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DIRECCIÓN DE NUEVAS CREACIONES

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Bogotá, D.C.,

Abogada
LUZ CLEMENCIA DE PAEZ

ASUNTO Radicación 09-007875
 Trámite 11
 Evento 378
 Actuación 519
 Oficio
 Folios 8 1 25 1 4

Patente de Invención ☒ Patente de Modelo de Utilidad

Via Nacional

Via PCT (Fase Nacional) ☒ Capítulo I ☒ Capítulo II

No. Solicitud Internacional PCT/EP2007/057710
Fecha de Presentación Internacional 26/Julio/2007

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:	Folios 94 a 177	28/Enero/2009
Reivindicaciones:	Folios 178 a 190	28/Enero/2009
Prioridad:	US 60 834 067	28/Julio/2006
	EP 60118159.0	31/Julio/2006

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.

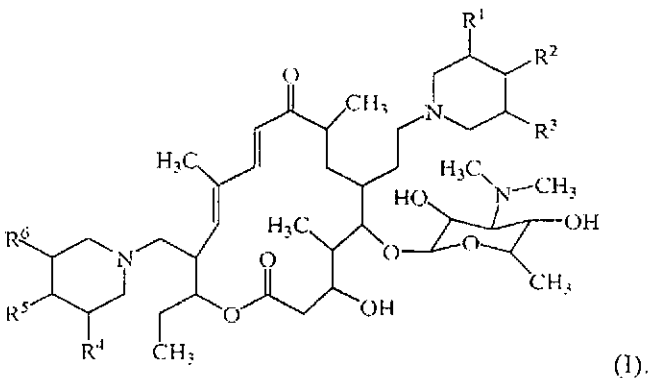
3. Objeto de la invención

Título de la solicitud: "PROCESO DE SINTESIS DE MACROLIDA"

El problema planteado en esta solicitud consiste en la necesidad de proporcionar

métodos de obtención de compuestos tipo macrólidas

Para resolver este problema técnico la solicitud en estudio proporciona un procedimiento para obtener un compuesto de la fórmula (I)



(Ver folio 100)

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C 07 H 017/008

4. Excepción a la Patentabilidad (Art 20 D 486 literal (d))

El objeto de la reivindicación 10 no es patentable de acuerdo con el Art citado.

5. De la Solicitud de Patente

Titulo

El título no corresponde con el objeto de la solicitud porque no define las características técnicas esenciales de la invención por cuanto los procesos para sintetizar compuestos macrolídicos ya son conocidos en el estado de la técnica, por lo cual se sugiere al Solicitante hacer la corrección necesaria.

Reivindicaciones (Art 30 D 486, Art 22 PCT)

El capítulo reivindicatorio no es claro porque no especifica las condiciones de reacción, ni cual disolvente polar es utilizado para formar el compuesto 20-piperidinil-tilosina, ni cual agente de activación es utilizado para obtener el compuesto activado, ni tampoco cual es el grupo saliente que comprende el compuesto activado. Se requiere hacer las aclaraciones necesarias.

La reivindicación 1 no es clara cuando menciona la etapa "hacer reaccionar un compuesto 20-piperidinil-tilosina con un ácido para formar un compuesto 23-O-micinosil-120-piperidinil-5-O-micaminosil-tilonolida" en tanto que se refiere a la obtención del compuesto 23-O-micinosil-20-piperidinil-5-O-micaminosil-tilonolida. Igualmente no especifica que ácido es utilizado en la reacción y no especifica si la reivindicación reclama la secuencia de pasos mencionada o la reacción de un compuesto activado con un compuesto piperidinilo de la fórmula (VII).

La reivindicación 6 no es clara cuando utiliza el término "por lo menos" el cual no es limitativo ni preciso. Se sugiere sustituirlo por un término preciso o un rango completo.

La reivindicación 10 se refiere a un método de tratamiento el cual no es patentable según la legislación aplicable (Art 20 D 486 literal (d))

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: Julio 28 de 2006, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Item	Documento	Título	Fecha de Publicación
D1	WO03027250	Monoclonal Antibodies to The Drug Tilmicosin and a Method for Detecting The Same	03/Abril/2003
D2	THE JOURNAL OF ANTIBIOTICS VOL. 56 NO. 5, MAY 2003 Facile Synthesis of Tilmicosin and Tylosin Related Haptens for Use as Protein Conjugates	Facile Synthesis of Tilmicosin and Tylosin Related Haptens for Use as Protein Conjugates	01/Mayo/2003
D3	US4629786	C-20- and C-23 Modified Macrolide Derivatives	16/Diciembre/1986
D4	US3459853	O-Mycaminosyl Tylonolide and A Process for The Preparation Thereof	05/Agosto/1969

D1
<http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=WO&NR=03027250A2&KC=A2&FT=D&ND=3&date=20030403&DB=worldwide.espacenet.com&locale=en> EP divulga (páginas 15 y 16) un proceso para obtener el derivado 23-yodo de 23- demycinosyl tilmicosina a partir de 23- demycinosyl tilmicosina.

D2
<http://naldc.nal.usda.gov/download/20320/PDF> divulga (páginas 481 a 487) un proceso de obtención de tilmicosina y tilosina

D3
<http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=US&NR=4629786A&KC=A&FT=D&ND=3&date=19861216&DB=worldwide.espacenet.com&locale=en> EP divulga (columnas 9 y 10, método 2) un proceso que comprende hidrólisis de tilosina modificada en los carbonos 20 y 23.

