q es $-(CH_2)_f-$, "e" es un número entero de 0 a 4 y "f" es un número entero de 0 a 3; y R_2 es fenilo-(alquilo C_{1-6}) o naftilo-(alquilo C_{1-6}).

10. Un compuesto según la reivindicación 1 ó 2, en el que

R_2 es hidrógeno, alquilo C_{1-6} , fenilo-(alquilo C_{1-6}) o naftilo-(alquilo C_{1-6});

R_3 es hidrógeno;

R_4 es hidrógeno o halógeno;

R_5 es hidrógeno, alquilo C_{1-6} , o trifluormetilo, o alcoxi C_{1-6} ;

R_6 es hidrógeno o alquilo C_{1-6} ; y

R_7 es hidrógeno, cloro, alcoxi C_{1-6} o alquilo C_{1-6} .

11. Un compuesto según la reivindicación 10, en el que R_1 es un grupo de la fórmula Y-1.

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12. Un compuesto según la reivindicación 11, en el que R_1 es un grupo de la fórmula Y-1 en la que R_{22} y R_{23} con independencia entre sí son hidrógeno, alquilo C_{1-6} o halógeno y R_{24} es hidrógeno, alquilo C_{1-6} o alcoxi C_{1-6} .

13. Un compuesto según la reivindicación 12, en el que R_1 es un grupo Y-1 en el que R_{24} es hidrógeno y R_2 es fenilo-(alquilo C_{1-6}) o naftilo-(alquilo C_{1-6}).

14. Un compuesto según la reivindicación 10, en el que R_1 es Y-2.

15. Un compuesto según la reivindicación 10, en el que R_1 es un grupo de la fórmula Y-3.

16. Un compuesto según la reivindicación 15, en el que R_1 es un grupo de la fórmula Y-3 en la que R_{25} es un grupo de la fórmula $R_{26}-(CH_2)_e-$, en la que R_{26} es alcoxi C_{1-6} , Q es $-(CH_2)_f-$, "e" es un número entero de 0 a 4 y "f" es un número entero de 0 a 3.

17. Un compuesto según la reivindicación 10, en el que R_2 es hidrógeno, alquilo C_{1-6} , fenilo-(alquilo C_{1-6}) o naftilo-(alquilo C_{1-6});

R_3 es hidrógeno;

R_4 es halógeno;

R_5 es hidrógeno;

R_6 es hidrógeno o alquilo C_{1-6} ; y

R_7 es hidrógeno, cloro, alcoxi C_{1-6} o alquilo C_{1-6} .

18. Un compuesto según una cualquiera de las reivindicaciones 1, 3 ó 4, en el que R_1 es un grupo de la fórmula Y-1 o un grupo de la fórmula Y-3;

R_2 es hidrógeno, alquilo C_{1-6} o fenilo o naftilo-(alquilo C_{1-6});

R_3 es hidrógeno, alquilo C_{1-6} , fenilo-(alquilo C_{1-6}) o naftilo-(alquilo C_{1-6});

R_4 es hidrógeno o halógeno;

R_5 es hidrógeno, alquilo C_{1-6} , o trifluormetilo;

R_6 es hidrógeno, alquilo C_{1-6} , (alquilo C_{1-6})-carboniloxi-(alquilo C_{1-6}) o un grupo de la fórmula P-3 o un grupo de la fórmula P-4; y

R_7 es hidrógeno o alquilo C_{1-6} .

19. Un compuesto según la reivindicación 18, en el que

R₂ es alquilo C₁₋₆, fenilo-(alquilo C₁₋₆) o naftilo-(alquilo C₁₋₆);

R₆ es hidrógeno, alquilo C₁₋₆, (alquilo C₁₋₆)-carboniloxi-(alquilo C₁₋₆) o un grupo de la fórmula P-3; y

R₇ es hidrógeno.

20. Un compuesto según la reivindicación 19, en el que R₁ es Y-1 en el que R₂₂ y R₂₃ son hidrógeno, halógeno o alquilo C₁₋₆ y R₂₄ es hidrógeno o alcoxi C₁₋₆, o bien R₁ es un grupo de la fórmula Y-3 en la que R₂₅ es un grupo de la fórmula R₂₆-(CH₂)_e-, en la que R₂₆ es alcoxi C₁₋₆, Q es -(CH₂)_f-, "e" es un número entero de 0 a 4 y "f" es un número entero de 0 a 3.

21. Un compuesto según la reivindicación 20, en el que R₂ es alquilo C₁₋₆; R₄ es hidrógeno; R₃ y R₅ son alquilo C₁₋₆ o trifluormetilo.

22. Un compuesto según la reivindicación 21, en el que R₆ es hidrógeno.

23. Un compuesto según la reivindicación 22, en el que R₁ es Y-1.

24. Un compuesto según la reivindicación 23, en el que R₂₂ y R₂₃ son halógeno o alquilo C₁₋₆.

25. Un compuesto según la reivindicación 24 que es N-[(2,6-diclorofenil)carbonil]-4-[1,6-dimetil-4-trifluormetil)-2-oxo-3-piridinil]-L-fenilalanina;

N-[(2-etil-6-metilfenil)carbonil]-4-[1,6-dimetil-4-trifluorometil)-2-oxo-3-piridinil]-L-fenilalanina;

N-[(2-(1-metiletil)-6-metilfenil)carbonil]-4-[1,6-dimetil-4-(trifluormetil)-2-oxo-3-piridinil]-L-fenilalanina;

N-[(2-cloro-6-metilfenil)carbonil]-4-[1,6-dimetil-4-(trifluormetil)-2-oxo-3-piridinil]-L-fenilalanina;

N-[(2,6-diclorofenil)carbonil]-4-[1,4-dimetil-6-(trifluormetil)-2-oxo-3-piridinil]-L-fenilalanina; o

N-[(2-cloro-6-metilfenil)carbonil]-4-[1,4-dimetil-6-(trifluormetil)-2-oxo-3-piridinil]-L-fenilalanina.

26. Un compuesto según la reivindicación 22 en el que R_1 es Y-3.

27. Un compuesto según la reivindicación 26, que es N-[[1-(2-metoxietil)ciclopentil]carbonil]-4-[1,6-dimetil-4-(trifluormetil)-2-oxo-3-piridinil]-L-fenilalanina.

28. Un compuesto según la reivindicación 21 en el que R_6 es alquilo C_{1-6} .

29. Un compuesto según la reivindicación 28 que es éster etílico de la N-[(2,6-diclorofenil)carbonil]-4-[1,6-dimetil-4-(trifluormetil)-2-oxo-3-piridinil]-L-fenilalanina; éster etílico de la N-[(2,6-diclorofenil)carbonil]-4-[1,4-dimetil-6-(trifluormetil)-2-oxo-3-piridinil]-L-fenilalanina; éster etílico de N-[(2-cloro-6-metilfenil)carbonil]-4-[1,6-dimetil-4-(trifluormetil)-2-oxo-3-piridinil]-L-fenilalanina o

éster etílico de N-[(2-cloro-6-metilfenil)carbonil]-4-[1,4-dimetil-6-(trifluormetil)-2-oxo-3-piridinil]-L-fenilalanina.

30. Un compuesto según la reivindicación 21 en el que R_6 es (alquilo C_{1-6})-carboniloxi-(alquilo C_{1-6}).

31. Un compuesto según la reivindicación 30 que es éster 1-(acetoxi)etílico de la N-[(2,6-diclorofenil)carbonil]-4-[1,6-dimetil-4-(trifluormetil)-2-oxo-3-piridinil]-L-fenilalanina;

éster 1-(acetoxi)etílico de la N-[(2,6-diclorofenil)carbonil]-4-[1,4-dimetil-6-(trifluormetil)-2-oxo-3-piridinil]-L-fenilalanina;

éster 1-(acetoxi)etílico de la N-[(2-cloro-6-metilfenil)carbonil]-4-[1,6-dimetil-4-(trifluormetil)-2-oxo-3-piridinil]-L-fenilalanina o

éster 1-(acetoxi)etílico de la N-[(2-cloro-6-metilfenil)carbonil]-4-[1,4-dimetil-6-(trifluormetil)-2-oxo-3-piridinil]-L-fenilalanina.

32. Un compuesto según la reivindicación 21 en el que R_6 es un grupo de fórmula P-3, en la que R_{32} es hidrógeno, R_{33} y R_{34} son alquilo C_{1-6} , uno de "h" y "g" es distinto de 0.

33. Un compuesto según la reivindicación 32 que es éster 2-[(N,N-dietil)amino]etílico de la N-[(2,6-dicloro-fenil)carbonil]-4-[1,6-dimetil-4-(trifluormetil)-2-oxo-3-piridinil]-L-fenilalanina;

éster 2-[(N,N-dietil)amino]etílico de la N-[(2,6-diclorofenil)carbonil]-4-[1,4-dimetil-6-(trifluormetil)-2-oxo-3-piridinil]-L-fenilalanina;

éster 2-[(N,N-dietil)amino]etílico de la N-[(2-cloro-6-metilfenil)carbonil]-4-[1,6-dimetil-4-(trifluormetil)-2-oxo-3-piridinil]-L-fenilalanina o

éster 2-[(N,N-dietil)amino]etílico de la N-[(2-cloro-6-metilfenil)carbonil]-4-[1,4-dimetil-6-(trifluormetil)-2-oxo-3-piridinil]-L-fenilalanina.

34. Un compuesto según la reivindicación 20 en el que R₆ es hidrógeno.

35. Un compuesto según la reivindicación 34, en el que R₂ es alquilo C₁₋₆; R₃ es hidrógeno; R₄ es hidrógeno o halógeno y R₅ es hidrógeno o alquilo C₁₋₆.

36. Un compuesto según la reivindicación 35, en el que R₁ es Y-1.

37. Un compuesto según la reivindicación 36, en el que R₂₂ y R₂₃ son alquilo C₁₋₆ o halógeno y R₂₄ es hidrógeno.

38. Un compuesto según la reivindicación 37 que es N-[(2-cloro-6-metilfenil)carbonil]-4-(5-cloro-1-metil-2-oxo-3-piridinil)-L-fenilalanina;

N-[(2-cloro-6-metilfenil)carbonil]-4-(1-metil-2-oxo-3-piridinil)-L-fenilalanina;

N-[(2-cloro-6-metilfenil)carbonil]-4-(1,4-dimetil-2-oxo-3-piridinil)-L-fenilalanina.

39. Un compuesto según la reivindicación 36 en el que R₂₂ y R₂₃ son halógeno y R₂₄ es hidrógeno.

40. Un compuesto según la reivindicación 39 que es la N-[(2,6-diclorofenil)carbonil]-4-(1,4-dimetil-2-oxo-3-piridinil)-L-fenilalanina.

41. Un compuesto según la reivindicación 36 en el que R₂₂ y R₂₃ son halógeno o hidrógeno y R₂₄ es alcoxi C₁₋₆.

42. Un compuesto según la reivindicación 41 que es N-[(2-bromo-5-metoxifenil)carbonil]-4-(1-metil-2-oxo-3-piridinil)-L-fenilalanina o N-[(2-bromo-5-metoxifenil)carbonil]-4-(5-bromo-1-metil-2-oxo-3-piridinil)-L-fenilalanina.

43. Un compuesto según la reivindicación 35, en el que R_1 es Y-3.

44. Un compuesto según la reivindicación 43 que es la 4-(5-cloro-1-metil-2-oxo-3-piridinil)-N-[[1-(2-metoxietil)ciclopentil]carbonil]-L-fenilalanina.

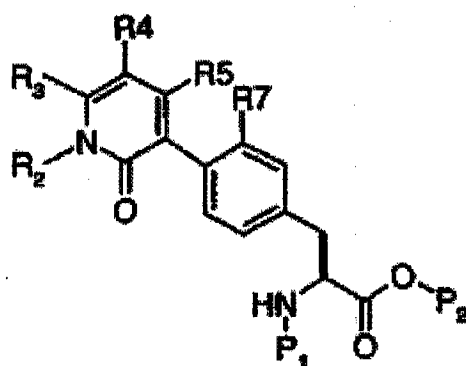
45. Un compuesto de la reivindicación 19, en el que R_{22} y R_{23} son halógeno o alquilo C_{1-6} , R_{24} es hidrógeno o alcoxi C_{1-6} , R_{25} es un grupo de la fórmula $R_{26}-(CH_2)_e-$, en la que R_{26} es alcoxi C_{1-6} , Q es $-(CH_2)_f-$, "e" es un número entero de 0 a 4 y "f" es un número entero de 0 a 3.

46. Un compuesto según la reivindicación 45 en el que R_2 es fenilo-(alquilo C_{1-6}) o naftilo-(alquilo C_{1-6}); R_3 es hidrógeno, R_4 es halógeno y R_5 , R_6 y R_7 son hidrógeno.

47. Un compuesto según la reivindicación 46 en el que R_1 es Y-1.

48. Un compuesto según la reivindicación 47 que es la 4-(1-bencil-5-cloro-2-oxo-3-piridinil)-N-[(2-cloro-6-metilfenil]carbonil]-L-fenilalanina; o la 4-(1-bencil-5-cloro-2-oxo-3-piridinil)-N-[[1-(2-metoxietil)ciclopentil]carbonil]-L-fenilalanina.

49. Un compuesto de la fórmula II

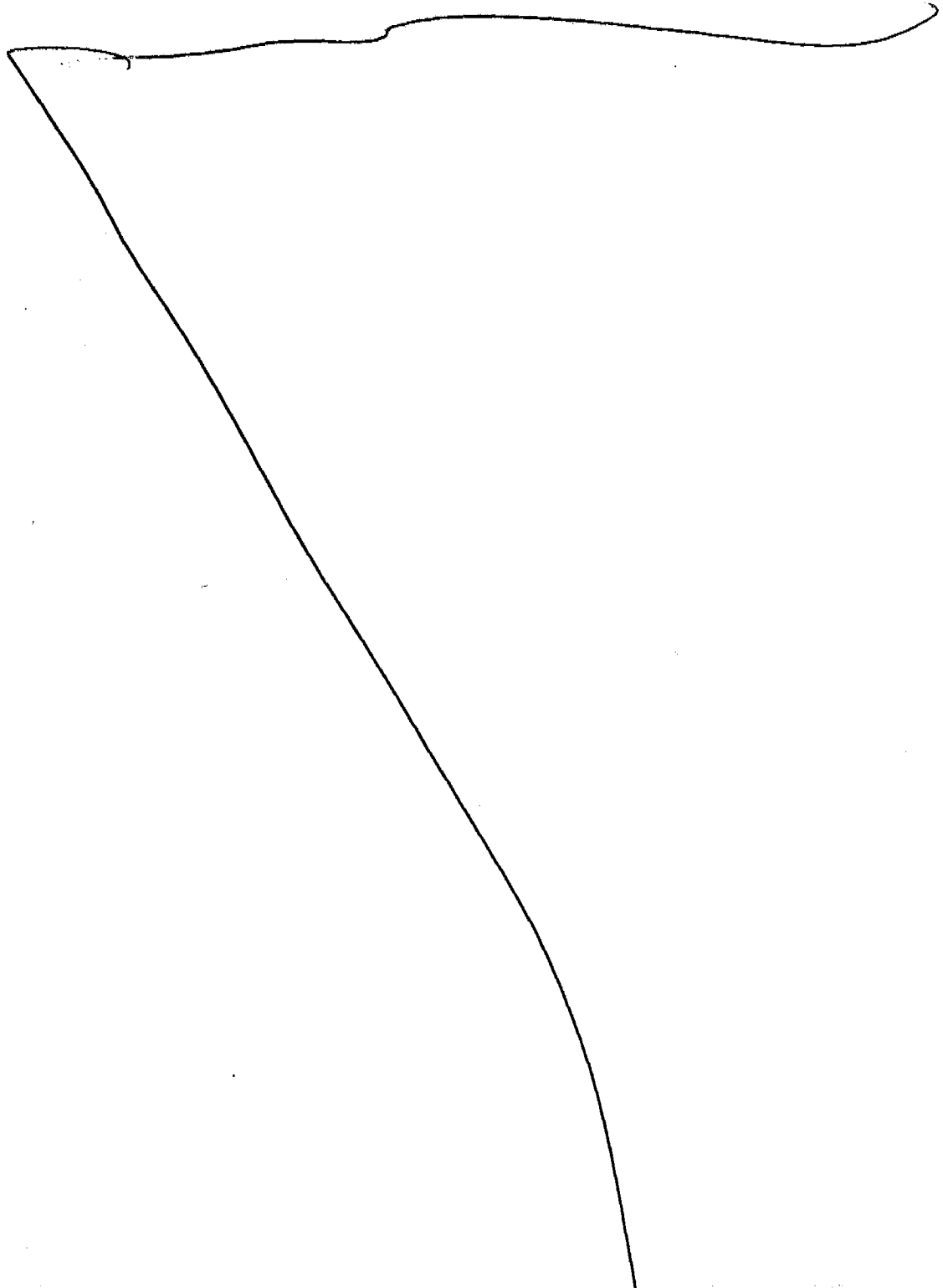


en la que R_2-R_5 y R_7 tienen los significados definidos en la reivindicación 1 y P_1 es un grupo Boc o carbobenciloxi y P_2 es un alquilo C_{1-6} o alquilo C_{1-6} sustituido.

50. Una composición farmacéutica que consta de un compuesto según una cualquiera de las reivindicaciones 1-48 o una sal farmacéuticamente aceptable del mismo y un excipiente farmacéuticamente aceptable.

51. Un procedimiento de fabricación de una composición farmacéutica que consiste en incorporar un compuesto según una cualquiera de las reivindicaciones 1-48 o una sal farmacéuticamente aceptable del mismo y un excipiente farmacéuticamente aceptable a una forma de administración galénica.

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PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07C 235/60, 233/87, 237/30, C07D 295/18, 213/61, 213/81, A61K 31/195, 31/455	A1	(11) International Publication Number: WO 99/43642 (43) International Publication Date: 2 September 1999 (02.09.99)
(21) International Application Number: PCT/GB99/00589 (22) International Filing Date: 26 February 1999 (26.02.99) (30) Priority Data: 9804161.9 26 February 1998 (26.02.98) GB 9826668.7 3 December 1998 (03.12.98) GB (71) Applicant (for all designated States except US): CELLTECH THERAPEUTICS LIMITED [GB/GB]; 216 Bath Road, Slough, Berkshire SL1 4EN (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): ARCHIBALD, Sarah, Catherine [GB/GB]; 5 College Glen, Maidenhead, Berkshire SL6 6BL (GB). HEAD, John, Clifford [GB/GB]; 4 Dorchester Close, Maidenhead, Berkshire SL6 6RX (GB). WAR- RELOW, Graham, John [GB/GB]; Oakside, 4 Wieland Road, Northwood, Middlesex HA6 3QU (GB). PORTER, John, Robert [GB/GB]; 7 Farm Place, Henton, Chinnor, Ox- fordshire OX9 4AD (GB). (74) Agents: MERCER, Christopher, Paul et al.; Carpmails & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.
(54) Title: PHENYLALANINE DERIVATIVES AS INHIBITORS OF ALPHA4 INTEGRINS		
(1)		
(57) Abstract Phenylalanine derivatives of formula (1) are described: wherein R is a carboxylic acid or a derivative thereof; L¹ is a linker atom or group; Ar is an optionally substituted aromatic group; and the salts, solvates, hydrates and N-oxides thereof. The compounds are able to inhibit the binding of alpha4 integrins to their ligands and are of use in the prophylaxis and treatment of immune or inflammatory disorders.		

- r and s, which may be the same or different, is each zero or an integer 1;
 R^a and R^b , which may be the same or different is each an atom or group
 $-L^2(CH_2)_pL^3(R^c)_q$ in which L^2 and L^3 is each a covalent bond or a linker
atom or group, p is zero or the integer 1, q is an integer 1, 2 or 3 and R^c is
5 a hydrogen or halogen atom or a group selected from straight or branched
alkyl, $-OR^d$ [where R^d is a hydrogen atom or an optionally substituted
straight or branched alkyl group], $-SR^d$, $-NR^dR^e$, [where R^e is as just
defined for R^d and may be the same or different], $-NO_2$, $-CN$, $-CO_2R^d$,
 $-SO_3H$, $-SO_2R^d$, $-OCO_2R^d$, $-CONR^dR^e$, $-OCONR^dR^e$, $-CSNR^dR^e$, $-COR^d$,
10 $-N(R^d)COR^e$, $N(R^d)CSR^e$, $-SO_2N(R^d)(R^e)$, $-N(R^d)SO_2R^e$, $-N(R^d)CONR^eR^f$
[where R^f is a hydrogen atom or an optionally substituted straight or
branched alkyl group], $-N(R^d)CSNR^eR^f$ or $-N(R^d)SO_2NR^eR^f$;
 Alk^2 is a straight or branched alkylene chain;
m is zero or an integer 1;
15 R^2 is a hydrogen atom or a methyl group;
 R^3 is a hydrogen atom or a straight or branched alkyl group;
Ar is an optionally substituted aromatic group;
and the salts, solvates, hydrates and N-oxides thereof.

- 20 It will be appreciated that compounds of formulae (1) and (1a) may have
one or more chiral centres. Where one or more chiral centres is present,
enantiomers or diastereomers may exist, and the invention is to be
understood to extend to all such enantiomers, diastereomers and mixtures
thereof, including racemates. Formulae (1) and (1a) and the formulae
25 hereinafter are intended to represent all individual isomers and mixtures
thereof, unless stated or shown otherwise.

- In the compounds of formulae (1) and (1a), derivatives of the carboxylic
acid group R include carboxylic acid esters and amides. Particular esters
30 and amides include $-CO_2Alk^4$ and $-CON(R^4)_2$ groups as described herein.

- When in the compounds of formulae (1) and (1a) L^1 is present as a linker
atom or group it may be any divalent linking atom or group. Particular
examples include $-O-$ or $-S-$ atoms or $-C(O)-$, $-C(O)O-$, $-C(S)-$, $-S(O)-$,
35 $-S(O)_2-$, $-N(R^4)-$ [where R^4 is a hydrogen atom or a straight or branched
alkyl group], $-CON(R^4)-$, $-OC(O)N(R^4)-$, $-CSN(R^4)-$, $-N(R^4)CO-$,

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-N(R⁴)C(O)O-, -N(R⁴)CS-, -S(O)N(R⁴)-, -S(O)₂N(R⁴)-, -N(R⁴)S(O)-, -N(R⁴)S(O)₂-, -N(R⁴)CON(R⁴)-, -N(R⁴)CSN(R⁴)-, -N(R⁴)SON(R⁴)- or -N(R⁴)SO₂N(R⁴)- groups. Where the linker group contains two R⁴ substituents, these may be the same or different.

5

Alk² in the compounds of formulae (1) and (1a) may be for example a straight or branched C₁₋₃alkylene chain. Particular examples include -CH₂-, -CH(CH₃)-, -C(CH₃)₂- and -(CH₂)₂-.

- 10 When R³ and/or R⁴ in the compounds of formula (1) is a straight or branched alkyl group it may be a straight or branched C₁₋₆ alkyl group, e.g. a C₁₋₃ alkyl group such as a methyl or ethyl group.

- 15 When Alk¹ in compounds of formula (1) is an optionally substituted aliphatic chain it may be an optionally substituted C₁₋₁₀ aliphatic chain. Particular examples include optionally substituted straight or branched C₁₋₆ alkylene, C₂₋₆ alkenylene, or C₂₋₆ alkynylene chains.

- 20 Heteroaliphatic chains represented by Alk¹ include the aliphatic chains just described but with each chain additionally containing one, two, three or four heteroatoms or heteroatom-containing groups. Particular heteroatoms or groups include atoms or groups L⁴ where L⁴ is as defined above for L¹ when L¹ is a linker atom or group. Each L⁴ atom or group may interrupt the aliphatic chain, or may be positioned at its terminal
- 25 carbon atom to connect the chain to the atom or group R¹.

- Particular examples of aliphatic chains represented by Alk¹ include optionally substituted -CH₂-, -CH₂CH₂-, -CH(CH₃)-, -C(CH₃)₂-, -(CH₂)₂CH₂-, -CH(CH₃)CH₂-, -(CH₂)₃CH₂-, -CH(CH₃)CH₂CH₂-,
- 30 -CH₂CH(CH₃)CH₂-, -C(CH₃)₂CH₂-, -(CH₂)₄CH₂-, -(CH₂)₅CH₂-, -CHCH-, -CHCHCH₂-, -CH₂CHCH-, -CHCHCH₂CH₂-, -CH₂CHCHCH₂-, -(CH₂)₂CHCH-, -CC-, -CCCH₂-, -CH₂CC-, -CCCH₂CH₂-, -CH₂CCCH₂-, or -(CH₂)₂CC- chains. Where appropriate each of said chains may be optionally interrupted by one or two atoms and/or groups L⁴ to form an
- 35 optionally substituted heteroaliphatic chain. Particular examples include

for example ethylchloroformate, prior to reaction with the amine of formula (3).

Intermediates of formulae (2), (3) and (4), or compounds of formula (1),
5 may be manipulated to introduce substituents to aromatic or heteroaromatic groups or modify existing substituents in groups of these types. Typically, such manipulation may involve standard substitution approaches employing for example alkylation, arylation, heteroarylation, acylation, thioacylation, halogenation, sulphonylation, nitration, formylation
10 or coupling reactions. Alternatively, existing substituents may be modified for example by oxidation, reduction or cleavage reactions. Particular examples of such reactions are given below. Where these are particularly described in relation to the generation of the group $R^1(Alk^1)_r(L^1)_s$, it will be appreciated that each reaction may also be used to introduce or modify
15 R^5 and/or R^6 substituents in for example Ar groups as appropriate.

Thus in one example, a compound wherein $R^1(Alk^1)_r(L^1)_s$ is a $-L^1H$ group may be alkylated, arylated or heteroarylated using a reagent $R^1(Alk^1)_rX$ in which R^1 is other than a hydrogen atom and X is a leaving atom or group
20 such as a halogen atom, e.g. a fluorine, bromine, iodine or chlorine atom or a sulphonyloxy group such as an alkylsulphonyloxy, e.g. trifluoromethylsulphonyloxy or arylsulphonyloxy, e.g. p-toluenesulphonyloxy group.

The reaction may be carried out in the presence of a base such as a
25 carbonate, e.g. caesium or potassium carbonate, an alkoxide, e.g. potassium t-butoxide, or a hydride, e.g. sodium hydride, in a dipolar aprotic solvent such as an amide, e.g. a substituted amide such as dimethylformamide or an ether, e.g. a cyclic ether such as tetrahydrofuran.

30 In another example, a compound where $R^1(Alk^1)_r(L^1)_s$ is a $-L^1H$ group is a hydrogen atom may be functionalised by acylation or thioacylation, for example by reaction with a reagent $R^1(Alk^1)_rL^1X$ [wherein L^1 is a $-C(O)-$, $C(S)-$, $-N(R^4)C(O)-$ or $N(R^4)C(S)-$ group], in the presence of a base, such
35 as a hydride, e.g. sodium hydride or an amine, e.g. triethylamine or N-methylmorpholine, in a solvent such as a halogenated hydrocarbon, e.g.

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dichloromethane or carbon tetrachloride or an amide, e.g. dimethylformamide, at for example ambient temperature, or by reaction with $R^1(Alk^1)_rCO_2H$, $R^1(Alk^1)_4COSH$ or an activated derivative thereof, for example as described above for the preparation of esters of formula (2).

5

In a further example a compound may be obtained by sulphonylation of a compound where $R^1(Alk^1)_r(L^1)_s$ is an $-OH$ group by reaction with a reagent $R^1(Alk^1)_rL^1Hal$ [in which L^1 is $-S(O)-$ or $-SO_2-$ and Hal is a halogen atom such as chlorine atom] in the presence of a base, for example an inorganic base such as sodium hydride in a solvent such as an amide, e.g. a substituted amide such as dimethylformamide at for example ambient temperature.

10

In another example, a compound where $R^1(Alk^1)_r(L^1)_s$ is a $-L^1H$ group, may be coupled with a reagent R^1OH (where R^1 is other than a hydrogen atom) or R^1Alk^1OH in a solvent such as tetrahydrofuran in the presence of a phosphine, e.g. triphenylphosphine and an activator such as diethyl, diisopropyl- or dimethylazodicarboxylate to yield a compound containing a $R^1(Alk^1)_rO-$ group.

15

20

In a further example, ester groups $-CO_2R^4$ or $-CO_2Alk^4$ in compounds of formula (1) may be converted to the corresponding acid $[-CO_2H]$ by acid- or base-catalysed hydrolysis depending on the nature of the group R^4 or Alk^4 . Acid- or base-catalysed hydrolysis may be achieved for example by treatment with an organic or inorganic acid, e.g. trifluoroacetic acid in an aqueous solvent or a mineral acid such as hydrochloric acid in a solvent such as dioxane or an alkali metal hydroxide, e.g. lithium hydroxide in an aqueous alcohol, e.g. aqueous methanol.

25

30 In a second example, $-OR^7$ [where R^7 represents an alkyl group such as methyl group] groups in compounds of formula (1) may be cleaved to the corresponding alcohol $-OH$ by reaction with boron tribromide in a solvent such as a halogenated hydrocarbon, e.g. dichloromethane at a low temperature, e.g. around $-78^\circ C$.

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Expert Opinion

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VLA-4 antagonists

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Evidence has accumulated over recent years for an important role for VLA-4 in inflammatory disease. Work with antibodies directed against VLA-4 or its primarily ligand VCAM-1 and peptides that mimic the Leu-Asp-Val (LDV) VLA-4 binding epitope on fibronectin suggest that asthma, rheumatoid arthritis, multiple sclerosis, atherosclerosis and inflammatory bowel disease are amenable to treatment with a VLA-4 antagonist. Several types of molecules, including humanised monoclonal antibodies, VCAM antisense polynucleotides, peptide mimetics of the LDV sequence and 4-substituted N-acetylphenylalanine derivatives, constitute the major compound classes currently under development. Clinical trials with several compounds are underway with early reports of efficacy with the monoclonal antibody natalizumab in multiple sclerosis and Crohn's disease.

Keywords: $\alpha_4\beta_1$, $\alpha_4\beta_7$, anti-inflammatory drugs, cell adhesion, MAdCAM, VCAM, VLA-4

Expert Opin. Ther. Patents (2002) 12(7):991-1008

1. Introduction

The integrins constitute a large class of heterodimeric, cell surface molecules consisting of α and β chains, each of which has a large extracellular domain and a short cytoplasmic tail. There are at least 14 different α chains and eight β chains known, which combine in a restricted manner, depending on cell type, giving ~ 23 members of the integrin family, each of which bind specific peptide ligands. Integrins mediate a variety of cell functions, including adhesion, migration, activation and survival. Lymphocytes and leukocytes with the exception of neutrophils constitutively express the integrin $\alpha_4\beta_1$, very late activating antigen-4 (VLA-4, CD49d/CD29) and are capable of expressing the closely related integrin, $\alpha_4\beta_7$ (LPAM, lymphocyte Peyer's patch cellular adhesion molecule) [1]. Mouse and rat neutrophils are also capable of expressing low levels of VLA-4, at least during reperfusion [2]. VLA-4 interacts with both vascular cell adhesion molecule-1 (VCAM-1, CD106) and the alternatively spliced portion of the Type III connecting segment of fibronectin known as the CS-1 region. The predominate form of VCAM-1 contains seven extracellular immunoglobulin domains, a transmembrane domain and a short cytoplasmic tail. There is homology between domains 1 - 3 and 4 - 6 suggesting that they arose from a gene duplication event. The critical epitope of VCAM-1 for recognition of VLA-4 or $\alpha_4\beta_7$ is the sequence -QID(40)SPL- in domain 1, of which Asp40 is essential [3-8]. Crystal structures of a fragment containing the two N-terminal domains show that this sequence lies on an exposed area of the C-D loop [7,8]. A related sequence on the CS-1 segment of fibronectin ...LDV... is responsible for its interaction with VLA-4 [9-11]. Compounds based on these sequences have fuelled much of the effort in small molecule VLA-4 antagonists.

A recent report stating that VLA-4 also binds to the N-terminal fragment of osteopontin resulting from thrombin cleavage, but not full-length osteopontin, may also be of significance as both osteopontin and thrombin are present at sites of inflammation. The osteopontin cleavage fragment (OPN 17-168) or either of the two peptides ELVTFPTDLPAT (OPN 131-143) and SVVYGLR (OPN 162-168), identified by fragment screening are independently able to mediate adhesion of

VLA-4 antagonists

VLA-4 expressing cells [12]. Intracellular adhesion molecule (ICAM)-4, is a member of the intracellular adhesion molecule subgroup of the immunoglobulin superfamily and is expressed on erythroid cells. Unlike other members of the class, ICAM-4 lacks the I/LETP/S consensus motif and binds the VLA-4 integrin via an, as yet undefined epitope [13].

The integrin $\alpha_4\beta_7$ also interacts with the same epitope as VLA-4 in both VCAM and the CS-1 region of fibronectin and with the mucosal addressin cell adhesion molecule-1 (MAdCAM). However, the opposite is not true; VLA-4 does not interact with MAdCAM. The expression of MAdCAM is limited to high endothelial venules of gut associated lymphoid organs and lamina propria venules and is involved in the trafficking of $\alpha_4\beta_7$ bearing lymphocytes to these mucosal tissues [14].

Integrins are involved in two-way signalling. The cytoplasmic tail of VLA-4 is linked to intracellular cytoskeletal proteins and kinases [15-17]. While the details of this signalling process are largely unknown, the affinity of VLA-4 for its ligands can be modulated by intracellular pathways linked to chemokine interactions with G-protein-coupled receptors on leukocytes in a process referred to as inside-out signalling [18,19]. In addition, binding of leukocytes to VCAM or fibronectin causes marked changes in cell morphology and is crucial for regulating cell motility (outside-in signalling) [20]. Both the α and β subunits are required for ligand binding, with affinity determined by their juxtaposition under control of their contacts with proteins associated with the cytoskeleton [21].

Until recently, little was known about the structure of integrins. Structural studies, including electron microscopy, support an extended extracellular domain with a bulbous head domain [22]. A β propeller motif in which the seven N-terminal repeats are arranged in a torus has been proposed for the α subunit [23] and a series of epidermal growth factor (EGF) repeats have been postulated on the stalk portion of the β chain [24]. A homology model of the putative metal ion dependent adhesion site (MIDAS) domain of the β chain based on known structures of the comparable domains of CD11b together with a model for ligand binding, have recently been proposed [25]. In an exciting development for the field, a 3.1 Å resolution crystal structure of the related $\alpha_v\beta_3$ integrin appeared confirming the presence of the proposed structural features. [26] A structure of a complex of $\alpha_v\beta_3$ with a cyclic peptide containing the signature Arg-Gly-Asp motif is now also available. This structure reveals that the ligand bridges the heterodimer with the aspartic acid moiety binding to a metal ion in the MIDAS domain on the β -subunit and the guanidine of the arginine binding two aspartic acids within the β -propeller domain of the α -subunit [27]. It is likely that comparable recognition elements exist on $\alpha_4\beta_1$ for its ligands. These studies, as well as mutagenesis and biochemical experiments, indicate that the subunits are conformationally mobile, moving between high and low affinity states in response to physiological stimuli [28,29].

2. Possible disease targets

In response to inflammatory stimuli, locally released chemokines and cytokines activate VLA-4 and the related integrin LFA-1 on the surface of circulating leukocytes and upregulate the expression of VCAM-1 on endothelial cells, respectively. While VLA-4 is constitutively expressed on leukocytes, CD4+ T cells [30] and eosinophils [31], it may be upregulated in response to inflammation. Contact between E-, L- and P-selectins and their carbohydrate ligands on leukocyte and endothelial surfaces, in concert with VCAM, cause leukocytes to stick and begin to roll when in contact with blood vessel walls [32-34]. This process provides an opportunity for firm adhesion to occur between these leukocytes and VCAM-1, expressed on the endothelial cell surface, and leads to a flattening of the cells, followed by their transmigration from blood vessels to the extracellular matrix. Here they bind fibronectin and possibly other elements of the extracellular matrix and migrate along a chemokine gradient to the site of inflammation. Since many inflammatory diseases are characterised by an influx of lymphocytes and leukocytes, there is keen interest in the pharmaceutical industry to find and test compounds with the potential to attenuate this process.

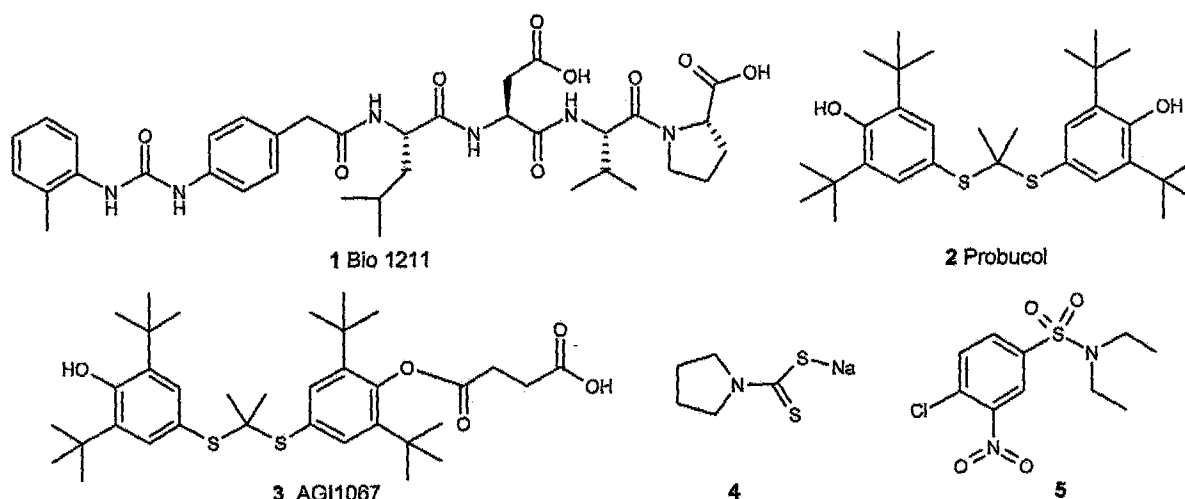
Although the ligand for LFA-1, ICAM-1 is also expressed on endothelial cells and is involved in mediating leukocyte adhesion, expression of ICAM-1 is not as tightly regulated and thus, the LFA-1/ICAM interaction is less specific. Compounds that inhibit this interaction are being targeted to neutrophil-mediated diseases, particularly psoriasis. The role of ICAM receptors in regulating cell trafficking has recently been reviewed [35,36].

2.1 Asthma

Asthma is an inflammatory disease characterised by accumulation of activated CD4+ T cells and eosinophils in lung airways, resulting in the restriction of airflow and hyper-reactivity of airway smooth muscle. VCAM expression and levels of soluble VCAM are increased in asthma patients [37,38]. Furthermore, antibodies directed against VLA-4 block eosinophil accumulation, hyper-reactivity and inflammation in mouse [39,40], rat [41] and guinea-pig [42] models of allergic asthma. More recently, the peptide VLA-4 antagonist Bio1211, (1) [43] was shown to block late phase airway response and to attenuate carbacol induced airway hyperresponsivity in a sheep model of allergic asthma.

2.2 Atherosclerosis

Recent data support an important role for upregulated endothelial VCAM-1 in monocyte migration into the subintimal space during the early stages of atherosclerosis [44-46]. Mice in which the fourth domain of VCAM-1 was knocked out (VCAM-1^{D4D/D4D}) are viable, but produce only 2 - 8% of the VCAM-1 or VCAM-1 mRNA levels produced in the wild type. When crossed with low density lipoprotein (LDL) receptor null mice, the resulting LDLR^{-/-} VCAM-1^{D4D/D4D}



animals were paired with LDLR^{-/-} VCAM^{+/+} littermates and fed a high cholesterol diet for 8 weeks. While lipoprotein and cholesterol levels were comparable in the two groups, the extent of the atherosclerotic lesions in the aorta was significantly reduced in the LDLR^{-/-} VCAM-1^{D4D/D4D} mice [47]. Treatment of normal or LDLR^{-/-} mice on an atherogenic diet with a peptide mimetic derived from the LDV sequence over the course of 3 weeks significantly reduced the amount of lipid accumulation in their aortic sinuses [48].

Protein expression levels of VCAM-1 and other pro-inflammatory proteins have been related to oxidative stress and it is suggested that antioxidants may be of benefit in disease states, such as atherosclerosis, mediated by the VCAM/VLA-4 interaction [49]. Probucol (2) is under development in Japan for restenosis [50] and closely related compounds, including the development candidate AGI 1067 (3), have been patented by AtheroGenics as inhibitors of MCP-1 and VCAM-1 gene expression [201,202]. Phase IIa clinical trials for restenosis have been completed in 305 patients treated with 70, 140 and 280 mg doses of AGI 1067 or probucol, comparing coronary artery lumen size 6 weeks after angioplasty, using intravascular ultrasound. The 280 mg dose proved effective and a larger, 1-year trial is now underway using this dose [301]. Workers from AtheroGenics have also described the inhibitory effect of dithiocarbamates, for example pyrrolidine dithiocarbamate (4) [51] and the nitrobenzene derivatives related to compound 5 [52] on VCAM-1 expression. The potential contribution to their overall pharmacological profile from VCAM-1 expression inhibition is not clear from the available data on these antioxidants; however, this area is worth watching.

2.3 Multiple sclerosis

Multiple sclerosis (MS) and Guillain-Barre syndrome are autoimmune diseases of the central and peripheral nervous systems, respectively characterised by an infiltration of activated T cells and demyelination leading to neuronal degeneration. Anti-VLA-4 and anti- β_1 antibodies have been shown to be effective in a number of animal models of experimental

autoimmune encephalomyelitis (EAE) [53], although the timing of treatment administration may be critical [54-57]. Recently, preliminary results of the first human trials of the anti- α_4 antibody, natalizumab, were reported and will be summarised in Section 3.2.

2.4 Rheumatoid arthritis

Treatments directed to VLA-4 and $\alpha_4\beta_7$ integrins may have a role in rheumatoid arthritis, inflammatory bowel disease and reperfusion injury. Fibroblast-like synovial cells express VCAM-1 and are involved in the chemotaxis and survival of B and T cells, which mediate rheumatoid arthritis [58-60]. Antibodies to VLA-4 were effective in rat models of adjuvant arthritis [61] and, as noted in the following sections, a few small molecule VLA-4 antagonists have also shown efficacy in animal models as well. The possible role of VCAM/VLA-4 in joint inflammation has recently been reviewed [62].

2.5 Inflammatory bowel disease and Crohn's disease

Inflammatory bowel disease is thought to be mediated, at least in part, by VCAM and MAdCAM expressed locally in gut mucosal tissues [63]. Antibodies against $\alpha_4\beta_7$ have been shown to reduce the spontaneous development of ulcerative colitis in the cotton top tamarin [64] and inflammation in mouse models of colitis [65,66]. Anti-VCAM is also active in the mouse dextran sulfate sodium (DSS)-induced colitis model [67]. As summarised below, the humanised anti- α_4 monoclonal antibody natalizumab has been shown to be effective in the treatment of Crohn's disease in a small Phase II clinical trial.

2.6 Reperfusion injury

Recent publications indicate a possible role for VLA-4 in cardiac [68,69], renal [70] and cerebral [71] reperfusion damage. In the former case, murine neutrophils were shown to express VLA-4 after penetrating the extracellular matrix. Although these studies suggest VLA-4 is not important for adhesion in this case, it may be involved in the coupling of superoxide release with cell-cell contact.

VLA-4 antagonists

2.7 Haematopoiesis and fetal development

In addition to the above, VCAM/VLA-4 interactions play a role in regulating the trafficking of haematopoietic cells [72,73] and may help render myeloma and lymphoma cells resistant to apoptosis [74-76]. VCAM expression is required for embryogenesis; VCAM knockout mice display placental and heart defects and, with the exception of a very small percentage who develop to adulthood, die by embryonic day 11.5 - 12.5 [77,78]. However, animals in which the VCAM-1 gene was deleted after birth are viable and show defects in the homing of haematopoietic cells to bone marrow [79,80]. Mice in which VCAM expression is markedly attenuated are viable [44] and while there is little published information on possible reproductive toxicity of compounds targeting VLA-4 or $\alpha_4\beta_7$, several companies have drugs undergoing chronic studies in Phase II, suggesting that these compounds are free of major reproductive liabilities.

3. VCAM/VLA-4 inhibitors

3.1 Antisense molecules

The VCAM-1 antisense oligonucleotide ISIS 18155, given at 8 mg/kg, i.v., inhibits the number of rolling lymphocytes and the inflammation scores in a rat model of indomethacin induced ileitis. Lower doses were not effective [81]. The orally-active oligonucleotide directed against α_4 , ISIS 107248 is being developed for MS by Antisense Therapeutics under license from ISIS Pharmaceuticals [302]. According to a company press release, clinical trials should start during 2002.

3.2 Antibodies as drugs - natalizumab

Natalizumab (AntegrenTM, Protein Design Labs) is a monoclonal antibody humanised from a murine anti- α_4 antibody and is currently under joint development by Biogen and Elan Pharmaceuticals [82]. Pharmacokinetic studies have been carried out in patients with MS at doses of 0.1 - 6 mg/kg, i.v. The compound exhibits a long half-life, 70 - 177 h at doses of 1 and 6 mg/kg and a dose-dependent area under curve (AUC). Only mild adverse effects were observed consisting of transient headache and fatigue [83,84].

