Supplementary information

Synthesis and pharmacology of glutamate receptor ligands: new isothiazole analogues of ibotenic acid

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General experimental

All materials were obtained from commercial suppliers and used without further purification unless stated otherwise. 3-Benzyloxyisothiazole, 2-(*N-tert*-butoxycarbonylimino)malonic acid diethyl ester and Thio-Ibo were prepared according to previously described methods.[1, 2] Ibo was purchased from Tocris. *i*PrMgCl and *n*BuLi was titrated prior to use.[3] Et_2O was dried over sodium. LDA was freshly prepared from *n*BuLi and *i*Pr_2NH in Et_2O at -78 ˚C under N_2, left stirring at rt for 30 min and then used in the reactions. All air-sensitive reactions were carried out under N_2. Microwave experiments were performed in sealed glass vials (capacity 10-20 mL) using an Emrys Optimizer (Personal Chemistry). Compounds were visualized on TLC (silica gel 60 F254 plates) using UV light, $FeCl_3$ or $KMnO_4$. Flash chromatography (FC) was performed on a glass column (silicagel 60, 0.040-0.063 mm), a FlashMaster^{TM} Personal one column (FP) (ISOLUTE^{R} SPE Columns) or a FlashMaster^{TM} (F) (ISOLUTE^{R} SPE Columns) apparatus. Preparative HPLC was performed on an XTerra Prep MS C_{18} column (10 × 300 mm, 10 μm) equipped with an XTerra guard column (10 × 10 mm) connected to a Jasco 880 pump, a Rheodyne 7125 injector, a 5 mL loop, a TSP UV100 spectrophotometer (210 nm), and a Hitachi D-2000 Chromato-Integrator. Melting points were measured in open capillary tubes by normal oil bath method or an OptiMelt MPA100 apparatus (SRS) and are all uncorrected. Accurate mass determination (HRMS) was performed on a Micromass Q-Tof mass spectrometer (MeCN/HCOOH, 8.74 μL min^{-1}) in electrospray (ES) mode. Elemental analyses were carried out at the Analytical Research Department, H. Lundbeck A/S, Denmark. GC-MS analyses were performed using electron ionization (EI). ^1H, ^{13}C and APT NMR spectra were recorded on a 300/75 MHz Varian (Gemini) instrument or on a 500/125 MHz Bruker Avance DRX500 instrument. Chemical shifts were measured in ppm (*J* values are given in Hz), TMS was used as internal reference for ^1H NMR spectra and $CDCl_3$ was used as internal reference standard for ^{13}C NMR spectra. The pK_a values were measured potentiometrically using a Sirius GLpKa Auto-titrator and the data were analyzed with Sirius pKaLOGP software (version 5.2). Multiple runs were performed using MeOH/H_2O mixtures. The derived pK_a values were extrapolated to zero organic solvent using Yasuda-Shedlovsky plots.[4] The samples were run from low pH to high pH using first HCl (aq) to obtain a pH of 2 or 3.5 and then titrated with KOH (aq).

Experimental procedures

(*RS*)-2-Amino-2-(3-hydroxy-5-isothiazolyl)propionic acid (1).

(*RS*)-2-(3-Benzyloxy-5-isothiazolyl)-2-(*N*-*tert*-butoxycarbonylamino)propionic acid (**6**) (313 mg, 0.9 mmol) was dissolved in AcOH (4 mL). 10% HBr in AcOH (4 mL) was added. After stirring for 3½ h the reaction was concentrated *in vacuo*. The solid was triturated with Et_2O and recrystallized from H_2O giving the zwitterion of **1** as light brown crystals (37 mg, 21%). Mp. >170 ˚C; (Found: C, 36.3; H, 4.5; N, 13.7. Calc. for $C_6H_8N_2SO_3 \cdot 0.67\ H_2O$: C, 36.0; H, 4.7; N, 14.0%); TLC (*n*BuOH/H_2O/AcOH/EtOAc 1:1:1:1) R_f 0.4; δ_H(DMSO-d_6) 1.57 (s, 3H), 6.54 (s, 1H).

(*RS*)-2-Amino-(4-bromo-3-hydroxy-5-isothiazolyl)acetic acid (2b).

(*RS*)-(3-Benzyloxy-4-bromo-5-isothiazolyl)-(*N*-*tert*-butoxycarbonylamino)acetic acid (**14b**) (272 mg, 0.6 mmol) was dissolved in AcOH (3 mL). 10% HBr in AcOH (3 mL) was added. After stirring for 4 h the reaction was concentrated *in vacuo*. The solid was triturated with Et_2O and recrystallized (*i*PrOH/Et_2O) giving the hydrobromide of **2b** as colorless crystals (176 mg, 86%). Mp. >140 ˚C; (Found: C, 16.4; H, 2.4; N, 8..0. Calc. for $C_5H_5BrN_2O_3S \cdot HBr \cdot 1.5H_2O$: C, 16.6; H, 2.5; N, 7.8%); TLC (*n*BuOH/H_2O/AcOH/EtOAc 1:1:1:1) R_f 0.4; δ_H(DMSO-d_6) 5.50 (s, 1H), 8.97 (br s, 3H).

(*RS*)-2-Amino-(3-hydroxy-4-methyl-5-isothiazolyl)acetic acid (2c).

The title compound was prepared according to the procedure described for **2a** starting with (*RS*)-(3-benzyloxy-4-methyl-5-isothiazolyl)-(*N*-*tert*-butoxycarbonyl-amino)acetic acid (**14c**) (109 mg, 0.3 mmol) and a reaction time of 6 h. The zwitterion of **2c** was isolated as colorless crystals (32 mg, 59%). Mp. >145 ˚C; (Found: C, 37.35; H, 4.3; N, 14.1. Calc. for $C_6H_8N_2O_3S \cdot 0.33\ H_2O$: C, 37.1; H, 4.5; N, 14.4%); TLC (*n*BuOH/H_2O/AcOH/EtOAc 1:1:1:1) R_f 0.4; δ_H(DMSO-d_6) 1.97 (s, 3H), 4.50 (s, 1H).

