Chemical Modification of Ansamitocins. II. Synthesis of 3-Epimaytansionoids via 3-Maytansinones

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As part of our recent search for new semisynthetic analogs of maytansinoids having a better therapeutic ratio than maytansine, we synthesized 3-epimaytansinoids (VIIIa—c) starting from ansamitocin P-3, a fermentation product of Nocardia sp., via maytansinol (I). A key intermediate, 3-epimaytansinol (VI), was synthesized by oxidation of I with pyridinium chlorochromate to 3-maytansinone (IV), followed by stereoselective reduction with NaBH₄. Esterification of VI with appropriate carboxylic acids gave the corresponding 3-epimaytansinoids (VIIIa—c) in high yields. These compounds did not show the biological activity characteristic of natural maytansinoids to any appreciable degree.

Keywords—ansamitocin; 3-epimaytansinoid; maytansinoid; 3-epimaytansinol; 3-maytansinone; oxidation; sodium borohydride reduction; stereoselectivity; tubulin polymerization; antitumor activity

The potent antitumor activity and relatively severe side effects of maytansine have prompted us to conduct chemical modification studies of maytansinoids with the aim of developing new semisynthetic analogs with a better therapeutic profile. Maytansinoids, especially those of natural origin, have a 19-membered macrocyclic ring with a conjugated diene (E, E), seven chiral centers and a number of functional groups including the C₃ ester side chain, the C₄—C₅ epoxide and the six-membered cyclic carbinolamide moiety (Fig. 1). Earlier studies have indicated that the last three functional groups are essential for the antitumor activity.

The previous paper from this laboratory reported the synthesis of 4,5-deoxymaytansinoids and demonstrated that their antiprotozoal and antitumor activities are almost the same as those of the corresponding maytansinoids. That finding suggests that the presence of the epoxy group is not essential for the biological activities of the maytansinoids. The next point to be considered is the contribution of the C₃ ester side chain to the antitumor activity. The importance of the presence of this group in the molecule is evident from the fact that

![Diagram](image)

Fig. 1
maytansinoids with no ester group at the C₃ position, such as normaysine, maysine and N-methylmaysenine, as well as maytansinol, lack the activity.⁴ Several modified C₃ esters were synthesized from maytansinol (I) in this laboratory and their structure-activity relationships were partially reported.⁷ Changes in the structure of the C₃ ester group of maytansinoids resulted in significant changes in their biological activities. However, the effect of a configurational change at the C₃ ester oxygen on their activity remains to be determined. This report deals with the synthesis and the inhibitory activity of 3-epimaytansinoids, which differ from the maytansinoids in the relative configuration of the hydroxyl or acyloxy group at the C₃ position.

3-Epimaytansinoids (VIII) were synthesized from maytansinol (I) by a process including oxidation of the C₃ hydroxyl to yield 3-maytansinone (IV), followed by reduction to 3-epimaytansinol (VI) and acylation of the C₃ hydroxyl group of VI, as depicted in Chart 1.

The C₉ hydroxyl of I was selectively protected by treating I with methanol in the presence of acid or with N,O-bis(trimethylsilyl)acetamide. Subsequently, the reaction of II with pyridinium chlorochromate under neutral conditions gave the C₉-protected C₃ ketone (C₉-protected 3-maytansinone, IIIa, b) which, on deprotection by acid hydrolysis, yielded 3-maytansinone (IV),⁸¹ a key intermediate, in excellent yield. Treatment of maytansinones (IIIa, b and IV) with sodium borohydride gave 3-epimaytansinol (VI) as the main product, although the acid-catalyzed deprotection of the C₉ ether groups was necessary with IIIa, b. The sodium borohydride reduction was repeated under various conditions (Table I); the choice of the starting material, IIIa, b or IV, markedly affected the stereochemistry of the reaction products, while the temperature had only a slight effect. Optimum stereoselectivity was obtained with the unprotected 3-maytansinone (IV). High-performance liquid chromatography (HPLC) revealed that the ratio of the C₃ β-hydroxy isomer (VI) to I in the reaction products was 9:1. The high preference for the C₃ β-hydroxy isomer over the corresponding
Table I. NaBH₄ Reduction of 3-Maytansinones

