

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
RAD: 13-252979- -3	FECHA: 2014-12-10 14:03:27
DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 21
ACT: 519 PRESENTACION	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
CARLOS R. OLARTE GARCIA

Asunto: Radicación: 13-252979- -3
 Trámite: 11
 Evento: 378
 Actuación: 519
 Oficio: 15227
 Folios: 21

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/IB2012/051977				
Fecha de Presentación Internacional	19/04/2012				

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Radicado No. 13-252979-00000-0000	24/Octubre/2013
Reivindicaciones:	Radicado No. 13-252979-00000-0000	24/Octubre/2013
Prioridad:	US 61 477 319	20/Abril/2011

2. Análisis de la Prioridad

Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

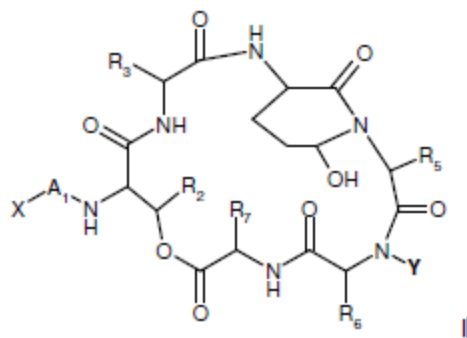
3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. En el presente caso solo se recibió el pago por las 10 primeras reivindicaciones.

Título de la solicitud: “Procesos para la elaboración de depsipéptidos macrocíclicos y nuevos intermediarios”.

El problema planteado en esta solicitud consiste en la necesidad de suministrar procesos de obtención de depsipéptidos cíclicos de la fórmula (I) con un mayor rendimiento y/o con buena pureza.

Para resolver este problema técnico la solicitud en estudio proporciona un método o proceso para la preparación de un compuesto de depsipéptido cíclico de la fórmula I:



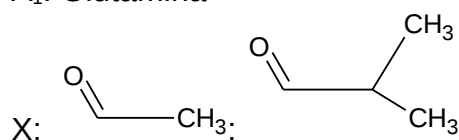
El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C07K11/02

4. De la Solicitud de Patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 1 no se encuentra totalmente soportada en la descripción porque la descripción solo soporta la obtención del depsipéptido donde:

A₁: Glutamina



R²: metilo

R³: Cadena lateral de leucina

R⁵: Cadena lateral de isoleucina o cadena lateral de fenilalanina

R⁶: Cadena lateral de tirosina

R⁷: Cadena lateral de isoleucina o cadena lateral de valina

Y: metilo

Por lo cual se sugiere hacer una generalización razonable de acuerdo a lo efectivamente soportado en la descripción, teniendo en cuenta que la palabra “especialmente” no restringe la reivindicación de ninguna manera, por lo cual se sugiere eliminarla del capítulo reivindicatorio para evitar confusiones al determinar el alcance del capítulo reivindicatorio.

La reivindicación 1 no es concisa porque no establece claramente cómo se realiza la desprotección selectiva del compuesto de fórmula II para llegar al compuesto de la fórmula III, no establece cual es el grupo protector, cuales son los grupos funcionales que se protegen ni los grupos protectores de los mismos, como se realiza la oxidación del compuesto de fórmula III para llegar al compuesto de fórmula IV, y cómo se remueven los grupos protectores restantes. Se sugiere identificar claramente los grupos funcionales, grupos protectores y reactivos usados en cada una de las etapas de acuerdo a lo soportado en la descripción de modo que sea posible determinar claramente el alcance de la solicitud. Los ejemplos soportan:

Grupo protector: difenil-silil-oxi-propil

Desprotección del compuesto de fórmula II: Por reacción de (S)-N¹-((3S,6S,9S,12S,15S,18S,19R)-6-(4-terbutoxi)-bencil)-3,9-di-((S)-sec-butil-12-(3-((terbutil-difenil-silil)-oxi)-propil)-15-isobutil-7,19-dimetil-2,5,8,11,14,17-hexaoxo-1-oxa-4,7,10,13,16-pentaazaciclon-onadecan-18-il)-2-isobutiramido-N⁵-tritol-pentandiamida con Et₃N(HF)₃ en tetrahidrofurano o una mezcla de tetrahidrofurano y metil-éter de terbutilo; o la des-sililación del compuesto (S)-2-acetamido-N¹-((3S,6S,9S,12S,15S,18S,19R)-9-bencil-6-(4-(terbutoxi)-bencil)-12-(3-(hidroxi)-propil)-15-isobutil-3-isopropil-7,19-dimetil-2,5,8,11,14,17-hexaoxo-1-oxa-4,7,10,13,16-pentaazaciclo-nonadecan-18-il)-N⁵-tritol-pentan-diamida en las mismas condiciones ya mencionadas

Grupos funcionales que se protegen: La amida de A₁ y el OH de R₆

Grupos protectores de los grupos protectores:

- De la amida de A₁: Trifenilmetil o tritol
- Del OH de R₆: terbutoxi

Oxidación del compuesto de fórmula III y remoción de grupos protectores restantes: Por reacción de (S)-N¹-((3S,6S,9S,12S,15S,18S,19R)-6-(4-terbutoxi)-bencil)-3,9-di-((S)-sec-butil-15-isobutil-7,19-dimetil-2,5,8,11,14,17-hexaoxo-12-(3-oxopropil)-1-oxa-4,7,10,13,16-pentaazaciclononadecan-18-il)-2-isobutiramido-N⁵-tritol-pentan-diamida con 1-óxido de 1-hidroxi-1,2-benzodioxol-3(1H)-ona (IBX) en una mezcla de tetrahidrofurano, y sulfóxido de dimetilo; y posterior reacción de (S)-N¹-((2S,5S,8S,11R,12S,15S,18S,21R)-2,8-di-(S)-sec-butil-21-hidroxi-5-(4-hidroxi-bencil)-15-isobutil-4,11-dimetil-3,6,9,13,16,22-hexaoxo-10-oxa-1,4,7,14,17-penta-azabibiciclo-[16.3.1]-docosan-12-il)-2-isobutiramido-pentano-diamida con ácido trifluoro-acético en dicloro-metano a 0°C (la síntesis del compuesto nostopeptina BN920 se realiza en condiciones similares).

La reivindicación 1 no es clara porque establece que el proceso comprende una etapa de convertir un análogo deshidratado de un compuesto de la fórmula I en el compuesto correspondiente de la fórmula I. Sin embargo, la palabra análogo podría generar confusión a la hora de determinar el alcance de la solicitud, por lo cual se sugiere modificar la reivindicación de tal forma que no genere confusión.

La reivindicación 2 no es clara, ni concisa, porque señala que la síntesis del compuesto de fórmula IV se realiza mediante una combinación de síntesis de péptidos en fase sólida y síntesis en fase de solución a partir de los aminoácidos de partida y precursores de cadena lateral correspondientes, sin establecer claramente cómo se llevan a cabo cada una de las etapas de la síntesis, no se establece cuáles son los aminoácidos de partida, ni cuáles son los precursores de cadena lateral correspondientes de acuerdo a lo soportado en la descripción. Se sugiere establecer claramente cómo se llevan a cabo cada una de las etapas del proceso de tal manera que no se presenten confusiones al momento de establecer el alcance de la solicitud.

La reivindicación 3 no cuenta con soporte descriptivo, porque no se encuentra en la descripción se presente algún ejemplo en el que la etapa reclamada se lleve a cabo. Se sugiere eliminar del capítulo reivindicatorio la materia que no cuenta con soporte descriptivo.

La reivindicación 3 no es concisa porque no establece cuáles son las opciones de grupo protector X^{**}, ni cómo se lleva a cabo la etapa de desprotección para retirar X^{**}. Adicionalmente, la reivindicación 3 no es clara porque menciona que C* es como se define para un compuesto de la fórmula II en la reivindicación 1, sin embargo, la reivindicación 1 no menciona ningún C*, y no es claro a que se refiere con un derivado activo del mismo, ya que no es claro si es un derivado del compuesto de la fórmula II o un derivado de C*. El término derivado puede incluir una cantidad indeterminada de

posibilidades por lo cual se sugiere eliminarlo del capítulo reivindicatorio. Se sugiere al solicitante modificar la reivindicación de tal forma que sea posible establecer su alcance claramente.

La reivindicación 4 no es clara, ni concisa porque no establece cual es la estructura del péptido precursor lineal del compuesto de la fórmula VI, no establece claramente cuales son las condiciones de reacción que permiten la formación de un enlace de amida a partir del grupo amino y el grupo carboxilo, y el término “de preferencia” no delimita la reivindicación al uso de fase de solución que es la que cuenta con soporte descriptivo. Se sugiere modificar la reivindicación estableciendo de forma clara los reactivos, estructuras y condiciones del proceso de tal manera que sea posible determinar su alcance de forma clara.

La reivindicación 5 no es clara, ni concisa porque se refiere a un compuesto de la fórmula VIIIA, cuya estructura no se encuentra en la reivindicación, ni tampoco en las reivindicaciones de las que depende, no establece cuales son las opciones de grupo protector que se puede disociar selectivamente sin afectar a otros grupos protectores presentes, y que es estable durante los pasos de desprotección durante la síntesis del péptido precursor lineal, y no establece como se realiza la remoción del grupo protector Prot* de acuerdo a lo soportado en la descripción. Se sugiere modificar la reivindicación de tal forma que sea posible determinar su alcance de forma clara.

La reivindicación 6 no es concisa porque no establece cómo se obtiene el compuesto de fórmula VII usando la síntesis de péptidos en fase sólida y la subsiguiente disociación a partir de la fase sólida empleada. Se sugiere modificar la reivindicación estableciendo cómo se llevan a cabo las etapas mencionadas de acuerdo a lo soportado en la descripción.

La reivindicación 7 no es clara porque no establece cuales son las posibilidades de enlazadores disociables de acuerdo a lo soportado en la descripción. La frase “o un derivado reactivo de este aminoácido” puede incluir una cantidad indeterminada de posibilidades, por lo cual se sugiere eliminarla del capítulo reivindicatorio. La reivindicación no menciona cuales son posibilidades de resinas incluidas de acuerdo a lo soportado en la descripción. Se menciona un compuesto de fórmula VIIIA cuya estructura no se presenta en el capítulo reivindicatorio, se sugiere incluirla la estructura menciona con el fin de evitar confusiones a la hora de determinar el alcance de la solicitud.

La reivindicación 8 no es clara, ni concisa porque no establece cual es la estructura del péptido precursor lineal del compuesto de la fórmula II, no establece claramente cuáles son las condiciones de reacción que permiten la formación de un enlace de amida a partir del grupo amino y el grupo carboxilo, y el término “de preferencia” no delimita la reivindicación al uso de fase de solución que es la que cuenta con soporte descriptivo. Se sugiere modificar la reivindicación estableciendo de forma clara los reactivos, estructuras y condiciones del proceso de tal manera que sea posible determinar su alcance de forma clara.

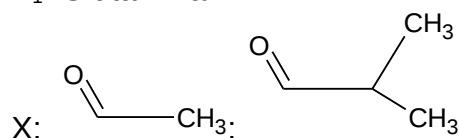
La reivindicación 10 no es concisa porque no establece las posibilidades de enlazador disociable, de resina sólida, un rango de posibles valor de n, y las posibilidades de grupo protector Prot** de acuerdo a lo soportado en la descriptivo. La reivindicación no es concisa porque no establece como se re realiza la remoción del grupo protector Prot**, ni la remoción del compuesto de fórmula XXV de la resina.

La reivindicación 11 no es concisa porque la frase “o un derivado activado de este aminoácido” cubre una cantidad indeterminada de compuestos no soportada en la descripción y no permite determinar el alcance de la solicitud. Adicionalmente, la reivindicación no establece las posibilidades para el enlazador disociable L, la resina sólida y un rango de posibilidades para n.

Las reivindicaciones 12 – 17 no son concisas porque la frase “o un derivado activado de este aminoácido” cubre una cantidad indeterminada de compuestos no soportada en la descripción y no permite determinar el alcance de la solicitud. Adicionalmente, las reivindicaciones no establecen las posibilidades para el enlazador dissociable L, la resina sólida y el grupo protector Prot^{***}, y un rango de posibilidades para n. Las reivindicaciones tampoco establecen como se puede disociar el grupo protector. Se sugiere modificarlas de tal manera que los reactivos y condiciones de reacción se identifiquen claramente de acuerdo a lo soportado en la descripción.

La reivindicación 18 no se encuentra enteramente soportada en la descripción porque la descripción soporta la obtención de compuestos donde:

A₁: Glutamina



R²: metilo

R³: Cadena lateral de leucina

R⁵: Cadena lateral de isoleucina o cadena lateral de fenilalanina

R⁶: Cadena lateral de tirosina

R⁷: Cadena lateral de isoleucina o cadena lateral de valina

Y: metilo

Por lo cual se sugiere hacer una generalización razonable de acuerdo a lo efectivamente soportado en la descripción, teniendo en cuenta que la frase “en especial” no delimita la reivindicación de ninguna manera, por lo cual se sugiere eliminarla del capítulo reivindicatorio para evitar confusiones al determinar el alcance del capítulo reivindicatorio.

La reivindicación 19 no es concisa porque no establece claramente cómo se realiza el proceso de conversión de los compuestos de fórmula V, V* o VA* en un compuesto de la fórmula I, ni cuáles son las opciones de ácido acuoso a usar como solvente reactivo de acuerdo a lo soportado en la descripción. Se sugiere modificar la reivindicación de tal modo que se identifiquen claramente las condiciones de reacción.

La reivindicación 20 no es clara, ni concisa, porque los términos “en especial” o “más especialmente” son imprecisos, por lo cual no permiten distinguir la invención del estado de la técnica. Se sugiere sustituirlos por un término preciso o un rango concreto.

5. Unidad de invención (Art 25 D 486)

La Solicitud no cumple con el Requisito de Unidad de Invención porque se refiere a 2, Grupos Inventivos.

Los Grupos Inventivos están definidos por las siguientes características **esenciales**:

1° Proceso de síntesis e intermediarios de las reivindicaciones 1 – 18, 21 y 22

2° Método de las reivindicaciones 19 y 20

No hay concepto común inventivo porque el proceso de la reivindicación 1 no es nuevo en vista del documento Tetrahedron 61 (2005) 1459–1480, y tampoco es inventivo en vista de los documentos Tetrahedron 61 (2005) 1459–1480 y WO2009024527, o WO2009024527 y Chem. Commun., 2010, 46, 8857–8859.

Se sugiere presentar las Solicitudes Divisionales que considere necesarias, ó limitar la solicitud inicial a una sola invención, eliminando de ella las otras invenciones.

6. Determinación del estado de la técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: Abril 20 de 2011 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	Tetrahedron 61 (2005) 1459–1480	Synthetic studies of the cyclic depsipeptides bearing the 3-amino-6-hydroxy-2-piperidone (Ahp) unit. Total synthesis of the proposed structure of micropeptin T-20	7/Febrero/2005
D2	WO2009024527	Cyclic depsipeptides	26/Febrero/2009
D3	Chem. Commun., 2010, 46, 8857–8859	Solid phase total synthesis of the 3-amino-6-hydroxy-2-piperidone (Ahp) cyclodepsipeptide and protease inhibitor Symplocamide A	22/Octubre/2010

D1 Divulga un proceso de síntesis total de un compuesto depsipéptido cíclico que presenta la unidad 3-amino-6-hidroxi-2-piperidona la cual se forma por medio de construcción oxidativa (Resumen).

D2 http://worldwide.espacenet.com/publicationDetails/biblio?CC=WO&NR=2009024527A1&KC=A1&FT=D&ND=3&date=20090226&DB=worldwide.espacenet.com&locale=en_EP

Divulga ciclodepsipéptidos de la misma estructura de los ciclodepsipéptidos de la fórmula I de la presente solicitud (Reivs. 1 y 7 y compuesto II) los cuales son producidos por una cepa de Chondromyces (Págs.26 y 27).

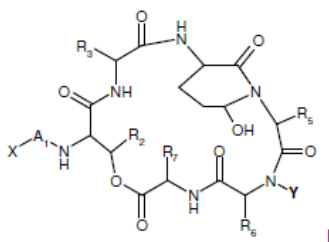
D3

Divulga la síntesis completa en fase sólida de AHP ciclodepsipéptidos, usando como modelo. La anterioridad señala que estudios sintéticos anteriores han demostrado que la unidad Ahp se forma espontáneamente a partir de un aldehído glutámico generado dentro del ciclodepsipéptido ensamblado correctamente (Esquema 1). La anterioridad señala que una ruta de síntesis general, por tanto, podría consistir en una síntesis en fase sólida de la columna vertebral del ciclodepsipéptido, seguido por la región y quimioselectiva generación de un aldehído glutámico (Pág. 8857, col. de la izquierda, último Párr.).

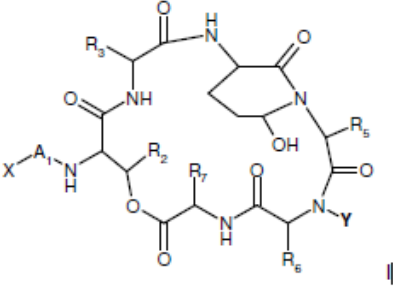
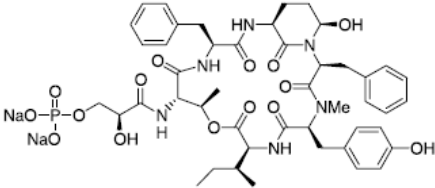
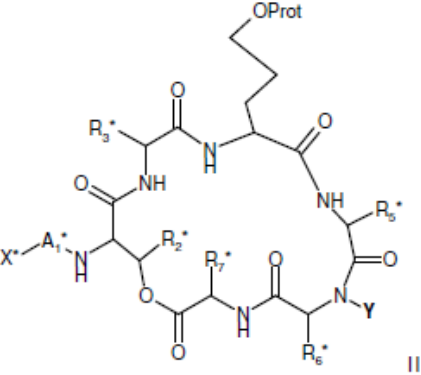
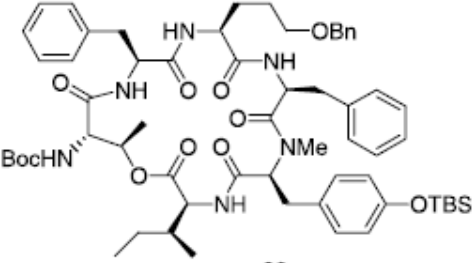
7. Examen de Patentabilidad (Art 14 D486)

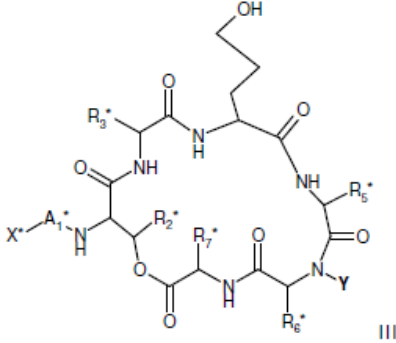
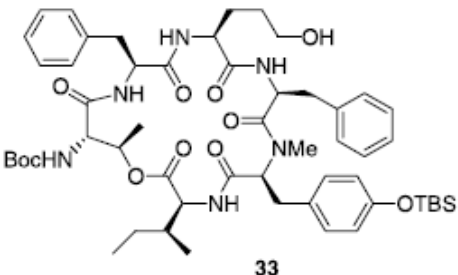
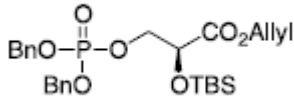
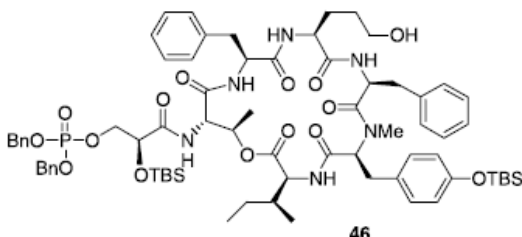
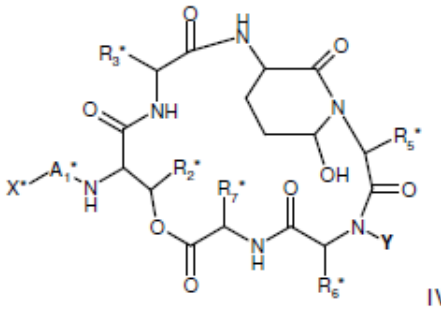
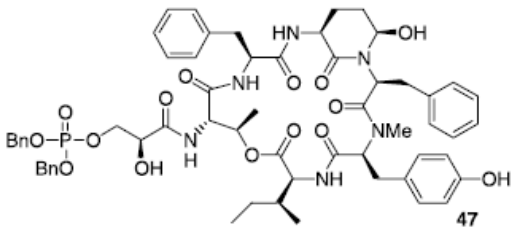
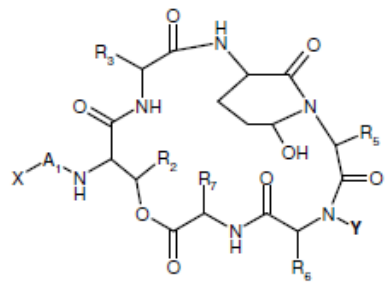
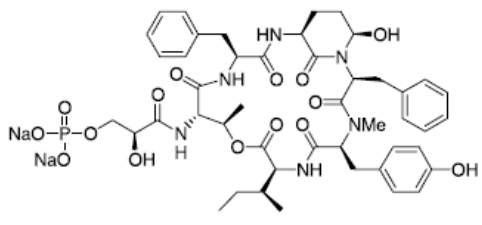
Novedad (Art 16 D486)

La reivindicación 1 consiste en un método o proceso para la preparación de un compuesto de depsipéptido cíclico de la fórmula I (Pág. 132, Radicado No. 13-252979-00000-0000):



Comparación de las características técnicas **esenciales**, con las del estado de la técnica:

Características esenciales	D1
Un método o proceso para la preparación de un compuesto de depsipéptido cíclico de la fórmula I:  En donde: A1: hidroxi-alcanoílo de 1 a 8 átomos de carbono fosforilado. X: Está ausente R2:Metilo R3:Es la cadena lateral de un aminoácido (fenilalanina) R5:Es la cadena lateral de fenilalanina R6:Es la cadena lateral de tirosina R7:Es la cadena lateral de isoleucina Y:alquilo de 1 a 8 átomos de carbono comprendiendo este método:	Proceso para preparar el depsipéptido de la fórmula (1):  Micropeptin T-20 (1)
Desproteger selectivamente un compuesto de la fórmula II:  En donde Prot es un grupo protector. Y es como se define para un compuesto de la fórmula I , y X*, A1*, R2*, R3*, R5*, R6*, y R7* corresponden a X, A1, R2, R3 , R5, R6, y R7 en la fórmula I, respectivamente, pero con la condición de que los grupos funcionales reactivos sobre estas fracciones están presentes en una forma protegida cuando menos si podrían participar en reacciones secundarias indeseadas.	Desproteger selectivamente un compuesto de la fórmula 32:  32 (Pág. 1464, Esquema 8)
Para dar como resultado un compuesto de la fórmula III:	Para dar como resultado un compuesto de la fórmula 33:

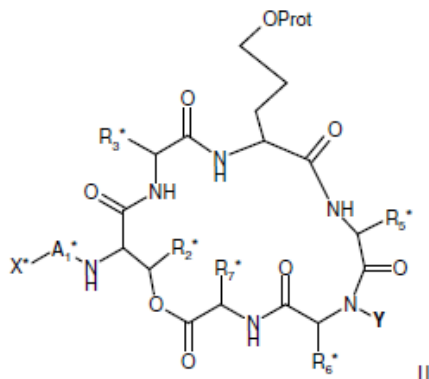
Características esenciales	D1
 III en donde X*, A1*, R2*, R3*, R5*, R6*, y R7* tienen los significados definidos anteriormente,	 33 El cual se hace reacción con:  en presencia de (PPh₃)₄Pd, morfolina y THF para obtener el compuesto de fórmula:  46 (Pág. 1467, esquema 11)
Hacer reaccionar el grupo hidroxilo libre bajo condiciones de oxidación para formar un compuesto de la fórmula IV:  IV	Hacer reaccionar el grupo hidroxilo libre bajo condiciones de oxidación para formar un compuesto de la fórmula:  47 (Pág. 1467, esquema 11)
Y remover los grupos protectores restantes para proporcionar un compuesto de la fórmula I, o una sal del mismo.  I	Y remover los grupos protectores restantes para proporcionar un compuesto de la fórmula (1):  Micropeptin T-20 (1) (Pág. 1467, esquema 11).

Las características **esenciales** del proceso de la reivindicación 1 habían sido divulgadas previamente en el documento D1, el cual enseña un proceso de síntesis total de un compuesto depsipéptido cíclico que presenta la unidad 3-amino-6-hidroxi-2-piperidona la cual se forma por medio de construcción oxidativa (Resumen).

En consecuencia, la reivindicación 1 carece de novedad, según lo divulgado en el documento D1.

Las reivindicaciones dependientes 2 – 18, 21 y 22 no mencionan alguna característica que haga nueva a la composición de la reivindicación 1, por lo cual se considera que no son nuevas.

La reivindicación 21 consiste en un compuesto de la fórmula II:



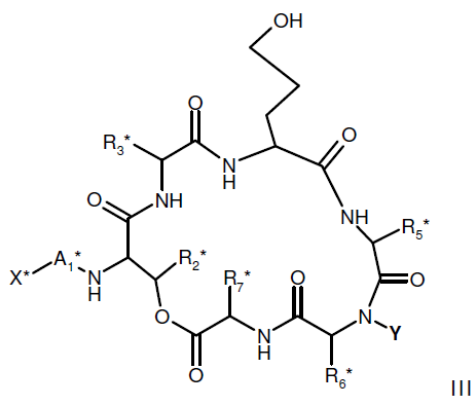
Comparación de las características técnicas **esenciales**, con las del estado de la técnica:

Características esenciales	D1
Un compuesto de la fórmula II: en donde Prot es un grupo protector, Y es como se define para un compuesto de la fórmula I en la reivindicación 1 o en la reivindicación 17, y X*, A1*, R2*, R3*, R5*, R6*, y R7* corresponden a X, A1, R2, R3, R5, R6, y R7 en la fórmula I como se define en la reivindicación 1 o en la reivindicación 17, respectivamente, sin embargo con la condición de que los grupos funcionales reactivos sobre estas fracciones están presentes en una forma desprotegida o protegida.	Un compuesto de la fórmula 32: (Pág. 1464, Esquema 8)

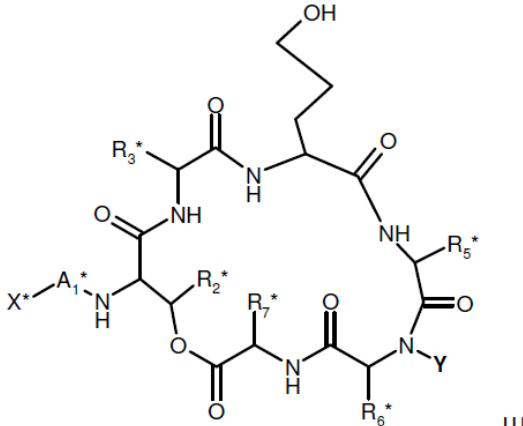
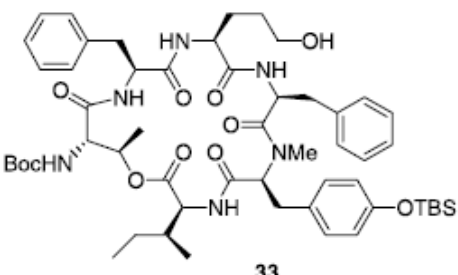
Las características **esenciales** del compuesto de la reivindicación 21 habían sido divulgadas previamente en el documento D1, el cual enseña el compuesto de la fórmula 32 (Pág. 1464, Esquema 8).

En consecuencia, la reivindicación 21 carece de novedad, según lo divulgado en el documento D1.

La reivindicación 22 consiste en un compuesto de la fórmula III:



Comparación de las características técnicas **esenciales**, con las del estado de la técnica:

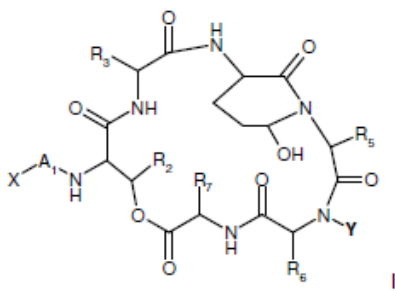
Características esenciales	D1
Un compuesto de la fórmula III:  III en donde Y es como se define para un compuesto de la fórmula I en la reivindicación 1 o en la reivindicación 17, y X*, A1*, R2*, R3*, R5*, R6*, y R7* corresponden a X, A1, R2, R3, R5, R6, y R7 en la fórmula I como se define en la reivindicación 1 o en la reivindicación 17, respectivamente, sin embargo con la condición de que los grupos funcionales reactivos diferentes del hidroxilo representado en la parte superior de la fórmula II I sobre estas fracciones, están presentes en una forma desprotegida o protegida.	Un compuesto de la fórmula 33:  33 (Pág. 1467, esquema 11)

Las características **esenciales** del compuesto de la reivindicación 22 habían sido divulgadas previamente en el documento D1, el cual enseña el compuesto de la fórmula 33 (Pág. 1467, esquema 11).

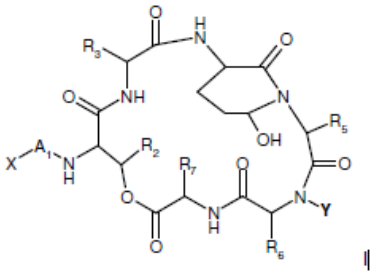
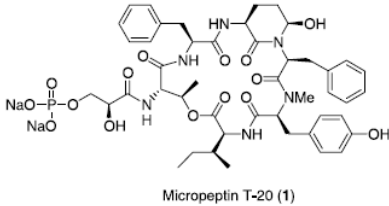
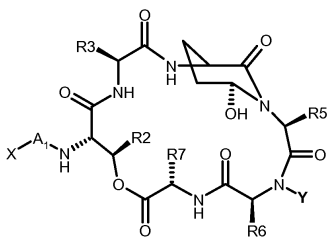
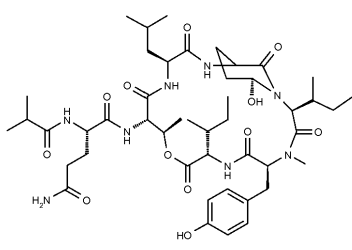
En consecuencia, la reivindicación 22 carece de novedad, según lo divulgado en el documento D1.

