Structural Studies of FR900359, a Novel Cyclic Depsipeptide from *Ardisia crenata Sims* (Myrsinaceae)

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The molecular structure and absolute configuration of FR900359, a novel cyclic depsipeptide from *Ardisia crenata sims*, has been determined by a combination of *X*-ray crystallographic analysis and g.c./m.s. study of the diastereomeric derivatives of its constituents. There are five intramolecular hydrogen bonds (or short contacts) in the FR900359 molecule. All the imino nitrogen and hydroxy oxygen atoms having a proton-donating ability efficiently participate in the hydrogen bond network. The FR900359 molecule contains two *cis* peptide bonds, in a conformation which can take part in the hydrogen bonds. This hydrogen bond network contributes to the stabilization of the overall structure of FR900359; constituents not restrained by this network are considered to be flexible. Since the *N*-methyldehydro-L-alanine residue falls within the unstable region of a Ramachandran (φ–ψ) plot, it is vulnerable to nucleophilic attack and may, therefore, be involved in the biological activity of FR900359.

FR900359 is a novel cyclic depsipeptide isolated from a methanol extract of the whole plant of *Ardisia crenata sims* (Myrsinaceae) as a result of a continuing search for potentially therapeutic compounds. It inhibits platelet aggregation *in vitro* and *ex vivo* in rabbits, decreases blood pressure, and shows dose-related hypotensive action in anaesthetized normotensive rats.[1] It is also cytotoxic in cultured rat fibroblasts and myelocytic leukemia cells.[1]

Efforts at structural determination combined chemical methods with ^{1}H- and ^{13}C-n.m.r. spectroscopic and mass spectrometric studies of the fragments after partial hydrolyses, and resulted in all the FR900359 constituents and their sequences being identified by M. Fujioka *et al.*[2] FR900359 seemed to have a novel amino acid constituent; *N*,*O*-dimethylthreonine (as yet unreported in Nature), and an uncommon amino acid, *N*-methyldehydroalanine (previously found only in a microcystis toxin from the blue-green alga, *Microcystis aeruginose*[3]). However, the presence of these novel constituents had been only indirectly confirmed because of their susceptibility to hydrolysis. Recently,[4] an *X*-ray crystallographic study established unambiguously the structure of FR900359. This is discussed here, together with the absolute configuration and the conformational characteristics of the compound.