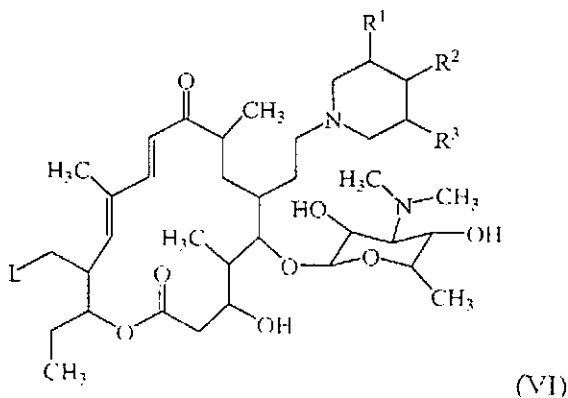
D4

<http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=US&NR=3459853A&KC=A&FT=D&ND=3&date=19690805&DB=worldwide.espacenet.com&locale=en> EP divulga (columna 2, líneas 49 a 55) un proceso que comprende hidrólisis de tilosina en condiciones ácidas

7. Examen de Patentabilidad (Art 14 D486)

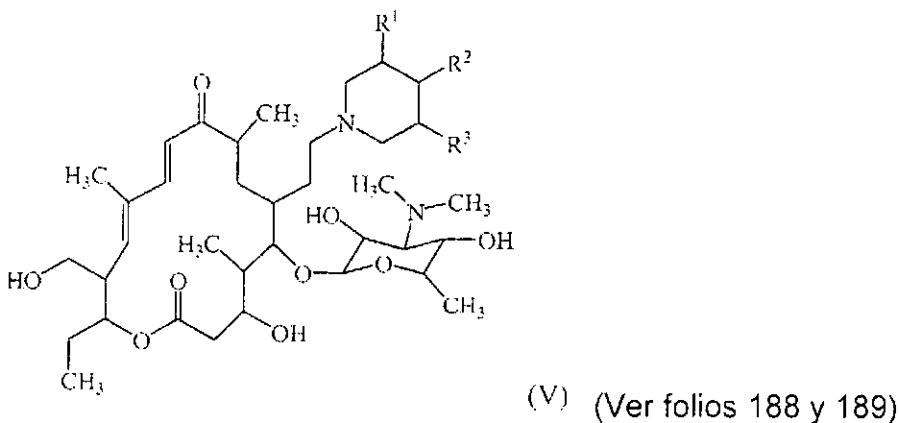
Novedad (Art 16 D486)

La reivindicación 14 consiste en un proceso para elaborar un compuesto activado o su sal, en donde el compuesto activado corresponde en estructura a la fórmula (VI):

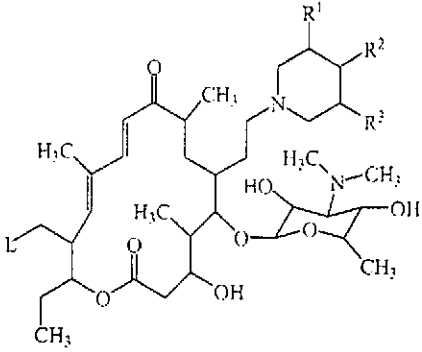
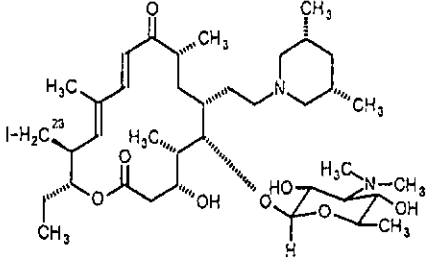
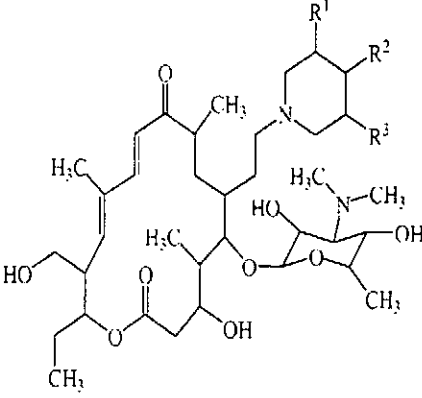
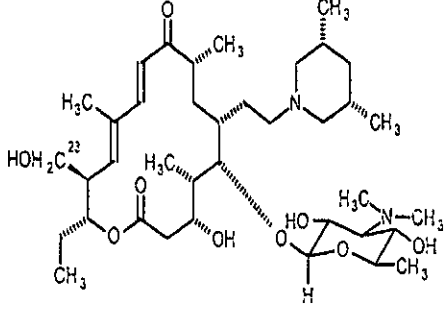


El proceso comprende activar un compuesto 23-hidroxi-20-piperidinil-5-O-micaminosil-tilonolida con un agente de activación;

El 23-hidroxi-20-piperidinil-5-O-micaminosil-tilonolida corresponde en estructura a la fórmula (V):



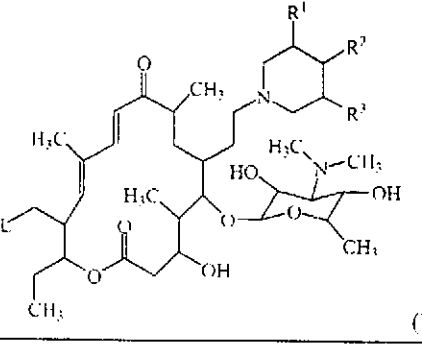
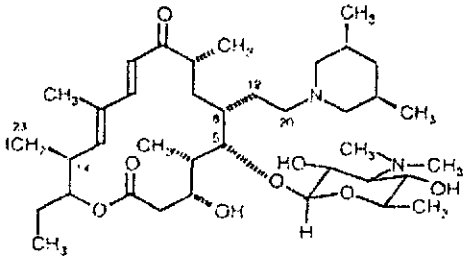
Comparación de las características técnicas esenciales, con las del Estado de la Técnica:

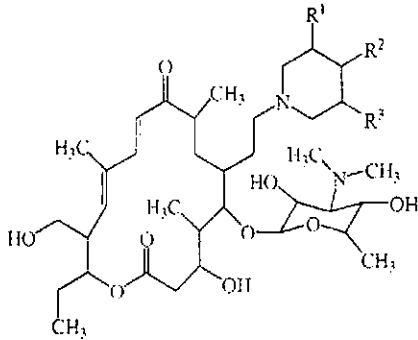
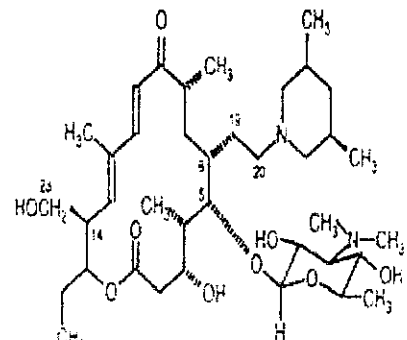
Características esenciales	D1
 (VI)	
Reacción de  con un agente de activación	Reacción de  disuelto en piridina anhidra, con trifenilfosfina y con yodo disuelto en piridina anhidra.

