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Tetrahedron 62 (2006) 8952–8958

Tetrahedron

Unusual sesquiterpene glucosides from *Amaranthus retroflexus*

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Received 10 April 2006; revised 15 June 2006; accepted 6 July 2006
Available online 28 July 2006

Abstract—Implementing the phytochemical study of the weed *Amaranthus retroflexus*, four new sesquiterpene glucosides were isolated from the methanolic extract of the plant. The structures of these metabolites are determined on the basis of the mass spectrometry, and 1D and 2D NMR spectroscopies (DQ-COSY, TOCSY, HSQC, HSQC–TOCSY, HMBC, and NOESY). Two compounds are characterized by a new aglycone and differed from the site of glucosylation. The other two compounds are dimeric diastereoisomers.
All the glucoside sesquiterpenes were tested on the wild species *Taraxacum officinale* to evaluate the role of this weed in the habitat and on the seed of *A. retroflexus* to verify the potential autotoxic effect of the plant.
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1. Introduction

One of the most useful aspects of allelopathy in manipulated ecosystems is the role in agriculture.[1] Many plants have been studied and a number of new phytotoxic secondary metabolites have been isolated and characterized.[2,3] Recent investigations are focused on the effects of weeds on crops,[4] crops on weeds,[5] and crops on crops.[6] The goal of these studies is the use of allelochemicals as growth regulators and natural pesticides to promote sustainable agriculture.[7,8]

In the search for new allelochemicals from plants of the Mediterranean area, we recently studied the weed *Amaranthus retroflexus*, known as redroot pigweed, a summer annual invasive plant, widely distributed in Italy and commonly found in cultivated lands. From the methanolic extract of the plant, we isolated and characterized new free and glucosylated nerolidol sesquiterpenes, named amarantholidols and amarantholidosides, respectively.[9] These compounds, when tested on the cultivated species *Lactuca sativa*, showed a moderate phytotoxic activity down to 10^{-9} M.

In this work we completed the study of the organic extract and described the isolation and characterization of four new nerolidol glucosides, named amarantholidosides IV–VII. Two of them, in fact, showed an unusual dimeric structure. The amarantholidosides I–VI have been tested on the wild species *Taraxacum officinale* to verify their impact on other weeds and on the seed of *A. retroflexus* to test their potential autotoxic effects.

2. Results and discussion

The EtOAc fraction of the MeOH fresh leaf extract of *A. retroflexus* L. was chromatographed on a silica gel column (flash chromatography) using $CHCl_3$–EtOAc and $CHCl_3$–MeOH mixtures as eluents in increasing polarity. The most polar fractions yielded seven compounds: four amarantholidols A–D and three amarantholidosides I–III (**1**–**3**).[9] Continuing the phytochemical study of the fraction we isolated four new glucosides **4**–**7**, of which two of them are dimeric (Figs. 1 and 2).

Compound **4**, named amarantholidoside IV, has been identified as (3*S*,6*E*,10*R*)-10-β-D-glucopyranosyloxy-3,11-dihydroxy-3,7,11-trimethyldodeca-1,6-diene.

Its molecular formula $C_{21}H_{38}O_8$ was deduced on the basis of its ESIMS spectrum, which showed the pseudomolecular peak at *m/z* 441 $[M+Na]^+$, and the elemental analysis.

The mass spectrum showed fragment ion at *m/z* 279 and 203, both diagnostic for the presence of a hexose moiety in the molecule. The ^{1}H NMR spectrum (Table 1) showed, in the downfield region, an ABX system as three double doublets centered at δ 5.91, 5.19, and 5.02, a partially overlapped signal at δ 5.21, and a double doublet at δ 3.40. The doublet at δ 4.44 (J=7.5 Hz) and two double doublets at δ 3.72 and

Keywords: *Amaranthus retroflexus*; Amaranthaceae; Nerolidol glucosides; Amarantholidosides; NMR analysis; Phytotoxic effect; Autotoxic effect; *Taraxacum officinale*.
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doi:10.1016/j.tet.2006.07.017

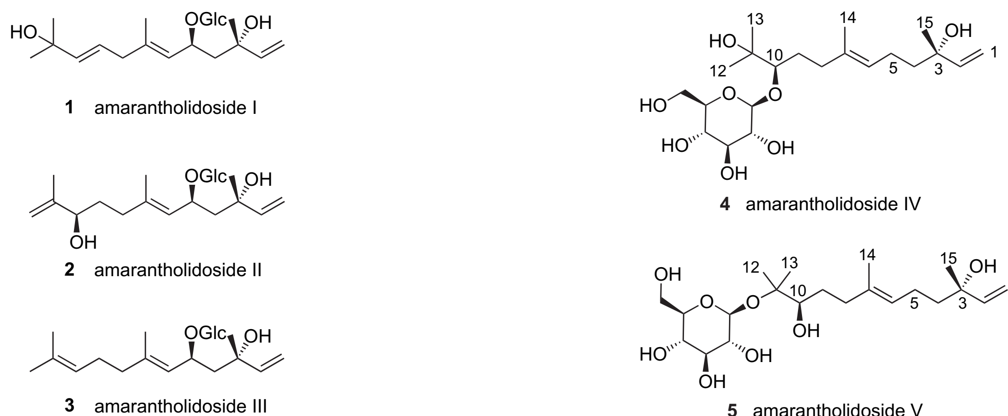

Figure 1. Structures of amarantholidosides I–III (**1**–**3**).

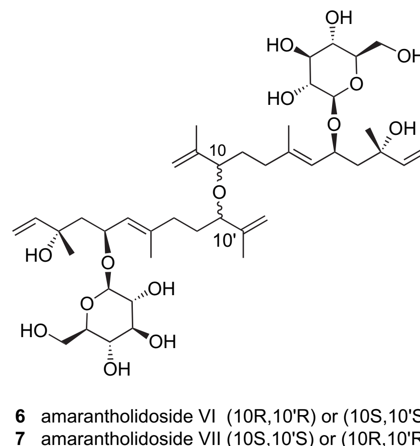

Figure 2. Structures of amarantholidosides IV–VII (**4**–**7**).