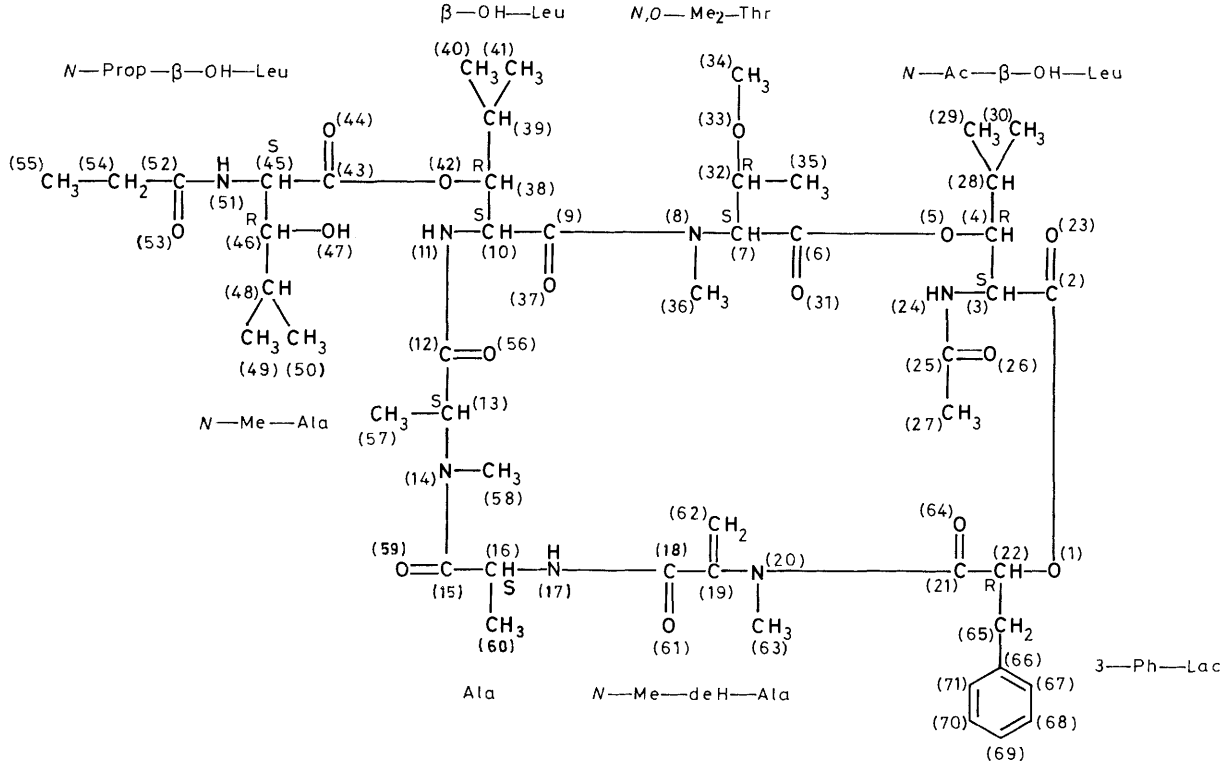

Figure 1. Chemical structure and atomic numbering of FR900359 molecule used in the *X*-ray investigation

Table 1. Fractional co-ordinates with e.s.d.s in parentheses

Atom	*x*	*y*	*z*	Atom	*x*	*y*	*z*
O(1)	0.412 0(5)	0.936 6(8)	0.906 3(5)	O(37)	0.291 5(3)	0.544 8(6)	0.731 3(4)
C(2)	0.372 5(7)	0.884 8(13)	0.971 6(10)	C(38)	0.111 0(5)	0.599 2(8)	0.713 2(6)
C(3)	0.309 9(6)	0.964 3(11)	1.003 0(8)	C(39)	0.039 5(5)	0.674 7(10)	0.699 1(8)
C(4)	0.259 9(6)	0.887 9(10)	1.061 9(7)	C(40)	0.023 0(7)	0.716 7(14)	0.805 1(10)
O(5)	0.230 1(4)	0.795 2(6)	0.997 5(4)	C(41)	−0.026 9(6)	0.602 1(14)	0.652 1(9)
C(6)	0.244 3(8)	0.684 0(12)	1.028 2(8)	O(42)	0.121 4(3)	0.559 2(5)	0.610 8(4)
C(7)	0.202 8(6)	0.596 2(9)	0.956 6(7)	C(43)	0.135 0(5)	0.443 3(9)	0.595 8(7)
N(8)	0.256 2(4)	0.558 9(7)	0.885 9(5)	O(44)	0.135 8(6)	0.370 6(7)	0.658 1(6)
C(9)	0.248 2(5)	0.585 4(8)	0.787 7(6)	C(45)	0.152 5(5)	0.423 6(8)	0.488 0(6)
C(10)	0.184 1(5)	0.669 1(8)	0.747 4(6)	C(46)	0.240 4(5)	0.414 4(8)	0.490 0(7)
N(11)	0.209 1(4)	0.734 4(6)	0.664 1(5)	O(47)	0.275 3(3)	0.519 5(6)	0.533 4(4)
C(12)	0.218 5(4)	0.851 7(8)	0.665 3(6)	C(48)	0.265 7(5)	0.403 0(9)	0.386 3(7)
C(13)	0.247 1(5)	0.901 9(8)	0.571 3(7)	C(49)	0.225 1(7)	0.301 3(12)	0.325 9(8)
N(14)	0.212 2(4)	0.836 2(7)	0.482 0(5)	C(50)	0.352 1(6)	0.381 2(13)	0.394 7(9)
C(15)	0.249 3(5)	0.775 1(8)	0.417 9(6)	N(51)	0.121 7(4)	0.509 7(7)	0.418 3(5)
C(16)	0.336 1(4)	0.786 5(9)	0.425 2(6)	C(52)	0.047 5(5)	0.511 7(10)	0.387 5(7)
