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SEVEN ACYLATED ANTHOCYANINS IN THE BLUE FLOWERS OF *HYACINTHUS ORIENTALIS*

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Key Word Index—*Hyacinthus orientalis*; Liliaceae; blue flower; acylated anthocyanin 3,5-diglucosides; pelargonidin; cyanidin; delphinidin; petunidin; *p-trans*-coumaric acid; *p-cis*-coumaric acid; caffeic acid; malonic acid; ^{1}H and ^{13}C NMR; FAB-MS.

Abstract—Two novel anthocyanins, delphinidin 3-*O*-(6-*O*-*cis*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside) and petunidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside), were isolated from the blue flowers of *Hyacinthus orientalis* cv. Delft Blue. Five known acylated anthocyanins, namely, delphinidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(6-*O*-β-D-glucoside), delphinidin 3-*O*-(6-*O*-*trans*-caffeoyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside), delphinidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside), cyanidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside) and pelargonidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside), were also isolated from these flowers. The complete structure of each compound was unambiguously determined by one- and two-dimensional NMR techniques.

INTRODUCTION

Hyacinthus orientalis L. is a popular ornamental plant with blue, red, pink, yellow or white flowers. The anthocyanins of the dark purple flowers of *H. orientalis* L. cv. Ivanhoe have been reported to be acylated delphinidin glycosides [1]. However, the complete structures of these anthocyanins have not been elucidated. We now report the determination of the complete structures of seven acylated anthocyanins, including two novel compounds, that are present in the blue flowers of *H. orientalis* L. cv. Delft Blue.

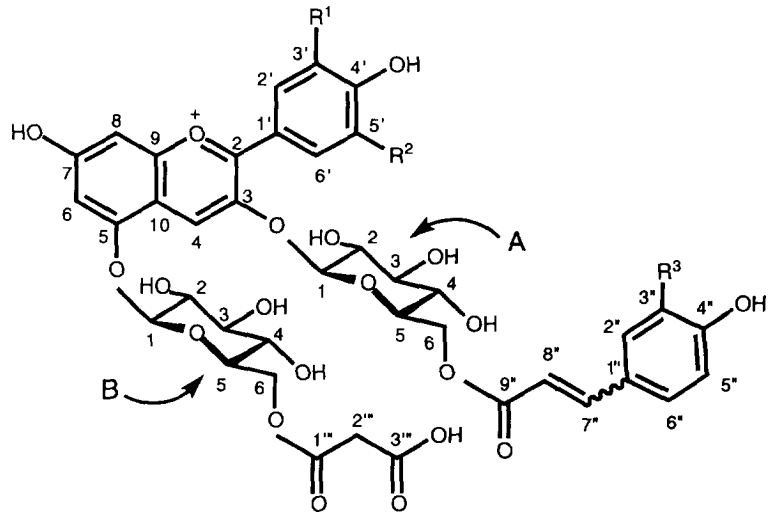
RESULTS AND DISCUSSION

Compounds **1**–**7** of blue flowers of *H. orientalis* were isolated by column chromatography on Amberlite XAD-7, with subsequent preparative HPLC. The UV–Vis and FAB-mass spectra of all seven compounds are shown in Table 1. In the UV–Vis spectra, the ratios of $E_{440}/E_{vis,max}$ were close to 0.10 for **1**–**6**, indicating the presence of 3,5-diglucosides [2]. The ratio for **7** was 0.19 and no significant shift upon addition of $AlCl_3$ was observed, an indication of the presence of pelargonidin 3,5-diglucosides [2]. All seven compounds exhibited characteristic absorbance peaks in their UV spectra between 301 and 328 nm, which suggested acylation with hydroxycinnamic acids [2].

Compound **4** was obtained as a major component. The FAB-mass spectrum included its molecular ion at *m/z* 859, in good agreement with the mass calculated for $C_{39}H_{39}O_{22}$. Fragment peaks were also observed at *m/z* 611 [M − 248(malonylhexose)]$^+$, 551 [M − 308 (coumaroylhexose)]$^+$ and 303 [aglycone]$^+$, indicating that **4** was composed of delphinidin, malonylhexose and coumaroylhexose.

