

Chemical recycling of polyhydroxybutyrate (PHB) into bio-based solvents and their use in a circular PHB extraction

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1. MHB and MMB synthesis and screening conditions

MHB from pure PHB and PHB inclusions

PHB, MeOH and catalyst in different molar ratio were charged in a closed cap glass reactor. The methanolysis reaction was carried out under different time and temperatures. After that, the reaction mixture was cooled to rt, an equimolar amount (respect to the catalyst) of NaOH (in the case of H₂SO₄ and TsOH) or HCl (in the case of NaOH and NaOMe) was added and the solution was stirred at rt for 15 min. The resulting solution was distilled to recover the unreacted methanol (room pressure) and HMB (75-80 °C under reduced pressure of 10 mbar).

Table S1. Optimization of reaction conditions for MHB synthesis from PHB.

Substrate	Catalyst	Cat. loading (mol%)	MeOH (eq.)	T (°C) ^a	Time (h)	Yield (%)
pure PHB	NaOMe	1	20	140	4	4
pure PHB	CaO	1	20	140	4	7
pure PHB	SnCl ₂	1	20	140	4	0
pure PHB	TsOH	1	20	140	4	77
pure PHB	Sn(Bu) ₂ (OAc) ₂	1	20	140	4	85
pure PHB	H ₂ SO ₄	1	20	140	4	88
pure PHB	H ₂ SO ₄	1	20	140	6.5	98
pure PHB	H ₂ SO ₄	1	10	140	7	81
pure PHB	H ₂ SO ₄	1	5	140	7	63
pure PHB	H ₂ SO ₄	0.5	15	140	7	95
pure PHB	H ₂ SO ₄	0.25	15	140	7	61
pure PHB	Sn(Bu) ₂ (OAc) ₂	0.5	15	140	7	87
pure PHB	TsOH	0.5	15	140	7	79
pure PHB	H ₂ SO ₄	0.5	15	70	7	3
SSC-57	H ₂ SO ₄	0.5	15	140	7	10 ^b
SSC-57	H ₂ SO ₄	5	15	140	7	53 ^b
SSC-57	H ₂ SO ₄	10	15	140	7	85 ^b

^a reaction conducted at 140 °C under autogenous pressure . ^b Yields are calculated respect to the PHB content inside bacterial cells.

H₂SO₄ recycle in MHB synthesis

To demonstrate the recyclability of the acidic catalyst (H₂SO₄), the reaction mixture **was not** quenched with NaOH at the end of the reaction: the solution was distilled to remove MeOH and then MHB, the distillation residue and the recovered MeOH were directly reused to re-perform methanolysis on PHB.

Table S2. Catalyst recycle in MHB synthesis.

Cycle	MHB yield (mass, g ; Yield, %) ^a	MeOH recovery (mass, g ; Yield, %) ^b	Distillation Residue (mass, g)
I	2.8; 82	12.8; 98.3	0.58
II	3.1; 91	12.95; 99.5	0.70
III	3.15; 93	12.85; 98.7	0.82
IV	3.23; 95	13; 99.8	0.90
V	3.28; 96	12.95; 99.5	0.95

^a Calculated on input PHB ^b **Input PHB** = 2.5 g; **MeOH** = 13.95g, 15 eq; **Theoretical consumption of MeOH** (if 100% MHB yield) = 930 mg; **recoverable MeOH** (if 100% MHB yield) = 13.02g

MMB from pure PHB and PHB inclusions

Synthesis of MC

Previously synthesized CA,¹ heterogeneous acidic catalyst (wt% respect to CA) and MeOH in different molar ratios were charged in a closed cap glass reactor. The esterification reaction was carried out under different times and temperatures. After that, the solution was filtrated to recover the heterogeneous catalyst. The filtrate was distilled using a Vigreux condenser to recover, firstly unreacted methanol mixed with water (produced during the reaction) at atmospheric pressure, and then MC (b.p.= 118-120 °C).

Table S3. Optimization of reaction conditions for MC synthesis from CA.

Substrate	Catalyst	Cat. Loading (wt %)	MeOH (eq)	T (°C) ^a	Time (h)	GC-MS conversion (%)	Yield (%)
CA	/	/	20	130	5	20	nd
CA	Amberlyst-15	10	15	reflux	24	50	nd
CA	Amberlyst-15	10	20	130	5	>99	nd
CA	Amberlyst-15	10	10	130	5	>99	nd
CA	Amberlyst-15	5	20	130	5	>99	nd
CA	Amberlyst-15	5	10	130	5	>99	nd
CA	Amberlyst-15	2.5	10	130	5	98	nd
CA	Amberlyst-15	5	5	130	5	97.5	nd
CA	Amberlyst-15	2.5	5	130	5	94	84
CA	Amberlyst-15	2.5	5	130	7	98	89
CA	C-SO ₃ H ^b	2.5	5	130	5	96	88
CA	C-SO ₃ H	2.5	5	130	7	>99	93
CA (SSC-57)	C-SO ₃ H	2.5	5	130	7	>99	91

^a reaction conducted at 130 °C under autogenous pressure . ^b C-SO₃ synthesized following a previously reported procedure.²

Synthesis of MMB

MeOH and catalyst were mixed under magnetic stirring in a round bottomed flask at rt for 15 min, then MC was added dropwise over 30 minutes and the solution stirred at different times and temperatures. After that time, the resulting solution was distilled to recover MeOH, unreacted MC and MMB (65-70 °C under reduced pressure of 10 mbar).