N(17)	0.370 6(4)	0.717 6(6)	0.509 5(5)	O(53)	0.005 5(4)	0.436 6(8)	0.417 3(6)
C(18)	0.433 8(5)	0.758 6(8)	0.563 9(6)	C(54)	0.019 0(6)	0.607 1(12)	0.315 1(9)
C(19)	0.467 6(5)	0.674 7(9)	0.645 5(7)	C(55)	−0.042 9(9)	0.578 5(27)	0.246 4(18)
N(20)	0.489 6(4)	0.732 0(8)	0.739 3(6)	O(56)	0.205 1(3)	0.911 9(6)	0.737 0(4)
C(21)	0.442 0(5)	0.810 9(10)	0.774 5(7)	C(57)	0.234 6(7)	1.031 8(9)	0.561 2(8)
C(22)	0.475 3(6)	0.885 2(12)	0.866 7(7)	C(58)	0.127 7(5)	0.835 1(10)	0.465 4(7)
O(23)	0.372 4(6)	0.778 7(11)	0.995 4(9)	O(59)	0.215 8(3)	0.715 6(6)	0.352 5(4)
N(24)	0.273 4(4)	1.027 7(7)	0.920 8(5)	C(60)	0.365 1(6)	0.741 3(12)	0.327 0(7)
C(25)	0.278 5(6)	1.146 7(9)	0.916 0(7)	O(61)	0.462 8(3)	0.851 8(6)	0.550 8(5)
O(26)	0.305 8(5)	1.202 1(7)	0.986 8(6)	C(62)	0.485 1(6)	0.567 4(9)	0.622 5(8)
C(27)	0.244 7(7)	1.200 0(10)	0.818 2(8)	C(63)	0.558 1(6)	0.682 6(11)	0.801 8(8)
C(28)	0.193 7(8)	0.957 2(12)	1.095 6(9)	O(64)	0.379 7(3)	0.827 3(7)	0.732 0(5)
C(29)	0.136 4(9)	0.877 1(14)	1.134 4(11)	C(65)	0.527 2(6)	0.980 1(13)	0.840 8(9)
C(30)	0.225 5(9)	1.040 9(14)	1.181 3(12)	C(66)	0.486 1(6)	1.085 3(11)	0.788 1(9)
O(31)	0.278 7(7)	0.654 8(8)	1.106 4(6)	C(67)	0.479 3(7)	1.190 3(14)	0.838 1(11)
C(32)	0.170 4(9)	0.491 5(12)	1.011 4(9)	C(68)	0.444 6(8)	1.282 7(13)	0.782 7(13)
O(33)	0.128 6(6)	0.428 1(9)	0.936 1(7)	C(69)	0.418 6(7)	1.274 4(13)	0.682 4(12)
C(34)	0.101 5(11)	0.320 0(16)	0.964 0(15)	C(70)	0.427 5(8)	1.174 1(12)	0.635 6(11)
C(35)	0.123 5(12)	0.536 2(16)	1.092 5(12)	C(71)	0.460 3(7)	1.080 3(12)	0.685 9(9)
C(36)	0.324 2(7)	0.489 7(10)	0.924 2(8)				

