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Mollenyne A, a Long-Chain Chlorodibromohydrin Amide from the Sponge *Spirastrella mollis*

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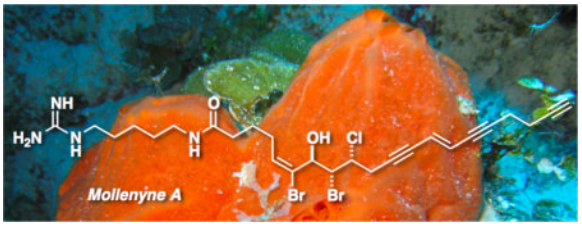
Abstract



The structure of mollenyne A, acytotoxic nitrogenous halogenated long-chain carboxamide from the sponge *Spirastrella mollis*, was elucidated by integrated spectroscopic analysis, including CD, and chemical conversion.

Brominated lipids commonly occur in several genera of marine sponges including *Petrosia*, *Xestospongia* and *Oceanapia*.[1] Unlike the Rhodophyta (red algae), which are replete with halogenated terpenes and lipids containing myriad combinations of Br, Cl and sometimes I, Porifera (sponges) – suprisingly – rarely contain compounds with *both* Br and Cl. We describe here mollenyne A (**1**) from the sponge *Spirastrella mollis* Verrill, 1907,[2] collected from the Bahamas (Plana Cays). Mollenyne A (**1**) is a chiral tri-halogenated cytotoxic C_{20} carboxamide, embodying three units: a triyne-ene terminus, an allylic alcohol flanked by halogenated carbons, and homoagmatine (decarboxyhomoarginine).[3]

The only other bromochloro natural products from sponges are axinellamine A[4] (**2**) from *Axinella* sp. and tetrabromostyloguanidine (**3**)[5] from *Stylissa caribica* (Figure 1), long-chain terminal vinyl bromochloro azirines (4*E*-**4** and *4Z*-**5**)[6] from *Dysidea fragilis*, and halogenated cyclohexenones from *Aplysina cavernicola* (e.g. 3-bromo-5-chloroverongiaquinol, **6**),[7] but none resemble **1**. Screening of crude extracts from a collection of Bahamian sponges and tunicates ($n = 180$) in assays with cultured cancer cells (human colon tumor, HCT-116) identified a highly cytotoxic extract from the red encrusting sponge *Spirastrella mollis* ($IC_{50} \leq 0.1$ μg/mL). Further purification of the CH_2Cl_2 partition from *S. mollis* by C_{18} flash chromatography and reversed phase HPLC gave a new halogenated lipid, mollenyne A (**1**).

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Supporting Information Available. Experimental procedures and full spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

HRESIMS analysis of **1** revealed the molecular formula of $C_{26}H_{36}O_2N_4Br_2Cl$ (*m/z* 629.0888 $[M+H]^+$) with 10 degrees of unsaturation and six exchangable hydrogens (LRESIMS in CD_3OD, *m/z* at 635 $[M+H]^+$). Interpretation of one and two dimensional NMR experiments allowed the assignment of five substructures (*A*–*E*, Figure 3 and Table 1). Substructure *A* contained a ^1H spin system of three contiguous methylene groups H2 through H4 (δ 2.23, t, *J* = 7.4 Hz, δ 1.79, and δ 2.23, respectively) and terminated with a vinyl proton H5 (δ 6.10, t, *J* = 7.7 Hz).

C5 (δ 137.3) was connected to a quaternary sp^2 carbon C6 (δ 128.5) by an HMBC cross peak from H5 to C6. COSY and TOCSY correlations showed the oxygenated methine H7 (δ 4.73, d, *J* = 9.7 Hz) was coupled to methine H8 (δ 4.57, m), and H9 (δ 4.57, m) was coupled to H10 (δ, 2.97, m) leading to substructure *B*.