Las características esenciales del proceso de la reivindicación 14 habían sido divulgadas previamente en D1, el cual da a conocer un proceso de obtención del derivado 23-yodo de 23- demycinosyl tilmicosina a partir de reacción entre 23- demycinosyl tilmicosina, yodo y trifenilfosfina en presencia de piridina anhidra (páginas 15 y 16).

En consecuencia, la reivindicación 14 no es nueva ó carece de novedad, según lo divulgado en el documento D1.

Comparación de las características técnicas esenciales, con las del Estado de la Técnica:

Características esenciales	D2
 (VI)	

Reacción de  con un agente de activación	Reacción de  Con yodo, trifenilfosfina, piridina y tetrahydrofurano.
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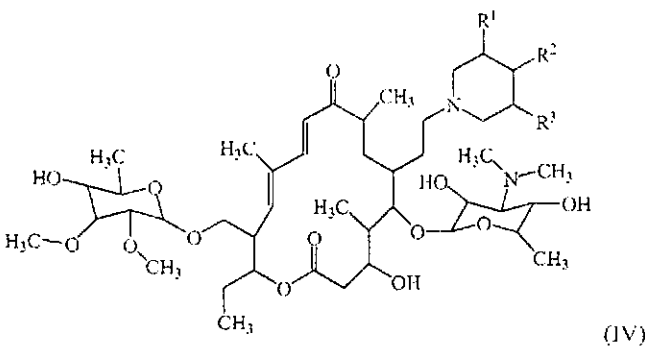
Las características esenciales del proceso de la reivindicación 14 habían sido divulgadas previamente en D2, el cual da a conocer un proceso de obtención del derivado 23-yodo de 23- demycinosyl tilmicosina a partir de reacción entre 23- demycinosyl tilmicosina, yodo y trifenilfosfina en presencia de piridina y tetrahydrofurano (página 483 esquema 1).

En consecuencia, la reivindicación 14 no es nueva ó carece de novedad, según lo divulgado en el documento D2.

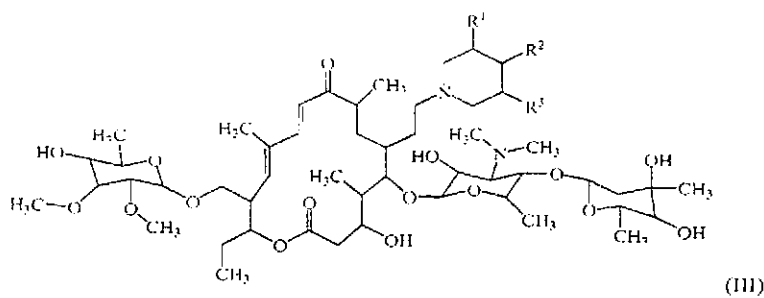
Nivel Inventivo (Art18 D486)

La reivindicación 12 consiste en un proceso para elaborar un compuesto 23-O- micinosil-20-piperidinil-5-O-micaminosil-tilonolida o su sal, en donde:

El compuesto 23-O-micinosil-20-piperidinil-5-O-micaminosil-tilonolida corresponde en estructura a la fórmula (IV):



El proceso comprende hacer reaccionar un compuesto 20-piperidinil-tilosina con HBr; el compuesto 20-piperidinil-tilosina corresponde en estructura a la fórmula (III)



(Ver folios 186 y 187).

Comparación de las características técnicas esenciales, con las del Estado de la Técnica:

SOLICITUD	D3	D4
Hidrólisis de 20-piperidinil tilosina para obtener 23-O-micinosil-20-piperidinil-5-O-micaminosil-tilonolida	Preparación de un C-20 Derivado de tilosina seguido de hidrólisis.	Hidrólisis de tilosina a pH 1.5-2.5

Los documentos D3 y D4 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 12 porque divulgan un proceso que comprende hidrólisis de tilosina y sus derivados.

La diferencia entre la invención, definida en la reivindicación 1 y el procedimiento de D3 consiste en que no divulga específicamente el derivado piperidinil de tilosina reclamada en la reivindicación 12

La diferencia entre la invención, definida en la reivindicación 1 y el procedimiento de D4 consiste en que no divulga específicamente el derivado piperidinil de tilosina reclamada en la reivindicación 12

El efecto que logra el realizar la hidrólisis de tilosina en condiciones ácidas es que permite encontrar un proceso alternativo para la obtención de macrólidas.

Por lo tanto, el Problema Técnico Objetivo que pretende resolver esta invención se puede formular así: "cómo modificar el procedimiento conocido en D3 para encontrar una ruta alterna de producción de macrólidas.

Sin embargo, la hidrólisis de tilosina en condiciones ácidas para obtener compuestos macrólidos ya está divulgada en el documento D4 que se refiere a un procedimiento para obtener O-micaminosil-tilonolida comprendiendo la hidrólisis de tilosina en condiciones ácidas (columna 2, líneas 49 a 55).

En consecuencia, la persona del oficio normalmente versada en la materia, incluiría la hidrólisis de tilosina en condiciones ácidas divulgada en D4 en el procedimiento de hidrólisis descrito en D3 para el compuesto derivado piperidinil de tilosina reclamado en la solicitud para llegar así al objeto de la reivindicación 12 de la solicitud. Por lo cual se considera obvia.

Puesto que las reivindicaciones 13 y 17 no presentan características inventivas

adicionales tampoco se pueden considerar inventivas.

En conclusión las reivindicaciones 12, 13 y 17 cumplen el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

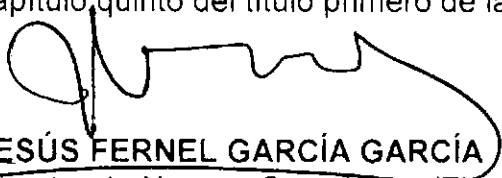
8. Conclusión

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

Item	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	WO03027250	14	Novedad
D2	Facile Synthesis of Tilmicosin and Tylosin Related Haptens for Use as Protein Conjugates	14	Novedad
D3	US4629786	12, 13, 17	Nivel Inventivo
D4	US3459853	12, 13, 17	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 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.