A group of 72 patients with relapsing-remitting or secondary progressive MS were treated with an i.v. infusions of 3 mg/kg natalizumab or placebo at weeks 0 and 4 and were followed for 24 weeks. Patients were evaluated for general well being and MS symptoms every two weeks for the first eight weeks and then every four weeks. Magnetic resonance imaging revealed a significant reduction in new active (1.8 vs 3.6 per patient) and new enhancing lesions (1.6 vs 3.3 per patient) during the first 12 weeks of the study, with no difference in the second 12 weeks. The number of treated patients with acute exacerbations was not different from placebo during the first 12 weeks, but was significantly higher during the 12 week follow-up (14 vs 3) [85,303]. A Phase III clinical trial is starting in which 2100 relapsing-remitting MS patients will be treated for 2 years with natalizumab either alone or in combination with IFN- β .

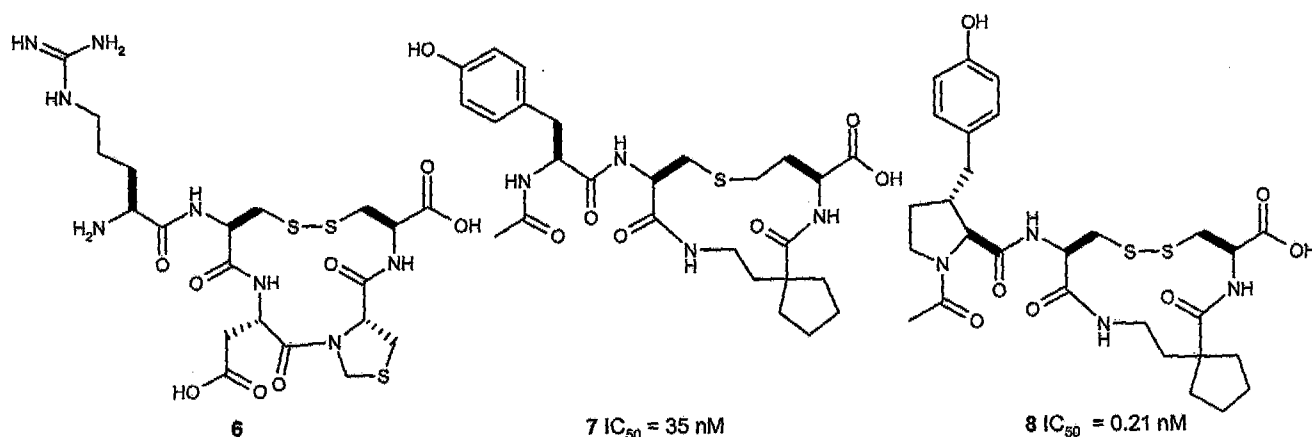
A Phase II study was carried out in a group of 244 patients with moderately severe Crohn's disease (CDAI, Crohn's Disease Activity Index, scores of 220 - 450). Treatment groups received placebo, a single 3 mg/kg infusion or two infusions of 3 or 6 mg/kg of natalizumab four weeks apart and were followed for 12 weeks. Patients were assessed by means of the CDAI and changes in quality of life as reflected in an inflammatory bowel disease questionnaire (IBDQ). The treatment group that received 3 mg/kg showed an improvement in the CDAI score of > 70 points by week two, which was maintained throughout the study. Remission as defined by a CDAI score of < 150 was achieved by 46% of the patients who received 3 mg/kg, compared with 27% in the placebo treated group. There were also significant responses as determined by IBDQ scores at weeks 6 and 12 in the patients who received two infusions of natalizumab [86,87,303]. Side effects were generally mild and included headache, nausea, asthenia and urinary tract infections. A Phase III trial was scheduled to begin at the end of 2001. Five of ten ulcerative colitis patients achieved a positive clinical response in a small safety trial of a single 3 mg/kg i.v. dose [88]. It is not clear whether further work in ulcerative colitis is ongoing with this drug. It is encouraging that patients exposed to natalizumab for up to six months do not exhibit significant side effects and that there is a suggestion of efficacy in MS and Crohn's disease.

3.3 Small molecules as drugs

There has been intense interest among pharmaceutical companies over the past decade in discovering and evaluating new candidate VCAM/VLA-4 inhibitors. The early work on peptide derivatives, mostly based on variants of the LDV sequence, has been published and led to a concerted effort particularly directed to phenyluriedo-LDV mimetics. Acyl-phenylalanines constitute the second major class of active compounds, many of which are capable of inhibiting both VLA-4 and $\alpha_4\beta_7$ mediated interactions with varying degrees of selectivity. In addition, there are individual reports of compounds of other chemical classes, which are summarised in Section 3.3.4. Although reports are starting to appear in peer-reviewed journals, many of the novel structures are only available through the patent literature and poster presentations at scientific meetings. In the sections that follow, the binding data cited are gathered under widely different protocols and are not useful for directly comparing compounds, except when they are members of a series from one organisation.

3.3.1 Cyclic peptide derivatives

An early publication from Tanabe Research [89] reported the cyclic peptide 6, which was presumably originally designed as a constrained RGD mimic and is a potent inhibitor of both the VLA-4 and $\alpha_5\beta_1$ integrins. NMR analysis indicates that the N-terminal amino acid is disordered, but that the ring system exists in a well-defined, relatively planar conformation [90]. Subsequently, in a series of papers, scientists



9 ZD-7349



10

from Genentech and Roche reported extensive structure-activity and structural studies around this compound, leading to the potent and simplified N-terminal tyrosine derivative, compound 7 [91,92]. A further enhancement in potency was gained by incorporation of constraint into the tyrosine to give compound 8, which is ~ 1000-fold more potent in a VCAM-VLA-4 ELISA type binding assay than the lead compound 6 [203].

From an extensive series of cyclic peptides built around the LDV motif, AstraZeneca chemists prepared the cyclic hexapeptide 9 (ZD7349), which potently inhibited the binding of MOLT-4 cells to VCAM ($IC_{50} = 260 \text{ nM}$) and to fibronectin ($IC_{50} = 330 \text{ nM}$) [93]. Further profiling of compound 10 in animal models indicates that it inhibits the development of ovalbumin-induced delayed type hypersensitivity (DTH) in mice when given via osmotic mini-pumps at a rate of 0.01 mg/kg/day s.c., as a 3 – 10mg/kg s.c. injection 4 h prior to challenge or as an i.v. bolus 20 h after challenge. ZD7349 given chronically to mice in a 14 day mini-pump at 10 mg/kg/day also inhibited the development of collagen-induced arthritis at 3 mg/kg/day and delayed the onset of symptoms in an EAE model.

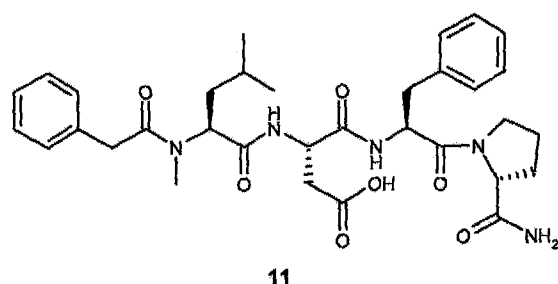
ZD7349 was chosen for development of a prototype depot formulation through incorporation into a 50:50 poly(d,l-lactide-co-glycolide) depot form suitable for s.c. implantation. Unlike some potent compounds in the series, for example, compound 10, which was unstable at pH 3 [94], compound 9 has adequate stability and is released from the polymer matrix at a steady-state over the course of 17 days [92].

3.3.2 Linear peptides – LDV mimics

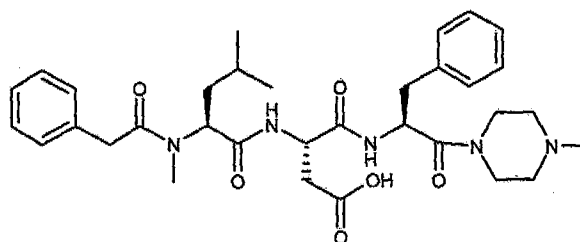
The 10-amino acid fibronectin CS-1 derived peptide GPEILDVPST inhibits the binding of ⁵¹Cr labelled Jurkat cells to the CS-1 25-mer ($IC_{50} = 150 \text{ } \mu\text{M}$). Arrhenius *et al.* examined a series of truncated analogues and showed that the minimal fragment retaining activity was the sequence ILDVP ($IC_{50} = 250 \text{ } \mu\text{M}$) [95]. Replacement of isoleucine with phenylalanine and inversion of the stereochemistry of the C-terminal proline lead to the sequence: FLDVp ($IC_{50} = 25 \text{ } \mu\text{M}$), prompting a more extensive investigation of analogues based on LDV and culminating in the identification of the N-terminal phenyl acetyl analogue compound 11 ($IC_{50} = 0.5 \text{ } \mu\text{M}$). Compound 11 at 6 mg/kg, was active in inhibiting oxazolone induced DTH in the mouse over 24 h [204]. It also inhibits both the early and late phase increase in bronchial resistance in sheep sensitive to ascaris suum, as well as the development of hyper-reactivity in these animals after b.i.d. treatment with 30 mg/kg of compound 11 by aerosol for 3 days and 30 min prior to and 4 h postchallenge on day 4 [96].

CY9701 (11) is an analogue in which an N-methyl group and C-terminal piperazines were added to improve proteolytic stability. CY9701 (100 mg/kg, intranasally, 5 min before ovalbumin challenge) decreased eosinophil influx to the lung, bronchial hyperresponsiveness and the concentration of IL-4, IL-5 and eotaxin in the bronchoalveolar lavage fluid in a mouse allergy model 72 h after antigen challenge [97].

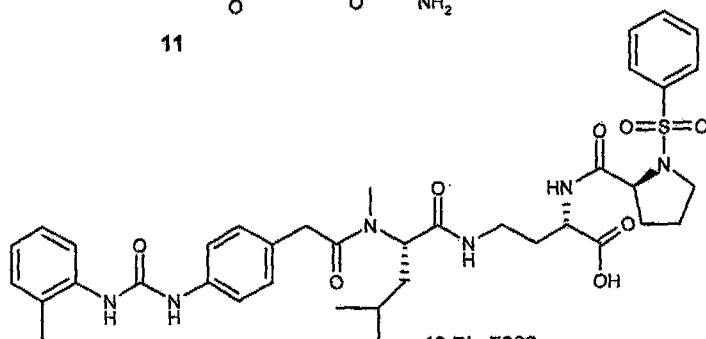
Biogen medicinal chemists systematically varied both ends of the molecule and discovered that addition of a urea terminating in 2-methylphenyl led to a marked increase in potency. The profound role of the phenylureidophenylacetyl-moiety is evident from its adoption into LDV mimics by many pharmaceutical companies. Addition of a C-terminal proline gave Bio1211 (1), which is a highly selective inhibitor of VLA-4 mediated binding ($IC_{50} = 4 \text{ nM}$). It is ~ 500-fold more selective for VLA-4 than $\alpha_4\beta_7$ integrins and is inactive in binding assays for inhibition of $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_{IIb}\beta_{IIIa}$ and $\alpha_L\beta_2$. After a single nebulised dose of 0.1 mg/kg to sheep sensitised to ascaris suum, Bio 1211 inhibited the early phase and virtually eliminated the late phase increase in bronchial resistance 24 h after challenge [43]. Bio 1211 was licensed to



11



12 CY-9701



13 Bio 7662

Merck and taken into the clinic for the treatment of asthma by aerosol administration; however, efficacy results in Phase II trials were disappointing.

A very interesting observation made by Biogen scientists working with ^3H -Bio1211 is that its dissociation constant and off-rate for binding to Jurkat cells is highly dependent on the affinity state of the integrin. *In vitro*, this is modulated by cation concentrations. In the presence of 1 mM Ca^{2+} and 1 mM Mg^{2+} the K_d of Bio1211 was 20 – 40 nM and improved to 100 pM in the presence of 2 mM Mn^{2+} . A further enhancement of K_d to 18 pM was observed when the binding experiments were run in the presence of 2 mM Mn^{2+} and 10 mg/ml of the activating antibody TS2/16. The effect is due exclusively to modulation of the off-rates; on-rates did not vary widely among experiments. Similar results were observed for Jurkat cells binding to the CS-1 peptide or to an Ig-VCAM conjugate suggesting that regulation of off-rates by metal ion concentrations and likely by the activation state of cells expressing VLA-4 may be general [98].

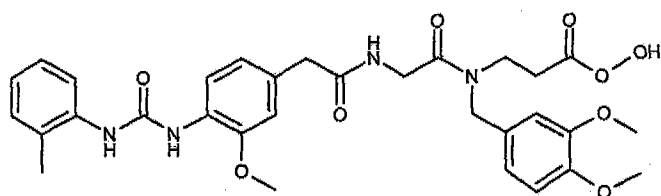
More recently the same group at Biogen discovered that binding of the related, more potent ligand, compound 13 (Bio7662) is not dependent on the activation status of the cells. Furthermore, there is a complex interplay between the binding affinity of Bio 7662, 13, and that of Ca^{2+} suggesting that conformational changes needed to accommodate ligand and metal ion binding are thermodynamically linked. Binding of Bio 7662 exposes an epitope associated with integrin activation, termed the ligand induced binding site (LIBS) suggesting that it may be capable of inducing signalling [99]. It will be very interesting to determine the significance of this behaviour and whether it is also exhibited by other compounds.

Several companies have pursued related phenylureidophenyl-LDV mimics; the most advanced of these is IVL-745 (14), which Aventis has taken into the clinic as an inhaled

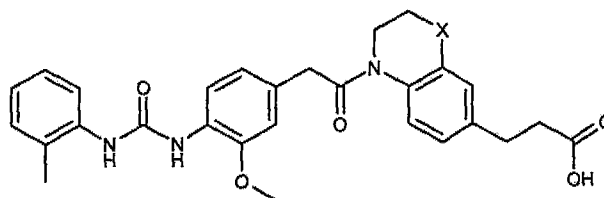
antiasthmatic agent. IVL-745 is a potent inhibitor of Ramos cell-fibronectin and -VCAM-1 binding with little effect on $\alpha_5\beta_1$ or $\alpha_4\beta_7$ -mediated adhesion [100]. VCAM-1 binding to human lymphocytes and eosinophils [101,102] and the proliferation of human T cells induced by culture in the presence of immobilised anti-CD3 and rh-VCAM-1 are inhibited in a concentration-dependent manner [103]. When administered 4 – 6 h prechallenge or $\leq$ 4 h postchallenge, inhaled 3 – 10 mg/kg doses of IVL-745 prevented typical lung histopathological changes and eosinophil accumulation in allergic Brown-Norway rats challenged with ovalbumin [104]. A number of related compounds reported in Aventis patents include compounds 15 – 18 [205-208].

The 3-fluoroprolinol (19) described in a Daiichi patent was active in VCAM-VLA-4 binding assays and demonstrated bioavailability of 16 and 59% in the mouse and the rat, respectively, after doses of 10 mg/kg, p.o. At a dose of 50 mg/kg, p.o., t.i.d for 2 days compound 19 inhibited eosinophil accumulation in bronchoalveolar lavage fluid of mice sensitive to ascaris suum by 46%. At a dose of 5.0 mg/kg, b.i.d., it also limited pulmonary eosinophilia in rats induced over 24 h by treatment with compound 48/80 [209]. AstraZeneca has also been active in this area; one representative compound is the diether 20 [210].

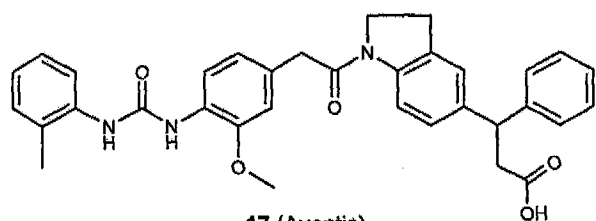
Groups from Pfizer, Novartis and Bayer have each published selective examples of their efforts in the area. As part of an effort to reduce the peptidic character, chemists from Novartis focused on substitution of the glycine nitrogen, leading to derivatives such as compound 21, which are essentially equipotent to the reference Bio 1211 and highly selective for VLA-4 over other integrins, including $\alpha_4\beta_7$ [105]. Compound 22 was one of the most interesting of a series of oxazole, isoxazole and thiazole derivatives prepared at Pfizer. It was ~ 7-fold more potent than 12 in a Jurkat cell based binding assay and was effective after s.c. or intra-tracheal administration



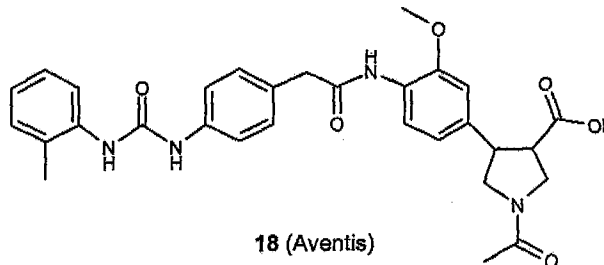
14 IVL-745 (Aventis)

15 X = CH₂ (Aventis)

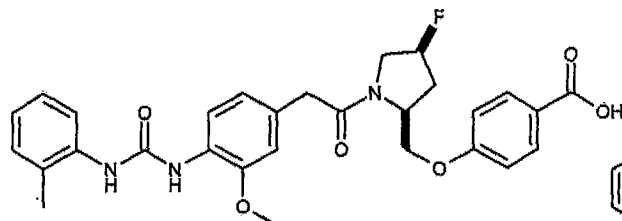
16 X = NH (Aventis)



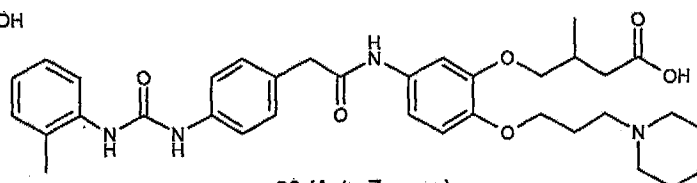
17 (Aventis)



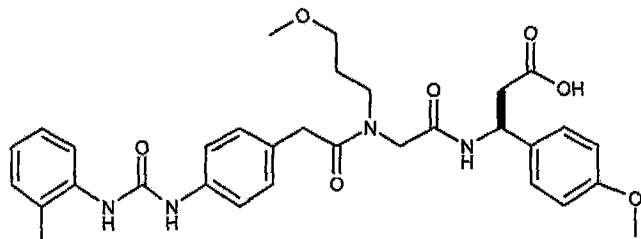
18 (Aventis)



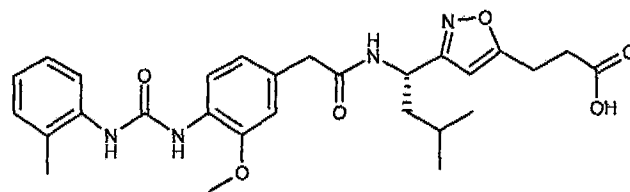
19 (Dalichi)



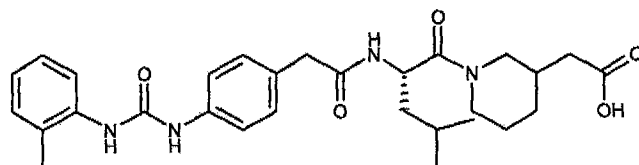
20 (AstraZeneca)



21 (Novartis)



22 (Pfizer)

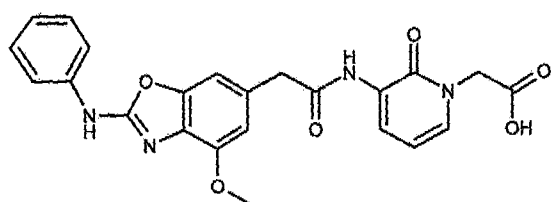


23 (Bayer)

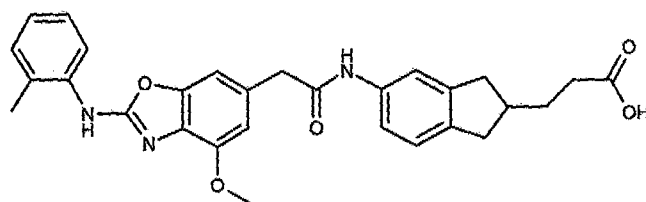
in a mouse allergen challenge model at doses at which Bio 1211 was inactive [106]. The piperidine acetic acid derivative 23 was selected from a series of analogues at Bayer for further evaluation based on its *in vitro* profile. Although it has a reasonable half-life, there were no detectable plasma levels after oral administration [107]. In a variation on the diphenylurea theme, the benzoxazoles 24 [211] and 25 [212] have been reported in the patent literature, but with insufficient biological data to assess their relevance.

Texas Biotechnology has reported a series of ureas, including compounds 26 – 29, which are very potent antagonists

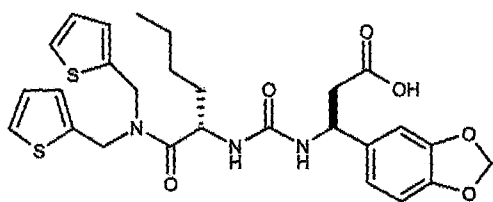
(IC₅₀ = 0.4 – 1.5 nM) of VLA-4 binding to the CS-1 peptide and > 1000-fold selective for VLA-4 over $\alpha_4\beta_7$ [108–110]. TBC3486 (26) appears to be the lead compound; 3 mg/kg intranasally and 3 and 10 mg/kg, i.p. were effective in a mouse lung inflammation model and compound 26 is also active in a mouse adjuvant arthritis model. The related analogue, compound 27 has an IC₅₀ value of 7 nM in the VLA-4/CS-1 binding assay [213]. Texas Biotechnology has licensed their VLA-4 programme to Schering-Plough and is collaborating with them on selecting candidates for development for the treatment of asthma.



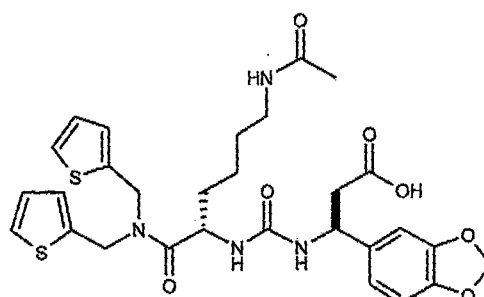
24 (AstraZeneca)



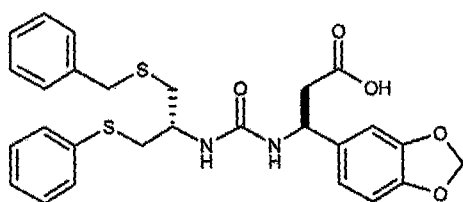
25 (Aventis)



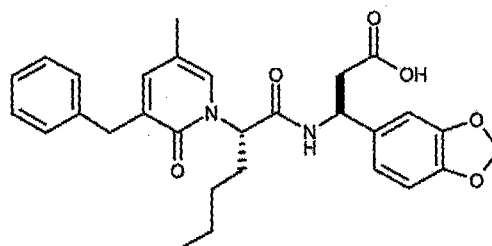
26 (Texas Biotech) TBC3486



27 (Texas Biotech)



28 (Texas Biotech) TBC 3342



29 (Texas Biotech)

3.3.3 Acylphenylalanines

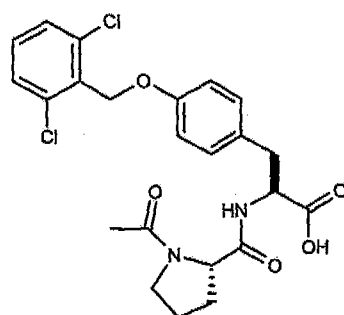
Acylphenylalanines constitute a second major class of VLA-4 antagonists. The acylphenylalanine core seems to be critical for activity but tolerates a remarkably broad array of substituents in the 4-position and on the phenylalanine nitrogen atom. Also, unlike the LDV mimics, which are generally highly selective for VLA-4, many members of the acylphenylalanine class are dual inhibitors of the VLA-4/VCAM and $\alpha_4\beta_7$ /MAdCAM interaction, depending on their particular substitution pattern. Since relative activities are determined using different cell lines that express VLA-4 (typically Ramos or Jurkat cells) or $\alpha_4\beta_7$ (RPMI 8866 cells), it is not possible to quantitate them, except by comparison of different compounds in the same assays. Several companies, including Roche [111] and Merck [112], have used combinatorial or high throughput chemistry effectively to elaborate early hit structures resulting from their screening efforts.

From a series of thiaproline-tyrosine esters and ethers, the 2,6-dichlorobenzyl ether, compound 30 (CT 5219) was among the most potent in a Jurkat cell-VCAM-1 binding assay ($IC_{50} = 35$ nM). It was inactive against other integrins, except $\alpha_4\beta_7$ ($IC_{50} = 190$ nM), and was highly stable towards plasma esterases. Given to sheep at doses of 0.03 – 3 mg/kg, i.v., CT 5219 inhibited both the early and late phase increases in airways resistance caused by administration of antigen in a dose-dependent manner, and also inhibited the increase in

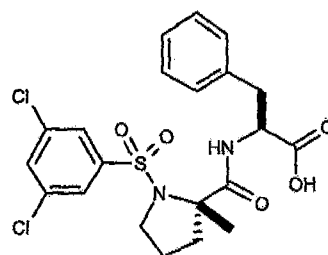
airways hyper-reactivity at the higher doses [113]. It has poor oral bioavailability in rats and sheep and is subject to rapid elimination, particularly in rats [114].

In an attempt to understand the factors driving the pharmacokinetics of members of the acylphenylalanine class, Kopka *et al.* [115] at Merck studied the pharmacokinetics of the simple derivative 31. Oxidation of the proline ring and hydroxylation of the aromatic ring were observed during *in vitro* metabolism experiments with rat liver microsomes and hepatocytes; however, the compound is excreted in the bile largely unchanged *in vivo*. The only major identifiable metabolite was the acyl glucuronide. Only those diastereomers derived from (S)-phenylalanine were active in a Jurkat cell binding assay. Side by side comparison of all four possible stereoisomers of compound 31 in the rat indicated that all four have moderate to good oral bioavailability (22 – 91%) and high clearance (30 – 84 ml/min/kg) as shown in Table 1. It is particularly interesting to note that clearance is not more closely linked to stereochemistry at either chiral centre. The results of further pharmacokinetic investigation of compound 31 in rat, dog, sheep and monkey are summarised in Table 2. These data suggest that oral bioavailability is achievable in this class but that clearance could be problematic [115].

Elan Pharmaceuticals and Wyeth Research, formerly American Home Products, have been collaborating on VLA-4/VCAM antagonists for some time. The piperidine (32,



30 (Celltech R&D) CT 5219



31 (Merck)

Table 1. Pharmacokinetic evaluation of all four diastereomers of 31 in rat*.

Isomer	VCAM-Ig/Jurkat IC ₅₀ nM	F(%) [‡]	C _L (ml/kg/min)	Vdss (l/kg)	t _{1/2} (h)
S,S (31)	1.0	59	30	1.25	1.0
R,S	2,055	26	60	3.5	0.7
R,R	28,450	91	62	5.6	2.6
S,R	152	22	84	2.0	0.8

*Reproduced with permission from [115].

[‡]doses: 1 mg/kg, i.v.; 2 mg/kg p.o.

Table 2. Pharmacokinetic evaluation of compound 31 in various species*.

Species	F(%) [‡]	C _L (ml/kg/min)	Vdss (l/kg)	t _{1/2} (h)
S-D Rat	59	30	1.25	1.0
Beagle dog	39	15.4	2.6	6.7
Rhesus monkey	23	16.3	2.7	7.9
Sheep	42	20	4.2	6.9

*Reproduced with permission from [115].

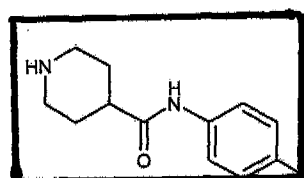
[‡]doses: 1 mg/kg, i.v.; 2 mg/kg p.o.

CT767) is an important lead compound. Introduction of the piperidine ring lowers protein binding as demonstrated by carrying out the Jurkat cell/HUVEC assay in the presence and absence of serum [116,117]. The collaboration has produced many patents covering members of the acylphenylalanine class, although their broad scope and absence of assay data makes their significance difficult to judge.

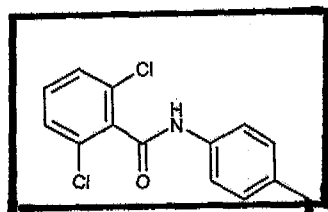
Many investigators have identified the 2,6-dichlorobenzoylamino moiety as a highly favourable substituent for the 4-position of various acyl phenylalanines. Among them are the camphoric acid derivative TR-9109 (33) from Tanabe Seiyaku [118], N-(1-substitutedcycloalkylcarbonyl)-derivatives 34 and 35 [214,119], the N-benzylpyro-L-glutamyl derivative 36 [120], N-(2,6-disubstitutedbenzoyl)- 37 [121] and the substituted ureido (38) [215] groups, all of which give low nanomolar inhibition of the Ramos cell-VCAM interaction. Crystal structures of analogues related to compounds 35 and 37 reveal compact gauche(-) conformations which are consistent with a bioactive conformational model of such compounds based on

the NMR structure of the cyclic peptide 6. Other 4-substituents which are associated with high potency in the same assay include the imide (39) [122] and the imidazolidinone (40). [216]. Hoffman-La Roche has demonstrated that the phenylalanine NH is not involved in receptor recognition with the publication of the activity of the benzolactam 41 [123]. Squaric acid analogues have been explored in place of the aroyl- or alkanoyl-moieties by a number of organisations, an example is the amide 42 [124].

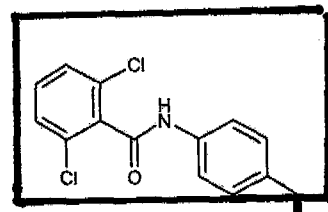
Several organisations have described carbamates derived from N-acyltyrosines. Typical among these compounds is the phenoxy-acetyl-leucyl analogue 43 [217] from GlaxoSmithKline. A series of N-prolyl derivatives, including compound 44 [125] from Merck and the squaric acid analogue 45 [218] from Wyeth Research, represent further examples of structure types associated with potent activity in cell-based assays. Compound 43 was reported to reduce both eosinophil infiltration and airway hyper-reactivity in a guinea-pig asthma model when administered intratracheally at 0.2 mg/kg 0.5 h before



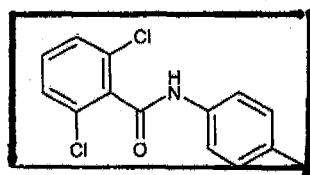
32 (Wyeth/Elan) CT767



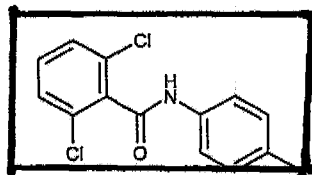
33 (Tanabe Seiyaku) TA9109



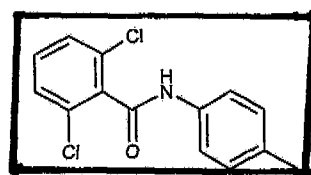
34 (Tanabe Seiyaku)



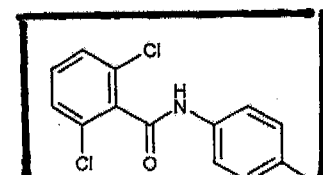
35 (Hoffmann-La Roche)



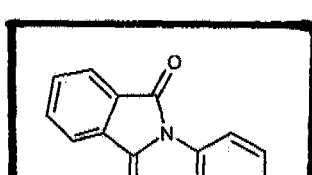
36 (Hoffmann-La Roche)



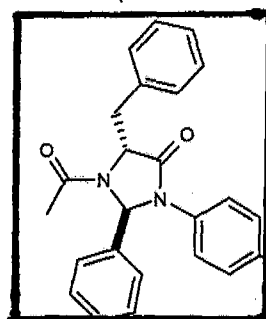
37 (Hoffmann-La Roche)



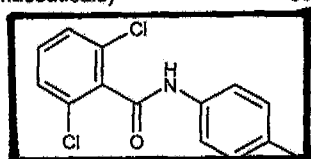
38 (Kaken Pharmaceuticals)



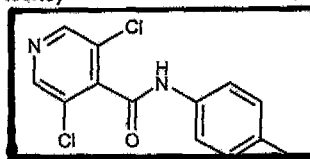
39 (Hoffmann-La Roche)



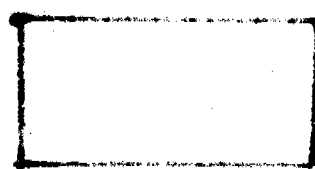
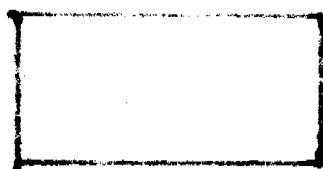
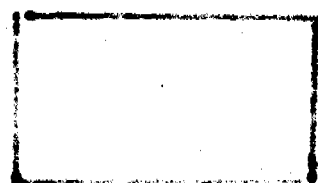
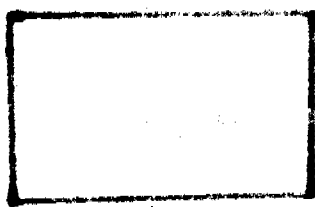
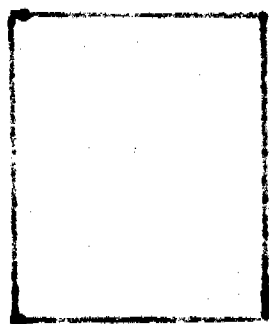
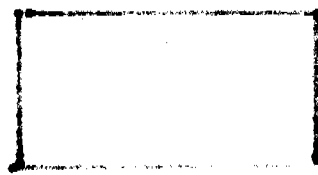
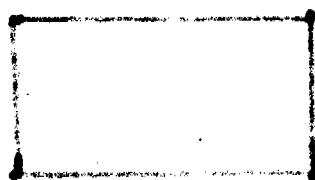
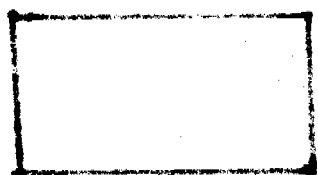
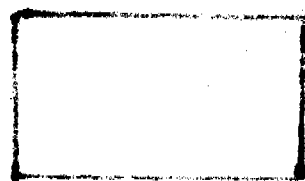
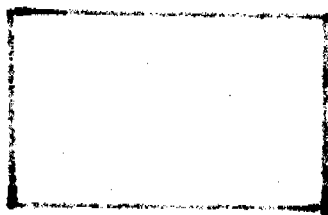
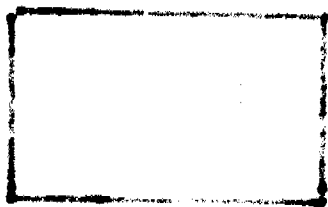
40 (Hoffmann-La Roche)

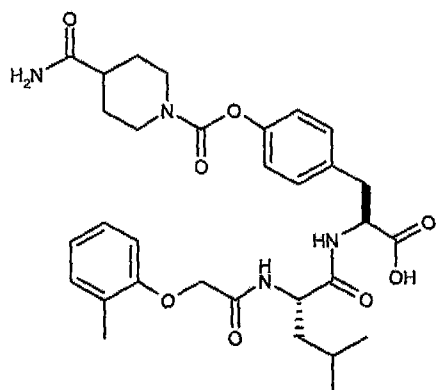


41 (Hoffmann-La Roche)

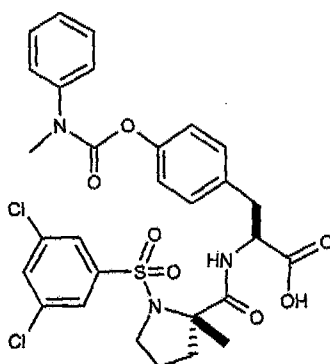


42 (Celltech R&D)

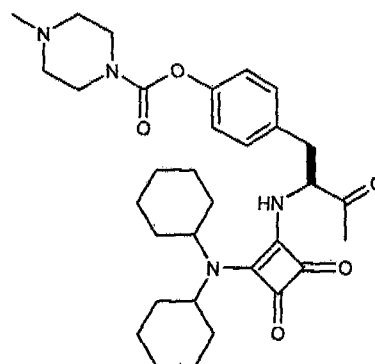




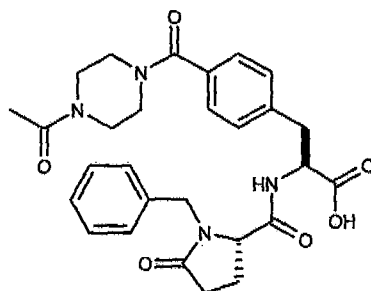
43 (GlaxoSmithKline)



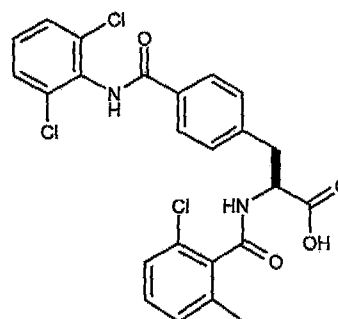
44 (Merck)



45 (Wyeth/Elan)



46 (Roche)



47 (Novartis)

and 6 h after ovalbumin challenge. GlaxoSmithKline has an inhaled VLA-4 antagonist in clinical trial (GW559090), which may be compound 43 or a close analogue. Both Roche and Novartis have disclosed 4-(substituted aminocarbonyl)-*N*-acylphenylalanines and their activities as VLA-4 antagonists in cell based assays. Representative compounds include 46 [126] and 47 [218]. In the case of Roche, it appears that the reversed amides prepared from 4-aminophenylalanines, such as compounds 32 – 38 are a more interesting class based on their relative potencies.

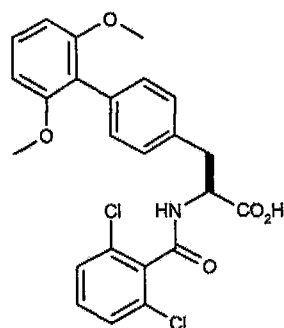
TR14035 (48) [220] represents the first example of a new variation on the acylphenylalanines in which the 2,6-dichlorobenzamide typical of many of the more potent analogues is replaced by a phenyl ring with a hydrogen bond acceptor in the ortho position. The increased lipophilicity thus gained translates into improved oral bioavailability. In rats, $F = 60\%$, $t_{1/2} = 5$ h and in dogs, $F = 25\%$, $t_{1/2} = 2.5$ h [127]. Like other acylphenylalanines, TR14035 is cleared rapidly, largely through biliary excretion. IC_{50} values in Jurkat cell-CS-1 and RPMI cell-CS-1 assays for VLA-4 and $\alpha_4\beta_7$ inhibitory activities were 56 and 6 nM, respectively, suggesting that TR14035 is somewhat selective for $\alpha_4\beta_7$ over VLA-4. Consistent with this finding, it also potently inhibits RPMI cell/VCAM and MAdCAM interactions.

In addition, TR14035 inhibited the DNFB delayed type hypersensitivity in the mouse at doses of 10 – 100 mg/kg, p.o. and MLN cell migration to the DSS induced inflamed colon of mice at doses of 10 – 25 mg/kg, p.o. A dose of 30 mg/kg, po, b.i.d. over 29 days caused a significant decrease in paw

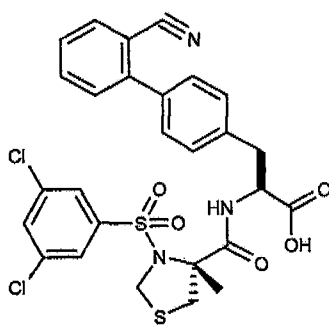
swelling relative to controls in a rat peptidoglycan/polysaccharide (PGPS) model of polyarthritis. Tanabe Seiyaku has licensed TR14035 to GlaxoSmithKline worldwide, except for parts of Asia, and it has been renumbered by the GlaxoSmithKline organisation SB-683698. This compound has completed Phase I human trials with Phase II expected to start in 2002 for asthma, inflammatory bowel disease, rheumatoid arthritis and MS [304].

Competing companies particularly Merck, have been actively pursuing biphenyl derivatives discovered from a combinatorial library follow-up to initial screening hits. One publication from Merck describes phenylsulfonylprolyl- and azacyclobutylbiphenylalanines, such as compounds 49 and 50. These compounds have IC_{50} values very close to those of Bio 1211 and TR14035 used as standards in a Jurkat cell-VCAM-Ig conjugate binding assay, and are at least 100-fold selective over $\alpha_4\beta_7$ /RPMI8866 cell binding. They have moderate to poor oral bioavailability and generally rapid clearance approaching hepatic blood flow in rats. Compound 49 stood out in this series as having a 33% oral bioavailability in rats and low clearance (5 ml/kg/min). Oral bioavailability in dog and monkey were low, 4 and 17%, respectively, and clearance was moderate, 25 and 12 ml/min/kg, respectively. The favourable clearance in the rat is the likely consequence of its high protein binding, which is $> 99.5\%$ in both rat and human plasma [128].

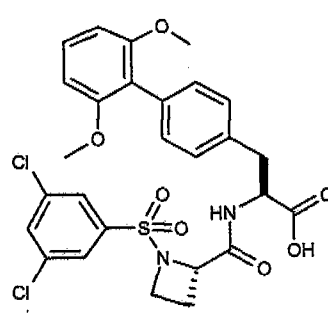
Further work from the Merck laboratories on the related thiaproline series characterised by compounds 51 and 52, indicated that inhibition of $\alpha_4\beta_1$ is relatively independent of



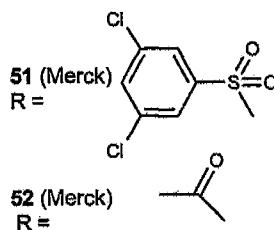
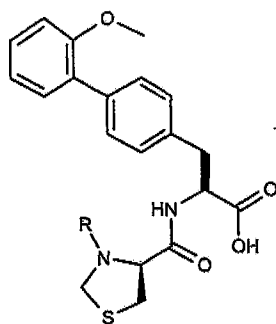
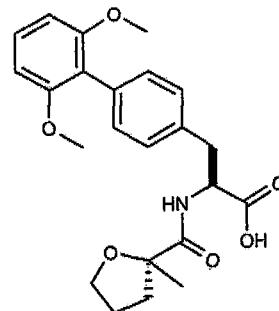
48 (Tanabe Seiyaku/GSK) TR14035



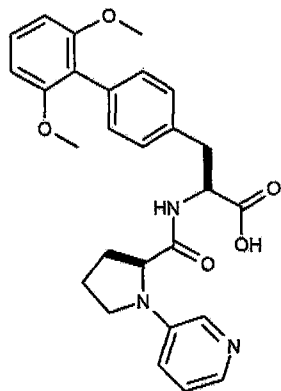
49 (Merck)



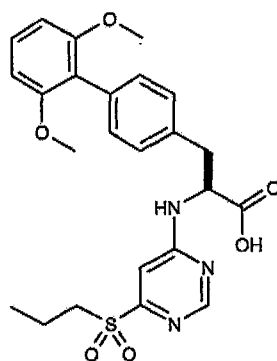
50 (Merck)

51 (Merck)
R =52 (Merck)
R =

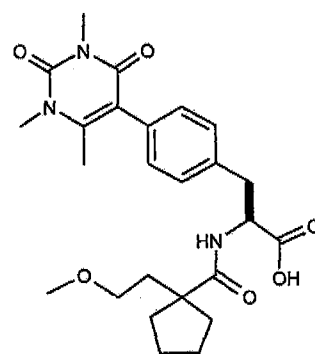
53 (Merck)



54 (Merck)



55 (Merck)



56 (Hoffman-La Roche)

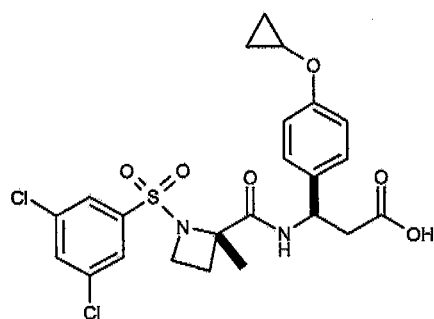
thiaproline stereochemistry and the size of the thiaproline acyl group. On the other hand, activity in the $\alpha_4\beta_7$ assay is sensitive to both stereochemistry and the size of the N-acyl group, suggesting the ratio of the two activities can be modulated by design. The N-acetyl-S-thiaproline derivative 52 was the most potent of the dual-acting inhibitors [129].

Merck chemists have also explored substitutions for the proline and thiaproline rings in this series. The tetrahydrofuran 53 combines good potency in the $\alpha_4\beta_1$ cell based binding assay, moderate selectivity over $\alpha_4\beta_7$ binding (24-fold) and moderately good pharmacokinetics in Sprague-Dawley rats ($F = 42\%$, $C_L = 58$ ml/min/kg, $t_{1/2} = 3.4$ h) [130]. Further work indicates that a wide variety of substituents are tolerated in the 1-position of the tetrahydrofuryl moiety [131] and that the oxygen atom of the tetrahydrofuran can be replaced with carbon [132]. Additional N-substitutions described by Merck

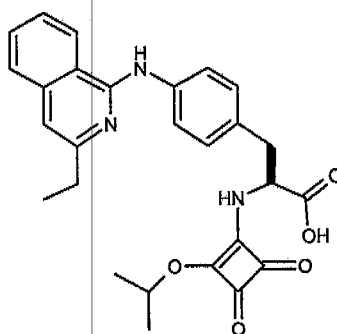
include the N-arylprolyl derivative 54 [133] and the N-pyrimidinyl analogue 55 [134]. Hoffman-La Roche has explored heterocyclic analogues of the biphenylalanines; compound 56 is an example of this class [221,222].