Substructure *C* of **1** is a *trans* disubstituted alkene (δ 5.91, dt, *J* = 16.1, 1.9 Hz, H13; 5.97, dt, *J* = 16.1, 2.0 Hz, H14). Substructure *D* contained the C19-C20 acetylene terminus (δ_H 2.31, t, *J* = 2.6 Hz, H20; δ_C 70.5, C20, CH; 94.6, Cq, C19) linked to a 1,2-disubstituted ethane, H17 (δ 2.54, td, *J* = 7.2, 2.0 Hz) and H18 (δ 2.39, td, *J* = 7.2, 2.6 Hz). The final substructure *E* was assigned as a 1,5-diamine unit.

Substructures *A*–*E* were assembled by further analysis of HMBC spectra (Figure 5.5). *A* and *E* were connected through an amide carbonyl group (δ 175.6, C1) which showed HMBC correlations to both H2 and H1′. Hydroxymethine H7 showed a correlation to C5 which established the link between substructures *A* and *B*. The C5–C6 double bond was assigned the *E* geometry based on NOESY correlation between H4 and H7. Both H10 and H13 showed correlations to sp carbons C11 (δ 89.9) and C12 (δ 82.6).

Substructure *D* was attached to *C* by a third C-C triple bond [C15 (δ 80.4) and C16 (δ 94.6)]. The left fragment *N*-terminal fragment of **1** was identified as a guanidine group based on the balance of the molecular formula and HMBC correlation from H5′ to C6′ with a characteristic C=N chemical shift (δ 158.6).

Placement of the halogens (two Br and one Cl) was supported by NMR and chemical conversion.[8] A vinyl bromide was favored over a vinyl chloride based on comparison of ^{13}C chemical shifts with known synthetic compounds.[9] The quaternary sp^2 carbon bearing a Br (~δ 125 ppm) is typically observed upfield of the protonated sp^2 carbon (~ δ 133 ppm) in trisubstituted vinyl bromides (‘heavy atom’ effect). In vinyl chlorides, the opposite trend is observed; the quaternary sp^2 carbon (~ δ 135 ppm) is observed downfield of the protonated sp^2 carbon (~ δ 125 ppm). Based on ^{13}C chemical shift and chemical transformation (see below) the remaining Br and Cl were placed at C8 and C9, respectively.

To assign the relative configuration of mollenyne A (**1**), we turned to *J*-based configurational analysis (JBCA), established by Murata and coworkers for hydroxylated polyketides.[10] The stereotriad of **1** resembles those found in chlorosulfolipids[11] isolated from Adriatic shellfish,[12] freshwater algae[13] and marine cyanobacteria.[14] Recently, the Carreira group completed a meticulous investigation of the homo- and hetero-nuclear two- and three-bond coupling constants in a series of chlorosulfolipid models[15] and demonstrated that subtle differences should be considered when analyzing spin systems of polychlorinated compounds compared to those of polyhydroxylated ketides. The former more reliable basis sets were used as a starting point for JBCA analysis of **1**.

The C8-C9 *threo* configuration was assigned by JBCA in a straightforward manner (Figure 5, conformer ***c***). H8 and H9 were oriented gauche based on a small vicinal homonuclear coupling (*J* = 1.6 Hz), however the low magnitude lead to inefficient magnetization transfer and we were unable to observe $^2J_{HC}$ and $^3J_{HC}$ coupling constants by HETLOC or HSQC-

HECADE experiments. Consequently, we turned to *J*-resolved HMBC that revealed the magnitudes of all heteronuclear couplings were small (Figure 5). The vicinal Br and Cl were oriented *anti* to H9 and H8, respectively, and C7 and C10 were assigned *gauche* to H9 and H8, respectively. H7 and H8 were oriented *anti* based on large H-H vicinal coupling (J = 9.0 Hz), and HSQC-HECADE revealed that both $^2J_{HC}$ H7-C8 and H8-C7 have large couplings. These data are accommodated by either diastereomer *threo* ***a*** or *erythro* ***b*** (Figure 5), an ambiguity that was resolved by chemical conversion as follows.

