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Onchidin B: A New Cyclodepsipeptide from the Mollusc *Onchidium* sp.

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Contribution from the Departamento de Química Orgánica, Facultad de Química and Instituto de Acuicultura, Universidad de Santiago, E-15706, Santiago de Compostela, Spain, Departamento de Química Pura y Aplicada, Facultad de Ciencias, Universidad de Vigo, E-36200, Vigo, Spain, and Centre ORSTON, B. P. A5, Nouméa, Cedex, New Caledonia

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Abstract: Onchidin B (**4**) is a cyclic depsipeptide isolated from the pulmonate mollusc *Onchidium* sp. Its structure was determined by extensive 2D-NMR, FABMS, tandem FAB MS/MS, selective hydrolysis, and synthesis. It contains four α-amino acids [two units of *N*-methyl valine (MeVal), two units of proline (Pro)], four α-hydroxy acids [two 2-hydroxyisovaleric acids (Hiv), two 2-hydroxy-3-methylpentanoic acid moieties (Hmp)] and two units of the new β-hydroxy acid: 3-hydroxy-2-methyloct-7-ynoic acid (Hymo)]. Selective hydrolysis and direct comparison by chiral GC-MS with authentic samples of the α-amino and α-hydroxy acids allowed us the assignment of the entire absolute stereochemistry of onchidin B. In this way, the α-hydroxy acids were found to be (*S*)-Hiv and (*S,S*)-Hmp, and the α-amino acids (*R*)-proline, (*S*)-proline, and (*R*)-MeVal. In order to establish the absolute configuration of the new β-hydroxy acid, Hymo, its four possible stereoisomers were stereoselectively synthesized using chiral *N*-propionyl oxazolidinones and hex-5-ynal as starting material. Comparison by HPLC-MS of the synthetic samples with the natural Hymo (all derivatized as esters of (−)-(*R*)-α-methoxy-α-(9-anthryl)acetic acid), affirmed its absolute stereochemistry as (*2R,3R*). Thus, onchidin B (**4**) is cyclo [(*R*)-MeVal-(*R,R*)-Hymo-(*S*)-Pro-(*S,S*)-Hmp-(*S*)-Hiv-(*R*)-MeVal-(*R,R*)-Hymo-(*R*)-Pro-(*S,S*)-Hmp-(*S*)-Hiv]. It is formed by a head-to-tail linkage of two halves, each one built by five units and identical sequence. The lack of symmetry of onchidin B is thus due to the presence of one (*S*)-Pro unit in one half and one (*R*)-Pro unit in the other. The structural similarity between onchidin B (**4**) and onchidin (**2**), both isolated from the same organism, and between the β-hydroxy acid Hymo (**5**) and the β-amino acid Amo (**3**) found in onchidin is noticed.

Introduction

Natural products derived from marine organisms have become an increasingly important source of biologically active compounds. Some of the most interesting ones are the cyclic depsipeptides. This class of metabolites frequently offer an unrivaled chemical diversity incorporating new amino and/or hydroxy acids. From a chemical point of view, this is one of the reasons why these compounds have attracted the effort of researchers resulting on the report of a fair number of new structures.[1] Most of the depsipeptides from marine organisms have been isolated from sponges; striking examples are the geodiamolides,[2] arenastatin,[3] and the antifungal and cytotoxic jasplakinolide (jaspamide).[4,5]

On the other hand, only three molluscs have been found to be a source of cyclodepsipeptides. Pettit and co-workers have isolated along the last ten years, more than a dozen dolastatins from *Dolabella auricularia*.[6] Particularly important from the pharmacological point of view is the highly cytotoxic dolastatin 10, the most potent antineoplastic agent known to date. It belongs to the selected group of marine natural products that are in clinical or preclinical stage as antitumoral compounds. Other marine molluscs that were reported to produce cyclic depsipeptides are *Elysia rufescens* and *Onchidium* sp., collected in New Caledonia, from which kahalalide F (**1**)[7] and onchidin (**2**)[8] were isolated, respectively. Onchidin constitutes the only example reported to date of a cyclic, symmetric depsipeptide from a mollusc. Its structure is composed of two identical halves: cyclo [(*S*)-*N*-methylvaline-(2*S*,3*S*)-3-amino-2-methyloct-7-ynoic acid-(*S*)-valine-(*S*)-hydroxyisovaleric acid-(*S*)-hydroxyisovaleric acid]$_2$ linked together in a head-to-tail way so onchidin has a C_2 axis of symmetry and, as a result, produces only half the expected NMR signals. In addition to known α-amino and α-hydroxy acids, onchidin (**2**) incorporates two identical units of the new β-amino acid, 3-amino-2-methyloct-7-ynoic acid (**3**, Amo).

We reported some time ago several polypropionate pyrones named onchitriols.[9] Those metabolites showed moderate antitumor activity and were isolated from the low polarity cytotoxic extracts of *Onchidium* sp. From this marine organism, we now

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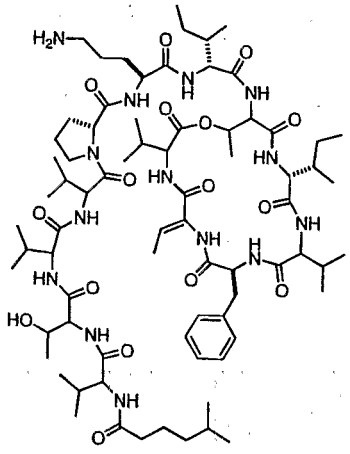
Chart 1



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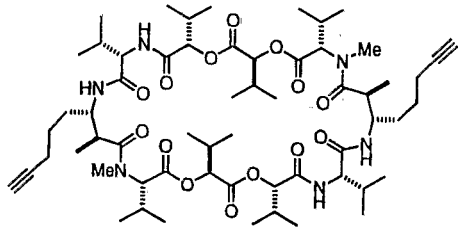

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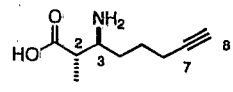

3

wish to report the bioassay guided isolation from the cytotoxic CCl_4 extracts (97% inhibition to Kb cells at 10 μg/mL) of another cyclic depsipeptide, which we have named onchidin B (**4**) (Chart 3), and that shares with onchidin (**2**) a great number of structural features.

Isolation and Structural Analysis

Onchidium sp. (Pulmonata, order: Stylommatophora, family: Onchidiidae) was collected at Chesterfield archipelago in the intertidal zone on "Ile Longue" (Mouillage isles, 450 NW of New Caledonia).

Kb (human epidermoid carcinoma cells) bioactivity-guided isolation by solvent partition and further chromatography of the methanolic extract afforded 3 mg of pure onchidin[8] (**2**) (P-388 cells, $IC_{50} = 8$ μg/mL) and 5 mg of onchidin B (**4**) [$IC_{50} = 8$ μg/mL to Kb cells, $[\alpha]^{25}_D$ −220.1° (*c* 0.1, $CHCl_3$)]. The (+) FABMS spectrum showed a molecular ion at *m/z* 1153, whose molecular formula $C_{62}H_{96}N_4O_{16}$ (17 unsaturations) was deduced on the basis of its HRFABMS peak (*m/z* 1153.6999 $[M + H]^+$, $\Delta + 9.9$ mmu). The edited DEPT and ^{13}C NMR spectra showed the presence of 62 carbons. Among them, the following signals could be easily identified: 10 amide or ester carbonyls (δ = 175.4−166.3 ppm), 14 methyl signals (δ = 20.1−9.4 ppm), 2 *N*-methyl signals (δ = 32.2 and 31.3 ppm), 10 methines, attached to oxygen or nitrogen (δ = 83.5−57.6 ppm), 2 methylenes at 46.7 and 46.6 ppm, assigned as nitrogen-bearing carbons, and another 4 signals (δ = 83.9, 82.8, 68.9, and 68.6 ppm) that were assigned to two terminal acetylenes (Table 1). The presence of two acetylene functional groups was confirmed by the presence of two high field methylene carbons at δ_C 18.2 (C-12) and 18.1 (C-42).

The ^1H NMR (500 MHz, $CDCl_3$) showed as the most relevant resonances: ten methines, corresponding to the α-amino and α-hydroxy acid protons at δ = 5.34 (H-9), 5.26 (H-2), 5.14 (H-32), 5.09 (H-27), 5.08 (H-21), 4.90 (H-39), 4.73 (H-57), 4.66 (H-16), 4.63 (H-51), and 4.18 (H-46) as well as two *N*-methyl singlets at δ 3.21 (MeVal 1) and 3.15 (MeVal 2). In addition, the two terminal acetylenic protons were detected as triplets (due to propargylic coupling), at δ = 1.94 (H-14, t, $J = 2.6$ Hz) and 1.91 (H-44, t, $J = 2.6$ Hz).

