Phenylalanine-derived β-lactam TRPM8 antagonists: revisiting configuration and new benzoyl derivatives

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1. Key intermediates for the preparation of β–lactam derivatives
2. 1H NMR spectra
3. Blockade of TRPM8-mediated responses evoked by menthol

New synthetic intermediates for the preparation of Phe-derived β–lactams

***N*-[(2*R*-Dibenzylamino-3-phenyl)prop-1-yl]-*N*-Ns-*L*-Phe-O^{t}Bu (6b)**

The title compounds was obtained in 68% yield (From **4** and **5b**) as a syrup. Eluent: 11% of EtOAc in hexane. HPLC: t_R = 5.17 min (gradient from 80% to 95% of A, in 10 min). ^{1}H-NMR (400 MHz), $CDCl_3$: δ 7.87 (m, 1H, Ar), 7.67 (m, 1H, Ar), 7.59 (m, 2H, Ar), 7.34 - 7.04 (m, 18H, Ar), 6.76 (m, 2H, Ar), 4.70 (m, 1H, α-H Phe), 3.94 (m, 1H, H_1), 3.80 (dd, J = 15.3, 3.6 Hz, 1H, H_1), 3.74 (d, J = 14.1 Hz, 2H, NCH_2), 3.65 (d, J = 14.0 Hz, 2H, NCH_2), 3.26 (m, 1H, H_2), 3.05 (dd, J = 14.0, 9.5 Hz, 1H, H_3), 2.92 (dd, J = 14.5, 3.7 Hz, 1H, β-H Phe), 2.87 - 2.73 (m, 2H, β-H Phe, H_3), 1.17 (s, 9H, ^{t}Bu). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 168.6 (COO), 148.4, 140.3, 139.9, 136.8, 133.9, 133.6, 132.0, 129.8, 129.3, 128.6, 128.3, 128.0, 127.0, 126.9, 125.9, 124.2 (Ar), 82.3 (C, ^{t}Bu), 61.9 (α-C Phe), 57.8 (C_2), 53.2 (NCH_2), 47.0 (C_1), 37.9 (β-C Phe), 35.4 (C_3), 27.8 (CH_3, ^{t}Bu). MS $(ES)^+$: 721.25 $[M+H]^+$.

***N*-[(2*R*-Dibenzylamino-3-phenyl)prop-1-yl]-*L*-Phe-O^{t}Bu (7b).**

The title compounds was obtained in 94% yield (From **6b**) as a syrup. Eluent: 2% to 5% of EtOAc in hexane. HPLC: t_R = 8.45 min (gradient from 15% to 95% of A, in 5 min). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.35 - 7.15 (m, 18H), 7.06 (m, 2H, Ar), 3.85 (d, J = 13.3 Hz, 2H, NCH_2), 3.45 (d, J = 13.4 Hz, 2H, NCH_2), 3.30 (m, 1H, α-H Phe), 3.11 (m, 2H, H_2, H_3), 2.96 (dd, J = 13.2, 8.8 Hz, 1H, β-H Phe), 2.86 (dd, J = 13.2, 4.5 Hz, 1H, β-H Phe), 2.77 (m, 2H, H_1), 2.39 (dd, J = 13.2, 8.8 Hz, 1H, H_3), 2.38 (s, 1H, NH), 1.35 (s, 9H, CH_3, ^{t}Bu). ^{13}C-NMR (75 MHz, $CDCl_3$): 173.7 (COO), 140.3, 139.9, 137.5, 130.0, 129.3, 128.7, 128.5, 128.4, 128.3, 127.1, 126.8, 126.0 (Ar), 81.0 (C, ^{t}Bu), 62.8 (α-C Phe), 59.9 (C_2), 53.2 (NCH_2), 47.3 (C_1), 40.0 (β-C Phe), 32.9 (C_3), 28.2 (CH_3, ^{t}Bu). MS $(ES)^+$: 536.28 $[M+H]^+$.