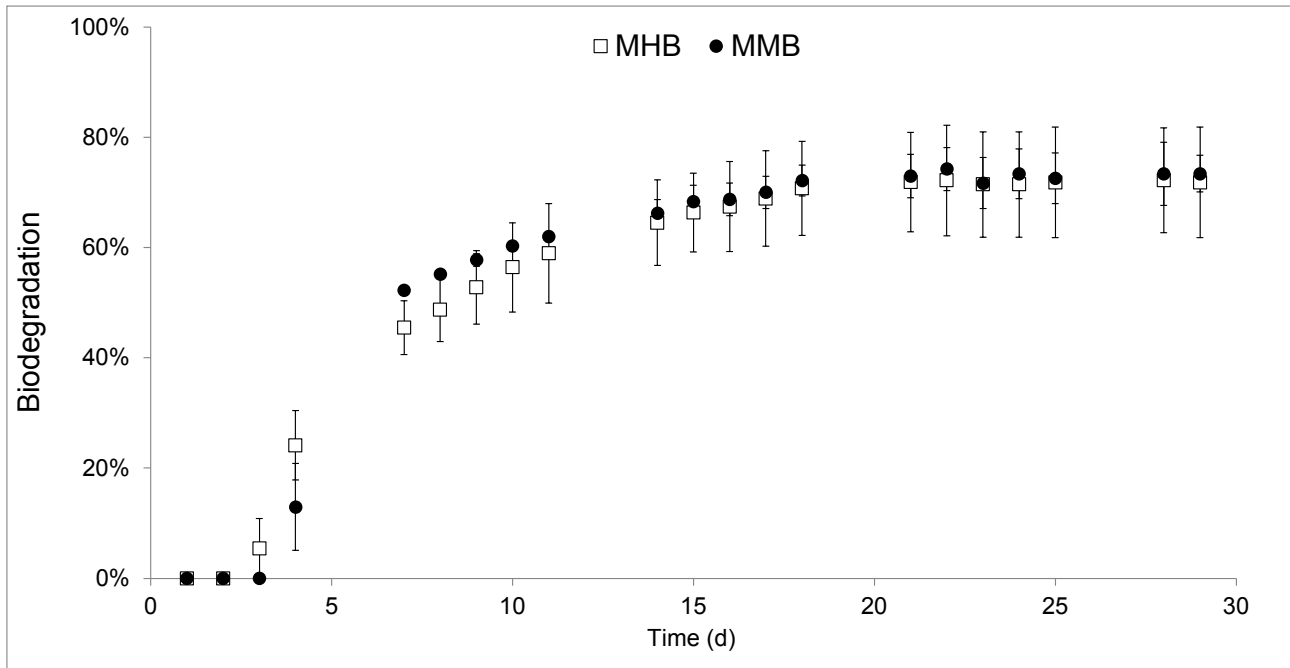
Table S4. Optimization of reaction conditions for MMB synthesis from MC.

Substrate	Catalyst	Cat. Loading (%)	MeOH (eq)	T (°C)	Time (h)	GC-MS conversion (%)	Yield (%)
MC	ZnCl ₂	10	2.5	r.t.	24	0	nd
MC	NaI	10	2.5	r.t.	24	0	nd
MC	Morpholine	10	2.5	r.t.	24	0	nd
MC	DBU	10	2.5	r.t.	24	46	nd
MC	DMCA	10	2.5	r.t.	24	0	nd
MC	CaO	10	2.5	r.t.	24	3	nd
MC	CuO	10	2.5	r.t.	24	0	nd
MC	PBu ₃	10	2.5	r.t.	24	17	nd
MC	Na ₂ CO ₃	10	2.5	r.t.	24	0	nd
MC	K ₂ CO ₃	10	2.5	r.t.	24	82	nd
MC	NaOH	10	2.5	r.t.	24	48	nd
MC	KOH	10	2.5	r.t.	24	42	nd
MC	NaOMe	10	10	r.t.	16	95	90
MC	NaOMe	10	2.5	r.t.	24	98	93
MC	NaOMe	10	2.5	60	24	87	nd
MC	NaOtBu	10	2.5	r.t.	24	97	94
MC	NaOMe	5	2.5	r.t.	24	90	nd
MC	NaOMe	5	2.5	r.t.	50	98	nd
MC	NaOMe	3	2	r.t.	50	98	92
MC	NaOMe	1.5	2	r.t.	50	85	nd
MC (SSC-57)	NaOMe	3	2	r.t.	50	95	90

2. MHB and MMB biodegradability test

Biodegradation test of HMB and MMB were conducted according to OECD guideline 301 f.³

Figure S1. MHB and MMB biodegradation over 28 days.



