Electronic Supplementary Information (ESI)

Comonomer distribution analysis of poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate)s using high-resolution MALDI-TOF mass spectrometry

Naoki Kanayama[a]*, Sayaka Nakamura[a], Shin Sano[b], Mami Tanaka[b], Noriko Takahara[a], Hiroaki Sato[a] and Hideaki Hagihara[a]

a. *Research Institute for Sustainable Chemistry, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central 5, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8565, Japan*
b. *Functional Chemical Materials Division, Research and Development Department, Tokyo Metropolitan Industrial Technology Research Institute (TIRI), 2-4-10, Aomi, Koto-ku, Tokyo 135-0064, Japan*

Contents:

S-1. ^{1}H-NMR spectra of PHBH samples

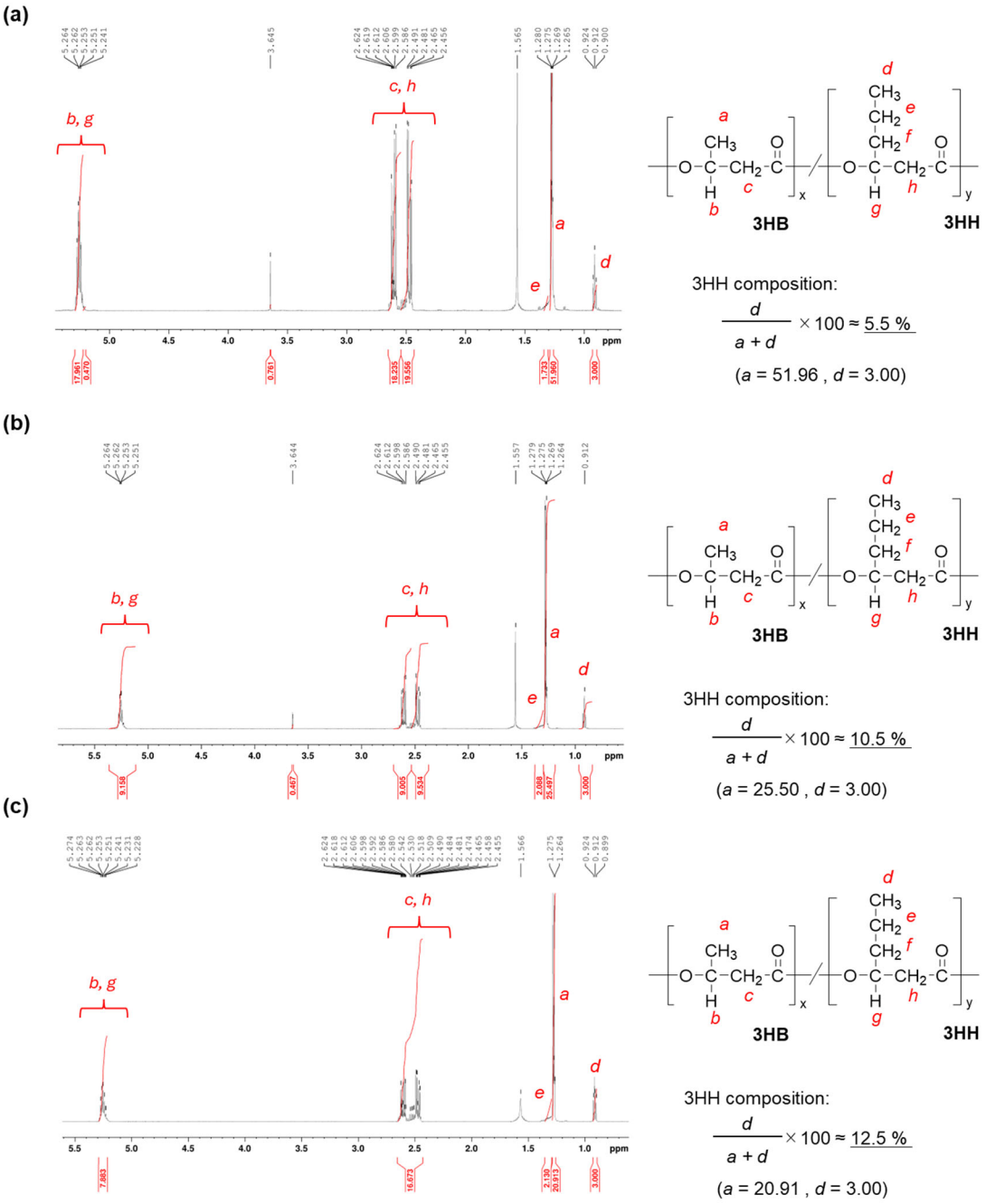


Fig. S1 ^{1}H-NMR (600 MHz) spectra of (a) PHBH6, (b) PHBH11, and (c) PHBH13 dissolved in $CDCl_3$ containing 0.05 wt% of TMS. Conc.: 3 mg·mL^{-1}. Scans: 8 times, Temp.: r.t.

S-2. SEC traces of PHBH samples

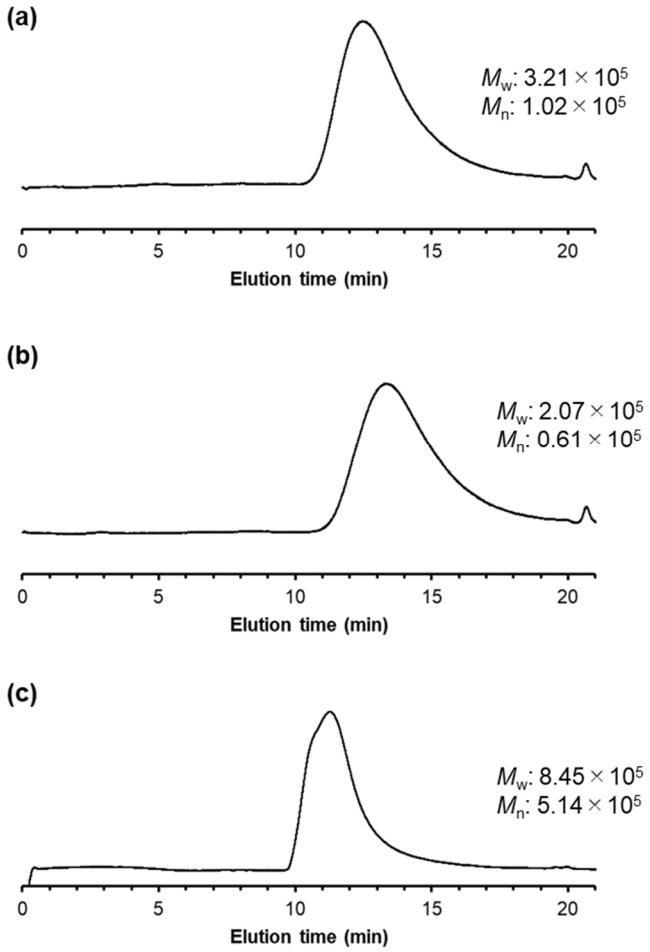


Fig. S2 SEC traces of (a) PHBH6, (b) PHBH11, and (c) PHBH13 dissolved in chloroform (2 mg/mL). SEC traces were recorded on an RI detector. For a mobile phase, chloroform was used at a flow rate of 1.0 mL·min^{-1} at 40 °C.