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 ~~17~~ **8 JUL. 2012**

Elaborado por: Rodolfo Cadena Amaya. *owet*
Revisado por: Ana Delia Pinzón.

Facile Synthesis of Tilmicosin and Tylosin Related Haptens for Use as Protein Conjugates

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(Received for publication January 20, 2003)

Synthesis is described for the haptens 23-demycinosyl-23-deoxy-23-(3-aminoprop-1-yl)-aminotilmicosin (**6**) from 5-(*O*-mycaminosyl)tylonolide (OMT) and for 23-demycinosyl-23-deoxy-23-(3-aminoprop-1-yl)-amino-20-dihydrotylosin (**10**) from demycinosyltylosin (DMT), respectively. The mild reaction conditions used to synthesize the second hapten, DMT derivative **10**, were necessary to overcome instabilities and acid lability of DMT. The haptens synthesized here may be further used to produce protein conjugates useful in developing antibodies against the antibiotics tilmicosin and tylosin.

Tilmicosin (**1**, Fig. 1) is a semi-synthetic macrolide antibiotic approved for veterinary use in the U.S. for cattle, swine, and poultry to combat respiratory disease. It also is used with sheep in various areas of the world. Tilmicosin (**1**) is derived from the macrolide antibiotic tylosin (**2**, Fig. 1), which is produced by *Streptomyces fradiae*. The synthesis of **1** from **2** has been described^{1,2)}. Most antimicrobial agents have limited ability for cellular penetration, but **1** was shown to accumulate in bovine lung tissue³. To investigate potential tissue binding sites of these molecules, it would be advantageous to produce monoclonal antibodies specific for **1** and **2**.

Previous attempts have been made to produce antibodies to these macrolides. JACKMAN *et al.* reported preparation of polyclonal antibodies against desmycosin, conjugated through the C20 aldehyde group, for use in determining residues of **1** in feeds and tissues⁴⁾. SILVERLIGHT *et al.* reported that such polyclonal antibodies had 96% cross-reactivity with **2**⁵⁾. WICKER *et al.* reported the use of polyclonal antibodies to **2** in a fluorescence immunoassay for detecting **2** in premix and feeds⁶⁾. The antibodies produced to **2** also cross-reacted with **1**. YAO and MAHONEY produced polyclonal antibodies to 23-deoxy-23-amino-OMT; however, these antibodies demonstrated reactivity

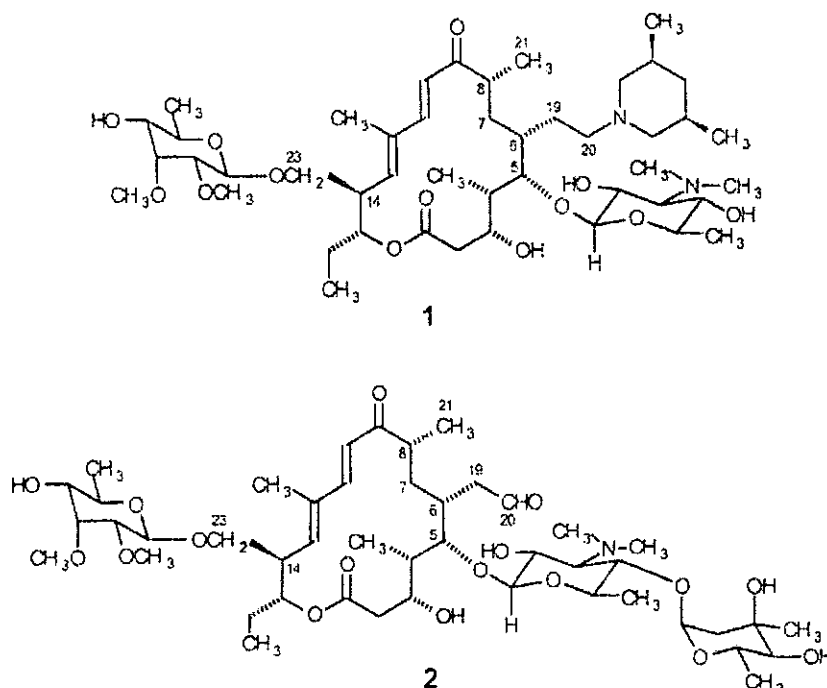
with 12-, 14-, and 16-membered macrolides possessing amino-substituted sugar moieties, regardless of the presence of neutral sugar residues⁷⁾.

For our experimental purposes, we required monoclonal antibodies that had high specificity. In order to develop antibodies specific for tilmicosin and tylosin, protein conjugates of derivatives **1** and **2** were required that would present immunologically unique portions of the molecules to the immune system. Therefore, the C20 aldehyde group would not be a good choice for the linkage site. Rather, we considered the C23 position to be a good linkage site that would present the best structural differences of **1** and **2** to the immune system. The selection of antibodies with the best specificity may then be accomplished through the monoclonal screening process.

We expected that the synthesis of analogues of **1** and **2** containing 1,3-diaminopropane linker arms would provide suitable haptens. The amino group was selected since it can be utilized with a number of standard synthetic strategies to produce protein conjugates suitable for antibody production, whereas the linker length also provides sufficient spacing to result in favorable antibody production. This paper describes the synthesis of 23-demycinosyl-23-deoxy-23-(3-aminoprop-1-yl)-

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Fig. 1. Chemical structures of tilmicosin (1), and tylosin (2).



aminotilmicosin (6) from 5-*O*-mycarinosyltylonolide (OMT) (3) and 23-demycinosyl-23-deoxy-23-(3-aminoprop-1-yl)-amino-20-dihydrotylosin (10) from the acid labile demycinosyltylosin (DMT) (7), which also contains a reactive aldehyde group.