Mollenyne A (**1**) underwent hydrogenation (H_2, Pd/C, MeOH, 3 days, Scheme 1), with concomitant hydrogenolysis of the vinyl bromide to give **7**. Exposure of the product **7** to base (K_2CO_3, MeOH) gave epoxide **8**[16] after neutralization (AcOH), and HPLC purification. The vicinal H7-H8 coupling constant (J = 2 Hz) confirmed a *trans* epoxide. Accounting for inversion at C8 during epoxide ring closure by S_N2 substitution, the relative configuration of the C7-C8 stereocenters in **1** was revealed as *erythro* ***b*** (Figure 5).

Assignment of the carbinol center C7 and completion of the absolute stereostructure of **1** were achieved by conversion of the latter to chromophoric derivatives and application of the exciton chirality CD (ECCD) method. Nakanishi reported[17] that cyclic and acyclic allylic alcohols can be assigned by simple interpretation of ECCD of their corresponding benzoate derivatives; the latter arises from exciton coupling between π–π* transitions of the *O*-benzoate and C=C double bond.

In principle, this analysis is applicable to an *O*-benzoyl-derivative of **1**, however, additional contributions were anticipated from long-range EC with a second chromophore: the C11-C16 conjugated yne-ene-yne.

Mollenyne A (**1**) was benzoylated under standard conditions (Bz-Cl, pyridine), and the CD spectrum of the HPLC-purified benzoate ester **9** was acquired in MeOH (Scheme 2 and Figure 6). Two positive Cotton effects (CEs) associated with the benzoate chromophore [λ 227 nm (Δε +6.1)], and yne-ene-yne chromophore[18] [λ 260 nm (Δε +7.5), 275 nm (Δε +6.5)] were observed, and ascribed to separate EC effects. The positive CE at λ 227 nm was assigned as the high-energy component of the exciton couplet arising from positive helicity between the double bond (λ ~190 nm) and the benzoate chromophore (λ = 227 nm)(Scheme 2). The negative CE expected from the C=C π–π* contribution, but was obscured by solvent (MeOH, cutoff ~200 nm). A positive split CE between the yne-ene-yne chromophore and the benzoate was assigned to a positive helicity, however, the expected negative CE at λ = 227 nm is canceled by the positive component of the allylic benzoate EC couplet.

In order to remove the ambiguity and ‘red-shift’ the ECCD interactions, mollenyne A (**1**) was derivatized with *para*–methoxycinnamoyl chloride to give **10** (Scheme 2). Gratifyingly, a clear bisignate CE emerged [Figure 6, λ 262 nm (Δε −6.6); ~296 nm (Δε +10.8)] that was assigned to a positive helicity of the allylic *O*-cinnamate ester. Consequently, **10** and **1** have the 7*S*,8*R*,9*R* configuration. The latter assignment was verified by calculation of mimimized structures of a truncated benzoate model of **9** (C4 to C12, Spartan, MMFF). The lowest energy conformer (65% of Boltzmann-weighted population) of **1** was consistent with the solution structure predicted from NMR and CD studies and fully supports the 7*S*,8*R*,9*R* assignment.

Few compounds have been reported from the genus *Spirastrella*;[20] including cytotoxic macrolides, spirastrellolides A-G,[20a–c] sphingosine sulfates[20d] and halogenated hydrocarbons.[20e] An account of the biosynthesis of **1**, the first natural products reported from *S. mollis*, must explain two uncommon features; the rare homoagmatine (1-amino-5-*N*-

guanidino) group[21] which likely arises from decarboxylation of arginine,[3] and the chlorodibromohydrin in the C_{20} carboxamide segment that is without precedent among sponge metabolites. Compound **1** exhibited significant cytotoxicity against human colon tumor cells (HCT-116; IC_{50} = 1.3 μg/mL; etoposide = 0.55 μg/mL).