The structure of **4** was determined by a detailed analysis of one- and two-dimensional NMR spectra. A combination of HMQC, COSY, TOCSY, and HMBC indicated the presence of ten independent spin systems: four amino acid units and six hydroxy acid units (Figure 1). Badly overlapped protons were resolved by observing TOCSY correlations. The α-methine proton of substructure **4a** δ 4.66 (H-16, Pro 1) was connected to a set of protons formed by H-17 (2.12/2.06), H-18 (1.84/1.62), and H19 (3.58). In the same way, the signal at δ 4.18

Table 1. ^1H NMR and ^{13}C NMR Data of Onchidin B (**4**) in $CDCl_3$

C no.	^1H, m (*J* in Hz)[a]	^{13}C[b]	C no.	^1H, m (*J* in Hz)[a]	^{13}C[b]
MeVal 1			MeVal 2		
1		171.9	31		173.2
2	5.26, d (10.0)	59.6	32	5.14, d (10.0)	60.8
3	2.15, m	28.2	33	2.19, m	29.3
4	0.88, d (6.5)	19.5	34	0.96, d (7.0)	20.1
5	1.01, d (7.0)	19.7	35	1.10, d (7.0)	19.9
NMe	3.21, s	31.3	NMe	3.15, s	32.2
Hymo 1			Hymo 2		
6		174.2	36		175.4
7	3.10, dq (2.0;7.0)	40.0	37	3.15, m	39.5
8	1.07, d (6.5)	15.0	38	1.13, d (7.0)	14.4
9	5.34, dddd (10.0; 10.0;5.0;2.0)	75.3	39	4.90, dddd (10.0; 10.0;5.0;2.0)	78.2
10	1.79, m	30.6	40	1.99, m	30.8
11	1.58, m	23.5	41	1.67, m	23.2
	1.46, m			1.54, m	
12	2.19, dddd (16.5; 7.0;7.0;2.5)	18.2	42	2.22, dddd (16.5; 7.0;7.0;2.5)	18.1
13		83.9	43		82.8
14	1.91, t (2.6)	68.6	44	1.94, t (2.6)	68.9
Pro 1			Pro 2		
15		171.4	45		168.9[c]
16	4.66, d (8.5)	57.6	46	4.18, d (9.0)	60.3
17	2.12, m	31.5	47	2.15, m	28.4
	2.06, m			2.06, m	
18	1.84, m	26.2	48	1.84, m	20.6
	1.62, m		49	3.57, m	46.6
19	3.58, m	46.7		3.48, m	
Hmp 1			Hmp 2		
20		168.9	50		167.3[c]
21	5.08, d (2.5)	77.2	51	4.63, d (11.5)	83.5
22	2.02, m	35.0	52	1.69, m	34.0
23	1.51, m	23.0	53	1.22, m	25.6
24	0.90, t (7.0)	11.4	54	0.89, t (7.0)	9.4
25	0.98, d (7.0)	15.8	55	0.91, d (7.0)	13.6
Hiv 1			Hiv 2		
26		166.3	56		169.4
27	5.09, d (3.5)	77.3	57	4.73, d (8.5)	79.2
28	2.38, m	30.8	58	2.20, m	29.2
29	0.95, d (7.0)	16.6	59	0.97, d (6.0)	17.9
30	1.02, d (7.0)	19.0	60	1.03, d (6.0)	18.8

[a] Assignments based on COSY and TOCSY. [b] Assignments based on HMQC and HMBC. [c] Assignments for these signals may be interchanged.

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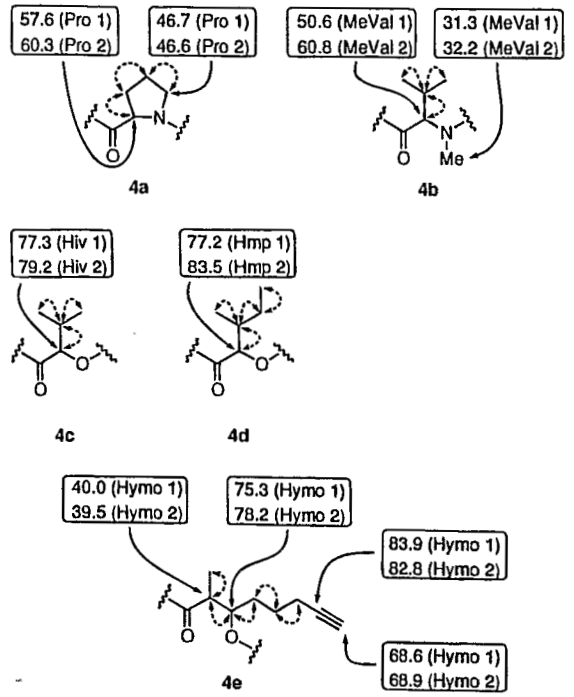

Figure 1. Partial structures **4a**–**e** and selected COSY correlations.

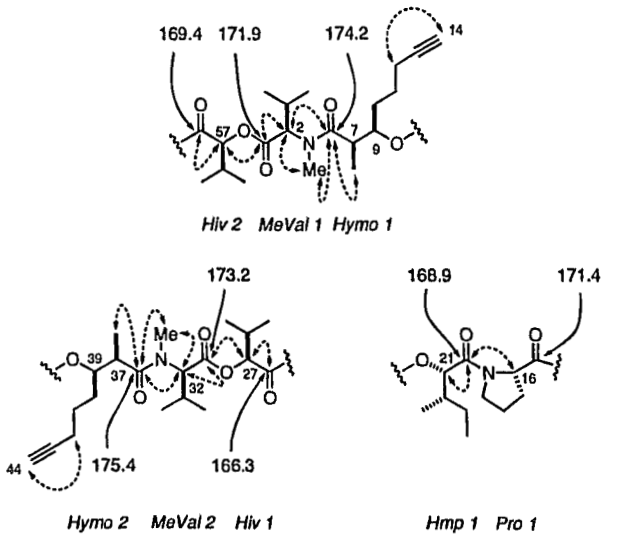

Figure 2. Selected HMBC correlations of onchidin B (**4**).

(H-46, Pro 2) was connected to those of H-47 (2.15/2.06), H-48 (1.84), and H-49 (3.57/3.48).

Two spin systems with *N*-methyl valine (MeVal), **4b**, were depicted as follows: ^{1}H NMR resonances at δ 5.26 (H-2, MeVal 1) showed COSY correlations to 2.15 (H-3) and this last signal to Me-4 and Me-5 (1.01 and 0.88, respectively). The same information was deduced from the following COSY correlation set: 5.14 (H-32, MeVal 2)/2.19 (H-33)/1.10–0.96 (Me-34, Me-35). One bond heteronuclear correlations allowed the identification of α-carbons, [δ 59.6 (MeVal 1, C-2) and 60.8 (MeVal 2, C-32)] and β-carbons [δ 28.2 (MeVal 1, C-3) and 29.3 (MeVal 2, C-33)] for both residues. HMBC correlations between the *N*-methyl signal at 3.21 and the α-carbon at 59.6 (MeVal 1) and between the *N*-methyl at 3.15 and the α-carbon at 60.8 established unambiguously the presence of the *N*-methyl group in the valines.

Another two similar patterns of correlations were detected in the COSY and TOCSY spectra for the 2-hydroxyisovaleric acids (Hiv). Thus, the α-methine protons H-27 and H-57 [δ 5.09 (Hiv 1) and 4.73 (Hiv 2), respectively] were connected to another two methines [δ 2.38 (H-28, Hiv 1) and 2.20 (H-58, Hiv 2)] bonded to two pairs of methyl groups [δ 1.02/0.95 (Me-34/Me-35, Hiv 1) and 1.03/0.97 (Me-59/Me-60, Hiv 2)]. Nevertheless, the chemical shifts of the α-carbons obtained from HMQC [δ 77.3 (Hiv 1) and 79.2 (Hiv 2)] were significatively downfield from those of the valines, allowing the unambiguous assignment of the signals for the two hydroxy acid units (**4c**).

COSY and TOCSY for two additional spin systems showed the following pattern of correlations: in each case, an α-methine proton [δ 5.08 (H-21, Hmp 1) and 4.63 (H-51, Hmp 2)] was connected to another methine [δ 2.02 (H-22, Hmp 1) and 1.69 (H-52, Hmp 2)]; this last methine was connected to both a methyl group [δ 0.98 (H-25, Hmp 1) and 0.91 (H-55, Hmp 2)] and a methylene group [δ 1.51 (H-23, Hmp 1) and 1.22 (H-53, Hmp 2)]; finally, this methylene was connected to a second methyl group [δ 0.90 (Hmp 1) and 0.89 (Hmp 2)]. HMQC spectrum allowed the assignment of the resonances of all the protonated carbons. The chemical shifts obtained for the α-carbons [δ 77.2 (C-21, Hmp 1) and 83.5 (C-51, Hmp 2)] were consistent with the presence of oxygen substituents. Thus, these two spin systems belong to 2-hydroxy-3-methylpentanoic acid units, Hmp (**4d**).

The remaining spin systems have the following connectivity pattern according to COSY, HMQC, and TOCSY analysis: each unit possessed a methine group ^{1}H/^{13}C NMR [δ 3.10/40.0 (C-7, Hymo 1) and 3.15/39.5 (C-37, Hymo 2)] connected to a methyl group [δ 1.07/15.0 (C-8, Hymo 1) and 1.13/14.4 (C-38, Hymo 2)] as well as to an oxygen-bearing methine [δ 5.34/75.3 (C-9, Hymo 1) and 4.90/78.2 (C39, Hymo 2)] which is bonded to a chain formed by three methylenes, the last one being connected to a terminal acetylene [δ 1.91/68.6 (C-14, Hymo 1) and 1.94/68.9 (C-44, Hymo 2)]. Further HMBC experiments reaffirmed the presence of the alkyne group: correlations from the signal at 2.19 ppm (H-12, Hymo 1) to carbons at 68.6 (C-14, Hymo 1) and 83.9 ppm (C-13, Hymo 1) and from the proton at 2.22 (H-42, Hymo 2) to carbons at 68.9 (C-44, Hymo 2) and 82.8 ppm (C-43, Hymo 2). This connectivity pattern is exactly the same as we have already found in the β-amino acid Amo (**3**), present in onchidin (**2**), but the chemical shift of the β-carbons are notably downfield from those of Amo. All these data led us to identify these two spin systems (**4e**) as belonging to a new β-hydroxy acid, 3-hydroxy-2-methyloct-7-ynoic acid, Hymo (**5**), that is reported for the first time in this work.