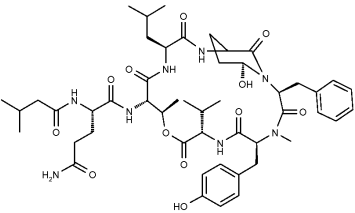
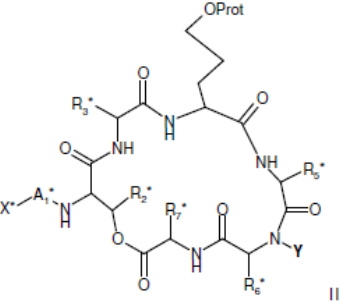
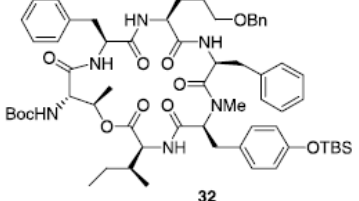
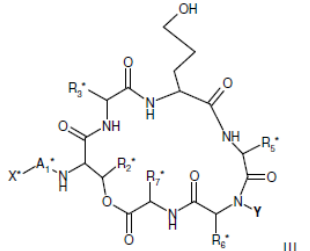
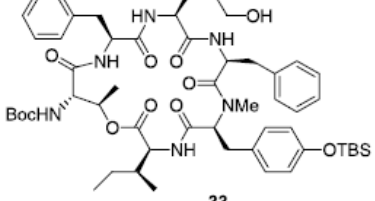
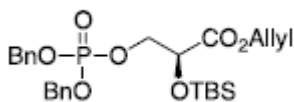
Nivel Inventivo (Art18 D486)

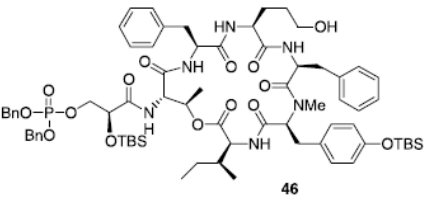
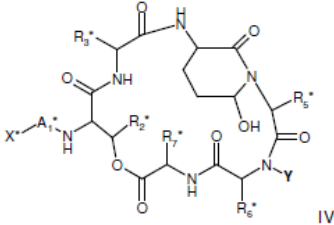
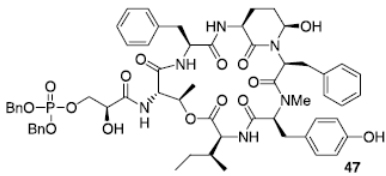
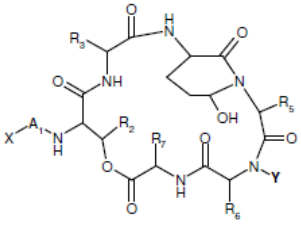
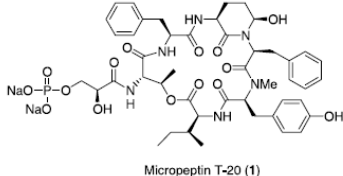
La reivindicación 1 consiste en un método o proceso para la preparación de un compuesto de depsipéptido cíclico de la fórmula I (Pág. 132, Radicado No. 13-252979-00000-0000):



Comparación de las características técnicas **esenciales**, con las del estado de la técnica:

Características esenciales	D1	D2
Un método o proceso para la preparación de un compuesto de depsipéptido cíclico de la fórmula I:  En donde: A₁: Fracción bivalente de un aminoácido, en especial glutamina o hidroxi-alcanoílo de 1 a 8 átomos de carbono fosforilado. X: Está ausente R₂:Metilo R₃:Es la cadena lateral de un aminoácido (fenilalanina) R₅: Es la cadena lateral de un aminoácido, de preferencia de fenilalanina, leucina, isoleucina o valina. R₆:Es la cadena lateral de tirosina R₇:Es la cadena lateral de isoleucina Y:alquilo de 1 a 8 átomos de carbono comprendiendo este método:	Proceso para preparar el depsipéptido de la fórmula (1):  Micropeptin T-20 (1)	Proceso para la preparación de un compuesto de depsipéptido cíclico de la fórmula I:  A En donde: A₁:Glutamina X: acilo R₂: Cadena lateral de un aminoácido en especial treonina R₃: Cadena lateral de leucina R₅:Es la cadena lateral de un aminoácido isoleucina o valina R₆: Es la cadena lateral de tirosina R₇:Es la cadena lateral de isoleucina Y: hidrógeno o metilo, en particular metilo (Reivs. 1 y 7). Ejemplo:  Formula (II)) (Pág. 25)

Características esenciales	D1	D2
		 Formula (VIII) (Pág. 26).
Desproteger selectivamente un compuesto de la fórmula II:  II En donde Prot es un grupo protector. Y es como se define para un compuesto de la fórmula I , y X*, A1*, R2*, R3*, R5*, R6*, y R7* corresponden a X, A1, R2, R3 , R5, R6, y R7 en la fórmula I, respectivamente, pero con la condición de que los grupos funcionales reactivos sobre estas fracciones están presentes en una forma protegida cuando menos si podrían participar en reacciones secundarias indeseadas.	Desproteger selectivamente un compuesto de la fórmula 32:  32 (Pág. 1464, Esquema 8)	El péptido de la fórmula (II) puede ser producido por una cepa de Chondromyces crocatus (DSM19329) (Pág. 26) El péptido de la fórmula (VIII) puede ser producido por una cepa de Chondromyces robustus (DSM19330) (Pág. 27).
Para dar como resultado un compuesto de la fórmula III:  III en donde X*, A1*, R2*, R3*, R5*, R6*, y R7* tienen los significados definidos anteriormente,	Para dar como resultado un compuesto de la fórmula 33:  33 El cual se hace reacción con:  en presencia de (PPh3)4Pd, morfolina y THF para obtener el compuesto de fórmula:	

Características esenciales	D1	D2
	 (Pág. 1467, esquema 11)	
Hacer reaccionar el grupo hidroxilo libre bajo condiciones de oxidación para formar un compuesto de la fórmula IV: 	Hacer reaccionar el grupo hidroxilo libre bajo condiciones de oxidación para formar un compuesto de la fórmula:  (Pág. 1467, esquema 11)	
Y remover los grupos protectores restantes para proporcionar un compuesto de la fórmula I, o una sal del mismo. 	Y remover los grupos protectores restantes para proporcionar un compuesto de la fórmula (1):  (Pág. 1467, esquema 11).	

Los documentos D1 y D2 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 1 porque:

El documento D2 enseña ciclodepsipéptidos de la misma estructura de los ciclodepsipéptidos de la fórmula I de la presente solicitud (Reivs. 1 y 7 y compuesto II) los cuales son producidos por una cepa de Chondromyces (Págs.26 y 27).

La diferencia entre la invención, definida en la reivindicación 1 y el método de obtención de los ciclodepsipéptidos del documento D2 consiste en que los ciclodepsipéptidos de la anterioridad son producidos por cepas de Chondromyces (Págs.26 y 27) y en la presente solicitud son obtenidos por métodos sintéticos.

El efecto que logra el incluir esta característica según lo establecido en la descripción es que permite obtener un mayor rendimiento y mayor pureza.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo modificar el procedimiento de obtención de los ciclodepsipéptidos divulgados en el documento D2 para llegar a un procedimiento que permita obtener un mayor rendimiento”.

Sin embargo, el documento D1 divulga un proceso de obtención de ciclodepsipéptidos, el cual es completamente sintético, donde la unidad 3-amino-6-hidroxi-2-piperidona se

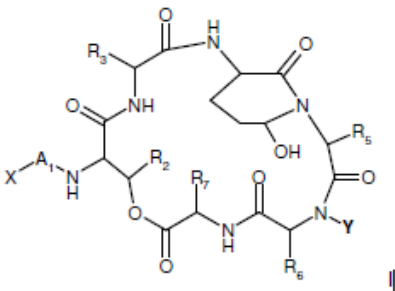
forma por una reacción de oxidación con IBX en dimetilsulfóxido (Esquemas 1, 8 y 11). El documento divulga buenos rendimientos tales como 84% y 68% (Pág. 1462, col. de la derecha, segundo Párr.; y Esquema 9).

En consecuencia, la persona del oficio normalmente versada en la materia, interesada en obtener un proceso de síntesis de mayor rendimiento y pureza al divulgado en el documento D2 para la síntesis de ciclodepsipéptidos se vería motivada por la información divulgada en el documento D1 a usar un método completamente sintético como el divulgado en dicha anterioridad, con la expectativa razonable de obtener buenos rendimientos. Por lo cual el objeto de la reivindicación se considera obvio.

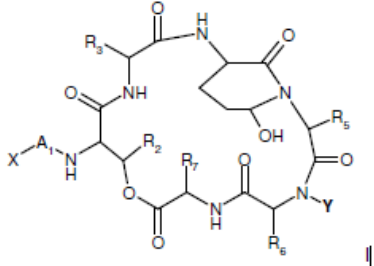
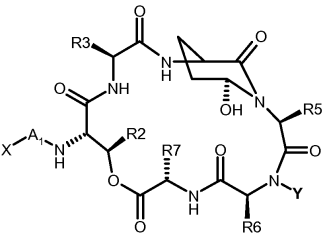
Conclusión:
 La reivindicación 1 no cumple el requisito de novedad (Art. 16), y tampoco cumple con el requisito de nivel inventivo (Art. 18), de la misma forma que sus reivindicaciones dependientes 2 – 18. Los compuestos intermediarios de las reivindicaciones 21 y 22 no son inventivos porque no hacen inventivo al proceso de la reivindicación 1.

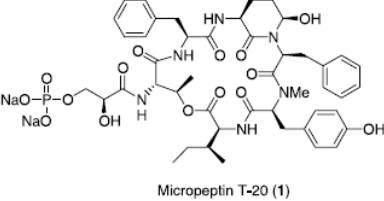
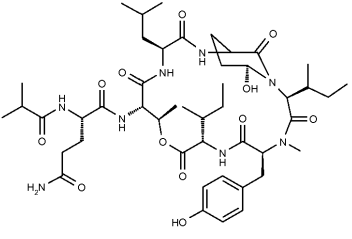
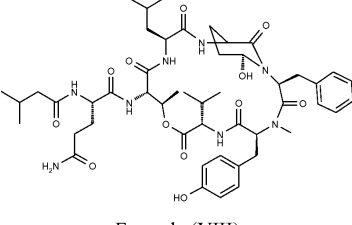
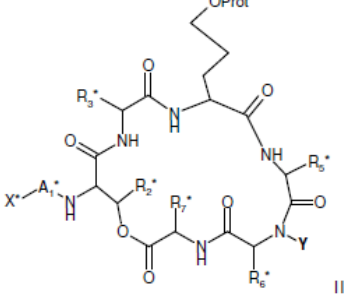
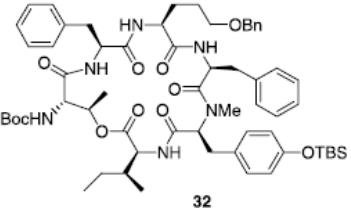
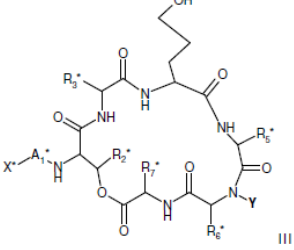
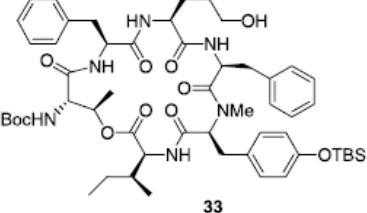
NIVEL INVENTIVO FRENTE A LOS DOCUMENTO D2 Y D3

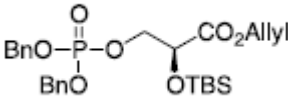
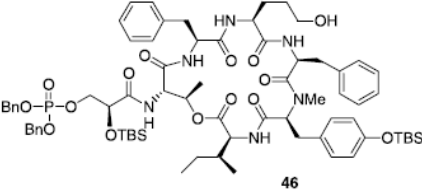
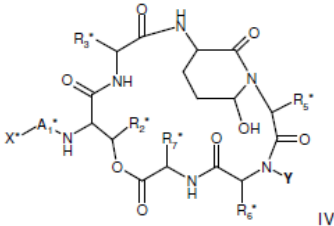
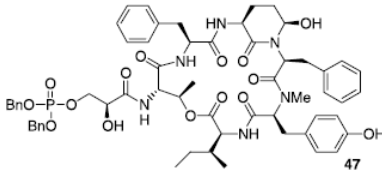
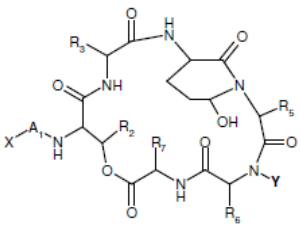
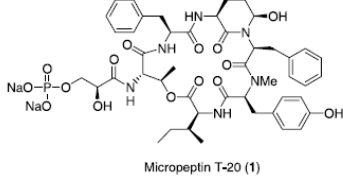
La reivindicación 1 consiste en un método o proceso para la preparación de un compuesto de depsipéptido cíclico de la fórmula I (Pág. 132, Radicado No. 13-252979-00000-0000):



Comparación de las características técnicas **esenciales**, con las del estado de la técnica:

Características esenciales	D3	D2
Un método o proceso para la preparación de un compuesto de depsipéptido cíclico de la fórmula I:  En donde: A₁: Fracción bivalente de un aminoácido, en especial glutamina o hidroxi-alcanoílo de 1 a 8 átomos de carbono fosforilado. X: Está ausente R₂:Metilo R₃:Es la cadena lateral de un aminoácido (fenilalanina) R₅: Es la cadena lateral de un aminoácido, de preferencia de fenilalanina, leucina, isoleucina o	Divulga la síntesis completa en fase sólida de AHP ciclodepsipéptidos, usando como modelo la Simplocamida A. La anterioridad señala que estudios sintéticos anteriores han demostrado que la unidad AHP se forma espontáneamente a partir de un aldehído glutámico generado dentro del ciclodepsipéptido ensamblado correctamente (Esquema 1). La anterioridad señala que una ruta de síntesis general, por tanto, podría consistir en una síntesis en fase sólida de la columna vertebral del ciclodepsipéptido, seguido por la regio y quimioselectiva generación de un aldehído glutámico (Pág. 8857, col. de la izquierda, último Párr.), haciendo referencia al documento tetrahedron 61 (2005) 1459–1480, el cual divulga un proceso para preparar el depsipéptido de la fórmula (1):	Proceso para la preparación de un compuesto de depsipéptido cíclico de la fórmula I:  A En donde: A₁:Glutamina X: acilo R₂: Cadena lateral de un aminoácido en especial treonina R₃: Cadena lateral de leucina R₅:Es la cadena lateral de un aminoácido isoleucina o valina R₆: Es la cadena lateral de tirosina R₇:Es la cadena lateral de isoleucina

Características esenciales	D3	D2
valina. R₆:Es la cadena lateral de tirosina R₇:Es la cadena lateral de isoleucina Y:alquilo de 1 a 8 átomos de carbono comprendiendo este método:	 Micropeptin T-20 (1)	Y: hidrógeno o metilo, en particular metilo (Reivs. 1 y 7). Ejemplo:  Formula (II)) (Pág. 25)  Formula (VIII) (Pág. 26).
Desproteger selectivamente un compuesto de la fórmula II:  II En donde Prot es un grupo protector. Y es como se define para un compuesto de la fórmula I , y X*, A1*, R2*, R3*, R5*, R6*, y R7* corresponden a X, A1, R2, R3 , R5, R6, y R7 en la fórmula I, respectivamente, pero con la condición de que los grupos funcionales reactivos sobre estas fracciones están presentes en una forma protegida cuando menos si podrían participar en reacciones secundarias indeseadas.	Desproteger selectivamente un compuesto de la fórmula 32:  32 (Pág. 1464, Esquema 8)	El péptido de la fórmula (II) puede ser producido por una cepa de Chondromyces crocatus (DSM19329) (Pág. 26) El péptido de la fórmula (VIII) puede ser producido por una cepa de Chondromyces robustus (DSM19330) (Pág. 27).
Para dar como resultado un compuesto de la fórmula III:  III en donde X*, A1*, R2*, R3*, R5*,	Para dar como resultado un compuesto de la fórmula 33:  33	

Características esenciales	D3	D2
R6*, y R7* tienen los significados definidos anteriormente,	El cual se hace reacción con:  en presencia de (PPh3)4Pd, morfolina y THF para obtener el compuesto de fórmula:  46 (Pág. 1467, esquema 11)	
Hacer reaccionar el grupo hidroxilo libre bajo condiciones de oxidación para formar un compuesto de la fórmula IV:  IV	Hacer reaccionar el grupo hidroxilo libre bajo condiciones de oxidación para formar un compuesto de la fórmula:  47 (Pág. 1467, esquema 11)	
Y remover los grupos protectores restantes para proporcionar un compuesto de la fórmula I, o una sal del mismo.  I	Y remover los grupos protectores restantes para proporcionar un compuesto de la fórmula (1):  Micropeptin T-20 (1) (Pág. 1467, esquema 11).	

Los documentos D3 y D2 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 1 porque:

El documento D2 enseña ciclodepsipéptidos de la misma estructura de los ciclodepsipéptidos de la fórmula I de la presente solicitud (Reivs. 1 y 7 y compuesto II) los cuales son producidos por una cepa de Chondromyces (Págs.26 y 27).

La diferencia entre la invención, definida en la reivindicación 1 y el método de obtención de los ciclodepsipéptidos del documento D2 consiste en que los ciclodepsipéptidos de la anterioridad son producidos por cepas de Chondromyces (Págs.26 y 27) y en la presente solicitud son obtenidos por métodos sintéticos.

El efecto que logra el incluir esta característica según lo establecido en la descripción es que permite obtener un mayor rendimiento y mayor pureza.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo modificar el procedimiento de obtención de ciclodepsipéptidos divulgado en el documento D2 para llegar a un procedimiento que permita obtener un mayor rendimiento”.

Sin embargo, el documento D3 divulga la síntesis completa en fase sólida de AHP ciclodepsipéptidos, usando como modelo la Simplocamida A. La anterioridad señala que estudios sintéticos anteriores han demostrado que la unidad Ahp se forma espontáneamente a partir de un aldehído glutámico generado dentro del ciclodepsipéptido ensamblado correctamente (Esquema 1, Pág. 8857, col. de izquierda, último Párr.). El documento D3 establece que una ruta de síntesis general, por tanto, podría consistir en una síntesis en fase sólida de la columna vertebral del ciclodepsipéptido, seguido por la regio y quimioselectiva generación de un aldehído glutámico (Pág. 8857, col. de la izquierda, último Párr.), haciendo referencia al documento tetrahedron 61 (2005) 1459–1480, el cual divulga un proceso de obtención de ciclodepsipéptidos, el cual es completamente sintético, donde la unidad 3-amino-6-hidroxi-2-piperidona se forma por una reacción de oxidación con IBX en dimetilsulfóxido (Esquemas 1, 8 y 11). El documento divulga buenos rendimientos tales como 84% y 68% (Pág. 1462, col. de la derecha, segundo Párr.; y Esquema 9).

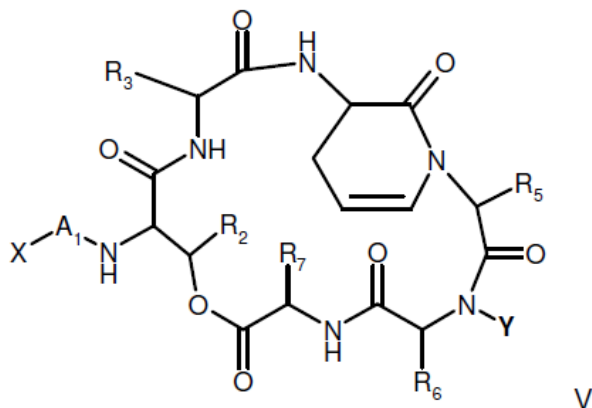
En consecuencia, la persona del oficio normalmente versada en la materia, interesada en obtener un proceso de síntesis de mayor rendimiento y pureza al divulgado en el documento D2 para la síntesis de ciclodepsipéptidos se vería motivada por la información divulgada en el documento D3 a usar la síntesis en fase sólida para la obtención del péptido precursor lineal y el método divulgado en el documento Tetrahedron 61 (2005) 1459–1480, citado en el documento D3, para la obtención del AHP ciclodepsipéptido, con la expectativa razonable de obtener buenos rendimientos y porque es una técnica usual en la síntesis de depsipéptidos cíclicos¹. Por lo cual el objeto de la reivindicación se considera obvio.

Conclusión:

La reivindicación 1 no cumple el requisito de novedad (Art. 16), y tampoco cumple con el requisito de nivel inventivo (Art. 18), de la misma forma que sus reivindicaciones dependientes 2 – 18. Los compuestos intermediarios de las reivindicaciones 21 y 22 no son inventivos porque no hacen inventivo al proceso de la reivindicación 1.

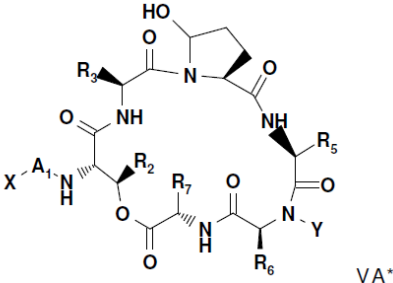
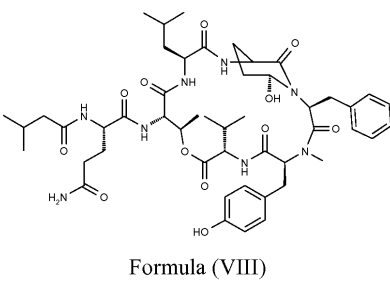
La reivindicación 19 consiste en un método o proceso para convertir un deshidrato de un compuesto de la fórmula I dado en la reivindicación 1, o con los sustituyentes como se definen en la reivindicación 18 en el compuesto correspondiente de la fórmula I, en donde el deshidrato tiene la fórmula V: (Pág. 155, Radicado No. 13-252979-00000-0000):

¹ Tetrahedron Letters 47 (2006) 8587–8590. A novel strategy for the solid-phase synthesis of cyclic lipodepsipeptides. Pág. 8588, Col. de la derecha.



Comparación de las características técnicas **esenciales**, con las del estado de la técnica:

Características esenciales	D1	D2
Un método o proceso para convertir un deshidrato de un compuesto de la fórmula I dado en la reivindicación 1, o con los sustituyentes como se definen en la reivindicación 18 en el compuesto correspondiente de la fórmula I, en donde el deshidrato tiene la fórmula V: en donde Y, X, A1, R2, R3, R5, R6 y R7 son como se definen para un compuesto de la fórmula I en cualquiera de las reivindicaciones 1 y 17; y/o su análogo de hemiaminal correspondiente con una estructura de cinco anillos en lugar de la estructura ahp en la fórmula I, que también se puede formar como un subproducto, y tiene la fórmula V*: en especial la fórmula VA*:	Menciona la influencia de agua para la estabilización de la mitad AHP (Pág. 1466, col. de la izquierda, primer Párr.). El documento menciona que la etapa de adición de agua al sistema solvente podría prevenir la deshidratación. La anterioridad establece que la adición de buffer ácido de fosfato a 0.1M (pH6) en THF fue suficiente para estabilizar la mitad AHP y prevenir la formación del deshidrato (Pág. 1466, col. de la izquierda, primer Párr.).	Proceso para la preparación de un compuesto de depsipéptido cíclico de la fórmula I: En donde: A1: Glutamina X: acilo R2: Cadena lateral de un aminoácido en especial treonina R3: Cadena lateral de leucina R5: Es la cadena lateral de un aminoácido isoleucina o valina R6: Es la cadena lateral de tirosina R7: Es la cadena lateral de isoleucina Y: hidrógeno o metilo, en particular metilo (Reivs. 1 y 7). Ejemplo: Formula (II)) (Pág. 25)

Características esenciales	D1	D2
 en donde Y, X, A1, R2, R3 , R5, R6 y R7 son como se definen para un compuesto de la fórmula I en cualquiera de las reivindicaciones 1 y 17, respectivamente; o un método o proceso para cambiar el equilibrio de una mezcla de un compuesto de la fórmula I, y su deshidrato correspondiente y/o hemiaminal en favor del compuesto de la fórmula I, comprendiendo este método o proceso utilizar un ácido acuoso como solvente reactivo para impulsar la reacción.		 Formula (VIII) (Pág. 26).

Los documentos D1 y D2 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 1 porque:

El documento D2 enseña ciclodepsipéptidos de la misma estructura de los ciclodepsipéptidos de la fórmula I de la presente solicitud (Reivs. 1 y 7 y compuesto II) los cuales son producidos por una cepa de Chondromyces (Págs.26 y 27).

La diferencia entre la invención, definida en la reivindicación 19 y el método de obtención de los ciclodepsipéptidos del documento D2 consiste en que los ciclodepsipéptidos de la anterioridad son producidos por cepas de Chondromyces (Págs.26 y 27) y no se establece un método para para convertir un deshidrato de un compuesto de la fórmula I en el compuesto de la fórmula I.

El efecto que logra el incluir esta característica según lo establecido en la descripción es que permite obtener un mayor rendimiento y mayor pureza.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo modificar el procedimiento de en el documento D2 para obtener un procedimiento que permita obtener un mayor rendimiento”.

Sin embargo, el documento D1 divulga la influencia de agua para la estabilización de la mitad AHP (Pág. 1466, col. de la izquierda, primer Párr.). El documento menciona que la etapa de adición de agua al sistema solvente podría prevenir la deshidratación. La anterioridad establece que la adición de buffer ácido de fosfato a 0.1M (pH6) en THF fue suficiente para estabilizar la mitad AHP y prevenir la formación del deshidrato (Pág. 1466, col. de la izquierda, primer Párr.).

En consecuencia, la persona del oficio normalmente versada en la materia, interesada en evitar o disminuir la formación del derivado deshidrato en la síntesis de los compuestos divulgados en el documento D2, se vería motivada por la información divulgada en el documento D1 a usar agua y ácido para estabilizar la mitad AHP y prevenir la formación del deshidrato. Por lo cual el objeto de la reivindicación 19 se considera obvio.

Conclusión:
 La reivindicación 19 cumple con el requisito de novedad (Art. 16), pero no cumple con

el requisito de nivel inventivo (Art. 18), de la misma forma que su reivindicación dependiente 20.

8. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

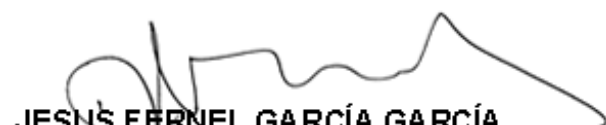
Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	Tetrahedron 61 (2005) 1459–1480	1 – 18, 21 y 22	Novedad
D1	Tetrahedron 61 (2005) 1459–1480	1 – 22	Nivel Inventivo
D2	WO2009024527	1 – 22	Nivel Inventivo
D3	Chem. Commun., 2010, 46, 8857– 8859	1 – 18, 21 y 22	Nivel Inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JESÚS FERNEL GARCÍA GARCÍA
Director de Nuevas Creaciones (E)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2014-12-16

Elaboró: Juanita Catalina Rico Valbuena
Revisó: Gloria Jacqueline Alfonso Roza

Synthetic studies of the cyclic depsipeptides bearing the 3-amino-6-hydroxy-2-piperidone (Ahp) unit. Total synthesis of the proposed structure of micropeptin T-20

Fumiaki Yokokawa,^{a,†} Akiko Inaizumi^a and Takayuki Shioiri^{b,*}

^aGraduate School of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan

^bGraduate School of Environmental and Human Sciences, Meijo University, Shiogamaguchi, Tempaku, Nagoya 468-8502, Japan

Received 14 October 2004; revised 5 November 2004; accepted 2 December 2004

Available online 18 December 2004

Abstract—The first total synthesis of a cyclic depsipeptide possessing the 3-amino-6-hydroxy-2-piperidone (Ahp) unit was successfully achieved in a convergent manner by the oxidative construction of the Ahp unit at the later stage of the synthesis. This synthetic work provides data indicating that the structure of the target Ahp-depsipeptide, micropeptin T-20, should be re-examined.

© 2004 Elsevier Ltd. All rights reserved.

1. Introduction

The 3-amino-6-hydroxy-2-piperidone (Ahp) unit has been recently characterized as a constituent in more than 60 cyclic depsipeptides derived from cyanobacteria.¹ These cyclic depsipeptides commonly consist of the 19-membered ring, and generally exhibit an interesting and significant inhibiting action against peptidic proteases. The unique unprecedented Ahp moiety will be biosynthetically derived from glutamate, and probably play an important role to exhibit biological activity because it will participate in converting the cyclic depsipeptide into a bioactive conformation due to the conformationally restricted structure and hydrogen bonding with the free hydroxyl group.

Micropeptin T-20 was isolated from the cyanobacterium *Microcystis aeruginosa* collected from the freshwater Kang Krachan Dam in Thailand, and its structure was determined to be **1** (Fig. 1) by Kaya and co-workers.^{1b} This cyclic depsipeptide strongly inhibits chymotrypsin with an IC₅₀ of 2.5×10^{-9} M, but weakly inhibits tyrosinase with an IC₅₀ of 5×10^{-3} M.

