

# Articles

## Isolation and Structure Determination of a New Bastadin from the Marine Sponge *Ianthella basta*

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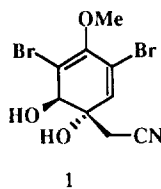
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A new metabolite **2** of bastadin class and the previously reported bastadins **3** and **4** were isolated from the sponge *Ianthella basta* collected in Indonesia.

### Introduction

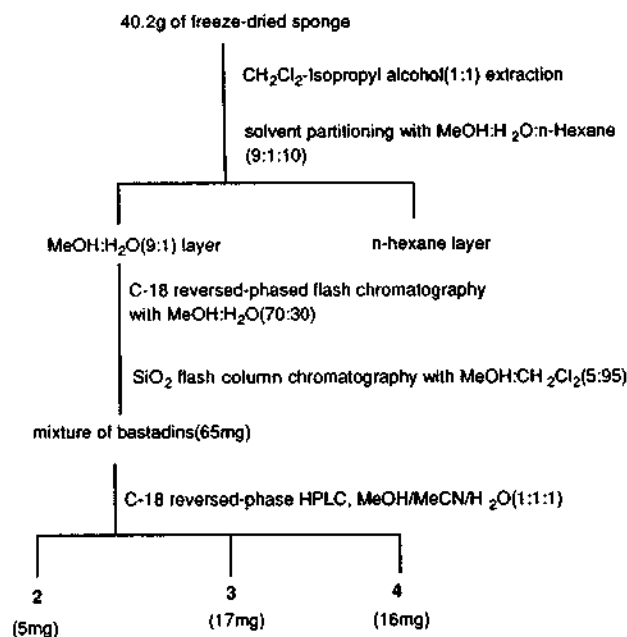
Sponges of the order Introduction Verongida contain brominated tyrosin-derived metabolites<sup>1,2</sup>. Most of these metabolites consist of modified tyrosines such as aeroplysinin-1 (**1**)<sup>3</sup>. The bastadins, macrocyclic derivatives of bromotyrosine, have previously been reported only from the sponge *Ianthella basta*<sup>4,5</sup>. We now report the isolation and structure determination of a new bastadin **2** and the previously reported compounds **3**<sup>5</sup> and **4**<sup>6</sup> from the sponge *Ianthella basta* collected in Oct., 1992 at Manado Bay, Sulawesi, Indonesia.



### Experimental

**General Experimental Procedures.** IR spectra were recorded on a Perkin-Elmer model 1420 spectrophotometer and UV spectra were obtained on a Hewlett-Packard Model 8452A diode array detector. NMR spectra were measured on a General Electric GN OMEGA 500 at 500 MHz (<sup>1</sup>H) and 125 MHz (<sup>13</sup>C). <sup>1</sup>H-NMR chemical shifts are reported in ppm with the chemical shift of the residual protons of the solvent used as internal standard. <sup>13</sup>C-NMR chemical shifts are reported in ppm by using the natural abundance of <sup>13</sup>C of the solvent as an internal standard. Mass spectra were provided by the University of Illinois mass spectrometry facility. YMC RP and Silica gel (230-400 mesh) were used for flash column chromatography. YMC ODS 30×10 mm column was used for the hplc separation.

**Collection and Isolation.** The sponge was collected on Oct., 1992 from Manado bay north along the coast of Sulawesi up to Bunaken Island in Indonesia. The sponge was identified by Dr. Peter Karuso, University of Macquarie. The freeze-dried sponge (40.2 g) was extracted for 2 days with 400 ml of CH<sub>2</sub>Cl<sub>2</sub>-iPrOH (1:1), and the residue (3.02



Scheme 1.

g) was partitioned between MeOH/H<sub>2</sub>O (9:1) and *n*-hexane. The MeOH/H<sub>2</sub>O layer yielded 2.03 g of red solid. The MeOH/H<sub>2</sub>O layer was subjected to RP-C<sub>18</sub> column chromatography and Si gel flash column chromatography sequentially using MeOH-H<sub>2</sub>O (7:3) and MeOH-CH<sub>2</sub>Cl<sub>2</sub> (5:95), yielding 65 mg of mixture of bastadins. The mixture was separated by C-18 reversed-phase hplc [ODS column, MeOH/H<sub>2</sub>O/MeCN (1:1:1), 2 ml/min] to give 5 mg of **2** as a pure white solid, 17 mg of **3**, and 16 mg of **4** as shown in scheme 1.