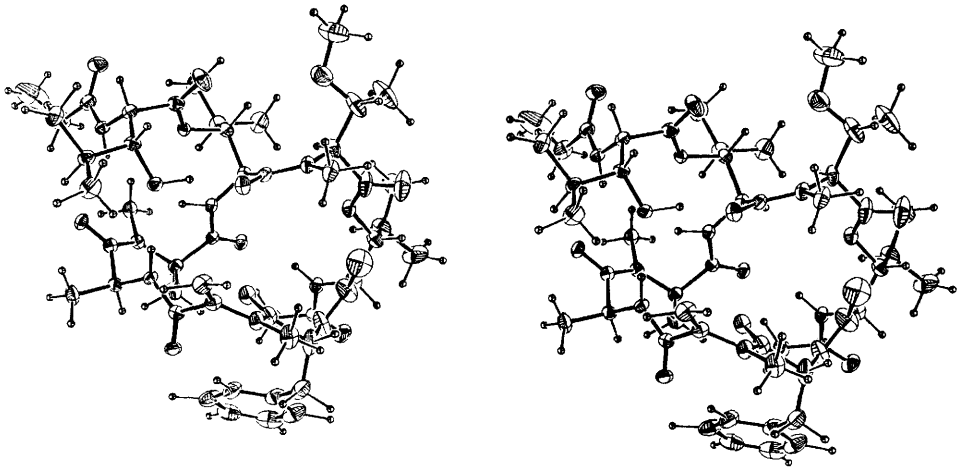

Figure 2. Stereographic view of the molecular conformation of FR900359 (ORTEP II)

Results and Discussion

An *X*-ray crystallographic study identified FR900359 as a cyclic depsipeptide with a 22-membered ring backbone and a side chain (see Figure 1). The backbone is composed of one lactic acid (3-Ph-Lac) and six amino acid residues (Ala, *N*-Me-Ala, *N*-Me-deH-Ala, β-OH-Leu, *N*-Ac-β-OH-Leu and *N*,*O*-Me_2-Thr) linked by amide or ester bonds. The side chain (*N*-Prop-β-OH-Leu) is bound to the backbone by an ester bond.

The molecule has 11 asymmetric carbon atoms. The alanine unit was assigned the L-configuration by a g.c./m.s. study, since the retention time for the diastereoisomeric derivative of the alanine residue with *N*-trifluoroacetyl-L-proline corresponded to that for the diastereoisomeric derivatives of authentic L-alanine with *N*-trifluoroacetyl-L-proline. The remaining optically active carbon atoms were assigned from the three-dimensional structure as determined by *X*-ray crystal analysis. The results were as