(*RS*)-2-Amino-(4-ethyl-3-hydroxy-5-isothiazolyl)acetic acid (2d).

The title compound was prepared according to the procedure described for **2a** starting with (*RS*)-(3-benzyloxy-4-ethyl-5-isothiazolyl)-(*N*-*tert*-butoxycarbonyl-amino)acetic acid (**14d**) (230 mg, 0.6 mmol) and a reaction time of 4½ h. The zwitterion **2d** was isolated as light beige crystals (62 mg, 52%). Mp. >130 ˚C; (Found: C, 37.1; H, 5.4; N, 12.3. Calc. for $C_7H_{10}N_2O_3S \cdot 1.33\ H_2O$: C, 37.2; H, 5.6; N, 12.4%); TLC (*n*BuOH/H_2O/AcOH/EtOAc 1:1:1:1) R_f 0.5; δ_H(DMSO-d_6) 1.06 (t, *J* 7, 3H), 2.41-2.46 (m, lies below DMSO peaks), 4.49 (s, 1H). δ_H(D_2O, NaOD) 1.06 (t, *J* 7, 3H), 2.42 (dq, *J* 2 and 7, 2H), 4.58 (s, 1H).

(*RS*)-2-Amino-(3-hydroxy-4-phenyl-5-isothiazolyl)acetic acid (2e).

The title compound was prepared according to the procedure described for **2a** starting with (*RS*)-(3-benzyloxy-4-phenyl-5-isothiazolyl)-(*N*-*tert*-butoxycarbonyl-amino)acetic acid (**14e**) (260 mg, 0.6 mmol) and a reaction time of 4½ h. The zwitterion **2e** was isolated as light beige crystals (103 mg, 70%). Mp. >165 ˚C; (Found: C, 52.2; H, 4.1; N, 11.0. Calc. for $C_{11}H_{10}N_2O_3S \cdot 0.167\ H_2O$: C, 52.2; H, 4.1; N, 11.1%); TLC (*n*BuOH/ H_2O/AcOH/EtOAc 1:1:1:1) R_f 0.5; δ_H(DMSO-d_6) 4.42 (s, 1H), 7.36-7.46 (m, 3H), 7.64-7.66 (m, 2H).

(*RS*)-2-Amino-(4-benzyl-3-hydroxy-5-isothiazolyl)acetic acid (2f).

The title compound was prepared according to the procedure described for **2a** starting with (*RS*)-(4-benzyl-3-benzyloxy-5-isothiazolyl)-(*N*-*tert*-butoxycarbonyl-amino)acetic acid (**14f**) (448 mg, 0.8 mmol) and a reaction time of 8 h. **2f** was purified using HPLC (2.5% MeOH in 15 mM AcOH (aq)) and recrystallized ($MeOH/H_2O$) to give the zwitterion as colorless needles (44 mg, 21%). Mp. 142.8-143.1 ˚C; (Found: C, 54.4; H, 4.7; N, 10.6. Calc. for $C_{12}H_{12}N_2O_3S$: C, 54.5; H, 4.6; N, 10.6%); TLC (*n*$BuOH/H_2O$/AcOH/EtOAc 1:1:1:1) R_f 0.5; δ_H(DMSO-d_6) 3.75-3.78 (m, 1H), 3.90-3.95 (m, 1H), 4.51-4.55 (m, 1H), 7.14-7.27 (m, 5H).

(*RS*)-2-Amino-(3-hydroxy-4-phenethyl-5-isothiazolyl)acetic acid (2g). The title compound was prepared according to the procedure described for **2a** starting with (*RS*)-(3-benzyloxy-4-phenethyl-5-isothiazolyl)-(*N*-*tert*-butoxycarbonylamino)acetic acid (**14g**) (267 mg, 0.57 mmol) and a reaction time of 8 h. HPLC (10% MeOH in 15 mM AcOH (aq)) and recrystallization ($MeOH/H_2O$) gave the zwitterion **2g** as colorless needles (78 mg, 49%). Mp. 142.8-143.1 ˚C; (Found: C, 55.95; H, 5.1; N, 10.0. Calc. for $C_{13}H_{14}N_2O_3S$: C, 56.1; H, 5.1; N, 10.1%); TLC (*n*$BuOH/H_2O$/AcOH/EtOAc 1:1:1:1) R_f 0.6; δ_H(DMSO-d_6) 2.64-2.79 (m, 4H), 4.57 (s, α-H, 1H), 7.17-7.31 (m, 5H).

2-(*N*-*tert*-Butoxycarbonylimino)propionic acid ethyl ester (3).

N-*tert*-butoxycarbonyl-triphenyliminophosphorane[2] (9.40 g, 25 mmol) was suspended in freshly distilled THF (20 mL). Freshly distilled ethyl pyruvate (2.5 mL, 2.60 g, 23 mmol) in dry THF (20 mL) was added and the reaction mixture was refluxed over night. The mixture was concentrated *in vacuo*. Triphenylphosphine oxide was removed using several volumes of Et_2O in order to precipitate the oxide. Kugelrohr distillation (100 ˚C, 0.5 mbar) yielded **3** as a clear oil (1.45 g, 30%). NMR showed a tautomeric mixture (~3:2, imine:enamine). The compound was found to be unstable and should be used within a few hours. $\delta_H(CDCl_3)$ 1.33 (t, *J* 7, CH_3, 1.8H), 1.54 (s, *t*Bu, 5.4H), 2.23 (s, CH_3, 1.8H), 4.24-4.36 (m, CH_2, 1.2H). 2-(*N*-*tert*-Butoxycarbonylamino)acrylic acid ethyl ester:[5] $\delta_H(CDCl_3)$ 1.33 (t, *J* 7, CH_3, 1.2H), 1.48 (s, *t*Bu, 3.6H), 4.24-4.36 (m, CH_2, 0.8H), 5.72 (s, H-C=, 0.4H), 6.13 (s, H-C=, 0.4H), 7.03 (br s, NH, 0.4H).

