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# Acetylenic Acids from the Aerial Parts of *Nanodea muscosa*<sup>1</sup>

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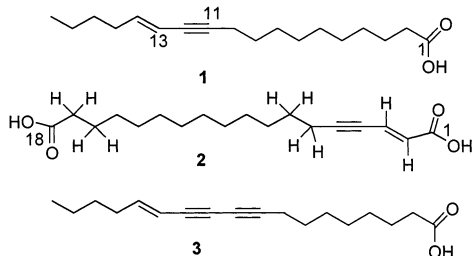
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Received October 31, 2002

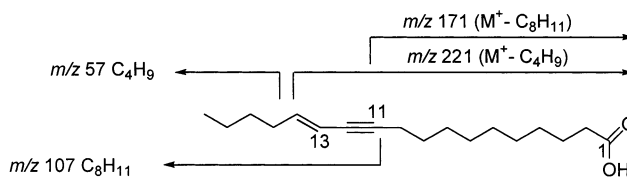
The aerial parts of *Nanodea muscosa*, collected in Chile, yielded two new acetylenic acids. Their structures were elucidated by spectroscopic analyses, including 2D NMR techniques, as (13*E*)-octadec-13-en-11-ynoic acid (**1**) and (2*E*)-octadec-2-en-4-ynedioic acid (**2**). Compound **2** constitutes the first example of a conjugated ene-yne fatty diacid isolated from a natural source. Compounds **1** and **2** did not exhibit toxicity toward a panel of DNA damage checkpoint defective yeast mutants or show affinity for the 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, D<sub>2</sub>, and H<sub>1</sub> receptors.

As part of a search for new bioactive compounds from species used in Chilean folk medicine,<sup>1–3</sup> *Nanodea muscosa* Banks (Santalaceae)<sup>4</sup> has been studied. This species is a small herb found in extreme southern regions of South America, including Patagonia, Tierra del Fuego, and the Falkland Islands. To the best of our knowledge, there have been no previous phytochemical studies on the genus *Nanodea*, but other phytochemical studies of various species belonging to the same family have revealed the presence of saponins,<sup>5</sup> alkaloids,<sup>6</sup> coumarins,<sup>7</sup> flavonoid glycosides,<sup>8</sup> lignans,<sup>9</sup> iridoids,<sup>10</sup> sesquiterpenes,<sup>11</sup> and fatty acids.<sup>12</sup>

Six fatty acids were isolated from the EtOH extract of the aerial part of *N. muscosa*, of which two, **1** and **2**, are new to the literature. This extract also yielded the known fatty acids arachidic acid, palmitic acid, myristic acid, and (13*E*)-octadec-13-ene-9,11-dienoic acid (**3**). This last compound has been previously reported by Gunstone et al. and was isolated from *Santalum acuminatum*,<sup>13</sup> but its <sup>1</sup>H NMR and <sup>13</sup>C NMR data were not published. For this reason, we have included its spectroscopic data in the Experimental Section. The structures of compounds **1** and **2** were determined by means of <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic studies, including heteronuclear correlations (HSQC and HMBC). Compound **2** constitutes the first example of an  $\alpha,\omega$  fatty diacid with a conjugated enyne system isolated from a natural source. Herein we also draw a hypothesis regarding its possible biosynthesis.



The polyunsaturated fatty acid **1** was isolated as a pale yellow oil and exhibited a structure similar to that of



