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Phlorethols and fucophlorethols from the brown alga *Cystophora retroflexa*

Birgit Sailler, Karl-Werner Glombitza*

Institut für Pharmazeutische Biologie, Nußallee 6, 53115 Bonn, Germany

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Abstract

The ethanolic extract of the brown alga *Cystophora retroflexa* has yielded three different classes of phlorotannins. Most substances belong to the classes of phlorethols and fucophlorethols. Only one, well known fucol, the difucol hexa-acetate, was isolated. Three new phlorethols and ten new fucophlorethols are described and characterized as their acetates: tetraphlorethol-E nona-acetate, pentaphlorethol-B undeca-acetate, hexaphlorethol-A trideca-acetate, fucotriphlorethol-G dodeca-acetate, fucotriphlorethol-H dodeca-acetate, fucotetraphlorethol-J tetradeca-acetate, fucotetraphlorethol-K tetradeca-acetate, fucopentaphlorethol-E hexadeca-acetate, bisfucoheptaphlorethol-A tricosa-acetate, difucofucotriphlorethol-A octadeca-acetate, difucofucotetraphlorethol-B icosa-acetate, terfucohexaphlorethol-B tetracosa-acetate and terfucoheptaphlorethol-A hexacosa-acetate. In addition, the following sixteen known compounds were identified: phloroglucinol tri-acetate, diphlorethol penta-acetate, triphlorethol-A hepta-acetate, tetraphlorethol-C nona-acetate, difucol hexa-acetate, fucophlorethol-B octa-acetate, fucodiphlorethol-D deca-acetate, fucotriphlorethol-B dodeca-acetate, fucotetra-phlorethol-B tetradeca-acetate, bisfucotriphlorethol-A pentadeca-acetate, bisfucotetraphlorethol-A heptadeca-acetate, bisfucopentaphlorethol-A nonadeca-acetate, bisfucopentaphlorethol-B nonadeca-acetate, difucophlorethol-A undeca-acetate, difucofucotetraphlorethol-A icosa-acetate, terfucopentaphlorethol-A docosa-acetate and terfucohexaphlorethol-A tetracosa-acetate.

Keywords: *Cystophora retroflexa*; Phaeophyceae; Cystoseiraceae; Phlorotannins; Phlorethols; Fucols; Fucophlorethols; Structural elucidation

1. Introduction

Many marine brown algae contain a particular class of phenolic compounds, the phlorotannins, which can be grouped into several structure subclasses according to the structural elements they contain. Two species of the genus *Cystophora* (family: Cystoseiraceae) have been the subject of investigation previously. The phlorotannin pattern of the alga *C. torulosa* turned out to be highly complex consisting of various phlorethols, fucols, fuhalols, fucophlorethols and hydroxyfucophlorethols (Glombitza, Hauperich, & Keusgen, 1997a). For *Cystophora congesta* only five substances, phloroglucinol, three phlorethols and one fucophlorethol, have been characterized so far (Koch & Gregson, 1984).

We herein report on the isolation and characterization of thirty phlorotannins from *C. retroflexa,* seventeen of which have been characterized before. 13 compounds represent novel structures, three of which belong to the subclass of phlorethols, the remaining ten are new fucophlorethols.

* Corresponding author.

2. Results and discussion

The frozen shredded thallus was extracted with ethanol, the concentrated extract shaken with petroleum ether, chloroform and ethyl acetate. The ethyl acetate fraction contained the phenolic compounds. They were peracetylated before isolation to protect them from oxidation. First this mixture was fractionated by HPLC using a semipreparative silica gel column and afterwards each fraction was purified in several HPLC steps using an analytical column.

By means of ^{1}H NMR spectra ($CDCl_3$) and comparison with published data phloroglucinol tri-acetate (Glombitza, Rösener, Vilter, & Rauwald, 1973), diphlorethol penta-acetate (Glombitza, Rösener, & Müller, 1975), triphlorethol-A hepta-acetate (Koch & Gregson, 1984), tetraphlorethol-C nona-acetate (Koch & Gregson, 1984), difucol hexa-acetate (Glombitza, Rösener, & Koch, 1976), fucophlorethol-B octa-acetate (Glombitza, Wiedenfeld, & Eckhardt, 1978), fucodiphlorethol-D deca-acetate (**4**) (Koch & Gregson, 1984), fucotriphlorethol-B dodeca-acetate (**7**) (Glombitza, Wegner-Hambloch, & Schulten, 1985), fucotetra-phlorethol-B

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tetradeca-acetate (Glombitza & Große-Damhues, 1985), bisfucotriphlorethol-A pentadeca-acetate (Glombitza, Schnabel, & Koch, 1981), bisfucotetraphlorethol-A heptadeca-acetate (Glombitza & Große-Damhues, 1985), bisfucopentaphlorethol-A nonadeca-acetate (Glombitza, Große-Damhues, & Schulten, 1983), bisfucopentaphlorethol-B nonadeca-acetate (Glombitza, Keusgen, & Hauperich, 1997b), difucophlorethol-A undeca-acetate (**15**) (Glombitza & Große-Damhues, 1985), difucofucotetraphlorethol-A icosa-acetate (**12**) (Glombitza et al., 1997a), terfucopentaphlorethol-A docosa-acetate (**16**) (Glombitza & Li, 1991) and terfucohexaphlorethol-A tetracosa-acetate (Glombitza & Li, 1991) were identified.

For all new compounds the chemical shifts for nearly all protons could be assigned by comparing the ^{1}H NMR data with the data of known phlorethols and fucophlorethols. The data of the three phlorethols are listed in Table 1. They fit well with the published data of each ring type. The data of the ten fucophlorethols are compared with the data of the structural homologues **4**, **7**, **12**, **15**, **16** (Tables 3 and 5–7).

Phlorethols are phlorotannins containing phloroglucinol nuclei exclusively linked by aryl ether bonds. The ring types A_1, B and D are characteristic (Fig. 1).

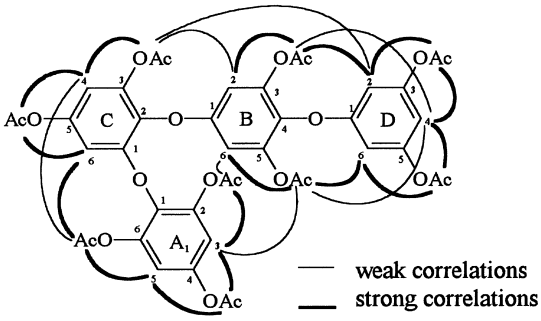

Fig. 1. Tetraphlorethol-E nona-acetate (**1**) with the correlations of the ^{1}H^{1}H ROESY NMR spectrum. The numbering of the carbons in this and all following figures is not in accordance with IUPAC-rules.