2-(3-Benzyloxy-5-isothiazolyl)-2-(*N*-*tert*-butoxycarbonylamino)propionic acid ethyl ester (5).

3-Benzyloxyisothiazole (**4)** (1.36 g, 7.1 mmol) in Et_2O (2 mL) was added to freshly prepared LDA (7.8 mmol) in Et_2O (4 mL) at -78 ˚C. The mixture was left for 15 min and **3** (1.64 g, 7.6 mmol) in Et_2O (1 mL) was added over 5 min. The mixture was left at -78 ˚C for 15 min. Satd. NH_4Cl (aq) was added and the mixture was slowly warmed to rt. The mixture was extracted with EtOAc (3×), washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. FC (petroleum ether/EtOAc 4:1) yielded **5** as a yellow oil (923 mg, 36%). TLC (petroleum ether/EtOAc 4:1) R_f 0.20; $\delta_H(CDCl_3)$ 1.28 (t, *J* 8, CH_3, 3H), 1.40 (s, *t*Bu, 9H), 1.88 (s, CH_3, 3H), 4.17-4.31 (m, CH_2, 2H), 5.35 (s, CH_2, 2H), 5.65 (br s, NH, 1H), 6.64 (s, H-4, 1H), 7.33-7.45 (m, 5H).

2-(3-Benzyloxy-5-isothiazolyl)-2-(*N*-*tert*-butoxycarbonylamino)propionic acid (6).

5 (450 mg, 1.24 mmol) was dissolved in THF and 2.5M LiOH (aq) (2 mL, 5 mmol) was added. After 2½ h the mixture was cooled to 0 ˚C and pH adjusted to ~2 with 1M HCl (aq). The mixture was extracted with EtOAc (3×), the organic phase was washed with brine and dried with $MgSO_4$. FC (CH_2Cl_2/MeOH/AcOH 100:5:2) gave **6** (313 mg, 75%) as an oil. TLC (CH_2Cl_2/MeOH/AcOH 100:5:2) R_f 0.25; $\delta_H(CDCl_3)$ 1.39 and 1.45 (br s and steep s, 9H), 1.91 (s, CH_3, 3H), 5.35 (s, CH_2, 2H), 6.68 (s, H-4, 1H), 7.26-7.42 (m, 5H).

3-Benzyloxy-4-bromoisothiazole (7b).

3-Benzyloxyisothiazole (**4**) (590 mg, 3 mmol) was dissolved in dry MeCN (10 mL) and NBS (588 mg, 3.3 mmol) was added. The mixture was left stirring for 7 d at rt. The mixture was concentrated to approx. 1 mL and EtOAc was added. The organic solution was washed with H_2O (2×), followed by brine and dried using $MgSO_4$. Concentration *in vacuo* followed by FC (petroleum ether/toluene 2:1 - 1:1) gave pure **7b** (729 mg, 88%) as a clear oil. (Found: 269.9597 [M+H$^+$]. Calc. for $C_{10}H_9BrNOS$: 269.9588 [M+H$^+$]); TLC (petroleum ether/toluene 1:1) R_f 0.4; $\delta_H(CDCl_3)$ 5.40 (s, 2H), 7.27-7.43 (m, 5H), 8.20 (s, 1H); $\delta_C(CDCl_3)$ δ. 71.0 (CH_2), 96.4 (C), 127.7 (CH), 128.0 (CH), 128.3 (CH), 135.9 (C), 146.0 (CH), 164.8 (C); m/z (EI) 269 (3%, M$^+$), 190, 106, 91.

3-Benzyloxy-4-methylisothiazole (7c).

7b (540 mg, 2 mmol), $PdCl_2(PPh_3)_2$ (140 mg, 0.1 mmol), $MeB(OH)_2$ (360 mg, 6 mmol) and dry K_2CO_3 (1.44 g, 10 mmol) was placed in a vial. DMF (10 mL) was added and the vial was sealed with a cap. The inhomogeneous mixture was heated using a microwave apparatus (160 ˚C, 5 min) and then cooled to 25 ˚C. Et_2O and H_2O was added. Four reactions under the above-mentioned conditions were run in parallel and combined. The organic and aqueous phases were separated and the aqueous phase was further extracted twice with Et_2O. The combined organic phases were washed with brine, dried with $MgSO_4$, concentrated *in vacuo* and purified by FC (petroleum ether/toluene 1:0 - 1:1) giving **7c** (531 mg, 32%) as a clear oil (the presence of 3-benzyloxyisothiazole was estimated to 3% from NMR). (Found: 206.0629 [M+H$^+$]. Calc. for $C_{11}H_{12}NOS$: 206.0640 [M+H$^+$]); TLC (petroleum ether/toluene 1:1) R_f 0.21; $\delta_H(CDCl_3)$ 2.08 (s, 3H), 5.41 (s, 2H), 7.28-7.43 (m, 5H), 7.95 (s, 1H); $\delta_C(CDCl_3)$ 11.40 (CH_3), 70.4 (CH_2), 121.8 (C), 127.9 (CH), 128.1 (CH), 128.6 (CH), 137.1 (C), 143.8 (CH), 167.7 (C); m/z (EI) 205 (11%, M$^+$), 188, 106, 91.