**Figure 1.** Mass spectral fragmentation of compound **1**.

compound **3**. It did not show the usual C-9/C-10 unsaturation present in most of acetylenic acids isolated from Santalaceae species. Its UV spectrum revealed a typical ene-yne chromophore<sup>14</sup> absorption [ $\lambda_{\max}$  (EtOH) 228 nm]. The main signals in its <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>) were a triplet at  $\delta$  0.88 ( $J$  = 6.6 Hz, 3H), assigned to a methyl group linked to a  $-\text{CH}_2-$  group, a broad singlet between  $\delta$  1.15 and 1.50 (18H) for  $9 \times -\text{CH}_2-$  groups, a quartet at  $\delta$  2.07 ( $J$  = 7.4 Hz, 2H), a triplet at  $\delta$  2.35 ( $J$  = 7.4 Hz, 4H), and a doublet of triplets at  $\delta$  6.04 ( $J$  = 15.8, 7.4 Hz, 1H). The doublet of triplets is characteristic of an olefinic hydrogen linked to a  $\text{CH}_2$  and *trans* to the other double bond hydrogen, which appears as a doublet at  $\delta$  5.44 ( $J$  = 15.8 Hz). In the HRMS, **1** showed the molecular formula C<sub>18</sub>H<sub>30</sub>O<sub>2</sub>, and a DEPT experiment revealed two quaternary carbons at  $\delta$  79.3 and 88.5, typical of acetylenic carbons. The position of the ene-yne system between C-11 and C-14 was established by the three-bond couplings observed in HMBC experiments and confirmed by major fragments detected in the MS spectrum, which are shown in Figure 1.

Unsaturated fatty acids can be produced via several biosynthetic routes,<sup>15</sup> but frequently they arise by desaturation of the corresponding alkanolic acids and further desaturations in subsequent steps. Double bonds at position 9 are rather common, but unsaturation can also occur at other positions of the chain. The position of the second and further desaturations depends very much on the particular organism. For instance, nonmammalian enzymes tend to introduce additional double bonds between the existing double bond and the methyl terminus.<sup>16</sup> Moreover, the acetylenic bonds are also predominantly produced by further desaturation of olefinic systems in fatty acid derived molecules.<sup>17</sup> Considering all this, it could be suggested that the *cis*-vaccenic acid [(11*Z*)-octadec-11-enoic acid] may be the precursor of **1**.

Compound **2** was isolated as an oil with a molecular formula C<sub>18</sub>H<sub>28</sub>O<sub>4</sub>. Its UV spectrum also showed the presence of a conjugated ene-yne system [ $\lambda_{\max}$  (EtOH) 245, 319

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<sup>†</sup> Dedicated to the memory of Prof. Antonio González-González.

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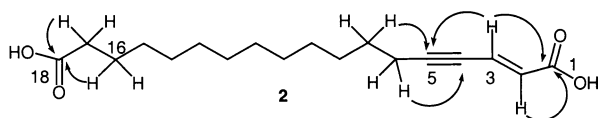


Figure 2. HMBC correlations of compound 2.

nm].<sup>14</sup> In its <sup>1</sup>H NMR spectrum were as main signals a doublet at  $\delta$  6.14 ( $J$  = 15.8 Hz, 1H) and a doublet of triplets at  $\delta$  6.84 ( $J$  = 15.8, 2.1 Hz, 1H). These signals are characteristic of the hydrogens of an  $\alpha,\beta$ -unsaturated carbonyl moiety. Its <sup>13</sup>C NMR spectrum showed the presence of two carboxylic carbons at  $\delta$  179.5 and 171.0, two olefinic carbons at  $\delta$  128.6 and 128.4, and two acetylenic carbons at  $\delta$  102.5 and 78.0, and the rest of the signals corresponded to aliphatic methylene units. The position of the ene-yne system at C-2 through C-5 was established by HMBC experiments, which showed the three-bond couplings depicted in Figure 2. In the COSY experiments, a long-range correlation was observed between H-3 and H-6. All the data presented above led us to establish the structure of compound 2 shown in Figure 2.

The  $\alpha,\omega$  diacids are compounds commonly found in the cutin of many plants.<sup>18</sup> Royal jelly also contains short-chained 8–10 carbon diacids.<sup>19</sup> These two examples show that nature offers saturated  $\alpha,\omega$  alkane dicarboxylic acids with up to 18 carbon atoms. To the best of our knowledge, 2 is the first example of an  $\alpha,\omega$  C<sub>18</sub> unsaturated diacid. Compound 2 could be derived from an oxidative cleavage of a double bond present in a monoacid with a greater number of carbons. Besides, it could also be produced by oxidation of an  $\omega$ -unsaturated fatty acid. For example, some compounds with a terminal double bond such as octadec-17-en-9-ynoic acid have been isolated from other species of Santalaceae.<sup>20</sup> Recently, a new cytochrome P450 (CYP94A5) from *Nicotiana tabacum*<sup>21</sup> has been characterized, which is able to catalyze the oxidation of fatty acids to the  $\omega$ -alcohol and to the corresponding diacid. Consistent with these findings, we offer yet another possible pathway to compound 2 from 16(*E*)-octadec-16-en-14-ynoic acid as precursor (Supporting Information).

