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Total Synthesis of Mololipids: A New Series of Anti-HIV Moloka'iamine Derivatives

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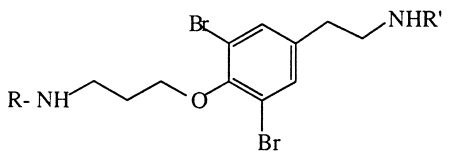
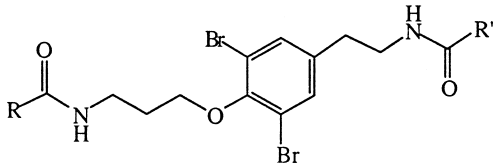
Abstract—A new family of bioactive bromotyrosine derivatives, termed mololipids, was recently isolated from a Hawaiian sponge, but could not be resolved into individual components by chromatography. To complete their structural characterization and better understand structure–activity relationships, the first pure samples of dimyristoyl, distearoyl, dioleoyl, and stearoyl/oleoyl mololipids have now been prepared by total synthesis, and their anti-HIV activity investigated. © 2000 Published by Elsevier Science Ltd.

Natural products from marine organisms often possess distinctive molecular frameworks and potentially useful biological properties. Sponges of the order Verongida, indigenous to Japanese and Hawaiian waters, are a particularly rich source of unusual new structures. Noteworthy among these is a series of bromotyrosine derivatives (Fig. 1) that includes ceratinamine **1** and moloka'iamine **2**.[1,2] Recently, a new series of lipophilic bromotyrosine derivatives displaying anti-HIV activity was isolated as a white waxy extract from a Verongid sponge collected off Moloka'i, Hawaii. Named 'mololipids', the naturally-occurring mixture was assigned general structure **3**, consisting of bis-amides between **2** and long chain fatty acids.[3] Here, we describe the first synthesis of pure samples of these structures, together with their physical and biological properties.

Mass spectrometric analysis of the extract indicated that the mololipids ranged in size from 750 to >1000 daltons. However, repeated attempts at HPLC purification failed to separate the components of the mixture, thus precluding the complete structural elucidation of individual mololipids.[3] One significant unresolved question concerned the constitution and point of attachment of specific fatty acids. Although hydrolysis of the mololipid mixture afforded a series of linear and branched fatty acids ranging in size from C_{14} to C_{20}, it was not possible to determine whether individual mololipids



1 R= COCN, R' = H
2 R, R' = H



3 R, R' = C_{13} - C_{19}, alkyl, alkenyl "mololipids"

Figure 1.

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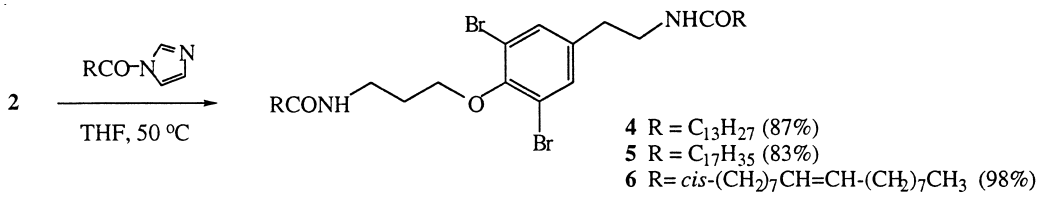
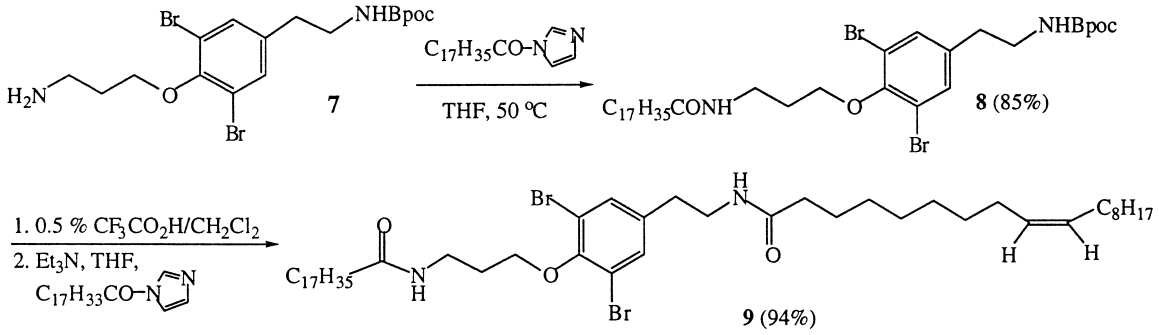


Scheme 1.