S-3. Molecular size distribution of the SEC-fractionated PHBH11 oligomers

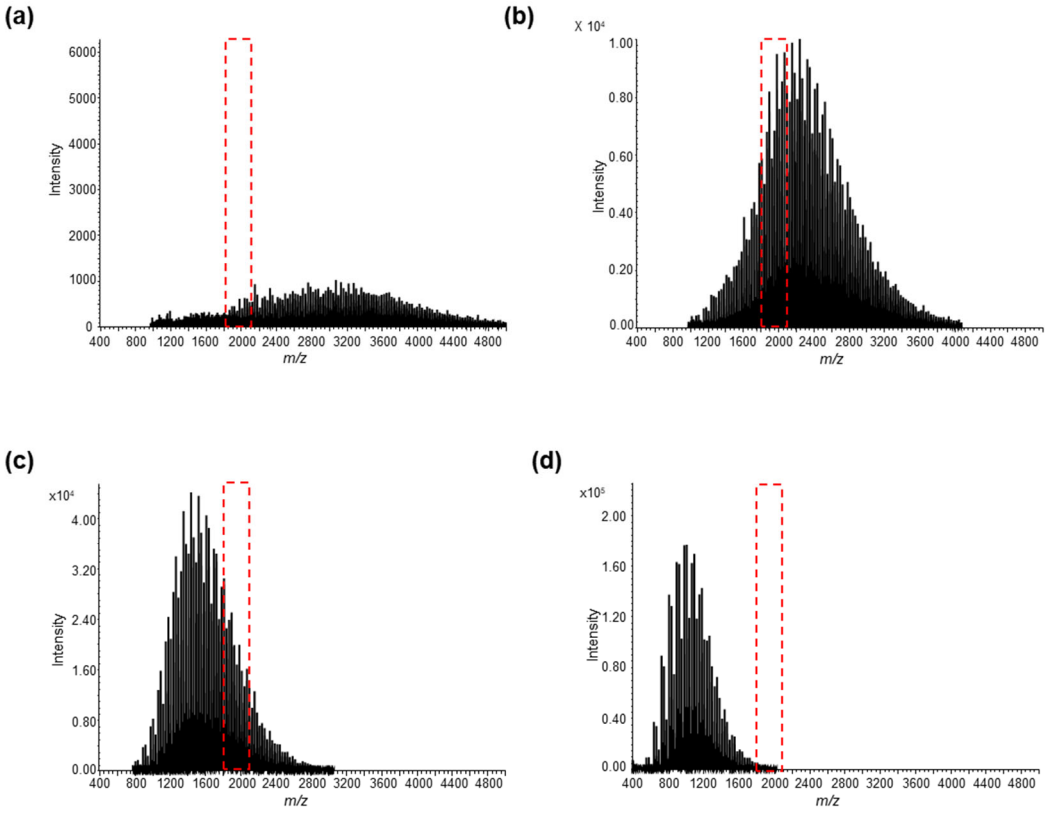


Fig. S3 Molecular size distribution of SEC-fractionated PHBH11 oligomer assessed by preliminary mass spectra measurements by MALDI spiral-TOF-MS. (a) Fraction #1 (elution time: 18.0–18.5 min), (b) fraction #2 (elution time: 18.5–19.0 min), (c) fraction #3 (elution time: 19.0–19.5 min), and (d) fraction #4 (elution time: 19.5–20.0 min). All mass spectra were acquired in the positive ion mode using THAP as a matrix. Signal intensities of the mass spectra were normalized according to a previous report,[S1] which utilizes the RI signal intensity of the corresponding elusion peak on the SEC trace. The red square indicates the *m/z* range (approximately 1800 to 2100) of interest in the mass spectral data analysis performed in this study.

Reference

[S1] H. Sato, N. Ichieda, H. Tao and H. Ohtani, *Anal. Sci.*, 2004, **20**, 1289–1294.

S-4. Chemical structures of molecular species presumed from the molecular formula

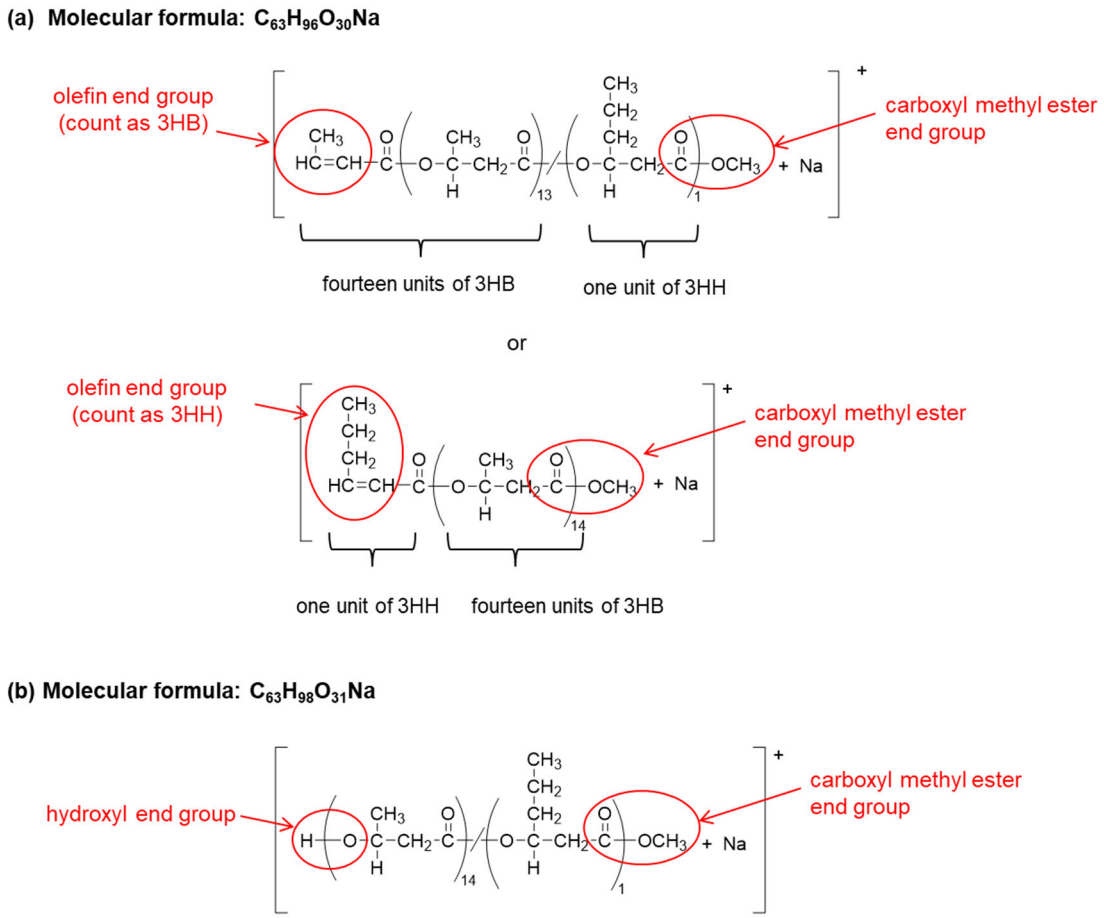


Fig. S4 Chemical structures of the molecular species (PHBH oligomers) corresponding to the molecular formulae (a) $C_{63}H_{96}O_{30}Na$ and (b) $C_{63}H_{98}O_{31}Na$. For (a), two possible chemical structures exist, both sharing the identical monomer composition (fourteen units of 3HB and one unit of 3HH).

S-5. Comparison of mass spectral data measured for PHBH11 oligomers prepared with amylene-stabilised chloroform and reagent-grade chloroform

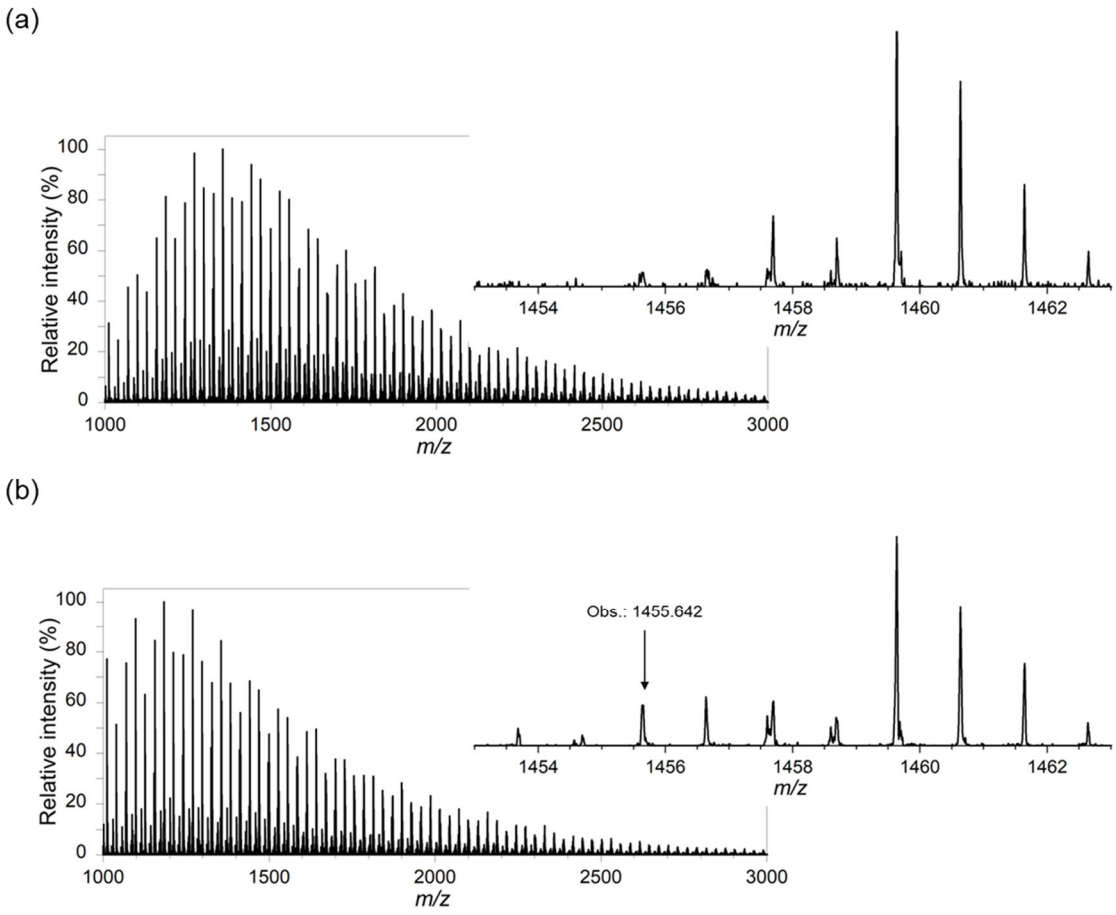


Fig. S5 High-resolution mass spectra of the PHBH11 oligomers prepared with (a) amylene-stabilised chloroform and (b) reagent-grade chloroform in the *m/z* range of 1000 to 3000. The inset shows the expansion of the *m/z* range, 1453 to 1463.