As a continuation of our interests in the synthetic studies of biologically active aquatic natural products,² we have investigated methods used for the synthesis of the Ahp peptides. Herein, we wish to report the total synthesis of micropeptin T-20 having the proposed structure **1**,³ which revealed that natural micropeptin T-20 was not identical

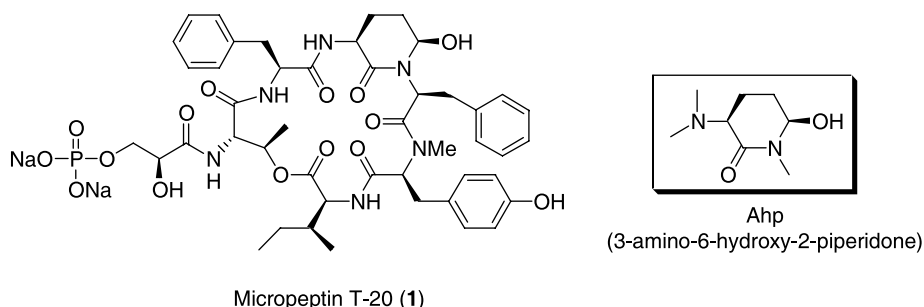
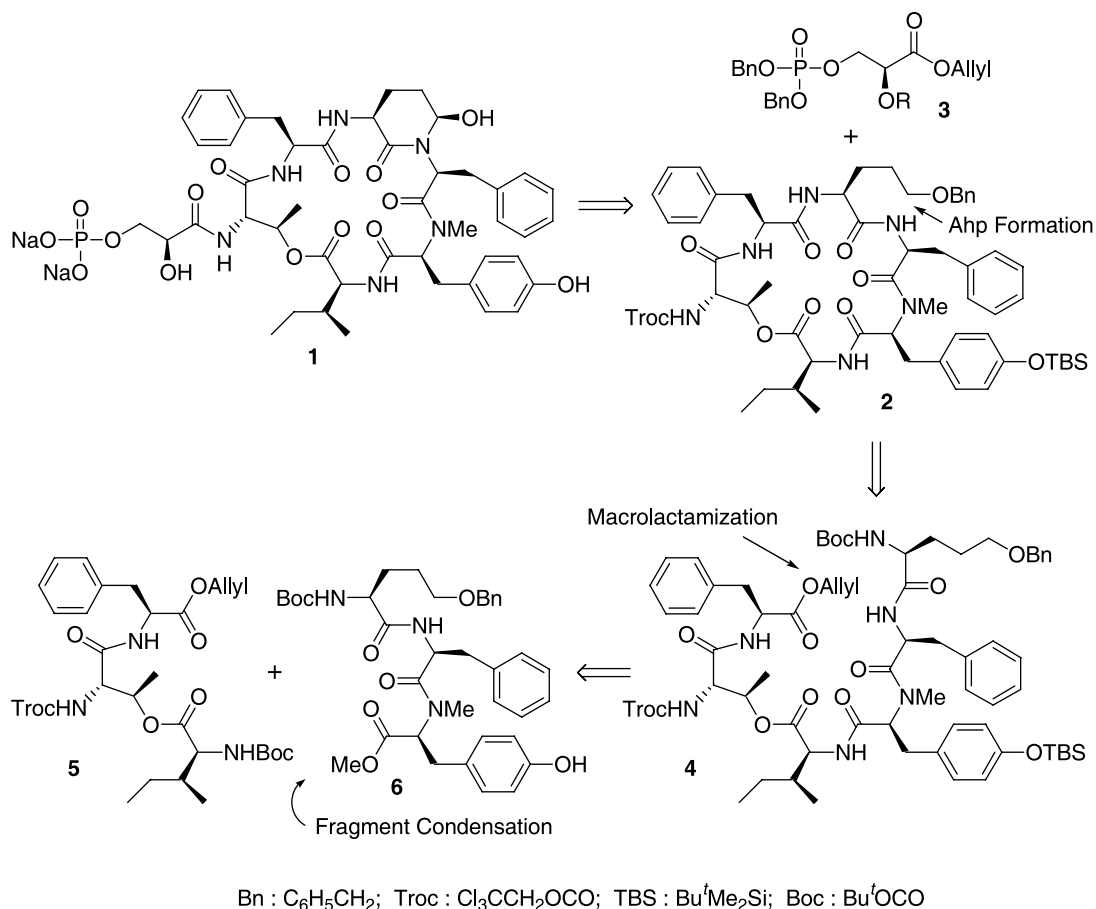


Figure 1. The proposed structure of micropeptin T-20 and the structure of the Ahp unit.

Keywords: Total synthesis; Cyclic depsipeptide; Macrolactamization; Hemiaminal; Cyanobacteria.

* Corresponding author. Tel./fax: +81 52 832 1555; e-mail: shioiri@ccmfs.meijo-u.ac.jp

[†] Present address: Tsukuba Research Institute, Novartis Pharma K. K., Ohkubo 8, Tsukuba, Ibaraki 300-2611, Japan



Scheme 1.

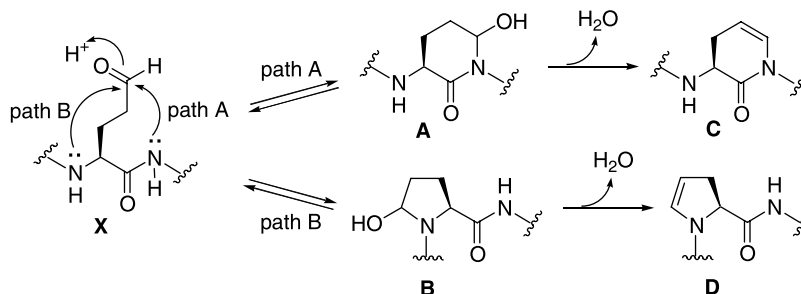
with the synthetic **1**. Thus the structure of micropeptin T-20 should be re-examined.

2. Synthetic strategy

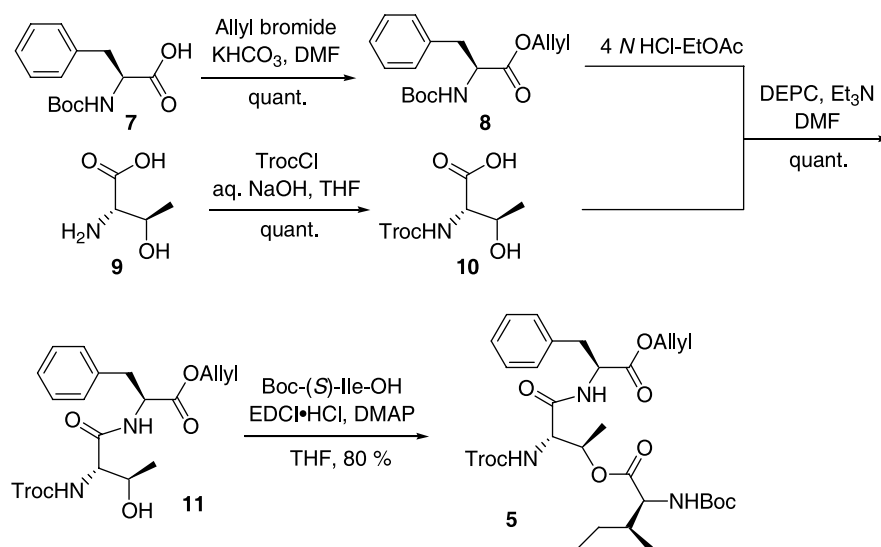
Our synthetic plan for constructing micropeptin T-20 (**1**) is depicted in [Scheme 1](#): (1) Preparation of the depsipeptide **5** and the tripeptide **6**, (2) fragment condensation of the depsipeptide **5** with the tripeptide **6**, (3) macrolactamization, (4) attachment of the side chain **3**, (5) oxidative cyclization to form the Ahp unit, and (6) deprotection. One of the most important problems to overcome in this synthesis lies in the formation of the hemiaminal-Ahp ring. The obvious starting material to construct the Ahp unit will be the aldehyde **X** prepared from glutamate, and aldehyde **X** will cyclize in

two ways to give the 6-hydroxy-2-piperidone ring **A** (path A in [Scheme 2](#)) or 5-hydroxypyrrolidine ring **B** (path B). Control of the stereochemistry of the newly formed hydroxyl group will be another problem to solve. Furthermore, the hemiaminal ring is generally labile under both strong acidic and basic conditions, and it will easily undergo dehydration to produce the dehydro derivatives **C** or **D**, respectively.⁴ Thus the construction of the Ahp unit should be done during the later stage of the synthesis.

As shown in the structure of micropeptin T-20 (**1**), it is a very polar compound because it has three hydroxyl groups in addition to the sodium phosphate function. Selective protection and deprotection of these polar groups will be another point to overcome. One of the most important problems to construct macrocyclic depsipeptides will be



Scheme 2.

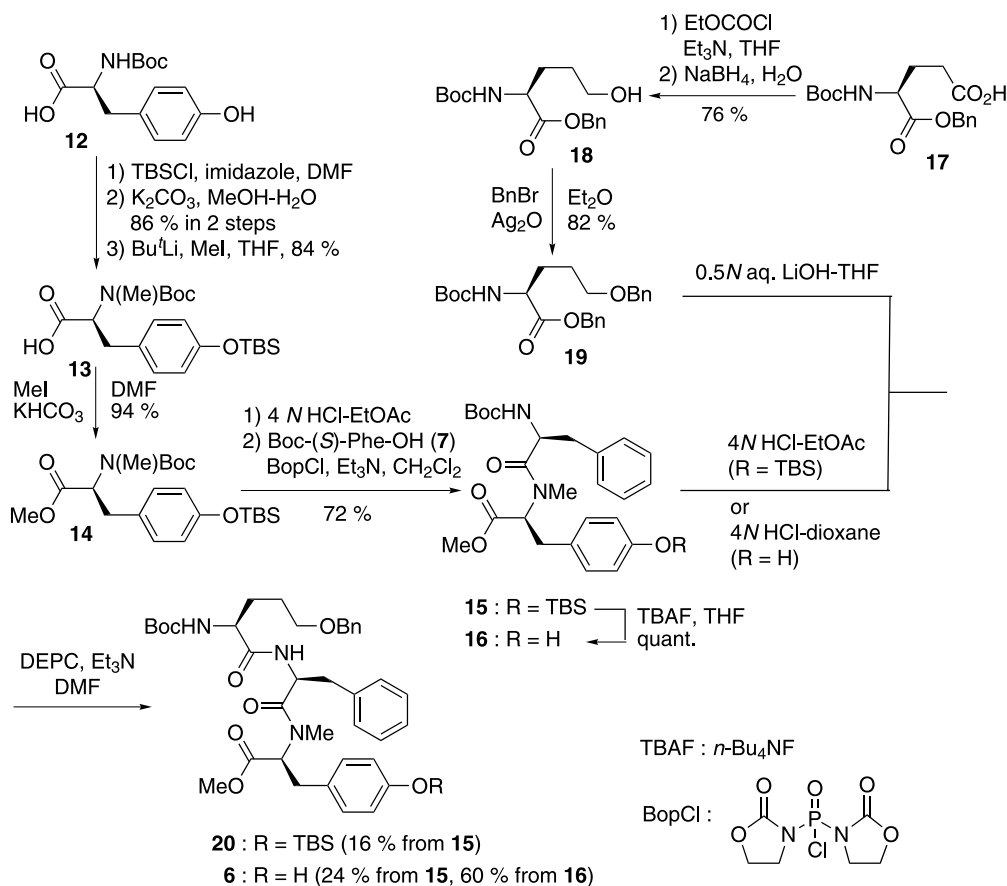


DEPC : $(\text{EtO})_2\text{P}(\text{O})\text{CN}$; EDCI : $\text{Me}_2\text{N}(\text{CH}_2)_3\text{N}=\text{C}=\text{NEt}$; DMAP : 4-(dimethylamino)pyridine

Scheme 3.

where the macrocyclization should be done and which will be better: macrolactamization or macrolactonization. Because the nucleophilicity of the amino group is generally higher than that of the hydroxyl group, macrolactamization will be preferred. An *N*-methylamino acid part in a linear

peptide will form a bent structure, and it should be situated in the middle part of a cyclization precursor. This led us to synthesize the depsipeptide **5** and the tripeptide **6**. Coupling of these peptide fragments and then macrolactamization of the linear peptide **4** will give the 19-membered cyclic



Scheme 4.

depsipeptide **2**. After addition of the side chain fragment **3**, construction of the Ahp moiety followed by deprotection will finally afford micropeptin T-20 (**1**).

3. Results and discussions

The synthesis of the depsipeptide **5** started from Boc-(*S*)-phenylalanine (**7**) by conversion to the corresponding allyl ester **8**, as shown in Scheme 3. After acidic deprotection of the *tert*-butoxycarbonyl (Boc) group of **8**, the resulting de-Boc derivative was coupled with trichloroethoxycarbonyl (Troc)-(*S*)-threonine (**10**) using diethyl phosphorocyanidate (DEPC, (EtO)₂P(O)CN) in the presence of triethylamine⁵ to quantitatively give the dipeptide **11**. Esterification of **11** with Boc-(*S*)-isoleucine with *N*-ethyl-*N'*-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDCI·HCl) afforded the depsipeptide **5**.

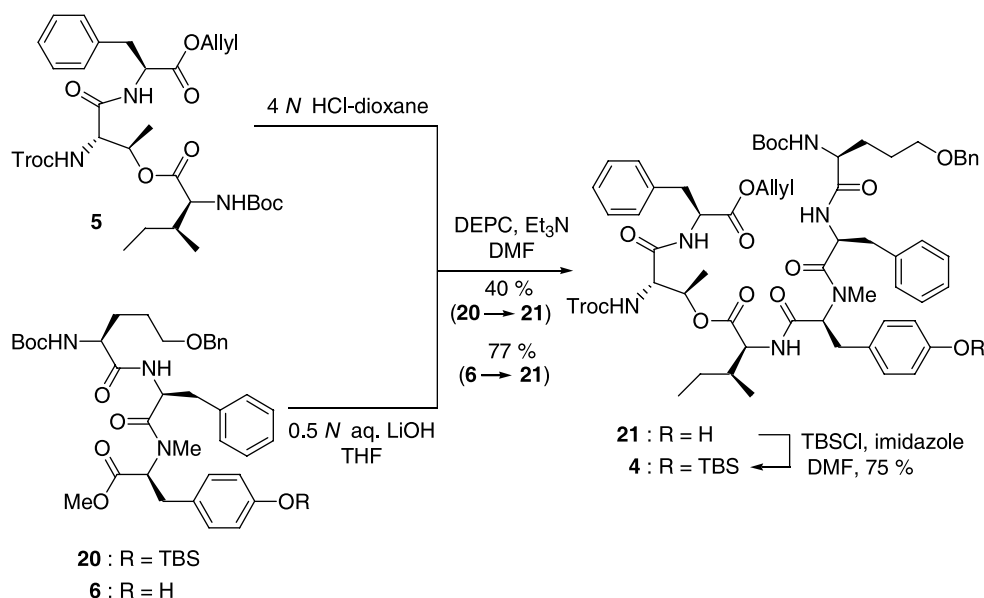
The required *N*-methyl-(*S*)-tyrosine derivative (**13**) was prepared from Boc-(*S*)-tyrosine (**12**) by conversion to its *O*-*tert*-butyldimethylsilyl (TBS) derivative, which afforded the *N*-methyl derivative **13** according to our synthesis of the corresponding (*R*)-derivative,⁶ as shown in Scheme 4. Esterification of the acid **13** gave the methyl ester **14**, whose Boc group was removed under acidic conditions. Coupling of the de-Boc derivative with Boc-(*S*)-phenylalanine (**7**) using bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BopCl)⁷ gave the dipeptide **15**.

The pentahomoserine derivative **19**, which will become a constituent of the Ahp unit, was prepared from Boc-(*S*)-glutamic acid benzyl ester (**17**) by its conversion to the mixed anhydride, reduction to the alcohol **18**,⁸ and then benzylation. After the acidic deprotection of the Boc group of **15** and saponification of **19**, respectively, coupling by the DEPC method afforded a mixture of the tripeptides **20** and **6** in 16 and 24% yields, respectively. In contrast, the dipeptide **16**, obtained by removal of the TBS group from **15** with TBAF (Bu₄NF), underwent acidic deprotection and

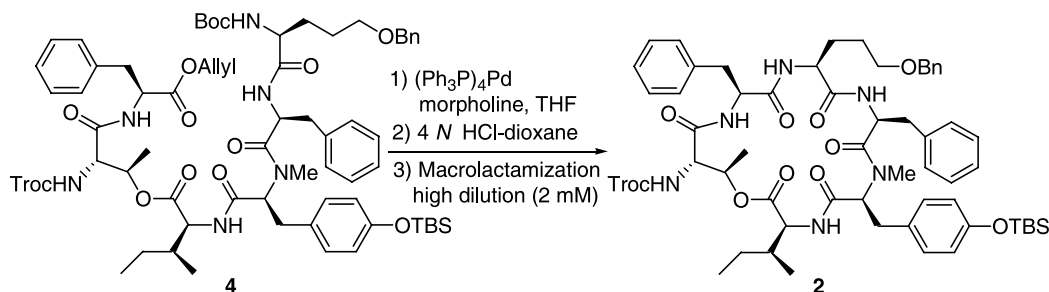
smoothly coupled with the carboxylic acid from **19** to give the tripeptide **6** in 60% yield,⁹ as shown in Scheme 4. Preparation of the hexapeptide, the cyclization precursor, was first tried by coupling of the depsipeptide **5** with the O-TBS derivative **20**. After deprotection of each compound as shown in Scheme 5, coupling by the DEPC method afforded the hexapeptide **21** without the TBS function in 40% yield. It should be noted that removal of the TBS group again occurred in this case.⁹ On the other hand, the coupling of **5** with the phenolic derivative **6** smoothly proceeded to give the hexapeptide **21** in 77% yield, which was converted to the O-TBS derivative **4**.

We are now at the stage of macrolactamization. After removal of the allyl group from the ester part of **4**, the Boc group was removed under acidic conditions. The resulting deprotected peptide underwent cyclization under high dilution conditions, as summarized in Table 1. So far, pentafluorophenyl diphenylphosphinate (FDPP, (C₆H₅)₂P(O)OCF₅)¹⁰ in the presence of diisopropylethylamine in dichloromethane furnished best result, giving the macrolactam **2** in 84% yield. Unexpectedly, the yield of **2** using the corresponding pentafluorophenyl ester was low (23%).

Since there is no precedent on the construction of the Ahp ring in peptides, some model experiments were carried out, as shown in Scheme 6. First, the tripeptide **20** underwent a catalytic debenzoylation to give the corresponding alcohol, which was subjected to oxidation with the Dess–Martin periodinane.¹¹ Neither the aldehyde **22** nor Ahp derivative was detected in the products. Next, the macrocyclic depsipeptide **2** underwent the catalytic removal of the benzyl function and then the product was analogously oxidized as described above. Again the aldehyde **23** was very labile and immediately transformed into a complex mixture. It was thought that the stability of the aldehyde might depend on the presence of the side chain which is attached at the *N*-terminal of threonine in the micropeptins and affects the conformation. Thus the construction of the



Scheme 5.

Table 1. Macrolactamization of the linear precursor **4**

Entry	Coupling conditions	Time (h)	Yield (%)
1	DPPA, NaHCO ₃ , DMF, 4 °C	72	52
2	DEPC, NaHCO ₃ , DMF, 4 °C	96	44
3	HATU, Pr ₂ NEt, DMF, rt	14	38
4	HATU, Pr ₂ NEt, DMF, 4 °C	96	59
5	FDPP, Pr ₂ NEt, DMF, rt	14	57
6	FDPP, Pr ₂ NEt, CH ₂ Cl ₂ , rt	17	84

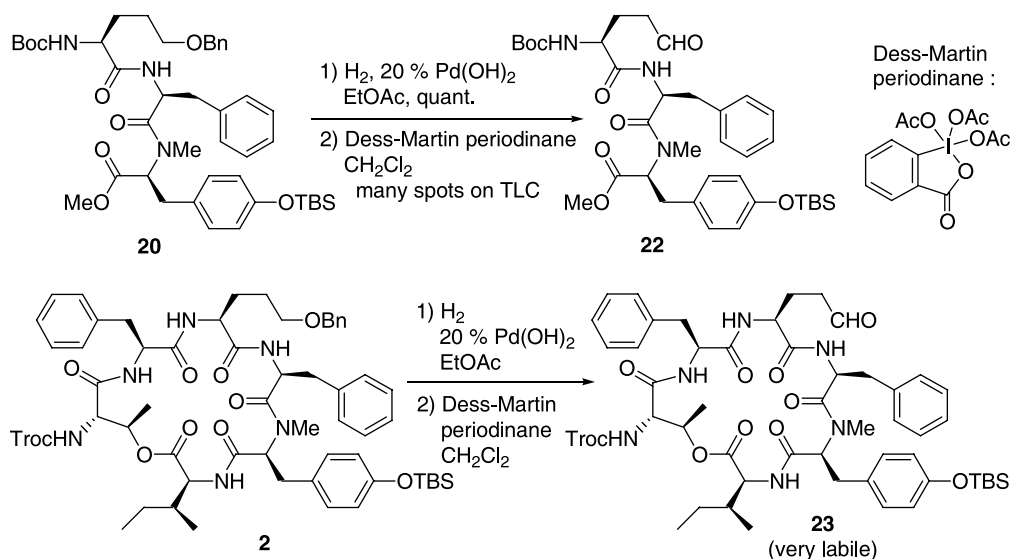
DPPA, (C₆H₅O)₂P(O)N₃; FDPP, (C₆H₅)₂P(O)OC₆F₅; HATU, PF₆⁻, NMe₂.

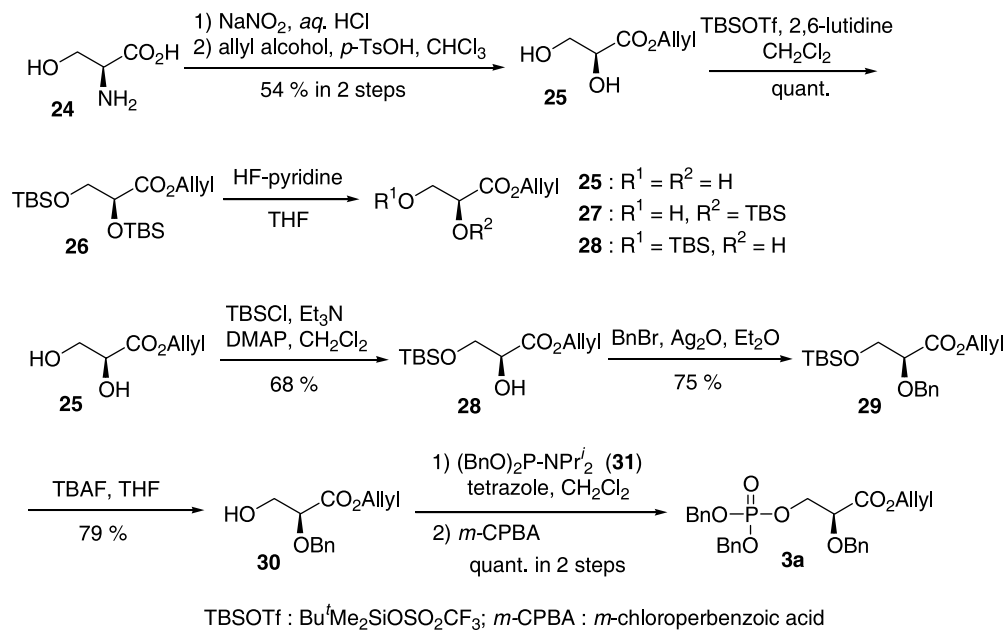
Ahp unit was postponed after the introduction of the side chain.

To prepare the side chain, (*S*)-glyceric acid allyl ester (**25**) obtained from (*S*)-serine (**24**)¹² was converted to the bis-TBS ester **26**, as shown in Scheme 7. The attempted selective deprotection of the bis-TBS ester **26** with HF-pyridine¹³ afforded a mixture of the primary alcohol **27**, secondary alcohol **28**, and diol **25**. Thus the diol **25** was first converted to the monosilylated compound **28**,¹⁴ which was transformed to the benzyl derivative **29**. Removal of the TBS group afforded the primary alcohol **30**. Phosphorylation of **30** failed using *o*-xylene *N,N*-diethylphosphoramidite¹⁵ or the Mitsunobu reaction with dibenzyl phosphate.¹⁶ The Mitsunobu reaction of the diol **25** with dibenzyl phosphate proceeded to give the mono phosphate

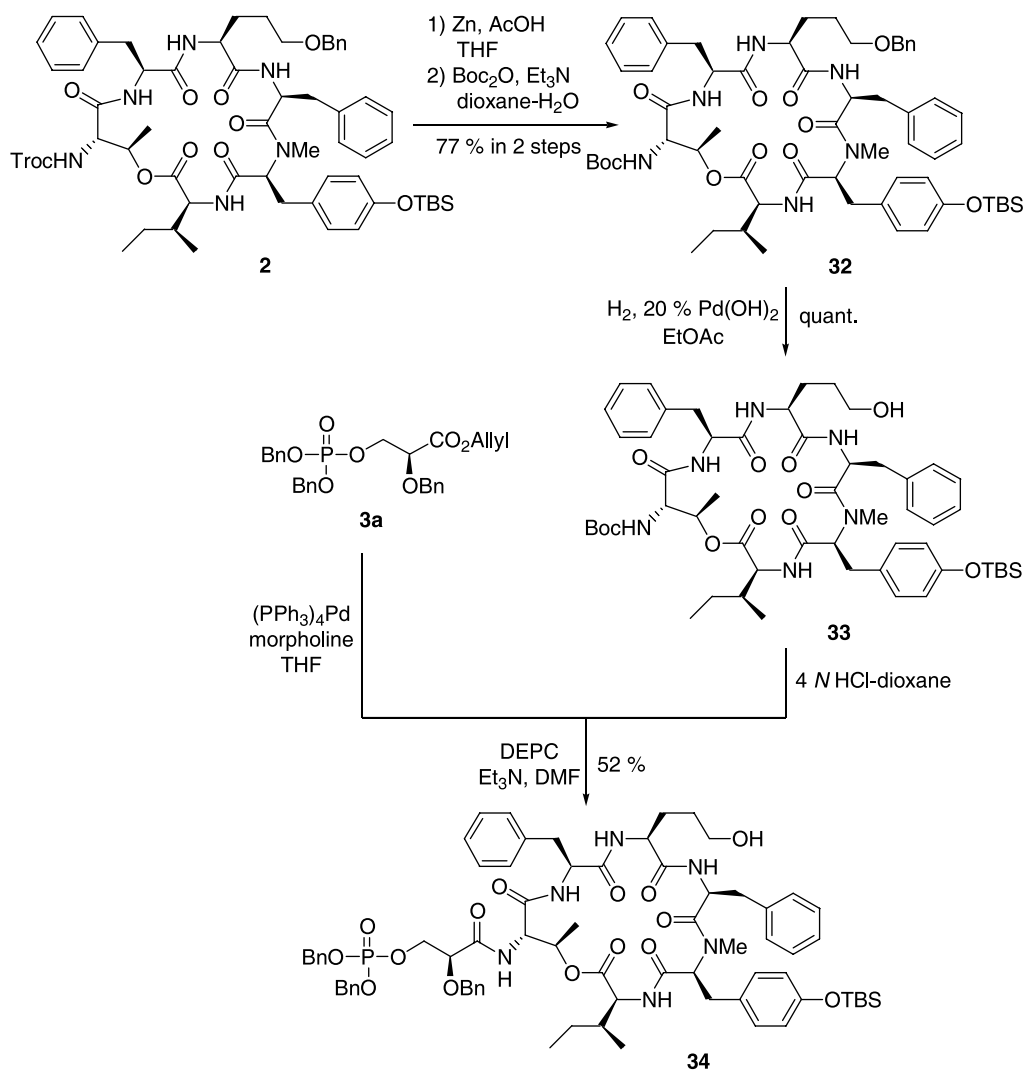
in low yield. Finally, the primary alcohol **30** was treated with dibenzyl *N,N*-diisopropyl phosphoramidite (**31**) to give the phosphite, which was oxidized with *m*-chloroperbenzoic acid (*m*-CPBA)¹⁷ to produce the desired side chain fragment **3a** in quantitative yield.