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Temp. (°C)</th>
<th>VI: I</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIIa</td>
<td>30</td>
<td>66:34</td>
<td>89</td>
</tr>
<tr>
<td>IIIa</td>
<td>0</td>
<td>67:33</td>
<td>90</td>
</tr>
<tr>
<td>IIIa</td>
<td>-20</td>
<td>69:31</td>
<td>86</td>
</tr>
<tr>
<td>IIIa</td>
<td>-55</td>
<td>71:29</td>
<td>85</td>
</tr>
<tr>
<td>IIIb</td>
<td>-20</td>
<td>69:31</td>
<td>81</td>
</tr>
<tr>
<td>IIIb</td>
<td>-55</td>
<td>70:30</td>
<td>80</td>
</tr>
<tr>
<td>IV</td>
<td>-40</td>
<td>90:10</td>
<td>78</td>
</tr>
<tr>
<td>IV</td>
<td>-55</td>
<td>90:10</td>
<td>77</td>
</tr>
</tbody>
</table>

For the determination of the ratio of 3-epimaytansinol (VI) to maytansinol (I), each sample was analyzed by HPLC (Waters Associates µ-Bondapak C₁₈ column, 3.9 mm x 30 cm; 40% ac., CH₃CN, 1 ml/min; detection at 254 nm). The retention times of VI and I were 3.8 and 4.2 min, respectively.

Fig. 2. Possible Transition States of Hydride Reduction

α-isomer (I) suggests that the C₉ α-hydroxyl group of maytansinone (IV) is important in determining the stereochemistry of the product. In accordance with this view, the reduction of IIIa and IIIb with sodium borohydride resulted in the formation of two diastereoisomers in approximately 7:3 ratio. Therefore, the reduction of IV is presumed to proceed via the formation of the molecular complex between the C₉ α-hydroxyl group and sodium borohydride, followed by intramolecular attack of hydride ion on the carbonyl group from the α-face of the macrocyclic ring, as illustrated in Fig. 2. In contrast, with C₉-protected maytansinones which are unable to form such a complex, the C₃ carbonyl suffers only intermolecular attack by hydride ion, so that the reaction proceeds less stereoselectively, being controlled by a small difference between the α- and β-faces of the molecule in steric hindrance toward the hydride ion.

Esterification of the C₃ hydroxyl group of 3-epimaytansinol (VI) and its C₉ ether (V) was carried out according to the method¹¹ originally established for the esterification of maytansinol (I). The reaction of these epimaytansinols (VI and V) with appropriate carboxylic acids in the presence of dicyclohexyl carbodiimide (DCCD) and 4-dimethylaminopyridine gave 3-epimaytansinoids (VIIIa—c) in high yields.

The structures of all the semisynthetic epimaytansinoids (V, VI, and VIIIa—c) were readily deduced from the spectral data. 3-Epimaytansinol 9-methyl ether (V) showed an
nuclear magnetic resonance (NMR) spectrum very similar to that of authentic maytansinol 9-methyl ether (IIa), but was characterized by a large downfield shift (0.38 ppm) of the doublet methine proton at the C₅ position (the epoxide ring proton; 2.98 ppm), as compared to the corresponding proton of IIa (2.60 ppm). The mass spectral fragmentation patterns of VI and VIIIA—c were very similar to those of I and the maytansinoids with the corresponding C₅ side chain esters, respectively. The observation of a weak parent peak ion and a characteristically strong ion peak at M⁺−61, which corresponds to the loss of H₂O and HNCO from the carbinolamide group, indicated that VI and VIIIA—c have the ansamacrolide ring and molecular weights consistent with the expected structures.

In contrast to the maytansinoids, which have the natural configuration at the C₅ position, these 3-epimaytansinoids (VIIIA—c) showed no appreciable antimicrobial activity against eukaryotic cells and no inhibition of ciliated tubulin polymerization in the deciliated protozoan Tetrahymena pyriformis W.⁹ These results suggest that the epimerization at C₅ in maytansinoids leads to loss of the capability for inhibition of tubulin polymerization, a characteristic biological property of the maytansinoids including maytansine and ansamitocin P-3.

Further chemical modifications of maytansinoids and biological testing are in progress.