4-Benzyl-3-(benzyloxy)isothiazole (7f).

The title compound was prepared according to the procedure described for **7d** starting with (3-benzyloxy-4-isothiazolyl)phenylmethanol (**9f**) (948 mg, 3.2 mmol). FC (FP, petroleum ether/toluene 1:1) gave **7f** as a yellow oil (485 mg, 54%). (Found: 282.0977 [M+H$^+$]. Calc. for $C_{17}H_{16}NOS$: 282.0953 [M+H$^+$]); TLC (petroleum ether/toluene 1:1) R_f 0.24; $\delta_H(CDCl_3)$ 3.80 (s, 2H), 5.41 (s, 2H), 7.20-7.36 (m, 10H), 7.88 (s, 1H); $\delta_C(CDCl_3)$ 32.5 (CH_2), 70.3 (CH_2), 125.7 (C), 126.4 (CH), 127.8 (CH),

128.0 (CH), 128.5 (CH), 128.6 (CH), 128.9 (CH), 136.9 (C), 139.2 (C), 144.7 (CH), 166.9 (C); m/z (EI) 281 (11%, M^+), 190, 106, 91.

3-Benzyloxy-4-(phenylethyl)isothiazole (7g).

The title compound was prepared according to the procedure described for **7d** starting with 1-(3-benzyloxy-4-isothiazolyl)-2-phenylethanol (**9g**) (255 mg, 0.8 mmol). FC (FP, petroleum ether/toluene 1:1) gave **7g** as a clear oil (157 mg, 65%). (Found: 296.1092 [$M+H^+$]. Calc. for $C_{18}H_{18}NOS$: 296.1109 [$M+H^+$]); TLC (petroleum ether/toluene 1:1) R_f 0.22; $\delta_H(CDCl_3)$ 2.79-2.90 (m, 4H), 5.42 (s, 2H), 7.11-7.43 (m, 10H), 7.91 (s, 1H); $\delta_C(CDCl_3)$ 28.1 (CH_2), 35.2 (CH_2), 70.3 (CH_2), 125.6 (C), 126.1 (CH), 127.8 (CH), 128.1 (CH), 128.5 (CH, 128.46), 128.5 (CH, 128.53), 137.0 (C), 141.3 (C), 143.8 (CH), 167.2 (C); m/z (EI) 295 (11%, M^+), 204, 106, 91.

(3-Benzyloxy-4-isothiazolyl)methanol (9c).

Method A: 3-Benzyloxy-4-isothiazolylcarbaldehyde (**10**) (1.00 g, 4.6 mmol) was dissolved in MeOH (18 mL) and cooled to 0 ˚C. $NaBH_4$ (0.42 g, 5.9 mmol) was added over 20 min. The mixture was left stirring for 1 h at 0 ˚C followed by the addition of H_2O and concentrated *in vacuo*. The mixture was redissolved in $CHCl_3$ and washed with brine. The organic phase was concentrated *in vacuo* giving crude **9c** (0.99 g). TLC (petroleum ether/EtOAc 1:1) R_f 0.6; $\delta_H(CDCl_3)$ 2.95 (br s, 1H), 4.49 (s, 2H), 5.38 (s, 2H), 7.29-7.40 (m, 5H), 8.25 (s, 1H); $\delta_C(CDCl_3)$ 57.1 (CH_2), 70.5 (CH_2), 125.6 (C), 128.0 (CH), 128.2 (CH), 128.5 (CH), 136.4 (C), 145.6 (CH), 166.2 (C).

Method B: 8 (200 mg, 0.6 mmol) was dissolved in dry THF (5 mL) under N_2. The mixture was cooled to -30 ˚C and *i*PrMgCl in THF (0.38 mL, 2M, 0.8 mmol) was added to the mixture. The mixture was slowly warmed to 0 ˚C. Paraformaldehyde (170 mg, 5.7 mmol) was added in one portion and the mixture was left stirring over night at rt. The reaction was quenched with satd. NH_4Cl (aq) (1 mL) and additional H_2O (4 mL). The pH was adjusted to pH ~ 7 using 1M HCl followed by extraction with EtOAc (3×). The combined organic phases were washed with brine and dried with $MgSO_4$. FC (toluene/EtOAc 9:1) gave pure **9c** (50 mg, 36%). The NMR data were consistent with the above-mentioned data.

(3-Benzyloxy-4-isothiazolyl)phenylmethanol (9f).

The title compound was prepared according to the procedure described for **9d** starting with **8** (1 g, 3.2 mmol) and benzaldehyde (0.35 mL, 368 mg, 3.5 mmol). FC (F, petroleum ether/EtOAc) gave **9f** as a yellow oil (811 mg, 86%). (Found: 298.0927 [$M+H^+$]. Calc. for $C_{17}H_{16}NO_2S$: 298.0902 [$M+H^+$]); TLC (petroleum ether/EtOAc 2:1) R_f 0.3; $\delta_H(CDCl_3)$ 2.98 (br s, 1H), 5.36 (s, 2H), 5.75 (s, 1H), 7.23-7.35 (m, 10H), 8.15 (s, 1H); $\delta_C(CDCl_3)$ 69.9 (CH), 70.5 (CH_2), 126.5 (CH), 127.8 (CH), 127.9 (CH), 128.1 (CH), 128.4 (CH), 128.5 (CH), 128.7 (C), 136.3 (C), 141.8 (C), 145.2 (CH), 165.5 (C).

1-(3-Benzyloxy-4-isothiazolyl)-2-phenylethanol (9g).