***N*-(2'*S*-Chloropropanoyl)-*N*-[(2*R*-dibenzylamino-3-phenyl)prop-1-yl]-*L*-Phe-O^{t}Bu (8b)**

The title compounds was obtained in 66% yield (From **7b** and 2*S*-chloropropionic acid) as a syrup. Eluent: 3% of EtOAc in hexane. HPLC: t_R = 9.18 min (gradient from 60% to 95% of A, in 10 min). ^{1}H-NMR (400 MHz, $CDCl_3$, rotamer's ratio M:m, 80:20) δ major rotamer 7.30 - 7.19 (m, 14H), 7.12 (m, 3H, Ar), 7.06 (m, 2H, Ar), 6.93(m, 1H, Ar), 4.56 (q, J = 6.5 Hz, 1H, $H_{2'}$), 3.68 (m, 1H, α-H Phe), 3.60 (d, J = 14.0 Hz, 2H, NCH_2), 3.55 (d, J = 14.1 Hz, 2H, NCH_2), 3.33 (dd, J = 13.4, 6.7 Hz, 1H, H_1), 3.28 (dd, J = 13.5, 6.3 Hz, 1H, H_1), 3.16 - 2.99 (m, 3H, β-H Phe, H_2), 2.88 - 2.77 (m, 2H, H_3), 1.60 (d, J = 6.4 Hz, 3H, $H_{3'}$), 1.36 (s, 9H,

CH_3, ^{t}Bu). ^{13}C-NMR (101 MHz, $CDCl_3$): δ major rotamer 169.1 (COO), 168.7 (CON), 139.6, 139.1, 139.0, 129.7, 129.5, 129.2, 128.6, 128.6, 128.5, 127.4, 126.6, 126.5 (Ar), 81.9 (C, ^{t}Bu), 63.8 (α-C Phe), 59.5 (C_2), 53.8 (NCH_2), 51.0 (C_1), 49.9 ($C_{2'}$), 35.2 (β-C Phe), 34.7 (C_3), 28.0 (CH_3, ^{t}Bu), 21.5 ($C_{3'}$). MS(ES)$^+$: 625.43 $[M]^+$.

_N_-(2'_R_-chloropropanoyl)-_N_-[(2_S_-Dibenzylamino-3-phenyl)prop-1-yl]-_L_-Phe-O^{t}Bu (8c)

The title compound was obtained in 72% yield (From **7a** and 2_R_-chloropropionic acid) as a syrup. Eluent: 2% of EtOAc in hexane. HPLC: t_R = 7.06 min (gradient from 60% to 95% of A, in 10 min). ^{1}H-NMR (400 MHz, DMSO-d_6, rotamer's ratio M:m, 8:1): δ major rotamer 7.32 (m, 2H, Ar), 7.29 - 7.30 (m, 10H, Ar), 7.05 (m, 6H, Ar), 6.99 (m, 2H, Ar), 4.56 (q, J = 6.4 Hz, 1H, $H_{2'}$), 3.47 (s, 4H, NCH_2), 3.42 (dd, J = 9.4, 5.5 Hz, 1H, α-H Phe), 3.36 (dd, J = 15.9, 3.9 Hz, 1H, H_1), 2.94 (m, 4H, H_1, β-H Phe, H_2), 2.79 (dd, J = 14.2, 4.9 Hz, 1H, H_3), 2.53 (dd, J = 14.2, 7.3 Hz, 1H, H_3), 1.28 (d, J = 6.3 Hz, 3H, $H_{3'}$), 1.24 (s, 9H, CH_3, ^{t}Bu). ^{13}C-NMR (75 MHz, DMSO-d_6): δ major rotamer 168.6 (COO), 167.9 (CON), 139.4, 139.1, 137.6, 129.6, 129.5, 128.3, 128.3, 128.2, 128.2, 127.0, 126.4, 126.2 (Ar), 80.3 (C, ^{t}Bu), 63.8 (α-C Phe), 60.6 (C_2), 53.1 (NCH_2), 51.5 (C_1), 49.8 ($C_{2'}$), 34.0 (β-C Phe), 33.6 (C_3), 27.6 (CH_3, ^{t}Bu), 20.9 ($C_{3'}$). MS(ES)$^+$: 625.43 $[M]^+$.