Table 2. Bond lengths (Å) with e.s.d.s in parentheses

O(1)–C(2)	1.325(18)	O(1)–C(22)	1.426(17)
C(2)–C(3)	1.534(20)	C(2)–O(23)	1.255(20)
C(3)–C(4)	1.531(17)	C(3)–C(24)	1.418(15)
C(4)–O(5)	1.433(13)	C(4)–C(28)	1.531(18)
O(5)–C(6)	1.353(16)	C(6)–C(7)	1.524(19)
C(6)–O(31)	1.203(20)	C(7)–N(8)	1.482(14)
C(7)–C(32)	1.551(20)	N(8)–C(9)	1.346(13)
N(8)–C(36)	1.488(14)	C(9)–C(10)	1.541(13)
C(9)–O(37)	1.232(12)	C(10)–N(11)	1.458(11)
C(10)–C(38)	1.553(13)	N(11)–C(12)	1.352(12)
C(12)–C(13)	1.527(13)	C(12)–O(56)	1.230(11)
C(13)–N(14)	1.493(13)	C(13)–C(57)	1.507(16)
N(14)–C(15)	1.339(12)	N(14)–C(58)	1.496(14)
C(15)–C(16)	1.544(13)	C(15)–O(59)	1.214(12)
C(16)–N(17)	1.459(12)	C(16)–C(60)	1.524(17)
N(17)–C(18)	1.355(11)	C(18)–C(19)	1.530(13)
C(18)–O(61)	1.207(11)	C(19)–N(20)	1.436(13)
C(19)–C(62)	1.313(15)	N(20)–C(21)	1.359(15)
N(20)–C(63)	1.511(16)	C(21)–C(22)	1.564(18)
C(21)–O(64)	1.205(14)	C(22)–C(65)	1.493(21)
N(24)–C(25)	1.367(13)	C(25)–O(26)	1.200(14)
C(25)–C(27)	1.512(16)	C(28)–C(29)	1.507(22)
C(28)–C(30)	1.555(21)	C(32)–O(33)	1.392(19)
C(32)–C(35)	1.533(27)	O(33)–C(34)	1.394(23)
C(38)–C(39)	1.534(15)	C(38)–O(42)	1.481(11)
C(39)–C(40)	1.562(20)	C(39)–C(41)	1.524(20)
O(42)–C(43)	1.368(11)	C(43)–O(44)	1.179(14)
C(43)–C(45)	1.533(14)	C(45)–C(46)	1.567(13)
C(45)–N(51)	1.426(12)	C(46)–O(47)	1.446(11)
C(46)–C(48)	1.518(14)	C(48)–C(49)	1.551(18)
C(48)–C(50)	1.551(18)	N(51)–C(52)	1.340(14)
C(52)–O(53)	1.234(15)	C(52)–C(54)	1.511(18)
C(54)–C(55)	1.396(33)	C(65)–C(66)	1.539(20)
C(66)–C(67)	1.389(20)	C(66)–C(71)	1.400(19)
C(67)–C(68)	1.398(23)	C(68)–C(69)	1.378(23)
C(69)–C(70)	1.327(22)	C(70)–C(71)	1.365(20)

follows: five chiral centres, C(4), C(22), C(32), C(38), and C(46), took *R*-configurations, while the other six chiral centres, C(3), C(7), C(10), C(13), C(16), and C(45), which were all α-carbons of amino acid residues, took *S*-configurations. Consequently, FR900359 has been named (3*S*,4*R*,7*S*,10*S*,13*S*,16*S*,22*R*)-3-acetamido-22-benzyl-10-{R-1-[(2*S*,3*R*)-3-hydroxy-4-methyl-2-propionamidopentanoyloxy]-2-methylpropyl}-4-isopropyl-7-(*R*-1-methoxyethyl)-19-methylene-8,13,14,16,20-pentamethyl-1,5-dioxa-8,11,14,17,20-penta-azacyclodocosane-2,6,9,12,15,-18,21-heptaone.

The final fractional atomic co-ordinates for non-H atoms are listed in Table 1 and the bond lengths, bond angles, and selected torsion angles are listed in Tables 2, 3, and 4, respectively. The stereoview of the molecular conformation of FR900359 drawn with the ORTEP II program [5] is presented in Figure 2.

There are five short distances suggesting the existence of intramolecular hydrogen bonds in the molecule, as shown in Figure 3 and Table 5. The hydrogen bond between NH(24) of *N*-Ac-β-OH-Leu and O(56) of *N*-Me-Ala, with a distance of 2.95(1) Å and an angle of 163(10)°, crosses the depsipeptide ring diametrically, and possibly stabilizes the loose-fitting ring structure. The other hydrogen bonds (or short contacts) are formed between Ala or β-OH-Leu of the depsipeptide ring and the *N*-Prop-β-OH-Leu side chain, *i.e.*, O(59)···NH(51) [3.08(1) Å, 142(10)°], NH(17)···O(47) [2.87(1) Å, 158(10)°], NH(11)···O(47) [3.32(1) Å, 138(9)°], and O(37)···OH(47) [2.66(1) Å, 166(10)°], which are important in fixing the exocyclic *N*-Prop-β-OH-Leu side chain to the depsipeptide backbone. There are five atoms in all which have proton donating ability in the FR900359 molecule, *i.e.*, the imino nitrogen atoms, N(11), N(17), N(24), and N(51) and the hydroxy oxygen atom, O(47). It is noteworthy that all these five atoms efficiently participate in the network of the intramolecular hydrogen bonds (or short contacts) especially the O(47) atom, which both donates a proton, H(47), to O(37) and accepts two protons from N(11) and N(17), and may play an important role as the key station in this network.