The title compound was prepared according to the procedure described for **9d**. Due to a low yield of 26% using the iodo compound **8** and freshly prepared phenylacetaldehyde[6], the bromo compound **7b** (2 g, 7.4 mmol) and commercially

available stabilized phenylacetaldehyde (934 mg, 7.8 mmol) was used instead. FC (F, petroleum ether/EtOAc) gave **9g** as a yellow oil (995 g, 43%). (Found: 312.1072 [M+H$^+$]. Calc. for $C_{18}H_{18}NO_2S$: 312.1058 [M+H$^+$]); TLC (petroleum ether/EtOAc 2:1) R_f 0.3; $\delta_H(CDCl_3)$ 2.67 (br s, 1H), 2.87 (dd, *J* 8 and 14, 1H), 3.10 (dd, *J* 4 and 14, 1H), 4.87 (dd, *J* 4 and 8, 1H), 5.39 (s, 2H), 7.08-7.39 (m, 10H), 8.15 (s, 1H); $\delta_C(CDCl_3)$ 43.5, 68.9, 70.5, 126.6, 127.9, 128.2, 128.4, 128.5, 128.6, 129.5, 136.5, 137.7, 144.6, 165.5.

3-Benzyloxy-4-isothiazolylcarbaldehyde (10).

The title compound was prepared according to the procedure described for **9d** starting with **8** (2.00 g, 6.3 mmol) and DMF (0.64 mL, 8.2 mmol). The mixture was left stirring at rt for 2 h. The reaction was worked-up according to **9d**. FC (petroleum ether/EtOAc 9:1) gave **10** as a white solid (1.13 g, 82%). Mp. 60-61 ˚C (EtOAc/petroleum ether); (Found: 220.0432 [M+H$^+$]. Calc. for $C_{11}H_{10}NO_2S$: 220.0432 [M+H$^+$]; TLC (petroleum ether/EtOAc 9:1) R_f 0.15; $\delta_H(CDCl_3)$ 5.53 (s, 2H), 7.36-7.49 (m, 5H), 9.21 (s, 1H), 9.90 (s, 1H); $\delta_C(CDCl_3)$ 71.2 (CH_2), 124.9 (C), 128.4 (CH), 128.7 (CH), 128.8 (CH), 136.0 (C), 155.5 (CH), 168.6 (C), 183.2 (C).

(3-Benzyloxy-4-isothiazolyl)methanesulfonic acid methyl ester (11).

9c (100 mg, 0.45 mmol) was dissolved in THF (2 mL) and cooled to 0 ˚C. Et_3N (0.08 mL, 0.59 mmol) was added followed by the addition of MsCl (0.04 mL, 0.54 mmol). The turbid white mixture was left stirring for 90 min at 0 ˚C and quenched with H_2O (2 mL). The mixture was extracted with Et_2O, the combined organic phases were washed with brine, dried with $MgSO_4$ and concentrated *in vacuo* giving crude **11** (141 mg). $\delta_H(CDCl_3)$ 2.86 (s, 3H), 3.60 (s, impurity), 5.12 (s, 2H), 5.54 (s, 2H), 7.31-7.44 (m, 5H), 8.58 (s, 1H); $\delta_C(CDCl_3)$ 37.8 (CH_3), 52.6 (impurity), 62.6 (CH_2), 70.9 (CH_2), 118.4 (C), 128.2 (CH), 128.4 (CH), 128.6 (CH), 136.1 (C), 150.3 (CH), 166.5 (C).

3-Benzyloxy-4-(chloromethyl)isothiazole (12).

9c (100 mg, 0.45 mmol) was placed in a dry flask. $SOCl_2$ (2.5 mL) was added. After 5 min at rt the reaction mixture was concentrated *in vacuo* giving crude **12** (108 mg). $\delta_H(CDCl_3)$ 4.47 (s, 2H), 5.44 (s, 2H), 7.30-7.45 (m, 5H), 8.41 (s, 1H); $\delta_C(CDCl_3)$ 36.6 (CH_2), 70.7 (CH_2), 122.2 (C), 127.9 (CH), 128.2 (CH), 128.6 (CH), 136.5 (C), 148.0 (CH), 166.1 (C).

2-(3-Benzyloxy-4-bromo-5-isothiazolyl)-2-(*N*-*tert*-butoxycarbonylamino)malonic acid diethyl ester (13b).

Freshly prepared LDA (2.4 mmol) in Et_2O (20 mL) was added to **7b** (511 mg, 1.9 mmol) in Et_2O (2 mL) over 30 min at -78 ˚C. The mixture was left stirring at -78 ˚C for 10 min. 2-(*N*-*tert*-Butoxycarbonylimino)malonic acid diethyl ester (626 mg, 2.3 mmol) in Et_2O (2 mL) was added over 3 min and the mixture was left at -78 ˚C for 1 h. The reaction was quenched with satd. NH_4Cl (aq) at -78 ˚C and worked-up according to the procedure described for **13a**. FC (petroleum ether/toluene/EtOAc 4:4:1) yielded **13b** as a yellow oil (596 mg, 56%). TLC (petroleum ether/toluene/EtOAc 4:4:1) R_f 0.3; $\delta_H(CDCl_3)$ 1.25 (t, *J* 7, 2×CH_3, 6H), 1.40 (s, *t*Bu, 9H), 4.21-4.35 (m, 2×CH_2, 4H), 5.43 (s, CH_2, 2H), 6.68 (br s, NH, 1H), 7.31-7.45 (m,

5H); δ_C($CDCl_3$) 14.0 (CH_3), 28.2 (CH_3), 64.0 (CH_2), 66.2 (C), 70.5 (CH_2), 81.2 (C), 95.8 (C), 127.7 (CH), 128.0 (CH), 128.4 (CH), 136.1 (C), 153.1 (C), 156.1 (C), 163.2 (C), 165.1 (C).