Analysis of the ^{1}H NMR spectrum of **4** revealed the presence of delphinidin, two glucose residues, malonic acid and *p*-coumaric acid (Table 2). In the *p*-coumaric acid moiety, the 7″- and 8″-protons had a large coupling constant (J = 16 Hz). Therefore, the olefinic part of the *p*-coumaric acid moiety was deduced to have the *trans* configuration. The signals from glucose moieties were observed in the region of δ3.45–5.50 and all the vicinal coupling constants of two glucose moieties were at 8.1–9.0 Hz. The chemical shifts of two anomeric protons with large coupling constants were δ5.50 (*d*, J = 8.1 Hz, glucose A) and 5.18 (*d*, J = 8.1 Hz, glucose B). Therefore, both glucose units must be β-D-glucopyranoside. By analysis of the proton network of the glucose moieties, the anomeric protons (δ5.50 and 5.18) of glucoses A and B were finally correlated to non-equivalent methylene protons of C-6 at δ4.41 and 4.52 and at δ4.21 and 4.53, respectively. The downfield shift of these methylene signals indicated that the coumaroyl/malonyl moieties were attached to the 6-OH of both glucoses.

In order to confirm the position of the ester linkage, a heteronuclear multiple-bond correlation (HMBC) spectrum was generated. A correlation between H-6 (δ4.41



	R1	R2	R3	7"/8"
2	OH	OH	OH	*trans*
3	OH	OH	H	*cis*
4	OH	OH	H	*trans*
5	OMe	OH	H	*trans*
6	OH	H	H	*trans*
7	H	H	H	*trans*

Table 1. Spectral properties of anthocyanins from blue flowers of *Hyacinthus orientalis* L. cv. Delft Blue

	UV–Vis (0.1% HCl–MeOH)					FAB-mass spectra
Anthocyanin	$\lambda_{vis,max}$ (nm)	$\lambda_{acyl,max}$ (nm)	$E_{acyl,max}/E_{vis,max}$	$E_{440}/E_{vis,max}$	$AlCl_3$ shift	$[M]^+$ & fragment ions
1	541	282	0.70	0.10	+	773 $[M]^+$, 611, 465, 303
		309	0.76			
2	539	281	0.91	0.11	+	875 $[M]^+$, 627, 551, 303
		304	0.95			
		328	1.00			
3	544	280	0.59	0.10	+	859 $[M]^+$, 551, 303
		305	0.53			
4	541	280	0.60	0.09	+	859 $[M]^+$, 611, 551, 303
		308	0.58			
5	537	281	0.71	0.11	+	873 $[M]^+$, 625, 565, 317
		301	0.70			
6	528	281	0.64	0.11	+	843 $[M]^+$, 595, 535, 287
		296	0.64			
		314	0.56			
7	509	288	0.66	0.19	–	827 $[M]^+$, 579, 519, 271
		316	0.55			

Table 2. ^{1}H NMR spectra of anthocyanins from *Hyacinthus* (in CD_3OD containing 10% CF_3COOD)