HMBC condition for **2**: concentration of sample, 5 mg/DMSO-d<sub>6</sub>, 0.6 ml, 256 of blocks×2048 data matrix with 64 of scan per *t*<sub>1</sub> increment, 3.0 delay period for long-range couplings, 100° shifted since bell squared filtering for *t*<sub>1</sub> and 5100 MHz line broadening for it.

HMBC condition for **4**: concentration of sample, 16 mg/DMSO-d<sub>6</sub>, 0.6 ml, 256 of blocks×2048 data matrix with 128

Table 1. NMR Data for 2 (DMSO-d<sub>6</sub>)

Position	<sup>13</sup> C	<sup>1</sup> H (J, Hz)	HMBC	NOE
1	27.42	3.55, 2H, s	2, 3, 36, 37, 38	36, 38
2(NOH)	151.42	11.65, 1H, s	1	
3	163.04		1, 5	
4(NH)		8.06, 1H, t (6.0)	3, 5	
5	40.58	3.28, 2H, m	3, 6, 7	
6	33.89	2.71, 2H, t (6.6)	5, 7, 8, 12	5, 8, 12
7	139.75		6	
8	133.24	7.59, 1H, s	6, 9, 10	
9	117.69		8	
10	146.56		8, 12	
11	117.69			
12	133.24	7.59, 1H, s		
14	144.07		OH-15, 16, 19	
15(OH)	144.26	9.25, 1H, s	14, 16, 17, 19	
16	116.55	6.81, 1H, d (8.2)	14, 15, 17, 18	
17	122.88	6.67, 1H, dd (8.2, 1.8)	15, 16, 18, 19	
18	129.49		16, 20	
19	112.52	6.17, 1H, d (1.8)	14, 15, 17, 18, 20	
20	33.27	2.67, 2H, t (6.6)	17, 18, 19, 21	17, 19, 21
21	40.38	3.28, 2H, m	18, 20, 23	
22(NH)		7.94, 1H, t (6.0)	21, 23	
23	163.28		25	
24(NOH)	150.68	11.86, 1H, s	25	
25	28.72	3.68, 2H, s	23, 24, 26, 27, 31	27, 31
26	137.78		25	
27	133.68	7.63, 1H, s	25, 28, 29, 30	
28	117.69		27	
29	146.11		27, 31	
30	117.19			
31	133.68	7.63, 1H, s		
33	141.98		OH-34, 38	
34(OH)	144.78	9.99, 1H, s	35, 36, 38	
35	109.82		OH-34, 36	
36	126.95	7.07, 1H, d (1.6)	1, 34, 35, 38	
37	128.19		1, 38	
38	112.75	6.15, 1H, d (1.6)	1, 33, 34, 36, 37	

s; singlet, d; doublet, t; triplet, dd; doublet of doublet, m; multiplet

of scan per  $t_1$  increment, 3.0 delay period for long-range couplings, 100° shifted sine bell squared filtering for  $t_1$  and 5300 MHz line broadening for it.

**Bastadin (2).** Hrfabms  $m/z$  [M+H]<sup>+</sup> 1018.7792 (calcd for C<sub>34</sub>H<sub>28</sub>N<sub>2</sub>O<sub>8</sub><sup>79</sup>Br<sub>3</sub><sup>81</sup>Br<sub>2</sub>, 1018.7783); hreims  $m/z$  499.8166 (calcd for C<sub>16</sub>H<sub>9</sub>N<sub>2</sub>O<sub>2</sub><sup>79</sup>Br<sub>2</sub><sup>81</sup>Br, 499.8194; ir  $\nu_{max}$  (Nujol) in cm<sup>-1</sup> 3500-3300, 1652, 1630, 1480, 1240, 1225; UV  $\lambda_{max}$  (MeOH) in nm (log  $\epsilon$ ) 244 (3.50), 240 (3.49), 280 (2.49), 294 (3.66); <sup>1</sup>H-NMR (in MeOH-d<sub>4</sub>),  $\delta$  7.54 (4H, s), 7.10 (1H, d,  $J=1.8$  Hz), 6.84 (1H, d,  $J=8.1$  Hz), 6.75 (1H, dd,  $J=8.1, 1.8$  Hz), 6.27 (1H, d,  $J=1.8$  Hz), 6.23 (1H, d,  $J=1.8$  Hz), 3.83 (2H, s), 3.66 (1H, s), 3.45-3.27 (4H, m), 2.75 (2H, t,  $J=7.0$  Hz), 2.67 (2H, t,  $J=7.0$  Hz); eims (rel. int.) 504 (3.3), 502 (3.1), 500 (4.0), 499 (2.3), 498 (3.0), 485 (10.0), 483 (17.0), 481 (8.6), 440 (32.7), 442 (17.5), 483 (15.7); <sup>1</sup>H- and <sup>13</sup>C-NMR see Table 1.

