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Design and synthesis of a bis(cycloisodityrosine) analogue of RA-VII, an antitumor bicyclic hexapeptide

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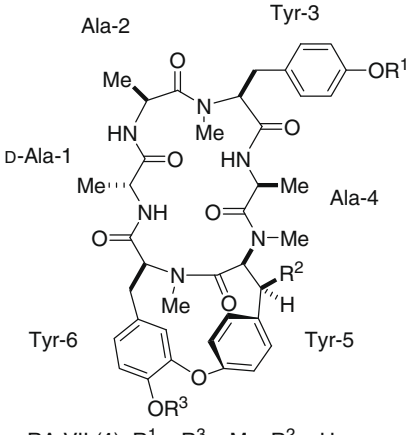
ABSTRACT

An analogue of an antitumor bicyclic hexapeptide RA-VII was prepared, in which the Ala-2 and Tyr-3 residues of RA-VII were replaced by a cycloisodityrosine unit. In the crystalline state, the peptide backbone structures and the side-chain conformations at Tyr-3, Tyr-5, and Tyr-6 of this analogue and of RA-II were very similar. This analogue, however, showed much weaker cytotoxicity against P-388 leukemia cells than parent RA-VII.

RA-VII (**1**),[1,2] isolated from *Rubia cordifolia* L. and *R. akane* Nakai (Rubiaceae) is a bicyclic hexapeptide with a potent antitumor activity (Fig. 1). The antitumor activities of this compound and also of bouvardin (NSC 259968, **2**)[3] from *Bouvardia ternifolia* (Cav.) Schltdl. (Rubiaceae) which is structurally closely related to **1** are considered to be due to inhibition of protein synthesis through interaction with eukaryotic ribosomes.[4,5] Recently, **1** was shown to cause conformational changes in F-actin and stabilization of actin filaments to induce G2 arrest.[6] Peptide **1** is known to exist as a mixture of two or three stable conformers in solution,[7,8] and the most populated conformer, having *trans*, *trans*, *trans*, *trans*, *cis*, and *trans* (*t*–*t*–*t*–*t*–*c*–*t*) configuration at the peptide bonds between D-Ala-1/Ala-2, Ala-2/Tyr-3, Tyr-3/Ala-4, Ala-4/Tyr-5, Tyr-5/Tyr-6, and Tyr-6/D-Ala-1, respectively, has been identified as an active conformer.[9–11] Of the three tyrosines at positions-3, -5, and -6 in peptide **1**, Tyr-5 and Tyr-6 form a cycloisodityrosine unit by forming a linkage between the phenolic oxygen of Tyr-5 and the C_ε of Tyr-6. Due to a planar amide bond and 1,3-disubstituted- and 1,4-disubstituted-phenyl rings included in the 14-membered ring of the cycloisodityrosine unit, the rotation of the side chains of those residues is restricted. The remaining side chain at Tyr-3 rotates about the C_α–C_β (χ_1) and C_β–C_γ (χ_2) bonds. Since the substituent at the zeta position of Tyr-3 is known to greatly relate to the activity,[12] the χ_1 and χ_2 angles of Tyr-3, defining the spatial orientation of the Tyr-3 phenyl ring, appear to play a critical role in the cytotoxicity. In the present study, to obtain information about the effect of the side-chain conformation of Tyr-3 upon the activity, we designed an analogue having a restricted Tyr-3 side-chain rotation, synthesized it, performed its X-ray crystallographic studies, and evaluated its cytotoxicity.



RA-VII (**1**): $R^1 = R^3 = Me$, $R^2 = H$
Bouvardin (**2**): $R^1 = Me$, $R^2 = OH$, $R^3 = H$
RA-II (**11**): $R^1 = R^2 = H$, $R^3 = Me$

Figure 1. Structures of natural RA-series peptides.