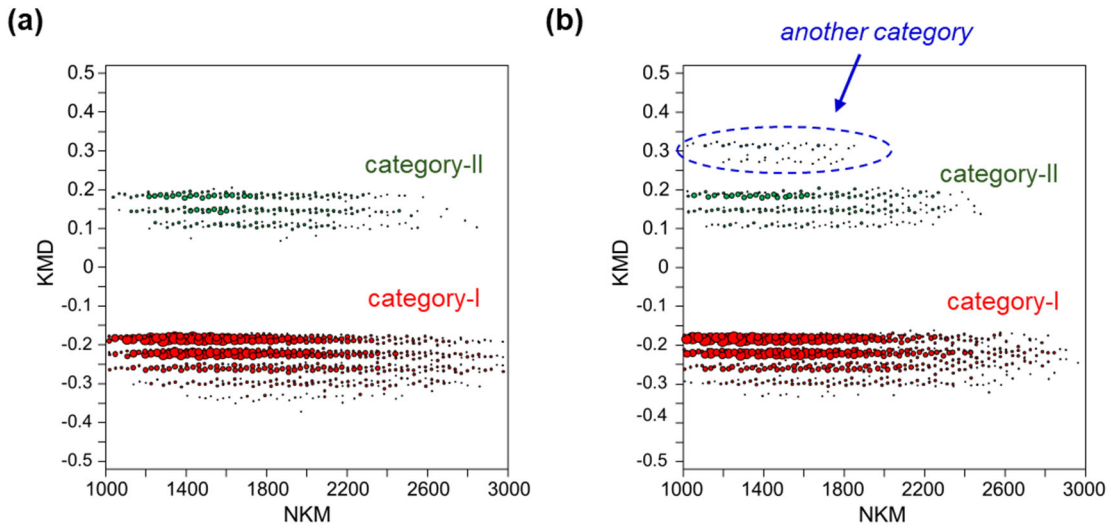


Fig. S6 Resolution-enhanced KMD plots processed from the mass spectral data of the PHBH11 oligomers prepared with (a) amylene-stabilised chloroform and (b) reagent-grade chloroform. For comparison, both the mass spectral data were subjected to KMD analysis under the identical conditions using the accurate mass of 3HB (86.036 Da) as a base unit and a divisor value (X) of $X = 89$. For clarity, the KMD values were uniformly shifted by +0.1.

Notably, in Fig. S6(b), the data points are separated into categories I and II, which are also shown in Fig. S6(a). A representative data point was selected for another category (NKM = 1506, KMD = 0.214) and the corresponding *m/z* value was confirmed to be 1455.642. This is consistent with the molecular formula $C_{68}H_{104}O_{32}Na$ (theoretical mass: 1455.640 Da), which can be assigned to the sodium adduct of the PHBH 16-mer comprising fifteen units of 3HB and one unit of 3HH with olefin/carboxy ethyl ester end groups. Two possible chemical structures are shown in Fig. S7.

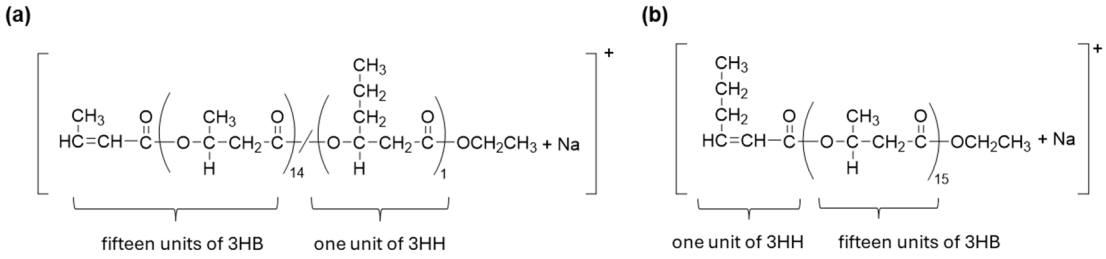
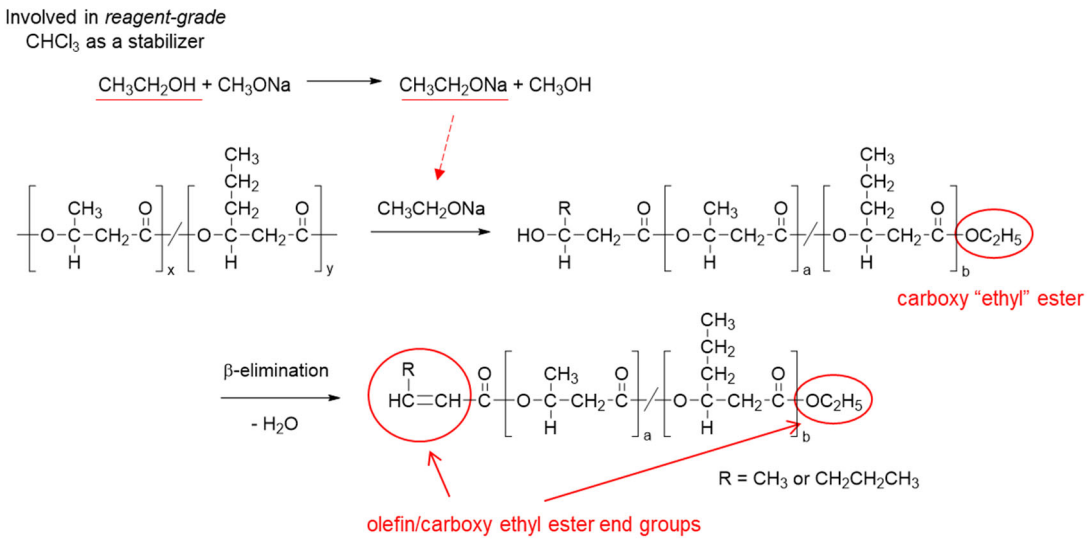


Fig. S7 Possible chemical structures of the PHBH oligomers corresponding to the molecular formula $C_{68}H_{104}O_{32}Na$.

Given that the use of ethanol-free grade chloroform (i.e., amylene-stabilised grade) did not result in the formation of unintended by-products, as demonsrated in Fig. S7, we formulated the generation scheme for the unintended PHBH11 oligomers with olefin/carboxy ethyl ester end groups, as illustrated in Scheme S1.



Scheme S1 Expected formation pathway of unintended PHBH11 oligomers with olefin/carboxyl ethyl ester end groups.

S-6. Molecular formulae of PHBH oligomers (16, 19, 21, and 24-mers) for all possible monomer compositions and end groups

Table S1-1 List of molecular formula estimated for PHBH 16-mer, 19-mer, 21-mer, and 24-mer with olefin/carboxyl methyl ester end-groups (type I)

16-mer			19-mer			21-mer			24-mer		
3HB (m)	3HH (n)	Molecular formula	3HB (m)	3HH (n)	Molecular formula	3HB (m)	3HH (n)	Molecular formula	3HB (m)	3HH (n)	Molecular formula
16	0	$C_{65}H_{98}O_{32}$	19	0	$C_{77}H_{116}O_{38}$	21	0	$C_{85}H_{128}O_{42}$	24	0	$C_{97}H_{146}O_{48}$
15	1	$C_{67}H_{102}O_{32}$	18	1	$C_{79}H_{120}O_{38}$	20	1	$C_{87}H_{132}O_{42}$	23	1	$C_{99}H_{150}O_{48}$
14	2	$C_{69}H_{106}O_{32}$	17	2	$C_{81}H_{124}O_{38}$	19	2	$C_{89}H_{136}O_{42}$	22	2	$C_{101}H_{154}O_{48}$
13	3	$C_{71}H_{110}O_{32}$	16	3	$C_{83}H_{128}O_{38}$	18	3	$C_{91}H_{140}O_{42}$	21	3	$C_{103}H_{158}O_{48}$
12	4	$C_{73}H_{114}O_{32}$	15	4	$C_{85}H_{132}O_{38}$	17	4	$C_{93}H_{144}O_{42}$	20	4	$C_{105}H_{162}O_{48}$
11	5	$C_{75}H_{118}O_{32}$	14	5	$C_{87}H_{136}O_{38}$	16	5	$C_{95}H_{148}O_{42}$	19	5	$C_{107}H_{166}O_{48}$
10	6	$C_{77}H_{122}O_{32}$	13	6	$C_{89}H_{140}O_{38}$	15	6	$C_{97}H_{152}O_{42}$	18	6	$C_{109}H_{170}O_{48}$
9	7	$C_{79}H_{126}O_{32}$	12	7	$C_{91}H_{144}O_{38}$	14	7	$C_{99}H_{156}O_{42}$	17	7	$C_{111}H_{174}O_{48}$
8	8	$C_{81}H_{130}O_{32}$	11	8	$C_{93}H_{148}O_{38}$	13	8	$C_{101}H_{160}O_{42}$	16	8	$C_{113}H_{178}O_{48}$
7	9	$C_{83}H_{134}O_{32}$	10	9	$C_{95}H_{152}O_{38}$	12	9	$C_{103}H_{164}O_{42}$	15	9	$C_{115}H_{182}O_{48}$
6	10	$C_{85}H_{138}O_{32}$	9	10	$C_{97}H_{156}O_{38}$	11	10	$C_{105}H_{168}O_{42}$	14	10	$C_{117}H_{186}O_{48}$
5	11	$C_{87}H_{142}O_{32}$	8	11	$C_{99}H_{160}O_{38}$	10	11	$C_{107}H_{172}O_{42}$	13	11	$C_{119}H_{190}O_{48}$
4	12	$C_{89}H_{146}O_{32}$	7	12	$C_{101}H_{164}O_{38}$	9	12	$C_{109}H_{176}O_{42}$	12	12	$C_{121}H_{194}O_{48}$
3	13	$C_{91}H_{150}O_{32}$	6	13	$C_{103}H_{168}O_{38}$	8	13	$C_{111}H_{180}O_{42}$	11	13	$C_{123}H_{198}O_{48}$
2	14	$C_{93}H_{154}O_{32}$	5	14	$C_{105}H_{172}O_{38}$	7	14	$C_{113}H_{184}O_{42}$	10	14	$C_{125}H_{202}O_{48}$
1	15	$C_{95}H_{158}O_{32}$	4	15	$C_{107}H_{176}O_{38}$	6	15	$C_{115}H_{188}O_{42}$	9	15	$C_{127}H_{206}O_{48}$
0	16	$C_{97}H_{162}O_{32}$	3	16	$C_{109}H_{180}O_{38}$	5	16	$C_{117}H_{192}O_{42}$	8	16	$C_{129}H_{210}O_{48}$
Carbon number		65–97	2	17	$C_{111}H_{184}O_{38}$	4	17	$C_{119}H_{196}O_{42}$	7	17	$C_{131}H_{214}O_{48}$
			1	18	$C_{113}H_{188}O_{38}$	3	18	$C_{121}H_{200}O_{42}$	6	18	$C_{133}H_{218}O_{48}$
			0	19	$C_{115}H_{192}O_{38}$	2	19	$C_{123}H_{204}O_{42}$	5	19	$C_{135}H_{222}O_{48}$
			Carbon number		77–115	1	20	$C_{125}H_{208}O_{42}$	4	20	$C_{137}H_{226}O_{48}$
						0	21	$C_{127}H_{212}O_{42}$	3	21	$C_{139}H_{230}O_{48}$
						Carbon number		85–127	2	22	$C_{141}H_{234}O_{48}$
									1	23	$C_{143}H_{238}O_{48}$
									0	24	$C_{145}H_{242}O_{48}$
									Carbon number		97–145