Relevant information pertaining to the determination of the bonding sequence was carried out using ^{13}C–^{1}H long range correlation experiments (HMBC), assigning the connectivities from carbonyl carbons to the adjacent amino or hydroxy substituted C_α–H protons. In this way, the presence of the structural segment Hiv 2-MeVal 1-Hymo 1 was established by the following correlations (Figure 2): CO (C-56, Hiv 2)/H-57 (Hiv 2); CO (C-1, MeVal 1)/H-57 (Hiv 2) and H-2 (MeVal 1); CO (C-6, Hymo 1)/H-2 (MeVal 1), NMe (MeVal 1), and Me-8 (Hymo 1). Similarly, two other skeletal segments were deduced from the correlations shown next: CO (C-20, Hmp 1)/H-16 (Pro 1) and H21 (Hmp 1) (segment Pro 1-Hmp 1), and CO (C-31, MeVal 2)/H-27 (Hiv 1) and H-32 (MeVal 2); CO (C-36 Hymo 2)/H-32 (MeVal 2), NMe (MeVal 2), and Me-38 (Hymo 2) (segment Hiv 1-MeVal 2-Hymo 2).[10]

The (+) FABMS of **4** showed, in addition to $[M + H]^+$ peak at *m/z* 1153, the parent ion peak at *m/z* 577 (100%). HRFABMS provided a value of *m/z* 577.3479 ($[C_{31}H_{48}N_2O_8 + H]^+$, Δ –

(10) HMBC shows also a strong correlation between C-26 (Hiv 1) and the multiplet at δ = 5.08–5.09 ppm. This multiplet is produced by the overlapping of the H-21 (Hmp 1) and H-27 (Hiv 1) resonances, and so, the connection between Hmp 1 and Hiv 1 could only be unambiguously established after MS and selective hydrolysis.

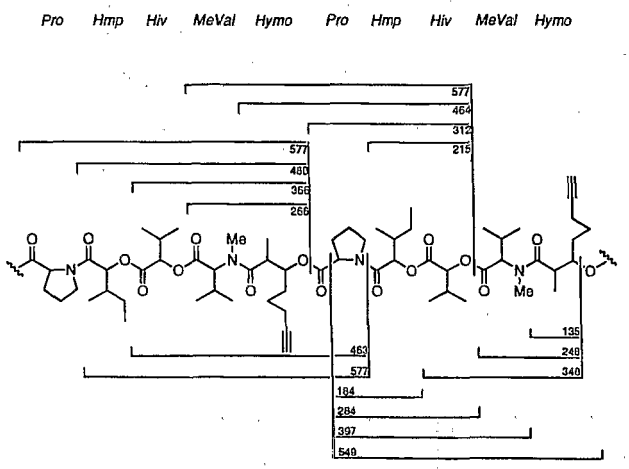

Figure 3. Onchidin B depsipeptide sequence determination by FABMS/MS.

0.9 mmu) that corresponds to the cleavage of the molecule into halves with identical mass. In order to shed light on the sequence, we decided to carry out $(MS)^n$ experiments. Thus, collisionally induced tandem FABMS (FABMS/MS) of the *m/z* 577 $[C_{31}H_{48}N_2O_8 + H]^+$ fragment gave ions at *m/z* 577 (Pro-Hmp-Hiv-MeVal-Hymo + H)$^+$, *m/z* 549 (Pro-Hmp-Hiv-MeVal-Hymo − CO + H)$^+$, *m/z* 480 (Hmp-Hiv-MeVal-Hymo + H)$^+$, *m/z* 464 (Hymo-Pro-Hmp-Hiv + H)$^+$, *m/z* 463 (Hiv-MeVal-Hymo-Pro + H)$^+$, *m/z* 397 (Pro-Hmp-Hiv-MeVal − CO + H)$^+$, *m/z* 366 (Hiv-MeVal-Hymo + H)$^+$, *m/z* 312 (Pro-Hmp-Hiv + H)$^+$, *m/z* 284 (Pro-Hmp-Hiv − CO +H)$^+$, *m/z* 266 (MeVal-Hymo + H)$^+$, *m/z* 215 (Hmp-Hiv + H)$^+$, and *m/z* 184 (Pro-Hmp − CO + H)$^+$ (Figure 3) whose assignment corroborates the NMR results and completes the linkages between the structural units, suggesting that onchidin B is composed by the sequence Pro-Hmp-Hiv-MeVal-Hymo-Pro-Hmp-Hiv-MeVal-Hymo. This accounts for only 16 unsaturation degrees and, therefore, to satisfy the molecular formula requirements (17 unsaturations), **4** must have the corresponding cyclic structure.

Stereochemistry of Onchidin B

Information about the absolute stereochemistry of onchidin B and additional evidence of its structure was achieved by GC-MS analysis of its components. Hydrolysis of **4** with 6 M HCl (110 °C, 24 h) followed by derivatization and GC-MS analysis on a chiral column (Chirasil-Val III) revealed the presence of *(S)*-Hiv, *(S,S)*-Hmp, *(R)*-MeVal, *(S)*-Pro, and *(R)*-Pro in a 2:2:2:1:1 ratio.

Furthermore, smooth basic hydrolysis (1 M NaOH/MeOH) of **4** cleaves the ester bonds and affords, after treatment with diazomethane, the expected three dipeptides (Figure 4): *(S,S)*-Hmp-*(S)*-Pro-OMe (**6**), *(S,S)*-Hmp-*(R)*-Pro-OMe (**7**), and Hymo-*(R)*-MeVal-OMe (**8**). **6** and **7** were identified by comparison (GC-MS) with authentic samples, unambiguously confirming the presence of both *(S)*-Pro and *(R)*-Pro in onchidin B (**4**). A third component (**8**) from the mixture was identified by EIMS proving the location of Hymo next to MeVal as previously suggested by NMR.

At this stage, the only remaining point to be solved was the absolute configuration of the two asymmetric centers of the β-hydroxy acid Hymo. No useful *J* values could be obtained due to massive overlapping in the ^{1}H NMR spectrum. A strong NOE was detected between H-7/H-9 and H-37/H-39, but it was not conclusive to distinguish between *eritro* or *threo* relative stereochemistries. To overcome this difficulty, we decided to prepare the four stereoisomers of Hymo and proceed to their

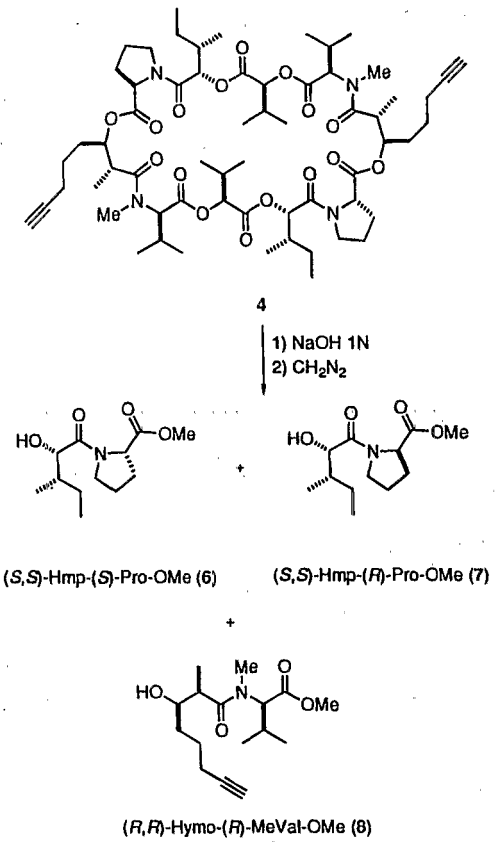

Figure 4. Basic hydrolysis of onchidin B (**4**).

comparison with the natural one, obtained by hydrolysis of onchidin B (**4**).

Diasteroselective Synthesis of Hymo

All four possible stereoisomers of 3-hydroxy-2-methyloct-7-ynoic acid (**5**) were synthesized in a diastereoselective mode. The synthetic strategy relies on the well-known reaction developed by Evans *et al.*,[11] between the enolborinate of optically pure *N*-propionyl oxazolidinones and aldehydes to obtain enantiomerically pure aldols and on the Mitsunobu reaction[12] which allows the inversion of configuration at the hydroxylated carbon of the aldol, yielding its epimer.

Reaction of *N*-propionyl oxazolidinone **9a**, derived from *(S)*-phenylalaninol,[13] with hex-5-ynal (prepared from hex-5-yn-1-ol by a Swern oxidation)[14] gives the aldol **10** with configuration (2*S*, 3*R*), whereas the reaction of oxazolidinone **9b**, obtained from (1*S*,2*R*)-norephedrine, with the same aldehyde provides aldol **11** with configuration (2*R*,3*S*).

Compounds **10** and **11** were obtained optically pure when the reactions were carried out with freshly opened bottles of dibutylborontriflate, since no other diastereomers were detected by NMR. Standard hydrolysis[15] of **10** and **11**, followed by diazomethane treatment, afforded β-hydroxy methyl esters **12b** (2*S*,3*R*) and **13b** (2*R*,3*S*), respectively.