3.85 were in accordance with an anomer and a hydroxymethylene H-6 of a sugar moiety, respectively, while the chemical shift of six carbon signals in the ^{13}C NMR was in good agreement with a glucose unit. Accordingly, the HSQC–TOCSY experiment (Table 1) showed heterocorrelation between these glycidic carbons and the H-1′ and H-6′ protons ranging from 3.40 to 3.24 ppm. In the upfield region of the spectrum four singlet methyls at δ 1.60, 1.28, 1.17, and 1.13 and eight protons as multiplets were present.

A DQ-COSY experiment showed homocorrelations among the olefinic protons of the ABX system, the proton at δ 5.21 and a multiplet centered at δ 2.03, which correlated with a double doublet at δ 1.51. Among the protons bonded to oxygen atoms, correlations between the doublet at δ 4.44 and a signal at δ 3.27 as well as interactions among the double doublet at δ 3.40 and an overlapped signal centered at δ 1.45 were evident.

The ^{13}C NMR spectrum (Table 1) showed 21 signals confirming the presence of a glycosilated sesquiterpene. All of the carbons were identified, on the basis of a DEPT experiment, as four methyls, six methylenes, eight methines, and three tetrasubstituted carbons. Their chemical shifts indicated the presence of a terminal and an internal double bonds, a glucose moiety, and three carbinol groups (one of them secondary). All of the carbons were correlated to the respective protons using an HSQC experiment as reported in Table 1. In the same table, the results of an HMBC and HSQC–TOCSY have also been reported. These experiments were crucial for the structure elucidation. On the basis of these correlations, the carbinol carbon at δ 90.3, bonded to the proton at δ 3.40, was attributed to the C-10 carbon. Its downshift value could be explained by hypothesizing a linkage with the glucose. The heterocorrelations, in the HMBC experiment, between the carbon and the anomeric proton at δ 4.44 and, vice versa, between the anomeric carbon at δ 106.4 and the proton at δ 3.40 confirmed the hypothesis. The glucose moiety was confirmed by GC analysis and the coupling constant value of the anomeric proton in the ^{1}H NMR indicated the presence of a β-glucose.

The absolute configuration to the tertiary C-3 and secondary C-10 carbinol carbons has been assigned using two different methods. The *R* configuration at the C-10 carbon has been determined using a modified Mosher's method on the aglycone of amarantholidol IV obtained from treatment with β-glucosidase.[10,11] The positive and the negative $\Delta\delta_{R-S}$ values for the H-9, H-12, and H-15 protons were found, respectively, on the right and the left sides of the MTPA plane indicating a *R* configuration for C-10 carbon.

The *S* absolute configuration at the C-3 of compound **4** has been deduced using a bidentate NMR chiral solvent, as described by Kishi and co-workers.[12,13] The absolute configuration of acyclic alcohols was established from analysis of the chemical shifts' behaviors of the adjacent carbons in (*R*,*R*)- and (*S*,*S*)-bis-α-methylbenzylpropandiammine (BMBA-*p*). The positive (+0.121) and negative (−0.098) $\Delta\delta_{R-S}$ values of the amarantholidoside IV were found on the left and right of the tertiary carbinol carbon, respectively, according to an α-orientation for the hydroxyl and a β-orientation for the methyl.

Compound **5**, named amarantholidoside V, showed a molecular formula $C_{21}H_{38}O_8$ in accordance with its ESI mass spectrum, which showed the pseudomolecular peak at *m*/*z* 441 $[M+Na]^+$, and the elemental analysis. This data suggested that it was an isomer of compound **4**.

The ^{1}H NMR spectrum showed the vinyl protons at δ 5.91, 5.18, and 5.02, the H-6 olefin proton at δ 5.21, the methine H-10 at δ 3.43, two multiplets at δ 2.25 and 2.03, a double doublet at δ 1.50, three singlet methyls at δ 1.24, 1.22, and

Table 1. NMR data of amarantholidoside IV (**4**) in CD_3OD

	^1H (δ)	J (Hz)	DQ-COSY	^{13}C (δ)	DEPT	HMBC (H→C)	HSQC–TOCSY (H→C)
1t	5.19 dd	17.4, 1.5	1c, 2	112.0	CH_2	2	1, 2
1c	5.02 dd	10.8, 1.5	1t, 2			2, 3	1, 2
2	5.91 dd	17.4, 10.8	1c, 1t	146.3	CH	1, 3, 4, 15	1, 2
3	—	—	—	74.8	C	—	—
4	1.51 dd	9.3, 7.5	5	43.5	CH_2	2, 3, 5, 6, 15	4, 5, 6, 14
5	2.03 m	—	4, 6	23.7	CH_2	3, 4, 6, 7	4, 5, 6, 14
6	5.21 ov	—	5	126.1	CH	4, 5, 8, 14	4, 5, 6
7	—	—	—	136.0	C	—	—
8	2.30 m	—	9	37.0	CH_2	6, 7, 9, 10, 14	8, 9, 10
	2.25 m		9			6, 7, 9, 10, 14	8, 9, 10
9	1.45 m	—	8, 10	30.7	CH_2	7, 8	8, 9, 10
10	3.40 dd	10.5, 1.5	9	90.3	CH	glu1, 8, 9, 11, 12, 13	8, 9, 10
11	—	—	—	73.8	C	—	—
12	1.17 s	—	—	23.7	CH_3	10, 11, 13	—
13	1.13 s	—	—	26.4	CH_3	10, 11, 12	—
14	1.60 s	—	6	16.1	CH_3	6, 7, 8	—
15	1.28 s	—	—	27.6	CH_3	1, 2, 3, 4, 5	—
glu1	4.44 d	7.5	glu2	106.4	CH	10, glu2, glu3	glu1, glu2, glu3, glu4, glu6
glu2	3.27 ov	—	glu1, glu3	76.0	CH	glu1, glu3, glu4	glu1, glu2, glu3, glu4, glu6
glu3	3.27 ov	—	glu2, glu4	77.9	CH	glu1, glu2, glu4	glu1, glu2, glu3, glu4, glu6
glu4	3.37 ov	—	glu3, glu5	71.4	CH	glu5, glu6	glu2, glu3, glu4, glu6
glu5	3.37 ov	—	glu4, glu6	78.4	CH	glu6	glu2, glu3, glu4, glu6
glu6	3.72 dd	11.7, 2.1	glu5, glu6	62.6	CH_2	glu4, glu5	glu3, glu4, glu6
	3.85 dd	11.7, 5.7	glu5, glu6			glu4, glu5	glu3, glu4, glu6

d=doublet; dd=double doublet; m=multiplet; ov=overlapped; s=singlet.

