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Bis-quaternary ammonium betulin-based dimethacrylate: synthesis, characterization, and application in dental restorative resins

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1. Characterization of betulin-based bromide intermediates and bis-quaternary ammonium betulin-based dimethacrylate derivatives (Bis-QADM-Bet)

The chemical structure of three betulin-based bromide intermediates (DEB-Bet, DBB-Bet, and DHB-Bet) and the corresponding Bis-QADM-Bet products (EBet, BBet, and HBet) were confirmed with ^{1}H NMR spectra (Fig. S1A, S2A, and S3A), ^{13}C NMR spectra (Fig. S1B, S2B, and S3B), and FT-IR spectra (Fig. S1C, S2C, and S3C). Their molecular weight was determined by HRMS (Fig. S1D, S2D, and S3D).

1.1. Characterization of DEB-Bet and EBet

Analysis of DEB-Bet. ^{1}H NMR (600 MHz, $CDCl_3$, see Fig. S1A): δ (ppm) = 4.65 (m, 2H), 4.57 (m, 1H), 4.42 (ddd, J = 13.3, 11.0, 1.9 Hz, 1H), 4.09 (m, 2H), 3.97 (m, 1H), 3.86 (m, 2H), 2.46 (td, J = 11.0, 5.8 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$, Fig. S1B): δ (ppm) = 167.79, 149.92, 110.05, 83.34, 77.26, 76.84, 64.79, 55.36, 50.23, 48.81, 47.70, 46.58, 42.72, 41.27, 40.89, 38.30, 37.63, 35.56, 34.43, 32.79, 31.60, 29.61, 27.93, 26.39, 25.11, 23.55, 22.67, 20.79, 19.12, 18.12, 16.44, 14.76. FT-IR (Fig. S1C): υ (cm^{-1}) = 3428 (OH), 1711 (C=O), 1380 (CH_3), 1250 (C-O), 513 (C-Br). HRMS (TOF MS ESI, CH_2Cl_2): calculated $C_{34}H_{52}Br_2O_4$ for 680.22 *m/z*; found 681.44 *m/z* $[M+H]^+$ (Fig. S1D).

Analysis of EBet. ^{1}H NMR (600 MHz, $CDCl_3$, Fig. S1A): *δ* (ppm) = 6.18 (d, J = 16.9 Hz, 2H), 5.72 (d, J = 9.7 Hz, 2H), 5.05 (d, J = 11.8 Hz, 1H), 4.91 (d, J = 17.9 Hz, 1H), 4.68 (m, 8H), 4.43 (s, 3H), 4.31 (d, J = 5.9 Hz, 1H), 4.09 (m, 2H), 3.74 (m, 12H), 2.42 (s, 1H). FT-IR (Fig. S1C): υ (cm^{-1}) = 3490 (NH_3), 3428 (OH), 1711 (C=O), 1380 (CH_3), 1250 (C-O), 513 (C-Br). HRMS (MALDI, CH_2Cl_2): calculated $C_{50}H_{82}Br_2N_2O_8$ for 919.12 *m/z*; found 919.52 *m/z* $[M-Br]^+$ (Fig. S1D).

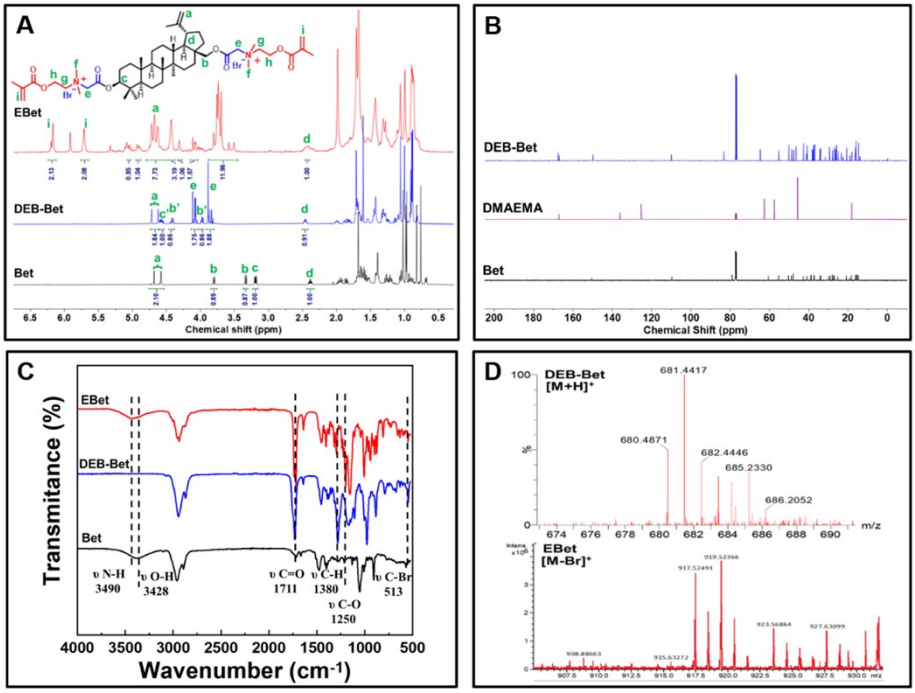


Fig. S1 ^{1}H NMR (A), ^{13}C-NMR (B), and FTIR (C) spectra of betulin, DEB-Bet, and EBet. HRMS (D) of DEB-Bet and EBet.

