Tremorgenic Mycotoxins from *Penicillium crustosum*. Structure Elucidation and Absolute Configuration of Penitrems B—F[1]

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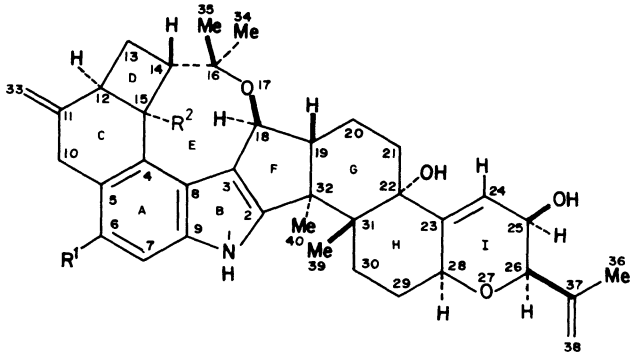
The assignment of structures (2)—(6) to penitrems B—F, tremorgenic mycotoxins isolated from cultures of *Penicillium crustosum*, is based on a detailed study of their high field ^1H and ^{13}C n.m.r. spectra. The relative configuration of penitrems B—F was deduced from the magnitude of the proton–proton coupling constants. The absolute configuration of these metabolites is based on comparisons with that of penitrem A and on the absolute configuration of penitrem D determined by the ' partial resolution ' method of Horeau.

In the preceding publication we report the isolation and physical characteristics of six tremorgenic metabolites, penitrems A—F, from cultures of *Penicillium crustosum*.[2] The structure of penitrem A was deduced mainly from very high field ^1H and ^{13}C n.m.r. data while the conformation of the metabolite was deduced from the proton–proton coupling constants and the observed proton–proton nuclear Overhauser effects (n.O.e.s) and thus, when the chirality of C-25 was determined by the Horeau method,[3] the absolute configuration of penitrem A followed as shown in structure (1). We now report the structure elucidation of the five remaining tremorgenic mycotoxins, penitrems B—F, which is based mainly on a detailed study of the high field ^1H and ^{13}C n.m.r. spectra of these compounds.

The molecular formulae, as determined by mass spectrometry for penitrem A (1) ($C_{37}H_{44}ClNO_6$), penitrem B (2) ($C_{37}H_{45}NO_5$), penitrem C (3) ($C_{37}H_{44}ClNO_4$), penitrem D (4) ($C_{37}H_{45}NO_4$), penitrem E (5) ($C_{37}H_{45}NO_6$), and penitrem F (6) ($C_{37}H_{44}ClNO_5$), indicate that these compounds share the same basic structure and differ from each other only in the nature of the substituents at certain carbon atoms.

The structure of penitrem E (5) should therefore differ from



(1) R^1 = Cl, R^2 = OH; 23α,24α-epoxide
(2) R^1 = R^2 = H; 23α,24α-epoxide
(3) R^1 = Cl, R^2 = H
(4) R^1 = R^2 = H
(5) R^1 = H, R^2 = OH; 23α,24α-epoxide
(6) R^1 = Cl, R^2 = H; 23α,24α-epoxide

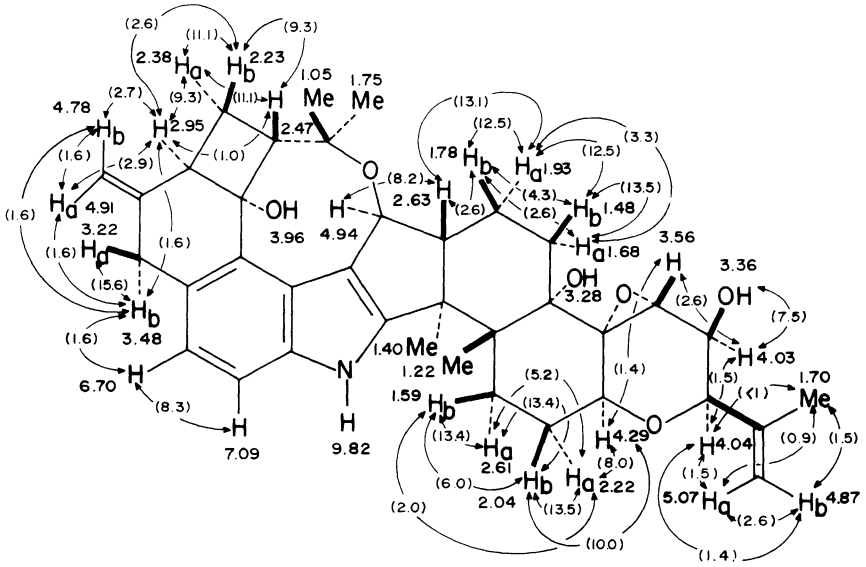


Figure 1. 500.14 MHz ^1H N.m.r. data for penitrem E (5)