**Experimental**

Melting points were measured on a Yanagimoto MP-S3 hot plate apparatus, and are uncorrected. Infrared (IR) spectra were recorded on a Hitachi 215 spectrometer. Mass spectra (MS) were determined with a JMS-01SC spectrometer equipped with a direct inlet system. Proton nuclear magnetic resonance (¹H-NMR) spectra were obtained using Varian XL-100-12 and Varian EM-360 instruments: chemical shifts (δ) are reported in ppm downfield from internal TMS. For analytical thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC) pre-coated Kieselgel 60 F₂₅₄ plates (E. Merck. Art. 5642) were used. HPLC was carried out on a Waters ALC/GPC 204 instrument and preparative column chromatography was done on Kieselgel 60 (E. Merck. Art. 7743).

**Material**—Maytansinol, the starting compound of this study, was prepared by reductive ester cleavage⁹ of ansamitocin P-3, a fermentation product of Nocardia species.¹⁰

**Maytansinol 9-Methyl Ether (IIa)**—A solution of maytansinol (I; 226 mg) and trifluoroacetic acid (0.4 ml) in MeOH (4 ml) was allowed to stand at room temperature for 3 h. The reaction mixture, after neutralization with Na₂CO₃, was poured into ice water and extracted with CHCl₃. The extract was dried over MgSO₄ and evaporated in vacuo, giving a pale yellow solid. This was chromatographed on silica gel with 2% MeOH in CHCl₃ as an eluent. After work-up, IIa (216 mg) was obtained as colorless needles, mp 171—173°C (ethyl acetate–ether). IR vmax cm⁻¹: 1705, 1640, 1580, 1080. MS m/e: 578 (M⁺), 502 (M⁺−76). NMR (90 MHz, CDCl₃) δ: 0.81 (3H, s), 1.23 (3H, d), 1.63 (3H, s), 2.60 (1H, d), 3.17 (3H, s), 3.26 (3H, s), 3.43 (3H, s), 3.94 (3H, s), 5.49 (1H, dd), 6.10 (1H, d), 6.38 (1H, dd), 6.77 (1H, s), 7.07 (1H, d).

**Maytansinol 9-Trimethylsilyl Ether (IIb)**—A mixture of I (195.5 mg), dry triethylamine (0.5 ml), and N,O-bis(trimethylsilyl)acetamide (0.22 ml) in dry CH₂Cl₂ (10 ml) was kept at room temperature for 2 h, then evaporated to dryness. The residue was chromatographed on silica gel using 0.8% MeOH in CHCl₃ to give the desired product (142.3 mg) as a colorless powder, mp 235—238°C (dec.) (ethyl acetate–ether). IR vmax cm⁻¹: 1710, 1630, 1580, 1085. MS m/e: 636 (M⁺).

3-Maytansinone 9-Methyl Ether (IIia)—A suspension of maytansinol 9-methyl ether (IIa; 214 mg), powdered sodium acetate (1.18 g), and pyridinium chlorochromate (400 mg) in dry CH₂Cl₂ (37 ml) was warmed at 39°C for 48 h under a nitrogen atmosphere with vigorous stirring. After cooling of the mixture, ethyl ether (74 ml) was added and the resulting inorganic precipitate was filtered off. The filtrate was evaporated in vacuo and the residue was chromatographed on silica gel with 0.5% MeOH in CHCl₃ to yield IIia (134 mg) as a pale yellow solid, mp 163—165°C (ether). IR vmax cm⁻¹: 1730, 1705 sh, 1630, 1575, 1460, 1335, 1090. MS m/e: 576 (M⁺), 544 (M⁺−32), 500 (M⁺−76). NMR (90 MHz, CDCl₃) δ: 1.02 (3H, s), 1.32 (3H, d), 1.67 (3H, s), 3.22 (3H, s), 3.27 (3H, s), 3.34 (3H, s), 3.96 (3H, s), 4.78 (2H, s).

3-Maytansinone 9-Trimethylsilyl Ether (IIib)—By means of a procedure similar to that described in the preceding section, IIib was synthesized from IIb (23.5 mg) as an amorphous powder (13.8 mg). IR vmax cm⁻¹: 1730, 1710, 1635, 1580, 1090. MS m/e: 634 (M⁺).