1.21, and a doublet methyl at δ 1.60. In the same spectrum, the sugar signals were present as a doublet at δ 4.49 (glu1), two double doublets at δ 3.81 and 3.64 (glu6), and a double doublet at δ 3.15 (glu2) besides other signals obscured by the solvent signal.

The main differences observed in the ^{13}C NMR spectrum, when compared to amarantholidoside IV, are for C-10 and C-11 carbons (Table 2). The sugar moiety was in good accordance with the presence of a glucose and the J value of the H-glu1 proton suggested the presence of a β-anomer. The 2D NMR experiments confirmed the same aglycone of compound **4**. In the HMBC experiment, the signal at δ 81.8, assigned to the C-11 carbon, showed correlations with the anomeric proton at δ 4.49, the H-12 and H-13 methyls and with the H-10 double doublet, which itself correlates, in the HSQC experiment, to the carbon at δ 78.1. This latter carbon correlated with the H-9 protons at δ 1.35 and 2.20 and with the H-12 and H-13 methyls. This data led to the hypothesize of the presence of a linkage between the C-glu1 of

Table 2. NMR data of amarantholidoside V (**5**) in CD_3OD

	^1H (δ)	J (Hz)	DQ-COSY	^{13}C (δ)	DEPT	HMBC (H→C)	HSQC–TOCSY (H→C)
1t	5.18 dd	17.4, 1.5	1c, 2	112.0	CH_2	2, 3	1, 2
1c	5.02 dd	10.5, 1.5	1t, 2			2, 3	1, 2
2	5.91 dd	17.4, 10.5	1c, 1t	146.3	CH	1, 3, 4, 15	1, 2
3	—	—	—	73.8	C	—	—
4	1.50 dd	9.3, 7.5	5	43.5	CH_2	2, 3, 5, 6, 15	4, 5, 6, 14
5	2.03 m		4, 6	23.7	CH_2	4, 6, 7	4, 5, 6, 14
6	5.21 ov		5	126.0	CH	3, 5, 7, 8, 14	4, 5, 6
7	—	—	—	135.9	C	—	—
8	2.30 m	—	9	37.8	CH_2	6, 7, 9, 10, 14	8, 9, 10
	2.25 m		9			6, 7, 9, 10, 14	8, 9, 10
9	2.20 m	—	8, 10	30.7	CH_2	7, 8	8, 9, 10
	1.35 m						8, 9, 10
10	3.43 dd	10.5, 1.5	9	78.1	CH	8, 9, 11, 12, 13	8, 9, 10
11	—	—	—	81.8	C	glu1, 10, 12, 13	—
12	1.21 s	—		21.3	CH_3	10, 11, 13	
13	1.22 s	—		23.8	CH_3	10, 11, 12	
14	1.60 d	0.9	6	16.0	CH_3	6, 7, 8	
15	1.24 s	—		27.6	CH_3	1, 2, 3, 4, 5	
glu1	4.49 d	8.1	glu2	98.6	CH	11, glu2, glu3	glu1, glu2, glu3, glu4, glu6
glu2	3.15 dd	9.0, 7.5	glu1, glu3	75.1	CH	glu1, glu3, glu4	glu1, glu2, glu3, glu4, glu6
glu3	3.36 ov		glu2, glu4	77.7	CH	glu1, glu2, glu4	glu1, glu2, glu3, glu4, glu6
glu4	3.37 ov		glu3, glu5	71.6	CH	glu5, glu6	glu2, glu3, glu4, glu6
glu5	3.26 dt	9.0, 8.4	glu4, glu6	77.7	CH	glu6	glu2, glu3, glu4, glu6
glu6	3.64 dd	11.7, 5.1	glu5, glu6	62.6	CH_2	glu4, glu5	glu3, glu4, glu6
	3.81 dd	11.7, 2.1	glu5, glu6			glu4, glu5	glu3, glu4, glu6

d=doublet; dd=double doublet; m=multiplet; ov=overlapped; s=singlet.

the glucose and the C-11 of the aglycone. The NOEs observed, in an NOESY experiment, among the H-glu1 and H-12 protons confirmed this hypothesis. The enzymatic hydrolysis with β-glucosidase afforded the same aglycone **4a** of the previous glucoside.

Compounds **6** and **7** have been identified as two diastereomers and named amarantholidosides VI and VII, respectively.

Compounds **6** and **7** had a molecular formula $C_{42}H_{70}O_{15}$, as resulted by the elemental analysis and the ESI mass spectrum, which showed the pseudomolecular peak at *m/z* 837 $[M+Na]^+$.

In the ^1H NMR spectrum of amarantholidoside VI (Table 3), the ABX system of H-1 cis, H-1 trans, and H-2 protons and the H-6 and H-12 olefinic protons was evident. In the region of the protons geminal to oxygen, the signals of the anomeric and of the hydroxymethyl glu6 of a sugar moiety were present, and the H-10 triplet at δ 4.22 and further four protons ranging from 3.10 to 3.40 ppm were also present. In the upfield region of the spectrum three methyls at δ 1.72, 1.67, and 1.28, a methylene as triplet at δ 2.06, and a double doublet centered at δ 1.96 were identifiable. This data together with the information from the ^{13}C NMR spectrum led to the hypothesize of the presence of a $\Delta^{1,6,11}$ nerolidol sesquiterpene bonded to a glucose unit at C-5. The presence of 21 carbon signals in the ^{13}C NMR and ESI mass spectrum indicated a high degree of symmetry in the molecule. The mass spectrum also showed fragments at *m/z* 438 $[M-C_{21}H_{35}O_7+Na]^+$ and 404 $[M-C_{21}H_{35}O_8-H_2O+Na]^+$ due to the cleavage of the ether bridge, and at *m/z* 488 $[M-2\times C_6H_{11}O_5]^+$ and 472 $[M-C_6H_{11}O_5-C_6H_{11}O_6]^+$ due to the loss of both the sugar unities. In accordance with the hypothesis of a dimeric structure, the C-10 value (δ 89.8) was downshifted with respect to the known amarantholidol II.[9] This value suggested ethereal bridge among the C-10 carbons.