_N_-(2'_R_-Chloropropanoyl)-_N_-[(2_R_-dibenzylamino-3-phenyl)prop-1-yl]-_L_-Phe-O^{t}Bu (8d)

The title compounds was obtained in 88% yield (From **7b** and 2_R_-chloropropionic acid). Eluent: 3% of EtOAc in hexane. HPLC: t_R = 5.05 min (gradient from 80% to 95% of A, in 10 min). ^{1}H-NMR (400 MHz, DMSO-d_6, rotamer's ratio M:m, 15:1): δ major rotamer 7.37 (m, 3H, Ar), 7.28 - 7.16 (m, 11H, Ar), 7.04 (m, 2H, Ar), 6.92 (m, 4H, Ar), 4.80 (q, J = 6.4 Hz, 1H, $H_{2'}$), 3.55 (d, J = 14.2 Hz, 2H, NCH_2), 3.48 (dd, J = 9.7, 4.6 Hz, 1H, α-Phe), 3.38 (dd, J = 14.3, 8.7 Hz, 1H, H_1), 3.21 (d, J = 14.2 Hz, 2H, NCH_2), 3.27 (dd, J = 14.3, 5.0 Hz, 1H, H_1), 3.06 (dd, J = 13.6, 9.8, 1H, β-Phe), 3.00 (m, 1H, H_2), 2.96 (m, 1H, β-Phe), 2.76 (m, 1H, H_3), 2.45 (m, 1H, H_3), 1.46 (d, J = 6.3 Hz, 3H, $H_{3'}$), 1.26 (s, 9H, CH_3, ^{t}Bu). ^{13}C-NMR (101 MHz, DMSO-d_6): δ major rotamer 168.0 (COO), 167.9 (CON), 139.6, 139.3, 138.1, 129.6, 129.4, 128.4, 128.1, 128.0, 127.9, 126.8, 126.6, 126.0 (Ar), 80.7 (C, ^{t}Bu), 62.6 (α-Phe), 56.7 (C_2), 52.8 (NCH_2), 49.4 (C_1), 49.4 ($C_{2'}$), 34.0 (β-Phe), 33.7 (C_3), 27.5 (CH_3, ^{t}Bu), 20.9 ($C_{3'}$). MS(ES)$^+$: 625.43 $[M]^+$.

