SUPPORTING INFORMATION

EVALUATION OF NHS CARBAMATES AS A HIGHLY POTENT AND SELECTIVE CLASS OF ENDOCANNABINOID HYDROLASE INHIBITORS

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I. SUPPLEMENTARY FIGURES

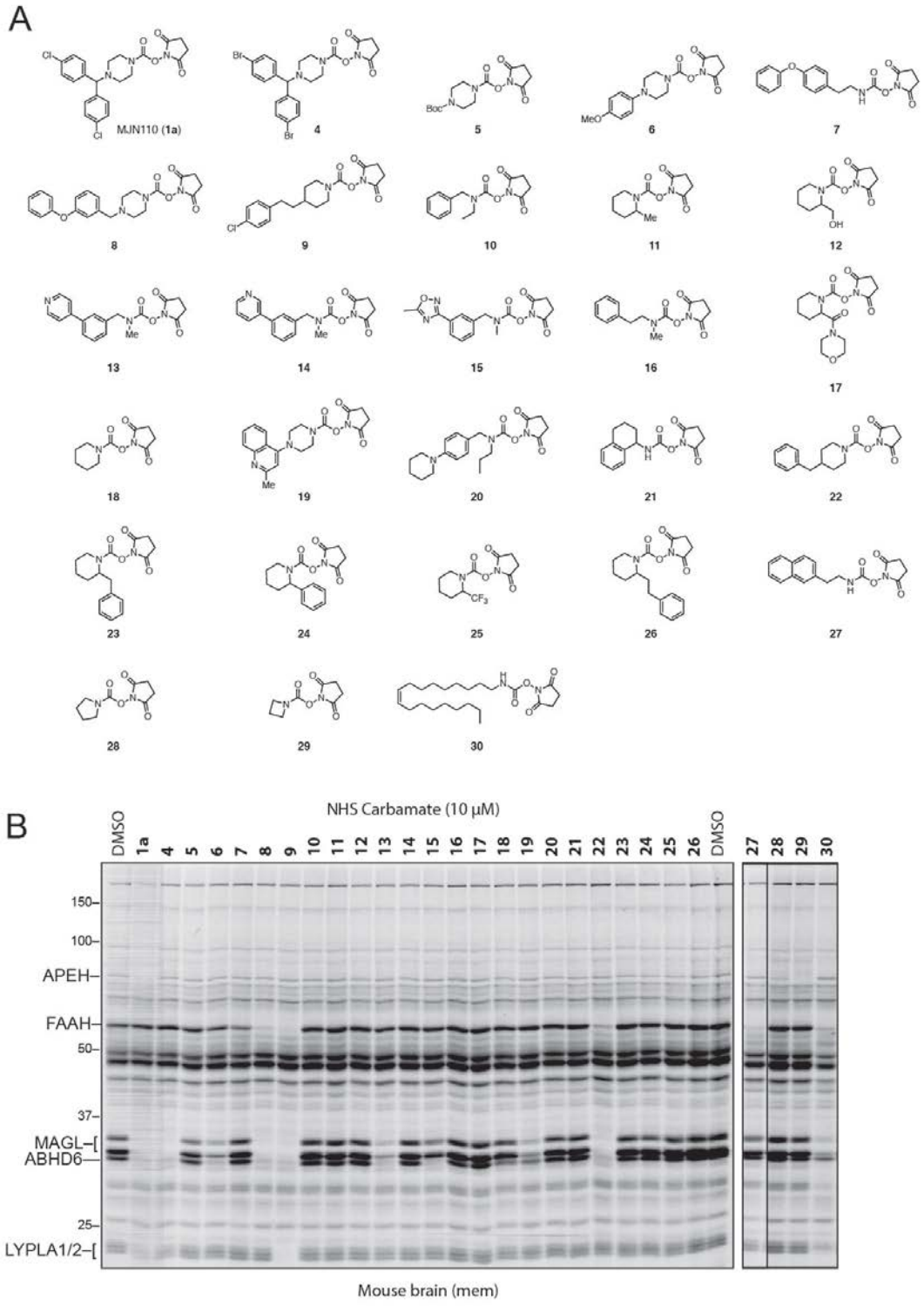


Figure S1. NHS-Carbamate Library competitive ABPP screen in mouse brain membrane proteomes. A) Structures of NHS carbamate library. B) *In vitro* competitive ABPP gel measuring serine hydrolase inhibitory activity in mouse brain membrane proteomes of NHS carbamate probes (10 μM) revealing inhibition of multiple serine hydrolases, including MAGL, ABHD6, FAAH, LYPLA1/2 and APEH.

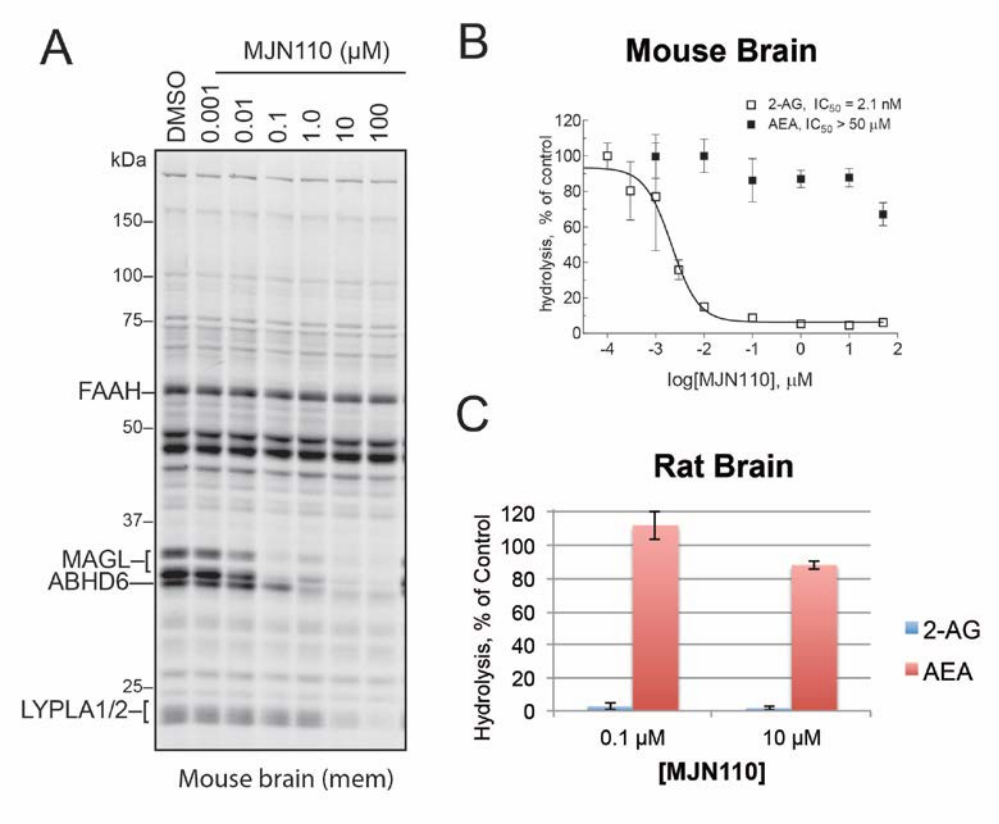


Figure S2. (A) *In vitro* competitive ABPP profiles of MJN110 in mouse brain proteomes. Inhibition of MAGL and FAAH by MJN110 in mouse (B) and rat (C) brain proteomes as measured by hydrolysis of 2-AG and AEA, respectively.