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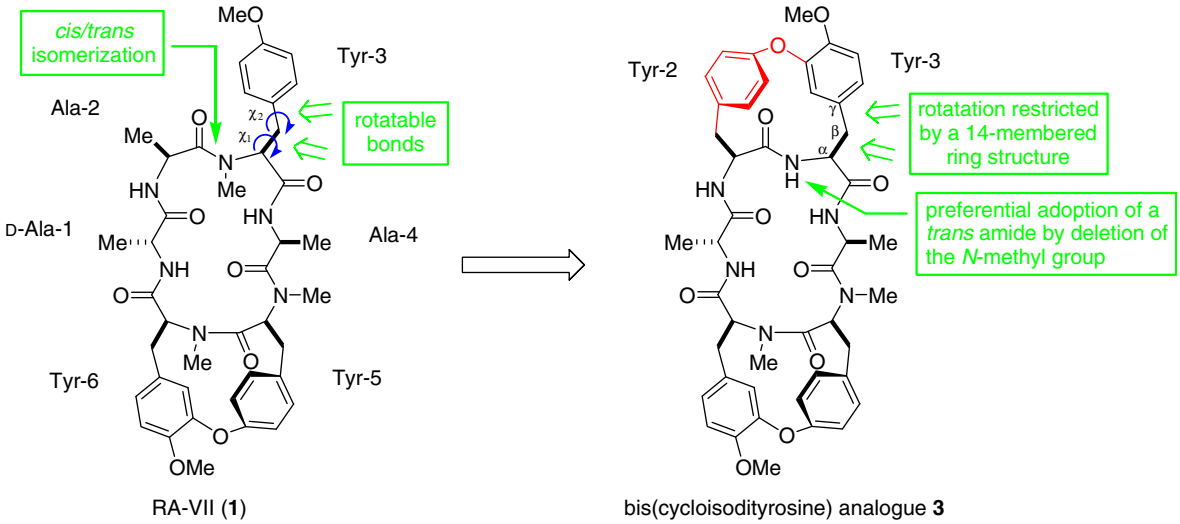
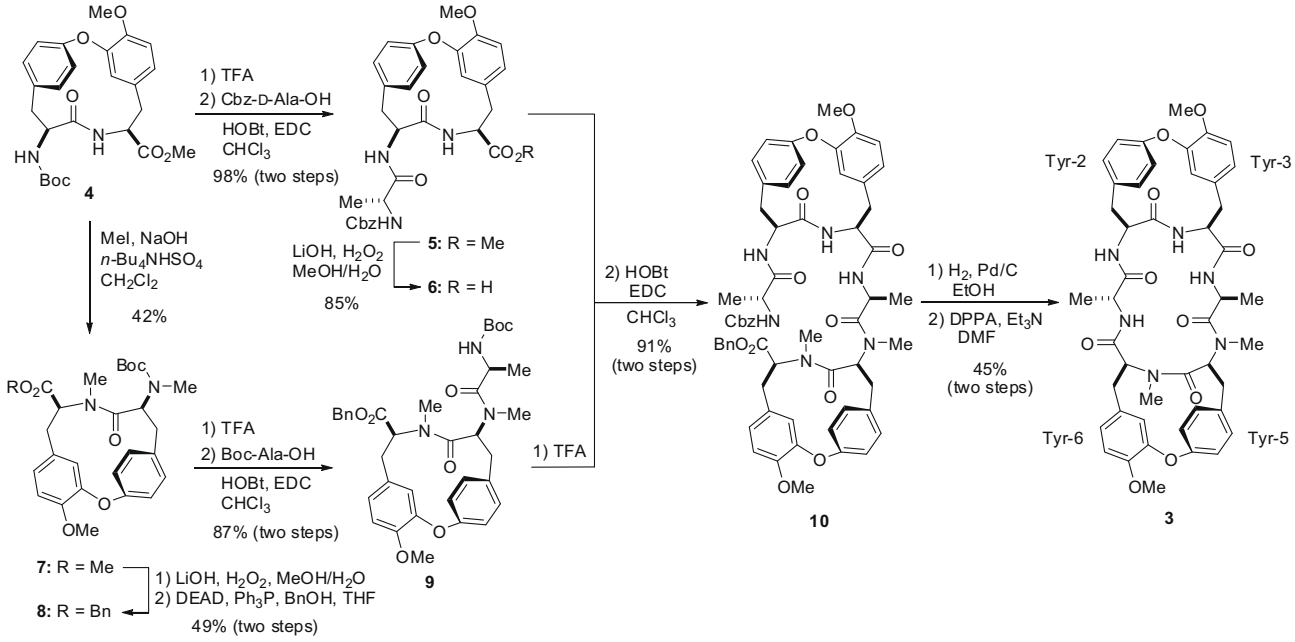


Figure 2. Design of conformationally restricted analogue of **1**.