3. MHB and MMB recyclability test

Table S5. MMB extraction cycle (PHB-to-solvent ratio = 26 mg/mL; Volume tested = 5 mL).

Cycle	PHB recovery (%)	Solvent recovery (%)
1	99	100
2	96	95
3	98	98
4	99	99
5	96	98
6	99	99
7	98	96
8	99	97
9	95	96
10	99	100
11	99	99
12	96	95

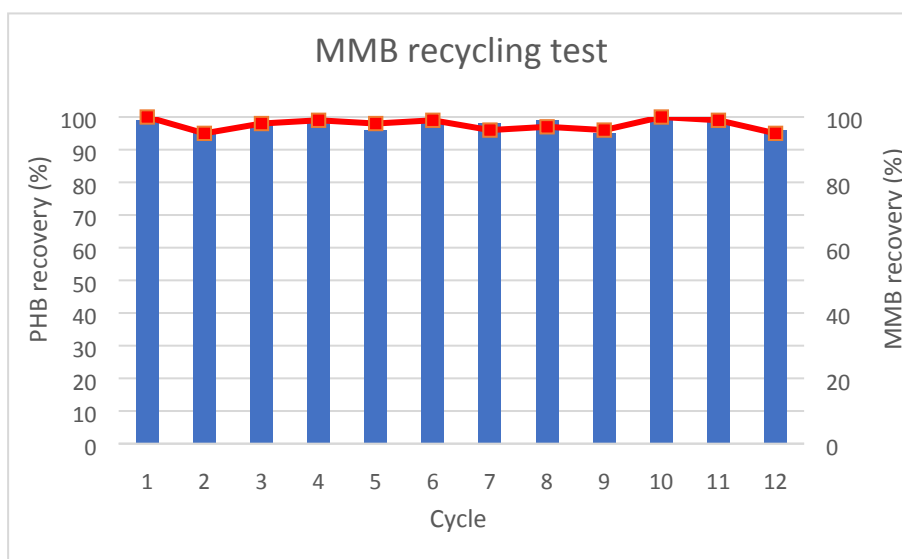
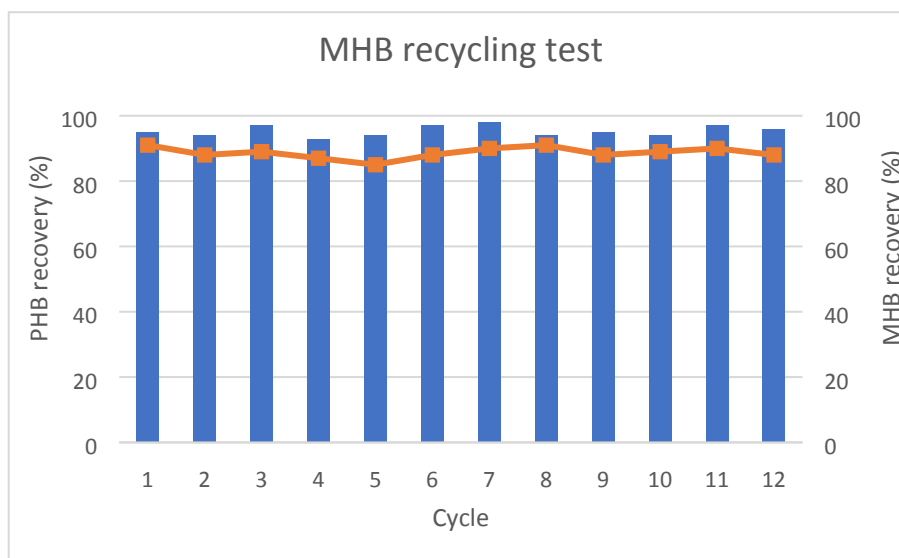
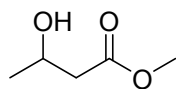


Table S6. MHB extraction recycle (PHB-to-solvent ratio = 26 mg/mL; Volume tested = 5 mL).

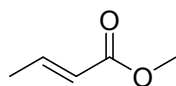
Cycle	PHB recovery (%)	Solvent recovery (%)
1	95	91
2	94	88
3	97	89
4	93	87
5	94	85
6	97	88
7	98	90
8	94	91
9	95	88
10	94	89
11	97	90
12	96	88



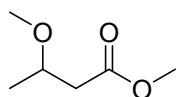
4. Characterization of synthesized products



MHB. Colorless liquid, b.p. = 173-174 °C. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 4.21 – 4.08 (m, 1H), 3.65 (s, 3H), 2.48 – 2.33 (m, 2H), 1.17 (d, $J = 6.3$ Hz, 3H). $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 173.16, 64.16, 51.62, 42.60, 22.42. **Anal. Calc.** for ($\text{C}_5\text{H}_{10}\text{O}_3$; 118.06): C, 50.84; H, 8.53; found: C, 50.79, H, 8.86.