A set of isogenic yeast strains defective for the G1/S and G2/M DNA damage checkpoints was used in order to detect the potential cytotoxicity specific for these genetic backgrounds of compounds 1 and 2.<sup>22</sup> Neither 1 nor 2 exhibited cytotoxicity for any of the eight yeast strains tested using concentrations ranging from 0 to 100  $\mu$ g/mL. As these strains are hypersensitive to DNA-damaging agents, we can therefore conclude that both compounds are not genotoxic. The viability of the wild-type strain was not affected by the treatment, suggesting that compounds 1 and 2 did not exhibit antifungal activity. Compounds 1 and 2 were also evaluated for binding to 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, D<sub>2</sub>, and H<sub>1</sub> receptors, but they showed low values.

## Experimental Section

**General Experimental Procedures.** UV spectra were collected in absolute EtOH on a JASCO V-560 spectrophotometer. IR spectra were taken on a Bruker IFS28/55 spectrophotometer. <sup>1</sup>H and <sup>13</sup>C spectra were recorded in CDCl<sub>3</sub> at 300 and 75 MHz, respectively, with TMS as internal reference. The 2D NMR experiments were conducted on a Bruker WP-400 SY NMR spectrometer in CDCl<sub>3</sub> at 400 MHz. High- and low-resolution mass spectra were obtained on a VG Autospec spectrometer. Macherey-Nagel polygram Sil G/UV<sub>254</sub> and preparative TLC Sil G-100UV<sub>254</sub> foils were used for TLC. Silica gel (0.2–0.63 mm) and Sephadex LH-20 were used for column chromatography.

**Plant Material.** The plant material was collected at Magallanes, Chile, in January 1993, and it was identified by the botanist A. Peñaloza. A voucher specimen is on file at the Herbarium of the Escuela de Química y Farmacia, Universidad de Chile.

**Extraction and Isolation.** Dried leaves of *Nanodea muscosa* (0.60 kg) were extracted with EtOH at room temperature for one week. The dried extract (0.017 kg) was chromatographed on silica gel and Sephadex LH-20 using as eluent mixtures of *n*-hexanes–EtOAc of increasing polarity. Five fractions, A–E, were separated, and A, B, and C were studied and chromatographed on Sephadex LH-20 eluted with *n*-hexanes–CHCl<sub>3</sub>–MeOH (2:1:1). Some of the eluted products were separated by preparative TLC. Melissyl alcohol<sup>23</sup> (14.7 mg), arachidic acid<sup>24</sup> (12.8 mg), myristic acid<sup>24</sup> (18.5 mg), and 3 (15 mg) were separated from fraction A. Fraction B yielded palmitic acid<sup>25</sup> (14.4 mg),  $\beta$ -sitosterol<sup>26</sup> (4.3 mg), and 1 (58.8 mg). Fraction C afforded ethyl *p*-hydroxy cinnamate<sup>27</sup> (20.3 mg), chlorophyll *b*<sup>28</sup> (4.3 mg), and 2 (93.0 mg).