1.2. Characterization of DBB-Bet and BBet

Analysis of DBB-Bet. ^{1}H NMR (600 MHz, $CDCl_3$, see Fig. S2A): δ (ppm) = 4.65 (m, 2H), 4.51 (dd, J = 10.1, 6.3 Hz, 1H), 4.31 (dd, J = 11.0, 1.9 Hz, 1H), 3.89 (d, J = 11.1 Hz, 1H), 3.49 (td, J = 6.4, 4.4 Hz, 4H), 2.53 (m, 4H), 2.46 (m, 1H), 2.20 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$, Fig. S2B): δ (ppm) = 174.61, 172.97, 150.08, 109.95, 81.20, 80.65, 77.26, 76.84, 67.93, 62.94, 55.39, 48.77, 47.72, 46.47, 42.71, 40.89, 38.98, 37.93, 34.74, 32.96, 29.78, 28.02, 27.94, 26.92, 25.29, 23.73, 22.68, 21.84, 20.80, 19.13, 18.76, 16.57, 14.75. FT-IR (Fig. S2C): υ (cm^{-1}) = 3426 (OH), 1710 (C=O), 1381 (CH_3), 1252 (C-O), 514 (C-Br). HRMS (TOF MS ESI, CH_2Cl_2): calculated $C_{38}H_{60}Br_2O_4$ for 740.70 *m/z*; found 741.29 *m/z* $[M+H]^+$ (Fig. S2D).

Analysis of BBet. ^{1}H NMR (600 MHz, $CDCl_3$, Fig. S2A): δ (ppm) = 6.16 (m, 2H), 5.69 (m, 2H), 4.71 (m, 5H), 4.61 (s, 1H), 4.49 (ddd, J = 16.1, 10.6, 5.7 Hz, 1H), 4.29 (m, 2H), 4.13 (m, 3H), 3.87 (d, J = 11.0 Hz, 1H), 3.54 (m, 16H), 2.53 (m, 5H), 2.44 (dt, J = 10.8, 5.4 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$, Fig. S2B): δ (ppm) =175.92, 172.69, 166.38, 150.07, 135.19, 127.56, 110.01, 109.92, 82.03, 81.22, 77.30, 76.87, 70.08, 68.73, 64.19, 63.45, 62.93, 61.18, 58.78, 55.35, 52.08, 50.56, 48.74, 47.68, 46.38,

44.16, 43.70, 42.68, 40.87, 38.32, 37.83, 34.55, 32.95, 30.96, 29.91, 28.07, 27.89, 25.10, 23.74, 22.18, 21.81, 20.78, 19.10, 18.31, 16.56, 14.73. FT-IR (Fig. S2C): υ (cm^{-1}) = 3492 (NH_3), 3426 (OH), 1710 (C=O), 1381 (CH_3), 1252 (C-O), 514 (C-Br). HRMS (MALDI, CH_2Cl_2): calculated $C_{54}H_{90}Br_2N_2O_8$ for 975.23 *m/z*; found 975.60 *m/z* $[M-Br]^+$ (Fig. S2D).