In conclusion an unusual optically active chlorodibromohydrin, mollenyne A (**1**) was obtained from the marine sponge *Spirastrella mollis*, and its complete stereostructure solved by an integrated approach employing NMR, MS, CD and chemical synthesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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16. HRESIMS *m/z* 487.3776 $[M+H]^+$ (calcd for $C_{26}H_{52}ClN_4O_2$ 487.3773). Sequential elimination of two molecules of HBr upon conversion of **1** to **8** confirmed the location of the halogens in **1**.

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18. CD and UV spectra were normalized using literature ε values for an ene–yne–ene chromophore. λ_{max} 261 nm (ε 32,000), 276 nm (ε 30,500). Crombie L, Jacklin AG. J Chem Soc. 1957:1632.

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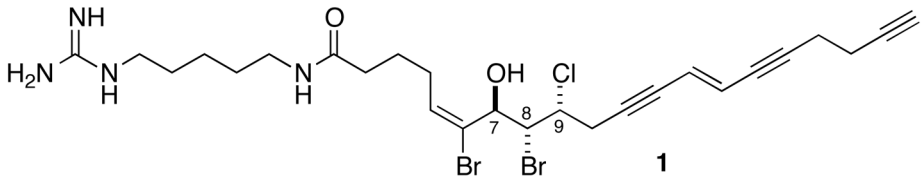


Figure 1.
Mollenyne A (**1**) from *Spirastrella mollis*.