**(13E)-Octadec-13-en-11-ynoic acid (1):** yellow oil; IR (CHCl<sub>3</sub>)  $\nu_{\max}$  2929, 2857, 2215, 2093, 1710, 1463, 1413, 1247, 955 cm<sup>-1</sup>; UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 228 (3.59) nm; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.88 (3H, t,  $J$  = 6.6 Hz, H<sub>3</sub>-18), 1.15–1.50 (18H, bs, H-3, H-4, H-5, H-6, H-7, H-8, H-9, H-16, H-17), 2.07 (2H, q,  $J$  = 7.4 Hz, H-15), 2.35 (4H, t,  $J$  = 7.4 Hz, H<sub>2</sub>-2, H<sub>2</sub>-10), 5.44 (1H, d,  $J$  = 15.8 Hz, H-13), 6.04 (1H, dt,  $J$  = 15.8, 7.4 Hz, H-14); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  14.0 (CH<sub>3</sub>, C-18), 19.3 (CH<sub>2</sub>, C-10), 22.5 (CH<sub>2</sub>, C-17), 24.6 (CH<sub>2</sub>, C-3), 28.6 CH<sub>2</sub>, 28.7 CH<sub>2</sub>, 28.8 CH<sub>2</sub>, 28.9 CH<sub>2</sub>, 29.2 CH<sub>2</sub>, 29.3 CH<sub>2</sub> (C-4, C-5, C-6, C-7, C-8, C-9), 31.6 (CH<sub>2</sub>, C-16), 32.9 (CH<sub>2</sub>, C-15), 33.9 (CH<sub>2</sub>, C-2), 79.3 (C, C-11), 88.5 (C, C-12), 109.7 (CH, C-13), 143.4 (CH, C-14), 179.7 (C, C-1); EIMS  $m/z$  278 [M<sup>+</sup>] (10), 221 (8), 150 (70), 107 (30), 79 (100), 57 (25); HREIMS  $m/z$  278.2249 (calcd for C<sub>18</sub>H<sub>30</sub>O<sub>2</sub>, 278.2246), 221.1541 (calcd for C<sub>14</sub>H<sub>21</sub>O<sub>2</sub>, 278.1542), 171.1379 (calcd for C<sub>10</sub>H<sub>19</sub>O<sub>2</sub>, 171.1385), 107.0866 (calcd for C<sub>8</sub>H<sub>11</sub>, 107.0861), 57.0699 (calcd for C<sub>4</sub>H<sub>7</sub>, 57.0704).

**(2E)-Octadec-2-en-4-ynedioic acid (2):** pale yellow oil; IR (CHCl<sub>3</sub>)  $\nu_{\max}$  2931, 2854, 2360, 2213, 1697, 1620, 1466, 1411, 1305, 1206, 1095, 940 cm<sup>-1</sup>; UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 319.4 (4.36), 245.2 (4.68) nm; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.35 bs (16H, H-8, H-9, H-10, H-11, H-12, H-13, H-14, H-15), 1.56 (2H, quint.,  $J$  = 7.0 Hz, H-7), 1.64 (2H, m, H-16); 2.36 (4H, t,  $J$  = 7.2 Hz, H-6, H-17), 6.14 (1H, d,  $J$  = 15.8 Hz, H-2), 6.84 (1H, dt,  $J$  = 15.8, 2.1 Hz, H-3); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  19.7 (CH<sub>2</sub>, C-6), 24.4 (CH<sub>2</sub>, C-16), 28.8 CH<sub>2</sub>, 28.7 CH<sub>2</sub>, 28.6 CH<sub>2</sub>, 28.5 CH<sub>2</sub>, 28.4 CH<sub>2</sub>, 28.2 CH<sub>2</sub>, 28.1 CH<sub>2</sub>, 28.0 CH<sub>2</sub> (C-6, C-7, C-8, C-9, C-10, C-11, C-12, C-13, C-14, C-15), 33.9 (CH<sub>2</sub>, C-17), 78.0 (C, C-4), 102.5 (C, C-5), 128.6 (CH, C-2), 128.4 (CH, C-3), 171.0 (C, C-1), 179.5 (C, C-18); FABMS  $m/z$  331 [M + Na]<sup>+</sup> (15), 325 (85), 192 (45), 296 (45), 282 (35), 280 (100), 270 (22), 267 (40), 252 (20), 154 (40), 136 (40), 73 (100); HRFABMS  $m/z$  331.1849 (calcd for C<sub>18</sub>H<sub>28</sub>O<sub>4</sub>Na, 331.1885).