2-(3-Benzyloxy-4-methyl-5-isothiazolyl)-2-(*N*-*tert*-butoxycarbonylamino)malonic acid diethyl ester (13c).

The title compound was prepared according to the procedure described for **13a** starting with (500 mg, 2.4 mmol) of **7c**. FC (FP, petroleum ether/toluene/EtOAc 0:1:0 - 4:4:1) gave **13c** as a yellow oil (593 mg, 51%). TLC (petroleum ether/toluene/EtOAc 4:4:1) R_f 0.22; δ_H($CDCl_3$) 1.25 (t, *J* 7, CH_3, 6H), 1.38 (s, *t*Bu, 9H), 2.07 (s, CH_3, 3H), 4.20-4.35 (m, 4H, 2×CH_2), 5.39 (s, CH_2, 2H), 6.48 (br s, NH, 1H), 7.30-7.44 (m, phenyl, 5H); δ_C($CDCl_3$) 10.5 (CH_3), 13.9 (CH_3), 28.1 (CH_3), 63.6 (CH_2), 66.2 (C), 69.7 (CH_2), 81.1 (C), 119.7 (C), 127.8 (CH), 127.9 (CH), 128.4 (CH), 137.0 (C), 153.3 (C), 154.9 (C), 165.9 (C), 166.2 (C).

2-(3-Benzyloxy-4-ethyl-5-isothiazolyl)-2-(*N*-*tert*-butoxycarbonylamino)malonic acid diethyl ester (13d).

7d (468 mg, 2.1 mmol) in Et_2O (2 mL) was added over 15 min to freshly prepared LDA (2.6 mmol) in Et_2O (22 mL) at -78 ˚C. The mixture was left stirring at -78 ˚C for 1 h 15 min. 2-(*N*-*tert*-Butoxycarbonylimino)malonic acid diethyl ester (642 g, 2.6 mmol) in Et_2O (2 mL) was added over 5 min and the mixture was left at -78 ˚C for 90 min. The reaction was quenched with satd. NH_4Cl (aq) at -78 ˚C and worked-up according to the procedure described for **13a**. FC (toluene/petroleum ether/EtOAc 4:4:1) yielded **13d** as a yellow oil (397 mg, 38%). Starting material **7d** was recovered (277 mg, 59%). TLC (petroleum ether/toluene/EtOAc 4:4:1) R_f 0.20. δ_H($CDCl_3$) 1.04 (t, *J* 7, CH_3, 3H), 1.27 (t, *J* 7, 2×CH_3, 6H), 1.40 (s, *t*Bu, 9H), 2.53 (q, *J* 7, CH_2, 2H), 4.20-4.38 (m, 2×CH_2, 4H), 5.41 (s, CH_2, 2H), 6.50 (br s, NH, 1H), 7.31-7.45 (m, phenyl, 5H); δ_C($CDCl_3$) 13.2 (CH_3), 14.1 (CH_3), 18.9 (CH_2), 28.3 (CH_3), 63.7 (CH_2), 66.1 (C), 69.7 (CH_2), 77.4 (C), 125.6 (C), 127.7 (CH), 127.8 (CH), 128.4 (CH), 137.0 (C), 154.3 (C), 166.1 (C), 166.3 (C).

2-(3-Benzyloxy-4-phenyl-5-isothiazolyl)-2-(*N*-*tert*-butoxycarbonylamino)malonic acid diethyl ester (13e).

7e (681 mg, 2.5 mmol) in Et_2O (1 mL) was added over 15 min to freshly prepared LDA (3.1 mmol) in Et_2O (25 mL) at -78 ˚C. 2-(*N*-*tert*-Butoxycarbonylimino)malonic acid diethyl ester (766 mg, 2.8 mmol) in Et_2O (2 mL) was added over 15 min and the mixture was left at -78 ˚C for 1 h and 45 min. The reaction was quenched with satd. NH_4Cl (aq) at -78 ˚C and worked-up according to the procedure described for **13a**. FC (toluene/petroleum ether/EtOAc 4:4:1) yielded **13e** as a yellow oil (850 mg, 62%). TLC (petroleum ether/toluene/EtOAc 4:4:1) R_f 0.22; δ_H($CDCl_3$) 1.22 (t, *J* 7, 2×CH_3, 6H), 1.34 (s, *t*Bu, 9H), 4.09-4.28 (m, 2×CH_2, 4H), 5.37 (s, CH_2, 2H), 5.80 (br s, NH, 1H), 7.02-7.36 (m, 10H); δ_C($CDCl_3$) 14.0 (CH_3), 28.3 (CH_3), 63.7 (CH_2), 66.3 (C), 69.7 (CH_2), 80.7 (C), 124.8 (C), 127.2 (CH), 127.6 (CH), 128.0 (CH), 128.2 (CH), 129.9 (CH), 132.4 (C), 137.0 (C), 152.9 (C), 157.4 (C), 164.8 (C), 165.9 (C).

2-(4-Benzyl-3-benzyloxy-5-isothiazolyl)-2-(*N*-*tert*-butoxycarbonylamino)malonic acid diethyl ester (13f).