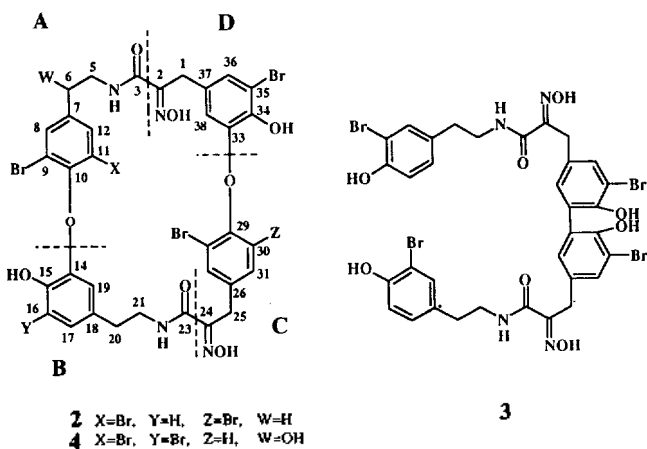
**Bastadin 3(3).** <sup>1</sup>H-NMR (in MeOH-d<sub>4</sub>);  $\delta$  7.36 (2H, d,  $J=2.2$  Hz), 7.28 (2H, d,  $J=2.2$  Hz), 7.12 (2H, d,  $J=2.2$  Hz), 6.89 (2H, dd,  $J=8.3, 2.2$  Hz), 6.72 (2H, d,  $J=8.3$  Hz), 3.80 (4H, s), 3.37 (4H, t,  $J=6.9$  Hz), 2.66 (4H, t,  $J=7.3$  Hz); <sup>13</sup>C-NMR,  $\delta$  165.91, 153.92, 153.74, 153.61, 134.16, 133.29, 133.01, 132.27, 130.31, 130.07, 128.80, 117.21, 114.09, 110.67, 42.09, 35.19, 28.83; Irradiation at  $\delta$  3.80 (H-1) induced nuclear Overhauser enhancement (nOe) at  $\delta$  7.28 and 7.12. Irradiation at  $\delta$  2.62 (H-6) induced nOe at  $\delta$  3.37, 7.36 and 6.89. Irradiation at  $\delta$  3.37 (H-5) induced nOe at  $\delta$  2.62, 7.36 and 6.89.

**Bastadin 12 (4).** <sup>1</sup>H-NMR (in DMSO-d<sub>6</sub>);  $\delta$  11.88 (N-OH, s), 9.91 (-OH, brs), 7.90 (22-NH, t,  $J=5.6$  Hz), 7.79 (4-NH, t,  $J=5.6$  Hz), 7.67 (H-8 and -12, brs), 7.48 (H-27, d,  $J=1.6$  Hz), 7.15 (H-36, d,  $J=1.6$  Hz), 7.13 (H-31, dd,  $J=8.2, 1.6$  Hz), 7.02 (H-19, d,  $J=1.7$  Hz), 6.76 (H-30, d,  $J=8.2$  Hz), 6.45 (H-38, d,  $J=1.6$  Hz), 6.11 (H-17, d,  $J=1.7$  Hz), 4.65 (H-6, m),