Scheme 2.

carried identical or nonidentical fatty acid chains, or whether certain fatty acids were uniquely or preferentially associated with either the aminoethyl or aminopropyl side chains in **3**.

Having previously developed short synthetic routes to **1** and **2**,[4] we decided to address the remaining structural questions surrounding the mololipids by synthesizing pure samples of the assigned structures, which would also make available larger quantities of these minor (0.005% w/w) sponge constituents. The fact that mololipids exhibit activity against HIV-1 without toxicity to human lymphocytes[3] provided an additional incentive to couple the synthetic effort with structure–activity studies on pure samples. Here we report the synthesis of four representative mololipids by a route that permits either direct bis-acylation of moloka'iamine **2** or stepwise condensation of a suitably protected precursor with different fatty acyl groups.

Using standard procedures (acid chloride, pyridine or Et_3N, various solvents), attempts to bis-acylate moloka'iamine **2** involved inhomogeneous reaction conditions and led to complex product mixtures. However, the corresponding myristoyl, stearoyl, and oleoyl imidazoles reacted smoothly with **2** (THF, 50 °C) to produce mololipids **4**–**6** in excellent yield (Scheme 1).[5]

Monoprotected diamine **7**, a key intermediate in our earlier synthesis of ceratinamine **1**, was useful in developing a synthetic approach to mixed mololipids such as **9** (Scheme 2).

Acylation of **7** with stearoyl imidazole as earlier described afforded **8** (85%).[6] Following removal of the acid-labile 2-(4-biphenylyl)-2-propyloxycarbonyl (Bpoc) protecting group in dilute trifluoroacetic acid,[7] reaction of the resulting free amine group with oleoyl imidazole afforded unsymmetrically diacylated mololipid **9** in 94% yield.[8]

The mechanism by which mololipids exert their anti-HIV-1 activity has not been elucidated. To shed light on that question, a series of synthetic mololipids was screened for gp120 binding by analyzing the interaction of mololipid monolayers with recombinant gp120 at the air–water interface. Using a previously described assay[9,10] that monitors the increase in surface pressure $\Delta\Pi$, isotherms recording the variation of surface pressure versus apparent molecular area at the air–water interface indicated that each of the synthetic mololipids formed monomolecular films. Satisfactory compressibility was observed at all film pressures, and no discontinuities in the isotherms were detected, indicating that the liquid expanded states of the monolayers were well-behaved up to film collapse. Recombinant gp120 (HIV-IIIB isolate) was then added to monolayers prepared at an initial pressure of 8–10 mN/m.

In the case of compounds **4**, **5**, **6**, and **9**, observed values of $\Delta\Pi_{max}$ were low (0, 3.8, 5.9, and 4.2 mN/m, respectively) compared to galactosylceramide, a known ligand for gp120.[9] Our results indicated that none of the synthetic mololipids interacted significantly with gp120. It thus seems unlikely that mololipids act by impairing HIV–glycolipid interactions on the plasma cell membrane. However, with an abundant synthetic supply of representative mololipids in hand, it now becomes possible to test pure mololipids as inhibitors of other enzymes involved in HIV-1 replication and infection.