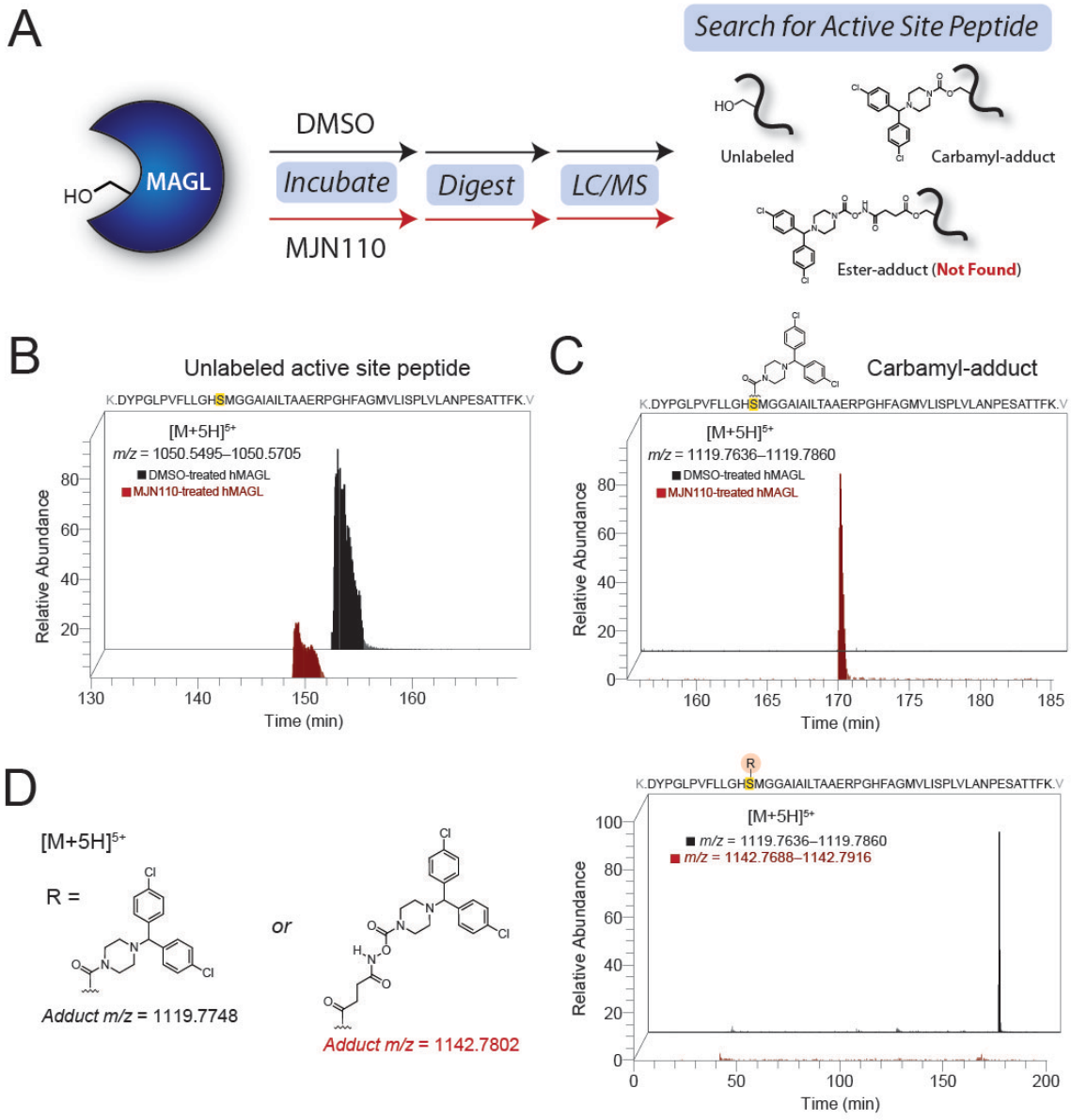


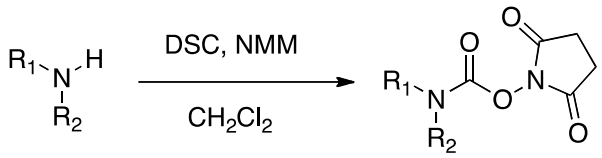
Figure S3. Detection of active-site peptide adducts. A) Experimental workflow for identification of active site labeled peptides of MAGL involving human recombinant MAGL being treated with either DMSO or MJN110 and subsequently subjected to proteolytic digestion and LC/MS analysis. MS data was then searched for unlabeled, carbamylated and esterified active site peptides based on their predicted m/z values. B) Extracted ion chromatograms (EIC) for mass of unlabeled active-site peptide showing a reduction of signal in MJN110-treated (red) vs. DMSO-treated (black) hMAGL. C) EIC for mass of carbamylated active-site peptide showing detection of carbamyl-adduct in MJN110-treated (red) but not in DMSO-treated (black) samples. D) EIC for mass of carbamylated active site peptide adduct (black) and esterified active site peptide (red) in MJN110-treated samples of hMAGL showing detection of carbamyl- but not esterified-adduct.

II. EXPERIMENTAL SECTION

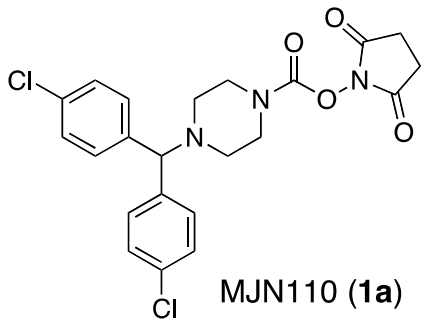
General Methods: All chemicals were obtained from commercial suppliers and were used without further purification. Anhydrous solvents were obtained by passing solvents through activated alumina columns. Merck silica gel TLC plates (0.25 mm, 60 F_{254}) were used to monitor reactions. Flash chromatography was performed using SiliaFlash F60 silica gel (40–63 μm, 60 Å). NMR spectra were recorded at room temperature on Bruker DRX-500, Varian Inova-400 or Bruker DRX-600 (5 mm DCH Cryoprobe) instruments. Chemical shifts are recorded in ppm relative to tetramethylsilane (TMS) with peaks being reported as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bm = broad multiplet), coupling constant (Hz). High-resolution mass spectra (HRMS) were obtained on an Agilent LC/MSD TOF mass spectrometer by electrospray ionization–time-of-flight (ESI-TOF). MJN110yne (**1b**) was synthesized according to previously reported methods.[1]

Determination of Compound Purity: Compound purity was determined by LC/MS on an Agilent 1100 series LC-MSD SL instrument with UV detection at 254 nm. Chromatographic separation was achieved with a Phenomenex Gemini C18 column (5 μm, 50 mm x 4.6 mm) in-line with a Phenomenex guard column cartridge (C18, 4 mm x 3 mm). Mobile phases A and B consisted of H_2O (0.1% HCO_2H) and CH_3CN (0.1% HCO_2H), respectively. Using a constant flow rate of 0.5 ml/min, the mobile phase gradient was as follows: 1.0 min, 10% B; 2.0 min, 10–98% B (linear gradient); 5.0 min, 98% B; 2.0 min, 10 % B. All final compounds (**1**–**30**) were determined to be ≥ 95% pure by this method.

General Procedure for Synthesis of NHS-carbamates (Procedure A):

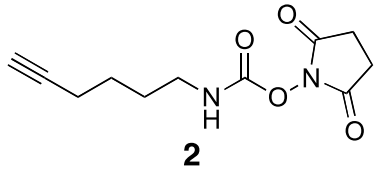


To a stirring solution of *N*,*N*'-disuccinimidyl carbonate (130 mg, 0.50 mmol, 1.0 equiv) and *N*-methylmorpholine (0.16 mL, 1.5 mmol, 3.0 equiv) in dry CH_2Cl_2 (5.0 mL) was added 1º or 2º amines (0.50 mmol, 1.0 equiv). The reaction mixture was stirred at room temperature for12 h. A stream of nitrogen was passed over the reaction mixture to remove the solvent and to the remaining residue was added EtOAc (20 mL). The resulting precipitate was filtered off and the filtrate was concentrated and purified by SiO_2 flash chromatography (EtOAc/hexanes) to give the pure NHS carbamate.