3.3.4 Miscellaneous

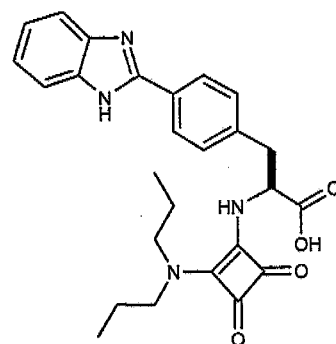
There are a number of individual reports of other chemical classes reported to inhibit VLA-4 VCAM-1 binding that should be monitored. Many are either very weak inhibitors relative to the LDV and acylphenylalanine derivatives noted in the previous sections, or they are described in the patent literature with no associated biological data. One exception is documented in a recent publication from Merck describing a series of orally bioavailable β -phenylalanines with good potency. An example is compound 57 ($F = 52\%$, $C_L = 26$ ml/min/kg, $IC_{50} = < 1$ nM in a Jurkat cell/VCAM binding assay) [135]. Typical



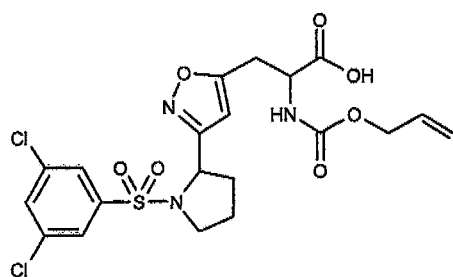
57 (Merck)



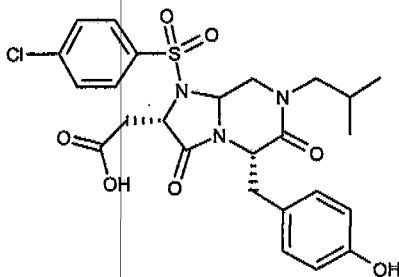
58 (Celltech R&D)



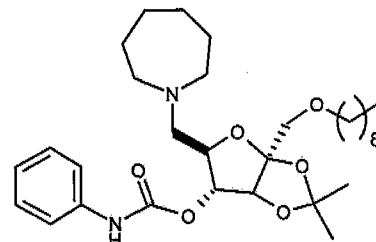
59 (Celltech R&D)



60 (Pfizer)



61 (Moleculometrics)



62 (Ranbaxy)

novel squaric acid-containing structures appearing in recent patents from Celltech R&D are compounds **58** [223,224] and **59** [225]. The alloc amino acid derivative **60**, reported in a recent Pfizer patent application, represents an interesting structural variation, although there is no comparative data with other, better characterised compound classes as yet [226]. The bicyclic peptide mimetic **61** and related compounds have been described as $\alpha_4\beta_1$ antagonists in patents from Moleculometrics [227,228]. Finally, a patent from Ranbaxy describes sugars such as compound **62**, which is reported to be active in a Jurkat cell/human VCAM binding assay ($IC_{50} = 7.5$ nM) and is also highly effective in the mouse DNFB delayed hypersensitivity assay when administered i.v., s.c. or p.o. [229]. It remains to be determined whether compounds of this class work largely or solely through effects on VLA-4 or whether other mechanisms are involved.

4. Expert opinion

There is a great deal of anticipation concerning the potential role of VLA-4/VCAM-1 inhibitors with and without concomitant inhibition of $\alpha_4\beta_7$ mediated interactions in the treatment of human inflammatory diseases. Early interest in this field focused on linear and cyclic peptides and some were considered for development. Currently, monoclonal antibodies, such as natalizumab, LDV mimics and acylphenylalanines are receiving the bulk of the attention. Since 1998, a large number of patents have been published

on small molecule inhibitors and these are now being followed up by publications that help to clarify the areas of true interest. Most of these publications are directed to the discovery of active molecules; there is little data on their efficacy in animal models of inflammatory disease. One area where a deeper understanding needs to be developed is the desired selectivity between $\alpha_4\beta_1$ and $\alpha_4\beta_7$ inhibition for safety and efficacy in different inflammatory diseases. In addition, the finding that the off-rate of certain compounds is dependent on the activation status of the target cells suggests that there may be an important difference between the pharmacodynamic and the pharmacokinetic effects of these compounds.

Clinical trials are presently underway, sponsored by Biogen/Elan (monoclonal antibody), Merck (a LDV mimic and possibly an acylphenylalanine), Aventis (at least two LDV mimics), GlaxoSmithKline (two acylphenylalanines) and Roche (acylphenylalanine). Other companies who may have compounds in or near clinical trials include Wyeth in collaboration with Elan and Texas Biotechnology in collaboration with Schering-Plough. Early reports, largely from Biogen/Elan suggest that patients tolerate chronic blockade of α_4 integrins and show signs of clinical efficacy in Crohns disease and MS. Natalizumab has a long half-life in humans, enabling once monthly dosing, whereas clearance and exposure after oral dosing are critical concerns judging from published work on the small molecule inhibitors described to date.

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VLA-4 Antagonists: Potent Inhibitors of Lymphocyte Migration

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DOI 10.1002/med.10044



Abstract: Circulating lymphocytes normally migrate through extravascular spaces in relatively low numbers as important members of the immunosurveillance process. That is until signals are received by endothelial cells that there is an underlying infection or inflammatory condition. These vascular surface cells in turn overexpress and present ligands to circulating lymphocyte adhesion molecules. Upon encountering this higher density of ligands, lymphocytes, which had been leisurely rolling along the vascular surface, now become more firmly attached, change shape, and migrate through tight junctions to the sites of infection or inflammation. If the initiating events are not resolved and the condition becomes chronic, there can be a sustained extravasation of lymphocytes that can exacerbate the inflammatory condition, which in turn will continue to recruit more inflammatory cells resulting in unwanted tissue destruction. It is for the attenuation of this cycle of sustained inflammatory cell recruitment that very late activating antigen-4 (VLA-4) antagonists are being developed. Most lymphocytes, except neutrophils, express VLA-4 on their surface and they interact with endothelial vascular cell adhesion molecule-1 (VCAM-1). It is this interaction that VLA-4 antagonists are intended to disrupt, thus, putting an end to the cycle of chronic inflammation, which is the hallmark of many diseases. This review will provide an update of VLA-4 antagonists that have appeared since early 2001 and will discuss some of the issues, both positive and negative, that may be encountered in their development. © 2003 Wiley Periodicals, Inc. *Med Res Rev*, 23 No. 3, 369–392, 2003

Key words: VLA-4; $\alpha_4\beta_1$; very late antigen 4; CD49D/CD29; VCAM-1

1. INTRODUCTION

The integrin VLA-4 ($\alpha_4\beta_1$, very late activating antigen-4) is a non-covalent heterodimer of the α_4 (155 kDa) and β_1 (150 kDa) subunits. VLA-4 is constitutively expressed on most mononuclear lymphocytes, including eosinophiles, monocytes, and basophils but is thought to be absent on circulating neutrophils. A counterligand for VLA-4 is vascular cell adhesion molecule-1 (VCAM-1),

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Medicinal Research Reviews, Vol. 23, No. 3, 369–392, 2003
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which is a member of the immunoglobulin superfamily, and is found on endothelial cells. Its expression is upregulated in response to pro-inflammatory cytokines and chemokines. A recent discussion of the biological aspects of the VLA-4/VCAM interaction is recommended.¹ It includes a comprehensive description of various immune defense mechanisms and a detailed discussion of the mechanisms of cell adhesion, cellular immune response, and the role of VLA-4 in this complex machinery. The following discussion on VLA-4 biology includes some of the basic concepts and terminology useful to medicinal chemists and is intended to complement recent reviews.¹⁻⁵ The major focus of this review is to provide an update of the many recent disclosures of novel VLA-4 inhibitors.

A. Integrins

Integrins are α , β heterodimeric, transmembrane receptor molecules consisting of α - and β -subunits found in certain α and β combination on every mammalian cell type except red blood cells. There are fifteen α -subunits and eight β -subunits that are non-covalently linked and expressed on the surfaces of cells in various combinations. The integrin name was derived from their role in "integrating" the extracellular matrix with the cytoskeleton. The integrin superfamily is divided into three families: the "very late activation" (VLA) family (β_1), the leucam family (β_2), and the cytoadhesin family (β_3). Beta subunits 4-8 are not yet classified into families.¹ The β_1 integrins were also known as VLA due to their delayed appearance on T-cells after activation.

In a highly simplified scenario, binding interactions between lymphocytes flowing in the blood stream and the endothelial surface are mediated by a variety of adhesion molecules, including the selectins and VCAM-1.⁶ These binding interactions serve to slow the flow of lymphocytes in a process described as rolling and tethering. At endothelial surfaces adjacent to inflammatory sites, the greater density of VCAM-1 expression appears to lead to firm attachment and arrest of rolling cells. This is followed by rapid cytoskeletal rearrangement of the bound lymphocyte, diapedesis through endothelial tight junctions, and migration to the inflammatory sites. VLA-4 also binds tightly to an alternatively spliced segment of fibronectin, termed CS-1. This interaction seems to provide firm adhesion within the extracellular matrix. This interaction with fibronectin has been linked in multiple functional processes such as progenitor lymphocyte differentiation, T-cell proliferation, and triggering of intracellular signals such as tyrosine phosphorylation of proteins in T- and B-cell.⁷

Another closely related α_4 integrin, the $\alpha_4\beta_7$ heterodimer, also interacts with the same epitopes as VLA-4 in VCAM-1, although the interaction is weaker.⁸ The primary counterligand for $\alpha_4\beta_7$ is the mucosal addressin cell adhesion molecule-1 (MadCAM) located in the gut. Both α_4 integrins ($\alpha_4\beta_1$ and $\alpha_4\beta_7$) interact with the spliced CS-1 region in plasma fibronectin.⁸ The desire to develop specific VLA-4 antagonists with appropriate pharmaceutical properties has been challenging. This review will concentrate primarily on selective inhibitors of the VCAM-1/VLA-4 interaction and their therapeutic potential in treating a variety of inflammatory diseases.

B. VLA-4/VCAM-1 Adhesion

As a part of immune surveillance process, lymphocytes are recruited to specific sites of infection and/or inflammation. Normally lymphocytes circulate in the blood stream with only low numbers extravasating through the endothelial wall to extravascular sites. At the sites of inflammation, the local release of inflammatory mediators such as cytokines (tumor necrosis factor alpha (TNF α) and interleukin-1 (IL-1)) and chemokines from activated T-cells and resident monocytes stimulate adjacent endothelial cells to overexpress cell adhesion molecules on their vascular surface. The elevated and prolonged expression of VCAM-1 had been observed *in vitro* as well as in the vasculature *in vivo*, both in experimental models and human diseases such as allograft rejection, atherosclerosis, allergic rhinitis, asthma, and cutaneous delayed hypersensitivity reaction.⁹ Rolling

lymphocytes begin to adhere more strongly *via* the selectins and their carbohydrate ligands and are arrested by stronger binding interactions with integrins. Interactions with integrins, apical chemokines, and shear forces applied from the ever flowing blood contribute to events that lead to integrin clustering, cytoskeletal rearrangement, and eventual migration into target tissues *via* tight intracellular junctions.^{10,11}

The mechanism of these dynamic events has been emerging over many years; the details of which are still not completely elucidated. An adhesion process has been proposed that lymphocytes extravasate by a sequence of tethering, rolling, arresting, and migration. Circulating lymphocytes normally conduct surveillance by a loosely "tether and roll" mechanism on the endothelium surface. Upon encountering stimulatory signals, a rapid conversion of the rolling behavior to firm "adhesion" seems to take place. A recent study demonstrated that surface-bound chemokines can signal and modulate the activity of lymphocyte integrins during initial contact with the vascular ligand.¹² In fact the activation of the integrin by the chemokines at the leukocyte-endothelium contact zone takes place within milliseconds. Chemokine signaling triggers the lymphocyte to cluster VLA-4 along the contact surface, which rapidly increases the effective concentration of VLA-4/VCAM-1 interaction (clustering). This study found that it is the type and expression level of the G protein-coupled receptors (GPCRs) that are activated, rather than the cell type upon which VLA-4 is expressed or the activation state of the VLA-4 that dictates the ability of the GPCR to enhance VLA-4 tether. On the other hand, it had been demonstrated earlier that high affinity VLA-4 subsets are required for the firm adhesion. A mutant VLA-4 with the reduced affinity supported rolling and tethering but failed in adhesion.¹³ Another recent study established that in T-lymphocytes or resting T-cell leukemia Jurkat cells p56^{lck} is responsible for upregulation of integrin affinity.¹⁴ The affinity regulation under physiological conditions was described in another report.¹⁵ The binding affinities and association and dissociation kinetics in real time were measured to examine affinity regulation in response to divalent cations and physiological mediators in two cell lines and blood leukocytes. The results support the idea that α_4 -integrin exhibit multiple affinity states and affinity changes are regulated by the dissociation rate and conformation. The changes in affinity occur within seconds and are reversible in response to chemokines and chemoattractants. The affinity change on different cell lines appears to be similar, with comparable affinities and off rates in response to different stimuli. However, the duration of the affinity change vary depending on the nature of the stimuli.

C. VLA-4 Activation States

Like most integrins, VLA-4 exists in "low" and "high" affinity states. It is believed that the initial contact of VLA-4 with VCAM-1 is through low affinity, multi-valent binding interactions.¹⁶⁻¹⁸ As mentioned above, activation of the lymphocyte is thought to affect a conformational change in VLA-4 to a stronger, higher affinity state. Activated states of VLA-4 may also be induced by various stimuli including antigens, anti-T-cell antibodies, phorbol esters, the divalent cation manganese (Mn^{2+}), and selected $\beta 1$ -specific antibodies.¹⁹⁻²¹

The terms "low" and "high" affinity states are relative terms and should not be construed to define two separate and distinct states of activation. Recent studies with a fluorescent labeled BIO1211 analog suggest that the physiologically relevant state of activation lies somewhere between a "low" or resting state and the Mn^{2+} or antibody activated state.²² The addition of Mn^{2+} to TS2/16, an activating antibody, produces an activated state that is higher than either Mn^{2+} or TS2/16 alone. Thus, there appears to be a continuum of activation states between resting or unactivated integrin and a series of "high" affinity states.

When comparing the potency of VLA-4 inhibitors from one laboratory to another, assay conditions must be taken into consideration. A compound that appears potent under Mn^{2+} activation may be much less potent versus the resting or Ca^{2+}/Mg^{2+} conditions. Even within a single

laboratory, the observed Kd values for BIO1211 ranged from 20 to 40 nM in the resting or Ca²⁺/Mg²⁺ state of the integrin to 100 pM in the activated state when binding was measured after activation by Mn²⁺ plus the integrin-activating monoclonal antibody TS2/16.²² Also, it is difficult to compare potencies from binding assays that employ a mono- or bivalent ligand with cell adhesion assays that may employ varying and undetermined densities of ligands (CS-1 or VCAM-1) on a plate. These ligand densities may in turn affect activation states. In terms of physiological relevance, given the role that shear forces may play in the activation of VLA-4 bearing cells, it is difficult to imagine that the static binding or cell adhesion assay conditions that are generally employed to assess inhibitor potencies will faithfully predict absolute potencies expected *in vivo* (serum protein binding and clearance mechanisms notwithstanding). Within a given laboratory, any assay will only give relative potencies among a series of inhibitors. Across laboratories, it would be useful if a "standard" inhibitor, such as BIO1211, were used as a positive control to make more useful comparisons among different inhibitor classes.

D. Therapeutic Potential for VLA-4 Antagonists

Although steroids and other anti-inflammatory drugs with broad-spectrum activities are effective in treating numerous inflammatory diseases, long-term usage is known to have unacceptable side effects, such as greater risk of infection caused by impairment of phagocytic leukocyte migration and function. Therefore, it is desirable to develop drugs that more selectively inhibit specific cellular functions without affecting normal immune surveillance in the treatment of chronic inflammatory disorders.

As discussed above, leukocyte recruitment into the inflamed tissue is an essential physiologic process. However, this beneficial response can also lead to a chronic and detrimental inflammatory process. Since it appears that leukocyte infiltration is a key factor in the pathogenic process of many allergic and inflammatory disorders, finding inhibitors that target VLA-4 has been the focus of many pharmaceutical companies. The association of VLA-4/VCAM-1 in numerous animal models of human diseases is well documented. The positive Phase II clinical results thus far reported for the anti- α_4 antibody, natalizumab (Antegren[®]), in multiple sclerosis and Crohn's disease confirm a role for VLA-4 (and/or $\alpha_4\beta_7$) in these diseases. Other potential human diseases for a VLA-4 antagonist for which there is supporting evidence in associated animal models are listed in Table I along with selected references.

Table I. Potential Therapeutic Targets and Associated Animal Models for VLA-4 Antagonists

Potential disease target	Animal model	Selected references
Multiple sclerosis	Experimental allergic encephalomyelitis in mice and rats	23–26
Asthma	Bronchial hyperresponsiveness in mice, rats, sheep, and guinea pigs	27–33
Inflammatory arthritis	Adjuvant-induced arthritis in rats	34–36
Type-1 diabetes	Adoptive autoimmune diabetes in the NOD mouse	37–39
Organ transplantation rejection	Cardiac allograft survival in mice	40, 41
Inflammatory bowel disease	Spontaneous chronic colitis in cotton-top tamarins	42
Contact dermatitis	Allergic contact hypersensitivity in mice	43, 44
Nephritis	Acute nephrotoxic nephritis in mice	45
Tumor metastasis	Melanocyte tumor progression in mice	46
Thyroiditis	Experimental autoimmune thyroiditis in mice	47
Restenosis	Ischemic tissue damage following arterial occlusion in rats	48
Allergies	Inhibition of TH2 T-cell cytokine production in mice?	49
Atherosclerosis	LDLR ^{-/-} vs. LDLR ^{+/+} mice fed high fat diet	50–52

2. VLA-4 ANTAGONISTS

A. Cyclic Peptides

Constrained peptide derivatives that contain the essential binding sequences found in VCAM-1 (IDS) and fibronectin (LDV) have afforded a variety of potent VLA-4 antagonists. Previous mutagenesis studies of VCAM-1 implicated Arg36 to be an important contact residue. Surprisingly, deletion of Arg in 1a (Fig. 1) actually improved affinity in 1b.⁵⁰ In addition to Arg, Asp has also been implicated as an essential binding contact residue for $\alpha_4\beta_1$ -mediated cell adhesion to fibronectin and VCAM-1. Again the aspartic acid residue in analog 2a could be replaced by alanine or serine as shown in 2b and 2c

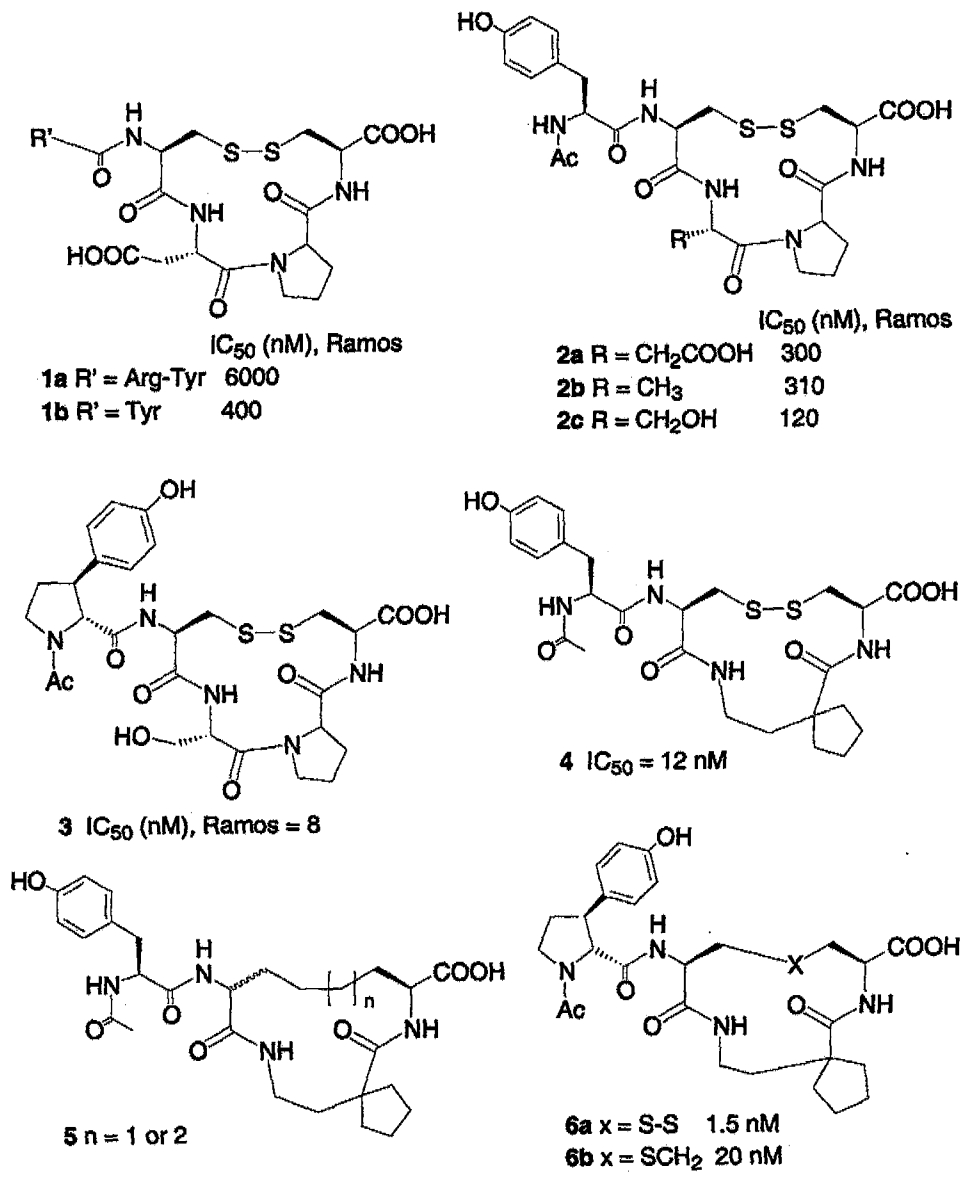


Figure 1. Cyclic peptides.

(Fig. 1). Although **2c** inhibited $\alpha_5\beta_1$ binding to fibronectin, it is over 1,000 times more potent as an inhibitor of the $\alpha_4\beta_1$ /VCAM interaction. More importantly, the *in vivo* activity of **2c** was demonstrated in a mouse model of lymphocyte gut homing to Peyer's patches. The lack of structural similarity between peptides **1** and **2** and the VCAM-1 CD-loop led to the conclusion that these cyclic peptides are not mimics of VCAM-1 and that they bind $\alpha_4\beta_1$ in a different orientation or at a different site relative to the VCAM-1 CD-loop. A constrained tyrosine introduced in **3** further improved affinity.

Although a loss of activity was observed when Asp-Pro were simultaneously replaced in the cyclic peptides,⁵³ the possibility of a replacement of the Asp-Pro moiety was investigated.⁵⁴ An NMR-based conformation model of **2a** was used as a starting point and each potential spacer was computed using the molecular modeling package SybylTM. Based on the modeling results, a series of compounds were then synthesized. As predicted by the modeling result, compound **4** was found to be approximately 17-fold more potent than **2a** while possessing a more simplified structure.

Concerned that the disulfide linker might be a metabolic liability, the corresponding carbacyclic structure **5** was also prepared, which led to a significant loss of activity.⁵⁵ However, the replacement of one of the sulfur atoms in the disulfide **6a** with a carbon atom, together with the incorporation of a constrained tyrosine as in **6b** was found to be well tolerated.⁵⁶

Cyclic peptides using the LDV binding motif present in the 25-amino acid fibronectin peptide CS-1 and the related binding epitope present in domain 1 of VCAM-1 within the region Arg-Thr-Gln-Ile-Asp-Ser-Pro-Leu-Asn have been reported, including the earlier examples of cyclo(Melle-Leu-Asp-Val-dAla-dAla) and Ac-cyclo(dLys-dIle-Leu-Asp-Val).^{57,58} They were reported to be active in an *in vivo* inflammation model at a dose of <0.1 mg/kg/day when administered continuously by a subcutaneous mini-pump. These compounds were found to be inactive after a bolus subcutaneous injection. In addition, their stabilities at pH 3 were less than desirable and they were quickly released from a polymer depot formulation.

Cyclic peptides were identified which were compatible with biodegradable polymer formulations, for example, poly(dl-Lactide-co-glycolide). The design principles were to use cyclo(Ile-Leu-Asp-Val-NH(CH₂)₅CO) and cyclo(Ile-Leu-Asp-Val-D-Ala-D-Ala) as starting points, keep the Leu, Asp, and Val residues intact, and replace the rest of the amino acids and linking-group residues with various hydrophobic and basic amino acid residues. The premise was that hydrophobic and basic groups may also bring additional interactions with the polymer chain and the free carboxyl end groups of the polymer. These additional interactions should in turn reduce the rate of drug release from the polymer. These efforts afforded many more potent inhibitors. Their improved stability was evident in that many new peptides were also found to be efficacious when administered by a bolus subcutaneous injection (0.1, 1.0, and 10 mg/kg) 4 hr before ovalbumin challenge, in the delayed-type hypersensitivity (DTH) model. The optimized compound cyclo(MePhe-Leu-Asp-Val-D-Arg-D-Arg) (ZD7349) was found to be active in two mouse models of inflammatory diseases: type II collagen-induced arthritis (CIA) and experimental autoimmune encephalomyelitis (EAE). ZD7349 significantly reduced the severity of CIA and delayed the onset of EAE. Finally, ZD7349 was tested in a prototype depot formulation and found to be released from the polymer matrix at a steady state over the course of 17 days. ZD7349 had been reported to be in preclinical development.⁵⁹

B. Linear Peptides-LDV Mimetics

The progression of the earlier ILDVP to the LDV analogs and more recently to the phenylureidophenacyl moiety as LDV mimetics was recently reviewed.² Several of these compounds demonstrated efficacy in animal models and were advanced into the clinic. Since the publication of the most recent review, extensive work to find less peptidic analogs has been investigated. In this section, only those publications and patents pertaining to linear LDV mimetics disclosed from 2001 to the present will be discussed.

The preparation and testing of various substituted imidazolidine derivatives as VLA-4 antagonists has been reported.⁶⁰ One of the more potent compounds **7** (Fig. 2) was reported to inhibit the interaction between U-937 cells and human VCAM with an IC_{50} of 0.85 nM. *In vivo* assay procedures such as leukocyte adhesion in rats, DTH in mouse, and anti-asthmatic action to the guinea-pigs were described, but biological results were not disclosed. Some of the potentially labile amide linkages were eliminated while maintaining potency.⁶¹ Urea **8** is among the more potent compounds (IC_{50} = 0.9 nM, Ramos cell assay) reported in this patent application.

These LDV peptidomimetics were divided into three regions: the aromatic cap, a suitable spacer, and a β -carboxylate mimetic of Asp36 as illustrated in **9**. A library consisting approximately 200 compounds was synthesized.⁶² Diamines were chosen as spacers in positioning the aromatic cap and the β -acid. The diphenyl urea cap was found to be essential for potency and that the para linked urea is superior to the corresponding meta analog. Consistent with the discovery of BIO1211, improvements in activity were observed upon incorporation of the *ortho*-methyl substituent and even more strikingly upon introduction of the 3-methoxy moiety in the phenylacetyl portion as in **9**. The central amide appears to be a key pharmacophore in this series or perhaps is imposing a particular conformation, which is essential for VLA-4 activity. Substitution at the α -position to the acid resulted in a tenfold improvement in potency.

Similarly, while keeping the bis-aryurea portion constant, the position of the acid on the phenyl ring and the distance between the acid and phenyl were varied.⁶³ The most potent compound **10** had an IC_{50} of 0.5 μ M in a Jurkat cell assay. While retaining the biaryl-urea, the possibility of finding antagonists comprising a more rigid and less polar acid head group was further explored. A variety of piperidinyl carboxylic acids with the associated conformational constraints were prepared.⁶⁴ One of the most potent compound **11** exhibited an IC_{50} of 0.30 μ M in a Jurkat assay against VLA-4 and is highly selective (not active for integrins $\alpha_9\beta_1$ or $\alpha_v\beta_3$, although, the activity for $\alpha_4\beta_7$ was not discussed). The unbound fraction was found to be 9.6% using intraperitoneal administration in mice. The systemic exposure was low after oral dosing.

An interesting variant to the more common phenyl phenylacetic urea moiety was discovered.⁶⁵ The potency of benzoxazole **12** is 0.5 μ M or less in the Ramos cell assay. It is interesting to note that although these compounds originated as LDV mimetics, the isopropyl side chain is not essential for potency and is generally absent. Related benzoxazole replacements for the bis-aryl urea had been reported earlier.^{66,67}

Diazepine derivatives **13** were claimed as VLA-4 antagonists (VCAM-Ig IC_{50} = 0.35 μ M).⁶⁸ These compounds offer a different constraint than that seen in **10**–**12**. They seem to place the carboxylic acid in a very different space from the piperidine analogs. They are reported to have excellent oral bioavailability.

Concerned with the peptidic nature of many of the earlier LDV mimics, the possibility of generating antagonists that would retain nanomolar potency while eliminating peptidic features was explored.⁶⁹ The most potent examples of this effort are **14a**–**14d**. The selectivity of these compounds against the integrin $\alpha_4\beta_7$ is far better than previously reported peptide analogs (more than 1,300-fold differential). In another patent, 24 analogs of this structure class were claimed without disclosure of any biological data.⁷⁰

In an effort to improve oral bioavailability of the LDV trimer, the aspartic acid moiety was incorporated into a heterocycle and the valine was removed, thus reducing the number of metabolically labile amide bonds.⁷¹ Compounds were screened for VLA-4 antagonist activity in a Jurkat cell ELISA assay and a human eosinophil cell-binding scintillation proximity assay (SPA). Isoxazole, isoxazoline, oxazole, and thiazole were equally potent (**15a**–**15d**). Modification of the length of the propionic acid side chain was found to be detrimental to potency. However, potency was again improved with the introduction of an *ortho*-methoxyphenylurea as in **15e**. Compounds **15a**, **15c**, and **15e** were also evaluated in an allergic mouse model, measuring the effect on bronchoalveolar lavage fluid eosinophil influx after antigen challenge. Compounds **15a**, **15c**, and **15e** were found to be

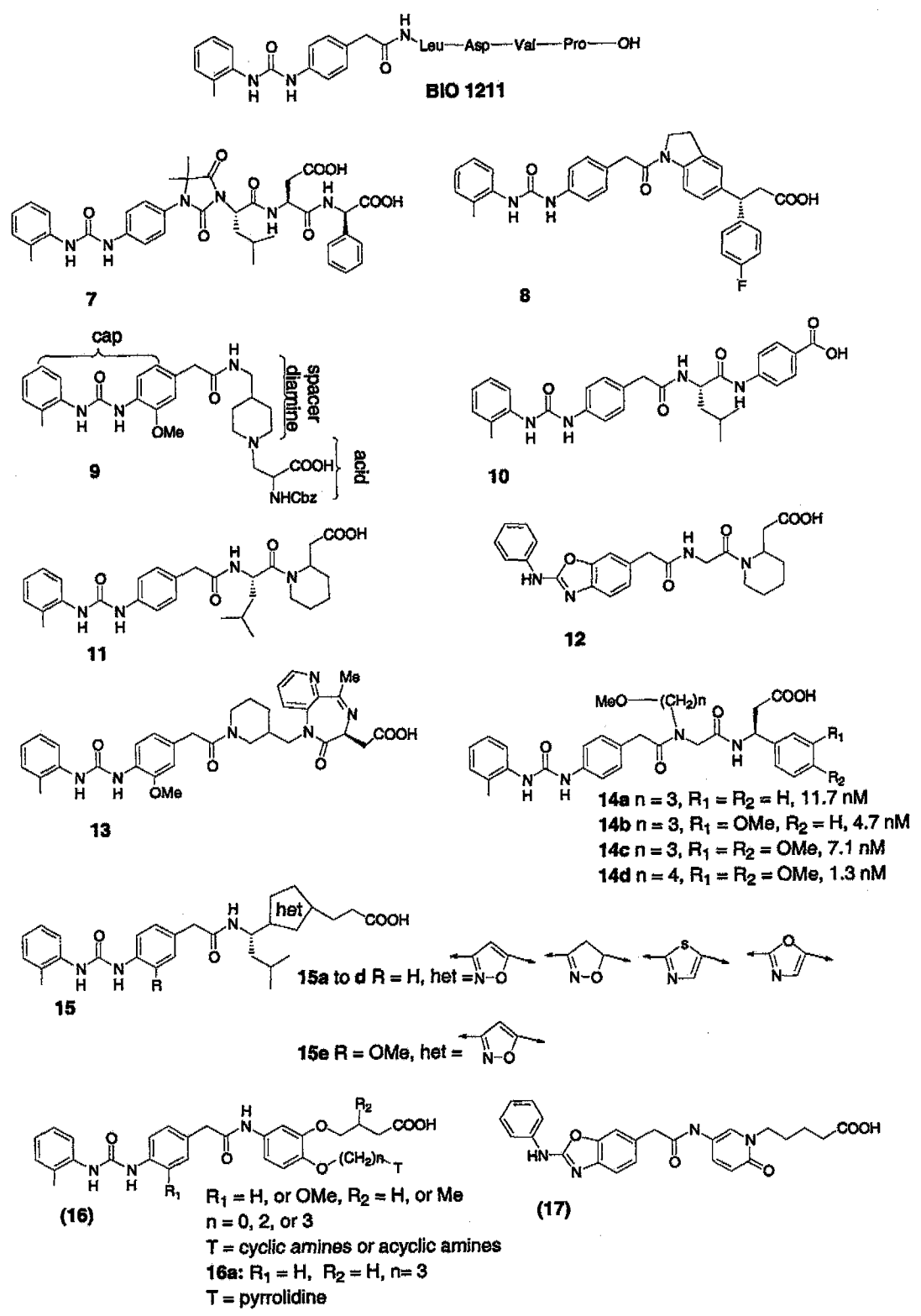


Figure 2. Linear Peptides-LDV Mimetics.

efficacious with an ED₅₀ of 20, 100, and 2 µg/kg, respectively, when dosed by intra-tracheal instillation. At a similar dose, **BIO1211** was inactive. Compound **15e** was found to be efficacious following systemic administration (58% inhibition @ 10 mg/kg, sc).

Ureas of general formula **16** were claimed as VLA-4 antagonists.⁷² The specifically claimed compound **16a** is stated to have inhibited MOLT-4 cell adhesion with an IC₅₀ value of 84 nM and a second derivative is stated to have shown an IC₅₀ of 87 nM in the same assay. Apparently, some potency was lost when the urea was replaced with anilinobenzoxazole.⁷³ One of the specified compounds **17** was stated to have IC₅₀ value of 1.1 µM in the same assay.

C. *N*-Acylphenylalanine Derivatives

The *N*-acylphenylalanines constitute a major new class of VLA-4 antagonists. As VLA-4 programs have progressed, the number of publications in this area continued to increase at an accelerated pace. As a result, only publications since 2001 are described in this next section. This class can be subdivided into phenylalanines having substituents in the 4-position as shown in Figure 3 and biphenylalanines as shown in Figure 4.

Many investigators have identified the 2,6-dichlorobenzoylamino moiety as a favorable substituent at the 4-position of various *N*-acylphenylalanines.⁷⁴ Approximately, 200 compounds were described in a patent application and tested for their VLA-4 antagonistic activity which ranged from 0.01 to 2,000 nM. One of the most potent compounds **18a** exhibited an IC₅₀ of 0.01 nM in a soluble VCAM-Ig assay.⁷⁵ In a later patent, new urea and thiourea derivatives were claimed.⁷⁶ One of the preferred structure **18b** had an IC₅₀ value of 0.014 nM for inhibiting HL-60 cell growth in a cell proliferation assay. The structure can be further simplified by eliminating the urea moiety as in **18c**, which still had an IC₅₀ value of 0.1 nM for inhibiting HL-60 cell growth.⁷⁷

Another example of the 2,6-dichlorobenzoylamino 4-substituted phenylalanines is the benzazepinone **19**.⁷⁸ The incorporation of benzazepinone in **19** was intended to mimic the bioactive conformation of its acyclic parent (ring-opened structure of **19** as shown). However, **19** is approximately sevenfold less potent than its conformationally more mobile parent.

Replacement of the 2,6-dichlorobenzoylamino moiety with 3,5-dichloro-pyridylamide as in **20a** enhances the affinity of VLA-4 binding.⁷⁹ However, rapid clearance of **20a** was observed and there was evidence pointing to an active transport mechanism. Postulating that the α-amido group was responsible for the rapid clearance of this series of compounds, its replacement became the focus of further investigation. Indeed the 3-cyclobutene 1,2-dione analog **20b** was found to be potent and had an improved pharmacokinetic profile. The 3,5-dichloropyridylamide was also incorporated into a series of bicyclic amino acid derivatives.⁸⁰ One of the most potent compounds **21** had IC₅₀ of 7 nM in a Ramos cell assay.

Another variant of this design feature is compound **22** which was found to be somewhat more specific for α₄β₇ (IC₅₀ = 1.2 nM) than for α₄β₁ (IC₅₀ = 27 nM).⁸¹ It is noteworthy that the corresponding 2,6-dichlorobenzyl ether derivatives were approximately 100-fold less potent than **22**. This structure was simplified to offer improved selectivity and retained potency.⁸² Compound **23** was one of the specifically claimed and had IC₅₀ values of 1.0 and 18 nM for α₄β₁ and α₄β₇, respectively.

In an effort to improve the pharmacokinetics profile of this class of compounds, elimination of the amide N-H *via* incorporation of the nitrogen atom into a cyclic imide was investigated.⁸³ The most potent compound **24** (IC₅₀ = 0.52 nM) is only twofold less potent than the corresponding acyclic 2,6-dichlorobenzoylamino parent. Reduction of the phthalimide to the corresponding lactam provided a compound that was 25-fold less active. The presence of the second carbonyl group of the imide may cause the phthalimido group to rotate with respect to the phenylalanine aromatic ring thus providing a comparable nonplanar conformation as in the 2,6-dichlorobenzoylamino group. Pyridones were also claimed in another patent.⁸⁴ One of the specifically claimed compounds was **25** which had an IC₅₀ values of less than 100 nM in VLA-4/VCAM-1 cell-based screening in both α₄β₁ and α₄β₇ assays.

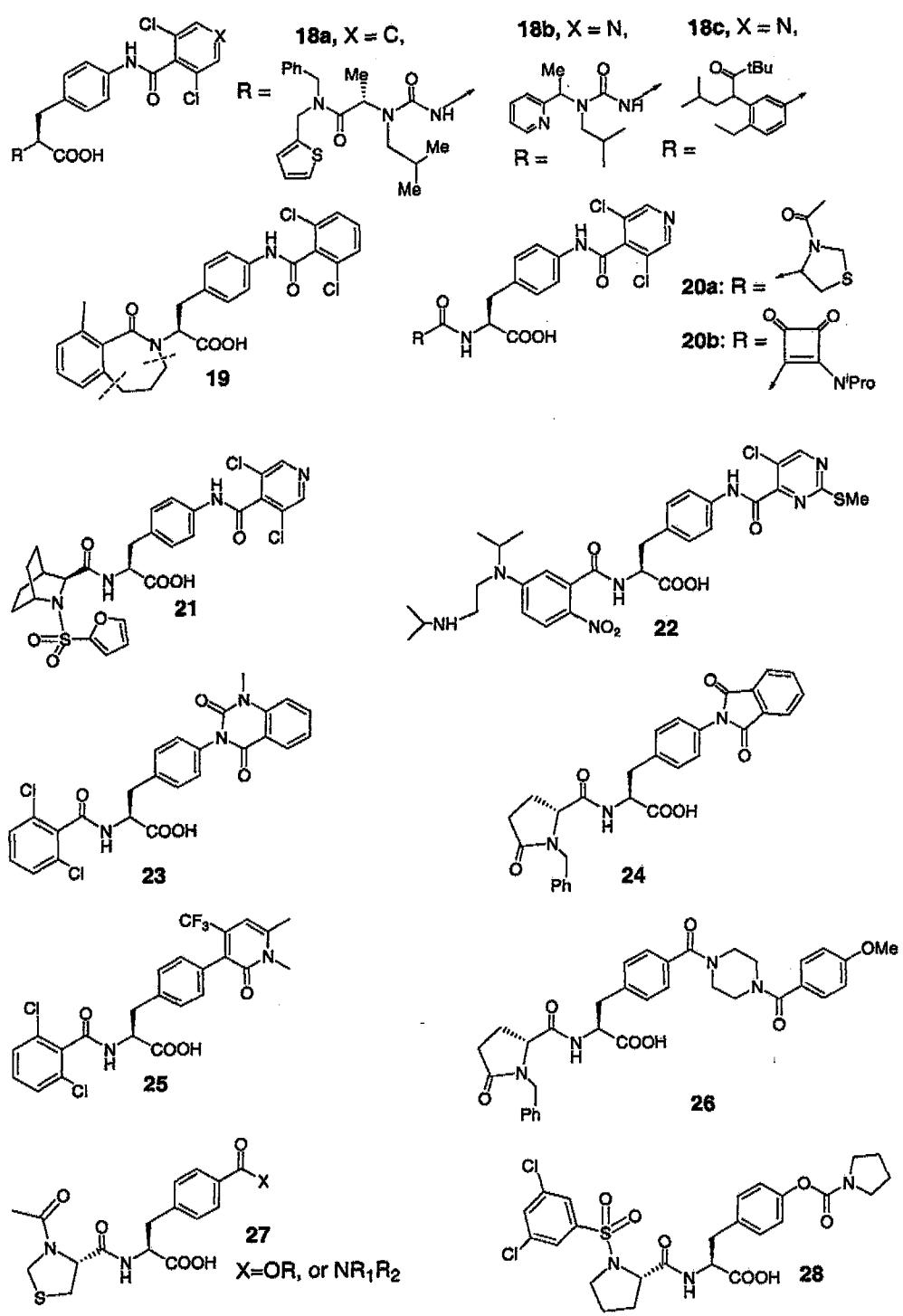


Figure 3. N-Acylphenylalanine derivatives.

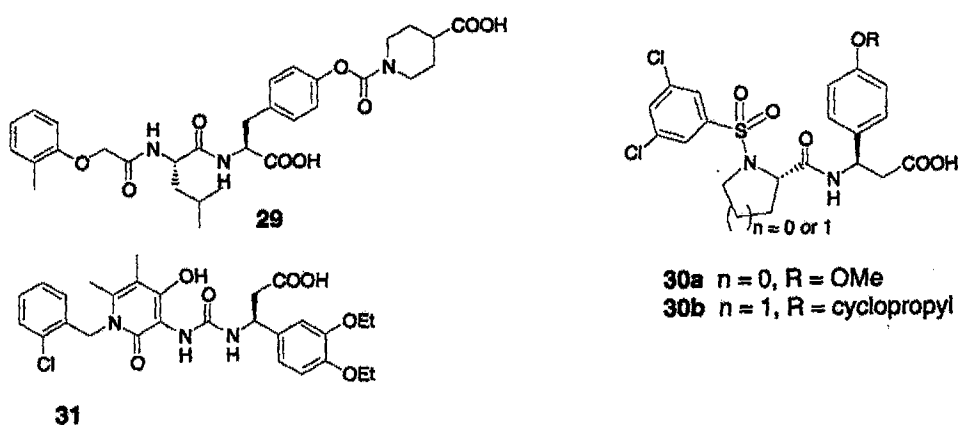


Figure 3. (Continued)

A series of 4-acylaminophenylalanine derivatives typified by 26 was also reported as potent VLA-4 antagonists.⁸⁵ The working hypothesis was that the cyclic peptide core (2a in Fig. 1) and 4-acylaminophenylalanine may share key pharmacophores, such that one can overlay the five-membered pyrrolidinone ring and the C-terminal carboxylic acid in structure 2a and 26 and then place the aromatic ring of 26 in the region of the disulfide bridge of the cyclic peptide. This hypothesis was supported in that 26 ($IC_{50} = 4$ nM) was reported to be equally potent to the cyclic peptide 2a.

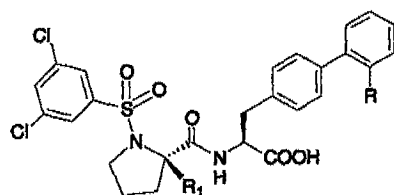
Related benzoate derivatives 27 were claimed as both $\alpha_4\beta_1$ and $\alpha_4\beta_7$ inhibitors.⁸⁶ Compounds of the invention inhibited VLA-4 binding *in vitro* with IC_{50} values as low as 1 nM. Introduction of a carbamate at the 4-position was reported to be key in maintaining potency for both $\alpha_4\beta_1$ and $\alpha_4\beta_7$.⁸⁷ For example, compound 28 was reported to be a potent dual $\alpha_4\beta_1/\alpha_4\beta_7$ antagonist ($IC_{50} = 0.13$ nM vs. $IC_{50} = 1.92$ nM).