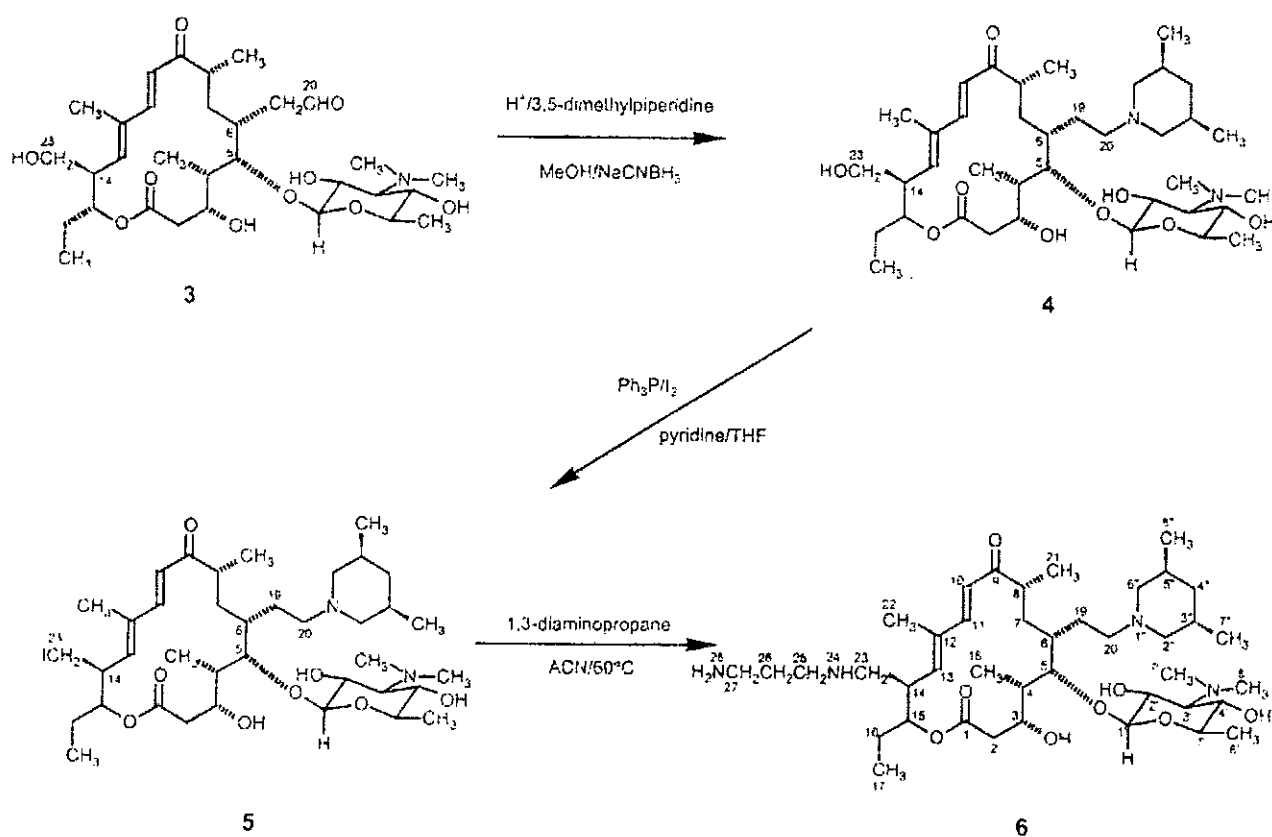
Results

The starting material for the synthesis of 6 was the fermentation product OMT (3) (Scheme 1), which was reductively aminated following the literature procedure of KIRST *et al.* using 3,5-dimethylpiperidine in MeOH at room temperature⁹. This gave 23-demycinosyl-tilmicosin (4), which was extracted into pH 4 buffer, treated with base, reextracted with CH₂Cl₂, and used without further purification. The product was converted to its 20-iodo analog (5) following the literature procedure of KIRST *et al.*⁹, employing a slow addition of iodine in dry THF at 0°C into triphenylphosphine in dry THF/dry pyridine. 23-Demycinosyl-23-deoxy-23-(3-aminoprop-1-yl)-aminotilmicosin (6) was produced by displacement of the 23-iodo group with 1,3-diaminopropane in ACN at room temperature in an overall yield of 14% according to the method of KIRST *et al.*⁹.

Synthetic manipulations of the macroide intermediate, DMT, to produce the desired hapten suitable for protein linkage proved to be a difficult endeavor for two reasons. The aldehyde group at C20 of 2 is reactive and can form unwanted by-products during the required chemical reactions or purification steps. In addition, the linkage between the carbohydrate residues (*i.e.*, between the amino sugar and the mycarosyl residue) is acid labile.

The *Streptomyces fradiae* GS48 fermentation product⁹, DMT (7), was reacted with triphenylphosphine and iodine according to a modified method of SAKAMOTO *et al.*¹⁰, employing pyridine in the presence of 4 Å molecular sieves at 0°C, to obtain 23-deoxy-23-iodo-DMT (8) (Scheme 2). There was some initial confusion during the iodination reaction using triphenylphosphine. The reaction was followed by ion spray mass spectral analysis, and showed a large peak for triphenylphosphine oxide (Ph₃P=O) at *m/z* 279 (Fig. 2). The spectrum also showed several dominant peaks at *m/z* 557 (Ph₃P=O)₂, *m/z* 579 (Ph₃P=O)₂Na⁺, *m/z* 588 (Ph₃P=O)₂OMe, *m/z* 602 (Ph₃P=O)₂Na⁺, and *m/z* 857 (Ph₃P=O)₃Na⁺ which dwarfed the product, 23-deoxy-23-iodo-DMT, peak at *m/z* 852. These peaks were evident whenever triphenylphosphine was used in the reaction. This result was verified by ion spray mass spectrometry of the isolated and crystallized Ph₃P=O. Product 8 was not

Scheme 1. Synthesis of 23-demycinosyl-23-deoxy-23-(3-aminoprop-1-yl)-aminotilmicosin (6) from OMT (3).



Scheme 2. Synthesis of 23-demycinosyl-23-deoxy-23-(3-aminoprop-1-yl)-amino-20-dihydrotylosin (10) from DMT (7).