3-Maytansinone (IV)—a From 3-Maytansinone 9-Methyl Ether (IIia): A solution of IIia (5.76 mg) in 25% aq. MeOH (1.0 ml) was treated with 2 N HCl (0.2 ml) at room temperature for 2 h. The reaction mixture was neutralized with saturated NaHCO₃ and extracted with CHCl₃. The combined extracts were dried over MgSO₄ and evaporated. Preparative thin-layer chromatography (PTLC) of this residue on Silica gel F-254 plates developed with 2% MeOH in CHCl₃ gave a major band (Rf = 0.39) which corresponded to that of the desired product. This portion
was collected and extracted with 10% MeOH in CHCl₃, giving IV (5.43 mg) as a pale yellow solid, mp 151–152°C (ethyl acetate–ether). IR ν<sub>max</sub> cm⁻¹: 1730, 1710, 1630, 1580, 1440, 1335, 1090. MS m/e: 562 (M⁺), 501 (M⁺ – 61). NMR (90 MHz, CDCl₃) δ: 1.02 (3H, s), 1.33 (3H, d), 1.68 (3H, s), 2.30 (3H, s), 3.40 (3H, s), 3.95 (3H, s), 4.80 (2H, s).

b) From 3-Maytaninosine 9-Trimethylsilyl Ether (I11b): In the same manner as described above, treatment of I11b (6.34 mg) with 2N HCl gave IV (5.35 mg). HPTLC, HPLC and MS data showed that this compound was identical with the sample obtained from IIIa.

HPLC Analysis of the Products Obtained by NaBH₄ Reduction of 3-Maytaninosines (IIIa, IIIb and IV)—A solution of 0.01 mmol of 3-maytaninosine (IV) or a protected 3-maytaninosine (IIIa, b) in MeOH (0.5 ml) was treated with NaBH₄ (5.0 mg) for 30 min at the temperature indicated in Table I. The reaction mixture, after being acidified with 2N HCl, was left standing at room temperature (25°C) for 2 h, then diluted with water and extracted with CHCl₃. The organic layer was dried over MgSO₄ and evaporated in vacuo, yielding a diastereomeric mixture of I and IV as a pale yellow solid. To determine the ratio of 3-epimaytaninosinol (IV) to maytaninosinol (I), each sample was analyzed by HPLC (μ-Bondapak C-18 column, 3.9 mm × 30 cm, 40% αq, CH₃CN). The results are summarized in Table I.

3-Epimaytaninosinol 9-Methyl Ether (V)—A solution of IIIa (5.76 mg) in MeOH (0.5 ml) was treated with NaBH₄ (5.0 mg) at room temperature for 30 min. After being quenched with 2N HCl, the mixture was poured into ice water and extracted with CHCl₃. The combined organic layer was purified by TLC on Silica gel F-254 developed with 1.0% MeOH in CHCl₃. The silica gel corresponding to the major band was collected and extracted with 10.0% MeOH in CHCl₃ to give V (3.45 mg) as a colorless powder, mp 147–149°C (ether). IR ν<sub>max</sub> cm⁻¹: 1710, 1655, 1580, 1430, 1085. MS m/e: 578 (M⁺), 546 (M⁺ – 32), 502 (M⁺ – 76). NMR (90 MHz, CDCl₃) δ: 0.72 (3H, s), 1.27 (3H, s), 1.65 (3H, s), 2.98 (1H, d), 3.18 (3H, s), 3.27 (3H, s), 3.40 (3H, s), 3.95 (3H, s), 5.58 (1H, dd), 6.22 (1H, s), 6.37 (1H, s), 6.73 (1H, dd), 6.80 (1H, d).

3-Epimaytaninosinol VI—A solution of V (5.78 mg) in 50% αq MeOH (1.0 ml) was treated with 2N HCl (0.2 ml) at room temperature for 2 h. The reaction mixture was neutralized and extracted with CHCl₃. The combined organic layer was evaporated in vacuo to yield VI (5.32 mg) as a colorless powder, mp 143–145°C (ethyl acetate–ether). IR ν<sub>max</sub> cm⁻¹: 1705, 1650, 1580, 1455, 1430, 1085. MS m/e: 564 (M⁺), 503 (M⁺ – 61). NMR (90 MHz, CDCl₃) δ: 0.75 (3H, s), 1.35 (3H, d), 1.68 (3H, d), 3.22 (3H, s), 3.34 (3H, s), 3.97 (3H, s), 5.60 (1H, dd), 6.75 (1H, sd), 6.78 (1H, sd).