^{1}H NMR spectra

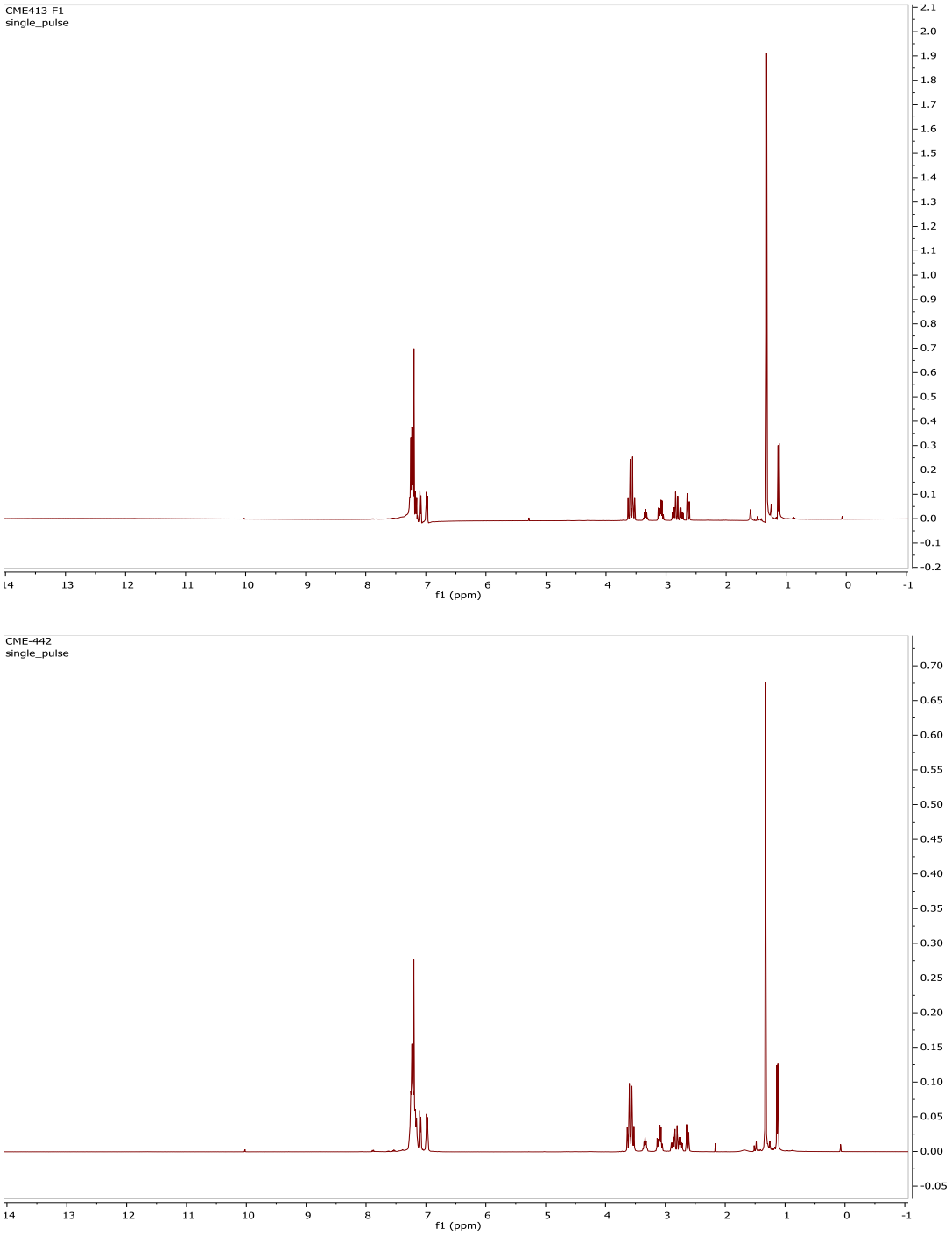


Figure S1. Comparison of ^{1}H NMR spectra between enantiomeric compounds **1b** (CME413) and **1c** (CME442)