Amarantholidoside VII NMR data overlapped with those of the previously described compound, except slight differences for C-6–C-14 carbon values (Table 3). Unfortunately, these differences could be due to a different configuration for the C-10 and C-10′ carbons. The isolated quantities of these compounds did not allow further spectroscopic investigations and we were not able to define the configurations at C-10 and C-10′ carbons.

The amarantholidosides I–VI have been tested on the wild species *T. officinale* to define their phytotoxic role in the habitat and on the seed of *A. retroflexus* to evaluate the potential autotoxic activity; the results are reported in Figures 3 and 4, respectively.

Table 3. NMR data of amarantholidosides VI (**6**) and VII (**7**) in CD_3OD

	^1H (δ)		J (Hz)		DQ-COSY	^{13}C (δ)		DEPT	HMBC (H→C)	HSQC–TOCSY (H→C)
	6	7	6	7		6	7			
1t/1′t	5.23 dd	5.23 dd	17.1, 1.5	17.4, 1.2	1c/1′c, 2/2′,	112.0	112.0	CH_2	2/2′, 3/3′	1/1′, 2/2′
1c/1′c	5.01 dd	5.01 dd	9.0, 1.5	10.8, 1.2	1t/1′t, 2/2′				2/2′, 3/3′	1/1′, 2/2′
2/2′	5.96 dd	5.96 dd	11.1, 1.2	17.4, 10.8	1c, 1t	146.4	146.4	CH	1/1′, 3/3′	1/1′, 2/2′
3/3′	—	—	—	—	—	74.0	74.0	C	—	—
4/4′	1.96 dd	1.96 dd	14.1, 7.8	14.7, 8.1	5/5′	48.1	48.1	CH_2	2/2′, 3/3′, 5/5′, 6/6′, 15/15′	4/4′, 5/5′, 6/6′
	1.65 ov	1.65 ov							2/2′, 3/3′, 5/5′, 6/6′, 15/15′	
5/5′	4.90 ov	4.90 ov	—	—	4/4′, 6/6′	71.3	71.3	CH	glu1/1′, 3/3′, 4/4′, 6/6′	4/4′, 5/5′, 6/6′
6/6′	5.11 dd	5.13 dd	9.6, 1.2	9.0, 0.9	5/5′	126.6	126.8	CH	4/4′, 5/5′, 7/7′, 8/8′, 14/14′	4/4′, 5/5′, 6/6′
7/7′	—	—	—	—	—	141.1	141.8	C	—	—
8/8′	2.06 t	2.06 t	7.5	7.5	9/9′	36.7	36.5	CH_2	6/6′, 7/7′, 9/9′, 10/10′, 14/14′	8/8′, 9/9′, 10/10′
9/9′	1.57 dd	1.61 dd	6.6, 1.8	6.6, 1.7	8/8′, 10/10′	30.1	30.1	CH_2	7/7′, 8/8′, 10/10′, 11/11′	8/8′, 9/9′, 10/10′
	1.52 dd	1.58 dd	6.6, 1.2	6.6, 1.2	8/8′, 10/10′				7/7′, 8/8′, 10/10′, 11/11′	
10/10′	4.22 t	4.22 t	6.6	6.3	9/9′	89.8	89.6	CH	8/8′, 9/9′, 11/11′, 12/12′, 13/13′	8/8′, 9/9′, 10/10′
11/11′	—	—	—	—	—	145.5	145.9	C	—	—
12/12′	4.94 s	4.94 s	—	—	—	114.2	114.1	CH_2	10/10′, 11/11′, 13/13′	—
13/13′	1.72 s	1.73 s	—	—	—	17.1	17.1	CH_3	10/10′, 11/11′, 12/12′	—
14/14′	1.67 s	1.68 s	—	—	6/6′	16.7	16.7	CH_3	6/6′, 7/7′, 8/8′	—
15/15′	1.28 s	1.28 s	—	—	—	28.6	28.6	CH_3	2/2′, 3/3′, 4/4′	—
glu1/1′	4.23 d	4.24 d	7.8	7.5	glu2/2′	100.0	100.1	CH	5/5′, 10/10′, glu2/2′	glu1, glu2, glu3, glu4, glu6
glu2/2′	3.15 dd	3.15 dd	8.9, 7.8	8.9, 7.8	glu1/1′, glu3/3′	78.1	78.1	CH	5/5′, glu1/1′, glu3/3′, glu4/4′	glu1, glu2, glu3, glu4, glu6
glu3/3′	3.30 m	3.30 m	—	—	glu2/2′, glu4/4′	75.1	75.1	CH	glu1/1′, glu2/2′, glu4/4′	glu1, glu2, glu3, glu4, glu6
glu4/4′	3.29 ov	3.29 ov	—	—	glu3/3′, glu5/5′	71.9	71.9	CH	glu5/5′, glu6/6′	glu2, glu3, glu4, glu6
glu5/5′	3.26 m	3.26 m	—	—	glu4/4′, glu6/6′	78.1	78.1	CH	glu6/6′	glu2, glu3, glu4, glu6
glu6/6′	3.85 dd	3.86 dd	11.7, 2.1	12.3, 2.1	glu5/5′, glu6/6′	62.9	62.9	CH_2	glu4/4′, glu5/5′	glu3, glu4, glu6
	3.66 dd	3.66 dd	11.7, 5.7	12.3, 6.3	glu5/5′, glu6/6′				glu4/4′, glu5/5′	glu3, glu4, glu6

d=doublet; dd=double doublet; m=multiplet; ov=overlapped; s=singlet; t=triplet.