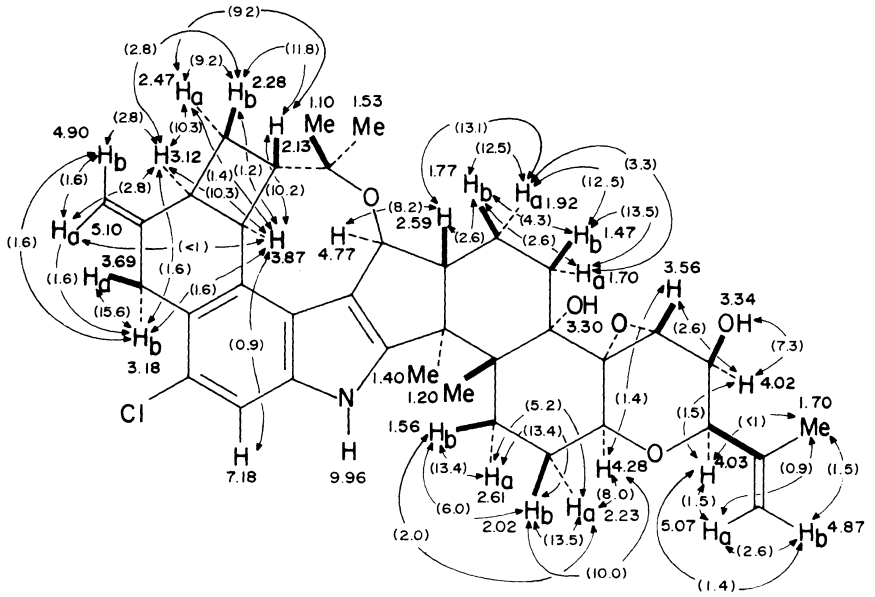


Figure 2. 500.13 MHz ^{1}H N.m.r. data for penitrem F (6)

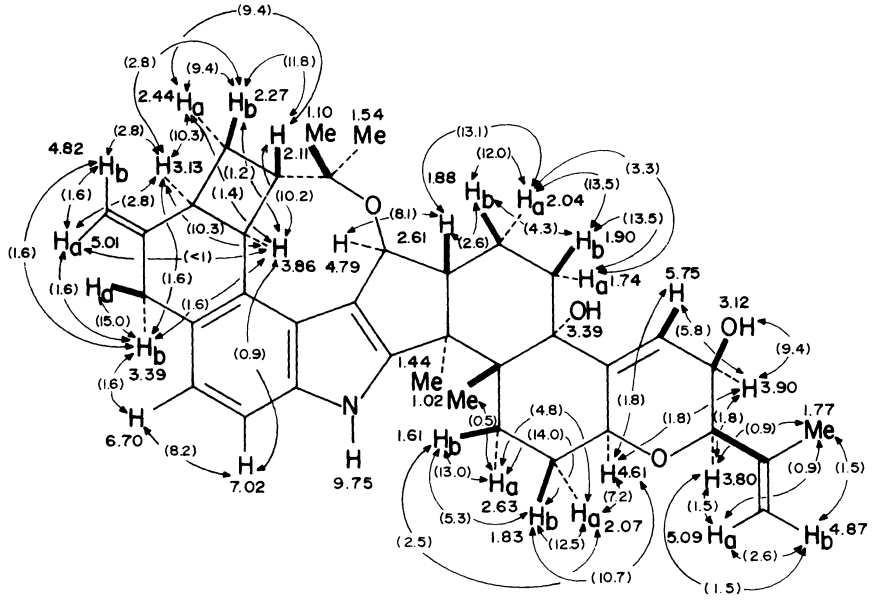


Figure 3. 500.14 MHz ^{1}H N.m.r. data for penitrem D (4)