Since both the hydroxyl and phosphate residues of the side chain **3a** were protected by the benzyl group, the benzyl group of the homoserine part of the cyclic depsipeptide **2** should be removed before the introduction of the side chain. Although the hydrogenolytic removal of the benzyl group from **2** proceeded using 20% Pd(OH)₂, attempted removal of the Troc group using zinc in acetic acid failed. In contrast, removal of the Troc group from **2** rapidly proceeded to give the corresponding amino derivative using zinc in acetic acid, but hydrogenolysis did not give the

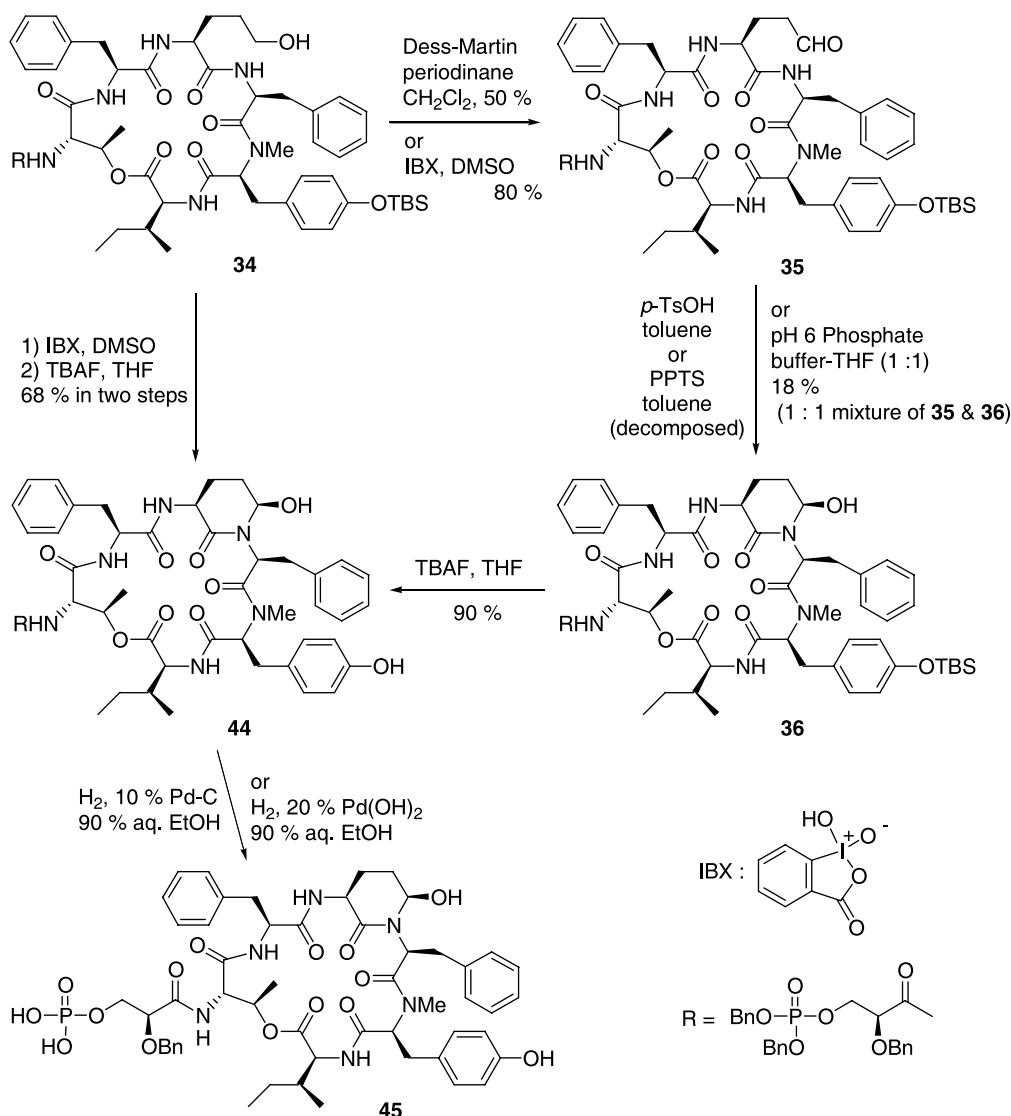
**Scheme 6.**



Scheme 7.



Scheme 8.



Scheme 9.

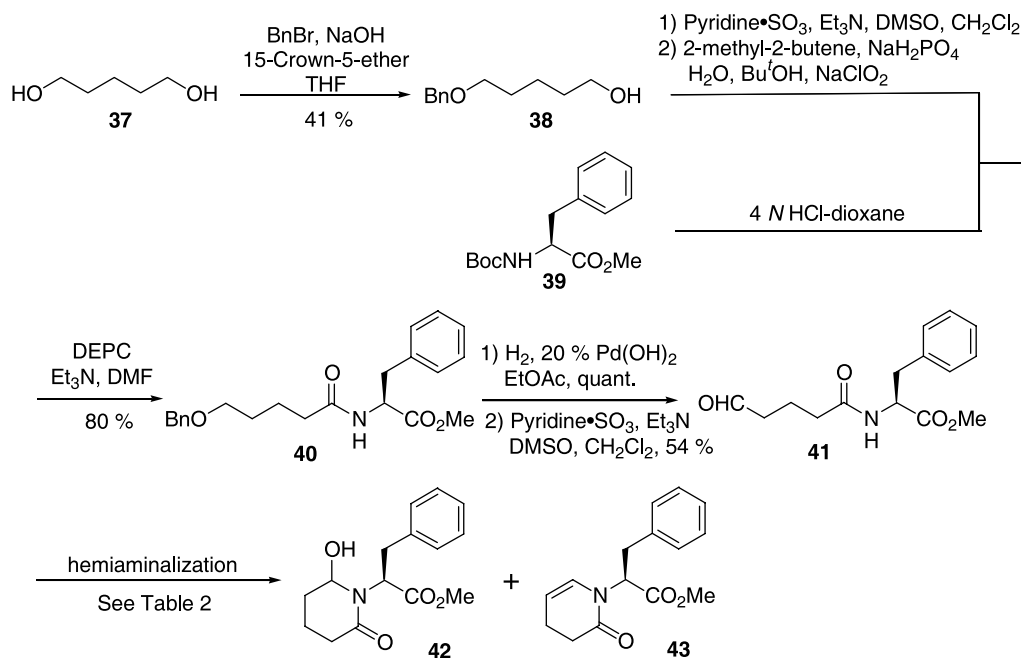
required *N,O*-deprotected compound under a variety of conditions. This might be due to the high polarity of the de-Troc product.

To overcome this difficulty, replacement of the Troc group with the less polar Boc group was undertaken. Thus, after removal of the Troc group of **2** with zinc in acetic acid as described above, the crude product was converted to the Boc derivative **32**, as shown in Scheme 8. The Boc derivative **32** smoothly underwent catalytic hydrogenolysis to give the Boc alcohol **33**, from which the Boc group was removed under acidic conditions. After removal of the allyl group from **3a**, the side chain fragment was coupled with the amine from **33** to give the Ahp precursor **34**.

The Ahp precursor **34** was oxidized with Dess–Martin periodinane to give the aldehyde **35** in 50% yield, as shown in Scheme 9. Alternatively, the use of 1-hydroxy-1,2-benziodoxol-3(1*H*)-one 1-oxide (IBX), a milder oxidant,¹⁸ afforded **35** in 80% yield, while oxidation with pyridinium dichromate resulted in the formation of a complex mixture. The aldehyde **35** was relatively stable compared to **23**. No

equilibrium between the aldehyde **35** and the hemiaminal **36** was detected, and attempted cyclization of **35** using *p*-toluenesulfonic acid or pyridinium *p*-toluenesulfonate (PPTS) caused the gradual decomposition of the starting aldehyde **35**.

We now returned to the model experiments on the cyclization of the aldehyde to the hemiaminal. Thus, 1,5-pentanediol (**37**) was transformed into the monobenzyl ether **38**,¹⁹ which was converted to the carboxylic acid and coupled with phenylalanine methyl ester from **39**, as shown in Scheme 10. The resulting amide **40** was converted to the aldehyde **41** through catalytic debenzoylation followed by the Parikh–Doering oxidation. Cyclization of the aldehyde **41** to **42** was carried out under a variety of conditions, as summarized in Table 2. Many Lewis acids were not effective and resulted in no reaction or decomposition of the starting aldehyde **41** (entries 1–6 in Table 2). Alumina was also not useful, and cyclization followed by dehydration occurred using silica gel at higher temperature to give the dehydro derivative **43** (entries 7–9). Both *p*-toluenesulfonic acid and PPTS were ineffective (entries



Scheme 10.

Table 2. Hemiaminalization of the model compound 41

Entry	Reagents	Solvents	Temperature (time)	Results
1	ZnCl ₂	CH ₂ Cl ₂	−78 °C (0.5 h) to rt (24 h)	No reaction
2	MgBr ₂	Et ₂ O	0 °C (1 h) to rt (48 h)	Trace
3	SnCl ₄	CH ₂ Cl ₂	0 °C (1 h) to rt (1 h)	Complex mixture
4	Sc(OTf) ₃	CH ₂ Cl ₂	0 °C (2.5 h)	Trace
5	TiCl ₄	CH ₂ Cl ₂	0 °C (1 h)	Trace
6	BF ₃ ·Et ₂ O	CH ₂ Cl ₂	−78 °C (4 h) to rt (2 h)	Trace
7	Al ₂ O ₃	CH ₂ Cl ₂	0 °C (1 h) to rt (2 h)	No reaction
8	Al ₂ O ₃	Toluene	80 °C (18 h)	Unknown product
9	SiO ₂	Toluene	rt (1 h) to 80 °C (12 h)	Dehydro product 43 (62%)
10	<i>p</i> -TsOH	CH ₂ Cl ₂	0 °C (1 h)	Trace (dehydro product 43: major)
11	PPTS	CH ₂ Cl ₂	0 °C (1 h) to rt (2.5 h)	Trace
12	0.1 M pH 6 phosphate buffer	THF	0 °C (0.5 h) to rt (20 h)	6-Hydroxy-2-piperidone 42 (66%)

10–11). We thought at this stage that the addition of water to the solvent system might prevent the dehydration. In fact, treatment of the aldehyde 41 in tetrahydrofuran containing 0.1 M phosphate buffer (pH 6) afforded the desired piperidone 42 as a single isomer in 66% yield (entry 12). In the synthetic route to micropeptin T-20, removal of both the TBS and benzyl groups will be necessary after the construction of the Ahp unit. Thus, the stability of the model compound 42 toward hydrogenolysis and TBAF was examined. The hemiaminal 42 was stable to hydrogenolysis but treatment with TBAF caused dehydration to give the dehydro derivative 43 in only 30 min, which suggested the requirement of the careful treatment with TBAF.

The results of the above experiments indicated that both aldehyde and hemiaminal structures were labile to acidic conditions and the strong oxidative conditions were not necessary. After oxidation of the alcohol 34 with IBX, treatment of the aldehyde 35 with a pH 6 phosphate buffer afforded a 1:1 mixture of the starting aldehyde 35 and the Ahp derivative 36 in low yield, as shown in Scheme 9. Since the mixture was unseparable and labile, desilylation of the mixture was carried out with TBAF to give the Ahp

derivative 44 as a single isomer, and the ¹H NMR spectrum of its Ahp part was completely identical with that of natural micropeptin T-20 (1), proving its stereochemistry as depicted. The conformational effect of the cyclic depsipeptide will govern the stereochemistry. The above experiments revealed that treatment of the aldehyde 35 with TBAF would give rise to the Ahp ring formation accompanied by desilylation. In fact, after oxidation of 34 with IBX, treatment with TBAF afforded the Ahp derivative 44 as a single isomer in good yield.

The stability of the Ahp structure will be due to cooperation of the resonance effect between the nitrogen and carbonyl group in the Ahp ring in addition to the anomeric and substituent effects. Since the dehydration in the Ahp nucleus will occur when the C5-axial hydrogen and the hydroxyl group are situated antiperiplanar, the freedom of the 6-membered chair conformation will affect the stability. This will be the reason for the different reactivities between the model compound 42 and the real substrate 44.

Debenzylation at the side chain of 44 was attempted under a hydrogen atmosphere by using 10% Pd–C or 20% Pd(OH)₂

Entry	Conditions	Temperature (time)	Results	Reference
1	3.5 M HF-pyridine, THF	rt (5 h)	Complex mixture	13
2	AcOH-H ₂ O-THF (13:7:3)	rt (9 h) −40 °C (4.5 h) to rt (11 h)	28%	21a
3	CF ₃ CO ₂ H-H ₂ O-THF (9:1:40)	0 °C (0.5 h) to rt (6 h)	54%	21a
4	Oxone, MeOH-H ₂ O (1:1) C	rt (6 h)	Trace	21b
5	CeCl ₃ ·7H ₂ O, NaI, MeCN	rt (15 h) to reflux (3 h)	62%	21c



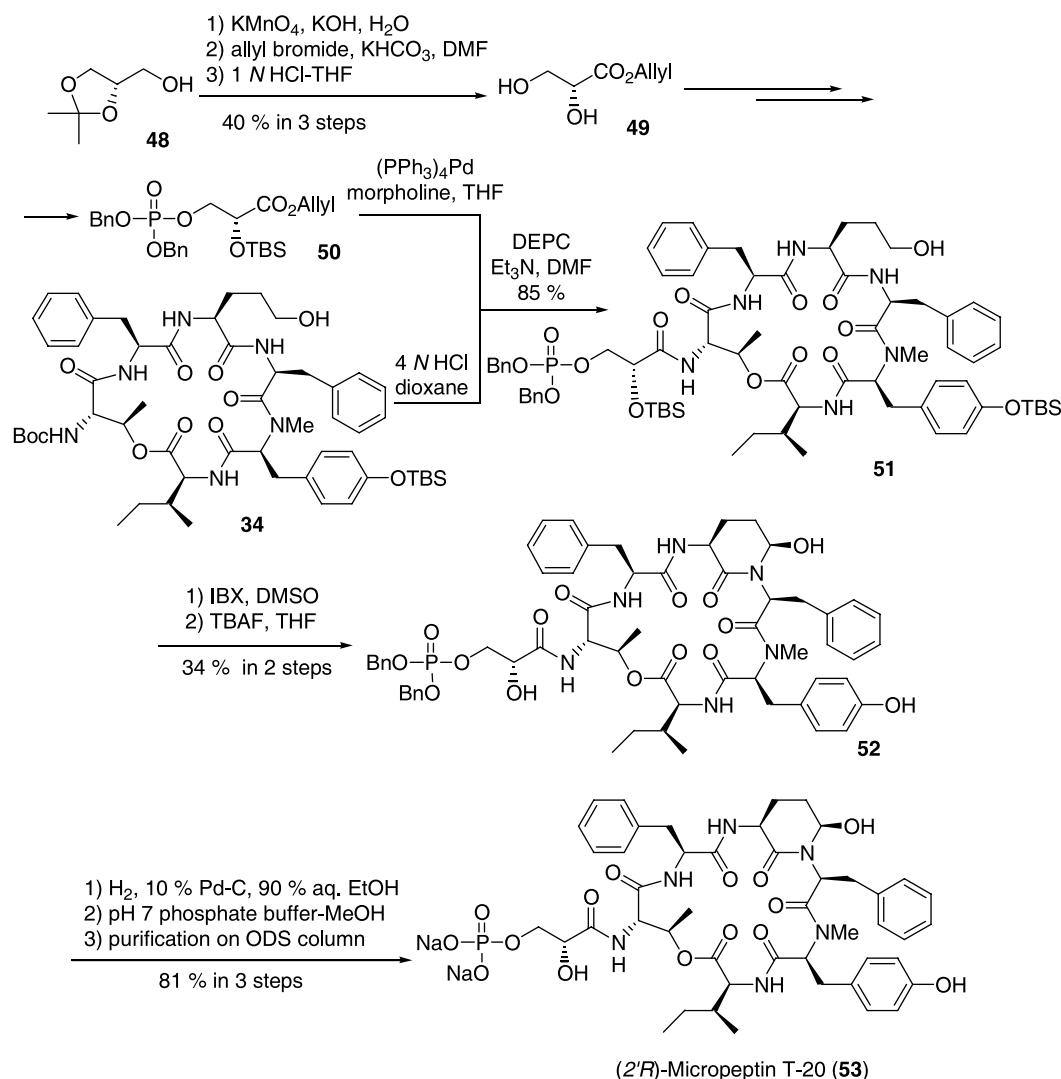
in 90% aqueous ethanol.^{17,20} The product was revealed to be the monobenzylated derivative **45** (Scheme 9). Although both of the benzyl functions at the phosphate part were removed, the benzyl group at the ether function could not be removed at all. The addition of acetic acid with a longer reaction time resulted in cleavage of the side chain.

We now needed to renew the protective group at the glyceric phosphate side chain, therefore, the protection with the TBS group was reinvestigated, the results of which are shown in Table 3. So far, a combination of cerium chloride and sodium iodide^{21c} toward **26** gave the best result that furnished the primary alcohol **27** in 62% yield. The alcohol **27** was analogously converted to the phosphate **3b** as the conversion of **30** to **3a** (See Scheme 7).

After introduction of the side chain **3b** to the cyclic depsipeptide **33**, oxidation of the alcohol **46** with IBX followed by treatment with TBAF afforded the Ahp peptide **47** as a single isomer. Catalytic removal of the benzyl function was easily accomplished over 10% Pd–C in 90% aqueous ethanol, as shown in Scheme 11. From the NMR spectral data, our synthetic micropeptin T-20 is apparently

not the same compound reported by Kaya and co-workers.^{1b} Comparison of the NMR spectra leads us to conclude that our synthetic compound has the structure **1** and it will be configurationally isomeric to natural micropeptin T-20. A complete similarity was observed in the spectral data of their cyclic depsipeptide part. However, a difference was seen at the methine proton at C2 of the glyceric acid part and methylene protons at C3; the ¹H signal of the synthetic material was 0.2 ppm higher than that of the natural one and the difference of 3 ppm was observed in their ¹³C NMR spectra. This obvious spectral difference at the side chain was also observed after treatment with a pH 7 phosphate buffer followed by purification on an ODS column. Furthermore, pH and concentration proved not to influence the chemical shift of the NMR spectra. This structural discrepancy between the synthetic and natural micropeptin T-20 led us to synthesize another micropeptin T-20 having the (*R*)-glyceric acid phosphate residue.

The alcohol **48** was submitted to oxidation, allylation, and then acidic treatment to give the (*R*)-glyceric acid allyl ester (**49**), which was converted to the (*R*)-glyceric acid phosphate **50**, as shown in Scheme 12. After removal of



Scheme 12.

the allyl group, coupling with the amine from the cyclic depsipeptide **34** afforded **51** with the (*R*)-side chain. IBX oxidation and treatment with TBAF afforded **52**, which was converted to micropeptin T-20 (**53**) having the (*R*)-side chain as described above. However, this compound **53** was again not identical with natural micropeptin T-20.

Based on the preceding analysis and synthesis, we conclude that our synthesis proceeded as intended to correctly provide molecules possessing structures **1** and **53**. Although the method for the synthesis of the Ahp-containing cyclic depsipeptide was established,²² this work strongly indicates that the structure of micropeptin T-20 should be revised. Unfortunately, however, since no natural micropeptin T-20 is presently available, structural re-assignment will require re-isolation.

4. Experimental

4.1. General information

Infrared spectra were recorded on a SHIMADZU FT IR-8100 spectrometer. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter with a sodium lamp ($\lambda = 589$ nm, D line) and are recorded as follows: $[\alpha]_D^{25}$ (C g/100 ml, solvent). ^1H NMR spectra were recorded on a JEOL EX-270 or α -500 spectrometer in deuterio solvent using tetramethylsilane (TMS) or CHCl_3 (δ 7.26 ppm) or CH_3OH (δ 3.30 ppm) as an internal standard. Data are described as follows: chemical shift, integration, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, br=broad, m=multiplet), coupling constants (Hz), and assignment. ^{13}C NMR spectra were recorded on a JEOL EX-270 (67.8 MHz) spectrometer with complete proton decoupling. Chemical shifts are described in ppm with the solvent as the internal standard (CDCl_3 : δ 77.0 ppm, CD_3OD : δ 49.0 ppm). Analytical thin layer chromatography was performed on Merck Art. 5715, Kieselgel 60 F₂₄₅/0.25 mm thickness plates. Visualization was accomplished with UV light, phosphomolybdic acid, or ninhydrin solution followed by heating. Mass spectra were obtained on a JEOL JMS-SX 102A (EI) and JMS-AX 505HA (FAB) spectrometer. Column chromatography was performed with silica gel BW-820 MH or BW-200 MH (Fuji Silysia Co.). Solvents for extraction and chromatography were reagent grade. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl. Diethyl ether (Et_2O) was distilled from lithium aluminum hydride (LiAlH_4). Dichloromethane (CH_2Cl_2) was distilled from calcium hydride. All other commercially available reagents were used as received.

4.1.1. Boc-(S)-Phe-OAllyl (8). To a solution of Boc-(S)-Phe-OH (**7**) (3.0 g, 11.3 mmol) in DMF (30 ml) was added KHCO_3 (2.3 g, 23.0 mmol) and allyl bromide (1.4 ml, 16.2 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 14 h. The reaction was quenched with 1 M aqueous KHSO_4 and the mixture was extracted with EtOAc. The extracts were successively washed with H_2O , saturated aqueous NaHCO_3 , H_2O , and brine, dried over Na_2SO_4 , and concentrated in vacuo to give **8** (3.6 g, quant.) as a colorless oil: $[\alpha]_D^{25} = +27.9$ (c 1.0, CHCl_3); IR $\nu_{\text{max}}^{\text{neat}}$ cm^{-1} 3375, 2978, 1743, 1716, 1498, 1367,

1167; ^1H NMR (270 MHz, CDCl_3) δ 1.41 (9H, s, *t*Bu), 3.02–3.14 (2H, m, Phe-3-*H*), 4.58–4.61 (3H, m, Phe-2-*H*, CO_2CH_2), 4.98 (1H, br, *NH*), 5.22–5.33 (2H, m, $\text{CO}_2\text{CH}_2\text{CHCH}_2$), 5.78–5.93 (1H, m, $\text{CO}_2\text{CH}_2\text{CHCH}_2$), 7.12–7.23 (5H, m, C_6H_5); ^{13}C NMR (67.8 MHz, CDCl_3) δ 28.1, 38.2, 54.3, 65.7, 79.7, 118.7, 126.8, 128.0, 129.0, 131.4, 135.9, 154.9, 171.4; Anal. calcd for $\text{C}_{17}\text{H}_{23}\text{NO}_4$: C, 66.86, H, 7.59, N, 4.59. Found: C, 66.72, H, 7.63, N, 4.65.

4.1.2. Troc-(S)-Thr-OH (10). To a solution of (S)-Thr-OH (**9**) (15.0 g, 0.12 mol) in THF (120 ml) and 2.5 N aqueous NaOH (120 ml) was added 2,2,2-trichloroethoxycarbonyl chloride (10 ml $\times$ 3, total 0.19 mol) three times every 1 h at 0 °C. The mixture was stirred at 0 °C for 6 h, and then concentrated. The residue was acidified with 1 M aqueous KHSO_4 and extracted with EtOAc ($\times$ 3). The extracts were washed with brine, dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc = 1:1 to 1:3 then EtOAc only) to give **10** (23.8 g, 65%) as a colorless amorphous solid: $[\alpha]_D^{25} = +0.67$ (c 1.0, CHCl_3); IR ν_{max} (CHCl_3) cm^{-1} 3424, 1717, 1525, 1408, 1113; ^1H NMR (270 MHz, CDCl_3) δ 1.23–1.35 (3H, m, Thr-4-*H*), 4.37–4.42 (1H, m, Thr-3-*H*), 4.48–4.51 (1H, m, Thr-2-*H*), 4.69 (1H, d, $J = 12.2$ Hz, OCH_2CCl_3), 4.76 (1H, d, $J = 11.8$ Hz, OCH_2CCl_3), 6.22 (1H, br, *NH*); ^{13}C NMR (67.8 MHz, CDCl_3) δ 19.2, 59.1, 65.7, 68.0, 74.6, 95.1, 155.4, 173.9; Anal. calcd for $\text{C}_7\text{H}_{10}\text{C}_3\text{NO}_5 \cdot 1/4\text{EtOAc} \cdot 1/2\text{H}_2\text{O}$: C, 29.34, H, 3.91, N, 4.39. Found: C, 29.13, H, 3.73, N, 4.70.

4.1.3. Troc-(S)-Thr-(S)-Phe-OAllyl (11). Boc-(S)-Phe-OAllyl (**8**) (1.00 g, 3.29 mmol) was treated with 4 N HCl–EtOAc (5 ml) at 0 °C and the mixture was stirred for 1.5 h. Removal of the solvent under reduced pressure afforded the crude hydrochloride salt as a colorless solid.

To a solution of the above crude amine salt and Troc-(S)-Thr-OH (**10**) (880 mg, 2.99 mmol) in DMF (6 ml) was added DEPC (0.72 ml, 4.74 mmol) and Et_3N (1.04 ml, 7.47 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 19 h. After dilution with EtOAc, the whole mixture was successively washed with 1 M aqueous KHSO_4 , H_2O , saturated aqueous NaHCO_3 , H_2O , and brine, dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc = 2:1 to 1:1) to give **11** (1.37 g, quant.) as a colorless amorphous solid: $[\alpha]_D^{25} = -8.8$ (c 1.0, CHCl_3); IR ν_{max} (CHCl_3) cm^{-1} 3324, 1740, 1662, 1538; ^1H NMR (270 MHz, CDCl_3) δ 1.17 (3H, d, $J = 6.2$ Hz, Thr-4-*H*), 2.79 (1H, br, exchangeable with D_2O , OH), 3.05 (1H, dd, $J = 7.0$, 13.8 Hz, Phe-3-*H*), 3.20 (1H, dd, $J = 5.9$, 14.0 Hz, Phe-3-*H*), 4.08–4.15 (1H, m, Thr-3-*H*), 4.25–4.35 (1H, m, Thr-2-*H*), 4.60–4.80 (4H, m, $\text{CO}_2\text{CH}_2\text{CH}$, OCH_2CCl_3), 4.84–4.92 (1H, m, Phe-2-*H*), 5.25–5.36 (2H, m, $\text{CO}_2\text{CH}_2\text{CHCH}_2$), 5.81–5.95 (1H, br, $\text{CO}_2\text{CH}_2\text{CHCH}_2$), 7.10–7.33 (5H, m, C_6H_5); ^{13}C NMR (67.8 MHz, CDCl_3) δ 17.9, 37.6, 53.3, 58.7, 66.2, 66.8, 74.7, 95.2, 119.2, 127.2, 128.5, 129.1, 131.2, 135.5, 154.9, 169.9, 170.0; Anal. calcd for $\text{C}_{19}\text{H}_{24}\text{Cl}_3\text{N}_2\text{O}_6$: C, 47.37, H, 4.81, N, 5.81. Found: C, 47.54, H, 4.84, N, 5.51.

4.1.4. Troc-(S)-Thr[Boc-(S)-Ile]-(S)-Phe-OAllyl (5). To a solution of dipeptide **11** (50 mg, 0.10 mmol) and

Boc-(S)-Ile-OH (35 mg, 0.15 mmol) in THF (0.5 ml) was added DMAP (1.2 mg, 0.01 mmol) and EDCI·HCl (28 mg, 0.15 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 12 h. After dilution with EtOAc, the whole mixture was washed with 1 M aqueous KHSO₄, saturated aqueous NaHCO₃, and brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-200 MH, hexane–EtOAc=4:1) to give **5** (63 mg, 80%) as a colorless amorphous powder: $[\alpha]_D^{24} = +18.7$ (c 1.0, CHCl₃); IR $\nu_{\max}$ (CHCl₃) cm⁻¹ 3437, 1747, 1716, 1697, 1684, 1506; ¹H NMR (270 MHz, CDCl₃) δ 0.88–0.93 (6H, m, Ile-5,6-H), 1.18–1.28 (5H, m, Ile-4-H, Thr-4-H), 1.49 (9H, s, *t*Bu), 1.67–1.80 (1H, m, Ile-3-H), 3.06–3.23 (2H, m, Phe-3-H), 4.02–4.10 (1H, m, Thr-2-H), 4.32–4.37 (1H, m, Ile-2-H), 4.59 (2H, d, *J*=5.6 Hz, CO₂CH₂CH), 4.73 (2H, s, OCH₂Cl₃), 4.84–4.92 (1H, m, Phe-2-H), 4.95–5.05 (1H, br, NH), 5.20–5.38 (3H, m, Thr-3-H, CO₂CH₂CHCH₂), 5.78–5.89 (1H, m, CO₂CH₂CHCH₂), 5.90–6.00 (1H, br, NH), 7.15–7.30 (6H, m, C₆H₅, NH); ¹³C NMR (67.8 MHz, CDCl₃) δ 11.2, 14.9, 15.2, 25.2, 28.3, 36.7, 37.7, 53.7, 56.7, 58.4, 65.9, 69.7, 74.6, 80.2, 95.2, 118.9, 127.0, 128.5, 129.1, 131.3, 135.9, 153.9, 156.0, 167.3, 170.7, 171.2; Anal. calcd for C₃₀H₄₂Cl₃N₃O₉: C, 51.84; H, 6.09; N, 6.05. Found: C, 51.55; H, 6.07; N, 5.77.

4.1.5. Boc-(S)-Tyr(TBS)-OH. To a solution of Boc-(S)-Tyr-OH (**12**) (3.0 g, 10.6 mmol) in DMF (20 ml) was added TBSCl (4.8 g, 31.9 mmol) and imidazole (4.3 g, 63.6 mmol) at 0 °C. The solution was stirred at 0 °C for 1 h, then at room temperature for 12 h. After dilution with EtOAc, the whole mixture was washed with 1 M aqueous KHSO₄, saturated aqueous NaHCO₃, brine, dried over Na₂SO₄, and concentrated in vacuo. K₂CO₃ (2.1 g, 15 mmol) in H₂O (30 ml) was added to a solution of the above crude disilylate in THF (60 ml) and MeOH (30 ml). The mixture was stirred at room temperature for 48 h. After dilution with Et₂O, the whole mixture was successively washed with 1 M aqueous KHSO₄, H₂O, and brine, dried over MgSO₄, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=3:1) to give Boc-(S)-Tyr(TBS)-OH (3.6 g, 86%) as a colorless amorphous solid: $[\alpha]_D^{20} = +30.2$ (c 1.1, CHCl₃) ($[\alpha]_D^{25} = -30.4$ (c 1.5, CHCl₃) for (*R*)-isomer⁶); IR $\nu_{\max}^{\text{neat}}$ cm⁻¹ 3367, 1716, 1512, 1257, 1167; ¹H NMR (270 MHz, CDCl₃) δ 0.18 (6H, s, SiMe₂), 0.97 (9H, s, Si-*t*Bu), 1.41 (9H, s, O-*t*Bu), 3.01–3.14 (2H, m, Tyr-3-H), 4.50–4.60 (1H, br, Tyr-2-H), 4.85–4.95 (1H, br, NH), 6.77 (2H, d, *J*=8.6 Hz, Tyr-6,8-H), 7.04 (2H, d, *J*=8.2 Hz, Tyr-5,9-H); ¹³C NMR (67.8 MHz, CDCl₃) δ -4.5, 18.1, 25.5, 28.2, 37.0, 54.2, 80.0, 120.0, 128.6, 130.3, 154.6, 155.3, 175.4; Anal. calcd for C₂₀H₃₃NO₅Si: C, 60.73; H, 8.41; N, 3.54. Found: C, 60.58; H, 8.31; N, 3.52.