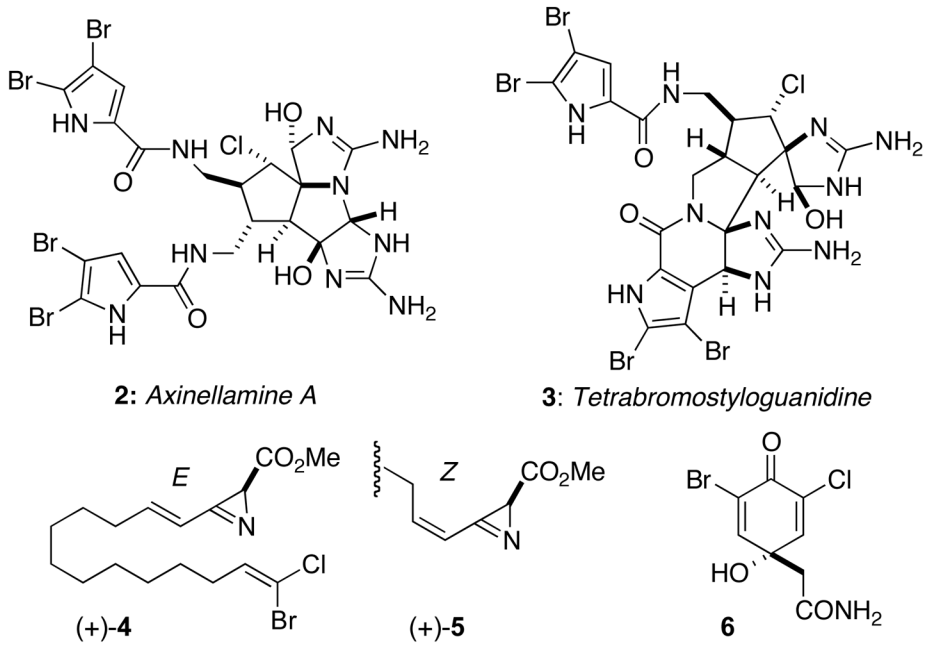


Figure 2.
Bromochloro-natural products from sponges

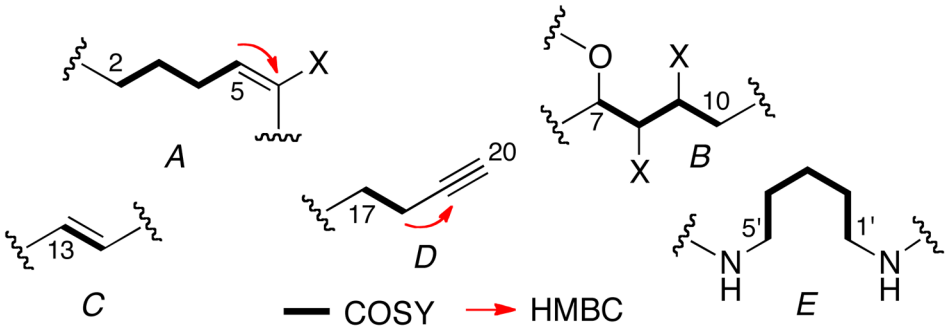


Figure 3.
Substructures of **1** (*A*–*E*) assigned from 2D NMR

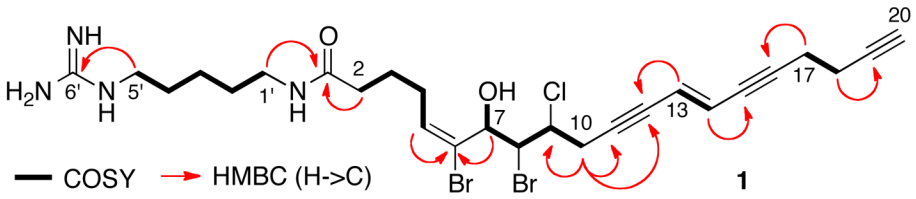
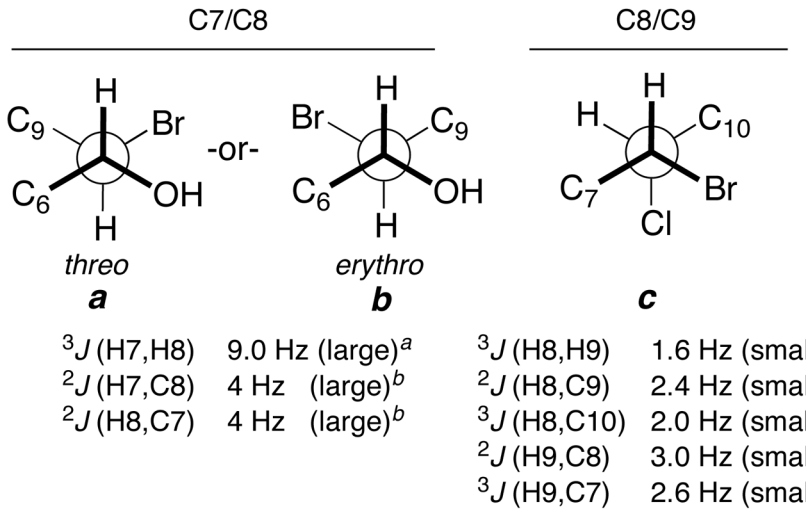


Figure 4.
Assembly of substructures A–E of **1** through COSY and HMBC correlations.



^3J (H7,H8)	9.0 Hz (large)[a]	^3J (H8,H9)	1.6 Hz (small)
^2J (H7,C8)	4 Hz (large)[b]	^2J (H8,C9)	2.4 Hz (small)[c]
^2J (H8,C7)	4 Hz (large)[b]	^3J (H8,C10)	2.0 Hz (small)[c]
		^2J (H9,C8)	3.0 Hz (small)[c]
		^3J (H9,C7)	2.6 Hz (small)[c]

[a] δ(H7,H8) only resolved in CD_3CN. [b]Measured from HSQC-HECADE. [c]Measured from *J*-resolved HMBC

Figure 5.
$^3J_{HH}$, $^2J_{HC}$, and $^3J_{HC}$ coupling constants for C7–C9 of **1** (600 MHz, CD_3CN).