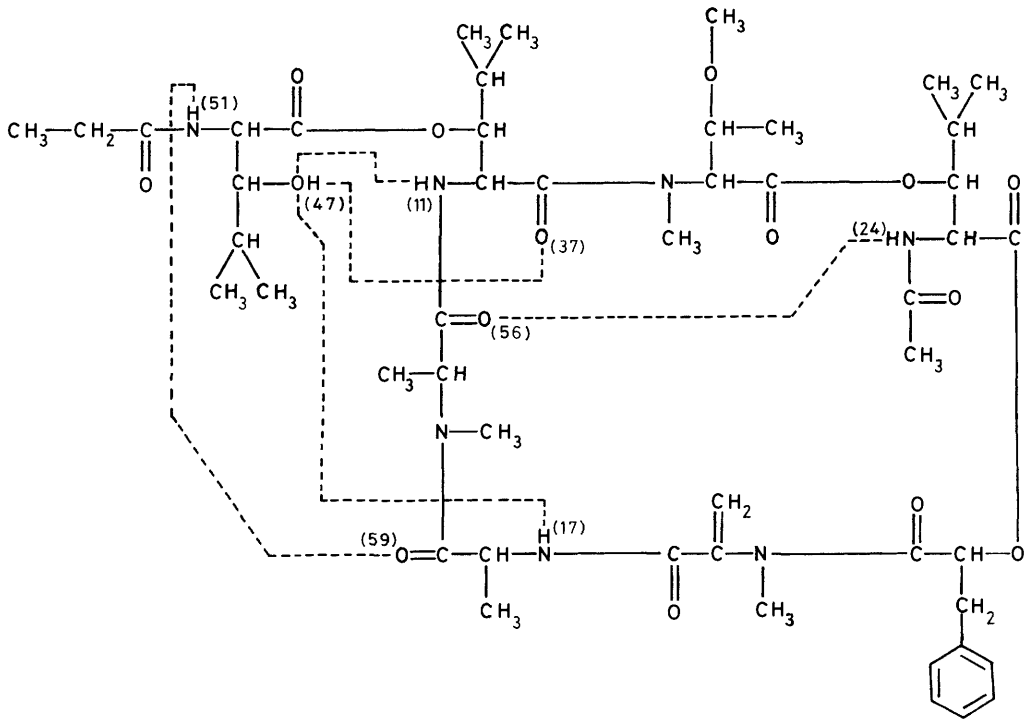

Figure 3. Intramolecular hydrogen bonds or short contacts of FR900359

Table 3. Bond angles (°) with e.s.d.s in parentheses

C(22)-O(1)-C(2)	124.3(11)	O(1)-C(2)-C(3)	111.9(12)
O(1)-C(2)-O(23)	128.1(14)	C(3)-C(2)-O(23)	118.8(13)
C(2)-C(3)-C(4)	106.6(11)	C(2)-C(3)-N(24)	112.0(11)
C(4)-C(3)-N(24)	116.9(10)	C(3)-C(4)-O(5)	107.9(9)
C(3)-C(4)-C(28)	111.7(10)	O(5)-C(4)-C(28)	108.5(9)
C(4)-O(5)-C(6)	117.9(9)	O(5)-C(6)-C(7)	111.5(11)
O(5)-C(6)-O(31)	126.0(14)	C(7)-C(6)-O(31)	122.2(13)
C(6)-C(7)-N(8)	107.1(10)	C(6)-C(7)-C(32)	112.9(11)
N(8)-C(7)-C(32)	112.5(10)	C(7)-N(8)-C(9)	123.9(8)
C(7)-N(8)-C(36)	119.1(8)	C(9)-N(8)-C(36)	116.9(8)
N(8)-C(9)-C(10)	118.6(8)	N(8)-C(9)-O(37)	120.9(9)
C(10)-C(9)-O(37)	120.6(8)	C(9)-C(10)-N(11)	108.1(7)
C(9)-C(10)-C(38)	110.3(7)	N(11)-C(10)-C(38)	110.8(7)
C(10)-N(11)-C(12)	123.2(7)	N(11)-C(12)-C(13)	114.6(8)
N(11)-C(12)-O(56)	122.1(8)	C(13)-C(12)-O(56)	123.3(8)
C(12)-C(13)-N(14)	109.1(8)	C(12)-C(13)-C(57)	112.7(8)
N(14)-C(13)-C(57)	112.3(8)	C(13)-N(14)-C(15)	126.1(8)
C(13)-N(14)-C(58)	116.5(8)	C(15)-N(14)-C(58)	117.4(8)
N(14)-C(15)-C(16)	118.5(8)	N(14)-C(15)-O(59)	121.4(9)
C(16)-C(15)-O(59)	120.0(8)	C(15)-C(16)-N(17)	109.7(8)
C(15)-C(16)-C(60)	110.9(9)	N(17)-C(16)-C(60)	112.1(9)
C(16)-N(17)-C(18)	119.0(7)	N(17)-C(18)-C(19)	114.0(7)
N(17)-C(18)-O(61)	124.8(8)	C(19)-C(18)-O(61)	121.3(8)
C(18)-C(19)-N(20)	113.0(8)	C(18)-C(19)-C(62)	120.2(9)
N(20)-C(19)-C(62)	125.8(9)	C(19)-N(20)-C(21)	119.3(9)
C(19)-N(20)-C(63)	116.2(9)	C(21)-N(20)-C(63)	123.4(9)
N(20)-C(21)-C(22)	116.2(10)	N(20)-C(21)-O(64)	121.1(10)
C(22)-C(21)-O(64)	122.5(11)	O(1)-C(22)-C(21)	106.0(10)
O(1)-C(22)-C(65)	108.8(11)	C(21)-C(22)-C(65)	113.6(11)
C(3)-N(24)-C(25)	121.3(9)	N(24)-C(25)-O(26)	120.8(10)
N(24)-C(25)-C(27)	114.9(9)	O(26)-C(25)-C(27)	124.3(10)
C(4)-C(28)-C(29)	111.2(11)	C(4)-C(28)-C(30)	108.0(11)
C(29)-C(28)-C(30)	108.9(12)	C(7)-C(32)-O(33)	104.7(12)
C(7)-C(32)-C(35)	110.0(14)	O(33)-C(32)-C(35)	113.7(14)
C(32)-O(33)-C(34)	116.1(13)	C(10)-C(38)-C(39)	113.8(8)
C(10)-C(38)-O(42)	104.3(7)	C(39)-C(38)-O(42)	104.3(8)
C(38)-C(39)-C(40)	107.4(10)	C(38)-C(39)-C(41)	109.9(10)
C(40)-C(39)-C(41)	109.2(11)	C(38)-O(42)-C(43)	118.7(7)
O(42)-C(43)-O(44)	124.5(10)	O(42)-C(43)-C(45)	109.9(8)
O(44)-C(43)-C(45)	125.5(10)	C(43)-C(45)-C(46)	107.6(8)
C(43)-C(45)-N(51)	114.6(8)	C(46)-C(45)-N(51)	111.6(8)
C(45)-C(46)-O(47)	109.6(7)	C(45)-C(46)-C(48)	112.8(8)
O(47)-C(46)-C(48)	106.5(8)	C(46)-C(48)-C(49)	112.1(9)
C(46)-C(48)-C(50)	109.9(9)	C(49)-C(48)-C(50)	108.6(10)
C(45)-N(51)-C(52)	120.6(8)	N(51)-C(52)-O(53)	120.0(11)
N(51)-C(52)-C(54)	117.3(10)	O(53)-C(52)-C(54)	122.7(11)
C(52)-C(54)-C(55)	116.1(16)	C(22)-C(65)-C(66)	113.6(12)
C(65)-C(66)-C(67)	121.1(13)	C(65)-C(66)-C(71)	120.5(12)
C(67)-C(66)-C(71)	118.2(13)	C(66)-C(67)-C(68)	117.0(14)
C(67)-C(68)-C(69)	123.4(16)	C(68)-C(69)-C(70)	118.6(15)
C(69)-C(70)-C(71)	120.8(14)	C(66)-C(71)-C(70)	122.0(13)

The FR900359 molecule contains two *cis* peptide bonds, between Ala and *N*-Me-Ala, and between β-OH-Leu and *N*,*O*-Me_2-Thr (Table 4); generally a *cis* conformation is less favoured energetically than its *trans* counterpart.[6] In the case of the FR900359 molecule, the O(59) and O(37) atoms involved in these *cis* peptide bonds can face the proton donating atoms, N(51) and O(47), respectively, to take part in the intramolecular hydrogen bonds (or short contacts). The *cis* peptide bond occurs very rarely in Nature except at the terminal nitrogen of proline[6] and tyrosine[7] residues, but in FR900359 these intramolecular interactions must contribute to overall molecule stabilization.