type I

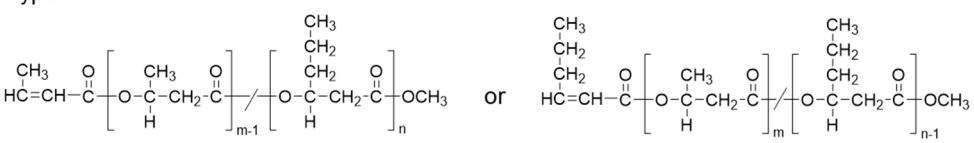
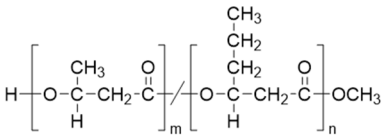


Table S1-2 List of molecular formula estimated for PHBH 16-mer, 19-mer, 21-mer, and 24-mer with hydroxyl/carboxyl methyl ester end-groups (type II)

16-mer			**19-mer**			**21-mer**			**24-mer**		
3HB (m)	3HH (n)	Molecular formula	3HB (m)	3HH (n)	Molecular formula	3HB (m)	3HH (n)	Molecular formula	3HB (m)	3HH (n)	Molecular formula
16	0	$C_{65}H_{100}O_{33}$	19	0	$C_{77}H_{118}O_{39}$	21	0	$C_{85}H_{130}O_{43}$	24	0	$C_{97}H_{148}O_{49}$
15	1	$C_{67}H_{104}O_{33}$	18	1	$C_{79}H_{122}O_{39}$	20	1	$C_{87}H_{134}O_{43}$	23	1	$C_{99}H_{152}O_{49}$
14	2	$C_{69}H_{108}O_{33}$	17	2	$C_{81}H_{126}O_{39}$	19	2	$C_{89}H_{138}O_{43}$	22	2	$C_{101}H_{156}O_{49}$
13	3	$C_{71}H_{112}O_{33}$	16	3	$C_{83}H_{130}O_{39}$	18	3	$C_{91}H_{142}O_{43}$	21	3	$C_{103}H_{160}O_{49}$
12	4	$C_{73}H_{116}O_{33}$	15	4	$C_{85}H_{134}O_{39}$	17	4	$C_{93}H_{146}O_{43}$	20	4	$C_{105}H_{164}O_{49}$
11	5	$C_{75}H_{120}O_{33}$	14	5	$C_{87}H_{138}O_{39}$	16	5	$C_{95}H_{150}O_{43}$	19	5	$C_{107}H_{168}O_{49}$
10	6	$C_{77}H_{124}O_{33}$	13	6	$C_{89}H_{142}O_{39}$	15	6	$C_{97}H_{154}O_{43}$	18	6	$C_{109}H_{172}O_{49}$
9	7	$C_{79}H_{128}O_{33}$	12	7	$C_{91}H_{146}O_{39}$	14	7	$C_{99}H_{158}O_{43}$	17	7	$C_{111}H_{176}O_{49}$
8	8	$C_{81}H_{132}O_{33}$	11	8	$C_{93}H_{150}O_{39}$	13	8	$C_{101}H_{162}O_{43}$	16	8	$C_{113}H_{182}O_{49}$
7	9	$C_{83}H_{136}O_{33}$	10	9	$C_{95}H_{154}O_{39}$	12	9	$C_{103}H_{166}O_{43}$	15	9	$C_{115}H_{186}O_{49}$
6	10	$C_{85}H_{140}O_{33}$	9	10	$C_{97}H_{158}O_{39}$	11	10	$C_{105}H_{170}O_{43}$	14	10	$C_{117}H_{190}O_{49}$
5	11	$C_{87}H_{144}O_{33}$	8	11	$C_{99}H_{162}O_{39}$	10	11	$C_{107}H_{174}O_{43}$	13	11	$C_{119}H_{194}O_{49}$
4	12	$C_{89}H_{148}O_{33}$	7	12	$C_{101}H_{166}O_{39}$	9	12	$C_{109}H_{178}O_{43}$	12	12	$C_{121}H_{198}O_{49}$
3	13	$C_{91}H_{152}O_{33}$	6	13	$C_{103}H_{170}O_{39}$	8	13	$C_{111}H_{182}O_{43}$	11	13	$C_{123}H_{202}O_{49}$
2	14	$C_{93}H_{156}O_{33}$	5	14	$C_{105}H_{174}O_{39}$	7	14	$C_{113}H_{186}O_{43}$	10	14	$C_{125}H_{206}O_{49}$
1	15	$C_{95}H_{160}O_{33}$	4	15	$C_{107}H_{178}O_{39}$	6	15	$C_{115}H_{190}O_{43}$	9	15	$C_{127}H_{210}O_{49}$
0	16	$C_{97}H_{164}O_{33}$	3	16	$C_{109}H_{182}O_{39}$	5	16	$C_{117}H_{194}O_{43}$	8	16	$C_{129}H_{214}O_{49}$
Carbon number		65–97	2	17	$C_{111}H_{186}O_{39}$	4	17	$C_{119}H_{198}O_{43}$	7	17	$C_{131}H_{218}O_{49}$
			1	18	$C_{113}H_{190}O_{39}$	3	18	$C_{121}H_{202}O_{43}$	6	18	$C_{133}H_{222}O_{49}$
			0	19	$C_{115}H_{194}O_{39}$	2	19	$C_{123}H_{206}O_{43}$	5	19	$C_{135}H_{226}O_{49}$
			Carbon number		77–115	1	20	$C_{125}H_{210}O_{43}$	4	20	$C_{137}H_{230}O_{49}$
						0	21	$C_{127}H_{214}O_{43}$	3	21	$C_{139}H_{234}O_{49}$
						Carbon number		85–127	2	22	$C_{141}H_{238}O_{49}$
									1	23	$C_{143}H_{242}O_{49}$
									0	24	$C_{145}H_{246}O_{49}$
									Carbon number		97–145