Patent applications claiming various formulation and delivery methods for 29 have appeared.⁸⁸ Compound 29 was reported to be an antagonist of both $\alpha_4\beta_1$ and $\alpha_4\beta_7$.⁸⁹ The methods claimed included dry powder formulations and a device for administering 29 as an inhaled aerosol for the treatment of inflammatory diseases, especially asthma. Aqueous suspensions of 29 suitable for nebulization are also described.^{90,91}

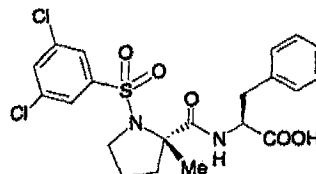
Through modification of the ring size and substitution of the phenyl ring, β -amino acids as illustrated by structure 30 were discovered as potent antagonists while maintaining a good pharmacokinetic profile (30a: $IC_{50} = 0.49$ nM, 30b: $IC_{50} = 0.85$ nM).⁹² A 4-substituent on the β -phenyl ring capable of hydrogen bonding seems to be preferred for potency. It was presumed that the unnatural β -amino acid derivatives may be less favorable substrates for carboxypeptidase. This was confirmed by the observation that no hydrolysis products were detected in rat plasma, bile, and urine samples unlike the corresponding α -amino acid derivatives.

A series of pyridone ureas as VLA-4 inhibitors has been claimed in a recent patent application.⁹³ TBC-4746 had been selected as the pre-clinical lead (structure not disclosed). Compound 31 was among one of the more potent compounds claimed and had an IC_{50} of 3 nM in Ramos cell assay.

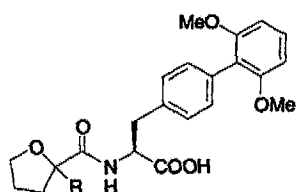
An aspartate residue, located on a β -turn connecting the C and D β -strands of VCAM-1, is critical for binding of VCAM-1 to VLA-4.^{94,95} The sequence LDV may share a receptor-binding site on VLA-4 with an RGD sequence, which has been exploited for the identification of potent inhibitors of other integrins, notably $\alpha_{2b}\beta_{3a}$ and $\alpha_v\beta_3$.^{96,97} These two motifs (LDV and RGD) are now widely accepted as a common binding feature among many integrin ligands. The aspartate residue is thought to be critical for binding due to its ability to direct coordination to a bound cation, notably calcium.⁹⁸ Screening of a carboxylate-containing library led to the discovery of a series of sulfonlated dipeptides such as 32 (Fig. 4).⁹⁹ The first and second amino acid preferred the (S) configuration. Many



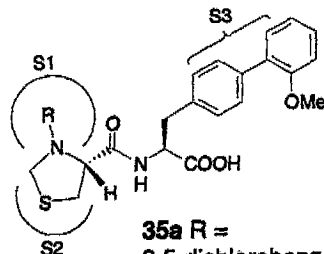
32a R = OMe, R₁ = H
32b R = OMe, R₁ = Me
32c R = CN, R₁ = Me



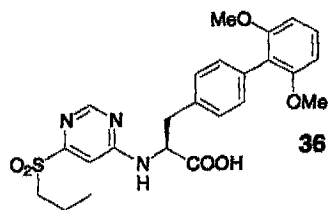
33



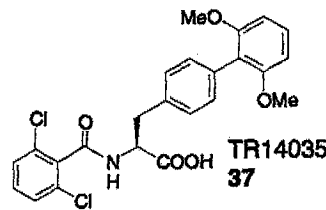
34a R = Me
34b R = CH₂CH₂OMe
34c R = morpholine amide



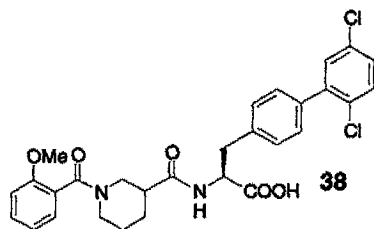
35a R =
 3,5-dichlorobenze
 nesulfonamide
35b R = acetyl



36



TR14035
37



38

Figure 4. Biphenylalanine derivatives.

polar substituents on the distal ring of the 4-biphenylalanines were potency enhancing. The optimization of the pharmacokinetic profile of these compounds proved to be much more challenging. When a methyl group on the central proline of **32a** was introduced, the resulting compound **32b** showed a reduced plasma clearance and better bioavailability, presumably due to minimized cleavage of the amide bond *in vivo*. The methoxy compound **32b** was potent and selective (IC₅₀ = 0.08 nM in a soluble VCAM-Ig binding assay). The introduction of a cyano group as in **32c** improved the pharmacokinetic profile still further in rats (F% = 33, Cl_p = 5 ml/kg/min, t_{1/2} = 1.5 hr). However, compound **32c** was found to be highly protein bound (> 99.5% in human and rat plasma) and much less potent in a plasma shift assay where plasma was added to the binding assay, suggesting that the attractive pharmacokinetic profile of **32c** was largely due to protein binding.

In an effort to reduce protein binding, a variety of substituted phenylalanine derivatives were prepared. The compounds showed excellent potency with a wide variety of substituents on the phenyl ring. Heteroaryl ring substitution for phenylalanine was also well tolerated. The unsubstituted

compound **33** was found to have modest to good bioavailability in all four species (rat, dog, monkey, and sheep).¹⁰⁰ The clearance rates across all species were moderate, with acceptably long half-lives. However, protein binding was still high (99.5% in human and rat plasma).

Tetrahydrofuroyl phenylalanine derivatives were also reported to be potent VLA-4 antagonists.¹⁰¹ In rhesus monkey, compound **34a** exhibited excellent oral bioavailability and low plasma clearance ($F = 51\%$, $Cl_p = 3$ ml/kg/min). Again, protein binding may be a factor contributing to the apparent low plasma clearance as indicated by a tenfold shift in the IC_{50} which was observed when the binding assay was run in the presence of 5% plasma. In an effort to reduce plasma protein binding and improve the specificity for VLA-4 versus $\alpha_4\beta_7$, a systematic study of substitution at the α -carbon of the tetrahydrofuran ring was undertaken.¹⁰² The ether analog **34b** was equipotent to the methyl compound and had a reduced plasma protein binding. However, this reduction in protein binding also resulted in a significant loss in rat pharmacokinetics ($F = 2\%$; $Cl_p = 81$ ml/kg/min). Substitution with acyl groups as typified by **34c** provided a significant increase in potency and a significant decrease in protein binding which again resulted in a poor pharmacokinetic profile.

In working with thioprolines, a hypothesis that one of the three major binding sites in VLA-4 is larger than that in $\alpha_4\beta_7$ was supported.¹⁰³ When R is large, for example, a 3,5-dichlorobenzene-sulfonyl group in **35a**, it is a potent ($IC_{50} = 0.3$ nM) VLA-4 antagonist but is less active against $\alpha_4\beta_7$ ($IC_{50} = 306$ nM) because the S1 site of $\alpha_4\beta_7$ is smaller than that of VLA-4 and unable to accommodate the large R group. When R is small, such as an acyl group in **35b**, the result is a dual VLA-4 ($IC_{50} = 0.20$ nM) and $\alpha_4\beta_7$ ($IC_{50} = 2.1$ nM) antagonist.

The central amide bond that is a common feature in almost all of the reported VLA-4 antagonists is also important in maintaining good potency. It was postulated that the amide carbonyl may be serving as a hydrogen bond acceptor and the amide nitrogen may be serving as a hydrogen bond donor. To test the hypothesis that the amide bond hydrogen was providing a key binding interaction with VLA-4 and to move away from peptide-like inhibitors of VLA-4, a series of aryl amines and heteroaryl amines were prepared and tested.¹⁰⁴ Pyrimidine **36** was found to be potent against VLA-4 ($IC_{50} = 0.4$ nM) and its potency was attributed to the aminopyridine hydrogen atom more effectively mimicking the amide N-H rather than binding interactions between substituents on the pyrimidine ring and the VLA-4. Despite the elimination of the amide bond, the pharmacokinetic profile of this compound was poor. Related pyridine, pyrimidine, and triazine derivatives afforded similar results.^{105,106}

Through systematic modifications at each of the three phenyl rings, the discovery of (TR-14035) **37** as a balanced dual $\alpha_4\beta_7/\alpha_4\beta_1$ antagonist was recently reported ($IC_{50} \alpha_4\beta_7/\alpha_4\beta_1 = 7/87$ nM).^{107,108} 2,6-dichloro substitution on the first ring was optimal. 2,6-disubstitution at the distal ring was also necessary to maintain potency implicating the importance of a twisted conformation of the biphenyl system. Attempts to replace the metabolically labile methoxy groups resulted in loss of potency. Compound **37** is highly selective when tested against $\alpha_5\alpha_1$, $\alpha_6\alpha_1$, $\alpha_v\alpha_3$, and $\alpha_I\alpha_2$. It has been shown to be orally absorbed in several animal species, was selected for clinical development, and has completed Phase I studies in healthy volunteers in Europe. The introduction of alkoxy substituents at the 4'-position of the biphenyl nucleus of **37** was found to reduce clearance and improve bioavailability.

The central *N*-sulfonyl-prolyl group of **32–33** was replaced with cyclic β -amino acids to afford VLA-4 and $\alpha_4\beta_7$ antagonists.¹⁰⁹ One of the preferred compounds **38** inhibited adhesion of Jurkat cells to VCAM-1 with an IC_{50} of less than 0.5 μ M.

D. Unique Structures With Undisclosed Biological Data

Enamine derivatives were claimed to be potent (IC_{50} less than 1 μ M) and selective (no or minimal inhibitory action on integrins of other subgroups) inhibitors of α_4 integrins.¹¹⁰ The enamines as illustrated by the general structure **39** (Fig. 5) are claimed to possess the additional advantage of good

pharmacokinetic properties, especially low plasma clearance, although no specific data were provided. Thiapiperidines of general formula **40** are claimed as VLA-4 antagonists but their potency was not disclosed.¹¹¹ Similar thioprolines or six-membered heterocycles were claimed as inhibitors of both $\alpha_4\beta_1$ and $\alpha_4\beta_7$.¹¹² One or more these compounds **41** were claimed to give an IC_{50} of less than 1 nM in a VCAM-Ig assay.

Recognizing that peptidyl inhibitors are prone to poor absorption, poor solubility, and are subject to metabolism *in vivo* (both systemically and locally when administered directly into the lung), efforts were devoted to disguise the central amide bond as an oxazole. Two structural classes of isoxazoles **42** were claimed.¹¹³ However, neither the potency nor the PK properties was disclosed. Squaric acid

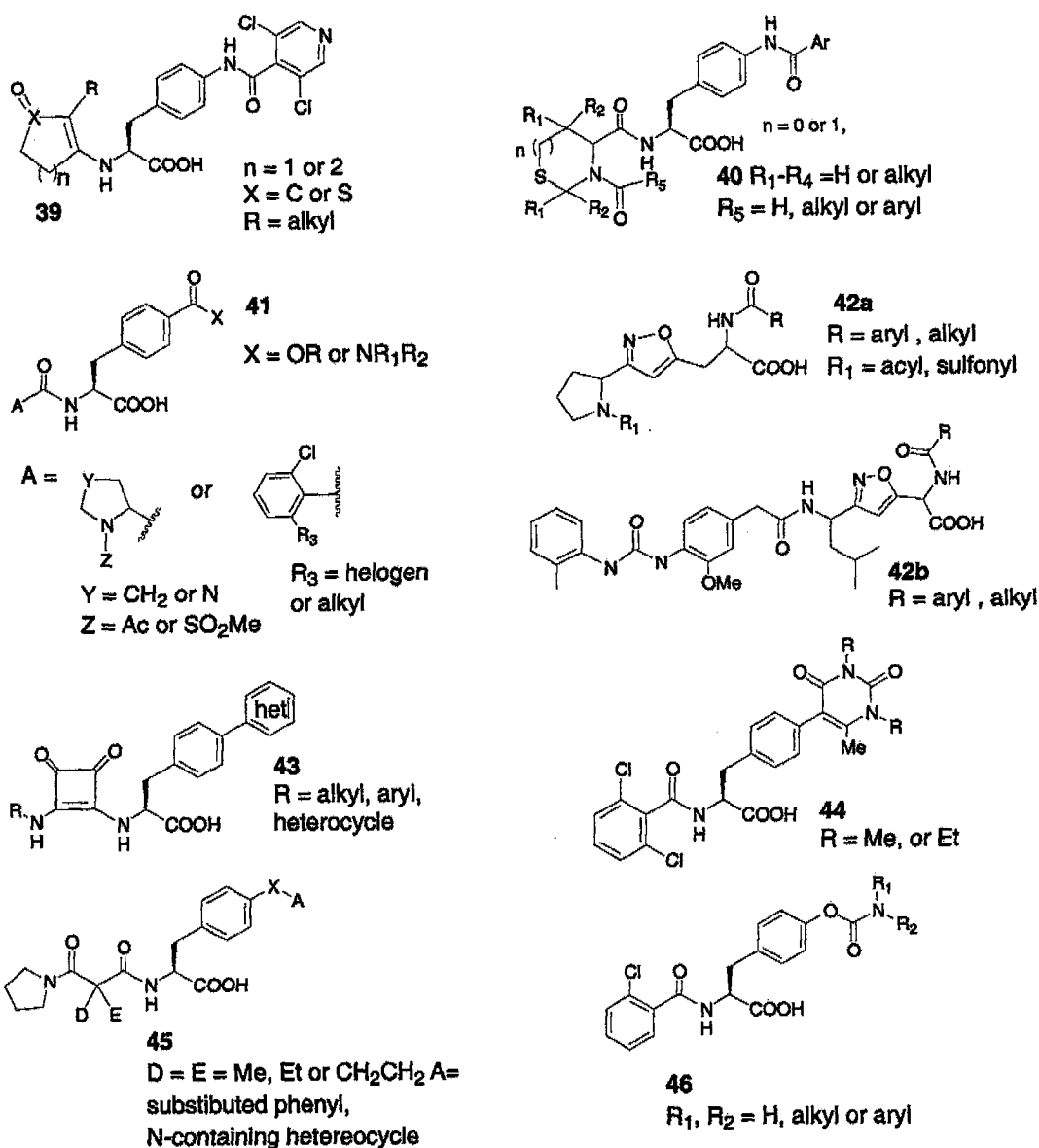


Figure 5. Unique structures with undisclosed biological data.

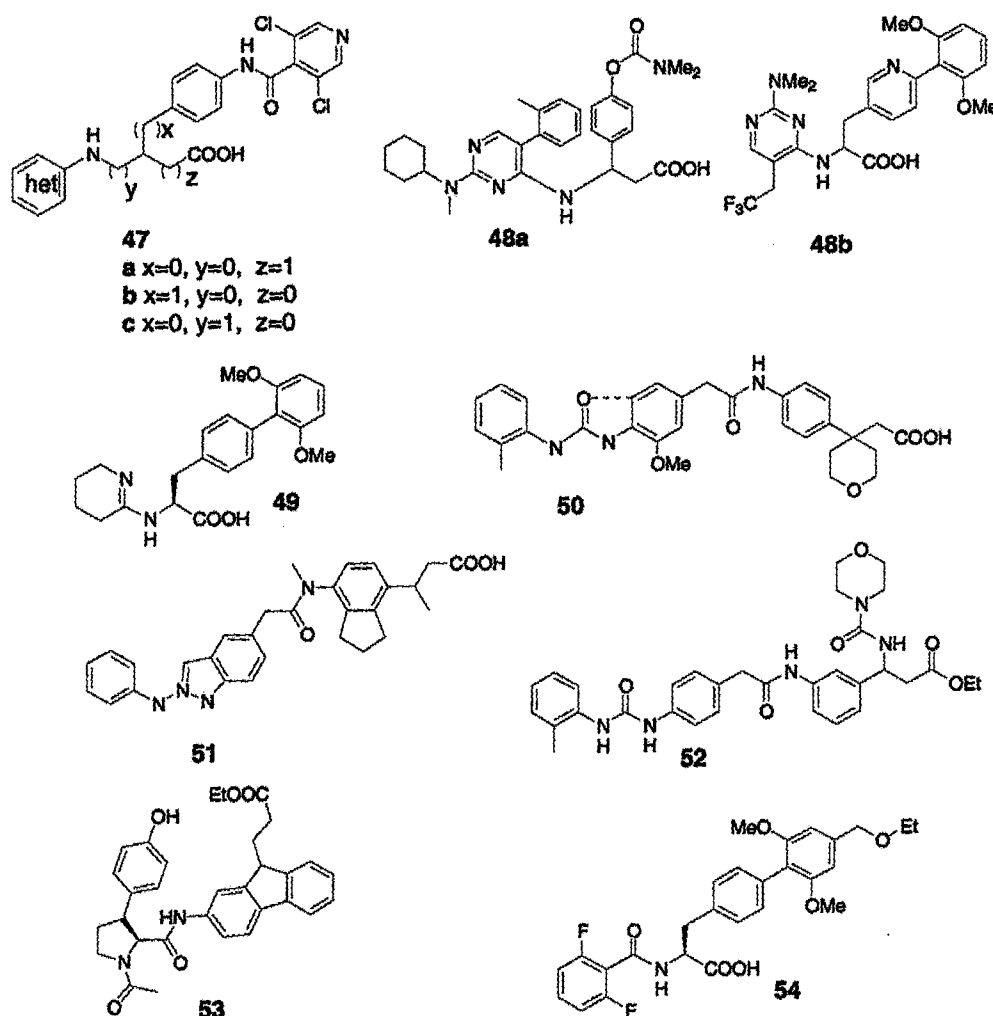


Figure 5. (Continued)

derivatives **43** have been reported as inhibitors for both $\alpha_4\beta_1$ and $\alpha_4\beta_7$.¹¹⁴ The Het group is an optionally substituted bicyclic fused ring, heteroaromatic group, benzimidazole, or substituted isoquinoline.¹¹⁴⁻¹¹⁶ The potency of these compounds was not discussed. However, it was emphasized in both patents that many peptidic and non-peptidic carboxylic acids of therapeutic interest are subject to high hepatic clearance from plasma. These squaric acids compare favorably with functionally equivalent integrin binding compounds in which the squaric acid framework is not present because they are not rapidly or extensively extracted by the liver and have low plasma clearance ($Cl_p \leq 10$ ml/min/kg in rats). Pyrimidinyl phenylalanines like the one illustrated in structure **44** are claimed to have an IC_{50} less than 100 nM for both $\alpha_4\beta_1$ in VCAM, VLA-4 Ramos cell assay and $\alpha_4\beta_7$ (MadCAM/RPMI cell assay).¹¹⁷

Approximately 400 examples of inhibitors of α_4 integrins were claimed in a patent application.¹¹⁸ One of the most potent compounds (IC_{50} is less than 0.02 μ M in VCAM-Ig assay) was **45** with equal potency to $\alpha_4\beta_7$. Tyrosine derivatives of the general formula **46** have been disclosed.¹¹⁹ They generally exhibit inhibitory activity against α_4 integrins with an IC_{50} of less than 1 μ M.

β -alanine derivatives 47a were claimed as α_4 integrin inhibitors.¹²⁰ It is interesting to note that in 47 and 48, the common amide bond is replaced by heteroaryls such as pyrimidines and triazines. The preferred compounds of 47a generally have IC_{50} values in the $\alpha_4\beta_1$ and $\alpha_4\beta_7$ assay of 1 μ M and below. Some selectivity was evident in that in the other assays featuring α integrins of other subgroups the same compounds had IC_{50} values of 50 μ M and above. The corresponding α -amino acid derivatives 47b were claimed in a separate patent and are expected to have IC_{50} values of 15 μ M or less.¹²¹ A third variation was claimed as represented by structure 47c which have IC_{50} value of 1 μ M or less in the $\alpha_4\beta_1$ and $\alpha_4\beta_7$ assay.¹²² Structures 48a and 48b represent the specified compound in two patents. There was no disclosure of specific biological data, but the compounds are stated to display IC_{50} values of less than 15 μ M in the binding assay.^{123,124}

The central amide bond was replaced by amidine 49 which was specifically claimed and exemplified by synthesis but with no specific biological data.¹²⁵ Novel alkanolic acids are claimed as LDV mimics.¹²⁶ Compounds are broadly divided into alkanolic acids linked via an aromatic unit to a phenyl urea or (2-methylphenylamino)benzoxazole. Compound 50 is one of the specifically claimed. Over 500 indane derivatives were claimed as VLA-4 inhibitor with IC_{50} values ranging from 100 nM to 100 μ M.¹²⁷ The compounds were assessed in an airway inflammation animal model but no results were presented. 51 is one of the five specifically claimed compounds.

β -Ureido- β -phenyl acid derivatives 52 were claimed in a patent application.¹²⁸ Biological data were not presented. Fluorenyl derivative 53 was one of the several specifically claimed VLA-4 antagonists from a patent application.¹²⁹ It is an interesting structure because it locks the two phenyl ring in a rigid planar conformation while other data suggest that the two distal aromatic rings need to be perpendicular to each other. However, no biological data were given to assess its potential. Finally, substituted biphenylalanine derivatives 54 were claimed as, α_4 inhibitors.¹³⁰ Biological data were not presented.

3. POTENTIAL LIABILITIES OF VLA-4 INHIBITION

A. Developmental Issues

Pharmacological antagonism of VLA-4 during certain stages of development may have dire consequences if the results of gene knockout experiments are recapitulated. Gene knockouts of the integrin α -subunit or the ligand VCAM-1 in homozygous null mice resulted in embryolethality.¹³¹⁻¹³⁵ This lethality apparently arose from the failure of specific tissue fusion events during two critical stages of development. Defects in chorio-allantoic fusion in the knockout mice prevented placentation. Furthermore, there were defects in epi-myocardial fusion during coronary vascular development. VLA-4 and its ligand VCAM-1 have been localized to the chorion and epicardium and the allantois and myocardium, respectively during critical stages of chorio-allantoic and epicardial-myocardial fusion. Inhibition of VLA-4 during these fusion events might be expected to cause severe disruption of placentation and organogenesis. Initiation of chorio-allantoic fusion occurs in the mouse at an early stage of development (gestation days GD9 through 10). In humans, the expected sensitive period for anti-VLA-4 affects on placentation and coronary vessel development would be early in the first trimester of pregnancy, probably before the pregnancy was confirmed.

Results of formal developmental and reproductive toxicity studies of VLA-4 antibodies or inhibitors have not been published. Disruption of chorio-allantoic fusion in an *ex vivo* model of rat embryo development with VLA-4 antibodies and inhibitors has recently been reported.¹³⁴ Early neurulating rat embryos (GD9 and 10) were cultured and exposed to exocoelomic microinjection of TA2, a VLA-4 blocking antibody, or cultured with a series of potent small molecule inhibitors of VLA-4. An inhibitor with affinity only for the Mn^{2+} -activated or "high affinity" state of VLA-4 did not cause any defects in chorio-allantoic fusion, whereas the antibody and dual inhibitors of both the Mn^{2+} -activated and unactivated or "low affinity" Ca^{2+} , Mg^{2+} -state of VLA-4 caused abnormal

fusion of these tissues. A dose titration of one particularly potent inhibitor (Mn^{2+} $\text{IC}_{50} = 70 \text{ pM}$; Ca^{2+} , Mg^{2+} $\text{IC}_{50} = 60 \text{ pM}$) had a no effect level (NOEL) of only 156 nM. The activation state selectivity of inhibitors, the contribution of protein binding, and further developmental issues were also discussed. This *ex vivo* model is not a replacement for *in vivo* studies since there are neither maternal barriers nor mechanisms for clearance of the compounds; the embryos are literally awash in inhibitors. Thorough developmental and reproductive toxicity studies are necessary to convincingly assess the potential for embryonic toxicities of VLA-4 inhibition.

B. Effects on Hematological Processes

VLA-4 plays a critical role in hematopoietic development of lymphocytes.^{135–138} VLA-4 is present on bone marrow-derived hematopoietic cells.^{139–141} Likewise, its ligands VCAM-1 and CS-1 are found on bone marrow stromal cells.¹⁴² VLA-4 and VCAM-1 expression are regulated during skeletal muscle differentiation and their interaction appears to be important in the generation of secondary myoblasts.¹⁴³ Hematopoietic processes have been blocked *in vitro* by antibodies to VLA-4 or VCAM.^{144,145} Increases (1.5–2-fold) in circulating hematopoietic stem cells have been observed *in vivo* after administration of antibodies to VLA-4 or VCAM-1.^{146–150} Chimeric mice that are deficient in the expression of the α_4 sub-unit also exhibit changes in hematopoietic processes.^{151,152}

It would appear that VLA-4 plays an important role in the normal development of lymphocytes, erythrocytes, and myocytes. Transient increases in white blood counts may be of little consequence given the normal, daily variation. However, the long-term consequences of sustained increases in lymphocyte counts for any individual are unknown. The impact on normal host defense processes will need to be evaluated in carefully controlled clinical studies of VLA-4 antibodies and small molecule inhibitors.

4. SUMMARY

Realization of the therapeutic potential of inhibiting the binding of integrins to their respective ligands has met with mixed success. Several $\alpha_{2b}\beta_{3a}$ antagonists have proved to be medical and commercial breakthroughs while others have been withdrawn from further consideration for lack of efficacy and/or unacceptable side effects. Effective control of coagulation *via* inhibition of platelet aggregation is clearly a delicate balance. It is still too early to assess the clinical potential of $\alpha_v\beta_3$ antagonists in osteoporosis, arthritis, and/or cancer as these vitronectin antagonists are just now entering more advanced clinical trials. Likewise, VLA-4 antagonists are only beginning to enter later efficacy trials in the clinic. The early success of natalizumab in the treatment of multiple sclerosis and Crohn's disease offers hope that inhibition of VLA-4 will be effective in treating these inflammatory conditions. The contribution of migrating lymphocytes to chronic inflammatory diseases as such may be found in asthma and arthritis is as yet unsubstantiated in clinical trials. The ongoing studies with advanced compounds will hopefully affirm the enormous effect that many researchers have contributed to the emerging field of VLA-4 antagonists.

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The Discovery of VLA-4 Antagonists

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Abstract: Starting with a cyclic peptide of moderate potency as a VLA-4 antagonist, highly potent and conformationally defined cyclic peptides were developed incorporating a constrained tyrosine and an achiral Asp-Pro spacer. N-Acyl phenylalanine derivatives were also discovered to have VLA-4 antagonist activity. During the course of development of this series, we found that the active acylphenylalanines mimic the pharmacophores present in the cyclic peptides and hypothesized that they bind to the same site on VLA-4. This insight guided our optimization strategy. Based on the emerging SAR, as well as insights from the recent X-ray crystal structure of the integrin $\alpha v \beta 3$ bound to a RGD containing cyclic peptide, we propose a binding model for these compounds.

INTRODUCTION

The integrin VLA-4 ($\alpha_4 \beta_1$) interacts with vascular cell adhesion molecule (VCAM), which is conditionally expressed on endothelial cells in the presence of inflammatory cytokines, as well as with the intercellular matrix protein fibronectin. VLA-4 is expressed on a variety of leucocytes including B-cells, T-cells, basophils and eosinophils and is involved in the recruitment, activation and survival of these cell types. Interest in modulation of the VCAM/VLA-4 integrin interaction developed in the Hoffmann-La Roche laboratories with the publication of several papers suggesting that antibodies against VLA-4 inhibited leukocyte trafficking and contact hypersensitivity in rodents [1-4] and were active in rodent models of rheumatoid arthritis [5-7], asthma [8,9], and inflammatory bowel disease. [10,11] Furthermore peptides that mimic the CS-1 connecting sequence of fibronectin were also known to be capable of inhibiting VCAM/VLA-4 binding and of suppressing the development of arthritis symptoms in rats. [12,13] The later results were particularly encouraging as they suggested that, like other integrin-integrin receptor interactions, the VCAM/VLA-4 interaction might be susceptible to interruption by small molecule inhibitors. More recently, positive results of two clinical trials of the humanized anti- α_4 antibody, Natalizumab, in Crohn's disease and multiple sclerosis were published, validating the potential for α_4 integrin antagonists in human disease. [14,15]

CYCLIC PEPTIDES

The publication of an early patent application by the Tanabe organization describing a series of cyclic peptides provided us with a starting point for a chemistry effort. In particular, Tanabe workers indicated that the peptide 1 was an effective antagonist of VCAM/VLA-4 binding and that related cyclic peptides inhibited the interaction of the integrin $\alpha_5 \beta_1$ with the matrix protein fibrinogen. [16,17]

Since workers from Tanabe demonstrated that VLA-4, like other integrins known at the time, including $\alpha_5 \beta_1$ and $\alpha_2 \beta_3$ recognized the peptide sequence Arg-Gly-Asp, [18] we believed that the activity of 1 was associated with this sequence as well.

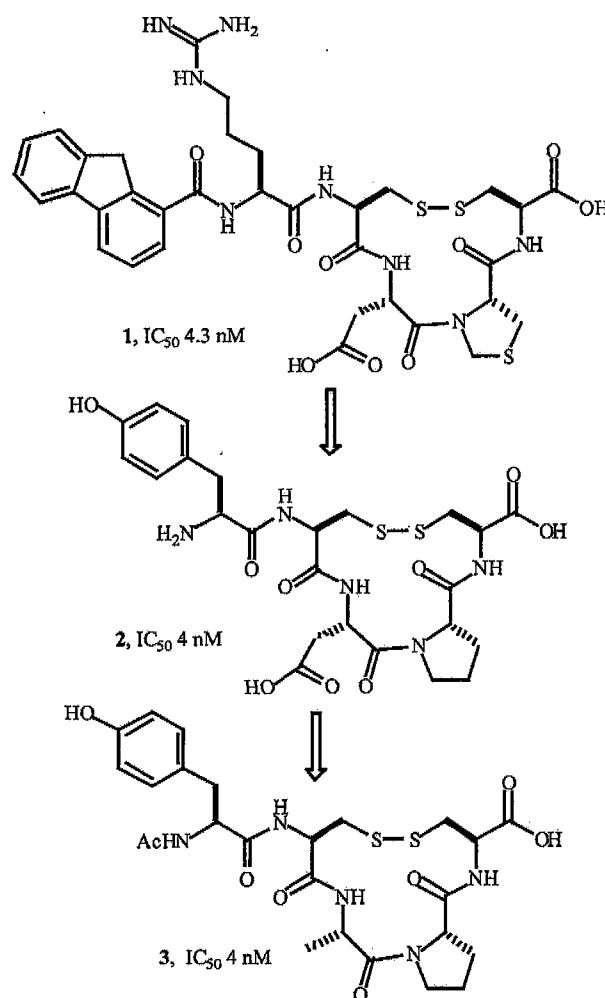


Chart 1.

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Together with our colleagues at Genentech, we initiated structure-activity studies on **1** in order to validate the above assumption and to map the essential pharmacophores responsible for VLA-4 inhibition. Compounds were assayed for VLA-4 antagonist activity using a solid-phase, dual-antibody-ELISA in which VLA-4 derived from Ramos cells was allowed to compete for bound recombinant human VCAM in the presence of serial dilutions of test compound. VLA-4 bound to VCAM-1 was detected by a complex of anti- β 1 antibody and HRP-conjugated anti-mouse IgG: chromogenic substrate (K-Blue). [19] A secondary, cell based assay was also established in which fluorescently labeled Ramos cells were allowed to compete for immobilized VCAM. As our work progressed, we noted that we needed to achieve potencies of ≤ 1 nM in the ELISA assay to observe consistent sub- μ M potency in the more stringent Ramos cell based assay where it is necessary to disrupt a matrix of interactions across the cell surface.

We established early that the N-terminal FCA-arginine present in **1** could be replaced with a surprisingly diverse array of acyl groups. One of the most potent examples of them was the tyrosine derivative, **2**. [20] Our Genentech coworkers further found that the aspartic acid moiety was also dispensable and could be replaced with a variety of amino acids and linear spacers. For example, the alanine analogue **3** is equipotent with the initial lead compound. Taken together, our structure-activity studies suggested that a free carboxylic acid at the C-terminus, one of a variety of N-acyl groups and a hydrophobic moiety, represented by the thiaproline in **1** and the prolines in **2** and **3**, were essential pharmacophores.

We were intrigued by the possibility of incorporating these results in the design of Asp-Pro dipeptide replacements that integrate both a hydrophobic portion capable of mimicking the proline interaction as well as a suitable spacer to maintain the appropriate geometry and conformational constraints of the cyclic peptide ring system. Cyclic peptides related to **1** are rather well defined conformationally. For example, NMR studies of **1** revealed that although the position of the N-terminal residue was indeterminate, the ring is well defined, forming essentially two families of conformers, which differ only in the directionality of the disulfide linkage as shown in Fig. (1a). The ring system is nearly planar with the two amide bonds within the ring oriented perpendicular to the ring plane in opposite directions to minimize the net dipole. As a consequence, both are highly solvent exposed.

Since the ring conformation seems well conserved, we were primarily concerned with the positions of the hydrophobic groups relative to the exocyclic bonds leading to the N-terminal residue and the C-terminal carboxyl group which are defined by the ring geometry. Thus, we modeled analogues by displaying a single member of the most populated conformer family of **1** and manually building in potential spacers using the molecular modeling package Sybyl. Each new structure was minimized, subjected to a short molecular dynamics run and reminimized using the maximin function within Sybyl. The suitability of individual mimetics was evaluated visually by inspecting their overall effect on ring conformation.

As a result of this effort, a series of 1-(aminoalkyl)cyclopentane carboxylic acids was designed as easily accessible, achiral dipeptide mimetics. As illustrated in Fig. (1b), modeling experiments suggested that a derivative incorporating the 1-(2-aminoethyl)cyclopentane carboxylic acid linker, **4** offered the best opportunity to preserve the ring conformation. Incorporation of shorter spacers caused a considerable shift in the key vectors and thus changed the relationship between the critical N-terminal tyrosine and the C-terminal carboxyl group. Thus a series of homologous 1-(aminoalkyl)cyclopentane carboxylic acid spacers was synthesized and incorporated into analogues of **3**. As shown in Table 1, the data support the modeling predictions that the aminoethyl derivatives **4** and **6** are preferred over their homologues **5** and **7**. [21] It is interesting to note that while **1** has a 14-membered ring, the ring in analogue **4** is one atom smaller.

An additional finding by the Jackson group at Genentech was that incorporation of a constraint in the N-terminal tyrosine provided an additional enhancement in potency as exemplified by **8**, which consists of a mixture of diastereomers. [20] Incorporation of this constraint into the peptide mimics described above led to the synthesis of **9** which had the expected enhancement in potency. Since **9** represents a relatively compact, low molecular weight structure with only three independent chiral centers and three amide bonds, we were intrigued to explore whether it could be further manipulated to improve its drug-like properties. We hypothesized that replacement of one of the sulfur atoms in the cyclic disulfide with a carbon atom, together with modification of the N-terminus of the cyclic core would reduce the metabolic liabilities that may be associated with a structure such as **9**, while retaining the desired biological activity. We also reasoned that since the geometry of the disulfide may be different from that of a thioether, the overall ring size might also affect the activity. However, molecular modeling suggested that carbon replacement would have minimal impact on the N- and C-terminal vectors as well as the position of the cyclopentyl moiety. We were thus encouraged to prepare the thioether analogues **10**, incorporating the best features discovered so far to determine their effect on the VCAM/VLA-4 interaction.

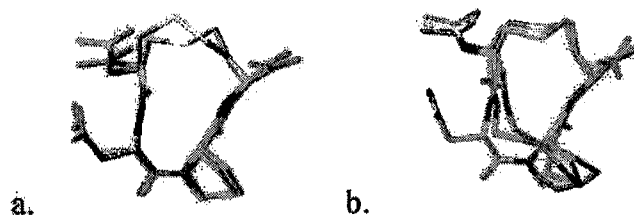
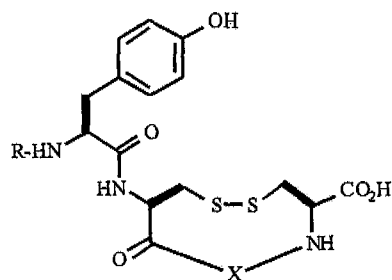
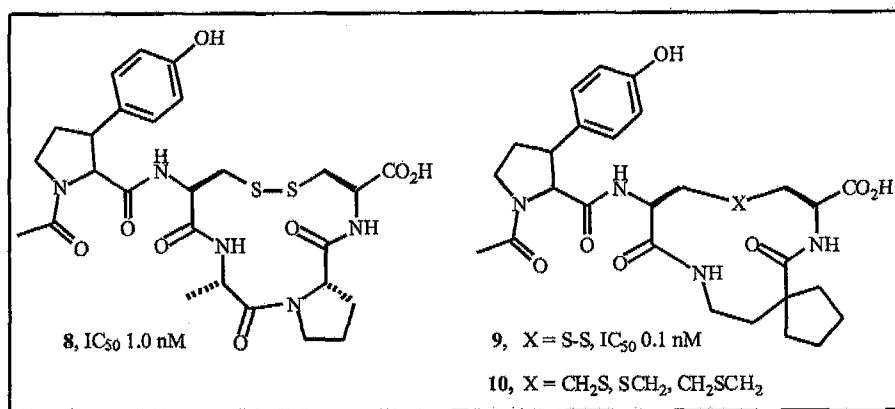


Fig. (1). (a) Overlay of the two most populated conformer families of **1** as determined by NMR. Of 42 lowest energy conformers, 28 are represented by the green structure and 13 by the grey. The major differences among family members are in the position of the N-terminal residues which are not shown. (b) Overlay of the NMR structure of core ring system of **1** (green) and two spirocyclic systems where the Asp-Gly is substituted with 1-(aminomethyl)cyclopentane carboxylic acid (light grey) or 1-(2-aminoethyl)cyclopentane carboxylic acid (orange).

Table 1. Structure-Activity of Cyclic Peptide Derivatives with Asp-Pro Spacers.



No.	R	X	Solid Phase Assay	Ramos Cell Assay
			IC ₅₀ (nM)	IC ₅₀ (nM)
1	Ac	Asp-Pro	1.8	200
4	Ac		0.70	12
5	H		5.0	-
6	H		0.50	24
7	H		19	480

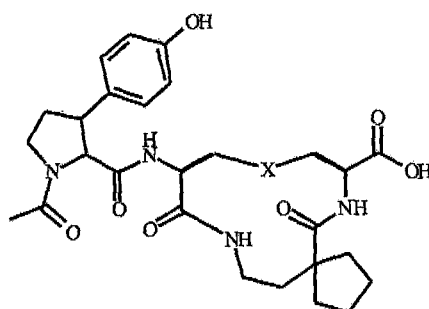


The results of this study are summarized in Table 2. Replacement of a single sulfur atom by carbon, without altering the ring size resulted in only a relatively modest decrease in potency. More interestingly, there was a significant difference between the effects of substitution of the N-terminal sulfur atom and the C-terminal sulfur. In fact, the thioether 13 was at least 5 fold more potent than its

positional isomer 11, while only 4-5 fold less potent than the parent disulfide 9 in the ELISA assay. [22] A similar trend is evident in the cell-based assay, although the differences are more pronounced. It is possible that the C-terminal sulfur atom makes a key hydrophobic contact with the receptor, and consequently, its replacement with a carbon atom results in a suboptimal interaction.

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Table 2. Activity of Cyclic Thioethers VCAM/VLA-4 Assays.

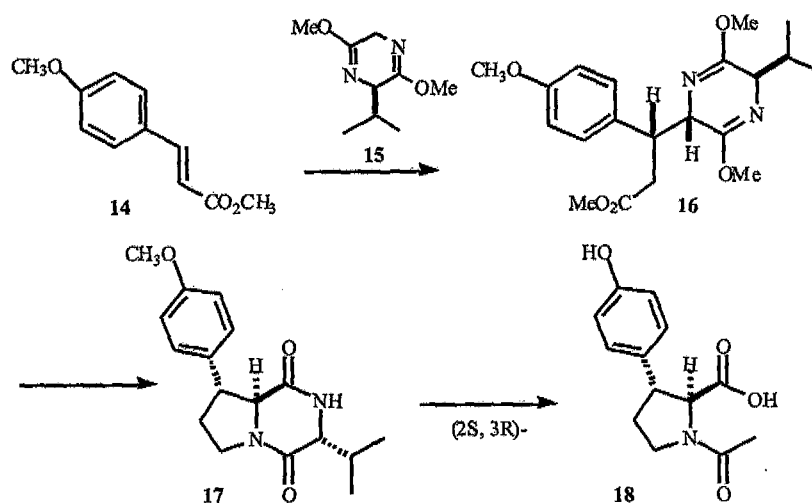


No.	X	Solid Phase Assay	Ramos Cell Assay
		IC ₅₀ (nM)	IC ₅₀ (nM)
9	S-S	0.1	1.5
11	CH ₂ S	2.7	220
12	CH ₂ SCH ₂	35	—
13	SCH ₂	0.5	20

These cyclic peptides are highly polar, likely a consequence of the conformation of the ring system placing the amide bonds in a highly solvent exposed orientation. Pharmacokinetic studies of selected compounds from this class indicated that they were rapidly cleared and would not be useful as orally active drugs. However, we were interested in exploring this series further as possible inhaled agents and to better define the potency limits achievable with a small molecule VLA-4 antagonist. To this end, we elected to investigate a stereo defined synthesis of the constrained tyrosine and its incorporation into analogues of **9**. An efficient synthesis of (2S,3R)-3-(4-hydroxyphenyl)proline **18** was developed employing as a key step, a stereo selective Michael addition of the Schöllkopf reagent **15** to the hydroxyphenylcinnamic ester **14** as shown in scheme 1. [23] This intermediate was employed in the synthesis of **20** and a similar route was used in the preparation of **21-23**.

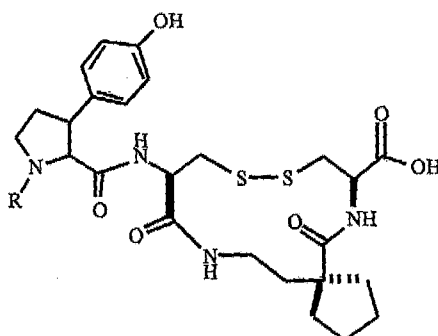
Assay data for this series of compounds is shown in Table 3. From this data, it is readily apparent that (+)-*trans*-isomers **20**, **22** and **23** are highly preferred over the (±)-*cis*- and (-)-*trans*- isomers, independent of the nature of substitution on the N-terminal nitrogen. The activity is remarkably sensitive to the presence of the 4-hydroxyl group. For example, the corresponding phenyl derivative **24** is more than 300-fold less potent than **20** in the Ramos cell based assay. Compounds **20**, **22** and **23** represent the most potent VLA-4 antagonists we have seen and represent a benchmark for our small molecule program.

In order to utilize this information in future design efforts, it would be necessary to localize the tyrosine hydroxyl group with respect to the key pharmacophores incorporated into the ring system. In an attempt to derive this information, we examined **20** by NMR. The seventeen lowest energy conformers are shown in Fig. (2a). As



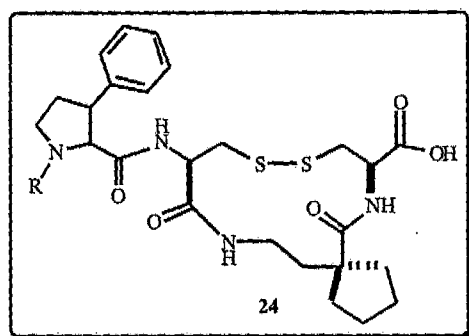
Scheme 1.

Table 3. Effect of Stereochemistry of Constrained Tyrosine Derivatives on Potency as VLA-4 Antagonist Activity.



Compd	Stereoisomer	R	ELISA IC ₅₀ nM	Ramos IC ₅₀ nM
1	-	-	3.4	65
19	(±)- <i>cis</i>	Ac	1.70	3.2
20	(+)- <i>trans</i>	Ac	0.14	0.24
21	(-)- <i>trans</i>	Ac	1.7	24
22	(+)- <i>trans</i>	H	0.19	0.15
23	(+)- <i>trans</i>	Pivaloyl	0.14	0.18

anticipated based on our results with 1, the ring system is conformationally well defined. Although the N-terminal residue has essentially free rotation about the exocyclic N-C bond, the position of the tyrosine hydroxyl group falls into a fairly narrow region relative to the other key pharmacophores. The effect of adding the constraint to the tyrosine is highlighted by comparison with the unconstrained analogue, 6 as illustrated in Fig. (2b). Careful examination of the 5-membered ring of the constrained tyrosine moiety reveals that it too exists in at least two rapidly interchanging conformations, which are not reflected in the conformations shown in Fig. (2a).



This work demonstrated that molecules of MW <600 are capable of exquisitely potent VLA-4 antagonist activity. Large potency gains are possible by optimizing the N-terminus of these molecules, but lacking additional information, development of a detailed understanding of the three-dimensional relationship of the pharmacophores is

extremely challenging. In the next section, we will describe our efforts to apply these learnings in the context of the acylphenylalanine class of VLA-4 antagonists.

ACYLPHENYLALANINES

At the same time the SAR studies on the cyclic peptide leads were initiated, and guided by the belief that the arginine and aspartic acid were important residues that constituted the binding epitope, we searched our collection for compounds that incorporated the elements of a guanidine and a carboxylic acid separated by a spacer. As shown in chart 2, the acid 25, which had been prepared as a member of a series of interleukin 2 antagonists, [24] was found to be a weak VLA-4 antagonist, IC₅₀ 7 μM. Since we had access to a large number of related compounds, we were able to quickly establish that the guanidine did not contribute to the activity and that potency could be improved by variation of both the N-acyl and the 4-substituents. The thiaproline and proline analogues 28 and 29 were prepared in recognition that the acylphenylalanines might be interacting with the same binding site as the cyclic peptides. Combining these modifications with the 4-carboxypentynyl- substitution present in 27 led to the relatively potent inhibitors 30 and 31. The sensitivity of potency to the structural features mentioned and consistency of these results provided us with confidence that the acylphenylalanines constituted a bona fide lead series.

To facilitate our SAR studies, we divided the lead molecule into three parts as shown in Fig. (3) and choose to investigate the "N-Cap", the "Core" and the "Distal" regions

a.



b.