The indices used for ring type A and later on for ring type F are necessary to explain different chemical shifts caused by typical ring combinations. In addition to these ring types the ^{1}H NMR spectrum of tetraphlorethol-E nona-acetate (**1**) Fig. 1 shows an AB-system belonging to a 1,2-diphenoxylated 3,5-diacetoxybenzene (ring type C). This ring must be connected with the 1-phenoxylated 2,4,6-triacetoxybenzene (ring A_1) since the signal for the aromatic protons of the ring A_1 is obviously shifted upfield to $\delta = 6.92$ ppm. Typically these protons give a

Table 1
^{1}H NMR spectral data of **1**–**3**

	1		**2**		**3**	
H	$CDCl_3$	d_6-Me_2CO	$CDCl_3$	d_6-Me_2CO	$CDCl_3$	d_6-Me_2CO
Ring A_1						
3,5	6.92	7.00	6.95	7.04	6.95	7.05
Ac-2, Ac-6	2.06	[a]	2.07	2.10	2.13	2.11
Ac-4	2.28	2.22	2.28	2.26	2.28	2.26
Ring C						
4	6.75	6.84				
6	6.54	6.49				
Ac-3	2.20	2.13				
Ac-5	2.24	2.17				
J_{AB} (Hz)	2.7	2.8				
Ring B						
2,6	6.68	6.73	6.70	6.76	6.67	6.76
Ac-3, Ac-5	2.06	2.03	2.05	[a]	2.02	[a]
Ring D						
2,6	6.56	6.54	6.55	6.59	6.56	6.60
4	6.63	6.64	6.62	6.64	6.68	6.71
C-3, Ac-5	2.24	2.18	2.24	2.21	2.24	2.23
J_{AB} (Hz)	2.2	2.0	2.1	2.0	1.9	2.0
Ring G						
4,6			6.35	6.36	6.30	6.31
Ac-5			2.19	2.16	2.18	2.16

[a] Hidden by solvent.

Table 2
^{13}C NMR spectral data of **1** (in $CDCl_3$)

C	Measured **1**	Calculated (Wegner-Hambloch & Glombitza, 1985)
Ring A_1		
1	136.3	136.8
2,6	143.4	143.6
3,5	115.0	113.8
4	148.8	146.2
Ring B		
1	154.5	153.1
2,6	109.3	108.3
3,5	144.2	143.9
4	134.2	134.7
Ring C		
1	150.7	150.6
2	133.7	133.7
3	144.4	143.9
4	112.2	111.8
5	147.4	147.8
6	108.6	108.3
Ring D		
1	158.4	158.2
2,6	107.4	107.3
3,5	151.5	151.6
4	110.3	110.7

signal at $\delta = 6.95$ ppm if ring A_1 is connected to a 1,4-diphenoxylated benzene (ring B). The structure of **1** was proven by a $^1H^1H$ ROESY NMR spectrum showing the expected correlations Fig. 1. The four ring types of this substance (A_1, B, C, D) were further confirmed by ^{13}C NMR. The measured values match the calculated data very well (Table 2).

The FAB-mass spectrum of **1** shows the molecular ion $[M+H]^+$ at m/z 877 ($C_{42}H_{36}O_{21}$) and a ketene elimination series 877→499 (down to the free phenol), corresponding to nine acetoxy groups.

The ^1H NMR spectra of pentaphlorethol-B undeca-acetate (**2**) (Fig. 2) and hexaphlorethol-A trideca-acetate (**3**) Fig. 2 are very similar. Both spectra show the signals for a ring D, two rings of type A_1 and one ring of type G. This benzene unit is phenoxylated in position 1,2 and 3 and has been observed frequently in fucophlorethols (Glombitza et al., 1981, 1997a; Glombitza & Große-Damhues, 1985). The singlets at $\delta = 6.35$ ppm for **2** and $\delta = 6.30$ ppm for **3** each representing two protons are assigned to the aromatic protons of this ring. Both substances must therefore be symmetric. They only differ in the number of the 1,4-diphenoxylated benzene units (ring B) and their position in the molecule. Substance **2** bears a single B ring connected to C-2 of ring G. Substance **3**

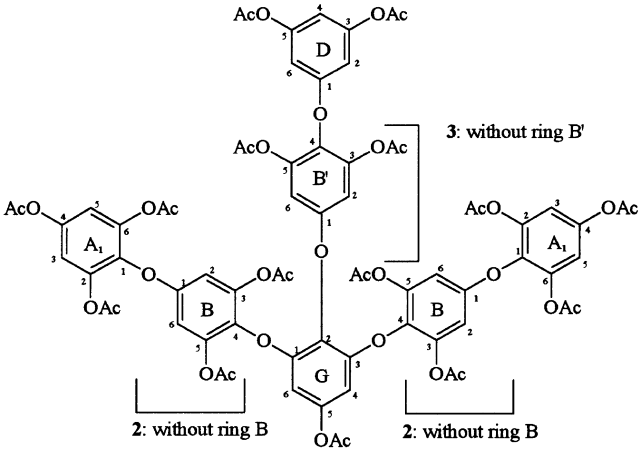

Fig. 2. Pentaphlorethol-B undeca-acetate (**2**) and hexaphlorethol-A trideca-acetate (**3**).

possesses two rings of this type. They are linked with the position 1 and 3 of the ring G. These positions are indirectly proven by the chemical shifts for the aromatic protons of the ring A. The signal at $\delta = 6.95$ ppm for **3** shows that the ring A must be connected with a 1,4-diphenoxylated benzene. The linkage of ring A and ring G in the case of **2** causes a high field shift ($\delta = 6.94$ ppm).

The expected M_r of 1084 ($C_{52}H_{44}O_{26}$) for **2** and 1292 ($C_{62}H_{52}O_{31}$) for **3** were confirmed by FAB-mass spectrum. The ketene elimination series of both compounds are not complete and terminate after nine eliminations.

The fucophlorethols contain aryl ether bonds and aryl–aryl bonds. They may contain the same ring types as the phlorethols (Figs. 1–2). Additionally there are five typical structural elements with the sequences A_4F_1, $A_4F_3A_4$ (Fig. 6), $F_1F_4A_4$, $A_4F_2A_2$ (Fig. 7) and F_5A_4D (Fig. 8). The three structures mentioned first have only aryl–aryl bonds whereas the last two have aryl–aryl and aryl–ether bonds. Predominantly the structures A_4F_1 and $A_4F_2A_2$ were found. The typical chemical shifts of the five elements are compared with the data of one published structure, respectively (Table 3 and Tables 5–7).

Fucotriphlorethol-G dodeca-acetate (**5**) (Fig. 3) is homologous to fucodiphlorethol-D deca-acetate (**4**) (Fig. 3). However, **5** bears an extra 1,4-diphenoxylated benzene unit (ring B). Fucotetraphlorethol-J tetradeca-acetate (**6**) (Fig. 3) contains the same ring types as **5** plus an additional 1,2-phenoxylated benzene (ring C). By comparing with fucotriphlorethol-B dodeca-acetate (**7**) (Fig. 4) (sequence: A_4–F_1–B–C–D) it was shown that this ring is linked to the element A_4–F_1 because the ^1H NMR spectrum shows different chemical shifts for the signals belonging to the aromatic protons of the ring C and D (ring C: $\delta = 6.75/6.49$ ppm for **6**, $\delta = 6.73/6.51$ ppm for **7**, ring D: $\delta = 6.62/6.56$ ppm for **6**, $\delta = 6.68/6.55$ ppm for **7**). Fucotetraphlorethol-K tetradeca-acetate (**8**) (Fig. 4) and fucopentaphlorethol-E hexadeca-acetate (**9**) (Fig. 4) have also one element A_4–F_1, one ring of the type A_1 and D.