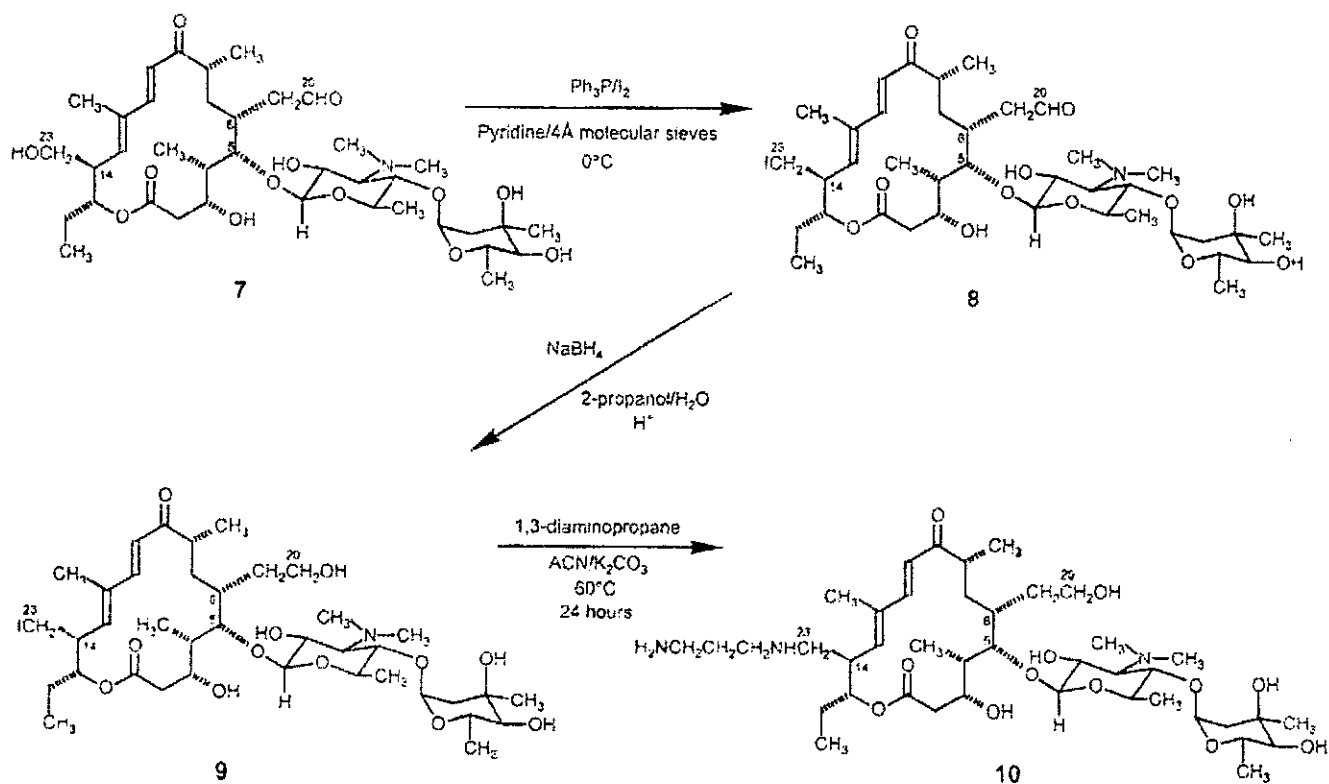


Fig. 2. ESI-TOF-MS of DMT iodination reaction products.

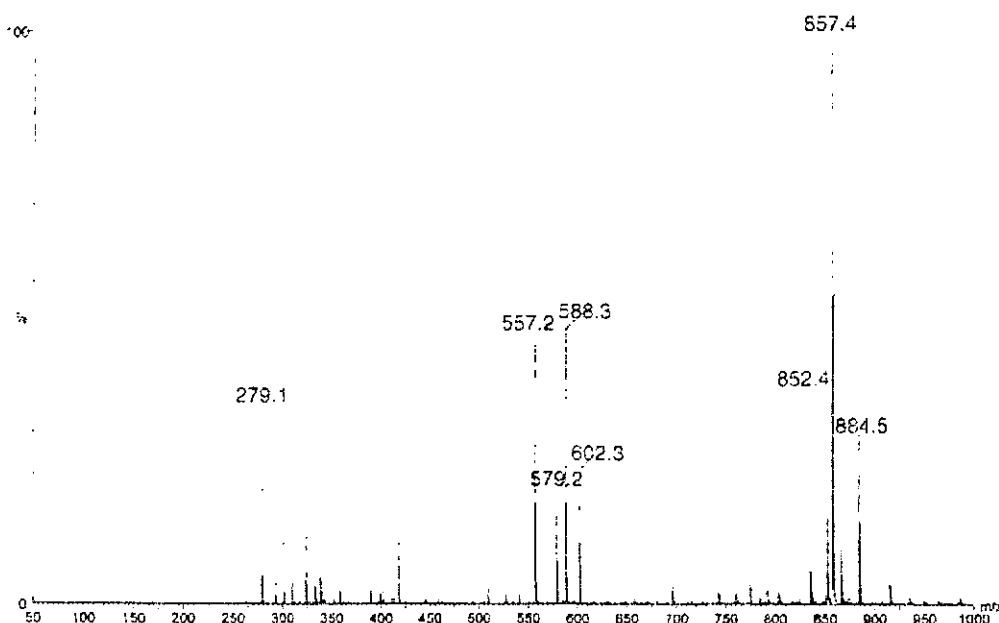
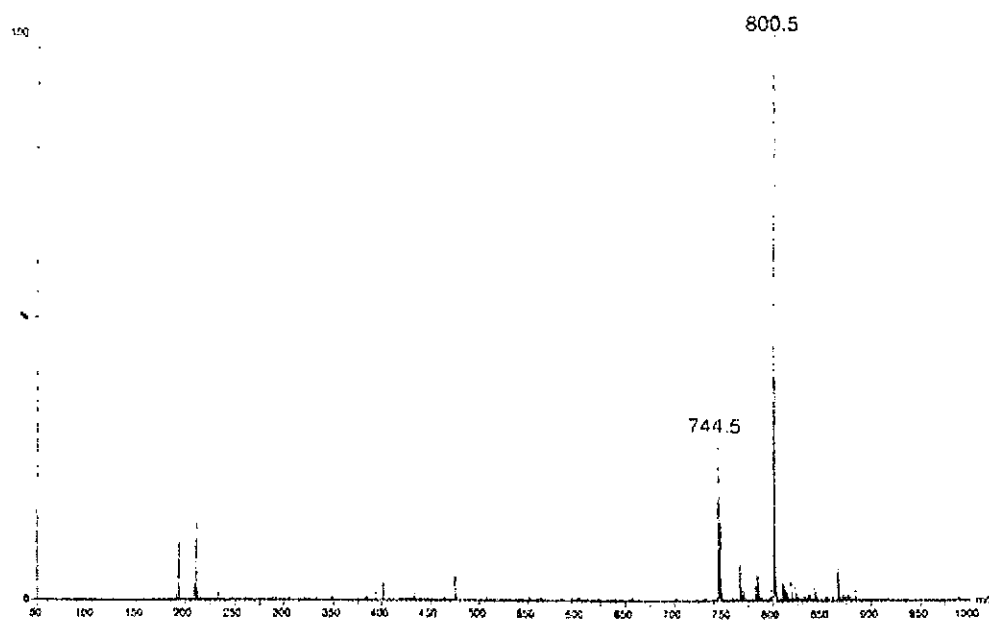