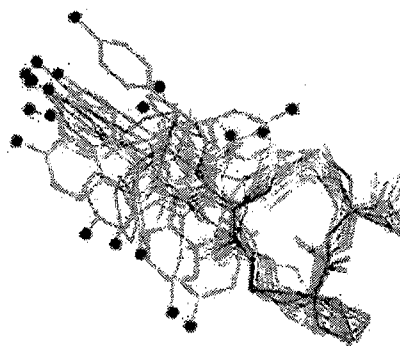


Fig. (2) (a) Overlay of the 17 lowest energy conformers of 20 as determined by NMR. (b) Overlay of the 20 low energy conformers of 6. In both Figs, the black balls represent the location of the tyrosine oxygen atoms.

separately. SAR studies on the "Core" as summarized in Fig. (4) indicated that this portion of molecule is very sensitive to modification. The carboxylic acid and S-stereochemistry are absolute requirements for activity and substitution of heterocyclic rings for the phenyl or addition of an alkyl group to either the *alpha*- or *beta*-carbons of phenylalanine led to a marked reduction in potency. Alkylation of the amide nitrogen atom also led to a loss of activity, but since this substitution has significant conformational effects, as indicated by our molecular modeling experiments, we were unable to conclude whether the amide NH was playing a role in receptor recognition.

We focused next on "N-Cap" optimization and chose to incorporate building blocks selected from a set of readily

available low molecular weight carboxylic acids as shown in scheme 2. The N-Fmoc-4-substituted phenylalanines, selected from intermediates available from our IL-2 program, were coupled to Wang resin under standard conditions. After removal of the protecting group, the carboxylic acids were coupled to the free amino group of the phenylalanine derivatives under standard conditions, followed by acid cleavage to provide 68 analogs of N-acylphenylalanine.

Compounds which had an average of greater than 80% inhibition at 10 μ M and MW < 500 were selected for IC₅₀ determination. The results indicate that both N-aroyl (37 and 39) and N-alkanoyl (35 and 36) analogues of phenylalanine showed mid-nanomolar inhibition. The most potent

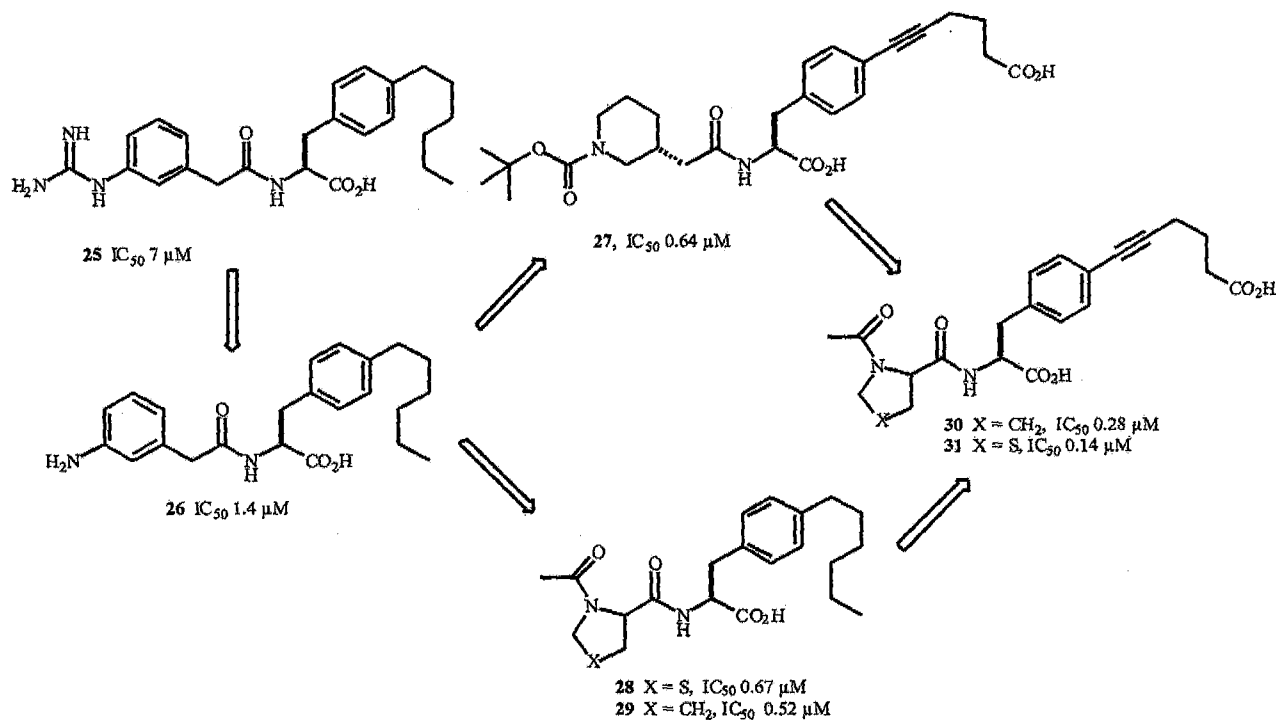


Chart 2. Early discovery work in the acylphenylalanine class of VCAM/VLA-4 antagonists.

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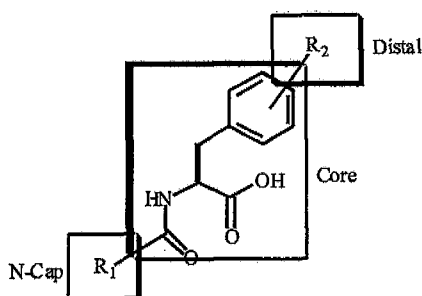


Fig. (3). Regions of the acylphenylalanine system considered separately for lead optimization.

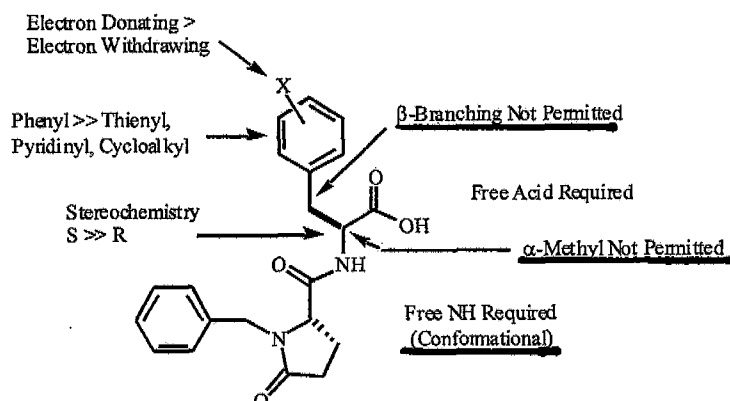


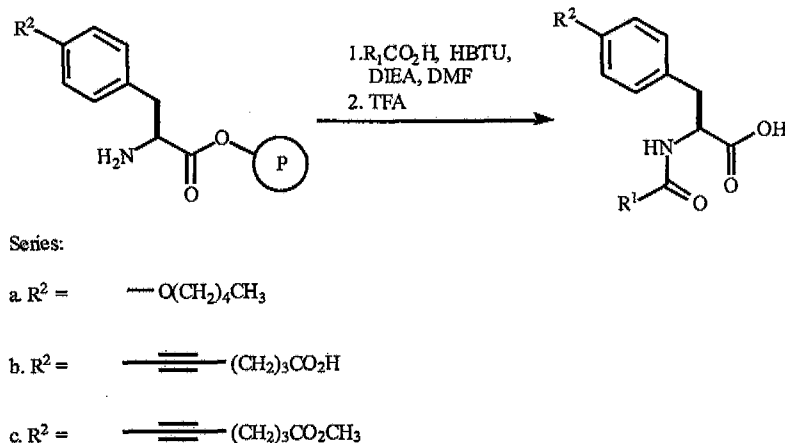
Fig. (4). Summary of structure-activity studies carried out on the acylphenylalanine core.

compound from this library was **35**, which was derived from N-benzylpyroglutamic acid, with an IC₅₀ of 46 nM. Combination of this acyl group with the 4-hexynoate substituent gave **40** with an IC₅₀ of 20 nM. [25] This “N-cap” was incorporated into our first generation of libraries intended to explore the “distal” region.

One intermediate, the 3-hydroxymethyl analogue **42** prepared as part of our follow up to this work, yielded an X-ray crystal structure shown in spacefilling form in Fig. (5b). This structure represents a low energy conformation that may reflect the bioactive conformation of compounds related to **35**. Interestingly, a comparison of the X-ray structure of

42 with the most populated ring conformer of the cyclic peptide **1** revealed a high degree of overlap with the 5-membered proline and pyroglutamyl rings and the carboxyl groups. In this overlay shown in Fig. (5c), the phenylalanine benzene ring extends to the area of the disulfide bond of **1**. This finding supports our hypothesis that the cyclic peptides related to **1** and the acylphenylalanines under investigation bind to the same site on VLA-4.

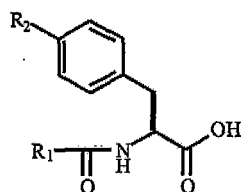
Combining our earlier observation that the disulfide is sensitive to substitution and hence, could be involved in binding with the observation that the benzene ring of the acylphenylalanines is intolerant of even conservative



Scheme 2

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Table 4. Inhibition Activity of VLA4 Antagonists.



Compd	R ₁	R ₂	VCAM/VLA-4 ELISA	
			% Inb (10 μ M)	IC ₅₀ (μ M)
34		$-\text{O}(\text{CH}_2)_4\text{CH}_3$	90	0.87
35		$-\text{O}(\text{CH}_2)_4\text{CH}_3$	97	0.046
36		$-\text{C}\equiv\text{C}-(\text{CH}_2)_3\text{CO}_2\text{CH}_3$	99	0.135
37		$-\text{C}\equiv\text{C}-(\text{CH}_2)_3\text{CO}_2\text{CH}_3$	94	0.317
38		$-\text{C}\equiv\text{C}-(\text{CH}_2)_3\text{CO}_2\text{H}$	80	0.187
39		$-\text{O}(\text{CH}_2)_4\text{CH}_3$		0.245
40		$-\text{C}\equiv\text{C}-(\text{CH}_2)_3\text{CO}_2\text{H}$		0.020
41		$-\text{O}(\text{CH}_2)_4\text{CH}_3$		0.060

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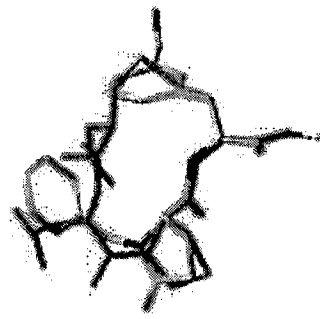
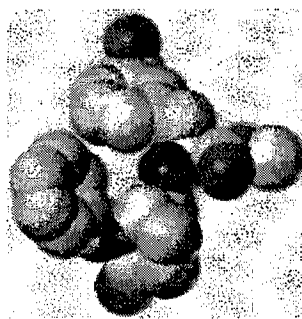
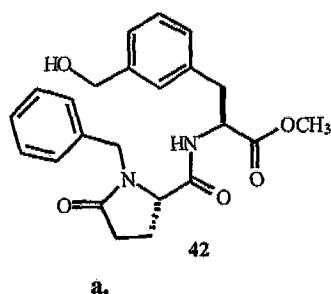


Fig. (5). (a) Structure of **42**. (b) Spacefilling view of the X-ray crystal structure of **42**. (c) Overlay of one of lowest energy conformational families of **1** with the X-ray derived conformation of **42**.

changes led to the conclusion that these moieties do contribute to the overall binding energy and are not mere scaffolding. If this conclusion is correct, then the 3- and 4-positions of the phenylalanine benzene ring point to the region of the N-terminal group present on the cyclic peptides which is critical for high potency. Based on these considerations, we focused our efforts into exploring and optimizing these substitutions.

A sample of some of the phenyl ring substitutions that were explored is shown in Chart 3. One of the most

interesting groups of compounds to emerge from this effort was the benzoylamino derivatives. For this series, substitution in the 4-position is preferred over the 3-position. [26] The data indicate that a number of these compounds combine sub-nM IC₅₀s in the ELISA assay with low nM activity in the Ramos cell assay. The most potent compounds feature an electron deficient aromatic rings with at least one bulky *ortho* substituent. The presence of these elements is more important than their identity. For example the 2-nitro- (**43**), the 2-methoxy-5-nitro- (**44**) and the 2,6-dichloro- (**45**) analogues all have similar profiles [27].

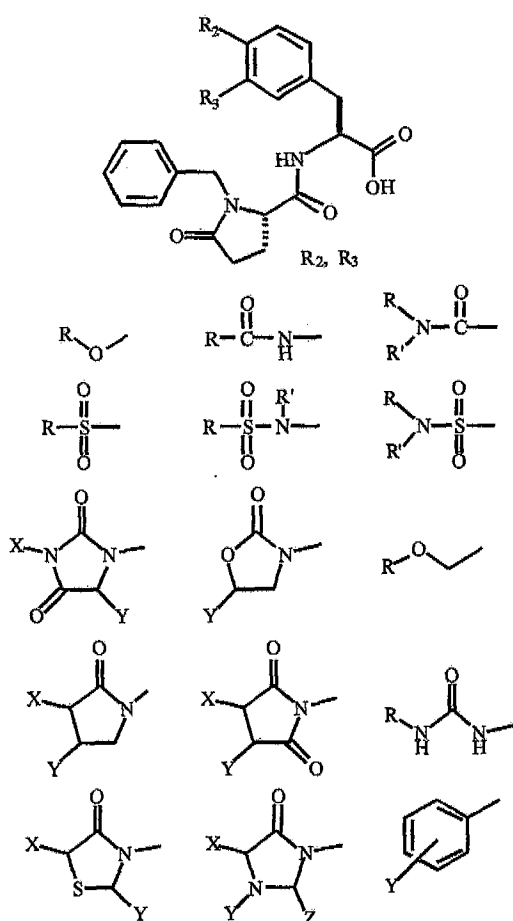


Chart 3. Compilation of phenylalanine substituents explored during SAR studies.

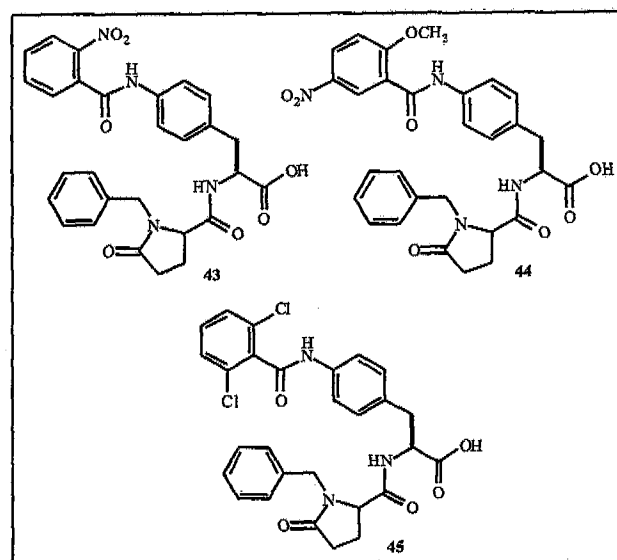


Table 5. Activity of N-benzylpyroglutamyl Phenylalanine Derivatives.

Compd	ELISA IC ₅₀ nM	Ramos Cells IC ₅₀ nM
43	0.87	33
44	0.82	28
45	0.37	12

Development of an ex vivo binding assay based on our standard ELISA assay enabled us to readily quantitate blood levels. To our disappointment, this assay procedure revealed

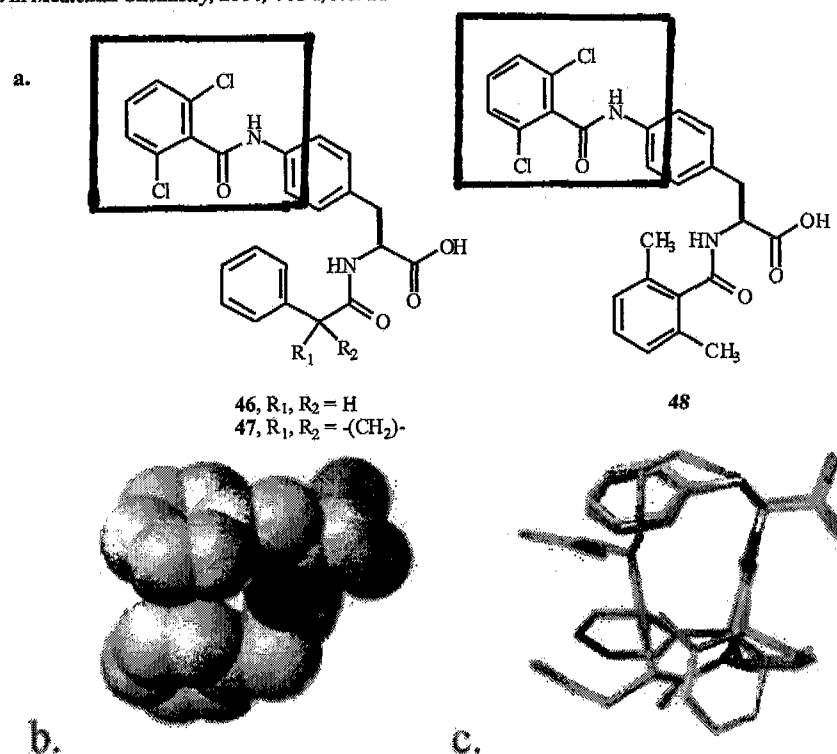


Fig. (6). a) structure of 46-48. b) Spacefill model of the X-ray crystal structure of 46. c) Overlay of modeled structure of the cyclic peptide 1 (47), and 48.

that compounds related to 43-45, all exhibited rapid clearance from mouse plasma after iv administration. More detailed experiments with one member of the series revealed that the molecule was cleared intact in the feces, suggesting that rapid metabolism was not the problem. All of these compounds are quite polar with LogD values around -2 at pH 7.4. Evaluation of a variety of compounds incorporating some of the other "distal" groups shown in Chart 3 in an attempt to increase lipophilicity without seriously compromising potency led to several compounds with lower molecular weight and higher LogD, but little improvement in

clearance. Thus we concluded that the required carboxyl group and the N-benzylpyroglutamic acid moiety itself was at least partly responsible for the observed fast clearance. This result prompted us to explore further optimization of "N-Cap" region of the molecule through the incorporation of more lipophilic acyl groups.

To this end, compounds 47 and 48 were designed based on the hypothesis that an intramolecular CH- π interaction may facilitate the *N*-acylphenylalanines adoption of a gauche (-) conformation which mimics the core structure of the potent peptide VCAM/VLA-4 inhibitors such as 1. The

Table 6. VCAM/VLA-4 Binding Inhibition of *N*-(1-alkyl)cycloalkanoyl-4-[(2,6-dichlorophenyl)carbonyl]amino-L-phenylalanine Derivatives.

Compd	R	ELISA IC ₅₀ nM	Ramos IC ₅₀ nM
49		1.0	134
50		0.26	12
51		0.61	17

(Table 6. Contd....)

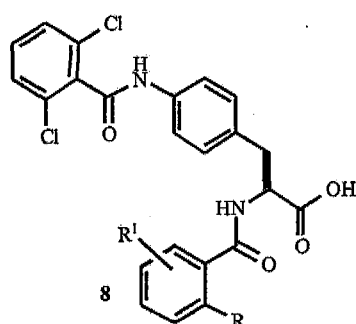
Compd	R	ELISA IC ₅₀ nM	Ramos IC ₅₀ nM
52		0.32	29
53		0.50	27
54		0.52	39
55		1.1	150
56		0.29	62
57		0.8	75
58		1.8	528
59		1.1	94
60		0.15	8

published X-ray crystal structure of *N*-phenylacetyl-L-phenylalanine (**46**) features a gauche (-) conformation of the phenylalanine and an edge-to-face interaction between the two benzene rings as shown in Fig. (6). [28] The methylene hydrogens of **46** were morphed into a cyclopentane ring and the resulting compound **47** was minimized using the Sybyl molecular modeling program. Both **47** and **48** proved to be potent VCAM/VLA-4 antagonists prompting us to expand the series to look more carefully at optimization of the "N-caps". [29]

The structure and activity of a selection of the homologous cycloalkyl [30] derivatives that were prepared during the course of this effort are shown in Table 5 and the

aryl [31] derivatives are depicted in Table 6. Many of these compounds are very potent and these results demonstrate that VLA-4 antagonists accommodate a variety of acyl substituents on the phenylalanine nitrogen. Some of these compounds have the anticipated improved clearance. The pharmacology of these compounds will be described in detail in future publications.

In order to provide more insight into the observed structure-activity relationships, we obtained crystal structures of the intermediate methyl esters **83** and **84** shown in Fig. (7) as space filling models. These structures are consistent with our general hypothesis that the acylphenylalanines adopt a gauche (-) conformation that mimics the pharmacophore

Table 7. VCAM/VLA-4 Binding Inhibition of *N*-aroyl-4-[(2,6-dichlorophenyl)carbonyl]amino]-L-phenylalanine Derivatives.

Compd	R	R'	ELISA IC ₅₀ nM	Ramos IC ₅₀ nM
61	Br	H	0.49	45
62	OCH ₃	H	8.6	6,800
63	NHCH ₃	H	1.9	1,000
64	CF ₃	H	16	650
65	SCH ₃	H	0.40	100
66	SO ₂ CH ₃	H	3.3	160
67	C(CH ₃) ₃	H	1.9	74
48	CH ₃	6-CH ₃	1.3	84
68	CH ₃	6-C ₂ H ₅	0.30	10
69	CH ₃	6- <i>n</i> -C ₃ H ₇	0.42	17
70	CH ₃	6- <i>i</i> -C ₃ H ₇	1.2	23
71	CH ₃	6-F	0.44	85
72	CH ₃	6-Cl	0.30	12
73	CH ₃	6-Br	0.20	9
74	<i>i</i> -C ₃ H ₇	6- <i>i</i> -C ₃ H ₇	11	460
75	F	6-F	1.2	67
76	F	6-CF ₃	0.40	85
77	Cl	3-CH ₃	1.4	1,300
78	Cl	5-CH ₃	11	460
79	Cl	4-OH	0.46	33
80	Cl	4-OCH ₃	1.9	350
81	Cl	4-SO ₂ CH ₃	0.25	9.5
82	Cl	4-Br	1.60	1,300

displayed by the cyclic peptides. There have also been a number of publications from other laboratories on VLA-4 antagonists from the acylphenylalanine class [32]. Compounds **84-87** [19,32-33] constitute a representative selection of key structures and emphasize the diversity of substitution tolerated on both the phenylalanine nitrogen and 4-positions. We have not yet succeeded in finding the elusive

hydroxyl binding site observed among the cyclic peptides related to **4** even though the search drove much of our exploration of diversity in the acylphenylalanine class. It remains to put these observations into the context of integrin structure and attempt to draw conclusions that may be useful in guiding the design of future antagonists.

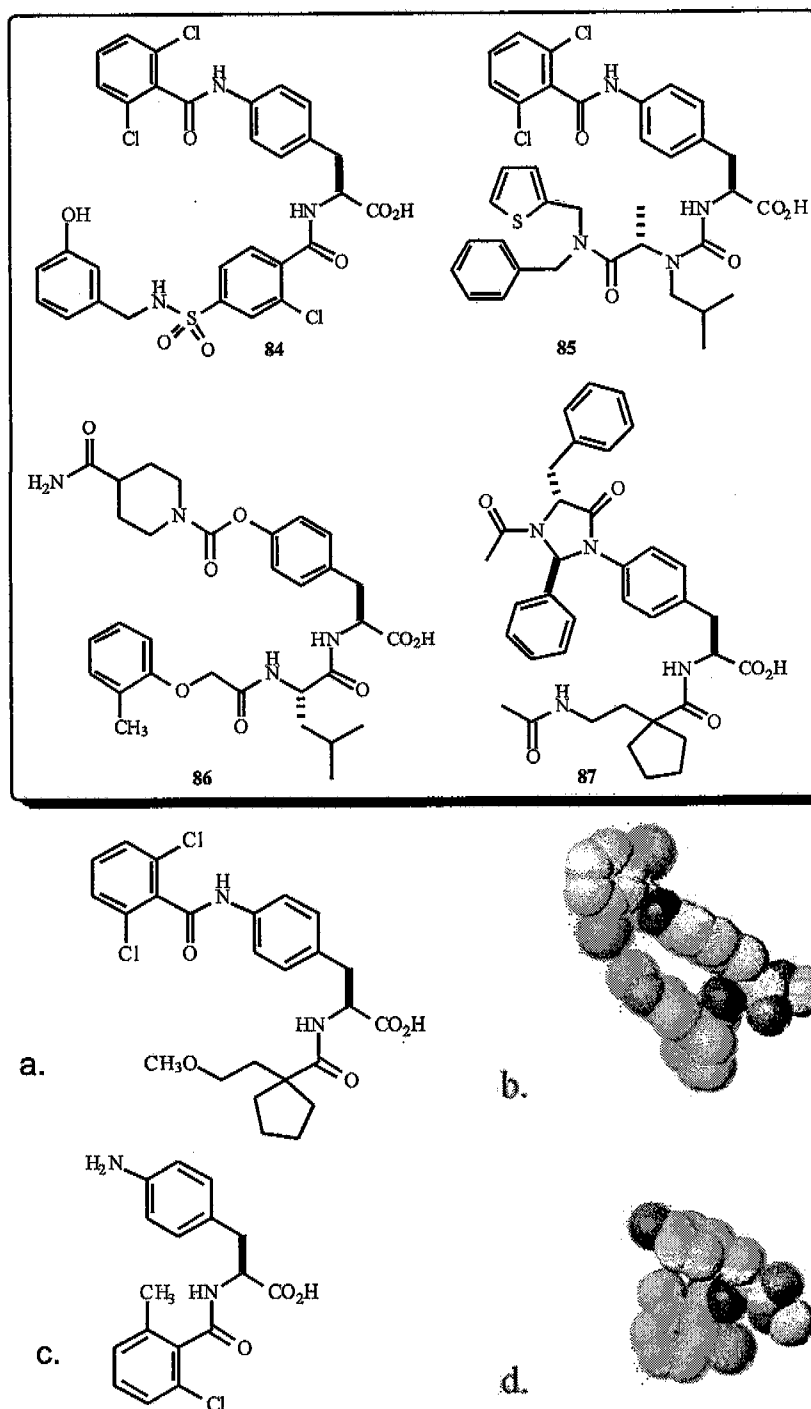


Fig. (7). (a and c) Structures of 83 and 84. (b and d) spacefilling representation of the X-ray crystal structure of 83 and 84 respectively.

It has long been hypothesized that integrin ligands bind at the interface between the alpha and beta chains. Recently the structure of a complex between the integrin $\alpha v \beta 3$ with a cyclic peptide containing the signature Arg-Gly-Asp motif became available. This structure confirms that the ligand bridges the heterodimer with the aspartic acid moiety associated with a metal ion in the MIDAS-like domain on the β -subunit and the guanidine of the arginine binding

two aspartic acids within the β -propeller domain of the α -subunit [34].

We hypothesize that ligands for VLA-4 bind in a similar manner to the RGD mimics. Thus the requisite carboxylic acid present in all peptide and phenylalanine antagonists could interact with a metal ion in the MIDAS-like domain on the beta chain. This domain could further provide an

associated hydrophobic pocket capable of accommodating both the small hydrophobic rings present in cyclic peptides and several classes of acylphenylalanines and the hydrophobic group in the *ortho*-position of aroylphenylalanines. Since a large variety of substitutions on the 1- or 2-positions of the hydrophobic rings or the 4-position of the aroyl moieties are permitted, these substituents may extend into solvent, away from the hydrophobic binding pocket. Differences in potencies among these substituents may be related to secondary interactions or solvation effects. The moiety on the 4-position of the phenylalanine could bind to appropriate recognition elements on the beta-propeller domain of the integrin alpha chain. The inherent conformational flexibility of the of the integrin chains, and to a lesser extend the ligands themselves, could permit these substituents access to a variety of binding sites within the beta-propeller domain and account for the diversity of structures capable of binding with high affinity. We eagerly await the availability of additional structural studies and particularly, data relating more directly to VLA-4 and its ligand interactions to verify the hypothetical binding mode.

ACKNOWLEDGEMENT

The authors thank our many Roche colleagues who contributed to this work and also to John Roberts for his helpful suggestions during the preparation of this manuscript.

ABBREVIATIONS

VLA-4 = Very late antigen 4

VCAM = Vascular cell adhesion molecule

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Catalytic Cross-coupling Reactions in Biaryl Synthesis

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Received 14 October 1997

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1. INTRODUCTION

1.1 General Aspects and Overview

Biaryls ($\text{Ar}^1\text{-Ar}^2$) and their homologues such as teraryls, oligoaryls and polyaryls are an important class of organic compound; the biaryl unit is represented in several types of compounds of current interest including natural products, polymers, advanced materials, liquid crystals, ligands and molecules of medicinal interest. In view of the tremendous importance of biaryls, a number of catalytic methods for forming these molecules from two monoaryl precursors in a cross-coupling reaction have been developed over the last two decades.

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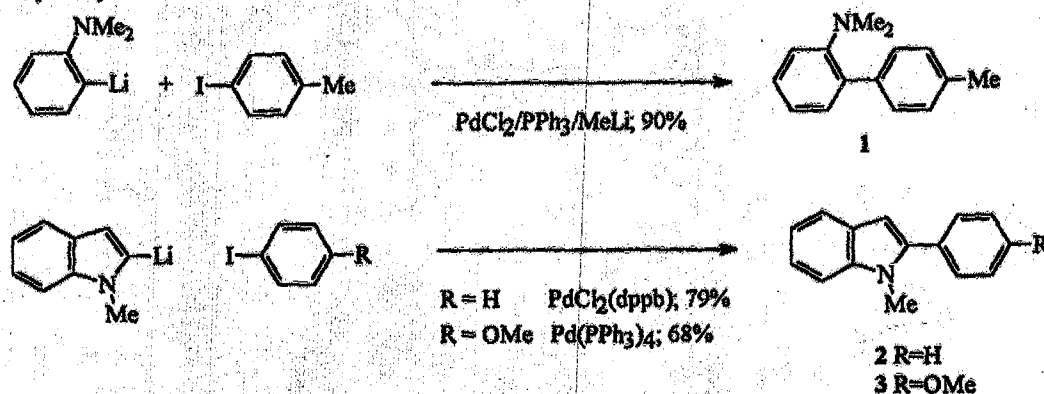
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This Report will review the development of modern *catalytic* methods in biaryl synthesis and will provide a broad perspective of the more important methodologies as well as reporting methods which have not yet found widespread use. Methods which are not catalytic are not covered in this Report and neither are methods which can only give homo-coupled products.

The four most commonly used catalytic methods in biaryl synthesis are the Kharasch, Negishi, Stille and Suzuki reactions. These reactions enable the preparation of both symmetrical and unsymmetrical biaryls in a cross-coupling reaction and invariably proceed using either nickel or palladium catalysts.

The Kharasch reaction began to achieve importance as a method for biaryl synthesis in the mid to late 1970's. In this reaction an aryl Grignard reagent (Ar^1MgX , X = halogen) is generally reacted with an aryl halide (Ar^2X) in the presence of an appropriate catalyst to yield the biaryl ($\text{Ar}^1\text{-Ar}^2$). Other functionalised aryls can also partner the Grignard reagent in this reaction, for example, phenolic derivatives such as triflates, mesylates and ethers have been used as well as thiophenolic derivatives such as sulphides and sulphones. One disadvantage of the Kharasch reaction is that the polar nature of the Grignard reagent precludes the use of several types of functional groups in the coupling partner such as aldehydes, ketones, esters and nitro groups.

The Negishi reaction utilises arylzinc reagents (Ar^1ZnX , X = halogen) and aryl halides or triflates (Ar^2X , X = halogen or triflate) and began to assume importance in the mid 1970's. Unlike the Kharasch reaction, functional groups such as aldehydes, ketones, esters, amines and nitro groups *etc* are tolerated in the coupling partner of the arylzinc reagent. Although arylmagnesium and arylzinc reagents are precursors to biaryls in the Kharasch and Negishi reactions respectively, aryllithiums are not generally used due to their highly polar and basic nature. There are however, a few isolated examples where aryllithiums have been used successfully in biaryl synthesis as illustrated by the formation of compounds 1-3 (Scheme 1).¹⁻³



Scheme 1

In the late 1970's the Stille reaction started to be used in biaryl synthesis and this reaction uses arylstannanes (Ar^1SnR_3 , R = Me, Bu) and aryl halides or triflates (Ar^2X , X = halogen or triflate) as the coupling partners. This reaction is extremely versatile, proceeds under neutral conditions and can tolerate a wide range of substituents on both coupling partners. Thus, substituents which are not compatible with the Kharasch and Negishi reactions are often tolerated in the Stille reaction. The major disadvantage of the Stille reaction is the toxicity of the organotin reagents and byproducts.

The early 1980's saw the advent of the Suzuki reaction. The Suzuki reaction, like the Stille reaction, has proved extremely versatile and has found extensive use in natural product synthesis. Boronic acids [$\text{Ar}^1\text{B(OH)}_2$] are the usual substrates in this reaction together with aryl halides or triflates (Ar^2X , X = halogen or triflate) although esters of boronic acids and arylboranes are frequently used.

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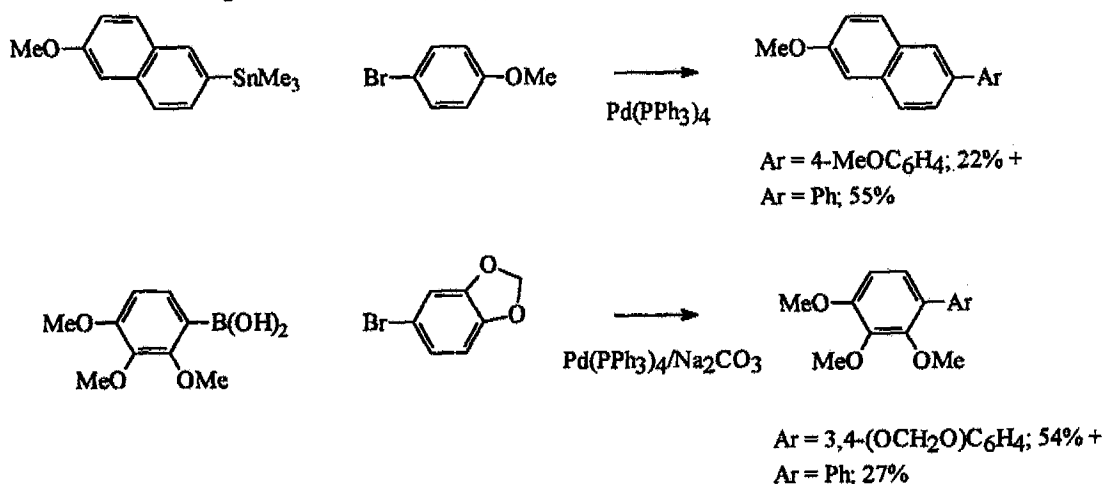
1.2 Catalysts and Catalytic Cycles

Numerous palladium and nickel catalysts have been employed in the Kharasch, Negishi, Stille and Suzuki reactions. The catalyst is frequently added as a metal(II) complex from which the catalytically active metal(0) species is generated *in situ*. The palladium(0) complex, $\text{Pd}(\text{PPh}_3)_4$ is one example of a preformed catalyst which has found widespread application in cross-coupling reactions. Palladium black has been successfully used as a catalyst on several occasions and water soluble catalysts have been developed for cross-coupling reactions in aqueous solution. Metals possessing chiral ligands have been used for the preparation of biaryls with axial chirality (atropisomers).

Most catalysts are associated with arylphosphine ligands and one of the consequences of using such ligands is the formation of 'scrambled' products in which the aryl group of the phosphine ligand has become incorporated into the product. In most cases this is not a major problem but two examples where considerable 'scrambling' has been observed in a Stille reaction⁴ and a Suzuki reaction⁵ are illustrated in Scheme 2. Arylphosphine ligands are often responsible for chain termination reactions in the synthesis of polyaryls and 'ligandless' catalysts have therefore been advocated for use in polymerisations.⁶

Other side reactions which are sometimes encountered to varying extents are homocoupling reactions and reductions and these reactions will be discussed at relevant points in the text.

The structures of ligands contained in this Report are given in Section 8.

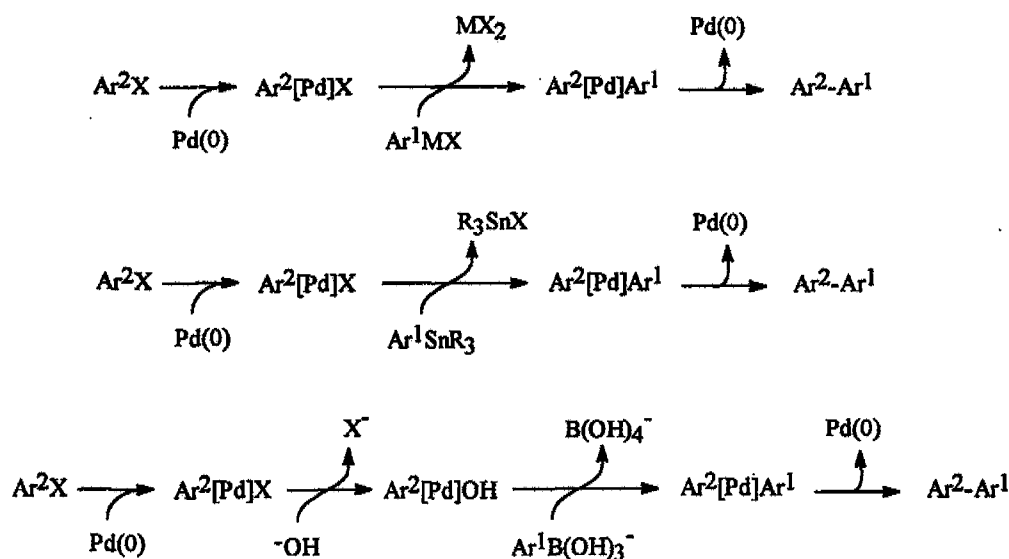


Scheme 2

The catalytic process associated with the Kharasch, Negishi, Stille and Suzuki reactions can be represented as shown in Scheme 3 which illustrates these reactions with an aryl halide (Ar^2X , X = halogen) as one of the coupling partners in a palladium catalysed reaction. Potential ligands of the palladium(0) intermediates have been omitted for clarity and the process depicted in Scheme 3 can obviously be extended to nickel catalysed reactions and substrates other than aryl halides, for example aryl triflates. When aryl triflates are used, lithium chloride is an essential additive in the Stille reaction.

All the reactions shown in Scheme 3 have three common steps; firstly an oxidative addition of the catalyst to the aryl halide giving an intermediate $\text{Ar}^2[\text{Pd}]\text{X}$; secondly a transmetallation step to yield a diarylated palladium moiety, $\text{Ar}^2[\text{Pd}]\text{Ar}^1$; and finally a reductive elimination from the diarylated palladium compound giving the biaryl product and the palladium(0) catalyst which re-enters the catalytic cycle.

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Scheme 3

2. THE KHARASCH REACTION

2.1 Chemoselectivity and Regioselectivity

The reaction of 4-bromochlorobenzene with phenylmagnesium bromide catalysed by $\text{PdPh}(\text{PPh}_3)\text{I}$ gave 4-chlorobiphenyl **6** (73%) showing that the bromo substituent can be selectively coupled in the presence of a chloro substituent.⁷ With these reagents and either NiCl_2 or $\text{Ni}(\text{dpe})\text{Cl}_2$ as the catalyst the mono phenylated product **6** (18-71%) has been reported together with the diphenylated product, *para*-terphenyl **13** (3-37%) with the mono: diphenylated ratio depending upon the reaction conditions and the reagent stoichiometries.⁸ 2-Tolylmagnesium bromide and 4-bromochlorobenzene gave higher yields (38-87%) of the biaryl **15** with various catalysts with very little of the corresponding teraryl product being formed (3-12%).⁸ This Grignard reagent and 2-bromochlorobenzene afforded only 2-chloro-2'-methylbiphenyl **14** (32-49%).⁸ The iodo substituent in 4-fluoroiodobenzene was also selectively replaced with phenylmagnesium bromide in a $\text{PdPh}(\text{PPh}_3)\text{I}$ catalysed reaction giving 4-fluorobiphenyl **8** (74%).⁷

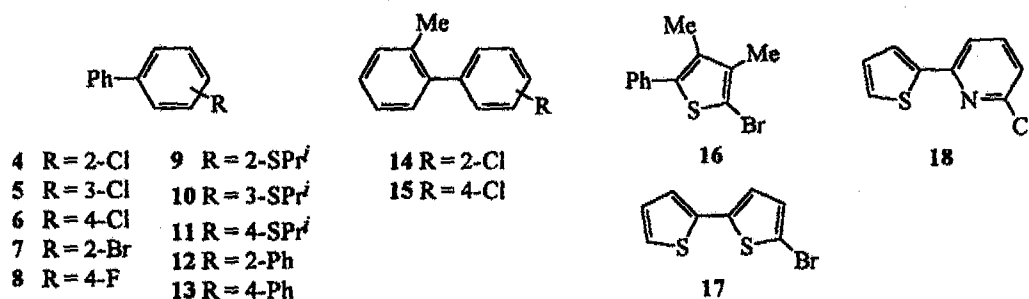
The preferential replacement of a methylthio group over an isopropylthio group has been reported as illustrated for the formation of biaryl **9** from phenylmagnesium bromide and the *ortho*-disulphide, 1,2-(MeS)(Pr^iS) C_6H_4 in the presence of $\text{Ni}(\text{PPh}_3)_2\text{Cl}_2$ as the catalyst.⁹ A chloro substituent can also be selectively replaced in the presence of an isopropylthio group as exemplified by the formation of all three isomeric biaryls **9-11** (65-84%) from 1,*n*-Cl(Pr^iS) C_6H_4 using $\text{Ni}(\text{PPh}_3)_2\text{Cl}_2$ as the reaction catalyst.¹⁰

In dihaloaryls, where both halogen substituents are in identical chemical environments, it is often possible to replace only one halogen substituent in the Kharasch reaction. Thus, in a $[\text{Ni}(\text{triphos})\text{Cl}]\text{PF}_6$ catalysed reaction, 1,2-dichlorobenzene and phenylmagnesium bromide gave 2-chlorobiphenyl **4** (53%) together with some of the diphenylated product, *ortho*-terphenyl **12** (17%).¹¹ Biaryl **4** (79%) has also been obtained from phenylmagnesium chloride and 1,2-dichlorobenzene with $\text{Pd}(\text{dppf})\text{Cl}_2$ as the catalyst and this Grignard reagent and 1,3-dichlorobenzene gave 3-chlorobiphenyl **5** (85%) in an analogous reaction.¹² Similarly, 1,2-dibromobenzene and phenylmagnesium bromide gave biaryl **7** (75%) with very little (2-3%) of *ortho*-terphenyl **12** being formed in a $\text{Pd}(\text{dppb})\text{Cl}_2$ catalysed reaction.¹³

Examples of the selective replacement of one halogen in heterocyclic dihalo compounds are provided by formulae 16-18.¹³⁻¹⁵ Thus, phenylmagnesium bromide reacted with 2,5-dibromo-3,4-dimethylthiophene in the

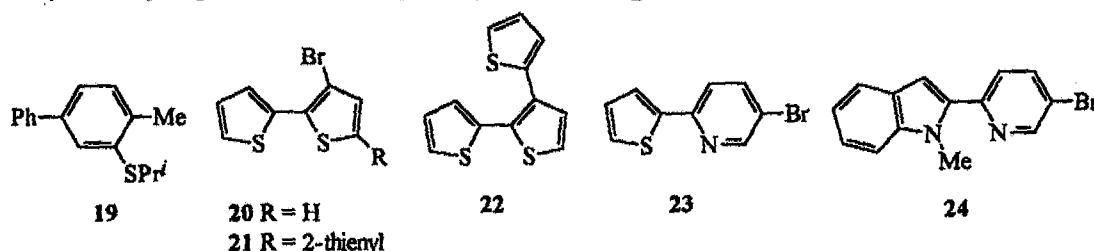
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presence of $\text{Pd}(\text{PPh}_3)_4$ giving product **16** (72%).^{13,13a} 2-thienylmagnesium bromide and 2,5-dibromothiophene yielded the bithienyl **17** (53%) in a $\text{Pd}(\text{dppf})\text{Cl}_2$ catalysed reaction¹⁴ and 2,6-dichloropyridine and 2-thienylmagnesium bromide gave the heterobiaryl **18** (55%) with $\text{Pd}(\text{dppb})\text{Cl}_2$ as catalyst.¹⁵ Other substituents besides halogens can also be replaced with some selectivity: 1,4-dimethoxybenzene and phenylmagnesium bromide gave a mixture of 4-methoxybiphenyl (33%) and *para*-terphenyl **13** (24%) in a $\text{Ni}(\text{PPh}_3)_2\text{Cl}_2$ catalysed reaction.¹⁶



2,4-Bis(isopropylthio)toluene reacted regiospecifically with phenylmagnesium bromide in benzene at 50°C [$\text{Ni}(\text{PPh}_3)_2\text{Cl}_2$ catalyst] yielding biaryl **19** (55%) in which the less sterically crowded isopropylthio group had been replaced.⁹ A 1:1 mixture of 2-thienylmagnesium bromide and 2,3-dibromothiophene afforded the bithienyl derivative **20** (82%) with $\text{Pd}(\text{dppf})\text{Cl}_2$ as the catalyst whereas with a 2:1 mixture of these reagents the terthiophene **22** (85%) was produced.¹⁴ A 3:1 mixture of this Grignard reagent and 2,3,5-tribromothiophene resulted in the displacement of only the 2- and 5- bromo substituents yielding the terthiophene **21** (89%).¹⁴

The 2-bromo substituent in 2,5-dibromopyridine can be selectively replaced as illustrated by the formation of the heterobiaryls **23** (95%) and **24** from 2,5-dibromopyridine and 2-thienylmagnesium bromide and 1-methyl-2-indolylmagnesium bromide respectively in $\text{Pd}(\text{dppb})\text{Cl}_2$ catalysed reactions.¹⁵



2.2 Steric Considerations

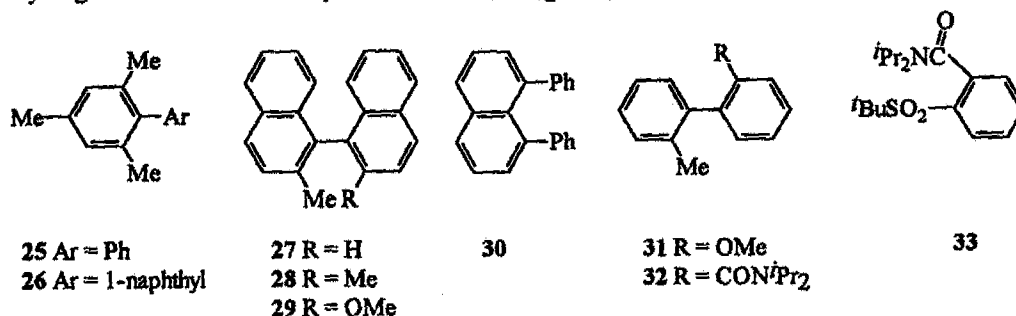
The palladium catalysed Kharasch reactions of mesitylmagnesium bromide and either bromobenzene or iodobenzene can give good yields of the biaryl **25**.^{3,7,17} With chlorobenzene as the coupling partner, only low yields of this product are obtained.^{17,18} Mesitylmagnesium bromide can also be coupled successfully with 2-bromonaphthalene in a $\text{Ni}(\text{dppe})\text{Cl}_2$ catalysed reaction giving biaryl **26** in moderate (45%) yield.¹⁸ Binaphthyl derivatives such as compounds **27-29** (55-79%) have been prepared from 2-methyl-1-naphthylmagnesium bromide and an appropriate bromonaphthalene coupling partner using $\text{Ni}(\text{PPh}_3)_2\text{Cl}_2$ as the catalyst.¹⁹

An excess of phenylmagnesium iodide reacted with 1,8-diiodonaphthalene giving 1,8-diphenylnaphthalene **30** in 70% yield. This reaction occurred in a mixture of ether and benzene at -10 to -15°C with $\text{Ni}(\text{acac})_2$ as the catalyst.²⁰

In cases where both coupling partners possess *ortho* substituents the yields of biaryls are generally not good. Thus, 2-methoxyphenylmagnesium bromide and 2-bromotoluene gave a low yield (8%) of biaryl **31** with 0.02 equivalents of $\text{Pd}(\text{PPh}_3)_4$ as the catalyst but the yield could be increased to 39% when a stoichiometric

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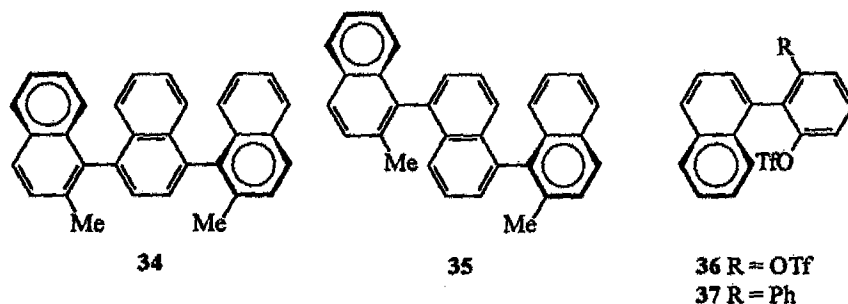
quantity of this catalyst was used.³ The biaryl **32** was formed (42%) from the reaction between 2-tolylmagnesium bromide and sulphone **33** in a $\text{Ni}(\text{acac})_2$ catalysed reaction in THF at 20°C.²¹



Several groups have reported the synthesis of the chiral binaphthyl **28** with a range of enantiomeric excesses (ee's). Early examples used chiral nickel catalysts and gave poor ee's as well as low yields^{17,22} whereas later examples gave good ee's (95%) and yields (69–77%).^{23,24,24a} With a chiral palladium catalyst, only low yields and poor ee's were obtained.²⁵

Chiral ternaphthyls have been prepared from 2-methyl-1-naphthylmagnesium bromide and dibromonaphthalenes using a mixture of NiBr_2 and the chiral ligand, (*S*)-(*R*)-PPFOMe, as the catalyst.²⁶ Thus, this Grignard reagent with 1,4-dibromonaphthalene afforded an 86:14 mixture (74%) of the ternaphthyl **34** (95% ee) and its corresponding meso isomer, and 1,5-dibromonaphthalene gave an 84:16 mixture (89%) of compound **35** (99% ee) and its meso isomer.