In order to prepare the other two stereoisomers (2*R*,3*R* and 2*S*,3*S*), the inversion of one of the chiral centers of both **10**

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(12) (a) Mitsunobu, O. *Synthesis* **1981**, 1−28. (b) Hugues, D. L. *Org. React.* **1992**, *42*, 335−656.
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(14) Freeman, F.; Kim, D. S. H. L.; Rodríguez, E. *J. Org. Chem.* **1992**, *57*, 1722−1727.
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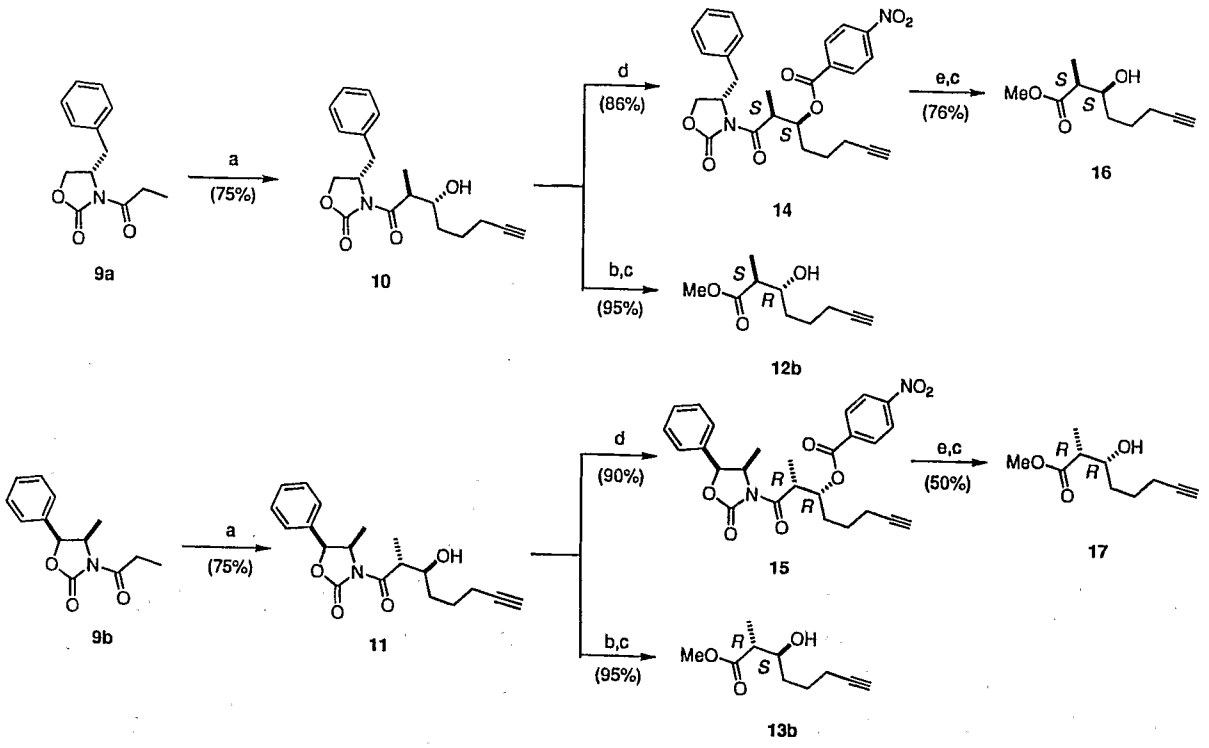
Scheme 1[a]



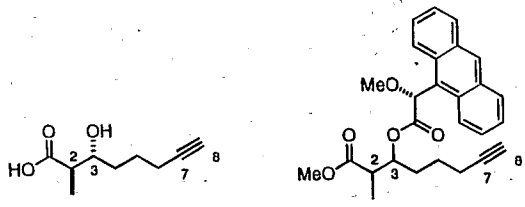
[a] (a) $(nBu)_2BOTf$, Et_3N, CH_2Cl_2, 0 °C and then hex-5-ynal, CH_2Cl_2, −70 °C; (b) LiOH, H_2O_2, THF−H_2O, 0 °C; (c) CH_2N_2, Et_2O; (d) diethylazodicarboxylate, triphenylphosphine, *p*-nitrobenzoic acid, benzene; (e) 30% H_2O_2, LiOH, 0 °C.

and **11** was needed. Since the configuration at position 2 could not be fully inverted easily, we decided to concentrate our efforts on the inversion of the configuration at carbon 3 making use of the Mitsunobu reaction. When β-hydroxy methyl ester **12b** was submitted to Mitsunobu conditions, no successful results were obtained. Nevertheless, a smooth inversion took place when aldols **10** and **11** were treated in similar conditions[16] and *p*-nitrobenzoate esters **14** and **15** were obtained in good yields. Simultaneous hydrolysis of the benzoate ester and oxazolidinone moieties, followed by diazomethane treatment, afforded the desired (2*S*,3*S*)-β-hydroxy methyl ester **16** and its (2*R*,3*R*) isomer **17**, in good yields.

Direct comparison of the methyl esters of the four synthetic stereoisomers with the methyl ester of Hymo, the natural component of onchidin B (**4**), by chiral GC and HPLC was not successful in spite of our efforts to find experimental conditions suitable for a good separation of the four isomers. This problem was finally overcome by HPLC-MS analysis of the 3-*O*-[(−)-(*R*)-α-methoxy-α-(9-anthryl)acetyl]-2-methyloct-7-ynoic methyl esters (**18**−**21**). These derivatives were prepared by standard treatment of the hydroxy esters with (−)-(*R*)-α-methoxy-α-(9-anthryl)acetic acid,[17] and were chosen because they provide four beautifully separated peaks in the HPLC chromatogram. In addition, they present strong and characteristic UV absorption and give clean CIMS fragmentations that enabled a safe comparison by coinjection with the natural Hymo.

Thus, reaction of the four synthetic β-hydroxy esters with (−)-(*R*)-α-methoxy-α-(9-anthryl)acetic acid, in the presence of DCC and DMAP, afforded the corresponding derivatives that showed the following retention times in HPLC-MS with the mass detector fixed at *m/z* 378: (*S*,*R*) 28.09 min, (*R*,*R*) 30.93 min, (*R*,*S*) 41.74 min, and (*S*,*S*) 46.54 min.

Chart 2



5

18 (2*S*,3*S*)
19 (2*R*,3*R*)
20 (2*S*,3*R*)
21 (2*R*,3*S*)

Onchidin B (**4**) was hydrolyzed with 6 M HCl at 110 °C for 24 h, and the mixture containing the free Hymo was esterified first with diazomethane and then with (−)-(*R*)-α-methoxy-α-(9-anthryl)acetic acid. HPLC-MS of that mixture showed a peak with identical t_R, UV, and MS as isomer (2*R*,3*R*)-3-O-[(−)-(*R*)-α-methoxy-α-(9-anthryl)acetyl]-2-methyloct-7-ynoic methyl ester. Therefore, the absolute configuration of the natural Hymo (**5**) is (2*R*,3*R*) and the structure of onchidin B (**4**) is cyclo [*(R)*-MeVal-*(R,R)*-Hymo-*(S)*-Pro-*(S,S)*-Hmp-*(S)*-Hiv-*(R)*-MeVal-*(R,R)*-Hymo-*(R)*-Pro-*(S,S)*-Hmp-*(S)*-Hiv].

Finally, in order to assign the *(R)* and *(S)* stereochemistries to the prolines, characteristic ^{13}C chemical shifts differences[18] and additional NOEs were considered. The linkage between Pro 1 and Hmp 1 is probably *trans*, as suggested by the value obtained for $\Delta\delta_{\beta\gamma} = 5.3$ ppm for C-17 and C-18, while the bond between Pro 2 and Hmp 2 is probably *cis* in accordance with the value $\Delta\delta_{\beta\gamma} = 7.8$ ppm for C-47 and C-48. A ROESY correlation between H-21 (Hmp 1) and H-19 (Pro 1) confirms the *trans* assignment for Pro 1.[19] These considerations, together with the NOE observed between H-21 (Hmp 1) and H-16 (Pro 1),[20] suggest that both hydrogens lie on the same side, so C-16

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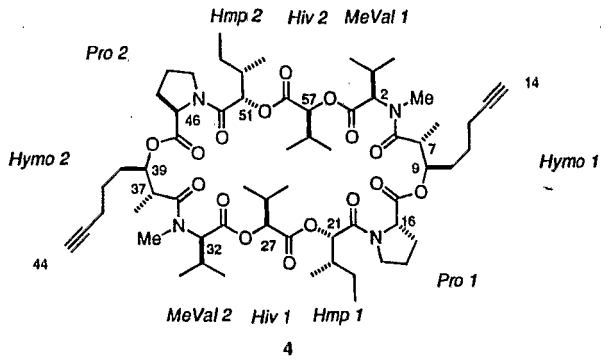
Chart 3



4

and C-21 must have identical configuration and Pro 1 should be *(S)*. Furthermore, the lack of NOE between H-46 (Pro 2) and H-51 (Hmp 2), in spite of the *cis* stereochemistry, suggests that both protons do not lie on the same side of the molecule, and so the absolute configuration of Pro 2 should be *(R)*.

Onchidin B (**4**) is then a cyclic depsipeptide formed by the head-to-tail bonding of two chains (MeVal-Hymo-Pro-Hmp-Hiv) with identical sequence. In spite of this common sequence, onchidin B (**4**) has no C_2 symmetry and shows signals for all the 62 carbons in the NMR spectra. This lack of coincidence of NMR signals is related to the presence of the two enantiomers of proline that renders the two halves of the molecule different.

Similar structural characteristics were found in onchidin (**2**), the other cyclodepsipeptide isolated from this organism, but, in that case, the repeating amino and hydroxy acids have the same configuration and so compound (**2**) has C_2 symmetry and presents only half the expected proton and carbon resonances.

A further point of interest is the presence in onchidin B (**4**) and onchidin (**2**) of the obviously related acetylenic β-hydroxy and β-amino acids, [Hymo (**5**) and Amo (**3**)], respectively. A β-hydroxy acid with the same skeleton but an additional methyl group at C-2 has recently been described by P. Scheuer as a component of the cyclodepsipeptide kulolide isolated from the mollusc *Philinopsis speciosa*.[21] Another related molecule is the β-hydroxy acid, *(2R,3S)*-7,7-dichloro-3-hydroxy-2-methyloctanoic acid, isolated from the marine sea hare *Dolabella auricularia*[16] that can be viewed as the dichlorinated derivative of a Hymo stereoisomer.