**(13E)-Octadec-13-en-9,11-diynoic acid (3):** IR and UV data, coincident with those published previously;<sup>13</sup> <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.88 (3H, t,  $J$  = 6.9 Hz, H<sub>3</sub>-18); 1.33 (12H, bs, H-4, H-5, H-6, H-7, H-16, H-17); 1.62 (2H, quint.,  $J$  = 6.9 Hz, H<sub>2</sub>-3), 2.12 (2H, q,  $J$  = 7.3 Hz, H<sub>2</sub>-15), 2.34 (2H, t,  $J$  = 7.4 Hz, H<sub>2</sub>-8), 2.77 (2H, t,  $J$  = 7.3 Hz, H<sub>2</sub>-2), 5.48 (1H, d,  $J$  = 15.9 Hz, H-13), 6.27 (1H, dt,  $J$  = 15.9, 7.3 Hz, H-14); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  13.8 CH<sub>3</sub> (C-18), 19.5 CH<sub>2</sub> (C-10), 22.1 CH<sub>2</sub> (C-17), 24.6 CH<sub>2</sub> (C-3), 28.2 CH<sub>2</sub> (C-6), 28.6 CH<sub>2</sub> (C-7 + C-8), 28.8 CH<sub>2</sub> (C-4), 30.6 CH<sub>2</sub> (C-16), 32.8 CH<sub>2</sub> (C-15), 33.8 CH<sub>2</sub> (C-2), 65.3 C (C-10), 72.8 C (C-11), 74.1 C (C-12), 83.4 C (C-9), 108.6 CH (C-13), 148.2 CH (C-14), 179.3 C (C-1); EIMS  $m/z$  274 (50), 245 (22), 231 (25), 217 (27), 201 (42), 146 (40), 131 (75), 117 (100), 105 (40), 91 (82); HREIMS  $m/z$  274.1959 (calcd for C<sub>18</sub>H<sub>26</sub>O<sub>2</sub>, 274.1933).

**Biological Assays. Yeast Strains.** All the strains used in this study have the W3031A genetic background (MATa, ade2-1, can1-100, his3-11, leu2-3, trp11-1, ura3.1). The rad9, rad17, rad24, mec3, and tel1 strains, harboring disruptions of the respective genes, have been described elsewhere.<sup>29</sup> The mec1-1 and rad53-11 yeast mutants have also been described previously.<sup>29</sup>

**Yeast Growth Assays.** Standard methods for yeast culture and manipulations were used.<sup>30</sup> To assess cell growth in the presence of increasing concentrations of each compound tested, mid-log cultures of each strain growing on liquid YPD medium were 10-fold serially diluted, and volumes of around 3  $\mu$ L were applied with a stainless steel replicator (SIGMA) on solid plates containing 2% Bacto-Agar (Difco) and YPD medium with 10-fold increasing doses of each product. Growth was recorded after 2–3 days in all cases.

**Receptor Binding Assays.** The receptor binding assays used for the evaluation of compounds **1**–**3** have been reported previously.<sup>31,32</sup>

**Acknowledgment.** This work has been conducted within the CYTED Program and was partly funded by the Spanish MCYT (project PPQ-2000-1655-C02-01). N.E.J. thanks Caja Canarias-Ull for a grant. We thank Dr. A. Orjales and FAES SA for carrying out the receptor binding assays.

**Supporting Information Available:** Schemes showing the possible formation of compounds **1** and **2**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References and Notes