	1	2	3	4	5	6	7
Aglycone							
4	8.81 *s*	8.86 *s*	8.52 *s*	8.78 *s*	8.92 *s*	8.89 *s*	8.98 *s*
6	6.94 *d* 1.5	6.97 *s*	6.83 *d* 1.2	6.92 *d* 1.8	6.99 *d* 1.8	6.97 *d* 1.8	6.99 *d* 1.8
8	6.80 *d* 1.5	6.92 *s*	6.72 *d* 1.2	6.82 *d* 1.8	6.95 *d* 1.8	6.92 *d* 1.8	7.00 *d* 1.8
2′	7.63 *s*	7.76 *s*	7.67 *s*	7.64 *s*	7.93 *d* 2.2	7.98 *d* 2.3	8.58 *d* 9.1
3′							7.05 *d* 9.1
5′						6.99 *d* 8.8	7.05 *d* 9.1
6′	7.63 *s*	7.76 *s*	7.67 *s*	7.64 *s*	7.77 *d* 2.2	8.21 *dd* 8.8, 2.3	8.58 *d* 9.1
3′-OMe					3.98 *s*		
Glucose A							
1	5.49 *d* 7.8	5.41 *d* 8.0	5.46 *d* 7.9	5.50 *d* 8.1	5.46 *d* 8.0	5.45 *d* 7.8	5.41 *d* 8.1
2	3.80 *dd* 9.2, 7.8	3.78 *dd* 9.0, 8.0	3.81 *dd* 9.3, 7.9	3.80 *dd* 8.6, 8.1	3.76 *dd* 9.5, 8.0	3.76 *dd* 9.2, 7.8	3.72 *dd* 8.9, 8.1
3	3.65 *t* 9.2	3.60 *t* 9.0	3.62 *t* 9.3	3.69 *t* 8.6	3.61 *t* 9.5	3.62 *t* 9.2	3.59 *t* 8.9
4	3.54 *t* 9.2	3.49 *t* 9.0	3.45 *t* 9.3	3.48 *t* 8.6	3.48 *t* 9.5	3.49 *t* 9.2	3.48 *t* 8.9
5	3.93 *ddd* 9.2, 7.1, 2.7	3.94 *ddd* 9.0, 7.8, 2.7	3.95 *ddd* 9.7, 9.3, 2.2	3.94–3.97 *m*	3.94–3.97 *m*	3.93–3.95 *m*	3.90–3.96 *m*
6	4.45 *dd* 12, 7.1	4.42 *dd* 12, 7.8	4.67 *d* 12, 9.7	4.41 *dd* 12, 8.3	4.45–4.48 *m*	4.43 *dd* 12, 7.9	4.43 *d* 12, 7.7
	4.51 *dd* 12, 2.7	4.51 *dd* 12, 2.6	4.35 *d* 12, 2.2	4.52 *dd* 12, 2.7	4.45–4.48 *m*	4.51 *dd* 12, 3.0	4.51 *d* 12, 1.5
Glucose B							
1	5.16 *d* 7.9	5.16 *d* 8.0	5.21 *d* 7.9	5.18 *d* 8.1	5.19 *d* 8.0	5.19 *d* 7.7	5.19 *d* 8.1
2	3.76 *dd* 9.3, 7.9	3.75 *dd* 8.8, 8.0	3.78 *dd* 9.2, 7.9	3.78 *dd* 9.0, 8.1	3.74 *dd* 9.5, 8.0	3.78 *dd* 9.3, 7.7	3.78 *dd* 8.9, 8.1
3	3.58 *t* 9.3	3.55 *t* 8.8	3.61 *t* 9.2	3.58 *t* 9.0	3.57 *t* 9.5	3.58 *t* 9.3	3.56 *t* 8.9
4	3.45 *t* 9.3	3.45 *t* 8.8	3.51 *t* 9.2	3.45 *t* 9.0	3.46 *t* 9.5	3.46 *t* 9.3	3.46 *t* 8.9
5	3.60 *ddd* 9.3, 6.3, 2.4	3.78–3.79 *m*	3.86 *ddd* 9.2, 6.4, 2.8	3.78–3.81 *m*	3.78 *m*	3.78–3.81 *m*	3.78–3.81 *m*
6	3.73 *dd* 12, 6.3	4.23 *dd* 12, 5.9	4.53 *d* 12, 6.4	4.21 *dd* 12, 6.1	4.23 *dd* 12, 6.1	4.22 *dd* 12, 6.1	4.23 *d* 12, 6.2
	3.98 *dd* 12, 2.4	4.55 *d* 12, 2.7	4.57 *d* 12, 2.8	4.53 *dd* 12, 1.6	4.53 *dd* 12, 2.1	4.53 *dd* 12, 2.7	4.52 *d* 12
Aromatic acid moiety							
2″	7.21 *d* 8.7	6.81 *d* 1.4	7.09 *d* 8.6	7.15 *d* 8.6	7.22 *d* 8.6	7.20 *d* 8.6	7.23 *d* 8.6
3″	6.71 *d* 8.7		6.31 *d* 8.6	6.67 *d* 8.6	6.72 *d* 8.6	6.70 *d* 8.6	6.72 *d* 8.6
5″	6.71 *d* 8.7	6.68 *d* 8.3	6.31 *d* 8.6	6.67 *d* 8.6	6.72 *d* 8.6	6.70 *d* 8.6	6.72 *d* 8.6
6″	7.21 *d* 8.7	6.73 *dd* 8.3, 1.4	7.09 *d* 8.6	7.15 *d* 8.6	7.22 *d* 8.6	7.20 *d* 8.6	7.23 *d* 8.6
7″	7.32 *d* 16	7.29 *d* 16	6.37 *d* 13	7.30 *d* 16	7.36 *d* 16	7.34 *d* 16	7.37 *d* 16
8″	6.19 *d* 16	6.19 *d* 16	5.72 *d* 13	6.19 *d* 16	6.23 *d* 16	6.22 *d* 16	6.23 *d* 16
Malonic moiety							
2‴		nd*	3.34 *s*	3.39 *s*	3.41 *s*	3.41 *s*	3.41 *s*