3.68 (H-25, q,  $J=13.1$  Hz), 3.59 (H-1, q,  $J=13.1$  Hz), 3.39 (H-5, m), 3.10 (H-21, m), 2.59 (H-20, t,  $J=7.6$  Hz);  $^{13}\text{C}$ -NMR (in DMSO- $d_6$ ),  $\delta$  162.98 (C-23), 162.92 (C-3), 151.22 (C-2), 151.10 (C-24), 151.06 (C-29), 146.62 (C-10), 145.00 (C-33), 144.16 (C-34), 144.00 (C-15), 141.78 (C-14), 141.02 (C-7), 134.24 (C-26), 133.48 (C-27), 130.68 (C-18), 130.13 (C-8 and 12), 130.09 (C-37), 129.72 (C-31), 128.08 (C-36), 126.13 (C-19), 119.64 (C-9, 11 and 30), 117.26 (C-38), 117.61 (C-35), 113.16 (C-28), 111.98 (C-17), 110.26 (C-16), 69.84 (C-6), 46.80 (C-5), 46.17 (C-21), 33.19 (C-20), 28.44 (C-25), 27.45 (C-1).

## Results and Discussion

The  $\text{CH}_2\text{Cl}_2/i\text{PrOH}$  (1:1) extract of the sponge showed cytotoxicity against KB<sup>7</sup> cell line (MIC of 1  $\mu\text{g}/\text{ml}$ , 3<sup>+</sup>). The extract was further partitioned between MeOH/ $\text{H}_2\text{O}$  (9:1) and *n*-hexane. Red solid was obtained from the MeOH/ $\text{H}_2\text{O}$  layer after concentration *in vacuo*. Separation of the mixture was accomplished sequentially by application of RP-C<sub>18</sub> flash column chromatography [MeOH/ $\text{H}_2\text{O}$  (7:3)], Si gel flash column chromatography [MeOH/ $\text{CH}_2\text{Cl}_2$  (5:95)], and C-18 reversed-phased high performance liquid chromatography [MeOH/ $\text{H}_2\text{O}/\text{MeCN}$  (1:1:1)] to give three different compounds 2, 3 and 4. The compounds 3 and 4 were identified as bastadins 3 and 12 by comparison of their spectral data with the literature values<sup>5,6</sup>.



A molecular formula of  $\text{C}_{34}\text{H}_{27}\text{Br}_5\text{N}_4\text{O}_8$  for 2 was established by hrfabms, which indicates that 2 was isomeric with bastadins 5 and 15<sup>8</sup>, but a preliminary inspection of  $^1\text{H}$ -NMR spectrum suggested that 2 was comprised of a trisubstituted and three tetrasubstituted aromatic rings (two symmetric and one unsymmetric). The  $^1\text{H}$ -NMR spectrum (DMSO- $d_6$ ) of 2 showed exchangeable proton signals at  $\delta$  11.65 (1H, s), 11.86 (1H, s), 9.99 (1H, s), 9.25 (1H, s), 8.06 (1H, t,  $J=6.0$  Hz), 7.94 (1H, t,  $J=6.0$  Hz), and the  $^{13}\text{C}$ -NMR spectrum displayed all of 34 carbon signals (Table 1). Exchangeable triplet signals at  $\delta$  7.94 (NH) and 8.06 (NH), and singlet signals at  $\delta$  11.65, 11.86, 9.25, and 9.99 were not shown in MeOH- $d_4$ .  $^1\text{H}$ -NMR data in MeOH- $d_4$  are shown in experimental section.

The H-C and C-C connectivities were established by HMQC and HMBC NMR experiments. An exchangeable triplet proton at  $\delta$  8.06 showed correlations to an amide carbonyl carbon ( $\delta$  163.04, C-3) and a carbon signal at  $\delta$  40.58 (C-5). A two-proton triplet at  $\delta$  2.71 also showed a correlation

to a two-carbon signal at  $\delta$  133.24 (C-8 and 12) as well as correlations to the methylene and an aromatic carbon signal at  $\delta$  40.58 (C-5) and 139.75 (C-7). A two-proton multiplet at  $\delta$  3.28 showed correlations to C-3 and C-7, which indicated the connectivity of the A and D subunits. The H-C correlation of a two-proton singlet at  $\delta$  7.59 to carbon signals at  $\delta$  33.89 (C-6), 117.69 (2C's, C-9 and 11), and 146.56 (C-10) and a comparison of chemical shifts for this portion of the molecule with those of bastadins 5 and 15<sup>8</sup> firmly secured the structure of the A portion.