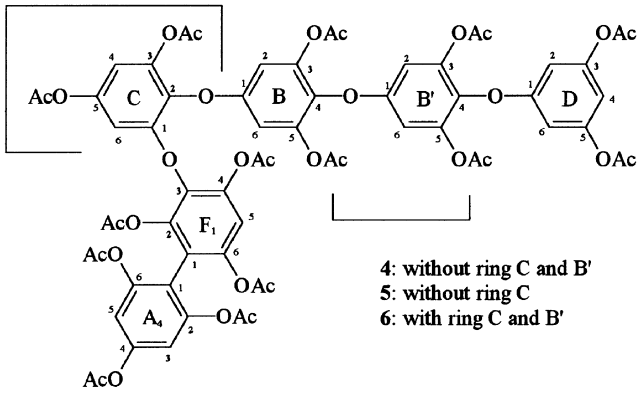

Fig. 3. Fucodiphlorethol-D deca-acetate (**4**), fucotriphlorethol-G dodeca-acetate (**5**) and fucotetraphlorethol-J tetradeca-acetate (**6**).

Both spectra show the AB-system of the ring type G. They only differ in the number of B-type rings. Compound **8** bears one and **9** two of these phloroglucinol units. The chemical shifts of nearly all corresponding aromatic protons are very similar, only shifts of the aromatic protons of the ring A_1 are different. The chemical shift $\delta = 6.94$ ppm for **8** shows that ring A_1 is linked to ring G whereas in **9**, the sequence is $GB'A_1$. The chemical shift $\delta = 6.95$ ppm is typical for an A_1-ring linked to a 1,4-diphenoxylated benzene (ring type B). The ring types of **9** are confirmed by the good correlation of the measured and the calculated ^{13}C NMR data (Table 4).

In the FAB-mass spectrum of **5** a six-fold ketene elimination series from $[M+H]^+$ 1127 ($C_{54}H_{46}O_{27}$) to 875 can easily be detected. An eight-fold ketene elimination series from $[M+H]^+$ 1543 ($C_{74}H_{62}O_{37}$) to 1207 was observed for **9**. FAB-mass-spectrum also confirmed the expected M_r of 1334 ($C_{64}H_{54}O_{32}$) for **6** and **8**.

Bisfucoheptaphlorethol-A tricosa-acetate (**10**) (Fig. 5) contains the same sequence A_4F_1BGD like compounds **8** and **9**. Furthermore two rings of the type B, one of the type C and a second fucol element A_4F_1 occur in **10**. These rings might be arranged in various manners. The chemical shifts of the signals belonging to the aromatic protons of ring G are shifted upfield ($\delta = 6.27/6.30$ ppm instead of $\delta = 6.38$ ppm). This is typical for the linkage of ring G at C-4 and C-6 with a 1,4-diphenoxylated benzene each. The sequence A_4F_1BCB was also derived from the correlations of the $^1H^1H$ ROESY NMR spectrum (Fig. 5). The presence of all ring types in **10** is proven by the ^{13}C NMR spectrum (Table 6).

Difucofucotriphlorethol-A octadeca-acetate (**11**) contains two different fucol elements, A_4F_1 and $A_4F_3A_4$, in addition to one ring G and D. Only a single structure proposal is possible for this compound (Fig. 6). The $A_4F_3A_4$-element is confirmed by the chemical shifts of **12** (Table 5). This compound has another second fucol element with the ring types $A_4F_2A_2$.

In FAB-mass-spectrum **10** caused a protonated molecular ion at 2210 (for $C_{106}H_{88}O_{53}$) and a thirteen-fold ketene elimination series from $[M+H]^+$ 2210→1664 was visible. The difference of 1 amu is caused by the high amount of ^{13}C in this large molecule. The FAB-mass spectrum of **11** shows the molecular ion $[M+H]^+$ at m/z 1627 ($C_{78}H_{66}O_{39}$) and the elimination of ten ketenes.

Fucotriphlorethol-H dodeca-acetate (**13**) (Fig. 7) shows the fucol element $A_4F_2A_2$ and the signals for one ring of type C and D each. Instead of the typical AB_2-system the ^1H NMR spectrum shows a system of higher order for the aromatic protons at position C-2, C-4 and C-6 of ring D. In addition to the $A_4F_2A_2$-structure difucofucotetraphlorethol-B icosa-acetate (**14**) (Fig. 7) contains a second fucol element $F_1F_4A_4$, up to now, this ring sequence has been found only in difucophlorethol-A undeca-acetate (**15**) (Fig. 7). Because of the two aryl bonds the signal of the aromatic proton at C-5 of ring F_4 is shifted extremely downfield ($\delta = 7.16$ ppm). The signal for the aromatic proton at C-5 of ring F_1 is shifted downfield ($\delta = 7.14$ ppm) too. The ^1H NMR spectrum shows an AB-system for the aromatic protons at C-3 and C-5 of the ring A_4 which is linked to ring F_4. This may be caused by the asymmetry of the molecule. The same effect is seen in the ^1H MR spectrum of **15**, which had been isolated from *Cystophora retroflexa*. Glombitza and Große-Damhues (1985) who isolated **15** first, described a singlet for the aromatic protons Table 7.

The M_r 1126 ($C_{54}H_{46}O_{27}$) for **13** and 1834 ($C_{88}H_{74}O_{44}$) for **14** was derived from FAB-mass spectra. The spectrum of **13** shows an eight-fold ketene elimination and the spectrum of **14** an eleven-fold ketene elimination.

Terfucohexaphlorethol-B tetracosa-acetate (**17**) (Fig. 8) and terfucoheptaphlorethol-A hexacosa-acetate (**18**) (Fig. 8) contain three fucol elements each. Consequently, ring G cannot be linked to a D-ring at position C-2 as for the most of the other fucophlorethols described here. **17** shows the signals of three different fucol elements. The positions of the signals belonging to the aromatic protons at C-4 and C-6 of the ring G are caused by the linkage of the fucol elements A_4F_1 and $A_4F_2A_2$ at C-1 and C-3. Therefore, ring B must be linked to C-2 of ring G and also be connected with the element F_5A_4D. This element was derived by comparison to the data of terfucopentaphlorethol-A docosa-acetate (**16**) (Table 7). The position of the B-ring could not be determined by $^1H^1H$ ROESY NMR because the yield of **17** was too low. The ^1H NMR spectrum of **18** shows two rings of type G, one of which is substituted symmetrically, obviously, the sequence must be $A_4F_1GF_1A_4$. The second ring of type G must be connected with the structural element $A_4F_2A_2$ and a ring D. The position of ring B is uncertain again. The signal of the aromatic protons at position C-2 and C-6 are shifted upfield ($\delta = 6.61$ ppm). Therefore, it may be located between both G-type rings, due to the low sample amount it was not possible to allow a definite assignment by the ^1H–^1H ROESY NMR experiment.