*Not detected.

and δ4.52) of glucose A and the carbonyl carbon (δ169.2) of *trans-p*-coumaric acid was observed, indicating that *trans-p*-coumaric acid was attached to 6-OH of glucose A via an ester bond. Similarly, malonic acid appeared to be attached to the 6-OH of glucose B, as indicated by the presence of a correlation between H-6 (δ4.21 and δ4.53) of glucose B and one of the carbonyl carbons (δ168.6) of malonic acid.

The positions of the glucosidic linkage were determined by HMBC and from the nuclear Overhauser effect (NOE) difference spectra. A correlation between the anomeric proton (δ5.50) of glucose A and C-3 (δ145.9) of delphinidin was observed, indicating that glucose A was attached to the 3-OH of delphinidin. Similarly, glucose B was deduced to be attached to 5-OH of delphinidin, from the presence of a correlation between the anomeric

Table 3. ^{13}C NMR spectra of anthocyanins from *Hyacinthus* (in CD_3OD containing 10% CF_3COOD)

	1	2	3	4	5	6	7
Aglycone							
2	164.3	164.8	163.5	164.1	164.7	164.7	165.5
3	145.9	146.1	146.0	145.9	146.2	145.9	145.9
4	134.7	134.3	132.3	133.9	134.9	134.9	136.0
5	156.7	156.5	156.1	156.4	156.6	156.5	156.7
6	106.2	106.2	104.8	106.2	106.2	106.2	106.3
7	169.7	169.5	169.0	169.5	169.6	169.6	169.9
8	97.5	97.3	96.8	97.3	97.4	97.4	97.5
9	156.8	156.9	156.4	156.7	157.1	156.9	157.3
10	113.1	113.1	112.8	113.0	113.3	113.2	113.5
1′	119.7	119.9	119.8	119.7	119.8	121.0	120.7
2′	112.9	113.0	112.9	112.9	109.6	118.6	136.2
3′	147.5	147.7	147.6	147.6	149.9	147.6	118.1
4′	145.5	145.7	145.6	145.7	146.3	156.7	167.4
5′	147.5	147.7	147.6	147.6	147.7	117.6	118.1
6′	112.9	113.0	112.9	112.9	114.2	128.9	136.2
-OMe					57.2		
Glucose A							
1	102.6	102.4	101.2	102.1	102.9	102.4	102.8
2	74.5	74.4	74.2	74.3	74.6	74.4	74.4
3	78.1	78.1	78.2	78.2	78.3	78.2	78.2
4	72.0	72.2	72.3	72.4	72.4	72.3	72.2
5	75.8	76.0	75.9	75.5	75.6	75.6	75.6
6	64.3	64.5	64.3	64.6	64.5	64.6	64.5
Glucose B							
1	102.7	102.9	101.5	102.9	102.6	102.9	102.9
2	74.9	74.8	74.7	74.8	74.8	74.8	74.8
3	78.0	77.8	77.7	77.8	77.7	77.8	77.7
4	71.4	71.0	71.4	71.0	71.0	71.0	71.0
5	78.9	75.5	75.9	76.0	76.0	76.0	76.0
6	62.7	65.1	65.2	65.2	65.2	65.2	65.2
Aromatic acid moiety							
1″	126.9	127.4	127.0	126.8	126.8	126.8	126.8
2″	131.4	115.9	133.3	131.4	131.4	131.4	131.4
3″	116.9	146.6	115.5	116.7	116.8	116.8	116.8
4″	161.2	149.5	159.5	161.2	161.3	161.3	161.3
5″	116.9	116.4	115.5	116.7	116.8	116.8	116.8
6″	131.4	122.8	133.3	131.4	131.4	131.4	131.4
7″	147.0	147.4	143.9	147.0	147.0	147.0	147.1
8″	114.7	114.8	115.8	114.8	114.7	114.8	114.8
9″	169.2	169.3	168.9	169.2	169.2	169.2	
Malonic moiety							
1‴		nd*	168.6	168.6	168.2	168.5	168.6
2‴		nd	nd	41.9	nd	41.9	nd
3‴		nd	170.6	170.6	nd	170.7	170.5