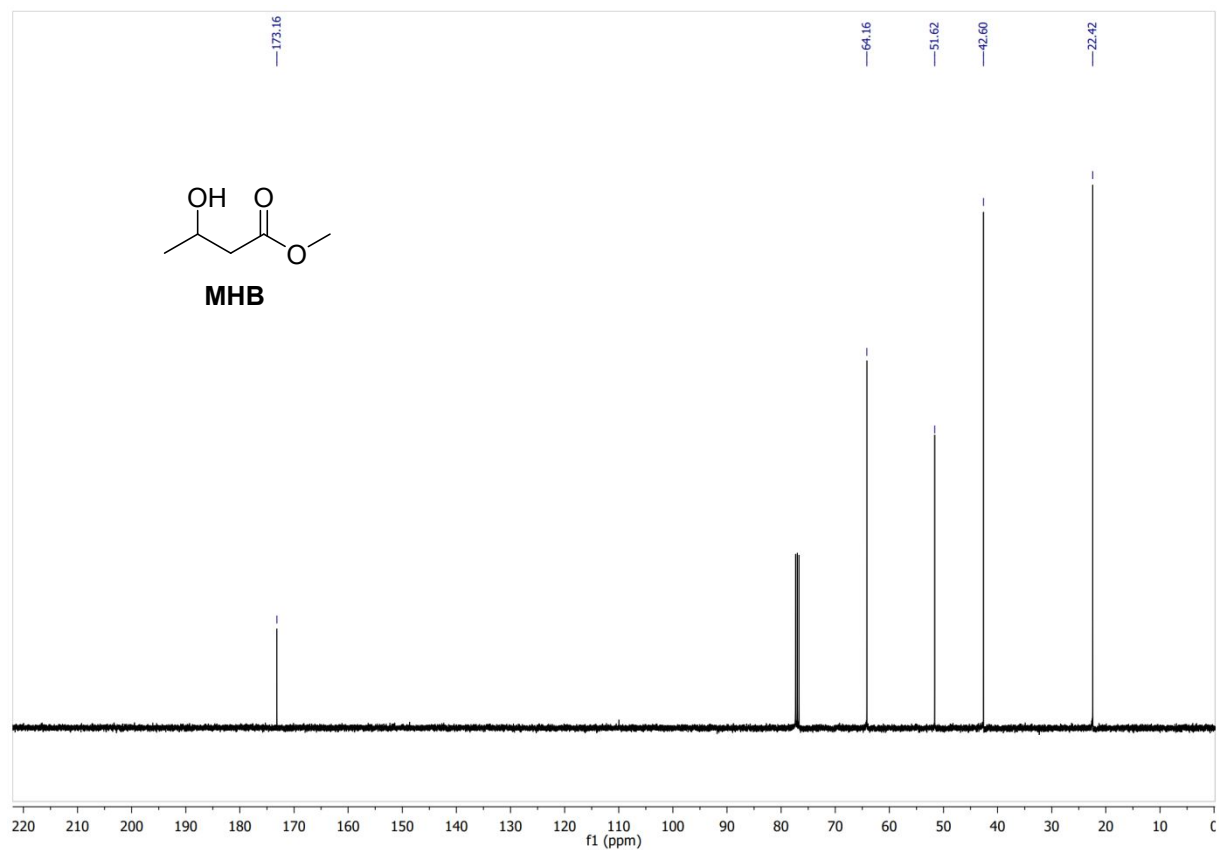
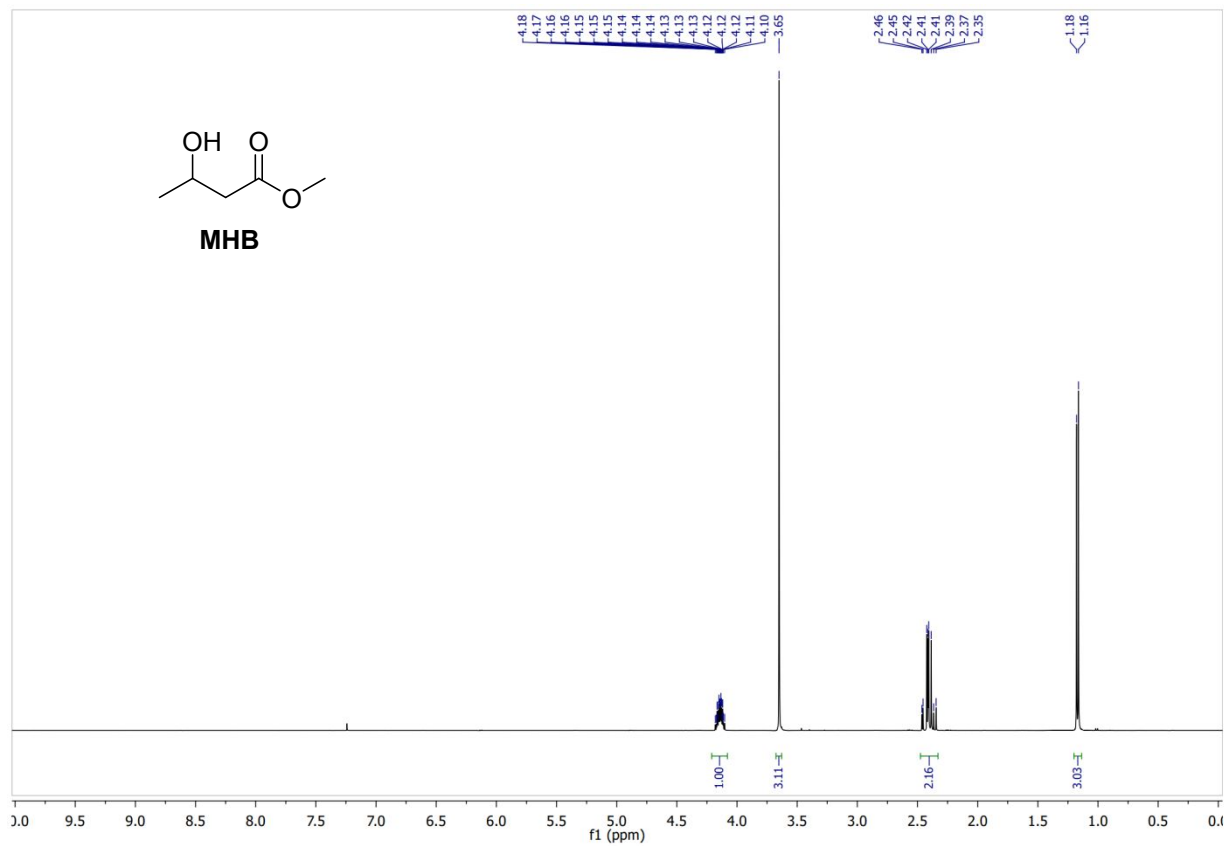
MC. Colorless liquid, b.p. = 118-120 °C. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 6.96 (dq, $J = 15.5, 6.9$ Hz, 1H), 5.83 (dq, $J = 15.6, 1.7$ Hz, 1H), 3.70 (s, 3H), 1.86 (dd, $J = 6.9, 1.7$ Hz, 3H). $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 166.98, 144.72, 122.35, 51.33, 17.94.