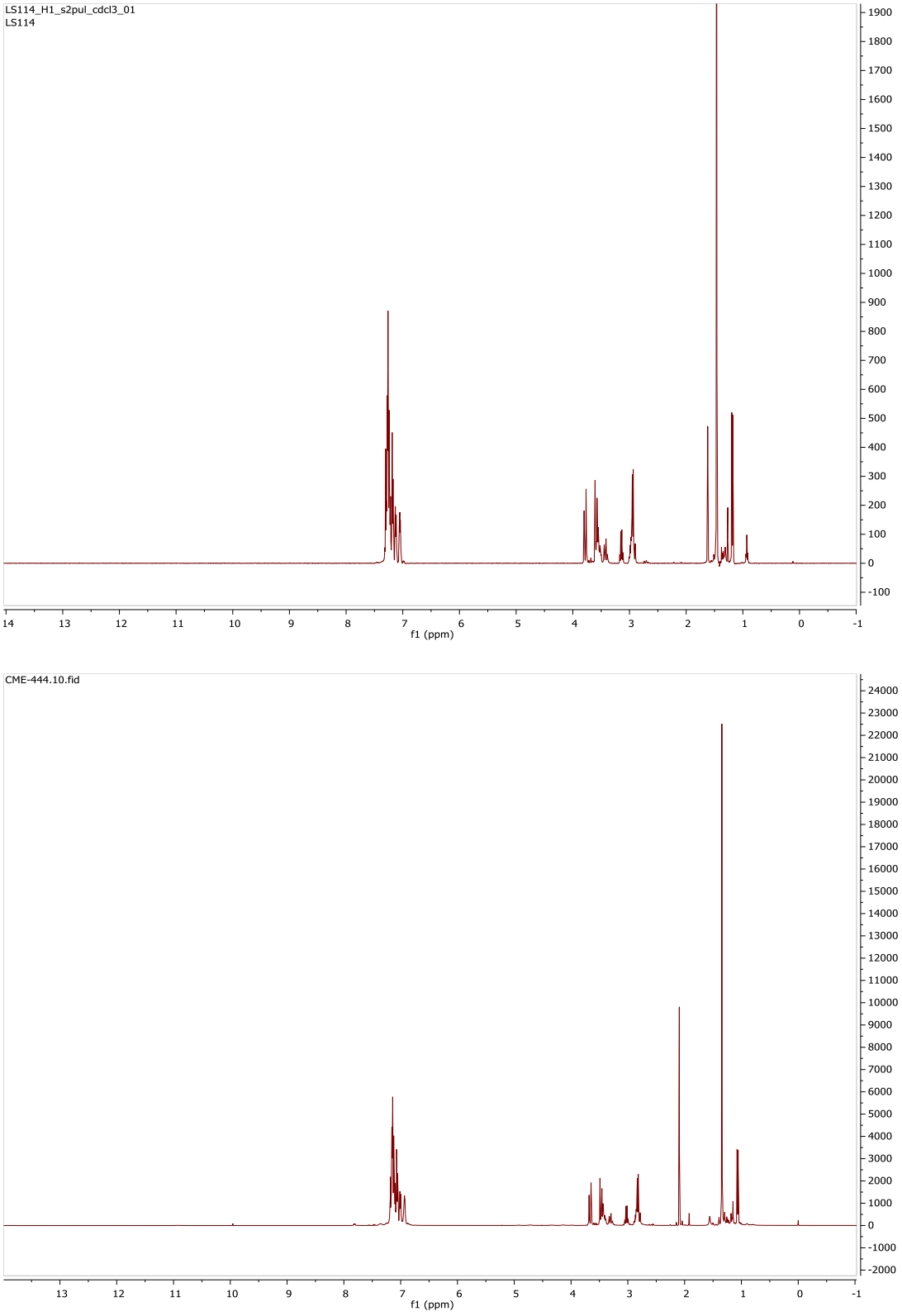
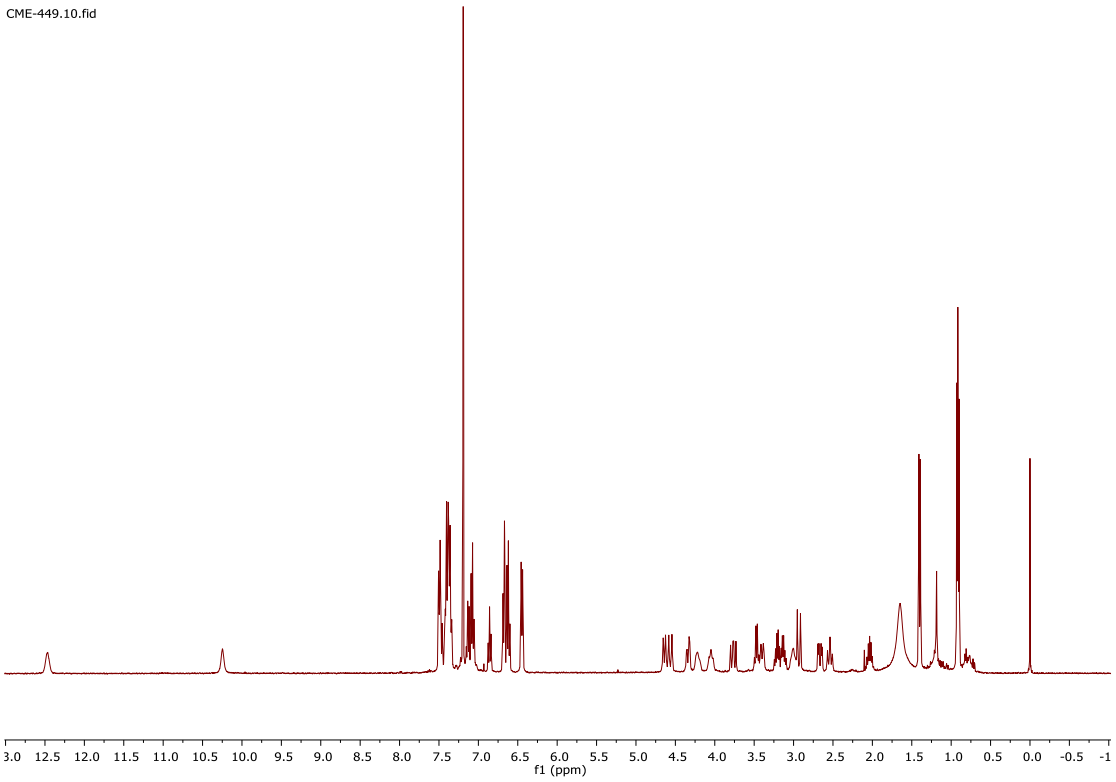