The FAB-mass spectra show a molecular ion $[M+H]^+$

Table 3
^{1}H NMR spectral data of **4**–**9**

H	**4**	**5**		**6**		**7**	**8**		**9**	
	$CDCl_3$	$CDCl_3$	d_6-Me_2CO	$CDCl_3$	d_6-Me_2CO	$CDCl_3$	$CDCl_3$	d_6-Me_2CO	$CDCl_3$	d_6-Me_2CO
Ring A_4										
3,5	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.01
Ac-2,Ac-6	2.06(4)[a]	2.06(3)[b]	1.97	2.04	1.97	2.02[e]	2.05	[f]	2.05	[f]
Ac-4	2.30	2.30	2.24	2.30	2.24	2.29	2.30	2.25	2.30	2.25
Ring F_1										
5	7.12	7.12	7.20	7.11	7.20	7.11	7.11	7.20	7.12	7.20
Ac-2	1.92	1.92	1.81	1.92	1.81	1.91	1.92	1.81	1.92	1.81
Ac-4	2.13	2.12	2.10	2.12	2.10	2.12	2.11	2.10	2.11	2.10
Ac-6	2.03	2.03	1.94	2.03	1.94	2.02	2.03	1.95	2.02	1.94
Ring C										
6				6.49	6.44	6.51				
Ac-3				2.20	2.13	2.14				
Ac-5				2.23	2.17	2.23				
$J_{4,6}$ (Hz)				2.6	2.5	2.3				
Ring B										
2,6	6.70	6.71	6.77	6.69[c]	6.75	6.68	6.69	6.75	6.69	6.74
Ac-3,Ac-5	2.05(7)[a]	2.05(9)[b]	[f]	2.06[d]	[f]	2.05[e]	2.04	1.97	2.03	[f]
Ring G										
4							6.32	6.29	6.30	6.27
6							6.29	6.26	6.28	6.25
Ac-5							2.18	2.12	2.18	2.12
$J_{4,6}$ (Hz)							2.6	2.6	2.6	2.6
Ring B′										
2,6		6.66	6.71	6.68[c]	6.75				6.67	6.72
Ac-3,5		2.12	2.06	2.05[d]	[f]				2.02	1.97
Ring A_1										
3,5							6.94	6.99	6.95	7.00
Ac-2,Ac-6							2.04	[f]	2.13	2.07
Ac-4							2.28	2.22	2.29	2.23
Ring D										
2,6	6.54	6.54	6.52	6.56	6.54	6.55	6.58	6.56	6.57	6.57
4	6.64	6.64	6.65	6.62	6.63	6.68	6.69	6.68	6.69	6.68
Ac-3,Ac-5	2.25	2.25	2.19	2.24	2.17	2.25	2.24	2.20	2.24	2.19
$J_{4,2,6}$ (Hz)	2.2	2.0	2.2	2.0	2.2	2.3	2.0	2.2	2.0	2.0

[a–e]Assignments with the same letters may be interchanged.
[f]Hidden by solvent.For some signals the chemical shift has been estimated to the approximated third decimal place (shown in parentheses) to distinguish between closely neighbored signals, which were nevertheless clearly separated by visual inspection.

at *m/z* 2252 ($C_{108}H_{90}O_{54}$) for **17** and at *m/z* 2460 ($C_{118}H_{98}O_{59}$) for **18**. From this parent ion **17** successively eliminates nine ketene fragments whereas **18** only shows an eight-fold ketene elimination. The signal of both compounds seems to be increased by 1 amu because of poor resolution of the isotopic envelope.

3. Experimental

3.1. EI-MS operation

Kratos MS-50 instrument, 70 eV, 200–300°C; positive ion FAB-MS: XE gun, 3-nitrobenzylalcohol as matrix.

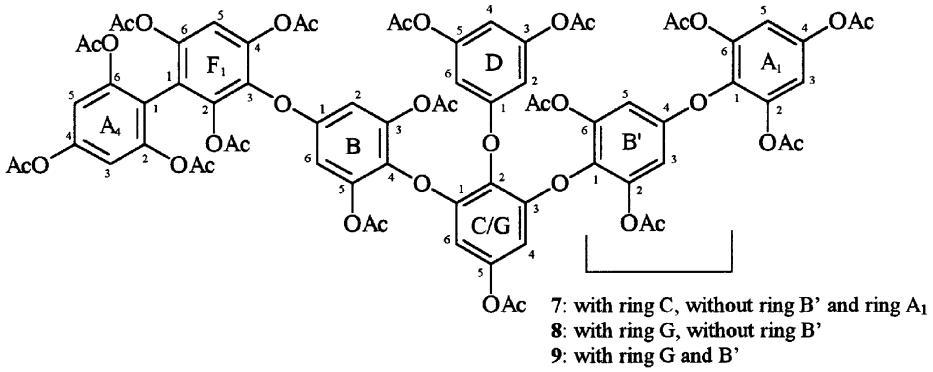

Fig. 4. Fucotriphlorethol-B dodeca-acetate (**7**), fucotetraphlorethol-K tetradeca-acetate (**8**) and fucopentaphlorethol-E hexadeca-acetate (**9**).

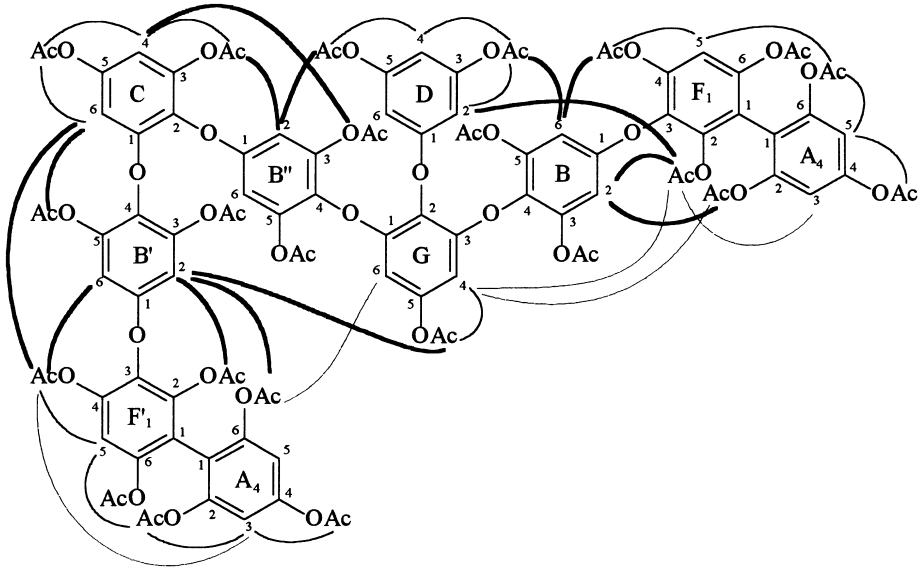

Fig. 5. Bisfucopheptaphlorethol-A tricosa-acetate (**10**) with the correlations of the $^{1}H^{1}H$ ROESY NMR spectrum.

3.2. ^{1}H NMR spectra

^{1}H NMR spectra (300 MHz, Varian XL 300), ^{1}H–^{1}H ROESY NMR (500 MHz, Bruker AMX) and ^{13}C NMR (400 and 500 MHz, Bruker AM 400/AMX) were recorded using solvents as int. standards.

3.3. Extraction and isolation

20 kg of frozen and shredded algae were extracted with 10 l EtOH in the presence of 50 g $K_2S_2O_5$ under N_2 for 2 h. The extract was concentrated in vacuo, the residue (5 l) was divided in two portions and each aliquot was extracted with petrol (4 × 1 l), $CHCl_3$ (4 × 1 l) and finally with EtOAc (4 × 1 l). The EtOAc fraction was dried in vacuo and immediately acetylated with 150 ml Ac_2O–pyridine (2:1 v/v). The yield was 27.5 g. 5 g portions of this material were fractionated by HPLC using a semi-preparative silica gel column (250 × 16 mm, Lichrosorb Si 60, 7 μm). Afterwards, each fraction was purified in several HPLC steps using an analytical column (250 × 8 mm, Lichrosorb Si 60, 5 μm). Different gradients with $CHCl_3$/$CHCl_3$–EtOH (97.5:2.5) as solvents were used.