The H-C correlations were observed between a broad two-proton singlet at  $\delta$  3.55, typical for benzylic protons adjacent to an oxime of the bastadins<sup>5,6,9,10</sup>, to the  $\alpha$ -oximino amide carbons (C-2 and 3) of the upper portion of the molecule. The benzylic protons also showed correlations to aromatic carbon signals at  $\delta$  128.19 (C-37), 126.95 (C-36), and 112.75 (C-38). A doublet at  $\delta$  7.07, meta-coupled to a doublet at  $\delta$  6.15, showed a H-C correlation to C-38, as well as to carbon signal at  $\delta$  144.78 (C-34). The proton at  $\delta$  6.15 also showed correlations to C-33 ( $\delta$  141.98) and C-34 ( $\delta$  144.78), thus establishing the 1, 3, 4, 5 substitution pattern of the D subunit and the structure of the upper part of the molecule.

A broad two-proton singlet at  $\delta$  3.68 arising from the benzylic protons adjacent to the other oxime showed correlations to the  $\alpha$ -oximino amide carbon signals of the lower portion of the molecule at  $\delta$  150.68 (C-24) and 163.28 (C-23), as well as to carbon signals at  $\delta$  137.78 (C-26) and 133.68 (C-27 and 31). A two-proton singlet at  $\delta$  7.63 showed correlations to carbon signals at  $\delta$  117.19 (2C's, C-28 and 30) and 146.11 (C-29). The structure of the C portion was confirmed by H-C correlation and a comparison of chemical shifts for this portion of the molecule with those reported for bastadin 6<sup>5</sup>.

An exchangeable proton signal at  $\delta$  7.94 (H-22) was correlated to an amide carbonyl at  $\delta$  163.28 (C-23) and a carbon signal at  $\delta$  40.38 (C-21). A two-proton triplet at  $\delta$  2.67 also showed correlations at C-21 ( $\delta$  40.38) and the aromatic carbon signals of subunit B at  $\delta$  129.49 (C-18), 122.88 (C-17), and 112.52 (C-19). A correlation of broad two-proton multiplet at  $\delta$  3.28 to C-23 and C-18 confirmed the connection of B and C subunits. A one-proton doublet at  $\delta$  6.81 ( $J=8.2$  Hz), ortho-coupled to a proton at  $\delta$  6.67 ( $J=8.2, 1.8$  Hz) which was meta-coupled to a proton at  $\delta$  6.17 ( $J=1.8$  Hz), also showed correlations to C-14 ( $\delta$  144.07), C-15 ( $\delta$  144.26), and C-18 ( $\delta$  129.46). A one-proton doublet at  $\delta$  6.17 ( $J=1.8$  Hz), meta-coupled to a proton at  $\delta$  6.67, was also correlated to carbon signals at  $\delta$  144.07 (C-14) and 129.49 (C-18). This H-C correlation securely established the structure of the B portion.

Irradiation of the benzylic protons in 2 [ $\delta$  3.55 (H-1)  $\delta$  3.65 (H-25)] induced nuclear Overhauser enhancement (nOe) in two aromatic resonances (H-36, 38 and H-27, 31), respectively, showing that the carbon containing the benzylic proton was adjacent to this ring.

Irradiation of the two-proton triplet signal at  $\delta$  2.71 (H-6) induced nOe in these two aromatic signals at  $\delta$  7.55 (H-8 and 12) and methylene proton signal at  $\delta$  3.28 (H-5), which demonstrated that the two-carbon fragment containing the benzylic proton was adjacent to this ring. The 9,11-dibromo ring A would have the symmetry as illustrated in the coalescence and nOe results described above.

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## Kinetic Studies on the Reaction of the Heterobimetallic Anion, (OC)<sub>5</sub>CrMn(CO)<sub>5</sub><sup>-</sup>M<sup>+</sup> (M<sup>+</sup>=Na<sup>+</sup>, PPN<sup>+</sup>) with CH<sub>3</sub>I

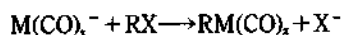
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The reaction of the heterobimetallic anion, (OC)<sub>5</sub>CrMn(CO)<sub>5</sub><sup>-</sup>M<sup>+</sup> (M<sup>+</sup>=Na<sup>+</sup>, PPN<sup>+</sup>) with CH<sub>3</sub>I was proven to be overall 1st order with respect to [(OC)<sub>5</sub>CrMn(CO)<sub>5</sub><sup>-</sup>]. This reaction mechanism may be described in terms of the consecutive reaction pathway in which Cr(CO)<sub>5</sub>(THF) may be an important intermediate, leading to the corresponding products such as MeMn(CO)<sub>5</sub> and ICr(CO)<sub>5</sub><sup>-</sup>, accordingly. The counterion effect on this reaction was also elucidated.