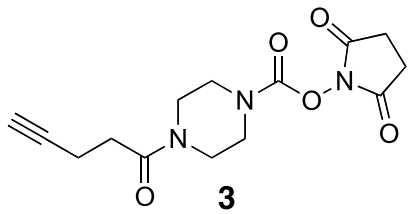
MJN110 (**1a**)

2,5-Dioxopyrrolidin-1-yl 4-(bis(4-chlorophenyl)methyl)piperazine-1-carboxylate (1a, MJN110**).**The title compound was synthesized according to **Procedure A** from 1-(bis(4-chlorophenyl)methyl)piperazine (160 mg, 0.50 mmol), DSC (130 mg, 0.50 mmol) and NMM (0.16 mL, 1.5 mmol). Purification of the crude product by flash chromatography (50% EtOAc/hexanes) provided the title compound (180 mg, 78%) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 – 7.29 (m, 4H), 7.28 – 7.25 (m, 4H), 4.24 (s, 1H), 3.63 (bs, 2H), 3.51 (bs, 2H), 2.79 (s, 4H), 2.42 (d, *J* = 4.8 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.14, 150.70, 140.44, 133.60, 129.42, 74.76, 51.45, 51.38, 45.54, 45.04, 25.89; HRMS (ESI-TOF+) *m/z* calc'd for $C_{22}H_{21}Cl_2N_3O_4$ $[M+H]^+$: 462.0987, found 462.0979.



2

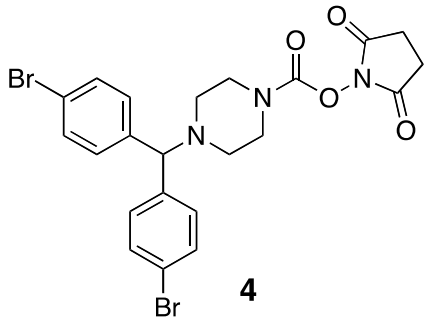
2,5-Dioxopyrrolidin-1-yl hex-5-yn-1-ylcarbamate (2). The title compound was synthesized according to **Procedure A** from 1-amino-5-hexyne (80 mg, 0.82 mmol), DSC (210 mg, 0.82 mmol) and NMM (0.27 mL, 2.5 mmol). Purification of the crude product by flash chromatography (80% EtOAc) provided the title compound (98 mg, 50%) as a white solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.67 – 5.63 (m, 1H), 3.27 (q, *J* = 6.6 Hz, 2H), 2.81 (s, 4H), 2.22 (td, *J* = 6.8, 2.6 Hz, 2H), 1.95 (t, *J* = 2.6 Hz, 1H), 1.71 – 1.66 (m, 2H), 1.60 – 1.53 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.90, 152.27, 84.59, 69.79, 42.36, 29.29, 26.31, 26.13, 18.83; HRMS (ESI-TOF+) *m/z* calc'd for $C_{11}H_{14}N_2O_4$ $[M+H]^+$: 239.1032, found 239.1024.



3

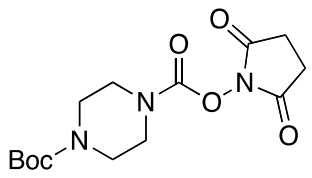
2,5-Dioxopyrrolidin-1-yl 4-(pent-4-ynoyl)piperazine-1-carboxylate (3). To a stirring solution of 4-pentynoic acid (75 mg, 0.76 mmol, 1.0 equiv), *N*-Boc-piperazine (156 mg, 0.84 mmol, 1.1 equiv) and NMM (0.097 mL, 0.84 mmol, 1.1 equiv) in dry CH_2Cl_2 (10 mL) was added EDCI (161 mg, 0.84 mmol, 1.1 equiv). After stirring at room temperature for 4 h, the reaction was quenched with a saturated solution of NH4Cl (50 mL) and extracted with CH_2Cl_2 (50 mL, 3x). The combined organic layers were washed once with brine (50 mL), dried over anhydrous $MgSO_4$ and concentrated to provide tert-butyl 4-(pent-4-

ynoyl)piperazine-1-carboxylate (187 mg, 92%) which was used without further purification. To a stirring solution of tert-butyl 4-(pent-4-ynoyl)piperazine-1-carboxylate (187 mg, 0.70 mmol) in CH_2Cl_2 (4.0 mL) was added TFA (1.0 mL). After 1 h, the reaction mixture was concentrated under a stream of N_2. The residue was redissolved in CH_2Cl_2 (5.0 mL) and concentrated under reduced pressure to remove residual TFA providing a crude colorless oil which was used without further purification. The title compound was synthesized according to **Procedure A** from the deprotected piperazine (123 mg, 0.44 mmol), DSC (110 mg, 0.44 mmol) and NMM (0.15 mL, 1.3 mmol). Purification of the crude product by flash chromatography (50% EtOAc/hexanes) provided the title compound (82 mg, 61%) as a white solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.77 – 3.48 (m, 8H), 2.82 (s, 4H), 2.56 (dd, *J* = 13.8, 5.9 Hz, 4H), 1.98 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.43, 151.29, 151.12, 83.99, 69.89, 45.66, 45.57, 41.80, 32.92, 26.34, 15.32; HRMS (ESI-TOF+) *m/z* calc'd for $C_{14}H_{17}N_3O_5$ $[M+H]^+$: 308.1246, found 308.1239.



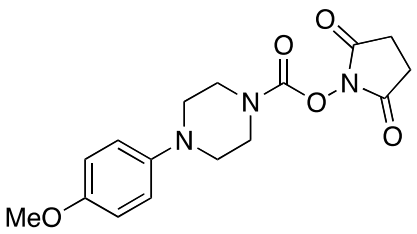
4

2,5-Dioxopyrrolidin-1-yl 4-(bis(4-bromophenyl)methyl)piperazine-1-carboxylate (4). The title compound was synthesized according to **Procedure A** from 1-(bis(4-bromophenyl)methyl)piperazine (45 mg, 0.11 mmol), DSC (28 mg, 0.11 mmol) and NMM (0.036 mL, 0.33 mmol). Purification of the crude product by flash chromatography (50% EtOAc/hexanes) provided the title compound (50 mg, 83%) as an off-white crystalline solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.42 (d, *J* = 8.32 Hz, 4H), 7.25 (d, *J* = 8.30 Hz, 4H), 4.21 (s, 1H), 3.64 (bs, 2H), 3.52 (bs, 2H), 2.81 (s, 4H), 2.44 (bs, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.71, 150.26, 140.45, 131.98, 129.32, 121.32, 74.50, 51.04, 50.97, 45.11, 44.60, 25.46; HRMS (ESI-TOF+) *m/z* calc'd for $C_{22}H_{21}Br_2N_3O_4$ $[M+H]^+$: 549.9977, found 549.9963.



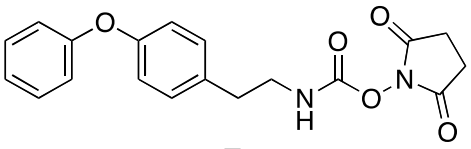
5

1-*tert*-Butyl 4-(2,5-dioxopyrrolidin-1-yl) piperazine-1,4-dicarboxylate (5). The title compound was synthesized according to **Procedure A** from *N*-Boc-piperazine (1.35 g, 7.25 mmol), DSC (1.86 g, 7.25 mmol) and NMM (2.39 mL, 21.7 mmol). Purification of the crude product by flash chromatography (50% EtOAc/hexanes) provided the title compound (1.70 g, 72%) as a white solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.60 (s, 1H), 3.50 (d, *J* = 10.0 Hz, 3H), 2.82 (s, 2H), 1.46 (d, *J* = 1.5 Hz, 5H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.46, 155.23, 151.28, 81.39, 45.65, 45.34, 44.37, 43.30, 29.20, 26.34; HRMS (ESI-TOF+) *m/z* calc'd for $C_{14}H_{21}N_3O_6$ $[M+Na]^+$: 350.1322, found 350.1315.