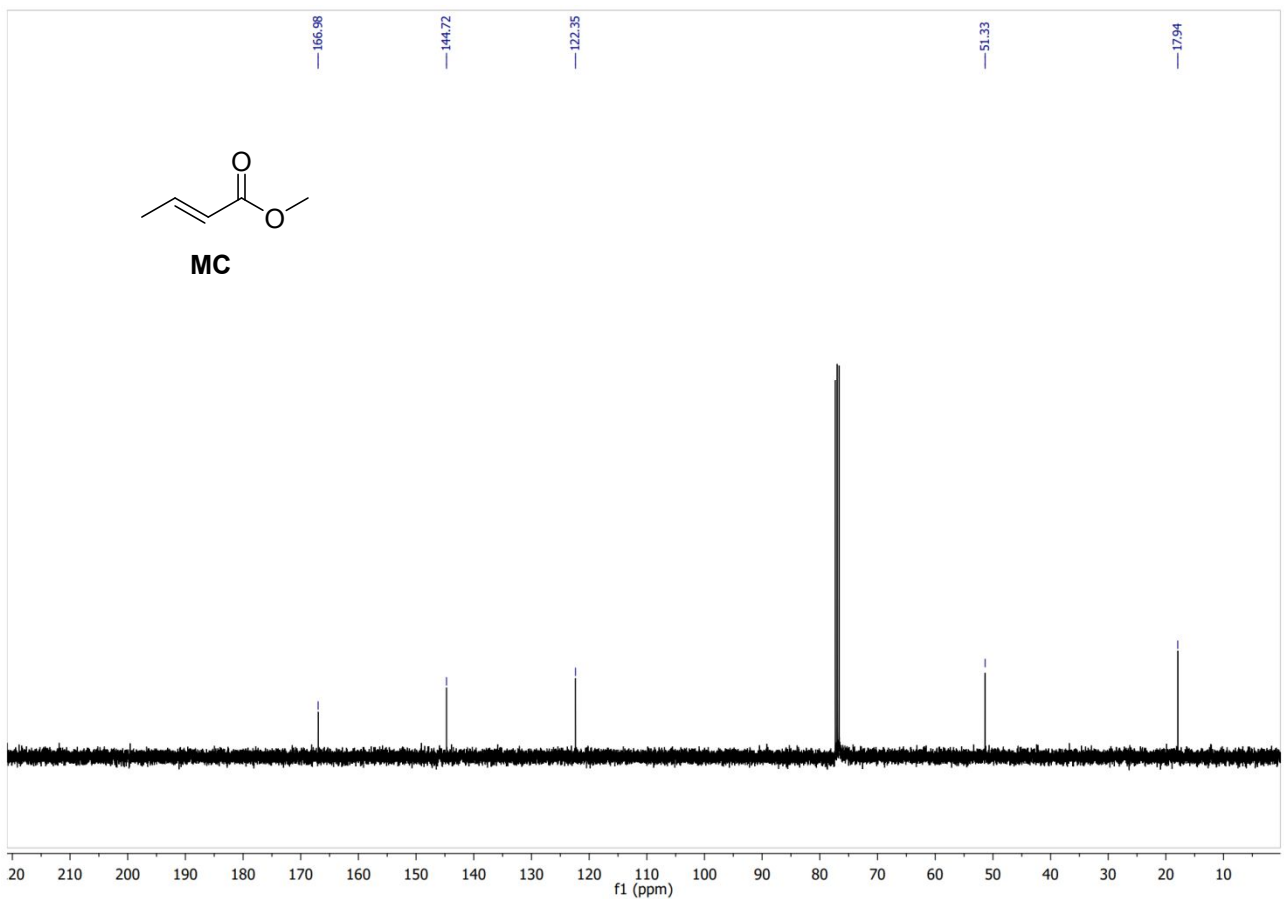
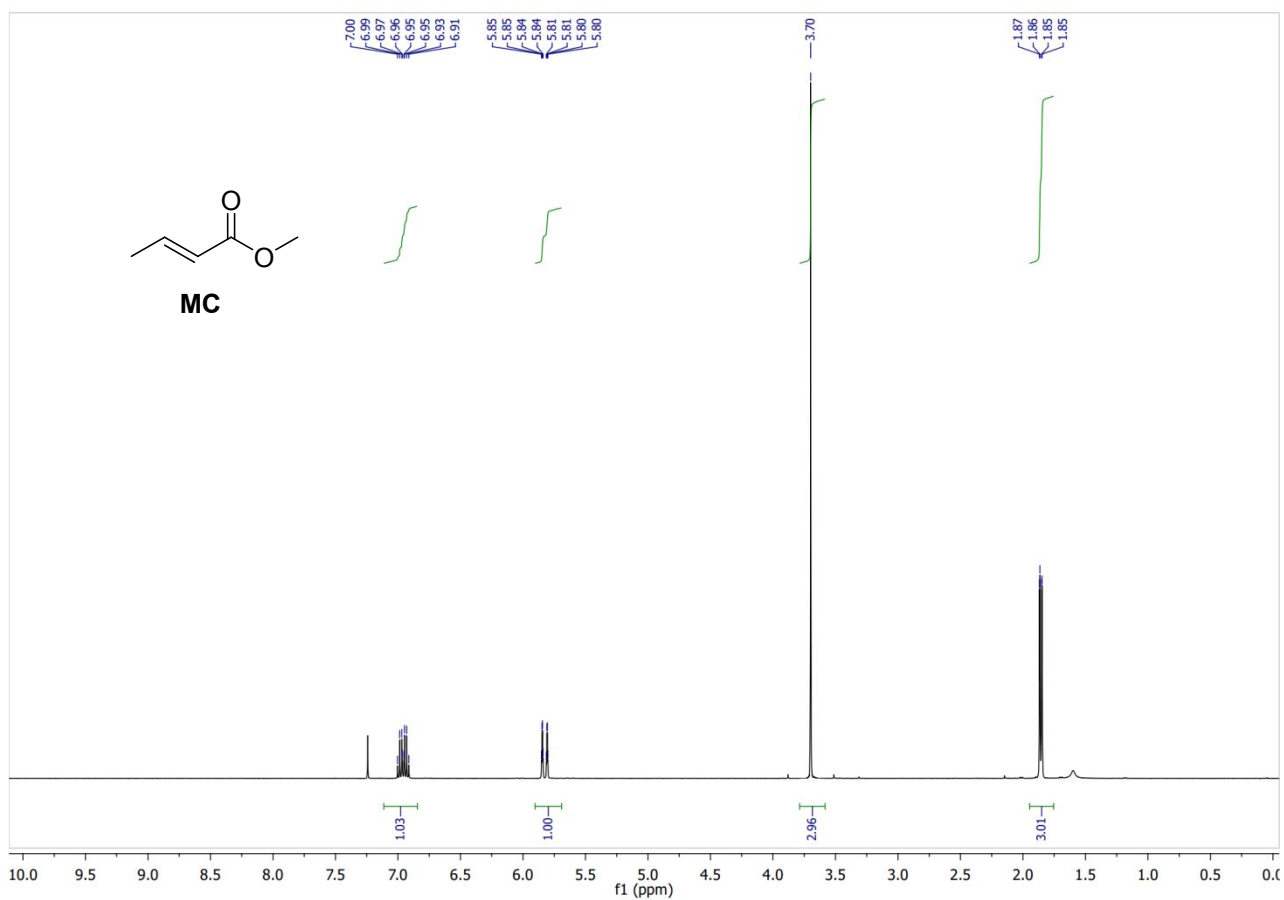
MMB. Colorless liquid, b.p. = 148-149 °C. $^1\text{H NMR}$ (401 MHz, CDCl_3) δ 3.77 – 3.67 (m, 1H), 3.64 (s, 3H), 3.29 (s, 3H), 2.53 (dd, $J = 15.1, 7.2$ Hz, 1H), 2.33 (dd, $J = 15.1, 5.7$ Hz, 1H), 1.16 (d, $J = 6.2$ Hz, 3H). $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 171.88, 73.51, 56.26, 51.52, 41.43, 19.10. **Anal. Calc.** for ($\text{C}_6\text{H}_{12}\text{O}_3$; 118.06): C, 54.53; H, 9.15; found: C, 54.55, H, 9.20.

5. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ Spectra of MHB, MC and MMB

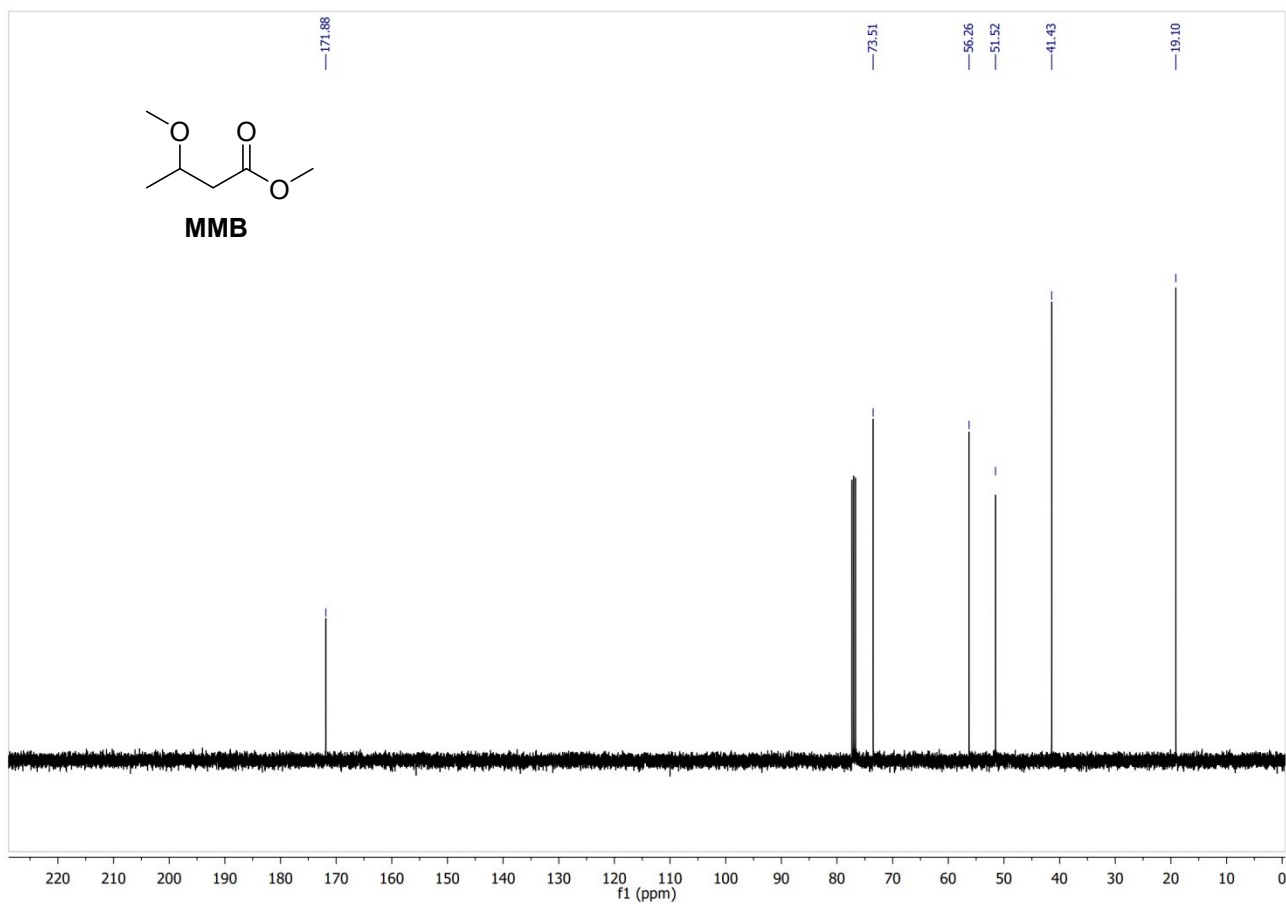
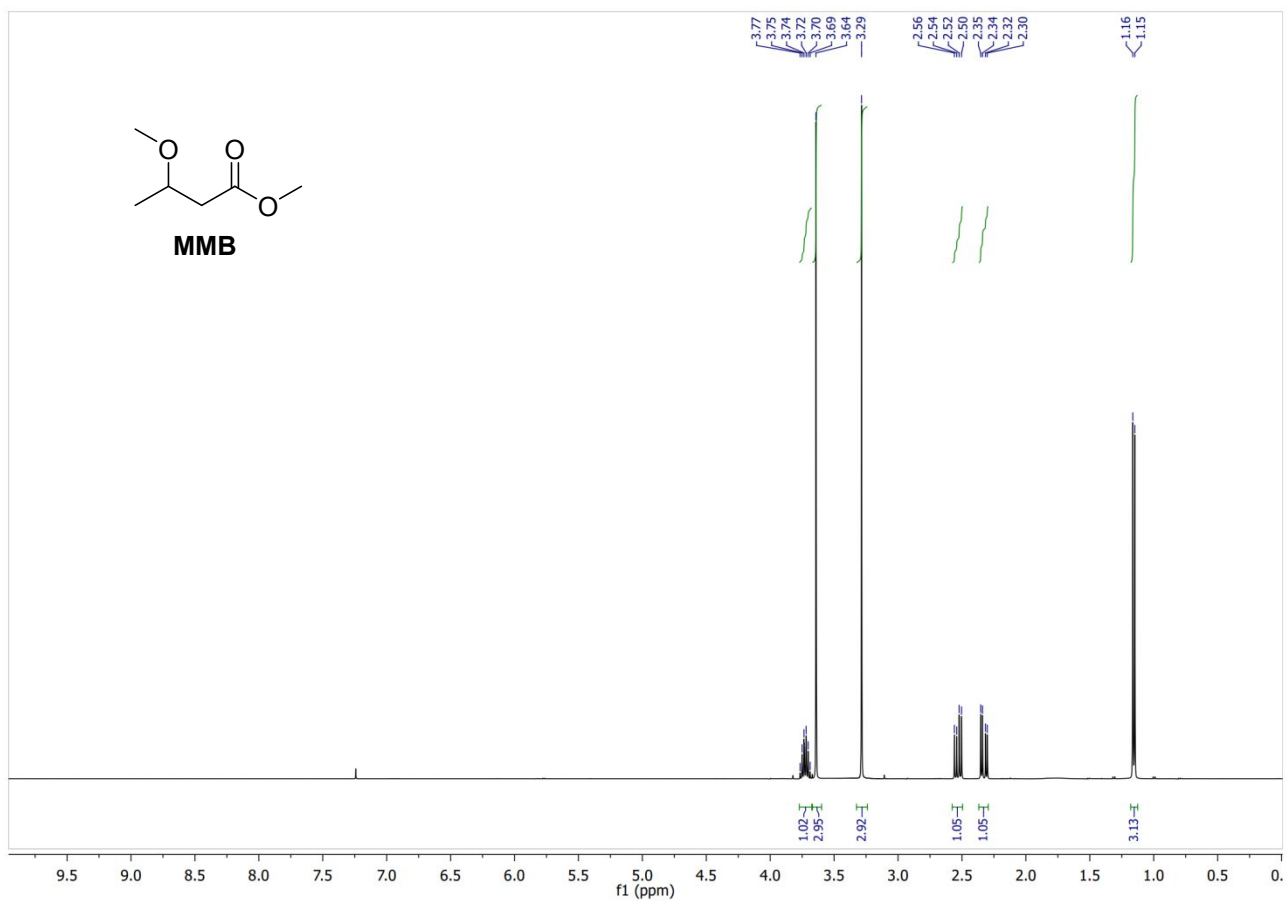
Figures S2 and S3. $^1\text{H-NMR}$ (up) and $^{13}\text{C-NMR}$ (down) of MHB.



Figures S4 and S5. ¹H-NMR (up) and ¹³C-NMR (down) of MC.



Figures S6 and S7. $^1\text{H-NMR}$ (up) and $^{13}\text{C-NMR}$ (down) of MMB.



6. References

- 1 A. Parodi, A. Jorea, M. Fagnoni, D. Ravelli, C. Samori, C. Torri and P. Galletti, *Green Chem.*, 2021, 3420–3427.
- 2 C. Samorì, A. Parodi, E. Tagliavini and P. Galletti, *J. Anal. Appl. Pyrolysis*, 2021, **155**, art. n° 105030.
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