Figure S2. Comparison of ^{1}H NMR spectra between enantiomeric compounds **1a** (LS114) and **1d** (CME444)



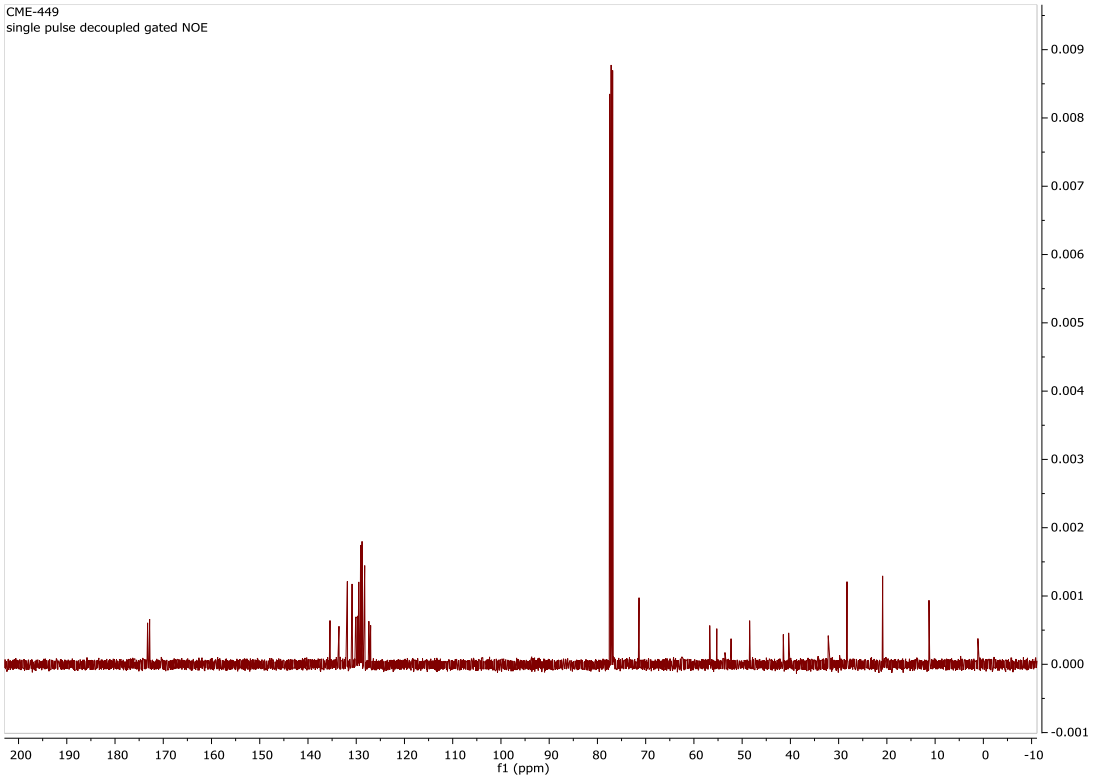


Figure S3. ^{1}H- and ^{13}C-NMR spectra of compound **9d.HCl** (CME499).