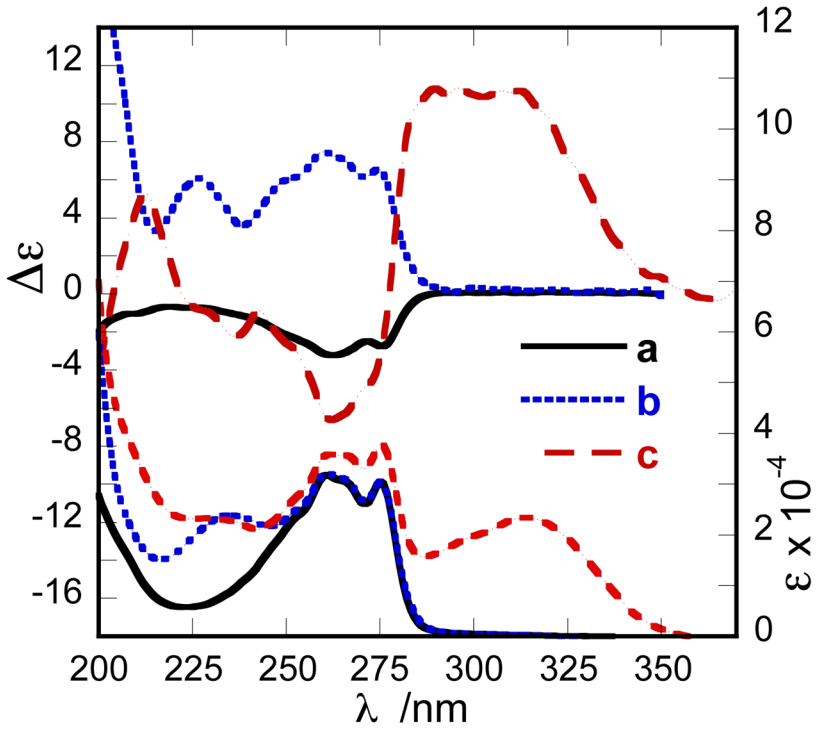
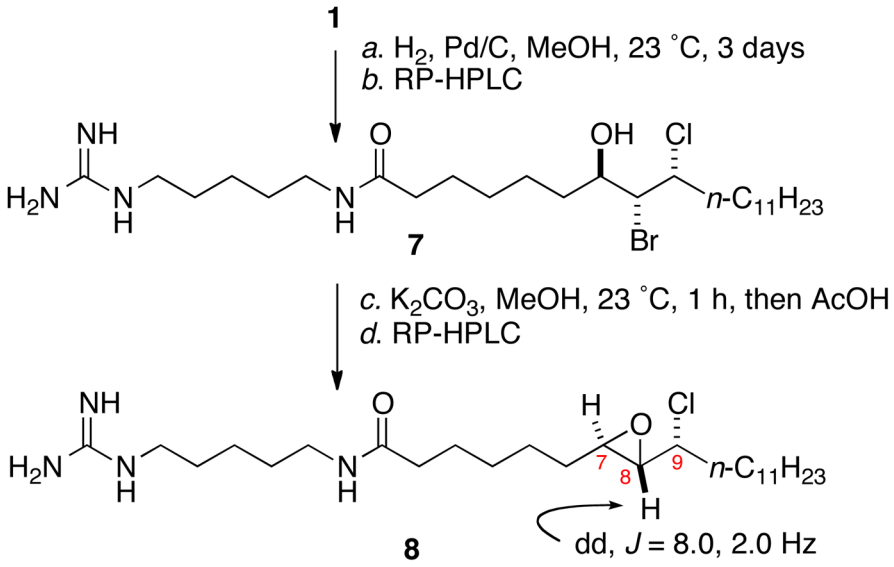
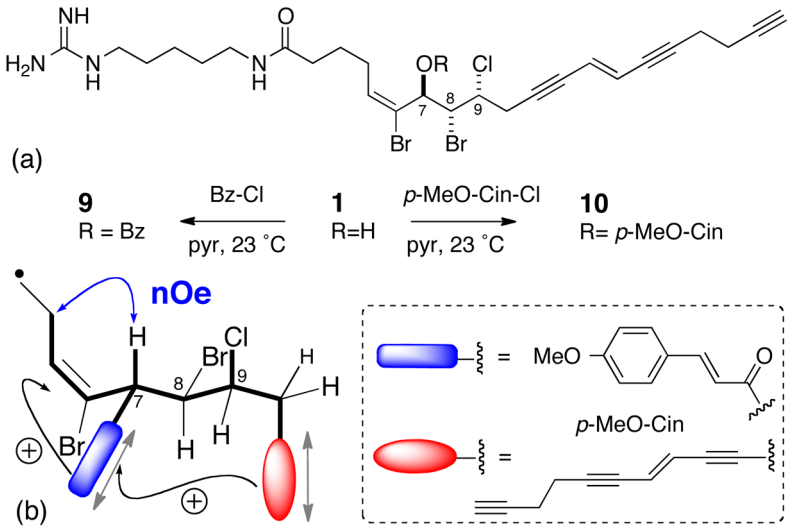