type II



S-7. High-resolution mass spectrum data of the PHBH6 oligomer

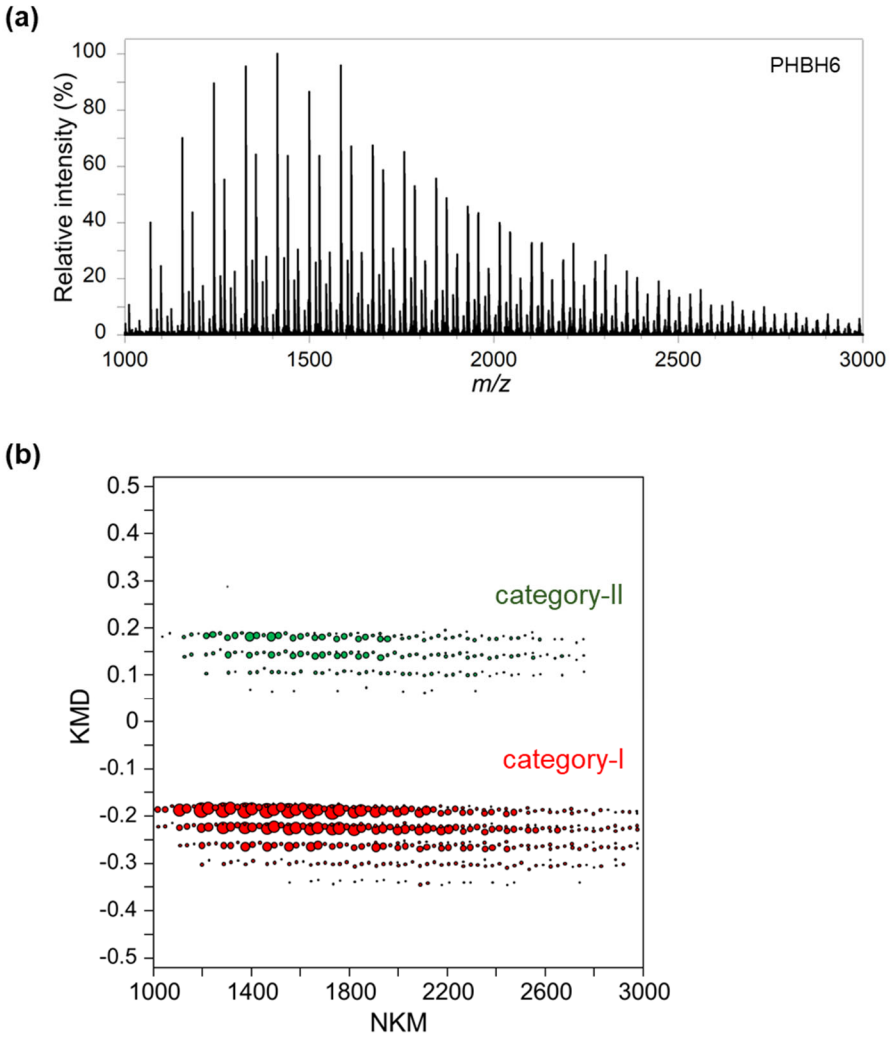


Fig. S8 (a) High-resolution mass spectrum of the PHBH6 oligomer acquired using the positive ion mode. THAP was used as a matrix. (b) Resolution-enhanced KMD plot processed from the mass spectral data of (a). The KMD analysis was conducted using the accurate mass of 3HB (86.036 Da) as a base unit and setting the divisor value (X) to $X = 89$. For clarity, the KMD values were uniformly shifted by +0.1.

Table S2 List of ion peaks observed for the PHBH6 oligomer corresponding to the calculated masses of the PHBH 21-mers

Symbol	Molecular formula*	*m/z* values		Error (ppm)	Peak area (–)
		calc.*	obs.		
Type I					
$B_{21}H_0$(I)	$C_{85}H_{128}O_{42}Na$	1843.777	1843.776	0.5	80430
$B_{20}H_1$(I)	$C_{87}H_{132}O_{42}Na$	1871.809	1871.808	0.5	74545
$B_{19}H_2$(I)	$C_{89}H_{136}O_{42}Na$	1899.840	1899.840	0	34576
$B_{18}H_3$(I)	$C_{91}H_{140}O_{42}Na$	1927.871	1927.871	0	15452
$B_{17}H_4$(I)	$C_{93}H_{144}O_{42}Na$	1955.902	1955.900	1.0	7001
Type II					
$B_{21}H_0$(II)	$C_{85}H_{130}O_{43}Na$	1861.788	1861.788	0	24193
$B_{20}H_1$(II)	$C_{87}H_{134}O_{43}Na$	1889.819	1889.822	1.6	22504
$B_{19}H_2$(II)	$C_{89}H_{138}O_{43}Na$	1917.850	1917.851	0.5	11584
$B_{18}H_3$(II)	$C_{91}H_{142}O_{43}Na$	1945.882	1945.882	0	5479

*The molecular formula and calculated mass are shown assuming that the corresponding molecular ion is a sodium adduct ($[M + Na]^+$).

S-8. High-resolution mass spectrum data of the PHBH13 oligomer

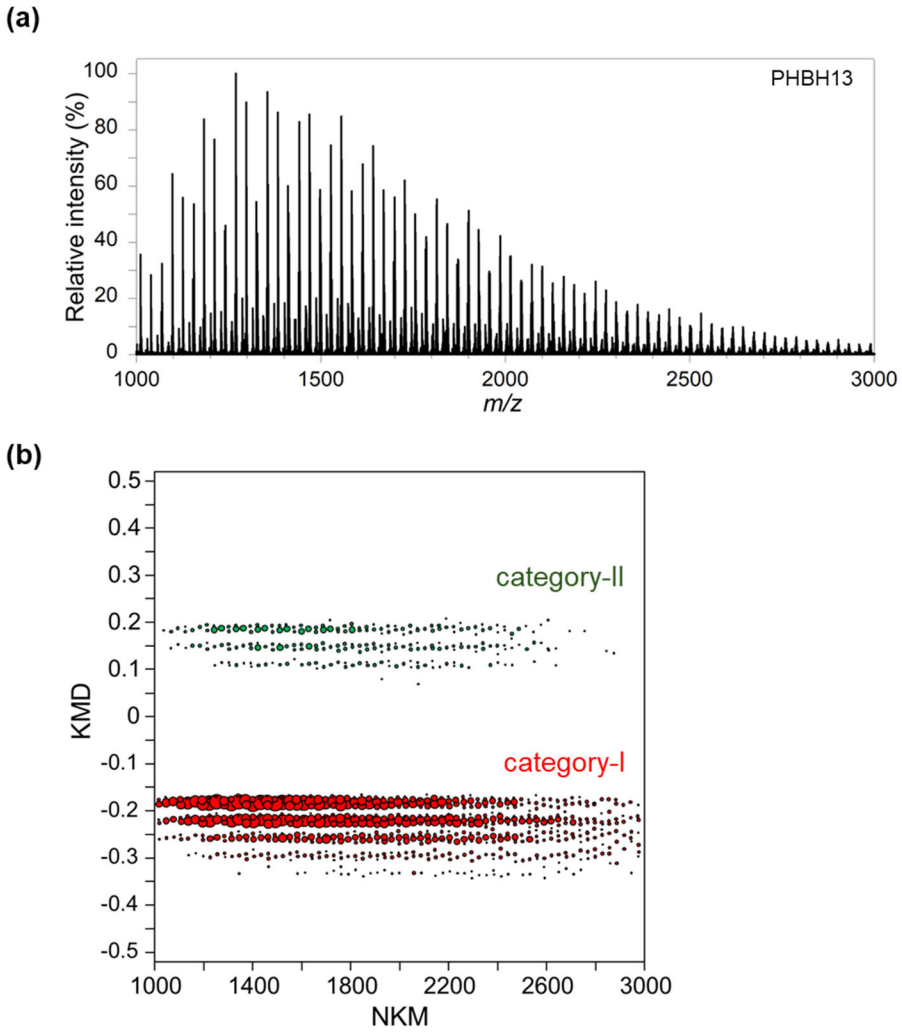


Fig. S9 (a) High-resolution mass spectrum of the PHBH13 oligomer acquired using the positive ion mode. THAP was used as a matrix. (b) Resolution-enhanced KMD plot processed from the mass spectral data of (a). The KMD analysis was conducted using the accurate mass of 3HB (86.036 Da) as a base unit and setting the divisor value (X) to $X = 89$. For clarity, the KMD values were uniformly shifted by +0.1.

Table S3 List of ion peaks observed for the PHBH13 oligomer corresponding to the calculated masses of PHBH 21-mers

Symbol	Molecular formula*	*m/z* values		Error (ppm)	Peak area (–)
		calc.*	obs.		
Type I					
$B_{21}H_0$(I)	$C_{85}H_{128}O_{42}Na$	1843.777	1843.777	0	16966
$B_{20}H_1$(I)	$C_{87}H_{132}O_{42}Na$	1871.809	1871.808	0.5	34159
$B_{19}H_2$(I)	$C_{89}H_{136}O_{42}Na$	1899.840	1899.839	0.5	47630
$B_{18}H_3$(I)	$C_{91}H_{140}O_{42}Na$	1927.871	1927.871	0	42224
$B_{17}H_4$(I)	$C_{93}H_{144}O_{42}Na$	1955.902	1955.901	0.5	29525
$B_{16}H_5$(I)	$C_{95}H_{148}O_{42}Na$	1983.934	1983.932	1.0	16060
$B_{15}H_6$(I)	$C_{97}H_{152}O_{42}Na$	2011.965	2011.967	1.0	8530
$B_{14}H_7$(I)	$C_{99}H_{156}O_{42}Na$	2039.996	2039.992	2.0	4733
Type II					
$B_{21}H_0$(II)	$C_{85}H_{130}O_{43}Na$	1861.788	1861.788	0	3458
$B_{20}H_1$(II)	$C_{87}H_{134}O_{43}Na$	1889.819	1889.820	0.5	8599
$B_{19}H_2$(II)	$C_{89}H_{138}O_{43}Na$	1917.850	1917.850	0	12474
$B_{18}H_3$(II)	$C_{91}H_{142}O_{43}Na$	1945.882	1945.882	0	11056
$B_{17}H_4$(II)	$C_{93}H_{146}O_{43}Na$	1973.913	1973.910	1.5	6191
$B_{16}H_5$(II)	$C_{95}H_{150}O_{43}Na$	2001.944	2001.949	2.5	3790

*The molecular formula and calculated mass are shown, assuming that the corresponding molecular ion is a sodium adduct ($[M + Na]^+$).

S-9. 3HH contents determined for original PHBH samples and PHBH 21-mers

Table S4 Comparison of 3HH contents determined for original PHBH samples and PHBH 21-mers

Code	Original PHBH sample (mol%)[a]	PHBH 21-mer (mol%)[b]
PHBH6	5.5	4.9 ± 0.1
PHBH11	10.5	10.1 ± 0.2
PHBH13	12.5	12.8 ± 0.2

a. Values from Table 1 in the main text (determined from ^{1}H-NMR spectrum (Fig. S1)). b. Determined from the number distribution data shown in Fig.5(b).