*Not detected.

proton (δ5.18) of glucose B and C-5 (δ156.4) of delphinidin. These conclusions were confirmed by NOE experiments, in which negative NOEs were observed for H-4 (δ8.78) and H-6 (δ6.92) of delphinidin upon irradiation of anomeric protons of glucose A and B, respectively. Thus, **4** is delphinidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside).

Compounds **1**, **2**, **3**, **5**, **6** and **7** were obtained from flowers as minor components. Compound **1** yielded a molecular ion at *m/z* 773 by FAB-mass spectrometry, in good agreement with the mass calculated for $C_{36}H_{37}O_{19}$. Fragment peaks were also observed at *m/z* 611 [M − 162 (glucose)]$^+$, 465 [M − 308 (coumaroylglucose)]$^+$ and 303 [delphinidin]$^+$. These data suggested that **1** corresponded to the des-malonyl derivative of **4**. The ^{1}H NMR spectrum of **1** was similar to that of **4**, apart from the signals of H-6 of glucose B. The rather high-field shift (δ3.98 and 3.73) of these protons indicated that 6-OH of glucose B was unsubstituted.

Compound **2** yielded a molecular ion at *m/z* 875 by FAB-mass spectrometry, which was 16 mass units larger than that of **4** and in good agreement with the mass calculated for $C_{39}H_{39}O_{23}$. In the aromatic region of the ^{1}H NMR spectrum of **2**, signals from a 1,2,4-trisubstituted benzene ring were found in place of the 1,4-disubstituted pattern due to *trans*-*p*-coumaric acid in **4**. These data strongly suggested the presence of caffeic acid in the molecule.

Compound **3** gave the same molecular peak as **4** at *m/z* 859 by FAB-mass spectrometry. The ^{1}H NMR spectrum of **3** was similar to that of **4**, with the exception of the signals due to the *p*-coumaric acid moiety. The coupling constant (*J*) between the α- and β-protons of **3** was 13 Hz, whereas $J_{\alpha,\beta}$ of **4** was 16 Hz. In addition, the chemical shifts of the α- and β-protons were 0.47 and 0.93 ppm higher than those of **4**, respectively. Therefore, the double bond of the *p*-coumarate moiety of **3** was deduced to have a *cis* configuration.

Compounds **5**–**7** gave molecular ions at *m/z* 873, 843 and 827 by FAM-mass spectrometry, respectively. The molecular ions were in good agreement with the masses calculated for $C_{40}H_{41}O_{22}$, $C_{39}H_{39}O_{21}$ and $C_{39}H_{39}O_{20}$, respectively. The ^{1}H NMR spectra of **5**–**7** were similar to those of **4**, apart from signals due to the B-ring of the aglycone moiety.