Scheme 1. Synthesis of analogue **3**.

Our approach to the application of restriction to the Tyr-3 side-chain rotation was to introduce a cycloisodityrosine unit in place of the Ala-2/Tyr-3 moiety of peptide **1**, that is, preparation of an analogue having two cycloisodityrosine units in one molecule as shown in **3** (Fig. 2). In this analogue **3**, the rotations about the C_α–C_β and C_β–C_γ bonds of Tyr-3 are restricted simultaneously as in the case of those in Tyr-6. However, Tyr-3 in the newly introduced second cycloisodityrosine unit bears no *N*-methyl group, so that, in contrast to the Tyr-5/Tyr-6 bond of the original cycloisodityrosine unit, the Tyr-2/Tyr-3 bond adopts a *trans* configuration as in the active conformer of **1**.

The route of synthesis of **3** is illustrated in Scheme 1. The starting material, cycloisodityrosine **4**, corresponding to Tyr-2 and Tyr-3 of **3**, was prepared from 3-iodo-L-tyrosine according to the procedure reported previously.[13] Compound **4** was deprotected and coupled with Cbz-D-Ala-OH to afford tripeptide **5**, which was then converted to acid **6**. Another portion of **4** was *N*,*N*′-dimethylated under phase-transfer catalysis conditions to prepare the other cycloisodityrosine unit, **7**, corresponding to Tyr-5 and Tyr-6 of the analogue. Subsequent conversion of the methyl ester functionality of **7** to a benzyl ester group afforded **8**, which, after removal of the Boc group, was coupled with Boc-Ala-OH to afford tripeptide **9**. After deprotection, tripeptide **9** was coupled with acid **6** to give hexapeptide **10**. Removal of the N- and C-terminal protecting groups of **10** and subsequent formation of the macrocycle with diphenylphosphoryl azide (DPPA) and triethylamine under high-dilution conditions in DMF (0.001 M) furnished **3** in 45% yield from **10**.

The solution structure of thus obtained analogue **3** was analyzed by the NMR experiments. In $CDCl_3$ at 300 K, analogue **3** gave a single set of sharp peaks (Fig. 3),[14] suggesting that only one single conformer was present in this solution. The amide configuration of this solution structure was determined by analysis of NOESY data. From the NOE correlations between D-Ala-1 H-α/Tyr-2 NH and be-

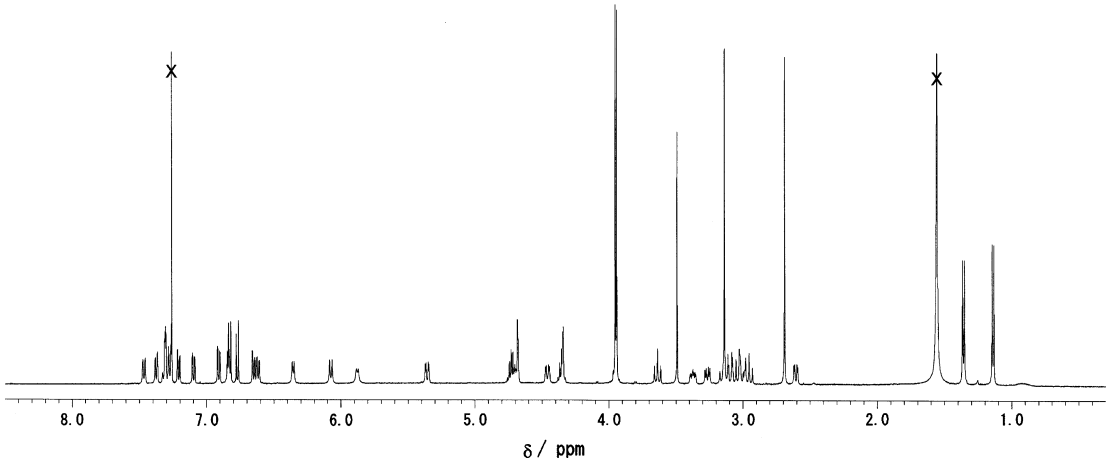


Figure 3. Five hundred megahertz ^{1}H NMR spectrum of analogue **3** in $CDCl_3$.