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5. (a) For **4**: mp 130–131 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.34 (s, 2H), 6.08 (1H), 5.54 (1H), 4.07 (t, 2H, J = 5.9 Hz), 3.61–3.55 (m, 2H), 3.49–3.43 (m, 2H), 2.75 (t, 2H, J = 7.0 Hz), 2.21–2.12 (m, 4H), 2.05 (tt, 2H, J = 5.9, 6.4 Hz), 1.68–1.56 (m, 4H), 1.25 (br. s, 40H), 0.90–0.85 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.3, 173.2, 151.4, 138.1, 133.0, 118.1, 72.0, 40.3, 37.3, 37.0, 36.8, 34.6, 31.9, 29.6, 29.5, 29.3, 29.3, 25.8, 22.7, 14.1; IR (film) 3300, 2920, 2840, 1640, 1550, 1470 cm^{-1}; FABMS m/z 771/773/775 (M + 1, 12%), 268 (100%). (b) For **5**: mp 124–126 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.35 (s, 2H), 6.07 (1H), 5.52 (1H), 4.07 (t, 2H, J = 5.9 Hz), 3.61–3.55 (m, 2H), 3.49–3.43 (m, 2H), 2.75 (t, 2H, J = 7.0 Hz), 2.21–2.12 (m, 4H), 2.05 (tt, 2H, J = 5.9, 6.4 Hz), 1.69–1.56 (m, 4H), 1.25 (br. s, 56H), 0.90–0.86 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.3, 173.2, 151.1, 138.0, 133.0, 118.2, 72.0, 40.4, 37.4, 37.0, 36.8, 34.6, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 25.8, 25.8, 22.7, 14.1; IR (film) 3280, 2920, 2840, 1640, 1550, 1470 cm^{-1}; FABMS m/z 883/885/887 (M + 1, 12%), 329 (100%). (c) For **6**: mp 97–99 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.34 (s, 2H), 6.09 (1H), 5.58 (1H), 5.41–5.26 (m, 4H), 4.07 (t, 2H, J = 5.9 Hz), 3.60–3.54 (m, 2H), 3.49–3.42 (m, 2H), 2.75 (t, 2H, J = 6.9 Hz), 2.21–2.12 (m, 4H), 2.09–1.91 (m, 10H), 1.69–1.57 (m, 4H), 1.40–1.19 (m, 40H), 0.90–0.85 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.2, 173.1, 151.4, 138.0, 132.9, 130.0, 129.9, 129.7, 118.1, 71.9, 40.3, 37.3, 36.9, 36.8, 34.5, 31.9, 29.7, 29.7, 29.6, 29.5, 29.3, 29.1, 27.2, 27.1, 25.8, 22.7, 14.1; IR (film) 3300, 2920, 2850, 1640, 1560, 1460 cm^{-1}; ESI-MS m/z 880/882/884 (M + 1).
6. For **8**: mp 103–105 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.60–7.55 (m, 4H), 7.45–7.40 (m, 4H), 7.36–7.32 (m, 3H), 6.09 (1H), 4.78 (1H), 4.06 (t, 2H, J = 5.9 Hz), 3.61–3.55 (m, 2H), 3.35–3.28 (m, 2H), 2.70 (t, 2H, J = 6.4 Hz), 2.18 (t, 2H, J = 7.5 Hz), 2.04 (tt, 2H, J = 5.9, 6.4 Hz), 1.79 (s, 6H), 1.67–1.57 (m, 2H), 1.35–1.17 (m, 28H), 0.88 (t, 3H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.2, 155.0, 151.4, 145.3, 140.8, 139.8, 138.0, 133.0, 128.7, 127.2, 127.1, 124.7, 118.1, 80.8, 72.0, 41.6, 37.4, 37.0, 34.8, 31.9, 29.7, 29.5, 29.4, 29.3, 29.3, 29.2, 29.0, 25.8, 22.7, 14.1; IR (film) 3300, 2910, 2840, 1700, 1650, 1560, 1540, 1460, 1220 cm^{-1}; ESI-MS m/z 873/875/877 ($M + NH_4^+$, 15%), 662/664/666 (100%).
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8. For **9**: mp 102–104 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.35 (s, 2H), 6.07 (1H), 5.50 (1H), 5.40–5.26 (m, 2H), 4.07 (t, 2H, J = 5.9 Hz), 3.61–3.55 (m, 2H), 3.49–3.43 (m, 2H), 2.75 (t, 2H, J = 7.0 Hz), 2.21–2.12 (m, 4H), 2.09–1.93 (m, 6H), 1.70–1.56 (m, 4H), 1.39–1.17 (m, 48H), 0.90–0.85 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.2, 151.4, 138.0, 132.9, 130.0, 129.7, 118.1, 72.0, 40.3, 37.3, 37.0, 36.8, 34.6, 31.9, 29.8, 29.7, 29.5, 29.4, 29.3, 29.3, 29.1, 27.2, 27.1, 25.8, 25.7, 22.7, 14.1; IR (film) 3290, 2920, 2840, 1640, 1560, 1470 cm^{-1}; ESI-MS m/z 882/884/886 (M + 1).
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10. The interaction between gp120 and the synthetic molo-lipids was measured with the μ-trough S tensiometer (Kibron Inc., Helsinki, Finland) and the data were analyzed with the FilmWare (version 2.3) software.