Fig. 3. ESI-TOF-MS of crude product 10.



purified due both to the reactivity of the C20 aldehyde group and the acid lability of the linkage between the two carbohydrate groups. Rather, reducing it to the alcohol immediately deactivated the aldehyde group at C20 of **8**¹⁰. Thus, product **8** was reacted with sodium borohydride in isopropanol and water to form the alcohol **9** according to

the method of KIRST *et al.*¹¹. Upon evaporation of CH₂Cl₂ from the product **9**, a glass formed, which was directly used in the next reaction. The desired hapten **10** was achieved in overall 11% yield from **7** via an iodide displacement reaction by employing a five-fold excess of 1,3-diaminopropane in ACN in the presence of potassium

carbonate, a modified method of KIRST *et al.*²⁹. The ESI-TOF-MS of the crude product **10** is shown in Fig. 3.

Since the resulting two haptens, **6** and **10** contain an NH₂ linker arm, they can be conjugated to proteins under a number of commonly used conditions. The length of the linker arm is appropriate for favorable recognition and antibody production by the immune system. Experiments to this end are on-going.

Experimental

General Analytical Methods

NMR data were acquired on a Bruker Avance 500 NMR spectrometer, and the following experiments were performed: 1D ¹H, 1D ¹³C, H-H 2D COSY, ¹H-¹H 2D ROESY, H-¹H 2D TOCSY, ¹H-¹³C 2D g-HSQC, and ¹H-¹³C 2D g-HMBC. Positive ion electrospray ionization (ESI) mass spectra were acquired using either a Micromass Quattro LC mass spectrometer or a Micromass LCT time-of-flight mass spectrometer. Most of the chemicals were purchased from Aldrich Chemical Company or prepared by the Eli Lilly and Company Natural Products Division and were used without further purification. Semi-preparative high performance liquid chromatography (HPLC) was accomplished using a Supelcosil LC-18-DB Semi-Prep 25 cm × 10 mm ID column by Supelco, Inc. (Bellefonte, PA) with the solvent system of ACN/MeOH/1% NH₄OAc buffer (56¹/₂:41:2¹/₂, v/v/v) at 4.75 ml/minute using Waters M-6000 pumps and a Waters 996 photodiode Array Detector (Waters Corporation, Milford, MA). Flash chromatography was carried out using silica gel EM Science Kieselgel 60 (230–400 mesh ASTM).

23-Demycinosyl-tilmicosin (**4**)

OMT (**3**; 3.0 g, 5.0 mmol) was dissolved in anhydrous methanol (30 ml). 3,5-dimethylpiperidine (1.3 ml, 9.8 mmol) was added followed by sodium cyanoborohydride (0.64 g, 10.1 mmol). This mixture was allowed to stir at room temperature for 24 hours and was monitored by HPLC. The mixture was then diluted with EtOAc and extracted with commercially available pH 4 buffer (3 times). The buffer was brought to pH 12 with a 50% NaOH soln. The aqueous layer was then extracted with CH₂Cl₂ (3 times). The CH₂Cl₂ layers were combined, washed with brine, and then dried over anhydrous sodium sulfate. The soln was then evaporated at room temperature under reduced pressure to give **4** as a pale yellow solid (2.7 g; 78% yield). This material was used without further purification.

23-Demycinosyl-23-deoxy-23-iodo-tilmicosin (**5**)

23-Demycinosyl-tilmicosin (**4**; 7.8 g, 11.2 mmol) was dissolved in anhydrous THF (40 ml). Triphenylphosphine (4.4 g, 16.8 mmol) was added to this soln followed by pyridine (10 ml), and the mixture was cooled on an ice bath. Iodine (4.2 g, 16.5 mmol) in anhydrous THF (15 ml) was added drop-wise to the ice-cold soln. When the addition was complete the mixture was allowed to stir at room temperature for 18 hours. The reaction was then quenched by addition of 5% sodium thiosulfate soln drop-wise until the iodine color dissipated. The mixture was then diluted with CH₂Cl₂ and washed with satd NaHCO₃. The CH₂Cl₂ was then washed with brine, dried over anhydrous sodium sulfate and evaporated at room temperature under reduced pressure to a small volume. This material was diluted with fresh CH₂Cl₂ and the resulting soln was extracted with 1N HCl. The aqueous soln was made alkaline with 5N NaOH and reextracted with EtOAc. The EtOAc soln was washed with brine, dried over anhydrous sodium sulfate and evaporated at room temperature under reduced pressure. This gave **5** as a light yellow solid (2.7 g, 30% yield). This material was used without further purification. HPLC showed that the product was approximately 68% pure.