3.4. Isolated compounds

Isolated compounds (yield: mg/kg alga); Phloroglucinol tri-acetate: 1.5 mg, ^{1}H NMR data as in Glombitza et al. (1973).

Diphlorethol penta-acetate: 2.0 mg, ^{1}H NMR data as in Glombitza et al. (1975).

Table 4
^{13}C NMR spectral data of **9** and **10** (in $CDCl_3$)

C	Measured **9**	Measured **10**	Calculated (Wegner-Hambloch & Glombitza, 1985)
Ring A_4,A'_4			
1	115.0	115.0(1)/115.0(3)	114.9
2,6	149.2	149.3	149.2
3,5	113.9	113,9	113.9
4	150.8	150.8	150.7
Ring F_1, F_1'			
1	118.3	118.3(0)/118.3(3)	118.1
2	143.2	143.3	143.0
3	137.1	137.1(2)/137.1(4)	136.5
4	142.8	142.8(6)/142.8(7)	142.5
5	116.2	116.2	116.0
6	145.2	145.2(2)/145.2(4)	145.1
Ring B,*B'*,*B''*			
1	153.6/153.8	153.5(6)/153.6(4)/154.6	153.1
2,6	109.6/109.7	109.2/109.6(5)/109.7(0)	108.3
3,5	143.9	143.9(2)/143.9(7)	143.9
4	133.9/134.0	133.4/133.6/134.0	134.7
Ring C			
1		150.8(9)[a]	150.6
2		134.4	133.7
3		144.3	143.9
4		112.0	111.8
5		147.4	147.8
6		108.3	108.3
Ring G			
1,3	150.9/151.0	150.9(5)[a]/151.0(4)	150.4[b]
2	130.1	130.0	130.3
4,6	104.7/104.9	104.6(7)/104.6(9)	103.2[b]
5	147.8	147.8	147.7
Ring A_1			
1	136.6		136.8
2,6	143.7		143.6
3,5	115.1		113.8
4	146.7		146.2
Ring D			
1	159.4	159.5	159.2
2,6	106.7	106.8	106.6
3,5	151.5	151.5	151.6
4	109.9	110.0	110.0

[a] Assignments may be interchanged.
[b] Data of a symmetrically substituted ring G.

Triphlorethol-A hepta-acetate: 174.0 mg, ^1H NMR data as in Koch and Gregson (1984).

Tetraphlorethol-C nona-acetate: 0.75 mg, ^1H NMR data as in Koch and Gregson (1984).

Difucol hexa-acetate: 0.05 mg, ^1H NMR data as in Glombitza et al. (1976).

Fucophlorethol-B octa-acetate: 0.38 mg, ^1H NMR data as in Glombitza et al. (1978).

Fucotetraphlorethol-B tetradeca-acetate: 0.15 mg, ^1H NMR data as in Glombitza and Große-Damhues (1985).

Bisfucotriphlorethol-A pentadeca-acetate: 0.8 mg, ^1H NMR data as in Glombitza et al. (1983).

Bisfucotetraphlorethol-A heptadeca-acetate: 0.23 mg, ^1H NMR data as in Glombitza and Große-Damhues (1985).

Bisfucopentaphlorethol-A nonadeca-acetate: 0.1 mg,

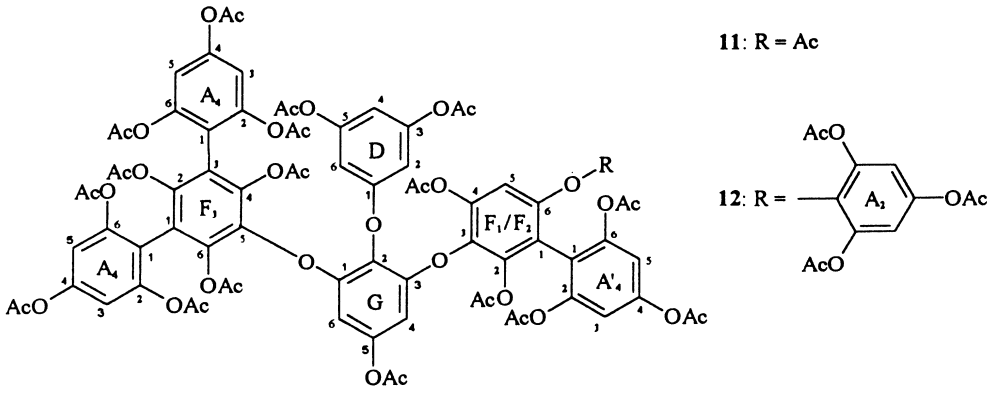

Fig. 6. Difucofucotriphlorethol-A octadeca-acetate (**11**) and difucofucotetraphlorethol-A icosa-acetate (**12**).

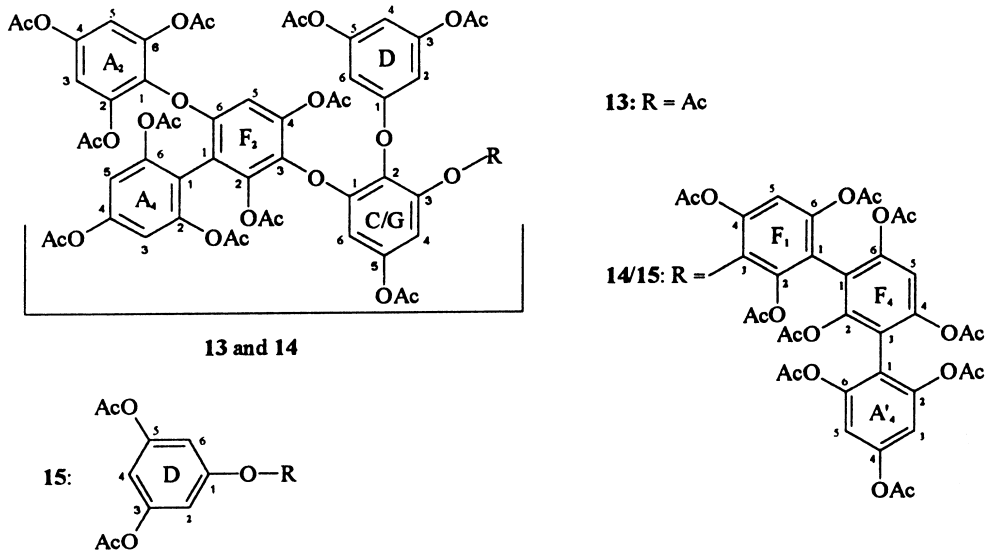

Fig. 7. Fucotriphlorethol-H dodeca-acetate (**13**), difucofucotetraphlorethol-B icosa-acetate (**14**) and difucophlorethol-A undeca-acetate (**15**).

^{1}H NMR data as in Glombitza and Große-Damhues (1985).

Bisfucopentaphlorethol-B nonadeca-acetate: 0.7 mg, ^{1}H NMR data as in Glombitza et al. (1981).

Terfucohexaphlorethol-A tetracosa-acetate: 0.25 mg, ^{1}H NMR data as in Glombitza et al. (1997b).

Fucodiphlorethol-D deca-acetate (**4**), 40.0 mg, ^{1}H NMR shown in Table 3.