S-10. High-resolution mass spectral data of the PHBH11 16-mer and 19-mer

Table S5 List of ion peaks observed for the PHBH11 oligomer corresponding to the calculated masses of PHBH 16-mers

Symbol	Molecular formula*	*m/z* values		Error (ppm)	Peak area (–)
		calc.*	obs.		
Type I					
$B_{16}H_0$(I)	$C_{65}H_{98}O_{32}Na$	1413.593	1413.593	0	74364
$B_{15}H_1$(I)	$C_{67}H_{102}O_{32}Na$	1441.625	1441.625	0	86639
$B_{14}H_2$(I)	$C_{69}H_{106}O_{32}Na$	1469.656	1469.656	0	81140
$B_{13}H_3$(I)	$C_{71}H_{110}O_{32}Na$	1497.687	1497.689	1.3	43438
$B_{12}H_4$(I)	$C_{73}H_{114}O_{32}Na$	1525.719	1525.718	0.7	22312
$B_{11}H_5$(I)	$C_{75}H_{118}O_{32}Na$	1553.750	1553.749	0.6	8459
Type II					
$B_{16}H_0$(II)	$C_{65}H_{100}O_{33}Na$	1431.604	1431.606	1.4	19794
$B_{15}H_1$(II)	$C_{67}H_{104}O_{33}Na$	1459.635	1459.636	0.7	21846
$B_{14}H_2$(II)	$C_{69}H_{108}O_{33}Na$	1487.667	1487.668	0.7	20214
$B_{13}H_3$(II)	$C_{71}H_{112}O_{33}Na$	1515.698	1515.697	0.7	11405
$B_{12}H_4$(II)	$C_{73}H_{116}O_{33}Na$	1543.729	1543.728	0.7	8023
$B_{11}H_5$(II)	$C_{75}H_{120}O_{33}Na$	1571.760	1571.751	5.7	3835

*The molecular formula and calculated mass are shown, assuming that the corresponding molecular ion is a sodium adduct ($[M + Na]^+$).

Table S6 List of ion peaks observed for the PHBH11 oligomer corresponding to the calculated masses of PHBH 19-mers

Symbol	Molecular formula*	*m/z* values		Error (ppm)	Peak area (–)
		calc.*	obs.		
Type I					
$B_{19}H_0$(I)	$C_{77}H_{116}O_{38}Na$	1671.704	1671.704	0	39009
$B_{18}H_1$(I)	$C_{79}H_{120}O_{38}Na$	1699.735	1699.736	0.6	48094
$B_{17}H_2$(I)	$C_{81}H_{124}O_{38}Na$	1727.766	1727.767	0.6	51674
$B_{16}H_3$(I)	$C_{83}H_{128}O_{38}Na$	1755.798	1755.798	0	38012
$B_{15}H_4$(I)	$C_{85}H_{132}O_{38}Na$	1783.829	1783.828	0.6	22341
$B_{14}H_5$(I)	$C_{87}H_{136}O_{38}Na$	1811.860	1811.860	0	10064
$B_{13}H_6$(I)	$C_{89}H_{140}O_{38}Na$	1839.891	1839.890	0.5	3790
Type II					
$B_{19}H_0$(II)	$C_{77}H_{118}O_{39}Na$	1689.714	1689.714	0	11528
$B_{18}H_1$(II)	$C_{79}H_{122}O_{39}Na$	1717.746	1717.747	0.6	14280
$B_{17}H_2$(II)	$C_{81}H_{126}O_{39}Na$	1745.777	1745.775	1.1	12740
$B_{16}H_3$(II)	$C_{83}H_{130}O_{39}Na$	1773.808	1773.808	0	12832
$B_{15}H_4$(II)	$C_{85}H_{134}O_{39}Na$	1801.839	1801.839	0	6956

*The molecular formula and calculated mass are shown, assuming that the corresponding molecular ion is a sodium adduct ($[M + Na]^+$).

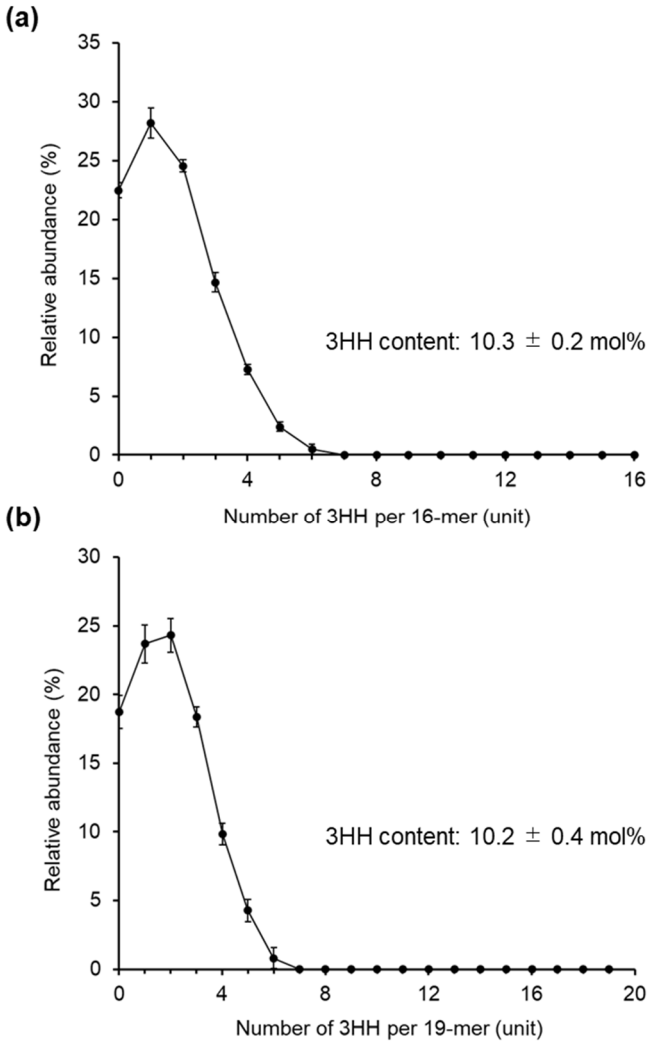


Fig. S10 Number distribution plots of 3HH within (a) PHBH11 16-mer and (b) PHBH11 19-mer. The plots were generated based on the mass spectral data acquired from five distinct sample spots (n = 5, ± S.D.). The values of 3HH content represented in each figure were estimated based on the number distribution data.

S-11. Biodegradation experiment of the PHBH films in seawater

Natural seawater was collected from the shoreline of Tokyo Bay using a bucket. The collected seawater was filtered through a 10 μm membrane filter (ADVANTEC Co., Ltd., Tokyo, Japan) and used for biodegradation experiments on the day of collection. The degree of biodegradation of PHBH films in seawater was evaluated using a microbial oxidative degradation analyser (MODA-MA) manufactured by Yahata-Bussan Co., Ltd. (Shizuoka, Japan). This system allows evaluation of the degree of biodegradation of the specimen in seawater at a controlled temperature by measuring the amount of CO_2 evolved during the test period. An outline of the MODA-MA system is presented in Fig. S11.

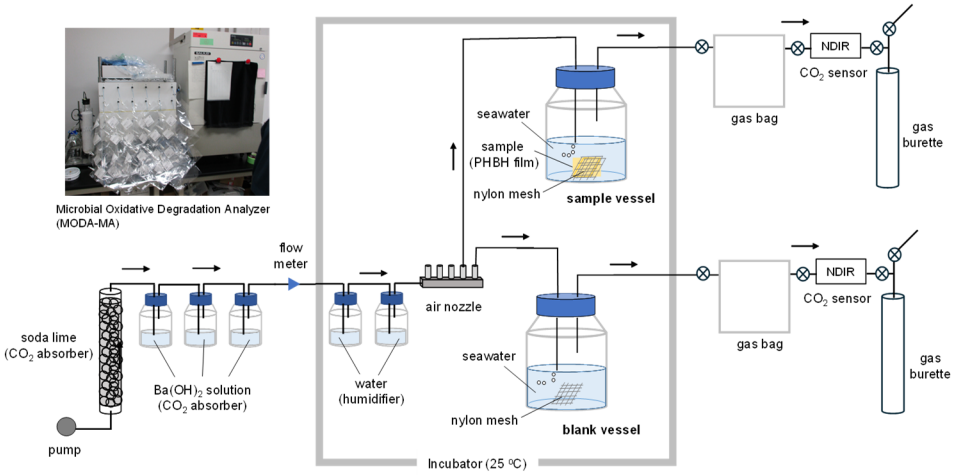


Fig. S11 Outline of the MODA-MA system used in this study.

PHBH film (size: 40 mm × 40 mm, thickness: 35 μm) sandwiched between a pair of nylon meshes (mesh size: 3.3 mm) and natural seawater (200 mL) were sealed in the 500-mL test vessel and incubated at 25 °C. A nylon mesh without the PHBH film was sealed with natural seawater in other test vessels as a blank. The CO_2 that evolved in each test vessel was collected separately in sampling bags by introducing CO_2-free and water-saturated air (approximately 0.3 mL·min^{-1}). The amount of CO_2 evolved in the test vessel was determined by periodically measuring the volume and CO_2 concentration of the collected air using a glass syringe and a non-dispersive infrared gas analyser (NDIR), respectively. The degree of biodegradation (*DB*) of the PHBH film specimens in seawater was evaluated as follows:

$$DB\ (\%) = (CO_{2\ sample} - CO_{2\ blank}) / CO_{2\ theor} \times 100$$

where $CO_{2\ sample}$ is the amount of CO_2 evolved in the sample vessel, and $CO_{2\ blank}$ is the amount of CO_2 evolved in the blank vessel during the immersion of the PHBH films in seawater. $CO_{2\ theor}$ is

the theoretical amount of CO_2 evolution determined based on the actual weight and elemental composition of the PHBH film specimen, assuming complete mineralisation to CO_2.