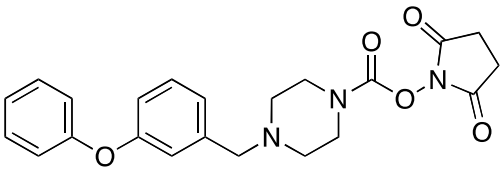
6

2,5-Dioxopyrrolidin-1-yl 4-(4-methoxyphenyl)piperazine-1-carboxylate (6). The title compound was synthesized according to **Procedure A** from 1-(4-methoxyphenyl)piperazine (100 mg, 0.52 mmol), DSC (130 mg, 0.52 mmol) and NMM (0.17 mL, 1.6 mmol). Purification of the crude product by flash chromatography (70% EtOAc/hexanes) provided the title compound (150 mg, 87%) as a colorless oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.90 (d, *J* = 8.5 Hz, 2H), 6.84 (d, *J* = 8.6 Hz, 2H), 3.78 (bm, 2H), 3.76 (s, 3H), 3.67 (bm, 2H), 3.10 (bm, 4H), 2.81 (bm, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.60, 155.43, 151.19, 145.96, 120.09, 115.37, 56.39, 51.50, 46.01, 45.57, 26.36; HRMS (ESI-TOF+) *m/z* calc'd for $C_{16}H_{19}N_3O_5$ $[M+H]^+$: 334.1403, found 334.1388.



7

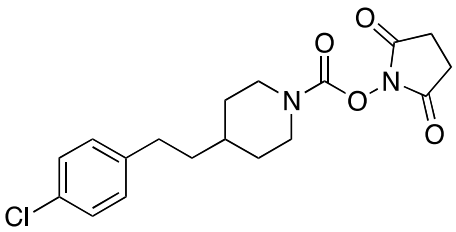
2,5-Dioxopyrrolidin-1-yl 4-phenoxyphenethylcarbamate (7). The title compound was synthesized according to **Procedure A** from 4-phenoxyphenylethylamine (122 mg, 0.57 mmol), DSC (147 mg, 0.57 mmol) and NMM (0.19 mL, 1.7 mmol). Purification of the crude product by flash chromatography (60% EtOAc/hexanes) provided the title compound (150 mg, 74%) as a colorless oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.32 (t, *J* = 7.7 Hz, 2H), 7.16 (d, *J* = 8.0 Hz, 2H), 7.09 (t, *J* = 7.3 Hz, 1H), 6.99 (d, *J* = 8.1 Hz, 2H), 6.95 (d, *J* = 8.1 Hz, 2H), 5.64 (t, *J* = 5.9 Hz, 1H), 3.47 (q, *J* = 6.8 Hz, 2H), 2.84 (t, *J* = 7.2 Hz, 2H), 2.80 (bm, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.85, 158.07, 156.91, 152.23, 133.52, 130.94, 130.60, 124.08, 120.04, 119.88, 119.65, 119.55, 44.10, 35.58, 26.32; HRMS (ESI-TOF+) *m/z* calc'd for $C_{19}H_{18}N_2O_5$ $[M+H]^+$: 355.1294, found 355.1285.



8

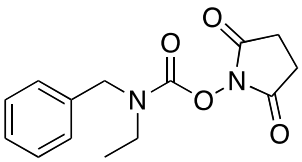
2,5-Dioxopyrrolidin-1-yl 4-(3-phenoxybenzyl)piperazine-1-carboxylate (8). The title compound was synthesized according to **Procedure A** from 1-(3-phenoxybenzyl)piperazine (112 mg, 0.42 mmol), DSC (110 mg, 0.42 mmol) and NMM (0.14 mL, 1.3 mmol). Purification of the crude product by flash chromatography (50% EtOAc/hexanes) provided the title compound (140 mg, 82%) as a colorless oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.31 (t, *J*

= 7.87 Hz, 2H), 7.24 (d, *J* = 7.60 Hz, 1H), 7.08 (t, *J* = 7.39 Hz, 1H), 7.02 (d, *J* = 7.56 Hz, 1H), 6.99 – 6.97 (m, 3H), 6.87 (dd, *J* = 2.41, 8.24 Hz, 1H), 3.61 (bs, 2H), 3.49 (s, 4H), 2.78 (s, 4H), 2.47 (bs, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.64, 158.24, 157.96, 151.16, 140.60, 130.61, 130.47, 124.61, 124.15, 120.10, 119.73, 118.49, 63.22, 53.08, 53.01, 45.95, 45.44, 26.35; HRMS (ESI-TOF+) *m/z* calc'd for $C_{22}H_{23}N_3O_5$ $[M+H]^+$: 410.1716, found 410.1720.



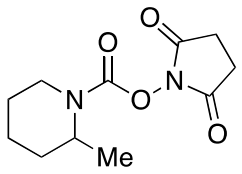
9

2,5-Dioxopyrrolidin-1-yl 4-(4-chlorophenethyl)piperidine-1-carboxylate (9). The title compound was synthesized according to **Procedure A** from 4-[2-(chloro-phenyl)-ethyl-piperidine (130 mg, 0.51 mmol), DSC (130 mg, 0.51 mmol) and NMM (0.17 mL, 1.5 mmol). Purification of the crude product by flash chromatography (30% EtOAc/hexanes) provided the title compound (130 mg, 70%) as a colorless oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.23 (d, *151* 7.6 Hz, 2H), 7.08 (d, *J* = 7.6 Hz, 2H), 4.18 (d, *J* = 13.2 Hz, 1H), 4.07 (d, *J* = 13.0 Hz, 1H), 2.97 (t, *J* = 13.1 Hz, 1H), 2.85 (d, *J* = 13.1 Hz, 1H), 2.79 (s, 4H), 2.59 (t, *J* = 8.4 Hz, 2H), 1.76 (d, *J* = 12.9 Hz, 2H), 1.56 (dd, *J* = 7.4 Hz, 2H), 1.47 (t, *J* = 9.7 Hz, 1H), 1.35 – 1.22 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.78, 151.19, 141.41, 132.35, 130.48, 129.33, 46.49, 45.68, 38.66, 35.76, 33.01, 32.56, 32.23, 26.36; HRMS (ESI-TOF+) *m/z* calc'd for $C_{18}H_{21}ClN_2O_4$ $[M+H]^+$: 365.1268, found 365.1264.



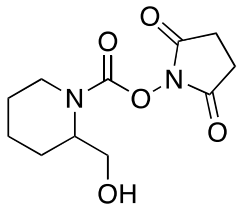
10

2,5-Dioxopyrrolidin-1-yl benzyl(ethyl)carbamate (10). The title compound was synthesized according to **Procedure A** from *N*-ethylbenzylamine (110 mg, 0.81 mmol), DSC (210 mg, 0.81 mmol) and NMM (0.27 mL, 2.4 mmol). Purification of the crude product by flash chromatography (50% EtOAc/hexanes) provided the title compound (210 mg, 94%) as a colorless oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 – 7.26 (m, 10H), 4.60 (s, 2H), 4.51 (s, 2H), 3.38 (q, *J* = 7.1 Hz, 2H), 3.31 (q, *J* = 7.2 Hz, 2H), 2.80 (s, 8H), 1.21 (t, *J* = 7.1 Hz, 3H), 1.11 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.72, 152.98, 151.92, 137.00, 136.94, 129.62, 128.73, 128.70, 128.52, 52.51, 51.05, 44.05, 42.77, 26.37, 14.10, 13.27; HRMS (ESI-TOF+) *m/z* calc'd for $C_{14}H_{16}N_2O_4$ $[M+Na]^+$: 299.1002, found 299.1006.



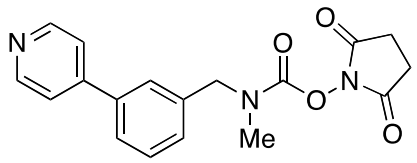
11

2,5-Dioxopyrrolidin-1-yl 2-methylpiperidine-1-carboxylate (11). The title compound was synthesized according to **Procedure A** from 2-methylpiperidine (700 mg, 7.1 mmol), DSC (1.8g, 7.1 mmol) and NMM (2.3 mL, 21 mmol). Purification of the crude product by flash chromatography (50% EtOAc/hexanes) provided the title compound (1.5 g, 88%) as a white solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.44 (bs, 1H), 3.96 (bs, 1H), 3.07 (bs, 1H), 2.81 (s, 4H), 1.83 – 1.73 (m, 1H), 1.69 (d, *J* = 13.1 Hz, 1H), 1.65 – 1.52 (m, 4H), 1.28 (bs, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.82, 151.42, 49.00, 41.04, 30.55, 26.35, 25.98, 19.00, 16.78; HRMS (ESI-TOF+) *m/z* calc'd for $C_{11}H_{16}N_2O_4$ $[M+H]^+$: 241.1188, found 241.1186.