Fucotriphlorethol-B dodeca-acetate (**7**), 0.4 mg, ^{1}H NMR shown in Table 3.

Difucofucotetraphlorethol-A icosa-acetate (**12**), 0.15 mg, ^{1}H NMR shown in Table 5.

Difucophlorethol-A undeca-acetate (**15**), 0.1 mg, ^{1}H NMR shown in Table 6.

Terfucopentaphlorethol-A docosa-acetate (**16**), 0.13 mg, ^{1}H NMR shown in Table 7.

*3.4.1. Tetraphlorethol-E nona-acetate: 2,4,3′,5-tetra-acetoxy-6-(2,4,6-triacetoxy-phenoxy)-4′-(3,5-diacet-oxyphenoxy)diphenylether (**1**)*

0.3 mg, FAB-MS ketene elimination series: *m/z* (915) $[M+K]^+$, 899 $[M+Na]^+ \rightarrow$857, 877 $[M+H]^+ \rightarrow$ 835 → 793 → 751 → 709 → 667 → 625 → (583) → (541) → (499). ^{1}H NMR is shown in Table 1 and ^{13}C NMR is shown in Table 2.

*3.4.2. Pentaphlorethol-B undeca-acetate: 4,3′,5′-triacetoxy-2,6-bis(2,4,6-triacet-oxyphenoxy)-4′-(3,5-diacetoxyphenoxy)diphenylether (**2**)*

0.13 mg, FAB-MS ketene elimination series: *m/z* 1122 $[M+K]^+$, 1107 $[M+Na]^+ \rightarrow$ 1065, 1085 $[M+H]^+ \rightarrow$ 1043 → 1001 → 959 → 917 → 875 → 833 → 792 → 749 → 708. ^{1}H NMR is shown in Table 1.

Table 5
^{1}H NMR spectral data of **10**–**12**

	10		**11**		**12**
H	$CDCl_3$	d_6-Me_2CO	$CDCl_3$	d_6-Me_2CO	$CDCl_3$
Ring A_4					
3,5	7.00	7.00(5)/7.00(2)	7.01	7.04	7.01
Ac-2,Ac-6	2.05	2.00(1)/1.98(9)	2.05	[a]	2.05
Ac-4	2.29	2.24	2.29	2.28	2.28
Ring F_3					
Ac-2			1.67	1.59	1.67
Ac-4,Ac-6			1.83	1.84	1.79
Ring F_1					
5	7.11(1)	7.20	7.12	7.23	
Ac-2	1.91(3)	1.81	1.78	1.79	
Ac-4	2.11	2.09	2.06	2.10	
Ac-6	2.02	1.98	2.01	1.97	
Ring F'_1					
5	7.10(8)	7.20			
Ac-2	1.90(8)	1.81			
Ac-4	2.11	2.09			
Ac-6	2.02	1.98			
Ring A'_4					
3,5			6.98	7.03	6.98
Ac-2,Ac-6			2.04	2.00	2.01
Ac-4			2.29	2.28	2.28
Ring F_2					
5					6.56
Ac-2					1.88
Ac-4					1.92
Ring A_2					
3,5					6.92
Ac-2,Ac-6					2.05
Ac-4					2.28
Ring B					
2,6	6.68	6.74			
Ac-3,Ac-5	2.01	1.94			
Ring G					
4	6.26	6.23	6.36	6.40	6.34
6	6.30	6.29	6.44	6.42	6.50
Ac-5	2.15	2.09	2.15	2.18	2.15
$J_{4,6}$ (Hz)	2.6	2.5	2.6	2.5	2.6
Ring D					
2,6	6.58	6.57	6.54	6.60	6.58
4	6.68	6.68	6.69	6.72	6.61
Ac-3,Ac-5	2.23	2.19	2.23	2.23	2.10
$J_{4,2,6}$ (Hz)	2.1	2.0	1.9	2.0	2.0
Ring B'					
2,6	6.67	6.74			
Ac-3,5	2.02	1.97			
Ring C'					
4	6.74	6.83			
6	6.48	6.44			
Ac-3	2.17	2.11			
Ac-5	2.22	2.17			
$J_{4,6}$ (Hz)	2.6	2.5			
Ring B''					
2,6	6.67	6.74			
Ac-3,5	2.02	1.97			

[a] Hidden by solvent.

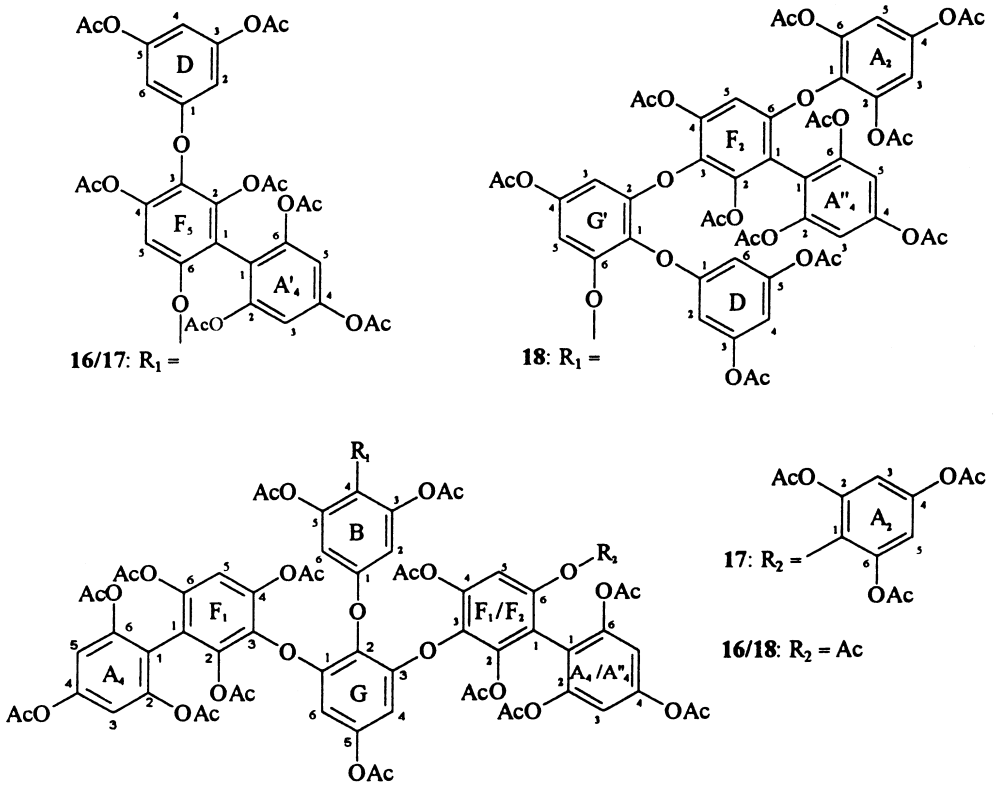

Fig. 8. Terfucopentaphlorethol-A docosa-acetate (**16**), terfucohexaphlorethol-B tetra-cosa-acetate (**17**) and terfucoheptaphlorethol-A hexacosa-acetate (**18**).