The φ- and ψ-angles of Table 4 are also summarized in Figure 4,[8] which shows that all (φ, ψ)-values other than that for *N*-Me-deH-Ala lie in the β-region. For *N*-Me-deH-Ala, steric inhibition between C_i^β and N_{i+1}, and between C_i^β and O_{i-1}, would be impossible because the methylene carbon C(62) deviates from the normal β-carbon position of the amino acid. However, it seems that C(62) is influenced by the N_{i+1} and O_{i-1} atoms because the distance between C(62) ··· N(17) is 2.95(1) Å

Table 4. Selected torsion angles (°)

[1] Amino acid residue

	φ	ψ	ω	$\chi^{2,1}$	$\chi^{2,2}$
Ala	−135	74	−10	—	—
N-Me-Ala	−122*	37	178	—	—
β-OH-Leu	−133	149	6	37†	−76
N-Prop-β-OH-Leu	−113	−23	—	69†	−50
N,*O*-Me_2-Thr	−113*	88	—	−65	173
N-Ac-β-OH-Leu	−117	52	—	67†	−53
N-Me-deH-Ala	33*	−135	−176	—	—

[2] Amido and ester bonds

3-Ph-Lac	C(22)-C(21)-N(20)-C(19):	−170
N-Prop-β-OH-Leu	C(45)-N(51)-C(52)-C(54):	179
N-Prop-β-OH-Leu	C(45)-C(43)-O(42)-C(38):	−174
N,*O*-Me_2-Thr	C(7)-C(6)-O(5)-C(4) :	175
N-Ac-β-OH-Leu	C(3)-C(2)-O(1)-C(22) :	178

ω: torsion angle of C_i^α–C_i–N_{i+1}–C_{i+1}^α
φ: [torsion angle of N_i–C_i^α–C_i–O_i] − 180°
ψ: [torsion angle of H_i–N_i–C_i^α–C_i] − 180°
$\chi^{2,1}$: torsion angle of N_i–C_i^α–C_i^β–O_i^γ
$\chi^{2,2}$: torsion angle of N_i–C_i^α–C_i^β–C_i^γ

* For *N*-methyl amino acid residues, the methyl carbon atom has replaced the H_i atom. † For β-OH-Leu, O_i is the O-atom bonded to the C(β)-atom.

Table 5. Intra-molecular hydrogen bonds or short contacts
D = donor, A = acceptor, the lengths (Å) and angles (°) with e.s.d.s in parentheses

Atoms	*d*(D ··· A)	*d*(H ··· A)	∢(D–H ··· A)
N(11)–H(11) ··· O(47)	3.32(1)	2.45(13)	138(9)
N(17)–H(17) ··· O(47)	2.87(1)	1.97(11)	158(10)
O(47)–H(47) ··· O(37)	2.66(1)	1.63(12)	166(10)
N(24)–H(24) ··· O(56)	2.95(1)	1.99(12)	163(10)
N(51)–H(51) ··· O(59)	3.08(1)	2.24(12)	142(10)

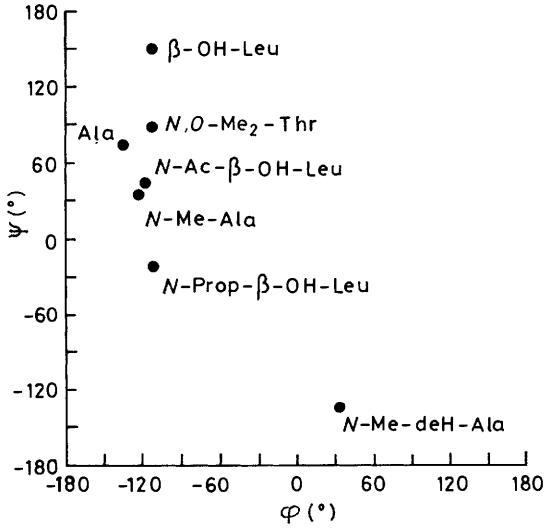

Figure 4. φ–ψ plot for crystalline FR900359 φ and ψ are torsion angles about the C(α)–N and C–C(α) bond, respectively