4.1.6. Boc-(S)-N-Me-Tyr(TBS)-OH (13). To a solution of Boc-(S)-Tyr(TBS)-OH (700 mg, 1.77 mmol) in THF (5.5 ml) was carefully added 1.54 N *t*-BuLi in pentane (2.5 ml, 3.89 mmol) at -78 °C. The solution was stirred at -78 °C for 30 min, then MeI (3.3 ml, 53.2 mmol) was added at 0 °C. After 1 h, the reaction mixture was allowed to warm to room temperature and stirred for 18 h. The reaction was quenched with saturated aqueous NaHCO₃ and the mixture was extracted with EtOAc. The extracts were

washed with 1 M aqueous KHSO₄ and brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=5:1 to 3:1) to give **13** (607 mg, 84%) as a pale yellow amorphous solid: $[\alpha]_D^{23} = -55.1$ (c 1.0, CHCl₃) ($[\alpha]_D^{25} = +57.7$ (c 1.1, CHCl₃) for (*R*)-isomer⁶); IR $\nu_{\max}^{\text{neat}}$ cm⁻¹ 3200, 1705, 1512, 1257; ¹H NMR (270 MHz, CDCl₃) δ 0.17 (6H, s, SiMe₂), 0.97 (9H, s, Si-*t*Bu), 1.36, 1.41 (9H, s×2, O-*t*Bu), 2.65, 2.73 (3H, s×2, NMe), 2.98–3.25 (2H, m, Tyr-3-H), 4.41–4.72 (1H, m, Tyr-2-H), 6.76 (2H, d, *J*=8.6 Hz, Tyr-6,8-H), 7.05 (2H, d, *J*=7.6 Hz, Tyr-5,9-H); ¹³C NMR (67.8 MHz, CDCl₃) δ -4.5, 18.0, 25.5, 28.0, 28.1, 32.4, 32.6, 33.8, 34.3, 60.0, 60.6, 80.3, 80.6, 120.0, 129.8, 154.2, 155.1, 156.1, 175.6; Anal. calcd for C₂₁H₃₅NO₅Si: C, 61.58; H, 8.61; N, 3.42. Found: C, 61.67; H, 8.68; N, 3.21.

4.1.7. Boc-(S)-N-Me-Tyr(TBS)-OMe (14). To a solution of Boc-(S)-N-Me-Tyr(TBS)-OH (**13**) (1.22 g, 2.98 mmol) in DMF (10 ml) was added KHCO₃ (0.60 g, 5.96 mmol) and MeI (0.26 ml, 4.17 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 12 h. The reaction was quenched with 1 M aqueous KHSO₄ and the mixture was extracted with EtOAc. The extracts were successively washed with H₂O, saturated aqueous NaHCO₃, H₂O, and brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=10:1 to 5:1) to give **14** (1.19 g, 94%) as a colorless oil: $[\alpha]_D^{23} = -46.5$ (c 1.0, CHCl₃); IR $\nu_{\max}^{\text{neat}}$ cm⁻¹ 1747, 1699, 1512, 1257, 1170; ¹H NMR (270 MHz, CDCl₃) δ 0.16 (6H, s, SiMe₂), 0.96 (9H, s, Si-*t*Bu), 1.36, 1.38 (9H, m, O-*t*Bu), 2.67 (3H, s, NMe), 2.89–3.30 (2H, m, Tyr-3-H), 3.73 (3H, s, CO₂Me), 4.40–4.44, 4.83–4.87 (1H, m, Tyr-2-H), 6.75 (2H, d, *J*=7.9 Hz, Tyr-6,8-H), 6.95–7.06 (2H, m, Tyr-5,9-H); ¹³C NMR (67.8 MHz, CDCl₃) δ -4.5, 18.0, 25.5, 28.1, 31.8, 32.7, 34.1, 34.6, 51.9, 59.5, 61.8, 79.7, 80.1, 120.0, 129.8, 154.3, 154.8, 171.5, 171.8; Anal. calcd for C₂₂H₃₇NO₅Si: C, 62.38; H, 8.80; N, 3.31. Found: C, 62.02; H, 8.80; N, 3.33.

4.1.8. Boc-(S)-Phe-(S)-N-Me-Tyr(TBS)-OMe (15). Boc-(S)-N-Me-Tyr(TBS)-OMe (**14**) (300 mg, 0.73 mmol) was treated with 4 N HCl–EtOAc (2.5 ml) at 0 °C and the mixture was stirred for 1 h. Removal of the solvent under reduced pressure afforded the crude hydrochloride salt as a colorless solid.

To a solution of the above crude amine salt and Boc-(S)-Phe-OH (**7**) (230 mg, 0.87 mmol) in CH₂Cl₂ (3 ml) was added Et₃N (0.25 ml, 1.82 mmol) and BopCl (220 mg, 0.87 mmol) at 0 °C. The reaction mixture was stirred at 4 °C for 15 h. After dilution with EtOAc, the whole mixture was successively washed with 1 M aqueous KHSO₄, saturated aqueous NaHCO₃, and brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-200 MH, hexane–EtOAc=5:1) to give **15** (300 mg, 72%) as a colorless amorphous solid: $[\alpha]_D^{24} = -47.4$ (c 1.0, CHCl₃); IR $\nu_{\max}$ (CHCl₃) cm⁻¹ 3324, 1743, 1713, 1645, 1510, 1255, 1170; ¹H NMR (270 MHz, CDCl₃) δ 0.15 (6H, s, SiMe₂), 0.95 (9H, s, Si-*t*Bu), 1.38 (9H, s, O-*t*Bu), 2.73 (3H, s, NMe), 2.80–2.90 (2H, m, Phe-3-H), 2.90–3.05 (1H, m, Tyr-3-H), 3.15–3.25 (1H,

m, Tyr-3-*H*), 3.68 (3H, s, CO₂Me), 4.68–4.78 (1H, m, Tyr-2-*H*), 5.08–5.15 (2H, m, Phe-2-*H*), 6.68–6.80 (2H, m, Tyr-6,8-*H*), 6.93–6.99 (2H, m, Tyr-5,9-*H*) 7.15–7.30 (5H, m, C₆H₅); ¹³C NMR (67.8 MHz, CDCl₃) δ –3.8, 18.7, 26.2, 28.9, 33.4, 34.4, 39.8, 52.2, 52.7, 59.6, 80.1, 120.7, 127.8, 128.9, 130.0, 130.2, 130.4, 137.0, 155.0, 155.5, 171.4, 172.6; Anal. calcd for C₃₁H₄₆N₂O₆Si·1/2H₂O: C, 64.22; H, 8.17; N, 4.83. Found: C, 64.57; H, 7.99; N, 5.13.

4.1.9. Boc-(S)-Phe-(S)-N-Me-Tyr-OMe (16). To a solution of dipeptide **15** (1.0 g, 1.75 mmol) in THF (1 ml) was added 1 N TBAF in THF (5.3 ml, 5.3 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h. After dilution with EtOAc, the whole mixture was washed with H₂O and brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=2:1) to give **16** (797 mg, quant.) as a colorless amorphous powder: [α]_D²⁴ = –19.6 (c 1.1, CHCl₃); IR ν_{max}^{neat} cm^{–1} 3324, 1741, 1698, 1646, 1516, 1250, 1169; ¹H NMR (270 MHz, CDCl₃) δ 1.37 (9H, s, *t*Bu), 1.70–1.90 (1H, br, OH), 2.19–2.26 (1H, m, Tyr-3-*H*), 2.75 (3H, s, NMe), 2.79–2.88 (2H, m, Tyr-3-*H*, Phe-3-*H*), 2.91–3.04 (1H, m, Phe-3-*H*), 3.67 (3H, s, CO₂Me), 4.73–4.81 (1H, m, Tyr-2-*H*), 5.13–5.24 (2H, m, Phe-2-*H*, NH), 6.66–6.72 (2H, m, Tyr-6,8-*H*), 6.82–7.00 (2H, m, Tyr-5,9-*H*), 7.17–7.28 (5H, m, C₆H₅); ¹³C NMR (67.8 MHz, CDCl₃) δ 15.1, 28.2, 31.5, 32.5, 33.7, 39.0, 51.5, 52.2, 59.0, 65.8, 79.9, 115.4, 126.7, 128.3, 129.4, 129.8, 136.1, 155.2, 170.8, 172.5.

4.1.10. Benzyl (S)-2-tert-butoxycarbonylamino-5-hydroxypentanoate (18). To a solution of Boc-(S)-Glu-OBn (**17**) (4.0 g, 11.8 mmol) in THF (60 ml) was added Et₃N (5.0 ml, 35.5 mmol) and ethyl chloroformate (3.4 ml, 35.5 mmol) at –10 °C under an argon atmosphere. The mixture was stirred at –10 °C for 1 h, and NaBH₄ (1.78 g, 47.2 mmol) in H₂O (60 ml) was added dropwise at –10 °C. The mixture was stirred at –10 °C for 1 h, then at room temperature for 2 h. The reaction was quenched with 1 M aqueous KHSO₄, and the mixture was extracted with EtOAc (×3). The extracts were washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=1:1) to give **18** (2.86 g, 76%) as a colorless oil: [α]_D²⁴ = –3.9 (c 1.0, CHCl₃); IR ν_{max}^{neat} cm^{–1} 3367, 1738, 1713, 1517, 1165; ¹H NMR (270 MHz, CDCl₃) δ 1.43 (9H, s, *t*Bu), 1.32–1.93 (5H, m, Phs-3,4-*H*, OH), 3.63 (2H, t, *J* = 6.1 Hz, Phs-5-*H*), 4.32–4.45 (1H, m, Phs-2-*H*), 5.11–5.23 (3H, m, CH₂C₆H₅, NH), 7.35 (5H, s, C₆H₅); ¹³C NMR (67.8 MHz, CDCl₃) δ 28.1, 29.1, 53.1, 61.7, 65.7, 66.9, 79.8, 128.3, 135.2, 155.4, 172.5; Anal. calcd for C₁₇H₂₅NO₅: C, 63.14; H, 7.79; N, 4.33. Found: C, 62.96; H, 7.63; N, 4.49.

4.1.11. Benzyl (S)-2-tert-butoxycarbonylamino-5-benzoyloxypentanoate (19). To a solution of the pentahomoserine derivative **18** (1.4 g, 4.31 mmol) in Et₂O (14 ml) was added Ag₂O (4.0 g, 17.2 mmol) and BnBr (3.6 ml, 30.1 mmol) at room temperature under an argon atmosphere. The mixture was stirred for 14 h. The mixture was filtered through a pad of celite and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-200 MH, hexane–EtOAc=3:1) to give **19** (1.46 g, 82%) as a colorless oil: [α]_D²² = –4.2 (c 1.0, CHCl₃); IR ν_{max}^{neat}

cm^{–1} 3367, 1743, 1714, 1498, 1161; ¹H NMR (270 MHz, CDCl₃) δ 1.42 (9H, s, *t*Bu), 1.58–2.05 (4H, m, Phs-3,4-*H*), 3.45 (2H, t, *J* = 6.0 Hz, Phs-5-*H*), 4.30–4.40 (1H, m, Phs-2-*H*), 4.46 (2H, s, CH₂OCH₂C₆H₅), 5.10–5.22 (3H, m, CO₂CH₂C₆H₅, NH), 7.29–7.34 (10H, m, C₆H₅×2); ¹³C NMR (67.8 MHz, CDCl₃) δ 25.4, 28.2, 29.3, 53.2, 66.8, 69.3, 72.7, 79.6, 127.4, 128.0, 128.2, 128.4, 135.3, 138.2, 155.3, 172.5; Anal. calcd for C₂₄H₃₁NO₅: C, 69.71; H, 7.56; N, 3.39. Found: C, 69.63; H, 7.68; N, 3.19.

4.1.12. Boc-(S)-Phs(Bn)-(S)-Phe-(S)-N-Me-Tyr-OMe (6) (Phs = pentahomoserine). Dipeptide **16** (700 mg, 1.22 mmol) was treated with 4 N HCl–dioxane (5 ml) at 0 °C and the mixture was stirred for 30 min. Removal of the solvent under reduced pressure afforded the crude hydrochloride salt as a colorless solid.

To a solution of the pentahomoserine derivative **19** (560 mg, 1.35 mmol) in THF (5 ml) was added 0.5 N aqueous LiOH (5 ml) at 0 °C. The mixture was stirred at room temperature for 12 h, then Et₂O was added. The separated aqueous layer was acidified with 1 M aqueous KHSO₄, salted out, and extracted with EtOAc (×2). The extracts were dried over Na₂SO₄, and concentrated in vacuo to give a crude carboxylic acid as a colorless oil which was used for the next step without further purification.

To a solution of the above crude amine salt and carboxylic acid in DMF (5 ml) was added DEPC (0.22 ml, 1.42 mmol) and Et₃N (0.42 ml, 3.05 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 18 h. After dilution with EtOAc, the whole mixture was successively washed with 1 M aqueous KHSO₄, H₂O, saturated aqueous NaHCO₃, H₂O, and brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=1:1) to give **6** (477 mg, 60%) as a colorless amorphous powder: [α]_D²⁴ = –2.6 (c 1.0, CHCl₃); IR ν_{max} (CHCl₃) cm^{–1} 3303, 1741, 1693, 1518, 1228, 1170; ¹H NMR (270 MHz, CDCl₃) δ 1.49 (9H, m, *t*Bu), 1.50–1.85 (5H, m, Phe-3,4-*H*, OH), 2.57 (3H, s, NMe), 2.68–2.95 (3H, m, Tyr-3-*H*, Phe-3-*H*), 3.20–3.27 (1H, m, Phe-3-*H*), 3.46–3.60 (2H, m, Phs-5-*H*), 3.70 (3H, s, CO₂Me), 3.90–4.05 (1H, m, Phs-2-*H*), 4.57 (2H, s, OCH₂C₆H₅), 4.80–4.89 (1H, m, Tyr-2-*H*), 5.00–5.10 (1H, m, Phe-2-*H*), 5.50–5.65 (1H, br, NH), 6.35–6.45 (1H, br, NH), 6.57 (2H, d, *J* = 8.2 Hz, Tyr-6,8-*H*), 6.88 (2H, d, *J* = 8.2 Hz, Tyr-5,9-*H*), 7.12–7.36 (10H, m, C₆H₅×2); ¹³C NMR (67.8 MHz, CDCl₃) δ 15.1, 25.4, 28.2, 30.0, 31.7, 33.6, 38.9, 50.0, 52.1, 54.0, 57.5, 65.7, 70.0, 73.1, 80.1, 115.4, 126.8, 127.1, 127.7, 127.9, 128.0, 128.2, 128.4, 129.4, 129.7, 130.2, 135.7, 137.4, 155.2, 170.7, 170.9, 171.4; Anal. calcd for C₃₇H₄₇N₃O₈·1/2EtOAc·1/2H₂O: C, 65.53; H, 7.33; N, 5.88. Found: C, 65.80; H, 7.07; N, 6.13.

4.1.13. Troc-(S)-Thr[Boc-(S)-Phs(Bn)-(S)-Phe-(S)-N-Me-Tyr-(S)-Ile]-(S)-Phe-OAllyl (21). Dipeptide **6** (482 mg, 0.69 mmol) was treated with 4 N HCl–dioxane (3 ml) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 2 h. Removal of the solvent under reduced pressure afforded the crude hydrochloride salt as a colorless amorphous solid.

To a solution of tripeptide **6** (409 mg, 0.63 mmol) in THF (3 ml) was added 0.5 N aqueous LiOH (3 ml) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 1 h. After acidification with 1 M aqueous KHSO₄, the mixture was extracted with EtOAc (×3). The extracts were dried over Na₂SO₄, concentrated in vacuo to give a crude carboxylic acid as a colorless oil which was used for the next step without further purification.

To a solution of the above crude amine salt and carboxylic acid in DMF (4 ml) was added DEPC (0.11 ml, 0.76 mmol) and Et₃N (0.22 ml, 1.58 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 14 h. After dilution with EtOAc, the whole mixture was successively washed with 1 M aqueous KHSO₄, H₂O, saturated aqueous NaHCO₃, H₂O, and brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=1:1) to give **21** (600 mg, 77%) as a colorless amorphous powder: $[\alpha]_D^{20} = -13.4$ (c 1.0, CHCl₃); IR $\nu_{\max}$ (CHCl₃) cm⁻¹ 3303, 1741, 1645, 1516, 1243; ¹H NMR (500 MHz, CDCl₃) δ 0.81–0.96 (6H, m, Ile-5,6-*H*), 1.11–1.27 (5H, m, Ile-4-*H*, Thr-4-*H*), 1.38, 1.44 (9H, s×2, *t*Bu), 1.58–1.90 (5H, m, Phs-3,4-*H*, OH), 1.95–2.06 (1H, br, Ile-3-*H*), 2.34–2.56 (1H, m, Tyr-3-*H*), 2.55–2.85 (5H, m, Phe(2)-3-*H*, NMe), 2.91–3.01 (1H, m, Tyr-3-*H*), 3.04–3.15 (2H, m, Phe(1)-3-*H*), 3.46–3.55 (2H, m, Phs-5-*H*), 3.96–4.05 (1H, br, Phs-2-*H*), 4.09–4.22 (1H, br, Thr-2-*H*), 4.37–4.51 (1H, br, Ile-2-*H*), 4.54–4.62 (6H, m, OCH₂Cl₃, OCH₂C₆H₅, CO₂CH₂CH), 4.65–4.89 (3H, m, Phe(1)-2-*H*, Phe(2)-2-*H*, Tyr-2-*H*), 5.20–5.28 (3H, m, Thr-3-*H*, CO₂-CH₂CHCH₂), 5.79–5.84 (1H, m, CO₂CH₂CHCH₂), 6.64–6.73 (2H, m, Tyr-6,8-*H*), 6.87–6.91 (2H, m, Tyr-5,9-*H*), 7.11–7.35 (15H, m, C₆H₅×3); ¹³C NMR (67.8 MHz, CDCl₃) δ 10.8, 14.1, 15.1, 20.9, 25.0, 28.2, 33.0, 35.8, 36.4, 37.5, 37.8, 50.1, 53.2, 53.8, 56.5, 57.3, 57.7, 65.7, 65.8, 69.9, 70.5, 74.5, 79.2, 95.3, 115.4, 118.5, 126.7, 127.5, 127.7, 127.8, 128.1, 128.2, 128.3, 128.6, 128.9, 129.0, 130.0, 131.2, 135.6, 135.8, 137.5, 154.0, 155.0, 169.5, 170.5, 170.9, 171.8, 173.1; Anal. calcd for C₆₁H₇₇Cl₃N₆O₁₄·EtOAc·H₂O: C, 58.66; H, 6.59; N, 6.32. Found: C, 58.56; H, 6.38; N, 6.25.

4.1.14. Troc-(S)-Thr[Boc-(S)-Phs(Bn)-(S)-Phe-(S)-N-Me-Tyr(TBS)-(S)-Ile]-(S)-Phe-O-Allyl (4). To a solution of hexapeptide **21** (960 mg, 0.78 mmol) in DMF (3 ml) was added imidazole (462 mg, 6.27 mmol) and TBSCl (473 mg, 3.13 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 12 h. After dilution with EtOAc, the whole mixture was successively washed with 1 M aqueous KHSO₄, H₂O, saturated aqueous NaHCO₃, H₂O, and brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=2:1) to give **4** (788 mg, 75%) as a colorless amorphous powder: $[\alpha]_D^{20} = -2.6$ (c 0.9, CHCl₃); IR $\nu_{\max}$ (CHCl₃) cm⁻¹ 3302, 1741, 1682, 1645, 1510, 1255, 910; ¹H NMR (270 MHz, CDCl₃) δ 0.00, 0.10 (6H, s×2, SiMe₂), 0.83–0.97 (6H, m, Ile-5,6-*H*), 0.86, 0.93 (9H, s×2, Si-*t*Bu), 1.14–1.28 (5H, m, Ile-4-*H*, Thr-4-*H*), 1.36, 1.39 (9H, s×2, O-*t*Bu), 1.60–2.05 (5H, br, Ile-3-*H*, Phs-3,4-*H*), 2.20–2.65 (2H, m, Phe(2)-3-*H*), 2.70–2.90 (4H, m, Tyr-3-*H*, NMe), 3.00–3.30 (3H, m, Tyr-3-*H*, Phe(1)-3-*H*), 3.38–3.70 (2H, m, Phs-5-*H*), 4.08–

4.40 (3H, m, Phs-2-*H*, Thr-2-*H*, Ile-2-*H*), 4.40–4.70 (6H, m, OCH₂Cl₃, OCH₂C₆H₅, CO₂CH₂CH), 4.70–5.00 (3H, m, Phe(1)-2-*H*, Phe(2)-2-*H*, Tyr-2-*H*), 5.19–5.30 (3H, m, Thr-3-*H*, CO₂CH₂CHCH₂), 5.70–5.90 (1H, m, CO₂CH₂-CHCH₂), 6.65–6.73 (2H, m, Tyr-6,8-*H*), 6.93–7.04 (2H, m, Tyr-5,9-*H*), 7.15–7.43 (15H, m, C₆H₅×3); ¹³C NMR (67.8 MHz, CDCl₃) δ -4.72, -4.62, 10.9, 15.2, 18.0, 25.5, 25.7, 28.3, 33.2, 36.6, 37.7, 49.8, 53.9, 58.1, 62.1, 65.8, 69.6, 70.5, 73.1, 74.6, 79.5, 95.3, 118.6, 120.1, 126.8, 126.9, 127.6, 127.7, 128.3, 128.5, 128.7, 129.0, 129.1, 129.6, 130.1, 131.3, 135.7, 136.1, 137.6, 153.8, 154.3, 155.0, 168.4, 169.9, 170.5, 171.6, 172.9; Anal. calcd for C₆₇H₉₁-Cl₃N₆O₁₄Si·EtOAc: C, 59.76; H, 6.99; N, 5.89. Found: C, 59.86; H, 6.98; N, 5.62.

4.1.15. Cyclo[Troc-(S)-Thr-(S)-Phe-(S)-Phs(Bn)-(S)-Phe-(S)-N-Me-Tyr(TBS)-(S)-Ile] (2) (entry 6 in Table 1). To a solution of hexapeptide **4** (2.07 g, 1.54 mmol) in THF (10 ml) was added (PPh₃)₄Pd (0.18 g, 0.15 mmol) and morpholine (0.20 ml, 2.25 mmol) at room temperature. The mixture was stirred for 2 h. After dilution with EtOAc, the whole mixture was washed with 1 M aqueous KHSO₄, and brine, dried over Na₂SO₄, and concentrated in vacuo to give a crude carboxylic acid as a yellow amorphous powder which was used for the next step without further purification.

The above carboxylic acid was treated with 4 N HCl–dioxane (20 ml) at 0 °C for 1.5 h. Removal of the solvent under reduced pressure afforded the crude hydrochloride salt as a yellow amorphous powder.

To a solution of the above deprotected hexapeptide in CH₂Cl₂ (700 ml) was added DIEA (1.30 ml, 7.50 mmol), and FDPP (1.15 g, 3.00 mmol) in CH₂Cl₂ (20 ml) at room temperature. The mixture was stirred at room temperature for 17 h, then concentrated. The residue was diluted with EtOAc, washed with 1 M aqueous KHSO₄, saturated aqueous NaHCO₃, and brine, dried over Na₂SO₄, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=1:1) to give cyclic peptide **2** (1.52 g, 84%) as a pale yellow amorphous powder: $[\alpha]_D^{24} = -27.1$ (c 0.9, CHCl₃); IR $\nu_{\max}$ (CHCl₃) cm⁻¹ 3303, 1739, 1645, 1512, 1259; ¹H NMR (270 MHz, CDCl₃) δ 0.01, 0.04 (6H, s, SiMe₂), 0.69–0.95 (6H, m, Ile-5,6-*H*), 0.88, 0.95 (9H, s×2, *t*Bu), 1.10–1.50 (5H, m, Ile-4-*H*, Thr-4-*H*), 1.50–1.90 (5H, br, Ile-3-*H*, Phs-3,4-*H*), 2.08–2.55 (2H, m, Phe(2)-3-*H*), 2.67, 2.80 (3H, s×2, NMe), 2.90–3.20 (2H, m, Tyr-3-*H*), 3.30–3.70 (4H, m, Phe(1)-3-*H*, Phs-5-*H*), 4.12–4.40 (3H, m, Phs-2-*H*, Thr-2-*H*, Ile-2-*H*), 4.40–4.60 (4H, m, OCH₂Cl₃, OCH₂C₆H₅), 4.60–4.80 (2H, m, Tyr-2-*H*, Phe(1)-2-*H*), 4.95–5.05 (1H, m, Phe(2)-2-*H*), 5.25–5.35 (1H, m, Thr-3-*H*), 6.65–6.72 (2H, m, Tyr-6,8-*H*), 6.88–6.93 (2H, m, Tyr-5,9-*H*), 7.01–7.34 (15H, m, C₆H₅×3); ¹³C NMR (67.8 MHz, CDCl₃) δ -4.7, -4.5, 10.7, 10.9, 14.9, 15.2, 17.9, 25.5, 25.6, 29.9, 30.7, 33.3, 36.5, 50.6, 51.3, 57.0, 57.5, 70.0, 70.4, 72.2, 74.7, 95.3, 120.1, 127.0, 127.2, 127.9, 128.5, 128.6, 128.9, 129.1, 129.7, 129.8, 130.0, 130.2, 135.9, 136.0, 137.6, 154.3, 154.4, 154.8, 168.7, 169.2, 170.2, 171.8, 172.1; Anal. calcd for C₅₉H₇₇N₆O₁₁Si·H₂O: C, 59.11; H, 6.64; N, 7.01. Found: C, 58.97; H 6.62; N, 7.01.

(Entry 1 in Table 1) To a solution of the deprotected peptide from **4** (70 mg, 0.054 mmol) in DMF (25 ml) was added DPPA (23 μ l, 0.11 mmol) in DMF (2.5 ml) and NaHCO₃ (45 mg, 0.54 mmol) at 0 °C. The mixture was stirred at 4 °C for 72 h, then concentrated. The residue was purified as described above to give **2** (33 mg, 52%).

(Entry 2 in Table 1) To a solution of the deprotected peptide from **4** (77 mg, 0.056 mmol) in DMF (25 ml) was added DEPC (17 μ l, 0.12 mmol) in DMF (3 ml) and NaHCO₃ (47 mg, 0.56 mmol) at 0 °C. The mixture was stirred at 4 °C for 96 h, then concentrated. The residue was purified as described above to give **2** (29 mg, 44%).

(Entry 3 in Table 1) To a solution of the deprotected peptide from **4** (76 mg, 0.058 mmol) in DMF (30 ml) was added HATU (67 mg, 0.18 mmol) and DIEA (50 μ l, 0.29 mmol) at 0 °C. The mixture was stirred at 0 °C for 3 h, and at room temperature for 10 h, then concentrated. The residue was purified as described above to give **2** (26 mg, 38%).

(Entry 4 in Table 1) To a solution of the deprotected peptide from **4** (53 mg, 0.04 mmol) in DMF (20 ml) was added HATU (31 mg, 0.08 mmol) and DIEA (28 μ l, 0.16 mmol) at 0 °C. The mixture was stirred at 4 °C for 96 h, then concentrated. The residue was purified as described above to give **2** (28 mg, 59%).

(Entry 5 in Table 1) To a solution of the deprotected peptide from **4** (208 mg, 0.16 mmol) in DMF (80 ml) was added FDPP (74 mg, 0.19 mmol) in DMF (5 ml) and DIEA (0.14 ml, 0.80 mmol) at room temperature. The mixture was stirred at room temperature for 14 h, then concentrated. The residue was purified as described above to give **2** (107 mg, 57%).

4.1.16. Allyl (S)-2,3-dihydroxypropanoate (25). To a solution of (S)-Ser-OH (**24**) (4.20 g, 40 mmol) in 0.4 M hydrochloric acid (200 ml) was added NaNO₂ (5.53 g, 80 mmol) in H₂O (100 ml) slowly at –10 °C. After addition, the mixture was allowed to warm to room temperature and stirred for 24 h, then concentrated. The residue was filtered to remove inorganic salt. Acetone–CHCl₃ (1:1) was added to the filtrate and the mixture was concentrated in vacuo. This work-up was repeated three times to remove H₂O completely. The mixture was filtered through a pad of celite and the filtrate was concentrated in vacuo to give crude (S)-glyceric acid (9.92 g) as a yellow oil which was used for the next step without further purification.

To a solution of the above crude carboxylic acid (3.0 g) in allyl alcohol–CHCl₃ (1:2, 40 ml) was added *p*-TsOH (114 mg, 0.6 mmol) at room temperature. The mixture was refluxed for 3 h, then concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=1:2) to give **25** (957 mg, 54% in 2 steps) as a colorless oil: $[\alpha]_D^{25} = -21.1$ (c 1.2, CHCl₃); IR $\nu_{\text{max}}^{\text{neat}}$ cm^{–1} 3389, 1740, 1207, 1120; ¹H NMR (270 MHz, CDCl₃) δ 2.40–2.70 (1H, br, exchangeable with D₂O, OH), 3.30–3.50 (1H, br, exchangeable with D₂O, OH), 3.80–3.95 (2H, br, CH₂OH), 4.30 (1H, br, CHOH), 4.70–4.73 (2H, m, CO₂CH₂), 5.26–5.40 (2H, m,

CO₂CH₂CHCH₂), 5.86–6.00 (1H, m, CO₂CH₂CHCH₂); ¹³C NMR (67.8 MHz, CDCl₃) δ 64.0, 66.1, 71.8, 118.8, 131.3, 172.6; Anal. calcd for C₆H₁₀O₄: C, 49.31; H, 6.90. Found: C, 49.05; H 6.92.