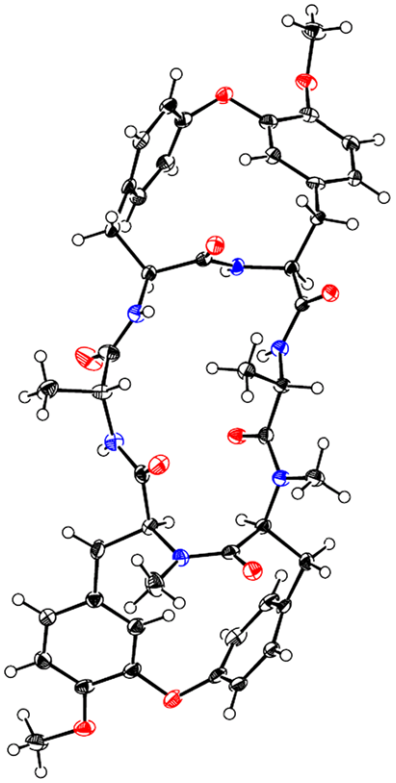


Figure 4. ORTEP representation of analogue **3**.

tween D-Ala-1 Me/Tyr-2 NH, the amide bond between D-Ala-1/Tyr-2 was determined to be *trans*, and from the correlations between Tyr-2 H-α/Tyr-3 NH, the amide bond between Tyr-2/Tyr-3 to be *trans*. Analogously, from the correlations between Tyr-3 H-α/Ala-4 NH, Ala-4 H-α/Tyr-5 NMe, Ala-4 Me/Tyr-5 NMe, Tyr-5 H-α/Tyr-6 H-α, and Tyr-6 H-α/D-Ala-1 NH, the amide bonds between Tyr-3/Ala-4, Ala-4/Tyr-5, Tyr-5/Tyr-6, and Tyr-6/D-Ala-1 were determined to be *trans*, *trans*, *cis*, and *trans*, respectively, to show the sequence of the amide configurations of **3** to be *t*–*t*–*t*–*t*–*c*–*t* as in the active conformer of **1**. In addition, an NOE correlation between D-Ala-1 H-α/Ala-4 Me was observed. Such a transannular cross-peak was also observed in the conformer of natural RAs having *t*–*t*–*t*–*t*–*c*–*t* configuration.[15] The structure in the solid state was obtained by the crystallography of **3** (Fig. 4).[16] The results showed that the crystal structure of **3** was basically identical to the solution structure of **3** in $CDCl_3$, derived from its NOESY experiments. The amide config-

Table 1
X-ray calculated backbone dihedrals (degree) in analogue **3**.

Residue		Dihedral angle	Residue		Dihedral angle
D-Ala-1	φ	136.3(3)	Ala-4	φ	−164.5(3)
	ψ	−129.2(3)		ψ	156.4(3)
	ω	178.3(3)		ω	176.3(3)
Tyr-2	φ	−141.7(3)	Tyr-5	φ	−115.5(3)
	ψ	116.6(3)		ψ	111.1(3)
	ω	−162.7(3)		ω	−21.3(4)
Tyr-3	φ	49.1(4)	Tyr-6	φ	−75.7(4)
	ψ	49.3(4)		ψ	−179.7(3)
	ω	−174.8(3)		ω	176.8(3)