*3.4.3. Hexaphlorethol-A trideca-acetate: 4,3′,5′-triacetoxy-2,6-bis[2,6-diacetoxy-4-(2,4,6-triacetoxyphenoxy)phenoxy]diphenylether (**3**)*

0.2 mg, FAB-MS ketene elimination series: *m/z* 1315 $[M+Na]^+$, 1293 $[M+H]^+ \rightarrow 1251 \rightarrow 1209 \rightarrow 1167 \rightarrow 1125 \rightarrow 1083 \rightarrow 1041 \rightarrow 957 \rightarrow 915 \rightarrow 873$. ^{1}H NMR is shown in Table 1.

*3.4.4. Fucotriphlorethol-G dodeca-acetate: 2,6,3′,5′-tetraacetoxy-4-(2,4,6,2′,4′,6′-hexaacetoxybiphenylyl-3-oxy)-4′-(3,5-diacetoxyphenoxy)di-phenylether (**5**)*

0.25 mg, FAB-MS ketene elimination series: *m/z* 1149 $[M+Na]^+$, 1127$[M+H]^+ \rightarrow 1085 \rightarrow 1043 \rightarrow 1001 \rightarrow$ 95917 $\rightarrow$ 875 $\rightarrow$ (833) $\rightarrow$ (791) $\rightarrow$ (749) $\rightarrow$ (707) $\rightarrow$ (665). ^{1}H NMR is shown in Table 3.

*3.4.5. Fucotetraphlorethol-J tetradeca-acetate: 2,4,3′,5′-tetraacetoxy-6-(2,4,6,2′,4′,6′-hexaacetoxybiphenylyl-3-oxy)-4′-[3,5-diacetoxy-4-(3,5-diacetoxyphenoxy)phenoxy]diphenylether (**6**)*

0.13 mg, FAB-MS ketene elimination series: *m/z* 1373 $[M+K]^+$, 1357 $[M+Na]^+ \rightarrow 1315 \rightarrow$ (1273), 1335 $[M+H]^+ \rightarrow 1293 \rightarrow 1251 \rightarrow 1209 \rightarrow 1167 \rightarrow 1125 \rightarrow 1083 \rightarrow 1041 \rightarrow 999 \rightarrow 958 \rightarrow 915 \rightarrow$ (873) $\rightarrow$ (831) $\rightarrow$ (789) $\rightarrow$ (747). ^{1}H NMR is shown in Table 3.

*3.4.6. Fucotetraphlorethol-K tetradecaacetate: 2,6,5′-triacetoxy-4-(2,4,6,2′,4′,6′-hexaacetoxybiphenylyl-3-oxy)-2′-(3,5-diacetoxyphenoxy)-3′-(2,4,6-triacetoxyphenoxy)diphenylether (**8**)*

0.3 mg, FAB-MS ketene elimination series: *m/z* 1373 $[M+K]^+$, 1357 $[M+Na]^+ \rightarrow$1315, 1335 $[M+H]^+ \rightarrow 1293 \rightarrow 1251 \rightarrow 1209 \rightarrow 1167 \rightarrow 1125 \rightarrow 1083 \rightarrow 1041 \rightarrow 999 \rightarrow 957$). ^{1}H NMR is shown in Table 3.

*3.4.7. Fucopentaphlorethol-E hexadeca-acetate: 2,6,5′-triacetoxy-4-(2,4,6,2′,4′,6′-hexaacetoxybiphenylyl-3-oxy)-2′-(3,5-diacetoxyphenoxy)-3′-[2,6-diacetoxy-4-(2,4,6-triacetoxyphenoxy)phenoxy]diphenylether (**9**)*

0.35 mg, FAB-MS ketene elimination series: *m/z* 1581 $[M+K]^+$, 1565 $[M+Na]^+$, 1543 $[M+H]^+ \rightarrow 1501 \rightarrow 1459 \rightarrow 1417 \rightarrow 1375 \rightarrow 1333 \rightarrow 1291 \rightarrow 1249 \rightarrow 1207 \rightarrow$ (1165) $\rightarrow$ (1123) $\rightarrow$ (1081) $\rightarrow$ (1039). ^{1}H NMR is shown in Table 3 and ^{13}C NMR in Table 4.

*3.4.8. Bisfucoheptaphlorethol-A tricosa-acetate: 2,6,5′-triacetoxy-4-{2,4-diacetoxy-6-[2,6-diacetoxy-4-(2,4,6,2′,4′,6′-hexaacetoxybiphenylyl-3-oxy)phenoxy]phenoxy}-2′-(3,5-diacetoxyphenoxy)-3′-[2,6-diacetoxy-4-(2,4,6,2′,4′, 6′-hexaacetoxy-biphenylyl-3-oxy)phenoxy]diphenylether (**10**)*

1.0 mg, FAB-MS ketene elimination series: *m/z* 2247 $[M+K]^+$, 2232 $[M+Na]^+ \rightarrow$2190, 2210 $[M+H]^+ \rightarrow$

Table 6
^{1}H NMR spectral data of **13**–**15**

	13		**14**		**15**	
H	$CDCl_3$	d_6-Me_2CO	$CDCl_3$	d_6-Me_2CO	$CDCl_3$	$CDCl_3$ (Glombitza & Große-Damhues, 1985)
Ring A_4						
3,5	6.99	7.01	6.98	7.02		
Ac-2			1.97[e]	1.95[f]		
Ac-2,Ac-6	2.00[a]	[l]				
Ac-4	2.28	2.23	2.29[d]	2.24[g]		
Ac-6			2.03[e]	[l]		
Ring F_2						
5	6.57	6.59	6.57	6.59		
Ac-2	1.91[a]	1.84[b]	1.87	1.80		
Ac-4	1.91[a]	1.82[b]	1.92[e]	1.83[f]		
Ring A_2						
3,5	6.92	6.97	6.93	6.97		
Ac-2,Ac-6	2.06[a]	[l]	2.06	1.98		
Ac-4	2.28	2.23	2.29[d]	2.23[g,h]		
Ring G						
4			6.33	6.35		
6			6.37	6.41		
Ac-5			2.17	2.15		
$J_{4,6}$ (Hz)			2.6	2.7		
Ring C						
4	6.80	6.85				
6	6.51	6.56[m]				
Ac-3	2.14	2.06				
Ac-5	2.23	2.20				
$J_{4,6}$ (Hz)	2.5	2.5				
Ring D						
2,6	6.56[m]	6.57[m]	6.58	6.59	6.55	6.55
4	6.57[m]	6.59[m]	6.59	6.61	6.66	6.67
Ac-3,Ac-5	2.13	2.09	2.11	2.09	2.25	2.23
$J_{4,2,6}$ (Hz)	2.0	1.9	1.6	2.0	1.9	2.0
Ring F_1						
5			7.14	7.18	7.14	7.13
Ac-2			1.79	1.78	1.89	1.87
Ac-4			2.06	[l]	2.06	2.06
Ac-6			2.05	[l]	2.04	2.03
Ring F_4						
5			7.15	7.18	7.16	7.15
Ac-2			1.70	1.60	1.72	1.69
Ac-4			2.03	1.98[n]	2.05	2.05
Ac-6			2.00	1.97	2.01	2.01
Ring A'_4						
3			7.01[e]	7.00[i]	7.02[k]	
5			7.00[e]	6.99[i]	7.01[k]	
3,5						7.00
Ac-2			2.03[d]	[l]		
Ac-2,Ac-6					2.08	2.07
Ac-4			2.29[d]	2.23[g,h]	2.29	2.27
Ac-6			1.93[e]	1.94[f]		
$J_{3,5}$ (Hz)			1.9	1.8	1.9	

[a–k] Assignments with the same letters may be interchanged.
[Δ] Signals are not separated.
[′] Hidden by solvent.