12

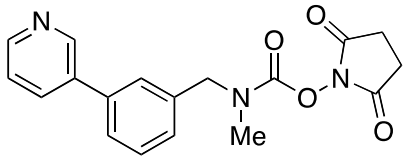
2,5-Dioxopyrrolidin-1-yl 2-(hydroxymethyl)piperidine-1-carboxylate (12). The title compound was synthesized according to **Procedure A** from 2-piperidinemethanol (120 mg, 1.1 mmol), DSC (270 mg, 1.1 mmol) and NMM (0.35 mL, 3.2 mmol). Purification of the crude product by flash chromatography (100% EtOAc) provided the title compound (140 mg, 51%) as a colorless oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.47 – 4.13 (m, 1H), 4.01 – 3.78 (m, 2H), 3.62 (dd, *J* = 11.6, 5.9 Hz, 1H), 3.18 – 2.94 (m, 1H), 2.78 (s, 4H), 1.79 – 1.44 (m, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.26, 152.52, 61.49, 55.47, 54.59, 41.93, 26.36, 25.77, 25.59, 19.77; HRMS (ESI-TOF+) *m/z* calc'd for $C_{11}H_{16}N_2O_5$ $[M+H]^+$: 257.1137, found 257.1134.



13

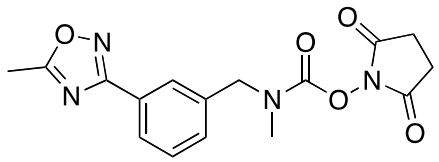
2,5-Dioxopyrrolidin-1-yl methyl(3-(pyridin-4-yl)benzyl)carbamate (13). The title compound was synthesized according to **Procedure A** from *N*-methyl-*N*-(3-pyridin-4-ylbenzyl)amine (110 mg, 0.55 mmol), DSC (141 mg, 0.55 mmol) and NMM (0.18 mL, 1.7 mmol). Purification of the crude product by flash chromatography (100% EtOAc) provided the title compound (140 mg,75%) as a colorless oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.66 (s,

4H), 7.72 (s, 1H), 7.64 – 7.45 (m, 9H), 7.42 (d, *J* = 7.7 Hz, 1H), 7.33 (d, *J* = 7.6 Hz, 1H), 4.66 (s, 2H), 4.57 (s, 2H), 3.05 (s, 3H), 2.94 (s, 3H), 2.84 (s, 8H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.58, 153.08, 152.19, 151.16, 148.55, 148.47, 139.74, 139.67, 137.49, 137.43, 130.54, 130.49, 129.27, 127.50, 127.46, 127.09, 126.97, 122.54, 122.52, 54.73, 53.50, 36.22, 34.91, 26.38; HRMS (ESI-TOF+) *m/z* calc'd for $C_{18}H_{17}N_3O_4$ $[M+H]^+$: 340.1297, found 340.1289.



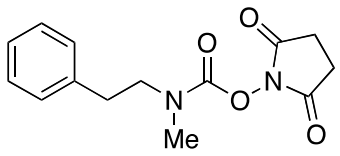
14

2,5-Dioxopyrrolidin-1-yl methyl(3-(pyridin-3-yl)benzyl)carbamate (14). The title compound was synthesized according to **Procedure A** from N-methyl-N-(3-pyridin-3-ylbenzyl)amine (110 mg, 0.55 mmol), DSC (141 mg, 0.55 mmol) and NMM (0.18 mL, 1.7 mmol). Purification of the crude product by flash chromatography (100% EtOAc) provided the title compound (160 mg, 87%) as a colorless oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.89 (bs, 1H), 8.84 (bs, 1H), 8.57 (bs, 2H), 7.97 (d, *J* = 7.7 Hz, 1H), 7.88 (d, *J* = 7.7 Hz, 1H), 7.65 (s, 1H), 7.56 – 7.42 (m, 5H), 7.39 – 7.34 (m, 3H), 7.28 (d, *J* = 7.6 Hz, 1H), 4.64 (s, 2H), 4.55 (s, 2H), 3.03 (s, 3H), 2.93 (s, 3H), 2.81 (s, 8H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.62, 153.07, 152.20, 149.47, 149.13, 149.09, 139.39, 139.31, 137.42, 137.38, 136.92, 136.85, 135.42, 135.35, 130.50, 130.44, 128.31, 127.55, 127.26, 127.13, 124.49, 54.74, 53.51, 36.21, 34.89, 26.37; HRMS (ESI-TOF+) *m/z* calc'd for $C_{18}H_{17}N_3O_4$ $[M+H]^+$: 340.1297, found 340.1294.



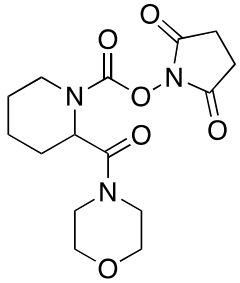
15

2,5-Dioxopyrrolidin-1-yl methyl(3-(5-methyl-1,2,4-oxadiazol-3-yl)benzyl)carbamate (15). The title compound was synthesized according to **Procedure A** from N-methyl-N-[3-(5-methyl-1,2,4-oxadiazol-3-yl)benzyl]amine (70 mg, 0.34 mmol), DSC (88 mg, 0.34 mmol) and NMM (0.11 mL, 1.0 mmol). Purification of the crude product by flash chromatography (70% EtOAc/hexanes) provided the title compound (110 mg, 93%) as a colorless oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.03 – 7.99 (m, 3H), 7.94 (s, 1H), 7.63 (d, *J* = 7.6 Hz, 1H), 7.53 (t, *J* = 7.7 Hz, 1H), 7.50 (t, *J* = 7.7 Hz, 1H), 7.44 (d, *J* = 7.7 Hz, 1H), 4.66 (s, 2H), 4.57 (s, 2H), 3.04 (s, 3H), 2.94 (s, 3H), 2.84 (s, 8H), 2.66 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.54, 177.53, 170.55, 170.53, 168.90, 168.89, 153.02, 152.18, 137.28, 137.26, 131.31, 131.23, 130.62, 130.57, 128.11, 127.92, 127.85, 127.82, 127.66, 54.59, 53.40, 36.17, 34.77, 26.37, 13.28; HRMS (ESI-TOF+) *m/z* calc'd for $C_{16}H_{16}N_4O_5$ $[M+H]^+$: 345.1199, found 345.1186.



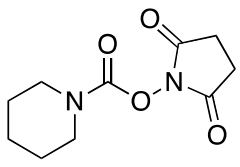
16

2,5-Dioxopyrrolidin-1-yl methyl(phenethyl)carbamate (16). The title compound was synthesized according to **Procedure A** from *N*-methyl-phenethylamine (140 mg, 1.0 mmol), DSC (270 mg, 1.0 mmol) and NMM (0.34 mL, 3.1 mmol). Purification of the crude product by flash chromatography (50% EtOAc/hexanes) provided the title compound (260 mg, 90%) as a colorless oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.33 – 7.26 (m, 4H), 7.26 – 7.19 (m, 6H), 3.58 (t, *J* = 7.6 Hz, 1H), 3.50 (t, *J* = 7.6 Hz, 1H), 3.01 (t, *J* = 7.5 Hz, 2H), 2.96 (s, 3H), 2.88 (t, *J* = 7.5 Hz, 2H), 2.81 (s, 3H), 2.78 (s, 4H), 2.77 (s, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.80, 170.79, 152.20, 152.16, 139.08, 139.02, 129.71, 129.68, 129.54, 129.52, 127.48, 127.45, 53.47, 52.12, 37.68, 35.93, 35.25, 34.34, 26.37, 26.34; HRMS (ESI-TOF+) *m/z* calc'd for $C_{14}H_{16}N_2O_4$ $[M+H]^+$: 277.1188, found 277.1184.