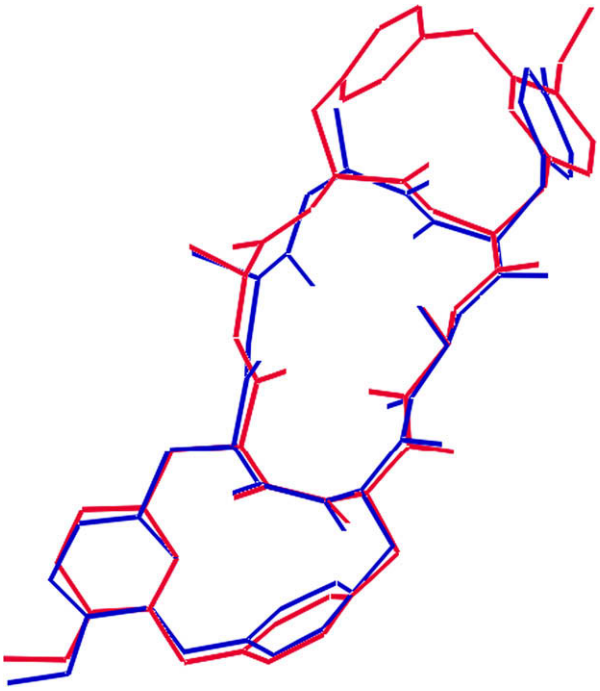


Figure 5. Superposition of the crystal structures of analogue **3** (red) and RA-II (**11**, blue).

uration of the crystal structure of **3** was also *t–t–t–t–c–t* (Table 1) and the distance between D-Ala-1 H-α and the nearest methyl hydrogen of Ala-4, responsible for the above mentioned significant NOE correlation in **3**, was 2.38 Å. This distance is reasonable for producing an NOE cross-peak.

The similarity in the three-dimensional structural features of **1** and **3** was highlighted by superimposing the crystal structure of **3** over that of RA-II (**11**),[17] whose conformational property is known to be identical to that of **1** and whose crystallographic data are available (Fig. 5).[18] The spatial positions of the phenyl rings of the three tyrosines and the peptide backbone conformation at residues 2–6 of these two peptides **3** and **11** are almost superimposable, which indicated that analogue **3** may effectively mimic one of the lowest-energy conformations in peptide **1** including the side chain of Tyr-3.

Analogue **3** and as reference, **1**, were evaluated for their cytotoxicity against P-388 leukemia cells. Their IC_{50} values were 7.5 and 0.0015 μg/mL, respectively. The result apparently does not agree with our hypothesis that the side-chain conformation at Tyr-3 of peptide **1**, as shown in the crystal structure of **11**, is a major factor which determines the cytotoxic activity of the compounds of this series. The bulky phenoxy tether connecting the C_β of Ala-2 and the C_ε of Tyr-3 in the present compound **3**, however, may be hampering its necessary close access to the relevant binding site, resulting in giving low cytotoxicity. Synthesis of further analogues and their analyses may give further information to this problem.