Figure 6.
UV (lower) and CD (upper) spectra (MeOH, 23 °C) for (a) **1**, (b) **9**, and (c) **10**. Δε for **1** and **9** were normalized to an ene–yne–ene chromophore[18] and **10** was normalized to *p*-methoxycinnamate.[19]



Scheme 1.
Conversion of **1** to chloroepoxide **8**.



Scheme 2.

Conversion of **1** to the corresponding benzoate (**9**) and *p*-methoxycinnamate (**10**) esters, and the major conformer accounting for the observed ECCD spectra.

Table 1

^{1}H and ^{13}C NMR data for **1** (CD_3OD, 600 MHz). See Supporting Information for NMR data in CD_3CN.

#.	δ_H, m (*J* in Hz)	δ_C[a]		DQF-COSY	HMBC[b]
1		175.6	C		
2	2.23, t (7.4)	36.4	CH_2	3	1, 3, 4
3	1.79 (m)	26.3	CH_2	2, 4	1, 2, 4, 5
4	2.23 (m)	30.3	CH_2	3, 5	2, 3, 5, 6
5	6.10, t (7.7)	137.3	CH	4	3, 4, 6, 7
6		128.5	C		
7	4.73, d (9.7)	70.8	CH	8	5, 6, 8, 9
8	4.57, m[d]	59.3[c]	CH	7	6, 7, 9, 10
9	4.57, m[d]	59.4[c]	CH	10	7, 8, 10, 11
10	2.97, m	29.6	CH_2	9, 13	8, 9, 11, 12, 13
11		89.9	C		
12		82.6	C		
13	5.91, dt (16.1, 1.9)	122.6	CH	10, 14	14
14	5.97, dt (16.1, 2.0)	120.7	CH	13, 17	13
15		80.4	C		
16		94.6	C		
17	2.54, td (7.2, 2.0)	20.4	CH_2	14, 18	15, 16, 18, 19
18	2.39, td (7.2, 2.6)	19.2	CH_2	17, 20	16, 17, 19, 20
19		83.3	C		
20	2.31, t (2.6)	70.5	CH	18	18
1′	3.18, t (7.2)	40.1	CH_2	2′	1, 2′, 3′
2′	1.54, p (7.2)	30.1	CH_2	1′, 3′	1′, 3′, 4′
3′	1.38, p (7.2)	24.9	CH_2	2′, 4′	1′, 2′, 4′, 5′
4′	1.61, p (7.2)	29.4	CH_2	3′, 5′	2′, 3′, 5′
5′	3.16, t (7.2)	42.4	CH_2	4′	3′, 4′, 6′
6′		158.6	C		

[a]Inferred from DEPT and HSQC, 600 MHz.

[b] HMBC correlations, optimized for 8 Hz. Correlations are from H→C.

[c] May be interchanged.

[d] Jobscured by overlapping multiplets.