In an enantio position-selective cross-coupling reaction, the pro-*R* triflate group in biaryl **36** was replaced by phenylmagnesium bromide yielding the teraryl **37** (87%, 93% ee) in a $\text{Pd}[(\text{S})\text{-phephos}]\text{Cl}_2$ catalysed reaction at -30°C.²⁷



2.3 General Survey of the Kharasch Reaction

The normal coupling partner for a Grignard reagent in the Kharasch reaction is an arylhalide but a number of other partners have been used. Thus, 2-phenylnaphthalene **38** has been prepared from phenylmagnesium bromide and the following compounds: 2-methoxynaphthalene **39** [77% yield, $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ ²⁸ or $\text{Ni}(\text{PPh}_3)_2\text{Cl}_2$ ¹⁶ as the catalyst]; tosylate **40** [60% yield, $\text{Ni}(\text{PPh}_3)_2\text{Cl}_2$ ¹⁶ as the catalyst]; phosphate **41** [75% yield, $\text{Ni}(\text{acac})_2$ ²⁹ as the catalyst] and the tetrazol ether **42** [55% yield, $\text{Ni}(\text{PPh}_3)_2\text{Cl}_2$ ³⁰ as the catalyst]. 1-Phenylnaphthalene **43** has been prepared from phenylmagnesium bromide and either 1-bromonaphthalene [91% yield, $\text{Ni}(\text{dppe})\text{Cl}_2$ ¹⁸ as the catalyst] or 1-methoxynaphthalene [70% yield, $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ ²⁸ or $\text{Ni}(\text{PPh}_3)_2\text{Cl}_2$ ¹⁶ as the catalyst]. 1-Naphthylmagnesium bromide and bromobenzene also gave compound **43** in excellent yield (98%) using $\text{Ni}(\text{dppe})\text{Cl}_2$ as the catalyst.¹⁸ 3,4-Dimethoxynaphthalene and phenylmagnesium bromide afforded 3,4-diphenylnaphthalene **44** (45%) in either nickel or palladium catalysed reactions.^{16,28}

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Carbamates have also been used as coupling partners with Grignard reagents as exemplified by the formation of biphenyls **45** (80%) and **47** (73%) from the reaction of carbamates **46** and **48** respectively with phenylmagnesium bromide/chloride in $\text{Ni}(\text{acac})_2$ catalysed reactions.³¹

The synthesis of 4-methylbiphenyl provides several examples of arylthiol coupling partners. Thus, 4-tolylmagnesium bromide reacted with both thiophenol and thioanisole in $\text{Ni}(\text{PPh}_3)_2\text{Cl}_2$ catalysed reactions giving 4-methylbiphenyl in 62% and 74% yields respectively,³² and with phenyl *tert*-butylsulfone giving this product (80%) in a $\text{Ni}(\text{acac})_2$ catalysed reaction.^{21,21a} Diphenylsulfide, diphenylsulfoxide and diphenylsulfone also gave this product (53–74%).³² Homocoupled and reduced byproducts are sometimes formed in significant quantities when aryl *tert*-butylsulfones are used as coupling partners with aryl-Grignard reagents.^{21,21a} The quaternary salt **49** and phenylmagnesium bromide also afforded 4-methylbiphenyl (31%) in a Kharasch reaction catalysed by $\text{Ni}(\text{dppp})\text{Cl}_2$ ³³ and this Grignard reagent and the tetrazol ether **50** gave 4-methoxybiphenyl in moderate yield (49%) with $\text{Ni}(\text{PPh}_3)_2$ as the catalyst.³⁰

Para-terphenyl **13** has been prepared from phenylmagnesium bromide and 1,4-disubstituted benzene derivatives such as 1,4-dibromobenzene (>80%),³⁴ 1,4-dimethoxybenzene (24%),^{16,28} the bissulphides **51** (89%),⁹ and **52** (84%)⁹ and the 1,4-bisphosphate **53** (80%).²⁹ The corresponding 1,2- and 1,3-bissulfide derivatives of compound **52** also yielded *meta*-terphenyl (77%) and *ortho*-terphenyl **12** (67%).⁹ Compound **12** has also been obtained from 2-iodobiphenyl and phenylmagnesium bromide (70–90%) with various nickel catalysts.¹⁶

Biphenyl has been prepared in high yield from iodobenzene and phenylmagnesium bromide using various palladium catalysts, including palladium black.³⁵ Phenylmagnesium bromide also coupled with 4-fluoriodobenzene giving 4-fluorobiphenyl **8** (92%, PdCl_2 catalyst) and 3-fluoriodobenzene similarly gave 3-fluorobiphenyl (85%). However, 2-fluoriodobenzene decomposed *via* benzyne formation when treated with this Grignard reagent.³⁵

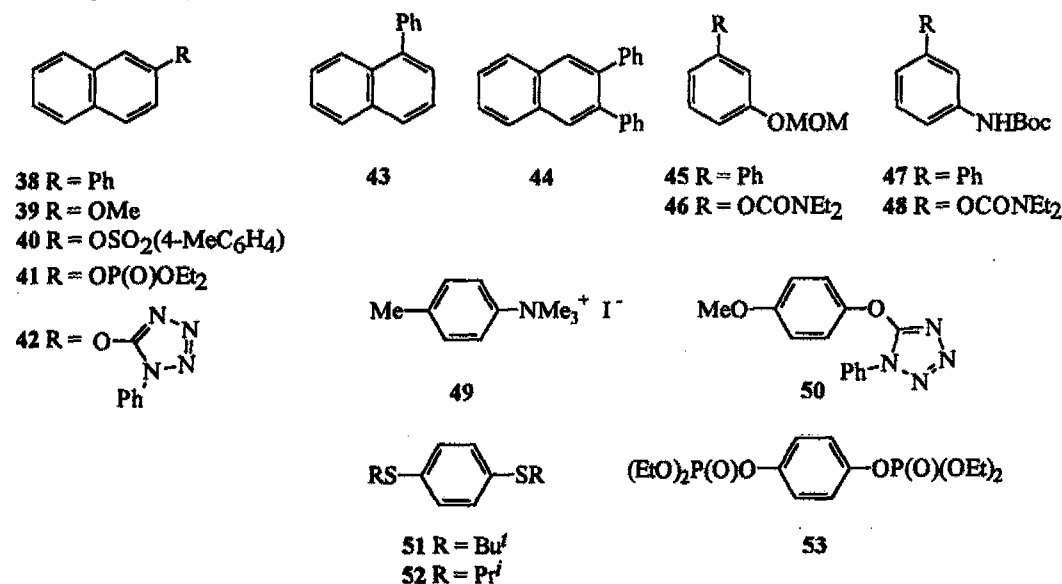
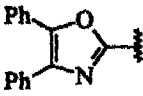
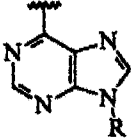
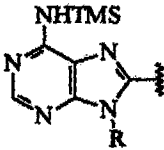


Table 1 illustrates some representative examples of the phenylation of heterocyclic compounds with phenylmagnesium bromide/chloride and other substituted Grignard reagents similarly gave arylated heterobiaryls. 2-Phenylthiophene has also been prepared from 2-thienylmagnesium bromide and bromobenzene [$\text{Ni}(\text{dppp})\text{Cl}_2$ catalyst, 56% yield] but with chlorobenzene the yield is poor (17%).³⁶ 1-Methyl-2-phenylpyrrole has been prepared (87%) from bromobenzene and 1-methyl-2-pyrrolylmagnesium bromide in the presence of $\text{Pd}(\text{dppb})\text{Cl}_2$ as the catalyst.²

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Table 1. Phenylation of Heterocycles using the Kharasch Reaction



Het	X	Catalyst	Yield (%)	Reference
2-thienyl	Br	Ni(dppp)Cl ₂	98	36
3-thienyl	Br	Ni(dppe)Cl ₂	80	37
2-furyl	Br	Ni(dppe)Cl ₂	82	38
3-furyl	Br	Ni(dppe)Cl ₂	93	38
	SMe	Ni(dppe)Cl ₂	90	39
2-benzothiazoyl	SH, SMe	Ni(dppp)Cl ₂	79-98	40
"	Cl	Ni(PPh ₃) ₂ Cl ₂	70	41
2-pyridyl	SH, SMe	Ni(dppp)Cl ₂	79-88	40
"	OCONEt ₂	Ni(acac) ₂	30-80	31
3-pyridyl	Br	Ni(PPh ₃) ₂ Cl ₂	54	42
"	OTf	Ni(acac) ₂	65	31
"	OCONEt ₂	Ni(acac) ₂	72	31
4-pyridyl	OCONEt ₂	Ni(acac) ₂	81	31
2,6-diphenyl-4-pyridyl	SMe	Ni(PPh ₃) ₂ Cl ₂	87	43
5/6/7/8-isoquinolyl	Br, Cl	Ni(dppp)Cl ₂	69-85	44
4,6-dimethylpyrimid-2-yl	SH, SMe	Ni(dppp)Cl ₂	43-94	40
				
R = H	SMe	Ni(dppp)Cl ₂	68	45
R = protected sugar	Cl	Ni(dppp)Cl ₂	40	46
	Br	Ni(PPh ₃) ₂ Cl ₂	15	47
R = protected sugar				

Numerous bithienyl and terthienyl derivatives have been prepared using the Kharasch reaction. These compounds are often precursors of oligo and polythiophenes which have been used extensively as conducting polymers. Terthiophenes also exhibit phototoxic properties and have thus received attention because of their biological activities. Thus, 2,2'-bithienyl **54** has been prepared in high yield (81-90%) from 2-thienylmagnesium bromide and bromothiophene in a Ni(dppp)Cl₂ catalysed reaction^{36,48} and the dimethyl derivative **55** (83%) has similarly been prepared.⁴⁸ 2,3'-Bithienyl (77-100%)^{36,49} and 3,3'-bithienyl (88%)⁴⁹ have also been prepared in nickel catalysed cross-coupling reactions.

Terthiophenes are often prepared from a dibromothiophene and a thiophene derived Grignard reagent and yields are generally excellent.^{14,49,49a} For example, the parent compound, 2,2':5',2"-terthiophene **56** (α-terthienyl) has been prepared (80-86%) in a Ni(dppp)Cl₂ catalysed reaction.^{36,48} The alkylated derivative **57**

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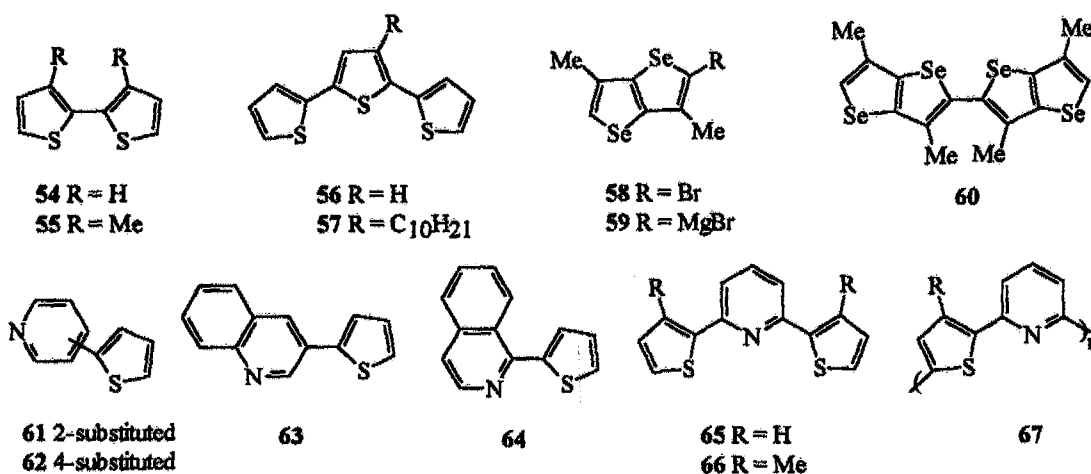
of α -terthiophene **56** has been prepared in excellent yield (92%) as a precursor to soluble sexithiophenes⁵⁰ and other alkylated bi- and terthienyls have been synthesised.⁵¹

The symmetrical selenium containing biaryl **60** has been prepared (85%) from the bromoaryl precursor **58** and the Grignard reagent **59** in a $\text{Pd}(\text{PPh}_3)_4$ catalysed reaction.⁵² Related sulfur containing heterocycles also underwent similar nickel catalysed cross-coupling reactions.⁵²

2,2'-Bipyridyl has been prepared in low yield (13%) from 2-bromopyridine and 2-pyridylmagnesium bromide with $\text{Ni}(\text{dppp})\text{Cl}_2$ as the catalyst.³⁶

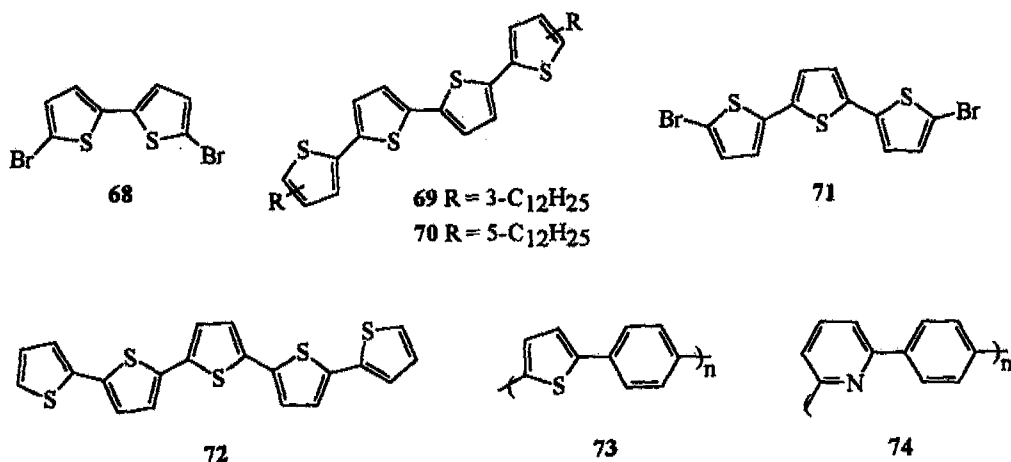
A number of heterobiaryls possessing two different types of heterocyclic ring have been prepared using the Kharasch reaction. Examples include the thienylpyridines **61** (78%)³⁶ and **62** (70%),⁵³ the thienylquinoline **63** (49%)³⁶ and the thienylisoquinoline **64** (69%),⁵⁴ all of which were synthesised from 2-thienylmagnesium bromide and the appropriate bromoaryl coupling partner in nickel catalysed reactions.

Numerous teraryls possessing three different types of heterocyclic ring have been prepared using two sequential Kharasch reactions.¹⁵ The bithienylpyridines **65** and **66** have been prepared in a nickel catalysed reaction in good yield from 2,6-dibromopyridine and 2-thienylmagnesium bromide or 3-methyl-2-thienylmagnesium bromide as appropriate. These teraryls have been prepared as intermediates in the synthesis of 'donor-acceptor' polymers **67**.⁵⁵



The quaterthiophenes **69** (75%)⁵⁶ and **70**,⁵¹ which possess alkyl substituents to enhance solubility, have both been prepared in a $\text{Ni}(\text{dppp})\text{Cl}_2$ catalysed reaction from the dibromo precursor **68** and the appropriate 3- or 5-alkylthienylmagnesium bromide. The quinquethiophene **72** (83%) has been synthesised from the terthiophene **71** and thienylmagnesium bromide in a $\text{Ni}(\text{dppe})\text{Cl}_2$ catalysed reaction.⁵⁷ A number of 'capped' oligothiophenes have also been prepared.^{53,58}

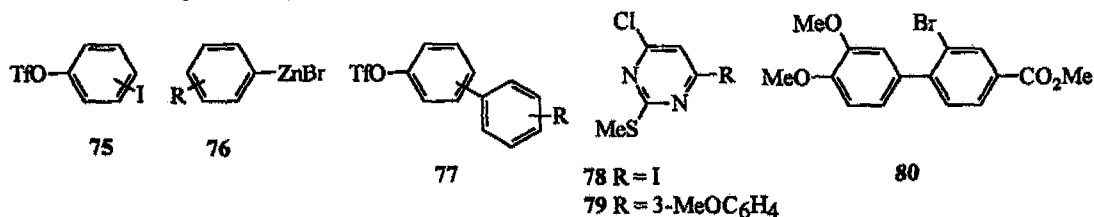
The Kharasch reaction has been used in polymer synthesis. Poly(*para*-phenylene) has been prepared from 1,4-dibromobenzene and magnesium metal via 4-bromophenylmagnesium bromide using $\text{Ni}(\text{bpy})\text{Cl}_2$ as the reaction catalyst in THF at reflux.⁵⁹ Alkylated poly(*para*-phenylene) derivatives have also been prepared.^{59a} 1,3-Dichlorobenzene and 4,4'-dibromodiphenylether were similarly polymerised and α -linked polythiophene has been prepared from 2,5-dibromothiophene using this methodology.⁶⁰ The bis-Grignard reagent, 1,4-C₆H₄(MgBr)₂, gave polymer **73** with 2,5-dibromothiophene and polymer **74** with 2,6-dibromopyridine.^{61,62}



3. THE NEGISHI REACTION

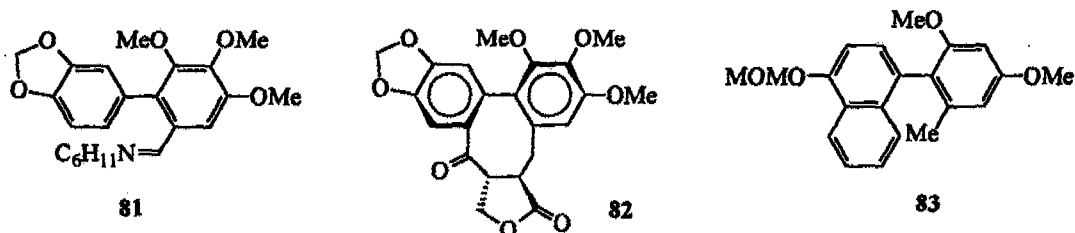
3.1 Chemoselectivity and Regioselectivity

All three isomers of the iodotriflate **75** reacted with a number of arylzinc compounds **76** at the iodo substituent in a palladium catalysed reaction yielding biaryls **77** (72-88%).⁶³ The pyrimidine derivative **78** gave biaryl **79** (79%) when reacted with 3-methoxyphenylzinc chloride in a Pd(PPh₃)₄ catalysed reaction.⁶⁴ In a regioselective reaction, 3,4-dimethoxyphenylzinc chloride reacted at the 4-bromo substituent of methyl 3,4-dibromobenzoate giving biaryl **80** (53%) in a palladium catalysed reaction.⁶⁵



3.2 Steric Considerations

The tolerance of the Negishi reaction to steric effects is illustrated by the formation of the biaryls **81** (80%)^{66,67} and **83** (50%)⁶⁸ in Ni(PPh₃)₄ and Pd(PPh₃)₄ catalysed reactions respectively. In these two biaryls, the left-hand fragment is derived from the corresponding arylzinc chloride and the right-hand fragment from the appropriate aryl iodide. Thus, *ortho* disubstituted aryl iodides undergo the Negishi reaction in useful yields. In the former reaction, the imine **81** is not isolated but is hydrolysed to the corresponding aldehyde which was then used in the synthesis of (-)-Steganone **82**. If the corresponding aryl bromide replaced the aryl iodide in the latter reaction, the yield of product **83** was low (16%).

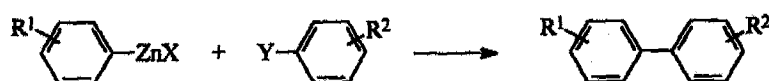


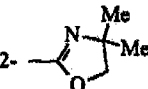
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3.3 General Survey of the Negishi Reaction

Table 2 illustrates some representative examples of the formation of simple biaryls using the Negishi reaction. The reactions generally proceed in good yield and substituents such as nitrile, ester and cyano groups, which are not tolerated in the Kharasch reaction, couple smoothly in the Negishi reaction. In addition to aryl halides as the usual coupling partner of the arylzinc reagent, other partners such as aryl triflates and fluorosulfonates have been used.

Table 2. Synthesis of Biphenyls using the Negishi Reaction



R ¹	R ²	X	Y	Catalyst	Yield (%)	Reference
H	H	Cl	OSO ₂ F	Pd(PPh ₃) ₄	95	69
H	4-OMe	Cl	OSO ₂ F	Pd(PPh ₃) ₄	56	69
H	4-OMe	Cl	I	Ni(PPh ₃) ₄	85	70
H	4-CHO	Cl	Br	Pd(PPh ₃) ₂ Cl ₂	92	71
H	4-CN	Cl	Br	Ni(PPh ₃) ₄	90	70
H	4-CO ₂ Me	Cl	Br	Ni(PPh ₃) ₄	70	70
H	4-NO ₂	Cl	I	a	90	70
4-Cl	3-NO ₂	Br	OTf	Pd(dba) ₂ /dppf	84	63
2-Me	4-NO ₂	Cl	Br	Pd(PPh ₃) ₄	78	72
3-Me	3-Me	Cl	I	Ni(PPh ₃) ₄	95	70
2-CN	3-CO ₂ Et	Br	Br	Pd(PPh ₃) ₄	93	73
4-CN	4-CN	Br	Br	Pd(PPh ₃) ₄	95	73
4-CN	2-CO ₂ Me	Br	I	b	84	63
4-CN	4-CO ₂ Et	Br	I	Pd(PPh ₃) ₄	82	73
3-CO ₂ Me	4-CN	Br	Br	Pd(PPh ₃) ₄	82	73
4-CO ₂ Et	4-CN	I	Br	Pd(PPh ₃) ₄	80	73
4-CO ₂ Et	4-CO ₂ Et	I	I	Pd(PPh ₃) ₄	94	73
2-CONMe ₂	3-CO ₂ Me	Cl	OTf	Pd(PPh ₃) ₄	81	74
2-CONMe ₂	3-NO ₂	Cl	OTf	Pd(PPh ₃) ₄	80	74
2-()	H	Cl	I	Pd(PPh ₃) ₄	75	74
"	2-Me	Cl	I	Pd(PPh ₃) ₄	66	74
"	4-CO ₂ Me	Cl	I	Pd(PPh ₃) ₄	70	74
"	3-NO ₂	Cl	I	Pd(PPh ₃) ₄	54	74

a Pd(PPh₃)₃Cl₂ + DIBAL, b Pd(dba)₂/ tris(2-furyl)phosphine

Simple biaryls possessing an amine substituent can also be prepared using Negishi methodology as exemplified by the preparation of 2-amino-4'-methyl-3-cyanobiphenyl [72% yield, Pd(dppf)Cl₂ catalyst] from 6-iodoanthranilonitrile and 4-tolylzinc bromide.⁷⁵

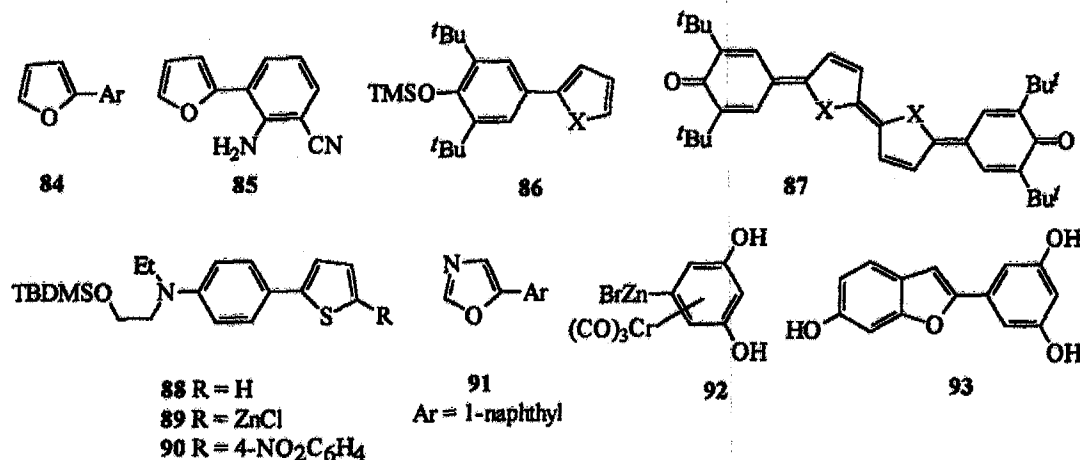
The Negishi reaction has been used to prepare a diverse variety of heterobiaryls. In furan chemistry, 2-furylzinc chloride has been coupled with aryl bromides,^{76,77} aryl triflates⁷⁸ and aryl fluorosulfonates⁶⁹ yielding products of general structure 84 in which the aryl group is a substituted benzene or naphthalene moiety. These reactions are usually catalysed by Pd(PPh₃)₄ and give good yields of products. Additionally, 2-phenylfuran has

been prepared from both 2-iodofuran and phenylzinc chloride (91%) and from iodobenzene and 2-furylzinc chloride (89%) in $\text{Pd}(\text{PPh}_3)_4$ catalysed reactions.⁷⁹ 2-Furylzinc bromide and 6-iodoanthranilonitrile gave the amine **85** (98%) in a $\text{Pd}(\text{dppf})\text{Cl}_2$ catalysed reaction.⁷⁵

A number of heteroquaterphenoquinones **87** have been prepared from biaryls **86** ($\text{X} = \text{O}, \text{S}, \text{Se}, \text{NR}$).⁸⁰ Biaryls **86** (75-79%) were synthesised from the appropriate iodophenol and heteroarylzinc chloride precursors in $\text{Pd}(\text{PPh}_3)_4$ catalysed reactions. The arylated thiophene **88** [63%, $\text{Pd}(\text{PPh}_3)_4$ catalyst] has been prepared from 2-thienylzinc bromide and the appropriate 4-bromoaniline derivative. Compound **88** was subsequently used in the synthesis of the teraryl **90** in a second Negishi reaction of the arylzinc derivative **89** and 4-bromonitrobenzene. This teraryl **90** was then used for the synthesis of non-linear optical (NLO) polyimides.⁸¹

A number of 1,3-oxazolylzinc chloride derivatives have been coupled with aryl iodides or triflates in palladium catalysed reactions.⁸² For example, 1,3-oxazo-5-ylzinc chloride with either 1-iodonaphthalene or its corresponding triflate afforded biaryl **91** in 78% and 83% yields respectively.

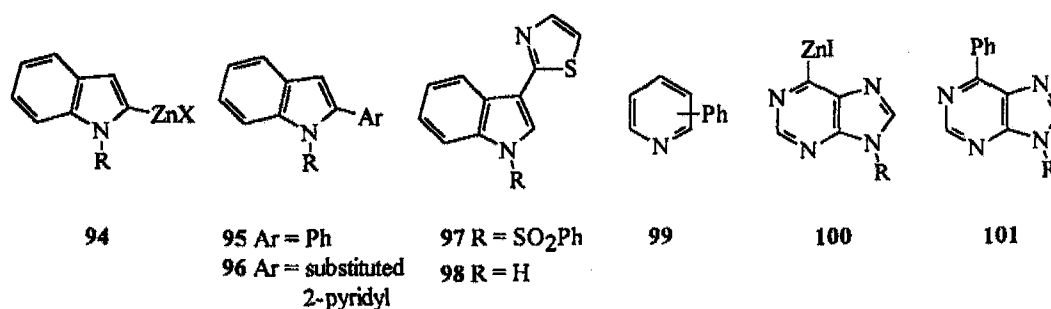
The organozinc reagent **92** was coupled in good yield [82%, $\text{Pd}(\text{PPh}_3)_4$ catalyst] with 5-hydroxy-2-iodobenzo[*b*]furan giving the chromium tricarbonyl complex of Moracin M **93**.⁸³



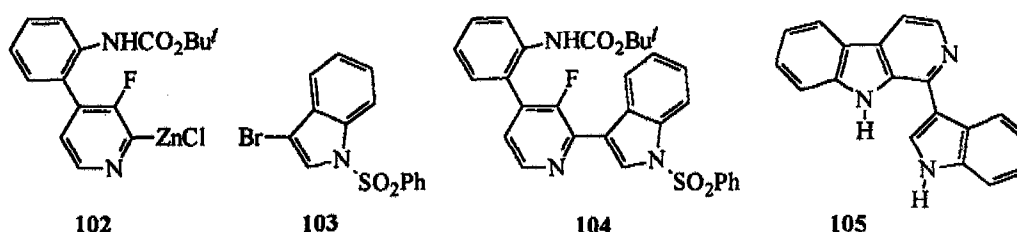
The phenylation of indolylzinc halides with aryl iodides has attracted considerable attention. Thus, the indolylzinc halides of general structure **94** ($\text{R} = \text{CH}_2\text{OMe}, \text{CO}_2\text{Bu}^t, \text{SO}_2\text{Ph}$ and CO_2Li) reacted with iodobenzene in a series of $\text{Pd}(\text{PPh}_3)_4$ catalysed reactions yielding the 2-phenylindole derivatives **95** generally in moderate to good yields. When $\text{R} = \text{CO}_2\text{Li}$, this group is lost on work-up giving the product **95** ($\text{R} = \text{H}$).⁸⁴⁻⁸⁶ Organozinc reagents **94** ($\text{R} = \text{SO}_2\text{Ph}$) have also been reacted with a wide range of substituted 2-pyridyl halides yielding products **96** in palladium catalysed reactions.^{87,88} 3-Aryl-1-phenylsulfonylindole derivatives have also been prepared using similar methodology,⁸⁶ including the thiazole derivative **97** which was the precursor to the natural product, Camalexin **98**.⁸⁴

Phenylpyridine derivatives **99** and substituted phenylpyridines have been successfully prepared using the Negishi reaction usually in good yield^{74,89,90,90a} and 3-phenylquinoline was synthesised (96%) from 3-quinolylzinc iodide and iodobenzene with $\text{Pd}(\text{PPh}_3)_4$ as the catalyst.⁹⁰ The purine derived zinc compounds **100** ($\text{R} = \text{CH}_2\text{Ph}$ or protected sugar) have been phenylated with iodobenzene (52-80%) giving products **101** using $\text{Pd}(\text{dba})_2$ as the catalyst in the presence of tris(2-furyl)phosphine.⁹¹ Substituted iodobenzenes similarly coupled with compound **100**.⁹¹

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A diverse range of biaryls in which both aryl fragments are heteroaryl groups have been prepared using the Negishi reaction. The synthesis of heteroaryl substituted indoles has already been discussed above and a further example is compound **104** which was obtained from precursors **102** and **103** in 47% yield in a $\text{Pd}(\text{PPh}_3)_4$ catalysed reaction.⁹² Biaryl **104** was subsequently used in the synthesis of Eudistomin U **105**.



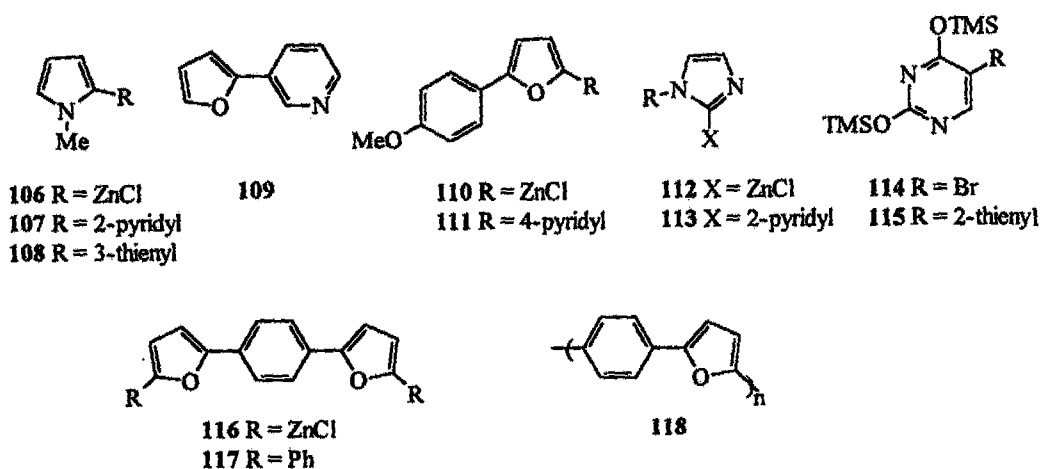
The two heteroaryl pyrrole derivatives **107** (71%) and **108** (73%) have been prepared from the zinc reagent **106** and the appropriate heteroaryl bromide using $\text{Pd}(\text{dppb})\text{Cl}_2$ as the catalyst.² 2-Furylzinc chloride and the appropriate pyridyl triflate afforded biaryl **109** (81%)⁷⁸ in a $\text{Pd}(\text{PPh}_3)_4$ catalysed reaction and the furylzinc chloride **110** and 4-chloropyridine gave the fluorescent heterocycle **111** (64%)⁷⁶ with $\text{Pd}(\text{dppb})\text{Cl}_2$ as the catalyst. The imidazole derivatives **113** [60–93%, $\text{Pd}(\text{PPh}_3)_4$ catalyst] were prepared from zinc reagents **112** ($\text{R} = \text{Me}, \text{CH}_2\text{OEt}, \text{SO}_2\text{NMe}_2$) and 2-bromopyridine.⁹³

Teraryl analogues of the selenium containing heterocycle **60** have been prepared in low yield by the Negishi reaction⁵² and the teraryl **65** has been synthesised in good yield (76%) from 2-thienylzinc chloride and 2,6-dibromopyridine in a $\text{Pd}(\text{PPh}_3)_4$ catalysed reaction.⁹⁴ 2-Thienylzinc bromide also reacted with the bromopyrimidine derivative **114** yielding the biaryl **115** [35%, $\text{Ni}(\text{PPh}_3)_4$ catalyst] which, after removal of the TMS groups, gave uracil analogues.⁹⁵

2,3'-Bipyridyl (81%) was synthesised from 3-pyridylzinc iodide and 2-bromopyridine in a $\text{Pd}(\text{PPh}_3)_4$ catalysed reaction.⁸⁹ Pyridylzinc halides have also been coupled with a number of halo quinolines. Thus, 2-pyridylzinc bromide and 3-iodoquinoline afforded 3-(2-pyridyl)quinoline in moderate yield (53%) in a $\text{Pd}(\text{PPh}_3)_4$ catalysed reaction and 3-pyridylzinc iodide and 2-iodoquinoline gave 2-(3-pyridyl)quinoline (73%) with this catalyst.⁹⁰ Several substituted pyridylzinc halides were reacted with 6-haloquinol-2-one derivatives in $\text{Pd}(\text{PPh}_3)_4$ catalysed reactions giving a series of substituted 6-pyridylquinol-2-ones (36–96%).⁹⁶

The bis-organozinc chloride **116** reacted with bromobenzene in a palladium catalysed reaction giving the oligomer **117** (87%) and with 1,4-dibromobenzene yielding the polymer **118** (94%). The thiophene analogue **73** of this polymer was also synthesised using a Negishi reaction.⁹⁷ Substituted α -linked polythiophenes^{98–100} and poly(*para*-phenylene)¹⁰⁰ have been prepared using the Negishi reaction. Pyridyl-capped thiophene oligomers have also been prepared in moderate yield.⁵³

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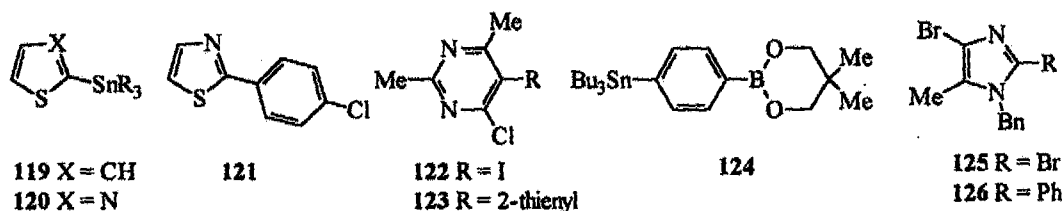


4. THE STILLE REACTION

4.1 Chemoselectivity and Regioselectivity

The thiazole derivative **120** (R = Me) reacted with 4-bromochlorobenzene selectively at the bromo substituent yielding biaryl **121** (80%) in a $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ catalysed reaction¹⁰¹ and the pyrimidine derivative **122** gave product **123** (60%) with stannane **119** (R = Bu) with this catalyst.¹⁰² The stannane **124** couples [$\text{Pd}(\text{PPh}_3)_4$ catalyst] selectively with halogenated pyrimidine and purine derivatives in good yield selectively at the stannyl group in a Stille reaction, and not at the boron moiety in a Suzuki reaction.^{103,104} The boron-containing biaryl products of these reactions are of potential use in ^{10}B neutron capture therapy.

The dibromoimidazole **125** reacted at the 2-bromo substituent with PhSnMe_3 giving heterocycle **126** (58%) in a $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ catalysed reaction.¹⁰⁵

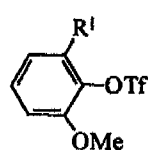


4.2 Steric Considerations

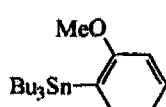
A number of *ortho* substituted biphenyls of general structure **129** have been successfully prepared in $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ catalysed Stille reactions of the triflates **127** and PhSnBu_3 or stannane **128**.^{106,107} For example, triflates **127** ($\text{R}^1 = \text{OMe}$) and **127** ($\text{R}^1 = \text{CO}_2\text{Me}$) gave biphenyls **129** ($\text{R}^1 = \text{OMe}$, $\text{R}^2 = \text{H}$) (74%) and **129** ($\text{R}^1 = \text{CO}_2\text{Me}$, $\text{R}^2 = \text{H}$) (87%) respectively with PhSnBu_3 . However, when the more sterically demanding stannane **128** was reacted with these triflates, only the former triflate gave a product **129** ($\text{R}^1 = \text{R}^2 = \text{OMe}$) which was isolated either in low yield (33%) or in moderate yield (49%)¹⁰⁸ when CuBr was present as a co-catalyst. 4-Methyl-2,2',6,6'-tetramethoxybiphenyl-4'-carbaldehyde **130** has also been obtained (25%) in a related Stille reaction.¹⁰⁸ Mesityl bromide has been successfully arylated in a Stille reaction¹⁰⁹ and the phenylation of both 2,4-dinitroiodobenzene and 2,4,6-trinitroiodobenzene has been achieved.¹¹⁰

It is of particular interest to note that compound **83** could not be synthesised using a Stille reaction whereas a Negishi reaction gave a moderate yield of this compound and a Suzuki reaction afforded a good yield.⁶⁸

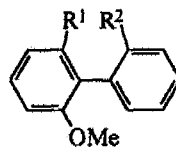
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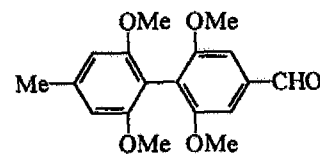
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4.3 General Survey of the Stille Reaction

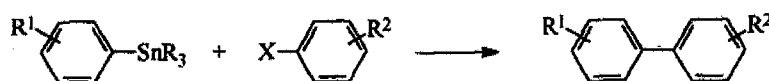
Arylstannanes possessing a tributyltin or trimethyltin moiety are the most commonly used biaryl precursors in the Stille reaction. Arylstannanes with fluorinated alkyl substituents have recently been developed for biaryl synthesis.¹¹¹ Arylstannoates, prepared from potassium hydroxide and ArSnCl_3 have been used to prepare biphenyl derivatives in good yield in an aqueous Stille reaction.^{112,113} The Stille reaction is often improved by the addition of copper salts¹¹⁴⁻¹¹⁶ and one explanation for this observation is that transmetalation of the arylstannane by the copper salt occurs yielding a more reactive organocopper reagent.