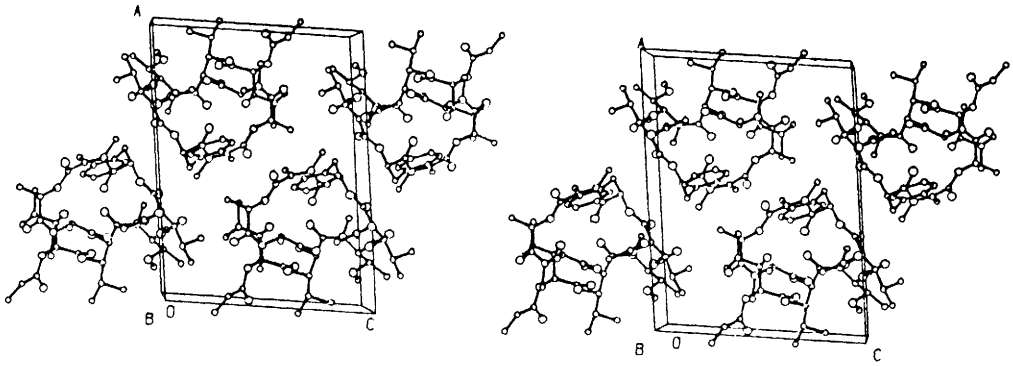

Figure 5. Crystal packing diagram of FR900359 viewed along the *b* axis (PLUTO)

and between C(62) · · · O(64) is 3.89(1) Å. The (φ, ψ)-value for the *N*-Prop-β-OH-Leu side chain indicates that it lies in the α-helix region.

The *N*,*O*-Me_2-Thr, 3-Ph-Lac and *N*-Me-deH-Ala residues excluded from the hydrogen bond network are considered to be flexible because most atoms in these residues have larger thermal parameters. The *N*-Me-deH-Ala residue may be especially unstable or reactive since it falls in the unstable region of the φ–ψ plot and its methylene group is susceptible to nucleophilic attack such as ammonolysis.[2] It may thus be inferred to be involved in the biological activity of FR900359.

Figure 5 shows the molecular packing diagrams of FR900359 viewed along the *b* axis.[9] Most methyl groups and the phenyl ring face outwards from the centre of the molecule, so that its surface can be considered to be hydrophobic. With no intermolecular hydrogen bonds in the FR900359 crystal, the packing force should be due solely to van der Waals contacts.

Experimental

FR900359 was prepared as described in ref. 2. Crystals were obtained from hexane solution as transparent prisms, crystal size for *X*-ray diffraction study was 0.10 × 0.20 × 0.25 mm.

Crystal Data.—$C_{49}H_{75}N_7O_{15}$, M = 1002.17, monoclinic, space group $P2_1$, Z = 2, a = 17.813(2), b = 11.444(1), c = 13.443(1) Å, β = 96.42(2)°, V = 2 723.1(5) Å^3 [by least-squares refinement using the automatic setting angles of 20 reflections, λ(Cu-K_α) = 1.5418 Å], D_c = 1.222 g cm^{-3}, μ(Cu-K_α) = 0.7146 mm^{-1}.

Data Collection.—The intensity of 4 886 independent reflections up to 2θ = 130°, surveyed by the 2θ-ω scan method, were collected on a Rigaku AFC-5 diffractometer with graphite monochromated Cu-K_α radiation. Corrections were applied for Lorentz and polarization factors, but not for absorption and extinction.

Structure Determination and Refinement.—The structure was solved by a combination of a direct method using the MULTAN 84 program[10] and a weighted Fourier technique, and refined by a block-diagonal matrix least-squares method.[11] The positions of all hydrogen atoms were determined from a difference Fourier synthesis; although some of the methyl hydrogen atoms were not successfully assigned to the peak-top of the Fourier map, they were assigned to reasonable positions. They were included in the last cycle of refinement. The final R value was 0.089 for 4 179 reflections with $F_o \geqq 3\sigma(F_o)$. Throughout the refinements, a unit weight was given to the intensity of each reflection. The atom scattering factors cited in International Table for *X*-ray Crystallography vol. IV.[12] All computations were carried out on FACOM M-150F or FACOM S-3300 computers.

Absolute Configuration of the Alanine Unit.—An evacuated and sealed tube containing FR900359 (200 μg) in constant boiling HCl (Pierce, USA; sequanal grade; 0.5 ml) was heated at 110 °C for 20 h. The hydrolysate was evaporated to dryness at reduced pressure, suspended in 10% HCl (0.5 ml) in anhydrous butanol (Regis, USA), sonicated at room temperature for 15 min and heated at 90 °C for 30 min in a sealed vial. The mixture was evaporated to dryness under reduced pressure, treated with *N*-trifluoroacetyl-L-prolyl chloride (0.2 ml) in chloroform (Regis, USA) at room temperature for 30 min, and was blown down under a stream of nitrogen. The residue was dissolved in chloroform (0.2 ml) and an aliquot of the solution was injected into g.c./m.s. For comparison, *ca.* 50 μg of D,L-alanine and L-alanine were converted into their respective *N*-trifluoroacetyl-L-prolyl-butyl ester derivatives, and used as authentic samples. A Shimadzu QP-1000 GC mass spectrometer operated at 70 eV and equipped with a capillary column (Chromato Packing Centre, Japan: ULBON HR-54, 0.32 mm × 25 m) was employed. The column temperature was raised from 90 to 250 °C at a rate of 10 °C/min.

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