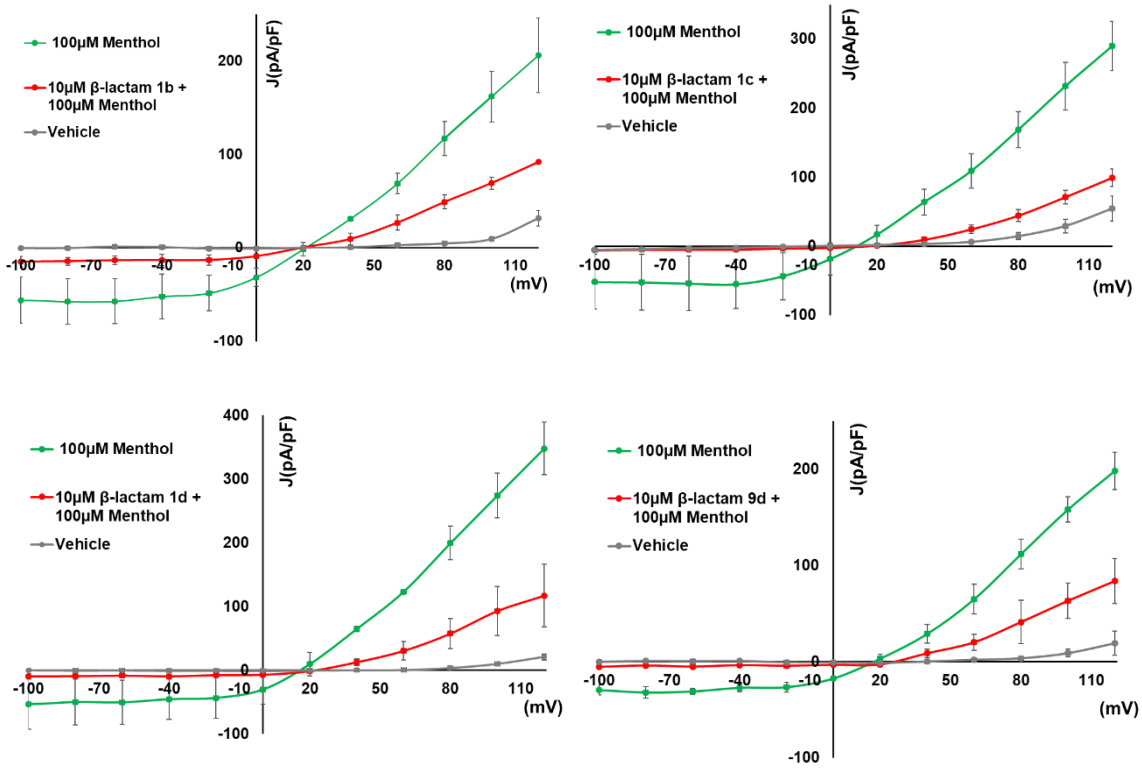


Figure S4. The indicated compounds block TRPM8-mediated responses evoked by menthol in rTRPM8-expressing HEK293 cells. Curves obtained after exposure to vehicle solution (grey trace), 100 μM menthol (green trace) and 100 μM menthol + 10 μM **1b** (**A**), **1c** (**B**), **1d** (**C**) and **9d** (**D**) (red trace). Peak current data were expressed as pA/pF (to allow comparison among different size cells). Each point is the mean ± SEM of n = 15.