The coupling partner of the arylstannane is usually an aryl halide or an aryl triflate. When triflates are used, lithium chloride is almost always necessary as a co-reagent. Other coupling partners have also been used less frequently; for example diazonium salts¹¹⁷ and hypervalent iodine reagents.¹¹⁸

Of particular current interest is the fact that the Stille reaction is also amenable to solid phase synthesis and the synthesis of biphenyl derivatives in solid phase reactions has been reported in several recent publications.¹¹⁹⁻¹²¹

Table 3 illustrates a selection of Stille reactions which have yielded biphenyls as products. A number of

Table 3. Synthesis of Biphenyls using the Stille Reaction



R ¹	R ²	R	X	Catalyst	Yield (%)	Reference
H	H	Ph	Br	$\text{BnPd(PPh}_3)_2\text{Cl}$	78	122
H	H	Bu	$\text{IPh}^+\text{BF}_4^-$	Pd/C	95	118
H	4-Me	Bu	$\text{N}_2^+\text{BF}_4^-$	a	59-66	117
H	4-NO ₂	Me	I	$\text{ArPd(PPh}_3)_2\text{I}$	83	110
H	4-COMe	Me	OTf	$\text{Pd(PPh}_3)_4$	85	123
H	4-COMe	Me/Bu	OTf	$\text{Pd}_2(\text{dba})_3/\text{AsPh}_3$	74-82	124
H	4-COMe	Bu	b	c	72	125
4-Cl	4-CHO	Bu	Br	$\text{Pd(PPh}_3)_4$	75	109
4-Cl	4-COMe	Bu	Br	$\text{Pd(PPh}_3)_4$	89	109
4-Me	4-CN	Bu	Br	$\text{Pd(PPh}_3)_4$	92	109
4-Me	4-NO ₂	Bu	Br	$\text{Pd(PPh}_3)_4$	68	109
4-Me	4-COMe	Bu	Br	$\text{Pd(PPh}_3)_4$	90	109
2-OMe	4-COMe	Bu	Tf	$\text{Pd}_2(\text{dba})_3/\text{AsPh}_3$	88	124
4-OMe	4-NO ₂	Bu	OMs	$\text{Pd(PPh}_3)_4$	48	126
4-OMe	4-NO ₂	Bu	OTf	$\text{Pd(PPh}_3)_4$	74	123
2-CHO	4-COMe	Bu	OTf	$\text{Pd}_2(\text{dba})_3/\text{AsPh}_3$	25	124

a Pd(OAc)_2 or Pd(dba)_2 . b $\text{OSO}_2(4\text{-FC}_6\text{H}_4)$. c $\text{Pd(OAc)}_2/\text{Ph}_2\text{PMe}$

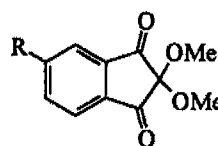
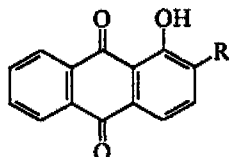
436

different palladium catalysts have been used and yields are generally good to excellent. Both 1-phenylnaphthalene **43** and 2-phenylnaphthalene **38** have been prepared in good yield [Pd(dba)₂/PPh₃ catalyst] from the appropriate naphthyl triflate and PhSnBu₃¹²⁷ and the triflates derived from salicylaldehyde¹²⁸ and substituted ethyl salicylates¹²⁹ have also been coupled with stannanes.

Byproducts resulting from the transfer of methyl or butyl groups from the stannanes ArSnMe₃ or ArSnBu₃ respectively to the coupling partner are frequently observed in the Stille reaction. One illustrative example¹³⁰ is the reaction of the anthraquinone triflate **131** with PhSnMe₃ in a Pd(dppf)Cl₂ catalysed reaction which gave a 2:1 mixture (85%) of phenylated product **134** and methylated product **132**. With PhSnBu₃ the reaction was more selective affording a 4:1 mixture (71%) of phenylated product **134** and butylated compound **133**. Other anthraquinone triflate derivatives were successfully phenylated.

5-Arylninhydrin derivatives with improved chromogenic and luminescent properties have been prepared using Stille and Suzuki methodologies. In one Stille reaction, bromide **135** reacted with 2-tributylstannylbenzo[*b*]thiophene [Pd(PPh₃)₄ catalyst] yielding product **136** (73%).¹³¹

131 R = OTf
132 R = Me
133 R = Bu
134 R = Ph

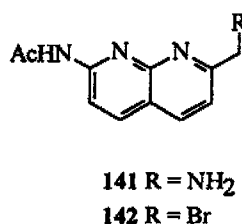
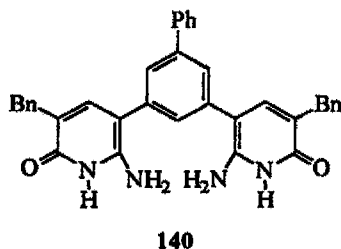
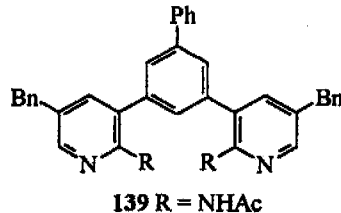
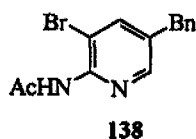
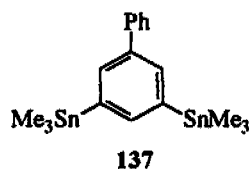


135 R = Br
136 R = 2-benzo[*b*]thienyl

Numerous heterobiaryls possessing one heterocyclic and one carbocyclic moiety have been successfully prepared using the Stille reaction as indicated in Table 4 (following page). The entries listed in Table 4 have been grouped into two types depending upon whether a heteroaromatic (method A) or carbocyclic aromatic (method B) stannane precursor was used and generally either synthetic method gave good yields.

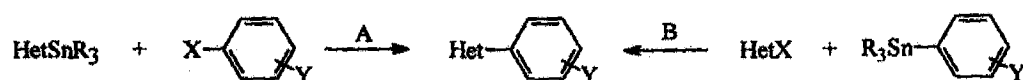
The arylstannane, PhSnMe₃, has also been generated *in situ* from bromobenzene and Me₃SnSnMe₃. In the presence of 2-pyridyl triflate and Pd(PPh₃)₄ as the catalyst, 2-phenylpyridine (55%) is produced. Other bromobenzene derivatives also gave moderate yields (35–50%) of substituted 2-phenylpyridines and 3-bromopyridine and 2-bromothiophene similarly yielded 2,3'-bipyridyl (53%) and 2-(2-thienyl)pyridine **61** (63%) respectively.¹³⁸

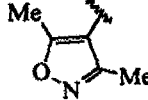
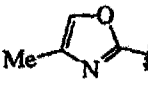
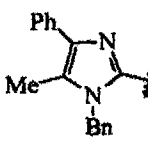
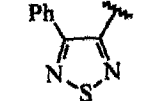
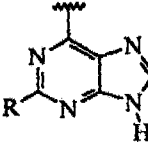
The Stille reaction has been used to synthesise reaction templates. Thus, the bis-stannane **137** and pyridine derivative **138** yielded the teraryl **139** [(60%), Pd(PPh₃)₂Cl₂ catalyst] which was then converted in three steps into the template **140**. This template was designed to hydrogen bond the substrates **141** and **142** prior to nucleophilic substitution of compound **141** with compound **142**.¹³⁹



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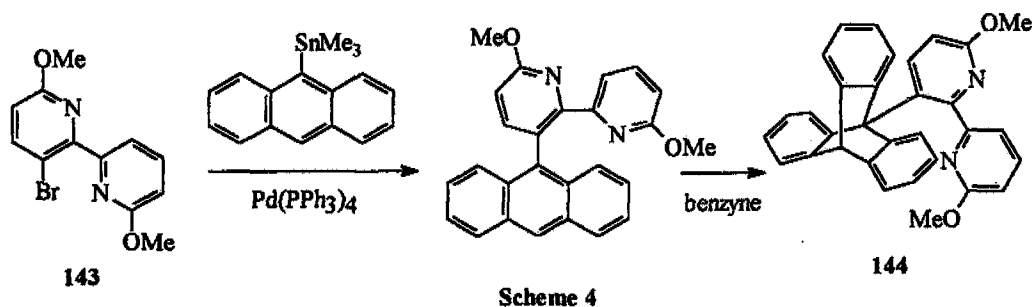
Table 4. Synthesis of Heterobiaryls using the Stille Reaction



A/B	Het	Y	R	X	Catalyst	Yield (%)	Ref.
A	2-thienyl	H	Bu	IPh ⁺ BF ₄ ⁻	PdCl ₂	87	118
B	2-thienyl	H	Bu	I	Pd/C	77	114
A	2-thienyl	2-CO ₂ Me	Bu	I	Pd(PPh ₃) ₂ Cl ₂	81	101
A	2-furyl	4-CO ₂ Me	Me	I	Pd(PPh ₃) ₂ Cl ₂	73	101
A	1-methyl-2-pyrrolyl	H	Me	I	Pd(PPh ₃) ₂ Cl ₂	54	101
B		H	Bu	I	Pd(PPh ₃) ₂ Cl ₂	58	132
A		H	Me	I	Pd(PPh ₃) ₄	80	133
A	"	4-COMe	Me	I	Pd(PPh ₃) ₄	100	133
A	1-methylimidazol-2-yl	H	Bu	Br	Pd(PPh ₃) ₂ Cl ₂	89	134
A	"	2/3/4-CH ₂ R ^a	Bu	Br	Pd(PPh ₃) ₂ Cl ₂	24-69	134a
B		H	Me	Br	Pd(PPh ₃) ₂ Cl ₂	60	105
B		4-Cl	Bu	Cl/Br/OTf	Pd(PPh ₃) ₄ or Pd(PPh ₃) ₂ Cl ₂	7-90	135
A	2-benzo[b]thienyl	4-COPh	Bu	I	Pd/C	60	114
A	2-benzothiazoyl	H	Bu	Br	Pd(PPh ₃) ₂ Cl ₂	75	134
B	2-benzothiazoyl	H	Bu	Cl	Pd(PPh ₃) ₂ Cl ₂	86	134
A	2-benzoxazolyl	H	Bu	Br	Pd(PPh ₃) ₂ Cl ₂	75	134
A	2-pyridyl	4-CO ₂ Me	Me	I	Pd(PPh ₃) ₂ Cl ₂	95	101
B	2-quinolyl	H	Bu	OTf	Pd(PPh ₃) ₂ Cl ₂	88	136
B	8-quinolyl	H	Bu	b	c	68	125
B	2-isoquinolyl	H	Bu	OTf	Pd(PPh ₃) ₂ Cl ₂	53	136
B		H	Bu	Cl	Pd(PPh ₃) ₂ Cl ₂	73-81	137

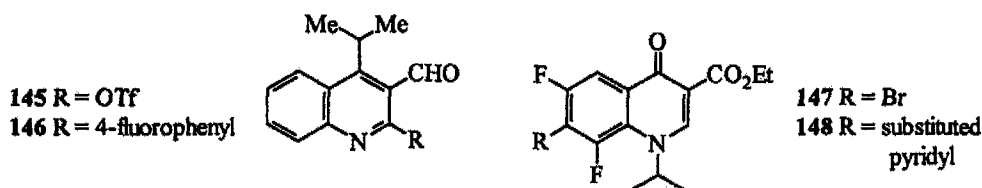
R = H, NH₂a R = P(O)OEt₂, b OSO₂(4-FC₆H₄), c Pd(OAc)₂/Ph₂PMe

Materials for use in the nanotechnology field have been prepared using Stille methodology. The 'molecular brake' **144** has been synthesised from the bipyridyl precursor **143** as shown in Scheme 4. The bipyridyl moiety of heterocycle **144** can coordinate to a suitable metal and thus activate the 'brake'.¹⁴⁰



The Stille reaction has been used to prepare a number of arylated quinoline derivatives of medicinal interest and two relevant examples include the preparation of compound **146** [95%, Pd(PPh₃)₄ catalyst], an intermediate in the synthesis of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) inhibitors, from triflate **145** and 4-FC₆H₄SnBu₃¹⁴¹ and the preparation of the anti-bacterial quinolones **148** [5–74%, Pd(PPh₃)₂Cl₂ catalyst] from bromides **147** and appropriate pyridylstannanes.¹⁴²

Several groups have reported the synthesis of 2-arylated indole derivatives in excellent yields from a 2-stannylated indole and an aryl halide.^{143–145} Numerous arylated pyrimidines,^{102,146,147,147a} pyrazines and pyrazine *N*-oxides^{148,149} have been prepared using a Stille reaction.



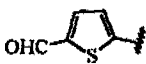
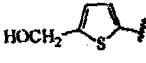
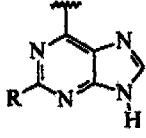
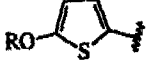
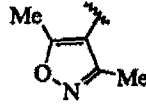
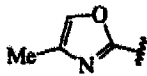
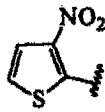
A diverse variety of biaryls in which both aryl fragments are heterocyclic moieties have been prepared using the Stille reaction and some representative examples are shown in Table 5 (following page). The yields of products are generally good to excellent and Pd(PPh₃)₄ or Pd(PPh₃)₂Cl₂ are usually employed as the reaction catalysts.

The fused tricyclic heteroaromatic compounds **149**¹⁵⁵, **150**¹⁵⁶ and **151**¹⁵³ have been prepared using the Stille reaction as shown in Scheme 5. The initially formed biaryls were not isolated but the adjacent aldehyde or protected aldehyde and substituted amine substituents underwent cyclisation giving the products. Other isomers of the heterocycles shown in Scheme 5 have also been prepared in a similar fashion.^{157,158} In the synthesis of compound **149**, it was noted that the yield was increased from 43% to 78% by the addition of CuO and the formation of compound **151** was promoted by the addition of Ag₂O.

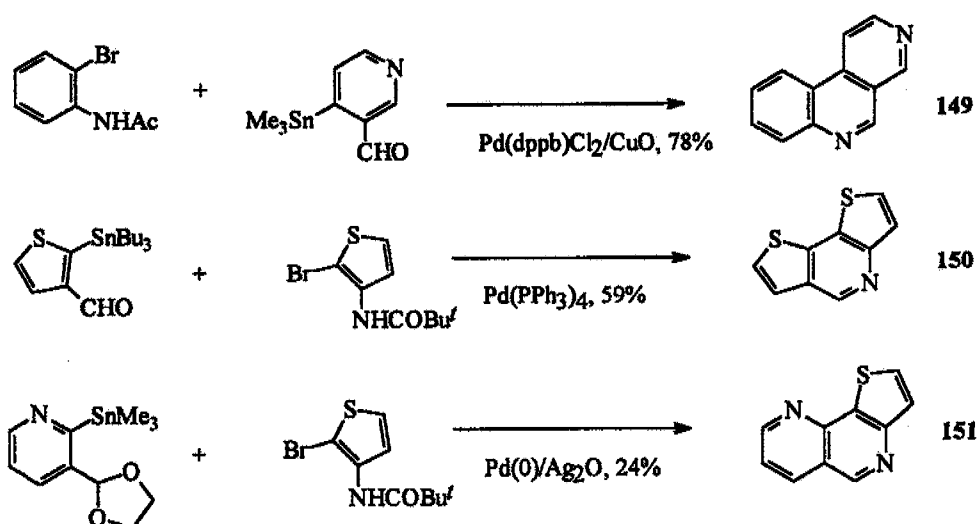
439

Table 5. Synthesis of Heterobiaryls using the Stille Reaction



Het ¹	Het ²	R	X	Catalyst	Yield (%)	Reference
2-thienyl	2-thienyl	Bu	I	Pd(PPh ₃) ₂ Cl ₂	80	150
2-thienyl	2-thienyl	Bu	IPh ⁺ BF ₄ ⁻	PdCl ₂	94	118
2-thienyl	2-quinolyl	Bu	OTf	Pd(PPh ₃) ₄	71	136
2-thienyl		Bu	I	Pd(PPh ₃) ₂ Cl ₂	71	150
2-thienyl		Bu	I	Pd(PPh ₃) ₂ Cl ₂	79	150
2-thienyl	 R = H, NH ₂	Bu	Cl	Pd(PPh ₃) ₂ Cl ₂	76-81	137
 R = Me, Bu ^t	2/3/4-pyridyl	Me	Br	Pd(PPh ₃) ₄	77-82	151
 X = O, S		Bu	I	Pd(PPh ₃) ₂ Cl ₂	84-85	132
2/4/5-thiazoyl	2/4/5-thiazoyl	Me	Br	Pd(PPh ₃) ₄	59-77	152
	3-pyridyl	Me	Br	Pd(PPh ₃) ₄	100	133
"	2-quinolyl	Me	Br	Pd(PPh ₃) ₄	92	133
2-pyridyl		Bu	Br	Pd(PPh ₃) ₄	51	153
2-pyridyl	2-thiazoyl	Bu	Br	Pd(PPh ₃) ₄	60	153
2-pyridyl	4-pyridyl	Bu	Br	Pd(PPh ₃) ₄	70	153
2/3/4-pyridyl	2/3/4-pyridyl	Me	Br	Pd(PPh ₃) ₄	59-77	154
3-quinolyl	2-pyridyl	Me	Br	Pd(PPh ₃) ₄	79	154

440



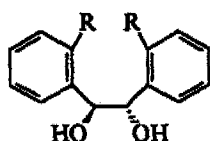
Scheme 5

The Stille reaction has also been successfully used in the preparation of teraryls, oligoaryls and polyaryls. In the thiophene field, α -terthiophene **56** (61%)¹⁵⁰ has been synthesised from 2,5-diiodothiophene and 2-tributylstannylthiophene; methoxy-substituted ter-, quater- and quinquethiophenes have been prepared;¹⁵⁹ thiophene tetramers and seximers possessing 2-hydroxyethyl substituents have been synthesised as potential precursors of water soluble thiophene oligomers^{160,161} and trimethylsilyl-capped oligothiophenes have been reported.¹⁶² Long-chain alkyl and alkoxy derivatives of polymer **73**^{163,164} have been reported and thiophene-containing photorefractive polymers have been prepared.¹⁶⁵ Several types of 'donor-acceptor' polymers have also been synthesised using the Stille reaction.¹⁶⁶

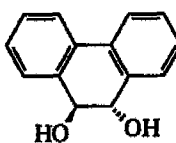
Dihalo- thiophenes, thiazoles and pyridines have been coupled to stannanes derived from furan, thiophene, selenophene and thiazole yielding a plethora of teraryls.¹⁶⁷ Terthiazoles,¹⁵² terpyridines,¹⁶⁸ quaterpyridines^{169,170} and oligopyridines¹⁷⁰ are also available using the Stille reaction.

The Stille reaction has enjoyed considerable success in the synthesis of a large number of natural products which possess a biaryl moiety. In the formulae which follow, the letters 'a' and 'b' have been used to denote the origin of the aryl-aryl bond in each of the natural product synthesis and the yields refer to the biaryl formation step. The chiral 9,10-dihydrophenanthrenediol unit **153** of pradimicins and benanomycins has been prepared (48-88%) from the precursors **152** (R = Br, I, OTf) in a Pd(PPh₃)₄ catalysed reaction in the presence of either (Me₃Sn)₂ or (Bu₃Sn)₂.^{170a} One of the R substituents in compounds **152** is converted into the appropriate stannyl group by the tin reagent and a subsequent Stille reaction then occurs. The pentacyclic benzo[*a*]naphthacenequinone natural products G-2N **154** (R = H) and G-2A **154** (R = CO₂H) have both been prepared from a common precursor, ester **154** (R = CO₂Me), which was synthesised in 44% yield using an intramolecular Stille reaction.¹⁷¹ In this reaction, the arylstannane coupling partner was generated *in situ* from the corresponding aryl bromide with (Me₃Sn)₂. The naphthylisoquinoline alkaloid, Pindikamine A **156** has been prepared in several steps from the precursor **155** which was synthesised using a Stille reaction.¹⁷² A stannyl analogue of compound **92** has been used in the synthesis of Moracin M **93**.⁸³

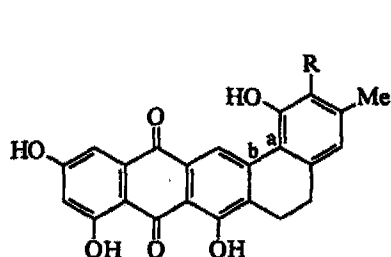
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152

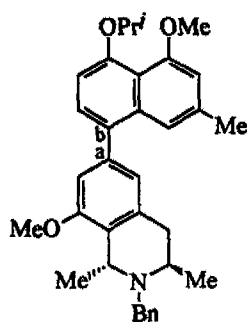


153



154

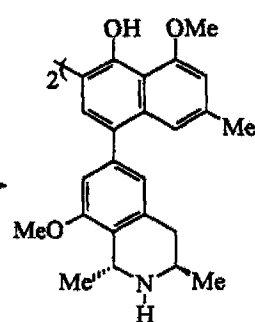
a=OTf, b=Br (see text)
(Me₃Sn)₂/ Pd(PPh₃)₄, 44%



155

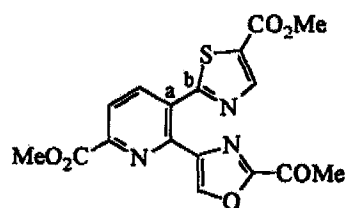
a=OTf, b=SnBu₃
Pd(PPh₃)₂Cl₂/ PPh₃/ CuBr, 63%

steps



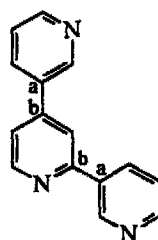
156

In the area of pyridine natural products, Dimethyl Sulfomycinamate **157**, the methanolysis product of the antibiotic Sulfomycin I has been prepared.^{173,174} The 2-stannylthiazole coupling partner of the pyridine triflate was prepared *in situ* from the corresponding bromothiazole in this coupling reaction. Several model coupling reactions were also investigated. The terpyridyl, Nicotelline **158** has been prepared from 3-bromopyridine and 2,4-bis(trimethylstannyl)pyridine.¹⁵⁴ The quinoline derivatives **159** and **161** have both been converted into the cytotoxic alkaloid Amphimedine **160**¹⁷⁵ and the non-natural isomer, Isoasididemin **163**,¹⁷⁶ of the pentacyclic alkaloid Ascididemin **162** respectively. The synthesis of compound **161** also provides another example of chemoselectivity in the Stille reaction; the quinolyl precursor to compound **161** reacted at the triflate group and not at the 6-bromo substituent. Continuing the theme of quinoline chemistry, the alkaloid Dubamine **164**¹²³ and the pyridylquinoline **165**¹⁷⁷ have also been prepared using Stille methodology. Compound **165** and related derivatives were prepared as analogues of the anti-tumor antibiotic Streptonigrin.



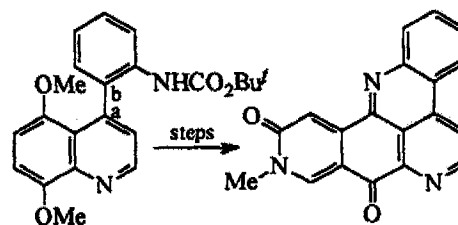
157

a=OTf, b=Br (see text)
(Bu₃Sn)₂/ Pd(PPh₃)₄/ Pd(PPh₃)₂Cl₂, 35%



158

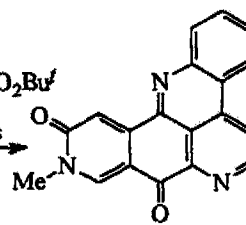
a=Br, b=SnMe₃
Pd(PPh₃)₄, 48%



159

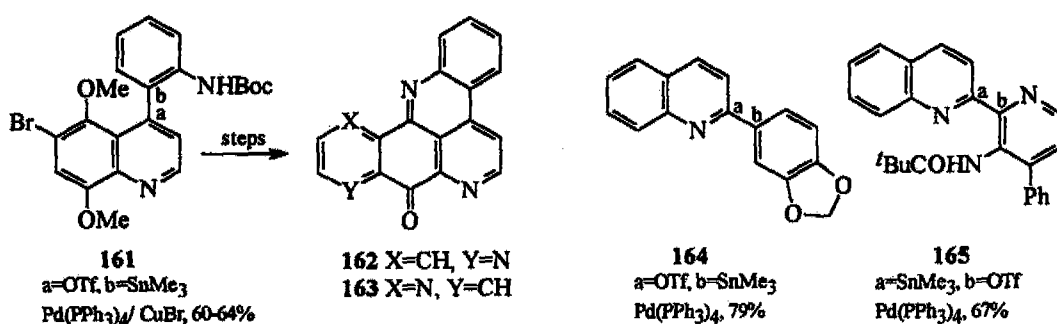
a=OTf, b=SnMe₃
Pd(PPh₃)₄, 87%

steps

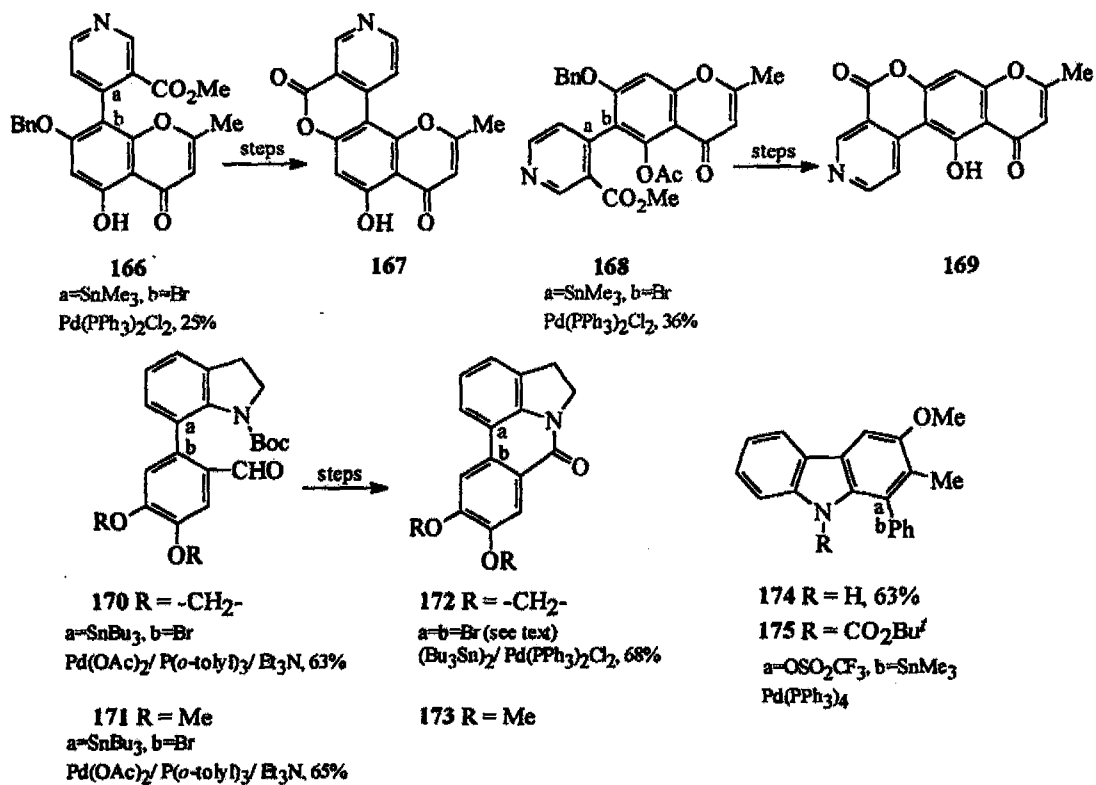


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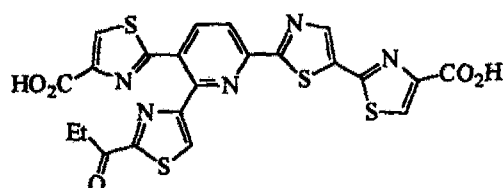
442



The lactones, Schumanniphytine **167** and Isoschumanniphytine **169** have both been prepared from the pyridylchromones **166** and **168** respectively.¹⁷⁸ The indole alkaloid, Hippadine **172** has been prepared by two methods. In one method,¹⁷⁹ the biaryl **170** was prepared using a Stille reaction and was subsequently converted into Hippadine **172** and in another method an intramolecular cyclisation (68% yield) of a dibromo precursor in the presence of $(\text{Bu}_3\text{Sn})_2$ (which transforms one bromo substituent into a tributylstannyl group) was used.¹⁸⁰ Pratosine **173** was also prepared from biaryl **170**.¹⁷⁹ Phenylation of the carbazole triflate precursor of compound **175** with PhSnMe_3 resulted in the loss of the CO_2Bu^t group giving Hyellazole **174** directly.¹⁸¹ Micrococcinic acid **176** has been prepared using Stille methodology to form all of the aryl-aryl bonds.¹⁸²



143

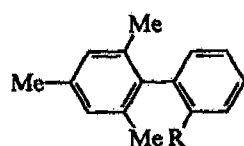


176 Micrococcinic acid

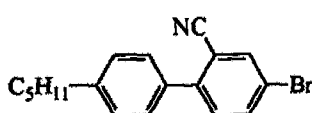
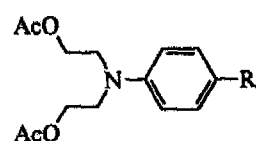
5. THE SUZUKI REACTION

5.1 Chemoselectivity and Regioselectivity

Phenylboronic acid reacted with 4-bromochlorobenzene exclusively at the bromo substituent giving 4-chlorobiphenyl **6** (74%) in a $\text{Pd}(\text{PPh}_3)_4$ catalysed reaction.¹⁸³ Mesitylboronic acid coupled with 2-chloriodobenzene (94%) and 2-bromiodobenzene (56%) [$\text{Pd}(\text{PPh}_3)_4$ catalyst, $\text{Ba}(\text{OH})_2$ base] giving the biphenyl derivatives **177** and **178** respectively.¹⁸⁴ Both 3-bromiodobenzene and 4-bromiodobenzene have been coupled sequentially with *meta*-tolylboronic acid and then phenylboronic acid yielding methylated *meta*- and *para*-terphenyl derivatives respectively in good yield.¹⁸⁵ 3-Bromo-5-iodobenzonitrile reacted with 4-(*n*-pentyl)phenylboronic acid at the iodo substituent [$\text{Pd}(\text{PPh}_3)_4$ catalyst] affording the biphenyl derivative **179** in 74% yield.¹⁸⁶ Other boronic acids similarly coupled with 3-bromo-5-iodobenzonitrile.^{186,187} Only a low selectivity was observed when boronic acids were reacted with 2,5-dibromobenzonitrile in a 1:1 ratio but with a 2:1 ratio of reactants coupling occurred at both bromo substituents giving terphenyls in moderate yield.¹⁸⁶ Although alkyl substituted derivatives of 4-bromophenylboronic acid polymerise yielding alkylated poly(*para*-phenylenes),¹⁸⁸ 4-bromophenylboronic acid and the iodoaniline **180** gave the biphenyl product **181** in a Suzuki reaction.¹⁸⁹ One bromo substituent in 1,3,5-tribromobenzene can be selectively replaced by phenylboronic acid giving 3,5-dibromobiphenyl in 67% yield.¹³⁹



177 R = Cl
178 R = Br

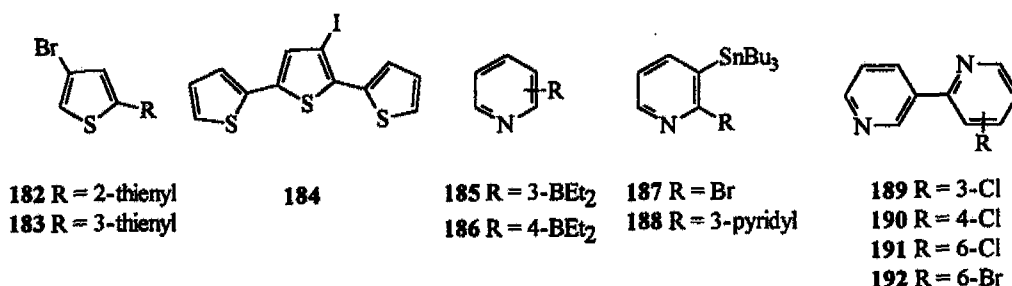
**179**

180 R = I
181 R = 4-BrC₆H₄

2,4-Dibromothiophene reacted regioselectively with both 2- and 3-thienylboronic acids giving the bithienyls **182** (35%) and **183** (65%) respectively in a $\text{Pd}(\text{PPh}_3)_4$ catalysed reaction.¹⁹⁰ 2,3,5-Triiodothiophene and 2-thienylboronic acid afforded the terthiophene derivative **184** (60%) in which two of the iodo substituents have been replaced.¹⁹⁰

The pyridylborane **185** reacted selectively [$\text{Pd}(\text{PPh}_3)_4$ catalyst] at the bromo substituent of pyridine derivative **187** in a Suzuki reaction rather than at the stannyl group in a Stille reaction giving the bipyridyl **188** in 86% yield.¹⁹¹ With 2,3-dichloropyridine and 2,4-dichloropyridine this borane **185** reacted selectively at the 2-positions affording bipyridyls **189** (92%) and **190** (57%) respectively.¹⁹¹ 2,6-Dichloropyridine can be mono-arylated with borane **185** giving bipyridyl **191**¹⁹² and 2,6-dibromopyridine gave bipyridyl **192** (67%) and 6-bromo-2,4'-bipyridyl (57%) with boranes **185** and **186** respectively.¹⁹³ 2,5-Dichloropyridine was selectively phenylated (71%) at the 2-position by phenylboronic acid yielding 2-chloro-6-phenylpyridine in a $\text{Pd}(\text{dppb})\text{Cl}_2$ catalysed reaction.^{194,195} In pyrimidine chemistry, the iodo substituent of compound **78** has been replaced in preference to the chloro substituent in a Suzuki reaction using phenylboronic acid.⁶⁴

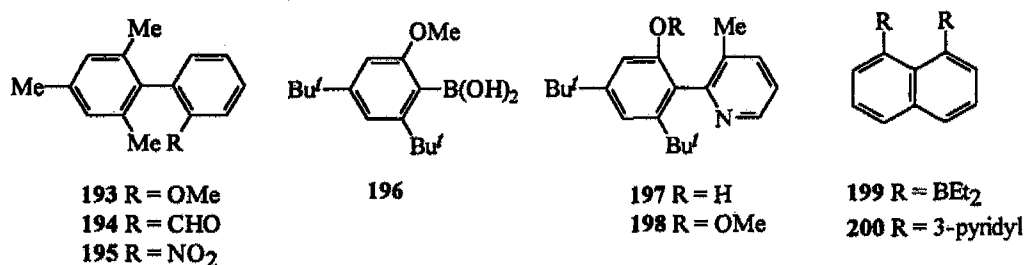
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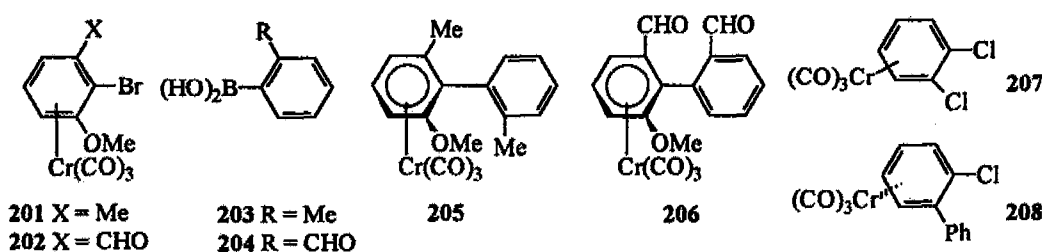
5.2 Steric Considerations

The synthesis of compounds 177 and 178 in a Suzuki reaction illustrates that biphenyl derivatives possessing three *ortho* substituents are readily available. The related biphenyl derivatives 193,¹⁸⁴ 194,^{184,196} and 195¹⁹⁷ have also been prepared using Suzuki methodology and further examples of the preparation of biphenyls possessing three *ortho* substituents in natural product chemistry are given below in Section 5.3. The most commonly used base in the Suzuki reaction is Na₂CO₃ and this base is often ineffective with sterically demanding coupling reactions. If Ba(OH)₂ or K₃PO₄ is used in sterically demanding reactions, good yields of products are usually obtained.¹⁹⁶

The boronic acid 196 and 2-bromo-3-methylpyridine gave the biaryl 198 [Pd(PPh₃)₄ catalyst, KOBu^t base] in 83% yield. This compound was subsequently demethylated affording the phenolic derivative 197 which, after resolution, was used as a chiral ligand for the enantioselective addition of Et₂Zn to arylaldehydes.¹⁹⁸ The bis-borane 199 and 3-bromopyridine gave product 200 in high yield in a Pd(PPh₃)₄ catalysed reaction.¹⁹⁹



Chromium tricarbonyl complexes of bromobenzene derivatives have been coupled with boronic acids yielding biaryls with axial chirality in Pd(PPh₃)₄ catalysed reactions in the presence of Na₂CO₃.²⁰⁰ Thus, compound 201 and boronic acid 203 afforded product 205 (96%) whereas compound 202 and boronic acid 204 gave biaryl 206 (43%). Other chromium tricarbonyl complexes and boronic acids similarly gave related biaryl products. Complex 207 reacted with phenylboronic acid in the presence of the chiral ligand, (*S*)-(*R*)-PPFA, yielding a 73:27 mixture (55%) of compound 208 (69% ee) and the corresponding achiral diphenylation product.²⁰¹ Similar results were obtained with 2-tolylboronic acid although the ee was lower.

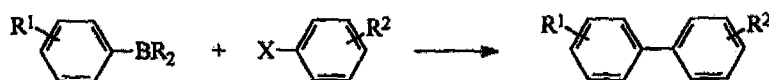


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5.3 General Survey of the Suzuki Reaction

The preparation of biphenyls using Suzuki methodology is well documented in the literature and some representative examples are given in Table 6. The yield of biphenyl products are invariably good to excellent.

Table 6. Synthesis of Biphenyls using the Suzuki Reaction



R ¹	R ²	R	X	Catalyst	Base	Yield (%)	Reference
H	H	OH	OTf	Pd(PPh ₃) ₄	K ₃ PO ₄	83	202
H	4-OH	OH	I	Pd(OAc) ₂	Na ₂ CO ₃	80	203
H	4-OMe	O(CH ₂) ₃ O	I	Pd(dppe)Cl ₂	TIOH	82	204
H	4-CHO	OH	Br	Pd/C+PPh ₃	Na ₂ CO ₃	96	205
H	2/3/4-CO ₂ H	OH	I	Pd(OAc) ₂	Na ₂ CO ₃	60-95	202, 206
H	4-CO ₂ Me	OH	Br	Pd(PPh ₃) ₄	Na ₂ CO ₃	94	183
H	2-NO ₂	OH	Br	Pd(PPh ₃) ₄	Na ₂ CO ₃	98	197
4-F	H	OH	I	Pd(OAc) ₂	Na ₂ CO ₃	>95	207
4-F	4-CHO	OH	Br	Pd/C+PPh ₃	Na ₂ CO ₃	84	205
4-Me	4-OMe	OH	OTf	Pd(PPh ₃) ₄	K ₃ PO ₄	85	208
4-Me	4-SO ₃ Na	OH	Br	a	Na ₂ CO ₃	78	209
4-Me	4-NO ₂	OH	OTf	Pd(PPh ₃) ₄	K ₃ PO ₄	82	202, 208
2-CHO	4-Me	OH	I	Pd(PPh ₃) ₄	Na ₂ CO ₃	54 ^b	196
2-CHO	4-Me	O(CH ₂) ₃ O	I	Pd(PPh ₃) ₄	K ₃ PO ₄	89	196
2-CHO	2-NO ₂	OH	Br	Pd(PPh ₃) ₄	Et ₃ N	82	210
2-CONPr ⁱ ₂	H	OH	Br	Pd(PPh ₃) ₄	Na ₂ CO ₃	82	211
2-CONPr ⁱ ₂	2-CONEt ₂	OH	Br	Pd(PPh ₃) ₄	Na ₂ CO ₃	44	211
3-NO ₂	H	OH	Br	Pd(PPh ₃) ₄	Na ₂ CO ₃	95	197
4-NO ₂	4-COMe	OBu	Br	Pd(PPh ₃) ₄	Tl ₂ CO ₃	90	204

a Pd[Ph₂P(3-SO₃H.C₆H₄)]₃, b deboronation also occurs giving benzaldehyde (39%)

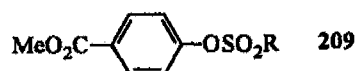
In addition to the biphenyls listed in Table 6, other more highly substituted biphenyls,^{212-218,218a} related compounds such as arylated naphthalenes,^{207,219} arylated binaphthyls²²⁰ and phenanthrenes²¹⁹ have also been prepared using the Suzuki reaction. Appropriately *ortho* substituted biphenyls have been synthesised as precursors to phenanthrols²¹⁴ and nitrofluorenones.²¹⁵ Biphenyl derivatives can also be prepared using solid phase synthesis.^{121,221,222}

Other applications of the Suzuki reaction in biphenyl chemistry include the preparation of butylated hydroxybiphenyls as anti-oxidants,²²³ 5-arylninhydrin derivatives from compound 135 and arylboronic acids,¹³¹ and liquid crystals.^{186,187,224,225} Biphenyl derivatives have also been synthesised for the medicinal chemistry field; examples include amino acid analogues²²⁶⁻²²⁸ and *N*-methyl-D-aspartate (NMDA) antagonists.²²⁹

Borates such as Ph₄BNa are frequently used as coupling partners of arylhalides in the Suzuki reaction and several biphenyl derivatives have been prepared using this methodology.^{203,230} Borates of general structure Ar¹XB(OR)₂⁻ Li⁺ (X = Me, Bu) gave biphenyls (Ar¹-Ar²) with mesylates (Ar²OMs) in Ni(PPh₃)₂Cl₂ catalysed reactions.²³¹

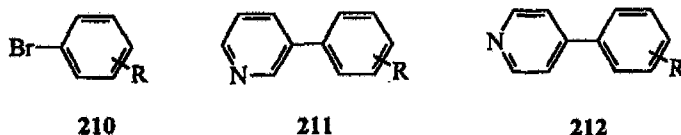
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The reaction of phenylboronic acid with esters of general structure 209 ($R = \text{Me}, \text{CF}_3, \text{Ph}, 4\text{-FC}_6\text{H}_4, 4\text{-MeC}_6\text{H}_4$) has been studied. The best yield of methyl biphenyl-4-carboxylate was obtained with the triflate 209 ($R = \text{CF}_3$).²³²



The fluoride anion can also be used as a base in the Suzuki reaction. Thus, phenylboronic acid has been reacted with a range of bromobenzene derivatives [$\text{Pd}(\text{PPh}_3)_4$ catalyst] in the presence of a variety of fluoride sources (Et_4NF , Bu_4NF , CsF or KF) and biphenyls were produced generally in good yield.²³³

There are many examples of the synthesis of heterobiaryls possessing one heterocyclic and one carbocyclic ring. In pyridine chemistry for example, borane 185 has been coupled [$\text{Pd}(\text{PPh}_3)_4$ catalyst] with bromoaryls 210 ($R = 2\text{- or }4\text{-Cl, Me, OMe, COMe, CO}_2\text{Me, NO}_2$) affording heterobiaryls 211 in 53-87% yield²³⁴ and borane 186 also gave heterobiaryls 212 ($R = 2\text{- or }4\text{-Me, CO}_2\text{Me, NO}_2$) in 47-77% yield.¹⁹³ Other arylated pyridines,^{185,202,204,208,209,211,235,236} pyrazines,^{185,194,236,237,238} pyrimidines,^{185,194} quinolines,^{194,239-241} imidazoles,¹⁰⁵ furans,^{241a} thiophenes,^{210,211} indoles,^{242-244,244a} and porphyrins²⁴⁵⁻²⁴⁷ have similarly been prepared using the Suzuki reaction.



The preparation of heterobiaryls using the Suzuki reaction in which both aryl moieties are heterocycles has attracted considerable attention and some illustrative examples of the synthesis of parent heterobiaryls are shown in Table 7. The catalyst in all of the reactions cited in Table 7 was $\text{Pd}(\text{PPh}_3)_4$ and the yields of products are generally good to excellent. Numerous substituted derivatives of the heterocycles listed in Table 7 have also been prepared.

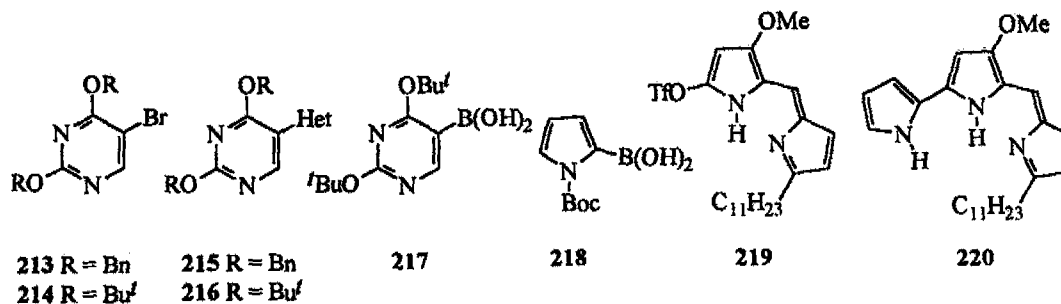
Table 7. Synthesis of Heterobiaryls using the Suzuki Reaction



Het ¹	Het ²	R	X	Base	Yield (%)	Reference
2-thienyl	3-thienyl	OH	Br	Na_2CO_3	40-70	248
3-thienyl	2-thienyl	OH	Br	Na_2CO_3	72	249
3-thienyl	3-thienyl	OH	Br	Na_2CO_3	70	248,249
2-thienyl	2/3/4-pyridyl	OH	Br	Na_2CO_3	57-68	250
3-thienyl	2/3/4-pyridyl	OH	Br	Na_2CO_3	69-74	250
5-indolyl	2/3-furyl	OH	Br	NaHCO_3	65-82	242
5-indolyl	2/3-thienyl	OH	Br	NaHCO_3	78-86	242
5-indolyl	2/3/4-pyridyl	OH	Br	NaHCO_3	65-90	242
3-pyridyl	2-pyridyl	Et	Cl/Br	KOH	82-85	192
4-pyridyl	2/3-thienyl	Et	Br	Na_2CO_3	66-67	193
4-pyridyl	3-pyridyl	Et	Br	Na_2CO_3	64	193
4-pyridyl	2/3-quinoloyl	Et	Br	Na_2CO_3	70-74	193

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A number of uracil analogues have been synthesised as potential anti-viral agents using the Suzuki reaction.^{95,251,252} For example, pyrimidine derivatives **213** and **214** have been coupled with heteroaryl boronic acids, HetB(OH)₂ (Het = 2/3-thienyl, 2/3-selenyl *etc*) giving biaryls **215** and **216** respectively which, after removal of the *O*-protecting group, give uracil analogues. Heteroaryl bromides can also be coupled with boronic acid **217** giving products **216**.⁹⁵ The pyrrole-containing immunosuppressive-agent undecylprodigiosine **220** has been synthesised using a Suzuki reaction of boronic acid **218** and triflate **219**.²⁵³ The Boc group of boronic acid **218** is lost during the coupling process.