Table 7
^{1}H NMR spectral data of **16**–**18**

	16	**17**		**18**	
H	$CDCl_3$	$CDCl_3$	d_6-Me_2CO	$CDCl_3$	d_6-Me_2CO
Ring A_4					
3,5	6.99	6.98	6.98	6.98	7.00
Ac-2,Ac-6	[j]	2.04	[i]	2.04	1.97
Ac-4	2.27	2.29	2.24	2.29	2.24
Ring F_1					
5	7.12	7.10	7.16	7.11	7.18
Ac-2	1.78	1.81	1.74	1.80	1.76
Ac-4	[j]	2.02[a]	1.98[e]	2.01	1.95
Ac-6	[j]	2.00[a]	1.97[e]	1.93	1.93
Ring G					
4		6.41	6.41		
6		6.31	6.39		
4,6	6.38			6.37	6.37
Ac-5	2.16	2.17	2.15	2.17	2.13
$J_{4,6}$ (Hz)		2.8	2.6		
Ring B					
2,6	6.63	6.71	6.77	6.61	6.69
Ac-3,Ac-5	[j]	2.07	[i]	2.06	[i]
Ring G′					
4				6.30	6.35
6				6.28	6.30
Ac-5				2.14	2.08
$J_{4,6}$ (Hz)				2.5	2.5
Ring F_5					
5	6.65	6.64	6.64		
Ac-2	1.90	1.91	1.85		
Ac-4	[j]	1.92	1.93		
Ring D					
2,6	6.59	6.58	6.59		6.61
4	6.71	6.68	6.68		6.62
2,4,6				6.61	
Ac-3,Ac-5	2.21	2.23	2.19	2.11	2.09
$J_{4,2,6}$ (Hz)	2.0	1.9	1.9		2.0
Ring A'_4					
3,5	6.99	6.99	6.99		
Ac-2,Ac-6	[j]	2.06	[i]		
Ac-4	2.27	2.29[b]	2.23[f]		
Ring F_2					
5		6.54	6.58	6.57	6.59
Ac-2		1.87	1.78	1.88	1.80
Ac-4		2.23[k]	2.19[k]	1.92	1.83
Ring A_2					
3,5		6.91	6.96	6.93	6.97
Ac-2,Ac-6		1.90[c]	1.92[g]	2.06	[i]
Ac-4		2.28[b]	2.23[f]	2.28	2.23
Ring A''_4					
3,5		6.98	6.98	6.98	7.01
Ac-2		1.90[c,d]	1.82[g,h]		
Ac-2,Ac-6				2.02	1.98
Ac-4		2.27[b]	2.23[f]	2.28	2.23
Ac-6		2.01[c,d]	1.97[g,h]		

[a–h] Assignments with the same letters may be interchanged.
[i] Hidden by solvent.
[j] Signals with the chemical shifts $\delta = 1.99$–2.03 ppm are not assignable undeniably.
[k] Assignment is not certain.

2167 → 2125 → 2084 → 2042 → 2000 → 1959 → 1917 → 1874 → 1832 → 1792 → 1751 → 1709 → 1664. ^{1}H NMR is shown in Table 5 and ^{13}C NMR in Table 4.

*3.4.9. Difucofucotriphlorethol-A octadeca-acetate: 4,3′,5′-triacetoxy-(2-2,4,6,2′, 4′,6′,2″,4″,6″-nonaaceto-oxy-1,1′:3′,1″-terphenylyl-5′-oxy)-6-(2,4,6,2′,4′, 6′-hexaacetoxybiphenylyl-3-oxy)diphenylether (**11**)*

0.08 mg, FAB-MS ketene elimination series: *m*/*z* (1665) $[M+K]^+$, 1649 $[M+Na]^+$ → 1607, 1627 $[M+H]^+$ → 1585 → 1543 → 1501 → 1459 → 1417 → 1375 → 1333 → 1291 → 1249 → 1207 → (1164) → (1122). ^{1}H NMR shown in Table 5.

*3.4.10. Fucotriphlorethol-H dodeca-acetate: 2,6,3′,5′-tetraacetoxy-3-(2,4,6-triacetoxyphenyl)-4-(2,4,6-tri-acetoxyphenoxy)-2′-(3,5-diacetoxyphenoxy) diphenylether (**13**)*

0.2 mg, FAB-MS ketene elimination series: *m*/*z* 1165 $[M+K]^+$, 1149 $[M+Na]^+$, 1127 $[M+H]^+$ → 1085 → 1043 → 1001 → 959 → 917 → 875 → 833 → 791. ^{1}H NMR shown in Table 6.

*3.4.11. Difucofucotetraphlorethol-B icosa-acetate: 2,6,5′-triacetoxy-3-(2,4,6-triacetoxyphenyl)-4-(2,4,6-tri-acetoxyphenoxy)-2′-(3,5-diacetoxyphenoxy)-3′-(2,4,6,2′,4′,6′,2″,4″,6″-nonaacetoxy-1,1′:3′,1″-terphenylyl-3″-oxy)diphenylether (**14**)*

0.05 mg, FAB-MS ketene elimination series: *m*/*z* 1873 $[M+K]^+$, 1858 $[M+Na]^+$ → (1815), 1835 $[M+H]^+$ → 1793 → 1751 → 1710 → 1668 → 1626 → 1583 → 1542 → 1499 → 1457 → 1415 → 1373. ^{1}H NMR is shown in Table 6.

*3.4.12. Terfucohexaphlorethol-B tetracosa-acetate: 4,3′,5′-triacetoxy-4′-[2,4,2′, 4′,6′-pentaacetoxy-3-(3,5-diacetoxyphenoxy)biphenylyl-6-oxy]-2-[2,6-diacetoxy-3-(2,4,6-triacetoxyphenyl)-4-(2,4,6-triacetoxyphenoxy)phenoxy]-6-(2,4,6,2′,4′,6′-hexaacetoxybiphenylyl)-3-oxy]diphenylether (**17**)*

0.08 mg, FAB-MS ketene elimination series: *m*/*z* 2290 $[M+K]^+$, 2274 $[M+Na]^+$ → 2231, 2252 $[M+H]^+$ → 2209 → 2169 → 2125 → 2085 → 2041 → 2002 → 1958 → 1918 → 1875. ^{1}H NMR is shown in Table 7.

*3.4.13. Terfucoheptaphlorethol-A hexacosa-acetate: 2,6,5′-triacetoxy-2′-(3,5-diacetoxyphenoxy)-3′-[2,4,2′,4′,6′-pentaacetoxy-6-(2,4,6-triacetoxy-phenoxy)biphenylyl-3-oxy]-4-[4-acetoxy-2,6-bis(2,4,6,2′,4′,6′-hexaacetoxybiphenylyl-3-oxy) phenoxy]diphenylether (**18**)*

0.4 mg, FAB-MS ketene elimination series: *m*/*z* 2497 $[M+K]^+$, 2482 $[M+Na]^+$ →2440, 2460 $[M+H]^+$ → 2418 → 2376 → 2334 → 2292 → 2249 → 2210 → 2168 → 2125. ^{1}H NMR is shown in Table 7.

In some FAB-MS, the signal of $[M+K]^+$ was not visible. Weak signals are put in parentheses.

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