## Introduction

The displacement of X<sup>-</sup> from RX (RX=organic halides) by anionic transition metal carbonylates usually follows a second order rate law, reaction rate=*k*<sub>2</sub>[M(CO)<sub>x</sub><sup>-</sup>][RX].



For the last two decades many chemists have studied the effects of ion pairing on the structure and reactivity of transition metal organic salts.<sup>1</sup>

Particularly much effort has been directed to the mononuclear carbonylates such as HFe(CO)<sub>4</sub><sup>-2</sup>, Mn(CO)<sub>5</sub><sup>-3</sup>, Co(CO)<sub>4</sub><sup>-4</sup>, CpMo(CO)<sub>3</sub><sup>-5</sup>, and CpW(CO)<sub>3</sub><sup>-6</sup>. For alkyl halides a "normal" counterion effect was observed; the less associated the anion is with its cation, the greater is its nucleophilicity. However, an "inverse" counterion effect was detected in the reactions of Co(CO)<sub>4</sub><sup>-</sup>, Mn(CO)<sub>5</sub><sup>-</sup>, and CpMn(CO)<sub>3</sub><sup>-</sup> with the activated organic halides such as benzyl halides or allyl halides.<sup>7</sup> In such cases, tight ion pairs were observed to enhance the corresponding reaction rates.

For several years we tried to understand the reactions using the anionic heterobimetallics such as L(CO)<sub>4</sub>CrMn(CO)<sub>4</sub>L<sup>-</sup>M<sup>+</sup> (M<sup>+</sup>=Na<sup>+</sup>, PPN<sup>+</sup> (bis-(triphenylphosphoranylidene) ammonium ion); L=CO, PR<sub>3</sub>).<sup>8,9</sup> In this paper, we would like to elucidate the counterion effect on the reaction of the heterobimetallic anion, (OC)<sub>5</sub>CrMn(CO)<sub>5</sub><sup>-</sup>M<sup>+</sup> (M<sup>+</sup>=Na<sup>+</sup>, PPN<sup>+</sup>)

with methyl iodide.

## Experimental

An inert-atmosphere glove box and Schlenk line and high-vacuum techniques were employed for most of sample transfers and manipulations. Infrared spectra were recorded on Perkin-Elmer 238B spectrophotometer. The temperature was controlled with Haake A81 thermostat. Most of weighing and transfers of compounds were done under an Ar atmosphere in the glove box (Vacuum Atmosphere Co.).

Photoreactions were performed using a 450 watt Hg vapor lamp covering a rather broad range of UV-VIS wavelengths. Solvents were distilled under N<sub>2</sub> atmosphere from appropriate drying and O<sub>2</sub> scavenging agents: tetrahydrofuran (THF) and toluene, Na<sup>0</sup>/benzophenone/diglyme; methylene chloride, P<sub>2</sub>O<sub>5</sub>; acetonitrile, CaH<sub>2</sub> followed by P<sub>2</sub>O<sub>5</sub>; ethanol, Mg<sup>0</sup>/I<sub>2</sub>. All reagents were purchased from ordinary vendors and used as received without further purification.

**Preparation of PPN<sup>+</sup>CrMn(CO)<sub>10</sub><sup>-</sup>.** The following modification of the preparation of this complex in the literature<sup>10</sup> was utilized to give good yields of the complex. To Na<sup>+</sup>Mn(CO)<sub>5</sub> (4.5 mmol) was added the THF solution (150 ml) of Cr(CO)<sub>5</sub>(THF) (4.5 mmol) freshly prepared from the photolytic reaction. This solution was stirred for more than 3 hr at room temperature. This reaction solution was then passed through a Celite column and concentrated to about 1/4 of its initial volume under vacuum. Hexane (50 ml) was

\*PPN<sup>+</sup> = bis(triphenylphosphoranylidene)ammonium ion