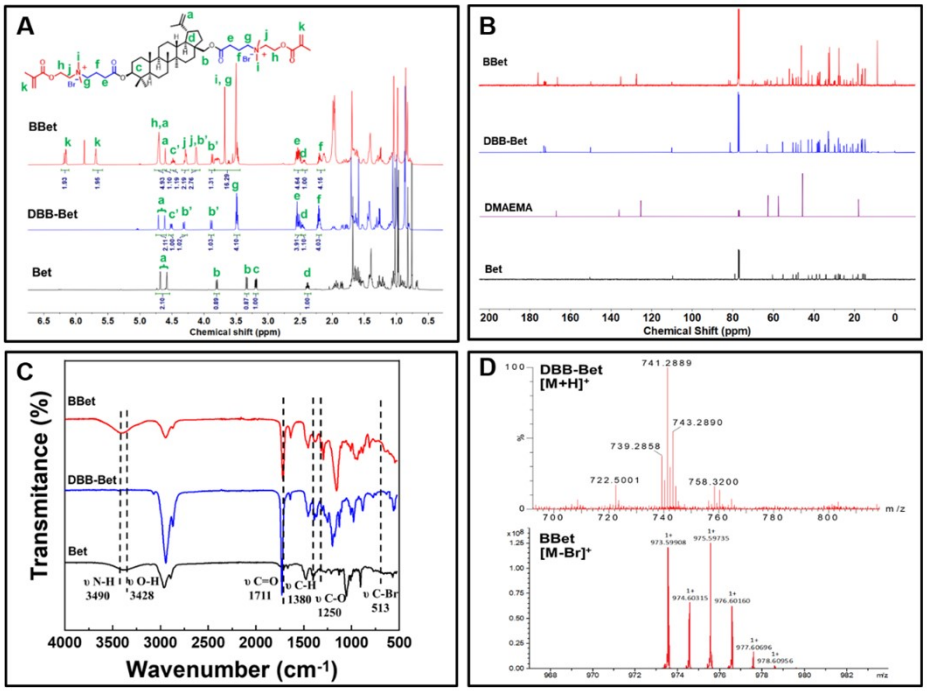


Fig. S2 ^{1}H NMR (A), ^{13}C-NMR (B), and FTIR (C) spectra of betulin, DBB-Bet, and BBet. HRMS (D) of DBB-Bet and BBet.

1.3. Characterization of DHB-Bet and HBet

Analysis of DHB-Bet. ^{1}H NMR (600 MHz, $CDCl_3$, Fig. S3A): δ (ppm) = 4.6 (m, 2H), 4.48 (dd, J = 10.6, 5.8 Hz, 1H), 4.28 (dd, J = 11.1, 1.9 Hz, 1H), 3.84 (d, J = 11.0 Hz, 1H), 3.41 (td, J = 6.8, 2.3 Hz, 4H), 2.44 (m, 1H), 2.34 (dt, J = 20.0, 7.4 Hz, 4H). ^{13}C NMR (151 MHz, $CDCl_3$, Fig. S3B): δ (ppm) = 173.91, 150.12, 109.92, 80.80, 77.27, 76.85, 67.54, 62.66, 55.37, 50.27, 48.77, 47.73, 46.40, 44.80, 43.00, 42.70, 40.89, 38.75, 37.84, 35.57, 34.95, 33.53, 32.54, 31.60, 29.80, 28.01, 27.71, 26.93, 25.29, 24.40, 23.74, 22.98, 21.87, 20.80, 19.13, 18.77, 16.59, 14.75. FT-IR (Fig. S3C): υ (cm^{-1}) = 3429 (OH), 1712 (C=O), 1379 (CH_3), 1251 (C-O), 510 (C-Br). HRMS (TOF MS ES$^+$, CH_2Cl_2): calculated $C_{42}H_{68}Br_2O_4$ for 796.81 *m/z*; found 797.35 *m/z* $[M+H]^+$ (Fig. S3D).

Analysis of HBet. ^{1}H NMR (600 MHz, $CDCl_3$, see Fig. S3A): δ (ppm) = 6.17 (m, 2H), 5.71 (m, 2H), 4.69 (m, 5H), 4.61 (s, 1H), 4.48 (m, 1H), 4.27 (m, 3H), 4.13 (m, 2H), 3.86 (d, J = 11.0 Hz, 1H), 3.61 (d, J = 72.8 Hz, 8H), 3.45 (dd, J = 35.6, 2.4 Hz, 8H), 2.45 (tt, J = 11.3, 5.4 Hz, 1H), 2.35 (dtd, J = 19.9, 7.3, 4.7 Hz, 4H). ^{13}C NMR (151 MHz, $CDCl_3$, Fig. S3B): δ (ppm) = 173.96, 166.46, 150.12, 135.22, 127.49, 109.94, 81.03, 80.80, 77.28, 76.86, 70.02, 65.13, 62.70, 61.09, 58.65, 56.15, 55.35, 54.46, 52.05, 50.63, 48.75, 47.71, 46.40, 44.82, 43.75, 42.69, 40.88, 38.34, 37.83, 34.57, 33.86, 32.41, 29.78, 28.04, 27.99, 26.41, 25.79, 24.44, 23.76, 22.94, 20.79, 19.11, 18.32, 16.61, 16.58, 16.15, 14.75. FT-IR (Fig. S3C): υ (cm^{-1}) = 3489 (NH_3), 3429 (OH), 1712 (C=O), 1379 (CH_3), 1251 (C-O), 510 (C-Br). HRMS (MALDI, CH_2Cl_2): calculated $C_{58}H_{98}Br_2N_2O_8$ for 1030.67 *m/z*; found 1030.71 *m/z* $[M\text{-}Br]^+$ (Fig. S3D).