that of penitrem A (1) only in that the chlorine atom is replaced by an hydrogen atom. This supposition was confirmed by the ^{1}H n.m.r. spectrum of penitrem E. The presence of two *ortho*-oriented aromatic protons was evident from the magnitude of the coupling constant [4] (8.3 Hz) observed for the resonances at δ 6.70 and 7.09. The only major differences in the ^{1}H n.m.r. data for penitrems A and E (see Figure 1) are observed for those protons in close proximity to the chlorine atom. The chemical shift difference for the C-10 protons [δ(10-H_a) − δ(10-H_b)] is 0.57 p.p.m. for penitrem A but −0.26 p.p.m. in the case of penitrem E. The chlorine atom therefore has a substantial influence on the chemical shift of these two protons owing to steric factors. In penitrem A the chemical shift equivalence of the C-25 and C-26 protons precluded the observation of (H,H) coupling between these protons. The small difference of 0.01 p.p.m. in the chemical shift of these two protons in penitrem E is sufficient to determine the value of the coupling constant as 1.5 Hz.

A comparison of the molecular formulae for penitrem A (1) and F (6) indicates that penitrem F lacks an oxygen atom. In the ^{1}H n.m.r. spectrum of (6) a new proton signal is observed at δ 3.87. The presence of additional (H,H) couplings between the cyclobutane protons as well as the changes in their chemical shift values show that the C-15 hydroxy group in penitrem A (1) is replaced by an hydrogen atom. The ^{1}H n.m.r. data for penitrem F are shown in Figure 2 and a comparison with the data for penitrem A reveals that the effect of replacing the C-15 hydroxy group with an hydrogen atom is especially pronounced for 18-H and the C-34 methyl protons owing to a decrease in steric crowding as all three of these groups are located below the plane of the eight-membered ring. The observed values for the coupling constants of the cyclobutane

Table 1. ^{13}C N.m.r. (125.76 MHz) data for penitrem A (1), penitrem B (2), penitrem E (5), and penitrem F (6)

Carbon atom	Penitrem A (1)	Penitrem B (2)					Penitrem E (5)		Penitrem F (6)			
	δ_C [a]	δ_C [a,b]		^1J	$>^1J$	$\Delta\delta$ [c]	δ_c [a]	$\Delta\delta$ [c]	δ_c [a]	$\Delta\delta$ [c]	ΔS_{Cl} [d]	ΔS_{OH} [d]
2	154.36	152.97	Sq		4.1	−0.154	153.48	−0.156	153.86	−0.160	0.89	0.51
3	120.64	119.40	Sd		5.8	−0.038	120.38		119.56	−0.038	0.20	1.03
4	133.29	128.84	Sbr				131.52	−0.036	130.76		1.88	2.61
5	125.80	128.13	Sq (br)		7.2		128.16		125.76		−2.36	
6	124.56	120.99	Dd	155.2	4.6		120.34		125.36		4.32	−0.73
7	111.86	110.26	D	158.3		−0.047	111.63	−0.046	110.60	−0.046	0.30	1.32
8	121.99	123.19	St		5.5	−0.040	122.75		122.33	−0.040	−0.82	−0.39
9	139.73	139.34	Sdd		9.8; 3.9	−0.147	140.16		138.81	−0.150	−0.49	0.87
10	35.06	38.75	DDdt	130; 125.3	11.6; 4.9		38.11		35.63		−3.10	0.61
11	149.48	150.22	Sbr				150.91		148.92		−1.34	0.64
12	47.01	35.04	Dm	136			47.41	−0.058	34.86		−0.25	12.26
13	24.67	26.69	Tm	133.2			24.72		26.61			−1.96
14	52.71	52.32	Dm	132			52.78		52.34			0.42
15	81.01	39.35	Ddt	134.6	8.2; 4.1		81.08	−0.121	39.54			41.66
16	76.09	75.47	Sbr				76.09		75.51			0.59
18	72.44	72.10	Dd	144.8	8.4		72.52		71.97		−0.14	0.45
19	58.79	59.12	Dm	124			58.86		59.13			0.30
20	18.56	18.61	Tm	128.4			18.63		18.55			
21	30.59	30.58	Tm	124		−0.062	e		30.49			
22	78.24	78.26	Sbr			−0.113	78.28	−0.113	78.23	−0.113		
23	66.11	66.16	Sm				66.17		66.10			
24	61.92	61.95	Dm	178.8		−0.026	61.95		61.93	−0.023		
25	66.31	66.33	Dt (br)	144.0	3.0	−0.096	66.34	−0.094	66.31	−0.097		
26	74.67	74.68	Dm	143			74.69		74.67			
28	71.99	72.03	Dm	147.6			72.05		71.97			
29	28.89	28.92	Tq (br)	128.7	3.2		28.94		28.88			
30	26.91	26.85	Tm	127			26.89		26.89			
31	43.55	43.60	Sm			−0.032	43.58	−0.030	43.55	−0.031		
32	50.08	49.68	Sbr				49.92		49.82		0.14	0.25
33	107.10	105.86	Tq	155.2	4.7		105.47		107.36		1.55	−0.33
34	20.32	18.61	Qqn	125.0	4.2		20.28		18.62			1.70
35	31.06	28.80	Qm	125.1			31.10		28.75			2.31
36	19.70	19.71	Qddd	126.2	11.4; 6.9; 1.8		19.70		19.70			
37	143.27	143.29	Sqn		6.1		143.31		143.26			
38	111.64	111.64	DDqd	159.8; 154.8	6.0; 3.9		111.62		111.65			
39	18.98	18.92	Qdd	125.2	6.8; 1.3		18.99		18.91			
40	21.35	21.24	Qd	127.1	5.6		21.53		21.06		−0.18	0.29