- Muñoz, O.; González, A. G.; Ravelo, A. G.; Estévez-Braun, A. Z. *Naturforsch.* **1999**, *54c*, 144–145.
- Muñoz, O.; Estévez-Braun, A.; Ravelo, A. G.; González, A. G. Z. *Naturforsch.* **2002**, *57c*, 108–209.
- Kennedy, M. L.; Cortés-Selva, F.; Pérez-Victoria, J. M.; Jiménez, I. A.; González, A. G.; Muñoz, O.; Gamarro, F.; Castanys, S.; Ravelo, A. G. *J. Med. Chem.* **2001**, *44*, 4668–4676.
- Muñoz, O.; Montes, M.; Wilkomirsky, T. *Plantas Medicinales de Uso en Chile*; Editorial Universitaria: Santiago, 2001.
- Forgacs, P.; Provost, J. *Phytochemistry* **1981**, *20*, 1689–1691.
- Huong, D.; Thi, T.; Martin, M. T.; Litaudon, M.; Sevenet, T.; Pais, M. *J. Nat. Prod.* **1998**, *61*, 1444–1446.
- Rizvi, S. H.; Kapil, R. S.; Shoeb, A. *J. Nat. Prod.* **1985**, *48*, 146.
- Sakurai, A.; Okada, K.; Okumura, Y. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 3051–3052.
- Wagner, H.; Feil, B.; Seligmann, O.; Petricic, J.; Kalogjera, Z. *Planta Med.* **1986**, *2*, 102–104.
- Shankaranarayana, K. H.; Venkatesan, K. R. *Indian Perfum.* **1981**, *25*, 3–31.
- Alpha, T.; Raharivelomanana, P.; Bianchini, J. P.; Faure, R.; Cambon, A. *Phytochemistry* **1997**, *44*, 1519–1522.
- Spitzer, V.; Bordignon, S. A.; Schenkel, E. P.; Marx, F. *J. Am. Oil Chem. Soc.* **1994**, *71*, 1343–1348.
- Gunstone, F. D.; Sealy, A. J. *J. Chem. Soc.* **1963**, 5772–5778.
- Bohlmann, F.; Burkhardt, T.; Zdero, C. *Naturally Occurring Acetylenes*; Academic Press: London, 1973; pp 4–21.
- Rawlings, B. *J. Nat. Prod. Rep.* **1998**, *15*, 275–308.
- Harwood, J. L. *Biochim. Biophys. Acta* **1996**, *1301*, 7–56.
- Kawaguchi, A.; Iwamoto-Kihara, A. *Comprehensive Natural Products Chemistry*; Elsevier: Amsterdam, 1999; Vol. 1, pp 23–59.
- Das, S.; Thakur, S. *Phytochemistry* **1989**, *28*, 509–511.
- Schmidt, J. O. *Bee Products: Chemical Composition and Applications*; Plenum Press: New York, 1996; pp 15–26.
- Powell, R. G.; Smith, C. R.; Glass, C. A.; Wolff, I. A. *J. Org. Chem.* **1966**, *31*, 528–533.
- Le Bouquin, R.; Skrabs, M.; Kahn, R.; Benveniste, I.; Salaün, J. P.; Schreiber, L.; Durst, F.; Pinot, F. *Eur. J. Biochem.* **2001**, *268*, 3083–3090.
- Simon, J. A.; Szankasi, P.; Nguyen, D. K.; Ludlow, C.; Dunstan, H. M.; Christopher, J.; Roberts, C. J.; Jensen, E. L.; Hartwell, L. H.; Friend, S. H. *Cancer Res.* **2000**, *60*, 328–333.
- Bhalerao, U. T.; Rao, S.; Tilak, B. D. *Tetrahedron Lett.* **1984**, *25*, 5439–5440.
- Marosi, L.; Schlenk, W. *Justus Liebigs Ann. Chem.* **1973**, 584–598.
- Bailey, A. V. *J. Am. Oil Chem. Soc.* **1971**, *48*, 775–777.
- Rubinstein, I.; Goad, L. J.; Clague, A. D. H.; Lawrence, J. M. *Phytochemistry* **1976**, *15*, 195–200.
- Kisiel, W.; Zielinska, K. *Fitoterapia* **2000**, *71*, 86–87.
- Risch, N.; Brockmann, H. *Tetrahedron Lett.* **1983**, *24*, 173–176.
- de la Torre-Ruiz, M. A.; Green, C. M.; Lowndes, N. F. *EMBO J.* **1998**, *17*, 2687–2698.
- Guide to Yeast Genetics and Molecular Biology*; Guthrie, C., Fink, G. R., Eds.; Academic: New York, 1991.
- Orjales, A.; Mosquera, R.; Labeaga, L.; Rodes, R. *J. Med. Chem.* **1997**, *40*, 586–593.
- Iriepa, I.; Villasante, F. J.; Gálvez, E.; Labeaga, L.; Innerarity, A.; Orjales, A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 189–192.

NP020513E