23-Demycinosyl-23-deoxy-23-(3-aminoprop-1-yl)-aminotilmicosin (**6**)

Crude 23-demycinosyl-23-deoxy-23-iodo-tilmicosin (**5**; 3.0 g, 1.9 mmol) was dissolved in ACN (50 ml). Commercially available 1,3-diaminopropane (780 µl, 9.4 mmol) was added to this soln and the mixture was stirred at 60°C for 19 hours. The reaction mixture was cooled to room temperature and evaporated under reduced pressure. The crude product was taken up between EtOAc and freshly prepared pH 4 buffer (Note: avoid commercial buffers containing formaldehyde). The phases were separated and the aqueous phase was washed with fresh EtOAc until no more UV active material was removed. The buffer was brought to pH 12 with 5N NaOH and reextracted with CH₂Cl₂. The CH₂Cl₂ was dried over anhydrous sodium sulfate and evaporated at room temperature at reduced pressure giving **6** (0.84 g, 60% yield based on purity of **5**). To obtain ultra pure material **6** (120 mg) was purified by semi-prep HPLC, as described in the general analytical methods section. The collected material was diluted with pH 10 NaHCO₃ buffer (75 ml), and extracted with fresh CH₂Cl₂ (3 × 30 ml). The combined CH₂Cl₂ layers were dried over sodium sulfate, filtered through Whatman #41 filter paper, and evaporated under vacuum at room temperature to obtain 54 mg **6** at greater than 99% purity. ESI-MS *m/z* 751.6 (M+1)⁺; H- and ¹³C-

Table 1. ¹H- and ¹³C-NMR data for product 6.

Position	¹³ C	¹ H	Position	¹³ C	¹ H
1	173.03	—	23	51.25	2.75, 2.69
2	40.17	2.30, 1.97	24	—	—
3	67.09	3.78	25	49.10	2.66
4	42.78	1.59	26	N.A.	N.A.
5	80.00	3.60	27	N.A.	N.A.
6	32.64	1.63	28	—	—
7	34.60	1.74, 1.48	1'	105.58	4.27
8	45.85	2.60	2'	71.25	3.37
9	203.72	—	3'	71.66	2.37
10	119.39	6.50	4'	71.82	3.08
11	148.12	7.21	5'	73.92	3.31
12	135.80	—	6'	18.24	1.21
13	145.14	5.71	7', 8'	42.04	2.51
14	45.94	2.93	1''	—	—
15	75.80	4.76	2''	59.76	2.87, 1.06
16	25.93	1.88, 1.60	3''	30.87	1.83
17	9.97	0.92	4''	43.48	1.70, 0.47
18	9.29	0.99	5''	30.66	1.85
19	25.21	1.71	6''	64.38	2.68, 1.41
20	55.40	2.76, 1.99	7''	19.88	0.97
21	17.91	1.19	8''	19.98	0.80
22	13.19	1.85			

N.A. = Not Assigned

NMR data are presented in Table 1.

23-Deoxy-23-(3-aminoprop-1-yl)-amino-20-dihydro-DMT (10)

DMT (7; 0.45 g, 0.61 mmol) was dissolved in anhydrous pyridine (3 ml). Triphenylphosphine (0.32 g, 1.2 mmol) was added, followed by 4 Å molecular sieves (pyridine just covered the molecular sieves) and this mixture was stirred for 80 minutes at room temperature under dry argon. The mixture was cooled on an ice bath, and a soln of I₂ (0.46 g, 1.8 mmol) in anhydrous pyridine (1 ml) was added dropwise via a syringe. The mixture, having a noticeable brown

I₂ color, was stirred for 80 minutes in the ice bath and then at room temperature for 1 hour. The mixture was filtered through a double-layered sandwich of Whatman #2 and #41 filter paper with the aid of CH₂Cl₂ (40 ml). The filtrate was washed with satd NaHCO₃ (75 ml), and extracted three successive times with fresh CH₂Cl₂ (40 ml, 2×30 ml), and the CH₂Cl₂ layers were combined. The combined layers were washed with 1 M sodium thiosulfate (80 ml), and the aqueous layer was reextracted with fresh CH₂Cl₂ (2×30 ml). The CH₂Cl₂ layers were combined, dried over anhydrous sodium sulfate, and evaporated under vacuum at room temperature to afford 23-deoxy-23-iodo-DMT (8).

This material was dissolved in 2-propanol (6 ml) and H₂O (4 ml), and sodium borohydride (5.8 mg, 0.15 mmol) was added in small portions. Forty-five minutes after the addition was complete, the pH of the reaction mixture was adjusted to 7.0 by addition of 0.1 N H₂SO₄. The neutralized soln was evaporated to a small volume to remove the isopropanol, and then treated with satd NaHCO₃ (30 ml). The mixture was extracted with fresh CH₂Cl₂ (3×20 ml), and the combined CH₂Cl₂ extracts were washed with satd NaCl (35 ml), then dried over anhydrous sodium sulfate, filtered, and evaporated under vacuum at room temperature to give 23-deoxy-23-iodo-20-dihydro-DMT (9). This material was dissolved in ACN (5 ml) in a 10 ml Reacti-vial (Pierce, Rockford, IL). Potassium carbonate (0.17 g, 1.2 mmol) was added to the reaction mixture, followed by 1,3-diaminopropane (1.7 mmol, 0.14 ml). The reaction mixture was stirred at 60°C, and the course of the reaction was followed by TLC on HPTLC-Fertigplatten, Kieselgel 60 F₂₅₄ with a concentration zone (EM Science, Gibbstown, NJ) using EtOAc/EtOH (80:20, v/v). After 24 hours at 60°C, the mixture was cooled to room temperature, and filtered through a sandwich filter of Whatman #2 and #41 to remove the potassium carbonate. CH₂Cl₂ was used to aid filtration. The filtrate was evaporated under vacuum at room temperature. The residue was taken up with fresh CH₂Cl₂ (40 ml) and washed with satd NaHCO₃ (75 ml). The NaHCO₃ layer was extracted with fresh CH₂Cl₂ (2×30 ml), and the combined CH₂Cl₂ layers were extracted with pH 3.0 buffer (100 ml). The buffer layer was then washed with fresh CH₂Cl₂ (4×40 ml). The pH was raised to 10 with 4 N NaOH, extracted with fresh CH₂Cl₂ (40 ml), twice more with fresh CH₂Cl₂ (30 ml), and the combined CH₂Cl₂ layers were dried over anhydrous sodium sulfate, filtered, and evaporated under vacuum at room temperature to afford 51 mg of 23-deoxy-23-(3-aminoprop-1-yl)-amino-20-dihydro-DMT (10) in an overall 11% yield from DMT (7). ESI-MS *m/z* 800.5 (M+1)⁺ (base), 744.5 (base- H₂NCH₂-CH=CH)⁺ (Fig. 3).

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