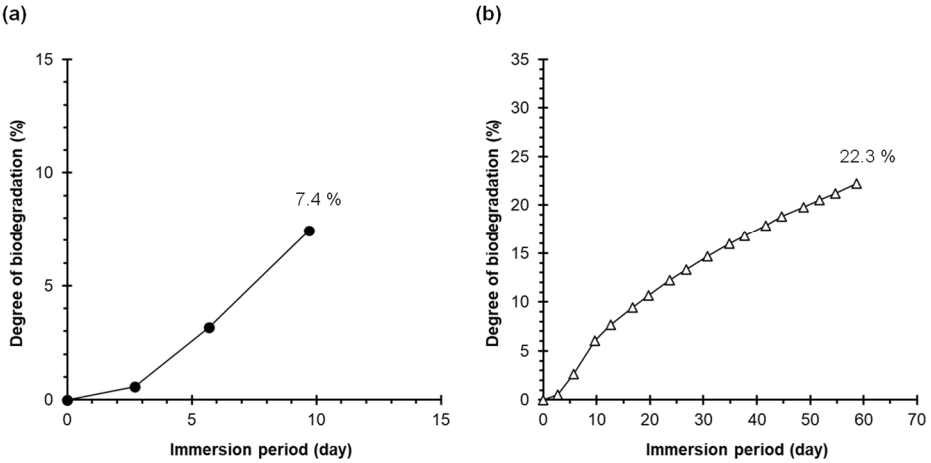


Fig. S12 Biodegradation profile of the PHBH11 film immersed in seawater at 25 °C. The PHBH11 film residues (R11-1 and R11-2) were obtained after the biodegradation experiment of (a) and (b), respectively.

After immersion in seawater, the PHBH film residue was removed from the seawater and sonicated three times for 30 s in approximately 200 mL of pure water to remove the extraneous matter adsorbed on its surface. After being rinsed with pure water, the PHBH film residue was dried in a desiccator containing silica gel for several days at room temperature. The degree of disintegration (*DD*) of the PHBH film was determined by weight loss after seawater immersion.

$$DD\ (\%) = (W_{before} - W_{after}) / W_{before} \times 100$$

where, W_{before} and W_{after} are the weights of the PHBH film before (pristine) and after (residual) immersion in seawater, respectively.

Table S7 Weights and degree of disintegration of the PHBH11 film residues resulting from biodegradation in seawater

Code	Immersion (days)	W_{before} (mg)	W_{after} (mg)	*DD* (%)
R11-1	10	67.0	46.6	27.5
R11-2	59	66.9	30.3	54.7

S-12. SEC traces of the PHBH11 film and biodegraded residues

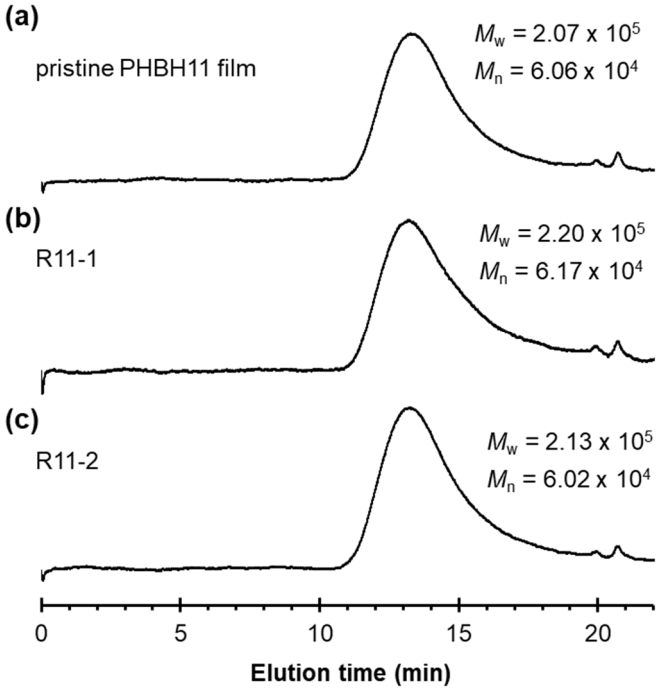


Fig. S13 SEC traces of (a) pristine PHBH11 film, (b) R11-1, and (c) R11-2 dissolved in chloroform. SEC traces were recorded on an RI detector. For a mobile phase, chloroform was used at a flow rate of 1.0 mL·min^{-1} at 40 ℃.

S-13. High-resolution mass spectral data of oligomers prepared from the PHBH11 film residues

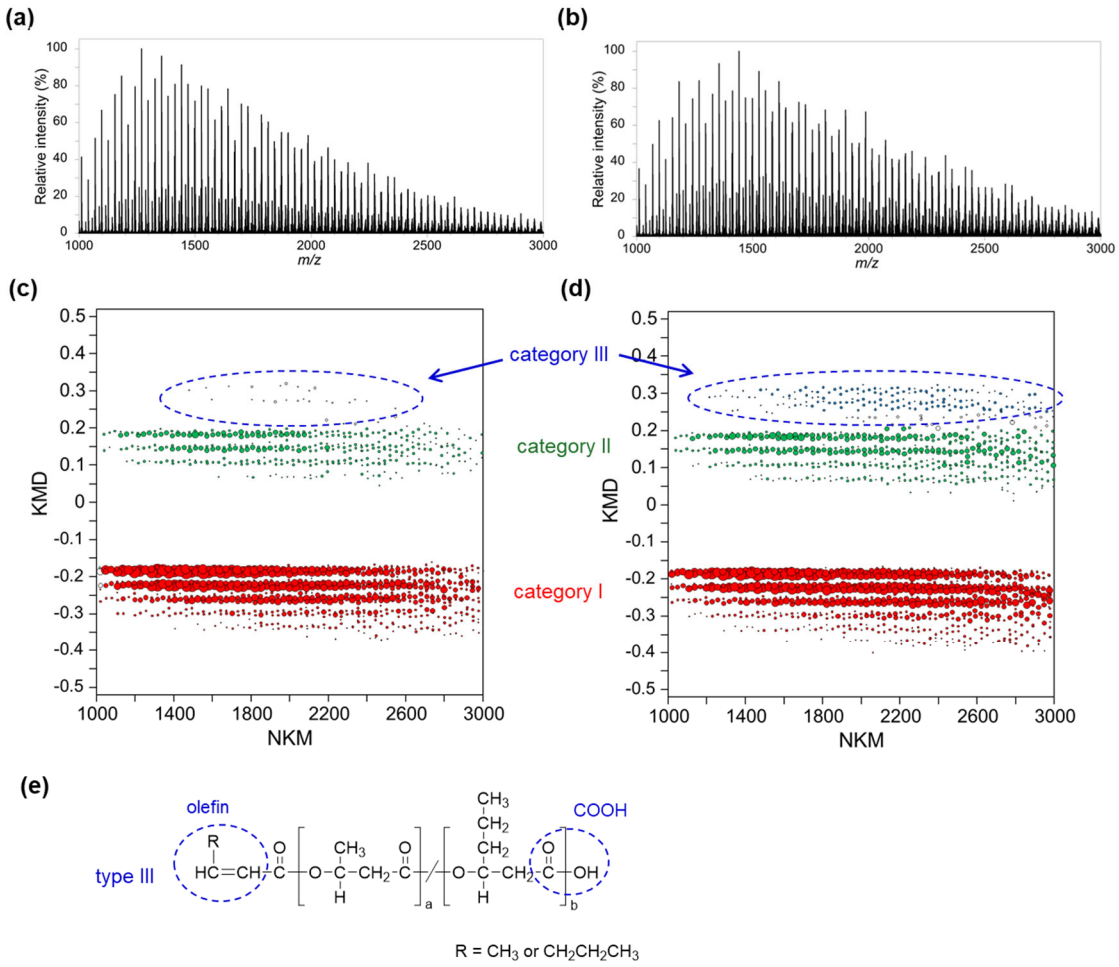


Fig. S14 High-resolution mass spectrum of (a) R11-1 oligomer and (b) R11-2 oligomer acquired using the positive ion mode. THAP was used as a matrix. Resolution-enhanced KMD plot processed from the mass spectral data of (c) R11-1 oligomer and (d) R11-2 oligomer, respectively. The KMD analysis was conducted using the accurate mass of 3HB (86.036 Da) as a base unit and setting the divisor value (X) to $X = 89$. For clarity, the KMD values were uniformly shifted by +0.1.

Both KMD plots revealed the presence of an additional terminal structure (category III) alongside types I and II. Based on the accurate mass values of the representative data points in category III, the chemical structures of the end groups were identified as olefin/carboxylic acid (Fig. S14(e)). Given that the carboxylic acid group was not formed via partial methanolysis of PHBH and was absent in the mass spectral data of the pristine PHBH11 sample, it must have originated from the enzymatically hydrolysed PHBH chains remaining on the surface of the biodegraded residue.