4.1.17. Allyl (S)-2-hydroxy-3-(tert-butyldimethylsiloxy)propanoate (28). To a solution of the allyl propanoate **25** (1.9 g, 13 mmol) in CH₂Cl₂ (40 ml) was added Et₃N (1.47 ml, 15.6 mmol), DMAP (63 mg, 0.52 mmol), and TBSCl (2.15 g, 14.3 mmol) at 0 °C. The mixture was stirred at 0 °C for 2 h, then at room temperature for 11 h. After dilution with Et₂O, the whole mixture was washed with 1 M aqueous KHSO₄, and brine, dried over MgSO₄, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=10:1) to give **28** (2.29 g, 68%) as a colorless oil: $[\alpha]_D^{25} = -7.6$ (c 1.0, CHCl₃); IR $\nu_{\text{max}}^{\text{neat}}$ cm^{–1} 3496, 2930, 1747, 1254, 1128; ¹H NMR (270 MHz, CDCl₃) δ 0.03, 0.05 (6H, s $\times$ 2, SiMe₂), 0.86 (9H, s, *t*Bu), 3.04 (1H, d, *J* = 7.9 Hz, OH), 3.83–3.98 (2H, m, CH₂OSi), 4.20–4.26 (1H, m, CHOH), 4.65–4.69 (2H, m, CO₂CH₂), 5.23–5.38 (2H, m, CO₂CH₂CHCH₂), 5.85–5.99 (1H, m, CO₂CH₂CHCH₂); ¹³C NMR (67.8 MHz, CDCl₃) δ –5.5, 25.6, 65.0, 65.9, 71.9, 118.7, 131.4, 172.3; Anal. calcd for C₁₂H₂₄O₄Si: C, 55.35; H, 9.29. Found: C, 55.17; H, 9.24.

4.1.18. Allyl (S)-2-benzyloxy-3-(tert-butyldimethylsiloxy)propanoate (29). To a solution of the 2-hydroxypropanoate **28** (1.22 g, 4.70 mmol) in Et₂O (20 ml) was added Ag₂O (3.27 g, 14.1 mmol) and BnBr (2.8 ml, 23.5 mmol) at room temperature under argon atmosphere. The mixture was stirred for 4 h. The mixture was filtered through a pad of celite and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-200 MH, hexane–Et₂O=20:1) to give **29** (1.24 g, 75%) as a colorless oil: $[\alpha]_D^{25} = -37.9$ (c 1.1, CHCl₃); IR $\nu_{\text{max}}^{\text{neat}}$ cm^{–1} 2955, 1751, 1450, 1257, 1132, 837; ¹H NMR (270 MHz, CDCl₃) δ 0.04 (6H, s, SiMe₂), 0.87 (9H, s, *t*Bu), 3.91 (2H, t, *J* = 5.2 Hz, CH₂OSi), 4.08 (1H, dd, *J* = 4.7, 5.8 Hz, COCH), 4.53 (1H, d, *J* = 11.8 Hz, CH₂C₆H₅), 4.62–4.66 (2H, m, CO₂CH₂), 4.76 (1H, d, *J* = 11.9 Hz, CH₂C₆H₅), 5.17–5.38 (2H, m, CO₂CH₂CHCH₂), 5.85–5.99 (1H, m, CO₂CH₂CHCH₂), 7.27–7.38 (5H, m, C₆H₅); ¹³C NMR (67.8 MHz, CDCl₃) δ –5.4, 18.1, 25.7, 64.1, 65.3, 72.4, 79.4, 118.5, 127.7, 127.9, 128.3, 131.7, 137.4, 170.5; Anal. calcd for C₁₉H₃₀O₄Si · 1/20 hexane · 1/20 Et₂O: C, 65.32; H, 8.77. Found: C, 65.68; H, 8.38.

4.1.19. Allyl (S)-2-benzyloxy-3-hydroxypropanoate (30). To a solution of the protected allyl propanoate **29** (460 mg, 1.31 mmol) in THF (2 ml) was added 1 N TBAF in THF (2.6 ml, 2.6 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h. After dilution with EtOAc, the whole mixture was washed with H₂O and brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=3:1) to give **30** (251 mg, 79%) as a colorless oil: $[\alpha]_D^{25} = -85.1$ (c 1.1, CHCl₃); IR $\nu_{\text{max}}^{\text{neat}}$ cm^{–1} 3453, 1747, 1454, 1190, 1122; ¹H NMR (270 MHz, CDCl₃) δ 2.10–2.25 (1H, br, OH), 3.90–3.95 (2H, m, CH₂OH), 4.12 (1H, dd, *J* = 3.6, 5.1 Hz, COCH), 4.52 (1H, d, *J* = 11.2 Hz, CH₂C₆H₅), 4.69 (2H, d, *J* = 5.9 Hz, CO₂CH₂), 4.84 (1H, d, *J* = 11.6 Hz, CH₂C₆H₅), 5.24–5.39 (2H, m, CO₂CH₂CHCH₂), 5.86–6.00

(1H, m, $\text{CO}_2\text{CH}_2\text{CHCH}_2$), 7.25–7.39 (5H, m, C_6H_5); ^{13}C NMR (67.8 MHz, CDCl_3) δ 63.3, 65.5, 72.6, 78.6, 118.6, 128.0, 128.1, 128.4, 131.5, 136.9, 170.1; Anal. calcd for $\text{C}_{13}\text{H}_{16}\text{O}_4 \cdot 1/5 \text{ EtOAc}$: C, 65.28; H, 6.99. Found: C, 65.49; H, 6.80.

4.1.20. 2-Propenyl (S)-3-[(dibenzoyloxyphosphinyl)oxy]-2-benzoyloxypropanoate (3a). To a solution of the 3-hydroxypropanoate **30** (20 mg, 0.08 mmol) in CH_2Cl_2 (1 ml) was added 1H-tetrazole (17 mg, 0.24 mmol) and *N,N*-diisopropyl dibenzyl phosphoramidite (**31**) (5.7 μl , 0.17 mmol) at room temperature. The mixture was stirred at room temperature for 1 h, then cooled to -30°C . *m*-CPBA (70%, 40 mg, 0.16 mmol) was added and the mixture was stirred at -30°C for 40 min. The reaction was quenched with saturated aqueous NaHCO_3 and the mixture was extracted with Et_2O . The extracts were successively washed with 1 M aqueous KHSO_4 , H_2O , and brine, dried over MgSO_4 , and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane– EtOAc =10:1 to 5:1 then 3:1) to give **3a** (40 mg, quant.) as a colorless oil: $[\alpha]_D^{22} = -28.4$ (c 1.1, CHCl_3); IR $\nu_{\text{max}}^{\text{neat}}$ cm^{-1} 1755, 1456, 1280, 1136, 1021; ^1H NMR (270 MHz, CDCl_3) δ 4.15–4.18 (1H, m, COCH), 4.21–4.36 (2H, m, CHCH_2OP), 4.53 (1H, d, $J=11.7$ Hz, $\text{CHOCH}_2\text{C}_6\text{H}_5$), 4.61–4.64 (2H, m, CO_2CH_2), 4.76 (1H, d, $J=11.6$ Hz, $\text{CHOCH}_2\text{C}_6\text{H}_5$), 4.99, 5.02 (4H, s $\times$ 2, $\text{P}(\text{OCH}_2\text{C}_6\text{H}_5)\times 2$), 5.21–5.35 (2H, m, $\text{CO}_2\text{CH}_2\text{CHCH}_2$), 5.81–5.93 (1H, m, $\text{CO}_2\text{CH}_2\text{CHCH}_2$), 7.26–7.36 (15H, m, $\text{C}_6\text{H}_5\times 3$); ^{13}C NMR (67.8 MHz, CDCl_3) δ 56.9, 67.2, 69.3, 72.7, 76.8, 119.0, 127.9, 128.0, 128.1, 128.4, 128.5, 131.4, 135.7, 136.9, 168.9; Anal. calcd for $\text{C}_{27}\text{H}_{29}\text{O}_7\text{P}$: C, 65.32; H, 5.89. Found: C, 65.05; H, 5.94.

4.1.21. Cyclo[Boc-(S)-Thr-(S)-Phe-(S)-Phs(Bn)-(S)-Phe-(S)-N-Me-Tyr(TBS)-(S)-Ile] (32). To a solution of cyclic peptide **2** (610 mg, 0.52 mmol) in THF (3 ml) was added Zn (900 mg) and AcOH (0.5 ml) at room temperature. The mixture was stirred for 4.5 h, and filtered through a pad of celite. The filtrate was concentrated in vacuo to give a crude amine salt as a colorless solid.

To a solution of the above crude amine salt in THF (3 ml) was added Boc_2O (340 mg, 1.55 mmol) and Et_3N (0.22 ml, 1.55 mmol) at 0°C . The mixture was stirred at 0°C for 1 h, then at room temperature for 5 h. After dilution with EtOAc , the whole mixture was successively washed with 1 M aqueous KHSO_4 , H_2O , and brine, dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane– EtOAc =1:1) to give **32** (438 mg, 77%) as a colorless solid: $[\alpha]_D^{23} = -42.0$ (c 0.5, CHCl_3); IR ν_{max} (CHCl_3) cm^{-1} 3303, 1732, 1682, 1654, 1645, 1510, 1253, 914; ^1H NMR (270 MHz, CDCl_3) δ 0.01, 0.03, 0.07 (6H, s $\times$ 3, SiMe_2), 0.65–0.90 (6H, m, Ile-5,6-*H*), 0.88, 0.95 (9H, s $\times$ 2, *Si-tBu*), 1.15–1.30 (5H, m, Ile-4-*H*, Thr-4-*H*), 1.41 (9H, s, *O-tBu*), 1.55–1.90 (5H, br, Ile-3-*H*, Phs-3,4-*H*), 2.35–2.75 (2H, m, Phe(2)-3-*H*), 2.66, 2.81 (3H, s $\times$ 2, *NMe*), 2.86–3.18 (2H, m, Tyr-3-*H*), 3.35–3.50 (2H, m, Phe(1)-3-*H*), 3.35–3.70 (2H, m, Phs-5-*H*), 4.03–4.12 (1H, br, Phs-2-*H*), 4.30–4.60 (4H, br, Thr-2-*H*, Ile-2-*H*, Phe(1)-2-*H*, Tyr-2-*H*), 4.46, 4.48 (2H, s $\times$ 2, $\text{CH}_2\text{C}_6\text{H}_5$), 4.85–5.05 (2H, br, Phe(2)-2-*H*, Thr-3-*H*), 6.64–6.72 (2H, m, Tyr-6,8-*H*), 6.85–6.96 (2H, m, Tyr-5,9-

H), 7.01–7.40 (15H, m, $\text{C}_6\text{H}_5\times 3$); ^{13}C NMR (67.8 MHz, CD_3OD) δ -4.5 , -4.4 , 11.3, 12.0, 15.1, 15.8, 17.6, 18.8, 26.1, 28.7, 30.4, 37.8, 51.2, 53.0, 56.7, 58.3, 72.8, 73.7, 80.7, 121.2, 127.5, 127.7, 128.5, 128.6, 128.7, 129.3, 129.4, 129.5, 129.9, 130.0, 130.3, 131.6, 137.5, 138.6, 139.6, 155.6, 157.3, 170.4, 171.6, 172.0, 173.2, 174.1, 174.3; Anal. calcd for $\text{C}_{61}\text{H}_{84}\text{N}_6\text{O}_{11}\text{Si}\cdot\text{H}_2\text{O}$: C, 65.22; H, 7.72; N, 7.48. Found: C, 65.61; H 7.64; N; 7.49.

4.1.22. Cyclo[Boc-(S)-Thr-(S)-Phe-(S)-Phs-(S)-Phe-(S)-N-Me-Tyr(TBS)-(S)-Ile] (33). To a solution of Boc protected cyclic peptide **32** (330 mg, 0.3 mmol) in EtOAc (2 ml) was added 20% $\text{Pd}(\text{OH})_2$ (100 mg) under an argon atmosphere. The black slurry was stirred under 1 atm of H_2 at room temperature for 3 h. The mixture was filtered through a pad of celite and the filtrate was concentrated in vacuo to give **33** (312 mg, quant.) as a colorless amorphous powder which was used for the next step without further purification. IR ν_{max} (CHCl_3) cm^{-1} 3303, 1730, 1682, 1651, 1645, 1634, 1512, 1454, 1257, 1170; ^1H NMR (270 MHz, CDCl_3) δ -0.02 , 0.04 (6H, s $\times$ 2, SiMe_2), 0.65–0.95 (6H, m, Ile-5,6-*H*), 0.87, 0.94 (9H, s $\times$ 2, *Si-tBu*), 1.18–1.30 (5H, br, Ile-3-*H*, Phs-3,4-*H*), 1.44 (9H, s, *O-tBu*), 1.70–2.00 (5H, br, Ile-3-*H*, Phs-3,4-*H*), 2.46–2.57 (2H, m, Phe(2)-3-*H*), 2.80, 2.85 (3H, s $\times$ 2, *NMe*), 2.95–3.20 (2H, m, Tyr-3-*H*), 3.36–3.40 (2H, m, Phe(1)-3-*H*), 3.45–3.48 (2H, br, Phs-5-*H*), 4.07–4.23 (1H, m, Phs-2-*H*), 4.25–4.65 (4H, m, Thr-2-*H*, Ile-2-*H*, Phe(1)-2-*H*, Tyr-2-*H*), 5.22–5.45 (2H, m, Phe(2)-2-*H*, Thr-3-*H*), 6.66–6.72 (2H, m, Tyr-6,8-*H*), 6.90–6.98 (2H, m, Tyr-5,9-*H*), 7.00–7.35 (10H, m, $\text{C}_6\text{H}_5\times 2$).

4.1.23. (S)-2'-Benzoyloxy-1'-cyclo[(S)-Thr-(S)-Phe-(S)-Phs-(S)-Phe-(S)-N-Me-Tyr(TBS)-(S)-Ile]-propanoate 3'-dibenzyl phosphate (34). Cyclic peptide **33** (30 mg, 30 μmol) was treated with 4 N HCl–dioxane (1 ml) at 0°C and the mixture was stirred at 0°C for 2 h, then at room temperature for 3 h. Removal of the solvent under reduced pressure afforded the crude hydrochloride salt as a colorless solid.

To a solution of phosphate **3a** (22 mg, 45 μmol) in THF (0.3 ml) was added $(\text{PPh}_3)_4\text{Pd}$ (5 mg, 4.5 μmol) and morpholine (6 μl , 68 μmol) at room temperature. The mixture was stirred for 3.5 h. After dilution with EtOAc , the whole mixture was washed with 1 M aqueous KHSO_4 , and brine, dried over Na_2SO_4 , and concentrated in vacuo to give a crude carboxylic acid as a yellow oil which was used for the next step without further purification.

To a solution of the above crude amine salt and carboxylic acid in DMF (0.5 ml) was added DEPC (7 μl , 45 μmol) and Et_3N (10 μl , 75 μmol) at 0°C . The mixture was stirred at 0°C for 1 h, then at room temperature for 12 h. After dilution with EtOAc , the whole mixture was successively washed with 1 M aqueous KHSO_4 , H_2O , saturated aqueous NaHCO_3 , H_2O , and brine, dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by thin layer chromatography (hexane– EtOAc =1:4) to give **34** (21 mg, 52%) as a colorless solid: $[\alpha]_D^{24} = -11.1$ (c 1.0, CHCl_3); IR ν_{max} (CHCl_3) cm^{-1} 3303, 1738, 1651, 1518, 1263, 1020; ^1H NMR (270 MHz, CDCl_3) δ -0.05 , -0.01 (6H, s $\times$ 2, SiMe_2), 0.68–0.98 (6H, m, Ile-5,6-*H*), 0.87, 0.91 (9H, s $\times$ 2, *Si-tBu*), 1.00–1.20 (3H, m, Thr-4-*H*), 1.20–1.55

(2H, m, Ile-4-*H*), 1.60–2.05 (5H, br, Ile-3-*H*, Phs-3,4-*H*), 2.50–2.90 (4H, m, Tyr-3-*H*, Phe(2)-*H*), 2.86 (3H, s, *NMe*), 3.00–3.35 (4H, m, Phs-5-*H*, Phe(1)-3-*H*), 3.50–3.70 (1H, br, *OH*), 3.90–4.20 (2H, br, Phs-2-*H*, Ga-2-*H*), 4.20–4.80 (6H, m, Thr-2-*H*, Ile-2-*H*, Phe(1)-2-*H*, Tyr-2-*H*, Ga-3-*H*), 4.52, 4.59 (2H, s×2, $\text{CHOCH}_2\text{C}_6\text{H}_5$), 4.80–5.15 (6H, m, Phe(2)-2-*H*, Thr-3-*H*, $\text{P}(\text{OCH}_2\text{C}_6\text{H}_5)\times 2$), 6.67–7.05 (4H, m, Tyr-5,6,8,9-*H*), 7.05–7.40 (20H, m, $\text{C}_6\text{H}_5\times 4$) 7.40–7.70 (5H, m, C_6H_5); ^{13}C NMR (67.8 MHz, CD_3OD) δ −4.4, −4.3, 12.2, 14.5, 15.0, 18.0, 18.9, 20.9, 26.1, 28.5, 29.7, 30.1, 30.5, 34.4, 37.0, 38.2, 51.4, 53.1, 56.2, 58.6, 61.5, 62.1, 63.7, 68.3, 70.7, 70.8, 70.9, 72.8, 74.0, 79.5, 79.7, 121.2, 127.5, 127.7, 129.0, 129.2, 129.3, 129.4, 129.5, 129.9, 130.0, 130.3, 131.0, 131.2, 131.6, 132.2, 136.8, 136.9, 137.6, 137.9, 138.4, 138.7, 155.6, 170.3, 170.6, 170.7, 171.5, 173.4, 174.1, 174.5; HRFABMS (*m*-nitrobenzyl alcohol) calcd for $\text{C}_{73}\text{H}_{93}\text{N}_6\text{O}_{15}\text{PSi}$ $[\text{M}+\text{H}]^+$: 1353.6284. Found: 1353.6309.

4.1.24. (S)-2'-Benzyloxy-1'-cyclo[(S)-Thr-(S)-Phe-(S)-Ahp-(S)-Phe-(S)-N-Me-Tyr(TBS)-(S)-Ile]-propanoate 3'-dibenzyl phosphate (44). To a solution of cyclic peptide **34** (35 mg, 26 μmol) in DMSO (0.7 ml) was added IBX (30 mg, 0.10 mmol) at room temperature. The mixture was stirred for 3 h. After dilution with EtOAc, the whole mixture was washed with H_2O ($\times 2$) and brine, dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, EtOAc only) to give aldehyde **35** (28 mg, 80%) as a colorless oil.

To a solution of the above aldehyde in THF (1 ml) was added 1 N TBAF in THF (20 μl) at 0 °C. The mixture was stirred at 0 °C for 20 min. After dilution with EtOAc, the whole mixture was washed with H_2O and brine, dried over Na_2SO_4 , and concentrated in vacuo to give **44** (25 mg, 85%) as a colorless oil: $[\alpha]_{\text{D}}^{27} = -130.1$ (c 0.1, CHCl_3); IR ν_{max} (CHCl_3) cm^{-1} 3389, 1732, 1675, 1670, 1667, 1651, 1634, 1517, 1456, 1261, 1020; ^1H NMR (270 MHz, CDCl_3) 0.74 (3H, t, $J=6.6$ Hz, Ile-5-*H*), 0.75 (3H, d, $J=7.3$ Hz, Ile-6-*H*), 0.88–0.95 (1H, m, Ile-4-*H*), 1.07 (3H, d, $J=6.4$ Hz, Thr-4-*H*), 1.40–1.55 (1H, m, Ile-4-*H*), 1.60–1.80 (2H, Ile-3-*H*, Ahp-5-*H*), 1.83–2.00 (2H, m, Ahp-4,5-*H*), 2.13–2.35 (1H, m, Tyr-3-*H*), 2.55–2.63 (1H, m, Ahp-4-*H*), 2.86 (3H, s, *NMe*), 2.88–3.00 (1H, m, Tyr-3-*H*), 3.05–3.45 (4H, m, Phe(1)-3-*H*, Phe(2)-3-*H*), 3.80 (1H, br, exchangeable with D_2O , *OH*), 3.85–3.95 (1H, m, Ahp-3-*H*), 4.00 (1H, br, Ga-2-*H*), 4.20–4.35 (2H, m, Ga-3-*H*), 4.38 (1H, br, exchangeable with D_2O , *OH*), 4.45 (1H, d, $J=9.9$ Hz, Thr-2-*H*), 4.56 (2H, s, $\text{CHOCH}_2\text{C}_6\text{H}_5$), 4.56–4.60 (1H, br, Ile-2-*H*), 4.78–4.90 (1H, br, Phe(1)-2-*H*), 4.90–4.95 (1H, br, Tyr-2-*H*), 5.00, 5.03 (4H, s×2, $\text{P}(\text{OCH}_2\text{C}_6\text{H}_5)\times 2$), 5.34 (1H, s, Ahp-6-*H*), 5.20–5.40 (2H, m, Phe(2)-2-*H*, Thr-3-*H*), 6.76 (2H, d, $J=8.4$ Hz, Tyr-6,8-*H*), 7.05 (2H, d, $J=8.3$ Hz, Tyr-5,9-*H*), 7.10–7.34 (25H, m, $\text{C}_6\text{H}_5\times 5$); ^{13}C NMR (67.8 MHz, CD_3OD) δ 11.5, 14.5, 16.5, 18.3, 18.9, 20.9, 26.2, 30.7, 31.6, 34.3, 36.4, 37.4, 38.8, 50.7, 52.5, 56.0, 56.2, 57.4, 61.5, 63.1, 70.7, 70.8, 70.9, 71.0, 74.0, 75.7, 116.6, 127.3, 127.5, 128.8, 129.0, 129.1, 129.2, 129.3, 129.5, 129.6, 130.0, 130.5, 131.7, 136.9, 137.0, 137.5, 138.0, 139.0, 157.5, 170.3, 170.8, 171.1, 172.8, 173.0, 174.5; HRFABMS (*m*-nitrobenzyl alcohol) calcd for $\text{C}_{67}\text{H}_{77}\text{N}_6\text{O}_{15}\text{P}$ $[\text{M}+\text{H}]^+$: 1237.5263. Found: 1237.5337.

4.1.25. 5-Benzyloxy-1-pentanol (38). To a solution of 1,5-pentanediol (**37**) (5.2 g, 50 mmol) in THF (100 ml) was added 15-crown-5-ether (0.3 ml, 3 mmol), finely grinded NaOH (12 g, 0.3 mol), and BnBr (6.0 ml, 50 mmol) at 10 °C. The mixture was stirred at 10 °C for 4 h. After dilution with EtOAc, the whole mixture was washed with brine ($\times 3$), dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=2:1 to 1:1) to give **38** (4.0 g, 41%) as a colorless oil: ^1H NMR (270 MHz, CDCl_3) δ 1.41–1.67 (7H, m, $\text{CH}_2\times 3$, *OH*), 3.48 (2H, t, $J=6.3$ Hz, $\text{CH}_2\text{OCH}_2\text{C}_6\text{H}_5$), 3.62 (2H, d, $J=6.3$ Hz, CH_2OH), 4.50 (2H, s, $\text{CH}_2\text{C}_6\text{H}_5$), 7.25–7.39 (5H, m, C_6H_5).

4.1.26. N-(5-Benzyloxypentanoyl)-(S)-phenylalanine methyl ester (41). To a solution of pentanol **38** (2.5 g, 13 mmol) in CH_2Cl_2 (15 mmol) was added Et_3N (9.1 ml, 65 mmol), and pyridine· SO_3 (10.3 g, 65 mmol) in DMSO (15 ml) slowly at 0 °C. The mixture was stirred at 0 °C for 20 min. The reaction was quenched with saturated aqueous NaHCO_3 and the mixture was extracted with Et_2O . The extracts were washed with H_2O and brine, dried over MgSO_4 , and concentrated in vacuo to give a crude aldehyde as a colorless oil which was used for the next step without further purification.

To a solution of the above crude aldehyde in H_2O (7 ml) and *t*-BuOH (28 ml) was added 2-methyl-2-butene (6.9 ml, 65 mmol), NaH_2PO_4 (2.34 g, 19.5 mmol), and NaClO_2 (1.76 g, 19.5 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h. The reaction was quenched with 1 M KHSO_4 and the mixture was extracted with EtOAc. The extracts were washed with brine, dried over Na_2SO_4 , and concentrated in vacuo to give a crude carboxylic acid as a colorless oil which was used for the next step without further purification.

Boc-(S)-Phe-OMe (**39**) (4.2 g, 15 mmol) was treated with 4 N HCl–dioxane at 0 °C and the mixture was stirred for 2 h. Removal of the solvent under reduced pressure afforded the crude hydrochloride salt as a colorless amorphous powder.

To a solution of the above crude carboxylic acid and amine salt in DMF (40 ml) was added DEPC (2.4 ml, 15.6 mmol) and Et_3N (4.2 ml, 32.5 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 2 h. After dilution with EtOAc, the whole mixture was successively washed with 1 M KHSO_4 , H_2O , saturated aqueous NaHCO_3 , H_2O , and brine, dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc=2:1 to 1:1) to give **40** (3.73 g, 80%) as a colorless oil: ^1H NMR (270 MHz, CDCl_3) δ 1.58–1.73 (4H, m, $\text{CH}_2\times 2$), 2.19 (2H, t, $J=7.2$ Hz, COCH_2), 3.00–3.17 (2H, m, Phe-3-*H*), 3.46 (2H, t, $J=6.1$ Hz, $\text{CH}_2\text{CH}_2\text{O}$), 3.71 (3H, s, CO_2Me), 4.47 (2H, s, $\text{OCH}_2\text{C}_6\text{H}_5$), 4.84–4.92 (1H, m, Phe-2-*H*), 5.90–5.95 (1H, br, *NH*), 7.06–7.33 (10H, m, $\text{C}_6\text{H}_5\times 2$).

4.1.27. N-(5-Formylbutanoyl)-(S)-phenylalanine methyl ester (41). To a solution of **40** (2.5 g, 6.8 mmol) in EtOAc (20 ml) was added 20% $\text{Pd}(\text{OH})_2$ (500 mg) under an argon atmosphere. The black slurry was stirred under 1 atm of H_2

at room temperature for 3 h. The mixture was filtered through a pad of celite and the filtrate was concentrated in vacuo to give the debenzylated alcohol (2.01 g, quant.) as a colorless oil which was used for the next step without further purification: IR $\nu_{\text{max}}^{\text{neat}}$ cm^{-1} 3306, 1745, 1649, 1534, 1219; ^1H NMR (270 MHz, CDCl_3) δ 1.51–1.58 (2H, m, CH_2), 1.64–1.75 (2H, m, CH_2), 2.23 (2H, t, $J=7.2$ Hz, COCH_2), 2.10–2.30 (1H, br, OH), 3.03–3.19 (2H, m, Phe-3-*H*), 3.60 (2H, t, $J=6.1$ Hz, CH_2OH), 3.73 (3H, s, CO_2Me), 4.86–4.93 (1H, m, Phe-2-*H*), 6.00–6.10 (1H, br, NH), 7.08–7.31 (5H, m, C_6H_5).

To a solution of the above alcohol (260 mg, 0.93 mmol) in CH_2Cl_2 (1.5 ml) was added Et_3N (0.65 ml, 4.65 mmol), and pyridine $\cdot \text{SO}_3$ (740 mg, 4.65 mmol) in DMSO (1.5 ml) slowly at 0 °C. The mixture was stirred at 0 °C for 1 h. The reaction was quenched with saturated aqueous NaHCO_3 and the mixture was extracted with Et_2O . The extracts were washed with H_2O and brine, dried over MgSO_4 , and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane– $\text{EtOAc}=1:2$) to give **41** (140 mg, 54%) as a colorless oil: IR $\nu_{\text{max}}^{\text{neat}}$ cm^{-1} 3302, 1743, 1724, 1651, 1539, 1217; ^1H NMR (270 MHz, CDCl_3) δ 1.88–1.96 (2H, m, $\text{CH}_2\text{CH}_2\text{CH}_2$), 2.22 (2H, t, $J=7.2$ Hz, COCH_2), 2.45 (2H, t, $J=7.1$ Hz, CH_2CHO), 3.10–3.20 (2H, m, Phe-3-*H*), 3.76 (3H, s, CO_2Me), 4.85–4.92 (1H, m, Phe-2-*H*), 5.85–5.92 (1H, br, NH), 7.07–7.32 (5H, m, C_6H_5), 9.72 (1H, s, CHO).

4.1.28. Methyl (2*S*)-2-(6-hydroxy-2-piperidone-1-yl)-3-phenylpropanoate (42). To a solution of aldehyde **41** (15 mg, 54 μmol) in THF (0.3 ml) was added 0.1 M pH 6 phosphate buffer (0.3 ml) at 0 °C. The mixture was stirred at 0 °C for 30 min, then at room temperature for 20 h. After dilution with EtOAc , the whole mixture was washed with H_2O and brine, dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane– $\text{EtOAc}=1:1$ to 1:3) to give **42** (10 mg, 66%) as a colorless oil: $[\alpha]_{\text{D}}^{22} = -78.4$ (c 0.82, CHCl_3); IR ν_{max} (CHCl_3) cm^{-1} 3389, 1738, 1629, 1300, 1234; ^1H NMR (270 MHz, CDCl_3) δ 1.45–1.73 (2H, m, piperidone-4-*H*), 1.86–2.10 (2H, m, piperidone-5-*H*), 2.20–2.50 (2H, m, piperidone-3-*H*), 3.10–3.25 (1H, br, exchangeable with D_2O , OH), 3.30–3.50 (2H, m, Phe-3-*H*), 3.81 (3H, s, CO_2Me), 3.98 (1H, dd, $J=4.7$, 9.1 Hz, piperidine-6-*H*), 4.07–4.15 (1H, m, Phe-2-*H*), 7.11–7.31 (5H, m, C_6H_5); ^{13}C NMR (67.8 MHz, CD_3OD) δ 16.4, 31.3, 33.1, 35.6, 52.6, 62.4, 83.0, 127.8, 129.6, 130.5, 139.5, 172.5, 172.8; Anal. calcd for $\text{C}_{15}\text{H}_{19}\text{NO}_4$: C, 64.97, H, 6.91, N, 5.05. Found: C, 65.24, H, 6.88, N, 5.02.