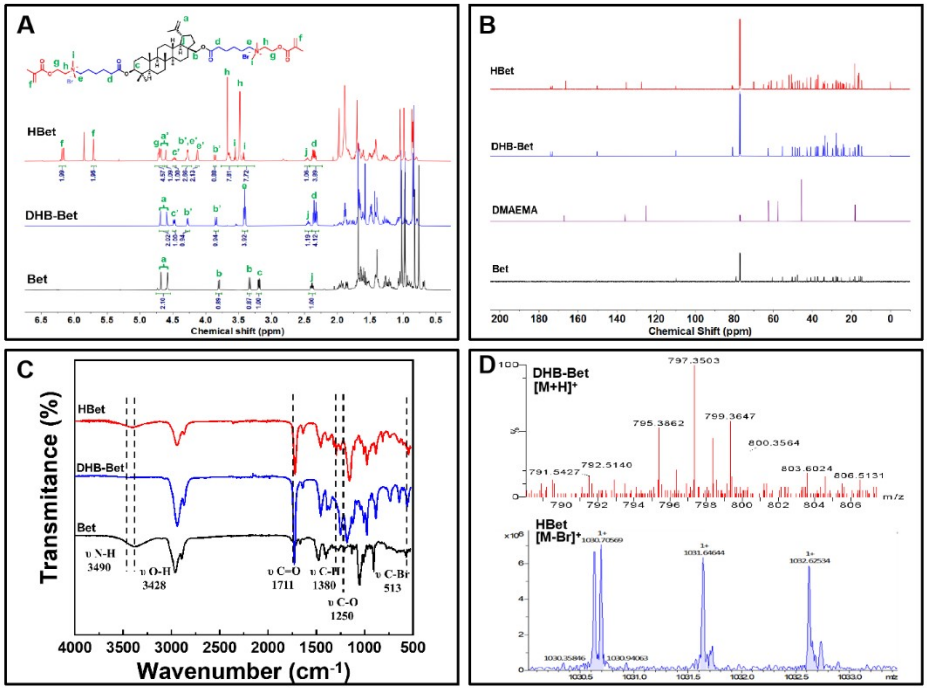


Fig. S3 ^{1}H NMR (A), ^{13}C-NMR (B), and FTIR (C) spectra of betulin, DHB-Bet, and HBet. HRMS (D) of DHB-Bet and HBet.

2. Design of the primer sequences

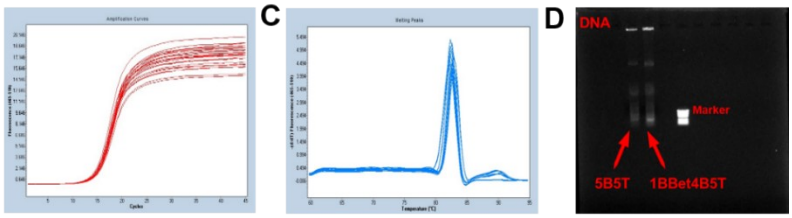
In this study, the primer sequences for RT-qPCR, and their standard amplification curves and melting curves are firstly collected in Fig. S4(A-C). A single peak at 83 °C in the melting curves (Fig. S4C) corresponds to a particular qPCR amplification product. Thus, the gene expression can be determined with a PCR system. As observed

from the gel electrophoresis image in Fig. S4D, two sharp bands are clearly found in 5B5T and 1BBet4B5T, indicating RNA was not degraded during the tests. Both RNA concentration and purity in the extracted samples were also analyzed by measuring the OD_{260}/OD_{280} ratio in the Nanodrop Microvolume UV-Vis spectrophotometer. The obtained OD_{260}/OD_{280} ratio for 5B5T (1.90) and 1BBet4B5T (1.91) ranges between 1.8 and 2.1 (Fig. S4E), which is an acceptable indicator of good quality RNA.[1] All these results are used to confirm the correctness of the primer sequences designed.

A

Gene name	Primer sequences
gtfB-F	TGCCGCAGTCCCTTCTTATTC
gtfB-R	GCCATGTATTGCCCGTCATCT
gtfC-F	GTGCGCCACCAATGACAGAC
gtfC-R	GCCTACTGGAACCCAAACACCTA
gtfD-F	TACCTTGGGCACCACAACACT
gtfD-R	TGCCGCCTTATCATCCTCACT

B C D



E

Dental resin	Concentration (mg/uL)	OD_{260}/OD_{280}
5B5T	57	1.90
1BBet4B5T	61	1.91

Fig. S4 The detailed primer sequences used for real-time quantitative polymerize chain reaction (RT-qPCR) analysis (A). The RT-qPCR amplification curves (B) and melting curves (C). The gel electrophoresis image (D), RNA concentration and OD_{260}/OD_{280} ratio (E) of *S. mutans* after coculture with 5B5T and 1BBet4B5T for 24 h.

References

[1] J. Kuang, X. Yan, A.J. Genders, C. Granata and D.J. Bishop, PLoS ONE, 2018, **13**, e0196438.