Freshly prepared LDA (2.2 mmol) in Et_2O (10 mL) was added to **7f** (478 mg, 1.7 mmol) in Et_2O (10 mL) over 10 min at -78 ˚C. The mixture was left at -78 ˚C for 5 min. 2-(*N*-*tert*-Butoxycarbonylimino)malonic acid diethyl ester (604 mg, 2.2 mmol) in Et_2O (2 mL) was added and the mixture left stirring for 1 h. The reaction was quenched with satd. NH_4Cl (aq) at -78 ˚C and worked-up according to the procedure described for **13a**. FC (toluene/petroleum ether/EtOAc 4:4:1) yielded **13f** as a yellow oil (580 mg, 62%). TLC (petroleum ether/toluene/EtOAc 4:4:1) R_f 0.20; $\delta_H(CDCl_3)$ 1.17 (t, *J* 7, 2×CH_3, 6H), 1.32 (s, *t*Bu, 9H), 3.93 (s, CH_2, 2H), 4.03 (dq, *J* 7 and 11, CH_2, 2H), 4.22 (m, CH_2, 2H), 5.35 (s, CH_2, 2H), 6.40 (br s, NH, 1H), 6.98-7.23 (m, 10H); $\delta_C(CDCl_3)$ 13.8 (CH_3), 28.1 (CH_3), 30.4 (CH_2), 63.6 (CH_2), 66.1 (C), 69.6 (CH_2), 81.1 (C), 122.0 (C), 125.8 (CH), 127.5 (CH), 127.7 (CH), 128.0 (CH), 128.2 (CH), 136.8 (C), 139.1 (C), 156.7 (C), 166.1 (C), 166.4 (C).

2-(3-Benzyloxy-4-phenethyl-5-isothiazolyl)-2-(*N*-*tert*-butoxycarbonylamino)malonic acid diethyl ester (13g).

Freshly prepared LDA (1.75 mmol) in Et_2O (10 mL) was added to **7g** (398 mg, 1.35 mmol) in Et_2O (10 mL) over 10 min at -78 ˚C. The mixture was left at -78 ˚C for 5 min. 2-(*N*-*tert*-Butoxycarbonylimino)malonic acid diethyl ester (479 mg, 1.75 mmol) in Et_2O (2 mL) was added and the mixture left stirring for 1 h. The reaction was quenched with satd. NH_4Cl (aq) at -78 ˚C and worked-up according to the procedure described for **13a**. FC (toluene/petroleum ether/EtOAc 4:4:1) yielded **13g** as a yellow oil (418 mg, 55%). TLC (petroleum ether/toluene/EtOAc 4:4:1) R_f 0.27; $\delta_H(CDCl_3)$ 1.24 (t, *J* 7, 2×CH_3, 6H), 1.40 (s, *t*Bu, 9H), 2.69-2.79 (m, 4H), 4.18-4.25 (m, CH_2, 2H), 4.32-4.36 (m, CH_2, 2H), 5.43 (s, CH_2, 2H), 6.58 (br s, NH, 1H), 7.17-7.47 (m, 10H); $\delta_C(CDCl_3)$ 13.9 (CH_3), 28.2 (CH_3), 28.4 (CH_2), 35.0 (CH_2), 63.7 (CH_2), 66.1 (C), 69.8 (CH_2), 81.3 (C), 123.7 (C), 126.0 (CH), 127.7 (CH), 127.9 (CH), 128.5 (CH), 137.1 (C), 142.0 (C), 155.0 (C), 166.1 (C), 166.3 (C).

(*RS*)-(3-Benzyloxy-4-bromo-5-isothiazolyl)-(*N*-*tert*-butoxycarbonylamino)acetic acid (14b).

The title compound was prepared according to the procedure described for **6** starting with **13b** (505 mg, 0.93 mmol) and a reaction time of 3 h. FC (CH_2Cl_2/MeOH/AcOH 100:5:2) gave **14b** as a light yellow foam (361 mg, 88%). TLC (CH_2Cl_2/MeOH/AcOH 100:5:2) R_f 0.23; $\delta_H(CDCl_3)$ 1.32 (br s, *t*Bu, 9H), 5.42-5.54 (m, CH_2, α-H, NH, 4H), 7.33-7.49 (m, 5H), 8.18 (d, *J* 4, COOH, 1H); $\delta_C(CDCl_3)$ 28.3 (CH_3), 53.9 (CH), 70.8 (CH_2), 83.4 (C), 97.7 (C), 127.9 (CH), 128.2 (CH), 128.5 (CH), 136.0 (C), 156.7 (C), 160.6 (C), 164.4 (C), 170.1 (C).

(*RS*)-(3-Benzyloxy-4-methyl-5-isothiazolyl)-(*N*-*tert*-butoxycarbonylamino)acetic acid (14c).

The title compound was prepared according to the procedure described for **6** starting with **13c** (362 mg, 0.76 mmol) and a reaction time of 4 h. FC (CH_2Cl_2/MeOH/AcOH 100:5:2) gave **14c** (285 mg, *quant.*). Recrystallization (EtOAc/petroleum ether) of a sample gave colorless crystals. Mp. 126.3-126.5 ˚C; (Found: C, 56.9; H, 6.1; N, 7.0. Calc. for $C_{18}H_{22}N_2O_5S$: C, 57.1; H, 5.9; N, 7.4%); TLC (CH_2Cl_2/MeOH/AcOH

100:5:2) R_f 0.3; $\delta_H(CDCl_3)$ 1.30 and 1.45 (2×br s, *t*Bu, 9H), 2.16 (s, CH_3, 3H), 5.38-5.62 (m, CH_2, α-H, NH, 4H), 7.32-7.45 (m, phenyl, 5H), 7.99 (br s, COOH, 1H).

(*RS*)-(3-Benzyloxy-4-ethyl-5-isothiazolyl)-(*N*-*tert*-butoxycarbonylamino)acetic acid (14d).