[a] Relative to internal Me_4Si; solvent $(CD_3)_2CO$. Measured from internal $(CD_3)_2CO$ and corrected by using the expression $\delta(Me_4Si) = \delta[(CD_3)_2CO] + 29.83$. [b] Capital letters refer to the pattern resulting from directly bonded (C,H) coupling [^1J(CH)] and lower case letters to that from (C,H) couplings over more than one bond [$>^1J$(CH)]. S = singlet, D or d = doublet, T or t = triplet, Q or q = quartet, qn = quintet, m = multiplet, and br = broad (no fine structure but the line is noticeably broadened). [c] Deuterium isotope shifts in p.p.m. (see text). [d] Average substituent shift in p.p.m. ΔS_{Cl} = average value of $\delta(1) - \delta(5)$, $\delta(6) - \delta(2)$, and $\delta(3) - \delta(4)$; ΔS_{OH} = average value of $\delta(1) - \delta(6)$ and $\delta(5) - \delta(2)$; $\Delta S_{oxirane}$ = average value of $\delta(6) - \delta(3)$ and $\delta(2) - \delta(4)$. [e] Obscured.

protons are in agreement with the values reported in the literature.[5]

The ^1H n.m.r. spectrum of penitrem B (2) proved that the structure of the metabolite differs from penitrem A (1) in that both the chlorine atom and the C-15 hydroxy group are replaced by hydrogen atoms. The chemical shifts and coupling constants for the protons of rings A—F are similar to those of penitrem D (4) (see Figure 3).

The most significant feature of the ^1H n.m.r. spectrum of penitrem D (4) compared to that of penitrem A is the presence of resonances at δ 6.70, 5.75, and 3.86 and the absence of the resonance at δ 3.56 which has been assigned to the C-24 oxirane proton.[2] This finding, in conjunction with the molecular formulae for penitrems A and E, suggests that once again the chlorine atom and the C-15 hydroxy group present in penitrem A have been replaced by hydrogen atoms. In addition a C(23)–C(24) double bond must be present in penitrem D. The data obtained from an analysis of the ^1H n.m.r. spectrum of penitrem D are presented in Figure 3. Penitrem C (3) differs from penitrem D (4) only by the presence of the chlorine atom at C-6, and the ^1H n.m.r. data of (3) for the protons of rings A—F are similar to those of penitrem F (6).