Experimental Section

General Methods. IR and UV spectra were obtained on MIDAC Prospect FTIR and 8452 A spectrophotometers, respectively, and optical rotations on a Jasco DIP-370 digital polarimeter. NMR spectra were recorded on Bruker AMX 500, Bruker AMX 300, or Bruker WM 250 spectrometers using $CDCl_3$ as solvent and internal standard. The $^1H-^1H$ COSY spectrum was acquired with 4K × 354 matrix and eight scans per increment. The TOCSY spectrum was acquired with 4K × 300 matrix and 16 scans per increment. The ROESY spectrum was acquired with 2K × 200 matrix and 32 scans per increment. The TOCSY and ROESY experiments were recorded using a mixing time of 0.070 and 0.280 ms, respectively. The HMQC[22] and HMBC[23] sequences were performed with 2K × 200 matrix and 64 scans per increment. FABMS were obtained on a Kratos MS-50 (employing Xe atoms at 7–9 KeV; glycerol and 2-hydroxyethyl disulfide + NaCl matrix) and FINIGAN 4000 (NBA matrix). Collisionally induced tandem mass spectra (MS/MS) in the FAB mode were obtained on a four sector tandem mass spectrometer VG 70-SE-4F. EIMS was obtained on a Hewlett-Packard HP59970 spectrometer.

HPLC separations were performed on a Waters Model 6000A using reversed-phase μ-Bondapack C_{18} (7.8 mm I.D. × 30 cm) and μ-porasil (7.8 mm I.D. × 30 cm). Gas chromatography analyses were performed using a Hewlett-Packard spectrometer with a Chirasil-Val III capillary column (50 m × 0.25 mm).

Cytotoxic activity was assessed *in vitro* using Kb cells.[24]

(1*S*,2*R*)-Norephedrine, (*S*)-phenylalanine, hex-5-yn-1-ol, and dibutylboron triflate were purchased from Aldrich Chemical Company and were used without further purification. All solvents were distilled under argon utilizing standard literature procedures. Flash column chromatography was performed using Kieselgel 60 230–400 mesh SiO_2 gel into glass columns. Synthetic products were purified by HPLC before optical rotation measurements. Synthetic yields were not optimized.

Extraction and Isolation. Animal Collection. *Onchidium* sp. is a Pulmonata, order: Stylommatophora, family: Onchidiidae, collected in July 1988 from Chesterfield archipelago in the intertidal zone on "Ile Longue" (Mouillage isles, 450 NW of New Caledonia), sample reference MG 332. Each animal weight was 300 g approximately. A voucher specimen is in deposit at the Museum National d'Histoire Naturelle in Paris, and it has been identified by Dr. Philippe Bouchet.

Extraction and Partitioning. Three kilograms of mollusc were freeze-dried (600 g dry weight) and then extracted with methanol (4 × 2 L). The methanol extract was decanted off and concentrated in vacuum. The viscous concentrate was partitioned between 400 mL of 10% aqueous methanol and hexanes (2 × 400 mL). The methanolic portion was made 20% aqueous and extracted with CCl_4 (2 × 400 mL). Then the methanolic portion was made 40% aqueous and extracted with CH_2Cl_2 (3 × 400 mL). The organic layers were concentrated in vacuum to yield 2.9 g of hexane extract (15% inhibition to Kb cells at 10 μg/mL), 1.2 g of CCl_4 extract (97% inhibition to Kb cells at 10 μg/mL), and 0.7 g of CH_2Cl_2 extract (99% inhibition to Kb cells at 10 μg/mL).

The CCl_4 extract was subjected to flash silica gel column chromatography (3 × 20 cm, silica gel 60, Merck 70–230 mesh) stepped gradient elution from 100% dichloromethane to 100% methanol giving five fractions: F1 (inactive), F2 (cytotoxic to Kb cells at 10 μg/mL: 93% inhibition), F3 (inactive), F4 (cytotoxic to Kb cells at 10 μg/mL: 95% inhibition), and F5 (inactive).

Isolation of Onchidin B. Fraction F2 was purified on a reversed-phase μ-Bondapack C_{18} HPLC column (30 cm × 7.8 mm, 93:7 MeOH: H_2O; flow rate: 2.0 mL/min) affording 5 mg (17×10^{-2} % from the CCl_4 extract) of onchidin B (**4**), t_R 12.7 min; $[\alpha]_{25}^{D}$ −220.1° (*c* 0.1, $CHCl_3$); FTIR (KBr) 3280, 2980, 2940, 2880, 1747, 1650, 1640 cm^{-1}; HRFABMS calcd for $C_{62}H_{97}N_4O_{16}$ *m/z* 1153.6899 $[M + H]^+$, found 1153.6999, Δ + 9.9 mmu; HRFABMS calcd for $C_{31}H_{49}N_2O_8$ *m/z* 577.3488 $[M + H]^+$, found 577.3479, Δ − 0.9 mmu; (+) FABMS (NBA matrix): *m/z* (% relative intensity): 1153 $[M + H]^+$ (7), 577 (100). (+) FABMS (2-hydroxyethyl disulfide + NaCl matrix), *m/z* (% relative intensity): 1175 $[M + Na]^+$ (1), 1153 $[M + H]^+$ (5), 577 (100). ^1H and ^{13}C NMR see Table 1.

Amino Acid Analysis by Chiral GC-MS. Onchidin B (**4**) (1 mg) was submitted to hydrolysis with 6 M HCl (0.5 mL) in a sealed tube at 110 °C for 24 h. The excess HCl was removed by passing a stream of N_2, and the residue was dried under vacuum. The hydrolyzates were diluted with water (1 mL), and the hydroxy acids were extracted with diethyl ether (3 × 1 mL).

The ethereal solution was dried under vacuum, and the residue was dissolved in 1 mL of ether and treated with diazomethane for 30 min. The excess of reagent was removed with a stream of dry N_2. Capillary

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(20) According to the literature, NOEs between H-16 and H-21 should be expected if the linkage Pro 1-Hmp 1 were *cis* and between H-19 and H-21 if *trans*. Models indicate that in **4** both NOEs are possible depending on the flexibility of the molecule and, in fact, we have observed them experimentally.

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(25) McDermott, J. R.; Benoiton, N. L. *Can. J. Chem.* **1973**, *51*, 1915–1919.

GC-MS analysis were carried out using a Chirasil-Val III column (50 m × 0.25 mm; carrier gas: He; flow rate: 1 mL/min; column temperature: 50 °C, 60 min isothermal) to show peaks at t_R 15.39 and 33.50 min. Standards (*S*)- and (*R*)-Hiv and (*S,S*)-, (*R,S*)-, (*R,R*)- and (*S,R*)-Hmp were also converted to the methyl derivatives by the same procedure. Retention times (minutes) were as follows: 15.51 [(*S*)-Hiv], 16.18 [(*R*)-Hiv], 31.10 [(*S,R*)-Hmp], 32.50 [(*R,S*)-Hmp], 33.69 [(*S,S*)-Hmp], and 35.25 [(*R,R*)-Hmp].

The water solution was dried under vacuum, and the resulting residue was divided into two equal portions. One of them was dissolved in dichloromethane (0.5 mL) and *N*-isopropyl isocyanate (1 mL) was added in a screwed-cap vial. After 60 min at 100 °C the excess of reagent was removed with a steady stream of dry N_2. Capillary GC-MS analysis were carried out using a Chirasil-Val III column (50 m × 0.25 mm; carrier gas: He; flow rate: 1 mL/min; column temperature 150 °C, 45 min isothermal, then programmed an increase of 5 °C/min to 200 °C) to show peak at t_R 33.11 min. Standards (*S*)- and (*R*)-MeVal [available by methylation of (*S*)- and (*R*)-Val][25] were also converted to the *N*-isopropyl isocyanates by the same procedure. Retention times (min) were as follows: 31.02 and 33.30 for (*S*)- and (*R*)-*N*-methyl valine, respectively.

The second portion was dissolved in *n*-BuOH (0.5 mL) and HCl (3 M, 0.5 mL) and heated in a sealed tube at 100 °C for 30 min. The product was evaporated, dissolved in trifluoracetic anhydride (0.5 mL) and CH_2Cl_2 (0.5 mL), reacted in a sealed tube at 150 °C for 10 min and evaporated in a stream of nitrogen. Capillary GC-MS analysis were carried out using the same column Chirasil-Val III column (50 m × 0.25 mm; carrier gas: He; flow rate: 1 mL/min; column temperature 50 °C, 30 min isothermal then programmed an increase of 2 °C/min to 200 °C) to show peaks at t_R 60.24 and 60.42 min. Standard (*S*)- and (*R*)-Pro were also derivatized by the same procedure. Retention times (min) were as follows: 60.36 and 60.55 for (*R*)- and (*S*)-proline, respectively.

Alkaline Hydrolysis of Onchidin B. Onchidin B (**4**) (0.5 mg) in MeOH (0.5 mL) was treated under an argon atmosphere with NaOH (1 M, 0.5 mL) at 0 °C. After 1 h the reaction mixture was diluted with CH_2Cl_2 (2 mL) and water (1 mL). The aqueous phase was acidified to pH 2 with 1 M HCl and extracted with CH_2Cl_2 (2 × 2 mL). The CH_2Cl_2 extract was dried with anhydrous Na_2SO_4 and concentrated in vacuum. The residue dissolved in ether (1 mL) was treated with an excess CH_2N_2 at room temperature for 30 min, and the excess of reagent was removed with a steady stream of dry N_2. Capillary GC-MS analysis were carried out using a Chirasil-Val III column (50 m × 0.25 mm; He as carrier gas; flow rate 1 mL/min; column temperature 150 °C, 45 min isothermal then programmed at 5 °C/min to 200 °C) to show peaks at t_R 62.86 min [(*S,S*)-Hmp-(*R*)-Pro-OMe EIMS *m/z* (% relative intensity): 211 (6), 155 (74), 127 (23)]; 63.97 min [(*S,S*)-Hmp-(*S*)-Pro-OMe EIMS *m/z* (% relative intensity): 211 (1), 155 (99), 127 (30)] and 69.37 min [(Hymo-MeVal-OMe EIMS *m/z* (% relative intensity): 267 (1), 238 (20), 230 (20), 201 (4), 144 (19), 86 (100)].