17

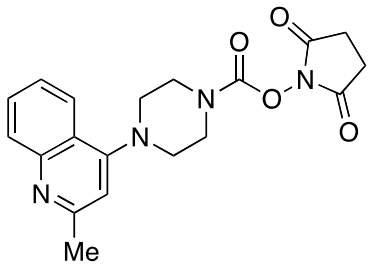
2,5-Dioxopyrrolidin-1-yl 2-(morpholine-4-carbonyl)piperidine-1-carboxylate (17). The title compound was synthesized according to **Procedure A** from morpholino(2-piperidinyl)methanone hydrochloride (63 mg, 0.27 mmol), DSC (69 mg, 0.27 mmol) and NMM (0.089 mL, 0.81 mmol). Purification of the crude product by flash chromatography (100% EtOAc) provided the title compound (42 mg, 46%) as a colorless oil (3:7 mixture of cis:trans carbamate isomers): ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.07 (s, 0.3H), 4.92 (d, *J* = 5.9 Hz, 0.7H), 4.07 (d, *J* = 12.7 Hz, 0.7H), 3.96 (d, *J* = 12.1 Hz, 0.3H), 3.82 – 3.35 (m, 9H), 2.81 (s, 4H), 1.96 – 1.55 (m, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.43, 168.86, 151.65, 150.58, 66.94, 66.74, 52.26, 51.46, 46.21, 43.79, 43.01, 42.54, 26.69, 26.39, 25.49, 24.77, 24.31, 19.37, 19.05; HRMS (ESI-TOF+) *m/z* calc'd for $C_{15}H_{21}N_3O_6$ $[M+H]^+$: 340.1508, found 340.1514.



18

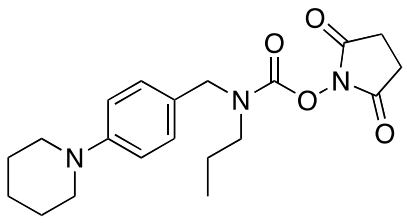
2,5-Dioxopyrrolidin-1-yl piperidine-1-carboxylate (18). The title compound was synthesized according to **Procedure A** from piperidine (150 mg, 1.7 mmol), DSC (450 mg, 1.7 mmol) and NMM (0.57 mL, 5.2 mmol). Purification of the crude product by flash

chromatography (50% EtOAc/hexanes) provided the title compound (310 mg, 79%) as a white solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.56 (s, 2H), 3.44 (s, 2H), 2.79 (s, 4H), 1.62 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.78, 151.24, 47.18, 46.44, 26.35, 26.31, 26.02, 24.74; HRMS (ESI-TOF+) *m/z* calc'd for $C_{10}H_{14}N_2O_4$ $[M+H]^+$: 227.1032, found 227.1028.



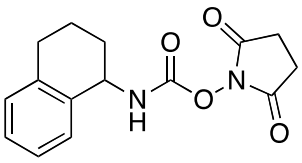
19

2,5-Dioxopyrrolidin-1-yl 4-(2-methylquinolin-4-yl)piperazine-1-carboxylate (19). The title compound was synthesized according to **Procedure A** from 2-methyl-4-piperazinoquinoline (86 mg, 0.38 mmol), DSC (97 mg, 0.38 mmol) and NMM (0.13 mL, 1.1 mmol). Purification of the crude product by flash chromatography (50% EtOAc/hexanes) provided the title compound (110 mg, 79%) as a white solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.2 Hz, 1H), 7.91 (d, *J* = 8.2 Hz, 1H), 7.62 (t, *J* = 6.7 Hz, 1H), 7.43 (t, *J* = 7.2 Hz, 1H), 6.74 (s, 1H), 3.91 (bs, 2H), 3.79 (bs, 2H), 3.24 (bs, 4H), 2.81 (s, 4H), 2.67 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.55, 160.37, 156.94, 151.33, 150.10, 130.21, 130.17, 125.89, 123.70, 122.47, 110.83, 52.41, 45.98, 45.58, 26.51, 26.35; HRMS (ESI-TOF+) *m/z* calc'd for $C_{19}H_{20}N_4O_4$ $[M+H]^+$: 369.1563, found 369.1572.



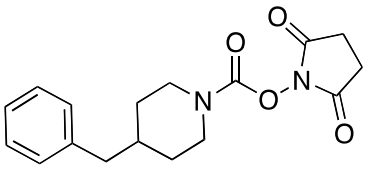
20

2,5-Dioxopyrrolidin-1-yl 4-(piperidin-1-yl)benzyl(propyl)carbamate (20). The title compound was synthesized according to **Procedure A** from *N*-(4-piperidin-1-ylbenzyl)-*N*-propylamine (23 mg, 0.10 mmol), DSC (25 mg, 0.10 mmol) and NMM (0.033 mL, 0.30 mmol). Purification of the crude product by flash chromatography (50% EtOAc/hexanes) provided the title compound (23 mg, 62%) as a colorless oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30 – 7.23 (m, 1H), 7.14 (d, *J* = 7.9 Hz, 1H), 6.92 (d, *J* = 8.1 Hz, 1H), 6.89 (d, *J* = 7.9 Hz, 1H), 4.51 (s, 1H), 4.42 (s, 1H), 3.23 (s, 1H), 3.19 – 3.12 (m, 5H), 2.82 (s, 4H), 1.72 – 1.52 (m, 8H), 0.86 (dt, *J* = 16.9, 7.5 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.69, 170.67, 153.00, 152.68, 152.66, 152.22, 129.86, 129.61, 127.02, 126.88, 117.25, 52.37, 51.29, 51.27, 50.88, 50.26, 48.97, 26.62, 26.39, 25.13, 22.03, 21.27, 12.03, 11.97; HRMS (ESI-TOF+) *m/z* calc'd for $C_{20}H_{27}N_3O_4$ $[M+H]^+$: 374.2080, found 374.2077.



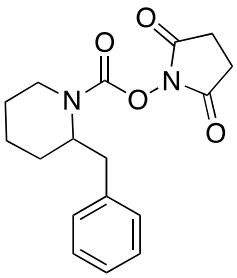
21

2,5-Dioxopyrrolidin-1-yl (1,2,3,4-tetrahydronaphthalen-1-yl)carbamate (21). The title compound was synthesized according to **Procedure A** from 1,2,3,4-tetrahydro-1-naphthylamine (270 mg, 1.8 mmol), DSC (460 mg, 1.8 mmol) and NMM (0.59 mL, 5.4 mmol). Purification of the crude product by flash chromatography (50% EtOAc/hexanes) provided the title compound (230 mg, 44%) as a white solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36 (d, *J* = 4.7 Hz, 1H), 7.23 – 7.20 (m, 2H), 7.12 – 7.10 (m, 1H), 5.37 (s, 1H), 4.91 (dd, *J* = 13.9, 6.4 Hz, 1H), 2.86 – 2.71 (m, 6H), 2.14 – 2.06 (m, 1H), 1.97 – 1.83 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.69, 151.83, 138.33, 135.99, 130.08, 129.57, 128.61, 127.35, 51.57, 30.82, 29.91, 26.32, 20.63; HRMS (ESI-TOF+) *m/z* calc'd for $C_{15}H_{16}N_2O_4$ $[M+Na]^+$: 311.1002, found 311.1002.



22

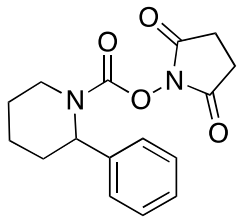
2,5-Dioxopyrrolidin-1-yl 4-benzylpiperidine-1-carboxylate (22). The title compound was synthesized according to **Procedure A** from 4-benzylpiperidine (690 mg, 3.9 mmol), DSC (1.0 g, 3.9 mmol) and NMM (1.3 mL, 12 mmol). Purification of the crude product by flash chromatography (60% EtOAc/hexanes) provided the title compound (1.1 g, 89%) as a white solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.28 (t, *J* = 7.4 Hz, 3H), 7.22 – 7.18 (m, 1H), 7.13 (d, *J* = 7.5 Hz, 2H), 4.18 (d, *J* = 13.1 Hz, 1H), 4.08 (d, *J* = 13.1 Hz, 1H), 2.94 (t, *J* = 12.9 Hz, 1H), 2.86 – 2.79 (m, 5H), 2.56 (d, *J* = 6.9 Hz, 2H), 1.77 – 1.67 (m, 3H), 1.41 – 1.22 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.73, 151.17, 140.59, 129.92, 129.20, 126.97, 46.56, 45.73, 43.68, 38.56, 32.46, 32.21, 26.35; HRMS (ESI-TOF+) *m/z* calc'd for $C_{17}H_{20}N_2O_4$ $[M+Na]^+$: 339.1315, found 339.1318.