The ^{13}C n.m.r. data for penitrem B (2), E (5), and F (6), together with the reported ^{13}C chemical shift values for penitrem A (1)[2] are collated in Table 1 and for penitrems C (3) and D (4) in Table 2. The data were obtained from broad-band proton-decoupled and single frequency nuclear Overhauser enhanced (n.O.e.) ^{13}C n.m.r. spectra. In the assign-

Table 2. ^{13}C N.m.r. (125.76 MHz) data for penitrem C (3) and penitrem D (4) *

Carbon atom	Penitrem C (3)					Penitrem D (4)		
	δ_C [a,b]		^1J	$>^1J$	$\Delta\delta$ [c]	δ_C [a]	$\Delta\delta$ [c]	$\Delta S_{oxirane}$ [d]
2	154.38	Sq		4.1	−0.157	153.49	−0.160	−0.52
3	119.33	Sd		5.6	−0.034	119.16		0.24
4	130.73	Sbr				128.80		
5	125.73	Sq (br)		5		128.09		
6	125.28	Sm				120.91		
7	110.56	D	163.9		−0.045	110.22	−0.044	
8	122.33	St		6	−0.040	123.18		
9	138.75	Sd		3.3	−0.147	139.27		
10	35.63	DDdd	132.0; 126.1	11.2; 6.7		38.75		
11	148.94	Sm				150.23		
12	34.86	Dm	134			35.04		
13	26.61	Tm	134.3			26.70		
14	52.37	Dm	134			52.36		
15	39.63	Ddt	134.8	8.4; 4.1		39.37		
16	75.49	Sm				75.44		
18	72.03	Dd	144.5	8.3		72.17		
19	58.89	Dm	124			58.87		0.25
20	19.06	Tm	126.0			19.11		−0.51
21	35.00	Tm	126		−0.060	35.08	−0.058	1.32
22	77.45	Sm			−0.116	77.48	−0.110	0.78
23	148.33	Sbr				148.44		−82.25
24	119.70	Dm	159.7			119.59		−57.71
25	64.27	Dm	142.7		−0.087	64.28	−0.088	2.04
26	74.36	Dm	143			74.40		0.30
28	80.38	Dm	137			80.39		−8.38
29	29.19	Tq	128.7	4.1		29.24		−6.14
30	27.71	Tm	129			27.68		−0.83
31	43.67	Sm			−0.025	43.71	−0.020	−0.12
32	49.91	Sm				49.77		
33	107.35	Tq	155.6	4.8		105.83		
34	18.64	Qqn	125.0	4.2		18.64		
35	28.77	Qm	125			28.81		
36	19.97	Qddd	125.7	11.3; 6.8; 1.8		19.98		−0.27
37	143.90	Sm				143.92		−0.64
38	110.78	Tqd	156.2	6; 4		110.75		0.88
39	20.10	Qd	125.9	8.0		20.11		−1.19
40	21.15	Qd	127.2	5.6		21.32		

* See Table 1 for footnotes.

ment of the different ^{13}C resonances extensive use was made of single frequency off-resonance proton-decoupled ^{13}C n.m.r. spectra and the reported ^{13}C chemical shifts and (C,H) coupling constants of penitrem A.[2]

The reported upfield deuterium isotope shifts[6] ($\Delta\delta$) observed in the broad-band proton-decoupled ^{13}C n.m.r. spectra of penitrems B—F (see Tables 1 and 2) are the separations between doubled signals when the exchangeable protons were partially exchanged with deuterium derived from deuterium oxide, apparently present as an impurity in the [$^2H_6$]acetone solvent. No external deuterium oxide was ever added to the samples. In superdry [$^2H_6$]acetone this effect was not observed. The deuterium isotope shifts correspond exactly with those observed for the corresponding carbon resonances in penitrem A when a small amount of [$^2H_4$]-methanol was added to the [$^2H_6$]acetone solution of penitrem A. No deuterium isotope shift was observed for the resonances due to C-12 and C-4 in penitrems B, C, D, and F as these compounds lack the C-15 hydroxy group. The observation of a deuterium isotope shift for C-4 in penitrem A was instrumental in defining the linkage between C-15 and C-4.

Changing the chlorine atom and the C-15 hydroxy group for hydrogen atoms, as well as going from an oxirane to a double bond moiety in the different penitrems, has a substantial influence on the ^{13}C chemical shifts of the neighbouring carbon atoms (see Tables 1 and 2). The effects are, however, largely restricted to the part of the molecule where the substitution takes place and very little interaction is observed between the different substituent changes. The influence of the chlorine atom is largely restricted to the carbon atoms of the indole ring as a result of electronic effects.[7] The C-15 hydroxy group has a deshielding δ-substituent effect[8] on both the C-34 and C-35 methyl groups which is, however, larger for the *anti* carbon atom, *i.e.* C-35. The effect of this moiety on the β- and γ-carbon atoms[9] of the cyclobutane ring is only significant for C-12. The ^{13}C chemical shift changes induced by the oxirane moiety can probably be associated with conformational changes on going from a double bond to an oxirane moiety.