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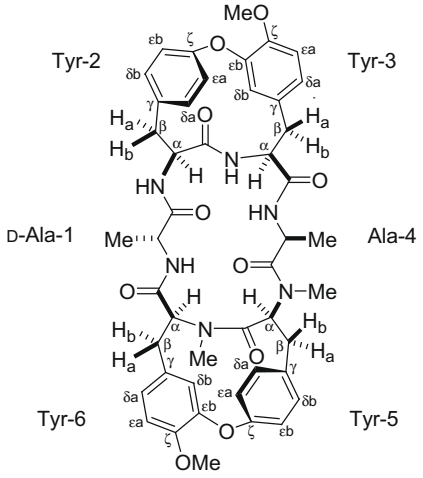
14. *Data for* **3**: colorless prisms, mp > 300 °C ($MeOH–H_2O$); ^1H NMR (500 MHz, 300 K, $CDCl_3$) δ 7.47 (1H, dd, *J* = 8.6, 1.8 Hz, Tyr-2 H-δb), 7.37 (1H, dd, *J* = 8.4, 2.2 Hz, Tyr-5 H-δb), 7.32 (1H, dd, *J* = 8.4, 1.8 Hz, Tyr-2 H-δa), 7.30 (1H, dd, *J* = 8.4, 2.0 Hz, Tyr-2 H-εa), 7.27 (1H, dd, *J* = 8.4, 2.2 Hz, Tyr-5 H-δa), 7.21 (1H, dd, *J* = 8.4, 2.3 Hz, Tyr-5 H-εb), 7.10 (1H, dd, *J* = 8.6, 2.0 Hz, Tyr-2 H-εb), 6.91 (1H, dd, *J* = 8.4, 2.3 Hz, Tyr-5 H-εa), 6.84 (1H, d, *J* = 7.2 Hz, Ala-4 NH), 6.83 (1H, d, *J* = 8.2 Hz, Tyr-6 H-εa), 6.77 (1H, d, *J* = 8.2 Hz, Tyr-3 H-εa), 6.65 (1H, dd, *J* = 8.2, 2.0 Hz, Tyr-3 H-δa), 6.62 (1H, dd, *J* = 8.2, 2.0 Hz, Tyr-6 H-δa), 6.36 (1H, d, *J* = 6.9 Hz, D-Ala-1 NH), 6.08 (1H, d, *J* = 9.2 Hz, Tyr-2 NH), 5.88 (1H, d, *J* = 6.4 Hz, Tyr-3 NH), 5.36 (1H, dd, *J* = 11.4, 3.3 Hz, Tyr-5 H-α), 4.73 (1H, m, Ala-4 H-α), 4.70 (1H, m, Tyr-2 H-α), 4.68 (1H, d, *J* = 2.0 Hz, Tyr-3 H-δb), 4.46 (1H, dd, *J* = 12.0, 3.6 Hz, Tyr-6 H-α), 4.35 (1H, d, *J* = 2.0 Hz, Tyr-6 H-δb), 4.35 (1H, quintet, *J* = 6.9 Hz, D-Ala-1 H-α), 3.95 (3H, s, Tyr-6 OMe), 3.94 (3H, s, Tyr-3 OMe), 3.64 (1H, t, *J* = 11.4 Hz, Tyr-5 H-βb), 3.37 (1H, m, Tyr-3 H-α), 3.27 (1H, dd, *J* = 12.8, 5.1 Hz, Tyr-2 H-βa), 3.16 (1H, m, Tyr-6 H-βa), 3.14 (3H, s, Tyr-5 NMe), 3.12–2.98 (2H, m, Tyr-3 H_2-β), 3.00 (1H, m, Tyr-6 H-βb), 2.95 (1H, t, *J* = 12.8 Hz, Tyr-2 H-βb), 2.69 (3H, s, Tyr-6 NMe), 2.61 (1H, dd, *J* = 11.4, 3.3 Hz, Tyr-5 H-βa), 1.36 (3H, d, *J* = 6.9 Hz, D-Ala-1 H_3-β), 1.14 (3H, d, *J* = 6.9 Hz, Ala-4 H_3-β); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$, δ) D-Ala-1 (171.8 C=O, 48.2 α, 21.1 β), Tyr-2 (170.6 C=O, 158.3 ζ, 133.6 γ, 131.1 δa, 131.0 δb, 127.3 εa, 125.0 εb, 56.3 α, 37.3 β), Tyr-3 (167.9 C=O, 152.0 εb, 146.7 ζ, 130.7 γ, 121.9 δa, 119.3 δb, 111.9 εa, 58.7 α, 56.2 OMe, 32.4 β), Ala-4 (171.7 C=O, 46.3 α, 19.0 β), Tyr-5 (169.5 C=O, 158.2 ζ, 134.9 γ, 132.9 δa, 130.7 δb, 126.0 εb, 124.4 εa, 53.9 α, 36.9 β, 30.6 NMe), Tyr-6 (170.5 C=O, 153.1 εb, 146.5 ζ, 128.1 γ, 120.9 δa, 113.3 δb, 112.3 εa, 57.6 α, 56.2 OMe, 35.1 β, 29.2 NMe).



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16. *Crystallography of* **3**: $C_{46}H_{49}N_6O_{10}\cdot CH_4O\cdot 3(O)$, *M* = 925.95, 0.19 × 0.12 × 0.09 mm, monoclinic, space group *C*2, *a* = 18.9568(18) Å, *b* = 14.9739(14) Å, *c* = 16.4613(15) Å, β = 93.3980(10)°, *V* = 4664.4(8) $Å^3$, *Z* = 4, D_X = 1.319 Mg m^{-3}, μ(Mo Kα) = 0.10 mm^{-1}, *T* = 100 K, 13,383 measured reflections, 9102 independent reflections, 7496 reflections with $I > 2\sigma(I)$, $R[F^2 > 2\sigma(F^2)] = 0.059$, $wR(F^2) = 0.155$, *S* = 1.05. The structure was solved by direct methods using SHELXS-97,[19] and refined by full-matrix least-squares on F^2 using SHELXL-97.[20] Crystallographic data for compound **3** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre under the reference number CCDC 698521. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif, or by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.
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