Table S8 List of ion peaks observed for the R11-1 oligomer corresponding to the calculated masses of PHBH 21-mers

Symbol	Molecular formula*	*m/z* values		Error (ppm)	Peak area (–)
		calc.*	obs.		
Type I					
$B_{21}H_0$(I)	$C_{85}H_{128}O_{42}Na$	1843.777	1843.778	0.5	23777
$B_{20}H_1$(I)	$C_{87}H_{132}O_{42}Na$	1871.809	1871.808	0.5	30026
$B_{19}H_2$(I)	$C_{89}H_{136}O_{42}Na$	1899.840	1899.840	0	31593
$B_{18}H_3$(I)	$C_{91}H_{140}O_{42}Na$	1927.871	1927.871	0	25187
$B_{17}H_4$(I)	$C_{93}H_{144}O_{42}Na$	1955.902	1955.904	1.0	16401
$B_{16}H_5$(I)	$C_{95}H_{148}O_{42}Na$	1983.934	1983.935	0.5	7040
$B_{15}H_6$(I)	$C_{97}H_{152}O_{42}Na$	2011.965	2011.966	0.5	3649
Type II					
$B_{21}H_0$(II)	$C_{85}H_{130}O_{43}Na$	1861.788	1861.788	0	6381
$B_{20}H_1$(II)	$C_{87}H_{134}O_{43}Na$	1889.819	1889.818	0.5	8371
$B_{19}H_2$(II)	$C_{89}H_{138}O_{43}Na$	1917.850	1917.849	0.5	8447
$B_{18}H_3$(II)	$C_{91}H_{142}O_{43}Na$	1945.882	1945.880	1.0	7384
$B_{17}H_4$(II)	$C_{93}H_{146}O_{43}Na$	1973.913	1973.914	0.5	5417
$B_{16}H_5$(II)	$C_{95}H_{150}O_{43}Na$	2001.944	2001.947	1.5	3414
Type III					
$B_{21}H_0$(III)	$C_{84}H_{126}O_{42}Na$	1829.762	N.D.**	–	–
$B_{20}H_1$(III)	$C_{86}H_{130}O_{42}Na$	1857.793	N.D.**	–	–
$B_{19}H_2$(III)	$C_{88}H_{134}O_{42}Na$	1885.824	1885.824	0	1837
$B_{18}H_3$(III)	$C_{90}H_{138}O_{42}Na$	1913.855	1913.851	2.1	2790

*The molecular formula and calculated mass are shown, assuming that the corresponding molecular ion is a sodium adduct ($[M + Na]^+$). **Not detected.

Table S9 List of ion peaks observed for the R11-2 oligomers corresponding to the calculated masses of PHBH 21-mers

Symbol	Molecular formula*	*m/z* values		Error (ppm)	Peak area (–)
		calc.*	obs.		
Type I					
$B_{21}H_0$(I)	$C_{85}H_{128}O_{42}Na$	1843.777	1843.777	0	25199
$B_{20}H_1$(I)	$C_{87}H_{132}O_{42}Na$	1871.809	1871.808	0.5	29737
$B_{19}H_2$(I)	$C_{89}H_{136}O_{42}Na$	1899.840	1899.842	1.1	34227
$B_{18}H_3$(I)	$C_{91}H_{140}O_{42}Na$	1927.871	1927.871	0	26761
$B_{17}H_4$(I)	$C_{93}H_{144}O_{42}Na$	1955.902	1955.904	1.0	14923
$B_{16}H_5$(I)	$C_{95}H_{148}O_{42}Na$	1983.934	1983.934	0	7467
$B_{15}H_6$(I)	$C_{97}H_{152}O_{42}Na$	2011.965	2011.962	1.5	3279
B_{14}H7(I)	$C_{99}H_{156}O_{42}Na$	2039.996	2039.992	2.0	1759
Type II					
$B_{21}H_0$(II)	$C_{85}H_{130}O_{43}Na$	1861.788	1861.788	0	11476
$B_{20}H_1$(II)	$C_{87}H_{134}O_{43}Na$	1889.819	1889.819	0	12655
$B_{19}H_2$(II)	$C_{89}H_{138}O_{43}Na$	1917.850	1917.850	0	12939
$B_{18}H_3$(II)	$C_{91}H_{142}O_{43}Na$	1945.882	1945.880	1.0	10929
$B_{17}H_4$(II)	$C_{93}H_{146}O_{43}Na$	1973.913	1973.915	1.0	5848
$B_{14}H_5$(II)	$C_{95}H_{150}O_{43}Na$	2001.944	2001.944	0	1777
Type III					
$B_{21}H_0$(III)	$C_{84}H_{126}O_{42}Na$	1829.762	1829.769	3.8	2818
$B_{20}H_1$(III)	$C_{86}H_{130}O_{42}Na$	1857.793	1857.795	1.1	4017
$B_{19}H_2$(III)	$C_{88}H_{134}O_{42}Na$	1885.824	1885.825	0.5	3621
$B_{18}H_3$(III)	$C_{90}H_{138}O_{42}Na$	1913.855	1913.858	1.6	2743
$B_{17}H_4$(III)	$C_{92}H_{142}O_{42}Na$	1941.887	1941.883	2.1	2217

*The molecular formula and calculated mass are shown, assuming that the corresponding molecular ion is a sodium adduct ($[M + Na]^+$).

S-14. ^{1}H-NMR spectra of the PHBH11 film residues

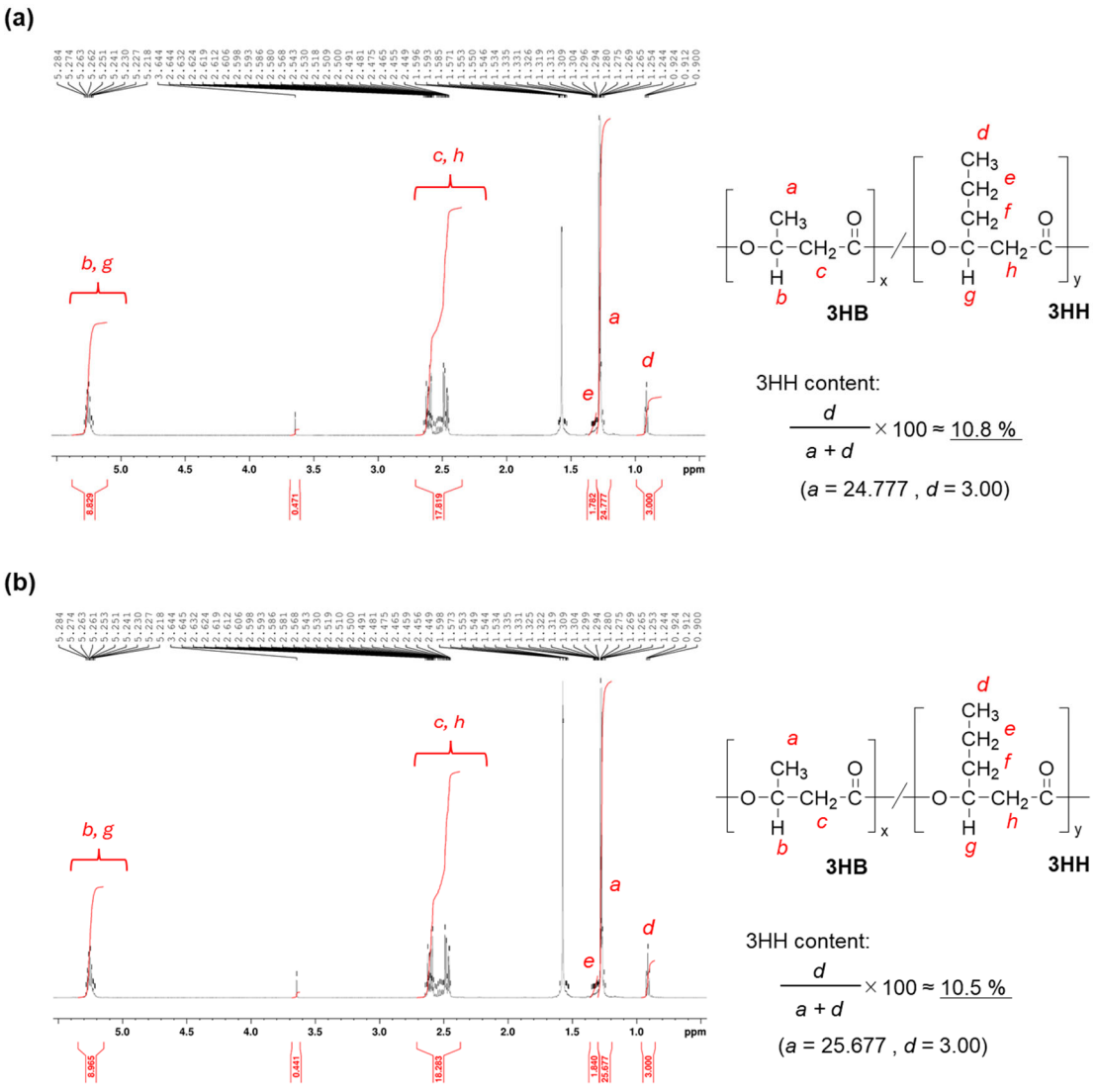


Fig. S15 ^{1}H-NMR (600 MHz) spectra of (a) R11-1 and (b) R11-2 dissolved in $CDCl_3$ containing 0.05 wt% of TMS. Conc.: 3 mg·mL^{-1}. Scans: 8 times, Temp.: r.t.