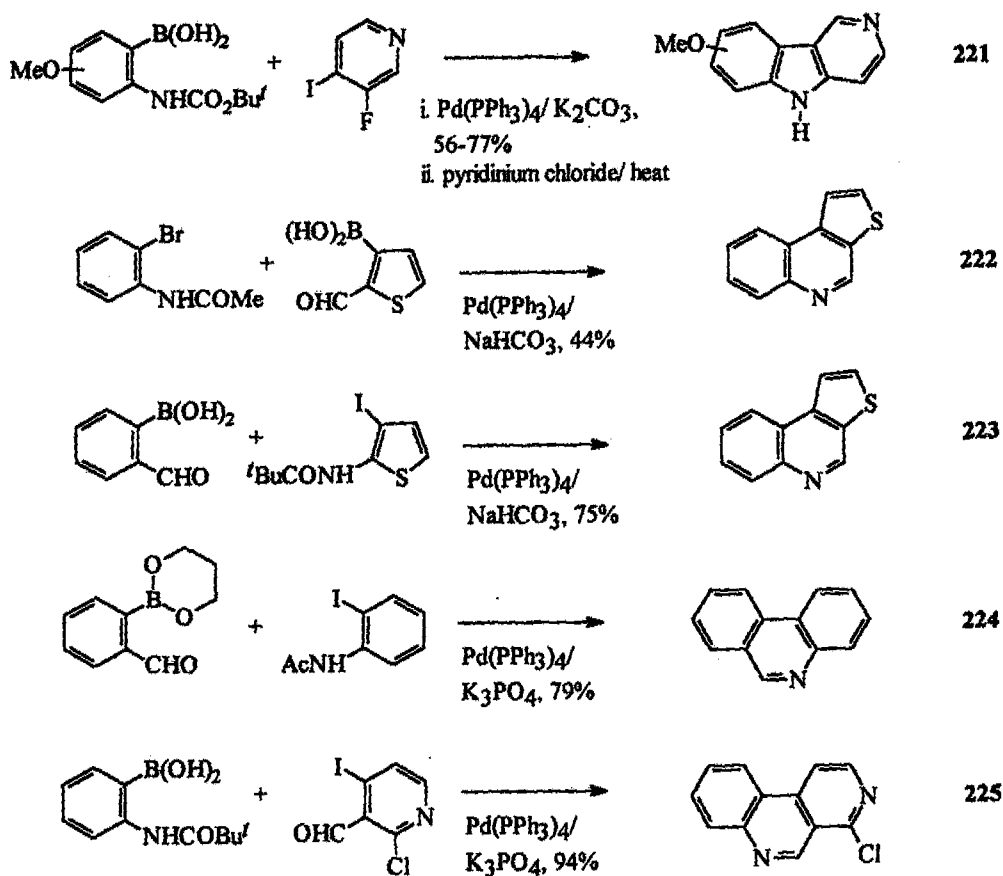


Scheme 5 illustrated how the Stille reaction could be applied to the synthesis of tricyclic heterocycles. The Suzuki reaction has also been employed in the synthesis of tricyclic heterocycles by cyclisation of biaryl precursors and heterocycles **221**,²⁵⁴ **222**,²⁵⁵ **223**,²⁵⁵ **224**¹⁸⁴ and **225**^{256,257} provide representative examples (**Scheme 6**). Substituted phenanthridines and phenanthridinones have also been prepared using similar methodologies.²⁵⁸

Terpyridyls,¹⁹³ quaterpyridyls¹⁶⁹ and a plethora of teraryl¹⁶⁷ with three heterocyclic rings have been prepared using the Suzuki reaction.

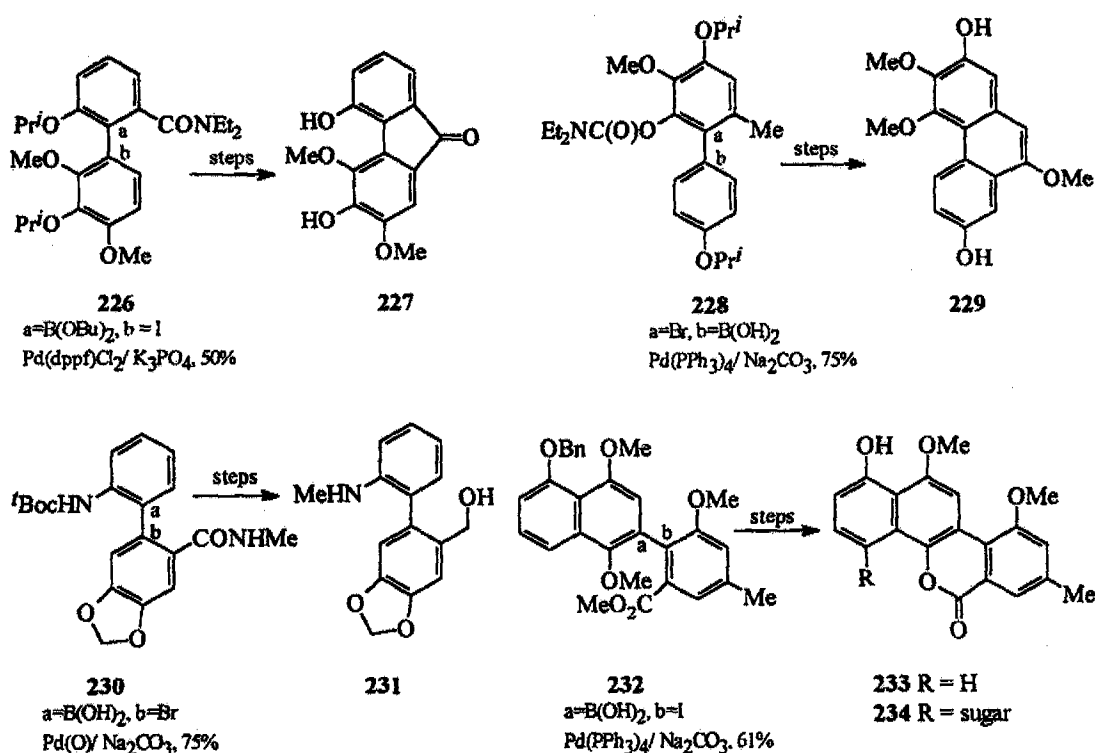
The Suzuki reaction has found widespread application in polymer chemistry. Poly(*para*-phenylene) derivatives,²⁵⁹ alkyl substituted poly(*para*-phenylenes),^{188,260–262} water soluble poly(*para*-phenylenes)²⁶³ and polyphenylene dendrimers^{264,265} have all been prepared. Polymers possessing alternating substituted biphenyl and binaphthyl moieties have also been prepared²⁶⁶ and the preparation of 'graphite ribbons' from alkynyl substituted poly(*para*-phenylenes) has been reported.²⁶⁷ A series of butylated oligonaphthalenes have been synthesised²⁶⁸ and chiral polynaphthols have been reported.²⁶⁹ In heterocyclic chemistry, alkylated polymers of 2-phenylpyrrole²⁷⁰ and trimethylsilyl-capped oligothiophenes have been prepared.¹⁶²

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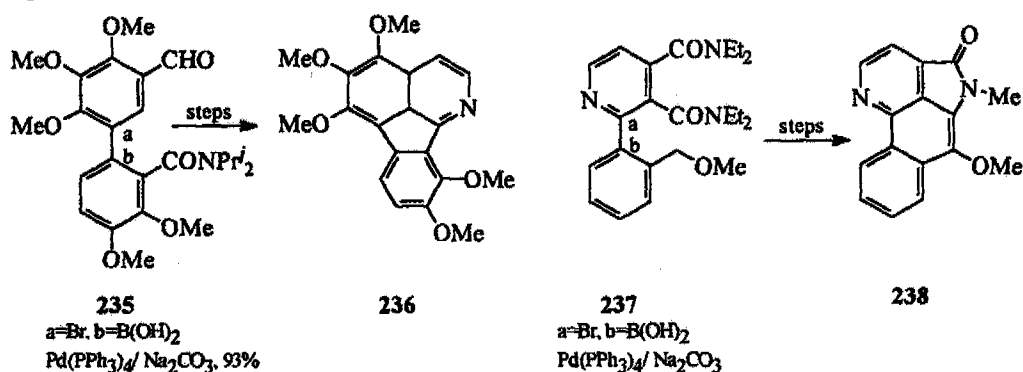
Scheme 6

The Suzuki reaction has found extensive application in natural product chemistry and some illustrative examples are now described. The fluorenone derived natural product, Dengibsinin **227** has been prepared from the biphenyl precursor **226** which was prepared using a Suzuki reaction.²⁷¹ The synthesis of biphenyl **226**, which possess three *ortho* substituents provides a further example of the tolerance of the Suzuki reaction to steric effects. The phenanthrene derived compound, Gymnopusin **229** has been prepared from biphenyl **228**²⁷² and Ismine **231** was synthesised from biphenyl **230**.²⁵⁸ The aglycone **233** of the benzonaphthopyrone antibiotics **234** has been synthesised from biaryl **232**²⁷³ and other lactones such as Autumnariol have similarly been prepared.²⁷⁴

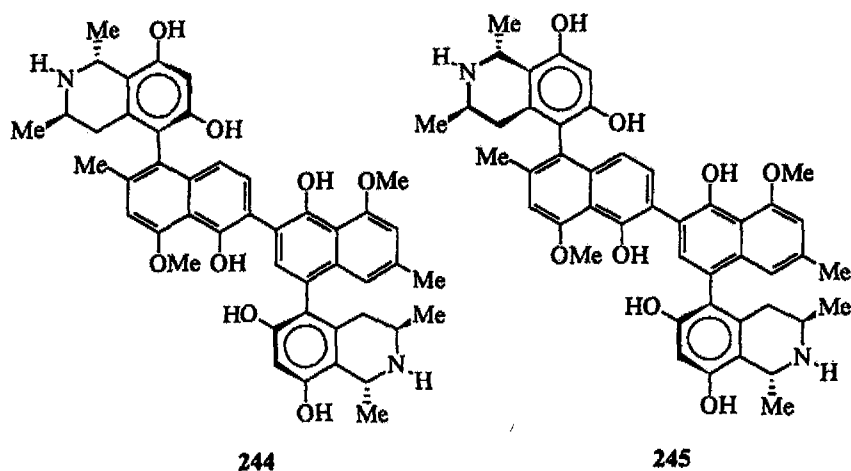
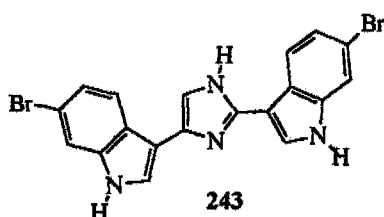
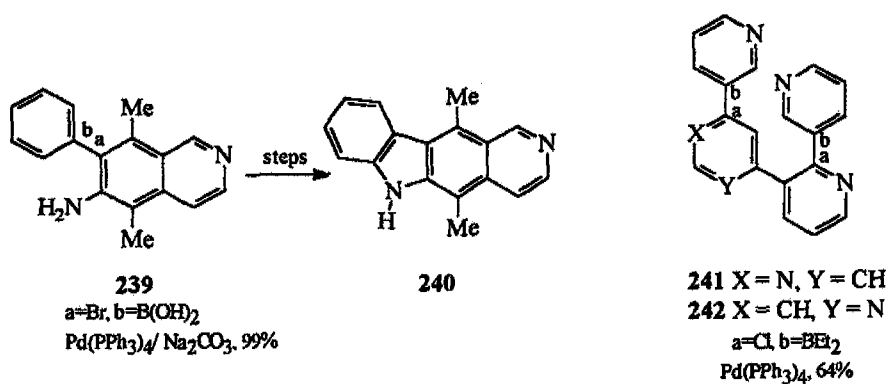


Examples of the synthesis of alkaloids using the Suzuki reaction include the preparation of Imeluteine **236** from biphenyl **235**,^{271,271a} Eupolauramine **238** from biaryl **237**,²⁷⁵ Ellipticine **240** from heterocycle **239**,²⁷⁶ the quaterpyridine **242**, an isomer of Nemertelline **241**²⁷⁷ and the teraryl, Nortopsentin A **243**.²⁷⁸ Nemertelline **241** has been prepared (68%) using Stille methodology from bipyridyls **188** and **190**.¹⁹¹ The quaterpyridine structure **242** had originally been assigned to Nemertelline **241** and its synthesis was undertaken for this reason. The alkaloids Dubamine **164** and Hippadine **172** have both been prepared using the Stille reaction as outline in Section 4.3 and the Suzuki reaction has also been applied to the synthesis of these two heterocycles^{194,240,279} as well as related alkaloids such as Ungerimine.²⁷⁹

The synthesis of Michellamines such as the atropisomers Michellamine A **244** and Michellamine B **245** has attracted considerable interest.^{68,280,281} In one synthesis of compounds **244** and **245**, a bis-Suzuki reaction was employed to form the quateraryl skeleton from tetrahydroisoquinolyl and binaphthyl precursors.²⁸⁰ Analogues of Michellamine B **245** have also been prepared.²⁸²



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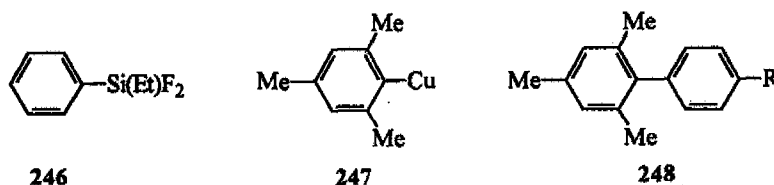
6. OTHER CROSS-COUPLING REACTIONS

Aryl silanes such as compound **246**²⁸³ and related compounds^{284,285} underwent a fluoride promoted cross-coupling reaction with aryl halides or triflates in the presence of a palladium catalyst yielding biaryls. For example, compound **246** and 2-iodoanisole afforded 2-methoxybiphenyl (45%) and 3-methoxybiphenyl (83%) was obtained from 3-iodoanisole and silane **246**.

Organomercury compounds (¹Ar₂Hg) have been coupled with aryl iodides (Ar²I) in the presence of a palladium catalyst giving biphenyl derivatives in good yield. 4-Nitrobiphenyl (98%) and 4'-X-4-nitrobiphenyls (X = Cl, Me, OMe) (97–99%) have been synthesised using this chemistry²⁸⁶ and heterobiaryls have similarly been prepared.²⁸⁷

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In copper chemistry, compound **247** reacted with a number of 4-substituted aryl iodides yielding products **248** ($R = Cl, OMe, COMe, CN, NO_2$) in good yield (70-90%).²⁸⁸ The catalyst was $PdPh(PPh_3)_2I$ and both the yield rate of reaction was accelerated by the presence of Bu_4NI . The role of the Bu_4NI was to form a more reactive organocopper intermediate, $[mesitylCuI]^- Bu_4N^+$.



7. REVIEW ARTICLES

There are a substantial number of review articles of various lengths and detail in the literature which have Sections describing catalytic cross-coupling reactions in biaryl synthesis.^{187,289-311} Many of these reviews consider cross-coupling reactions in a broader sense, for example the cross-coupling of aryl and vinyl moieties and of two different vinyl moieties but these reactions are usually mechanistically closely related to the reactions covered in this Review. Several of these reviews also give historical developments of catalytic cross-coupling reactions.

8. LIGAND ABBREVIATIONS AND STRUCTURES

The structure of ligands considered in this review are given in Table 8 (following page). It should however be appreciated that an extensive number of other ligands have been used in catalytic cross-coupling reactions.

9. CONCLUDING REMARKS

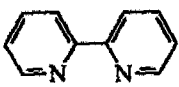
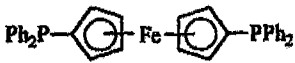
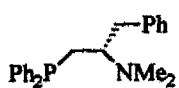
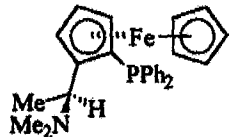
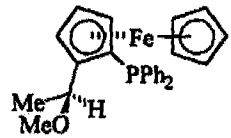
It is clear that the transition metal mediated coupling route to biaryls and heterobiaryls has come of age and is routinely employed in laboratories throughout the world. It is however appropriate within the context of this Review to broadly summarize the relative advantages and disadvantages of the Kharasch, Negishi, Stille and Suzuki reactions so that a judicious choice from these reactions can be made by the practitioner when contemplating the preparation of a biaryl molecule.

In terms of overall functional group compatibility, the Stille and Suzuki reactions tolerate a wide range of functional groups and biaryls possessing electronically different aryl fragments are amenable to synthesis using these methodologies. The Suzuki reaction is especially suited to sterically crowded biaryls. Both the Stille and Suzuki reactions can tolerate water and both arylstannanes and arylboronic acids are relatively easy to prepare.

For structurally uncomplicated biaryls possessing functional groups which can tolerate Grignard reagents, the Kharasch reaction is often a useful preliminary method to try if the Grignard reagent and its coupling partner are readily available.

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Table 8. Ligand Abbreviations and Structures

Abbreviation	Structure	Name
acac	$\text{MeCOCH}=\text{C}(\text{O}-)\text{Me}$	acetylacetonate
dba	$\text{PhCH}=\text{CHCOCH}=\text{CHPh}$	dibenzylideneacetone
bpy		2,2'-bipyridyl
dppe	$\text{Ph}_2\text{PCH}_2\text{CH}_2\text{PPh}_2$	1,2-bis(diphenylphosphino)ethane
dppp	$\text{Ph}_2\text{PCH}_2\text{CH}_2\text{CH}_2\text{PPh}_2$	1,3-bis(diphenylphosphino)propane
dppb	$\text{Ph}_2\text{PCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{PPh}_2$	1,4-bis(diphenylphosphino)butane
dppf		1,1'-bis(diphenylphosphino)ferrocene
(S)-phephos		(S)-2-(dimethylamino)-1-(diphenylphosphino)-3-phenylpropane
(S)-(R)-PPFA		(S)-N,N-dimethyl-[(R)-2-(diphenylphosphino)ferrocenyl]ethylamine
(S)-(R)-PPFOMe		(S)-1-[(R)-2-(diphenylphosphino)ferrocenyl]ethyl methyl ether
triphos	$(\text{Ph}_2\text{PCH}_2\text{CH}_2)\text{PPh}$	bis[2-(diphenylphosphino)ethyl]phenylphosphine

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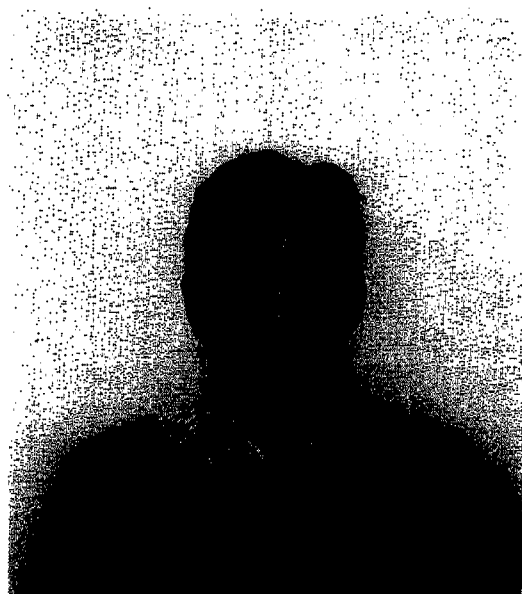
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Biographical sketch



Stephen Stanforth

Stephen Stanforth received both his Msc and PhD degrees from Sheffield University where he worked on the synthesis and cycloaddition reactions of heterocyclic mesomeric betaines with Professor W. D. Ollis, FRS. After one year's post-doctoral work in heterocyclic chemistry at Sheffield with Professor Ollis (1981–1982), he moved to the CNRS, Gif-sur-Yvette to work with Sir Derek Barton, FRS on organobismuth chemistry for one year. In 1983 he moved to the University of Northumbria where his current research interests are centred around heterocyclic chemistry.

Efficient Synthesis of 3-Aminocyclobut-2-en-1-ones: Squaramide Surrogates as Potent VLA-4 Antagonists

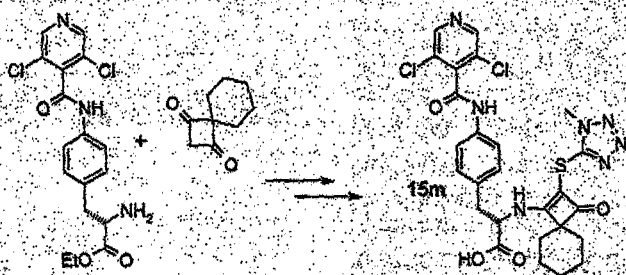
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Received April 28, 2003

ABSTRACT



A novel series of uniquely functionalized 3-aminocyclobut-2-en-1-ones has been prepared by facile condensation of a variety of cyclobuta-1,3-diones with a phenylalanine-derived primary amine. These systems subsequently lend themselves to substitution at C-2 by reaction with a variety of electrophilic reagents including *N*-halosuccinimides, sulfonyl chlorides, and Eschenmoser's salt. Compounds from this novel series are potent antagonists of VLA-4.

The integrins are a large family of heterodimeric, cell surface receptor proteins which consist of an α and a β subunit. Restricted association of these subunits can give rise to more than 24 characterized integrins, which bind specific peptide ligands of the extracellular matrix.¹ The integrin VLA-4 (very late activating antigen-4, $\alpha 4 \beta 1$, CD49d/CD29) is expressed on a variety of leukocytes and through interaction with its endothelial ligand VCAM-1 (vascular cell adhesion molecule) plays an important role in recruitment, trafficking, and infiltration of circulating lymphocytes to sites of inflammation.² Accumulating clinical evidence from the use of monoclonal antibodies (i.e., Antegren/Natalizumab) strongly suggests that pharmacological antagonism of VLA-4 is a viable method for alleviating the debilitating tissue damage which arises in autoimmune disorders including rheumatoid arthritis, asthma, multiple sclerosis, and Crohn's disease.³

VCAM-1 has been shown to bind VLA-4 through the sequences Ile-Asp-Ser.⁴ This binding motif was used as a starting point for early proteomimetic inhibitor design efforts, which initially gave rise to cyclic peptides such as 1 (Figure 1), being derived from this sequence.⁵ However, peptides typically make poor drug candidates as they frequently have sub-optimal absorption properties and low metabolic stability, factors which can significantly impede systemic exposure.

N-Acylated phenylalanine derivatives have subsequently emerged from several groups as a major class of potent VLA-4 antagonist.⁶ Notable members of this useful class of

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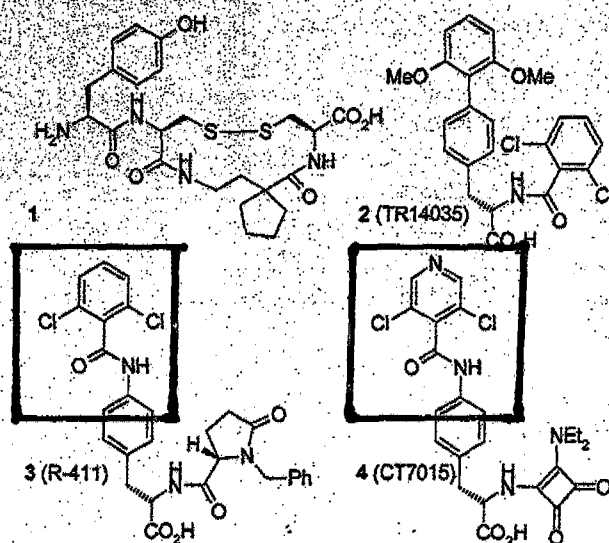


Figure 1. Potent small molecule VLA-4 antagonists.

small molecule are 2⁷ and 3,⁸ which are reportedly undergoing clinical evaluation for the treatment of asthma and are currently in phase 1 and phase 2, respectively.

Of the numerous inhibitors reported in this class, it is clear that a high degree of diversity in the *N*-acyl substituent is tolerated, although these are generally hydrophobic, and that the amino acid α -NH is important for activity. We have recently described the discovery of squaric acid derivatives including CT7015 (4) as VLA-4 antagonists⁹ that illustrate these observations.

As part of a search for a unique class of VLA-4 antagonists distinct from 4, we hypothesized that the combination of a hydrophobic spirocyclic substituent, such as that in 1, in the context of a rigid planar scaffold such as that provided by the 3,4-diamino-3-cyclobuten-1,2-dione (squaramide) group of 4, would provide potent compounds with an advantageous pharmacokinetic profile. We believed that the 3-amino-2-cyclobuten-1-one analogues typified by 5 would be the most appropriate compounds to test this "hybridization" hypothesis (Figure 2) and investigated the synthesis of these by condensation of an α -amino ester with a cyclobuta-1,3-dione or its enol ether.¹⁰

We felt that this intriguing structural element, thus far unutilized in the pharmaceutical arena, warranted further

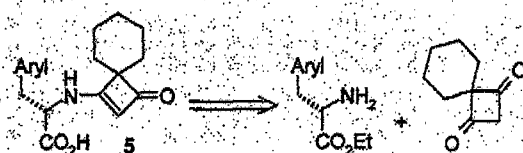
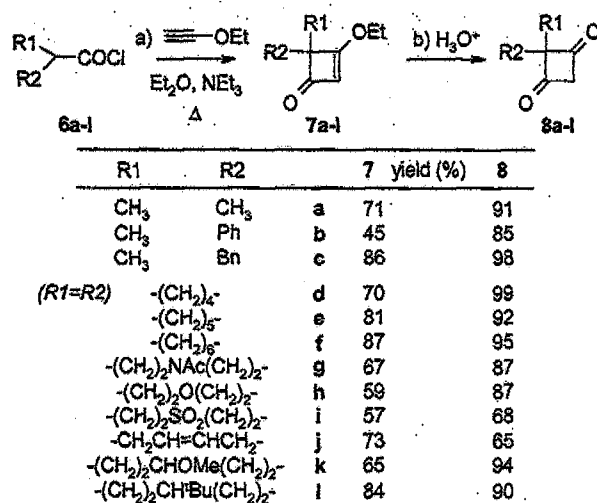


Figure 2. Proposed route to 3-aminocyclobut-2-en-1-ones.

investigation and embarked upon development of a robust method which would grant access to 5 and its analogues.

Accordingly, *gem*-disubstituted 3-ethoxy-2-cyclobutene-1-ones were readily accessed by the cycloaddition of a disubstituted ketene, generated in situ by dehydrohalogenation of the corresponding acid chloride, and ethoxyacetylene, according to a previously recognized phenomenon¹¹ (Scheme 1). For example, the reaction of cyclohexanecarbonyl

Scheme 1. Synthesis of Cyclobuta-1,3-diones



chloride and NEt₃ (1.5 equiv) in the presence of ethoxyacetylene¹² (2.0 equiv) in Et₂O afforded 3-ethoxyspiro[3.5]non-2-en-1-one (7e) in 81% yield.

The reaction time can be reduced from a reported 21 days at 5 °C^{10a} to less than 48 h by heating the reaction to reflux temperature. Although feasible, no apparent dimerization of the intermediate ketenes was observed at this elevated temperature. The novel spirocyclic examples 7g-l were prepared using an analogous transformation with comparable yields being obtained. In all cases examined, the cycloadducts were found to be stable and were conveniently purified by column chromatography on silica gel.

Various attempts to convert enoether 7e directly to the desired 3-amino derivative (i.e., 5) by substitution with L-phenylalanine ethyl ester as a test substrate were frustrat-

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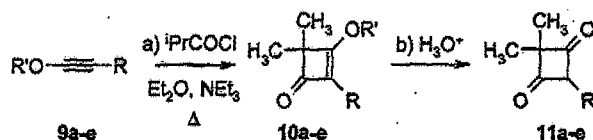
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(12) Ethoxyacetylene was purchased from Lancaster Chemical Co. as an approximately 50%w/w solution in hexanes. Yields 7a-l were calculated based upon the amount of acid chloride used.

ingly unproductive. We were consequently forced to examine formation of the desired vinylogous amides by combination of an amine with a cyclobuta-1,3-dione. To this end, cyclobutenones **7a-l** could be hydrolyzed to cyclobutadiones **8a-l** in good yield by treatment with 2 M HCl at 20 °C for 24 h. A notable aspect of these conversions is that hydrolysis proceeds without detriment to the four-membered carbocycle, in contrast to analogues lacking geminal substitution at C4 which undergo a facile ring-opening under these conditions to give β -ethoxycrotonic acids.^{10a}

In an effort to expand the scope of the ring-forming process, we attempted substitution at the 2-position of the cyclobutenone by employing a variety of substituted alkoxyacetylenes. Consequently, cycloaddition precursors **9a,b** were prepared directly by alkylation of the acetylide anion of ethoxyacetylene (1.1 equiv of ⁿBuLi, 2.2 equiv of HMPA) with benzyl bromide and *n*-hexyl iodide, respectively, according to literature procedure.¹³ Alkynes **9c-e** were prepared by reaction of chloroacetaldehyde dibutylacetal with 2.2 equiv LDA/HMPA followed by alkylation with the appropriate alkyl bromide, RBr.¹⁴ Alkynes **9a-e** were also found to undergo cycloaddition with dimethylketene to give enol ethers **10a-e** (Scheme 2), and these were hydrolyzed without incident under similar conditions to give diones **11a-e**.

Scheme 2. Synthesis of Trisubstituted Cyclobuta-1,3-diones



R'	R		10 yield (%)	11
Et	Bn	a	75	95
Et	ⁿ Hex	b	61	98
ⁿ Bu	CH ₃	c	45	85
ⁿ Bu	Et	d	56	70
ⁿ Bu	ⁿ Pr	e	51	64

In all cases described, the expected regiochemistry of the cycloaddition was complete and no regioisomeric material was detected.

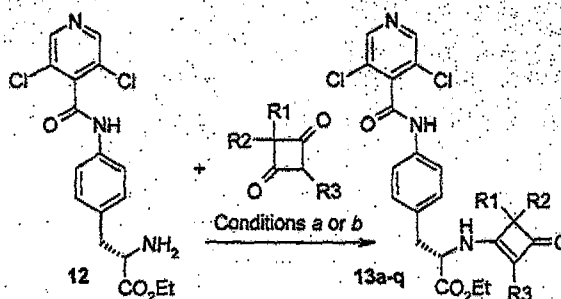
Subsequent reaction of cyclobuta-1,3-diones **8a-i** with the phenylalanine derivative **12** was found to proceed with surprising speed when mixed in THF at room temperature, to give the desired vinylogous amide derivatives **13a-l** in <24 h¹⁵ (Scheme 3, conditions a). Contrastingly, those reactions involving C-2 substituted cyclobuta-1,3-diones **11a-e** were wholly unreactive with **12** under these conditions and required heating at 100 °C in nitromethane for 48 h to achieve complete conversion to the corresponding analogues **13m-q** (Scheme 3, conditions b).

(13) Pons, J.-M.; Kocienski, P. *Tetrahedron Lett.* 1989, 30, 1833. **9a** and **9b** were prepared in 99% and 92% yields, respectively.

(14) A modification of the method reported in: Newman, M. S.; Geib, J. R.; Stalick, W. M. *Org. Prep. Proced. Int.* 1972, 4, 89.

(15) Contrastingly, the reaction of **4** with cyclopenta-1,3-dione required several days for completion under these conditions.

Scheme 3. Condensation of a Phenylalanine-Derived α -Amino Ester with Cyclobuta-1,3-diones



R1	R2	R3		yield (%)	13
CH ₃	CH ₃	H	a	a	93
CH ₃	Ph	H	a	b	59
CH ₃	Bn	H	a	c	89
-(CH ₂) ₄ -		H	a	d	86
-(CH ₂) ₅ -		H	a	e	95
-(CH ₂) ₈ -		H	a	f	86
-(CH ₂) ₂ NAc(CH ₂) ₂ -		H	a	g	67
-(CH ₂) ₂ O(CH ₂) ₂ -		H	a	h	91
-(CH ₂) ₂ SO ₂ (CH ₂) ₂ -		H	a	i	23
-CH ₂ CH=CHCH ₂ -		H	a	j	59
-(CH ₂) ₂ CHOMe(CH ₂) ₂ -		H	a	k	87
-(CH ₂) ₂ CH ^t Bu(CH ₂) ₂ -		H	a	l	84
CH ₃	CH ₃	Bn	b	m	59
CH ₃	CH ₃	ⁿ Hex	b	n	72
CH ₃	CH ₃	Me	b	o	70
CH ₃	CH ₃	Et	b	p	73
CH ₃	CH ₃	ⁿ Pr	b	q	75

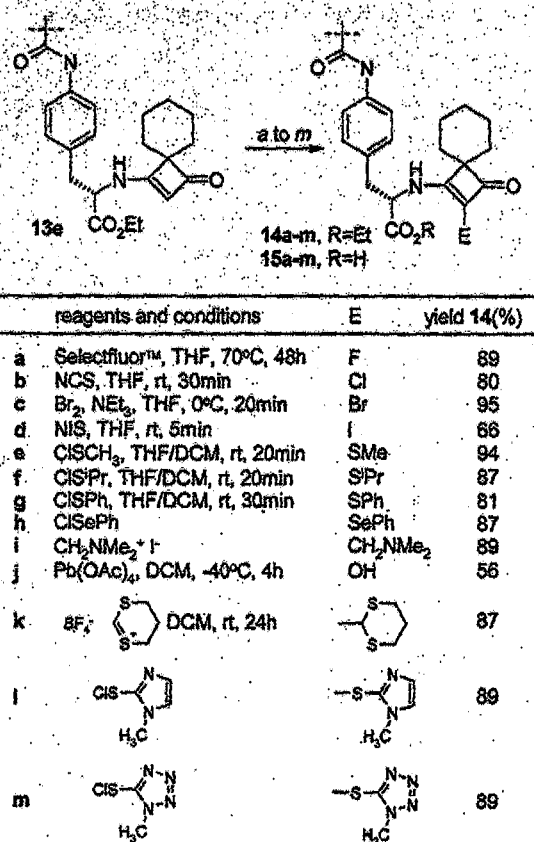
We speculate that this may either be a steric phenomenon or alternatively that the C2 substituent stabilizes the relatively unreactive enolic form of the dione, reducing the relative concentration of the reactive dicarbonyl tautomer. Interestingly, the condensation reaction of *meso*-diones **8b, c** and **k**, which contain prochiral centers, gave inseparable diastereomeric products with the reaction showing only weak diastereoselectivity (<30%). Contrastingly, dione **8l** reacted to give compound **13l** as a single isomer, the stereochemistry of which we were unable to determine conclusively.

With the intention of providing access to novel C-2 substituted versions of **5**, we surveyed the reactivity of **13e** to a variety of electrophiles and the results of this study are summarized in Scheme 4. In keeping with the observation that **7a** undergoes bromination,^{10a} vinylogous amide **13e** was found to be rapidly convertible to the 2-bromo analogue **14c** in 95% yield, by reaction with bromine or NBS in THF at room temperature (Scheme 4). Chlorination and iodination are achieved using NCS and NIS respectively and fluorination was performed using Selectfluor¹⁶ to give **14b, 14d**, and **14a**, respectively. The thioalkyl substituents of **14e,f** were introduced by reaction with the appropriate sulfonyl chlorides, which were produced in situ by reaction of the corresponding disulfide and SO₂Cl₂¹⁷ prior to addition of **13f**. Compound **14b** can arise as a minor byproduct of these reactions, presumably through chlorination of **13e** by un-

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Scheme 4. Reaction of 13e with Electrophiles



reacted SO₂Cl₂, although this side-reaction could be avoided by using an excess of disulfide during the sulfonyl chloride preparation step.

Compounds 14g,h were prepared in an analogous fashion by reaction with commercially available phenylsulfonyl and -selenenyl chloride. A dimethylaminomethyl group can be introduced onto 13e by reaction with Eschenmoser's salt [H₂C=N(Me)₂⁺ I⁻]. Hydroxylation of the unsubstituted vinylic carbon was achieved by oxidation with Pb(OAc)₄ to give compound 14j. π -Electrophiles are also found to react with the nucleophilic amino-cyclobutenone system as evidenced by reaction of 13e with 1,3-dithienium tetrafluoroborate to give 14k. The heterocyclic sulfonyl chlorides used in reactions l and m were prepared in-situ directly from the corresponding heterocyclic mercaptans by reaction with NCS in THF¹⁸ and these were found to react predictably with 13e to give 14l,m.

The ability of vinyl iodide 14d to act as a participant in a Stille-type reaction would allow more convenient parallel synthesis of C2-substituted compounds, thereby circumventing the need to synthesize individual acetylenes. To this end, 14d undergoes a Pd(PPh₃)₄-catalyzed Stille coupling with 3-tributylstannylpyridine and tributylstannyl-1,4-pyridazine in 41% and 53% yield, respectively, as representative examples of this strategy.

As a prelude to biological testing, ethyl esters 14a-m were required to be converted to their corresponding acids 15a-m, and this could be achieved by reaction with 1M LiOH in THF, in high yield (>85%). It is noteworthy that the aminocyclobutenone core of all compounds were found to be stable under these basic conditions (with the exception of 14j), an indication of the "amidic" stabilization of the enaminone moiety. The ability of carboxylic acids 15a-m to disrupt binding of VLA4 to VCAM was evaluated in a cell based assay using E6.1 Jurkat cells (human T-cell line) and immobilized VCAM-1 in the presence of 1%BSA (bovine serum albumin), and all demonstrated an IC₅₀ < 1 μ M. Notably compound 14m has an IC₅₀ of 3 nM in this assay. A fuller analysis of the biological properties of compounds 15a-m and their congeners shall be reported in due course.

In summary, we have developed the synthesis of a variety of highly functionalized 3-amino-2-cyclobutenones by condensation of an amine with a cyclobuta-1,3-dione as the key step. These uncommon functional groups have subsequently shown potential application as squaramide isosteres in a biological context.

Acknowledgment. We thank Dr. Richard Taylor and Barbara Mason for providing spectral and analytical data and Dr. Tony Shock and Paul Hale for cell assay data.

Supporting Information Available: Preparative methods and spectral and analytical data for compounds 7e-l, 13a-q, and 14a-m. This material is available free of charge via the Internet at <http://pubs.acs.org>.

OL034701N

(18) 1-Methyltetrazole-5-sulfonyl chloride has been previously prepared by chlorination of the mercaptan with chlorine. See: Postle, S. R. U.S. Patent 4 387 158, 1983.

**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES**

Radicación No. 02-105332

Clasificación Internacional (IPC): A61K31/4412, 31/198

Concepto Técnico No.

Patente de Invención



Vía Nacional



Fecha de Presentación Internal. 06/12/1999

Como resultado del examen definitivo, realizado con fundamento en el artículo 48 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

1.1. Descripción: Folios: 5 a 141 (como se radicó inicialmente)

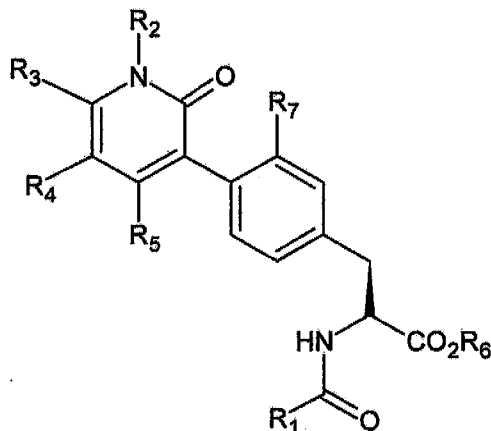
1.2 Reivindicaciones: Folios 142 a 155 (como se radicó inicialmente)

Folios 349 a 358 (con modificación)

1.3 Prioridad: 60/169090 Folios: 156 a 245, US

2 Objeto de la invención

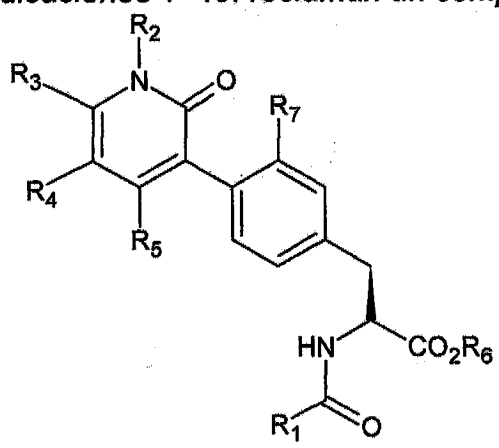
La presente solicitud cuyo título es "4-PIRIDIL-N-ACIL-L-FENILALANINAS," el objeto son los compuestos derivados de ácido (2S)-2-(benzoilamino)-3-[4-(2-oxo-1,2-dihidropiridin-3-yl)fenil]propanoico, de fórmula:



Útiles para el tratamiento de enfermedades cuyos síntomas y/o lesiones se atribuyen a la unión de la molécula ACAM-1 con las células de expresión del VLA-4.

El objeto de la invención se puede clasificar según la clasificación internacional como A61K 31/4412, 31/118.

Las Reivindicaciones 1-49; reclaman un compuesto de la fórmula:



Reivindicación 50 reclama composiciones farmacéuticas que contienen el compuesto de la fórmula referida.

Reivindicación 51 se refiere a un procedimiento de fabricación de una composición farmacéutica.

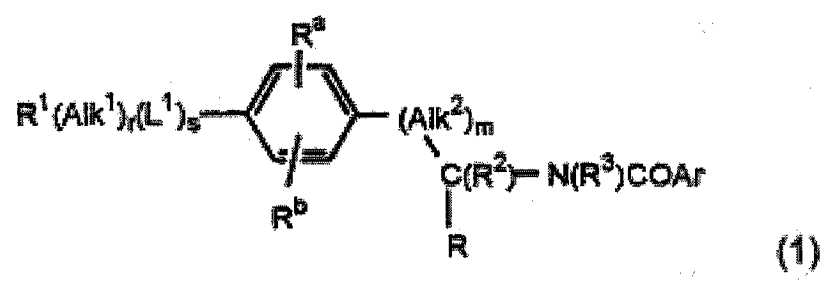
2 Determinación del Estado de la Técnica (art. 16 de la D 486 de la C.AN)

Item	Documento	Fecha de publicación	Título
D1	WO9943642	02-sep-1999	Phenylalanine derivatives as inhibitors of ALPH4 integrins

12. Análisis de patentabilidad (según requisito) (artículo 14 D486)

12.1 Novedad (artículo 16 D486)

- La anterioridad D1 enseña derivados de acilfenil alanina de formula 1:



Y composiciones usadas como inhibidores de integrinas ALPF4, especialmente en las pgs. 4 y 5; pg 12 renglón 25; pg 13 renglón 31; reivindicación 1 y 8. Donde se establece que R1 es un grupo heteroaromático opcionalmente sustituido, el grupo aromático es piridil, sustituido con hidroxil, que forma el tautometo ceto-enol,

sin embargo no se establece plenamente los sustituyentes tal como los compuestos que ahora se reclaman en la solicitud en estudio, por lo tanto esta oficina puede considerar que los compuestos son nuevos, cumpliendo con lo estipulado en el art. 16 de la Decisión 486 de la Comisión de la Comunidad Andina.

12.2 Nivel inventivo (artículo 18 D 486)

No se habían descrito con anticipación los derivados (2S)-2-(benzoilamino)-3-[4-(2-oxo-1,2-dihidropiridin-3-yl)fenil]propanoico, con los sustituyentes que se reivindican y si bien es cierto que la naturaleza química acil fenilalanina y sus analogos conservan los efectos farmacológicos como inhibidores de integrinas ALPF4, no se deriva de manera evidente del estado de la técnica que el grupo 2-oxo-3-piridinil se enlazara directamente a la posición 4-del fenilo, sin tener en medio otro sustituyente, lo cual no se deriva evidentemente del estado de la técnica más próxima. Por lo tanto se puede decir que la solicitud en estudio posee nivel inventivo.

12.3 Aplicación industrial (artículo 19 D 486)

Los compuestos derivados de (2S)-2-(benzoilamino)-3-[4-(2-oxo-1,2-dihidropiridin-3-yl)fenil]propanoico de la presente solicitud son susceptibles de la síntesis de los, en las cantidades requeridas por la industria farmacéutica, para producir medicamentos, por lo tanto se considera que tiene aplicación industrial.

12.4 Respecto a la reivindicación 51, referente a un procedimiento de fabricación de un medicamento, no aparece sustentado en la descripción ningún nuevo procedimiento para la fabricación de medicamentos, que involucre etapas, condiciones o cualquiera de sus características como procedimiento de fabricación, que sea un aporte al acervo tecnológico, por lo tanto la reivindicación en referencia no es aceptada, además de ser el proceso que universalmente debe ser aplicado, para obtener cualquier composición que consiste en incorporar a una mezcla el componente de interés.

13 Conclusión:

En consecuencia y por todo lo expuesto anteriormente, se recomienda **Conceder** las reivindicaciones: 1 a 50 (folios: 349 a 358) y **Negar**lo para la reivindicación 51.

Bogotá, D.C., enero 08 de 2008