(4*S*,2′*S*,3′*R*)-3-(3′-Hydroxy-2′-methyl-7′-octynoyl)-4-(phenylmethyl)-2-oxazolidinone (10). A stirred solution of *N*-propionyloxazolidinone **9a** (3.0 g, 12.9 mmol) in dry dichloromethane (30 mL) was cooled to 0 °C and sequentially treated dropwise via syringe with 1 M dibutylboron triflate (18.0 mL, 18.0 mmol in dichloromethane) and triethylamine (3.0 mL, 2.2 g, 21.8 mmol). The resulting solution was cooled to −70 °C, and a solution of hex-5-ynal (1.9 g) in dichloromethane (10 mL) was added dropwise via syringe. The resulting mixture was stirred at −60 °C for 20 min and then an additional hour at 0 °C. The reaction was quenched by addition of aqueous phosphate buffered solution (pH 7, 15 mL) and methanol (15 mL). Then a mixture methanol:30% H_2O_2 (2:1, 20 mL) was added, and the resulting solution was stirred for 45 min at room temperature. The solvent was evaporated under reduced pressure, the residue was redissolved in water (40 mL), and the resulting solution was extracted with EtOAc (3 × 20 mL). The combined organic phase was washed with 5% $NaHCO_3$ (40 mL) and brine (40 mL), dried over anhydrous $MgSO_4$, and concentrated under reduced pressure, yielding a viscous yellow oil. The crude was purified by flash chromatography (EtOAc:hexanes 1:3) and the aldol **10** was obtained as a colorless viscous oil (3.4 g, 80% yield). $[\alpha]_D^{25}$ +29.3° (*c* 1.2, $CHCl_3$). EIMS *m/z* (% relative intensity): 329 (M^+, 2), 296 (10), 244 (20), 233 (42), 196 (30), 178 (46), 153 (14), 148 (17), 134 (82), 91 (100), 85 (71), 57 (25). HREIMS: calcd for $C_{19}H_{23}O_4N$ *m/z* 329.1627 (M^+), found 329.1629. FTIR ($CHCl_3$): 3526, 3289, 2940, 2872, 1777, 1691, 1453, 1386, 1213, 1107, 972 cm^{-1}. ^1H NMR (250.13 MHz, $CDCl_3$): δ 1.22 (d, J = 7.1 Hz, 3 H), 1.47−1.75 (m, 4 H), 1.90 (t, J = 2.3 Hz, 1 H), 2.17−2.25 (m, 2 H), 2.74 (dd, J = 13.3, 9.4 Hz, 1 H), 2.91 (s broad, 1 H), 3.20 (dd, J = 13.3, 3.3 Hz, 1 H), 3.70 (m, 1 H), 3.93 (m, 1 H), 4.11−4.23 (m, 2 H), 4.65 (m, 1 H), 7.14−7.35 (m, 5 H). ^{13}C NMR (62.89 MHz, $CDCl_3$): δ 10.3 (c), 18.1 (t), 24.8 (t), 32.6 (t), 37.7 (t), 42.1 (d), 55.0 (d), 66.1 (t), 68.5 (s), 70.9 (d), 84.1 (d), 127.4 (d), 129.0 (d), 129.4 (d), 135.0 (s), 153.0 (s), 177.5 (s).

(4*R*,5*S*,2′*R*,3′*S*)-3-(3′-Hydroxy-2′-methyl-7′-octynoyl)-5-phenyl-4-methyl-2-oxazolidinone (11). Using the method described for the preparation of (4*S*,2′*S*,3′*R*)-3-(3′-hydroxy-2′-methyl-7′-octynoyl)-4-(phenylmethyl)-2-oxazolidinone (**10**), *N*-propionyl oxazolidinone **9b** (3.875 g, 16.63 mmol) was treated with 1 M dibutylborontriflate (18.3 mL, 18.3 mmol) and triethylamine (3 mL, 21.62 mmol) and the resulting enolborinate was allowed to react with hex-5-ynal (2.1 g). After workup and purification the aldol **11** was obtained as a white solid (mp 97−100 °C), (4.1 g, 75% yield). $[\alpha]_D^{25}$ +8.3 (*c* 1.6, $CHCl_3$). EIMS *m/z* (% relative intensity): 262 (M^+, 9), 234 (15), 233 (100), 178 (20), 134 (36), 188 (42), 107 (60), 91 (21), 57 (25). HREIMS: calcd for $C_{19}H_{23}NO_4$ *m/z* 329.1627 (M^+) found 329.1630. FTIR ($CHCl_3$): 3521, 3292, 2940, 2875, 1779, 1694, 1455, 1351, 1231, 1197, 1120, 960 cm^{-1}. ^1H NMR (300.13 MHz, $CDCl_3$): δ 0.89 (d, J = 6.6 Hz, 3 H), 1.25 (d, J = 6.9 Hz, 3 H), 1.54−1.79 (m, 4 H), 1.96 (t, J = 2.3 Hz, 1 H), 2.22−2.27 (m, 2 H), 2.78 (s broad, 1 H), 3.78 (m, 1 H), 4.00 (m, 1 H), 4.80 (m, 1 H), 5.69 (d, J = 7.3 Hz, 1 H), 7.26−7.46 (m, 5 H). ^{13}C NMR (75.45 MHz, $CDCl_3$): δ 10.6 (c), 14.7 (c), 18.6 (t), 25.3 (t), 33.1 (t), 42.6 (d), 55.1 (d), 69.0 (s), 71.4 (d), 79.3 (d), 84.5 (d), 126.0 (d), 129.1 (d), 129.2 (d), 133.4 (s), 153.0 (s), 177.7 (s).

Methyl (2*S*,3*R*)-3-Hydroxy-2-methyl-7-octynoate (12b). A solution of the aldol **10** (1.24 g, 3.76 mmol) in THF:H_2O (4:1, 20 mL) was cooled to 0 °C. To this solution were sequentially added 30% H_2O_2 (1.53 mL, 15.04 mmol) and a solution of LiOH (252 mg, 6.0 mmol) in water (5 mL). The solution was stirred for 1 h. A solution of Na_2SO_3 (1.89 g, 15.04 mmol) in water (12 mL) was added, and the resulting solution was stirred for another 15 min. The organic solvent was concentrated under reduced pressure, and the resulting aqueous phase (pH 12) was extracted with dichloromethane (3 × 40 mL). Then the aqueous phase was cooled to 0 °C, acidified to pH 1 with 1.5 M HCl, and extracted with EtOAc (5 × 40 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The β-hydroxy acid **12a** was obtained as a viscous colorless oil (610 mg, 95% yield). The dichloromethane extract was dried over $MgSO_4$ and filtered, and the solvent was removed under reduced pressure. In this way the chiral oxazolidinone auxiliary was recovered as a white solid (565 mg, 85% yield). $[\alpha]_D^{25}$ +6.2 (*c* 2.0, $CHCl_3$). EIMS *m/z* (% relative intensity): 170 (M^+, 1), 137 (4), 124 (4), 103 (35), 85 (43), 74 (100), 57 (21). HREIMS: calcd for $C_9H_{14}O_3$ *m/z* 170.0942 (M^+) found 170.0937. FTIR ($CHCl_3$) 3453, 3292, 2943, 1709, 1456, 1408, 1210, 1078, 958 cm^{-1}. ^1H NMR (300.13 MHz, $CDCl_3$): δ 1.20 (d, J = 7.2 Hz, 3 H), 1.52−1.79 (m, 4 H), 1.96 (t, J = 2.6 Hz, 1 H), 2.21−2.26 (m, 2 H), 2.59 (dd, J = 7.2, 3.3 Hz, 1 H), 3.98 (m, 1 H), 6.51 (s broad, 2 H). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 10.7 (c), 18.6 (t), 25.2 (t), 32.9 (t), 44.6 (d), 69.1 (d), 71.6 (s), 84.4 (d), 181.4 (s).

N-Nitroso-*N*-methyl urea (150 mg) was added to a two-phase 50% aqueous KOH:ether system (30 mL, 1:2). When the gas evolution ceased, the organic phase was carefully decanted and added to a solution of the β-hydroxy acid **12a** (57 mg, 0.33 mmol) in ether (10 mL) at room temperature. After 30 min the solvent was evaporated with an argon flow, yielding the β-hydroxy methyl ester **12b** as a yellowish oil. $[\alpha]_D^{25}$ +14.3 (*c* 0.4, $CHCl_3$). (+) FABMS (glycerol matrix) *m/z* (% relative intensity): 185 [M + H]$^+$ (89), 167 (15), 153 (35), 125 (11), 107 (78). HREIMS: calcd for $C_{10}H_{16}O_3$ *m/z* 184.1099 (M^+), found 184.1099. FTIR ($CHCl_3$) 3507−3465, 3294, 2946, 1728, 1448, 1434, 1368, 1264, 1201, 1167, 1087, 1046 cm^{-1}. ^1H NMR (300.13 MHz, $CDCl_3$): δ 1.19 (d, J = 7.2 Hz, 3 H), 1.51−1.64 (m, 3 H), 1.70−1.80 (m, 1 H), 1.95 (t, J = 2.6 Hz, 1H), 2.23 (m, 2 H), 2.51−2.59 (dq, J = 7.2, 3.5, 1 H), 3.71 (s, 3 H), 3.91 (m, 1 H). ^{13}C NMR (62.89

MHz, $CDCl_3$): δ 10.6 (c), 18.1 (t), 24.8 (t), 32.7 (t), 44.3 (d), 51.7 (c), 68.6 (d), 71.3 (d), 84.1 (s), 176.5 (s).

Methyl (2*R*,3*S*)-3-Hydroxy-2-methyl-7-octynoate (13b). Aldol **11** was hydrolyzed using the method described for the hydrolysis of **10**. Thus, aldol **11** (888 mg, 2.7 mmol) was treated with 30% H_2O_2 (1.1 mL, 10.84 mmol) and LiOH (182 mg, 4.3 mmol). After workup and purification the chiral auxiliary oxazolidinone was recovered (425 mg, 88% yield), and the β-hydroxy acid **13a** was obtained as a colorless viscous oil (440 mg, 95% yield). $[\alpha]_D^{25}$ −6.0 (*c* 1.3, $CHCl_3$). NMR and MS spectroscopic data are identical to those obtained for **12a**.