23

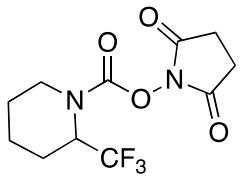
2,5-Dioxopyrrolidin-1-yl 2-benzylpiperidine-1-carboxylate (23). The title compound was synthesized according to **Procedure A** from 2-benzylpiperidine (180 mg, 1.0 mmol), DSC (260 mg, 1.0 mmol) and NMM (0.34 mL, 3.1 mmol). Purification of the crude product by

flash chromatography (50% EtOAc/hexanes) provided the title compound (210 mg, 65%) as a colorless oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.32 – 7.24 (m, 2H), 7.24 – 7.15 (m, 3H), 4.48 – 4.35 (m, 1H), 4.11 – 3.95 (m, 1H), 3.24 – 2.87 (m, 3H), 2.77 (s, 4H), 1.76 – 1.51 (m, 6H) ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.74, 151.65, 138.95, 130.03, 129.44, 127.35, 55.18, 54.82, 42.10, 41.55, 37.12, 36.60, 26.66, 26.37, 25.81, 19.09; HRMS (ESI-TOF+) *m/z* calc'd for $C_{17}H_{20}N_2O_4$ $[M+H]^+$: 317.1501, found 317.1499.



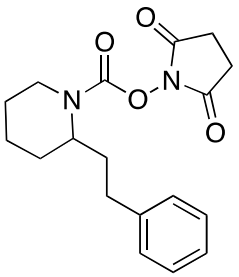
24

2,5-Dioxopyrrolidin-1-yl 2-phenylpiperidine-1-carboxylate (24). The title compound was synthesized according to **Procedure A** from 2-phenylpiperidine (117 mg, 0.73 mmol), DSC (190 mg, 0.73 mmol) and NMM (0.24 mL, 2.2 mmol). Purification of the crude product by flash chromatography (50% EtOAc/hexanes) provided the title compound (140 mg, 64%) as a colorless oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 – 7.22 (m, 5H), 5.47 (bs, 1H), 4.05 (bs, 1H), 2.99 (bs, 1H), 2.78 (s, 4H), 2.41 (d, *J* = 14.3 Hz, 1H), 2.06 – 1.97 (m, 1H), 1.72 – 1.57 (m, 3H), 1.57 – 1.45 (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.76, 152.30, 138.66, 129.73, 127.90, 127.38, 55.72, 42.46, 28.30, 26.38, 26.00, 19.79; HRMS (ESI-TOF+) *m/z* calc'd for $C_{16}H_{18}N_2O_4$ $[M+Na]^+$: 325.1159, found 325.1155.



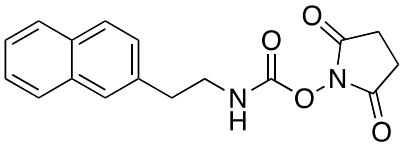
25

2,5-Dioxopyrrolidin-1-yl 2-(trifluoromethyl)piperidine-1-carboxylate (25). The title compound was synthesized according to **Procedure A** from 2-trifluoromethylpiperidine (170 mg, 1.1 mmol), DSC (280 mg, 1.1 mmol) and NMM (0.37 mL, 3.3 mmol). Purification of the crude product by flash chromatography (30% EtOAc/hexanes) provided the title compound (160 mg, 49%) as a colorless oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.73 – 4.67 (m, 1H), 4.66 – 4.58 (m, 1H), 4.16 (d, *J* = 13.6 Hz, 1H), 4.08 (d, *J* = 13.2 Hz, 1H), 3.21 (t, *J* = 13.3 Hz, 1H), 3.07 (t, *J* = 13.4 Hz, 1H), 2.78 (s, 8H), 2.04 (d, *J* = 14.7 Hz, 2H), 1.89 – 1.49 (m, 10H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.32, 152.44, 151.66,126.56 (q, *J* = 285 Hz), 126.37 (q, *J* = 285 Hz), 53.04 (p, *J* = 30.4 Hz), 43.25, 42.86, 26.29, 24.84, 24.64, 23.54, 23.23, 19.47; HRMS (ESI-TOF+) *m/z* calc'd for $C_{11}H_{13}F_3N_2O_4$ $[M+H]^+$: 295.0905, found 295.0899.



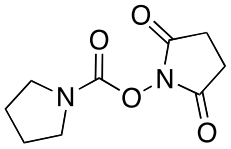
26

2,5-Dioxopyrrolidin-1-yl 2-phenethylpiperidine-1-carboxylate (26). The title compound was synthesized according to **Procedure A** from 2-phenethylpiperidine (120 mg, 0.53 mmol), DSC (140 mg, 0.53 mmol) and NMM (0.18 mL, 1.6 mmol). Purification of the crude product by flash chromatography (50% EtOAc/hexanes) provided the title compound (130 mg, 74%) as a colorless oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30 – 7.14 (m, 5H), 4.37 – 4.26 (m, 1H), 4.10 – 3.94 (m, 1H), 3.11 – 2.96 (m, 1H), 2.79 (s, 4H), 2.75 – 2.56 (m, 2H), 2.14 (bs, 1H), 1.85 – 1.51 (m, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.81, 150.82, 141.49, 128.30, 128.28, 125.82, 52.75, 52.20, 40.59, 40.12, 32.36, 31.61, 28.19, 25.44, 25.21, 24.97, 18.52, 18.47; HRMS (ESI-TOF+) *m/z* calc'd for $C_{18}H_{22}N_2O_4$ $[M+Na]^+$: 353.1472, found 353.1472.



27

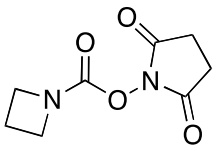
2,5-Dioxopyrrolidin-1-yl (2-(naphthalen-2-yl)ethyl)carbamate (27). The title compound was synthesized according to **Procedure A** from 2-(2-naphthyl)-ethylamine (160 mg, 0.93 mmol), DSC (240 mg, 0.93 mmol) and NMM (0.31 mL, 2.8 mmol). Purification of the crude product by flash chromatography (100% EtOAc) provided the title compound (130 mg, 45%) as a white solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ (d, *J* = 7.8 Hz, 3H), 7.67 (s, 1H), 7.46 (t, *J* = 8.3 Hz, 2H), 7.33 (d, *J* = 8.3 Hz, 1H), 5.54 (t, *J* = 6.2 Hz, 1H), 3.57 (d, *J* = 7.1 Hz, 2H), 3.05 – 3.00 (m, 2H), 2.75 (s, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.76, 152.24, 136.14, 134.39, 133.18, 129.36, 128.50, 128.45, 128.27, 127.85, 127.09, 126.54, 43.85, 36.33, 26.27; HRMS (ESI-TOF+) *m/z* calc'd for $C_{17}H_{16}N_2O_4$ $[M+H]^+$: 313.1188, found 313.1183.



28

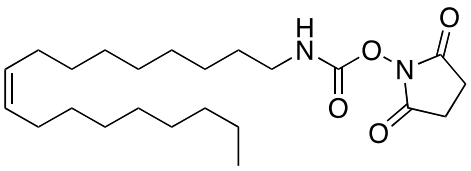
2,5-Dioxopyrrolidin-1-yl pyrrolidine-1-carboxylate (28). The title compound was synthesized according to **Procedure A** from pyrrolidine (330 mg, 4.7 mmol), DSC (1.2 g, 4.7

mmol) and NMM (1.5 mL, 14 mmol). Purification of the crude product by flash chromatography (50% EtOAc/hexanes) provided the title compound (720 mg, 72%) as a white solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.56 (t, *J* = 6.8 Hz, 2H), 3.45 (t, *J* = 6.8 Hz, 2H), 2.81 (s, 4H), 1.96 (p, *J* = 6.6 Hz, 2H), 1.90 (p, *J* = 6.6 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.85, 150.41, 48.33, 46.91, 26.67, 26.34, 25.48; HRMS (ESI-TOF+) *m/z* calc'd for $C_9H_{12}N_2O_4$ $[M+H]^+$: 213.0875, found 213.0868.