3-Epimaytaninosinol 9-Methyl Ether 3-Isobutyrate (VII)—A mixed solution of 3-epimaytaninosinol 9-methyl ether (V; 25 mg), isobutyric acid (37.8 mg), 4-dimethylaminopyridine (10.8 mg), and DCCD (89 mg) in dry CH₂Cl₂ (1.72 ml) was stirred at room temperature for 3 h. The white precipitate that formed was removed by filtration and the filtrate was evaporated to dryness in vacuo. Chromatography of the residue on silica gel with CHCl₃ as an eluent gave VIIa (27 mg) as a colorless powder. MS m/e: 648 (M⁺).

3-Epimaytaninosinol 3-Isobutyrate (VIIa)—A solution of VIIa (14 mg) in 50% αq MeOH (2.2 ml) was treated with 2N HCl (0.2 ml) at room temperature for 3 h. The reaction mixture was neutralized with saturated NaHCO₃ and extracted with CHCl₃. The organic layer was dried over MgSO₄ and evaporated in vacuo. Chromatography of the residue on silica gel using 2% MeOH in CHCl₃ as an eluent gave VIIa (13 mg) as colorless needles, mp 145–147°C (ethyl acetate). IR ν<sub>max</sub> cm⁻¹: 1730, 1660, 1580, 1460, 1085. MS m/e: 634 (M⁺), 573 (M⁺ – 61). NMR (90 MHz, CDCl₃) δ: 0.67 (3H, s), 1.75 (3H, s), 3.07 (3H, s), 3.37 (3H, s), 3.95 (3H, s), 4.87 (1H, dd), 6.82 (1H, d), 6.98 (1H, d).

3-Epimaytaninosinol 3-n-Octanoate (VIIIb)—In the same manner as described above, 3-epimaytaninosinol 9-methyl ether (V; 5.78 mg) was esterified by treatment with n-octanoic acid in the presence of 4-dimethylaminopyridine and DCCD. Deprotection with 2N HCl gave VIIIb (4.05 mg) as a colorless powder, mp 116–118°C (ether). IR ν<sub>max</sub> cm⁻¹: 1740 sh, 1730, 1710, 1660, 1580, 1460, 1435, 1085. MS m/e: 690 (M⁺), 629 (M⁺ – 61). NMR (90 MHz, CDCl₃) δ: 0.78 (3H, s), 0.87 (3H, s), 1.10–1.50 (13H, m), 1.66 (3H, bs), 3.13 (3H, s), 3.38 (3H, s), 3.97 (3H, s), 6.60 (1H, s), 6.83 (1H, s).

3-Epimaytaninosinol–Phenylacetate (VIIIc)—A mixture of 3-epimaytaninosinol (VI; 5.64 mg), phenylacetic acid (13.6 mg), 4-dimethylaminopyridine (2.44 mg), and DCCD (20.6 mg) in dry CH₂Cl₂ (0.5 ml) was stirred at room temperature for 30 min. The white precipitate that formed was removed by filtration and the filtrate was evaporated to dryness in vacuo. PTLC of the residue on silica gel plates, developed with 2% MeOH in CHCl₃, followed by workup, gave VIIIc (4.32 mg) as colorless prisms, mp 154–156°C (ethyl acetate–ether). IR ν<sub>max</sub> cm⁻¹: 1740, 1710, 1660, 1585, 1460, 1085. MS m/e: 682 (M⁺), 621 (M⁺ – 61). NMR (90 MHz, CDCl₃) δ: 0.77 (3H, s), 1.25 (3H, d), 1.68 (3H, brs), 3.15 (3H, s), 3.40 (3H, s), 3.94 (3H, s), 6.68 (1H, s), 6.87 (1H, s), 7.25 (5H, brs).

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References and Notes


8) A. I. Meyers, P. J. Reider, and A. L. Campbell, *J. Am. Chem. Soc.*, **102**, 6597 (1980). This communication, describing the total synthesis of (±)-maytansinol, reports the production of an epimeric mixture of the four compounds (the diastereoisomers at C_3 and C_{10}) on reduction of the epimeric mixture of (±)-3-maytansinones synthesized by macro lactam ring closure. No comment was made on 2-epimaytansinol.