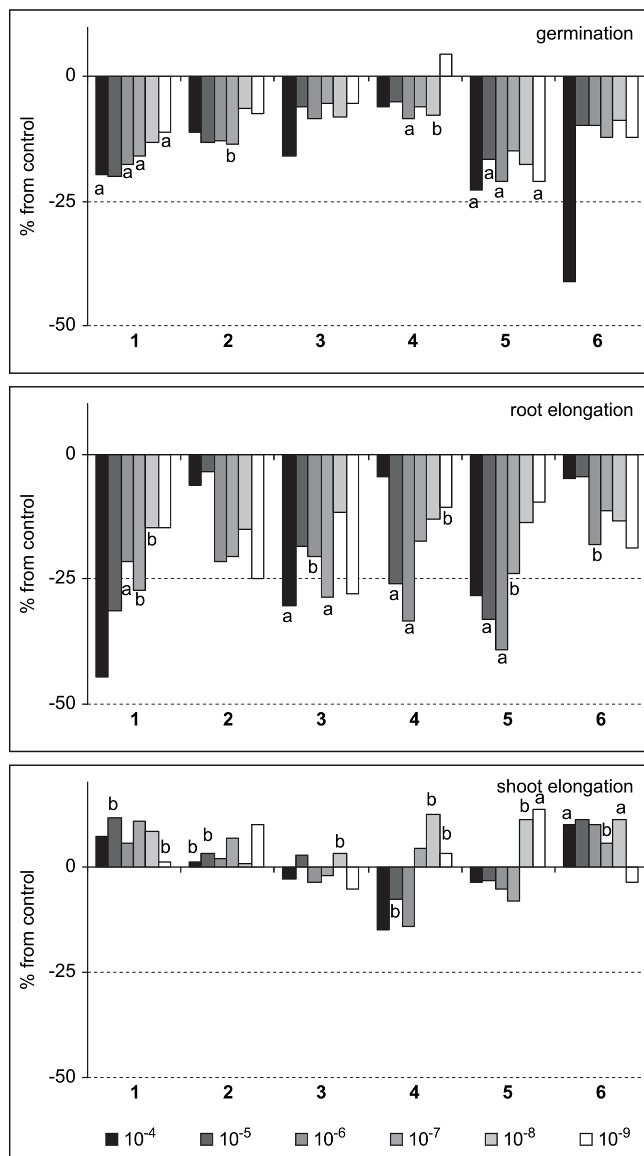

Figure 3. Bioactivity of compounds **1–6** on the germination, root elongation, and shoot elongation of *Taraxacum officinale*. Values are presented as percentage differences from control and they are significantly different with $P>0.05$ for Student's *t*-test: (a) $P<0.01$; (b) $0.01<P<0.05$. A positive percentage represents stimulation while negative values represent inhibitions.

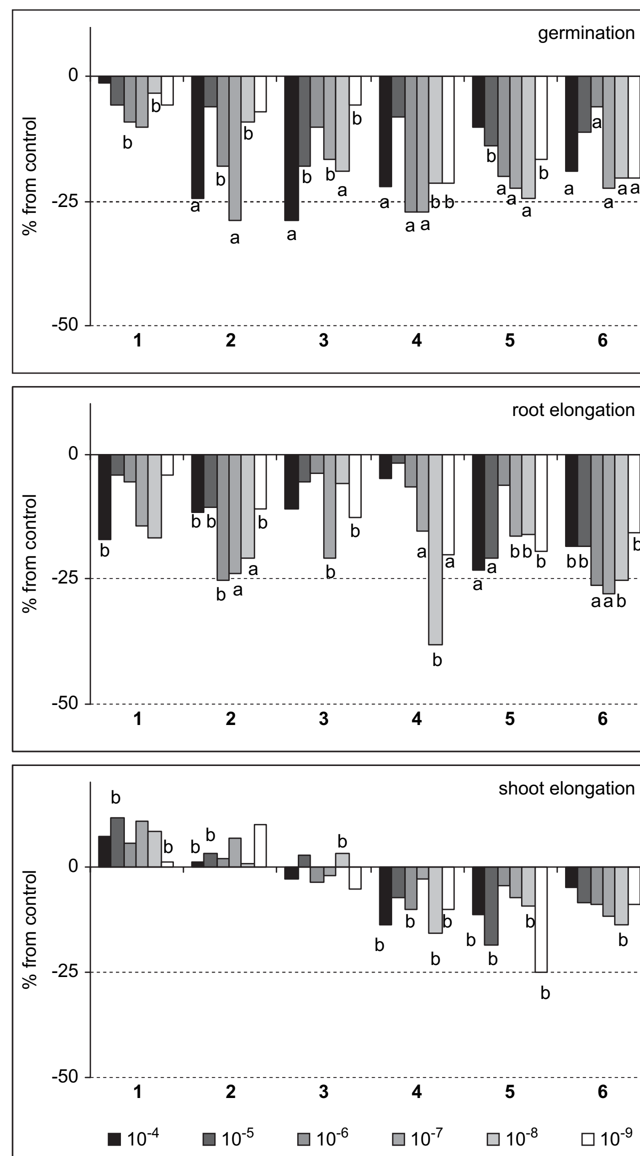

Figure 4. Bioactivity of compounds **1–6** on the germination, root elongation, and shoot elongation of *Amaranthus retroflexus*. Values are presented as percentage differences from control and they are significantly different with $P>0.05$ for Student's *t*-test: (a) $P<0.01$; (b) $0.01<P<0.05$. A positive percentage represents stimulation while negative values represent inhibitions.

On *T. officinale*, the greatest effects were registered on the root elongation. Compound **1** was more active: the chemical inhibited the root development of about 50% at the highest concentration tested. The effects on the germination were modest and with exception of compound **1** it seemed independent from the concentration. Furthermore, the autotoxicity, evident in both germination and root elongation (Fig. 4), was moderate. The results showed slow stimulating and/or inhibiting effects on shoot elongation of the test species.

To the best of our knowledge, this is the first report that describes the isolation of dimeric nerolidol derivatives from natural sources. The presence of secondary and tertiary chiral alcohols on aglicone moiety led us to use two different methods to assign the absolute configuration.