The title compound was prepared according to the procedure described for **6** starting with **13d** (397 mg, 0.81 mmol) and a reaction time of 4 h. FC (CH_2Cl_2/MeOH/AcOH 100:5:2) gave **14d** (245 mg, 78%). Recrystallization (EtOAc/petroleum ether) of a sample gave colorless crystals. Mp. 142-143 ˚C; (Found: C, 58.05; H, 6.2; N, 7.0. Calc. for $C_{19}H_{24}N_2O_5S$: C, 58.15; H, 6.2; N, 7.1%); TLC (CH_2Cl_2/MeOH/AcOH 100:5:2) R_f 0.4; $\delta_H(CDCl_3)$ 1.19 (t, *J* 7, CH_3, 3H), 1.33 and 1.46 (2×br s, *t*Bu, 9H), 2.59-2.73 (m, CH_2, 2H), 5.42-5.64 (m, CH_2, α-H, NH, 4H), 7.30-7.45 (m, phenyl, 5H), 8.09 (d, *J* 2, COOH, 1H); $\delta_C(CDCl_3)$ 13.4 (CH_3), 19.0 (CH_2), 28.4 (CH_3), 52.8 (CH), 70.0 (CH_2), 83.0 (C), 125.8 (C), 127.8 (CH), 128.0 (CH), 128.5 (CH), 136.9 (C), 156.7 (C), 158.3 (C), 167.0 (C), 170.9 (C).

(*RS*)-(3-Benzyloxy-4-phenyl-5-isothiazolyl)-(*N*-*tert*-butoxycarbonylamino)acetic acid (14e).

The title compound was prepared according to the procedure described for **6** starting with **13e** (728 mg, 1.35 mmol) and a reaction time of 4½ h. FC (CH_2Cl_2/MeOH/AcOH 100:5:2) gave **14e** (581 mg, 98%). Recrystallization (EtOAc/petroleum ether) of a sample gave colorless crystals. Mp. 146.5-147.5 ˚C; (Found: C, 62.6; H, 5.5; N, 6.2. Calc. for $C_{23}H_{24}N_2O_5S$: C, 62.7; H, 5.5; N, 6.4%); TLC (CH_2Cl_2/MeOH/AcOH 100:5:2) R_f 0.4; $\delta_H(CDCl_3)$ 1.22 and 1.42 (2×br s, *t*Bu, 9H), 5.40-5.62 (m, CH_2, α-H, NH, 4H), 7.26-7.46 (m, 8H), 7.60-7.62 (m, 2H), 7.93-7.94 (br s, COOH, 1H); $\delta_C(CDCl_3)$ 28.4 (CH_3), 52.7 (CH), 70.2 (CH_2), 83.1 (C), 125.2 (C), 127.6 (CH), 127.8 (CH), 128.4 (CH), 129.8 (CH), 131.5 (C), 136.7 (C), 156.5 (C), 160.8 (C), 165.6 (C), 170.0 (C).

(*RS*)-(4-Benzyl-3-benzyloxy-5-isothiazolyl)-(*N*-*tert*-butoxycarbonylamino)acetic acid (14f).

The title compound was prepared according to the procedure described for **6** starting with **13f** (550 mg, 1.00 mmol) and a reaction time of 4 h. FC (toluene/EtOH/AcOH 100:5:2) gave **14f** (448 mg, 99%). Recrystallization (EtOAc/petroleum ether) of a sample gave colorless crystals. Mp. 142.8-143.1 ˚C; (Found: C, 62.4; H, 5.75; N, 5.7. Calc. for $C_{24}H_{26}N_2O_5S{\cdot}0.5\ H_2O$: C, 62.2; H, 5.9; N, 6.0%); TLC (toluene/EtOH/AcOH 100:5:2) R_f 0.12; $\delta_H(CDCl_3)$ 1.24 and 1.43 (2×br s, *t*Bu, 9H), 4.00 (m, CH_2, 2H), 5.36-5.65 (m, CH_2, α-H, NH, 3.4H), 7.19-7.31 (m, 10H), 7.83 (br s, COOH, 0.5H).

(*RS*)-(3-Benzyloxy-4-phenethyl-5-isothiazolyl)-(*N*-*tert*-butoxycarbonylamino)acetic acid (14g). The title compound was prepared according to the procedure described for **6** starting with **13g** (384 mg, 0.68 mmol) and a reaction time of 4 h. FC (toluene/EtOH/AcOH 100:5:2) gave **14g** (267 mg, 84%). TLC (CH_2Cl_2/MeOH/AcOH 100:5:2) R_f 0.4; $\delta_H(CDCl_3)$ 1.32 and 1.44 (2×br s, *t*Bu, 9H), 2.87 (br s, 2×CH_2, 4H), 5.20-5.42 (m, CH_2, α-H, NH, 4H), 7.12-7.45 (m, 10H).

References

1. S. N. Lewis, G. A. Miller, M. Hausman and E. C. Szamborski, *J. Heterocycl. Chem.*, 1971, **8**, 571-580; L. Bunch, P. Krogsgaard-Larsen and U. Madsen, *J. Org. Chem.*, 2002, **67**, 2375-2377.
2. P. Calí and M. Begtrup, *Synthesis*, 2002, 63-66.
3. H. Lin and L. A. Paquette, *Synth. Commun.*, 1994, **24**, 2503-2506; J. Suffert, *J. Org. Chem.*, 1989, **54**, 509-510.
4. T. Shedlovsky and R. L. Kay, *J. Phys. Chem.*, 1956, **60**, 151-155; R. Ruiz, C. Ràfols, M. Rosés and E. Bosch, *J. Pharm. Sci.*, 2003, **92**, 1473-1481.
5. ^{1}H NMR in accordance with L. Van den Broek, M. L. Breuer, R. M. J. Liskamp, H. C. J. Ottenheijm, *J. Org. Chem.* 1987, **52**, 1511-1517
6. L. De Luca, G. Giacomelli and A. Porcheddu, *Org. Lett.*, 2001, **3**, 3041-3043.