13b was obtained by treatment of β-hydroxy acid **13a** with excess diazomethane as described previously for β-hydroxy methyl ester **12b**. NMR spectroscopic data are identical to those obtained for **12b**. $[\alpha]_D^{25}$ −13.0 (*c* 0.7, $CHCl_3$). (+) FABMS (glycerol matrix) *m/z* (% relative intensity): 185 $[M + H]^+$ (93), 167 (23), 153 (41), 125 (18), 107 (71). HREIMS: calcd for $C_{10}H_{16}O_3$ *m/z* 184.1099 (M^+), found 184.1099. ^1H NMR (300.13 MHz, $CDCl_3$): δ 1.19 (d, *J* = 7.3 Hz, 3 H), 1.51−1.61 (m, 2 H), 1.75 (m, 1 H), 1.95 (t, *J* = 2.6 Hz, 1 H), 2.23 (m, 2 H), 2.53−2.57 (m, 2 H), 3.71 (s, OMe, 3 H), 3.91 (m, 1 H). ^{13}C NMR (62.89 MHz, $CDCl_3$): δ 10.6 (c), 18.1 (t), 24.8 (t), 32.6 (t), 44.3 (d), 51.7 (c), 68.5 (d), 71.2 (d), 84.1 (s), 176.5 (s).

(4*S*,2′*S*,3′*S*)-3-[2′-Methyl-3′-[(4″-nitrobenzoyl)oxy]-7′-octynoyl]-4-phenylmethyl-2-oxazolidinone (14). A solution of diethylazodicarboxylate (1.48 g, 8.47 mmol) in benzene (5 mL) was added to a solution of aldol **10** (400 mg, 1.21 mmol), triphenylphosphine (2.22 g, 8.47 mmol), and *p*-nitrobenzoic acid (1.42 g, 8.47 mmol) in benzene (40 mL) at room temperature. The resulting solution was stirred for 17 h at room temperature. The solvent was removed under reduced pressure, and the reaction crude was purified by flash chromatography (EtOAc:hexanes 1:7), yielding the nitrobenzoate ester **14** as a colorless viscous oil (500 mg, 86%). $[\alpha]_D^{25}$ +72.1 (*c* 0.4, $CHCl_3$). EIMS *m/z* (% relative intensity): 478 (M^+, 2), 382 (1), 327 (1), 311 (5), 302 (11), 296 (10), 283 (2), 272 (4), 244 (27), 231 (3), 178 (5), 150 (100), 135 (51), 134 (22), 117 (22), 104 (28), 91 (38). HREIMS: calcd for $C_{26}H_{26}N_2O_7$ *m/z* 478.1740 (M^+), found 478.1745. FTIR ($CHCl_3$) 3291, 2945, 1780, 1720, 1604, 1528, 1449, 1387, 1350, 1274, 1214, 1110, 1020 cm^{-1}. ^1H NMR (300.13 MHz, $CDCl_3$): δ 1.33 (d, *J* = 7.0 Hz, 3 H), 1.66 (m, 2 H), 1.88 (m, 1 H), 1.95 (t, *J* = 2.6 Hz, 1 H), 2.05 (m, 1 H), 2.26 (m, 2 H), 2.74 (dd, *J* = 13.3, 9.5 Hz, 1 H), 3.27 (dd, *J* = 13.3, 3.3 Hz, 1 H), 3.97 (t, *J* = 8.2 Hz, 1H), 4.12 (dd, *J* = 9.1, 2.6 Hz, 1 H), 4.30 (dd, *J* = 8.6, 7.0 Hz, 1 H), 4.55 (m, 1 H), 5.55 (ddd, *J* = 8.2, 8.2, 3.2 Hz, 1 H), 7.14−7.17 (m, 2 H), 7.25−7.33 (m, 3 H), 8.16−8.20 (m, 2 H), 8.26−8.30 (m, 2 H). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 14.6 (c), 18.6 (t), 23.9 (t), 30.7 (t), 38.1 (t), 41.6 (d), 55.6 (d), 66.5 (t), 69.5 (d), 76.3 (d), 83.9 (s), 124.0 (d), 127.9 (d), 129.4 (d), 129.8 (d), 131.2 (d), 135.2 (s), 135.7 (s), 151.0 (s), 153.5 (s), 164.1 (s), 174.2 (s).

(4*R*,5*S*,2′*R*,3′*R*)-3-[3′-[(4″-Nitrobenzoyl)oxy]-2′-methyl-7′-octynoyl]-5-phenyl-4-methyl-2-oxazolidinone (15). Aldol **11** (270 mg, 0.82 mmol) was treated with triphenylphosphine (1.5 g, 5.74 mmol), *p*-nitrobenzoic acid (960 mg, 5.74 mmol), and diethylazodicarboxylate (0.9 mL, 5.74 mmol) in the manner described for the preparation of nitrobenzoate ester **14**. After workup and purification ester **15** was obtained as an oil together with a small amount of another product which could not be separated by HPLC (350 mg, 90% yield). $[\alpha]^{25}_D$ −8.4 (*c* 0.7, $CHCl_3$). EIMS *m/z* (% relative intensity): 478 (M^+, 1), 382 (1), 311 (3), 302 (2), 272 (2), 244 (3), 178 (6), 150 (100), 135 (27), 134 (40), 118 (13), 104 (26), 91 (21). HREIMS: calcd for $C_{26}H_{26}N_2O_7$ *m/z* 478.1740 (M^+), found 478.1730. FTIR ($CHCl_3$) 3292, 2942, 2869, 1781, 1720, 1611, 1526, 1453, 1348, 1274, 1233, 1198, 1115, 1078 cm^{-1}. ^1H NMR (300.13 MHz, $CDCl_3$): δ 0.85 (d, *J* = 6.6 Hz, 3 H), 0.95 (d, *J* = 6.6 Hz, 3 H), 1.61−1.72 (m, 2 H), 1.84−1.95 (m, 1 H), 1.95 (t, *J* = 2.7 Hz, 1 H), 2.00−2.08 (m, 1 H), 2.23−2.34 (m, 2 H), 4.29 (d, *J* = 6.6 Hz, 1 H), 4.65 (q, *J* = 6.8 Hz, 1 H), 5.44 (d, *J* = 7.3 Hz, 1 H), 5.55 (td, *J* = 8.6, 8.6, 3.3 Hz, 1 H), 7.20−7.31 (m, 2 H), 7.35−7.42 (m, 3 H), 8.27−8.31 (m, 2 H), 8.26−8.30 (m, 2 H). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 14.5 (c), 18.6 (c), 23.9 (t), 27.4 (t), 30.7 (t), 41.8 (d), 55.3 (d), 69,5 (d), 76.3 (d), 79.3 (d), 83.8 (s), 124.0 (d), 125.9 (d), 129.2 (d), 131.2 (d), 133.3 (d), 135.8 (s), 137.6 (s), 151.0 (s), 153.0 (s), 164.2 (s), 174.2 (s).

Methyl (2*S*,3*S*)-3-Hydroxy-2-methyl-7-octynoate (16). A 0 °C cooled solution of nitrobenzoate ester **14** (414 mg, 0.86 mmol) in THF:H_2O (40 mL, 4:1) was sequentially treated with 30% H_2O_2 (0.43 mL, 3.78 mmol) and a solution of LiOH (120 mg, 2.86 mmol) in H_2O (1 mL). The mixture was stirred for 1 h at 0 °C and then for a period of 18 h at room temperature. Aqueous 10% Na_2SO_3 solution (5 mL) was added, and the organic solvent was removed under reduced pressure. The resulting aqueous phase was extracted with dichloromethane (4 × 30 mL), acidified to pH 1 with 6 M HCl, and reextracted with EtOAc (4 × 30 mL). The combined EtOAc extract was washed with brine (50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was redissolved in ether (15 mL) and was treated with an ethereal solution of diazomethane. The solvent was evaporated with an argon flow, and the crude was purified by flash chromatography (EtOAc:hexanes 1:8), yielding the β-hydroxy methyl ester **16** as a colorless viscous oil (120 mg, 76%). $[\alpha]_D^{25}$ +2.5 (*c* 0.5, $CHCl_3$). (+) FABMS (glycerol matrix) *m/z* (% relative intensity): 185 $[M + H]^+$ (100), 167 (6), 153 (30), 125 (13), 107 (59). EIMS *m/z* (% relative intensity): 173 (1), 151 (2), 143 (2), 117 (56), 107 (7), 97 (9), 88 (100). HREIMS calcd for $C_{10}H_{16}O_3$ *m/z* 184.1099 (M^+), found 184.1099. ^1H NMR (300.13 MHz, $CDCl_3$): δ 1.21 (d, *J* = 7.2 Hz, 3 H), 1.45−1.83 (m, 4 H), 1.94 (t, *J* = 2.6, 1 H), 2.22 (m, 2 H), 2.52 (q, *J* = 7.1, 1 H), 2.66 (d, *J* = 6.4, 1 H), 3.67 (m, 1 H), 3.70 (s, 3 H). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 14.7 (c), 18.6 (t), 24.8 (t), 33.9 (t), 45.6 (d), 52.2 (c), 69.0 (d), 73.2 (d), 84.6 (s), 176.8 (s).