3. Experimental

3.1. General procedures

NMR spectra were recorded at 300 MHz for ^{1}H and 75 MHz for ^{13}C on a Varian 300 spectrometer Fourier transform NMR in CD_3OD at 25 °C. Proton-detected heteronuclear correlations were measured using a gradient heteronuclear single-quantum coherence (HSQC), optimized for $^{1}J_{HC}=$ 140 Hz, a gradient heteronuclear multiple bond coherence (HMBC), optimized for $^{n}J_{HC}=8$ Hz, and a gradient heteronuclear single-quantum coherence–total correlation spectroscopy (HSQC–TOCSY), optimized for $^{n}J_{HC}=8$ Hz and for a mixing time=0.09. Optical rotations were measured on a Perkin–Elmer 141 in MeOH solution. Electrospray mass spectra were recorded using a Waters Z-Q mass spectrometer equipped with an electrospray ionization (ESI) probe

operating in positive or negative ion mode. The scan range was 80–2000 *m/z*.

The HPLC apparatus consisted of a pump (Shimadzu LC-10AD), a refractive index detector (Shimadzu RID-10A), and a Shimadzu Chromatopac C-R6A recorder. Preparative HPLC was performed using NH_2 (Luna 10 µm, 250×10 mm i.d., Phenomenex) column. Analytical HPLC was performed using RP-8 (Luna 5 µm, 250×4.6 mm i.d., Phenomenex) or Polar-RP-80A (Synergi 4 µm, 250×4.6 mm i.d., Phenomenex) columns. Analytical TLC was performed on Merck Kieselgel 60 F_{254}, RP-18 F_{254}, or RP-8 F_{254} plates with 0.2 mm layer thickness. Spots were visualized by UV light or by spraying with H_2SO_4–AcOH–H_2O (1:20:4). The plates were then heated for 5 min at 110 °C. Preparative TLC was performed on Merck Kieselgel 60 F_{254} plates, with 0.5 or 1 mm film thickness. Flash column chromatography (FCC) was performed on Merck Kieselgel 60 (230–400 mesh) at medium pressure. Column chromatography (CC) was performed on Merck Kieselgel 60 (70–240 mesh). Filtrations on solid phase extraction (SPE) cartridge were performed on Waters NH_2 or C8 Sep-Pak Cartridge.

3.2. Plant material, extraction, and isolation of the metabolites

Plants of *A. retroflexus* L. were collected in May 2003, in the vegetative state, in Recale, near Caserta (Italy), and identified by Dr. Assunta Esposito of the Second University of Naples. A voucher specimen (CE118) has been deposited at the Herbarium of the Dipartimento di Scienze della Vita of Second University of Naples.

Fresh leaves of *A. retroflexus* (42 kg) were extracted with MeOH–H_2O (1:9) for 48 h at 20 °C and then in MeOH for 5 days. The methanolic extract was dissolved in water and then extracted with EtOAc. The organic fraction was dried with Na_2SO_4 and concentrated under vacuum, yielding 18.4 g of crude residue.

The EtOAc fraction was chromatographed on silica gel, with $CHCl_3$–EtOAc and $CHCl_3$–MeOH solutions, to give four fractions A–D.

Fractions A and B, eluted with EtOAC–$CHCl_3$ (1:9), give the free nerolidols previously described. Fraction C eluted with MeOH–$CHCl_3$ (1:9) was chromatographed on Sephadex LH-20 eluting with hexane–$CHCl_3$–MeOH (2:1:1) to obtain three fractions: the first, rechromatographed on RP-18 column, eluting with MeOH–MeCN–H_2O (2:1:2), furnished pure **3** (9.0 mg); the second fraction was chromatographed by NH2 HPLC eluting with MeCN–H_2O (9:1), which was purified by RP-8 HPLC, eluting with H_2O–MeOH–MeCN (13:3:4) to give pure **1** (3.0 mg) and **2** (2.0 mg); the third fraction was chromatographed on RP-18 column eluting with H_2O–MeOH–MeCN (2:1:2) to have a fraction that was first filtered on NH_2 Sep-Pak with H_2O–MeCN (2:23) and then purified by analytical RP-8 HPLC, eluting with H_2O–MeOH–MeCN (13:3:4) to give pure glucosides **6** (6.0 mg) and **7** (2.0 mg). Fraction D eluted with MeOH–$CHCl_3$ (1:1) was chromatographed on Sephadex LH-20 eluting with hexane–$CHCl_3$–MeOH (1:1:1), collecting fractions of 25 ml volumes. The second fraction was chromatographed on RP-18 column eluting with H_2O–MeOH–MeCN (5:3:2) to have pure glucoside **4** (30.1 mg) and a mixture, which was chromatographed by FCC on SiO_2, eluting with $CHCl_3$–MeOH–0.1% TFA–H_2O (100:20:3:1). The eluate collected from 50 to 90 ml was purified on RP HPLC, using the Polar-RP-80A column and eluting with H_2O–MeOH–MeCN (15:1:4) to have pure **5** (3.0 mg).

3.3. Characterization of the metabolites 4–7

Amarantholidoside IV [(3*S*,6*E*,10*R*)-10-β-D-glucopyranosyloxy-3,11-dihydroxy-3,7,11-trimethyldodeca-1,6-diene] **4**. Colorless oil; IR ($CHCl_3$) ν_{max} cm^{-1}: 3400, 2932, 1577; ^1H NMR (300 MHz, CD_3OD): Table 1; ^{13}C NMR (75 MHz, CD_3OD): Table 1; ESIMS *m/z* 441 $[M+Na]^+$, 279 $[M-glc+Na]^+$, 203 $[glc+Na]^+$; $[\alpha]_D^{25}$ −12.2 (*c* 0.16, MeOH). Anal. Calcd for $C_{21}H_{38}O_8$: C, 60.27; H, 9.15. Found: C, 60.31; H, 9.17.

Amarantholidoside V [(3*S*,6*E*,10*R*)-11-β-D-glucopyranosyloxy-3,10-dihydroxy-3,7,11-trimethyldodeca-1,6-diene] **5**. Colorless oil; IR ($CHCl_3$) ν_{max} cm^{-1}: 3389, 2921; ^1H NMR (300 MHz, CD_3OD): Table 2; ^{13}C NMR (75 MHz, CD_3OD): Table 2; ESIMS *m/z* 441 $[M+Na]^+$, 279 $[M-glc+Na]^+$; $[\alpha]_D^{25}$ −14.6 (*c* 0.12, MeOH). Anal. Calcd for $C_{21}H_{38}O_8$: C, 60.27; H, 9.15. Found: C, 60.30; H, 9.18.