4.1.29. Methyl (2*S*)-2-(3,4-dihydro-2-pyridone-1-yl)-3-phenylpropanoate (43). A colorless oil: $[\alpha]_{\text{D}}^{24} = -55.4$ (c 0.17, CHCl_3); IR ν_{max} (CHCl_3) cm^{-1} 1741, 1670, 1378, 1215; ^1H NMR (270 MHz, CDCl_3) δ 2.00–2.50 (4H, m, pyridone-3,4-*H*), 2.99–3.08 (1H, m, Phe-3-*H*), 3.35–3.43 (1H, m, Phe-3-*H*), 3.73 (3H, s, CO_2Me), 5.13–5.17 (1H, m, Phe-2-*H*), 5.27–5.34 (1H, m, pyridone-5-*H*), 6.04 (1H, d, $J=7.8$ Hz, pyridone-6-*H*), 7.15–7.28 (5H, m, C_6H_5); ^{13}C NMR (67.8 MHz, CDCl_3) δ 19.8, 31.3, 35.8, 52.5, 56.6, 77.2, 106.8, 126.7, 127.0, 128.3, 128.9, 136.4, 159.9, 169.2, 170.7. HRMS calcd for $\text{C}_{15}\text{H}_{17}\text{O}_3\text{N}$: 259.1208. Found: 259.1210.

4.1.30. Allyl (S)-2,3-bis(tert-butyldimethylsiloxy)propanoate (26). To a solution of allyl propanoate (**25**) (1.55 g, 10.6 mmol) in CH_2Cl_2 (20 ml) was added 2,6-lutidine (5.0 ml, 42.4 mmol) and TBSOTf (7.3 g, 31.8 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h. After dilution with Et_2O , the whole mixture was successively washed with 1 M aqueous KHSO_4 , H_2O , and brine, dried over MgSO_4 , and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane– $\text{Et}_2\text{O}=15:1$) to give **26** (3.78 g, quant.) as a colorless oil: $[\alpha]_{\text{D}}^{23} = -16.6$ (c 1.1, CHCl_3); IR $\nu_{\text{max}}^{\text{neat}}$ cm^{-1} 2955, 2930, 2858, 1759, 1472, 1257, 1148, 1128; ^1H NMR (270 MHz, CDCl_3) δ 0.05 (6H, s, SiMe_2), 0.08, 0.09 (6H, s $\times 2$, SiMe_2), 0.88 (9H, s, *t*Bu), 0.90 (9H, s, *t*Bu), 3.73–3.87 (2H, m, CH_2OSi), 4.23 (1H, t, $J=5.3$ Hz, CHOSi), 4.62 (2H, d, $J=5.6$ Hz, CO_2CH_2), 5.22–5.37 (2H, m, $\text{CO}_2\text{CH}_2\text{CHCH}_2$), 5.85–5.97 (1H, m, $\text{CO}_2\text{CH}_2\text{CHCH}_2$); ^{13}C NMR (67.8 MHz, CDCl_3) δ -5.3, -4.7, 18.4, 25.8, 65.0, 65.7, 72.7, 118.7, 131.5, 172.3; Anal. calcd for $\text{C}_{12}\text{H}_{24}\text{O}_4\text{Si} \cdot 1/2\text{H}_2\text{O}$: C, 56.35; H, 10.25. Found: C, 56.72; H, 10.07.

4.1.31. Allyl (S)-2-(tert-butyldimethylsiloxy)-3-hydroxypropanoate (27). To a solution of bis(TBS)propanoate **26** (100 mg, 0.26 mmol) in CH_3CN (2.5 ml) was added $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$ (150 mg, 0.39 mmol) and NaI (40 mg, 0.26 mmol) at room temperature. The mixture was stirred at room temperature for 15 h, and refluxed for 3 h. The reaction was quenched with 1 N aqueous HCl, then concentrated. After dilution with EtOAc , the whole mixture was washed with H_2O and brine, dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane– $\text{EtOAc}=5:1$) to give **27** (43 mg, 62%) as a colorless oil: IR $\nu_{\text{max}}^{\text{neat}}$ cm^{-1} 3496, 2958, 1759, 1615, 1255, 1140; ^1H NMR (270 MHz, CDCl_3) δ 0.10, 0.14 (6H, s $\times 2$, SiMe_2), 0.92 (9H, s, *t*Bu), 2.15–2.24 (1H, br, OH), 3.80–3.86 (2H, br, CH_2OH), 4.33 (1H, t, $J=4.6$ Hz, CHOSi), 4.65 (2H, d, $J=5.6$ Hz, CO_2CH_2), 5.24–5.38 (2H, m, $\text{CO}_2\text{CH}_2\text{CHCH}_2$), 5.85–5.99 (1H, m, $\text{CO}_2\text{CH}_2\text{CHCH}_2$).

4.1.32. Allyl (S)-2-(tert-butyldimethylsiloxy)propanoate 3-dibenzyl phosphate (3b). To a solution of 3-hydroxypropanoate **27** (150 mg, 0.57 mmol) in CH_2Cl_2 (2.0 ml) was added 1*H*-tetrazole (120 mg, 1.71 mmol) and *N,N*-diisopropyl dibenzyl phosphoramidite (0.23 ml, 0.69 mmol) at room temperature. The mixture was stirred at room temperature for 1.5 h, then cooled -30 °C. *m*-CPBA (70%, 280 mg, 1.14 mmol) was added and the mixture was stirred at -30 °C for 1 h. The reaction was quenched with saturated aqueous NaHCO_3 and the mixture was extracted with Et_2O . The extracts were washed with 1 M aqueous KHSO_4 , H_2O , and brine, dried over MgSO_4 , and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane– $\text{EtOAc}=5:1$ to 3:1) to give **3b** (303 mg, quant.) as a colorless oil: $[\alpha]_{\text{D}}^{21} = -12.8$ (c 1.2, CHCl_3); IR ν_{max} (CHCl_3) cm^{-1} 2955, 2930, 1759, 1456, 1283, 1260, 1153, 1018; ^1H NMR (270 MHz, CDCl_3) δ 0.07, 0.09 (6H, s $\times 2$, SiMe_2), 0.89 (9H, s, *t*Bu), 4.11–4.18, 4.24–4.32 (2H, m, CH_2OP), 4.37–4.40 (1H, m, CHOSi), 4.60 (2H, d, $J=5.6$ Hz, CO_2CH_2), 5.01, 5.03 (2H, s $\times 2$, $\text{CH}_2\text{C}_6\text{H}_5$), 5.04, 5.06 (2H, s $\times 2$, $\text{CH}_2\text{C}_6\text{H}_5$), 5.21–5.34 (2H, m, $\text{CO}_2\text{CH}_2\text{CHCH}_2$), 5.83–5.95

(1H, m, CO₂CH₂CHCH₂), 7.33 (10H, s, C₆H₅×2); ¹³C NMR (67.8 MHz, CDCl₃) δ −5.2, −4.9, 18.4, 25.7, 65.9, 68.9, 69.3, 69.4, 71.6, 71.7, 118.8, 127.8, 128.3, 128.4, 131.4, 135.5, 135.6, 169.9; Anal. calcd for C₂₆H₃₇O₇PSi: C, 59.98; H, 7.16. Found: C, 59.89; H, 7.26.

4.1.33. (S)-2'-(tert-Butyldimethylsiloxy)-1'-cyclo[(S)-Thr-(S)-Phe-(S)-Phe-(S)-Phe-(S)-N-Me-Tyr(TBS)-(S)-Ile]-propanoate 3'-dibenzyl phosphate (46). Cyclic peptide **33** (270 mg, 0.27 mmol) was treated with 4 N HCl–dioxane (2 ml) at 0 °C and the mixture was stirred at 0 °C for 2.5 h. Removal of the solvent under reduced pressure afforded the crude hydrochloride salt as a colorless solid.

To a solution of phosphate **3b** (208 mg, 0.40 mmol) in THF (1.5 ml) was added (PPh₃)₄Pd (46 mg, 0.04 mmol) and morpholine (52 μl, 0.60 mmol) at room temperature. The mixture was stirred for 1.5 h. After dilution with EtOAc, the whole mixture was washed with 1 M aqueous KHSO₄ and brine, dried over Na₂SO₄, and concentrated in vacuo to give a crude carboxylic acid as a yellow oil which was used for the next step without further purification.

To a solution of the above crude amine salt and carboxylic acid in DMF (2 ml) was added DEPC (62 μl, 0.4 mmol) and Et₃N (93 μl, 0.66 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 17 h. After dilution with EtOAc, the whole mixture was successively washed with 1 M aqueous KHSO₄, saturated aqueous NaHCO₃, and brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc = 1:5 to CHCl₃–MeOH = 20:1) to give **46** (211 mg, 57%) as a colorless solid: [α]_D²² = −20.3 (c 1.0, CHCl₃); IR ν_{max}(CHCl₃) cm^{−1} 3389, 3238, 2950, 2932, 1738, 1682, 1660, 1645, 1539, 1255, 1020; ¹H NMR (270 MHz, CDCl₃) δ 0.01, 0.08 (12H, s×2, SiMe₂×2), 0.64–0.93 (6H, m, Ile-5,6-*H*), 0.82, 0.84 (18H, s×2, *t*Bu×2), 1.03–1.44 (5H, m, Ile-4-*H*, Thr-4-*H*), 1.60–2.00 (5H, m, Ile-3-*H*, Phe-3,4-*H*), 2.50–2.95 (4H, m, Tyr-3-*H*, Phe(2)-3-*H*), 2.78, 2.84 (3H, s×2, *N*Me), 3.05–3.45 (2H, m, Phe(1)-3-*H*), 3.50–3.80 (2H, m, Phe-5-*H*), 4.00–4.80 (7H, m, Phe-2-*H*, Ga-2-*H*, Thr-2-*H*, Ile-2-*H*, Tyr-2-*H*, Ga-3-*H*), 4.83–5.15 (6H, m, Phe(1)-*H*, Phe(2)-*H*, P(OCH₂C₆H₅)×2), 5.20–5.30 (1H, br, Thr-3-*H*), 6.68–6.73 (2H, m, Tyr-6,8-*H*), 6.83–7.36 (22H, m, Tyr-5,9-*H*, C₆H₅×4); ¹³C NMR (67.8 MHz, CD₃OD) δ −5.1, −4.6, −4.5, −4.3, 12.1, 15.1, 17.9, 18.9, 26.1, 27.4, 29.7, 30.6, 34.4, 38.3, 51.3, 53.1, 56.1, 56.9, 58.6, 62.1, 63.7, 70.8, 70.9, 72.0, 72.8, 121.2, 127.6, 127.7, 127.8, 129.0, 129.2, 129.3, 129.4, 129.5, 129.6, 129.8, 130.1, 130.3, 131.3, 131.6, 136.9, 137.0, 137.7, 138.6, 155.7, 170.4, 170.5, 171.4, 171.5, 171.7, 173.4, 173.6, 174.6; HRFABMS (*m*-nitrobenzyl alcohol) calcd for C₇₂H₁₀₁N₆O₁₅PSi₂ [M+H]⁺: 1377.6679. Found: 1377.6696.

4.1.34. (S)-2'-Hydroxy-1'-cyclo[(S)-Thr-(S)-Phe-(S)-Ahp-(S)-Phe-(S)-N-Me-Tyr-(S)-Ile]-propanoate 3'-dibenzyl phosphate (47). To a solution of cyclic peptide **46** (25 mg, 18 μmol) in DMSO (0.4 ml) was added IBX (21 mg, 75 μmol) at room temperature. The mixture was stirred for 3 h. After dilution with EtOAc, the whole mixture was washed with H₂O (×2) and brine, dried over Na₂SO₄,

and concentrated in vacuo. The residue was purified by thin layer chromatography (CHCl₃–MeOH = 10:1) to give aldehyde (9 mg, 36%) as a colorless solid.

To a solution of the above aldehyde in THF (0.4 ml) was added 1 N TBAF in THF (20 μl, 20 μmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then concentrated. The residue was purified by thin layer chromatography (CHCl₃–MeOH = 15:1) to give Ahp product **47** (5.5 mg, 95%) as a colorless solid: [α]_D²⁴ = −29.6 (c 0.21, CHCl₃); IR ν_{max}(CHCl₃) cm^{−1} 3389, 2930, 1732, 1681, 1651, 1635, 1539, 1456, 1294, 1140, 1022; ¹H NMR (270 MHz, CD₃OD) δ 0.72–0.95 (6H, m, Ile-5,6-*H*), 1.18–1.28 (5H, m, Ile-4-*H*, Thr-4-*H*), 1.57–1.90 (4H, m, Ile-3-*H*, Ahp-4,5-*H*), 1.95–2.08 (1H, m, Tyr-3-*H*), 2.45–2.70 (2H, m, Tyr-3-*H*, Ahp-4-*H*), 2.81 (3H, s, *N*Me), 2.85–3.05 (2H, m, Phe(2)-3-*H*), 3.30–3.56 (2H, m, Phe(1)-3-*H*), 3.80–3.87 (1H, m, Ahp-3-*H*), 4.20–4.25 (3H, br, Ga-2,3-*H*), 4.47 (1H, d, *J* = 5.7 Hz, Ile-2-*H*), 4.53 (1H, br, Thr-2-*H*), 4.61–4.70 (1H, m, Phe(1)-*H*), 4.95–5.15 (6H, m, Tyr-2-*H*, Phe(2)-*H*, P(OCH₂C₆H₅)×2), 5.15 (1H, br, Ahp-6-*H*), 5.44–5.53 (1H, br, Thr-3-*H*), 6.81 (2H, d, *J* = 8.2 Hz, Tyr-6,8-*H*), 6.88 (2H, d, *J* = 6.3 Hz, Tyr-5,9-*H*), 7.06–7.18 (10H, m, C₆H₅×2), 7.35 (10H, s, P(OCH₂C₆H₅)×2); ¹³C NMR (67.8 MHz, CD₃OD) δ 11.4, 16.5, 18.4, 22.4, 26.2, 30.7, 31.7, 34.2, 36.4, 37.3, 38.7, 50.1, 50.7, 50.8, 52.6, 56.1, 57.5, 63.1, 70.7, 70.8, 70.9, 71.0, 72.0, 73.6, 75.7, 116.7, 127.4, 127.5, 128.9, 129.1, 129.3, 129.6, 129.8, 130.3, 130.5, 131.7, 136.9, 137.1, 137.5, 138.9, 157.7, 170.5, 170.7, 171.3, 172.7, 173.0, 174.6; HRFABMS (*m*-nitrobenzyl alcohol) calcd for C₆₀H₇₁N₆O₁₅P [M+Na]⁺: 1169.4613. found 1169.4620.

4.1.35. Micropeptin T-20 (1). To a solution of Ahp product **47** (10 mg, 4.3 μmol) in 90% aqueous EtOH (1 ml) was added 10% Pd–C (10 mg) under an argon atmosphere. The black slurry was stirred under 1 atm of H₂ at room temperature for 1 h. The mixture was filtered through a pad of celite and the filtrate was concentrated in vacuo to give a deprotected product (7.8 mg, 89%) as a pale yellow solid.

The above deprotected product was treated with pH 7 phosphate buffer–MeOH (2:3, 1 ml) and the mixture was stirred at room temperature for 1 h. After concentration, the residue was purified by ODS silica gel column chromatography (BW-300 MH, H₂O then MeOH) to give **1** (6.8 mg, 77%) as a pale yellow solid: [α]_D²³ = −24.6 (c 0.15, CH₃OH); ¹H NMR (500 MHz, CD₃OD) δ 0.87 (3H, t, *J* = 7.4 Hz, Ile-5-*H*), 0.91 (3H, d, *J* = 6.7 Hz, Ile-6-*H*), 1.07–1.15 (1H, m, Ile-4-*H*), 1.27 (3H, d, *J* = 6.4 Hz, Thr-4-*H*), 1.28–1.35 (1H, m, Ile-4-*H*), 1.60–1.66, 1.68–1.75 (2H, m, Ahp-5-*H*), 1.77–1.89 (2H, m, Ahp-4-*H*, Ile-3-*H*), 1.98–2.04 (1H, m, Tyr-3-*H*), 2.56–2.66 (1H, m, Ahp-4-*H*), 2.66–2.80 (2H, m, Phe(1)-3-*H*, Phe(2)-3-*H*), 2.85 (3H, s, *N*Me), 2.92–2.98 (1H, m, Tyr-3-*H*), 3.36–3.45 (2H, m, Phe(1)-3-*H*, Phe(2)-3-*H*), 3.83 (1H, ddd, *J* = 6.7, 9.4, 12.2 Hz, Ahp-3-*H*), 4.00–4.07 (1H, m, Ga-3-*H*), 4.13–4.18 (2H, m, Ga-2,3-*H*), 4.53 (1H, br, Thr-2-*H*), 4.65–4.71 (2H, m, Ile-2-*H*, Phe(1)-2-*H*), 4.90–4.96 (1H, m, Tyr-2-*H*), 5.03–5.06 (1H, m, Phe(2)-2-*H*), 5.16 (1H, br, Ahp-6-*H*), 5.46–5.52 (1H, m, Thr-3-*H*), 6.82 (2H, d, *J* = 8.5 Hz, Tyr-6,8-*H*), 6.89 (2H, d, *J* = 7.1 Hz, Tyr-5,9-*H*), 7.08–7.21 (10H, m, C₆H₅×2); ¹³C NMR (500 MHz, CD₃OD) δ 11.6, 16.7, 18.2, 22.4, 26.2,

30.6, 31.6, 34.3, 36.4, 37.4, 39.1, 50.8, 52.5, 55.8, 56.1, 57.4, 63.2, 68.3, 73.5, 73.7, 75.8, 116.7, 127.5, 127.7, 129.1, 129.4, 129.5, 130.1, 130.7, 131.8, 137.7, 139.0, 157.7, 190.9, 171.2, 171.5, 172.9, 173.3, 174.3, 174.6; HRFABMS (*m*-nitrobenzyl alcohol) calcd for $C_{46}H_{57}N_6Na_2O_{15}P [M+H]^+$: 1011.3492. Found: 1011.3533.

4.1.36. Allyl (*R*)-2,3-dihydroxypropanoate (49). To a solution of alcohol **48** (1.0 g, 7.56 mmol) in H_2O (30 ml) was added KOH (1.0 g, 15.1 mmol) in H_2O (20 ml) and $KMnO_4$ (1.8 g, 11.3 mmol) in H_2O (30 ml) at 0 °C. The mixture was stirred at 0 °C for 2 h. The mixture was filtered through a pad of celite and the filtrate was concentrated in vacuo. The residue was acidified to pH 4 with 1 M aqueous $KHSO_4$, salted out, and extracted with EtOAc ($\times 3$). The extracts were dried over Na_2SO_4 , concentrated in vacuo to give a crude carboxylic acid as a colorless oil which was used for the next step without further purification.

To a solution of the above crude carboxylic acid in DMF (20 ml) was added $KHCO_3$ (1.5 g, 15.0 mmol) and allyl bromide (1.03 ml, 11.4 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 10 h. After dilution with Et_2O , the whole mixture was successively washed with 1 M aqueous $KHSO_4$, H_2O , saturated aqueous $NaHCO_3$, H_2O , and brine, dried over $MgSO_4$, and concentrated in vacuo to give a crude allyl ester as a colorless oil which was used for the next step without further purification.

The above crude ester was treated with 1 N aqueous HCl–THF (1:1, 20 ml) at room temperature. The mixture was stirred for 5.5 h, then concentrated. After dilution with EtOAc, the whole mixture washed with brine, dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, hexane–EtOAc = 1:1) to give **49** (440 mg, 40% in 3 steps) as a colorless oil: $[\alpha]_D^{22} = +21.6$ (*c* 1.1, $CHCl_3$); IR $\nu_{max}^{neat} cm^{-1}$ 3389, 2945, 1748, 1615, 1205, 1120, 1067; ^1H NMR (270 MHz, $CDCl_3$) δ 2.60–2.74 (1H, br, OH), 3.47–3.57 (1H, br, OH), 3.85–4.00 (2H, m, CH_2OH), 4.27–4.37 (1H, m, $CHOH$), 4.72 (2H, d, $J=5.6$ Hz, CO_2CH_2), 5.26–5.39 (2H, m, $CO_2CH_2CHCH_2$), 5.85–6.00 (1H, m, $CO_2CH_2CHCH_2$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 64.0, 65.5, 71.6, 119.1, 131.1, 172.5; Anal. calcd for $C_6H_{10}O_4 \cdot 1/6H_2O$: C, 48.32; H, 6.98. Found: C, 48.43; H, 7.03.

4.1.37. Allyl (*R*)-2,3-bis(*tert*-butyldimethylsiloxy)propanoate. Obtained from **49** according to the preparation of **26**. A colorless oil: $[\alpha]_D^{23} = +16.4$ (*c* 1.0, $CHCl_3$); IR $\nu_{max}^{neat} cm^{-1}$ 2955, 2930, 2887, 1759, 1472, 1257, 1148, 1128; ^1H NMR (270 MHz, $CDCl_3$) δ 0.05 (6H, s, $SiMe_2$), 0.08, 0.09 (6H, s $\times 2$, $SiMe_2$), 0.88 (9H, s, *t*Bu), 0.90 (9H, s, *t*Bu), 3.73–3.87 (2H, m, CH_2OSi), 4.29 (1H, t, $J=5.3$ Hz, $CHOSi$), 4.62 (2H, d, $J=5.6$ Hz, CO_2CH_2), 5.22–5.37 (2H, m, $CO_2CH_2CHCH_2$), 5.86–5.97 (1H, m, $CO_2CH_2CHCH_2$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ –5.3, –4.9, 18.4, 25.8, 25.9, 65.4, 66.0, 74.0, 118.4, 131.8, 172.5. Anal. calcd for $C_{12}H_{24}O_4Si$: C, 57.70; H, 10.22. Found: C, 57.46; H, 10.36.

4.1.38. Allyl (*R*)-(*tert*-butyldimethylsiloxy)-3-hydroxypropanoate. Obtained from the above bis(TBS)propanoate according to the preparation of **27**. A colorless oil: IR ν_{max}^{neat}

cm^{-1} 3474, 2932, 1755, 1615, 1257, 1142; ^1H NMR (270 MHz, $CDCl_3$) δ 0.10, 0.14 (6H, s $\times 2$, $SiMe_2$), 0.92 (9H, s, *t*Bu), 2.15–2.24 (1H, br, OH), 3.80–3.86 (2H, br, CH_2OH), 4.32 (1H, t, $J=4.6$ Hz, $CHOSi$), 4.65 (2H, d, $J=5.4$ Hz, CO_2CH_2), 5.24–5.38 (2H, m, $CO_2CH_2CHCH_2$), 5.85–5.97 (1H, m, $CO_2CH_2CHCH_2$).

4.1.39. Allyl (*R*)-2-(*tert*-butyldimethylsiloxy)propanoate 3-dibenzyl phosphate (50). Obtained from the above mono(TBS)propanoate according to the preparation of **3b**. A colorless oil: $[\alpha]_D^{22} = +12.6$ (*c* 0.6, $CHCl_3$); IR $\nu_{max}^{neat} (CHCl_3) cm^{-1}$ 2955, 2930, 1759, 1456, 1283, 1260, 1153, 1018; ^1H NMR (270 MHz, $CDCl_3$) δ 0.07, 0.09 (6H, s $\times 2$, $SiMe_2$), 0.89 (9H, s, *t*Bu), 4.11–4.18 (1H, m, CH_2OP), 4.24–4.32 (1H, m, CH_2OP), 4.37–4.40 (1H, m, $CHOSi$), 4.60 (2H, d, $J=5.7$ Hz, CO_2CH_2), 5.01, 5.03 (2H, s $\times 2$, $CH_2C_6H_5$), 5.04, 5.06 (2H, s $\times 2$, $CH_2C_6H_5$), 5.21–5.34 (2H, m, $CO_2CH_2CHCH_2$), 5.83–5.95 (1H, m, $CO_2CH_2CHCH_2$), 7.33 (10H, s, $C_6H_5 \times 2$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ –5.2, –4.9, 18.4, 25.7, 65.9, 68.9, 69.0, 69.3, 69.4, 71.6, 71.7, 118.8, 127.8, 128.3, 128.4, 131.4, 135.5, 135.6, 169.9. Anal. calcd for $C_{26}H_{37}O_7PSi \cdot 1/2Et_2O$: C, 60.30; H, 7.59. Found: C, 60.42; H, 7.45.

4.1.40. (*R*)-2'-(*tert*-Butyldimethylsiloxy)-1'-cyclo[(*S*)-Thr-(*S*)-Phe-(*S*)-Phs-(*S*)-Phe-(*S*)-N-Me-Tyr(TBS)-(*S*)-Ile]-propanoate 3'-dibenzyl phosphate (51). Cyclic peptide **34** (100 mg, 0.10 mmol) was treated with 4 N HCl–dioxane (2 ml) at 0 °C and the mixture was stirred at 0 °C for 1 h. Removal of the solvent under reduced pressure afforded the crude hydrochloride salt as a colorless solid.

To a solution of phosphate **50** (80 mg, 0.15 mmol) in THF (1 ml) was added $(PPh_3)_4Pd$ (18 mg, 15 μ mol) and morpholine (20 μ l, 0.22 mmol) at room temperature. The mixture was stirred for 1 h. After dilution with EtOAc, the whole mixture was washed with 1 M aqueous $KHSO_4$ and brine, dried over Na_2SO_4 , and concentrated in vacuo to give a crude carboxylic acid as a yellow oil which was used for the next step without further purification.

To a solution of the above crude amine salt and carboxylic acid in DMF (1 ml) was added DEPC (24 μ l, 0.15 mmol) and Et_3N (35 μ l, 0.25 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then at room temperature for 13 h. After dilution with EtOAc, the whole mixture was successively washed with 1 M aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc = 1:5) to give **51** (118 mg, 85%) as a colorless solid: $[\alpha]_D^{22} = -2.9$ (*c* 0.3, $CHCl_3$); IR $\nu_{max}^{neat} (CHCl_3) cm^{-1}$ 3389, 3282, 2957, 2990, 1735, 1682, 1649, 1510, 1259, 1021; ^1H NMR (270 MHz, $CDCl_3$) δ –0.06, –0.02 (6H, s $\times 2$, $SiMe_2$), 0.10, 0.12 (6H, s $\times 2$, $SiMe_2$), 0.68–0.93 (6H, m, Ile-5,6-*H*), 0.83, 0.93 (18H, s $\times 2$, *t*Bu $\times 2$), 1.00–1.44 (5H, m, Ile-4-*H*, Thr-4-*H*), 1.70–1.90 (5H, m, Ile-3-*H*, Phs-3,4-*H*), 2.55–2.67 (2H, m, Tyr-3-*H*), 2.82, 2.86 (3H, s $\times 2$, *N*Me), 2.75–2.96 (2H, m, Phe(2)-3-*H*), 3.03–3.28 (2H, m, Phe(1)-3-*H*), 3.58–3.68 (2H, m, Phs-5-*H*), 3.95–4.16 (1H, m, Phs-2-*H*), 4.18–4.33 (1H, m, Ga-2-*H*), 4.35–4.55 (4H, m, Ga-3-*H*, Ile-2-*H*, Thr-2-*H*), 4.55–4.65 (1H, m, Phe(1)-2-*H*), 4.93–5.10 (6H, m, Tyr-2-*H*, Phe(2)-2-*H*, $P(OCH_2C_6H_5) \times 2$), 5.20–5.30 (1H,

br, Thr-3-*H*), 6.66 (2H, d, $J=8.3$ Hz, Tyr-6,8-*H*), 6.94 (2H, d, $J=8.4$ Hz, Tyr-5,9-*H*), 7.15–7.31 (20H, m, $C_6H_5 \times 4$); ^{13}C NMR (67.8 MHz, CD_3OD) δ –5.0, –4.4, –4.3, 12.4, 14.9, 17.9, 18.3, 18.9, 19.0, 26.1, 26.4, 27.7, 29.8, 30.4, 34.4, 38.2, 38.5, 51.3, 52.9, 56.2, 56.6, 58.5, 62.1, 63.5, 70.8, 70.9, 73.2, 73.9, 121.2, 127.6, 129.1, 129.2, 129.3, 129.4, 129.6, 129.7, 129.9, 130.1, 131.1, 131.6, 133.0, 133.7, 136.9, 137.0, 137.7, 138.4, 155.6, 170.4, 170.5, 171.4, 172.0, 173.6, 174.3, 174.6; FABMS (*m*-nitrobenzyl alcohol) m/z 1380 $[M+H]^+$.

4.1.41. (R)-2'-Hydroxy-1'-cyclo[(S)-Thr-(S)-Phe-(S)-Phe-(S)-Phe-(S)-N-Me-Tyr(TBS)-(S)-Ile]-propanoate 3'-dibenzyl phosphate (52). To a solution of cyclic peptide **51** (20 mg, 14 μ mol) in DMSO (0.4 ml) was added IBX (16 mg, 58 μ mol) at room temperature. The mixture was stirred for 4 h. After dilution with EtOAc, the whole mixture was washed with H_2O ($\times 2$) and brine, dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by thin layer chromatography ($CHCl_3$ –MeOH=10:1) to give aldehyde (9 mg, 45%) as a colorless solid.