Methyl (2*R*,3*R*)-3-Hydroxy-2-methyl-7-octynoate (17). Following the procedure described above for the preparation of β-hydroxy methyl ester **16**, benzoate ester **15** (273 mg, 0.57 mmol) was treated with 30% H_2O_2 (0.25 mL, 2.28 mmol) and LiOH (76 mg, 1.8 mmol). After workup, the mixture of acids was esterified with excess of diazomethane. Purification gave the β-hydroxy methyl ester **17** as a colorless viscous oil (77 mg, 74% yield). $[\alpha]_D^{25}$ −2.6 (*c* 0.5, $CHCl_3$). (+) FABMS (glycerol matrix) *m/z* (% relative intensity): 185 $[M + H]^+$ (74), 167 (5), 153 (20), 125 (7), 107 (44). EIMS *m/z* (% relative intensity): 173 (1), 151 (2), 143 (2), 117 (56), 107 (7), 97 (9), 88 (100). HREIMS calcd for $C_{10}H_{16}O_3$ *m/z* 184.1099 (M^+), found 184.1099. ^1H NMR (300.13 MHz, $CDCl_3$): δ 1.21 (d, *J* = 7.2 Hz, 3 H), 1.48−1.80 (m, 4 H), 1.94 (t, *J* = 2.6, 1 H), 2.22 (m, 2 H), 2.52 (q, *J* = 7.0, 1 H), 2.66 (d, *J* = 6.4, 1 H), 3.66 (m, 1 H), 3.70 (s, 3 H). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 14.7 (c), 18.6 (t), 24.8 (t), 33.9 (t), 45.6 (d), 52.2 (c), 69.0 (d), 73.2 (d), 84.6 (s), 176.8 (s).

Preparation of 3-*O*-[(−)-(*R*)-α-Methoxy-α-(9-anthryl)acetyl]-2-methyloct-7-ynoic Methyl Esters 18−21. General. The esters **18**−**21** were prepared by treatment of the corresponding β-hydroxy ester (3 mg) with (−)-(*R*)-α-methoxy-α-(9-anthryl)acetic acid (5 mg) in the presence of DCC (4 mg) and DMAP (catalytic) in CH_2Cl_2 at room temperature. After 12 h the solvent was evaporated, and the crude was sequentially purified by flash chromatography (CH_2Cl_2) and HPLC (μ-porasil, 30 cm × 7.8 mm; AcOEt:hexane 7:93; flow rate: 2.0 mL/min).

(a) (2*S*,3*S*)-3-*O*-[(−)-(*R*)-α-methoxy-α-(9-anthryl)acetyl]-2-methyloct-7-ynoic Methyl Ester (18). $[\alpha]_D^{25}$ −119.1 (*c* 0.2, $CHCl_3$). EIMS *m/z* (% relative intensity): 432 (M^+, 16), 221 (100), 206 (15), 205 (11), 178 (16). HREIMS calcd for $C_{27}H_{28}O_5$ *m/z* 432.1936 (M^+), found 432.1963. ^1H NMR (300.13 MHz, $CDCl_3$): δ 0.45 (m, 2 H), 1.05 (d, *J* = 7.2, 3 H), 1.12 (m, 1 H), 1.29 (m, 1 H), 1.44 (m, 2 H), 1.69 (t, *J* = 2.6, 1 H), 2.58, (q, *J* = 7.1, 1 H), 3.44 (s, 3 H), 3.59 (s, 3 H), 5.09 (m, 1 H), 6.22 (s, 1 H), 7.44−7.56 (m, 4 H), 8.00−8.03 (m, 2 H), 8.47−8.52 (m, 3 H). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 12.9 (c), 17.6 (t), 22.8 (t), 29.5 (t), 43.2 (d), 52.1 (c), 57.9 (c), 68.7 (d), 75.1 (d), 77.3 (d), 83.7 (s), 124.7 (d), 125.5 (d), 126.9 (d), 127.6 (s), 129.5 (d), 129.6 (d), 130.9 (s), 131.8 (s), 171.1 (s), 174.0 (s).

(b) (2*R*,3*R*)-3-*O*-[(−)-(*R*)-α-Methoxy-α-(9-anthryl)acetyl]-2-methyloct-7-ynoic Methyl Ester (19). $[\alpha]_D^{25}$ −87.6 (*c* 0.2, $CHCl_3$). EIMS *m/z* (% relative intensity): 432 (M^+, 23), 221 (100), 206 (19), 205 (16), 178 (13). HREIMS calcd for $C_{27}H_{28}O_5$ *m/z* 432.1936 (M^+), found 432.1963. ^1H NMR (300.13 MHz, $CDCl_3$): δ 0.42 (d, *J* = 7.1, 3 H), 1.52−1.68 (m, 2 H), 1.95 (t, *J* = 2.6, 1 H), 2.19, (m, 2 H), 2.33 (q, *J* = 7.0, 1 H), 2.94 (s, 3 H), 3.42 (s, 3 H), 5.15 (m, 1 H), 6.26 (s, 1 H), 7.45−7.57 (m, 4 H), 8.00−8.03 (m, 2 H), 8.48−8.57 (m, 3 H). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 11.8 (c), 18.4 (t), 24.4 (t), 29.6 (t), 42.7 (d), 51.5 (c), 57.8 (c), 69.2 (d), 75.2 (d), 77.5 (d), 84.0 (s), 124.8 (d), 125.4 (d), 126.9 (d), 127.5 (s), 129.5 (d), 129.6 (d), 130.9 (s), 131.8 (s) 171.0 (s), 173.2 (s).

(c) (2*S*,3*R*)-3-*O*-[(−)-(*R*)-α-Methoxy-α-(9-anthryl)acetyl]-2-methyloct-7-ynoic Methyl Ester (20). $[\alpha]_D^{25}$ −74.5 (*c* 0.1, $CHCl_3$). EIMS *m/z* (% relative intensity): 432 (M^+, 5), 221 (100), 208 (18), 206 (15), 205 (12), 180 (16), 178 (19), 152 (12). HREIMS calcd for $C_{27}H_{28}O_5$ *m/z* 432.1936 (M^+), found 432.1963. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 0.43 (d, *J* = 7.1 Hz, 3 H), 1.19−1.67 (m, 4H), 1.96, (t, *J* = 2.6 Hz, 1 H), 2.18−2.27 (m, 3 H), 2.94 (s, 3 H), 3.41 (s, 3 H), 5.15 (q, *J* = 6 Hz, 1 H), 6.28 (s, 1 H), 7.45−7.57 (m, 4 H), 8.01−8.03 (m, 2 H), 8.48−8.59 (m, 3 H). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 11.7 (c), 18.5 (t), 24.7 (t), 31.2 (t), 43.0 (d), 51.5 (c), 57.8 (c), 69.3 (d), 75.0 (d), 77.4 (d), 84.1 (s), 124.9 (d), 125.4 (d), 126.8 (d), 127.3 (s), 129.5 (d), 129.6 (d), 130.9 (s), 131.8 (s) 170.9 (s), 173.5 (s).

(d) (2*R*,3*S*)-3-*O*-[(−)-(*R*)-α-Methoxy-α-(9-anthryl)acetyl]-2-methyloct-7-ynoic Methyl Ester (21). $[\alpha]_D^{25}$ +20.1 (*c* 0.1, $CHCl_3$). EIMS *m/z* (% relative intensity): 432 (M^+, 18), 221 (100), 206 (14), 205 (9), 178 (18). HREIMS *m/z* 432.1963 for $C_{27}H_{28}O_5$ (calcd 432.1936). ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 0.50 (m, 2 H), 1.01 (d, *J* = 7.0, 3 H), 1.08 (m, 1 H), 1.22−1.30 (m, 1 H), 1.39−1.53 (m, 2 H), 1.69 (t, *J* = 2.6, 1 H), 2.55, (dd, *J* = 7.1, 5.2, 1 H), 3.44 (s, 3 H), 3.64 (s, 3 H), 5.13 (m, 1 H), 6.26 (s, 1 H), 7.45−7.56 (m, 4 H), 8.00−8.03 (m, 2 H), 8.47−8.54 (m, 3 H). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 12.4 (c), 17.9 (t), 23.7 (t), 30.7 (t), 43.5 (d), 52.6 (c), 58.2 (c), 69.0 (d), 74.9 (d), 77.8 (d), 84.0 (s), 124.7 (d), 125.7 (d), 127.2 (d), 127.8 (s), 129.8 (d), 129.9 (d), 131.0 (s), 132.0 (s), 171.5 (s), 174.6 (s).

(e) Separation of the Mixture of 3-*O*-[(−)-(*R*)-α-Methoxy-α-(9-anthryl)acetyl]-2-methyloct-7-ynoic Methyl Esters (18−21) by HPLC-MS. A mixture of the synthetic isomers was separated by HPLC-MS (μ-porasil, 30 cm × 7.8 mm; AcOEt:hexane 7:93; flow rate: 2.0 mL/min) showing the following retention times: (*S*,*R*) 28.09 min, (*R*,*R*) 30.93 min, (*R*,*S*) 41.74 min, and (*S*,*S*) 46.54 min.

Determination of Absolute Configuration of Hymo (5). Onchidin B (0.5 mg) was submitted to hydrolysis with 6 M HCl (0.5 mL) in a sealed tube at 110 °C for 24 h. The excess HCl was removed by passing a stream of N_2, and the residue was dried under vacuum. The hydrolyzates were diluted with water (1 mL), and the hydroxy acids were extracted with diethyl ether (3 × 1 mL). The ethereal solution was dried under vacuum, and the residue was dissolved in 1 mL of ether and treated with diazomethane for 30 min. The excess of reagent was removed with a stream of dry N_2. The hydroxy methyl ester was treated with (−)-(*R*)-α-methoxy-α-(9-anthryl)acetic acid (0.5 mg), DCC (0.5 mg), and DMAP (catalytic) in CH_2Cl_2 at room temperature. After 12 h the solvent was evaporated, and the residue was subjected to HPLC-MS (μ-porasil, 30 cm × 7.8 mm; AcOEt:hexane 7:93; flow rate: 2.0 mL/min) to show peak at t_R 30.69 min.

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, COSY, TOCSY, HMQC, ROESY spectra, and expansions of HMBC spectra, MS (FABMS and FABMS/MS) spectra of **4** (12 pages). See any current masthead page for ordering and Internet access instructions.

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