Amarantholidoside VI [(3*S*,3′*S*,5*S*,5′*S*,6*E*,6′*E*,10ξ,10′ξ)-10,10′-oxybis(5-β-D-glucopyranosyloxy-3-hydroxy-3,7,11-trimethyldodeca-1,6,11-triene] **6**. Colorless oil; IR ($CHCl_3$) ν_{max} cm^{-1}: 3397, 2932; ^1H NMR (300 MHz, CD_3OD): Table 3; ^{13}C NMR (75 MHz, CD_3OD): Table 3; ESIMS *m/z* 837 $[M+Na]^+$, 488 $[M-2\times C_6H_{11}O_5]^+$, 472 $[M-C_6H_{11}O_5-C_6H_{11}O_6]^+$, 438 $[M-C_{21}H_{35}O_7+Na]^+$, 404 $[M-C_{21}H_{35}O_8-H_2O+Na]^+$, 354 $[C_{16}H_{27}O_7+Na]^+$; $[\alpha]_D^{25}$ −26.4 (*c* 0.26, MeOH). Anal. Calcd for $C_{42}H_{70}O_{15}$: C, 60.30; H, 8.66. Found: C, 60.32; H, 8.69.

Amarantholidoside VII [(3*S*,3′*S*,5*S*,5′*S*,6*E*,6′*E*,10ξ,10′ξ)-10,10′-oxybis(5-β-D-glucopyranosyloxy-3-hydroxy-3,7,11-trimethyldodeca-1,6,11-triene] **7**. Colorless oil; IR ($CHCl_3$) ν_{max} cm^{-1}: 3394, 2931; ^1H NMR (300 MHz, CD_3OD): Table 3; ^{13}C NMR (75 MHz, CD_3OD): Table 3; ESIMS *m/z* 837 $[M+Na]^+$, 488 $[M-2\times C_6H_{11}O_5]^+$, 472 $[M-C_6H_{11}O_5-C_6H_{11}O_6]^+$, 438 $[M-C_{21}H_{35}O_7+Na]^+$, 404 $[M-C_{21}H_{35}O_8-H_2O+Na]^+$, 354 $[C_{16}H_{27}O_7+Na]^+$; $[\alpha]_D^{25}$ −43.3 (*c* 0.14, MeOH). Anal. Calcd for $C_{42}H_{70}O_{15}$: C, 60.30; H, 8.66. Found: C, 60.28; H, 8.29.

3.3.1. Enzymatic hydrolysis of amarantholidol IV. To a solution of pure amarantholidoside IV (10 mg) in acetate buffer (0.5 M, pH 5.0, 5 ml), 40 mg of β-glucosidase (Sigma) was added. After 24 h at 37 °C in stirring, the mixture was extracted with EtOAc (5 ml×2), dried over Na_2SO_4, and evaporated in vacuo. The crude extract was chromatographed on SiO_2 [hexane–$CHCl_3$–MeOH (3:6:1)] to have pure aglycone (3*S*,6*E*,10*R*)-3,10,11-trihydroxy-3,7,11-trimethyldodeca-1,6-diene (**4a**): ^1H NMR spectral data (300 MHz, CD_3OD): δ 5.91 (1H, dd, *J*=17.1, 10.5 Hz, H-2), 5.18 (1H, dd, *J*=17.1, 1.5 Hz, H-1 cis), 5.19 (1H, dd, *J*=9.3, 1.2 Hz, H-6), 5.02 (1H, dd, *J*=10.5, 1.5 Hz, H-1 trans), 3.22 (1H, dd, *J*=10.8, 1.8 Hz, H-10), 1.61 (3H, s, H-14), 1.24 (3H, s, H-15), 1.15 (3H, s, H-12), 1.12 (3H, s, H-13); ^{13}C NMR (75 MHz, CD_3OD) δ 145.0 (CH, C-2), 136.0 (C, C-7), 125.8 (CH, C-6),

112.0 (CH_2, C-1), 78.9 (CH, C-10), 73.8 (C, C-11), 74.8 (C, C-3), 43.4 (CH_2, C-4), 37.8 (CH_2, C-8), 30.7 (CH_2, C-9), 27.5 (CH_3, C-15), 26.2 (CH_3, C-13), 23.9 (CH_2, C-5), 23.7 (CH_2, C-12), 16.5 (CH_3, C-14); EIMS *m/z* 256 $[M]^+$, 238 $[M-H_2O]^+$, 223 $[M-CH_3-H_2O]^+$; $[\alpha]_D^{25}$ −14.6 (*c* 0.12, MeOH); $[\alpha]_D^{25}$ +7.8 (*c* 0.10, MeOH). Anal. Calcd for $C_{15}H_{28}O_3$: C, 70.27; H, 11.01. Found: C, 70.61; H, 10.96.

3.3.2. Preparation of (*S*)- and (*R*)-MTPA esters of 4a. (*R*)-(−)-MTPA chloride (5 μl, 26 μmol) was added to a solution of pure compound (1.5 mg) in dry pyridine (50 μl). After 6 h under magnetic stirring at room temperature, EtOAc (5 ml) and H_2O (5 ml) were added to the reaction mixture. The organic layer, separated by centrifugation at 4000 rpm for 10 min, gave a crude extract, which was purified by preparative TLC eluting with hexane–$CHCl_3$–MeOH (5:4:1).

The (*S*)-MTPA ester of **4a** had the ^1H NMR spectral data (300 MHz, CD_3OD): δ 5.90 (1H, dd, J=17.3, 10.5 Hz, H-2), 5.12 (1H, dd, J=17.3, 1.8 Hz, H-1 trans), 4.99 (H-10, detected by DQ-COSY spectrum), 4.92 (H-1 cis, detected by DQ-COSY spectrum), 1.59 (3H, s, H-14), 1.51 (H-9, detected by DQ-COSY spectrum), 1.24 (3H, s, H-15), 1.19 (3H, s, H-12), 1.15 (3H, s, H-13). The (*R*)-MTPA ester of **4a** had the ^1H NMR spectral data (300 MHz, CD_3OD): δ 5.89 (1H, dd, J=17.1, 10.5 Hz, H-2), 5.18 (1H, dd, J=17.1, 1.2 Hz, H-1 trans), 5.05 (H-1 cis, detected by DQ-COSY spectrum), 5.00 (H-10, detected by DQ-COSY spectrum), 1.76 (3H, s, H-14), 1.58 (H-9, detected by DQ-COSY spectrum), 1.24 (3H, s, H-15), 1.11 (3H, s, H-12), 1.07 (3H, s, H-13).