The molecular ion of **5** obtained by FAB-mass spectrometry was 14 mass units larger than that of **4**. In the ^{1}H NMR spectrum of the aglycone of **5**, the 1,2,3,5-tetrasubstituted pattern was found for the B-ring and two aromatic protons were non-equivalent. In the HMBC spectrum, a correlation between the methyl signal (δ3.98) and C-3′ (δ149.9) of the B-ring was observed. Thus, the aglycone of **5** is petunidin.

The ^{1}H NMR signals for the B-ring part of the aglycone of **6** revealed a 1,2,4-trisubstituted pattern. These data strongly suggested that the aglycone of **6** is cyanidin. In the case of **7**, a 1,4-disubstituted pattern for the B-ring of the aglycone was found and, hence, the aglycone is pelargonidin.

Thus, **1**–**3** and **5**–**7** are delphinidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-β-D-glucoside, delphinidin 3-*O*-(6-*O*-*trans*-caffeoyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside), delphinidin 3-*O*-(6-*O*-*cis*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside), petunidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside), cyanidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside) and pelargonidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside), respectively.

The complete assignments of the ^{1}H and ^{13}C signals, as deduced by one- and two-dimensional techniques are shown in Tables 2 and 3.

Compounds **3** and **5** are novel anthocyanins. Compound **3** contains *cis*-*p* coumaric acid as an acylglucosyl moiety. This type of anthocyanin, containing a *cis* cinnamate derivative, has only been reported to date in the purple leaves of *Perilla ocimoides* L. var. *crispa* [3]. Anthocyanidins acylated with *p*-coumaroylglucoside at the 3-OH group and with malonylglucoside at the 5-OH group have been reported previously [3, 4], but the corresponding petunidin derivative has not previously been identified.

EXPERIMENTAL

Plant material. Bulbs (*ca* 6 cm in diameter) of *H. orientalis* L. cv. Delft Blue were obtained from Kaneko Shubyo Co. (Saitama, Japan), in September 1990, and then grown in an experimental field. Fresh blue perianths were collected from March to April 1991 and freeze-dried.

Isolation of anthocyanins. Freeze-dried flowers (100 g) were extracted with a mixt. of EtOH–HOAc–H_2O (10:1:9) at 4°. The concd extract was loaded onto a column of Amberlite XAD-7 and washed with 5% HOAc. The anthocyanins were eluted by 50% MeOH that contained 5% HOAc. For further purification, the crude anthocyanins were purified by prep. HPLC on a Chromatorex-ODS (Fuji Silysia Chemical Ltd, Aichi, Japan) column at a flow rate of 5–8 ml min^{-1}, with monitoring of anthocyanins at 535 nm. Two solvent systems used for elution: 25–60% MeCN–HOAc–H_2O (5:4:11) containing 0.5% TFA and then 10–25% MeOH containing 15% HOAc and 0.5% TFA. To replace the counter anion of anthocyanins with TFA, the concd frs were adsorbed on a cartridge of activated Sep-Pak tC18 (Waters Associates, Milford, MA, U.S.A.). The cartridge was washed with 0.5% aq. TFA and then eluted with MeOH that contained 0.5% TFA. The solns of purified anthocyanins were concd to dryness under N_2. Each residue was dissolved in a small amount of 1% TFA and then freeze-dried to give, in all, 7 anthocyanins as powders (**1**, 7.8 mg; **2**, 2.0 mg; **3**, 5.6 mg; **4**, 222.0 mg; **5**, 3.1 mg; **6**, 8.8 mg **7**, 2.8 mg).

Spectral analysis. UV–visible spectra were recorded in MeOH that contained 0.1% HCl. FAB-mass spectra were obtained in a positive mode with glycerol as a matrix. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz)

spectra were obtained in 10% CF_3COOD–CD_3OD as a solvent.

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