The ^1H and ^{13}C n.m.r. data of penitrems B—F are consistent with the structures (2)—(6) proposed for these metabolites and also with that of penitrem A (1). The relative and absolute

configurations of penitrems B—F were deduced as outlined below using the known absolute configuration of penitrem A as well as the method of Horeau.[3] Penitrem E (5) differs from penitrem A only in the absence of the C-6 chlorine atom, and the same absolute configuration is therefore indicated for this metabolite. Penitrem B (2) is simply the 6-dechloro derivative of penitrem F (6) and both these compounds differ from penitrem A (1) in that they lack the C-15 hydroxy group. The relative configuration of the C-15 proton in penitrems B and F follows from the proton–proton coupling constants and also from the stereochemical requirement of *cis*-fusion between the six- and four-membered rings. The C-12 and C-15 protons therefore have the *cis*-configuration and penitrems B and F must have the same absolute configuration as penitrem A, *i.e.* 12*S*,14*S*,15*S*,18*S*,19*R*,22*S*,23*S*,24*R*,25*S*,-26*R*,28*S*,31*R*,32*S*. The change in descriptors for some chiral centres compared with the corresponding chiral centre in penitrem A is merely the result of the sequence rules of the Cahn–Ingold–Prelog system.

Penitrem C (3) is the 6-chloro derivative of penitrem D (4). The relative configuration of rings C—E in penitrem D was deduced from the proton–proton coupling constants and is the same as for penitrem B and F, and consequently the same as the relative and absolute configurations of penitrem A. The relative configuration of rings F—I in penitrem D follows similarly from the proton–proton coupling constants and by comparison with the other penitrems. The chirality of the C-25 hydroxy group in penitrem D and thus the absolute configuration of the molecule was determined by the ' partial resolution ' method of Horeau.[3] Esterification of penitrem D with racemic α-phenylbutyric acid anhydride and 4-dimethylaminopyridine instead of pyridine proceeded smoothly, leading quantitatively to the 25-*O*-α-phenylbutyrate. The mass spectrum of the ester (M^+, 713) indicated the presence of only the monoester. The recovered α-phenylbutyric acid had $[\alpha]_D^{23}$ + 2.35° (*c* 2.90, benzene). Penitrem D must therefore have the 25*R*-configuration [3,10,11] and consequently the absolute configuration as shown in (4) and Figure 3, *viz.* 12*S*,14*S*,15*S*,18*S*,19*R*,22*S*,25*R*,26*R*,28*S*,31*R*,32*S*.

The availability of penitrems B—F in addition to penitrem A proved invaluable in the structure elucidation of these chemically labile and fairly inaccessible metabolites. The biogenesis of penitrem A is discussed in the following paper.

Experimental

For general directions see ref. 2. The isolation of penitrems B—F (2)—(6) from cultures of *Penicillium crustosum* and the physical characteristics of these metabolites have been reported by us.[2]

Absolute Configuration of Penitrem D (4).—A solution of α-phenylbutyric acid anhydride (100 mg, 0.323 mmol), penitrem D (70 mg, 0.123 mmol), and 4-dimethylaminopyridine (76 mg) in dichloromethane (15 ml) was stirred at room temperature for 2 h (t.l.c. control). The excess of anhydride was destroyed by adding water (10 ml) and stirring the reaction vigorously for 1 h. The aqueous dichloromethane mixture was extracted with 6M-sodium hydrogen carbonate (2 × 10 ml), and the organic phase washed with 6M-HCl (1 × 10 ml), water (2 × 10 ml), dried and evaporated. The residual ester (86 mg) contained no starting material and had M^+ 713.

The combined sodium hydrogen carbonate extracts were acidified (6M-HCl) and extracted with dichloromethane to yield α-phenylbutyric acid (58 mg), $[\alpha]_D^{23}$ +2.35° (*c* 2.90, benzene) (theoretical $[\alpha]_D$ +22.8° [10]). The optical yield therefore was 10.3% (+), based on an esterification yield of 100%.

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