To a solution of the above aldehyde in THF (0.3 ml) was added 1 N TBAF in THF (23 μ l, 23 μ mol) at 0 °C. The mixture was stirred at 0 °C for 1 h, then concentrated. The residue was purified by thin layer chromatography ($CHCl_3$ –MeOH=10:1) to give Ahp product **52** (5.0 mg, 75%) as a colorless solid: ^1H NMR (270 MHz, CD_3OD) δ 0.85–0.94 (6H, m, Ile-5,6-*H*), 1.03–1.20 (2H, m, Ile-4-*H*), 1.25 (3H, d, $J=6.6$ Hz, Thr-4-*H*), 1.58–1.90 (4H, m, Ile-3-*H*, Ahp-4,5-*H*), 1.92–2.02 (1H, br, Tyr-3-*H*), 2.55–2.80 (3H, m, Ahp-4-*H*, Phe(1)-3-*H*, Phe(2)-2-*H*), 2.84 (3H, s, *NMe*), 2.85–3.02 (1H, m, Tyr-3-*H*), 3.20–3.40 (2H, m, Phe(1)-3-*H*, Phe(2)-3-*H*), 3.79–3.86 (1H, m, Ahp-3-*H*), 4.03–4.13, 4.18–4.27 (2H, m, Ga-3-*H*), 4.28–4.45 (1H, br, Ga-2-*H*), 4.52 (1H, br, Thr-2-*H*), 4.60 (1H, d, $J=6.0$ Hz, Ile-2-*H*), 4.65–4.70 (1H, m, Phe(1)-2-*H*), 4.95–5.13 (6H, m, Tyr-2-*H*, Phe(2)-2-*H*, $P(OCH_2C_6H_5) \times 2$), 5.16 (1H, br, Ahp-6-*H*), 5.46–5.53 (1H, br, Thr-3-*H*), 6.80 (2H, d, $J=8.4$ Hz, Tyr-6,8-*H*), 6.88 (2H, d, $J=6.4$ Hz, Tyr-5,9-*H*), 6.98–7.25 (10H, m, $C_6H_5 \times 2$), 7.37 (10H, s, $P(OCH_2C_6H_5) \times 2$).

4.1.42. (2'R)-Micropeptin T-20 (53). To a solution of Ahp product **52** (5.0 mg, 4.3 μ mol) in 90% aqueous EtOH (0.4 ml) was added 10% Pd–C (4 mg) under an argon atmosphere. The black slurry was stirred under 1 atm of H_2 at room temperature for 1 h. The mixture was filtered through a pad of celite and the filtrate was concentrated in vacuo to give a deprotected product (4.5 mg, quant.) as a colorless solid.

The above deprotected product was treated with pH 7 phosphate buffer–MeOH (2:3, 0.5 ml) and the mixture was stirred at room temperature for 0.5 h. After concentration, the residue was purified by ODS silica gel column chromatography (BW-300 MH, H_2O then MeOH) to give **53** (3.5 mg, 81%) as a colorless solid: $[\alpha]_D^{24} = -11.9$ (c 0.1, CH_3OH); ^1H NMR (500 MHz, CD_3OD) δ 0.87 (3H, t, $J=7.5$ Hz, Ile-5-*H*), 0.91 (3H, d, $J=6.7$ Hz, Ile-6-*H*), 1.07–1.15 (1H, m, Ile-4-*H*), 1.24 (3H, d, $J=6.4$ Hz, Thr-4-*H*), 1.30–1.37 (1H, m, Ile-4-*H*), 1.60–1.90 (5H, m, Ahp-4,5-*H*, Ile-3-*H*), 1.98–2.05 (1H, m, Tyr-3-*H*), 2.56–2.62 (1H, m, Ahp-4-*H*), 2.68–2.79 (2H, m, Phe(1)-3-*H*, Phe(2)-3-*H*), 2.85

(3H, s, *NMe*), 2.92–2.98 (1H, m, Tyr-3-*H*), 3.35–3.44 (2H, m, Phe(1)-3-*H*, Phe(2)-3-*H*), 3.80–3.87 (2H, m, Ahp-3-*H*, Ga-3-*H*), 4.08–4.16 (1H, m, Ga-3-*H*), 4.30–4.33 (1H, m, Ga-2-*H*), 4.52 (1H, br, Thr-2-*H*), 4.65 (1H, d, $J=5.5$ Hz, Ile-2-*H*), 4.70 (1H, dd, $J=4.2$, 10.5 Hz, Phe(1)-2-*H*), 4.93–4.96 (1H, m, Tyr-2-*H*), 5.02–5.07 (1H, m, Phe(2)-2-*H*), 5.16 (1H, br, Ahp-6-*H*), 5.46–5.50 (1H, m, Thr-3-*H*), 6.84 (2H, d, $J=9.4$ Hz, Tyr-6,8-*H*), 6.89 (2H, d, $J=7.0$ Hz, Tyr-5,9-*H*), 7.09–7.20 (10H, m, $C_6H_5 \times 2$); ^{13}C NMR (500 MHz, CD_3OD) δ 11.6, 16.6, 18.1, 22.3, 26.3, 30.6, 31.7, 34.2, 36.4, 37.3, 39.0, 50.8, 52.6, 55.8, 56.1, 57.4, 63.2, 68.3, 73.8, 73.9, 75.8, 116.7, 127.6, 127.7, 129.1, 129.4, 129.6, 130.0, 130.6, 131.8, 137.7, 138.9, 157.7, 170.9, 171.2, 171.6, 172.9, 173.3, 174.2, 174.6; FABMS (*m*-nitrobenzyl alcohol) m/z 1011 $[M+H]^+$.

Acknowledgements

This work was financially supported in part by a Grant-in-Aid for Research at Nagoya City University (to F. Y.), Uehara Memorial Foundation (to F. Y.), and Grant-in-Aids from the ministry, Education, Science, Sports and Culture, Japan. We thank Dr. Kunimitsu Kaya for providing us with the ^1H and ^{13}C NMR spectra of micropeptin T-20. The high resolution FAB mass spectrum was measured by Dr. Akito Nagatsu (Nagoya City University), to whom the authors would like to give thanks.

References and notes

- For a few representative examples, see (a) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Dufresne, C.; Cerny, R. L.; Herald, D. L.; Schmidt, J. M.; Kizu, H. *J. Am. Chem. Soc.* **1989**, *111*, 5015–5017. (b) Okano, T.; Sano, T.; Kaya, K. *Tetrahedron Lett.* **1999**, *40*, 2379–2382 and references therein. (c) Nogle, L. M.; Williamson, R. T.; Gerwick, W. H. *J. Nat. Prod.* **2001**, *64*, 716–719. For a recent review on peptides from freshwater cyanobacteria, see (d) Harada, K. *Chem. Pharm. Bull.* **2004**, *52*, 889–899. For a review on serine protease inhibitory peptides derived from cyanobacteria, see (e) Radau, G. *Pharmazie* **2000**, *55*, 555–560.
- (a) For a review, see Shioiri, T.; Hamada, Y. *Synlett* **2001**, 148–201. (b) For a recent achievement from our laboratories, see Yokokawa, F.; Asano, T.; Okino, T.; Gerwick, W. H.; Shioiri, T. *Tetrahedron* **2004**, *60*, 6859–6880.
- Preliminary accounts of this work were already published: (a) Yokokawa, F.; Inaizumi, A.; Shioiri, T. *Tetrahedron Lett.* **2001**, *42*, 5903–5908. (b) Yokokawa, F.; Inaizumi, A.; Shioiri, T. *Peptide Sci.* **2002**, *2001*, 33–36.
- Dehydrodolastatin 13 was also isolated together with dolastatin 13, see Ref. 19.
- Takuma, S.; Hamada, Y.; Shioiri, T. *Chem. Pharm. Bull.* **1982**, *30*, 3147–3153 and references cited therein.
- Shioiri, T.; Imaeda, T.; Hamada, Y. *Heterocycles* **1997**, *46*, 421–442. cf. Grieco, P. A.; Perez-Medrano, A. *Tetrahedron Lett.* **1988**, *29*, 4228–4255.
- Tung, R. D.; Rich, D. H. *J. Am. Chem. Soc.* **1985**, *107*, 4342–4343.

8. Ishizumi, K.; Koga, K.; Yamada, S. *Chem. Pharm. Bull.* **1968**, *16*, 492–497.
9. The reason of this difference dependent on the substrates is not clear.
10. Chen, S.; Xu, J. *Tetrahedron Lett.* **1991**, *32*, 6711–6714.
11. (a) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155–4156. (b) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277–7287.
12. Lok, C. M.; Ward, J. P.; Dorp, D. A. *Phys. Lipids* **1976**, *16*, 115.
13. Danishefsky, S. J.; Armistead, D. M.; Wincott, F. E.; Selnick, H. G.; Hungate, R. *J. Am. Chem. Soc.* **1987**, *109*, 8117–8119.
14. Chaudhary, S. K.; Hernandez, O. *Tetrahedron Lett.* **1979**, *20*, 99–102.
15. Watanabe, Y.; Komoda, Y.; Ebisuya, K.; Ozaki, S. *Tetrahedron Lett.* **1990**, *31*, 255–258.
16. (a) Mitsunobu, O.; Eguchi, M. *Bull. Chem. Soc. Jpn* **1971**, *44*, 3427–3430. (b) For a review, see Mitsunobu, O. *Synthesis* **1981**, 1–28.
17. Bannwarth, W.; Trzeciak, A. *Helv. Chim. Acta* **1987**, *10*, 175–186.
18. For oxidative ring closure of an amino alcohol to an amina, see Bose, D. S.; Srinivas, P.; Gurjar, M. K. *Tetrahedron Lett.* **1997**, *38*, 5839–5842.
19. Bessodes, M.; Boukarim, C. *Synlett* **1996**, 1119–1120.
20. Konami, Y.; Osawa, T.; Jeanloz, R. W. *Biochemistry* **1971**, *10*, 192–196.
21. (a) Nelson, T. D.; Crouch, R. D. *Synthesis* **1996**, 1031–1069 and references therein. (b) Sabitha, G.; Syamala, M.; Yadav, J. S. *Org. Lett.* **1999**, *1*, 1701–1703. (c) Bartoli, G.; Basco, M.; Marcantoni, E.; Sambri, L.; Torregiani, E. *Synlett* **1998**, 209–211.
22. For another synthesis of the Ahp-containing cyclic depsipeptide, see Yokokawa, F.; Shioiri, T. *Tetrahedron Lett.* **2002**, *43*, 8673–8677.

Solid phase total synthesis of the 3-amino-6-hydroxy-2-piperidone (Ahp) cyclodepsipeptide and protease inhibitor Symplocamide A†‡

Sara C. Stolze,^{ab} Michael Meltzer,^b Michael Ehrmann^b and Markus Kaiser^{*ab}

Received 29th July 2010, Accepted 27th September 2010

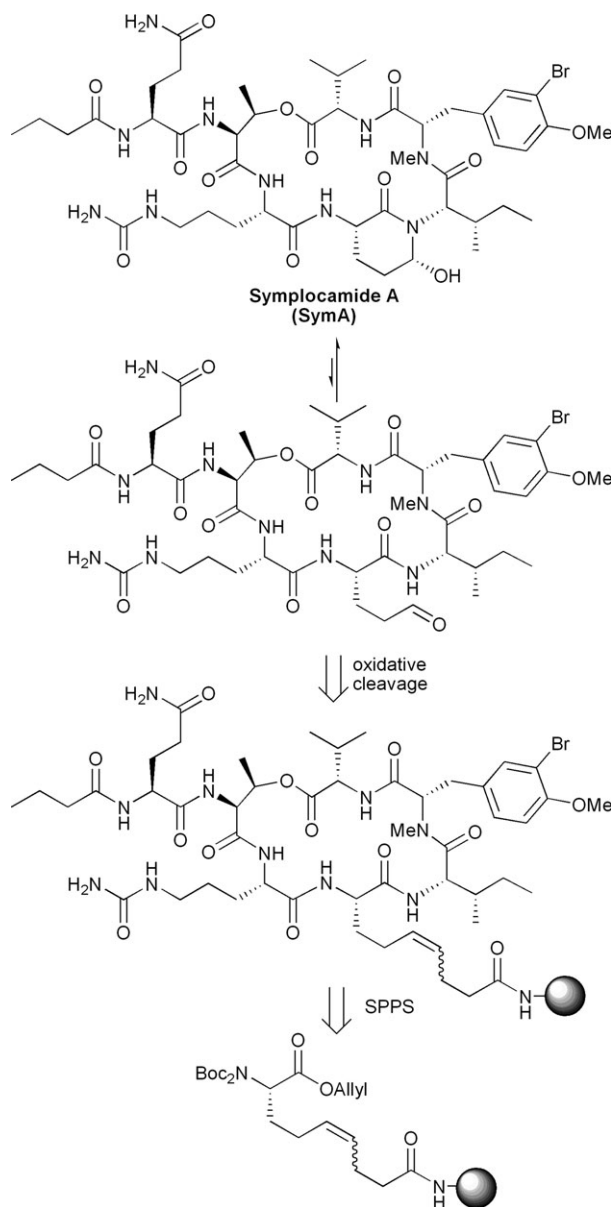
DOI: 10.1039/c0cc02889d

The solid phase total synthesis of the marine cyanobacterial Ahp-cyclodepsipeptide Symplocamide A is reported as a model for a general route for the synthesis of tailor-made non-covalent serine protease inhibitors.

Freshwater and marine cyanobacteria produce a wide variety of secondary metabolites with diverse activities.¹ Among them, the class of 3-amino-6-hydroxy-2-piperidone (Ahp) containing cyclic depsipeptides is of particular interest due to their potent, selective and most intriguingly non-covalent inhibition of S1 (trypsin- and chymotrypsin-like) serine proteases.^{2,3} These interesting biological activities suggest that this class of natural products might serve as a promising lead for serine protease inhibitor design.²

However, limited availability interferes with the wider application of Ahp cyclodepsipeptides as protease inhibitors in chemical biology research. Isolation usually yields very low amounts and chemical synthesis has been to date only reported for two Ahp cyclodepsipeptides *i.e.* Somamide A and Micropeptin T20, both generated after extensive multi-step solution phase chemistry.⁴ To facilitate and speed up their synthesis and more importantly to gain access to custom-made Ahp cyclodepsipeptides, a versatile and straight-forward solid phase synthesis route would therefore be highly desirable. To establish such a methodology, we considered the Ahp cyclodepsipeptide natural product and strong chymotrypsin inhibitor Symplocamide A (SymA) as a suitable model target structure (Scheme 1).⁵ To date, no synthesis of SymA has been reported and SymA furthermore introduces two interesting new features to the Ahp natural product class, *i.e.* a citrulline residue at the P1 site and the unusual and rarely found *N,O*-dimethyl-3-bromotyrosine residue in the macrocyclic part of the molecule.

Previous synthetic studies have shown that the Ahp moiety forms spontaneously from a glutamic aldehyde generated within the correctly assembled precursor cyclodepsipeptide (Scheme 1).⁴ A general synthetic route could therefore consist of a solid phase synthesis of the cyclodepsipeptide backbone, followed by the regio- and chemoselective generation of a glutamic aldehyde. Consequently, such a strategy would



Scheme 1 A solid phase-based retrosynthesis of Symplocamide A (SymA), using an unsaturated building block as a masked glutamic aldehyde equivalent.

require a suitable masked aldehyde equivalent that should (a) be compatible with Fmoc- as well as Boc-protecting group strategies; (b) generate the glutamic aldehyde residue upon cleavage from the resin, thereby spontaneously forming also the hemiaminal of the Ahp unit in a convenient one-pot procedure; and (c) represent a suitable anchoring group for

^a Chemical Genomics Centre der Max-Planck-Gesellschaft, Otto-Hahn-Str. 15, 44227 Dortmund, Germany

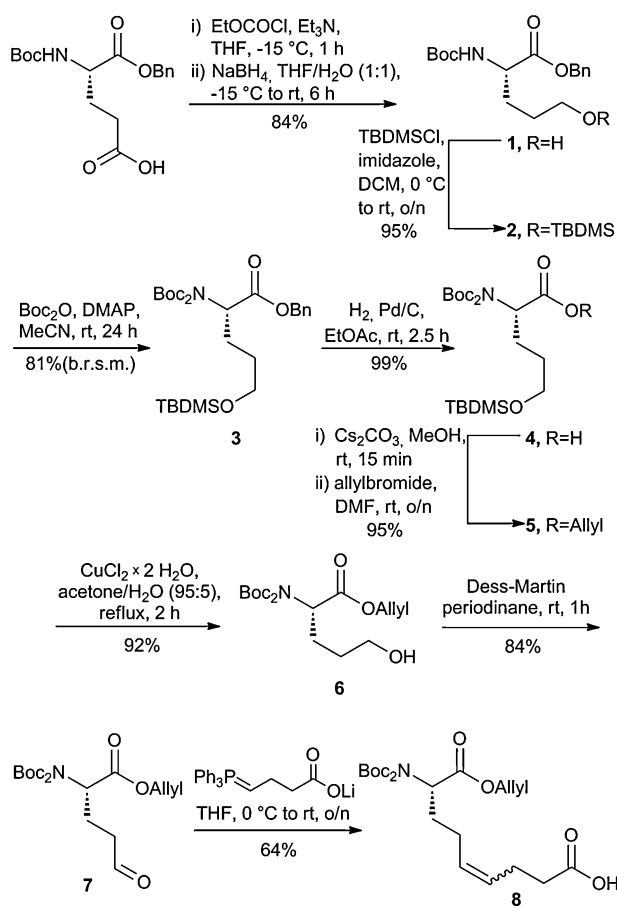
^b Zentrum für Medizinische Biotechnologie, Fakultät Biologie und Geographie, Universität Duisburg-Essen, Universitätsstr. 2, 45117 Essen, Germany. E-mail: markus.kaiser@uni-due.de; Fax: +49 201 1833135; Tel: +49 201 1832969

† This article is part of the 'Enzymes and Proteins' web-theme issue for ChemComm.

‡ Electronic supplementary information (ESI) available: Experimental details of the synthesis of compounds 1–13 and SymA on solid support. See DOI: 10.1039/c0cc02889d

the attachment to the solid support that still allows subsequent chemical modification of the N- and C-termini of the glutamic aldehyde equivalent. We envisioned that a novel amino acid consisting of a double bond as a masked aldehyde function and a free carboxylic acid for linking to the resin *via* its side chain could serve these requirements, as the Meldal group has previously shown that oxidative cleavage of double bonds on solid support efficiently generates aldehydes.⁶

Consequently, our synthetic investigations started with the solution phase synthesis of the Ahp precursor molecule **8** (Scheme 2). To this end, *N*-Boc protected glutamic acid benzyl ester was activated as a mixed anhydride and then reduced with sodium borohydride using the protocol by Shioiri *et al.*,⁴ thereby yielding alcohol **1**. **1** was then protected as a silyl ether and the resulting intermediate **2** was converted to the di-Boc protected derivative **3**.⁷ Removal of the benzyl ester protecting group by hydrogenation led to the acid **4**,⁸ which was reprotected as an allyl ester using the well-established protocol by Kunz *et al.*,⁹ thereby obtaining the fully protected intermediate **5**. The subsequent removal of the silyl ether to obtain alcohol **6** emerged to be demanding, as the standard cleavage conditions using fluoride or mild acidic conditions led to the loss of one of the two Boc protecting groups. This issue was solved with the very mild copper-mediated cleavage conditions described by Wang *et al.*¹⁰ The following oxidation of **6** to

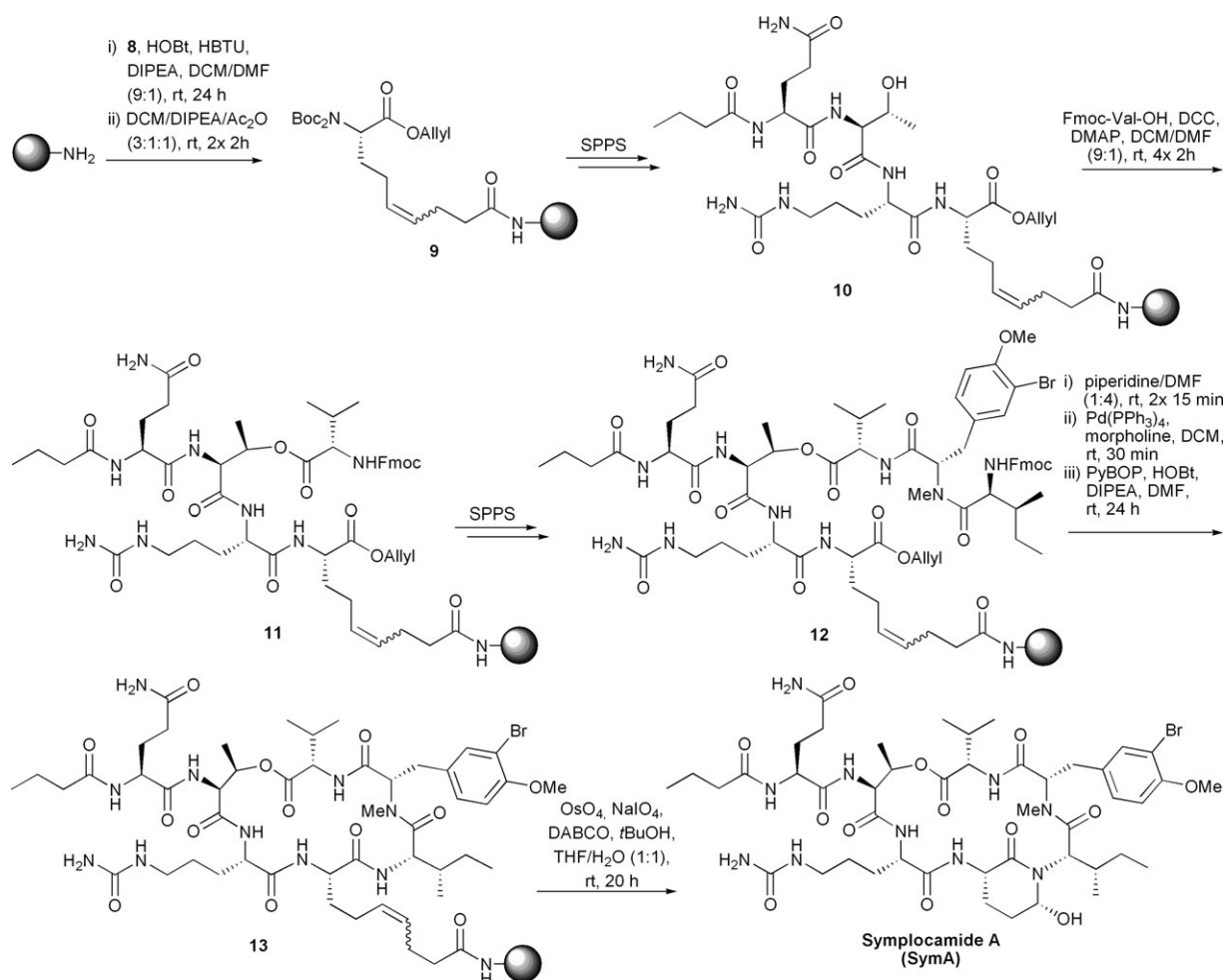


Scheme 2 Solution synthesis of building block **8**, the general precursor for the solid phase synthesis of Ahp containing cyclodepsipeptides.

aldehyde **7** under Dess–Martin conditions proceeded smoothly in 84% yield. **7** was then immediately converted by a Wittig reaction to obtain building block **8** for the subsequent solid phase synthesis.

With **8** in hand, the assembly of the cyclodepsipeptide on solid support could be initiated (Scheme 3). To prevent unwanted side reactions during the on resin macrolactamization, the reaction conditions were optimized towards a low loading (0.24 mmol g^{−1} resin) of **8** to a NovaPEG amino resin, resulting in **9**. Deprotection of the Boc-protecting groups with 50% TFA in DCM sets the stage for the subsequent amino acid couplings. Couplings were performed with HBTU (3.5 eq.) and HOBT (4 eq.) as the coupling reagents, DIPEA (3 eq.) as the base and the corresponding Fmoc- or Boc-protected amino acids (4 eq.). First, Fmoc-citrulline was coupled, followed by Fmoc-Thr-OH, Boc-Glu-OH and finally butyric acid, leading to intermediate **10**. For cleavage of the Fmoc- or Boc-protecting groups, either 20% piperidine in DMF or 50% TFA in DCM were used. The adjacent esterification of the threonine hydroxyl group side chain with Fmoc-Val-OH (10 eq.), thereby yielding **11**, was achieved with DMAP (1 eq.) and di-isopropyl carbodiimide (10 eq.) as the activating reagents. Fmoc-deprotection followed by coupling with *N*-Boc-*N,O*-dimethyl-3-bromotyrosine using standard coupling conditions and subsequent Boc-deprotection yielded an *N*-methyl intermediate, to which Fmoc-isoleucine (5 eq.) was coupled, using PyBOP (4.9 eq.) as the coupling reagent and DIPEA (10 eq.) as the base and a reaction time of 24 h. This intermediate **12** was deprotected at the N-terminus by Fmoc-cleavage and the C-terminus by removal of the allyl protection group by the protocol of Vaz and Brunsfeld¹¹ led to the cyclization precursor. Macrolactamization was obtained by PyBOP (4 eq.)/HOBT (8 eq.)/DIPEA (12 eq.) activation and a reaction time of 24 h, thereby yielding **13**. The final conversion of the double bond of **13** into the aldehyde moiety for establishing the Ahp residue in SymA and for simultaneous cleavage from the resin was achieved with OsO₄ and NaIO₄ as oxidation reagents and DABCO to prevent the formation of hydroxymethyl ketones.⁶ To the best of our knowledge, this is the first time that these reagents are used for cleaving aldehydes from a resin. Subsequent chromatographic purification of the crude product yielded the desired natural product Symplocamide A (SymA). The resulting ¹H and ¹³C NMR spectra of the synthetic compound corresponded well to the data published for the natural product.⁵ Moreover, the synthetic SymA demonstrated potent chymotrypsin inhibition, displaying a *K_i* value of 0.32 ± 0.09 μM, in accordance with the literature report (lit.,⁵ IC₅₀ = 0.38 ± 0.08 μM).

We have established the first solid phase synthesis of an Ahp cyclodepsipeptide by synthesizing the natural product Symplocamide A. Essential for the overall synthesis strategy was the development of the masked aldehyde precursor **8** that proved to be a useful building block to install the Ahp moiety at the last stage of the synthesis. The established synthetic route holds promise for the synthesis of custom-made Ahp depsipeptides, thereby opening the possibility to generate non-covalent S1 serine protease inhibitors as chemical biology tools in a fast and efficient manner for protease research.



Scheme 3 The solid phase synthesis of Symplocamide A as a model synthesis for establishing a general route towards the synthesis of tailor-made Ahp containing cyclodepsipeptides.

Notes and references

- M. Namikoshi and K. L. Rinehart, *J. Ind. Microbiol. Biotechnol.*, 1996, **17**, 373–384.
- G. Radau, *Curr. Enzyme Inhib.*, 2005, **1**, 293–307. For some examples see: E. N. Zainuddin, R. Mentel, V. Wray, R. Jansen, M. Nimtz, M. Lalk and S. Mundt, *J. Nat. Prod.*, 2007, **70**, 1084–1088; E. von Ehlert, L. Oberer, P. Merkel, T. Huhn and J. F. Blom, *J. Nat. Prod.*, 2005, **68**, 1324–1327; K. Taori, S. Matthew, J. R. Rocca, V. J. Paul and H. Luesch, *J. Nat. Prod.*, 2007, **70**, 1593–1600; E. Zafrir and S. Carmeli, *J. Nat. Prod.*, 2010, **73**, 352–358; J. F. Blom, B. Bister, D. Bischoff, G. Nicholson, G. Jung, R. D. Süßmuth and F. Jüttner, *J. Nat. Prod.*, 2003, **66**, 431–434; U. Matern, L. Oberer, R. A. Falchetto, M. Ehrhard, W. A. König, M. Herdman and J. Weckesser, *Phytochemistry*, 2001, **58**, 1087–1095.
- A. Y. Lee, T. A. Smitka, R. Bonjouklian and J. Clardy, *Chem. Biol.*, 1994, **1**, 113–117; M. A. McDonough and C. J. Schofield, *Chem. Biol.*, 2003, **10**, 898–900; U. Matern, C. Schleberger, S. Jelakovic, J. Weckesser and G. E. Schulz, *Chem. Biol.*, 2003, **10**, 997–1001.
- F. Yokokawa, A. Inaizumi and T. Shioiri, *Tetrahedron Lett.*, 2001, **42**, 5903–5908; F. Yokokawa and T. Shioiri, *Tetrahedron Lett.*, 2002, **43**, 8673–8677; F. Yokokawa, A. Inaizumi and T. Shioiri, *Tetrahedron*, 2005, **61**, 1459–1480.
- R. G. Linington, D. J. Edwards, C. F. Shuman, K. L. McPhail, T. Matainaho and W. H. Gerwick, *J. Nat. Prod.*, 2008, **71**, 22–27.
- T. E. Nielsen and M. Meldal, *Org. Lett.*, 2005, **7**, 2695–2698; T. E. Nielsen, S. Le Quement and M. Meldal, *Org. Lett.*, 2007, **9**, 2469–2472; S. Le Quement, T. E. Nielsen and M. Meldal, *J. Comb. Chem.*, 2007, **9**, 1060–1072.
- E. A. Englund, H. N. Gopi and D. H. Appella, *Org. Lett.*, 2004, **6**, 213–215.
- H. Sajiki, T. Ikawa, K. Hattori and K. Hirota, *Chem. Commun.*, 2003, 654–655.
- H. Kunz, H. Waldmann and C. Unverzagt, *Int. J. Pept. Protein Res.*, 1985, **26**, 493–497.
- Z. P. Tan, L. Wang and J. B. Wang, *Chin. Chem. Lett.*, 2000, **11**, 753–753.
- E. Vaz and L. Brunsveld, *Org. Lett.*, 2006, **8**, 4199–4202.