3.3.3. ^{13}C NMR of amarantholidol IV in the chiral solvent *R,R*- and *S,S*-BMBA-*p*. Chiral NMR solvent (*R*,*R*)-BMBA-*p* [or (*S*,*S*)-BMBA-*p*] was prepared with small modifications of method reported by Kobayashi et al.[13] 1,3-Diiodopropane (10.3 mmol, 1 equiv) was added dropwise over 7 min to (*R*)-α-phenetylamine [or (*S*)-α-phenetylamine] (41.3 mmol, 4 equiv) at 130 °C. After stirring for 30 min, the mixture was cooled to 80 °C and then poured into aqueous 50% NaOH solution (300 ml). The resulting free amines were extracted with EtOAc (300 ml) and the organic layer was washed with brine, dried over anhydrous K_2CO_3, filtered, and evaporated to give the crude oil. The pure BMBA-*p* was obtained by distillation as reported by Hulst et al.[14] (*R*,*R*)-BMBA-*p* (colorless oil) had ^1H NMR spectral data (300 MHz, $CDCl_3$): δ 7.40–7.20 (5H, overlapped), 3.71 (2H, q, J=6.6 Hz), 2.54 (2H, dt, J=6.6, 5.1 Hz), 2.47 (2H, dt, J=6.6, 5.1 Hz), 1.62 (2H, m), 1.34 (6H d, J=6.6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.3 (C), 128.3 (CH), 126.6 (CH), 126.4 (CH), 58.3 (CH), 46.3 (CH_2), 29.9 (CH_2), 24.1 (CH_3); $[\alpha]_D^{20}$ +66.2 (*c* 0.25, $CHCl_3$). (*S*,*S*)-BMBA-*p* (colorless oil) had ^1H NMR spectral data (300 MHz, $CDCl_3$): δ 7.40–7.20 (5H, overlapped), 3.71 (2H, q, J=6.6 Hz), 2.54 (2H, dt, J=6.6, 5.1 Hz), 2.47 (2H, dt, J=6.6, 5.1 Hz), 1.62 (2H, m), 1.34 (6H, d, J=6.6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.3 (C), 128.3 (CH), 126.6 (CH), 126.4 (CH), 58.3 (CH), 46.3 (CH_2), 29.9 (CH_2), 24.1 (CH_3); $[\alpha]_D^{20}$ −66.2 (*c* 0.25, $CHCl_3$).

A Varian Mercury 300 spectrometer (75 MHz) was used to collect the ^{13}C NMR data of amarantholidoside IV (**4**, 10 mg) in the chiral solvent BMBA-*p* (350 μl)–CD_3OD (300 μl), with readout of NMR spectra being adjusted to 0.001 ppm/point (sw=23980.8, fn=524288).

3.4. Phytotoxicity test

Seeds of *T. officinale* and *A. retroflexus*, collected during 2005, were obtained from Herbiseed (Twyford, UK).

All undersized or damaged seeds were discarded and the assay seeds were selected for uniformity.

The test solution (10^{-4} M) was prepared using 2-[*N*-morpholino]ethanesulfonic acid (MES; 10 mM, pH 6) and the rest (10^{-5}–10^{-9} M) was obtained by dilution. Parallel controls were performed. After adding 10 seeds and 1.0 ml of test solutions, Petri dishes were sealed with Parafilm® to ensure closed-system models. Seeds were placed in a growth chamber KBW Binder 240 at 27 °C in the dark. Germination percentage was determined daily for 7 days (no more germination occurred after this time).

After growth, the plants were frozen at −20 °C to avoid subsequent growth until the measurement process. Data are reported as percentage differences from control. Thus, zero represents the control, positive values represent the stimulation of the parameter studied, and negative values represent inhibition.

Statistical treatment. The statistical significance of differences between groups was determined by a Student's *t*-test, calculating mean values for every parameter (germination average, shoot, and root elongation) and their population variance within a Petri dish. The level of significance was set at $P<0.05$.

References and notes

1. Duke, S. O.; Romagni, J. G.; Dayan, F. E. *Crop Prot.* **2000**, *19*, 583–589.
2. D'Abrosca, B.; Della Greca, M.; Fiorentino, A.; Monaco, P.; Natale, A.; Oriano, P.; Zarrelli, A. *Phytochemistry* **2000**, *66*, 2681–2688.
3. Macìas, F. A.; Velasco, R. F.; Castellano, D.; Galindo, J. C. G. *J. Agric. Food Chem.* **2005**, *53*, 3530–3539.
4. Jefferson, L. V.; Pennacchio, M. *J. Arid Environ.* **2003**, *55*, 275–285.
5. Turk, M. A.; Tawaha, A. M. *Crop Prot.* **2003**, *22*, 673–677.
6. Kato, T.; Saito, N.; Kashimura, K.; Shinohara, M.; Kurahashi, T.; Taniguchi, K. *J. Agric. Food Chem.* **2003**, *50*, 6307–6312.
7. Walter, M. W. *Nat. Prod. Rep.* **2002**, *19*, 278–291.
8. Vyvyan, J. R. *Tetrahedron* **2002**, *58*, 1631–1646.
9. D'Abrosca, B.; De Maria, P.; Della Greca, M.; Fiorentino, A.; Golino, A.; Izzo, A.; Monaco, P. *Tetrahedron* **2006**, *62*, 640–646.
10. Yokosuka, A.; Mimaki, Y.; Sakagami, H.; Sashida, Y. *J. Nat. Prod.* **2002**, *65*, 283–289.
11. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
12. Kobayashi, Y.; Hayashi, N.; Kishi, Y. *Org. Lett.* **2002**, *4*, 411–414.
13. Kobayashi, Y.; Hayashi, N.; Kishi, Y. *Tetrahedron Lett.* **2003**, *44*, 7489–7491.
14. Hulst, R.; de Vries, N. K.; Feringa, B. L. *Tetrahedron: Asymmetry* **1994**, *5*, 699–708.