29

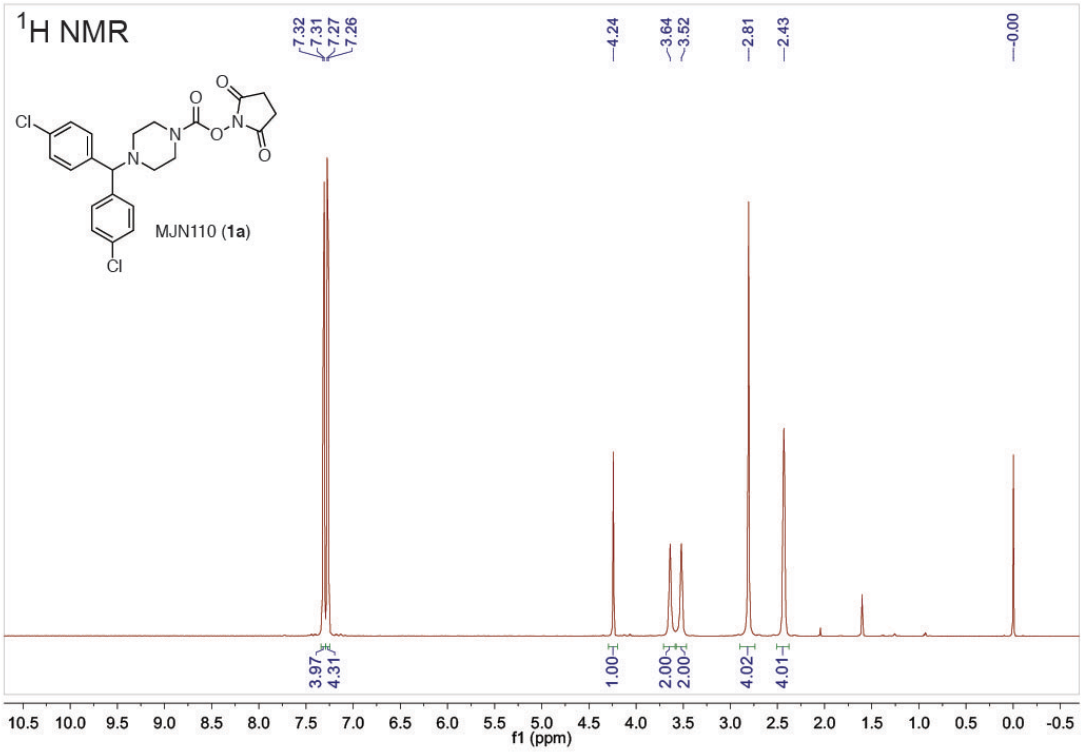
2,5-Dioxopyrrolidin-1-yl azetidine-1-carboxylate (29). The title compound was synthesized according to **Procedure A** from azetidine (150 mg, 2.6 mmol), DSC (670 mg, 2.6 mmol) and NMM (0.87 mL, 7.9 mmol). Purification of the crude product by flash chromatography (50% EtOAc/hexanes) provided the title compound (340 mg, 65%) as a white solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.30 – 4.26 (m, 2H), 4.15 – 4.10 (m, 2H), 2.79 (s, 4H), 2.36 (p, *J* = 7.8 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.70, 150.99, 51.43, 50.83, 26.32, 17.26; HRMS (ESI-TOF+) *m/z* calc'd for $C_8H_{10}N_2O_4$ $[M+H]^+$: 199.0719, found 199.0710.

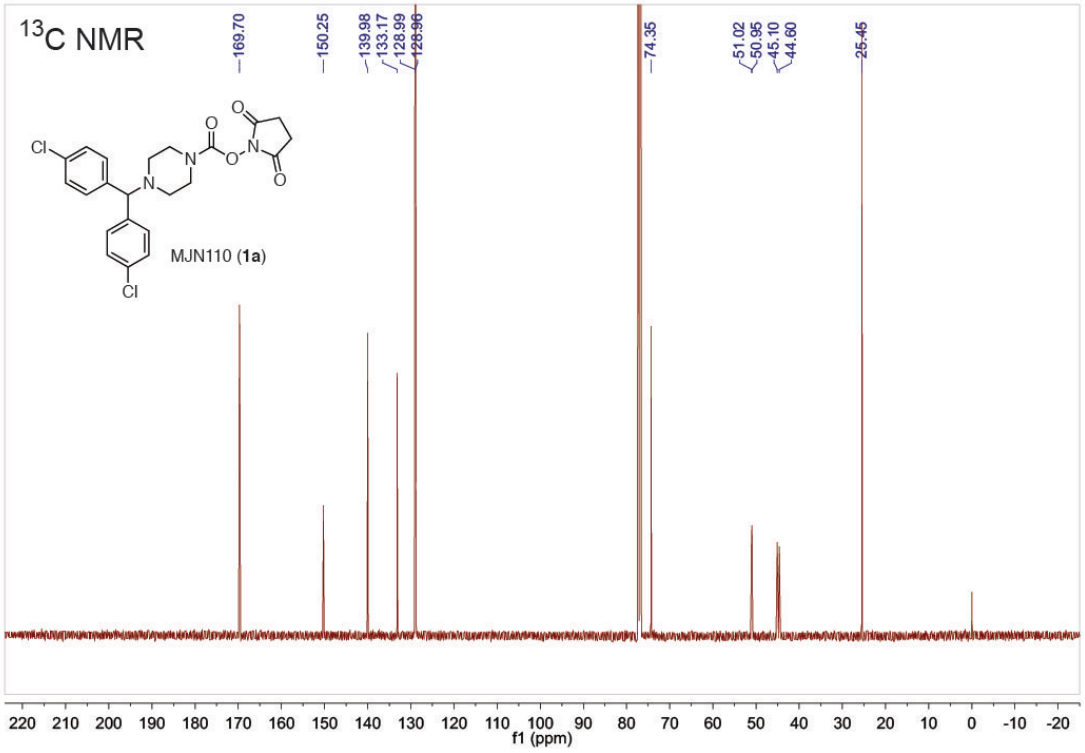


30

(*Z*)-2,5-Dioxopyrrolidin-1-yl octadec-9-en-1-ylcarbamate (30). The title compound was synthesized according to **Procedure A** from oleylamine (250 mg, 0.93 mmol), DSC (240 mg, 0.93 mmol) and NMM (0.31 mL, 2.8 mmol). Purification of the crude product by flash chromatography (50% EtOAc/hexanes) provided the title compound (150 mg, 39%) as a white solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.64 (t, *J* = 5.6 Hz, 1H), 5.40 – 5.30 (m, 2H), 3.22 (q, *J* = 6.8 Hz, 2H), 2.81 (s, 4H), 2.05 – 1.92 (m, 4H), 1.54 (p, *J* = 7.1 Hz, 2H), 1.32 – 1.23 (m, 22H), 0.87 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.96, 152.20, 130.80, 130.61, 42.93, 32.75, 30.61, 30.58, 30.37, 30.34, 30.29, 30.25, 30.16, 30.07, 30.01, 28.06, 28.04, 27.45, 26.31, 23.53, 14.97; HRMS (ESI-TOF+) *m/z* calc'd for $C_{23}H_{40}N_2O_4$ $[M+H]^+$: 409.3066, found 409.3062.

MJN110 NMR SPECTRA





References

1. Chang, J. W.; Cognetta, A. B., 3rd; Niphakis, M. J.; Cravatt, B. F., Proteome-Wide Reactivity Profiling Identifies Diverse Carbamate Chemotypes Tuned for Serine Hydrolase Inhibition. *ACS Chem Biol* **(ASAP)**.